POSSIBLE TRIGGERING MECHANISMS FOR THE COLLAPSE OF *Aphanizomenon flos-aquae* (L.) RALFS BLOOMS

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Master of Science

by

Annette Marie Coulombe

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INTRODUCTION

In freshwaters, the planktonic blue-green algae as a group generally exhibit characteristic cyclic growth; they bloom in large masses usually during the summer and they often spontaneously die off and lyse (Shilo, in Padan and Shilo, 1973). These sudden declines are often associated with a change in the dominant genus of the blue-green alga (Reynolds, 1975). Mechanisms such as photooxidation, O_2 toxicity, development of pathogens, autoinhibition, along with many other possibilities have been suggested as possible causes for such declines.

Collapsing *Aphanizomenon flos-aquae* (L.) Ralfs blooms and the resulting O_2 depletion in the water column cause massive fish kills in shallow eutrophic lakes across the North American prairie region and are a serious setback for the fisheries industry (Barica, 1978). The purpose of this investigation has been to examine some of the possible triggering mechanisms for the collapse of such blooms in small pothole lakes in the Erickson-Elphinstone area, Southwestern Manitoba, Canada. Photooxidation, O_2 toxicity and cyanophage infection were considered.

CHAPTER 1

LITERATURE REVIEW

LITERATURE REVIEW

Summer fish kills in shallow, landlocked lakes of the Erickson-Elphinstone area, southwestern Manitoba, have been reported to be caused by the collapses of heavy *Aphanizomenon flos-aquae* (L.) Ralfs blooms, and subsequent oxygen depletion (Barica, 1975). Nutrient cycling and regeneration studies (Barica, 1974;1975) have demonstrated that the major release of nutrients, namely NH_3-N (ammonia) and SRP (soluble reactive phosphorus), takes place regularly in late winter during anoxic conditions under the ice. The magnitude of release was found to be two to three times greater in so-called "summerkill" lakes than in "non-summerkill" lakes. In the summer, nonsummerkill lakes show relatively stable chlorophyll a, nutrient and DO (dissolved oxygen) levels, while in summerkill lakes they oscillate, with chlorophyll a increasing exponentially to over 100 µg/l within one to two weeks. This may be followed within one to several weeks by a sudden collapse of the algal population. The algal collapse has always been preceded by a peak in the chlorophyll a level and DO concentrations and consequently minimum Secchi disc transparency and nutrient levels (Barica, 1975).

A partial collapse has been defined (Barica, 1978) as a rapid reduction of the algal biomass by at least 70 µg/l chlorophyll a per week, causing no dangerous oxygen depletion (not below 5 mg/l DO). A total collapse has

been defined as an algal collapse accompanied by a substantial oxygen depletion (below 5 mg/l DO) causing only partial fish mortalities (50-80%), and having a reduction of algal biomass by more than 70 µg/l per week of chlorophyll a with usually less than 20 µg/l of chlorophyll a remaining. A total collapse with fish kill is an algal collapse as above but accompanied by an oxygen depletion below the level required by rainbow trout (3.0 mg/l) resulting in fish mortalities greater than 90%.

Attempts have been made to study the influence of changing weather conditions on the collapse of *Aphanizomenon* blooms (Barica, 1978; Van Nguyen and Woods, 1979). Barica concluded that algal collapses coincided with a significant drop in air temperature and that sudden cooling with accompanying overcast after hot sunny periods triggered the algal collapse. Since a collapse can result in no lake anoxia, anoxia only after a time lag, or immediate oxygen depletion, bloom collapses and lake anoxia should not be thought of as a single event (Papst et al. in press).

In a more recent study (Papst et al. in press) it has been demonstrated that the occurrence of lake anoxia is dependent on the erosion of thermal stability at times coincident with or subsequent to a period of algal bloom collapse and further, the thermal stability of the shallow prairie lakes is a function of the amount of solar radiation, the air temperature and the wind stress. The hypothesis that both thermal instability and algal bloom col-

lapse are necessary conditions for lake anoxia, can be used to explain partial collapses which occur when a period of algal biomass decline, sufficient to cause lake anoxia, is coincidental with a period of thermal stability. Observed time lags between algal bloom collapse and lake anoxia result when a period of thermal stability occurs after the collapse of the bloom.

Four blooms of *Aphanizomenon* were observed in detail by Healey and Hendzel (1976). In two instances, L885 and L587, in 1974, the period of algal exponential growth was followed by a collapse of the bloom within a week of the end of the most rapid growth. In L885, during the previous year, termination of exponential growth was followed by a prolonged period of stability before the collapse occurred. In a fourth case, L154, in 1974, no major collapse occurred during the period of observation. The course of the bloom in L885 during the summer of 1974 could be divided into three phases on the basis of population growth (represented as chlorophyll a). Following the appearance of *Aphanizomenon* colonies, in the first week of June, the population remained relatively low until early July. During at least part of this period, the algae showed some characteristics of nutrient deficiency. During the first half of July, higher values of SRP and NH_3-N were found. This nutrient input may have been responsible for the ensuing exponential growth of the alga over a period of three weeks. During the early

part of this growth period, N and P deficiency were lessened. However, as rapid growth continued the characteristics of nutrient deficiency reappeared. By the time growth ceased and collapse was imminent, the low P content and high P debt indicated P deficiency. At the same time, there were indications of N deficiency.

The bloom in L154, 1974 differed from the other three studied by Healey and Hendzel(1976), in that no major collapse occurred during the period of observation. During late June, the population increased exponentially, approximately doubling every three days and then remained relatively constant through July and into August. Following the termination of their observations, the bloom gradually declined. Unlike the other three blooms studied, there was no indication of nutrient deficiency in L154 when rapid growth ceased. Exponential growth seems to have been stopped by something other than P or N limitation. This bloom eventually became as nutrient deficient as blooms that collapsed, although RNA and protein to carbohydrate ratios were marginally higher.

The authors of this paper state that although neither indicators of impending collapse nor the factors triggering the collapse were identified, the results showed that general characteristics of nutrient deficiency were developed before each collapse. P-deficiency and to a lesser degree N-deficiency were probably important in setting the stage for the collapse.

In freshwaters, the blue-green algae as a group generally exhibit characteristic cyclic growth; they bloom in large masses usually during the summer and they often spontaneously die off and lyse (Shilo, in Padan and Shilo, 1973). These declines are often associated with a change in the dominant genus of blue-green alga (Reynolds, 1975). Mechanisms such as photooxidation, O_2 toxicity, development of pathogens, autoinhibition, along with many other possibilities, have been suggested as possible causes for these declines.

Oxygen Toxicity

As the *Aphanizomenon* blooms in these small prairie lakes progress, the DO in the upper layers increases (Barica, 1975). High oxygen concentrations are known to be toxic (Haugaard, 1968). Although oxygen is the oxidizing agent with the highest oxidation potential, it is at the same time one of the most sluggish of oxidants (Barron, in Haugaard, 1968). The bond between the oxygen atoms is so strong that oxidation by oxygen must be started by the formation of free radicals from the oxidizable substrate. This can occur at high temperatures, or under the influence of agents such as light, heavy metals or ionizing radiation. There is considerable evidence that free radicals are formed on oxidation of certain tissue constituents by molecular O_2 (in Haugaard, 1968). Presumably the cellular concentration of such

radicals is increased when the O_2 tension is raised. These highly reactive molecules may inactivate enzymes or destroy other vital tissue components by combining with or oxidizing sulfhydryl groups and other cellular moieties. Alternatively, it may be proposed that free radicals formed during hyperbaric oxygenation interfere with the action of such free radicals that are normally produced in enzyme catalysis.

Examples of the types of compounds oxidized include glutathione, lipoic acid and coenzyme A. Although the more precise role of glutathione in cell function is unknown, it is generally believed that the compound is of importance in regulation of oxidative and reductive processes in the cell. Reduced glutathione activates a number of enzymes. It is present mainly in the reduced form and any large change towards oxidation can be expected to lead to severe disturbances of cell function.

Much of the support for the concept that free radicals are intermediates in the toxic effects of oxygen comes from the similarities that exist between the damaging effects of excess pressures of oxygen and radiation (Haugaard, 1968).

Sirenko et al. (1968), in their laboratory studies of *Aphanizomenon*, have observed that this alga apparently has specific oxidation-reduction systems that regulate the level of their oxygen saturation. The authors noted a negative correlation between the oxygen content of the

water and that of the algal clusters. An example of such an oxidation-reduction system is that formed by reduced ascorbic acid and compounds containing sulfhydryl groups. The hydrogen liberated on transition to disulfide groups has considerable reducing potential. A decrease in the reduced ascorbic acid content was accompanied by a decrease in the oxygen saturation of the algal clusters. Sirenko et al. (1968) state that the mucilaginous coating of blue-greens, which is rich in compounds of the latter type, may be an adaptive mechanism that came about with the appearance of photosynthesis and the concommitant evolution of O_2.

An enzyme known to protect the cell from high oxygen tension is superoxide dismutase (SOD). This enzyme catalyses the following reaction : $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. O_2^- can react further : $O_2^- + H_2O_2 \rightarrow OH^- + OH^\cdot + H_2O$, which vastly amplifies the potential dangers of O_2. Catalases and peroxidases may also protect the cell from oxygen tension since catalase catalyzes the following reaction: $2H_2O_2 \rightarrow 2H_2O + O_2^\cdot$ and peroxidase catalyzes : $AH_2 + H_2O_2 \rightarrow 2H_2O + A$.

High oxygen tension is known to inhibit both CO_2 and N_2 fixation (Stewart and Pearson, 1970). Paerl (1976, 1978a,b, 1979) and Paerl and Kellar (1977, 1978, 1979) have conducted several investigations of such processes in the blue-green alga *Anabaena* in O_2 supersaturated waters and have made the following observations:

(1) In the bloom-forming alga *Anabaena*, both CO_2 and N_2

fixation share a dependence on light. During daylight, *Anabaena* reduces direct competition between these processes for light-generated reductant by optimizing carbon fixation during late morning while optimizing N_2 fixation during afternoon hours. Biochemically, it is sound to optimize carbon prior to nitrogen fixation, due to the higher sensitivity of the former to afternoon increases in dissolved oxygen levels which commonly occur. It is also advantageous to first assure adequate supplies of fixed carbon prior to incorporating fixed N_2 (Paerl, 1979, Paerl and Kellar, 1979).

(2) *Anabaena* is commonly colonized by bacteria, especially during N_2 fixing blooms. The algal N_2 fixing abilities appear to be increased in the presence of such bacteria. It seems likely that polar regions of the heterocysts are susceptible to O_2 intrusion, especially during periods of photosynthetically elevated ambient concentration. Since most of the attached bacteria were found to occur in this region, reductive microzones induced by such associations may be effective insurance against O_2 intrusion. Algal excretions, some of which occur in polar regions, may serve to maintain the O_2 consuming bacteria (Paerl, 1978a, Paerl and Kellar, 1977, 1978). An association between the heterocysts of *Aphanizomenon* and attached bacteria has also been observed by Paerl (1976) and by Caldwell and Caldwell (1978).

(3) An indigenous mechanism allowing recovery of nitro-

genase activity during O_2 supersaturation has been observed (Paerl and Kellar, 1977, Paerl, 1978) in two species of N_2 fixing *Anabaena* . The process is light mediated and appears to employ photoreduction as a means of overcoming O_2 inhibition. Reductant in cells is considered to be photogenerated and to be accompanied by elevated rates of phosphorylation. This mechanism is proposed to represent optimization of radiant energy in that the algae can direct photoreductant from inhibiting O_2 evolving photosynthesis to energy demanding nitrogen fixation.

(4) Changes in pigment composition, in a nitrogen fixing species of *Anabaena,* were observed to occur with increasing oxygen tension (Paerl and Kellar, 1979). Phycocyanin, an accessory pigment associated with O_2 evolving photosystem II, was found to decrease when O_2 supersaturation and concommitant inhibition of photosystem II activity are maintained. If O_2 elevation was sustained for at least 12 hours, the cellular carotenoid content increased while the chlorophyll a content remained unchanged. Field observations were similar. As a bloom of *Anabaena spiroides* proceeded, accompanied by increased O_2 supersaturation, the ratio of carotenoid to chlorophyll a increased and the phycocyanin content tended to decrease. There is a two-fold purpose to such a decrease; namely that phycocyanin serves as a N_2 source under nitrogen stress and secondly that this reduction aids in minimizing O_2 production (by the inhibition of photosystem II activity, since phycocyanin is a

component of this system).

The permanent form of *Aphanizomenon* is the flake (O'Flaherty and Phinney, 1970), consisting of upwards of several hundred trichomes, each of a hundred or more cells (Horne, 1979). The ephemeral form consists of thousands of flakes in a ball often several cm. in diameter, which is easily broken by separation of the surrounding water. Horne (1979) proposes that at night the center of a large colony of respiring cells may be depleted in oxygen, which would both enhance N_2 fixation in the heterocysts and perhaps enable N_2 fixation in the vegetative cells.

When considering oxygen toxicity as a triggering factor for the collapses of the *Aphanizomenon* blooms described, it is important to note that there may occur vertical differences in oxygen concentration. Sirenko et al. (1968), in laboratory experiments with *Aphanizomenon*, have shown that clusters of the algae move from the more oxygen saturated surface layers of the water to the bottom layers and back again over the course of a day. Any diurnal variations in dissolved oxygen at the various depths must therefore also be considered.

In summary, O_2 toxicity has been proposed as one possible triggering mechanism for the collapses of the *Aphanizomenon* blooms described. However, studies on *Aphanizomenon* and other N_2 fixing blue-green algae have revealed many mechanisms for dealing with high oxygen concentrations.

Photooxidation

Another possible triggering mechanism for the collapse of the *Aphanizomenon* blooms described is photooxidation. The term "photooxidative death" refers to the lethal effect to cells exposed to light in the presence of oxygen and sensitized by internal or external dyes (Abeliovich and Shilo, 1972). Carotenoids were found to protect certain organisms from the photooxidative effects at physiological temperatures. The frequent observations of sudden and spontaneous disintegration of the blue-green algal blooms which appear in the summer in Israeli fish ponds, raised the question of whether a photooxidative effect was involved in this phenomenon, since conditions of oxygen supersaturation and high light intensities prevailed (Abeliovich and Shilo, 1972). A characteristic feature preceding massive die-offs in these ponds was the low CO_2 content (as expressed by the elevated pH values during the light period) of the highly alkaline waters. Three neighbouring fishponds having different diurnal pH curves were observed. A comparison of the pH in each pond to the respective algal mortalities indicated to the authors that viability depends on the presence of available CO_2 under conditions of high light and high O_2 concentrations. The role of CO_2 in preventing photooxidative death of *Anacystis nidulans* indicated that the protective mechanism was associated with the photosynthetic activity of the cell. Addition of DCMU, which blocks photosystem II, led to photooxidative death even in

complete CO_2 containing medium. Abeliovich and Shilo (1972) speculated that photooxidation at physiological temperatures in the absence of CO_2 may involve a peroxide or superoxide radical produced by the direct reduction of oxygen by some reduced electron carrier that accumulates when photosynthesis is inhibited by lack of CO_2.

It follows that a possible enzyme taking part in prevention of photooxidative death is superoxide dismutase (SOD), since this enzyme catalyzes a reaction that reduces the concentration of oxygen free radicals. Levels of this enzyme, in a cell-free extract of *Anacystis nidulans* have been studied by Abeliovich et al.(1974). The specific activity of the extract increased with the age of the cultures, reaching a maximum at the end of logarithmic growth. Cultures grown in an illuminated incubator under constant flow of nitrogen containing 5% CO_2 showed no detectable SOD activity. Four hours after these cells were shifted to an atmosphere of air, the SOD activity of the extract reached its normal values. Chloramphenicol (at a concentration that inhibits protein synthesis) or incubation in the dark prevented the appearance of this enzyme.

Shifting of the cultures grown under an atmosphere of air to an atmosphere of pure oxygen in a medium devoid of CO_2 caused photooxidative death of the cells after a lag of six to eight hours. The SOD activity began decreasing early in this period and was gradually depleted to

10% of its initial value by the sixth hour. It was found that the lag phase before photooxidation could be prolonged to 40 hours by growing the cells under at atmosphere of air enriched with 5% CO_2 prior to shifting to photooxidative conditions. Here, the SOD activity was found to be depleted to 10% of its original level after 40 hours. On the other hand, in cells devoid of SOD (after growth in pure nitrogen) when shifted to photooxidative conditions, death commenced after a brief lag of from 2 to 4 hours. In all cases, photooxidative death occurred when the SOD activity was depleted to 10% of its normal value.

The induction of SOD synthesis in cells previously grown under an atmosphere of nitrogen was found to take over 4 hours, whereas the recovery of the original enzyme activity in cells exposed for 6 hours to photooxidative conditions took only 30 minutes. Abeliovich et al. (1974) proposed that it may be that a number of components are necessary for activity whereas inactivation due to photooxidative conditions is incomplete or does not affect them all.

Since the loss of SOD activity preceeds the onset of photooxidative death and cells remain viable as long as the enzyme level does not drop below 10% of the initial value of normal cells, Abeliovich et al. (1974) propose that it seems unlikely that the depletion of SOD activity per se is an expression of irreversible processes taking place during photooxidative death. It could, however, be

connected with the primary effects of damage leading to death.

Photooxidation of cyanobacteria in natural conditions has been studied by Eloff et al.(1976). Photodynamic effects were demonstrated and assayed under field conditions in a number of different laboratory strains and pond isolates of cyanobacteria. SOD levels dropped drastically in all laboratory strains upon the onset of photooxidation. Among all of the strains tested, a pond sample of *Microcystis* was the only form that retained all of the initial SOD after exposure to photooxidative conditions. The various levels of resistance shown by different cyanobacterial strains to photooxidative effects in the field and in the laboratory seemed to be correlated with the rate of loss of SOD activity on exposure rather than with the initial SOD level.

In a more recent study of *Anabaena variabilis* bloom collapses in fish ponds (Boyd et al.,1978) photooxidation was concluded to be the triggering mechanism. All algal die-offs were found to occur on warm still afternoons. The authors concluded that light injury to cells in surface scums probably caused die-offs.

A study of the occurrence of photooxidation in Lake Mendota was conducted by Fallon and Brock (1979a). Observations on clear sunny days showed that surface light intensities were 1800 to 2100 uE/m^2/sec. during midday. Dissolved oxygen concentrations occasionally reached 200% saturation in surface waters. On such occasions, surface

concentrations of blue-green algae often developed. Although generally healthy in appearance at 0800 h small areas of bleached colonies appeared by 1030 to 1100 h and bleaching at the surface scums was also quite extensive by 1300 h. Small patches of blue appeared by 1000 h indicating that chlorophyll was lost first. Further exposures of these colonies to high light intensities resulted in complete color loss. During this latter phase, the algal cells began to lyse. Lysis was indicated by the presence of a blue opalescent sheen in the surface, resulting from the release of phycocyanin pigments and gas vesicles from the lysing algae. Such bleaching events were usually very localized, confined to areas protected from the wind. Only under very calm conditions was bleaching widespread. Normally, wind-induced turbulence appeared to prevent the algae from remaining at the surface long enough to be bleached. Based on observations made in 1976 and 1977 estimates were made for critical wind speed and solar radiation values required for widespread bleaching to occur. The average wind speed had to be less than 3 m/sec. and daily radiation had to be greater than 3.4×10^3 to 3.9×10^3 E/m^2,day. Algae which formed larger clumps were less influenced by turbulence and therefore more prone to exposure. Fallon and Brock (1979a) concluded that only 24 days in 1976, and 11 days in 1977 had wind and light conditions for lake-wide bleaching to occur.

Laboratory experiments done by Fallon and Brock (1979a) with natural populations indicated that exposure to intense

sunlight should result in a more rapid loss of photosynthetic activity than of chlorophyll a.

Field observations on Lake Mendota on those days when widespread bleaching was possible showed that only a portion of the algae in the upper cm. of the water column were exposed to light intensities high enough to cause bleaching. Algae below this were protected by self-shading. Estimates from the integrated water column chlorophyll a showed that such exposed surface layers were likely to represent 5% or less of the total lake chlorophyll a.

Fallon and Brock (1979a) concluded that high light intensities, especially under conditions of high oxygen concentrations and low carbon dioxide concentrations will eventually lead to death as a result of photooxidation. Although the Lake Mendota population appeared to be resistant to photooxidation, experiments did show that carbon fixation ability was rapidly lost by exposure to high light. In Lake Mendota, physical factors such as wind turbulence and self-shading in association with the relatively resistant population tended to minimize photooxidative effects.

Photooxidative death of *Aphanizomenon* blooms have been reported in Clear Lake, California (Horne, 1979).

Factors such as light, temperature, N, P, and O_2 may show more or less marked gradients with depth (Fogg et al., 1973). The effects of these factors are interrelated so that with limiting nutrient concentrations, optimum growth conditions are obtained at low light intensities and lower

temperatures. With higher concentrations of nutrients, light and temperature optima might be expected to increase, so that the population would be better suited higher in the water column. Conversely, the population would find optimum conditions low in the water column if nutrients decreased. It is in fact generally observed that downward movement of population maxima occurs in the summer as light intensities increase and nutrient concentrations decrease (Reynolds, 1975; Fogg et al., 1973).

Normally, population maxima of blue-green algae tend to occur towards the bottom of the photic zone, where the light intensity is 1% of that at the surface. If the respiration is low, such intensities might still suffice to support active growth by normal photosynthesis. In addition, there is the possibility that growth is partially sustained by photoassimilation of organic substances and perhaps chemoassimilation as well. The biological advantage of photoassimilation in dimly lit situations is that by starting out with an already reduced source of carbon, the limited assimilatory power from the photochemical reaction may be used to provide for a much greater amount of growth than would be possible with CO_2 as the carbon source (Fogg et al., 1973).

Most species of bloom forming blue-green algae are gas-vacuolated (Reynolds and Walsby, 1975). Blue-green algae can regulate their buoyancy in response to light intensity: when algal colonies are exposed to high light

intensities some of the gas vesicles collapse and the colonies may sink (Konopka et al., 1978). The ability of a species to be near the surface and thus to gain maximum energy for photosynthesis and the energetically costly N_2 fixing process is dependent on its ability to avoid death from irradiation and this in turn appears to be dependent on both environmental and intracellular factors (Walsby, personal communication, in Horne, 1979). *Aphanizomenon* populations in Lake Mendota, have been reported to form subsurface population maxima during calm weather, in the early afternoon because of gas vesicle collapse (Konopka et al., 1978).

Reynolds and Walsby (1975) use the term "water-bloom" to refer to the accumulation of planktonic blue-greens at the surface of lakes and reservoirs. Intense surface blooms are likely to result only when the majority of the algae are unable to correct overbuoyancy during passage to the surface in calm conditions.

Changes in turbulence can be related to bloom formation (Reynolds and Walsby, 1975). If the euphotic zone is approximately equal to the zone of mixing the average exposure to light permits net production without permitting much excess photosynthesis so that the algae remain, on average, neutrally buoyant. As turbulence weakens, algae are left distributed throughout the euphotic zone and should be able to adjust their buoyancy according to the position in which they are left on the vertical light gradient, and

move to the optimum depth at a speed largely determined by colony size. If the euphotic zone is less than the zone of mixing , the high proportion of dark water within the mixed zone reduces the average light intensity experienced by the algae and these respond by increasing their buoyancy. With the withdrawal of turbulence, the algae start to regulate their buoyancy according to the light regime but in this case they tend to float upwards while the buoyancy adjustment is being made. If they spend this period of time close to the surface they may be subjected to high light intensities such that the photosynthesis needed to develop the necessary rise in turgor pressure to disrupt gas vesicles is inhibited.

Surface bloom formation may be related to senescence (Reynolds and Walsby, 1975), since cessation of blue-green algal growth is often accompanied by increases in gas-vacuole content. The ultimate increase in buoyancy might be partly attributed to a reduced rate of dilution of new gas vacuoles and partly to a reduction in disruption of gas vacuoles because of a decrease in turgor pressure. The poorer physiological state of nutrient deficient blue-green algal populations probably also interferes with their capacity to develop high turgor pressure. A reduction in turbulence allows such algae to float upwards since they have little chance of controlling their buoyancy.

Walsby and Booker (in Reynolds and Walsby, 1975) have suggested that surface bloom formation in natural

waters may also result from the depletion of dissolved CO_2. They propose that where CO_2 falls to limiting concentrations turgor pressure is prevented from rising and collapsing the gas vacuoles. This would explain why in calm weather algae may continue to rise to the surface over a period of a week or more (Reynolds, 1971) in which time there should have been ample opportunity for the buoyancy control response to have operated.

Schindler et al.(1972), have shown that a low concentration of dissolved inorganic carbon does not itself prevent the formation of a large standing crop of algae as long as sufficient N and P are present in the lake water. However, the rate of photosynthesis can be limited by CO_2. If so then it is likely that the gas vesicle regulating response, which depends on there being excess photosynthesis may also be limited.

In summary, for photooxidation to play a major role in blue-green algal decline events, it is necessary that solar radiation be high, wind stress be low, dissolved oxygen concentrations be high, carbon dioxide concentrations be low and that a sufficient proportion of the population be exposed to such conditions.

Cyanophages

In 1963, Safferman and Morris first reported virions known to attack and destroy blue-green algae. Since then, the probable influence of cyanophages on the geographical and seasonal distribution, as well as population dynamics, of blue-green algae, and their possible uses in biological control of blue-green algae has added a further dimension to blue-green algal ecology (Padan and Shilo, 1973).

Cyanophages isolated to date have been named according to their known hosts. Accordingly, cyanophage groups were designated by the initials of the generic name of the hosts, to which arabic numerals have been added to designate serological subgroups (in Padan and Shilo, 1973). For example, the LPP-1 cyanophage causes lysis of *Lyngbya*, *Plectonema* and *Phormidium*. Crowie and Prager (1969, in Padan and Shilo, 1973), found that nucleotide homologies exist among the DNAs of these algae and they suggested that these hosts of LPP-1 were closely related.

A virus infection of *Aphanizomenon* , in Lake Erken, Sweden, has been reported to regulate the termination of water blooms of this alga (Granhall, 1972). Nitrogen fixation by phytoplankton, in Lake Erken, was found to be well correlated with the growth, bloom and sudden disappearance of *Aphanizomenon*. Preliminary electron microscope studies, suggested a virus infection as a cause of algal degradation. Electron microscopy, sterile filtrations and plaque techniques were used the following season to correlate the

fluctuations of the virus, termed Ap-1, with the bloom and degradation of *Aphanizomenon*.

A lytic agent, as determined by plaque techniques, specific against *Aphanizomenon* was apparently present in concentrated algal samples during the whole growth period. However, infected cells of *Aphanizomenon* and extracellular virions could not be detected by electron microscopy in the lake samples until the late stage of the bloom. The cells were filled with fully expanded gas vesicles, the nuclear region was uniform and no viruslike particles were seen externally or internally. In infected cells, the nuclear region was granular, and the cell wall was often broken and the vegetative cells lysed. The heterocysts were unaffected and no particles of any kind were adsorbed onto their cell walls.

The virus particles, when observed at high magnification were often found to be aggregated into chains oriented against the cell wall. Virus particles were observed to be polyhedral, with a head of 50-60 nm and a contractile tail sheath of 20-30 nm.

At the end of the growth period, virus particles were recognized in the nuclear region. Gas vesicles were rare. Most particles in the nuclear region were surrounded by a low electron dense region, or a halo, indicating a degradation of the DNA matrix. The matured virions were hexagonal and had tails.

The morphology and behaviour of the Ap-1 virus were

found to be similar to those of the An-1 virus of *Anabaena variabilis* (Granhall and Hofsten, 1969). Some charecteristics displayed by these viruses are adsorption by the tail, injection of the nucleic acid, multiplication in the nuclear region of the host, degradation of the host DNA and finally lysis.

The abundance of *Aphanizomenon* was extremely low in Lake Erken the year after the noted virus disease.

Padan and Shilo (1973) have reported that the pattern of the infection cycle of cyanophages is very similar to that of bacteriophages. However, it is much slower, lasting 13 hours at 26 °C for LPP-1G or 50 hours in SM-1 whereas most bacteriophages complete their reproduction in tens of minutes. They propose that this may be the reason that intermediates of viral assembly can be detected.

Shortness of filaments due to breakage following the random infection of cells, as well as the presence of swollen cells in cultures of *Plectonema* indicate periods of lysis (Cowlishaw and Mrsa, 1975). A several day delay in fragmentation was also observed. Safferman (1967) has reported that in virus-infected *Plectonema*, as lysis progressed, the filaments fragmented into smaller units until only scattered cells were evident in the preparation.

Viral infection affects the physiological state of the host alga (Lindmark, 1979). Infection of *Plectonema*

by LPP-1G effects a rapid and complete cessation of CO_2 photoassimilation (Padan and Shilo, 1973). Infection with a single LPP-1G virion per *Plectonema* cell was found to inhibit CO_2 fixation completely. Wu and Shugarman (1967), in their studies of *Plectonema* have observed a 50% decrease in photosynthesis and a threefold increase in respiration in infected cells. Nitrogen fixation has been observed to decrease upon infection of *Anabaena variabilis* by An-1 (Granhall and Hofsten, 1969).

The interaction of cyanophage with its host is markedly influenced by environmental conditions (Padan and Shilo, 1973). For example, the tolerance of the cyanophages to alkalinity corresponds with the alkalinity range of *Plectonema*. Temperature affects the survival of free phage and cyanophage development in hosts. Two LPP isolates from nature (LPP-1 and LPP-1G) are temperature sensitive (Padan et al., 1971). They multiply normally in the 26 to 29 $^{\circ}$ C range, but produce only early symptoms of infection in hosts (invaginations of the photosynthetic lamellae, and cessation of CO_2 photoassimilation) above 31 $^{\circ}$C. Padan and Shilo (1973) report that growth of host cells is not required for LPP multiplication, and that this differs from that of most bacteriophages which only develop efficiently in growing cells.

The rate of LPP-1 cyanophage replication and lysis of *Plectonema* has been studied in relation to pH alterations by CO_2/ air addition, nutrient concentrations, and

algal host culture age and density (Lindmark, 1979). It was found that a lowering of the pH by CO_2/ air addition to a culture of *Plectonema* containing the cyanophage induced a rapid lysis of the host population despite the fact that the algae could grow well at low pH in the absence of cyanophage. Addition of Na_2CO_3 to lysed cultures to raise the pH accelerated a regrowth of *Plectonema* while addition of a nutrient solution had no effect on regrowth. Laboratory experiments showed that *Plectonema* in its exponential growth phase showed the strongest physiological resistance to lysis. Field observations revealed that a combined reduction in pH and the addition of nutrients (N and P) accelerated the blue-green algal collapse. Algae in the lag phase of growth were found to induce a rapid increase in cyanophage, thus it was concluded that variation in algal density was less important for viral replication than the physiological state of the host.

Cowlishaw and Mrsa (1975), in quasi-continuous culture studies of *Plectonema* and LPP observed that two rounds of lysis occurred, and that regrowth of the cells after the first lysis was fast. This regrowth was used by back extrapolation to estimate the concentration of resistant cells during the first round of lysis. Estimates done this way gave frequencies too high to be the frequency of phage-resistant mutants in the culture and seemed to indicate that cell viability during the first round of lysis was due to physiological differences.

The authors proposed that when the factor causing physiological resistance was diluted out more cells lysed resulting in the second round of lysis.

For temperate phages, factors such as temperature, ultraviolet light or unbalanced growth conditions, which are known to influence the formation of lysogenic cultures and the lytic cycle, might have considerable signifigance on the algal population balance in nature (Padan and Shilo, 1973). If conditions were such as to cause induction a decline in the population would be seen. Once conditions were not such, regrowth might occur.

Algae known to be susceptible to cyanophages are rarely dominant in natural waters, for example, *Plectonema* (Safferman and Morris, 1963). The survival and tolerance of the LPP cyanophages apparently enables them to control their algal hosts in nature and thus eliminate the development of blooms (Lindmark, 1979).

Stewart and Brown (1969, in Brown, 1972), report frequent lysis of algae by nonviral agents which mimic the infection cycle; they therefore stress that morphological evidence is essential for the verification of a virus infection. To establish the viral nature of a particle seen in an electron micrograph, repeated isolation of the particles, demonstration of their infectivity, together with biochemical characterization would be necessary.

In summary, cyanophages have been reported to play a role in the population dynamics of blue-green algae. This role varies in extent. For example, the LPP viruses control the size of their host populations to the extent that the host never becomes the dominant alga in nature. In other instances, the host population builds up to bloom proportions, infection spreads and the bloom terminates. An example is the *Aphanizomenon* bloom collapse described by Granhall (1972). Though not described for field situations, there must certainly be interactions midway between these two extremes.

Other Possible Pathogens

Bacterial pathogens of freshwater blue-green algae are known to occur. Daft and Stewart (1971) have provided evidence that heterotrophic bacteria which are pathogenic to certain blue-green algae occur in freshwater habitats in Britain (denoted as CP-1, CP-2, CP-3 and CP-4). Studies of CP-1 have shown that for lysis of the algal cell to occur, bacterial cells must be present, extracellular products alone are insufficient. Shilo (1970) concludes from studies on a myxobacter, that contact of the bacteria with the algae is necessary for lysis. Daft and Stewart's microscopical observations (1971) supported this contention. As with the myxobacter, lysis of the vegetative cells of the blue-green alga occurs but heterocysts appear unaffected. As heterocysts have cellulose cell

walls while vegetative cells have not, this suggested that the bacteria were unable to degrade cellulose. The host range of the 4 bacterial strains that Daft and Stewart isolated were slightly dissimilar but wide and many species of Cyanophyta were found to be susceptible. These included strains of *Anabaena flos-aquae*, *Anabaena circinalis*, *Aphanizomenon flos-aquae*, and *Microcystis aeruginosa*.

In a later study, the abundance of algal-lysing bacteria in various freshwater habitats was examined and found to be correlated directly with the abundance of Cyanophyceae in eutrophic waters (Daft et al., 1975). The authors concluded that it is probable that in most ecosystems the algae and bacteria co-exist, although if the equilibrium changes markedly as a result of a change in environmental conditions, situations may arise where the bacteria may play an important role in the lysis of algal blooms. Ecological evidence suggested that this is likely to be a rare occurrence in natural ecosystems.

These bacteria were particularly abundant in waters where Cyanophycean blooms occur, not because of any specificity for the Cyanophyceae (they grow perfectly well on complex organic material in the absence of blue-green algae and are common in alkaline soils and in sewage works where blue-green algae are rarely abundant) but probably both require similar environmental conditions for optimim growth. The following factors were found to favor the bacteria:

1) highly oxygenated waters since the bacteria are aerophilic,
2) if the algae are being affected adversely by other pathogens or grazers than the bacteria could contribute to the overall lysis of the bloom, even if they were incapable of causing lysis alone.

Examples of the factors that would shift equilibrium in favor of the algae are deoxygenation of the water or low organic content in the water.

Lytic organisms were shown to increase in numbers in Lake Mendota in response to the seasonal development of blue-green algae (Fallon and Brock, 1979a). Lytic organisms were quantified as numbers of plaques appearing on lawns of *Anacystis*. Results from 1976 and 1977 showed a tendency for the number of plaque forming units in the deep water samples to be lower by a factor of between 10 and 100 from those in the surface waters especially in midsummer.

Microscopic examination of the plaques indicated the presence of both bacteria and protozoa. Fungal hyphae were never observed. Two bacterial strains were isolated from the plaques and partially characterized. They were gram-negative rods which were obligately aerobic and had a temperature optimum of 30 °C.

The significance of the lytic activity in relationship to the midsummer chlorophyll decline events proved

difficult to evaluate; although lytic organisms appeared to play a primary role in causing declines, no definite conclusions were made. The question as to whether the lytic bacteria were acting as pathogens and thus were a primary cause for declines, or were acting as saprophytes decomposing dead algal material resulting from other processes, remains.

Phytoplankton and bacterial relationships in the eutrophic Lake Bysjon, Sweden have been studied (Coveney et al., 1978). Maxima in bacterial numbers followed immediately after blue-green algal collapses. Phytoplankton autolysis products and detritus appeared to be the most signifigant sources of organic material for the development of the bacterial maxima. Bacterial numbers also increased during the early stages of the phytoplankton blooms, indicating phytoplankton extracellular products as possible nutrient sources. Measurement of net phytoplankton extracellular release showed low levels which could, however, reflect rapid bacterial utilization of algal products rather than true low release. Bacterial numbers decreased during an *Aphanizomenon* bloom which was considered to indicate inhibitory algal products. The possibility of the bacteria acting as pathogens was not considered in this study.

Many algae are vulnerable to attacks of fungal parasites belonging to the orders Chytridiales and Blastocladiales (in Reynolds and Walsby, 1975). Fungal attacks may

reach epidemic proportions and bring about changes in the succession of dominant algae. The collapse of natural populations of *Aphanizomenon* and *Anabaena*, wrought respectively by ameboid (*Pelomyxa*) and ciliate (*Ophryoglena atra*) protozoa, has been noted by Reynolds (1971).

It may be that algal bloom formation could be regulated to some degree by the extent to which algae outstrip or are outstripped by the growth of algal pathogens.

Autoinhibition

Autoinhibition has also been considered as a mechanism leading to algal bloom collapse. Fogg (1969) reported that there is clear evidence that certain algae in laboratory cultures produce substances toxic to themselves in the course of their metabolism and that the accumulation of these may eventually bring exponential growth to a standstill. A growth modifying substance may be stimulating to a specific reaction in low concentrations and inhibitory in high concentrations. The higher concentration of inhibiting substances observed in old cultures may result from an increased rate of production by the cells; it may represent the accumulation of large quantities of the material from prolonged growth of the alga; or it may result from the release of larger quantities of cellular substances through changes in permeability of the cell membrane or autolysis.

There are difficulties associated with the extrapo-

lation of generalizations based on laboratory culture studies to conditions existing in natural waters. Excreted substances which remain in fairly concentrated form in confined culture media may become diluted below their limit of physiological activity in an unconfined situation. The activities of associated freshwater bacteria may result in the rapid decomposition of substances which remain active for long times in bacteria free cultures. Active materials may also be rendered inactive in natural waters by the formation of complexes.

CHAPTER 2

Collapses of *Aphanizomenon flos-aquae* (L.) Ralfs blooms: Possible contributions of photooxidation and O_2 toxicity

ABSTRACT

The declines of *Aphanizomenon flos-aquae* (L.) Ralfs populations in three shallow eutrophic lakes (L885, L958 and L522) in 1979 and one (L958) in 1978 in Southwestern Manitoba, Canada were examined. Algal declines of differing rates and durations were observed to occur. Three of the four populations studied endured collapses that resulted in severe dissolved oxygen depletion, namely L885, 1979 after July 19; L958, 1979, after August 19; and L958, 1978 after August 14. The severity of O_2 depletion appeared to depend not only on the actual amount of decrease in algal biomass (expressed as cell volume) but also on the depth of the anoxic layer prior to bloom decline and subsequent or concommitant lake mixing.

Throughout the summer, these lakes alternated between periods of lake thermal instability and stability. During thermally stable conditions, the upper zone developed features conducive to photooxidative death and death due to O_2 toxicity. Decline events were often intiated during such periods (eg L885, 1979 after July 19; L885, 1979 after August 20; L522, 1979 after July 17 and L958, 1979 after August 19). Photooxidation and death due to O_2 toxicity were likely occurring at such times to a significant portion of the population. Decline events were also observed to occur during periods of lake thermal instability when conditions would not promote photooxidation or death due

to O_2 toxicity (eg L958, 1978 after August 14 and L958, 1979 from August 7 to August 14).

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INTRODUCTION

Summer fish kills in shallow eutrophic lakes of the Erickson-Elphinstone area, Southwestern Manitoba, Canada, have been reported to be caused by collapses of heavy *Aphanizomenon flos-aquae* (L.) Ralfs blooms, and subsequent O_2 depletion (Barica, 1975). In summer, so-called "non-summerkill lakes" show relatively stable chlorophyll a (chl a), nutrient and dissolved oxygen (DO) levels while in "summerkill lakes" these parameters oscillate, with chl a increasing exponentially to over 100 µg/l within one to two weeks. This may be followed within one to several weeks by a sudden collapse of the algal population. The algal collapse has always been preceded by a peak in the chl a level and DO concentrations, and consequently minimum Secchi disc transparency and nutrient levels (Barica, 1975).

Four such blooms examined by Healey and Hendzel (1976) developed characteristics of P deficiency and to a lesser degree N deficiency. In spite of their similar characteristics, three of the blooms collapsed dramatically while the fourth did not. It was concluded that P deficiency seems to play an important part in setting conditions for a collapse but it does not seem to be the factor that triggered the collapse.

In Lake Mendota, (Konopka and Brock, 1978), the decline of *Aphanizomenon* that occurred in July, 1978, was preceded

by a decrease in the specific photosynthetic rate. The population peristed for two to three weeks after the decrease in specific photosynthetic rate, which Konopka and Brock (1978) speculated to indicate that either a lesser degree of photosynthesis was sufficient to maintain the algal density, or that the algae had synthesized enough reserve material to maintain themselves for that period of time. Primary production measurements done on the *Aphanizomenon* blooms in the Erickson-Elphinstone prairie lakes (Papst et al., in press) support the prediction that algal primary production is nutrient limited at the time of collapse.

The purpose of this investigation was to examine the conditions that accompanied the onset and occurrence of declines of *Aphanizomenon* populations in 3 lakes of the Erickson-Elphinstone area. Furthermore, since high O_2 tension may inactivate enzymes and destroy vital tissue components (Haugaard, 1968), and since high O_2 concentrations are known to inhibit both CO_2 and N_2 fixation (Stewart and Pearson, 1970; Paerl and Kellar, 1979) a further purpose was to investigate the possible contribution of O_2 toxicity to algal decline. Superoxide dismutase (SOD) activity was also to be assayed, since this enzyme is known to afford protection against high O_2 tension (Abeliovich and Shilo, 1972).

The term "photooxidation" refers to the lethal effect on cells exposed to light in the presence of oxygen and sensitized by internal dyes. Abeliovich et al.,(1974)

have demonstrated that the combination of high light, high pH, low CO_2 concentration and a high DO concentration causes photooxidative death in blue-green algae. The essential difference between O_2 toxicity and photooxidation is merely that photooxidation requires a high light intensity. For photooxidative death to contribute significantly to bloom decline it is therefore necessary that a significant proportion be exposed to high light intensities; that is, a surface bloom must occur. A third purpose was to assess the contribution of photooxidation to bloom collapse.

MATERIALS AND METHODS

The 3 lakes involved in the study (lakes 885, 958 and 522) are located in the Erickson-Elphinstone area of southwestern Manitoba (50°30' N, 100°10' W). The area and many of the lakes have been described by Sunde and Barica (1975) and Barica (1975). L885 has an area of 2.4 ha, a maximum depth of 3.0 m and a mean depth of 1.9 m. L958 has an area of 5.7 ha, a maximum depth of 2.7 m and a mean depth of 1.7 m (Sunde and Barica, 1975). L522 is a larger and deeper lake. The depth at the selected sampling station was 3 m.

Integrated phytoplankton samples were collected by way of a flexible plastic tube and discrete depth samples with a 2-litre VanDorn sampler. All samples were taken from the approximate centre of each lake.

Temperature profiles were measured using a YSI ther-

mometer. Light intensity in the water column was measured using a Li-cor quantum meter (model LI-185 A) equipped with an underwater quantum sensor (model LI-192S). Total daily photosynthetically active radiation (PAR, approximately 400-700 nm) was recorded and wind stress values were determined by squaring average wind speed values recorded on an anemometer mounted just above the surface of L885.

In 1978, DO concentrations were measured using the Winkler method as outlined by Stainton et al,(1974). In 1979, an oxygen probe (YSI 54) was used. pH was measured using unfiltered water samples and ammonia (NH_3-N) and soluble reactive phosphorus (SRP) analyses were performed as outlined by Stainton et al., (1974).

For the estimation of chl a and carotenoids (C) 100 to 300 ml of each sample were filtered through a Whatman GFC glass fibre filter. The filter was placed in a petri plate, wrapped in foil, and stored frozen. In 1978, chl a levels were determined fluorometrically after extraction in 90% acetone as outlined by Stainton et al., (1974). In 1979, chl a (corrected for phaeopigments) and C levels were determined as outlined by Strickland and Parsons (1968). Absorbances were measured using an SP6-500 Pye Unicam UV spectrophotometer.

A second portion (100-300 ml) of each water sample was filtered through a Whatman GFC glass fibre filter for the quantification of phycocyanin (phy). The filters were stored in petri plates wrapped in foil and frozen. Phy

levels were determined using the method outlined by Healey and Hendzel (1976).

A subsample of each 0-2 m water sample was preserved with Lugol's iodide solution and used for the estimation of cell volumes and heterocyst frequencies of *Aphanizomenon*. The Utermohl (1958) sedimentation procedure was used. Once the average diameter of the *Aphanizomenon* filaments was determined, the cell volume per microscope field could be calculated using the formula of Olson (1950). The number of fields counted for each sample was that necessary for the required degree of accuracy (±20%) and precision ($p \leq 0.05$). At the same time that the cell volume was estimated, the total number of *Aphanizomenon* cells and attached heterocysts within the fields observed were enumerated. From this, heterocyst frequency as percentage of total cells, was calculated.

N_2 fixation assays were carried out using the acetylene reduction technique as outlined by Flett et al., (1975). Three replicates of a 0.5 m water sample were incubated in syringes horizontally suspended at a depth of 0.5 m. Incubations were terminated by the removal of the vapor phase. Two blanks for each incubation period (to correct for any ethylene added with the acetylene as an impurity and/or any ethylene in the lake water) were prepared. Gas-chromatographic analysis (flame-ionization detection) was performed on the same day that the incubations were carried out. Three 0.2 ml subsamples of each replicate and blank were

injected into the column (Poracil C phenylisocyanate packing) run at 30 °C. Good separation of acetylene and ethylene was obtained. The amount of ethylene produced was quantified using an ethylene standard that was freshly prepared for each run. The mean value of the ethylene contained in the blanks was subtracted from the mean value of the incubated samples. No attempt was made to convert amounts of ethylene produced to the actual amounts of nitrogen fixed.

Algae to be used for the estimation of SOD levels were obtained from 0-2 m water samples. Flakes of *Aphanizomenon* were concentrated by filtration through a 250 μ mesh. A suspension of the algae in deionized water was frozen. On the same day that the enzyme assays were run, the algal suspensions were allowed to thaw and potassium phosphate buffer (pH 7.0) was added to give a final molarity of .01. The algal cells were broken up by sonication using a Bronwill Sonicator at 80S for 5, 30 s bursts. A subsample of the suspension was then observed microscopically. Generally only heterocysts and akinetes were left intact. The suspensions were centrifuged for 10 min. at 10,000 x g, twice in an RC-5 Superspeed Refrigerated Centrifuge. The supernatant was used for the determination of the specific activity of SOD. Total protein was determined by the method of Lowry et al., (1951) after the sample had been boiled for 10 min. in 1N NaOH. The enzyme assay used was that proposed by Misra and Fridovich (1972). In this system, SOD is quanti-

fied in terms of its ability to inhibit the reaction rate of the autooxidation of epinephrine to adrenochrome, at a pH of 10.2. The reaction rate was determined by recording the increase of absorbance at 480 nm, using a Gilford Model 2400-S Automatic Recording Spectrophotometer, with attached temperature control set at 30 °C. Three replicates of the control reaction rate (no SOD) were determined for each run. Three replicates of each sample were measured. A standard curve using Bovine SOD and % inhibition of control reaction rate was constructed for each run. The specific activity in terms of ngSOD/ug protein could then be calculated.

RESULTS

L885, 1979

Two periods of decline in cell volume of differing severity were noted (Fig. 1a). The first decline was observed after July 19 and the second after August 20. Heterocyst frequencies were observed to vary (Fig. 1a), although decreases in heterocyst frequencies accompanied the algal declines.

By comparing surface and bottom temperatures, the summer could be divided into periods of thermal stability (A,C, &E) and instability (B,D, &F)(Fig. 1b). DO at the surface and 1m were high during periods of thermal stability and lower during periods of thermal instability (Fig. 1c). Throughout most of the summer the lake bottom was anoxic.

During period A, NH_3-N and SRP were low (Fig. 1d). During period B, there was an initial increase in both nutrients, however by the onset of period C the levels were again low. Concentrations of both nutrients began to increase after August 20.

During period A, declines in both C and chl a levels were observed (Fig. 1e). C and chl a were relatively constant throughout period B. During period C, levels of chl a, C and phy initially increased. After July 19 a decrease was observed. Very high phy levels were

recorded on July 19. A second peak for C was recorded on July 24. Chl a and phy increased throughout period D. C were initially high; levels decreased until the middle of period D and then increased in step with chl a and phy for the remainder of the period. Chl a and C followed the same pattern of increase in period E with a decrease occurring after August 21. Phy levels were initially seen to increase in period E, Low concentrations of both chl a and C were observed on August 25.

A detailed description of the events in period C is presented in Fig. 2 and Table 1; and of period E in Fig. 3 and Table 2. Field observations did not reveal the formation of a surface bloom of *Aphanizomenon* at the onset of either collapse. Microscopic examination of the phytoplankton sample taken on July 20 revealed the presence of many single cells or short filaments of *Aphanizomenon*, and isolated heterocysts.

L522, 1979

Cell volumes recorded for L522 reached a much higher peak than those recorded for L958 and L885 (Fig. 4a). A drastic decline was observed after July 17. A decrease in heterocyst frequency accompanied this decline.

The sampling period for L522 can also be divided into periods of thermal stability (C & E) and instability (D) (Fig. 4b). During period C, DO was initially high

(Fig. 4c). With the decline of the algal population, an accompanying DO depletion occurred, however not to extremely low levels. This lake differed from both L958 and L885 in that the extent of the anoxic or O_2 depleted layer in L522 was always less.

The levels of SRP were always very high in L522 (100 ug/l, Fig. 4d). The peak value of SRP occurred after the period of mixing subsequent to the decline recorded in period C. At the onset of period C, NH_3-N levels were low (Fig. 4d). An increase occurred after the decline of *Aphanizomenon* in period C.

Like the cell volume maximum, the chl a maximum was observed on July 17 (Fig. 4e). Chl a levels did not drop sharply until subsequent mixing of the lake occurred after the cell volume decline observed in period C. High C levels were observed on July 17 and 23. High levels of phy were observed on July 20.

A detailed description of the events occuring in period C is presented in Fig. 5 and Table 3. Field observations revealed the formation of a surface bloom of *Aphanizomenon* from July 17 to approximately July 20.

L958, 1979

Three declines in cell volume, of varying severity were observed in L958 (Fig. 6a). The first decline occurred from July 31 to August 1 and was accompanied

by a slight decrease in heterocyst frequency. The third decline occurred after August 19. Heterocyst frequencies were low prior to and on August 19, however an increase was apparent by August 21.

The sampling period for L958 can also be divided into periods of lake thermal stability (C & E) and thermal instability (D & F)(Fig. 6b). DO at the surface and 1m were high during periods of thermal stability and lower during instability (Fig. 6c). Throughout most of the summer the lake bottom was anoxic.

NH_3-N and SRP concentrations were initially low in period C (Fig. 6d). An increase in both nutrients was noted on August 2. At the end of period D, SRP levels were low and NH_3-N levels were moderate. NH_3-N and SRP levels were high on August 25.

An increase in cell volume was recorded from July 24 to July 31 (Fig. 6a), however decreases in chl a, C and phy were observed during the same time interval (Fig. 6e). A decrease in cell volume was recorded from July 31 to August 1 yet an increase in pigments was observed. With the onset of period E, C levels increased. Very high levels of phy were recorded on August 19. After Aug. 19, chl a, C and phy concentrations decreased.

A detailed description of events occurring from July 31 to August 1 is given in Fig. 7 and Table 4. The second decline observed occurred during a time of thermal instability (period D). Total PAR inputs throughout this

period were low (Appendix 1). A detailed description of events as they occurred in period E is given in Fig. 8 and Table 5. Field observations revealed the formation of a surface bloom of *Aphanizomenon* on August 1 and on August 19 and 20.

L958, 1978

A major decline in cell volume occurred after August 14 (Fig. 9a). Heterocyst frequency on August 14 was low (Fig. 9a). A major decline in chl a also occurred after August 14 (Fig. 9b).

Temperatures at the surface and 2m varied during the last three weeks of August (Fig. 9c). Peak surface and 2m temperatures were recorded on August 11. On this date, the lake can be considered to have been thermally stable. Mixing of the lake occurred sometime between August 11 and 14. Total PAR inputs (Appendix 2) throughout this period were moderate to low.

The highest DO levels during this time interval were recorded on Aug. 8 (Fig. 9d). From then on a steady decline in DO occurred with lake anoxia occurring on August 22.

Low levels of SRP at the surface were recorded until after August 14 (Fig. 9e). 0-2m levels of SRP indicated that a release of nutrients occurred on or near August 8. From here the SRP levels declined with a slight increase observed at the end of August. Surface and 0-2m levels

of NH_3-N began to increase after August 11 (Fig. 9f).

On August 11, chl a profiles and field observations revealed that the algal population was localized at the surface (Fig. 9g). By August 14 the algae were evenly distributed throughout the water column.

SOD ASSAYS

The specific activites recorded for SOD are given in Table 6. Although a limited number of samples were tested, the evidence suggests that the levels of activity do vary.

DISCUSSION

In determining a possible triggering mechanism for the collapse of *Aphanizomenon* blooms it is important that the time of onset of the algal collapse be clearly recognized. In previous studies, the algal collapse and lake O_2 depletion were sometimes considered synonymous (Barica, 1978; Van Nguyen and Woods, 1979). Later studies (Papst et al., in press) have pointed out that algal bloom collapse and periods of DO depletion must be considered as separate events. DO depletion does not always immediately follow bloom collapse; there may be a lag of several days. Papst et al., (in press) have suggested that a decline in algal biomass as well as a concommitant or subsequent period of thermal instability are necessary conditions leading

FIGURE 1 L885, 1979

a: Cell volume of *Aphanizomenon* (●——●), x 10^7 u^3/ml and heterocyst frequency (▮) as the % of total cells in the 0-2m integrated water sample.

b: Surface (△——△) and bottom (▲——▲) water temperatures in °C, which were used to characterize the time of sampling into periods of lake thermal instability and stability: Period A - thermally stable, Period B - thermally instable, Period C - thermally stable, Period D - thermally instable, Period E - thermally instable, and Period F - thermally instable.

c: Surface (■-----■), 1m (□——□) and bottom (●——●) dissolved oxygen (DO) concentrations in mg/l.

d: Nutrient concentrations in the 0-2m integrated water sample: Ammonia (NH_3-N,○——○) and Soluble Reactive Phosphorus (SRP, ●——●) in ug/l.

e: Pigment concentrations in the 0-2m integrated water sample: Carotenoids (C, ◇——◇) in m-SPU/m^3, Chlorophyll a (Chl a.○——○) in mg/m^3 and Phycocyanin (Phy, ◆----◆) in mg/m^3.

L885

1979

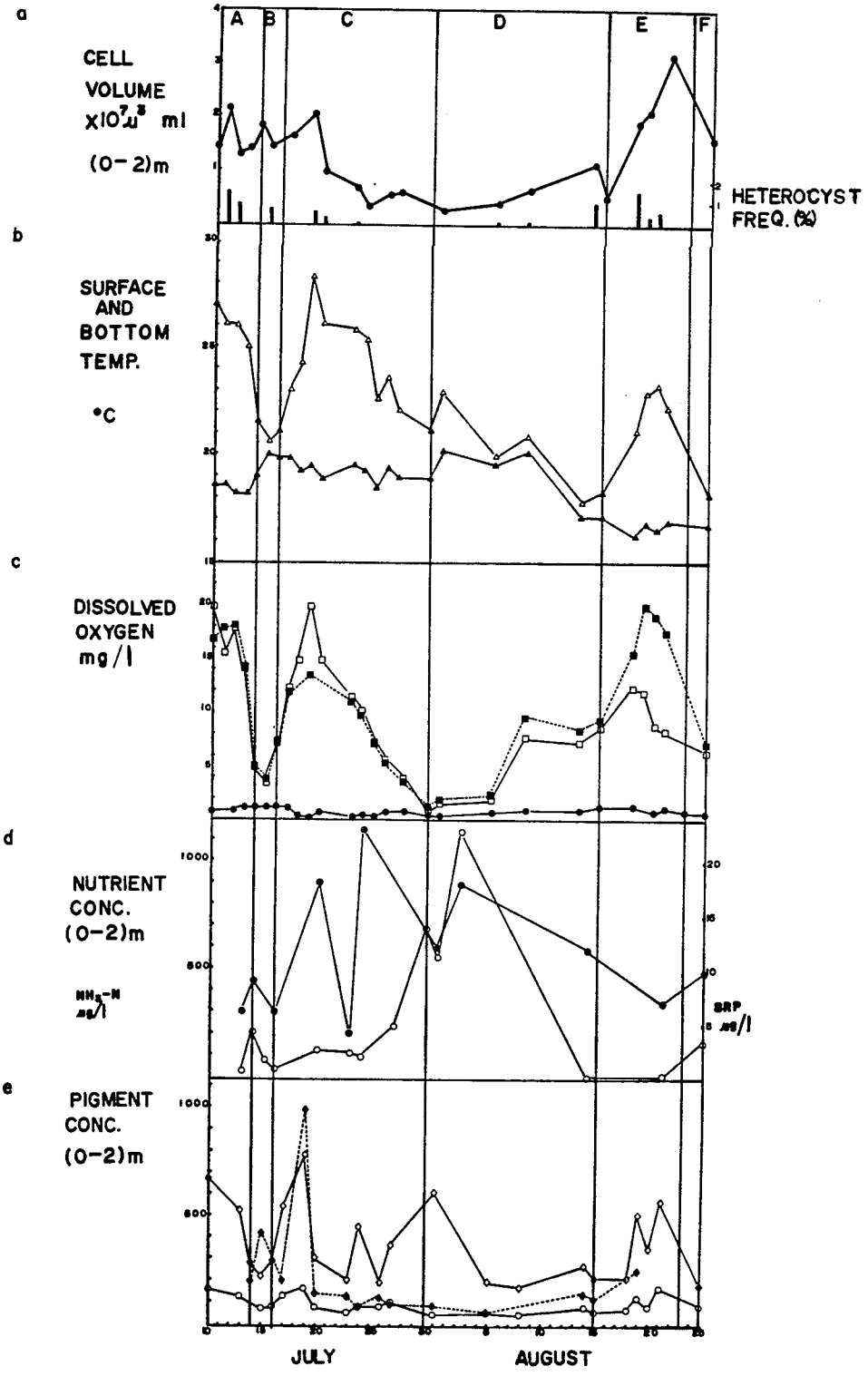
a
A
B
C
D
E
F
CELL VOLUME X10⁷ μ³ ml (0-2)m
HETEROCYST FREQ. (%)
b
SURFACE AND BOTTOM TEMP. °C
c
DISSOLVED OXYGEN mg/l
d
NUTRIENT CONC. (0-2)m
NH₃-N μg/l
SRP μg/l
e
PIGMENT CONC. (0-2)m
JULY
AUGUST

FIGURE 2 EVENTS ACCOMPANYING THE ONSET AND OCCURRENCE OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L885, 1979 AFTER JULY 19.

L885,1979

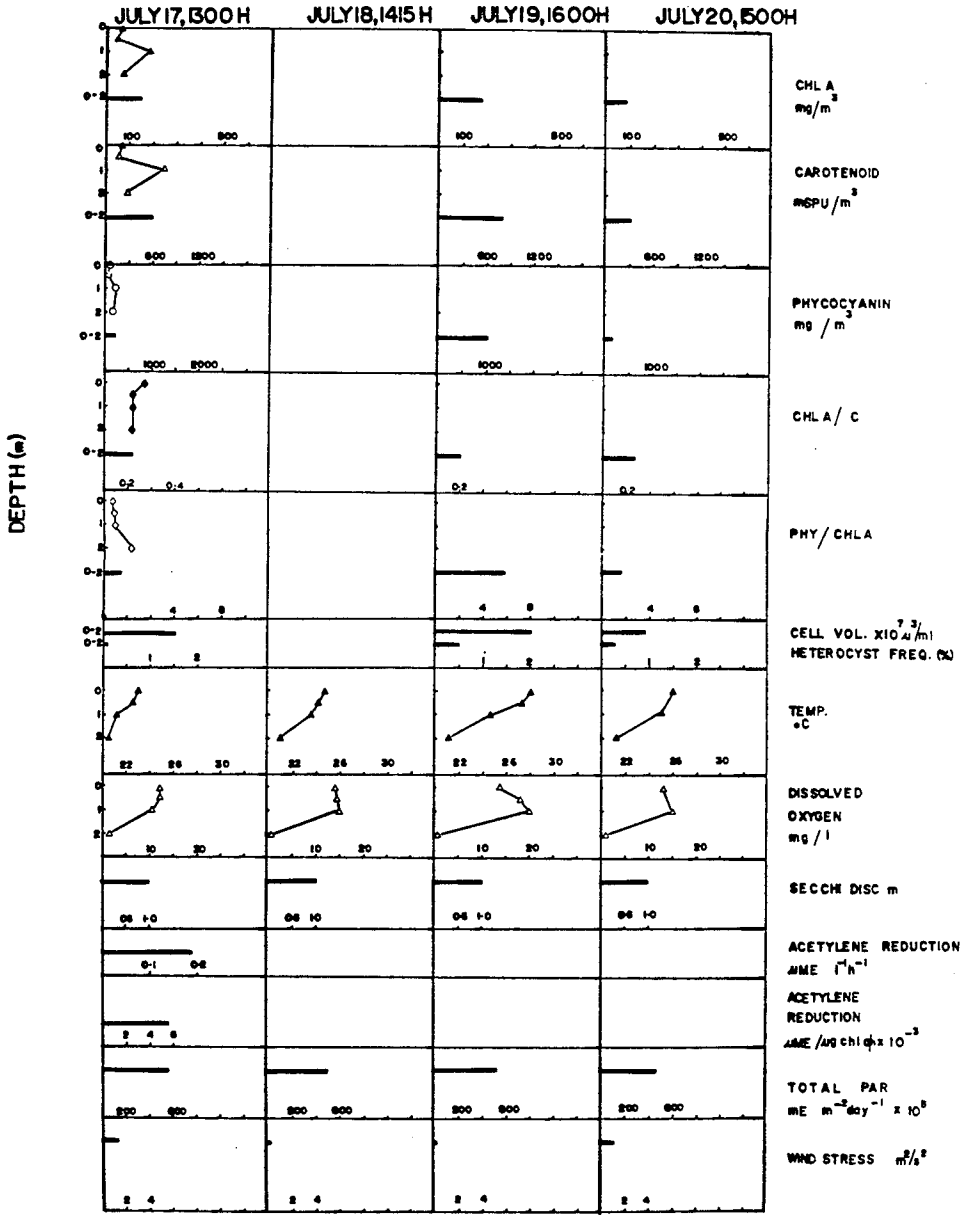
JULY 17, 1300 H
JULY 18, 1415 H
JULY 19, 1600 H
JULY 20, 1500 H
DEPTH (m)
CHL A mg/m3
CAROTENOID mSPU/m3
PHYCOCYANIN mg/m3
CHL A/C
PHY/CHLA
CELL VOL. X10 7.3/ml
HETEROCYST FREQ. (%)
TEMP. °C
DISSOLVED OXYGEN mg/l
SECCHI DISC m
ACETYLENE REDUCTION
ACETYLENE REDUCTION
TOTAL PAR
WIND STRESS

TABLE 1 SUMMARY OF CONDITIONS OCCURRING AT THE TIME OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L885, 1979 AFTER JULY 19.

DATE	CHL a MAX.(m)	DO	TEMP.	TOTAL PAR	WIND STRESS
July 17, 1300 h	1	m	m	h	1
July 18, 1415 h		h	m	h	1
July 19, 1600 h		h	m	h	1
July 20, 1500 h		h	h	m	1

DEFINITION OF TERMS FOR TABLES 1 TO 6.

CHL a MAX.(m) : is the depth at which the maximum chlorophyll a level was recorded.

DO : $h=x \geq 15$, $m=10 \leq x < 15$, $1=x < 10$, mg/l

TEMP. : $h=x \geq 25$, $m=20 \leq x < 25$, $1=x < 20$, ^{o}C

pH : $h=x \geq 9$, $m=8.5 \leq x < 9$, $1=x < 8.5$

TOTAL PAR : $h=x \geq 500$, $m=450 \leq x < 500$, $1=x < 450$, $x10^{5}$ $mE/m^{2}/d$

WIND STRESS : $h=x \geq 5$, $m=2 \leq x < 5$, $1=x < 2$, m^{2}/sec^{2}

LIGHT INTENS. ; $h=x \geq 600$, $m=100 \leq x < 600$, $1=x < 100$, $mE/m^{2}/sec$

* DO, TEMP. and pH are determined by the levels of such at the depth of the chl a maximum if known or at 1m if unknown.

FIGURE 3 EVENTS ACCOMPANYING THE ONSET AND OCCURRENCE OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L885, 1979 AFTER AUGUST 20.

L885,1979

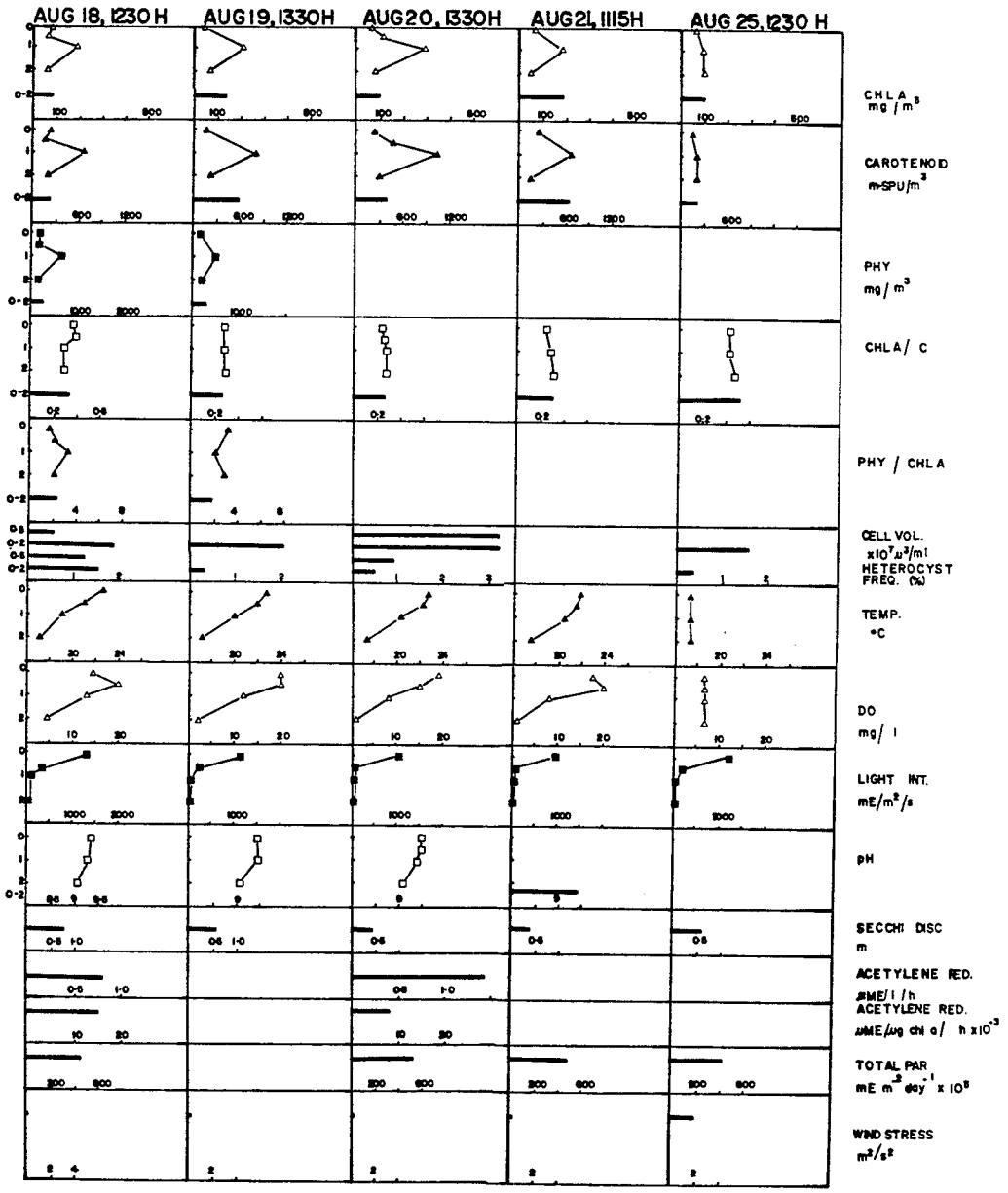
AUG 18, 1230 H
AUG 19, 1330H
AUG 20, 1330H
AUG 21, 1115H
AUG 25, 1230 H
CHLA mg / m3
CAROTENOID m-SPU/m3
PHY mg / m3
CHLA / C
PHY / CHLA
CELL VOL. x10^7 μ3/ml
HETEROCYST FREQ. (%)
TEMP. °C
DO mg / l
LIGHT INT. mE/m2/s
pH
SECCHI DISC m
ACETYLENE RED. μME/ l / h
ACETYLENE RED. μME/μg chl a/ h x10-3
TOTAL PAR mE m-2 day-1 x 10^3
WIND STRESS m2/s2

TABLE 2 SUMMARY OF CONDITIONS OCCURRING AT THE TIME OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L885, 1979 AFTER AUGUST 20.

DATE	CHL A MAX. (m)	DO	TEMP.	pH	TOTAL PAR	WIND STRESS
August 18, 1230h	1	h	m	h	m	1
August 19, 1330h	1	m	m	h		1
August 20, 1330h	1	m	m		h	1
August 21, 1115h	1	m	m		m	1
August 25, 1230h	2	1	1		1	

FIGURE 4 L522, 1979

a: Cell volume of *Aphanizomenon* (◆——◆), x 10^7 u^3/ml and heterocyst frequency (▮) as the % of total cell number in the 0-2m integrated water sample.

b: Surface (◇——◇) and bottom (◆——◆) water temperatures in ^oC, which were used to characterize the time of sampling into periods of thermal stability and instability: Period C - thermally stable, Period D - thermally instable and Period E - thermally stable.

c: Surface (△——△), 1m (▲——▲) and bottom (○——○) dissolved oxygen (DO) concentrations in mg/l.

d: Nutrient concentrations in the 0-2m integrated water sample: Ammonia (NH_3-N, ◆—◆), Soluble Reactive Phosphorus (SRP, ◇—◇) in ug/l.

e: Pigment concentrations in the 0-2m integrated water sample: Carotenoids (C, ◇—◇), in m-SPU/m^3, Chlorophyll a (chl a, □-□) in mg/m^3 and Phycocyanin (Phy, ■-■) in mg/m^3.

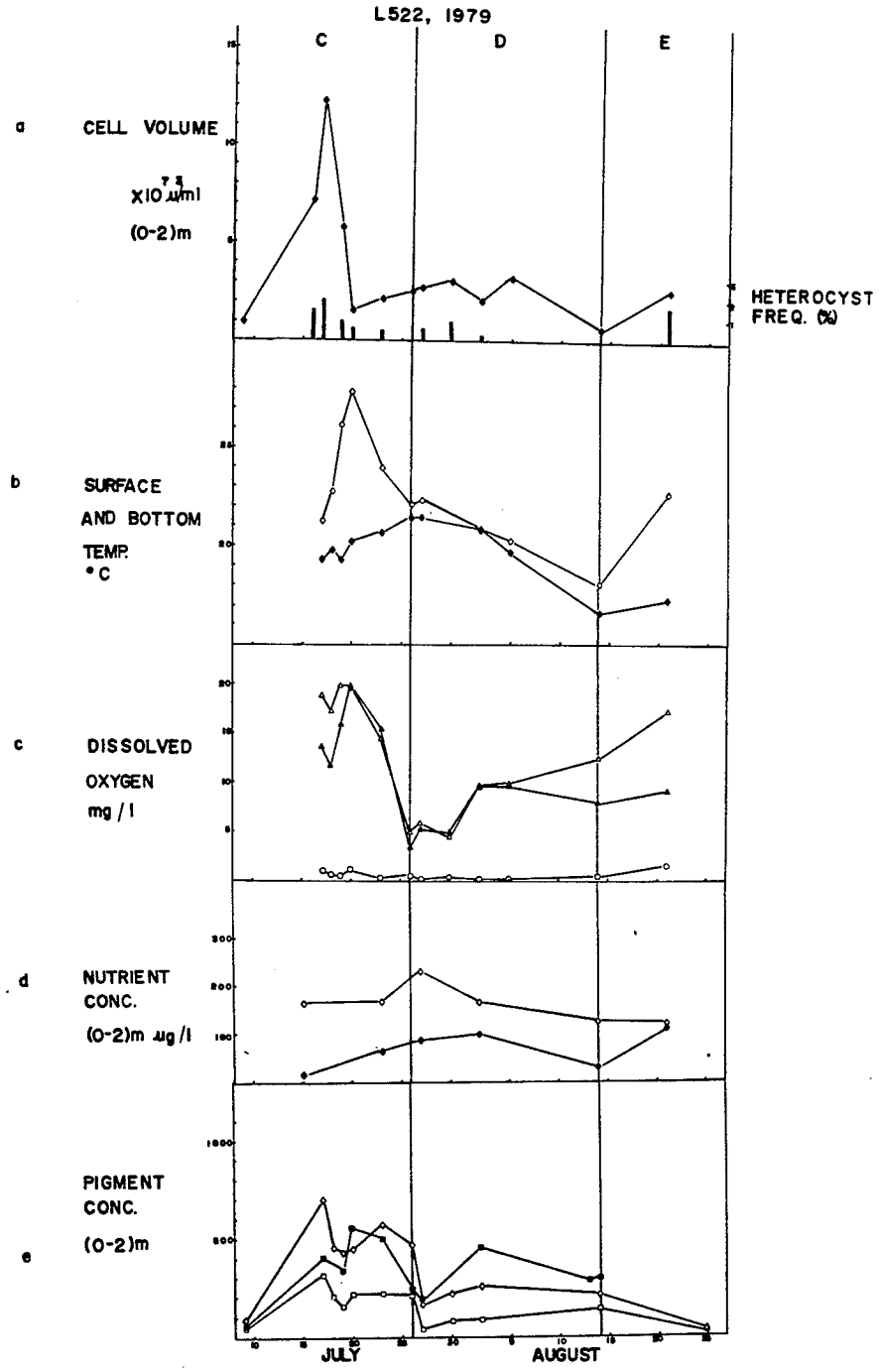
L522, 1979
C
D
E
a
CELL VOLUME
X10 μm³/ml
(0-2)m
HETEROCYST
FREQ. (%)
b
SURFACE
AND BOTTOM
TEMP.
°C
c
DISSOLVED
OXYGEN
mg / l
d
NUTRIENT
CONC.
(0-2)m μg / l
PIGMENT
CONC.
e
(0-2)m
JULY
AUGUST

FIGURE 5 EVENTS ACCOMPANYING THE ONSET AND OCCURRENCE OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L522, 1979 AFTER JULY 17.

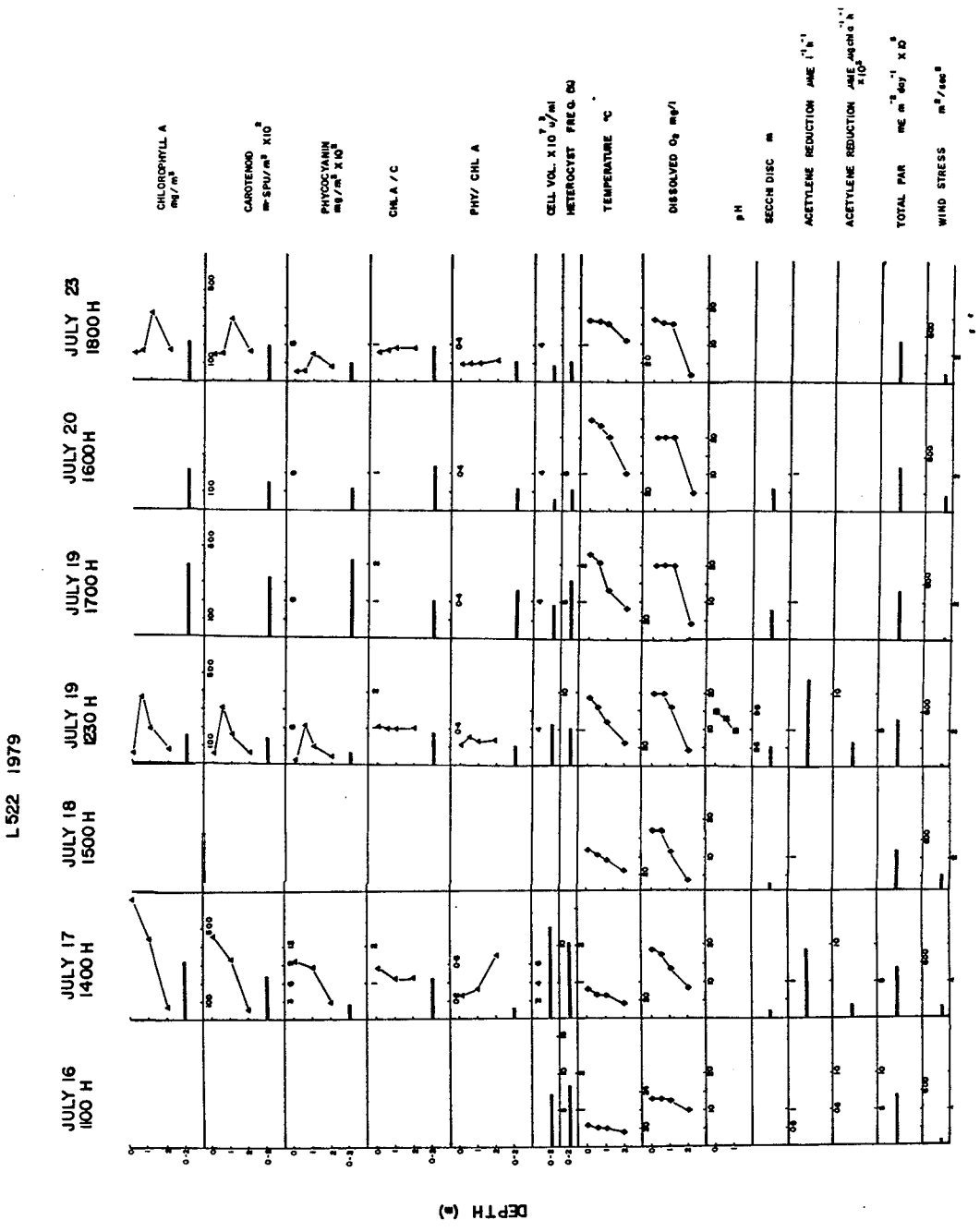
L522 1979
JULY 16 1100 H
JULY 17 1400 H
JULY 18 1500 H
JULY 19 1230 H
JULY 19 1700 H
JULY 20 1600 H
JULY 23 1800 H
CHLOROPHYLL A mg/m³
CAROTENOID m-SPU/m³ X10³
PHYCOCYANIN mg/m³ X10³
CHL A / C
PHY/ CHL A
CELL VOL. X10⁻⁹ µ³/ml
HETEROCYST FREQ. (%)
TEMPERATURE °C
DISSOLVED O₂ mg/l
pH
SECCHI DISC m
ACETYLENE REDUCTION nME l⁻¹ h⁻¹
ACETYLENE REDUCTION nME µgchl a⁻¹ h⁻¹ x10³
TOTAL PAR mE m⁻² day⁻¹ X10⁻³
WIND STRESS m²/sec²
DEPTH (m)

TABLE 3 SUMMARY OF CONDITIONS OCCURRING AT THE TIME OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L522, 1979 AFTER JULY 17.

DATE	CHL A MAX. (m)	DO	TEMP.	pH	TOTAL PAR	WIND STRESS
July 16, 1100h		m	m		h	1
July 17, 1400h	0	h	m		h	1
July 18, 1500h		m	m		h	1
July 19, 1230h	0.5	h	h	h	h	1
July 19, 1700h		h	m		h	1
July 20, 1600h		h	h		m	1
July 23, 1800h	1	h	m		h	1

FIGURE 6 L958, 1979

a: Cell volume of *Aphanizomenon* (■—■) x 10^7 u^3/ml and heterocyst frequency (▌) as the % of the total cell number in the 0-2m integrated water sample.

b: Surface (▲—▲) and bottom (△—△) water temperatures in °C, which were used to characterize the time of sampling into periods of thermal stability and instability: Period C - thermally stable, Period D - thermally instable, Period E - thermally instable and Period F - thermally instable.

c: Surface (◆—◆). 1m (◇—◇) and bottom (▲—▲) dissolved oxygen concentrations in mg/l.

d: Nutrient concentrations in the 0-2m integrated water sample: Ammonia (NH_3-N, △—△) and Soluble Reactive Phosphorus (SRP, ▲—▲) in ug/l.

e: Pigment concentrations in the 0-2m integrated water sample: Carotenoids (C, ◆—◆), in m-SPU/m^3, Chlorophyll a (Chl a, ○-○) in mg/m^3 and Phycocyanin (Phy, ■—■) in mg/m^3.

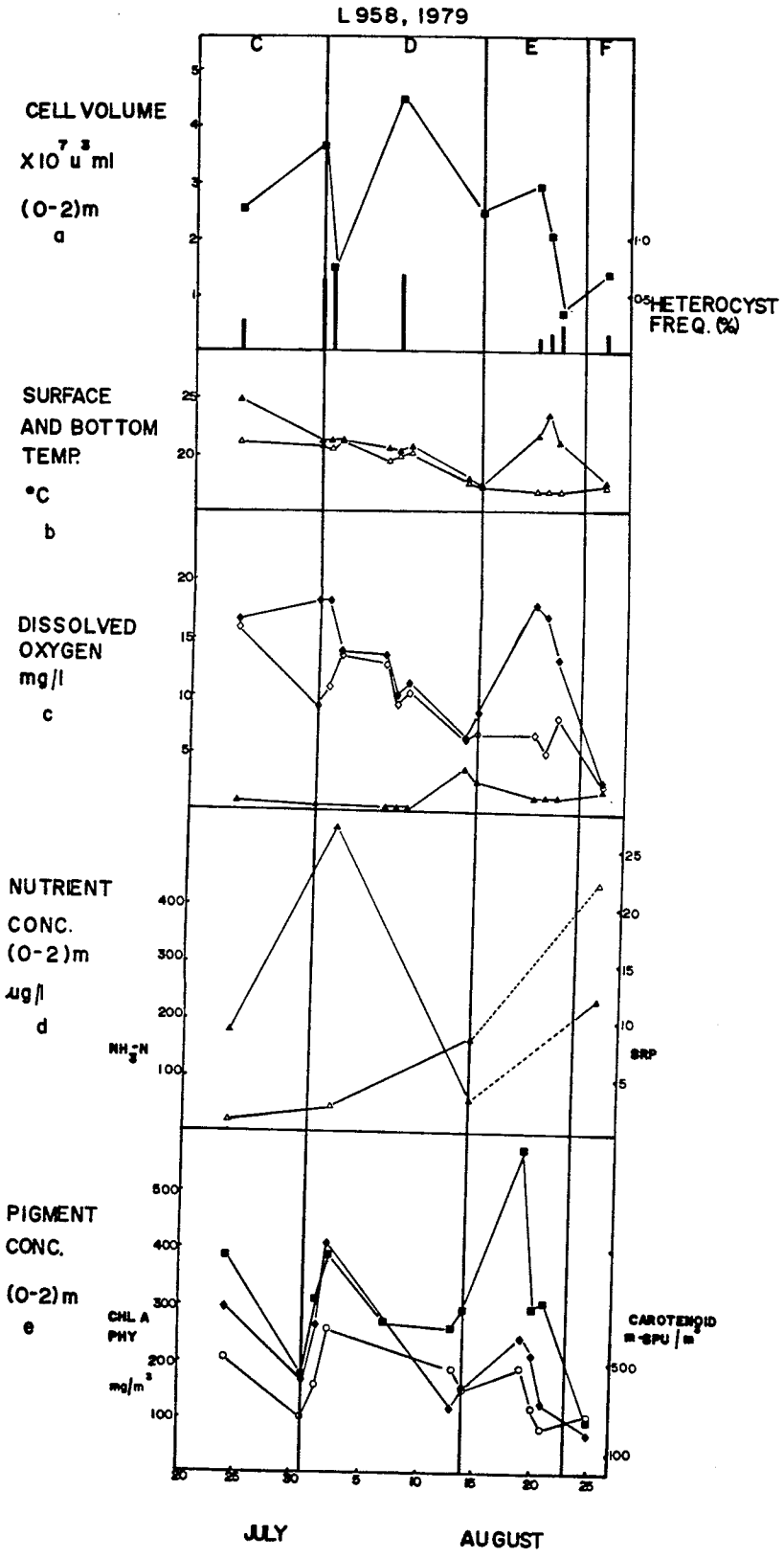
L 958, 1979
C
D
E
F
CELL VOLUME
X10⁷ u³ ml
(0-2)m
a
HETEROCYST FREQ. (%)
SURFACE AND BOTTOM TEMP.
°C
b
DISSOLVED OXYGEN
mg/l
c
NUTRIENT CONC.
(0-2)m
µg/l
d
NH₃-N
SRP
PIGMENT CONC.
(0-2)m
e
CHL A PHY
mg/m³
CAROTENOID
JULY
AUGUST

FIGURE 7 EVENTS ACCOMPANYING THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L958, 1979 FROM JULY 31 TO AUGUST 1.

L 958, 1979

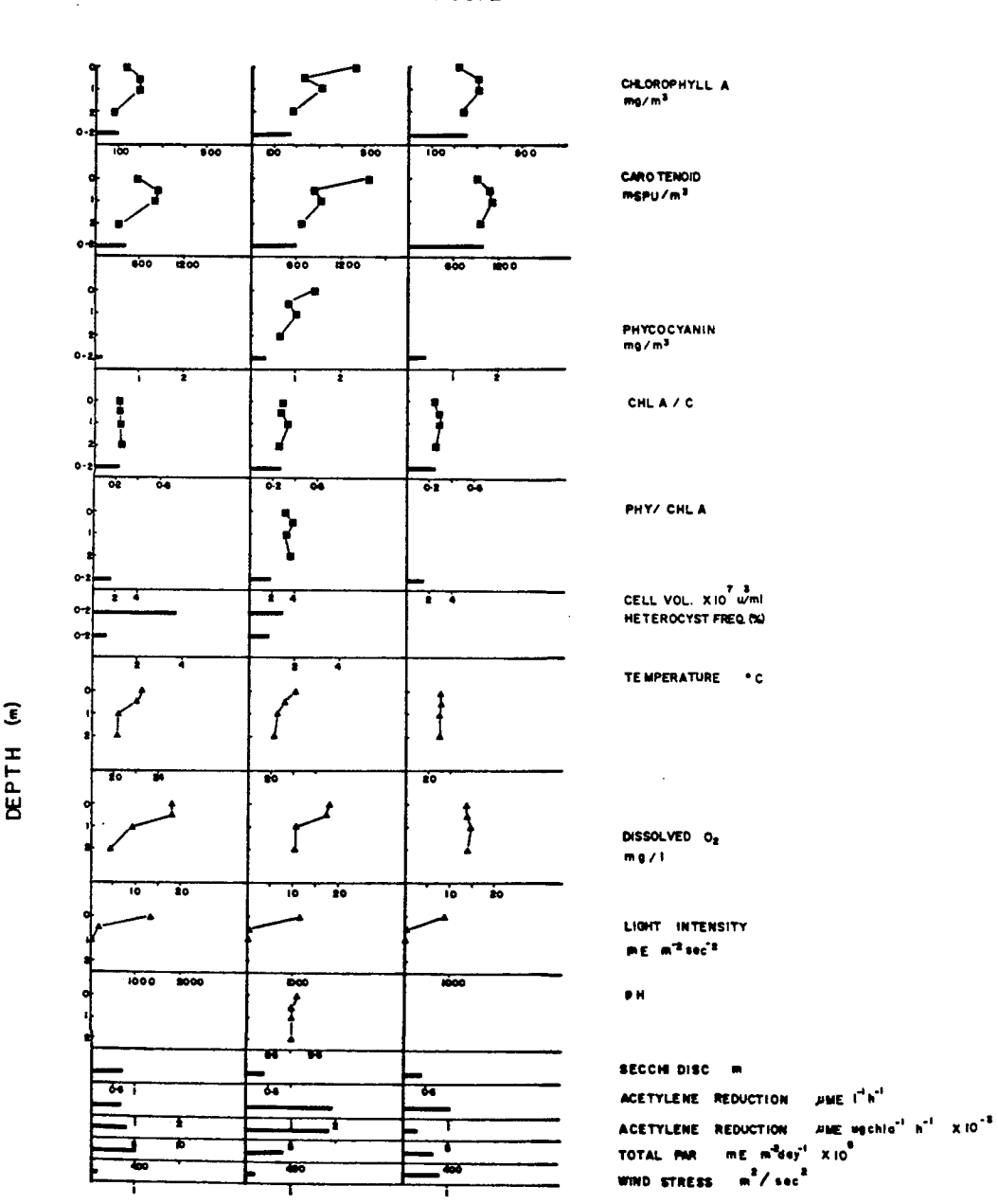
JULY 31
AUG. 1
AUG. 2
DEPTH (m)
CHLOROPHYLL A mg/m³
CAROTENOID mgSPU/m³
PHYCOCYANIN mg/m³
CHL A / C
PHY/ CHL A
CELL VOL. X10⁷ u³/ml
HETEROCYST FREQ (%)
TEMPERATURE °C
DISSOLVED O₂ mg/l
LIGHT INTENSITY μE m⁻²sec⁻¹
PH
SECCHI DISC m
ACETYLENE REDUCTION μME l⁻¹h⁻¹
ACETYLENE REDUCTION μME μgchla⁻¹ h⁻¹ X10⁻⁵
TOTAL PAR mE m⁻²day⁻¹ X10⁶
WIND STRESS m²/sec²

TABLE 4 SUMMARY OF CONDITIONS OCCURRING AT THE TIME OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L958, FROM JULY 31 TO AUGUST 1,1979.

DATE	CHL A MAX.(m)	DO	TEMP.	pH	LIGHT INT.	TOTAL PAR	WIND STRESS
July 31	0.5	h	m		m	1	1
August 1	0	h	m	h	h	1	1
August 2	1	m	m		1	1	1

FIGURE 8 EVENTS ACCOMPANYING THE ONSET AND OCCURRENCE OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L958, 1979 AFTER AUGUST 19.

L 958 ,1979

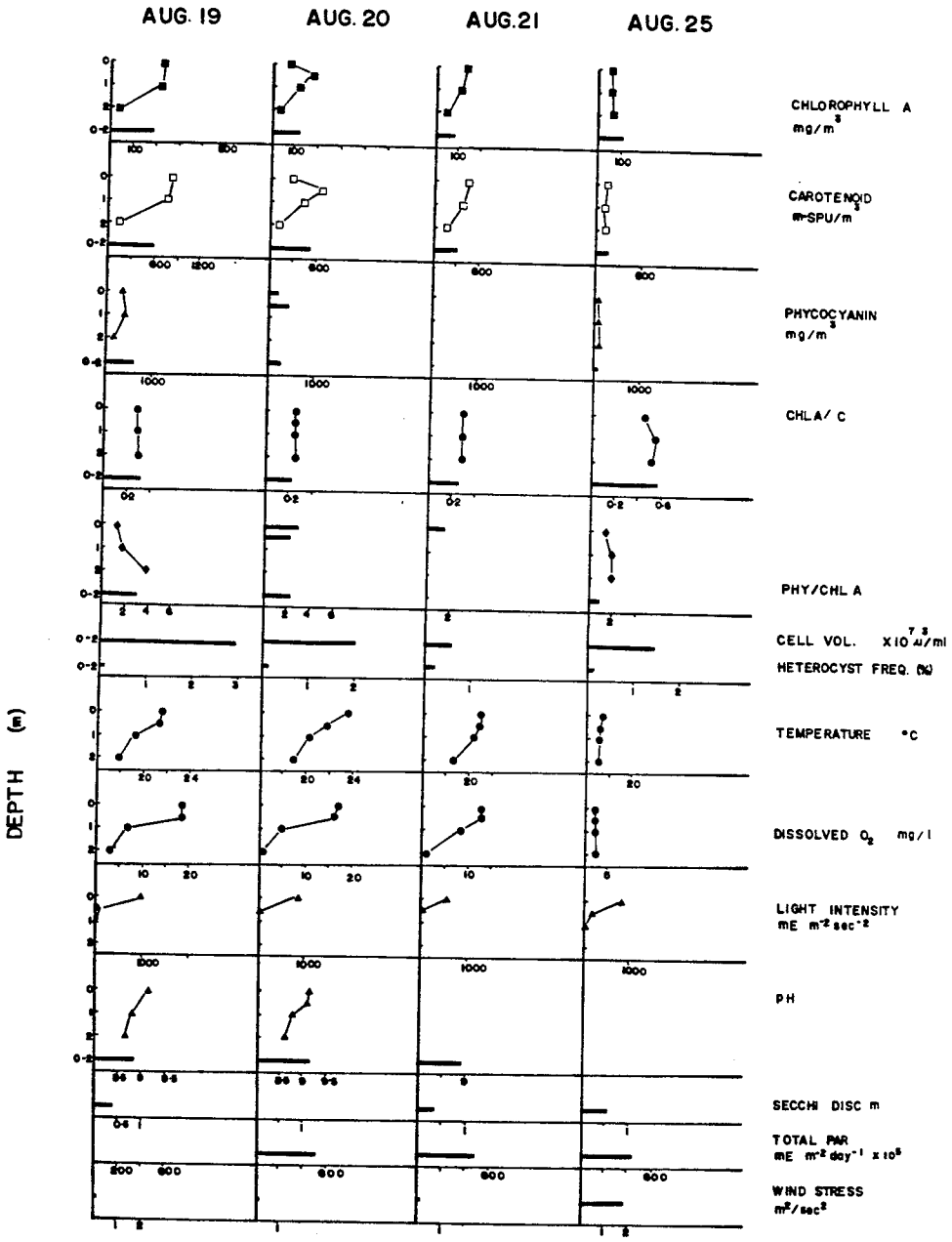
AUG. 19
AUG. 20
AUG.21
AUG. 25
DEPTH (m)
CHLOROPHYLL A mg/m3
CAROTENOID m-SPU/m3
PHYCOCYANIN mg/m3
CHLA/ C
PHY/CHL A
CELL VOL. X10 7.3 µ/ml
HETEROCYST FREQ. (%)
TEMPERATURE °C
DISSOLVED O2 mg/l
LIGHT INTENSITY mE m-2 sec-2
PH
SECCHI DISC m
TOTAL PAR mE m-2 day-1 x 10 5
WIND STRESS m2/sec2

TABLE 5 SUMMARY OF CONDITIONS OCCURRING AT THE TIME OF THE DECLINE IN CELL VOLUME OF *Aphanizomenon* OBSERVED IN L958, AFTER AUGUST 19, 1979.

DATE	CHL A MAX. (m)	DO	TEMP.	pH	TOTAL PAR	WIND STRESS
August 19	0	h	m	h		1
August 20	0.5	h	m	h	h	1
August 21	0	m	m		m	1
August 25	2	1	1		1	

FIGURE 9 L958, 1978

a: Cell volume of *Aphanizomenon* (■——■) x 10^7 u^3/ml and heterocyst frequency (▌) as the % of total cell number in the 0-2m integrated water sample.

b: Chlorophyll a (Chl a) concentration (■——■) in mg/m^3 and pH (▌) in the 0-2m integrated water sample.

c: Surface (■—■) and 2m water temperatures (□---□) in ^oC recorded in August.

d: Surface (■—■) and 2m (□---□) dissolved oxygen (DO) concentrations in mg/l, recorded in August.

e: Surface (■——■) and 0-2m integrated sample (▲----▲) Soluble Reactive Phosphorus (SRP) concentrations in ug/l, recorded in August.

f: Surface (■——■) and 0-2m integrated sample (▲----▲) Ammonia (NH_3-N) concentrations in ug/l, recorded in August.

g: Chlorophyll a profiles (▲——▲) in mg/m^3 demonstrating the occurrence of a surface bloom on August 11 with redistribution of the algae occurring by August 14.

L 958 • 1978

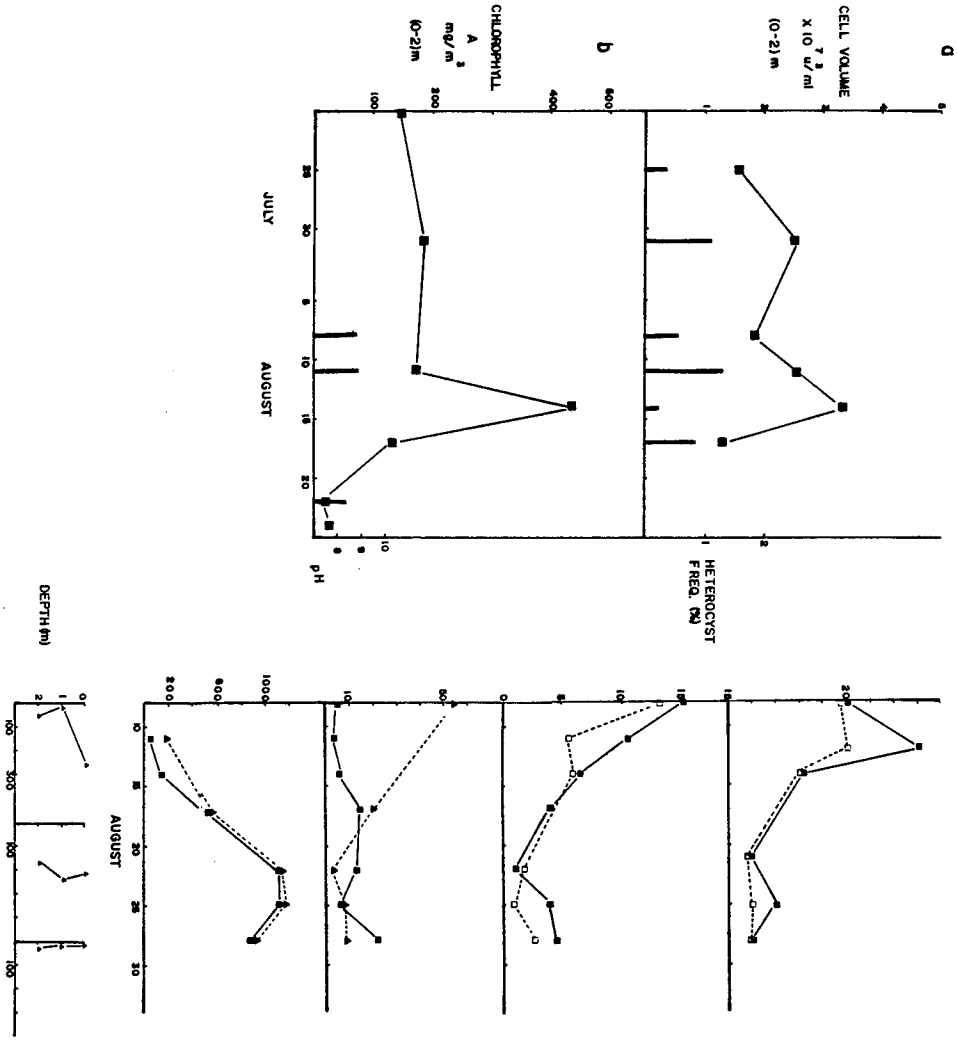
a
CELL VOLUME
x 10 μ³/ml
(0-2) m
b
CHLOROPHYLL
A
mg/m³
(0-2)m
JULY
AUGUST
pH
HETEROCYST
FREQ. (%)
c
TEMPERATURE
°C
d
DISSOLVED O₂
mg/l
e
SRP
μg/l
f
NH₃-N
μg/l
AUGUST
g
DEPTH (m)
CHL A
mg/m³
AUG. 11
AUG. 14
AUG. 22

TABLE 6 SUPEROXIDE DISMUTASE ASSAYS (1979)

LAKE	DATE	SPECIFIC ACTIVITY $\frac{\text{ng SOD}}{\text{ug protein}}$
522	August 2	5.98
522	August 13	.81
522	August 14	1.85
885	July 24	4.47
885	August 25	5.79
958	August 1	1.51
958	August 7	2.68
958	August 14	4.56
958	August 25	1.54

to severe lake oxygen depletion.

In 1885, 1979, a time lag was observed to occur between the onset of bloom collapse (July 19) and the period of severe DO depletion (July 31)(Fig. 1a and 1c). There did not appear to be a simple relationship between the actual amount of cell volume decline and the severity of DO depletion. For example, the loss in cell volume in L522 after July 17 was very high (Fig. 4a), however even after the following period of thermal instability, DO was not severely depleted (Fig. 4c). The depth of the anoxic layer prior to bloom decline and concommitant or subsequent mixing appeared to be important in determining the extent of DO depletion. Even though the cell volume declines observed in L958 (Fig. 6a) and L885 (Fig. 1a) were similar, DO in L958 (Fig. 6c) after subsequent mixing was much lower than in L885 (Fig. 1c). Prior to the decline, a greater proportion of L958 than L885 was anoxic or at least low in DO.

Cell volumes and chl a levels did not necessarily coincide. Cell volumes sometimes decreased before chl a levels and continued to decrease while chl a levels remained relatively stable (for example, L522, 1979, period C, Fig. 1a and 1e). On other occasions, cell volumes continued to increase after chl a levels were stationary or decreasing (for example, L958, 1979, period C, Fig. 6a and 6e).

The first of these situations may be partially due to

an inability to differentiate between intracellular chl and detrital chl by the methods employed in this study. It is likely that detrital chl could adsorb onto the flakes of algae on the filter. Poor extraction of chl a from blue-green algal cells is sometimes encountered when using acetone as an extraction medium (Holm-Hansen and Reiman, 1978). Once lysis was occurring, higher measured chl a levels could be due to a greater efficiency of extraction, since chlorophyll from small particles of algal material would be more easily extracted. It is generally thought that the initial breakdown of the chlorophyll molecule requires the presence of molecular O_2. Since the 0-2m water sample often contained anoxic water, it is possible that the breakdown of detrital chl was hindered. Kinetic analysis of the decomposition of natural blue-green algal populations under laboratory conditions has revealed that the rate of decrease of photosynthetic ability was greater than decrease in algal volume which was greater than particulate chlorophyll which was greater than particulate protein (Fallon and Brock, 1979b). Further experiments led Fallon and Brock (1979b) to assume that under dark conditions, algal autolysis takes place, leading to the loss of morphological integrity and that autolyzed material remains in particulate form, where it then undergoes decomposition. The high chl a levels sometimes measured after a cell volume decline, in the current study, may therefore be due to a slow rate of decomposition of such particulate

chlorophyll.

Reynolds (1975) has reported a tendency for the chl a content of blue-green algal cells to decrease with stage of growth. It is likely that intracellular chl a levels would decrease before cell volume decrease. This tendency may account for the second type of discrepancy in cell volume and chl a levels.

For the *Aphanizomenon* populations studied, cell volume appears to serve as a more accurate estimate of biomass than chl a.

Another problem encountered in estimating the total *Aphanizomenon* population was related to considering the 0-2m integrated water sample as representative of the entire lake population. Under different environmental conditions, the proportion of the total population below 2m was found to vary.

CHARACTERIZATION OF THERMALLY STABLE PERIODS

Lakes with surface temperatures of 25 to 30 °C, only need bottom water 3 to 4 °C cooler to be stable, while those with surface waters of 15 to 20 °C need bottom water about 6 to 8 ° C for stability under most wind conditions (Goldman and Horne, in Horne, 1978). During thermally stable periods the lakes can be considered to be composed of two distinct layers. The upper zone can be characterized as having high DO, pH and temperature and low nutrient levels. Light intensity at the surface

during periods of thermal instability was often high, although attenuation was rapid. Chl a profiles sometimes revealed a sharp localization of the algal population. It has been suggested that this sharp localization may be related to the limited flexibility of the blue-green algae resulting from lack of enzyme repression and induction (Fogg et al., 1973). Population maxima tended to occur towards the bottom of the photic zone, where the light intensity was low. The chla/C ratios for algae from this upper zone was often low, resulting in yellow-green cells.

The lower zone of the lakes was either anoxic of low in DO. pH and temperature were lower than those of the upper zone. Nutrient concentrations were high. The morphology of the algal flakes within this layer differed. Flakes of *Aphanizomenon* were generally shorter, jagged at the edges and a darker green (higher chl a/C ratios).

Laboratory investigations of *Aphanizomenon* have shown that clusters of the algae migrate from the more oxygen saturated surface layers of the water to the bottom layers and back again over the course of a day (Sirenko et al., 1968). Results in the current study indicate that passage of the algae from the upper zone into the lower zone was hindered or limited during periods of thermal stability. If passage to the nutrient rich lower waters occurred regularly, it is not likely that there would have occurred a difference in the flake morphology between the two zones or that algae in the upper zone would become nutrient deficient.

CHARACTERIZATION OF THERMALLY INSTABLE PERIODS

With the breakdown of thermal stratification, gradients in temperature, DO, pH and nutrient concentrations are lessened. Generally, DO, temperature and pH decreased and nutrients increased. The algae became more evenly distributed; the portion of the population below 2m increased. Light intensities although low at the surface, were attenuated at a lower rate than under thermally stable conditions. Often periods of thermal instability were accompanied by increases in the chl a/C ratios. The morphology of the algal flakes seen at this time was variable.

ALGAL RESPONSES EXPECTED TO OCCUR UNDER CONDITIONS CONDUCIVE TO PHOTOOXIDATION AND DEATH DUE TO OXYGEN TOXICITY

As O_2 supersaturation proceeds, and as pH levels rise, a decrease in the cellular phycocyanin contents is expected (Paerl and Kellar, 1979). A concurrent decrease in the chl a/C ratio would be expected since carotenoids may function to protect the cell from photooxidation. Chl a/C ratios for L522 were generally higher than those recorded for L885 and L958. As O_2 supersaturation in the upper zone proceeded during periods of thermal stability chl a/C ratios were found to decrease resulting in yellow-green cells. It was not apparent whether cells responded by decreasing chlorophyll contents, as is suggested by Jones and Myers (1965), or

by increasing carotenoid levels as suggested by Paerl and Kellar, (1979) or by a combination of both. Chl a/C ratios increased once algal declines were on their way and whenever mixing of the lake occurred.

Phy/chl a ratios proved more difficult to interpret. The major problem encountered was that intracellular and extracellular phycocyanin levels were not distinquished by the methods employed. On thermally stable days, patches of blue-green algae surrounded by rings of blue-green coloration were noted at the lake surface. Filtration of such water onto a glass fibre filter resulted in measurements of very high phycocyanin levels, since the filters were not washed before being frozen. The undersides of such filters were bright blue. High phycocyanin measurements in this study appeared to coincide with times of algal lysis. Similarly, in Lake Mendota, the appearance of opalescence and blue pigmentation in the water was assumed to indicate that a significant amount of lysis occurred (Fallon and Brock, 1979a). In the current study, the fact that the bluish-green scums were noted is evidence that before lysis occurs, phycocyanin was still found in the *Aphanizomenon* cells.

Even though heterocysts appear to represent a morphological specialization to permit CO_2 and N_2 fixation to occur in the same algal filament, nitrogen fixation in heterocystous blue-green algae has been reported to be sensitive to increases in diurnal oxygenation (Stewart and Pearson.1970;

Paerl and Kellar, 1977), which commonly occur during freshwater blooms. However, Paerl (1976, 1978a, 1978b) and Paerl and Kellar (1977, 1978, 1979) have observed several mechanisms or processes employed by N_2 fixing species of *Anabaena* in O_2 supersaturated waters to overcome the inhibitory effect of high O_2 tensions. N_2 fixation assays were difficult to interpret since bloom declines were often accompanied by apparent decreases in heterocyst frequencies, therefore decreases in acetylene reduction rates observed could not necessarily be assigned to inhibition by high O_2. Heterocysts have only a limited life span and upon senescence are broken off the algal filaments (Tischer, in Fay, 1973). The decreases in heterocyst frequencies observed could be due to a widespread senescence of heterocysts with a reduced rate of induction of heterocysts occurring.

SOD levels have been shown to vary with stage of growth in cultures of *Anacystis nidulans* grown under an atmosphere of air (Abeliovich et al., 1974). The specific SOD activity was found to increase with age of the cultures, reaching a maximum at the end of the logarithmic phase of growth. Unfortunately, in the current study of natural *Aphanizomenon* populations, no samples at the end of logarithmic growth were analyzed, so that the maximum specific activities are unknown.

Levels of resistance shown by different blue-green algae to photooxidative effects in the field and in the laboratory

seemed to be correlated with the rate of loss of SOD activity on exposure rather than to the initial SOD level (Eloff et al., 1976). Photooxidative death was found to occur after SOD levels dropped to 10% of the initial level (Abeliovich et al., 1974: Eloff et al., 1976). Measurements of SOD from L958 showed that enzyme levels from the same population do vary. Not enough evidence was available for any further speculation.

POSSIBLE CONTRIBUTION OF PHOTOOXIDATION AND O_2TOXICITY TO DECLINE EVENTS

L885, 1979

Although total PAR inputs were high and wind stress values were low during period C, it is unlikely that photo-oxidative death played a major role in the decline observed after July 20, since field observations revealed that a surface bloom did not occur during this period. Light intensities experienced by the majority of the algae were low, however DO and temperature were moderate to high. High temperatures have a similar effect as high light intensities in that both increase the rate of free radical formation and thus the damaging effects of high O_2 (Haugaard, 1968). It is possible that O_2 toxicity played a role in triggering this collapse.

Although total PAR inputs were moderate to high, and wind stress values were low during period E, it is unlikely that photooxidation played a major role in triggering this

collapse. Field observations and chl a profiles demonstrated that a surface bloom did not occur. The majority of the algae were however, exposed to high to moderate DO, moderate temperatures and high pH. It is possible that death due to O_2 toxicity was a major mechanism for the decline observed in period E.

L522, 1979

The decline observed in period C, was likely largely due to the photooxidative death of cells. A surface scum of the algal population occurred on July 17. At the same time, surface light intensities and DO, temperature and pH values throughout the upper zone were moderate to high. Total PAR inputs were high and recorded wind stress values low. It is of interest to note, that an algal collapse occurred in L522, even though SRP levels were high, and presumably the cells were not P deficient.

L958, 1979

The slight decline observed in cell volume from July 31 to August 1 may be due to some extent to the photooxidative death of cells. Conditions conducive to photooxidative cells were evident for at least a portion of August 1.

Since total PAR inputs, temperature, DO and pH were not high during period D, it can be concluded that photooxidative death did not contribute to the decline observed between August 7 and 14.

The algal decline that occurred after August 20 was preceded by high DO, moderate temperatures, high pH and calm sunny conditions. Since high levels of chl a were recorded near the surface it is likely that photooxidation contributed significantly to this decline.

L958, 1978

Judging by both cell volumes and chl a levels the collapse of the algal population began after August 14. On August 11, the algal population was concentrated at the surface. However, at this time, DO, temperature and pH as well as total PAR (Appendix 2) were not high. Perhaps photooxidative death occurred to a portion of the population, but it is unlikely that photooxidation or O_2 toxicity played an important role in triggering algal collapse.

APPENDIX 1

TOTAL DAILY PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR), 1979, $mE/m^2/day \times 10^5$

DATE	TOTAL PAR	DATE	TOTAL PAR
July 1	108	July 31	402
July 2	391	Aug. 1	346
July 3	425	Aug. 2	272
July 4	425	Aug. 3	207
July 5	421	Aug. 4	491
July 6	533	Aug. 5	465
July 7	542	Aug. 6	431
July 8	465	Aug. 7	487
July 9	478	Aug. 8	424
July 10	559	Aug. 9	344
July 11	534	Aug. 10	488
July 12	456	Aug. 11	430
July 13	528	Aug. 12	330
July 14	277	Aug. 13	NR
July 15	316	Aug. 14	257
July 16	566	Aug. 15	206
July 17	559	Aug. 16	397
July 18	506	Aug. 17	439
July 19	528	Aug. 18	463
July 20	439	Aug. 19	NR
July 21	441	Aug. 20	536
July 22	446	Aug. 21	491
July 23	538	Aug. 22	488
July 24	333	Aug. 23	509
July 25	325	Aug. 24	381
July 26	502	Aug. 25	426
July 27	507	Aug. 26	326
July 28	462	Aug. 27	541
July 29	100	Aug. 28	467
July 30	308	Aug. 29	533

APPENDIX 2

TOTAL PHOTOSYNTHETICALLY ACTIVE RADIATION (PAR), 1978, $mE/m^2/day \times 10^5$

DATE	TOTAL PAR
Aug. 9	440
Aug. 10	NR
Aug. 11	453
Aug. 12	446
Aug. 13	NR
Aug. 14	NR
Aug. 15	357
Aug. 16	424
Aug. 17	241
Aug. 18	457
Aug. 19	443
Aug. 20	348
Aug. 21	127
Aug. 22	NR
Aug. 23	74
Aug. 24	410
Aug. 25	285
Aug. 26	401
Aug. 27	386
Aug. 28	393
Aug. 29	443
Aug. 30	396
Aug. 31	411

APPENDIX 3

WIND STRESS VALUES MEASURED AT THE CENTRE OF L885, 1979

DATE	WIND STRESS m^2/sec^2
July 10	1.23
July 10 - July 11	.92
July 11	1.20
July 11 - July 12	.58
July 12	9.86
July 12 - July 13	1.93
July 13	11.36
July 13 - July 14	12.04
July 14 - July 15	2.82
July 15 - July 16	.28
July 17 - July 18	1.30
July 18 - July 19	.06
July 19 - July 23	.85
July 23 - July 24	.06
July 24 - July 26	.28
July 26 - July 27	.02
July 30 - July 31	.18
July 31 - Aug. 5	.83
Aug. 5 - Aug. 14	1.17
Aug. 14 - Aug. 15	1.06
Aug. 15 - Aug. 18	1.37
Aug. 18 - Aug. 19	.10
Aug. 19 - Aug. 21	.17
Aug. 21 - Aug. 25	1.93

CHAPTER 3

Collapses of *Aphanizomenon flos-aquae* (L.) Ralfs blooms: Possible contribution of cyanophages

ABSTRACT

Morphological evidence for the occurrence of virus-like particles within cells of *Aphanizomenon flos-aquae* (L.) Ralfs has been provided for populations studied in two shallow eutrophic lakes (L958, 1978, L958, 1979, L885, 1979) in Southwestern Manitoba. The virus-like particles (vlps) observed were polyhedral, and of a diameter of approximately 50-60 nm. Vlps were observed to occur both in the nucleoplasm and on the inside periphery of the cell wall. Although a limited number of samples were taken from each population evidence is suggestive that cyanophages contribute to bloom decline. Since transmission and isolation of the vlps has not been substantiated, the verification of a virus infection of the *Aphanizomenon* populations studied is not possible.

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INTRODUCTION

Collapsing *Aphanizomenon flos-aquae* (L.) Ralfs blooms and the resulting O_2 depletion in the water column cause massive fish kills in shallow eutrophic lakes across the North American prairie region and are a serious setback for the fisheries industry (Barica, 1978). Numerous mechanisms have been suggested to explain such sudden collapses, among them being photooxidation, O_2 toxicity, autoinhibition and cyanophage attack. In a previous paper (Coulombe and Robinson, 1980), the possible contributions of photooxidation and O_2 toxicity were examined. The algal declines examined were at times initiated during periods of thermal stability when conditions were conducive to photooxidation and death due to O_2 toxicity. However, some of the algal declines observed were initiated during periods of lake thermal instability, when conditions were such that photooxidation and O_2 toxicity could be ruled out as causal factors for the decline. Granhall (1972) has reported results that indicated that cyanophages regulated the termination of an *Aphanizomenon* bloom in Lake Erken, Sweden. In this paper, the possibility that a cyanophage attack may contribute to the decline of the *Aphanizomenon* blooms observed in small prairie lakes was considered.

Three *Aphanizomenon* populations, namely those of L958, 1978, L958, 1979, and L885, 1979, were examined for the morphological evidence of virus-like particles (vlps) using

electron microscopy. These lakes are located in Southwestern Manitoba, central Canada and are within the study area of a Rainbow Trout Aquaculture Project.

MATERIALS AND METHODS

A 0-2m integrated water sample was used to obtain a mass of the alga for examination. Flakes of *Aphanizomenon* were concentrated with a 250 μ mesh and fixed by suspension in a 3% glutaraldehyde solution buffered with Na cacodylate (0.1M) for 1.5 hours at room temperature. Four, 15 minute washes with 0.1M Na cacodylate buffer followed. A suspension of the alga was then embedded in a film of 2% noble agar. The solidified agar was cut into cubes less than $1mm^2$ in dimensions. Post fixation was carried out in a 1% OsO_4 solution buffered with Na cacodylate (0.1M) for 2 hours at 0 ^{o}C. A 1 hour rinse with distilled water followed. The cubes were stained in a 5%/H_2O uranyl acetate solution overnight. Dehydration through an ethanol series was carried out the next morning. The samples were then frozen.

After thawing (1 hr. room temperature), and 2, 30 min. rinses with absolute alcohol the cubes were embedded in Spurr's resin (1969), and sections cut at approximately 600 A^{o}. Sections were stained with Pb citrate (Reynolds, 1963) for 2 to 5 minutes in an atmosphere of N_2 and examined with either an AEI-EM6B or AEI-801S electron

microscope.

RESULTS

Virus-like particles (vlps) were observed in a fraction of the *Aphanizomenon* cells from all 3 populations examined. The vlps observed were polyhedral and of a diameter of approximately 50-60 nm (Fig.1). Higher magnification photographs revealed the presence of subunits (Fig. 2). Chains of vlps oriented perpendicularly to the cell wall were seen (Fig. 2). The vlps sometimes occurred in the nucleoplasm (Fig. 3) and sometimes along the inside periphery of the cell wall (Fig.4). Particles, which may be defined as ghost particles were observed to occur (Fig. 5). Cells containing vlps appeared empty while cells without vlps were dense and possessed abundant gas vesicles (Fig. 6). The photosynthetic lamellae of normal vegetative cells did not occur in peripheral bands but rather intrathylakoidal spaces were wide (Fig. 6). Some of the vlps observed had an inner less dense region. When individual cells of a filament contained vlps it was likely that other cells along the filament also contained vlps. Extracellular virions were not seen. Cells containing vlps and with broken cell walls were not observed.

Light microscope observations of phytoplankton samples taken at the onset of decline of *Aphanizomenon* often revealed the presence of many single cells or short

filaments of empty-looking cells. Isolated heterocysts were abundant in such samples.

Table 1 summarizes the possible relationship between the observation of intracellular vlps and the bloom declines reported in a previous paper (Coulombe and Robinson, 1980).

DISCUSSION

Morphological evidence of vlps within *Aphanizomenon* cells has been presented. The dimensions of such vlps were similar to those of the Ap-1 virus reported within *Aphanizomenon* in Lake Erken (Granhall, 1972). The Ap-1 viruses were reported to be polyhedral with a head of 50-60 nm and a contractile sheath of 20-30 nm. Some characteristics displayed by the Ap-1 virus are adsorption by the tail, a contractile tail sheath, injection of the nucleic acid, multiplication in the nuclear region of the host, degradation of the host DNA and finally lysis (Granhall, 1972).

In the current study and Granhall's study (1972) vlps were found to be agregated into chains oriented perpendicularly against the cell wall. Granhall proposed the possibility that this may be an artefact due to the fixation process.

Smith et al.,(in Brown, 1972) have reported that certain electron micrographs have suggested that virus particles may synthesize the tail assembly and immediately

FIGURE 1: *Aphanizomenon* cell containing polyhedral virus-like particles (vlps) of a diameter of approximately 50-60 nm. Note the clusters of gas vesicles forming gas vacuoles (gv).

1

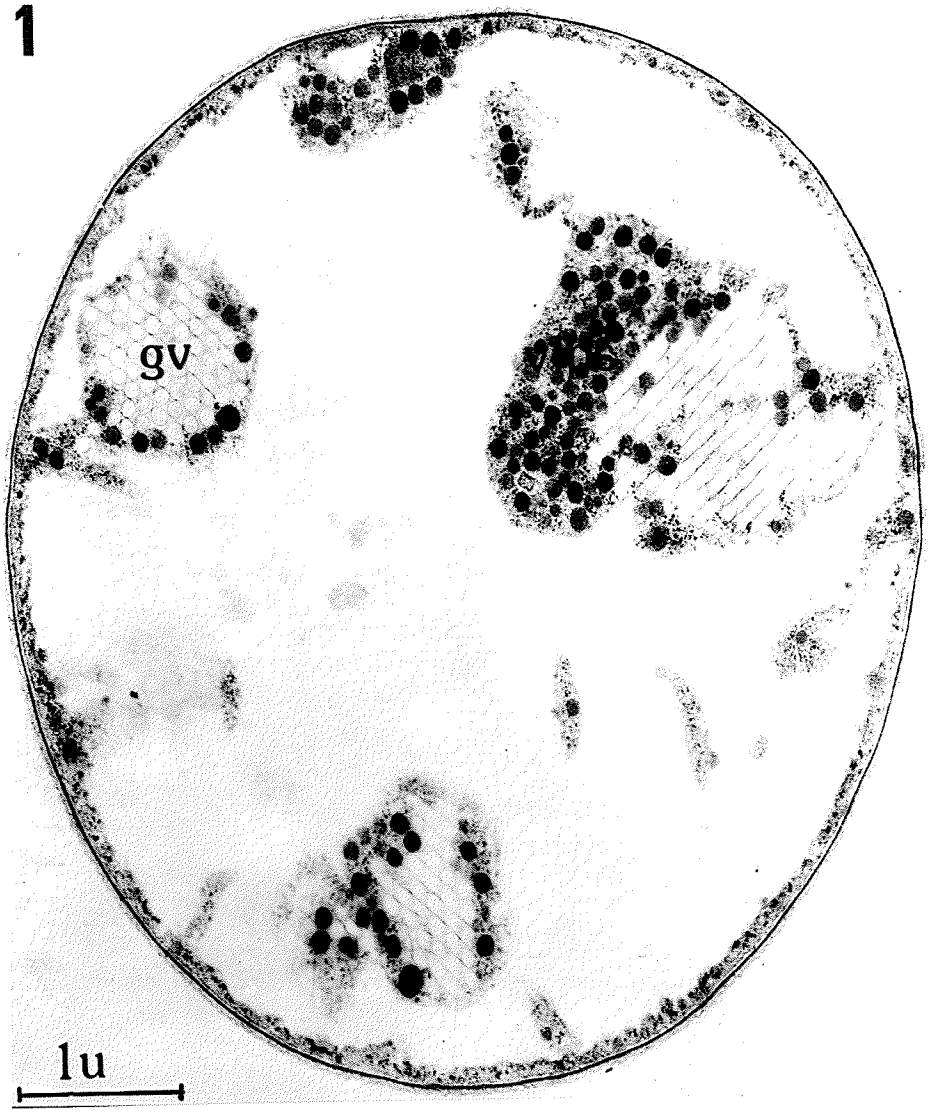
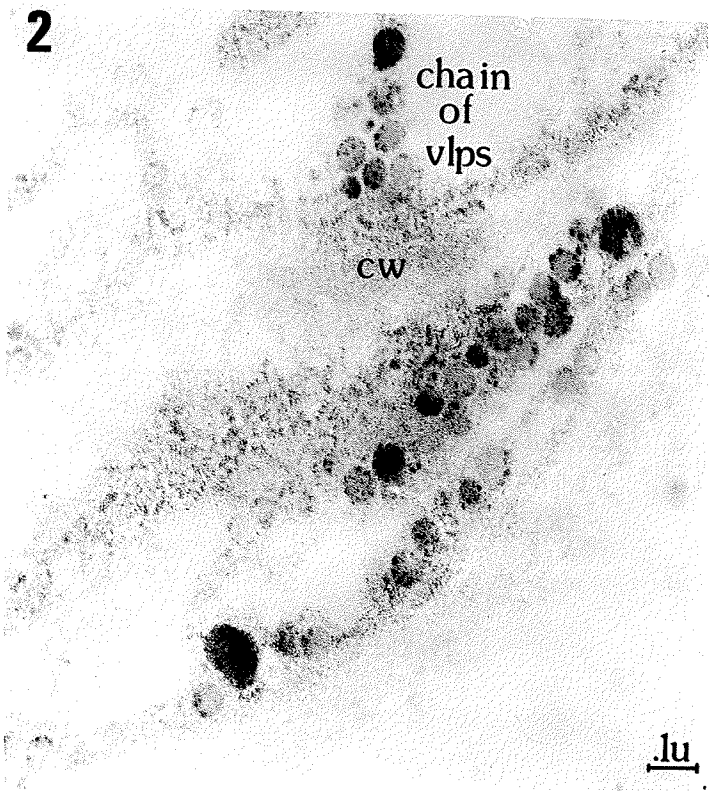


FIGURE 2: *Aphanizomenon* cell containing chains of vlps oriented perpendicularly to the cell wall (cw) or along the periphery of the cell wall. Note that the vlps appear to be composed of subunits.

FIGURE 3: *Aphanizomenon* cell containing a cluster of vlps in the nucleoplasm (nuc).


2
chain
of
vlps
cw
.1u

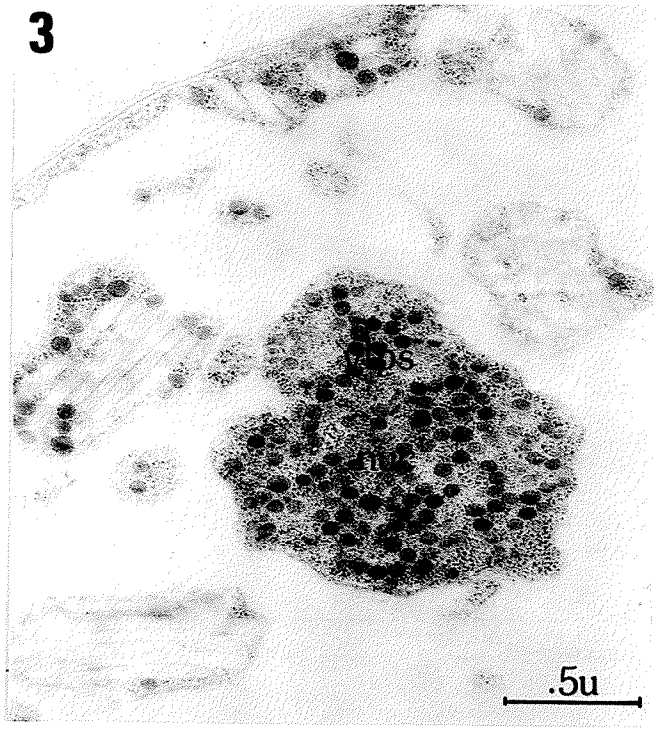
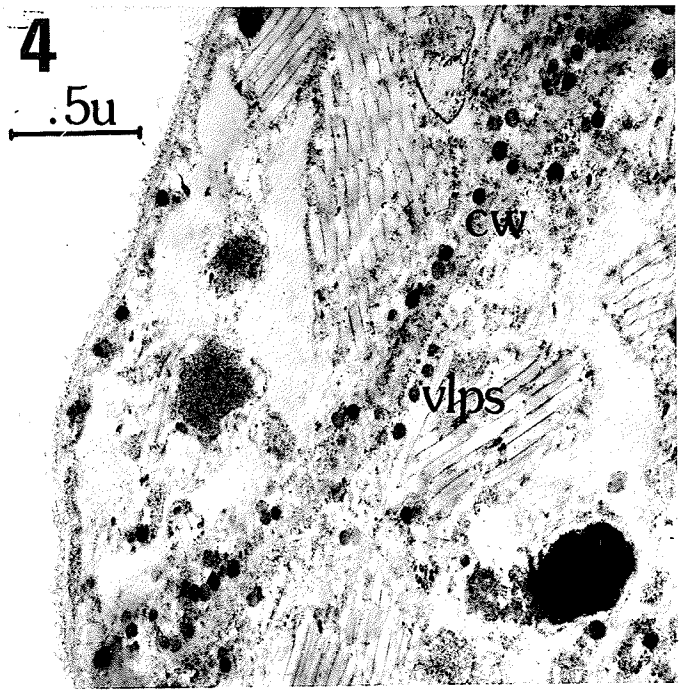

3
.5u

FIGURE 4 : Two adjacent *Aphanizomenon* cells both containing vlps along the inside periphery of their cell walls (cw).

FIGURE 5: *Aphanizomenon* cell at the end of a filament with attached ghost particles (gp).


4
.5u
cw
vlps

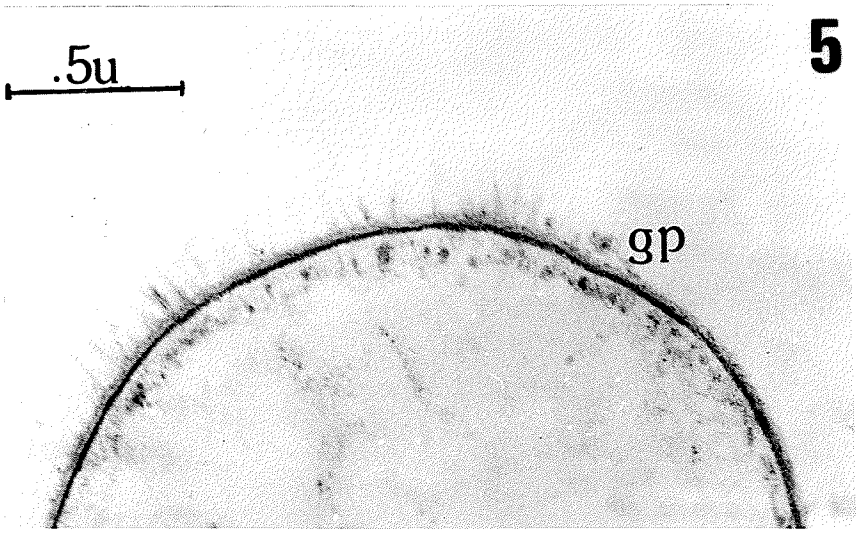
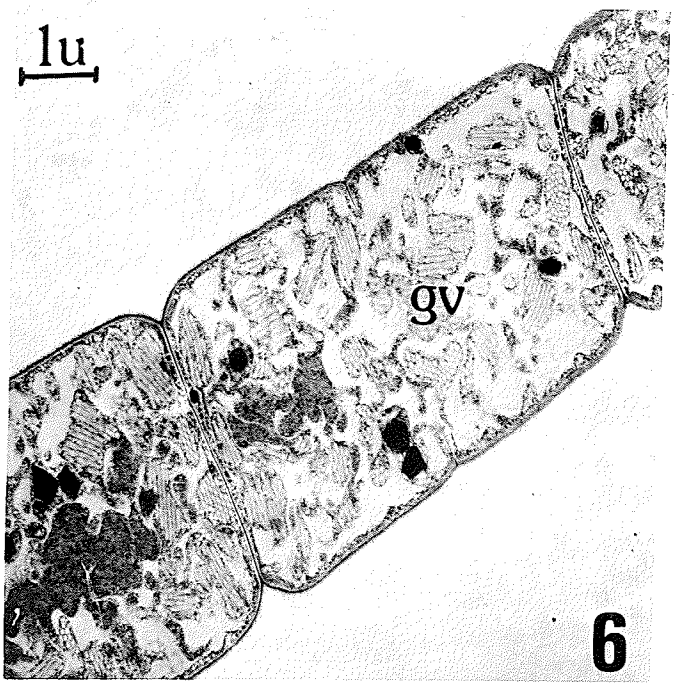

.5u
5
gp

FIGURE 6: Two photographs of normal *Aphanizomenon* filaments. Note that these cells are much denser than cells containing vlps. Gas vacuoles (gv) are abundant. The photosynthetic lamellae (pl) do not occur in peripheral bands but rather intrathylakoidal spaces are wide.


1u
gv
6

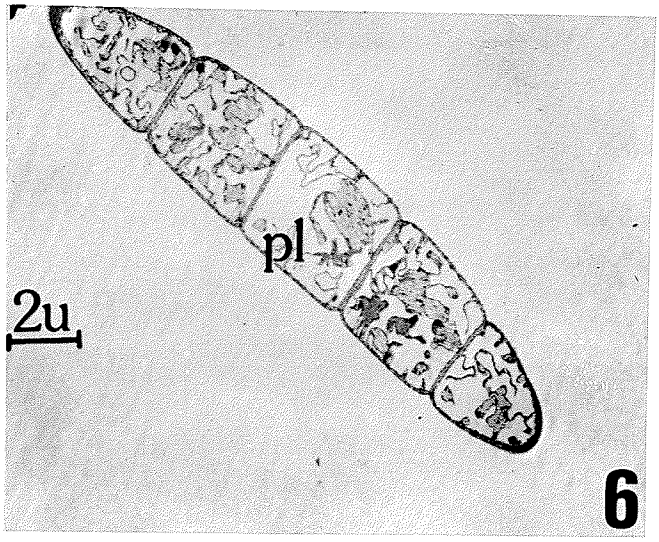

pl
2u
6

TABLE 1

POSSIBLE RELATIONSHIP BETWEEN THE OBSERVATION OF INTRACELLULAR VLPS AND THE *Aphanizomenon* DECLINES OBSERVED

PERIOD OF DECLINE IN CELL VOLUME	LAKE THERMAL CONDITIONS AT ONSET OF DECLINE	HETEROCYST FREQUENCY (increase) or (decrease)	DATES SAMPLED FOR ELECTRON MICROSCOPY	LAKE THERMAL CONDITIONS ON THIS DATE	VLPS
L885, 1979					
July 19-31	stable	(-)	July 14	instable	(-)
Aug. 20-25	stable	(-)	Aug. 8	instable	(+)
L958, 1978					
Aug. 14-17	instable	(-)	Aug. 14	instable	(+)
			Aug. 22	instable	(+)
L958, 1979					
July 31-Aug. 1	instable	(+)	July 24	stable	(+) 20%
Aug. 7-14	instable	(-)	Aug. 6	instable	(-)
			Aug. 13	instable	(+) 20%
Aug. 19-25	stable	-	Aug. 25	instable	(+) 80%

DEFINITION OF TERMS

Thermal Conditions: Stable and Instable (as defined in Coulombe and Robinson, 1980).
Heterocyst Frequency: (-) if decline accompanied by decrease, (+) if decline accompanied by increase, - if low prior to onset of decline
Virus-Like Particles: VLPS, (+) if observed, (-) if not seen, % infected given if known.

inject DNA into the adjacent cells of the filament prior to lysis. This would result in an intracellular infection and might explain the occurrence of vlps along the cell wall and the occurrence of vlps in adjacent cells in the algal filaments as observed in the current study.

The fact that the vlps in one cell did not appear to be exactly at the same stage and that intermediates of viral assemble could be detected may be the result of the prolonged duration of the cyanophage reproduction cycle. Padan and Shilo (1973) have reported that the pattern of the infection cycle of cyanophage is very similar to that of bacteriophages, although much slower, lasting 13 hours at 26 °C in LPP-1G or 50 hours in SM-1 whereas most bacteriophages complete their reproduction in tens of minutes.

Brown (1972) has reported that the first sign of an infected *Plectonema boryanum* cell is the displacement of the parietal and concentric photosynthetic membranes. This proved difficult to observe in *Aphanizomenon* since the photosynthetic lamellae do not ordinarily appear in such peripheral bands, as reported by Wildman et al.,(1975).

The fact that extracellular virions were not observed may be a function of the preparatory methods. Since the flakes of algae were washed many times and embedded in agar during the fixation procedure it is possible that such particles were washed away.

Cells containing vlps and with broken cell walls

were not observed in this study although Granhall (1972) reported their occurrence. This may have been due to a lack of preservation of such cells. In this connection, it should be noted that the presence of intracellular viruses may markedly effect the physiology of the host cell prior to lysis. Infection of *Plectonema* by LPP-1G effects a rapid and complete cessation of CO_2 photoassimilation (Padan and Shilo, 1973). Wu and Shugarman (1967) in their studies of *Plectonema* have observed a 50% decrease in photosynthesis and a threefold increase in respiration in infected cells. N_2 fixation has been observed to decrease upon infection of *Anabaena variabilis* by An-1 (Granhall and Hofsten, 1969). Therefore, it is likely that the presence of intracellular vlps effects the physiology of the host *Aphanizomenon* cell.

If one considers the possible relationship of vlps to bloom decline, observations of many apparently empty cells and short filaments of cells at the time of collapse is what one would intuitively expect to see following a cyanophage attack. Investigations on the LPP virus and *Plectonema* have revealed that during periods of lysis, the *Plectonema* filaments are very short due to breakages following the random infection and lysis of cells (1 to 5 cell fragments) and that there may be a several day delay in fragmentation (Cowlishaw and Mrsa, 1975). Similarly, Safferman (1967) has reported that in virus-infected *Plectonema* as lysis progressed, the filaments

fragmented into smaller units until only scattered cells were evident in the preparation. The presence of isolated heterocysts in some of the samples taken at the time of collapse onset and the decreases in heterocyst frequency which often accompanied algal declines is also what one would expect to observe after a widespread cyanophage attack, since heterocysts are known to be resistant to cyanophage attack (Granhall and Hofsten, 1969; Granhall, 1972).

The interaction of cyanophage with its host is markedly influenced by environmental conditions (Padan and Shilo, 1973). Vlps were often reported in *Aphanizomenon* cells taken from the lakes during periods of thermal instability. Such periods are accompanied by decreases in pH, as well as an increase of nutrients throughout the water column (Coulombe and Robinson, 1980). Lindmark (1979) has reported that the lowering of the pH by the addition of CO_2/air to a culture of *Plectonema* containing LPP-1 induced a rapid lysis of the *Plectonema* population despite the fact that the algae could grow well at low pH in the absence of cyanophage. Field observations revealed that a combined reduction of pH and the addition of nutrients (N and P) accelerated blue-green algal collapse (Lindmark, 1979). It may be speculated that the onset of thermal instability in L958 and L885 favored the cyanophage.

Laboratory experiments have shown thay *Plectonema* in the exponential growth phase possessed the strongest

physiological resistance to lysis by LPP-1 and that algae in the lag phase of growth were found to induce a rapid increase in cyanophage, thus it was concluded that variation in algal density was less important for viral replication than the physiological stage of the host (Lindmark, 1979). Perhaps vlps would be present in a portion of the *Aphanizomenon* population during the lag phase, stationary phase or whenever the cells were physiologically susceptible. Whether or not a noticeable algal decline would ensue may depend on the size of the initial susceptible population.

Granhall (1972) reported that in the year following the noted virus infection the abundance of *Aphanizomenon* was dramatically less. In this study, vlps were observed in samples from L958, in both 1978 and 1979, however the density in terms of cell volume of *Aphanizomenon* did not decrease from 1978 to 1979 (Coulombe and Robinson, 1980). Furthermore, in L885, at least 2 collapses were observed to occur in 1979. It may be speculated that in L885, 1979, 2 rounds of viral lysis occurred, and that regrowth of the cells after the first lysis was fast. Cowlishaw and Mrsa (1975) have reported two rounds of lysis in continuous culture studies of *Plectonema* and LPP and speculate that since regrowth was so rapid, that cell viability during the first round of lysis was due to some physiological factor. It was proposed that when the factor causing this physiological resistance was diluted out more cells lysed in the

second round of lysis (Cowlishaw and Mrsa, 1975).

If the vlps seen to occur within *Aphanizomenon* cells in this study were temperate phages, factors such as temperature, uv light, or unbalanced growth conditions, which are known to influence the formation of lysogenic cultures and the lytic cycle, might have considerable signifigance on the algal population balance in nature (Padan and Shilo, 1973). If environmental conditions were such to cause induction a decline in the algal population would result. Once conditions were changed, regrowth might occur.

To establish the viral nature of the vlps seen here, demonstration of their infectivity and subsequent recovery from an infected cell, together with biochemical characterization would be necessary. Verification of a viral infection of the *Aphanizomenon* populations reported here, is therefore not possible.

CONCLUDING DISCUSSION

The objective of this study was to examine some of the possible triggering mechanisms for the collapse of *Aphanizomenon* blooms. Several mechanisms leading to the decline of blue-green algal populations have been proposed, among them being photooxidation, O_2 toxicity, development of pathogens and autoinhibition. In the determination of a possible triggering mechanism it is important to determine the actual time of the onset of the collapse. Cell volume of *Aphanizomenon* appeared to serve as a better estimator of biomass than chlorophyll a for this purpose.

Several of the declines observed were initiated during periods of lake thermal stability when conditions conducive to photooxidation and O_2 toxicity were operable. Surface bloom formation sometimes occurred during such periods.

Other declines were initiated during periods of thermal instability. Such periods were accompanied by decreases in pH and dissolved oxygen and an increase of nutrients (NH_3-N and SRP) throughout the water column. These declines could not have been caused by photooxidation or by death due to O_2 toxicity. In these declines, the development of pathogens should be considered. Morphological evidence of virus-like particles within *Aphanizomenon* cells was often observed at such times. It may be that the decrease in pH and increase in nutrients favored cyanophage

development, as has been reported for *Plectonema* and LPP cyanophage by Lindmark (1979). Microscopical observations did not reveal the presence of fungal parasites. Bacteria were abundant along the *Aphanizomenon* filaments especially around the heterocysts, however, whether bacteria were ever actually responsible for algal lysis is unknown.

Declines of *Aphanizomenon* of differing rates and durations were observed to occur. Not all declines initiated carried on to extremely low cell volume levels. Presumably, for photooxidation and O_2 toxicity to cause a severe decline in the *Aphanizomenon* population several consecutive calm, sunny days would be necessary. Often declines initiated during periods of thermal stability continued with the onset of thermal instability. It may be that initially a fraction of the population was lost through photooxidation and O_2 toxicity. As thermal stability persisted it may be that the algal cells became progressively more nutrient deficient and presumably their resistance to cyanophage infection would be lessened. The onset of thermal instability accompanied by an increase of nutrients may then have favored cyanophage development and thus allowed the decline to continue.

SUMMARY

1) Declines of *Aphanizomenon* of differing rates and durations were observed to occur. Not all declines continued downward to extremely low biomass levels or resulted in severe O_2 depletion.
2) The severity of O_2 depletion appeared to depend not only on the actual amount of decrease in algal biomass (expressed as cell volume) but also on the depth of the anoxic layer prior to bloom decline and subsequent or concommitant mixing.
3) During periods of lake thermal stability, each lake was divided into essentially two zones. The upper zone developed features conducive to photooxidation and death from O_2 toxicity. Surface bloom formation sometimes occurred.
4) Since, decreases in heterocyst frequency often accompanied algal declines it was difficult to relate acetylene reduction rates to the possible inhibitory effects of high DO.
5) SOD levels taken from the same populations at different times throughout the summer were found to vary.
6) Decline events were at times initiated during periods of lake thermal instability when conditions were such that photooxidation and O_2 toxicity could be dismissed as causal factors. Morphological evidence of virus-like particles within *Aphanizomenon* cells was often

observed at such times. Since transmission and isolation of the vlps have not been substantiated the verification of a virus infection of the *Aphanizomenon* populations studied is not possible.

7) It is possible that photooxidation, O_2 toxicity and cyanophage infection may contribute to the same decline.

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