interesting to the systematist, since its peculiar structural characters oblige him to place it alone in the family Holopedidae of the cladoceran tribe Ctenopoda, while of this genus itself but two species are known: these are the familiar *Holopedium gibberum* and the little known *H. amazonicum,* described by Stingelin from material collected at the mouth of the Amazon[1] and recorded since then from only one additional locality, namely, Lake Charles, Louisiana.[2] Stingelin himself remarked upon the peculiarity of the fact that this characteristically boreal cold-stenothermous form should have its nearest relative in tropical waters: the circumstance is the more singular from the very close relationship which exists between the two species, and which induced Stingelin to construct a carefully tabulated key to their diagnostic features.[1]

Recently, in examining material collected in certain small lakes near St. Andrew's (New Brunswick), I have found the typical form of the species *H. gibberum* accompanied by forms which show all degrees of variation in respect of these very diagnostic characters used by Stingelin in the separation of the two species.[3] It looks very much as if we have here the beginning of a gradation towards the *amazonicum* type, and it is highly desirable to examine material from as many freshwater lakes and ponds as possible, especially along the eastern fringe of the United States, in order to see whether anything like a true connecting series between the two species can be established. Should this be possible, we should have a case among the Cladocera exactly parallel with that of the two waterbeetles *Deronectes depressus* and *D. elegans,* recently elucidated by Balfour Browne[4] and with that of *Gyrinus natator* and *G. substriatus,* on which another British entomologist is now working.[5] Such north-to-south gradations are of the highest interest in relation to the study of species-evolution and distribution, and it is, therefore, with confidence that I appeal to American biologists for collections of freshwater plankton containing *Holopedium,* especially from the eastern Atlantic States. Such collections can easily be made by towing a plankton-net of bolting cloth from a boat, or even with a hand-net: the catch should be diluted with 4 per cent. formalin containing a few drops of glycerin for efficient preservation. Any specimens sent to me will be gratefully acknowledged and expenses of mailing defrayed.

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[1] Th. Stingelin, *Revue Suisse de Zoologie,* 12: 53–64, 1904.

[2] E. A. Birge, Chapter on "Cladocera" in Ward & Whipple's "Freshwater Biology," 693, 1918.

[3] An account of these variations is included in a paper on "The Cladoceran Plankton of the Chamcook Lakes," shortly to be published in *Contributions to Canadian Biology.*

[4] F. Balfour Browne, *Scottish Naturalist,* November–December, pp. 172–188, 1930.

[5] J. Omer-Cooper, *Nature,* February 14, 1931.

MANGANESE AND THE GROWTH OF LEMNA MINOR[1]

IT has recently been shown by the author[2] that the unicellular alga Chlorella will not grow without manganese although this organism makes apparently normal growth when only a minute amount of this element is added to the manganese-free culture solution. It has further been demonstrated[3] that a number of other "rare" elements for plant growth are unable to replace manganese in the nutrition of this organism. The results reported on the necessity of manganese for Chlorella are based on determination of the dry weight of cells produced. Since then counts of the numbers of cells in plus and minus manganese cultures have been made. These counts show as high as 64,000,000 cells per culture in some cases in two weeks' time in cultures containing manganese in a concentration of 1:5,000,000. While in minus manganese cultures there is a decrease in the number of cells used for inoculation. This furnishes a striking confirmation of the data already reported. Manganese is therefore not a "stimulant" but an essential element for the growth of Chlorella. Other experiments not yet published using another unicellular green alga (species undetermined) have also shown the same clear cut results as with Chlorella. The investigation was then extended to the common duckweed, *Lemna minor,* and here again it has been found that manganese is indispensable for growth.

Clark and Fly[4] have studied the relation of manganese to Lemna and come to the conclusion that "there is no indication that manganese is an essential element in the nutrition of the plant." Since, however, my work has shown it to be absolutely essential for certain unicellular green algae and others have demonstrated it to be necessary for the normal development of a large number of seed plants it seems plausible that the duckweeds should require it also.

The experiments on *Lemna minor* were all carried out in pure culture. After many trials with various disinfectants the following procedure was successfully used in freeing the fronds from microorganisms: A

[1] The investigation upon which this article is based was supported by a grant from the Heckscher Foundation for the Advancement of Research established by August Heckscher at Cornell University.

[2] SCIENCE, 72: 609–610, 1930.

[3] E. F. Hopkins, "Manganese an Essential Element for a Green Alga," *Am. Jour. Bot.,* 17: 1047, 1930.

[4] N. A. Clark and C. L. Fly, "The Rôle of Manganese in the Nutrition of Lemna," *Plant Physiology* 5: 241–247, 1930.

number of healthy plants were obtained and washed thoroughly with sterile water. They were then immersed for an instant, one at a time, in 95 per cent. alcohol followed by one minute in $HgCl_2$ solution 1:1,000 and then rinsed in several changes of sterile acid phosphate solution. From this the plants were transferred to Knop's solution plus 0.5 per cent. of dextrose. In this manner six or eight pure cultures were obtained. It is suggested that this method may overcome the difficulties mentioned by Saeger[5] in connection with the direct disinfection of the fronds.

Experiments to determine the relation of manganese to the growth of Lemna were made on solutions freed from manganese in two ways. In the first case the chemicals used in the preparation of Knop's solution were recrystallized three times from conductivity water. In the other case manganese was removed by absorption on calcium phosphate at pH 8.0 as in the work with Chlorella. This latter solution which contained dextrose was acidified and its calcium content increased by adding to it a sterile solution of recrystallized calcium nitrate and potassium hydrogen phosphate. This procedure was resorted to because of the difficulty encountered in removing manganese from glucose by recrystallization. Known amounts of iron (1:500,000) were added to each culture from a solution prepared from recrystallized ferric alum and recrystallized sodium citrate. For plus manganese cultures manganese sulphate was added to give a concentration of Mn of 1:5,000,000.

In the first experiments without dextrose the controls showed no development of fronds and no root formation while with manganese 1:500,000 there was good development of green fronds and healthy root growth. In fact the fronds planted in the control flasks finally became chlorotic and died. In table I the results of such an experiment are given.

TABLE I

THE NECESSITY OF MANGANESE FOR THE GROWTH OF *Lemna minor* (WITHOUT DEXTROSE)

	Green fronds (54 days)
+ Mn	127 (5 cultures)
− Mn	9 (5 cultures)

In the later experiments the presence of dextrose (0.5 per cent.) made it possible to obtain results in a shorter time. The summarized data from one of these experiments is given in Table II.

The necessity of manganese for Lemna is clearly shown in the above data both with and without iron being added. In the latter experiment after the second transfer the plants without manganese showed no development at all and finally died. The plants with both iron and manganese were very healthy in appearance with large deep green fronds. Those with manganese and without iron were smaller and chlorotic. Since the dry weights of the plants agree closely with the counts of the number of fronds these data are not given here. An interesting occurrence is the development of definite symptoms of manganese deficiency consisting of depressed root formation and of light brownish to white necrotic areas on the fronds in the case of those cultures without manganese.

TABLE II

THE NECESSITY OF MANGANESE FOR THE GROWTH OF *Lemna minor* (WITH DEXTROSE)

Treatment	Per cent. increase in no. of fronds in 21 days	Per cent. increase in 15 days after transferring one plant to another set of culture solutions
− Fe − Mn	222 (5)*	0 (5)
− Fe + Mn	769 (5)	810 (5)
+ Fe − Mn	90 (5)	0 (5)
+ Fe + Mn	843 (5)	1160 (5)

* The figures in parenthesis refer to the number of cultures in each case.

I believe that these experiments which have been repeated a number of times with the same result show conclusively that manganese is an essential element for Lemna and suggest that it is undoubtedly necessary for the growth of all green plants.

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[5] Albert Saeger, "A Method of Obtaining Pure Cultures of *Spirodella polyrhiza*," *Bull. Torrey Bot. Club* 57: 117–122, 1930.

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