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Project Summary

The Potential for Biological Controls of *Cladophora glomerata*

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The purpose of this research program was to determine whether or not natural biological controls of *Cladophora glomerata* could be developed. Two avenues of research were pursued. One was to study macroscopic organisms that were known to occur in the Great Lakes area to see if any of them would prefer *C. glomerata* as a food source and under what conditions the desirability of *C. glomerata* could be increased. The second approach was to study microorganisms, particularly fungi, that commonly occurred in aquatic ecosystems to determine if they might function as contols for *C. glomerata*.

The results of the first avenue of research show that *C. glomerata* was a poor food for *Physa heterostropha, Orconectes propinquus, Rana pipiens, Ictalurus punctatus,* and *Pimephales promelas*. Not only were these species averse to eating the food, but when they did, it appeared to have a deleterious effect. In the studies with snails, egg production was greatly curtailed on the *C. glomerata* diet.

The unialgal *C. glomerata* was less preferred than the *C. glomerata* plus epiphytes. The main effect on organisms other than *Physa heterostropha* was loss in weight. The crayfish did not molt even when the eyestalks were removed, and the frog tadpoles did not show any signs of metamorphosing. All organisms steadily lost weight during the experiments.

An examination of the chemical constituents of cells of *C. glomerata* and of diatoms showed that they differed considerably from diatom-dominated periphyton, which was the preferred diet in all experiments. These differences were mainly that the diatoms had larger amounts of amino acids, such as serine, aspartic acid, and glutamic acid. Also the free and combined fatty acids of diatoms were different from those of *Cladophora*. The diatoms were dominated by C_{16} and C_{20} combined fatty acids, whereas the unsaturated C_{18} fatty acid, particularly linolenic acid, was common in *C. glomerata*. Of the free fatty acids, the diatom-dominated periphyton contained mainly C_{14} and C_{16} isomers, whereas *C. glomerata* contained mostly C_{18}. Of the short-chain fatty acids C_{12} acids, particularly lauric acid, was present in *C. glomerata* and absent from the periphyton. It is known that lauric acid is toxic to several organisms.

The second avenue was to study the effect of fungi as parasites on *C. glomerata*. One fungus, *Acremonium kiliense* (Fungi Imperfecti) was found to have an antagonistic effect on *C. glomerata*. The effect varied in various experiments, those carried out in the summer months showing the greatest antagonistic effect. It may be that the lesser effect in the fall was due to temperature and other environmental conditions or to the fact that *A. kiliense* had reduced virulence. A toxin was present in the supernatant derived from cultures of *C. glomerata* that had been damaged by this pathogen. It is water soluble and can withstand heat up to 80°C.

This Project Summary was developed by EPA's Environmental Research Laboratory, Duluth, MN, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

In this research, two approaches were pursued in trying to identify a biological control of *Cladophora glomerata.* One of these was to study macroorganisms common to the Great Lakes region that select algae as a considerable portion, if not the main portion, of their diets. The other avenue of research was to find a microorganism which, by parasitism, would destroy *C. glomerata.* It was realized that this organism must be specific for *C. glomerata,* and, if present in fairly large amounts, would not destroy other algae that were desirable food sources.

The organisms selected for the macroorganism studies were the pond snail, *Physa heterostropha;* the crayfish, *Orconectes propinquus;* the tadpole *Rana pipiens;* the channel catfish, *Ictalurus punctatus;* and the fathead minnow, *Pimephales promelas.* All of these organisms feed on algae. Some of these, such as *Tilapia aurea,* are known to be prodigious algal feeders. In these experiments most of the work was done on *Physa heterostropha, Orconectes propinquus,* and *Pimephales promelas.*

Three diets were tested on each species: Unialgal Cladophora, Cladophora plus epiphytes, and diatom-dominated periphyton. All experiments were run in the natural soft waters of White Clay Creek, Pennsylvania. Conditions under which tests were run are detailed in the full report. For all tests oxygen was maintained above 7 ppm, pH between 7 and 8.2 and ammonia less than 40 $\mu g/l$. Growth and respiration were measured for all test species, and fecundity was determined for snails.

All studies showed that *C. glomerata* was not a satisfactory diet. Diatom-dominated periphyton was a more satisfactoy diet, as the organisms had a better rate of growth on that diet than on *Cladophora* plus epiphytes. The *Cladophora* plus epiphytes tended to be a better food source than unialgal *Cladophora.* In the experiments using *P. heterostropha,* some snails were starved. Their decline in weight and their respiration rates tended to be greater when starved than when fed unialgal *C. glomerata.*

The results of the experiments with *P. heterostropha* showed that the relative value of the three diets presented were diatom-dominated periphyton > *Cladophora* plus epiphytes > unialgal *Cladophora.* The respiration rates of snails fed *Cladophora* or *Cladophora* plus epiphytes were less than those on the periphyton diet. There was not much difference between diets of *Cladopohora* plus epiphytes and unialgal *Cladophora.*

The reproductive success was much greater on the diatom-dominated periphyton diet and was very poor on the unialgal *Cladophora* diet. Egg-laying greatly decreased in a relatively few days (11-15 days) after the experiments started. However, the eggs produced from all three diets had similar caloric content. These experiments were repeated at various seasons of the year and showed similar results.

The experiment with *Orconectes propinquus* showed that none of the diets was very satisfactory, but that the diatom-dominated periphyton was much better than the other two diets. The crayfish molted for only a short time after the experiment started. This may be due to the normal life cycle, as crayfish tend to hibernate in the cooler months. However, the temperature at which the experiments were run was similar to summer temperatures. Molting was forced by removing the eyestalks and associated X organ-sinus gland. Following this operation, the females on the periphyton diet all molted. The respiration rates on all three diets declined during the experiments and the respiration rates of the females tended to be greater than those of the males.

The experiments with the tadpoles of *Rana pipiens* resulted in increases in size, and there were signs of metamorphosis on the diatom-dominated periphyton diet and on the *Cladophora* plus epiphyte diet. Those fed on unialgal *Cladophora* grew very little and showed no signs of metamorphosis. The respiration rates for tadpoles were similar on the periphyton and *Cladophora* plus epiphyte diets and were much lower for tadpoles on the *Cladophora* diet. These tadpoles were very small and weighed very little.

The experiments with the channel catfish *(Ictalurus punctatus)* showed a similar pattern . The diatom-dominated diet was more satisfactory than the unialgal *Cladophora* diet. However, none of the diets was as satisfactory as the prepared fish food diets.

The fathead minnow *(Pimephales promelas)* ingested and assimilate much more of the diatom-dominate periphyton than the *Cladophora* plu epiphytes or the unialgal *Cladophora.* Th fish fed the last two diets decreased i weight, with those on unialga *Cladophora* showing the greate decrease. The respiration rates wei variable for fish on the periphyton diet. was highest for those fed on *Cladophoi* plus epiphytes. On both the *Cladophoi* diets, the cal/mg/hr consumed wei higher than on the periphyton diet.

These results led to an examination the relative food value of *Cladophoi glomerata* and diatom-dominated pei phyton. Calories/mg were similar. A analysis of the chemical composition diatoms and *Cladophora* showed son interesting differences. The amino acic present in larger amounts in the diaton were serine, aspartic acid, glutamic aci and a small amount of cystine, which wa absent from *C. glomerata.*

The combined fatty acids in diaton were dominated by C_{16} and C_{20}. Th amount of unsaturated C_{18} fatty acid particularly linolenic acid, was muc smaller in diatoms. The composition of (*glomerata* was more like that of oth green plants, having C_{18} unsaturate fatty acids, particularly linolenic acid, ar low concentrations of C_{16} and C_{12} acid

Periphyton contained more free fat acids than *C. glomerata, with the majori being* C_{14} and C_{16} isomers, whereas thos present in *Cladophora were mostly* C_1 Of the short chain free fatty acids C (lauric acid) was present in *Cladophoi* and absent from periphyton, and C (capric acid) was in periphyton but not i *Cladophora.* Lauric acid has been show from many reports to be toxi Experiments with yeasts *(Saccharomyc cervisiae)* showed that the extract *Cladophora* containing this acid was tox to yeast. This suggests that the presen of this acid may be in part the reason wh *C. glomerata* was an unsatisfactory foo

C. glomerata contained 4.5% solub carbohydrates, whereas the periphyto contained 21.6%. There was also mo phosphorus in the periphyton.

The second approach was to determir whether fungi commonly occurring in th aquatic environment would have a antagonistic effect on *C. glomerata.* Or isolate, *Acremonium kiliense* (Fun imperfecti) was found to have this effec This fungus was isolated from the sto culture of *Cladophora* where it wa present in small amounts. This fungus flask cultures deleteriously affected th cell condition of *C. glomerata* and cause a reduction in chlorophyll *a* when the stock cultures of *Cladophora* were used. *Cladophora* isolated from Lititz Creek was more susceptible to the fungus than *Cladophora* from stock cultures. In the Lititz Creek community experiments, the correlation of loss of chlorophyll *a* with damage to the cells was not as good as that from stock cultures. This may be due to the fact that small amounts of algae were present that were not damaged by *A. kiliense* and thus would obscure the loss of chlorophyll *a*. Therefore, it was decided that direct microscopic examination of the cells was the most reliable way to determine the damage of this fungus.

Supernatant culture medium from the flasks containing damaged and undamaged *Cladophora* cells also had deleterious effects on the algae. This antagonistic substance from *A. kiliense* is water soluble and does not seem to be damaged by heating to 80°C. Cultures of *A. kiliense* were raised on Czapek-Dox broth, and *Cladophora* was inoculated with conidia, but these did not produce damage.

Microcosm experiments were then set up in which the *C. glomerata*-dominated communities were exposed to varying concentrations of *A. kiliense.*

In exposures carried out in the summer months, there was a striking effect of this fungus on the *Cladophora,* although diatoms and Ulothrix living in the microcosms were unaffected. In fact, the *Ulothrix* divided rapidly and occupied the habitats formerly occupied by *C. glomerata. Scenedesmus* and *Spirogyra* were also present, as were protozoans and rotifers. They did not seem to be affected by this fungus. Static microcosms produced similar results. However, experiments repeated in the fall showed far less effect.

Conclusions

Experiments in which a number of different consumer organisms were fed *C, glomerata* showed that the alga was either not eaten or caused deleterious physiological changes.

C. glomerata is lower in overall nutritive value (less protein, lipid and phosphorus per unit dry weight) than many other readily eaten algae. It contains low concentrations of steroids and free polyunsaturated fatty acids. Although low in sulfur-containing amino acids, *Cladophora* is not unique in this respect, and it appears to contain most of the other essential amino acids in adequate quantities.

C. glomerata contains substances that are toxic, based on yeast growth inhibition. One of these substances is free lauric acid, which is present in the organism at a concentration of about 170 ppm.

The microbial pathogen, *Acremonium kiliense,* may provide a useful tool for controlling extensive growths of *C. glomerata* in nature. The results of these experiments, though striking in some instances, have been variable, and more study is needed. Nevertheless, *Cladophora* from both stock cultures and field sites were adversely affected in laboratory experiments.

The results of these experiments showed that unialgal *Cladophora* and *Cladophora* plus epiphytes were unsatisfactory diets for the flathead minnow, *P. promelas.* These data, plus those on snails, showed the importance of chemical analysis of the food value of *C. glomerata.*

Cladophora plus epiphytes were unsatifactory diets for the fathead minnow, *P. promelas.* These data, plus those on snails, showed the importance of chemical analysis of the food value of *C. glomerata.*

The fungus *Acremonium kiliense* may be a potential biological control. However, a good deal more experimentation has to be done to understand the conditions under which it develops and what the toxin is.

The results from the experiments with megaorganisms as biological controls of *C. glomerata* produced negative results. However, these experiments did turn up some very interesting information as to the nutritional value of *C. glomerata.* This alga has a very different assortment of free and combined fatty acids, as well as different amino acid proportions than diatom-dominated periphyton, which was obviously the preferred diet.

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***Nelson Thomas** is the EPA Project Officer (see below).*
The complete report, entitled "The Potential for Biological Controls of Cladophora glomerata,*" (Order No. PB 83-251 298; Cost: $17.50, subject to change) will be available only from:*
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