have been eliminated by fertilization over a period of three to five years and in which the bottom muds contain considerable organic matter. It would also appear that its use may be successful in ponds following the killing of underwater weeds by herbicides, as these dead plants should furnish the required carbohydrate basis for nitrogen fixation.

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OBSERVATIONS ON THE FACTORS INVOLVED WITH FISH MORTALITY AS THE RESULT OF DINOFLAGELLATE "BLOOM" IN A FRESHWATER LAKE

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ABSTRACT

Complete fish mortality associated with the development of high populations of dinoflagellates (*Gymnodinium* spp.) was observed in 1960 in a 9.5 acre fresh-water lake at Baton Rouge, Louisiana. Toxicity of the water samples containing the algae appeared to be related to the increased pH, length of exposure to sunlight and concentration of algal cells. Filtration with activated carbon removed the toxic effects. Laboratory tests offered data to explain the course of the fish mortality in the lake.

INTRODUCTION

Large-scale mortality of fish as the result of "blooms" of dinoflagellate algae have been reported numerous times in brackish waters (Galtsoff, 1948; Ray and Wilson, 1957; Davis, 1948; Ingersoll, 1882). Such die-offs in freshwater are either relatively infrequent or unrecognized since only Jurgens (1953) described such an occurrence in a freshwater lake in Texas. Mortality of warm-blooded animals from toxic concentrations of blue-green algae in freshwater have been reported more frequently (Palmer, 1959; Rose, 1953; Ingram and Prescott, 1954). Toxic effects on fishes of blue-green algae are difficult to separate from lack of dissolved oxygen, but several publications have reported such data (Ingram and Prescott, 1954; MacKenthun *et al.*, 1948).

From May 1st through May 30th, 1960, a dense, greenish algal-bloom developed in a 9.5 acre Campus Lake located at Louisiana State University, Baton Rouge, Louisiana (91°11'-30° 29'). Eight dead gizzard shad (*Dorosoma cepedianum*) were noted floating in the lake on May 28th and several hundred gizzard shad were found dead the next day. On May 30th gizzard shad, threadfin shad (*Dorosoma petenense*), channel catfish (*Ictalurus punctatus*), black bullhead (*Ictalurus melas*), and orange-spotted sunfish (*Lepomis humilis*) were found dead and dying from 8:00 A.M. until sunset. During the following three days the apparent color of the lake water became yellowish-brown and sunfish and catfish were seen in distress on the water surface after 8:00 A.M. On June 3rd dead freshwater mussels were seen floating in the lake and dead and dying eels (*Anguilla rostrata*) were observed. Fish activity was not observed in Campus Lake after June 3rd and subsequent sampling two months later with seines failed to reveal any fish. On June 5th the apparent color of the water was becoming greener as the result of increasing numbers of *Scenedemus opoliensis* and *Anabaena flos-aquae*. By June 7th dense floating clumps of *Anabaena flos-aquae* had developed and clumps of *Anacystis cyanea* were developing. Large concentrations of these species of blue-green algae existed until cool weather of fall.

When large numbers of fish started to die on May 30th, Winkler tests for available dissolved oxygen were conducted by the Louisiana Wild Life and Fisheries Commission, Water Pollution Control Division Laboratory. Available surface dissolved oxygen was 8.9 parts per million at 8:00 A.M. and 17.0 parts per million at 4:30 P.M. The pH increased from 9.5 at 8:00 A.M. to 11.0 at 4:30 P.M. Free dissolved carbon dioxide was zero parts per million at 8:00 A.M. and 4:30 P.M. Total alkalinity of the Campus Lake water was 85 parts per million.

Since the course of the fish mortality did not follow that of a typical disease pattern nor that of an oxygen-depletion, other causes were suspected. It was thought that insecticides or other toxic substances which may have been introduced into the lake would cause continuous fish mortalities throughout the 24-hour period. Instead fish mortalities seemed to occur from several hours after sunrise until late afternoon. Examination of the lake water under magnification (X430) revealed large numbers of motile and encysted dinoflagellates as well as some green and blue-green algae. The numbers of dinoflagellates were estimated to exceed 1,000 per milliliter in surface water samples. Green algae and blue-green algae were estimated to be present in numbers below 100 per milliliter. Samples of the algae present in the "bloom" sent to Dr. C. Mervin Palmer were identified as *Gymnodinium bohemicum* and *Gymnodinium* spp. (probably *G. aeruginosum* and *G. varians*). Other algae present were *Chodatella subsalsa*, *Scenedesmus opoliensis* and *Anacystis* sp. The yellowish-brown color of the water appeared to be the result of red masses in part of the lumen of encysted dinoflagellates, especially *Gymnodinium bohemicum*.

Results of Bioassay

Samples of the surface water collected on May 30th at 11:00 A.M. were taken into the laboratory for bioassay tests at 20°C with bluegill

sunfish (*Lepomis macrochirus*). Five small bluegill (1.5 to 2.5 inches) lived in an aerated three-gallon aquarium for only 3.5 hours. The water in the aquarium was buffered with 200 milliliters of 0.02 H Cl from pH 11.0 to 7.9. After the pH was lowered, bluegill lived in the buffered water for over five days. Samples of the lake water filtered through activated carbon on filter paper (Whatman No. 42) were not toxic to bluegill sunfish, even if the pH which was lowered to 6.7 by filtering was buffered with Na_2CO_3 up to a pH of 10.5.

Plankton contents of water samples were increased by adding the plankton residues removed by filtering lake water (Whatman No. 42) to one-tenth the original water volume. A bluegill died in 55 minutes in the concentrated plankton (pH 10.4). A water sample collected from the lake at 1:00 P.M. on June 2nd was filtered (Whatman No. 42) and the algae residue washed back into one-fourth the original volume of lake water (pH 9.5). A bluegill lived only 30 minutes in the aerated concentrated sample. One bluegill, placed in the aerated filtrate (pH 9.5) from the solution filtered to increase the filterable algae by four times, lived for five hours. One bluegill placed in an unconcentrated water sample (pH 9.5) died in 45 minutes.

A water sample collected on May 30th at 11:00 A.M. was concentrated by centrifuging at 2,000 r.p.m. for 10 minutes and pouring off the water from the top of the test tubes. Algae precipitated from 10 times the original volume of water sample were treated several ways. A bluegill lived past the 24-hour test period in a portion of the algal cell subjected to sonic energy with a 50-watt nine-kc, Raytheon Magnetostriction Oscillator and placed in tap water (pH 8.6). A bluegill placed in another similarly treated solution of tap water buffered with Na_2CO_3 to pH 10.3 died in less than 18 hours. Bluegills placed in water from the top of the centrifuge tubes lived past the end of the test 24 hours later. Algae in water samples collected June 2nd at 1:00 P.M. were concentrated approximately 12 times by centrifuging. A bluegill in the concentrated precipitate (pH 9.5) died in 20 minutes. A mosquitofish (*Gambusia affinis*) placed in the sample immediately after removal of the bluegill died in 19 minutes. A bluegill placed in the solution from the top of the centrifuge tubes died in 41 minutes.

Large containers of lake water collected for bioassay on bluegill were left in the laboratory overnight and subsequently samples were tested by placing bluegill in them. A bluegill placed in water collected on May 30th and tested on May 31st (pH 8.6) lived past the 24-hour test period. One bluegill and mosquitofish placed in water samples collected on June 2nd and tested on June 3rd (pH 9.0) lived through the 24-hour test period. Also, lake water collected at 9:00 A.M. on June 3rd (pH 9.2) caused no mortality for three different bluegills in concentrated (10 times) and unconcentrated solutions. However, water from the same sample when left in the sunlight until 12 noon (pH 9.6) resulted in the death of a bluegill in 135 minutes. Another water sample collected June 3rd from Campus Lake at 1:45 P.M. (pH 9.9) resulted in the mortality of a bluegill in 75 minutes and a mosquitofish in 70 minutes.

DISCUSSION

Several indications are suggested by the mortality of the test animals in the plankton and water samples from Campus Lake. The length of time that bluegill sunfish or mosquitofish lived in the water samples appeared to be related to the concentration of the plankton, pH of the water, and the previous exposure of the algae to sunlight. The toxicity of the dinoflagellates appeared to occur only after photosynthesis had used up most free carbon dioxide in the water and raised the pH to 9 or more. Several studies have indicated that the culture and toxicity of some species of dinoflagellates is related to the pH of the culture media and the test solutions (Ray and Wilson, 1957; Shilo and Aschner, 1953; McLaughlin, 1956). Shilo and Aschner (1953, p. 336) stated "Adequate illumination of the cultures was important for cell proliferation and maintenance of culture toxicity. Light affected toxicity more quickly than cell number." Ray and Wilson (1957) found that growth phase

and concentrations of the algae influenced survival time of test animals. In Campus Lake, *Gymnodinium* spp. appeared to be toxic to fish just prior to and during the period of rapid encysting and color changes.

Filter paper appeared to remove the *Gymnodinium* spp. cells intact, thus reducing the toxic effects of the filtrate. Similar results were reported by Ray and Wilson (1957). However, as has been reported for the toxic effects of blue-green algae, some of the toxic material(s) is free in the water (Olson, 1952 and Fitch *et al.*, 1934). Shilo and Aschner (1953) secured a cell-free toxin solution of *Prymnesium parvum* by centrifugation at 10,000 r.p.m. for 15 minutes. Activated carbon appeared to be effective in removing the *Gymnodinium* spp. cells as well as the toxic substance(s) in the water. Wheeler *et al.* (1942) reported toxic substances of *Microcystis aeruginosa*[1] adsorbed by activated carbon and filtrate to be non-toxic. Shilo and Ashner (1935) reported activated charcoal as an effective absorbent. Many of the observations made in this short study fit very closely observations made by Shilo and Ashner (1953) and observations by Ray and Wilson (1957) on dinoflagellates.

Field observations on "fish-kills" as the result of toxic dinoflagellate populations (Jurgens, 1953; Galtsoff, 1948) revealed mortality of fish lasting over several days. The course of fish mortality in the present study can be explained by the factors apparently affecting the toxicity of the dinoflagellates. Photosynthesis taking place in the surface waters would cause the greatest pH change and concentration of organisms in this region. Gizzard shad and small sunfish would be the species most likely to be affected first. As concentrations of dinoflagellates increase and start to sink in the encysted stages, deeper water levels would become more toxic. Thus, species of fish inhabiting the deeper waters would be affected last. Campus Lake was small in volume. The dinoflagellates produced near the surface were apparently able to eventually produce toxic effects throughout the lake resulting in the complete destruction of the fish population. If other factors did not affect the toxic effects produced by the dinoflagellates, the course of fish mortality in small bodies of water would be very rapid and complete.

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THE PENETRATION OF LIGHT AND THE CONCENTRATION OF DISSOLVED OXYGEN IN FERTILIZED POND WATERS INFESTED WITH *MICROCYSTIS*

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ABSTRACT

Weekly measurements were made of light intensity, dissolved oxygen concentration, and water temperature at selected depths in five earthen experimental ponds. Measurements were made on a given pond on the same day between 7:00 a.m. and 8:45 a.m. and again between 10:00 a.m. and 11:45 a.m.

The depth at which the average light intensity, as measured with submersible Weston Photronic photoelectric cells, was less than 1 per cent incident radiation varied from 2.5 to 7.5 feet among the ponds, depending on the degree of *Microcystis* infestation. Generally, at depths where the average light intensity was not in excess of 1 per cent incident radiation, the average dissolved oxygen concentration was not in excess of 1 ppm. The average dissolved oxygen concentration in the pond with the most dense growth of *Microcystis* was usually less than 1 ppm. below 5 feet and less than 1 ppm below 7.5 feet in the ponds with the least amount of *Microcystis*.

Generally, the decrease in water temperature with increased depth was directly related to the abundance of *Microcystis*.

This study suggests that dense growths of scum-forming algae, such as *Microcystis*, limit the water depth at which the dissolved oxygen concentration is in excess of 1 ppm by limiting light penetration and by contributing to thermal stratification by heat absorption in the dense blooms near the water surface.

INTRODUCTION

In Alabama, fertilization of farm ponds has been advocated since the early work of Smith and Swingle (1939, 1941, 1942) and Swingle (1947). The application of inorganic fertilizers has been advantageous not only for increasing fish production but also in controlling obnoxious growths of submersed aquatic vegetation by inducing abundant growths of phytoplankton.

In contrast, the death of fish has been attributed to the depletion of oxygen caused by death and decay of dense growths of phytoplankton