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JOHN D. MADSEN

US Army Corps of Engineers
Waterways Experiment Station

Aquatic Plant Control Research Program

Proceedings, 27th Annual Meeting, Aquatic Plant Control Research Program

16-19 November 1992
Bellevue, Washington

Approved For Public Release; Distribution Is Unlimited

Prepared for Headquarters, U.S. Army Corps of Engineers

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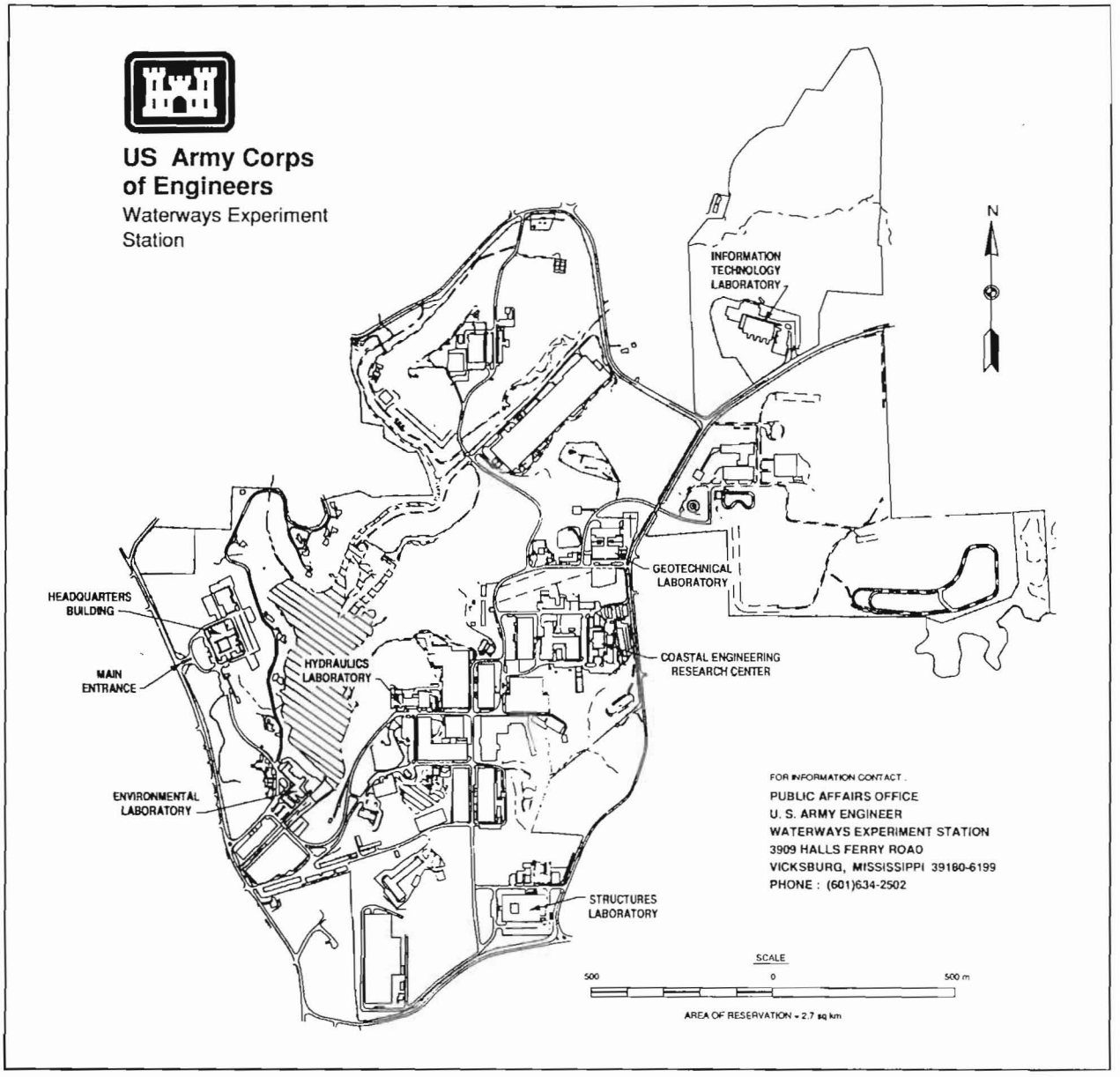

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Preface

The 27th Annual Meeting of the U.S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP) was held in Bellevue, WA, on 16-19 November 1992. The meeting is required by Engineer Regulation 1130-2-412, paragraph 4c, and was organized by personnel of the APCRP, which is managed under the Environmental Resources Research and Assistance Programs (ERRAP) of the Environmental Laboratory (EL), U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS.

The organizational activities were carried out and presentations by WES personnel were prepared under the general supervision of Mr. J. L. Decell, Program Manager, ERRAP, EL. Mr. Robert C. Gunkel, Assistant Program Manager, ERRAP, was responsible for planning the meeting. Dr. John Harrison was Director, EL, WES. Ms. Denise White was Technical Monitor for the Headquarters, U.S. Army Corps of Engineers.

Ms. Billie F. Skinner, ERRAP, was responsible for coordinating the necessary activities leading to publication.

At the time of publication of this report, Director of WES was Dr. Robert W. Whalin. Commander was COL Bruce K. Howard, EN.

Agenda

Monday, 16 November 1992

1:00 p.m. - 5:00 p.m.	***Registration*** Ballroom Foyer
3:00 p.m. - 5:00 p.m.	***Federal Aquatic Plant Management Working Group*** Boardroom II
6:00 p.m. - 7:30 p.m.	***Reception*** Marymoor Room

Tuesday, 17 November 1992

7:30 a.m. - 5:00 p.m.	***Registration*** Ballroom Foyer
8:00 a.m. - 5:00 p.m.	***Poster and Demonstration Session*** Lakehills Room
8:00 a.m. - 1:45 p.m.	***General Session*** Idylwood Room
8:00 a.m.	Call to Order and Announcements * Robert C. Gunkel, U.S. Army Engineer Waterways Experiment Station (WES) Vicksburg, Mississippi
8:05 a.m.	Welcome to Seattle District * LTC Rex N. Osborne, Deputy Commander U.S. Army Engineer (USAE) District Seattle, Washington
8:15 a.m.	Comments from Headquarters, U.S. Army Corps of Engineers (HQUSACE) * Darrell E. Lewis, Chief, Natural Resources Management Branch Washington, DC
8:30 a.m.	What Are We Managing? * J. Lewis Decell, Program Manager, Environmental Resources Research and Assistance Programs, WES

9:00 a.m.	Aquatic Plant Control Operations Support Center (APCOSC) Update * Wayne T. Jipsen, USAE District Jacksonville, Florida
9:15 a.m.	Lewisville Aquatic Ecosystem Research Facility (LAERF) Update (32733) * R. Michael Smart, WES-LAERF Lewisville, Texas
9:30 a.m.	Long Lake Project: Chemical Control Technology Transfer * Kurt D. Getsinger, WES
9:45 a.m.	***Break***
10:15 a.m.	Review of Eurasian Watermilfoil Biocontrol by the Milfoil Midge * Ben Kangasniemi, British Columbia Ministry of Environment Victoria, British Columbia, Canada
10:30 a.m.	Economic Evaluations of Aquatic Plant Control (32729) * Jim E. Henderson, WES
10:45 a.m.	Master Plan: Guntersville Reservoir Aquatic Plant Management * Joe Morrison, USAE District Nashville, Tennessee
11:00 a.m.	Aquatic Macrophyte Assessment - Joint Agency Guntersville Project * David H. Webb, Tennessee Valley Authority (TVA) Muscle Shoals, Alabama

Simulation Technology

Presiding: R. Michael Stewart, WES

11:15 a.m.	Overview of Simulation Studies * R. Michael Stewart, WES
11:30 a.m.	Plant Growth Model Investigations (32440) * R. Michael Stewart, WES
11:45 a.m.	Status of *Hydrellia pakistanae* Modeling Efforts and Approach for Future Developments to the INSECT Simulation Procedure (32438) * William A. Boyd, WES
12:00 noon	***Lunch***
1:00 p.m.	Studies for Calibrating Fate/Effects Algorithms in HERBICIDE Version 2.0 (32439) * John H. Rodgers, Jr., University of Mississippi Oxford, Mississippi
1:15 p.m.	Field Studies for Testing Control Technology Simulations (32438, 32439) * R. Michael Stewart, WES
1:30 p.m.	Computer Assisted Procedures for Aquatic Plant Mapping and Monitoring (32506) * M. Rose Kress, WES
1:45 p.m.	***Adjourn General Session***

2:00 p.m. - 5:00 p.m. ***USAE Division/District Working Session***
Factoria Room

Wednesday, 18 November 1992

8:00 a.m. - 12:00 noon ***Poster and Demonstration Session***
Lakehills Room

8:00 a.m. - 11:30 a.m. ***General Session***
Idylwood Room

Biological Technology
Presiding: Alfred F. Cofrancesco, WES

8:00 a.m. Overview of Biological Control Studies
* Alfred F. Cofrancesco, WES

8:15 a.m. Biocontrol of Milfoil and Hydrilla with Pathogens (32200, 32202, 32735)
* Judy F. Shearer, WES

8:30 a.m. Development of Commercial Microbial Herbicides and New Pathogen Approaches (32202)
* Edwin A. Theriot, WES

8:45 a.m. Foreign Research on Insect Biocontrol Agents (32730)
* Gary R. Buckingham, USDA
Gainesville, Florida

9:00 a.m. Quarantine Biocontrol Operations (31799, 32730)
* Chris A. Bennett, University of Florida
Gainesville, Florida

9:15 a.m. Biocontrol of Hydrilla in Texas, Louisiana, and Alabama with Insects (31799, 32734)
* Michael J. Grodowitz, WES

9:30 a.m. ***Break***

10:00 a.m. Release and Establishment of New Hydrilla Biocontrol Agents in Florida and Georgia (31799)
* Ted D. Center, U.S. Department of Agriculture (USDA)
Fort Lauderdale, Florida

10:15 a.m. Insects on Eurasian Watermilfoil (32739)
* Sally P. Sheldon, Middlebury College
Middlebury, Vermont

10:30 a.m. Biocontrol of Pistia (32406)
* F. Allen Dray, University of Florida
Fort Lauderdale, Florida

10:45 a.m. Alleopathy (32408)
* Harvey L. Jones, WES

11:00 a.m.	Biocontrol of Water Chestnut (*Trapa*) * Alfred F. Cofrancesco, WES
11:15 a.m.	Collection, Age, and Growth of Triploid Grass Carp (32738) * Phil Kirk, WES
11:30 a.m.	***Lunch***
1:00 p.m.	Field Trip to Hiram M. Chittenden Locks and Lake Washington Sites
3:30 p.m.	Boat Departs on Site Cruise and Dinner
8:00 p.m.	Return to Red Lion Hotel

Thursday, 19 November 1992

8:00 a.m. - 2:45 p.m.	***General Session*** Idylwood Room

Chemical Technology

Presiding: Kurt D. Getsinger, WES

8:00 a.m.	Overview of Chemical Control Studies * Kurt D. Getsinger, WES
8:15 a.m.	Phenology of Aquatic Plants (32441) * John D. Madsen, WES-LAERF
8:30 a.m.	Concentration/Exposure Time Relationships (32352) * Michael D. Netherland, WES
8:45 a.m.	Herbicide-Induced Stress in Aquatic Plants (32352) * Susan L. Sprecher, WES
9:00 a.m.	Application Techniques for Flowing Water (32354) * Allison M. Fox, University of Florida Gainesville, Florida
9:15 a.m.	Herbicide Delivery Systems (32437) * E. Glenn Turner, WES
9:30 a.m.	Plant Growth Regulators (32578) * Linda S. Nelson, WES
9:45 a.m.	***Break***
10:15 a.m.	Bioassays of Plant Growth Regulator Activity (32578) * Carole A. Lembi, Purdue University West Lafayette, Indiana
10:30 a.m.	Field Evaluations of Aquatic Herbicides - Pend Oreille River (32404) * Kurt D. Getsinger, WES

Ecological Technology
Presiding: John W. Barko, WES

10:45 a.m.	Overview of Ecological Studies * John W. Barko, WES
11:00 a.m.	Effects of Light on *Vallisneria* Growth in Relation to Sediment Fertility (32351) * Anne Kimber, Iowa State University Ames, Iowa
11:15 a.m.	Sediment Factors Influencing the Growth of *Vallisneria* (32351) * Dwilette G. McFarland, WES
11:30 a.m.	An Evaluation in the Field of Environmental Factors Affecting *Vallisneria* Growth (32351) * Sara J. Rogers, U.S. Fish and Wildlife Service Onalaska, Wisconsin
11:45 a.m.	Convective Hydraulic Circulation: Implications for Solute Transport (32405) * William F. James, WES-Eau Galle Research Laboratory Spring Valley, Wisconsin
12:00 noon	***Lunch***
1:00 p.m.	Nutrient Allocation and Uptake Efficiency in Hydrilla and Potamogeton in Relation to Supply: Implications for Management (32405) * Nancy J. Waters, Lafayette College Easton, Pennsylvania
1:15 p.m.	Aquatic Plant Competition Studies (32577, 32736) * R. Michael Smart, WES-LAERF
1:30 p.m.	Preemption as a Factor in Plant Competition (32577) * R. Michael Smart, WES-LAERF
1:45 p.m.	Competitive Ability of Selected Aquatic Macrophytes in *Lyngbya*-Dominated Littoral Zones of Guntersville Reservoir (32736) * Robert D. Doyle, WES-LAERF
2:00 p.m.	Evaluation of Submersed Macrophyte Invasions and Declines (32805) * Craig S. Smith, WES
2:15 p.m.	Microdistribution of Fishes in Relation to Submersed Aquatic Vegetation (32505) * Eric Dibble, WES
2:30 p.m.	Report on Tuesday's Division/District Working Session * William C. Zattau, USAE District Jacksonville, Florida
2:45 p.m.	***Adjourn 27th Annual Meeting***
3:00 p.m. - 5:00 p.m.	**FY94 Civil Works R&D Program Review** (Corps of Engineers Representatives Only) Newport Room

Posters And Demonstrations

Design of a Mesocosm Facility for Conducting Aquatic Herbicide Evaluations (32352)
* Gary O. Dick, WES-LAERF

GPS/GIS Applications to Chemical Control Scheduling and Monitoring (32506)
* Tommy Berry, WES

Fish and Aquatic Plant Interactions (32806)
* Jan Hoover, WES

Methodology for Applying AMUR/STOCK Model for Determining Potential Levels of Control from Proposed Stocking Strategies (32438)
* R. Michael Stewart, WES

Grass Carp Stocking Rate Model (AMUR/STOCK) (32438)
* R. Michael Stewart, WES

Aquatic Vegetation and Water Quality in Lake Marion Prior to Triploid Grass Carp
* Phil Kirk, WES

Effects of Temperature on Hydrellia (32734)
* Ramona H. Warren, WES

Aquatic and Wetlands Research and Development Support Facility
* Jan E. Freedman, WES

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Conversion Factors, Non-SI to SI Units of Measurement

Non-SI units of measurement used in this report can be converted to SI units as follows:

Multiply	By	To Obtain
acres	4,046.873	square meters
acre-feet	1,233.489	cubic meters
degrees (angle)	0.01745329	radians
feet	0.3048	meters
gallons (U.S. liquid)	3.785412	liters
inches	2.54	centimeters
miles (U.S. statute)	1.609347	kilometers
ounces (mass)	28.34952	grams
pound (mass)	0.4535924	kilograms
quarts (U.S. liquid)	0.9463529	liters
square feet	0.09290304	square meters
tons (mass) per acre	0.22	kilograms per square meter

Introduction

The Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP) requires that a meeting be held each year to provide for professional presentation of current research projects and to review current operations activities and problems. Subsequent to these presentations, the Civil Works Research and Development Program Review is held. This program review is attended by representatives of the Civil Works and Research Development Directorates of the Headquarters, U.S. Army Corps of Engineers; the Program Manager, Environmental Resources Research and Assistance Programs (ERRAP); and representatives of the operations elements of various CE Division and District Offices.

The overall objective of this annual meeting is to thoroughly review the Corps aquatic plant control needs and establish priorities for future research, such that identified needs are satisfied in a timely manner.

The technical findings of each research effort conducted under the APCRP are reported to the Manager, ERRAP, U.S. Army Engineer Waterways Experiment Station, each year in the form of periodic progress reports and a final technical report. Each technical report is distributed widely in order to transfer technology to the technical community. Technology transfer to the field operations elements is effected through the conduct of demonstration projects in various District Office problem areas and through publication of Instruction Reports, Engineer Circulars, and Engineer Manuals. Periodically, results are presented through publication of an APCRP Information Exchange Bulletin which is distributed to both the field units and the general community. Public-oriented brochures, movies, and speaking engagements are used to keep the general public informed.

The printed proceedings of the annual meetings are intended to provide all levels of Corps management with an annual summary to ensure that the research is being focused on the current nationwide operational needs.

The contents of this report include the presentations of the 27th Annual Meeting held in Bellevue, WA, 16-19 November 1992.

What Are We Managing?

by
J. L. Decell[1]

Historical

During the 1960s, Aquatic Plant Control's basic goal was eradication. Each time a problem developed to significant proportions, the idea of eliminating it once and for all was often the goal. It became, in most cases, an unwritten objective for operational programs. Such an approach inferred that we were all willing to "put ourselves out of business." When we abandoned eradication, some might have thought that it was because we decided we wanted to stay in business. Not so! Our shift to the objective of control was the result of realizing that eradication was an impossible task, given the limited resources and technology available to those charged with the responsibility.

As we embarked toward the goal of controlling the plants, we were soon overtaken by a surge of environmental concerns; and, recognizing that the existence of a plant population did not, in itself, constitute a problem, we began to reflect on the idea of control as it related to the emerging environmental initiatives. Through this reflection, it became apparent that managing plant populations would be our most worthy and attainable objective, and that in so doing, we not only could manage the problem, but also could possibly do so with environmental compatibility.

We soon recognized that while we could determine the benefit/cost relation between the interfering plant population and the result of the proposed management action, the public did not necessarily place any value on our solution. In fact, they often placed more value on the very thing that we planned to reduce!

We were just beginning to understand what was meant by "environmentally compatible management" and its effect on what and how we do our business—and along comes the concept of Sustainable Development.

Sustainable Development

There has been much written and discussed on this subject, and most people intuitively agree on what is meant by sustainable development. However, they also agree that defining sustainable development in terms that everyone can relate to, and subsequently implement, is quite another matter. As is the nature of most concepts, it seems we can agree on what something is about—and at the same time, agree that we cannot really understand what it is!

One notion is that sustainable development means "meeting the needs of the present without compromising the ability of the future generations to meet their own needs." This is not so easy to translate into implementable methods when we attempt to determine what the future generation's needs are going to be. Is their future standard what we feel is right and pure for our time? Based on experience gained from interactions with my own children, I can attest that my values of certain things in my time were not readily adopted by my children during their time—especially as these related to freedom, cars, and money! And these are much less complex elements than those involved with the environment.

One definition of sustainable development is that it is "improving the quality of human life while living within the carrying capacity

[1] Program Manager, Environmental Resources Research and Assistance Programs.

of the supporting ecosystem." To the biologist, it is simply "living within the carrying capacity of the environment." Pure biological solutions are not the answer. If they were, and man were willing to adjust his values and demands, we would not have most of the environmental problems we have today.

Policy makers are struggling with the problem of how to translate this broadest of concepts, sustainable development, into specific guidelines and concrete actions. At the same time, environmental science is wrestling with the problem of evaluating consequences of specific human activities and technologies on such things as carrying capacity.

In *A Sand County Almanac*, Leopold states it simply that we should "examine each question in terms of what is ethically and esthetically right!" I think that each of us agree and try to do that, knowing that it is a concept like eradication—not totally attainable, but worthy of constant focus.

Technological Edge

Regardless of the exact approach, we must maintain a technological edge—not a competitive edge over some other scientific field or effort, but the edge that keeps us ahead of our former ways of thinking about the total nature of the problems. Part of this edge will be to identify the critical technologies that will be needed. We will have to identify what we want from our investment; identify real world constraints and drivers; define a set of needed capabilities, and selectively invest in those technologies that show the best promise of obtaining the needed capabilities. Sound familiar? It should. It is the basic strategy that we have tried to follow for the past 15 years when identifying our research for the Aquatic Plant Control Research Program (APCRP).

In implementing the concept of sustainable development, it will be more important in the future to listen and gain the advice of the experienced members of the public sector.

How does this relate to the Aquatic Plant Control Program of the Corps of Engineers, and more specifically the answer to the title of this talk? During the past few years, you may have heard the phrase, the Greening of the Corps. Believe it or not, it has been and is continuing to happen. The Aquatic Plant Control Program of the Corps can take due credit for a significant contribution to this overall change.

In the past, policy allowed the Corps to basically ignore consideration of fish and wildlife, certain other environmental aspects, and recreation on an equal footing with flood control and navigation. That is changing and the Corps is making progress toward a new policy that reflects equal consideration.

The APCRP has done an excellent job of considering the ecology of the plant equally with the development of control technologies. Successfully initiating these basic studies was not an easy task. Convincing the field elements and the "upper management old guard" that it was the right thing to do took a great deal of time; but faced with the proof, they finally saw the merit. It has been successful because it was the right thing to do—and was done right!

What are we managing? For almost the last 20 years we have been managing aquatic plant populations, and have done well. We probably have the strongest relationship between research and the operating field elements that exists in the Corps of Engineers today. The field elements have done an outstanding job of applying the developed technology, and have done so in an environmentally compatible manner. They have provided the proof.

In the final analysis, most everyone that I personally know and have worked with is an environmentalist at heart. I think that they embrace the emerging concept of sustainable development whether they fully understand the meaning or not! I submit that we have an unrestricted opportunity and, more so, a

responsibility to admit that what we are managing today, and will be in the future, is aquatic habitat.

I do not mean to imply that we have not properly considered the environmental consequences of our plant control actions on the aquatic habitat. But I believe, in the context of the emerging concept, that we must and can do better in the future.

Our future APCRP research must, by design, reflect the fact that we recognize the plant population as a part of the larger interactive aquatic community. Operations personnel will have the unenviable task of convincing the public that a longer term approach, and not a quick fix, is the best way to proceed for the environment and, in so doing, for them.

Several years ago, I initiated research in the APCRP to determine the beneficial aspects of the plants. One outgrowth of that work is the work we have been doing to develop the relationships between the plants and fish. The plants are, among other things, a fisheries habitat. Fisheries are the reason that fishermen do their thing, and fishing is a major recreation component on our reservoirs. If you are in operations, you are aware that fishermen are sensitive to what is done to manage plants.

We in aquatic plant management have a stake in this business of sustainable development. We have our part to do. The transition from concept to concrete actions that the policy makers are struggling with will not be achieved by them. It will be achieved by you, the environmental scientists. We must be willing to use our vested authority, in a responsible manner, to implement the concept within our science, by doing what we intuitively know is right.

In the future, our work units will reflect the fact that we are managing habitat, or we will not conduct them. We will work closer with the Field Review Group, the Districts, and the public to ensure that we are also reflecting their needs and views.

There is a notion that if everyone can be truly environmentally sensitive, the job will be easier and life will be better. It is my opinion that we will find our tasks to be more complex and therefore harder, but very much more rewarding. As a result, life will be better.

The Aquatic Plant Control researchers and operations personnel of the Corps of Engineers have provided the national lead for other Federal agencies and the state agencies that have brought us to this point. We now have an opportunity to continue to sustain our excellence as we develop an even, new direction.

Annual Report — Aquatic Plant Control Operations Support Center

by
Wayne T. Jipsen[1]

In October 1980, the Jacksonville District was designated by Headquarters, U.S. Army Corps of Engineers (HQUSACE), as the Aquatic Plant Control Operations Support Center (APCOSC) in recognition of the District's knowledge and expertise gained through the administration of the largest and most diverse aquatic plant management program in the Corps. The APCOSC personnel assist other Corps elements and other Federal and state agencies in the planning and operational phases of aquatic plant control.

The specific duties and relationships with other Corps Aquatic Plant Control (APC) programs and guidelines for utilization of the APCOSC, as outlined in Engineer Regulation (ER) 1130-2-412 are as follows:

- Provide operational guidance to Corps Districts in the planning phases of APC programs.
- Provide technical guidance to Corps Districts in the operational phases of APC programs.
- Provide operational expertise and/or personnel and/or equipment to respond to localized, short-term critical situations created by excessive growths of aquatic plants.
- Provide assistance to HQUSACE and Division offices for the training and certification of Corps application personnel.
- Assist the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, in the field application and evaluation of newly developed control techniques or procedures.
- Provide assistance to HQUSACE in the development and administration of a comprehensive Corps-wide APC program.

The demand for and type of services performed by the Center vary from year to year, based on the type of problems encountered by Corps elements and other agencies. Four basic types of information are requested: planning, operations, research, and training. Planning assistance includes determinations of water body eligibility and allowable costs, computation for benefit-cost ratios, methods of data acquisition, and other factors that enter into the process of planning an APC program. Operations assistance involves most aspects of chemical, mechanical, biological, and integrated technology. The Center provides data, information, and recommendations relating to operational activities. Information on research activities is provided to requestors if available, or the requests are referred to WES. Training assistance includes providing materials for use in educational and training programs and presentation of the Pesticide Applicators Training Course and the Aquatic Plant Management Course by Center staff.

During fiscal year (FY) 1992, the Center responded to 132 requests for assistance. A breakdown of these activities appears in Table 1. Figure 1 indicates the types of information requested; Figure 2 provides a breakdown regarding the source of information requests.

[1] U.S. Army Engineer District, Jacksonville; Jacksonville, FL.

Table 1
APCOSC—Support Assistance, FY 1992

Type Assistance	Corps				Other Federal	Foreign Country	State/Local	Industry	Private	Total
	HQUSACE	WES	Division	District						
Planning	10	5	6	10	1	1	4	2	0	39
Operations	5	1	11	14	9	0	11	6	9	66
Research	1	9	0	3	2	0	5	0	0	20
Training	0	3	0	1	1	0	1	0	1	7
Totals	16	18	17	28	13	1	21	8	10	132

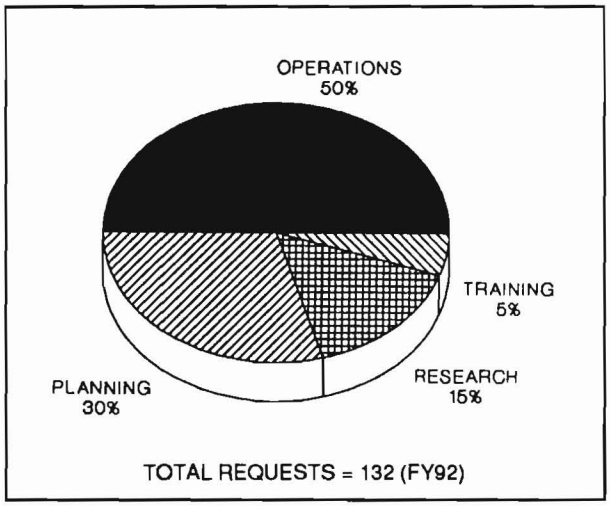

Figure 1. Types of information requested

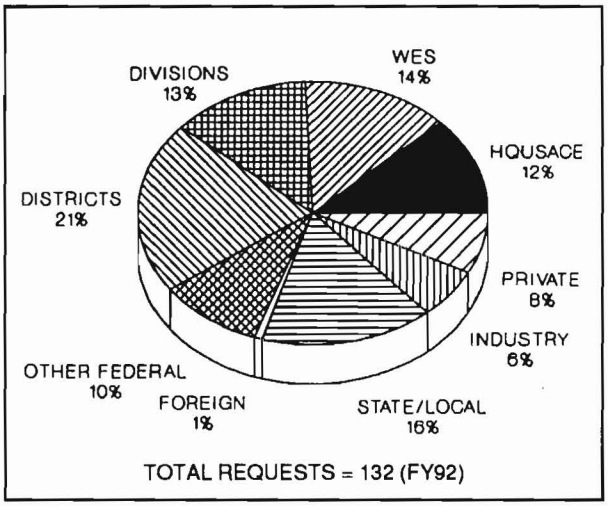

Figure 2. Sources of requests

While on temporary duty at HQUSACE, Bill Zattau worked on the revision of ER 1132-2-412 as well as assisting in the review process for reconnaissance reports from California, Minnesota, and Mississippi.

Operational support activities during the report period included a wide range of activities. Site visits conducted by the Center during the year included a trip to the Memphis District to evaluate aquatic plant problems in the St. Francis Basin Project and a comprehensive survey of the canal system in the City of Jacksonville (Florida), followed by an evaluation of the City's aquatic plant control program. The annual collection and shipment of alligatorweed flea beetles was canceled in FY 1992 because of a lack of insects in the donor areas. This activity will be resumed in FY 1993 if insect populations are adequate.

The Center conducted and/or participated in a number of training activities including the following: (a) conducting the "Pesticide Applicators Course" for the New Orleans District, (b) developing and teaching a new section, "Control of Exotic Vegetation on Spoil Sites," at the Wetlands Restoration Prospect Course, (c) teaching the aquatic plant identification and control portion of the Navy's "Pest Control Training Course," and (d) providing a history and overview of the Corps of Engineers role in aquatic plant control during a Florida applicators workshop. In addition to these formal training activities, Center staff also participated in a variety of activities at the Aquatic Plant Management Society's (APMS) International Symposium and annual meetings held by the MidSouth and Florida APMS chapters.

Further Developments at the Lewisville Aquatic Ecosystem Research Facility

by
R. Michael Smart[1]

Introduction

The Lewisville Aquatic Ecosystem Research Facility (LAERF) is being developed and operated under the Aquatic Plant Control Research Program (APCRP) to support studies of the biology, ecology, and control of aquatic plants. The LAERF receives partial funding (currently about 15 percent of the operating budget) directly from the APCRP. This funding is being used to assist in the renovation and development of the facility to meet the needs of the APCRP for an intermediate-scale research facility. The remainder of the funds are provided by research projects of the resident staff and from fees charged to users of the ponds.

The objective of this article is to provide an update on the renovation, development, and operation of the LAERF. Topics to be covered include facilities, research, and future plans.

Facilities

An onsite analytical laboratory has been developed to process and analyze water, sediment, and plant tissue samples. This laboratory is providing analytical support to many of the APCRP research projects conducted at the LAERF. We have also initiated a water quality monitoring program to obtain basic information on pond water quality on a regular basis. This information is available to researchers using the ponds. We recently acquired five additional Hydrolab data sondes, expanding our capabilities for continuous monitoring of dissolved O_2, pH, conductivity, and temperature. These data sondes can be deployed in different locations, depths, or ponds to provide investigators with a more detailed record of temporal or spatial variations in water quality of experimental ponds.

In addition to the ponds, the LAERF also includes other research facilities. These include a 1,300-sq ft greenhouse tank facility, flowing water raceway facilities, and a chemical control mesocosm system. The greenhouse facility has been expanded to include twenty 1,200-L fiberglass tanks equipped with individual temperature control units. These tanks can be filled with artificial or natural lake water, and we recently added a system to provide filtered, alum-treated pond water as well. This facility is in continuous use for culturing or propagating native species to be used in pond studies or for conducting short-term, controlled studies to supplement longer term studies conducted in ponds. Another improvement is the addition of a 600-sq ft support building housing drying ovens, balances, plant-grinding equipment, and four incubators with programmable temperature and light regimes. These incubators are being used to study environmental requirements for seed germination and tuber sprouting in aquatic plant species.

One of the raceway facilities has been covered with a greenhouse and is being used for maintaining populations of waterhyacinths and other plants during winter periods. Both raceway facilities are being used for holding/culturing aquatic plants and can be used for conducting research under flowing water conditions.

[1] U.S. Army Engineer Waterways Experiment Staion, Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

We recently completed Phase 1 of a chemical control mesocosm system. This system consists of twenty-two 6,000-L fiberglass tanks, a lined water supply pond to provide filtered, alum-treated pond water, a sediment preparation area, a growout pond to produce plants for testing, a 600-sq ft support building, and a treated-water collection pond. This system will primarily be used to test the efficacy of different chemical control/management strategies and will supplement concentration/exposure time studies conducted in controlled environment chambers located at the U.S. Army Engineer Waterways Experiment Station (WES) in Vicksburg, MS. The larger, outdoor system at the LAERF will allow testing of mature plants, longer exposure times, and consideration of seasonal/phenological cycles. Design features and operating characteristics of the system are described in the **Posters and Demonstrations** section.

We are continuing to renovate the ponds based on demand and availability of funds. During FY92, 5 of the 54 ponds were used for culture/study and 22 were used for research. Eight additional ponds have been reserved for FY93, for a total anticipated use of 35 ponds. Three ponds are still in need of repair. This leaves 16 ponds currently available for use. Excess ponds will be made available to other Corps research programs and to researchers at other Federal and state agencies and universities.

Research

All major technology areas of the APCRP are benefitting from research conducted at the LAERF. Biological control is represented by studies of the efficacy of microbial pathogens for control of submersed aquatic plants. Chemical control studies included an evaluation of the effects of a plant growth regulator (PGR) on growth, morphology, and physiology of *Hydrilla verticillata*, *Myriophyllum spicatum*, *Potamogeton nodosus*, and *Vallisneria americana*. Applications technology is being advanced in studies of the phenology or life cycle of waterhyacinth, *Hydrilla*, and Eurasian watermilfoil in both ponds and greenhouses. Simulation technology is benefitting from the collection of additional growth rate/biomass data on several species to improve/validate simulation models. A greenhouse study was also conducted to obtain critical information on *Hydrilla* tuber sprouting and regrowth from root crowns under different low light conditions, including complete darkness. Within the ecology area, studies are being conducted to understand fish-plant interactions, including changes in fish density and behavior in relation to the distribution/density of submersed plants and open water. Additional studies within the ecology area include both pond and greenhouse studies of competitive interactions among introduced weeds and beneficial, native species.

In addition to the APCRP-sponsored research, the facility is also supporting the Corps' Wetlands Research Program (WRP). Studies currently in progress include a collaborative effort between WES and Texas A&M University to evaluate moist soil management strategies for wildlife habitat enhancement. We are also conducting a wetlands mesocosm experiment on atrazine processing in a constructed wetland operated under different hydrological regimes. Investigators from the Hydraulics Laboratory of WES are studying the effects of submersed aquatic and emergent wetland vegetation on water flow and sedimentation. These studies are being conducted in a section of our concrete-lined drainage system that has been modified to function as a flume.

In addition to the Corps' APCRP and WRP, the LAERF is also providing research on aquatic plants and wetlands in support of other Federal agencies. One project, funded by the U.S. Environmental Protection Agency, Region 2 as a Clean Lakes Program Demonstration site, is concerned with identifying methods for reestablishing aquatic and wetland vegetation as an ameliorative treatment in chronically polluted Onondaga Lake. We are conducting mesocosm bioassays to determine the potential of historical, current, and predicted future water chemical compositions to support the growth of different species of

aquatic plants on infertile, oncolitic (calcareous) sediments. Results of the bioassays are being used to select suitable species for field trials to test the feasibility of using aquatic vegetation as part of a remediation plan for this lake. This research relies on methods developed earlier under the APCRP and complements similar APCRP work now being conducted.

We have also been involved in a cooperative effort with the Fort Worth District planning the development of artificial wetlands at Lake Ray Roberts, Texas. We have recently been contracted by the U.S. Environmental Protection Agency, Region 6 to conduct an experimental planting of aquatic and wetland vegetation at this site.

We have also initiated a study of the potential impacts of herbivorous turtles on the reestablishment of submersed aquatic vegetation in Guntersville Reservoir. This effort is funded by the Tennessee Valley Authority.

Future Plans

Renovation and development of the LAERF will continue during FY93. Due to excellent performance of this system (see **Posters and Demonstrations** section) and a resultant high demand, we are expanding the chemical control mesocosm system from 22 to 30 tanks. We are also developing a deepwater mesocosm system for studying the abilities of submersed aquatic plants to regrow, under low light conditions, from different depths of water. This system will allow us to collect data necessary for understanding and ultimately predicting the regrowth potential of different species growing under different light/depth conditions. This information will be used in the further development of plant growth models that will predict the efficacy of various aquatic plant management options under different environmental conditions.

The deepwater mesocosm system consists of nine 14,000-L fiberglass tanks measuring 10 ft deep by 8.5 ft in diameter. The insides of these tanks are finished in black to minimize reflected radiation to more closely approximate natural, low light conditions at depth. This system is adjacent to the chemical control mesocosm system and will share the water supply and growout ponds.

During FY93, we anticipate an additional increase in activity associated with APCRP research at the facility. We also anticipate additional WRP and reimbursable research efforts.

Acknowledgments

Many people have contributed to the continuing development of the LAERF. During the past year, the contributions of the following, in particular, are gratefully acknowledged: J. T. Alewine, Ken Howell, Allen Martin, and Larry Prestien of the Lewisville Lake Project Office; Michael Crouch, Gary Dick, Robert Doyle, David Holland, David Honnell, Chris Houchens, John Madsen, Kimberly Mauermann, Rita Pierson, Joe Snow, and Stephen Wood of LAERF; Kurt Getsinger, Richard Price, and Mike Stewart of WES.

Long Lake Project: Chemical Control Technology Transfer

by
K. D. Getsinger[1]

Background

The Long Lake Project is an excellent example of citizens, government, and private industry working together to solve a complex environmental problem using state-of-the-art technology generated through research and development. In this case, the problem was the invasion and subsequent dominance of Long Lake by the noxious submersed plant Eurasian watermilfoil (hereafter called milfoil). Milfoil was first reported in the shallow, 330-acre Long Lake, located near the town of Lacey in Thurston County, WA, in 1987. Long Lake is almost encircled by residential dwellings and is heavily used as a recreational resource (fishing, swimming, boating, etc.) via a public access area. Although some organisms can survive in a milfoil-dominated aquatic community, the biodiversity of a system is highly reduced as native plants and their associated fauna dwindle because of milfoil's aggressive nature. Recognizing the potential negative impact of a milfoil infestation on the Long Lake system, the local Lake Improvement Association (LIA) began to investigate milfoil control/eradication alternatives, such as mechanical harvesting, grass carp, bottom screens, herbicides, etc.

By 1991, the exotic invader had covered nearly 70 percent of Long Lake's surface and had completely infested a smaller, downstream water body, Lake Lois. Since Lois and Long Lakes are part of an interconnected lacustrine system that eventually flows into Puget Sound (Figure 1), local lake managers feared that an unchecked milfoil infestation in them could lead to the establishment of the troublesome weed in nearby watersheds. That year, a draft environmental impact statement (DEIS) reviewing various control techniques was completed. The DEIS recommended a whole-lake herbicide treatment, using the aqueous suspension (AS) formulation of fluridone (Sonar AS, manufactured by DowElanco), as the only realistic alternative for the first phase of the 5-year, integrated eradication plan. Additional phases of the plan included the use of hand-harvesting, diver-dredging, bottom screening, and herbicide spot-treatment techniques to remove any milfoil surviving the whole-lake fluridone treatment. In many situations, eradication is not a realistic target for an aquatic plant management plan; however, the relatively small size and isolation of the milfoil infestation and the potential of the plant to invade numerous other watersheds prompted the LIA to select eradication as a goal.

Management activities of the Long Lake Project were coordinated by the LIA. Project cooperators included the following:

a. Property owners, who planned to contribute up to $1.2 million for implementing the 5-year plan.

b. Thurston County Public Works (TCPW), who would implement the eradication strategies.

c. Washington Department of Ecology (WADOE), supplying $64,000 for watershed nutrient control.

d. U.S. Environmental Protection Agency (USEPA), contributing $220,000 for treatments and surveys.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

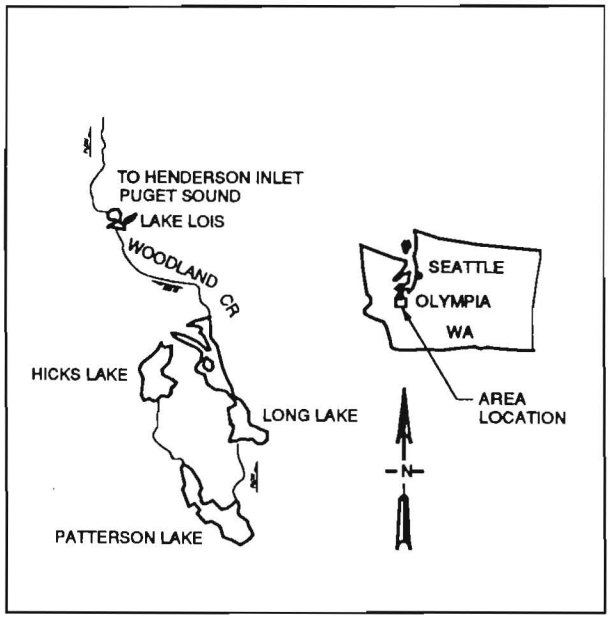

Figure 1. Long Lake watershed, Thurston County, WA

e. Washington Department of Wildlife, providing environmental monitoring efforts.

f. U.S. Army Engineer District, Seattle, contributing $90,000 for the first phase herbicide treatment.

These diverse groups were chosen as cooperators to ensure that treatment strategies implemented during the project would represent the most recent, environmentally compatible technology available and that the long-term impacts of these strategies, negative or positive, would be monitored.

Initial Treatment Strategy

In the winter of 1991, WADOE requested the U.S. Army Engineer Waterways Experiment Station (WES) Chemical Control Technology Team (CCTT) to design a fluridone treatment strategy to maximize the control and/or eradication of milfoil in the lake system. After reviewing all available morphometric, flow, and plant community data on the Long Lake system, the CCTT recommended a sequential, block-treatment strategy. This strategy involved applying the herbicide in series of 5- to 10-acre blocks over a period of time to maintain 15 to 25 ppb (μg/L) of fluridone in the water of the entire lake for 10 to 12 weeks. Past field experience and results from fluridone concentration/exposure time (CET) evaluations conducted at WES were the basis of this sequential block-treatment approach. Efficacy of fluridone is critically tied to length of exposure time. If the proper contact time can be achieved, using an extremely low level of fluridone is possible. Field applications and CET evaluations have repeatedly demonstrated that by maintaining a fluridone water concentration of 15 to 25 ppb (6 to 10 times less than the USEPA potable water tolerance of 150 ppb), greater than 98-percent milfoil control can be achieved (Netherland 1992; Netherland, Getsinger, and Turner 1993).

Concurrent use of the inert water-tracing dye rhodamine WT was recommended during the fluridone applications to aid in the selection of subsequent treatment blocks and to reduce the number of expensive fluridone water residue tests that would be required to track the movement of the herbicide. Recent studies have shown that rhodamine WT mimics the dispersal of fluridone in water with a correlation coefficient of 0.99 (Fox, Haller, and Schilling 1991).

Mid-May was recommended as the optimum time for the whole-lake treatment. Historically, water flow through the system decreased at that time, and newly sprouting milfoil would be most vulnerable to fluridone exposure. Also, desirable, nontarget submersed plants (pondweeds, elodea, etc.) would be least susceptible to a fluridone treatment in May. Finally, it was recommended that no fluridone treatment sites would be required in downstream Lake Lois.

Treatment Strategy Concerns and Constraints

Elimination of rhodamine WT

After reviewing the CCTT treatment strategy proposal, the Thurston County Board of Health denied the use of rhodamine WT in

conjunction with the fluridone application because of fears over its toxicity. These fears were unfounded since rhodamine WT is used routinely throughout the United States (including the state of Washington) for water-tracing studies and was proposed for use in Long Lake at 50 percent less than the USEPA potable water tolerance level of 10 ppb. In addition, active potable water intakes are not located in Long Lake.

By eliminating the dye treatment, an additional $15,000 of project funds were required for water residue sampling and, more importantly, the real-time, fine-tuning of fluridone treatment blocks was hindered. Final analysis of residue samples clearly indicated that the use of rhodamine WT as an indicator of herbicide movement would have resulted in a 50-percent reduction in the amount of fluridone used during the treatments, saving the project approximately $85,000.

Delayed fluridone treatment

The Department of Wildlife required that the fluridone treatment be postponed until June to minimize any adverse effects on salmonid reproduction. Although scientific evidence did not exist to suggest that low rates of fluridone would influence the reproductive cycle of the salmon, this 2- to 4-week delay in treatment still provided a near optimum window for milfoil susceptibility and nontarget plant tolerance to fluridone exposure.

However, a small (but vocal) group of environmental activists used public forums (the media, hearings, etc.) to play on the fears of local politicians and to question the environmental risks of the Long Lake herbicide treatment. Even though all of the potential environmental concerns had been thoroughly addressed in the DEIS, the local activists were successful in delaying the fluridone application until July 2. This delay caused serious ecological and economic consequences to the project:

a. By July, milfoil plants were mature and healthy, with sufficient physiological reserves to withstand herbicide assaults, and herbicide damage to nontarget submersed plants was more likely to occur.

b. Since the entire submersed plant community (milfoil and nontargets) would be impacted by a July fluridone treatment, the possibility for nuisance algal blooms was dramatically increased, and the potential for a rapid reestablishment of native submersed species was decreased.

c. Since the Board of Health required that the lake be closed following the final application until fluridone water levels were below 101 ppb (an arbitrary requirement from a label and toxicological standpoint), the recreation season on the lake was substantially shortened.

All of these occurrences caused many additional project and private dollars to be spent or lost and threatened the success of the project.

Modified Treatment Strategy

Adjusting for the various constraints and delays, the CCTT recommended a modified whole-lake treatment strategy that would result in 165 acres (50 percent) of the lake being treated over a 2- to 6-week period. This strategy would maintain a predicted fluridone concentration throughout the lake of 15 to 25 ppb for 8 to 10 weeks following the initial application and would be accomplished by the following protocol:

a. Treat 25 percent of the lake (82.5 acres) on Day 1 in 12 separate blocks at the maximum label rate of fluridone. Most of these blocks would be located in the upstream portion of the lake.

b. In lieu of dye information, the location of subsequent treatment blocks would be based on fluridone water residue samples collected from 12 stations around the lake 1 week after each treatment.

c. Using this strategy, treatment of downstream Lake Lois would not be necessary.

Aquatics Unlimited, a local firm certified for aquatic herbicide work, was selected by TCPW to apply the Sonar AS (fluridone) formulation. The initial application of 12 blocks was made on July 2, 1991, with subsequent treatments on July 17 (5 blocks), July 31 (4 blocks), and August 14 (3 blocks). Treatment dates, block location, and fluridone concentrations, 6 to 9 days following each application, are presented in Figure 2.

During the sequential treatments (July 11 - August 23), fluridone levels ranged from 25 to 43 ppb, somewhat greater than the target levels of 15 to 25 ppb. Residue analysis presented in Table 1 shows that fluridone concentrations in the lake were maintained above 25 ppb through posttreatment Day 92 (13.1 weeks). These higher fluridone levels suggested lower flows in the watershed than predicted and underscored the advantage of instantaneous water movement readings using tracer dyes versus using 1-week-old herbicide water residue data. The concurrent use of the fluridone-simulating rhodamine WT dye would have provided an accurate, real-time measurement of fluridone dissipation/degradation in the lake and allowed for significantly less herbicide to be used during the treatment period (while still providing a sufficient fluridone dose to the system).

Table 1
Fluridone Water Residues (ppb) from Surface and Bottom of Long Lake, WA, 1991

	Fluridone Residues, Days After Treatment					
	Pre	7	14	28	42	92
Surface	<5	48	35	43	38	26
Bottom	<5	39	29	34	36	25

Note: Fluridone treatment dates were July 2, 17, 31, and August 14. Data represents mean of 12 sampling locations.

Fluridone Residues Along Woodland Creek

One major concern of the environmental activists was the issue of fluridone residues in water carried downstream from Long Lake via Woodland Creek (Figure 1). This group predicted that the off-target residues would

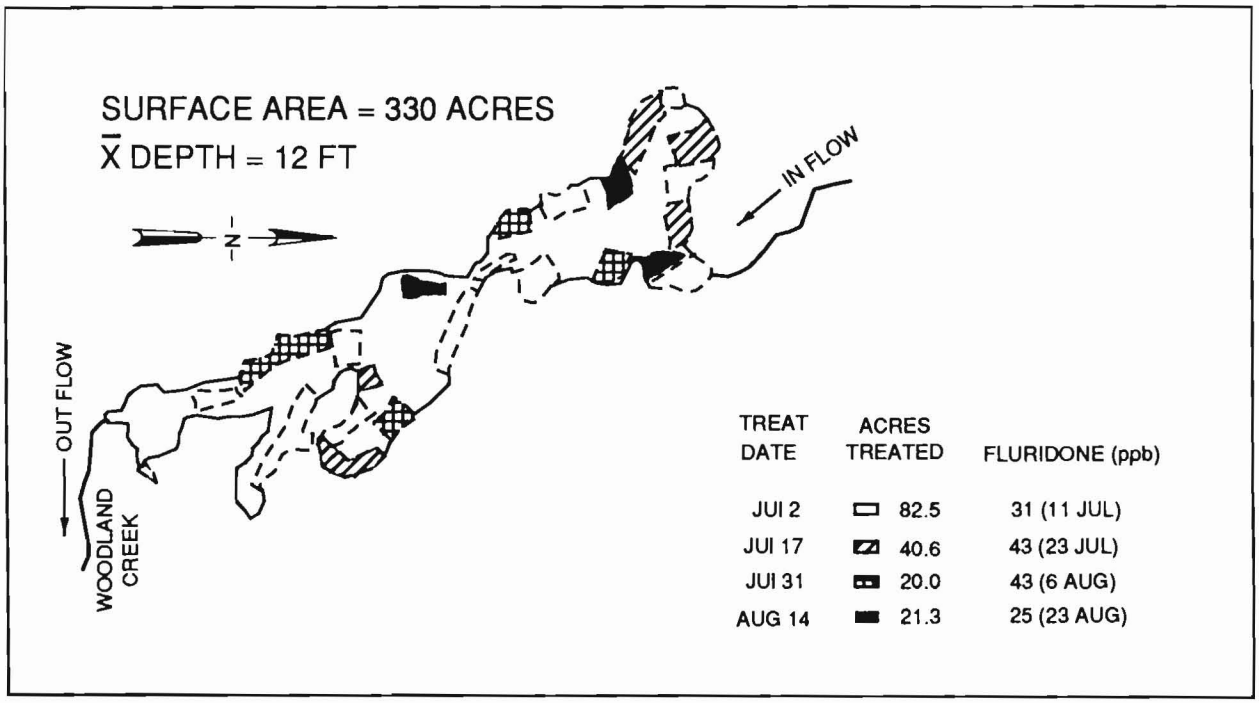

Figure 2. Fluridone split-treatment dates, blocks, and water residues from Long Lake, WA, 1991. Dates in parentheses indicate time of water sample collection

destroy desirable wetland vegetation along the watershed and in Henderson Inlet, an embayment of Puget Sound. To address this issue, water samples were collected for fluridone residues at nine sites along Woodland Creek downstream from the Long Lake treatment blocks. Figure 3 depicts fluridone levels measured in these samples at six of the sites, from the most upstream (Long Outlet) to the most downstream (31st Ave) location. Fluridone concentrations ranged from 15 to 48 ppb in water collected from Long Outlet (outfall from Long Lake), Lois Outlet (outfall from Lake Lois, 1.1 miles downstream from Long Lake), and Martin Way (1.8 miles downstream from Long Lake) through 92 days posttreatment. Water residues at these three downstream stations followed a pattern similar to the water residues measured in Long Lake: high enough to affect milfoil, but well below potable water tolerance levels.

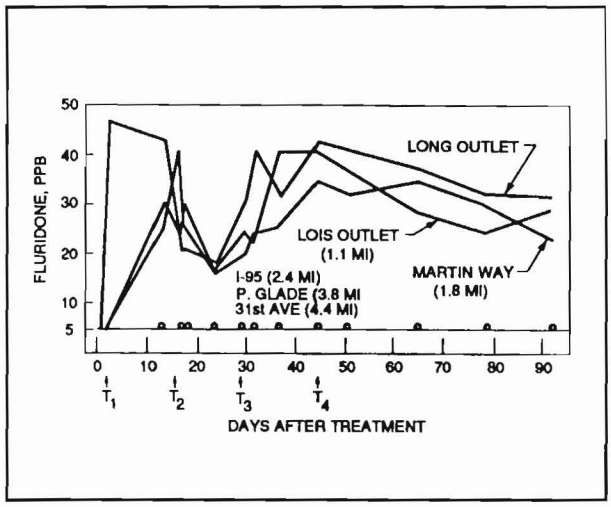

Figure 3. Fluridone water residues from selected sampling sites on Woodland Creek, WA, 1991. T_x represents Long Lake fluridone applications (T_1 = July 2; T_2 = July 17; T_3 = July 31; T_4 = August 14)

However, water residues measured at the sites farthest downstream, I-95, P. Glade, and 31st Ave (2.4, 3.8, and 4.4 miles from Long Lake, respectively) remained below detection (<5 ppb) for the duration of the sampling period. Large springs entering the surface water below Martin Way may have been partially responsible for the dilution of the fluridone-treated water at the lower three sampling locations.

One-Year Posttreatment Results

One year after the whole-lake fluridone application process, data collected by SCUBA divers on previously established transects indicated that greater than 98 percent of the milfoil had been eradicated in Long Lake. Milfoil was also eradicated in downstream Lake Lois. Long Lake residue data showed that fluridone levels had been maintained in the water at 25 ppb or higher for 92 days. This information verifies results from CET evaluations conducted at WES where milfoil control was greater than 98 percent when fluridone water concentrations were maintained at 24 ppb for 90 days.

The majority of the surviving milfoil was located in the inflow channel at the south end of Long Lake, where fluridone contact time had been negligible, as predicted. Hand-harvesting techniques were employed to remove any viable milfoil plants from that region of the system to eliminate a potential source of reinfestation.

Transect data also showed that the initial severe fluridone damage to nontarget submersed vegetation that occurred during the 1991 growing season, primarily because of the forced, delayed treatment schedule, had been mitigated by the 1992 growing season, and several native species (e.g., Nitella, pondweeds, and elodea) were spreading throughout the lake.

Comparison of extensive pretreatment and posttreatment surveys of Long Lake, Lake Lois, and the entire length of Woodland Creek to Henderson Inlet and Puget Sound showed no adverse impact on associated wetland vegetation, water quality, or nontarget organisms (Farone and McNabb 1993). Also, fluridone water residues were below detection 2.5 miles downstream of the treatment sites.

Long Lake Project Results: Summary

At 18 months posttreatment, greater than 98 percent of the established milfoil has been controlled in the Long Lake watershed, and the system is being naturally restored to a desirable, diverse submersed plant community via the self-establishment of native species. This release and reestablishment of native plants, in conjunction with persistent and effective nonchemical techniques for removing any surviving milfoil, can make the goal of milfoil eradication possible in the watershed. As predicted, there were no adverse impacts on wetland vegetation, water quality, or nontarget organisms. Since associated wetlands were undamaged by the herbicide treatment, hundreds of thousands of project dollars that were earmarked for wetlands mitigation remain unspent. Furthermore, public use of the lake reached near record levels during the 1992 recreation season.

By using a sequential block-treatment approach, 50 percent less fluridone than allowed by the label maximum was used than for a single, whole-lake application, thereby saving the project over $170,000 in chemical costs alone. In addition, fluridone water concentrations were maintained at 70 percent below the potable water tolerance level established by the USEPA. Both of these factors translated into minimal loading of herbicide into the environment without sacrificing efficacy.

Over the next several years, findings from the Long Lake Project (including efficacy on milfoil and nontarget plants, herbicide residues in water and sediment, reestablishment of native submersed plant communities, and treatment impacts on water quality and associated wetland vegetation) will be documented in reports and in the scientific literature. These documents will serve as a source of information for future milfoil control/eradication projects across the Nation.

Technology Transfer

While the Long Lake Project demonstrates the ability of diverse groups to solve an environmental dilemma, it also clearly illustrates the term "technology transfer." All of the individuals and organizations involved in the project possess a sense of satisfaction and pride in having accomplished the successful transfer of technology, which can be summarized as follows:

> Results from a research and development program sponsored by the Corps of Engineers were used to solve an operational problem in an environmentally compatible and cost-effective manner.

Acknowledgments

Pertinent contributions to the design and conduct of the Long Lake Project sequential block-treatment strategy provided by numerous aquatic weed control specialists, including members of the CCTT, are greatly appreciated.

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Review of Eurasian Watermilfoil Biocontrol by the Milfoil Midge

by
Ben Kangasniemi,[1] Helmut Speier,[2] and Peter Newroth[1]

Introduction

Eurasian watermilfoil (*Myriophyllum spicatum*) has been recognized in British Columbia since 1972. Presently, this plant is restricted to the southern portion of the province and occupies approximately 1,500 ha of which about 300 ha are subject to plant management programs (Figure 1). Roughly 70 percent of plant management is accomplished by derooting and rototilling methods that effectively eliminate most plants for one or more seasons (Newroth 1990). These methods are becoming increasingly effective as technical improvements to the machinery are made. As a result, public expectations for nuisance plant control in beach areas are very high. This technology represents formidable competition in high use areas; however, biocontrol is a promising option in other areas where intensive mechanical methods are environmentally inappropriate or too expensive.

Eurasian Water Milfoil Herbivores

During the late 1970s, detailed surveys of the health of representative nonmanaged *M. spicatum* populations were initiated. These surveys were in part a response to reports of declining populations elsewhere in North America and local observations of reduced plant height at some sites. Ten sites in five Okanagan Valley lakes were studied. Surveys conducted during 1980 and 1981 demonstrated that *M. spicatum* was host to three groups of herbivorous insects: a caddis fly, *Triaenodes tarda*, several weevils, primarily *Euhrychiopsis lecontei*, and a new midge species subsequently described as *Cricotopus myriophylli*, the milfoil midge (Kangasniemi 1983; Kangasniemi and Oliver 1983; Oliver 1984).

Cricotopus myriophylli and *E. lecontie* had much more impact on Eurasian watermilfoil growth than did *T. tarda*; however, *C. myriophylli* had the greatest impact at more sites and over a longer period of time than *E. lecontei*. Although weevil damage was observed on both submerged plant populations and surfacing plant populations, the greatest numbers of weevils and the most impact was observed at flowering sites.

Milfoil Midge Impact

During 1990 and 1991, the same Okanagan Valley sites sampled in the early 1980s were revisited. These surveys confirmed that the same insects were still causing considerable grazing pressure and that the milfoil midge was still the primary herbivore (Figure 2). When milfoil midge densities are sufficient, this insect impacts the plant populations by reducing overall height and preventing surfacing and flowering. Relatively little Eurasian watermilfoil biomass is consumed, but substantial biomass is prevented from developing. This is because the midge spends all of its larval life building and maintaining an attached case in the apical meristem region and eventually pupating. Most shoots supporting larvae are prevented from growing either because of the destruction of the meristems or stems. Vigorous lateral shoot production can sustain growth of the plant population; however, where midge densities are high enough,

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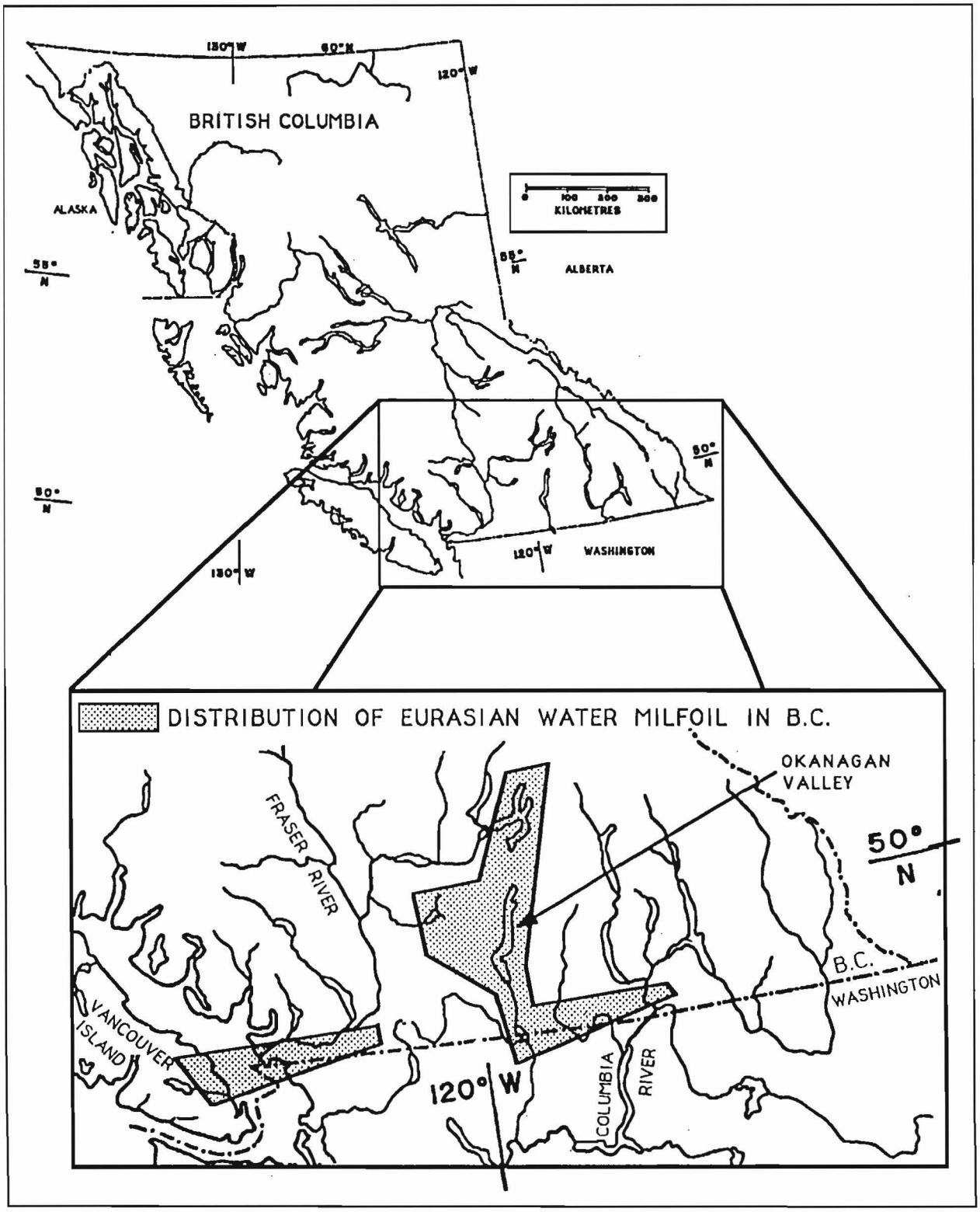

Figure 1. Distribution of Eurasian watermilfoil in British columbia

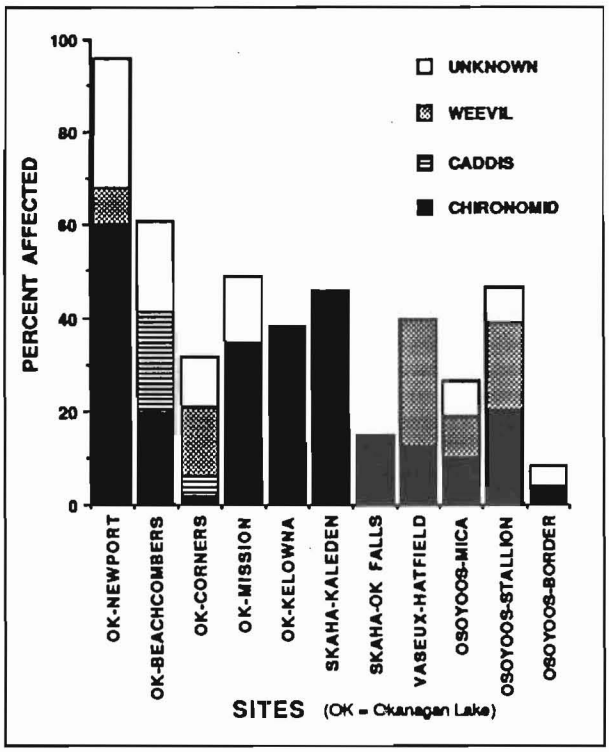

Figure 2. Percent Eurasian watermilfoil shoots affected by three herbivores in the Okanagan Valley lakes

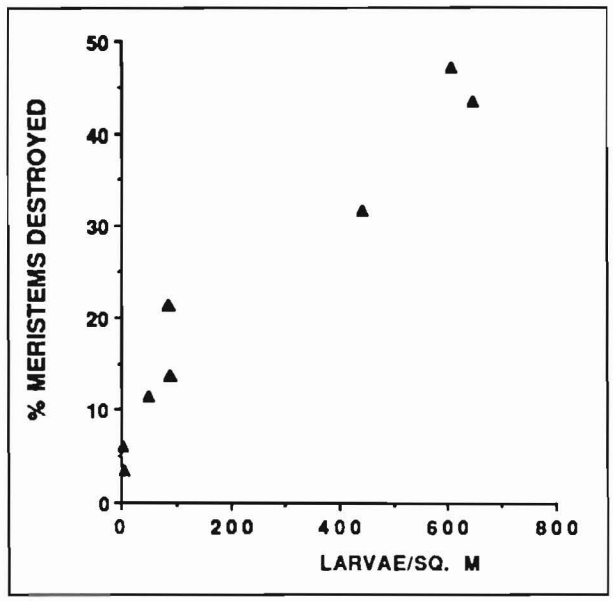

Figure 3. Percent apical meristems destroyed by the milfoil midge in comparison with larval density at eight Okanagan Valley sites

plants remain a meter or more below the surface throughout the year.

Cricotopus myriophylli density at most sites ranged from about 100 to 650 larvae per square meter. It is estimated that densities of about 500 larvae per square meter can prevent milfoil populations from surfacing (Figure 3). This is the case where plant biomass usually approaches 500 to 600 g/m^2 dry weight. Laboratory studies have demonstrated that a single larva prevented all new growth of individual shoots over a 10-day study period (MacRae, Winchester, and Ring 1990).

Identification and Biology

Adult milfoil midges can be difficult to distinguish from other members of the sylvestris group. The larval case, constructed of haphazardly arranged leaflets cut to varying lengths, is usually attached to the stem near the apex. The case is a useful diagnostic feature for identification. Other morphological features such as the presence of seven pairs of lateral setal brushes and the shape of the mentum can be used for taxonomic confirmation.

The life cycle is about 30 days and is multivoltine. Typical of midges, the adult males form swarms during dusk. Columnar swarms have been observed in clearings within shoreline vegetation (poplar trees and shrubs). The swarms use various objects as markers and can be very difficult to see, as they can occur at heights exceeding 3 m. The swarms also tend to move up out of reach at the sight of a sweep net.

Instars II, III, IV and pupae are found in the milfoil canopy throughout the year. The complete life history of the first instar is not known. In laboratory culture, the female usually lays 250 eggs; however, occasional clutches of 400 have been observed. Eggs have not been found in the field to date.

One site in Wood Lake was studied in detail during the winter and spring warm-up period to document winter behavior and the temperature when pupation and active feeding starts. Overwintering larvae were located on milfoil shoots in an inactive state throughout

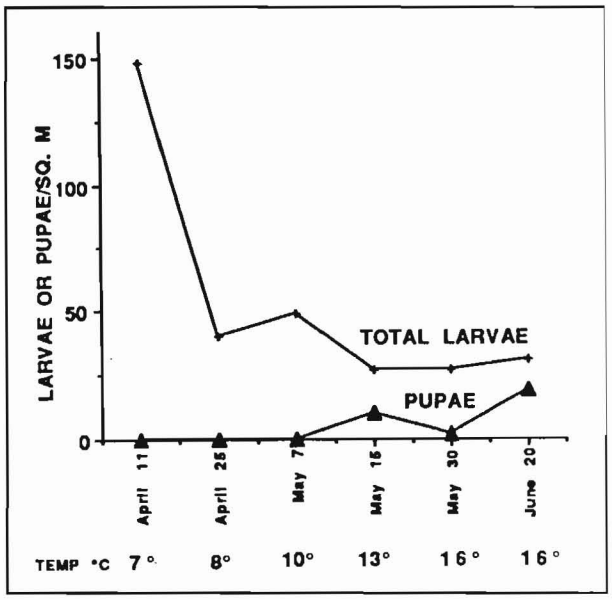

Figure 4. Milfoil midge density on Eurasian watermilfoil during spring in Wood Lake

the winter, surviving several weeks of ice cover. Larval development to pupation begins around April as lake water approaches 10 to 13 °C. A surprising finding was that larval density dropped dramatically before pupation at the study site (Figure 4). This is probably due to spring mortality of late instars, larvae that have survived and continued to develop since the previous October (Speier et al., In Preparation).

Plant Preferences and Native Host

Starvation trials with other plants reported by MacRae (1988) showed that the milfoil midge fed and/or built cases on 3 of the 12 aquatic plants tested: *Ranunculas aquatilis, Potamogeton natans*, and *Myriophyllum exalbescens* (=*sibiricum*). The strongest response occurred with *Myriophyllum sibiricum*, which supported feeding and case building.

During 1992 surveys, the milfoil midge was documented on *M. sibiricum* in Windermere Lake and Dragon Lake; neither of these lakes have *M. spicatum*. Dragon Lake is located 300 km north of the nearest population of *M. spicatum* in a sub-boreal biogeoclimatic region, whereas Windermere Lake is located 130 km east of the nearest *M. spicatum* population in the same warm/dry biogeoclimatic region as the Okanagan Valley. The preliminary conclusion from these findings is that *C. myriophylli* was not introduced along with *M. spicatum* as initially assumed, but is native to British Columbia, and the original host plant is northern milfoil, *M. sibiricum*.

With the introduction of *M. spicatum, C. myriophylli* has apparently taken advantage of a suitable new host plant. *Cricotopus myriophylli* has been found established in most locations where *M. spicatum* is found in British Columbia. The distribution of *M. spicatum* is restricted to southern British Columbia and overlaps with *M. sibiricum; M. sibiricum*, however, is found throughout British Columbia, including the northern boreal and sub-boreal biogeoclimatic regions.

Future efforts to document the distribution of the milfoil midge should therefore emphasize collections at *M. sibiricum* sites. The apparent absence of the milfoil midge in most of the United States may be due to the fact that previous surveys focused on *M. spicatum* populations. Specimens collected by Balciunas (1982) in an 11-state United States survey, which were initially identified as belonging to the *Cricotopus sylvestris* group, were subsequently reported not to include *Cricotopus myriophylli* (Oliver 1984). However, *C. myriophylli* is established on *M. spicatum* throughout Osoyoos Lake, including the southern basin, which is largely within Washington State.

Biocontrol Potential and Research Objectives

Based on field and laboratory studies of the biology of the milfoil midge, continued work on assessing its biocontrol potential seems warranted. Table 1 summarizes some of the major advantages and disadvantages of using this insect. A major advantage of this insect is the efficiency with which it prevents milfoil from reaching the surface. Without consuming large amounts of biomass, this insect retards shoot elongation by feeding on meristematic tissue.

Table 1
Summary of Advantages and Disadvantages of Using Milfoil Midge as a Biocontrol Agent for Eurasian Watermilfoil

Advantages	Disadvantages
Native insect—available for immediate testing	Native insect—not a classical approach
Destroys meristem—stops shoot growth	Substantial submerged biomass remains
Can be regulated by scale and frequency of releases	May require repeated releases
Prefers *Myriophyllum*	Feeds on *Myriophyllum sibiricum*
No known nuisance potential	Cannot currently be cultured

The most significant disadvantage encountered is our inability to establish a laboratory culture. Nonclassical biocontrol using this insect will depend on the ability to mass rear and inundate target plant populations each spring. Other strategies include augmentation of sparse milfoil midge populations and introduction to new habitats within British Columbia or beyond. All of these strategies depend on laboratory culturing. Furthermore, mass culturing must be inexpensive enough to enable inundation at a cost comparable with other control methods presently in use.

Establishing a Laboratory Culture

The establishment of a culture has been the primary objective during the last 2 years of research. To date, we have demonstrated that all larval stages collected from the field will readily develop, pupate, and emerge under simple laboratory conditions and can be sustained on whole plant material, slurry, or tissue culture material. Adults also can be maintained in a healthy and active state in relatively confined quarters for several days during which mating activity and egg laying occurs. Unfortunately, the proportion of eggs that complete embryogenesis to the larval stage in vitro is extremely small. Poor laboratory reproduction, using relatively low numbers as compared with the field conditions, suggests this insect has low fecundity. Males have very low fertility, as indicated by the very low proportion of viable sperm in most individuals, whether they have been reared in the laboratory or are from field collections.

These symptoms may be indicative of genetic abnormalities. Preliminary karyotyping results suggests that there are several potential genetic abnormalities; further detailed study of the relevance of these genetic abnormalities to laboratory culturing is planned.

We have also investigated the possibility that this low fertility may be due to a protozoan parasite, possibly a Trypanazoan. This possibility has been recently discounted. The role of laboratory conditions, including light, dimensions of the mating cages, temperature, humidity, food source, water quality, and potential sources of disease and parasites continues to be assessed. Further experimentation with these variables should be pursued.

Future of Program

The shortage of good biocontrol candidates for Eurasian watermilfoil makes it desirable to continue work on this species. Adequate funding to continue this work is not assured beyond March 1993. It is hoped that other agencies or institutions with interests in the milfoil midge may be able to do certain aspects of the research or support ongoing work through a cooperative arrangement with the British Columbia Ministry of Environment, Lands and Parks. Determination of the optimum culturing requirements should remain the primary objective. A culturing capability will facilitate field testing and will enable transport of adequate quantities of material to establish this effective herbivore outside British Columbia.

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Economic Evaluations of Aquatic Plant Control

by
Jim E. Henderson[1]

Introduction

Making decisions on aquatic plant control programs is complicated by limited budgets and the need to balance sometimes diverse public interests. These conditions have increased interest in economic evaluations as a means to better evaluate and sort out all the relevant considerations for plant control programs. The Guntersville Joint Agency Project has begun gathering data on economic values to demonstrate how economic evaluations can be used with other information to make decisions on aquatic plant control programs.

There are three questions that economic analyses can help answer for control programs; these are as follows:

- Are the benefits of the control efforts economically justified; that is, are the benefits worth the costs of the control program?
- What are the economic costs and benefits associated with the alternative control programs?
- How do perceptions of aquatic plants and preferences for control programs differ among user groups?

Economically Justified Plant Control Alternatives

Deciding on the appropriate level, i.e., the amount and distribution, of aquatic plants requires an evaluation of whether increased levels of control result in sufficient benefits to justify additional costs. That is, the question is "Do the benefits at least equal the costs?" Determining whether additional plant control is economically justified requires an understanding of the relationship of different aquatic plant control levels to total public benefits that accrue from the different control levels. This relationship is formalized in an economist's utility function shown in Figure 1.

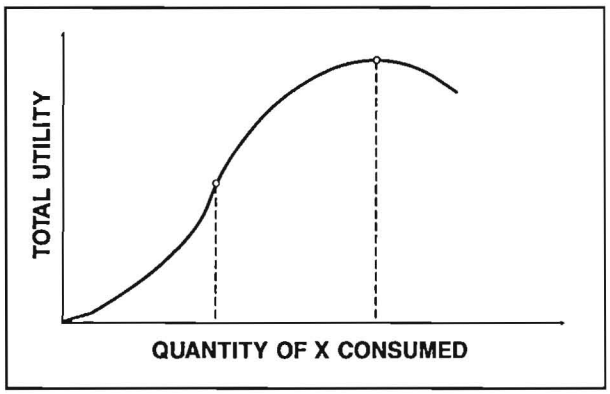

Figure 1. Total utility function

In considering the utility function and aquatic plant control, the y-axis represents "Total Utility" but can be thought of as a measure of value as Total Societal Good, Monetary Value, or some other measure of welfare. The x-axis shows "Quantity of X Consumed" with X being an environmental good such as recreation days, water quality, or, in this case, aquatic plant control. This generalized function shows the relation of providing public goods to the value those goods provide, starting from a zero level of the public good and increasing the quantity of the good consumed.

Starting at the zero level and increasing the quantity, it is observed that value rises rapidly with each marginal increase of the good. This is consistent with conditions of high demand for the good where little of the good is available. Where there is no aquatic plant control available, conditions may be such that

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

boaters are not able to launch their boats; or plant densities are so heavy that boat houses and slips are completely blocked. Given these conditions, a minimal quantity of control will be highly valued and will provide a high level of utility. At the highest levels of plant control, the total utility actually begins to decrease with additional plant control, perhaps because important fish habitat has been removed and other benefits from the plants are lost.

Determining whether control programs or additional control efforts are economically justified requires looking at the middle portion of the utility function. Examining the intermediate part of the graph shows that increasing the control still produces an increase in utility and value, but the rate of increase becomes more constant. This is consistent with an intermediate level of a service; the areas and users that produce the highest public value have been accommodated, and further increases in control still produce higher overall value.

At some point, the benefits produced by additional control exactly equal the costs of providing the additional control. Past that point, the benefits from additional control efforts are less than additional costs; i.e., the additional costs are not economically justified.

From an economic benefits standpoint, plant control should be at the level where the additional or marginal benefits of an additional amount of control exactly equals the costs of providing the extra control—the point where the marginal costs equal the marginal benefits. This is the so-called point of diminishing marginal returns. Providing levels of control past this point would result in the increased costs not producing an equal amount of benefit. This point, however, is not the same as the point where utility is maximized.

Conceptually, it is easy to identify the level of control where marginal benefits equal marginal costs, but the nature of aquatic plant control makes implementing this difficult. First of all, plant control is not implemented as if it is continuous, to be turned off at any particular point. Decisions are made on providing different levels of control. Control programs are formulated to manage for different percentages of lake coverage, e.g., 20 percent, or other specific levels of plant biomass. Secondly, each of the groups using a water resource may have a different utility function, and the utility of particular plant levels may be entirely different for different groups.

These considerations affect evaluations of alternative plant control programs. Because specific control alternatives are evaluated, the utility associated with each control level is determined. For decisions on the goal or appropriate control level, the question is posed in terms of "What level of benefits or utility is associated with each of the control alternatives under consideration?" This question becomes "What is the most cost-effective way to achieve the desired level of plant control?

To summarize, evaluation of plant control alternatives to determine if they are economically justified requires knowing the benefit or utility associated with the various plant control alternatives under consideration. Decisions on the alternative control plan leads to an examination of the most cost-effective control strategy to achieve the desired control level.

Economic Benefits

Economic benefits accruing from water resource developments are affected by the presence, density, and distribution of aquatic plants. Benefit categories include navigation, flood control, hydropower generation, water supply, irrigation, and recreation. The effect that aquatic plants have on a benefit category is determined by how the plants support or interfere with production of a particular economic service. For instance, increased amounts of aquatic plants may improve the fishery habitat to support recreation, but the increased plants may reduce the storage capacity for flood control or water supply.

The work at Lake Guntersville will be providing economic information on recreation and land use values. The methods used at

Lake Guntersville can be readily applied to other systems to develop benefit estimates. The other benefit categories, e.g., flood control, are being addressed under another work effort.

Recreation Benefits

The work at Lake Guntersville on recreation benefits utilized a contingent valuation survey to determine the willingness-to-pay of recreation users for different plant control alternatives. The contingent valuation method presents a number of different alternatives or scenarios to a respondent and then elicits willingness-to-pay values for the scenarios.

Alternatives

A series of five alternatives were presented to users in a mail survey showing different levels of plant management (Table 1). The alternatives presented ranged from basically minimal or no control of the plants (34,000 acres) to complete eradication or no plants (near 0 acres). The alternatives between the two extremes represented 30, 20, and 10 percent of the reservoir. Additionally the three mid-range alternatives correspond to plant levels of 3 recent years within the memory of most recreationists.

Table 1
Plant Control Alternatives—
Lake Guntersville

Alternative	Plant Coverage, acres	Year
Minimum control	34,000	
Alternative A	20,000	1988
Alternative B	14,200	1989
Alternative C	8,000	1990
Alternative D	Near "0"	

To graphically illustrate the alternatives to respondents, artist depictions were prepared of aquatic plant conditions at different types of recreation settings under the different alternatives. The recreation settings were as follows:

- Public boat launch.
- Public recreation area.
- Undeveloped shoreline.
- Marina.
- Developed shoreline.
- Middle of lake.

The artist depictions were prepared from aerial photography and showed the distributions of plants well below the surface of the lake, just below the surface, and topped-out plants. Figures 2 and 3 show the developed shorelines for Alternative B, 14,200 acres of plants and Alternative C, 8,000 acres.

Willingness-to-pay

With the series of six artist illustrations and alternative descriptions as information, respondents were asked how their behavior in number of recreation trips and willingness-to-pay would change in response to each of the scenarios. The user was asked the following question (using Alternative B), using values from $10 to $4,500:

> "Think for a moment about all the money you spent last year to use Lake Guntersville for outdoor recreation. If the total costs to you of using Lake Guntersville increased by $ ___ per year (from the total you spent last year), would you still use the lake if Management Alternative B (14,200 acres of aquatic plants) existed instead of the 'Minimum Control' alternative (34,000 acres of aquatic plants)?" (check one)
>
> ____ YES About how many trips would you take each year in this situation? (fill in blank) ____ TRIPS
> ____ NO

Benefit Calculation

The responses to the contingent valuation question yield measures of the willingness-to-pay values that are obtained from the different alternatives. To calculate benefits, it is necessary to determine the costs associated with use of Lake Guntersville. To do this, an

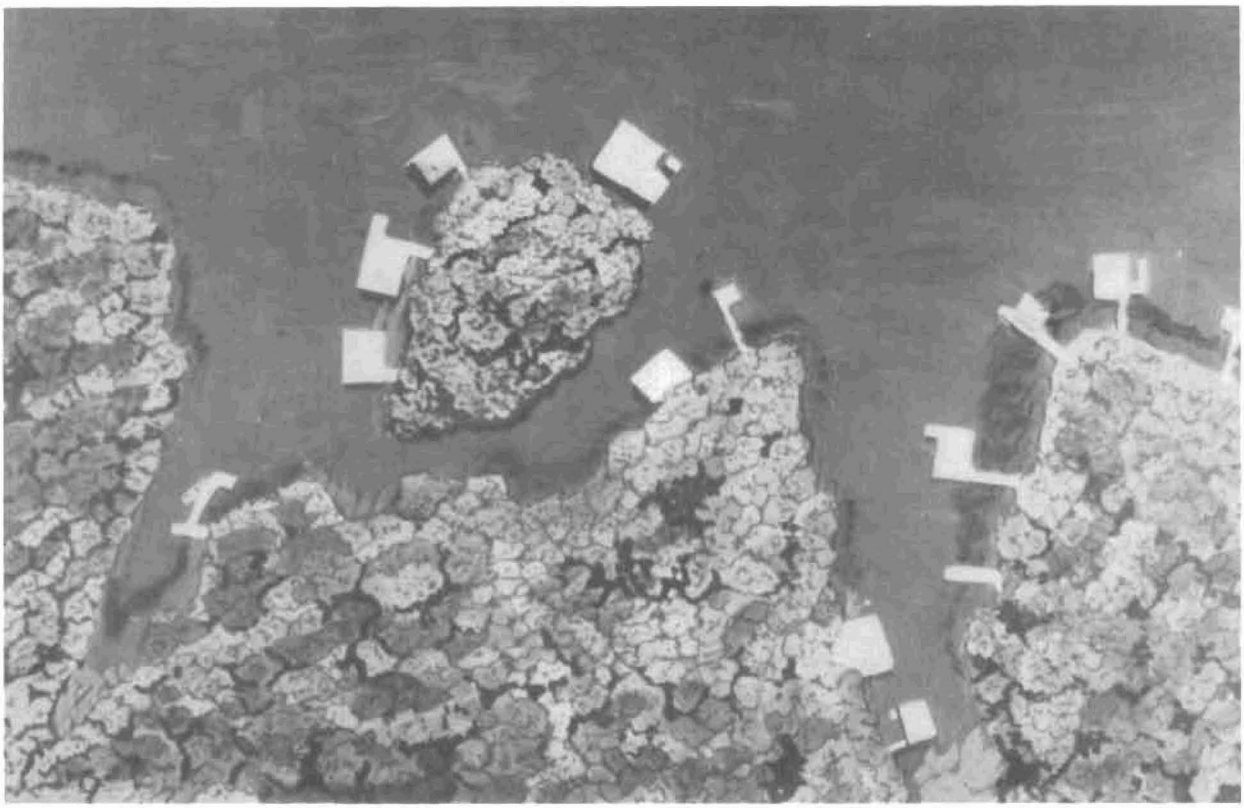

Figure 2. Lake Guntersville aquatic plant Alternative B, developed shoreline

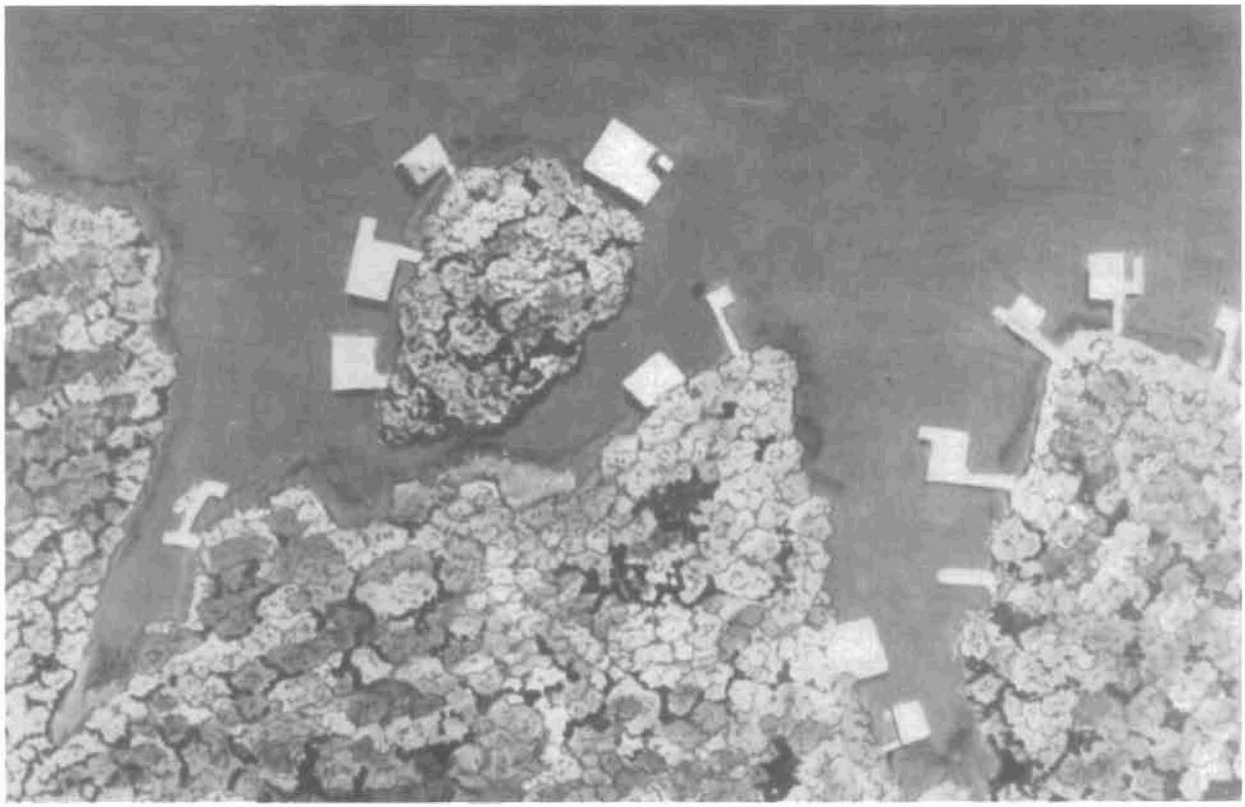

Figure 3. Lake Guntersville aquatic plant Alternative C, developed shoreline

expenditure survey was implemented along with the use survey. The results of the Guntersville expenditure survey are not yet available. However, the results of a recent nationwide Corps of Engineers expenditure survey collected expenditure data on the following:

Annual	Trip
Camping Equipment	Lodging
Boating Equipment	Food and Beverages
Fishing Equipment	Transportation
Vehicles	Entertainment
Hunting	Miscellaneous

The average per party trip expenditures for boaters was $248.30 and $159.24 per party for nonboaters. Thinking about calculating benefits from the willingness-to-pay values (benefits = willingness-to-pay costs (expenditures)), the difference in the costs for boaters and nonboaters will yield different magnitudes of benefits for the same willingness-to-pay values.

Public Preferences and Perceptions

Valuation of aquatic plant control by the public is determined by preferences, expectations, perceptions, and experiences. Since different recreation and other users can be affected differently by aquatic plants, it is important to identify the preferences and perceptions of major user groups. Rather than a single "public," there are actually multiple publics that are affected.

The existence of multiple user groups complicates decision making on plant control programs. Different groups may have conflicting preferences for plant levels or distributions. The value of plant control efforts also differs between user groups. Developing a plan for plant control requires accounting for how different groups are affected, considering preferences and valuations, and balancing the effects on the different groups.

At Lake Guntersville, a series of questions were asked to elicit perceptions and preferences regarding aquatic plants and plant control. The results presented here represent the preliminary, unweighted results of the onsite survey of approximately two thousand recreators. The survey results were analyzed in four user groups depending on whether the respondent boated and fished.

Respondents were asked to do the following: "In the area that you typically recreate in, describe the aquatic plant coverage." For the boat fishermen (FISH/BOAT), over 35 percent identified plant coverage as Moderate or Heavy (Figure 4). Approximately 65 percent of boat fishermen described plant coverage as Slight or Not Noticeable. Thirty-five percent of the bank fishermen (FISH/NONBOAT) identified coverage as Moderate or Heavy with over 60 percent responding as Slight or Not Noticeable. Thirty percent of the pleasure boaters, those boaters that are not fishers (NONFISH/BOAT), described plant coverage as Moderate or Heavy, and 35 percent said coverage was Slight.

To determine preferences for plant coverage, recreators were asked "What Amount of Plant Coverage Would You Like to See?" Sixty percent of the boat fishermen want More or As Much as Possible, and 35 percent are satisfied with current conditions (Same as Now) (Figure 5). The same proportion of bank fishermen prefers current conditions (35 percent), while approximately 27 percent want More or As Much as Possible. Of the pleasure boaters and skiers (NONFISH/BOAT), over 40 percent prefer fewer plants than current conditions, and a significant proportion, approximately 25 percent, want No Coverage. Of the day users, those recreators that neither boat or fish (NONFISH/NONBOAT), 30 percent prefer plant conditions that are less than the existing plant biomass and distributions.

To ascertain perceptions of the plant's effects on recreation, the question "How would you describe the aquatic plant impact on your main activity?" was asked. Close to 50 percent of the boat fishers said the plants were a Help (Figure 6). Of the bank fishers, over 50 percent responded that the plants had No Effect and approximately 20 percent said the plants

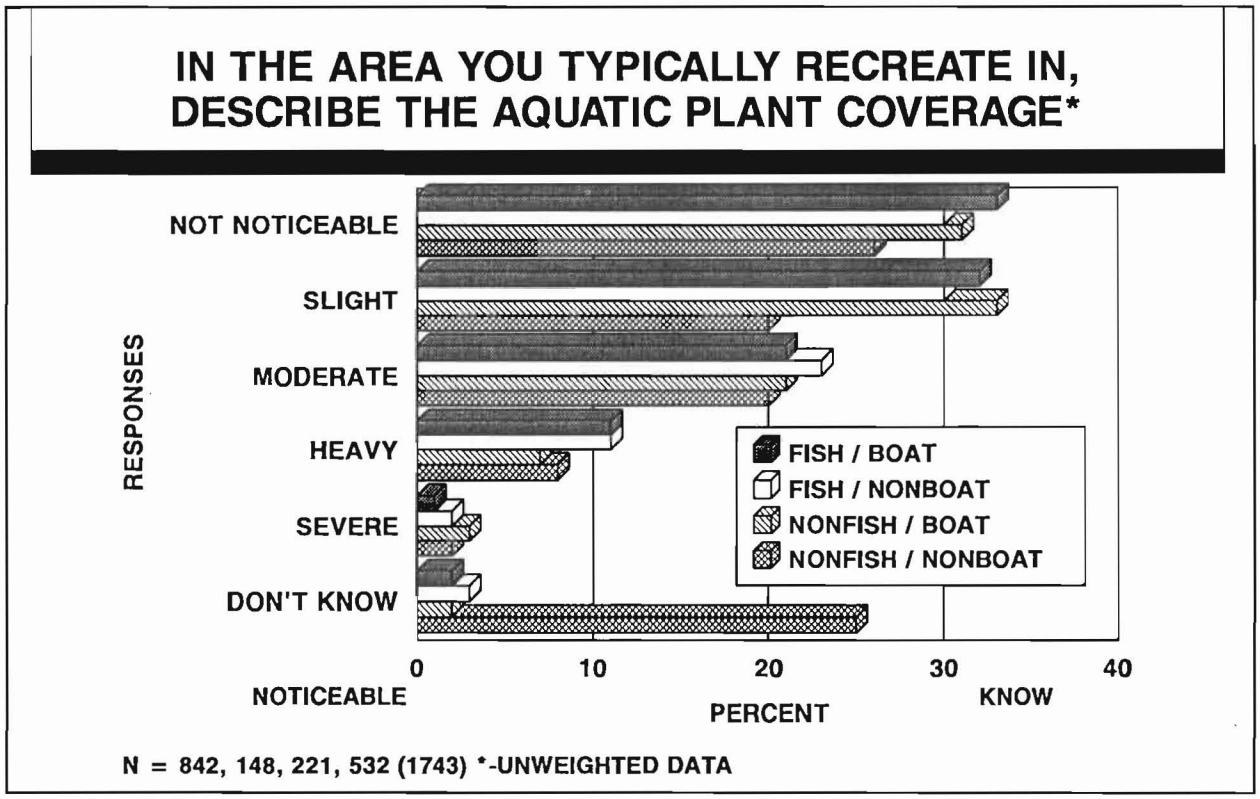

Figure 4. Perceptions of extent of aquatic plant coverage in Lake Guntersville

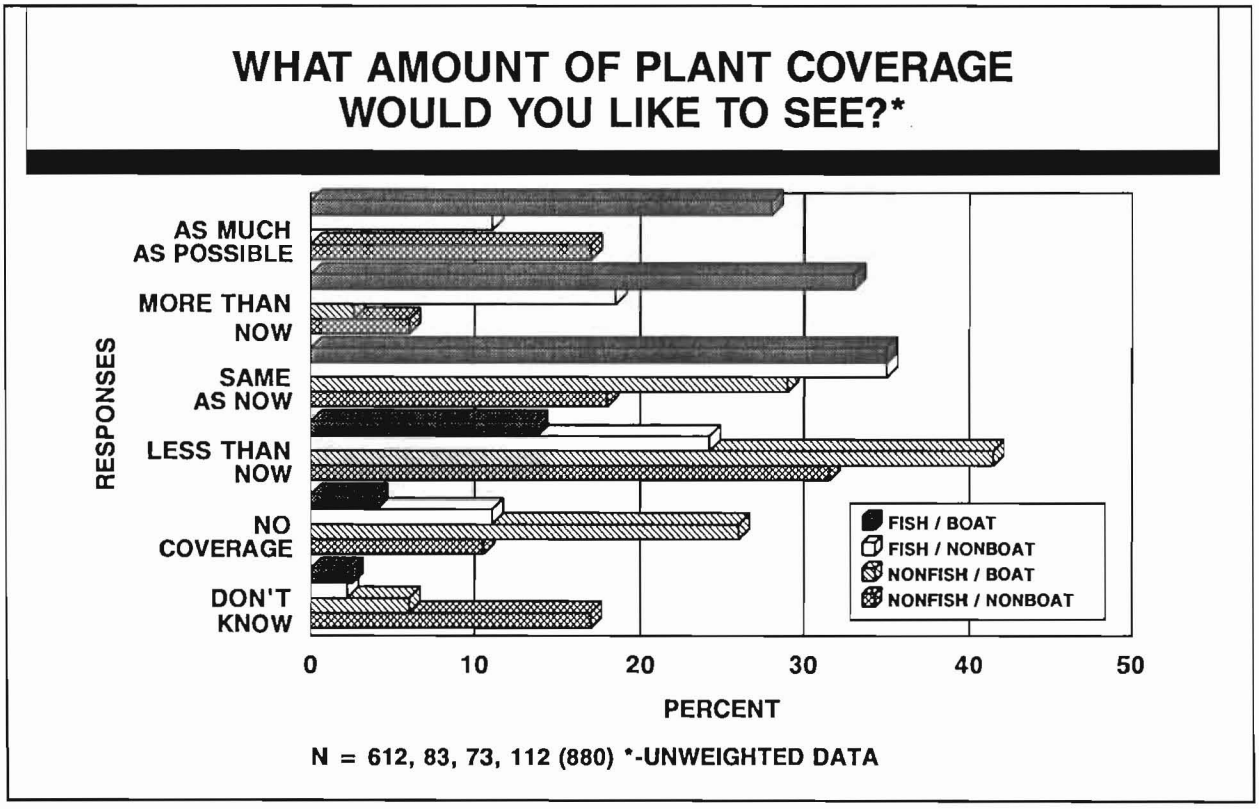

Figure 5. Preferred amounts of plants at Lake Guntersville

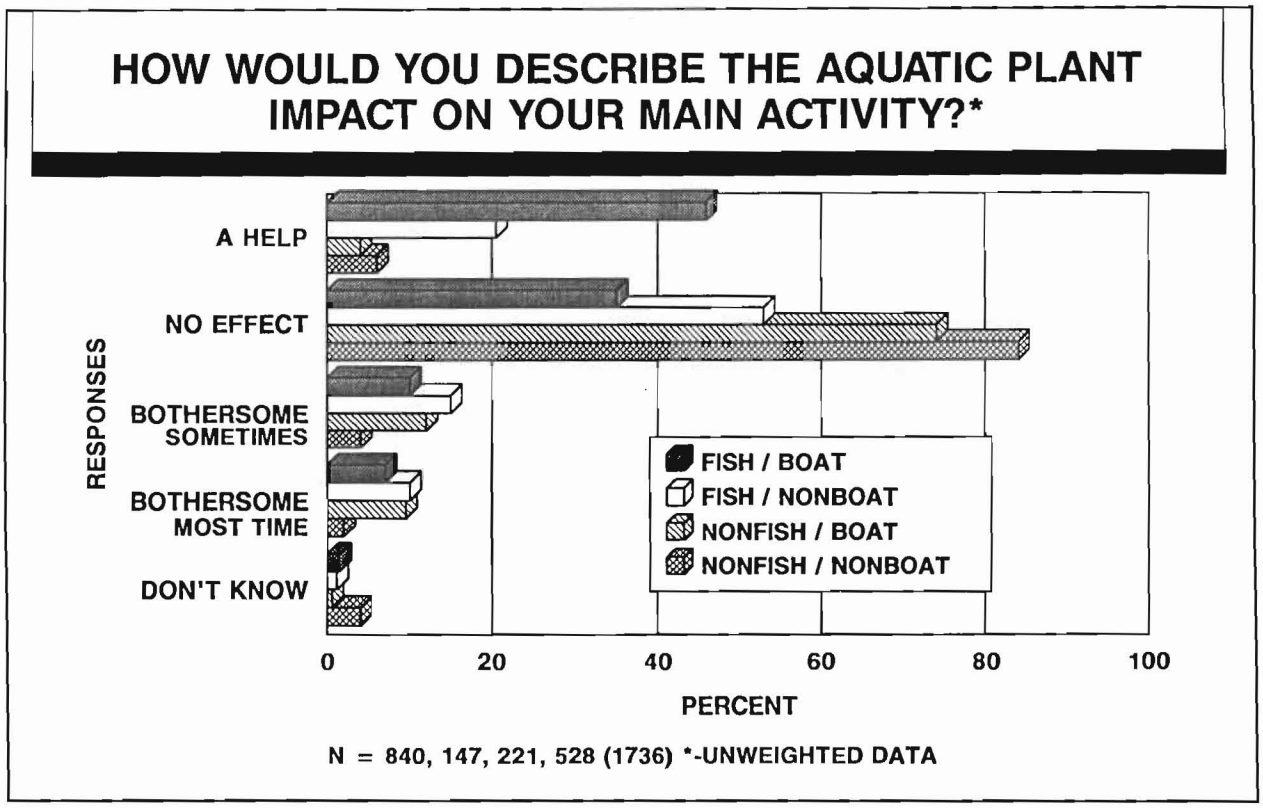

Figure 6. Perceptions of aquatic plant impact on recreation

were a Help. Over 75 percent of the skiers and pleasure boaters and 90 percent of the day users responded that the plants had No Effect.

The preference and perception data is valuable in evaluating how different groups may respond to control alternatives. Differences in group valuations must be considered along with preferences and perceptions in evaluating or comparing plans. To assist in accounting for different preferences and perceptions, an evaluation framework is being developed.

Summary

Economic information can be used to improve evaluation of aquatic plant control plans. The economic benefits and costs associated with plant control can be evaluated with existing economic methods. The evaluation of benefits and costs is limited by data gaps concerned with how aquatic plants affect the production of valued goods and services. Complicating the planning and evaluation of plant control programs is that user groups have differing perceptions of plant infestation levels and hold differing preferences for plant biomass levels and plant distributions. The valuation of plant control efforts also varies with user groups.

Work is being conducted to better value plant control efforts. Data collected at Lake Guntersville is providing information on the value to recreation of aquatic plant control and the preferences of different user groups. An evaluation framework is being developed to assist integration of different public preferences and diverse interests in a decision-making framework.

Guntersville Aquatic Plant Management Master Plan

by
Joe Morrison, Jr.[1]

Introduction

Lake Guntersville is a 75-mile-long reservoir, impounded and operated by the Tennessee Valley Authority (TVA), on the main stem of the Tennessee River in northeastern Alabama and southeastern Tennessee. The area is characterized by rugged topography, with the reservoir being located southeast of the Cumberland Plateau and northwest of Sand Mountain. Except for the towns of Guntersville and Scottsboro, the area is governed by a rural economy. Guntersville is a multipurpose project authorized for flood control, navigation, and power generation. Secondary benefits include public recreation, water supply, and fish and wildlife habitat.

Impounded in 1936, the reservoir contains a surface area of 67,900 acres at normal maximum pool elevation. The normal annual pool fluctuation, minimum to maximum, is approximately 2 ft.

History of Aquatic Plants at Guntersville

Historically, Guntersville has been more heavily colonized by aquatic plants than any other reservoir in the TVA system. Nearly two-thirds of the reservoir, or about 44,000 acres, is less than 18 ft deep and provides a potential habitat for aquatic plants.

Problems with submersed aquatic plants began in the 1950s with the introduction of the exotic Eurasian watermilfoil. Aquatic plants increased dramatically during the regional drought of 1984 to 1988. Coverage peaked at approximately 21,000 acres in 1988. The increase in Hydrilla coverage during this time was especially significant—from 75 acres in 1984 to 2,900 acres in 1988.

TVA has been actively involved in mosquito control and the management of aquatic plants at Guntersville since the reservoir was first impounded. The reservoir has been divided into 18 management units that TVA monitors and treats as necessary to manage aquatic plants. (Historically, treatment has been through the use of aquatic herbicides and reservoir drawdown.)

The management of aquatic plants in Guntersville Reservoir has become a very controversial issue over the years. Marina operators and recreational boaters favor management, complaining that aquatic plants restrict access, fowl boat props, interfere with waterskiing, etc. Likewise, homeowners exclaim that boathouse access is restricted and property values are reduced by unsightly aquatic plants. On the other side of the issue, it is widely accepted that aquatic plants do provide an excellent habitat for waterfowl and fisheries production. In fact, according to TVA studies, Guntersville is the most productive bass fishery in Alabama and one of the most noted bass fisheries in the United States. A 1991 Creel Survey revealed Largemouth Bass catch rates at Guntersville average from five and one-half to six fish/hour, while national catch rates for the same species average two and one-half fish/hour. Needless to say, TVA has had pressure from both sides; from the fishermen, to restrict aquatic plant management and from the marina and property owners, to increase management of aquatic plants.

[1] U.S. Army Engineer District, Nashville; Nashville, TN.

The Joint Agency Guntersville Project

Because of the strong interest from both sides regarding aquatic plant management at Guntersville Reservoir, Congressman Tom Bevill and former Congressman Ronnie Flippo were instrumental in obtaining congressional authorization and funding for the Joint Agency Guntersville Project (JAGP). This 5-year comprehensive project to study the management of aquatic plants in Guntersville Reservoir was authorized by Congress in 1989. This effort, led by TVA in cooperation with the U.S. Army Engineer Waterways Experiment Station and the Nashville District, has as its primary goal to test and demonstrate innovative aquatic plant management technologies. The Master Plan for Aquatic Plant Management is a key part of the JAGP delegated to the Nashville District for implementation.

Master Plan Concept

Our intent was to use the same Master Plan methodology the Corps typically employs for the management of recreational lands and apply it to the management of aquatic plants. The Nashville District, funded by TVA, contracted with the consulting firm of Harza Engineering Co. of Chicago, IL, to prepare the actual master plan document. Mr. David Pott served as Project Manager for Harza, and Mr. Leon Bates served as our point of contact with TVA.

The primary objective of the master plan was to describe long-range aquatic plant management strategies for the 18 management units within Guntersville Reservoir (where and how much to manage, as opposed to how—the how will be forthcoming from the other study units within the JAGP). The final plan attempts to balance the many competing demands placed on the reservoir. The needs and desires of the communities surrounding the reservoir, as well as users of the lake, were taken into account in the preparation of this master plan.

Specifically, the master plan describes existing operation, management, and public use of project lands, waters, resources, etc. This includes a database of project resources organized into a Geographic Information System (GIS) format.

Public Participation

Two public workshops were held in the project area in January of 1991 to obtain input on management strategies (where plants should be managed and how much area should be managed). Comments obtained at these meetings helped give direction to the master plan effort. Subsequently, a draft master plan was distributed to the public, and two additional meetings were held in May of 1992 to receive comments on the draft plan. Concurrent with public meetings, other state, local, and Federal agencies were invited to provide input and response to the master plan.

In addition, a technical advisory panel made up of representatives from academia, natural resource agencies, and the private sector was convened to provide additional guidance to the master planning team.

Applied Master Plan Methodology

Our first step was to evaluate the existing project resources/uses. Guntersville's natural resources make it one of the most popular areas in the region for water-oriented recreation. As previously mentioned, the Black Bass fishery brings this lake national recognition. Waterfowl production has increased as well, with the largest Gadwall harvests having been in the Jackson County area of the reservoir. The lake also supports several threatened and endangered species, including bald eagles and the Indiana and gray bats.

We also considered the socioeconomic impact of the project. Although the existing data is somewhat limited, clearly the reservoir and its natural resources have had a major impact on the local and regional economies. A separate component of the JAGP will be the study of this relationship in more detail (future data can be used in updating the master plan).

We also analyzed recreation use for the project. Besides fishing, the reservoir also provides for pleasure boating, sailing, swimming, waterskiing, and sightseeing. Recreational use is also a component of the JAGP presently being studied under a separate contract.

One of the key features of this master plan is the assembly of various base information into a GIS. Much of this base information was furnished by TVA, including the following: water uses, water supply points, specific recreation features (swimming beaches, launch ramps, etc.), shoreline data, and historic aquatic plant coverage. TVA's 18 existing management units established for aquatic plant control under TVA's annual aquatic plant control work program were also included in this database. Existing land uses mapped into the system include land use/land cover within one-quarter mile of shore. Land uses/cover types (for surrounding lands) was the dominant variable in determining suitability of the reservoir area for various uses.

Reservoir Use Suitability

One of the key elements of this master plan is the concept of reservoir use suitability. Obtained through application of the GIS and based largely upon surrounding land uses, we compiled maps that indicate the likely best uses for different portions of the lake. These reservoir use suitability maps ultimately became the basis for determining actual aquatic plant management zones and the intensity of management. It was not our intent to restrict usage on the lake or to dictate usage for any specific areas, but to define which uses were most appropriate for given uses, based on factors such as adjacent land use, existing aquatic plant coverage, depth of water, etc.

Aquatic Plant Management Maps

As a result of the reservoir use suitability evaluation, we were able to compile actual aquatic plant management maps covering the entire reservoir. In addition to highlighting TVA's existing treatment areas, these maps define "potential aquatic plant management areas" or areas that should be considered for future aquatic plant management. As a confirmation of this methodical process and TVA's work to date, these potential aquatic plant management areas were found to coincide with the existing treatment areas currently administered by TVA.

We also compiled National Environmental Policy Act documentation, in the form of an Environmental Assessment with accompanying Finding of No Significant Impact (FONSI) to cover the master plan action. (The FONSI ruling was due to the fact that the master plan and the resultant potential treatment areas did not differ significantly from TVA's existing treatment program.)

Conclusions

We confirmed that aquatic plants are considered to be an integral part of the ecosystem with demonstrated benefits for fish and wildlife production. We also realize that some uses cannot reasonably be accommodated without some effort being given to the management of aquatic plants.

The results of this master plan in the form of aquatic plant management potential treatment areas is not significantly different from TVA's current program of treatment, since both plans are based on adjacent shoreline land uses.

The master plan has been published and is available for distribution in three volumes (an Executive Summary, Public Participation Appendix, and Main Report) from TVA's Department of Aquatic Biology, located in Muscle Shoals, AL.

Recommendations

To keep the master plan a current, flexible, and dynamic document, it is recommended that it be reviewed and updated periodically as new information becomes available or as needs and uses change. Specifically, it is recommended that the master plan should be updated to include the results of the applicable JAGP studies, once all are completed.

Aquatic Macrophyte Assessment—Joint Agency Guntersville Project

by
David H. Webb[1]

Introduction

Guntersville Reservoir is a 27,500-ha impoundment of the Tennessee River in northeastern Alabama and southeastern Tennessee and is one of several impoundments operated by the Tennessee Valley Authority (TVA). Exotic submersed macrophytes such as Eurasian watermilfoil (*Myriophyllum spicatum*), spinyleaf naiad (*Najas minor*), hydrilla (*Hydrilla verticillata*) and native species such as southern naiad (*Najas guadalupensis*), coontail (*Ceratophyllum demersum*), American pondweed (*Potamogeton nodosus*), small pondweed (*Potamogeton pusillus*), muskgrass (*Chara zeylandica*), and other aquatic macrophytes have created reservoir-use conflicts requiring management in selected areas of the reservoir. The TVA uses drawdowns and herbicides to manage nuisance populations of aquatic macrophytes along developed shorelines, marinas, public-use sites, and commercial recreation areas (Burns, Bates, and Webb 1992).

In the late 1980s, aquatic macrophytes colonized about 8,200 ha or about 29 percent of the reservoir's surface area. Hydrilla, which was first discovered in Guntersville Reservoir in 1982, colonized about 1,160 ha and was considered a long-term threat to multiple uses of Guntersville Reservoir, as well as several other reservoirs within the TVA system.

As a result of public concerns, TVA and the U.S. Army Corps of Engineers were asked to develop a 5-year plan for reducing submersed aquatic plants in Guntersville Reservoir and to develop and demonstrate more efficient and effective methods of managing aquatic vegetation (Bates, Decell, and Swor 1991). The project, referred to as the Joint Agency Guntersville Project (JAGP), was initiated in 1990 and will continue through 1994.

One component of the JAGP is a large-scale demonstration with grass carp for hydrilla control and reduction in aquatic macrophyte populations. After preparation of an environmental assessment (TVA 1990), 100,000 triploid grass carp were released in Guntersville Reservoir from April to July 1990. A monitoring program began in 1990 to document changes in coverage and composition of aquatic macrophyte communities to provide an assessment of grass carp herbivory and to provide supportive data for other assessments and projects associated with the JAGP.

Materials and Methods

Quantitative sampling of aquatic macrophyte communities has been conducted since 1990 at several locations in Guntersville Reservoir from Tennessee River Mile (TRM) 356.0 to TRM 394.2. Aerial photography has been acquired annually since the late 1970s by TVA's aquatic plant management program and is used as supportive baseline data. Representative data from selected sampling sites (Figure 1) have been included in this report with a generic description of methods. A detailed discussion of methods and data will be included in interim and final project reports.

Coverage of aquatic macrophytes was determined from large-scale (1:7200), color aerial photography acquired annually during September or early October when biomass is typically at its peak. Aquatic macrophyte

[1] Tennessee Valley Authority, Muscle Shoals, AL.

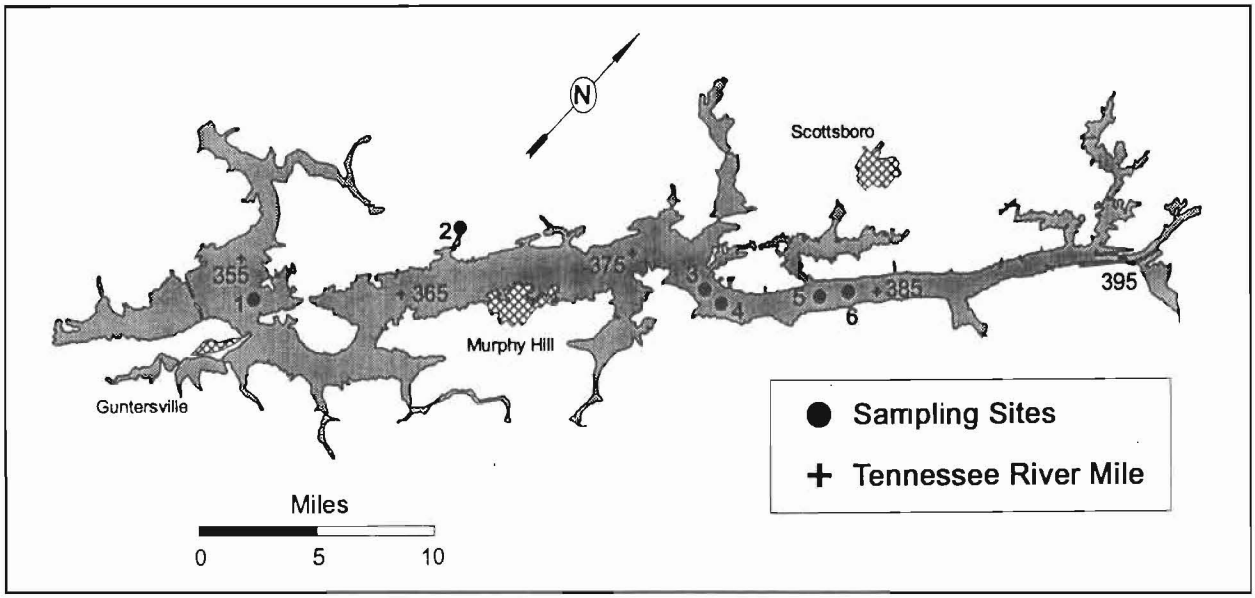

Figure 1. Location of aquatic macrophyte sampling sites on Guntersville Reservoir (1 = Conner's Islands, 2 = Mill Creek, 3 = Powerline Milfoil, 4 = Powerline Hydrilla, 5 = Chisenhall Exclosure, 6 = Brewster Hydrilla)

colonies were delineated on mylar overlays attached to the photographic prints and labeled by species. Area of delineated colonies was obtained using an electronic planimeter.

Aboveground biomass was collected using an open-ended plexiglass box sampler with a sampling area of 0.25 m^2, a hydraulically operated aquatic plant sampler developed for the U.S. Army Corps of Engineers Aquatic Plant Control Research Program (Sabol 1984) having a sampling area of 0.39 m^2, or with a mechanical harvester with a cutting head width of 1.6 m. The box sampler was used in shallow water sites that were generally less than 1 m deep. The hydraulically operated sampler and the mechanical harvester were used to sample deeper water sites (1 to 4 m). Wet weights of plant samples were obtained after spinning the samples in a washing machine for 6 min.

The aquatic plant community at many of the shallow water sites frequently was composed of a mixture of species such as spinyleaf naiad, southern naiad, muskgrass, small pondweed, and horned pondweed (*Zannichellia palustris*). Because of the excessive amount of time required to separate individual species, wet weights for this group of plants were determined collectively for each sample. They have been labeled annuals for purposes of this report although species such as horned pondweed may be a perennial plant. Species such as Eurasian watermilfoil, hydrilla, American pondweed, and coontail were individually separated and weighed, but their wet weights frequently were summed and collectively referred to as perennials.

Exclosures were constructed in the late winter or early spring of 1992 from wire or block nets having openings or mesh size small enough to exclude grass carp.

Results

Macrophyte coverage

Coverage of submersed aquatic macrophytes on Guntersville Reservoir declined from about 7,800 ha in 1988 to about 2,000 ha in 1991 (Figure 2). Several species such as spinyleaf naiad, southern naiad, small pondweed, muskgrass, and hydrilla, which formerly were widespread and abundant, occurred only in small colonies at scattered localities within the reservoir in 1991. Eurasian watermilfoil coverage

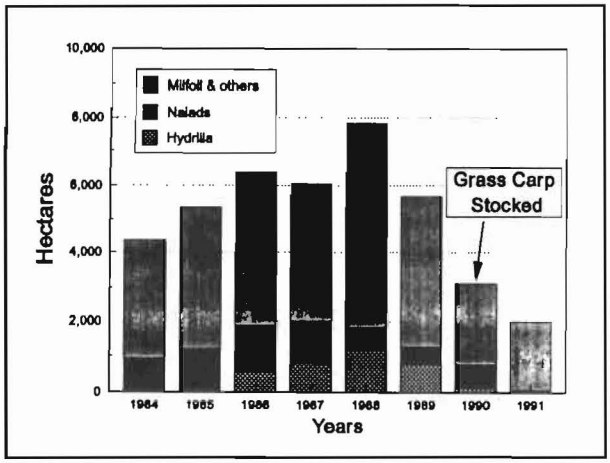

Figure 2. Submersed aquatic macrophyte coverage determined from aerial photography on Guntersville Reservoir from 1984 to 1991

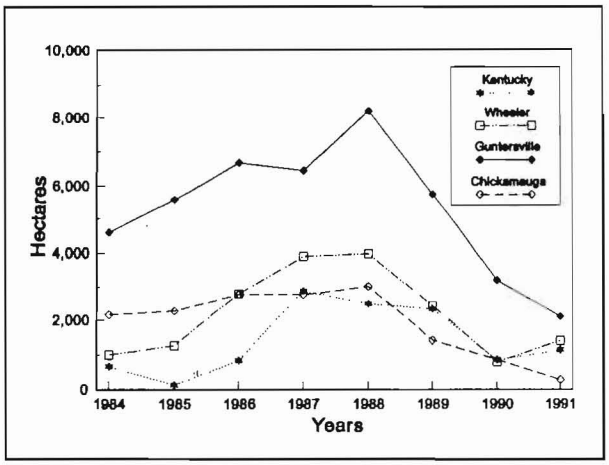

Figure 3. Aquatic macrophyte coverage determined from aerial photography on Kentucky, Wheeler, Guntersville, and Chickamauga Reservoirs from 1984 to 1991

in 1991 was about 95 percent of the 2,000 ha of submersed vegetation compared with about 80 percent of the approximate 5,700 ha of submersed macrophytes in 1989. From 1990 to 1991, several large colonies of Eurasian watermilfoil "disappeared" from the portion of the reservoir downstream of TRM 373. Hydrilla declined from about 1,160 ha in 1988 to about 750 ha in 1989 to about 120 ha in 1990. The 120 ha of hydrilla in Guntersville Reservoir in 1990 was primarily in overbank habitats along the old river channel from TRM 378 to TRM 384. The only hydrilla colonies visible at the surface in Guntersville Reservoir in 1991 and 1992 were about 1 ha in coverage and occurred as a narrow band in the vicinity of TRM 390.5.

A decline in aquatic macrophyte coverage comparable with Guntersville Reservoir occurred in other mainstream TVA reservoirs such as Kentucky, Wheeler, and Chickamauga Reservoirs from 1988 to 1990 (Figure 3). The increase in macrophyte coverage from 1984 to 1988 was probably related to optimum growth conditions (low flow, clear water) associated with record drought years, while the decline since 1988 was caused largely by higher flows and increased turbidities.

Biomass

A comparison of biomass from 1990 to 1992 of various macrophyte species from shallow water sampling sites showed a loss or significant decline in spinyleaf naiad, southern naiad, muskgrass, and small pondweed, which typically dominate the annual aquatic plant community. This occurred not only in Mill Creek embayment (Figure 4), but at other sampling sites in North Sauty Creek and Mud Creek embayments. Field observations from other portions of Guntersville Reservoir during 1990 to 1992 indicated the decline of these species to be reservoir wide. An exception was shallow water habitats that were only a few centimeters deep and small coves that had beaver dams or other physical barriers to fish movement.

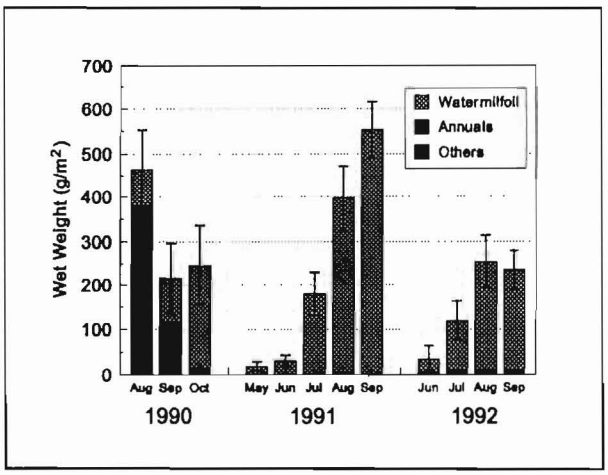

Figure 4. Mean aboveground biomass with 95-percent confidence limits of aquatic macrophytes at the Mill Creek sampling site from 1990 to 1992

Sampling sites in deeper water (1 to 2.5 m) from TRM 378.5 to TRM 384 that were colonized by near monospecific colonies of Eurasian watermilfoil in 1990 (Powerline Milfoil site) remained relatively stable (Figure 5). In

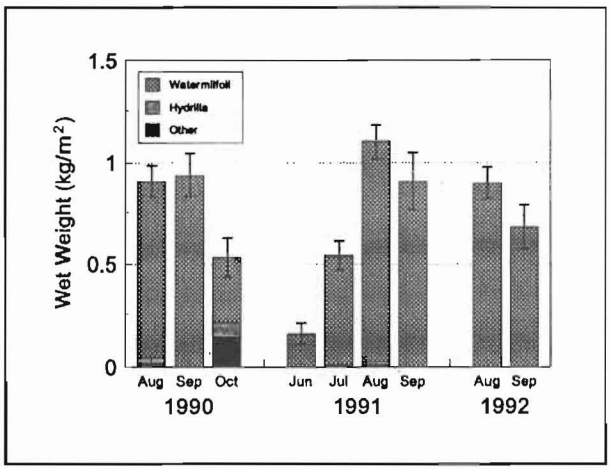

Figure 5. Mean aboveground biomass with 95-percent confidence limits of aquatic macrophytes at the Powerline Milfoil sampling site from 1990 to 1992

contrast, sampling sites dominated by hydrilla in 1990 (Powerline Hydrilla site) had significant declines in this species (Figure 6). Eurasian watermilfoil became the dominant submersed macrophyte at these sites and was present in nearly monospecific stands by the end of the 1991 growing season. At the two sampling sites with hydrilla in 1990, peak biomass declined from 3.566 kg/m^2 at the Powerline Hydrilla site to 1.263 and 0.904 kg/m^2 in 1991 and 1992, and from 4.307 kg/m^2 at the Brewster Hydrilla site to 1.020 and 0.885 kg/m^2 in 1991 and 1992 when Eurasian watermilfoil became the dominant macrophyte. While hydrilla almost totally declined in overbank areas from TRM 378 to TRM 384 from 1990 to 1991, hydrilla was present in 1991 in 1,000-m^2 exclosures constructed for studies with *Hydrellia pakistanae* (Grodowitz and Snoddy 1992) that were in the same area.

Exclosures

With a few exceptions, there were significant differences in biomass and/or species composition of aquatic macrophytes within the exclosures compared with contiguous areas with similar habitat. Biomass within the exclosure at Conner's Islands in 1992 was about 775 g/m^2 at peak biomass compared with less than 20 g/m^2 outside the exclosure (Figure 7). Horned pondweed, southern naiad, and small pondweed were the dominant species in the annual group, and Eurasian watermilfoil was the most abundant perennial within the exclosure. The Conner's Islands site was located in the downstream portion of Guntersville that had only minimal amounts of submersed macrophytes.

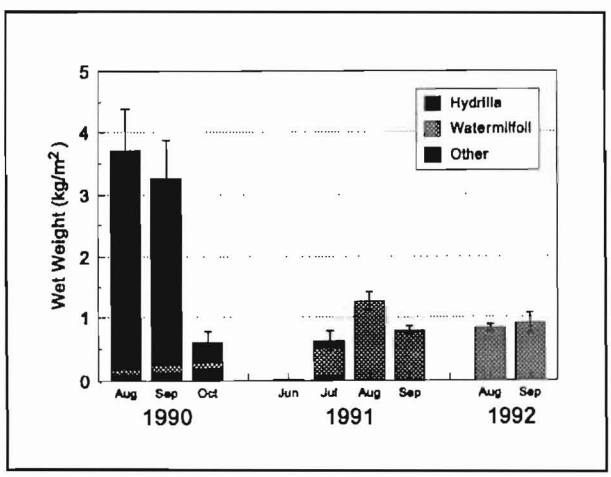

Figure 6. Mean aboveground biomass with 95-percent confidence limits of aquatic macrophytes at the Powerline Hydrilla sampling site from 1990 to 1992

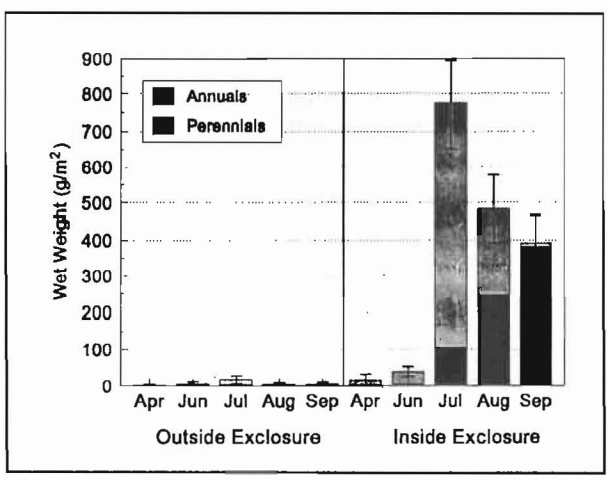

Figure 7. Mean aboveground biomass with 95-percent confidence limits inside and outside of exclosure at the Conner's Islands sampling site in 1992

At an upstream site (Chisenhall Exclosure) where Eurasian watermilfoil was abundant and widespread, total biomass of aquatic macrophytes was comparable inside and outside the net exclosure (Figure 8). Spinyleaf naiad, an annual species, was 15 to 33 percent of the total biomass during August and September within the exclosure, but was less than 4 percent of the total biomass in a contiguous area outside the exclosure having similar depth and substrate characteristics. Eurasian watermilfoil was more than 95 percent of the total biomass outside the exclosure during peak months.

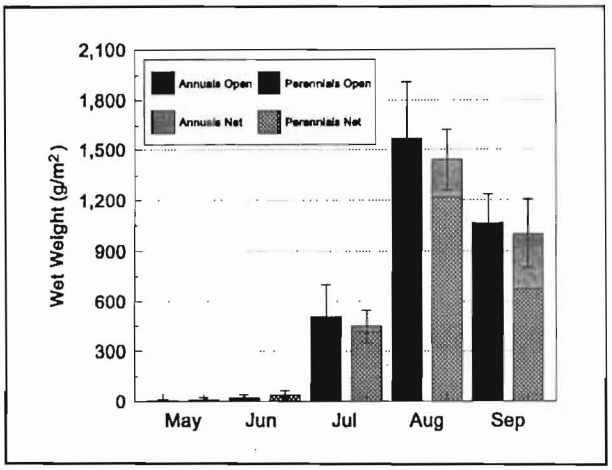

Figure 8. Mean aboveground biomass with 95-percent confidence limits inside and outside of net exclosure at the Chisenhall Exclosure sampling site in 1992

Discussion

Submersed macrophyte coverage on Guntersville Reservoir declined from about 5,700 ha in 1989 (the year prior to grass carp stocking) to about 2,000 ha in 1991. A large percentage of the decline probably was due to factors other than grass carp. Similar declines occurred on other mainstream reservoirs within the TVA system during the same period. Peak coverage occurred in 1988 at the end of record drought years. Factors associated with drought conditions such as clear water, reduced flows, and elevated water temperatures are conducive to rapid macrophyte growth and spread (Smith and Barko 1990). The decline since 1988 has been concomitant with higher flows during 1989 to 1991.

Grass carp are believed to have been a major factor in the reduction of 120 ha of hydrilla in the overbank from TRM 378 to TRM 384 from 1990 to 1991. Large numbers of grass carp were observed feeding in "topped out" hydrilla colonies in the late summer and early fall of 1990. The presence of hydrilla during the early summer of 1991 (Grodowitz and Snoddy 1992) in small exclosures constructed for *H. pakistanae* biocontrol studies also strongly supports this conclusion.

Although other herbivores such as turtles may have been a factor in some areas, the reservoir-wide decline of spinyleaf naiad, southern naiad, muskgrass, and small pondweed from 1990 to 1991 is believed to primarily have been a result of feeding by grass carp. These species, in addition to hydrilla, rank high on the grass carp's food preference list (Miller and Decell 1984; Leslie et al. 1987). Grass carp are known to selectively feed on preferred food plants before consuming less desirable species such as Eurasian watermilfoil. The abundance of spinyleaf naiad and several other macrophytes within exclosures constructed in 1992 on Guntersville Reservoir supports selective feeding behavior by grass carp.

Even though Eurasian watermilfoil is not a preferred species of grass carp (Miller and Decell 1984; Leslie et al. 1987), grass carp will consume it when more preferred species are unavailable. This may account for the absence of Eurasian watermilfoil outside the exclosure at the Conner's Islands site, which is in the downstream portion of the reservoir where submersed macrophytes are in small scattered colonies.

The Valley-wide decline in aquatic macrophytes within the TVA system during the past few years has made it difficult to determine the amount of decline that is attributable to grass carp and that resulting from other factors. Perhaps some insight can be derived from long-term monitoring that compares macrophyte

coverage and community composition on Guntersville with other TVA reservoirs without large grass carp populations.

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Synopsis of the District/Division Aquatic Plant Management Operations Working Session

by
William C. Zattau[1]

The sixth annual Working Session was held 17 November 1992 during the Aquatic Plant Control Research Program Review. Representatives from Headquarters, 2 Division Offices, 17 District and Project Offices, and the U.S. Army Engineer Waterways Experiment Station attended, as did other Federal, state, local, university, and industry representatives. A total of 46 people participated.

Topics discussed included the status of herbicide registration and re-registration; updates on 11 cost-shared District Aquatic Plant Control (APC) programs and three O&M programs; and Division and Headquarters updates.

Re-registration updates were provided for copper, dichlorobenil, diquat, endothal, fluridone, glyphosate, and 2,4-D, as was information on Dupont's decision to drop registration efforts for Mariner.

District personnel discussed the large-scale grass carp releases at Lake Marion, SC, and Lake Istokpoga, FL, and ongoing active dispersal efforts of insect biocontrol agents in Galveston, Mobile, and Jacksonville Districts by Corps and local cooperator personnel.

Reports were given on the APC Program Evaluation Document (PEG), the summer meeting of the Field Review Group (FRG), and the fiscal year (FY) 92 District/Division operations survey. The PEG, which has been reviewed Corps-wide, is expected to be ready for use in FY93. The FRG met this past summer to review several proposed research units designed to investigate relationships between fish, aquatic vegetation and aquatic plant management activities. Questionnaires for completing the District/Division FY92 APC surveys are expected to be mailed out during the second quarter FY93.

Prospects of using remote sensing technology were discussed. The main concerns expressed regarded standardization, compatibility, and cost. Participants agreed that this technology appears to be applicable to a number of operational activities besides aquatic plant control.

Other discussion included public education activities and the usual public perception that APC operations are "environmentally unsafe." Efforts should be initiated at the project and District levels to develop effective public education efforts in this area.

The situation in regard to new start programs, the status of local cooperative agreements, and the revision of Engineer Regulation 1130-2-412 was discussed.

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Simulation Technology

An Overview of Simulation Technology Development

by
R. Michael Stewart[1]

Introduction

This paper provides an overview of current work within each of the four Simulation Technology Development work units. The four work units are Plant Growth Models (WU# 32440), Biological Control Simulations (WU# 32438), Chemical Control Simulations (WU# 32439), and Aquatic Plant Databases (WU# 32505).

Goal of Simulation Technology

The long-term goal of Simulation Technology in the Aquatic Plant Control Research Program is technology transfer to Corps of Engineers (CE) operations elements. This technology transfer is facilitated through development and distribution of personal computer-based, user friendly software packages that allow systematic evaluation of how selected aquatic macrophytes and control agents/techniques will interact under site-specific environmental conditions. Through providing information that supports a better understanding of how environmental conditions influence these interactions, these simulation procedures should help aquatic plant managers make more informed and sound decisions related to design and implementation of operational aquatic plant control efforts.

Stewart (1992) describes the progression of steps in simulation development that are required before the technology transfer goal is achieved. These steps or work activities can be grouped into two categories: (a) synthesis of information and (b) testing and evaluation. Synthesis of information activities include literature reviews, development of a conceptual framework for the simulation, and development of a first-generation simulation procedure. Testing and evaluation activities determine the accuracy of the simulation procedure and, consequently, identify processes that are improperly represented in the simulation and require further research.

Development activities currently in progress in each of the four Simulation Technology work units are briefly described herein. A more detailed summary of our current research is provided in the following papers included in this section of these proceedings.

Overview of Current Research Activities

Plant growth simulations

Plant growth simulation procedures are being developed to evaluate how growth of nuisance plant infestations will respond under different site conditions with regard to generalized environmental parameters. Simulations will be available for waterhyacinth, Eurasian watermilfoil, and hydrilla, the three exotic aquatic macrophytes that most often reach nuisance levels in CE-managed water bodies. These plant growth simulations will provide information useful for answering "What if?" questions such as "Considering milfoil has established itself in this area of the reservoir, what seasonal growth pattern can I expect to occur under this set of environmental conditions?" Environmental conditions that can be considered for a simulation are beginning plant biomass, photoperiod, temperature, solar irradiance, secchi depth, and water depth.

First-generation capabilities have been developed for each of the three targeted nuisance aquatic plant species. The waterhyacinth plant growth simulation has been incorporated into

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the INSECT biocontrol simulation procedure (Akbay, Wooten, and Howell 1989). An operation guide for this simulation procedure is provided in Stewart and Boyd (1992). Development activities for simulation procedures of milfoil and hydrilla growth were summarized in Wooten and Stewart (1991). Current work on these two simulations is focused on improving relationships for plant regrowth and survivability under light-limiting conditions (Stewart and Monteleone 1993).

Biological control simulations

The objective of this task area is to develop empirically derived simulation procedures of aquatic plant biocontrol techniques that utilize introduced insect and herbivorous fish species. The simulations consider the effects of site conditions, including relevant descriptions of the target plant infestation, on the behavior and "daily-based" population dynamics of biocontrol agents. For biocontrol agents for which there are required, empirically derived relationships, estimates of the daily level of herbivory exerted on the target plant infestation under consideration are generated.

During fiscal year (FY) 92, an Instruction Manual (Stewart and Boyd 1992) for the *Neochetina* and waterhyacinth biocontrol system simulation procedure (INSECT Version 1.0) was completed. This will allow release of this software package and user documentation during FY93. Additionally, a temperature-driven, populations dynamics model has been completed for *Hydrellia pakistanae*, an exotic fly species introduced for biocontrol of hydrilla (Boyd and Stewart 1993). Current work has also led to a revision of the U.S. Army Engineer Waterways Experiment Station Grass Carp Stocking Rate Model, originally described by Miller and Decell (1984). The updated version, most recently discussed by Boyd and Stewart (1992), is currently being used to evaluate triploid grass carp stockings for aquatic macrophyte control in Guntersville Reservoir, AL, and Lake Marion, SC. An instruction manual for the updated software package (AMUR/STOCK Version 1.5) is in preparation.

Chemical control simulations

Research in this task area will lead to the development of process driven, "mass-balanced" fate and effects simulation procedures for the active ingredient fractions of selected herbicide formulations used operationally in aquatic plant management (Stewart 1992). The fate portions of the simulations, through consideration of the combined effects of site conditions and herbicide formulation properties, will estimate active ingredient concentrations through time within water and target plant tissue partitions. "Exposure-based" and "dose-based" target plant mortality relationships will be developed for these two separate partitions for each combination of target plant and active ingredient.

Current work (Stewart 1993) is focusing on determination of "dose-based" (i.e., tissue load) mortality relationships to complement "exposure-based" (i.e., water concentration) mortality relationships being developed under ongoing Chemical Control Technology work units. Additionally, state-of-the-art hydraulics-based modeling techniques will be evaluated during FY93 to determine their applicability in simulating water flow through submersed vegetation.

Aquatic plant databases

Digital database products are being developed to support execution and testing of simulation procedures developed under this technology area. A large portion of this work effort involves compiling climatic data needed for initializing the software packages for different geographic regions of the United States (Kress and Holt 1993). Additionally, Kress and Causey (1992) have demonstrated procedures for utilizing Geographic Information Systems techniques to aid in visualizing simulation outputs on a spatial scale for improved interpretation.

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Investigation of Submersed Plant Regrowth under Low Light Levels

by
R. M. Stewart[1] *and S. A. Monteleone*[2]

Introduction

Background

Planning efficient and effective operational management plans for aquatic macrophytes is difficult partly because of the extreme variability in both spatial and temporal patterns of plant growth within a given water body over successive years. This variability in plant growth occurs in both well-established and newly colonized plant infestations and is in large part due to changes in environmental conditions within the infested water body.

Because environmental variables (e.g., temperature and light levels) that determine levels of aquatic macrophyte growth are extremely variable from year to year, aquatic plant managers need the capability of evaluating how aquatic plants will grow under different sets of these environmental conditions. This capability will help them determine priority treatment areas within their water bodies before the infestations reach nuisance levels. Without such a capability, treatment strategies are based on historical occurrences of weed infestations or are not designed until after nuisance levels are attained each year. Further, proper pretreatment evaluation of the potential effects of applying long-term control techniques, such as the stocking of herbivorous fish, should include consideration of the natural annual variability in target plant regrowth success, which can signficantly alter plant distributions and areal coverage.

Overview of submersed plant models

Recently developed first-generation simulation procedures for Eurasian watermilfoil and hydrilla growth have been described by Wooten and Stewart (1991). These simulations are being developed to satisfy the information needs discussed above by providing daily estimates of plant biomass for a single growing season. The simulation considers site conditions by using daily values for temperature, solar radiation, water turbidity (sechhi depth), and photoperiod and is responsive to initial conditions of water depth and plant biomass. The user is allowed to initialize the model for these site conditions during execution through a series of screen prompts.

Testing of the MILFOIL and HYDRILLA models has included comparison of simulation outputs for plant biomass with estimates derived from field measurements made at Guntersville Reservoir, AL, during 1990 through 1992. These comparisons tests indicate that the MILFOIL model provides realistic outputs of plant biomass estimates for milfoil infestations growing in water depths less than 2.0 m. MILFOIL simulations for water depths greater than 2.0 m, however, predict spring regrowth rates and maximum plant biomass levels much higher than actually observed in Guntersville Reservoir.

Subsequent scrutiny of the MILFOIL and HYDRILLA simulation procedures led to the

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recommendation that plant regrowth should be investigated under control conditions at light levels below 10 percent full sun. This lower light level range corresponds with light measurements in Guntersville Reservoir at water depths of 2.0 m or greater. The remainder of this paper summarizes a preliminary investigation of hydrilla regrowth under such low light levels.

Objectives and scope

The objectives of this study were to measure regrowth responses of different plant growth structures of hydrilla under light levels approximating 10, 5, 1, and 0 percent full sunlight. Growth structures tested were intact root crowns and apical and subapical stem sections from cultured hydrilla shoots.

The Methods section of this report includes brief descriptions of test facilities, preparatory test procedures, and experimental design. The results section is limited to a preliminary presentation for root crown treatments only. Summarization of results for apical tips and subapical stem sections had not been completed at the time this paper was written.

Methods

Test facilities

Experiments were conducted in a greenhouse facility at the Lewisville Aquatic Ecosystem Research Facility (LAERF) within 1,200-L fiberglass tanks. Tank water was maintained at 30 °C, and each tank was covered with a combination of commercial shade fabrics to attain one of the desired light levels (i.e., 10, 5, 1, or 0 percent full sunlight). Profiles of actual light levels achieved through this setup were measured with LICOR quantum sensors and are illustrated in Figure 1. As shown in Figure 1a, ambient light levels in

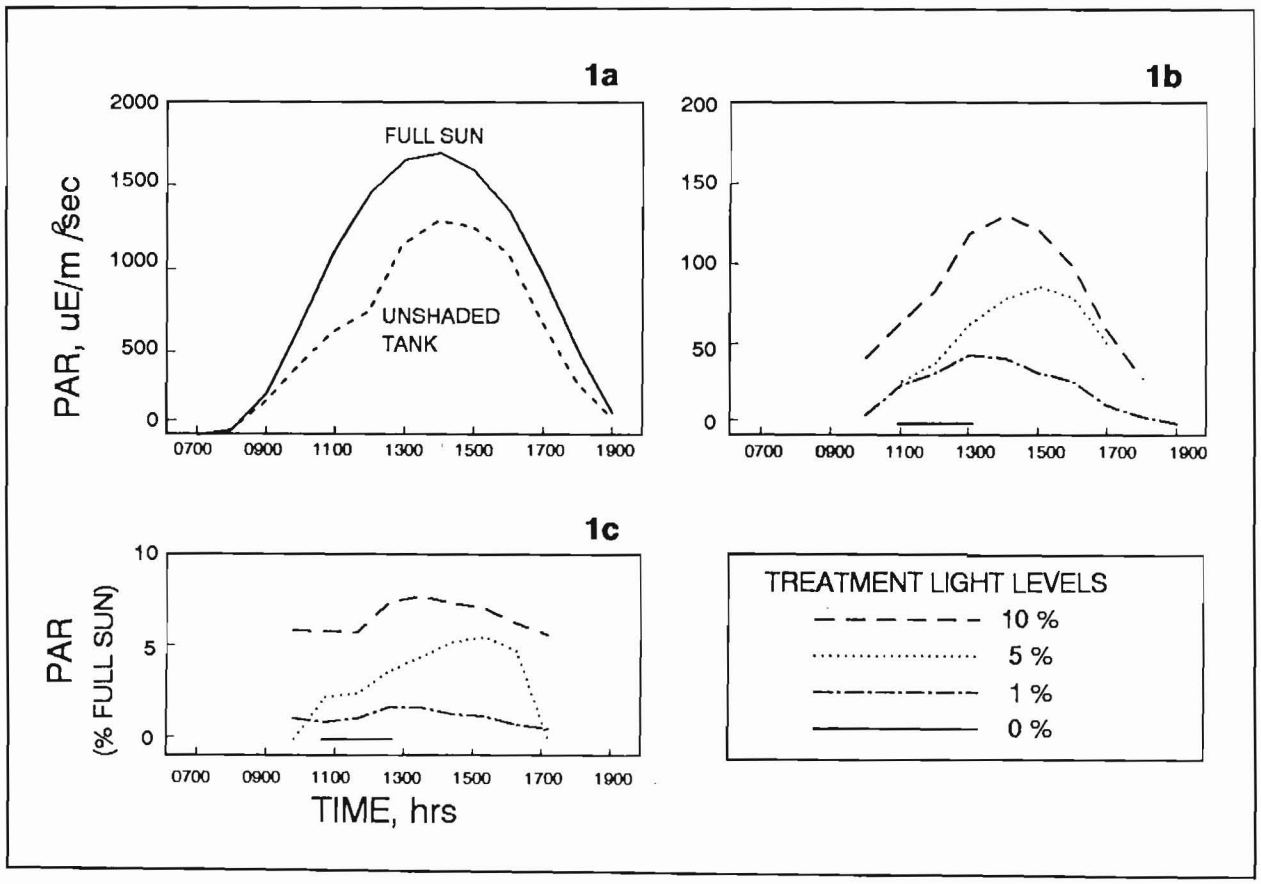

Figure 1. Profiles of ambient and experimental light conditions typical of those measured during this study

the greenhouse (i.e., unshaded tank) during afternoon hours were approximately 25 percent lower than light levels outside the greenhouse (i.e., full sun). Typical daily light profiles attained by the use of different selections of shade fabrics for each treatment tank are illustrated in Figure 1b. Figure 1c shows the percent of full sunlight that was measured within treatment tanks on a typical day during the study.

Preparation of test plant material

Hydrilla plant material used in this study was cultured in a flowing water raceway at the LAERF. Apical shoots were planted individually in 0.82-L pots, placed in a flowing water raceway, and allowed to grow for 8 weeks. After that time, pots were removed from the raceway culture containers, and representative plant tissues for the three test plant structures (i.e., root crowns, apical tips, and subapical stems) were prepared as described below.

Root crowns. All shoot material higher than 1.0 cm above the sediment layer in each culture pot was removed. Remaining intact root crowns were used for the root crown experiments.

Apical tips. Apical tip sections were obtained from the shoot material removed from the root crown containers. Apical tip sections were trimmed to approximately 10-cm length, and all subapical meristems occurring along this length were removed. Apical stem sections were individually replanted within a 0.82-L pot, with only the top 4 cm remaining above the substrate. After planting, granular silicon was layered over the sediments to prevent nutrient leaching and sediment resuspension into the tank water during the study.

Subapical stems. Subapical stem sections were also obtained from shoot material removed from root crowns. Individual subapical stem sections were measured to 20 cm and and laid on top of pond sediment within a 0.82-L pot. The sediment surface and stem section within each pot was covered with granular silicon to prevent nutrient leaching and sediment resuspension into the tank water.

Experimental design

The experimental design consisted of seven replications for each combination of initial plant growth structure, experimental light level, and sampling time shown in Table 1. Plant growth parameters measured at each sampling time were selected to provide an estimate of (a) dominant shoot growth in each treatment and (b) overall plant growth in each treatment. Dominant shoot growth measurements included length, number of meristems, and dry weight mass. From these values, estimates of mass per unit length were calculated for the dominant shoot in each test pot. Overall plant growth measurements included total aboveground mass, total belowground mass, and number of stems. Aboveground and belowground mass were summed for an estimate of total dry weight mass.

Table 1
Test Treatments for *Hydrilla verticillata* Regrowth Study

Treatments	Root Crowns	Apical Tips	Sub-apical Stems
Target light levels, %	10, 5, 1, 0	5, 0	5, 0
Sampling time, weeks	2, 5, 9	5, 9	5, 9

Preliminary Results: Root Crowns

Dominant shoot growth

Measurements of dominant shoot regrowth from hydrilla root crowns under the four different light levels are graphically summarized in Figure 2. These data show a noticeable reduction in dominant shoot regrowth arising from hydrilla root crowns at light levels below 5 percent full sunlight.

Measurements of dominant shoot length (Figure 2a) show similarity in maximum elongation for 5- and 10-percent light treatments. Attainment of maximum elongation, however,

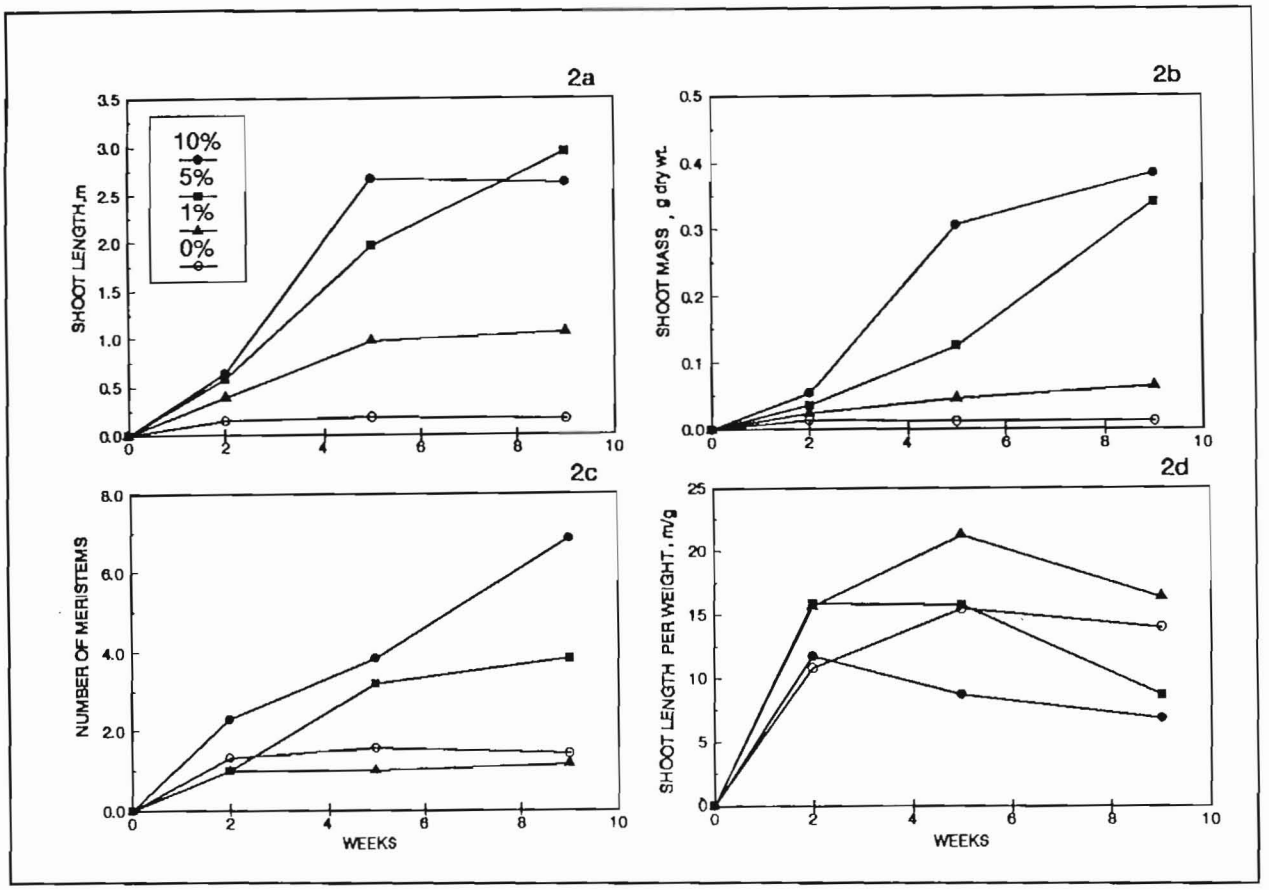

Figure 2. Measurements of dominant shoot regrowth from root crowns at Weeks 2, 5, and 9. Point values represent means of seven replicate samples

was delayed until after Week 5 in the 5-percent treatment. In comparison, elongation was greatly reduced in the 1- and 0-percent light treatments, with maximum values of 1.0 and 0.1 m, respectively. Further, no increases in elongation occurred in the 0 percent treatment after Week 2.

Similar trends occurred in biomass measurements (Figure 2b) for dominant shoots. As with elongation, maximum biomass values were similar for 5- and 10-percent treatments. Further, attainment of the maximum biomass level was again delayed in the 5-percent treatment. In the 1-percent treatment, dominant shoot biomass increased slightly at each sampling time, but maximum values were approximately 20 percent less than in higher light treatments. As with elongation, essentially no increases were observed in dominant shoot biomass in the 0-percent light treatment after Week 2.

Numbers of meristems per dominant shoot increased at successive sampling times for the 10- and 5-percent light treatments (Figure 2c). At Week 9, however, the 10-percent light treatment had produced approximately 1.5 times more meristems than the 5-percent treatment. Both the 1- and 0-percent treatments produced less than two meristems per dominant shoot during the 9 weeks of the study, showing essentially no increases in this variable subsequent to Week 2.

Dominant shoots from the different light treatments were also compared according to their length per mass ratios (Figure 2d). Consistent reductions in this ratio were observed in the 10- and 5-percent treatments following Week 2, while this ratio continued to increase through Week 5 in the 1- and 0-percent treatments.

Overall plant growth

Measurements of overall plant growth from hydrilla root crowns under the four light levels are summarized in Figure 3. These data indicate noticeable trends toward overall reductions in the measured parameters at each reduction in light level below 10 percent full sunlight.

For aboveground biomass (Figure 3a), mean values for the 10-percent treatment were consistently highest, while they were lowest for the 0-percent treatment. Mean values for the 5- and 1-percent treatments were similar through Week 5, but were considerably higher for the 5-percent treatment at Week 9.

Mean belowground biomass values (Figure 3b) showed similar and consistent decreases through Week 5 for all treatments. Week 9 values continued declining for all treatments except 10 percent light, which showed a greater than two-fold increase.

Total biomass values (i.e., aboveground + belowground) consistently increased between sampling times subsequent to Week 2 in the 10-percent treatment, but remained fairly constant through Week 5 in other treatments. A slight increase was observed in total biomass in the 5-percent treatment from Week 5 to Week 9.

The final parameter for overall shoot growth was the number of stems that grew from the original root crown. Stem numbers were similar in all treatments through Week 2, with mean values near two stems per root crown. Following Week 2, stem numbers remained fairly constant in 1- and 0-percent treatments, but continued to increase in higher light level treatments. At 9 weeks, stem numbers in the 5-percent treatment were approximately twice

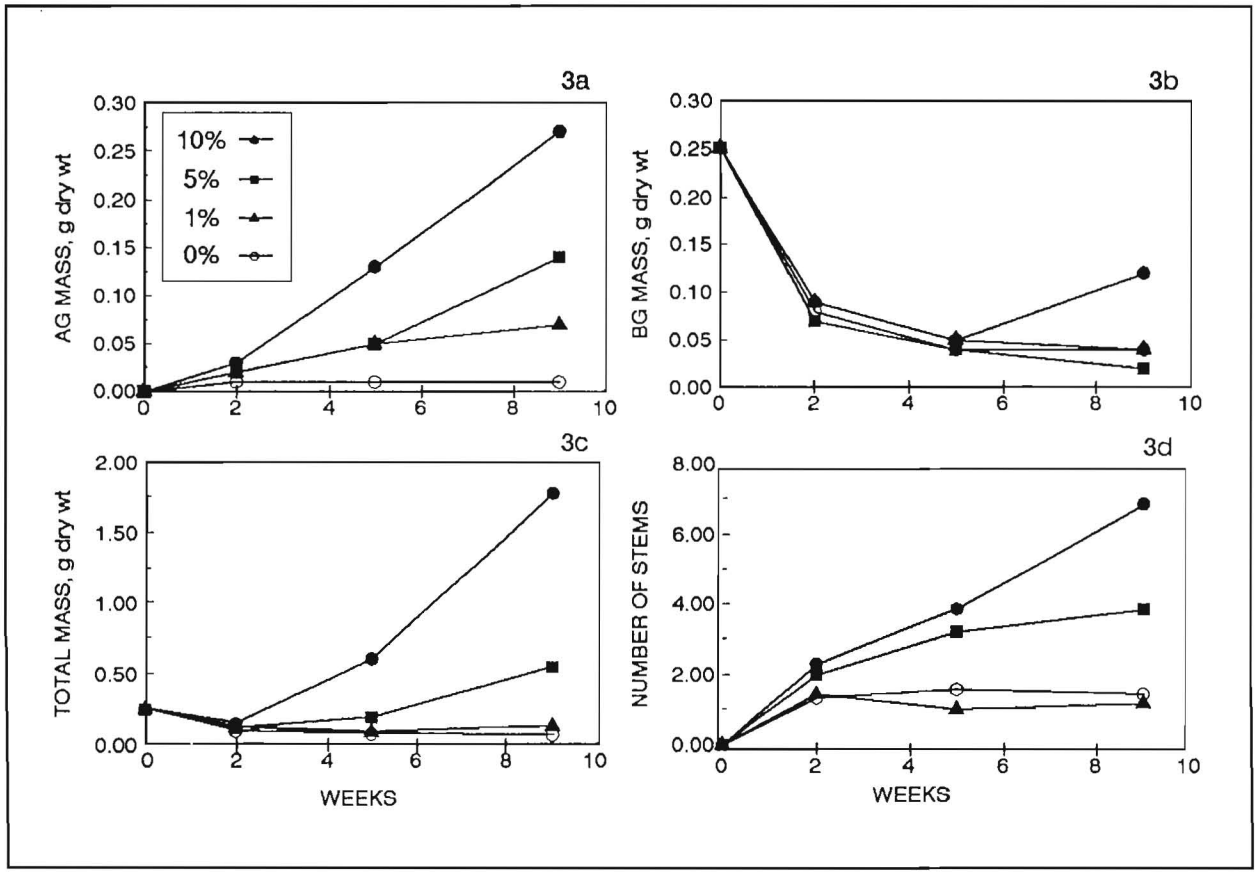

Figure 3. Measurements of overall plant regrowth from root crowns at Weeks 2, 5, and 9. Point values represent means of seven replicate samples

that in the 1- and 0-percent treatments. Likewise, stems numbers in 10-percent treatments were approximately twice that in 5-percent treatments.

Discussion

Results from these preliminary experiments indicate that light levels below 5 percent full sunlight can greatly reduce regrowth from hydrilla root crowns in comparison with regrowth at higher light levels. Reductions were measured both in terms of overall biomass production, shoot density, and dominant shoot elongation and meristem production. These results provide an extension to the information in Barko and Smart (1981), who reported increases in hydrilla dominant shoot elongation with decreases in light levels down to 5 percent full sunlight.

Information of this type is important in understanding regrowth patterns of hydrilla and other submersed aquatic plants under actual field conditions. Johnstone and Robinson (1987) attributes significant changes in the spatial distribution of hydrilla in a New Zealand lake to water quality changes that reduced light penetration. In this New Zealand study, changes in the spatial distribution of hydrilla were shown to coincide with changes in the areal extent of the littoral zone that received 5 percent or greater light penetration at full depth.

Though the effects of light on plant regrowth are often confounded or overshadowed by other factors, such as in situ levels of sediment nutrients and temperature (Barko and Smart 1981; Chambers and Kalff 1985), the results of this study indicate that light levels below 5 percent full sunlight may delay, or possibly even prevent, successful regrowth. Obviously, the ability of submersed plants to regrow in light-limiting environments is enhanced by their tendency to elongate at lower light levels, and thereby reach a position in the water column having more favorable light conditions.

In addition to in situ environmental conditions affecting shoot elongation during regrowth, biological considerations are equally important in determining regrowth success. For example, Bowes et al. (1977) has shown that the ability of hydrilla to successfully regrow from tubers under light-limiting conditions is affected by initial tuber mass. The differences in shoot elongation and survivability observed for different tuber weight classes were attributed by these researchers to differences in energy and nutrient reserves. Similarly, differences in regrowth success under light-limiting conditions can be expected among different types of regrowth structures (e.g., root crowns, stem fragments, and turions) and different "phenological stages" (Madsen 1992) of the same type of regrowth structure.

Recommendations

During fiscal year 1993, studies will be conducted to continue the investigation of hydrilla and Eurasian watermilfoil regrowth under low light conditions. These studies will further consider the effects of initial regrowth structure, temperature, and sediment composition. At least a portion of the studies will be conducted in deep water tanks that will allow "simulation" of realistic light gradients. This setup will enable us to consider effects of light quality in addition to light quantity.

Acknowledgments

The authors extend thanks to Dr. R. Michael Smart for advice and technical comments in regard to experimental design, and to Ms. Revellia Davis for technical assistance during data collection.

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Status of *Hydrellia pakistanae* Modeling Efforts and Approach for Future Development of the INSECT Simulation

by
William A. Boyd[1] *and R. Michael Stewart*[1]

Introduction

As part of the Aquatic Plant Control Research Program (APCRP), the U.S. Army Engineer Waterways Experiment Station (WES) develops simulation procedures that provide information needed to systematically evaluate how environmental conditions affect the population dynamics and life cycle processes of various insect biocontrol agents. At present, a development time model for *Hydrellia pakistanae*, first mentioned in Boyd and Stewart (1992), has been developed for incorporation into this overall simulation capability.

Hydrellia pakistanae is a leaf-mining fly in the family Ephydridae, subfamily Notiphilinae, tribe Hydrelliini. As a biocontrol agent, it is considered to be a valid alternative or complement to other control methods for hydrilla, *Hydrilla verticillata*. Characteristics of *Hydrellia pakistanae*, as reported in Buckingham, Okrah, and Thomas (1989), include the female ovipositing on hydrilla leaves and stems at or mostly above the water's surface and laying an average of 68.4 eggs during her lifetime. As eggs develop into larvae, these begin to mine the hydrilla leaves. A single larvae will damage from 10 to 17 leaves in its lifetime.

Hydrellia pakistanae Development Model

Status

There is very limited published information available on the population dynamics and development of *Hydrellia pakistanae*; therefore, much of the information used in the *Hydrellia pakistanae* model was obtain from unpublished information furnished by United States Department of Agriculture (USDA) and the University of Florida, at Gainesville (see Buckingham and Okrah, In Preparation). This information is based on intensive biology and host range tests conducted by USDA prior to making field releases of *Hydrellia pakistanae* in October 1987.

The *Hydrellia pakistanae* developmental time model is driven solely by temperature and allows consideration of factors such as the number of generations and the time of development over a specified period. These factors are essential in determining overall impact of *Hydrellia pakistanae* on hydrilla.

Development of the different life stages is based on the degree-day concept. Degree-days are calculated as the difference between the average daily temperature and the developmental threshold temperature. Table 1 shows the lower developmental threshold temperature and the cumulative degree-day requirement for development of each life stage as determined by USDA for studies conducted at a constant temperature of 27 °C (see Buckingham and Okrah, In Preparation). As the cumulative number of degree-days reaches the requirement, development proceeds from one life stage to the next. While development time differs by life stage, the developmental threshold remains constant at 13 °C. These requirements for development, as well as the developmental threshold temperature were incorporated into the *Hydrellia pakistanae* module.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Table 1
Development Time of *Hydrellia pakistanae*

Life Stage	Threshold Temperature for Development, °C	Required Degree-Days
Egg – 1st Larvae	13	45
1st Larvae – 2nd Larvae	13	53
2nd Larvae – 3rd Larvae	13	50
3rd Larvae – Pupae	13	77
Pupae – Adult	13	93
TOTAL		**318**

Further studies were conducted during the fiscal year (FY) 1992 at WES by the Environmental Laboratory's Ecological Research Division (ERD). In these studies, development times for *Hydrellia pakistanae* were recorded at constant temperatures of 20, 25, and 27 °C (see Warren 1992). To test the accuracy of our computer model against these laboratory data, temperatures used by the model were set constant at each of the three temperatures. Comparisons of the outputs obtained from the model and those from the laboratory are shown in Figure 1. As shown in Figure 1, development times were different for most life stages; however, when comparing the overall development time obtained from the model with laboratory data at each temperature, there was relatively little difference in *Hydrellia pakistanae* development times.

Applications

To illustrate the effect of temperature on the number of generations and time of development, 1986 average daily temperatures recorded for two geographical areas, southeast Florida and north Alabama, were used to initialize the model. As shown in Figure 2, average daily temperatures recorded for southeast Florida are significantly higher than those for north Alabama. Average daily temperatures recorded for southeast Florida are above the developmental threshold of 13 °C throughout the year, while those recorded for north Alabama fall below this threshold during periods at the beginning and end of the year.

Figure 3 presents comparative simulations of the development of *Hydrellia pakistanae* during a year using the southeast Florida and north Alabama weather data. Because average daily temperatures recorded for southeast Florida are above the developmental threshold temperature throughout the year (Figure 2), first

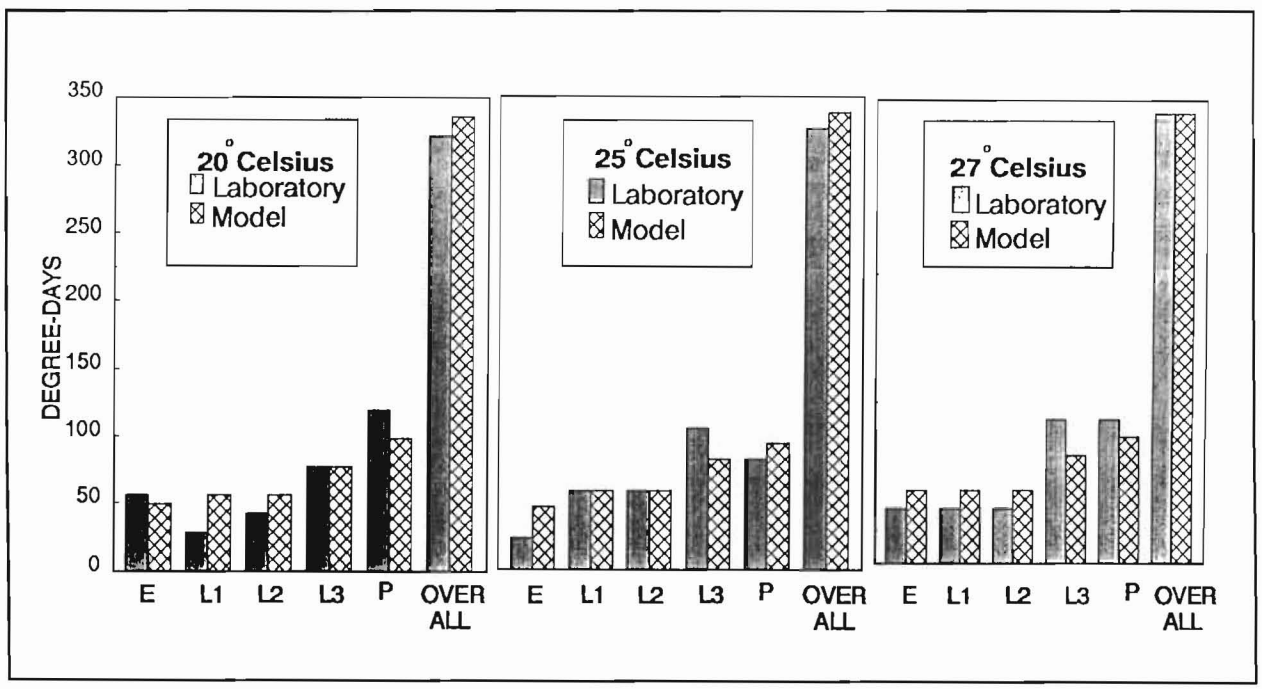

Figure 1. Development times for Hydrellia pakistanae at 20, 25, and 27 °C
Laboratory versus computer model outputs

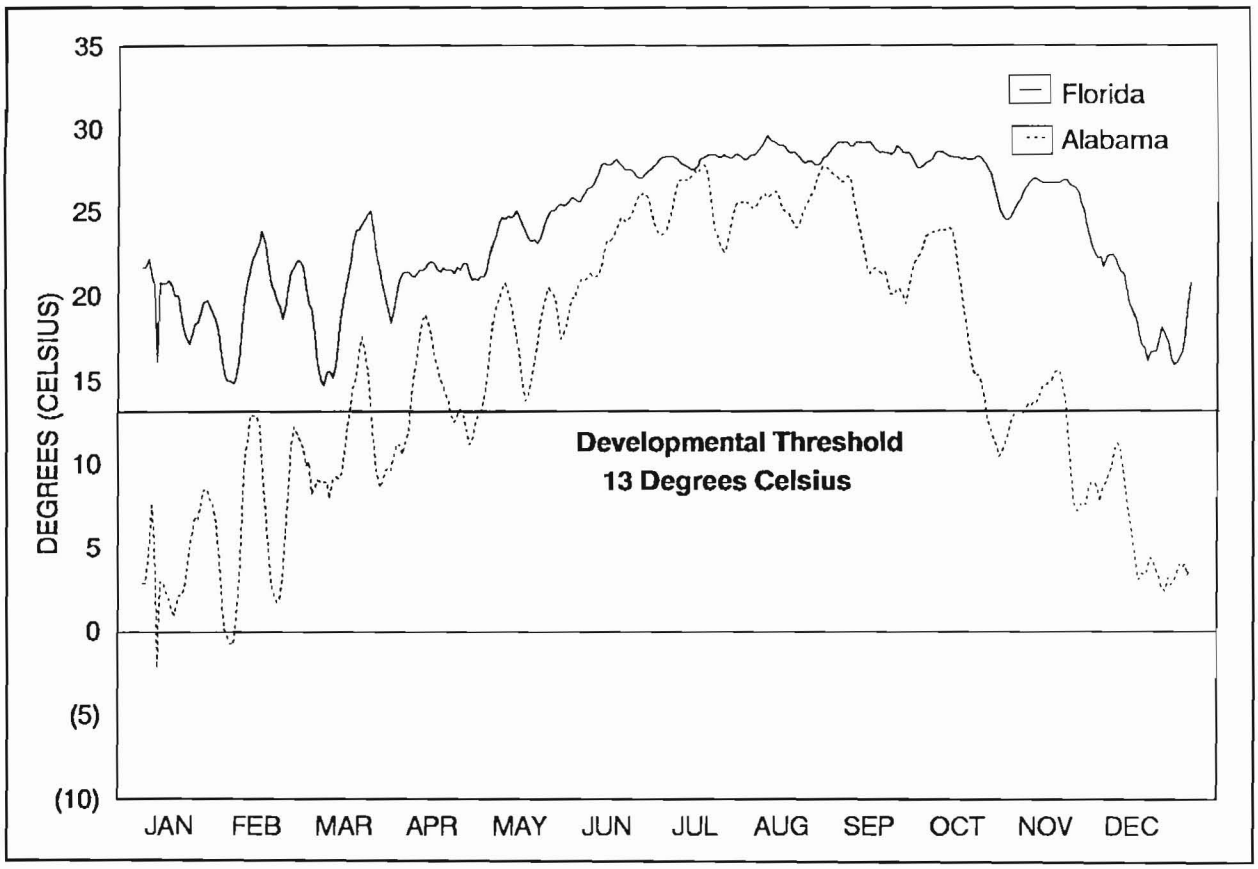

Figure 2. Average daily temperatures recorded for southeast Florida (West Palm Beach, FL) and north Alabama (Guntersville, AL)

generation larval development for the Florida simulation (Figure 3) begins in January, and development of successive generations continues throughout the entire year. As a result, larvae reach the 13th generation by the year's end with no break occurring in development. Since average temperatures recorded for north Alabama stay below the developmental threshold temperature into the month of March (Figure 2), development of the first generation of larvae in the north Alabama simulation does not begin until April. Six generations are shown to occur during the year, with development ceasing near the end of the year when average daily temperatures again fall below the developmental threshold temperature.

In the simulations, there were also significant differences in the peak number of individual larvae occurring in populations during the course of the year. Figure 4 shows a comparison of these numbers for both southeast Florida and north Alabama. In each case, the module was initialized with 10 eggs. Initialization was made on Julian Day 1 using the southeast Florida weather data while it was made on Julian Day 72 (first day average temperatures were above the developmental threshold of 13 °C) using the north Alabama weather data file. Peak numbers of larvae using the southeast Florida weather data file are shown to reach the tens of millions with no breaks in development occurring during the year. Results obtained using the north Alabama weather data show the peak number of larvae reach only into the tens of thousands. This is a direct result of development beginning later in the year than southeast Florida and ending before the year is complete.

It is recognized that other relationships can either directly or indirectly influence population dynamics as well as number of generations that occur under real world conditions.

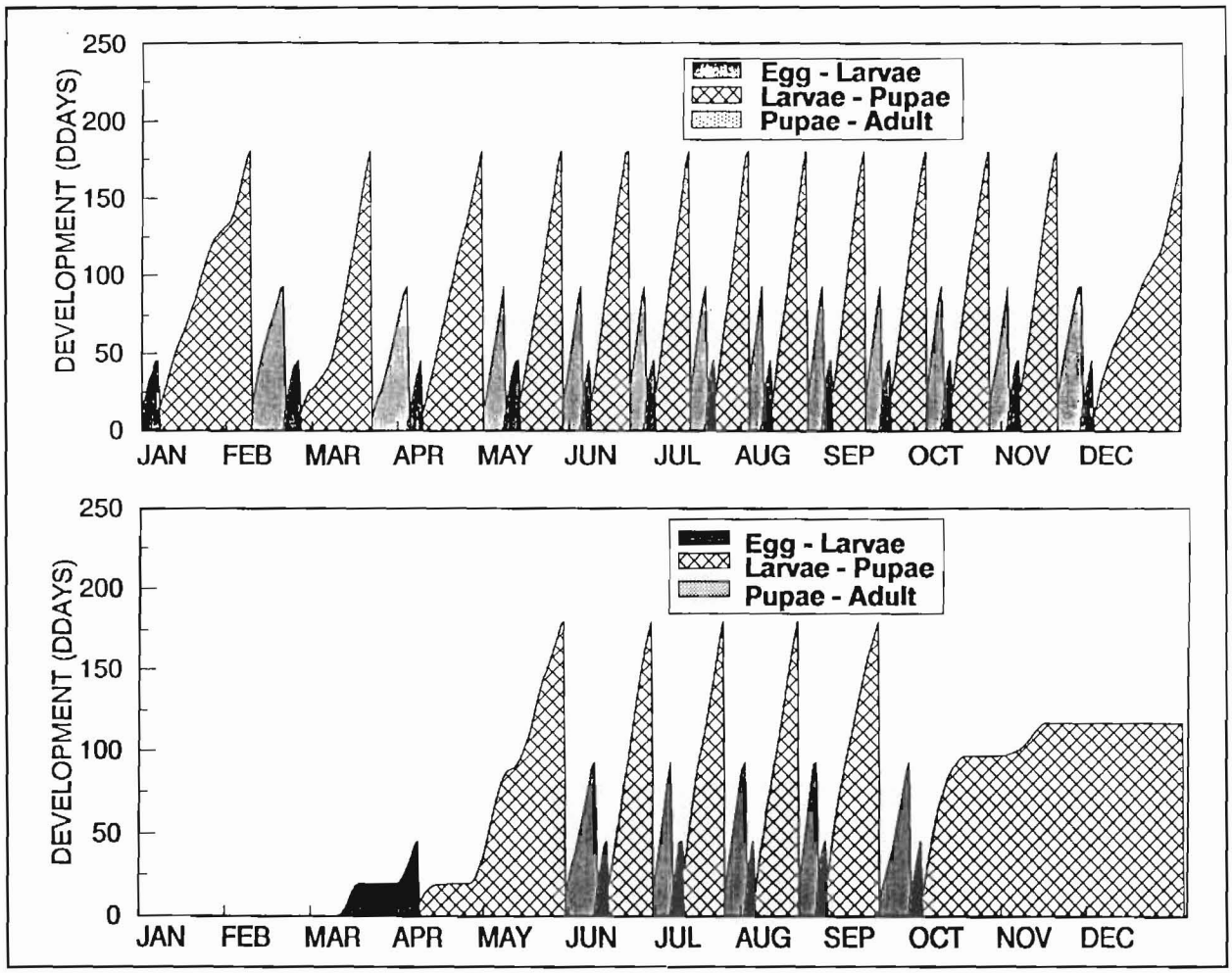

Figure 3. Development through successive life stages and generations of Hydrellia pakistanae during a year using daily temperatures recorded for southeast Florida and north Alabama

Because of limited information on *Hydrellia pakistanae* for these other relationships, the results herein are in no way to be interpreted as the actual number of generations or individuals that occur in the field; however, these results do indicate the significant impact temperature alone can have on the size of a particular insect biocontrol agent population in a single growing season. Further, this information will be useful when interpreting results of comparative efficacy studies of this biocontrol agent in different geographic areas.

INSECT Simulation

Future development

Further testing of the *Hydrellia pakistanae* developmental time model is needed at other temperatures. As results from studies conducted by ERD at temperatures below 20 °C and above 27 °C become available, these data will be compared with model outputs. Necessary adjustments to the model will be made accordingly. As determined in Buckingham and Okrah, In Preparation, the threshold temperature at which development of *Hydrellia pakistanae* occurs is 13 °C. Below this temperature, no development occurs; however, the model currently uses no upper lethal temperature limit. As further results from laboratory studies are provided at temperatures above 27 °C, it is hoped that an upper lethal temperature limit can be determined for *Hydrellia pakistanae* development.

Existing code for *Hydrellia pakistanae* development will be expanded to include other

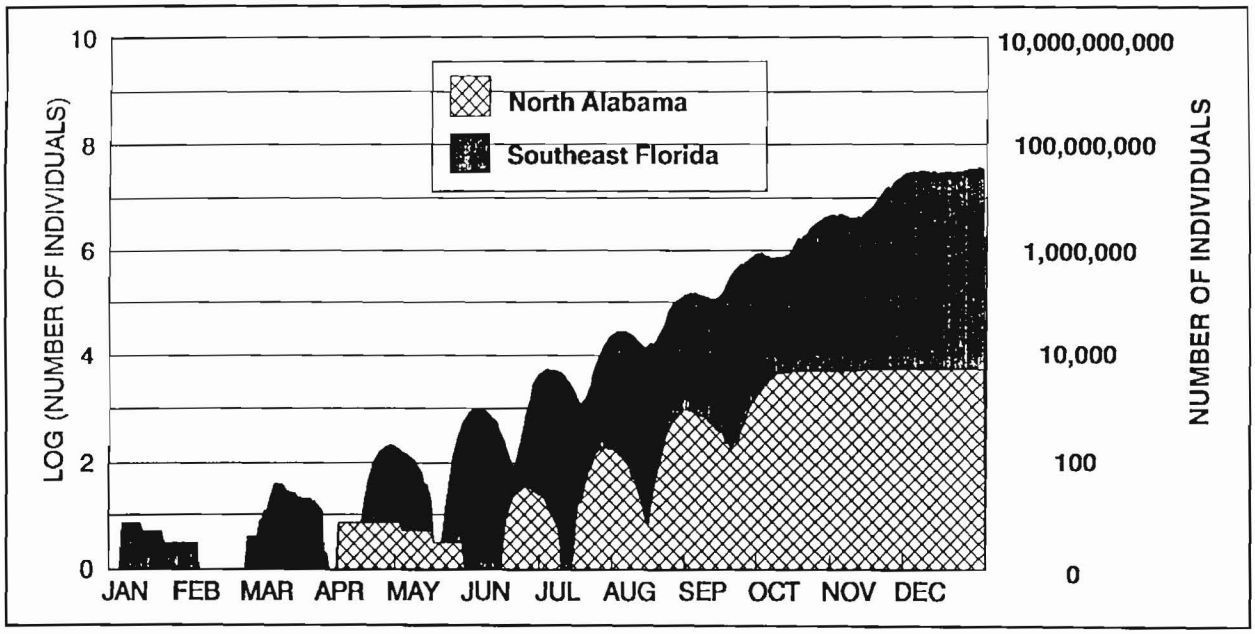

Figure 4. Number of individual larvae occurring during a year using average daily temperatures recorded for southeast Florida and north Alabama

insect biocontrol agents. This will be accomplished by generalizing the existing INSECT simulation code (see Akbay, Howell, and Wooten (1991) and Stewart and Boyd (1992)). In this way, the user will have the option of either accepting default values for relationships used in the model or inputting user-specified values for these relationships as they relate to a specific insect biocontrol agent. Such relationships include development rates, mortality and migration rates, and the effects of temperature, insect density, and other factors on fecundity limitation.

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Field Studies for Existing Control Technology Simulations

by
R. Michael Stewart[1]

Introduction

Simulation procedures are being developed for the Aquatic Plant Control Research Program (APCRP) to provide a consistent basis for systematic evaluation of the effects of site conditions on aquatic plant growth and control technique effectiveness. Control techniques considered by the simulations include selected aquatic herbicides, and, among other biocontrol agents, triploid White Amur. Stewart (1992) provides information on the overall role of simulation technology in the APCRP and describes the series of work activities that are undertaken during development of these simulation procedures. This paper describes field studies conducted during fiscal year (FY) 1992 that address "testing and evaluation activities" of the chemical control simulation procedure, HERBICIDE.

Overview of HERBICIDE

Structure of HERBICIDE

The HERBICIDE simulation model currently under development (Rodgers, Clifford, and Stewart 1991) is a decision support software package that provides data useful for designing effective aquatic herbicide application strategies. The generalized structure of the HERBICIDE model includes three interactive modules that estimate or predict (a) the postapplication fate of the active ingredient of the herbicide formulation, (b) the effectiveness of the herbicide treatment on the target plant infestation, and (c) the posteffect response or regrowth of the target plant. The following briefly descibes herbicide fate considerations of the fate module.

HERBICIDE fate considerations

Various types of fate processes effect significant reductions to initial concentrations of aquatic herbicide active ingredients following their application or release into aquatic systems. These fate processes, working collectively, produce time-varying levels of the active ingredients within different "partitions" of an aquatic system. Aquatic system partitions considered by HERBICIDE are the water, the tissues of the target plant, and the sediment.

The HERBICIDE fate module allows consideration of the effects of major fate processes on herbicide formulation active ingredients. The effects that various fate processes have are dependent upon both site conditions and the properties of the aquatic herbicide formulation (Reinert and Rodgers 1987; Westerdahl and Getsinger 1988). Failure to consider the effects that major transfer and transformation processes have on aquatic herbicides when designing aquatic herbicide applications often results in attainment of lower levels of control than desired. The HERBICIDE model provides time-dependent simulation outputs for concentrations of the herbicide active ingredients within the different "partitions" in consideration of the effects of the major fate processes. Rates of herbicide transfer and transformation because of the various fate processes are calculated based on user response to the input requirements shown in Table 1.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Table 1
Input Requirements for Calibration of Herbicide Fate Process Algorithms in HERBICIDE

Transfer Processes	Input Requirements
Drift	Percent loss of active ingredient
Dilution	Application rate of formulation Percent active ingredient fraction Release half-life of formulation Average depth of treated area Water flow rate of treated area
Sorption	Herbicide sediment layer partition coefficient Total suspended solids Sedimentation rate Depth of active sediment layer Sediment water content (percent) Sediment diffusion exchange rate
Volatilization	Volatilization half-life in water
Bioaccumulation	Bioaccumulation factor of active ingredient
Transformation Processes	**Input Requirements**
Oxidation	Oxidation half-life in water Oxidation half-life in sediment
Hydrolysis	Hydrolysis half-life in water Hydrolysis half-life in sediment
Photolysis	Photolysis half-life in water Photolysis half-life in sediment
Biodegradation	Biodegradation half-life in water Biodegradation half-life in sediment

Source: Rodgers, Clifford, and Stewart (1988).

Calibration Studies for Triclopyr

Objectives

During FY92, Chemical Control Technology (CTT) researchers conducted flume verification studies (Turner et al. 1993) of laboratory-derived "concentration/exposure time" (CET) relationships for triclopyr control of Eurasian watermilfoil (Netherland 1992). These CTT studies included measurements of postapplication concentrations of triclopyr in flume water and estimates of milfoil mortality. To the experimental design developed for these studies, we incorporated additional data collection to provide measurements of the postapplication partitioning of triclopyr into plant tissues and sediments. Collectively, these data will be used during FY93 to calibrate fate and effects relationships in HERBICIDE for triclopyr and Eurasian watermilfoil.

Methods

As part of the overall CTT flume verification study, one of the experiments consisted of a continuous "metered" input of triclopyr into the flume water for maintenance of a target water concentration of 0.25 ppm triclopyr for three different exposure durations, 24, 48, and 72 hr. To this study design, we added plant tissue and sediment sample collections for determination of triclopyr concentrations in these two partitions after the three exposure durations. Nine plant tissue samples were collected from the flume after each exposure duration using a long-handle rake. Attached filamentous algae and other associated debris were removed from plant samples by washing with tap water using a standard garden hose spray nozzle. After washing, plant samples were placed in a nylon mesh bag and "spun dry" by hand. Fresh weights were measured and recorded to the nearest gram. Each sample was wrapped in aluminum foil and frozen until being analyzed for triclopyr levels (analytical detection limit for plant samples was 0.05 ppm). For sediment samples, aluminum trays measuring 23 by 27 by 6 cm depth were filled with lake sediments and placed on the bottom of the flume. Three trays were removed from the flume after each of the three exposure durations. From each tray, the surface area was marked to divide the sample into three equivalent portions (i.e., 23 by 9 cm). For eight of these nine "subsamples," the sediment was removed to approximately 2 cm depth and placed in a 250-ml glass sample jar, which was then sealed with a polypropolene-lined screw cap. Samples were weighed to the nearest gram (fresh weight) and frozen until being analyzed for triclopyr levels (analytical detection limit for sediment samples was 0.01 ppm).

Summary of results

Summarizations of plant and sediment sample fresh weights and percent moisture levels are presented in Table 2. Triclopyr concentrations in plant tissues (Figure 1) were at peak levels after 24 hr exposure. As did water concentrations (Turner et al. 1993), plant tissue

Table 2
Mean Values for Plant Sample[1] and Sediment Sample[2] Fresh Weights and Percent Moisture Content

Exposure Time hr	Plant Fresh Weight g	Plant Moisture %	Sediment Fresh Weight g	Sediment Moisture %
24	367.1	85.68	360.3	62.18
48	322.7	87.67	362.0	65.61
72	343.6	88.51	343.8	66.37

[1] Mean values for n = 9.
[2] Mean values for n = 8.

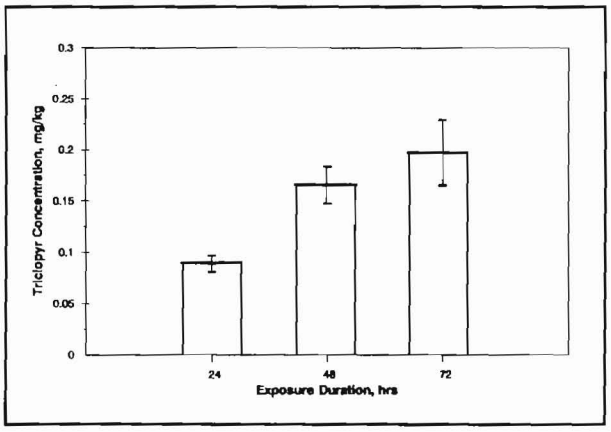

Figure 1. Mean triclopyr concentrations (mg/kg dry weight) in plant samples at exposure durations of 24, 48, and 72 hr. Analytical detection limit for triclopyr in plant tissues was 0.05 mg/kg. Vertical lines through mean values indicate ±1 standard deviation(n = 9)

concentrations were fairly consistent at each of the three 24-hr sampling times throughout the 72-hr treatment. In comparison, however, plant concentrations, on a dry weight basis, were approximately 30 to 40 times higher than water concentrations. Calculated concentration ratios (plant:water) were 40.4, 35.4, and 33.1 for 24-, 48-, and 72-hr samples, respectively. Data in Figure 2 show that triclopyr concentrations in sediments increased from 0.089 mg/kg at 24 hr to 0.197 mg/kg at 72 hr. Based on visual observations, it appeared that measured increases in triclopyr within the sediments through time probably resulted from deposition of suspended organic material (e.g., filamentous algae, sloughed milfoil leaves) containing sorbed triclopyr. However, since sedimentation rates were not measured during the study, there is no way to quantify individual routes of triclopyr partitioning into the sediments with these data.

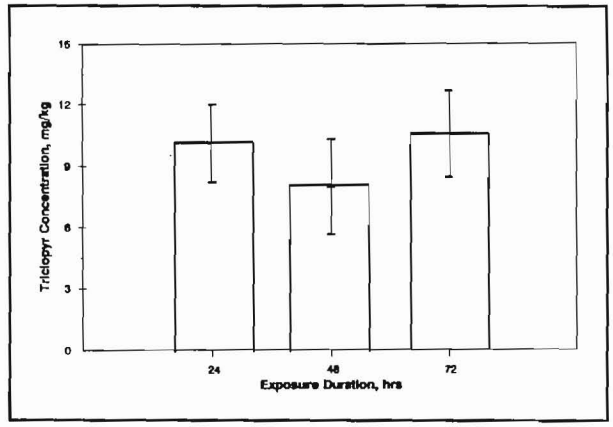

Figure 2. Mean triclopyr concentrations (mg/kg dry weight) in sediment samples at exposure durations of 24, 48, and 72 hr. Analytical detection limit for triclopyr in sediment samples was 0.01 mg/kg. Vertical lines through mean values indicate ±1 standard deviation(n = 8)

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Digital Mapping and Database Developments in Support of Simulation Modeling

by
M. Rose Kress[1] *and Janet L. Holt*[1]

Introduction

An important long-term goal of the simulation technology research area is to integrate validated numerical models into the aquatic plant control planning and decision-making process. To effectively support this goal, efforts to develop appropriate digital database procedures must address data collection and management needs at several levels. Regional level databases support simulation research and development by providing the input data necessary for assessing the sensitivity of numerical models to broad environmental factors such as temperature and solar radiation. Site or project level databases contain environmental data characterizing individual water bodies or management areas such as water depth, plant distribution patterns, and past treatment activities. Recent work at both the regional and site level are discussed below.

Regional Weather Database

Boyd and Stewart (1993) illustrate the importance of weather data, especially temperature, on insect biocontrol simulations. Other weather factors influencing biocontrol predictions are precipitation, day length, and daily solar radiation. Two regional level weather databases were developed for use in development and testing of the simulation models, one for the northwestern United States and one for the southeastern United States.

Each data set contains 10 years of historical daily weather data for each of six recording stations in the respective regions. Figure 1 shows the location of the recording stations in the northwest region, and Figure 2 shows those in the southeast region. Table 1 lists the name, station identification number, and geographic location for each station. Stations selected were close to large water bodies, had 24-hr recording stations, and at least 10 years of record.

Table 2 lists the factors included in the regional weather data sets. Maximum temperature, minimum temperature, and precipitation were extracted from National Climatic Data Center archives. Figure 3 is a plot of the 1980 maximum and minimum daily temperatures for Lake Guntersville, AL, as extracted from the southeast regional weather data set.

Day length, a function of Julian day and latitude, was numerically calculated. A physics-based numerical model, developed and validated by the U.S. Department of Agriculture (Richardson and Wright 1984), was used to estimate total daily solar radiation for each station. Figure 4 is a plot of the total daily solar radiation (1980, Lake Guntersville) as calculated by the model.

These regional weather data sets provide 10 years of specific weather profiles (e.g., cloudy spring or mild winter) for use in simulation model development and other task areas. For instance, the long-term data for Lake Guntersville will be valuable for investigating the influence of weather factors on the pattern of invasions and declines in that water body.

Project Level Database

For simulation technology to function as a tool in the aquatic plant control decision-making process, project level or site-specific databases containing the appropriate model

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

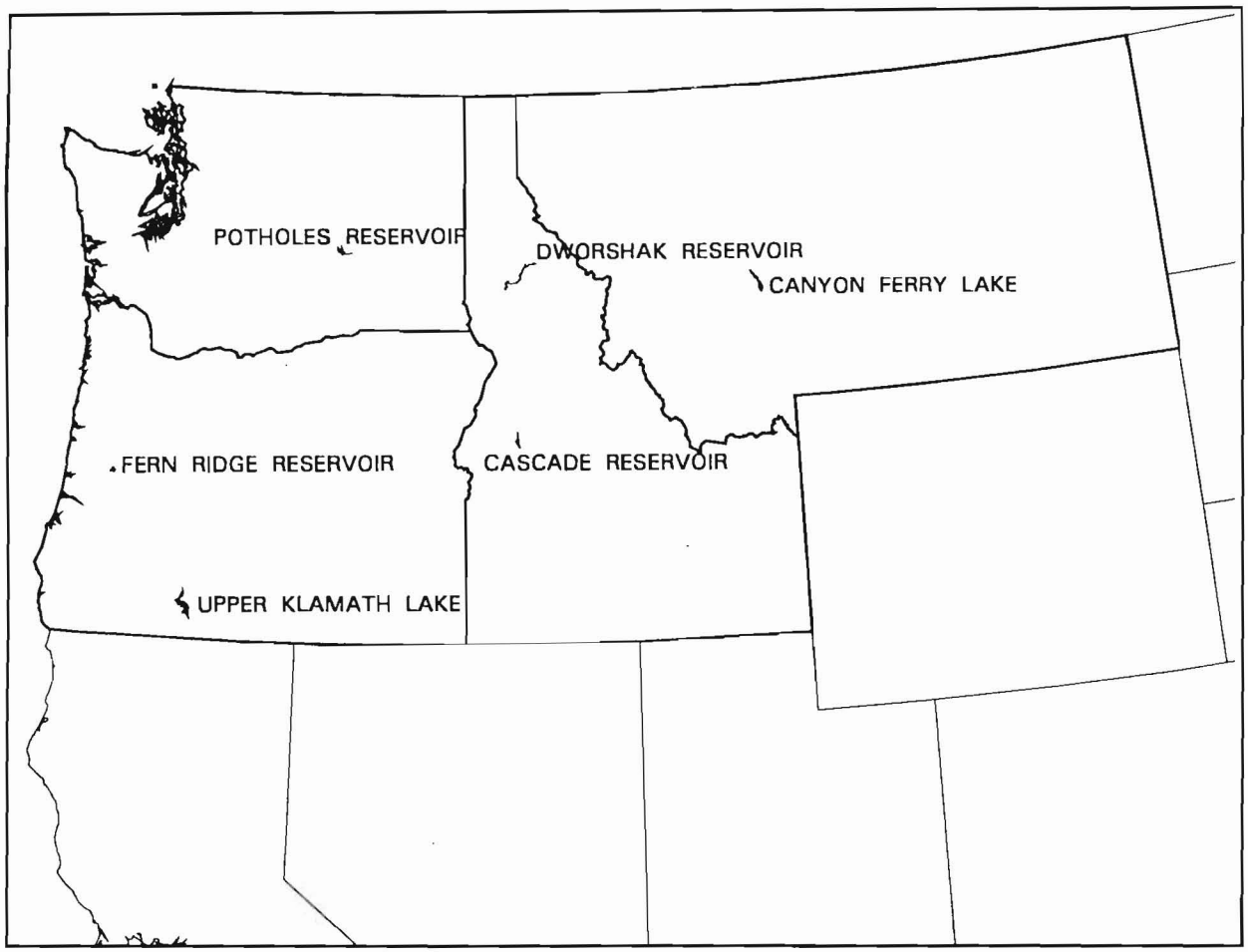

Figure 1. Locations of weather recording stations, northwest region

inputs are needed. These project level databases must characterize local water body and environmental conditions and conform to the input requirements of the simulation models. Recent efforts in this area have focused on integrating simulation models with digital database management technologies such as geographic information systems (GIS) and relational databases. Kress and Causey (1992) describe how the use of geographic information system technology can significantly enhance the operational use of simulation models.

To effectively use any computer-based decision aids, local managers must be able to generate and update properly formatted input data without undue reliance on outside contract services. The most basic component of the project level information base is an accurate assessment of the aquatic plant infestation distribution. Traditionally, aquatic plant distribution maps are compiled from aerial photography using accepted photogrammetric methods (Welch and Remillard 1991; Kress, Causey, and Ballard 1990). This procedure, although well-tested and accurate, is expensive, time-consuming, and generally not under the direct control of the local resource manager. Often the magnitude of the infestation is estimated by visual inspection. Figure 5 illustrates the difficulty in estimating plant infestation acreage by visual inspection.

Procedures for developing accurate, timely plant distribution and acreage information using a combination of GIS and global positioning system (GPS) technologies are being developed. GPS is a digital surveying technology based on the ability to receive and process thousands of satellite signals per second. From these signals, the geographic coordinates of the receiver's location are derived.

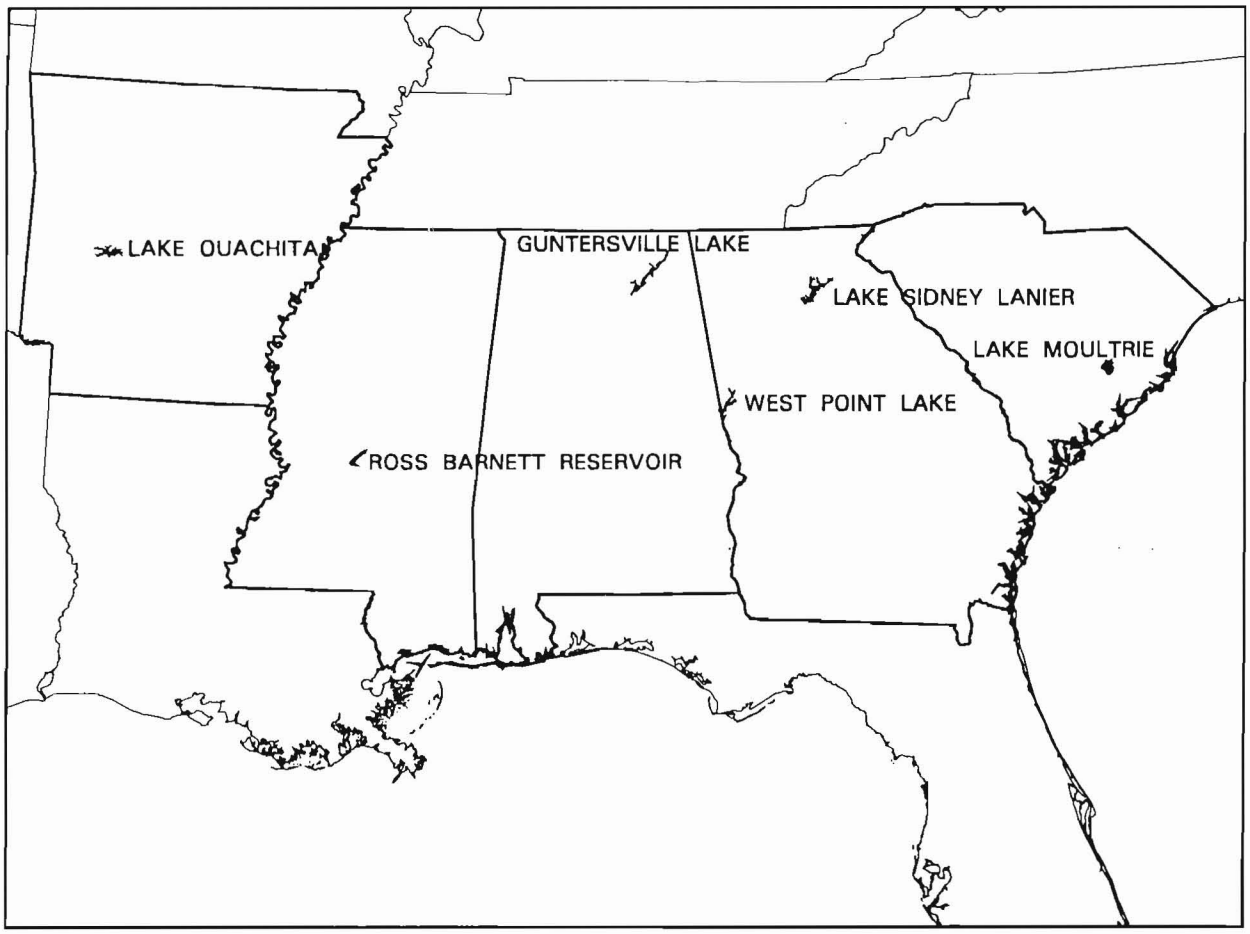

Figure 2. Locations of weather recording stations, southeast region

Table 1
Weather Stations Included in Regional Weather Database

Water Body	Weather Station Identification Number	Latitude	Longitude
Dworshak Reservoir, ID	2845	46°30′N	116°18′W
Cascade Reservoir, ID	1514	44°32′N	116°03′W
Fern Ridge Reservoir, OR	2867	44°07′N	123°18′W
Upper Klamath Lake, OR	4506	42°12′N	121°47′W
Canyon Ferry Lake, MT	1470	46°39′N	111°44′W
Potholes Reservoir, WA	7727	46°50′N	119°40′W
West Point Lake, GA	9291	32°52′N	85°11′W
Lake Sidney Lanier, GA	3621	34°18′N	83°51′W
Lake Moultrie, SC	6893	33°15′N	79°59′W
Guntersville Lake, AL	7304	34°41′N	86°03′W
Ross Barnett Reservoir, MS	4472	32°19′N	90°05′W
Lake Ouachita, AR	764	34°34′N	93°12′W

Table 2
Weather Variables Stored in Regional Weather Database

Year	1980-1991
Julian Day	1 - 365
Maximum Daily Temperature	°F
Minimum Daily Temperature	°F
Precipitation, Daily Total	inches
Length of Daylight	hours, minutes
Solar Radiation, Total Incoming	Langleys

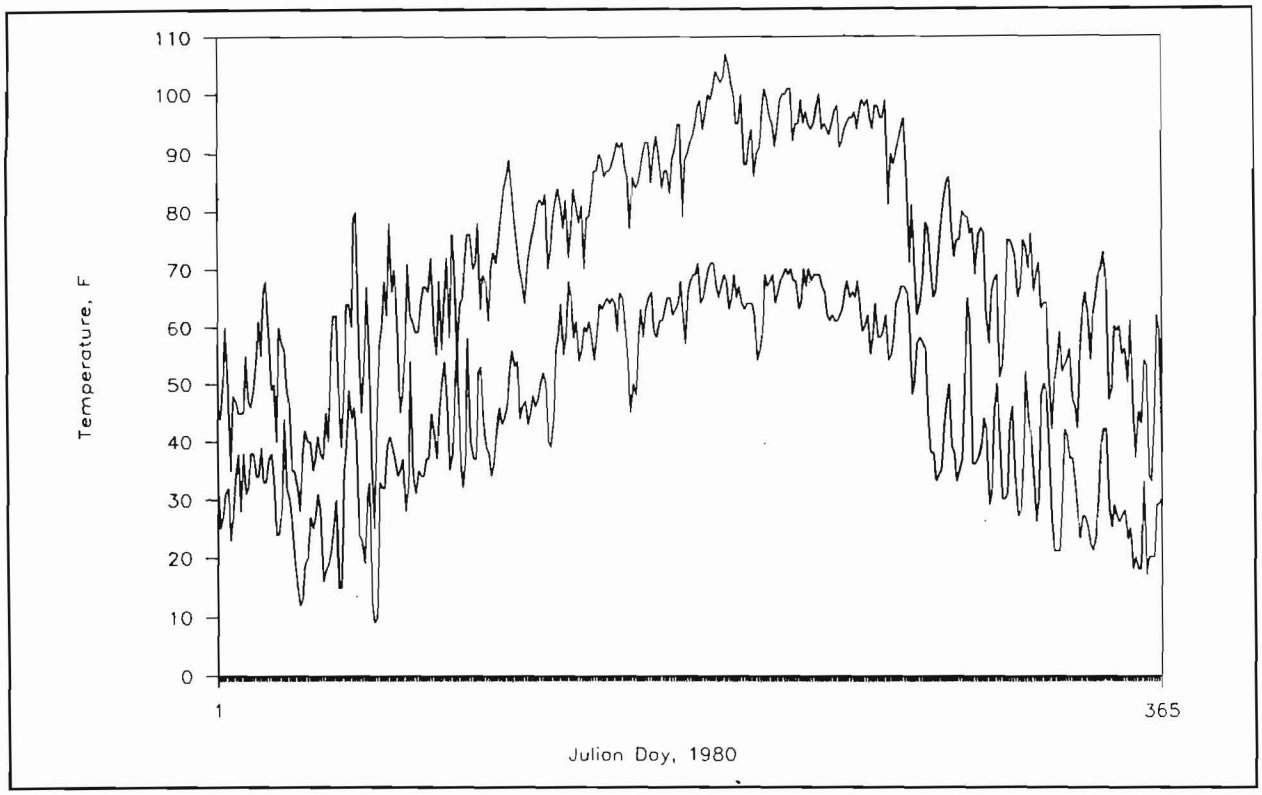

Figure 3. Daily minimum and maximum air temperatures for 1980 at Lake Guntersville, AL (these data are stored in the APCRP regional weather data set)

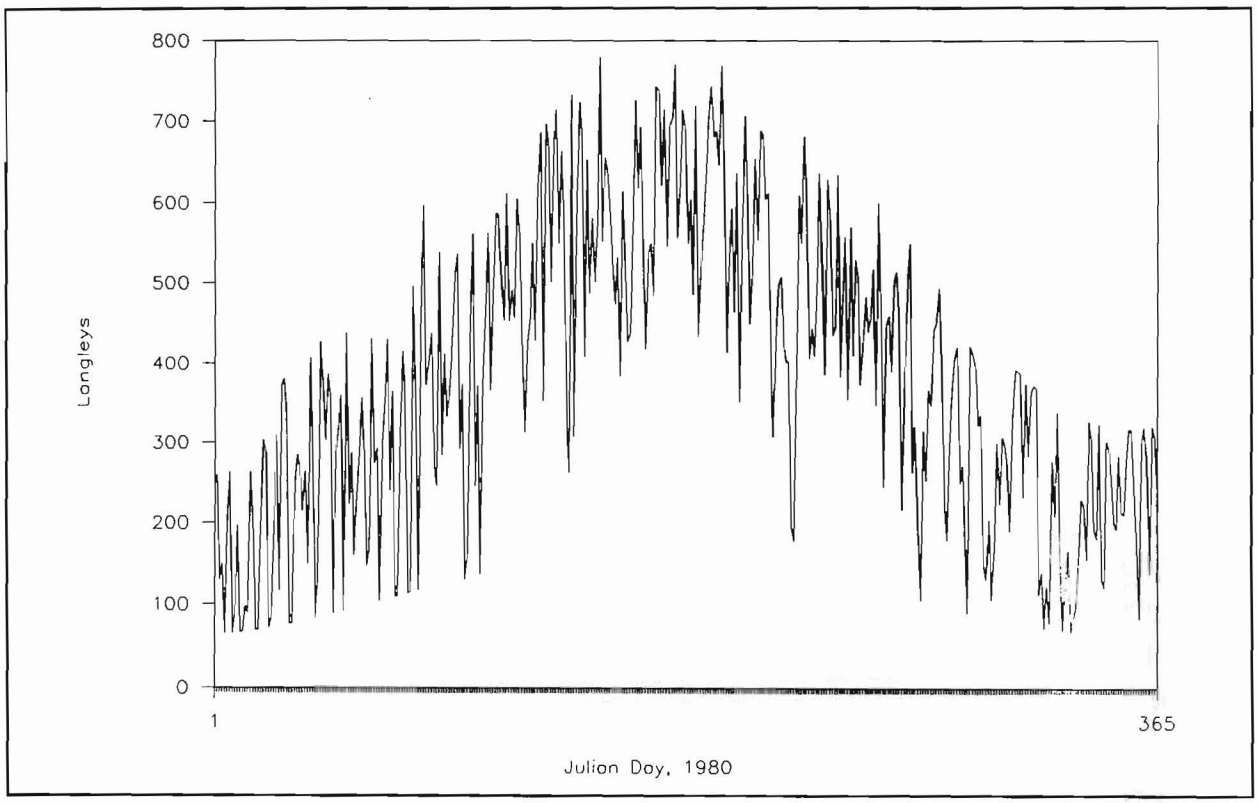

Figure 4. Total estimated daily solar radiation for 1980 at Lake Guntersville, AL

Figure 5. Typical view of operator when attempting to estimate acres of aquatic plants by visual inspection from a boat

Figure 6 shows a GPS receiver mounted on an airboat. As the operator drives the airboat along the edge of the aquatic plant infestation (Figure 7), the GPS records and stores the geographic coordinates along the airboat path.

Figure 6. GPS receiver mounted on airboat

Figure 7. Operation of airboat-mounted GPS receiver along aquatic plant infested shoreline

While delineating the boundary of plant infestations, the operator may record information describing the plant type and condition or other environmental data needed such as water and Secchi disk depth, density, or condition. These data are then transferred into the GIS database thus delineating the boundary of the infestation for mapping, analysis, simulation, planning, and monitoring purposes.

Figure 8 illustrates how using GPS and GIS technologies together allow accurate estimates of the important basic acreage and location information needed for control program planning and monitoring. The plant-water interface, shown as a dashed line in Figure 8, is delineated by the GPS mounted on an airboat. The plant type (cutgrass and torpedo grass) information is entered into the GPS data recorder in the field by the operator. The GIS data analysis capabilities allow the manager to display the plant distribution map on the computer screen, calculate total acreages of each plant type, determine the length of the plant water interface, or compare information from different years.

The GPS and GIS technologies can also play a role in execution of control programs and in long-term monitoring of the effectiveness of different control strategies. During execution of the control program, GPS receivers mounted on the spray boat record the geographic coordinates of areas treated. The actual boundaries of

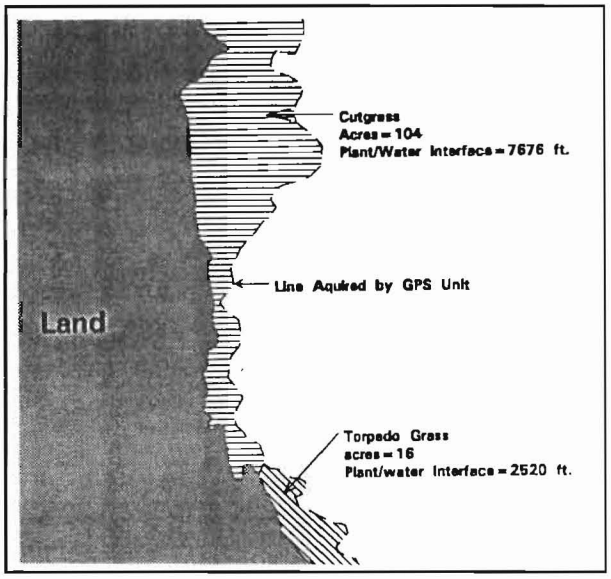

Figure 8. Example of estimating acreages of plant distributions using a combination of GPS and GIS technologies

the treated areas are stored in the GIS database. The resource manager is then able to return periodically to specific treated areas to monitor the effectiveness of the treatment.

Lake Seminole, Florida and Georgia (Figure 9), has been selected for development of a project level database designed to be compiled and updated using a combination of GPS and GIS capabilities. The database will be used to support planning, execution, and monitoring of the aquatic plant control program conducted by the local Resource Management Office. Two field tests of the airboat-mounted GPS have been conducted with good success. Procedures for the smooth transfer of GPS data into the GIS database have been developed and tested.

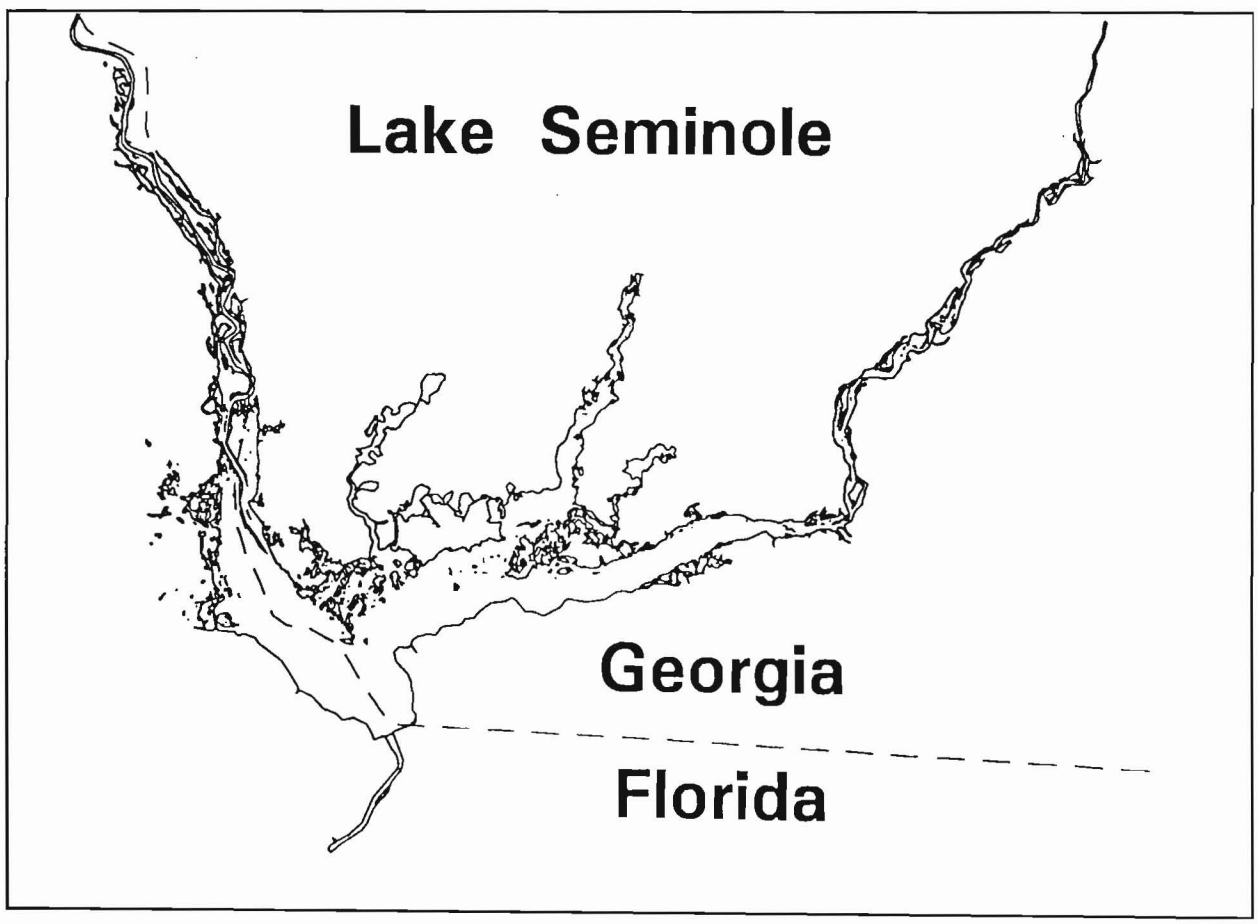

Figure 9. Shoreline of Lake Seminole as depicted in the project level digital database

Summary and Conclusions

Digital database procedures to address data collection, management, and analysis at several levels of detail are being developed. These procedures are planning and operations tools as well as research and development aids. Historical regional weather data sets were compiled for the Northwest and Southeast United States. A strategy for applying GIS and GPS technologies to mapping and monitoring aquatic plant infestations was devised and tested at Lake Seminole.

Digital mapping (GPS) and data analysis (GIS) technologies have potential applications throughout the Aquatic Plant Control Research Program (APCRP). Once the procedures for the efficient use of these technologies have been developed, they can be transferred to resource managers responsible for planning and executing control operations at the local level.

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Biological Control Technology

Biological Control

by
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To date, biocontrol projects have been initiated in over 70 countries on more than 100 species of weeds (Julien 1987). The first utilization of insect biocontrol agents to manage a noxious plant in the United States was in 1902 when *Aerenicopsis championi*, a beetle, was released in Hawaii to control Lantana (Weber 1956).

Most problem plants in the United States, particularly in the aquatic and wetland environments, are exotic species. The plants (weeds) usually have been introduced into favorable environments without their natural enemies. These exotic plants have the ability to increase rapidly, outcompeting the native vegetation for habitat and resources (Harley and Forno 1992). Biological control is the use of a parasite, predator, or disease organism to effect a degree of suppression on a target peat population.

There are two general control techniques or strategies used in biological control. The inundation or augmentation strategy is one where weak pathogens or other organisms are released in mass to cause a suppression of the target population. The other is the classical biological control strategy where natural enemies, such as arthropods or pathogens, are introduced into the new range of the target organism.

The inundation or augmentation strategy usually requires that a number of releases of high numbers of the biocontrol agent be released on the target. In the management of aquatic plants this is most often accomplished by the release of pathogens in the form of a mycoherbicide. A number of phases are involved in the development of this type of biocontrol agent. The first phase centers on the selection of an agent.

At the present time, the most successful biocontrol programs to manage aquatic plant growth in the United States have utilized insects from the native range of the problem plant.

The majority of biocontrol projects employed to control weeds utilize the concept known as "Classical Biological Control." The classical approach is based on the concept that the target plant has natural control agents present in its native range, and the introduction of these natural enemies will reestablish the pressure that the noxious plant normally experienced. In this approach, control agents (natural enemies) are introduced into areas that are not part of their native range to manage an introduced noxious plant (Harley and Forno 1992). In general, these agents are host specific arthropods, nematoda, or plant pathogens.

The process of introducing biocontrol agents can be divided into four phases. The first phase is the overseas surveys. These surveys are conducted in the problem plant's native range, and potential biocontrol agents are identified. During the second phase, overseas research is conducted on potential agents to determine the agents' general specificity to plant species. After these studies indicate that an agent is generally specific, a request is made to the U.S. Department of Agriculture, Animal Plant Health Inspection Service (USDA-APHIS) Technical Advisory Group on Biological Control of Weeds (TAG) to have the agent introduced into a quarantine facility. The Tag is composed of 13 members from state, Federal, and private agencies that review the petitions for content, experimental procedures, and conflict of interest. In the third phase, insects are shipped into a United States quarantine facility where intensive host specificity testing is conducted

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and studies are performed to determine other key preference factors of the agent. If the agent is specific to only the target plant, a petition to release the agent is requested from the USDA-APHIS-Plant Protection and Quarantine (PPQ). If the agent is approved for release, the USDA-APHIS-PPQ will issue a release permit. Once the release permit is received, the fourth phase is initiated; the agent is then released into the field, and its population development and dispersal are monitored.

Although the four-step process appears simple, the tasks outlined are quite difficult. Often, when a project is begun, conflicting reports occur regarding the country of origin for the noxious weed. The overseas surveys often take 3 to 4 years, and researchers are often faced with extremely primitive research facilities during the overseas portion of the project. After the overseas facility is established, the researcher must decide which of the many potential agents should be screened first. These agents are often only present on a seasonal basis and may be difficult to find; therefore, adjustments to the program are often needed (2 to 3 years). If the agent does show potential, it is sent to a quarantine facility where it will undergo further testing (2 to 4 years). Once the petition is submitted for release of the agent into the United States, it may take 1 to 2 years to receive the approval for release and the proper permits. In general, when a new biocontrol project begins for a noxious plant, it will take between 8 to 12 years before the first agent is released.

The introduction process is long and involved; however, the introduction of an agent that may attack a nontarget host could be devastating. Agriculture crops and the natural environment could be severely impacted; therefore, great care is taken in the introduction process.

Biological control methods are worth the time and effort that is required for their development. They are extremely cost-effective. Once a host specific agent is released and established, it will maintain itself in the environment; only minimal costs will be incurred to monitor the population. As the population of the biocontrol agent develops, it will respond to the population growth of the noxious plant but will never completely eliminate the plant. Researchers attempt to reduce the population of the problem plant below problem levels by introducing a complex of agents that attack various aspects of the plant or its life stages.

The development of biocontrol technology for noxious plants in the aquatic and wetland habitats began in 1959 when the U.S. Army Corps of Engineers and the USDA entered a cooperative study to manage exotic aquatic plants. In the first attempt, classical biological approaches were utilized. Researchers traveled to the country of origin of the plant and looked for natural enemies.

The first plant targeted for research with biocontrol technology was the alligatorweed (*Alternanthera philoxeroides*), a native of South America. This plant species grows primarily as an emersed aquatic plant rooted to bottom soils with the major portion of the plant foliage growing above water; however, the plant can also grow in the terrestrial habitat (Godfrey and Wooten 1979). In aquatic systems, the plant would produce large mats composed of hollow plant stems that would severely impact the use of the waterway.

In 1960, a USDA laboratory was established in Argentina as part of the cooperative effort of the USDA and U.S. Army Corps of Engineers to develop biocontrol agents for alligatorweed (Coulson 1977). During the initial surveys, over 40 insects were found that feed on alligatorweed. As testing progressed, the number of potential agents was reduced to five insects (Vogt 1960, 1961; Maddox et al. 1971). Additional testing reduced the number of possible insect biocontrol agents to three (Maddox et al. 1971), and all were petitioned for release.

The first insect released was the alligatorweed flea beetle (Agasicles hygrophila). In 1964, initial releases were made in California and South Carolina (Coulson 1977; Cofrancesco 1988). This insect has a short life cycle

of 30 days, and both the adults and larvae feed on alligatorweed. The impact to populations of alligatorweed by this insect occurred rapidly and the insect was eventually released in 11 states (Cofrancesco 1988).

The next insect released in the United States as a biocontrol agent of alligatorweed was the alligatorweed thrips (*Amynothrips andersoni*). Initial releases were made in 1967 in California, South Carolina, Florida, and Georgia (Coulson 1977). This insect has a life cycle of approximately 28 days (Maddox and Mayfield 1979), and both adults and larvae feed on the plant with their sucking mouthparts. The feeding insect causes the alligatorweed leaves to dry and curl; however, the impact of this agent has not been widespread throughout the United States, even though it was released in seven states (Cofrancesco 1988).

The last insect biocontrol agent released for alligatorweed was the alligatorweed stem borer (*Vogtia malloi*). The first releases of this insect were made in 1971 in Florida, Georgia, North Carolina, and South Carolina. The insect's life cycle is approximately 39 days, and only the larvae feed on the plant. The feeding process begins at the apical portion of the plant where the larvae hatch and bore into the hollow stem (U.S. Army Corps of Engineers 1965). The impact caused by this insect is significant especially in the northern range of alligatorweed. This insect was only released in five states; and in a 1981 survey it was found widely distributed in seven states (Cofrancesco 1988).

At the present time, only North Carolina has a small program to treat alligatorweed with herbicides in ditches. All the other states rely on the biocontrol agents to provide enough impact to keep the population level of alligatorweed below problem levels. In 1963, there were over 97,000 problem acres of alligatorweed in the United States; but in 1981, there were less than 1,000 problem acres of alligatorweed (Cofrancesco 1988).

The second plant targeted for biocontrol technology was waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), an aggressive floating plant species native to South America. Waterhyacinth was introduced into the United States at the 1884 Cotton States Exposition, New Orleans, LA (Sanders,Theriot, and Perfetti 1985). Since its introduction, waterhyacinth has spread or been distributed throughout the southern United States and California (Godfrey and Wooten 1979). The ability of waterhyacinth to infest a wide range of freshwater habitats and its tremendous growth rate (Penfound and Earle 1948; Center and Spencer 1981) have made it one of the most troublesome aquatic plants in the United States.

Overseas surveys were conducted in South America to find potential biocontrol agents of waterhyacinth. A number of insects were found that feed on waterhyacinth in its native range, and studies were initiated to determine which insects would be good biocontrol agents. These studies were conducted at the USDA laboratory in Argentina. Three potential insect biocontrol agents were identified and introduction permits were requested (Sanders, Theriot, and Perfetti 1985).

In 1972, the mottled waterhyacinth weevil (*Neochetina eichhornia*) was the first insect approved for release as a biocontrol agent of waterhyacinth. The initial releases were conducted in Florida; however, this insect has now been released in four other states. Both the adults and the larvae feed on the plant. Adults remove the upper leaf surface, and larvae penetrate the petiole and feed on internal tissues. As the larvae grow, feeding proceeds down the petiole to the plant crown. The generation time ranges from 90 to 120 days depending on temperature and other factors (DeLoach and Cordo 1976a). These insects stress the plant; however, their true impact to the plant population takes years to become apparent.

The second biocontrol agent released on waterhyacinth was another weevil, the chevroned waterhyacinth weevil (*Neochetina bruchi*). The first release of this insect occurred in Florida in 1974 (Sanders, Theriot, and Perfetti 1985). The chevroned waterhyacinth weevil occupies very similar habitats in the

plant to the mottled waterhyacinth weevil, and its impact to the plant is similar. The chevroned waterhyacinth weevil has a shorter generation time (60 to 90 days) than the mottled waterhyacinth weevil (Deloach and Cordo 1976b).

The last biocontrol agent released on waterhyacinth in the United States was the Argentine waterhyacinth moth (*Sameodes albiguttalis*) native to South America. The initial release was made in Florida in 1977. This insect has a life cycle of approximately 30 days (Deloach and Cordo 1978; Center 1981a). The larvae are the only life stage that feeds on the plant, and they are usually found on the smaller more bulbous plants (Center 1981b). The impact caused by these insects varies between sites; often, well-established populations of these insects will move from locations for no apparent reason (Sanders, Theriot, and Perfetti 1985).

In general, problem areas of waterhyacinth still exist, and chemical spray control operations continue. The biocontrol insects are having a significant impact on the waterhyacinth populations, but this is occurring in areas where the insect population levels are allowed to build. Most of the impact that has been documented has been attributed to the weevils. These insects have longer life cycles, so population buildup is slow. Dramatic declines in the acreage of waterhyacinth have occurred in Louisiana, where prior to the insects being released, the acreage of waterhyacinth reached 1.2 million acres. Presently, there are only 200 to 300 thousand acres in the state (Cofrancesco 1985). Similar declines were also noted in Florida and Texas. Although waterhyacinth problems still exist, the biocontrol agents are stressing the plants; and research is underway to develop better management procedures for these agents (Cofrancesco 1987).

Another problem aquatic plant that has been studied is waterlettuce (*Pistia stratiotes*). This plant is distributed mainly in the southeastern United States and has presented problems in areas where waterhyacinth populations are declining (Dray, Center, and Habeck 1989). In addressing this problem, researchers built upon work conducted by the Australians, who had a management program for waterlettuce for a number of years.

The first insect released on waterlettuce was *Neohydronomus affinis*, a weevil native to South America that the Australians have been using since 1982 (Julien 1987). Additional testing of this insect was conducted prior to its release in 1987 in Florida (Dray et al. 1990). The adults feed and penetrate the leaf while the larvae mine inside the leaf (Thompson and Habeck 1989). The insect's life cycle is approximately 30 days, which allows its population to develop rapidly (Habeck et al. 1988). Eighteen months after the release of the weevils at a site in Lake Okeechobee, FL, the entire 75-acre mat of waterlettuce was eliminated. Weevils began to migrate to adjacent control plots prior to the elimination of the test site (Center and Dray 1990).

Another insect, *Namangana pectinicornia*, a moth from Thailand, has been released in the United States as a biocontrol of waterlettuce. The releases were conducted in February 1991 in Florida. This moth has a short life cycle of approximately 35 days. Only the larvae feed on the plant; however, the damage that is caused is extensive with feeding occurring on the upper and lower leaf surface and at times girdling the leaves.

Biocontrol research has also been conducted on submersed aquatic plants. Research has been conducted using both insects and pathogens to manage hydrilla and Eurasian watermilfoil. Hydrilla is a submersed aquatic plant that clogs waterways and impedes navigation (Schardt and Schmidtz 1989). The plant was introduced into the United States by business as a plant for fish aquariums. The plant has spread rapidly throughout the southern United States and along the east coast as far north as Delaware; in addition, populations of the plant are found in California. Research began in 1980 to determine the area of origin for this problem plant. Surveys were conducted throughout Africa, Australia, and parts of Asia (Balciunas 1982, 1983, 1984, 1985, 1987; Balciunas and Dray 1985). Biocontrol agents

were most abundant in India and Australia. In 1984, the USDA established a research facility in Australia with the support of the Corps of Engineers.

The first insect released on hydrilla was a weevil (*Bagous affinis*) from Pakistan. Releases were made in 1987 in Florida (Center 1989; Center and Dray 1990); however, this insect feeds on the tubers of the hydrilla plant when water has receded from the plants (Buckingham and Bennett, In Preparation). While this situation is common in Pakistan, it is very uncommon in Florida except when lakes are drained. This type of life cycle has made it difficult to establish field populations. This insect is being used in the canal systems in California, which have annual periods of drawdown. Initially, problems occurred in establishing field protection; however, by mid-1990, field populations of the weevil were established in Florida (Center and Dray 1990).

The second insect released in the United States as a biocontrol of hydrilla is *Hydrellia pakistanae*, an ephydrid fly from Pakistan. This insect was released in 1987 in Florida. The larvae mine the leaves, and are the only life stage of the insect that impacts the plant. The life cycle is short, approximately 20 days (Center and Dray 1990). Insect populations have established and are widespread in Florida.

The third biocontrol insect released in the United States for hydrilla was *Hydrellia balciunasi*, an ephydrid fly from Australia. This fly causes damage similar to that of *H. pakistanae*. The first field release of this insect occurred in September 1989 in Broward County, FL (Center and Dray 1990).

Additional insect biocontrol agents are being studied as biocontrol agents of hydrilla. A weevil from Australia (*Bagous* n. sp.) was released in Florida and Georgia in 1991. Studies are also being conducted on moth and midge larva, which are potential biocontrol agents (Center and Dray 1990).

Pathogens have also been explored as biocontrol agents of hydrilla. A fungal pathogen associated with hydrilla populations in Texas has demonstrated potential as a biological control agent for hydrilla (Joye 1990). Additional testing on host specificity and the development of a commercial formation is still needed.

Eurasian watermilfoil is the most extensive aquatic problem plant in the United States. It has been reported from over 30 states. Biocontrol research on this plant dates back to 1967 with work in Yugoslavia. The major emphasis has been pathogen to control this plant because many of the European insects were already found in the United States.

A fungus, *Mycoleptodiscus terrestris*, was isolated from plants in western Massachusetts prior to 1979 (Gunner 1983). Testing at the University of Massachusetts funded by the Corps of Engineers indicated that this pathogen had potential as a biocontrol agent of Eurasian watermilfoil. At the present time, EcoScience Laboratories is developing a commercial formulation of this product.

Purple loosestrife (*Lythrum salicaria*) is one of the most severe problem plant species in the wetland habitat. This plant was accidentally introduced from Eurasia and has increased exponentially across the North American wetlands. In some areas, purple loosestrife has replaced over 50 percent of the native vegetation and has produced large changes in habitat value. At present, it has been reported from all states north of the 35th parallel except Alaska. Three insect biocontrol agents have been approved by the USDA-APHIS-PPQ for release on purple loosestrife. The first releases were made in 1992 at four nursery sites across the United States, and insects should be ready for dispersal in 1993.

Melaleuca quinquenervia (Cav.) Blake (also known as paperbark, punk tree, cajeput) is a large, woody plant introduced into Florida from Australia in the early 1900s. It is well adapted to the flooded, saturated soils of Florida's Everglades, but also successfully invades drier habitats when sufficient water is available. *Melaleuca* infests over 13 percent of the wetlands that are vital for recharge and

water storage in southern Florida. It also displaces estuarine species such as red and black mangroves. This tree grows rapidly, produces millions of viable seeds, and is extremely resistant to fire. It has been projected that *melaleuca* will cover two-thirds of all Everglades wetlands in Florida by the year 2000. These species, along with others such as Australian Pine, Brazilian pepper, *Mimosa pigra*, and Chinese Tallow, pose a severe threat to the remaining United States wetlands.

Over the last 33 years, the United States research efforts on biological control agents of aquatic and wetland plants have been conducted on six continents, in over 30 countries, and has involved eight overseas laboratories. To date, 10 biocontrol agents have been released on four plant species. Some or all of the agents have been established in 11 states. Five more agents are in quarantine or overseas research laboratories. Three endemic pathogens have also been identified, and they are at various stages of testing. In addition, 11 other countries have utilized the technology United States researchers have developed in biocontrol of aquatic and wetland plants.

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Biocontrol of Hydrilla And Milfoil Using Plant Pathogens

by
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Pathogens on Eurasian Watermilfoil

The pathogen biocontrol program for *Myriophyllum spicatum* L., Eurasian watermilfoil, has for the past 12 years been a joint effort between the U.S. Army Engineer Waterways Experiment Station (WES) and a private company, EcoScience Corporation of Worcester, MA. In the late 1970s, an endemic pathogen, *Mycoleptodiscus terrestris* (Gerdemann) Ostazeski, (Mt), was isolated from milfoil collected in Massachusetts (Gunner 1983). The company since that time has been responsible for development, strain improvement, and formulation of the fungus into a mycoherbicide, Aqua-Fyte. WES has been responsible in part for testing different formulations of the fungus in the laboratory and in field trials.

In a series of temperature tests, a calcium alginate pellet formulation of *M. terrestris* was evaluated for its effect on milfoil grown in 150-cm-tall by 13.75-cm-diam tubes. Optimum application time of the mycoherbicide was determined to be when water temperature reached at least 20 °C but did not exceed 30 °C. The most significant reduction in aboveground biomass was achieved in laboratory tests at 20 and 25 °C (Shearer 1992). Although the fungus grows vigorously on standard laboratory media at 30 °C, its impact on milfoil at 30 °C was difficult to quantify because the plant naturally senesces and fragments at high-water temperatures.

The most serious drawback to a spheroid-shaped formulation was the inability to remain attached to vegetative tissues of the plant. The pellets tumbled through the water column until coming to rest on the sediment surface. In a field situation, the problem would intensify by water currents passing through plant beds dislodging the pellets from the vegetation. Because *M. terrestris* is a contact pathogen, a period of residence time on plant tissues is imperative to initiate a disease epidemic.

EcoScience modified the calcium alginate formulation into a string shape in the spring of 1991. Theoretically, strings approximately 2 by 15 mm would become better entangled in the milfoil vegetation and increase contact time of the pathogen with the host plant. Observational studies of applications made to test plots in a milfoil-planted pond at the USACE Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX, in the spring of 1991, indicated a string formulation performed better than pellets in terms of plant coverage. An increase in coverage and contact time should enhance pathogen performance. It was noted at the time that neither formulation produced a plant disease epidemic in the test plots, and there were no visible discernable differences between the formulations regarding their impact on the target plant. The trials would seem to indicate there were serious problems with the mycoherbicide either with the formulation matrix or with the viability and virulence of the fungus itself.

In December 1991, EcoScience received an Experimental Use Permit from the U.S. Enviornmental Protection Agency allowing application of the mycoherbicide to bodies of water greater than 1 acre. A permit to transport and apply the fungus in Alabama was approved by Alabama state officials and the U.S. Department of Agriculture, Animal Plant Health Inspection Service in the late fall of 1991.

Four replicate test plots were set up on a 62-acre milfoil-infested pond at Tennessee

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Valley Authority's Guntersville Reservoir Aquatic Ecosystem Facility, Guntersville, AL, in the summer of 1992. The string formulation was applied to the plots at a rate of 70 lb active ingredient (dry wt)/acre in early July after the milfoil had grown to the water surface and topped out. Biomass samples were collected prior to the mycoherbicide application and 1 month postapplication. Additional plant samples were collected to determine the number of fungal colony forming units (CFU's) of *M. terrestris* that colonized milfoil plant tissues.

One month postapplication, there were no visible differences between the Aqua-Fyte-treated plots and the untreated controls. One would have expected the treated plots to be noticeably free of topped-out milfoil at the water surface. The effects were the same as had been noted at Lewisville the previous year. Collected plant samples confirmed there were no significant differences in aboveground biomass between the control and Aqua-Fyte-treated plots. Laboratory analysis revealed the fungus had not become well established in milfoil stem tissues.

The failure of the mycoherbicide to control milfoil in the field test could be attributed to problems with the fungus and/or the formulation. The fungus is known to be a weak pathogen, and the strategy with this particular mycoherbicide was to overwhelm the plant with a high-dose rate to achieve a quick and massive dieback. The viability and virulence of the fungus could well account for poor field performance. The use of a pellet or string formulation in aquatic systems had been in question because contact time and retention rates of the mycoherbicide on submersed vegetation had been poor. Innovations used in formulations for terrestrial weed control cannot be assumed to be equally effective for aquatic weed control. Formulation technology specific for packaging microbes for delivery in aquatic systems must be forthcoming before widespread success using plant pathogens can be achieved for submersed aquatic weeds.

The attributes of the mycoherbicide alone may not completely account for the lack of success in field trials. Physical, chemical, and biological factors in aquatic systems can alter the effectiveness of a biological control agent. The competitive nature of the native microflora on plants in aquatic systems has not been well researched. Resident microflora could inhibit or attack the applied microbe and significantly reduce its effect. More field applications and subsequent research will need to be undertaken to assess the effects of biocontrol pathogens in aquatic systems.

Pathogens on Hydrilla

A pathogen identified as *Macrophomina phaseolina* (Tassi) Goid. was isolated from *Hydrilla verticillata* (L. fil.) Royle (hydrilla) collected at Lake Houston, TX, in 1987. Identification of the organism was tentative because the isolate had never been induced to sporulate on laboratory media. The fungus in a preliminary series of tests proved to be one of the most effective of the potential mycoherbicides for use on submersed plants (Joye 1990).

In 1991, during a series of platings of the pathogen onto excised plant tissue of hydrilla, sporodochia and asexual spores developed on diseased areas of the stem where the fungus had been applied. The spores were characteristic of *Mycoleptodiscus terrestris* (Gerdemann) Ostazeski. Subsequent plating of all isolates labeled *M. phaseolina* preserved in cryovials, test tubes, or agar plates at WES proved to be *M. terrestris*. The identification was confirmed by Dr. B. C. Sutton at the International Commonwealth Institute, Kew, Surrey, England. Misidentification of these two organisms is commonly made because isolates look much alike in culture, and it is only when sporulation occurs that a positive identification can be made. Ostazeski (1967) noted that the appearance of the sclerotia of *M. terrestris* were suggestive of those generated by *M. phaseolina*.

A real concern was that the original isolate was a strain of *M. phaseolina*, and it had become contaminated with *M. terrestris* since both organisms were being concurrently tested in the biocontrol laboratory. The isolate

labeled *M. phaseolina* was grown from agar plugs preserved in cryoculture. When applied to hydrilla, results were similar to those described by Joye (1990). As an additional check, plant material was collected at Sheldon Reservoir near the field test site described in the 1990 paper. Isolations made from stem tissue of hydrilla produced 25 cultures that were positively identified as *M. terrestris*. These isolates were highly pathogenic on hydrilla.

The *M. terrestris* isolates from Sheldon Reservoir applied at high rates as a mycelial matrix destroy aboveground biomass of hydrilla in 10 to 14 days. In one preliminary test, an application rate as low as 160 CFUs/ml produced disease in laboratory-grown hydrilla. Application rates are presently being tested to determine titers for efficacy.

Future Research

The effectiveness of endemic pathogens developed for biocontrol use on hydrilla and milfoil in field tests must be determined. While it has been established that fungal inoculum will destroy aboveground biomass of hydrilla and milfoil in laboratory experiments, duplication of results must be achieved in the field, or determinations must be made that explain reduced effectiveness in the field. Concentration on the development of a formulation that will work effectively in aquatic systems needs to be initiated. The task may prove difficult and of long duration because formulation research to date has totally concentrated on delivery systems specific for terrestrial weed problems and has ignored the problems presented by an aqueous environment.

Biocontrol effects of insects and pathogens on hydrilla and milfoil have been independently studied. Preliminary research has been initiated at WES to assess synergistic effects of insects and pathogens on target plants. Efforts for biocontrol of submersed aquatics using a combination of insects and pathogens may prove much more effective than either organism used alone.

It is envisioned that WES should be the site of a repository for pathogens of aquatic plants. Without expending any additional funds, the requisite facilities and expertise to initiate development of a repository are already in place in the biocontrol laboratory. WES is an ideal location because it is a facility that is well recognized for its ongoing research addressing various aspects of control of aquatic weeds.

Diseased plants are routinely brought back from the field or sent to WES by field personnel, and isolations of disease-causing organisms are made in laboratory facilities on station. Plant pathogens are identified and deposited in a cryofreezer for long-term storage. By preserving innumerable strains of aquatic plant pathogens, future efforts for biocontrol of aquatic plants will be enhanced as new and improved mycoherbicides are developed.

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Pathogen Biocontrol Research for Aquatic Plant Management

by
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Background

Research on the utilization of plant pathogens as a tool for management of problem aquatic plants began in the early 1970s. Unexplained declines had occurred in the United States that indicated plant disease organisms could be impacting these plants. Initial research efforts under the Aquatic Plant Control Research Program (APCRP) dealt only with endemic opportunistic fungi in an attempt to isolate the causative agents of these declines. It is also true that working with endemic organisms poses very little risk of impacting nontarget species, and restrictions on the introduction of exotic pathogens were extensive and prohibitive.

Endemic plant pathogens did not need the approval of the Working Group on Biological Control of Weeds (WGBCW), a quarantine facility, or overseas cooperators. In contrast, exotic plant pathogens required the approval of the WGBCW (no approval had ever been given), testing in a pathogen quarantine facility (which was not available at the time in the United States), cooperation with overseas scientists, and coordination with the U.S. Environmental Protection Agency (EPA) and the U.S. Department of Agriculture (USDA).

Endemic Pathogens

The first endemic pathogen studied as a potential biocontrol agent for a problem aquatic plant was *Cercospora rodmanii*. The fungus was isolated in 1971 from waterhyacinth collected at Lake Rodman, FL, by researchers from the University of Florida under contract with the U.S. Army Engineer Waterways Experiment Station (WES). Testing was conducted to determine efficacy of the fungal pathogen to waterhyacinth. WES relinquished the rights to the organism, and the University of Florida applied for and received a patent for this potential biocontrol agent. Laboratory and greenhouse studies were subsequently conducted to determine the impact of this fungal pathogen on other plants growing in association with waterhyacinth. Abbott Laboratory was given the rights to commercially develop a formulation of the pathogen. WES worked cooperatively with Abbot Laboratory to evaluate the commercial formulation. A small-scale field test was conducted with the formulated fungus in 1980 in Louisiana. Infection was observed on waterhyacinths; however, the formulation had a short shelf life and low viability. In 1981 and 1982, large-scale field tests were conducted in Louisiana to establish the proper treatment rate of the modified formulation. The new formulation was much more homogenous, but the infection rate was extremely low. Further modifications of the Abbott Laboratory formulation were promised, but none were forthcoming.

In 1979, researchers at the University of Massachusetts isolated a fungal pathogen from milfoil. The Corps of Engineers (CE) APCRP supported the development of this potential biocontrol agent. The fungal pathogen was identified as *Mycoleptodiscus terrestris* (Mt). A joint testing program of the pathogen on Eurasian watermilfoil was initiated between the University of Massachusetts and WES. The pathogen appeared generally specific for milfoil, and impact to plants in laboratory and greenhouse tests was significant. The University of Massachusetts gave the rights to the organism to EcoScience

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Corportation laboratory. The laboratory was to research and develop a formulation and the CE would test the formulation in laboratory, greenhouse, and field studies.

To meet Federal requirements, field testing was restricted to enclosed systems less than 1 acre. Ponds at the USACE Lewisville Aquatic Ecosystem Research Facility were utilized and treatments were applied. The shallow ponds accumulated heat rapidly, and water temperatures exceeded the temperature tolerance limits of the pathogen. As a result, no significant differences were observed between the treated and control plants. A fall treatment of the pathogen was applied to milfoil in the Lewisville ponds in 1991. Sampling the following spring indicated the pathogen could overwinter in plant tissue. Modifications of the formulations to improve contact time were recommended to EcoScience; however, these modifications have not yet occurred.

New Federal regulations require researchers to obtain an Experimental Use Permit (EUP) to conduct a large-scale field test. Data were compiled by EcoScience and WES, and EcoScience submitted documentation to the EPA. After a number of meetings, EPA issued an EUP for the use of *Mycoleptodiscus terrestris* as a potential biocontrol agent of Eurasian watermilfoil. Permits were requested from the USDA-Animal Plant Health Inspection Service-Plant Protection and Quarantine (USDA-APHIS-PPQ) to move a commercial formulation of Mt into Alabama and Oregon and conduct releases in lakes where high-water temperatures would not adversely affect the pathogen. Scientists and cooperators in Oregon prepared an Environmental Assessment, obtained state permission, and prepared a Finding of No Significant Impact that was signed. Researchers in Alabama obtained state permission for the release of Mt. The USDA-APHIS-PPQ approved the release of Mt in Alabama but not in Oregon. The rationale was that Mt had been isolated from plants collected in Alabama but had not been documented as occurring in Oregon. The Alabama test was initiated in July 1992. Surveys are being conducted in Oregon to determine if Mt occurs in the state.

The CE surveyed populations of *Hydrilla verticillata* for disease-causing organisms, and a number of pathogenic candidates were identified. Laboratory testing was conducted to evaluate the impact of the pathogens and determine their specificity. The most promising organism, identified initially as *Macrophomina phaseolina*, was utilized in laboratory and small-scale greenhouse studies to evaluate the impact on hydrilla. The fungal pathogen significantly reduced plant biomass by as much as 80 percent. Small-scale field testing was conducted at Lake Sheldon, TX, in 1989. Mycelium of the fungus was cultured for WES at the USDA fermentation laboratory in Peoria, IL. After 1 month, a two-thirds reduction in hydrilla biomass occurred in the treatment areas. Microscopy studies were undertaken to document the mode of entry of the pathogen into the plant. Questions were raised regarding the identification of the fungus, and a reevaluation of the organism confirmed that it was not *Macrophomia phaseolina* but *Mycoleptodiscus terrestris*. Presently, studies are being conducted to determine if the fungal pathogen impacting hydrilla is a different pathovar of the agent impacting Eurasian watermilfoil.

The lack of success thus far in the development of endemic pathogens is more a reflection of potential profit margins rather than technical potential. Endemic pathogens that impact problem plants can be effective and can be developed as bioherbicides, based on successes in terrestrial efforts. However, often, companies that would be interested in commercially formulating the pathogens hesitate to incur the increased cost of registration in light of the limited potential market. It appears that the research agency should maintain control of potential agents through development of the formulation. This will allow completion of a field-useable formulation, reducing the cost for registration and making the venture more attractive to potential investors.

Exotic Pathogens

In addition to the development of endemic plant pathogens as biocontrol agents of problem plants, exotic plant pathogens are also

being given consideration. Many of the problems that were prohibitive in 1970 to the use of exotic pathogens to control aquatic plants are no longer valid. The Technical Advisory Group has approved the release of exotic plant pathogens; the United States has a pathogen quarantine research facility located at Fredrick, MD, and many overseas cooperators are available to assist in the research. The utilization of exotic pathogens follows the same procedures outlined for the successful use of exotic insect agents. Because exotic pathogens would be isolated from targeted plants in their native range, a major benefit would be the potential for host specificity in their impact. In addition, the CE is working cooperatively with at least three overseas USDA laboratories on searches for insect biocontrol agents. We could leverage our efforts to include searches for pathogens for a minimal increase in funding.

Recommendations

Based on the material outlined above, it has become clear that the pathogen portion of the biocontrol program should be restructured and the following changes are recommended:

- The CE take more responsibility for the development of the final formulation or work with industry under a Cooperative Research and Development Agreement to do so.
- Exotic plant pathogens be included in the arsenal for the development of an overall management program for aquatic plants.
- WES develop the capability as a national repository for aquatic plant pathogens.

Foreign Research on Insect Biocontrol Agents

by
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The discovery of hydrilla (*Hydrilla verticillata* (L. fil.) Royle) near Washington, DC, in 1982 and subsequent concern about its potential invasion into other northern locations led to the initiation of a cooperative project in the People's Republic of China for surveys and research on insects that attack hydrilla and Eurasian watermilfoil (*Myriophyllum spicatum* L.) in temperate climates. Our cooperators in China are in the Sino-American Biological Control Laboratory (SABCL), which is part of the larger Biological Control Laboratory at the Chinese Academy of Agricultural Sciences (CAAS), Beijing. Four rooms in the basement of the Biological Control Laboratory are reserved for the SABCL, which also has projects on terrestrial weeds and insect pests. Dr. Wang Ren is in charge of the SABCL. We have had almost annual changes in personnel assigned to our project, but this past summer a newly graduated entomologist was hired specifically for our project. Mr. Chen Zhi Qun studied English at the University, and he improved rapidly throughout the summer by speaking with us. He is enthusiastic and is interested in establishing a career in this field. During the winter, he will take intensive English lessons.

Dr. Joe Balciunas from the U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS) laboratory, Townsville, Australia, began surveys in China in 1989. He continued in 1990 and 1991 with my help. This year he left the project, and I was joined by Ms. Christine Bennett, University of Florida, Gainesville. Our Chinese colleagues at SABCL during these years were Dr. Wang Ren, Dr. Chen Ping-Ping, Mr. Wang Yuan, Ms. Jiang Hua, Mr. Chen Zhi Qun, Ms. Liu Wei-Zhen, Mr. Fan Zhong-nan, Mr. Yan Ming, and Mr. Han Nan-Ping. We also had a myriad of cooperators throughout China who assisted the field surveys. Although it has not been possible to survey all areas of China, representative areas have been surveyed throughout the country. Provinces visited include Guangdong, Heilongjiang, Hubei, Hunan, Inner Mongolia, Jiangsu, Liaoning, Sichuan, and Xinjiang.

The most important potential biocontrol agents discovered were two species of leaf-mining *Hydrellia* flies, stem-tip-boring midges and a stem-boring Bagous weevil on hydrilla and one or two species of seed-eating *Phytobius* weevils and stem-tip-boring midges on Eurasian watermilfoil. The two *Hydrellia* flies have been colonized in our Gainesville, FL, quarantine facility since 1990; and one of them, *H. pakistanae*, was released from quarantine in 1991.

The goals for this summer were (a) to confirm the host relationship of a *Bagous* weevil, whose larvae were found associated with hydrilla collected in 1991 at Harbin, Heilongjiang Province, north of Beijing on the Russian border, (b) to survey hydrilla and Eurasian watermilfoil near Harbin for additional insects, (c) to survey hydrilla and milfoil near Shenyang, Liaoning Province, north of Beijing, (d) to establish the field host range of a hydrilla leaf-miner *Hydrellia* n. sp. silver-face, near Beijing, and (e) to extend the surveys to Japan.

I arrived in Beijing at the end of June and spent the first week of July collecting hydrilla and other submersed species at our previous sites in and around Beijing. The material was searched visually for larvae and pupae of *Hydrellia* that were held for emergence. From 8-13 July, I surveyed near Harbin accompanied

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by Mr. Chen Zhi Qun, SABCL, Mr. Han Nan-Ping, CAAS, our interpreter, and Dr. Robert Pemberton, USDA/ARS Asian Parasite Laboratory, Seoul, Korea, who has a project on biocontrol of water chestnut, *Trapa natans* L. Our hosts in Harbin were Professor Chen Tie-bao, Plant Protection Institute of Heilongjiang, CAAS, and his staff. We were unable to find the small lake where the *Bagous* larvae were found in 1991 associated with hydrilla. However, we collected in various water bodies in the immediate area without finding *Bagous* larvae or adults. Feeding damage similar to that of *Bagous hydrillae* O'Brien (= *Bagous* n. sp. Z) adults from Australia was observed on the leaves, but that damage could not be distinguished definitively from damage by snails or by *Hydrellia* leaf-miners that were present. Species of Bagous were observed on plants associated with hydrilla, and it is possible that the larvae associated with hydrilla in 1991 were contaminants from other plants.

Our survey proceeded west of Harbin to QiQiHar through grasslands that extend to Inner Mongolia. Milfoil in Shi-er-li Pao marsh near Wo-Li-Tun was infested with larvae, pupae, and a newly formed adult of *Bagous* sp., a weevil that I had discovered in Kashmir, India, in 1985 but had been unable to obtain. This weevil was the target of my unsuccessful survey in the western province of Xinjiang in 1991. Larvae bore in the stems well below the waterline. Damaged stems become semi-transparent. The entire life cycle of this species appears to be spent in the water, unlike the hydrilla weevil *Bagous hydrillae*, which pupates on shore. Adult feeding, presumably by this weevil, was even found on short basal shoots about 1.5 m below the water surface. Milfoil flower stalks at this and other sites were attacked by *Phytobius* sp. weevils. These weevils have been collected at sites throughout China since 1989. Adults and larvae feed on the flowers and seeds, and the larvae also bore into the stems beneath the flower stalks where they pupate. One or two species attack *M. spicatum* and one species attacks *M. verticillatum* L. One specimen of a related weevil, *Eubrychius* sp., was collected on submersed stems of *M. verticillatum*. It might be the same species collected in 1991 in Xinjiang Province.

From Harbin, we traveled by train on 14 July to Shenyang, Liaoning Province, where our hosts were Professor Guan Guang-qing and his staff from Shenyang Agricultural University. Dr. Pemberton returned to Beijing from Harbin. We initially surveyed sites near Shenyang that I had visited in 1990 with Professor Guan. *Phytobius* sp. was found in small numbers on stems of *M. spicatum*, which were not flowering, and small populations of *Hydrellia* spp. were mining hydrilla leaves. Leaf beetles were collected on *Trapa* for Dr. Pemberton. At two sites along a drainage canal, hydrilla was attacked by grub-like larvae of an unidentified donaciine leaf beetle. Larvae were attached to roots and basal portions of stems that had new growth with very small leaves. Internodes near the stem tips were very compact. The plants were in shallow water nearshore. An uninfested site in the same canal between the two infested sites had lush normal-looking hydrilla. Those were the only two sites where I have found that type of hydrilla growth, which suggests that the larvae were causal agents. Unfortunately, no adult beetles were found and no larvae pupated even though they lived on moist hydrilla for several weeks in the Beijing laboratory.

We flew to Beijing on 18 July with plant samples and the live insects collected during our survey. Some plant samples were examined visually at the laboratory, and others were placed into a heat extraction funnel to collect insects that fled from the plants as they slowly dried. We also air dried plants on screens on sunny days in an attempt to collect insects in water containers beneath the screens. Usually 1 to 2 hr of collecting in the field required at least 1.5 days of visual examination by several persons in the laboratory to process the plant material.

Ms. Chris Bennett arrived in Beijing on 20 July, and with SABCL personnel we collected and processed hydrilla and associated plant species from nearby sites until I left for

Japan on 27 July. Hydrilla was collected to obtain *Hydrellia* flies that Chris would carry with her to Gainesville. Associated plants were collected to determine if *Hydrellia* n. sp. silver-face attacked them. These included species of *Potamogeton* (6 sp), *Vallisneria*, and *Najas* (2 sp). Sites were the same ones discussed in previous reports: "August 1st" Lake, Tsing Hua University drainage canal, Qiao Zhuang drainage pond, Sleeping Buddha Park lotus pond, and San Jia Dian reservoir. Chris and SABCL personnel continued weekly sampling until 14 August when she returned to Gainesville. Emergence from hydrilla is greatly underestimated because the majority of larvae and pupae were placed in colony-rearing jars without recording daily emergences and deaths. Obviously, hydrilla is the host plant of *Hydrellia* n. sp. silver-face, but flies did emerge from other species. Usually numbers this small indicate only incidental attack, especially considering the many hours of visual examination of each plant species that was necessary to collect the flies. Unfortunately, many immatures died or produced parasites instead of flies. Because we do not know the species of those immatures, we do not know if the true numbers of *Hydrellia* n. sp. silver-face were actually higher.

The hydrilla stem-tip-boring midges were present at the Beijing sites again this year. Because their numbers were small and because we concentrated on *Hydrellia*, we did not study them.

In Japan, I surveyed two lakes near Misawa and two near Niigata. Hydrilla was found at only one site, and Eurasian watermilfoil was not found. The hydrilla was undamaged, but leaf beetles were found on *Trapa* sp. I then met Dr. Pemberton in Kobe on 2 August where we surveyed for 1 week with Professor Y. Kadono, a botanist at Kobe University. Professor Kadono is an aquatic plant specialist who was able to lead us to sites with our target plants. Hydrilla was attacked by two species of *Hydrellia*, which are probably the same species as in China, although one might be a new species and a stem-tip-boring midge. Eurasian watermilfoil was found at two sites in rivers, and the only associated insects were caddis flies.

Additional foreign research was conducted this year in Australia, where Dr. Dale Habeck, University of Florida, Gainesville, studied several hydrilla moths. The moths had been studied earlier by Dr. Balciunas, who suggested that additional field studies be made and that the moths be imported into quarantine. As a result of his studies, Dr. Habeck sent eggs of *Aulacodes siennata* Warren and *Nymphula eromenalis* Snellen into quarantine in August for biology and host range studies.

Future Studies

China will again be the focus of our foreign research program. Attempts will be made to obtain a *Bagous* weevil that has been reported to spend its entire life cycle in submersed stems of hydrilla. It would complement the introduced Australian stem-borer *B. hydrillae* O'Brien, which pupates onshore. Additional germ plasm of the milfoil stem-borer *Bagous* n. sp. will be collected near Harbin and its field biology and host range studied. Attempts will be made to collect adults of the donaciine leaf beetle larvae on hydrilla roots at Shenyang and to study the field host range. We are hoping that Mr. Chen Zhi Qun, SABCL, will be able to visit Gainesville for training during winter, so that on his return he will be able to conduct additional field studies in Beijing with the hydrilla leaf-mining flies and the hydrilla stem-tip-boring midge.

Quarantine Biocontrol Operations

by
Christine A. Bennett[1]

Introduction

The aquatic weed biological control quarantine program is located at the Florida Biological Control Laboratory, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, FL. This quarantine facility was built in the early 1970s through a cooperative agreement with the Florida Department of Agriculture, U.S. Department of Agriculture, and the University of Florida. Personnel from all three agencies use these facilities (Figure 1) working with biological control agents of citrus pests, mole crickets, filth flies, and aquatic weeds.

Figure 1. Biological control quarantine operations staff. Left to right: Billy Talton, Robert Lowen, Christine Bennett, Yuvora Nong, and Jason Etchart

The majority of our aquatic weed work as in past years has concentrated on the submersed plant *Hydrilla verticillata* (L.f.) Royle. Since 1982, two weevils and two ephydrid flies have been imported into the Florida Biological Control Laboratory, tested, cleared, and released against hydrilla. The lab continues to import new germ plasm of these agents.

Studies continued this year with insects from temperate climates in China, as hydrilla continues to spread into colder regions of the United States. Studies also continued with Australian insects when two species of Australian moths that feed on hydrilla in streams and rivers were imported into quarantine. Stem-tip damage of hydrilla at Crystal River, FL, was investigated.

A new project was started on another submersed weed, Eurasian watermilfoil. A stem-boring weevil was imported from China into quarantine. Work was also started on the tree *Melaleuca quinquenervia* Cav. S.T. Blake, which is fast becoming a major problem in South Florida. Two insects from Australia were imported into quarantine.

Hydrilla Leaf-Mining Flies

This year we have continued to conduct laboratory host range and biology studies with the Chinese and Indian populations of *Hydrellia* n. sp. silver-face that our cooperator Dr. D. Deonier, the foremost taxonomist in this group, has labeled CH 1. Even though Dr. Deonier has tentatively identified these populations as the same species, we continue to study both because of host range and biology differences. These flies are close relatives of the two *Hydrellia* species that have been released previously as biological control agents of hydrilla in the United States. We are especially interested in the Chinese population, because it comes from a cold climate and might be more effective in the colder areas of hydrilla's range than the Indian *H. pakistanae* Deonier released in 1987.

The adult flies are similar in appearance to the Australian fly *Hydrellia balaciunasi*

[1] University of Florida, IFAS, Department of Entomology and Nematology, Gainesville, FL.

Bock released in 1989 except *H. balciunasi* (Figure 2) has a gold face and *Hydrellia* n. sp. CH 1 usually has a silver face (Figure 3). The biologies are also similar. Eggs are laid on the underside of emergent leaves usually near the water surface. Eggs of the China silver-face hatch in 2 to 2.5 days compared with 3 days for the Indian silver-face. Larvae of both mine the leaves. The larval period of China silver-face is 10 to 11 days compared with 12 to 13 days for the Indian silver-face.

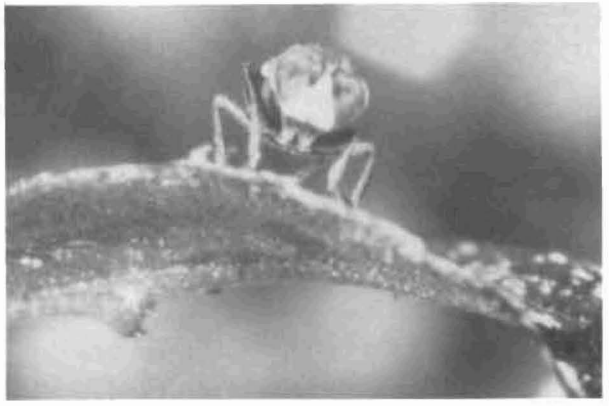

Figure 2. Gold-faced Hydrellia balciunasi adult on hydrilla

Figure 3. Silver-faced Hydrellia n. sp. CH 1 adult on hydrilla

The puparia are formed inside the leaves at the leaf axils. The mature larvae move to the base of the leaf and insert spiracular spines into the stem through which the puparia obtain oxygen. China silver-face adults emerge in approximately 4 to 5 days compared with 6 to 7 days for the Indian silver-face. All biology studies were conducted at 27 °C.

Laboratory host range tests were conducted in 35-ml glass culture tubes containing Barko's solution. One sprig of plant material 8 to 10 cm long was added in no-choice tests and one sprig of test plant and one of hydrilla in choice tests. Two to five mature eggs were added to each tube. Tubes were checked for attack at 7 days and for adults at 10 to 14 days. The number of puparia on each plant was counted in choice tests.

Laboratory host plants of China silver-face were as follows: *Elodea canadensi* Michx., *Najas quadalupensis* (Spreng.) Magnus, *Potamogeton crispus* L., *P. foliosus* Raf, *P. illinoensis* Morong, *P. nodosus* C. and S., *P. pusillus* L., and *Vallisneria americana* Michx. Percent adult emergence in our tests ranged from a low 2 percent from*Vallisneria* to a high 76 percent from *P. pusillus*. This is compared with 60 to 70 percent adult emergence from hydrilla.

Laboratory host plants of Indian silver-face were as follows: *Najas quadulpensis*, *P. crispus*, *P. foliosus*, *P. hillii* Morong, *P. illinoensis*, and *P. pusillus*. No adults emerged from *Vallisneria* and *Elodea*. Percent adult emergence from these plants was much lower. For example, only 18 percent emerged from *P. pusillus*. No adults emerged from *Vallisneria* and *Elodea*. Percent adults from the hydrilla controls was about 60 to 70 percent.

In choice tests, the same plants were attacked as in no-choice tests, but the percent emergence was at least half as much.

We will not apply for permission to field release China silver-face unless the releases of the China populations of *H. pakistanae* in the northern areas are unsuccessful or we have additional field data about the host range. In the case of Indian silver-face, even though the host range is not as broad, we do not have field data to support the laboratory data. We currently have no means to obtain this data, and will not apply for permission to field release this insect either.

Shipments into Quarantine

Much of our time this year has been spent colonizing imported insects. Table 1 lists the insects that have been imported into the quarantine facility. Each shipment took about 3 months of work from the time we received the insect until we had enough insects to ship to a cooperator. Each colony was usually checked for disease by a U.S. Department of Agriculture (USDA) insect pathologist before release.

Table 1
Hydrilla Insects Imported and Colonized Florida Biocontrol Laboratory Autumn 1991-Autumn 1992

Insect	Origin	Shipped To
Bagous n. sp.	Australia	ARS-Fort Lauderdale
Hydrellia balciunasi	Australia	WES
Hyrellia pakistanae	China (2)	WES, ARS-Fort Lauderdale
H. n. sp. CH 1	China (2)	Quarantine
H. n. sp. CH 1	Japan	Quarantine

Note: (#) = Number of shipments. WES = U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Australian Moths

In September, we received 9,000 eggs of two pyralid moths from Dr. D. H. Habeck, University of Florida, who was in Australia on sabbatical leave. From these, we obtained approximately 100 adults of *Aulacodes siennata* Warren and 20 to 30 adults of *Nymphula eromenalis* Snellen (Figure 4). The larvae of both of these stream dwellers feed on the leaves of hydrilla and defoliate the stems. Larvae of both species web leaves together to form their cases (Figure 5). Larvae of *A. siennata* also feed on the stem (Figure 6).

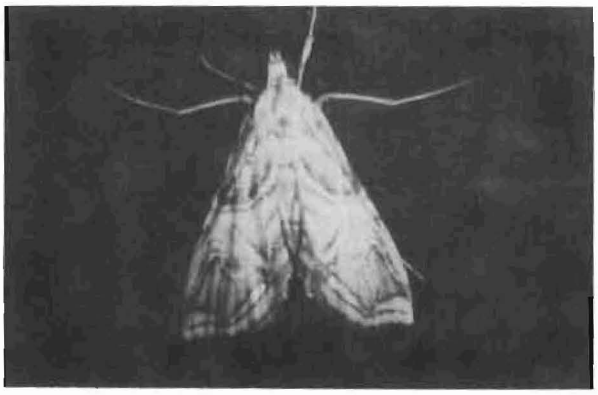

Figure 4. Adult of "Nymphula eromenalis"

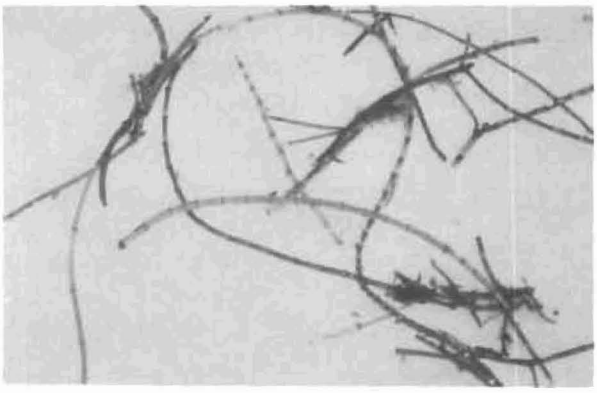

Figure 5. Damage and larval cases of "Aulacodes siennata"

Figure 6. Larva of "Aulacodes siennata"

We have reared both species to the F1 generation and are continuing to rear them.

Florida Hydrilla Midge

In June, Dr. Pat Greany, USDA research entomologist at Gainesville, called our attention to the hydrilla at Crystal River. Crystal River is a spring-fed river that flows to the Gulf of Mexico. Dr. Greany owns property on the river and noticed that the hydrilla was not reaching the surface and forming the thick mats as in past years. He asked us to come

and look at the plants and offered to set up a microscope on his boat so we could examine the plants as we collected them. We sampled three different sites. At each site, eight samples of five apical portions of the stem were collected by enclosing the stems in plastic bags underwater. Additional stems were collected in mass in the same way. The apical buds, lateral buds, and portions of the stems of hydrilla had been damaged by midge larvae. Amount of damaged varied between sites, and the number of larvae found were small. Two species of midge larvae, *Cricotopus sylvestris* group sp. and *Dicrotendipes* sp. A (an undescribed species), were associated with the damage. We were unable to confirm these species as the causal agents by transferring them to undamaged hydrilla; they do not survive handling well.

Watermilfoil Weevil

Eleven *Bagous* n. sp. (Figure 7) adults collected in northern China by Dr. G. R. Buckingham on *Myriophyllum spicatum* L. were hand carried by me into quarantine this summer. Larvae fed inside the stem, damaging four or five nodes before pupating inside the stem. Eggs are laid in the stem. We have reared 24 F1 adults, and 4 F2 adults have emerged thus far.

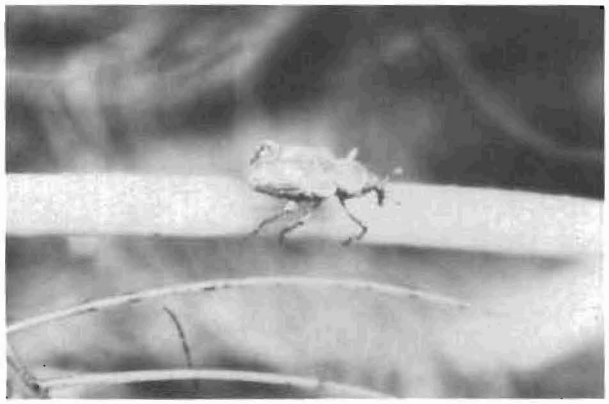

Figure 7. Adult of Bagous n. sp. on a milfoil stem

Melaleuca Insects

Melaleuca quinquenervia is major problem in south Florida especially in the Everglades. Dr. Joe K. Balciunas, USDA/Agricultural Research Service (ARS) has been working in Australia on biological control of this tree and has several candidates.

In May, we received a shipment of 30 prepupae of the sawfly *Lophyrotoma zonalis* Rohwer (Figure 8). After a month in quarantine, 14 females and 4 males emerged. We attempted to raise the sawfly through one generation on Florida melaleuca, because there was some concern in Australia that the sawfly would not accept Florida melaleuca as a host. Submittal of a request for permission to introduce it for host range testing was contingent on determining that Florida melaleuca was a host.

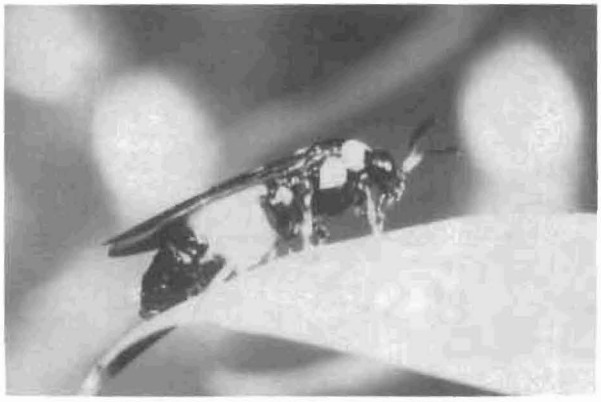

Figure 8. Female Lophyrotoma zonalis on melaleuca

Eggs were laid in rows along the margins of older leaves. After 21 days, the eggs hatched. The first instars moved to the top of the leaf and then grazed down the leaf scraping the epidermis. They moved together as a herd. The larvae are very gregarious until the fourth instar. Older larvae fed on the entire leaf surface and quickly defoliated small saplings in quarantine (Figure 9).

We obtained 11 males, but no females.

The second melalueca insect arrived in July, the weevil *Oxyops vitiosa* (Figure 10). Adults prefer young, soft, lush growth for food and oviposition sites. Eggs are laid on the stems and leaves. Larvae feed on the

Figure 9. Larvae of Lophyrotoma zonalis feeding on melaleuca

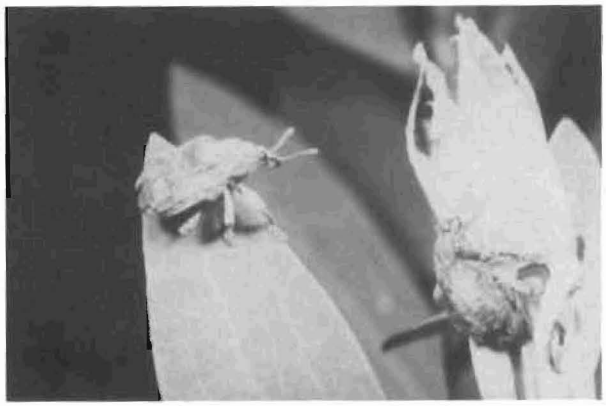

Figure 10. Adults of Oxyops vitiosa sitting on melaleuca

young growth also scraping the epidermis (Figure 11). Pupation occurs in the soil.

Future Plans

During the next year, we plan to finish studies of the biologies of the Indian and Chi-

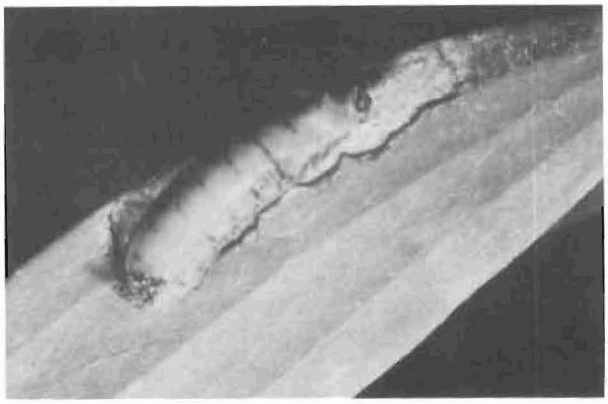

Figure 11. Larva of Oxyops vitiosa scraping a melaleuca leaf

nese populations of *Hyrellia* n. sp. We will conduct host range tests with the two Australian moths. If specific, these two could help control hydrilla in flowing-water situations where other controls are often not successful.

Plans are to rear large enough numbers of *Bagous* n. sp. on milfoil to start host range and biology tests. If our foreign travel plans are approved, I will travel to China to collect field data and import new germ plasm.

Dr. Balciunas will continue to ship field-collected *Oxyops vitiosa* to quarantine. We will conduct host range tests with these and continue our rearing studies. If *Lophyrotoma zonalis* (Figure 9) is approved for long-term testing in quarantine, Dr. Balciunas will be asked to ship it.

Status of the Release and Establishment of Insect Biocontrol Agents of Hydrilla

by
Michael J. Grodowitz,[1] *Ted Center,*[2] *Ed Snoddy,*[3] *and Elisa Rives*[3]

Introduction

Beginning in 1980, the U.S. Department of Agriculture, Agricultural Research Service (USDA, ARS) and the U.S. Army Engineer Waterways Experiment Station (WES) cooperatively initiated overseas surveys and research to identify potential insect biocontrol agents of hydrilla in the India, Pakistan, and Australia areas (Buckingham 1988). This work built upon surveys begun in the 1970s by other researchers (Sankaran and Rao 1972; Baloch and Sana-Ullah 1974; Baloch, Sannah-Ullah, and Ghani 1980). It was recognized through these surveys that several insect species offered potential as successful biocontrol agents of hydrilla. In 1983, the first of four insect species were brought into the United States quarantine facility in Gainesville, FL, for further host specificity testing. Host specificity proved to be highly satisfactory, and the first insects released from quarantine in the United States for control of hydrilla occurred rapidly (Buckingham, Okrah, and Thomas 1989). In 1987, the tuber-feeding weevil *Bagous affinis* and the leaf-mining fly *Hydrellia pakistanae* were released at sites in southern Florida. Other hydrilla specific insects followed quickly; and currently, four species have been released including the former two species, *B. affinis* and *H. pakistanae*, as well as the Australian leaf-mining fly *B. balciunasi* and the stem-feeding weevil *Bagous hydrillae* (formerly *Bagous* new species Z; Center 1992).

The major objective of this paper is to summarize the present status of the insect biocontrol agents released in the United States for hydrilla control in terms of release sites, numbers of individuals released, establishment success, and the status of the hydrilla at selected sites.

Present Status

Numbers of insect biocontrol agents released in the United States have been tremendous; over 1 million individuals of the four species have been released in 5 states and 53 locations since 1987 (Table 1). A large share of the credit for releasing high numbers is due to the technological advances in insect rearing made at the mass rearing facilities at the USDA, ARS Aquatic Plant Control Research Facility, Fort Lauderdale, FL, WES Biomanagement Team rearing facility at Vicksburg, MS, and the rearing facility located at Muscle Shoals, AL, operated by the Tennessee Valley Authority (TVA). By far the greatest number of individuals released is for the leaf-mining fly *H. pakistanae* with >80 percent of the total released in four southeastern states (Table 1). This is followed by *H. balciunasi*, *B. hydrillae*, and *B. affinis* with approximately 9, 6, and 1 percent of the total, respectively. Releases are continuing, and new release techniques using insect-infested hydrilla from small fish hatchery ponds are being tested at the rearing facility at Muscle Shoals, AL. These new techniques have the capability of drastically increasing our ability to release large numbers of individuals over a relatively short time interval, thereby, saving time and money normally associated with laboratory rearing. Establishment success is greatly dependent on our ability to release large numbers of highly competitive individuals.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
[2] USDA/ARS, Aquatic Plant Control Research Unit, Fort Lauderdale, FL.
[3] Tennessee Valley Authority, Muscle Shoals, AL.

Table 1
Release of Biocontrol Agents of Hydrilla in the United States up to October 1992

Species	No. of States	States	Locations	Number of Individuals	Release Year
Bagous affinis	2	FL, CA	11	10,695	1987
Bagous hydrillae	2	FL, GA	7	61,416	1991
Hydrellia balciunasi	2	FL, TX	8	92,003	1989
Hydrellia pakistanae	4	FL, GA, AL, LA	37	853,006	1987
Total	6		53	1,017,120	

Bagous affinis, the tuber-feeding weevil

Bagous affinis is a small, mottled, black and brown weevil approximately 3 to 4 mm in length (Bennett and Buckingham 1991). It was imported from Pakistan and first released in southern Florida in 1987. Adults feed on many different portions of the hydrilla plant including leaves, stems, turions, and tubers; but they appear to prefer the stem tissue. The adult lays eggs in a variety of substrates preferring waterlogged wood and other organic material. However, it has been observed to oviposit in hydrilla stems and tuber rhizomes. It is essential that low-water levels exist for extended periods for adequate success of *B. affinis* populations since the immatures require access to tubers via exposed sediment surfaces. First instars exit the oviposition substrate and rapidly burrow into the exposed sediment searching for hydrilla tubers. The larvae enter the tuber where they feed, develop, and essentially destroy the tuber. Developmental times range from 18 to 29 days depending on temperature.

As indicated previously, *B. affinis* was first released at Lake Tohopekaliga, FL, in 1987. Currently, approximately 11,000 individuals of various life stages have been released at 11 south Florida locations in 26 separate releases (Table 1; Center and Dray 1991). Limited postrelease surveys have been conducted, and evidence from Rodman Reservoir near Gainesville, FL, indicated establishment was occurring as indicated by the collection of several insect-damaged tubers. However, as has happened for other release sites, water levels were increased suddenly and subsequently eliminated *B. affinis* from the release area. Lakes in the southeastern United States are rarely subjected to drawdown conditions because of the length of the hydro period prevalent in the region; future releases were suspended, and research and release efforts were directed toward the stem-feeding weevil *B. hydrillae* and the leaf-mining flies *H. balciunasi* and *H. pakistanae*. Researchers in California have recently begun experiments in the use of *B. affinis* and have had some success with establishment and overwintering at selected California locations.[1] In many instances, water levels at many California canals and lakes can be controlled to a larger extent than those in the southeastern United States. *Bagous affinis* could, therefore, prove to be an important insect biocontrol agent for hydrilla management in California.

Bagous hydrillae (formerly *Bagous* new species Z), the stem feeding weevil

Bagous hydrillae is a small (3 to 4 mm) mottled brown weevil with indistinct whitish spots on the elytra. It was introduced from Australia and was first released at Lake Osborne, FL, during the spring of 1991 (Table 1; Center 1992). Adults feed on various portions of the hydrilla plant, most importantly the stem and often the leaf tissues (Balciunas and Purcell 1991). Eggs are oviposited in the stem where the larvae feed and develop entirely within the stem tissue. During the course of larval feeding, the stem fragments and separates

[1] Personal communication, L. Anderson, USDA, ARS.

from the mat. The fragmented stem pieces float to the shore either by wave or wind action where the mature larvae exit the stem and pupate within the soil or decaying plant material. Total developmental time is approximately 14 days depending on temperature.

Releases of *B. hydrillae* have been made in seven locations in both Florida and Georgia with approximately 61,000 individuals released. To release >60,000 individuals, more than 100,000 weevils were reared at the USDA, ARS Aquatic Plant Control Research Unit, Fort Lauderdale, FL, over the last 2 years. The ability to mass produce *B. hydrillae* has increased dramatically. For example, in the first year of release (i.e., 1991), only 3,427 individuals were released at three Florida locations. The number of individuals released increased tremendously during 1992 with a 17-fold increase, or >57,000 individuals released. These increases in numbers are due to several important technological changes in the mass-rearing procedure. These include the consistent use of higher quality hydrilla, reduction of crowded conditions, complete standardization of the rearing process, and better control of humidity in the rearing containers. Recently, successful rearing has been accomplished under more controlled field conditions using recently developed techniques. These new procedures may prove to be better than existing procedures for establishing *B. hydrillae*.

Establishment has not yet been verified at any site, mainly because of changes in site characteristics. One of the major release sites in southern Florida, Bulldozer Canal, is a good example. This site is a small drainage canal with large quantities of hydrilla throughout its length. The site was chosen because of its persistent quantities of stranded hydrilla in various states of desiccation on the shoreline. It is believed to be important for hydrilla to be present on the shoreline for successful establishment of *B. hydrillae*. Individuals were released consistently for several months at this site. After the initial releases, weevils were collected relatively frequently from the site. This provided us with hope that establishment was occurring. However, at about this time, severe changes in water level occurred when irrigation water from surrounding farmlands was pumped into the canal. The water level was raised approximately 2 to 3 ft. With this increase in water, the hydrilla was almost completely flushed from the banks and within the canal. Changes in water level have also occurred at release sites in Lake Seminole with similar disruption of establishment. While these changes would not influence established populations, such changes are devastating during the critical initial establishment periods. Releases of *B. hydrillae* are continuing; and as indicated previously, new rearing and release techniques are being tested using somewhat controlled field conditions to increase the success of establishment.

Hydrellia balciunasi, the Australian hydrilla leaf-mining fly

Hydrellia balciunasi is a small (2 to 3 mm) fly that was released into southern Florida from Australia in 1989 (Table 1; Center 1992). The adult is rather short-lived, having a life span of approximately 10 days. It is not known exactly what the adults feed on, but it is assumed that they feed on various fungi and algae as well as dead insects. The females oviposit from 100 to 200 eggs during their life, presumably on various types of emergent vegetation, but especially on topped-out hydrilla. The eggs hatch in 3 to 4 days depending on temperature, and the emerging larvae descend into the water column in search of hydrilla. The larvae enter into the leaf tissues and feed on the internal cellular material, the mesophyllic tissues. Each larvae is capable of damaging from 8 to 12 leaves during its feeding period. The larvae form pupae in specialized cases (i.e., puparia), which they attach to the stem usually in a stem axil. The adults emerge from the puparium and reach the surface in an air bubble that originates in the puparium. Total developmental time is from 20 to 30 days and is dependent on temperature.

Hydrellia balciunasi has been released at six Florida and two Texas locations as of

October 1992 (Table 2). Approximately, 92,000 individuals have been released. To date, only one site has verified establishment of *H. balciunasi*; i.e., Sheldon Reservoir in Northeast Houston, TX. About 80 percent of the flies collected from Sheldon Reservoir during 1992 were *H. balciunasi*, 5 percent were *H. pakistanae*, and the remaining were mixtures of mainly *H. bilobifera* and *H. discursa*, two native species commonly found in association with hydrilla. The 80- and 5-percent mixtures of *H. balciunasi* and *H. pakistanae* are very close to the mixture released during 1991 and early 1992. The population of *H. balciunasi* and, to a lesser extent, *H. pakistanae* at Sheldon Reservoir is increasing with approximately 5 percent of the leaves examined during an October sampling having obvious signs of fly damage. The remaining sites where *H. balciunasi* was released have not yet become established. All the south Florida release sites (i.e., six) have no establishment, but all have robust populations of *H. pakistanae*. It is possible that *H. pakistanae* is better adapted to survive the climatic conditions found in south Florida. Releases of *H. balciunasi* are continuing at these and other south Florida locations. The other Texas release site is Lake Raven, located in Huntsville State Park in Huntsville, TX, approximately 70 miles north of Houston. Only limited information on establishment is available since *H. balciunasi* has only recently been released at this site; releases are continuing presently. Other releases are planned for sites in Texas and Florida during 1993.

Table 2
Release Information by State for *Hydrellia balciunasi* as of October 1992

State	Number of Locations	Numbers Released	Sites Established %
FL	6	34,000	0
TX	2	58,003	50
Total	8	92,003	50

Hydrellia pakistanae, the hydrilla leaf-mining fly

Hydrellia pakistanae is very similar in morphology and biology to that described for *H. balciunasi*. It was first released from quarantine into south Florida in 1987 (Table 1; Center 1992). Adults of the two species can only be distinguished by variations in male and female genitalia, and such determinations should be left to highly trained personnel. No reliable characters presently exist for separating the immature life stages. Information on general biology is essentially similar to that reported for *H. balciunasi*.

To date, H. pakistanae has been released in 32 locations in four southeastern states (Tables 1 and 3; Center 1992). The highest number of individuals has been released in Florida with >45 percent of the total 853,000 flies. This is followed by Georgia, Alabama, and Louisiana with 28.1, 15.9, and 10.4 percent of the total, respectively. Establishment is proceeding exceptionally well with 91 percent of the original release with verifiable establishment. It is generally believed that it is well established throughout all of the southern two-thirds of the Florida peninsula; i.e., every hydrilla site visited in this region contained at least some levels of *H. pakistanae*. Of 45 sites surveyed over the last year, 80 percent had well-established populations whether or not they had been inoculated with *H. pakistanae*. This indicated that rapid dispersal of *H. pakistanae* has occurred throughout Florida. Distinct changes or declines in the hydrilla commonly occurs after establishment. For example, 28 of the 45 sites surveyed have been observed periodically, and 75 percent of these 28 sites has had significant changes in the status of the hydrilla.

Table 3
Release Information by State for *Hydrellia pakistanae* as of October 1992

State	Number of Locations	Number of Individuals	Percent of Total
FL	27	388,166	45.5
GA	1	240,101	28.1
AL	3	136,003	15.9
LA	1	88,736	10.4
Total	32	853,006	100.00

To better illustrate these changes, we have delineated the surveyed sites into four distinct

categories or groupings based on changes in the hydrilla status (Table 4). As indicated previously, establishment success has been high with all but 7 percent of the sites becoming established. Only 18 percent of the sites has had establishment with no significant changes observed in the hydrilla status. A majority of the sites have had significant declines in the hydrilla. For example, 75 percent of the sites has had dramatic changes in the hydrilla infestation. This is approximately equally divided between sites with well-verified *H. pakistanae* populations and those sites with only limited-*H. pakistanae* establishment but having significant hydrilla declines. We do not understand how certain sites could have declines with only limited establishment, but the coincidence is too high to ignore the possible role of the flies. Other complicating factors, such as plant pathogens or nutritional problems, may interact with the stress placed upon the plant by the insect feeding. We are currently initiating other studies to elucidate such possible interactions.

Table 4
***H. pakistanae* Site Status Categories and Associated Percentage of Surveyed Sites Within Each as of October 1992. (Surveyed sites (n = 45) may or may not have received shipments of flies)**

Site Category	Percent of Sites
Released but no establishment	7
Well established by no change in hydrilla status	18
Well established with hydrilla decline	39
No or limited establishment with hydrilla decline	36

Future Directions

We are planning to continue releases of the leaf-mining flies mainly in Texas, Alabama, and Georgia locations with minimal number of releases in Florida. We hope to have larger fly populations at Sheldon Reservoir during 1993 and use these for future Texas releases; if available, we will move some individuals to Florida locations. We are currently trying to mass rear a new strain of *H. pakistanae* from Beijing, China, a more temperate region than the area from which we originally collected *H. pakistanae*. If this strain adjusts to laboratory-rearing conditions, we may begin releasing it in the temperate regions of North and South Carolina.

Continued efforts will be placed in releasing and establishing *B. hydrillae* at Florida and Georgia locations. If previously mentioned release techniques appear promising, we may utilize these in several larger reservoirs in Texas where *B. hydrillae* may prove to be a valuable management tool.

As higher numbers of sites become established with insect biocontrol agents of hydrilla, research efforts will be directed on a larger scale toward understanding the biocontrol agents effects and to developing operational-level procedures for gauging such effects.

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The Effect of the Weevil *Euhrychiopsis lecontei* on Eurasian Watermilfoil: Results from Brownington Pond and Norton Brook Pond

by
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Introduction

There has been a growing interest in using the nearctic weevil *Euhrychiopsis lecontei* (Dietz) as a biological control agent for Eurasian watermilfoil *Myriophyllum spicatum* L. in North America. *Euhrychiopsis lecontei* has been found associated with declining watermilfoil populations in northeastern North America (Creed and Sheldon 1991a,b; 1992a). Weevil adults and larvae have also been found to suppress watermilfoil growth (adults and larvae), remove significant amounts of leaf tissue (adults), weaken the stem and remove vascular tissue (larvae), and reduce watermilfoil buoyancy (adults and larvae) in laboratory experiments (Creed and Sheldon 1991a,b; 1992a,b; Creed, Sheldon, and Cheek 1992). However, no field experiments have yet been conducted that have evaluated the effect of this weevil on Eurasian watermilfoil. In this paper, we present the results of surveys and a pond-enclosure experiment conducted in Brownington Pond, VT, that support the hypothesis that herbivory by weevils and not changes in environmental characteristics (e.g., sediment and water chemistry) was largely responsible for producing the observed watermilfoil declines. We also present the results of an experiment in which weevils were introduced into enclosures in Norton Brook Pond, a water body in Vermont that contained Eurasian watermilfoil but no *E. lecontei*.

Materials and Methods

Brownington Pond

Watermilfoil surveys

Pond surveys. Since the first summer of this project, we have been qualitatively mapping the positions of any watermilfoil beds in Brownington Pond (Creed and Sheldon 1991a,b; 1992a). The information for these maps has been gathered by snorkeling and boat surveys (Creed and Sheldon 1991a,b). We surveyed the pond in a similar fashion in the summer of 1992.

Plant transects. The initial survey of Brownington Pond in 1990 found that watermilfoil was restricted to water approximately 2.0 to 3.5 m deep. This absence of watermilfoil from shallower water seemed atypical as *M. spicatum* is often reported to grow throughout the littoral zone up to the depth at which light is limiting (Aiken, Newroth, and Wile 1979; Titus and Adams 1979; Smith and Barko 1990; Madsen, Hartleb, and Boylen 1991). To document this pattern of watermilfoil distribution, we began a program of sampling all submersed macrophytes along transects perpendicular to shore.

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In 1990, scuba divers sampled plants along transects in the vicinity of the two watermilfoil beds, the South Bed and the West Bed (see Creed and Sheldon 1991a,b). The transects in 1990 were not permanent. Three transects were sampled per bed on two dates, 17 July and 15 August. Only the data from 15 August will be presented here. Samples were taken at depths ranging from 0.5 to 3.5 m using a 0.25-m^2 quadrat. All plants within a quadrat were clipped at sediment level and then placed in sealable plastic bags. Plants were separated by species in the laboratory and then dried to a constant weight at 80 °C. For clarity of data presentation dry weights for native macrophytes were lumped together in the category "other." (See Creed and Sheldon (1991a) for a list of common native plants in Brownington Pond).

To see if this pattern of watermilfoil distribution by depth persisted in 1991 and 1992, we established three permanent transects through both of the main beds. The transects were evenly spaced across the length of the beds. Along each transect, locations were selected at half-meter depth intervals ranging from 0.5 to 3.5 m deep, for a total of 21 sample points in the vicinity of each bed. At each sample point, two polyvinyl chloride (PVC) pipe T's were pushed into the sediment at right angles to one another to form a cross. The four ends of the T's were numbered from 1 to 4. To ensure that the areas sampled in 1992 were not affected by the 1991 sampling, each transect was shifted 4.5 m; the direction that the transect was shifted was randomly determined.

The permanent transects were sampled on three dates in 1991 and 1992. For each point to be sampled, one of the four numbers from the T's was selected at random from the remaining possible numbers prior to sampling. The points were sampled by scuba divers. The divers inserted a 2-m-long piece of PVC pipe into the appropriate numbered opening (sampling a quadrat 2 m from the T's minimized the disturbance of the area to be sampled by the diver when reading the numbers on the PVC T's). A 0.25-m^2 quadrat was then placed on the bottom at the end of the pipe. All above sediment plant biomass within the quadrat was clipped and placed into a numbered plastic bag. Upon returning to the laboratory, plants from each sample were sorted according to species and dried to a constant weight at 80 °C. For clarity of data presentation, dry weights for native species were lumped together in the category "other." Data presented are from the last sample dates in 1990 and 1992 and from the midsummer samples in 1991.

Permanent grids. In addition to determining the location of watermilfoil beds in the littoral zone, we initiated a program to record finer scale expansions and contractions of *M. spicatum* beds using permanent grids. Four grids were established in the pond in 1990, two in each bed. The grids cover an area of 8 by 6 m with buoys placed every 2 m in a 4 by 5 array. The grids were placed on either the ends or the nearshore edges of the beds, as we believed that watermilfoil would be more likely to spread laterally and into shallow water. The grids did not extend into deep water, as watermilfoil abundance is probably limited on the deep edge of beds by light availability. Percent cover of watermilfoil was determined by a diver using a 0.5- by 0.5-m quadrat subdivided into 25 subunits. In 1990, percent cover was evaluated for four contiguous quadrats in the center of each 2- by 2-m block, i.e., readings were taken along three lines in each grid. The quadrat was held in position by a snorkeler. In 1991 and 1992, placement of the quadrat across the bed was determined using a transect "line" made of PVC pipe with openings placed every 0.5 m into which extensions from the quadrat were inserted. Percent cover was evaluated along four transects for each grid in 1991 and 1992. The position of the transects corresponds to the four lines of buoys that run along the longer dimension of each grid, i.e. A - E (see Figures 1 and 2). For all 3 years, the number of quadrat subunits more than half-filled with watermilfoil plants was then recorded. This technique generates percent cover values ranging from 0 to 100 percent. For clarity of data presentation, we grouped the percent cover values into five categories—(a) 0 percent, (b) 1 to 25 percent,

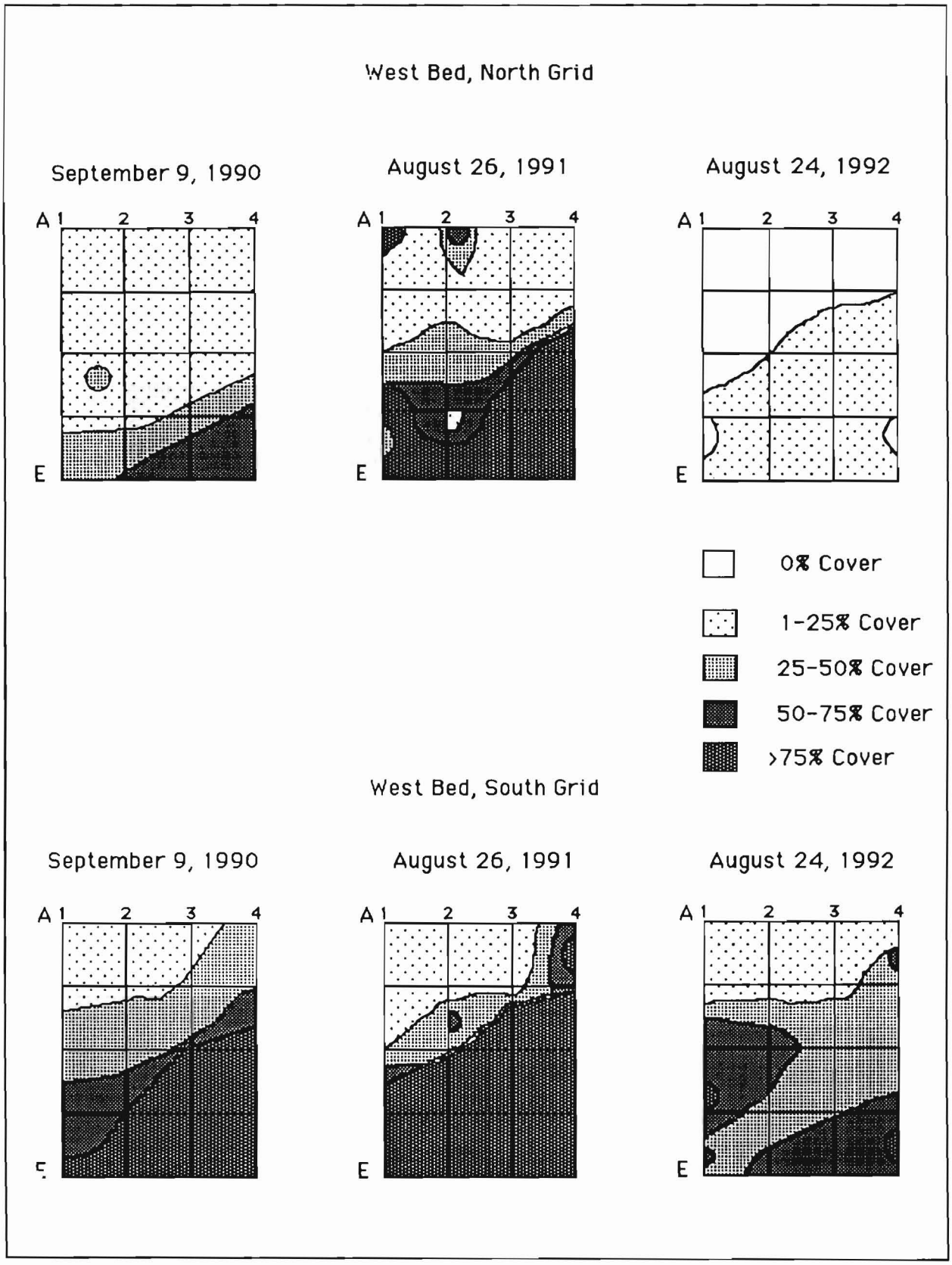

Figure 1. Maps of percent cover of Eurasian watermilfoil in the two West Bed grids for last sample date of each summer

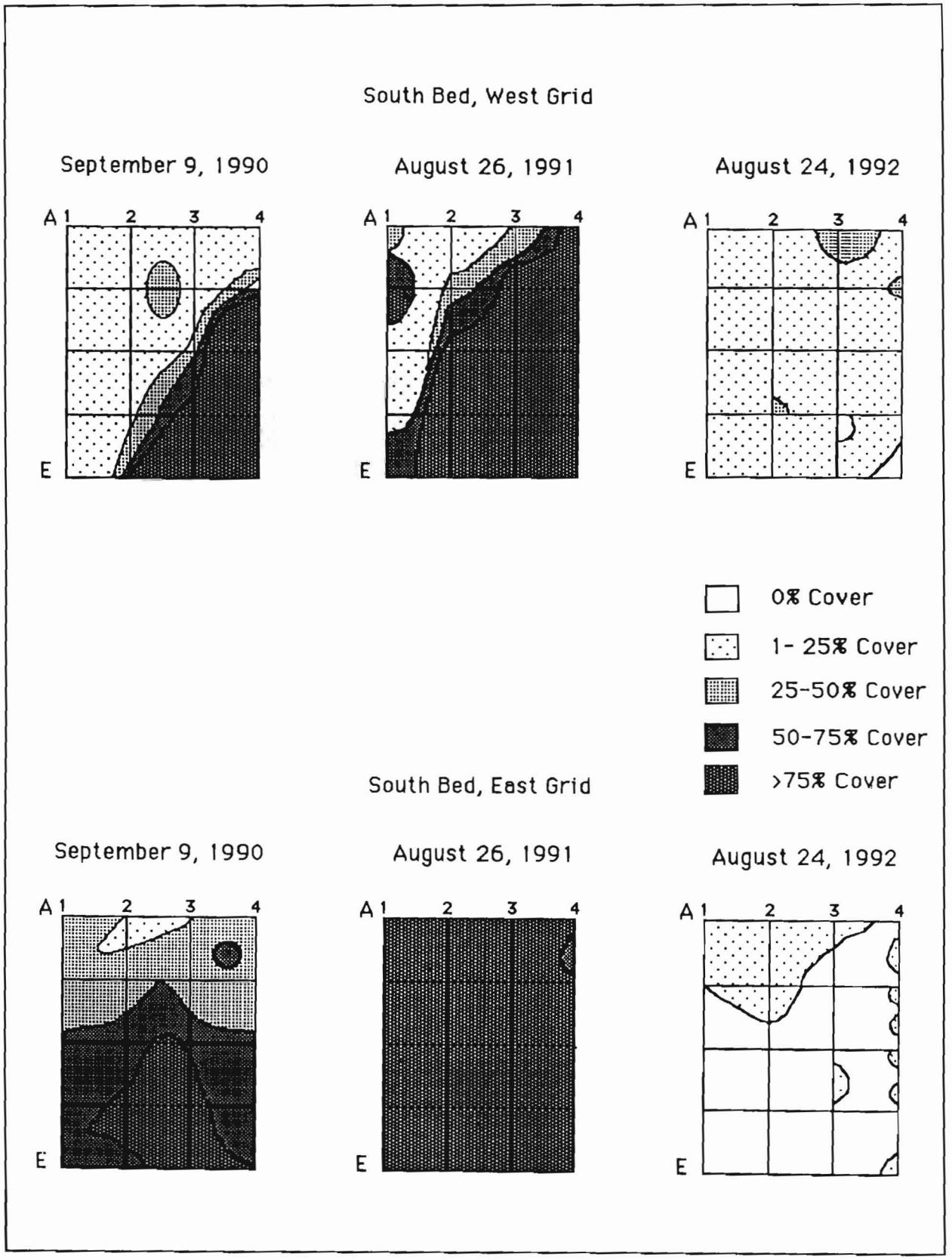

Figure 2. Maps of percent cover of Eurasian watermilfoil in the two South Bed grids for last sample date of each summer

(c) 25 to 50 percent, (d) 50 to 75 percent, and (e) greater than 75 percent. The grids were swum only once in 1990 on 9 September. The grids were swum in mid-June (18 and 21 June), late July (24-26 July), and late August (26 August) during the 1991 growing season. In 1992, the grids were swum on 15 June, 13 July and 24 August. We will only present grid data for the last sample of each of the three field seasons.

Weevil surveys

Quantitative samples of watermilfoil and the associated invertebrates were taken in the South and West Beds to describe the watermilfoil invertebrate assemblage. Samples were collected using a smaller version of the Mobile Invertebrate Sampler (MIS), developed by Smith and Sheldon (unpublished manuscript), that was designed for sampling a single stem of watermilfoil. The sampler was a long plastic tube with a removable, mesh-covered lid (500-µm Nitex mesh) that was employed by a scuba diver. A plant to be sampled was chosen haphazardly. The sampler was then slid over the plant as the diver descended. Plants were cut near the sediment surface; the lid was attached, and then the sample was returned to the surface. All samples were placed in sealable plastic bags. Samples were picked on the day of collection, and all animals removed were preserved in 70 percent ethanol (ETOH). Invertebrates were identified under a dissecting scope. We will only discuss the abundance of the weevil *E. lecontei* here. Samples were collected on six dates in 1990 (mid-July through late August), eleven dates in 1991 (taken weekly from mid-June through August), and twelve dates in 1992 (taken weekly from the beginning of June through August).

Water and sediment chemistry

Water chemistry. Surveys of nutrients (nitrate, nitrite, and orthophosphate) in the water column were made on 25 June 1991 and 30 June and 27 August 1992. Samples were collected from three sites. One set of samples was collected on the east side of the pond in an area with mixed native plant (*Heteranthera dubia* (Jacq.) MacM. and *Potamogeton amplifolius* Tuckerm.) cover. The other two sets of samples were collected in the South and West watermilfoil beds. Instead of sampling a fixed point, three or more locations were chosen to sample a broader array of potential microhabitats within a site. Water samples were collected using a Kemmerer sampler. Pairs of samples, one shallow (just below the surface) and one deep (just above the bottom) were taken at each point. Upon finishing a collection, samples were placed on ice and transported to the laboratory of the Vermont Department of Environmental Conservation for analysis.

Sediment chemistry. Sediment samples were taken in Brownington Pond on 11 August 1992. Samples were taken in (a) the West Bed, (b) a watermilfoil-free area adjacent to the West Bed (West Shallow), (c) in the South Bed, (d) in a watermilfoil-free area adjacent to the South Bed (South Shallow), and (e) on the east side of the pond in an area dominated by *H. dubia* and *P. amplifolius*. Pond sediment was collected by a scuba diver. A 3.8-L sealable plastic bag was filled with sediment below the water-sediment interface. The bag was sealed and then returned to the surface. All samples were kept cool and sent to the U.S. Army Engineer Waterways Experiment Station (WES),Vicksburg, MS, for analysis. Samples were sent to Mississippi within 48 hr of collection. Sediment data were analyzed using an analysis of variance (ANOVA), and means for each site were compared using Tukey's HSD (honest significant difference) test (Sokal and Rohlf 1981).

Enclosure experiment

The enclosures used in this experiment were 3-m-tall plexiglass tubes (20 cm outside diameter) that were composed of two parts. The bottom section (1-m-tall) was driven into the sediment. The upper portion of the chamber (2-m-tall) was then bolted to the bottom section. Along the sides of the upper portion were four pairs of ports covered with 202-µm Nitex mesh, which allowed for water exchange between the enclosures and the water column. A lid also covered with 202-µm Nitex mesh was bolted on the top of each tube. There

was a centimeter scale on the outside of the upper portion of each tube.

The bottom sections of 10 enclosures were placed in the pond on the nearshore side of the South Bed by a scuba diver on 17 June 1992. Because of the depth of the water, the tops of the enclosure bases were not flush with the sediment surface. Extra sediment that was free of other plants was added to each base. The sediment came from the middle of the South Bed. We collected a number of small (approximately 40-cm-long shoots) watermilfoil plants from the West Bed on 17 June. The plants were cleaned of obvious macroinvertebrates and any weevil eggs. The plants were then sorted into 13 groups of 6 and weighed (blotted wet weight) to standardize initial biomass. Ten of the groups of plants were randomly assigned to the enclosures; the remaining three groups were dried at 80 °C for an initial estimate of dry weight. Six plants per enclosure is equivalent to 181 plants/m^2. This value is well within the range of densities determined by surveys of watermilfoil in the two beds during 1990 (Creed and Sheldon 1991a). The initial mean wet weight (± 1 S.E.) of plants placed in the tubes was 5.61 ± 0.16 g.

On 18 June, the plants were planted in the tube bottoms by a scuba diver. Plants were gently pushed down into the sediments until the roots were buried. The upper portion of the tube was then bolted to the bottom. The lids were then bolted onto each enclosure top. Four days (22 June) after the plants had been placed into the enclosures, the maximum height of each plant in each tube was recorded by a scuba diver. The height of plants (the six original stems plus any new lateral stems) inside the enclosures was measured weekly until the end of the experiment.

The original plan had been to allow the watermilfoil plants inside the enclosures to grow for 3 weeks before adding the adult weevils. However, during the first 3 weeks of the experiment, larval weevil damage was observed on a single stem in four of the enclosures. These four enclosures were designated as the weevil treatment. As the plants had been randomly assigned to tubes, we assumed that the distribution of this treatment across enclosures was also random. On 9 July, we added four adult weevils (two males and two females) to these four enclosures. Another three enclosures had been contaminated by single *Acentria* larvae (Lepidoptera, Pyralidae), so we designated these three enclosures as an additional *Acentria* control treatment. These larvae appear to have entered the enclosures after the watermilfoil had been planted. We assumed that contamination of the three enclosures by *Acentria* were also random events. The remaining three enclosures were considered uncontaminated controls. At the time the adult weevils were added, the larval weevils and *Acentria* had not had a significant effect on mean plant height in these enclosures when they were compared with the uncontaminated controls. During the experiment, the enclosures were periodically cleaned of external periphyton.

The enclosures were sampled on 20 August. First, the upper portion of the enclosure was removed from the base. The plants were then clipped at sediment level. The shoots either floated or were gently pushed into the upper portion of the enclosure, which was then sealed with a screen-covered bottom. The upper portion of the enclosure was then returned to a boat. The tube was lifted out of the water, and all of the plant material was collected on the bottom screen. The plants were removed from the tubes and placed in sealable plastic bags. The roots were gently removed from the sediments, gently shaken to clean off any adhering sediment, and then bagged. In the laboratory, shoots were separated into the six original stems (i.e., the plant tissue produced prior to the adult weevil introduction) and the newer lateral stems. Roots were cleaned of any organic debris. Shoots and roots were dried to a constant weight at 80 °C. Weevil larvae were not found in one of the weevil enclosures, so this enclosure was not included in the analysis. Thus, N = 3 for all treatments. Treatment effects were analyzed using an ANOVA, and treatment means were compared using Tukey's HSD test (Sokal and Rohlf 1981).

Norton Brook Pond

Norton Brook Pond is a small (<8 ha) impoundment in Bristol township, Vermont. *Myriophyllum spicatum* was first identified in the pond in 1985 and, currently, is the dominant (percent cover, biomass) macrophyte in the pond. Currently, a dense bed of *M. spicatum* rings the pond. No other submerged plant species were seen in Norton Brook Pond.

Weevil surveys

Before weevils were introduced, samples of watermilfoil were collected to determine whether *E. lecontei* was already present in the pond. Three transects, oriented perpendicular to shore, were sampled in the area where the enclosures were to be placed. On each transect, the apical portion of five pairs of *M. spicatum* plants, one with an intact apical meristem and one without a meristem, were collected. In the laboratory, all invertebrates were removed from the stems and preserved in 70 percent ETOH. Invertebrates were identified and counted. The plants were examined for weevil-feeding damage.

Enclosure experiment

Cylindrical enclosures were used for the weevil addition experiment. The 30.5-cm-diam, 2.5-m-tall enclosures were constructed from impermeable 4-µm polyethylene sheeting held open by external rings. The tops and bottoms of the enclosures were held open by approximately 8-cm-tall PVC rings. The tops of the enclosures were covered with 200-µm Nitex mesh and were held at the water surface by floats. Six enclosures were placed in a line running north to south on the 2.1-m depth contour. The enclosures were placed over dense *M. spicatum*, and the bottom ring was pushed into the lake sediment enclosing 730 cm^2 of sediment. Fifty adult *E. lecontei* were placed in every other enclosure. No weevils were added to controls. Enclosures were examined weekly, and dissolved oxygen was measured at mid-day at the bottom of the enclosures three times over the course of the experiment with a dissolved oxygen meter.

After 36 days in situ, a diver pulled the bottom of each enclosure out of the sediments, cut all plants at sediment level, clamped a 200-µm mesh sieve to the bottom of the enclosure; and all of the material was brought to the surface. All plants and invertebrates were washed into sealable bags and preserved in 70 percent ETOH. To quantify the effects of enclosures, three similar samples were also taken from the adjacent watermilfoil bed on the day the enclosures were removed. The areas to be sampled were selected haphazardly between enclosure sites on the 2.1-m depth contour.

Samples were returned to the laboratory where watermilfoil meristems were removed and examined under a dissecting microscope (7-15X magnification) for weevil eggs and early instar larvae. All macroinvertebrates were removed, identified, and enumerated. Plants were placed in a drying oven for >4 days at 80 °C and dry weights recorded. The data were analyzed using a one-way ANOVA, and treatment means were compared using Tukey's HSD test.

Results

Brownington Pond

Watermilfoil surveys

Pond survey. The watermilfoil population in the pond declined substantially over the winter of 1991-1992 (Figure 3A and B). In June of 1992, there were no areas of the pond that had dense watermilfoil beds that reached the surface. The decline was most dramatic in the South Bed; the bottom of the pond in the area that once supported the South Bed was devoid of any watermilfoil growth. Scattered plants were present in the West Bed. Some of these were taller shoots (approximately 1.5 m high), which were probably survivors from the previous season; most were shorter shoots (<0.5 m) that appeared to have just begun to grow (Creed, personal observation). By the end of the summer, four areas of moderately dense watermilfoil growth were present (Figure 3B). These included the southern portion

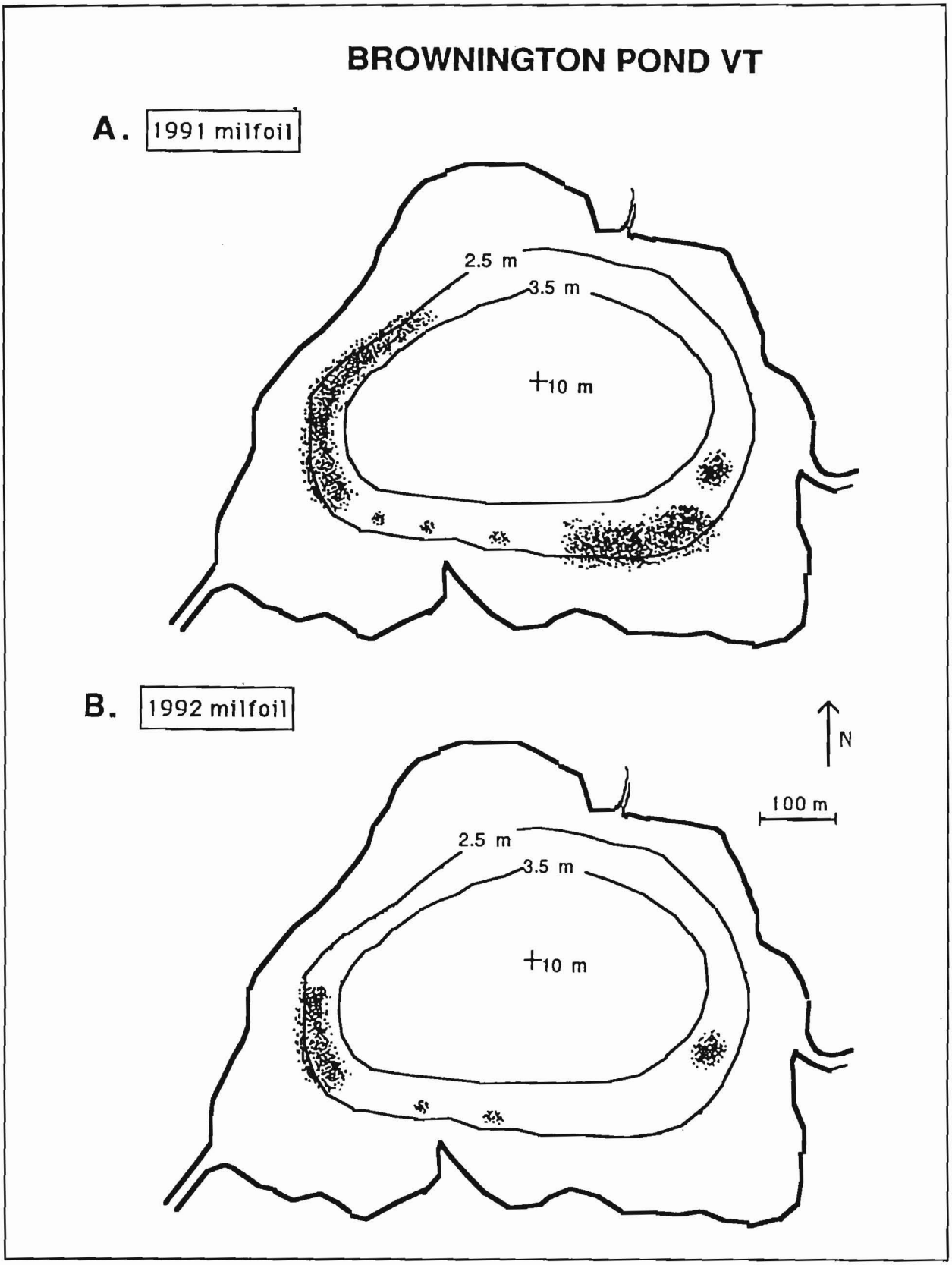

Figure 3. Distribution of Eurasian watermilfoil beds at the end of the summer in Brownington Pond, VT, in 1991 and 1992. Size of beds is not drawn to scale

of the West Bed and three scattered, small patches located along the southern shore of the pond. Watermilfoil only approached the surface in the West Bed; the tops of these plants were still almost 1-m below the surface. Only scattered, small plants were present in the vicinity of the former South Bed by the end of the summer.

Plant transects. The plant transect data also show that a watermilfoil decline occurred between 1991 and 1992 (Figures 4 and 5). There was a 4- to 6-fold reduction in watermilfoil biomass in the center of the West Bed (Figure 4). There was a 15- to 30-fold reduction in watermilfoil biomass in the center of the South Bed (Figure 5).

Permanent grids. The permanent grid data also indicated that a watermilfoil decline had occurred (Figures 1 and 2). The four grids displayed varying degrees of watermilfoil cover at the end of 1991; heavy watermilfoil cover (>50 percent) on the grids ranged from 40 percent (North Grid, West Bed) to almost 100 percent of the cover on the East Grid, South Bed. At the end of 1992, three of the four grids (the North Grid, West Bed, and both grids on the South Bed) had cover values that rarely exceeded 25 percent (a few small patches of cover >25 percent were present on the West Grid of the South Bed). In the case of two grids (the East Grid, South Bed and the North Grid, West Bed), anywhere from one-half to three-quarters of the grid area had 0-percent watermilfoil cover. The decline was most striking on the East Grid, South Bed (Figure 2). This grid had had essentially 100-percent watermilfoil cover over the entire grid at the end of 1991. Little watermilfoil cover was present on this grid in 1992. Only the South Grid from the West Bed had substantial watermilfoil cover by the end of the summer of 1992; approximately 30 percent of the watermilfoil cover on this grid exceeded 50 percent.

Weevil surveys

Weevil abundances were fairly low in both watermilfoil beds during 1990 (Figures 6 and 7). In general, weevil abundance increased through early 1992 and then began to decrease. When watermilfoil abundance is plotted for the same period, it is apparent that the increase in weevil abundance coincides with the pronounced decrease in watermilfoil abundance. The peak in weevil abundance occurs approximately 1 year after the peak in watermilfoil abundance.

Water and sediment chemistry

Concentrations of orthophosphate, nitrite, and nitrate varied little in 1991 and 1992. Concentration of orthophosphate rarely deviated from 0.002 mg/L; concentrations of nitrite and nitrate were always 0.01 mg/L.

Ammonium was the only sediment nutrient that varied significantly among sites (Table 1). Interstitial water ammonium concentrations were significantly lower in the South Bed than those for sediments from the native plant sediments or the West Bed sediments. Exchangeable ammonium in the South Bed sediments was significantly lower than only the West Bed sediments.

Enclosure experiment

Weevils had a significant effect on watermilfoil biomass and plant height in the enclosure experiment. Total biomass was significantly greater in the control and the *Acentria* treatments compared with the weevil treatment (Figure 8). The differences in total biomass were attributable to differences in root weight and lateral stem weight; there was no significant difference in the weight of the original stems (Figure 8). The damaged, original stems in the weevil treatment tended to collapse during the experiment. While the mean height of these weevil-damaged, original stems in the water column was usually lower than that of the original stems in the control treatment, the difference was not significant until the last 3 weeks of the experiment (Table 2). The difference in the mean height of original stems between the weevil treatment and the control treatments for this period ranged from 10 to 25 cm (Table 2). The weevil-damaged stems were often supported by the tubes. In the absence of the tubes, the difference in the height of weevil-

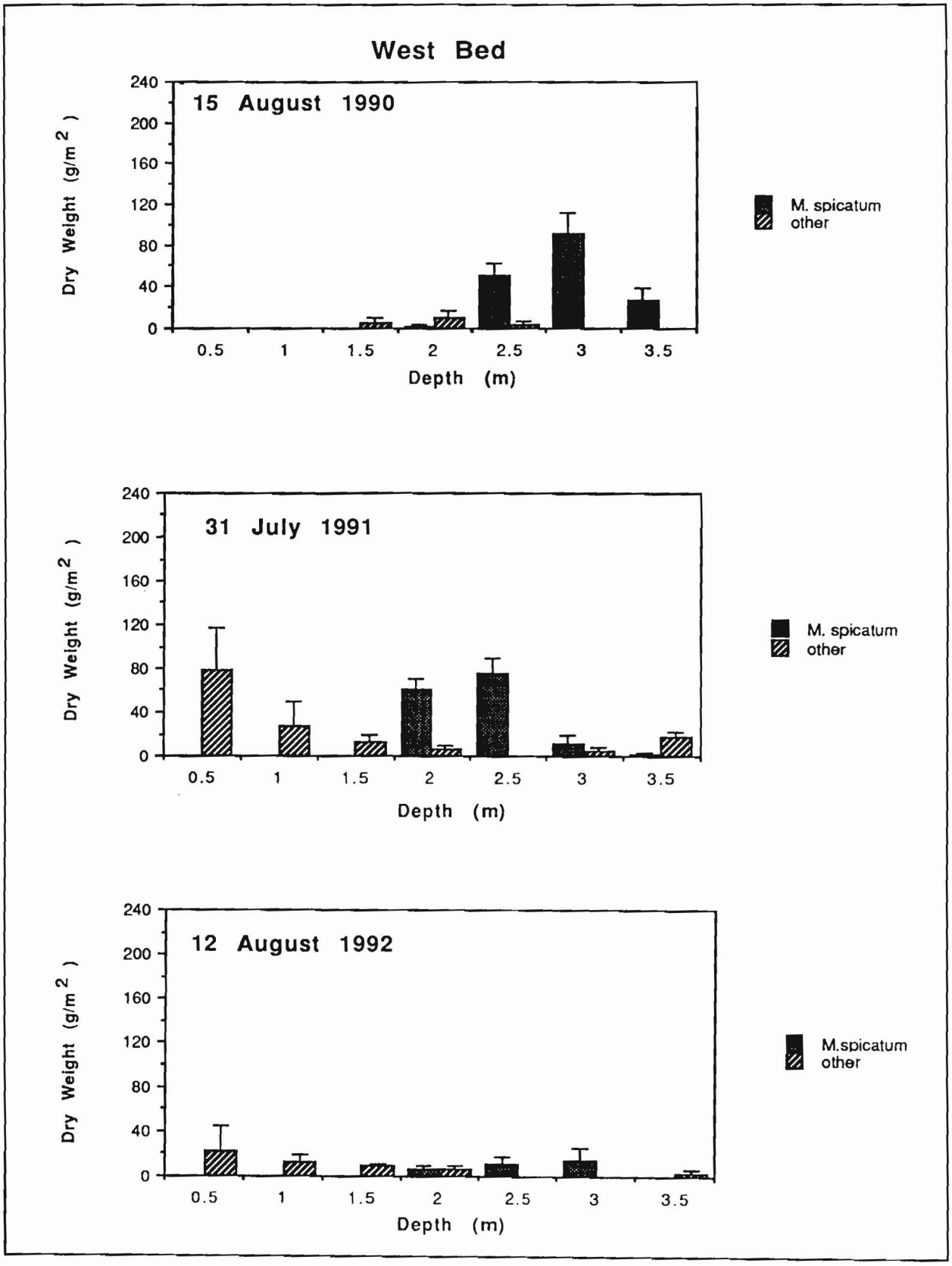

Figure 4. Results of plant transects for the West Bed, 1990-1992. Bars represent mean biomass (± 1 S.E.) of either Eurasian watermilfoil or combined native macrophyte species (=other)

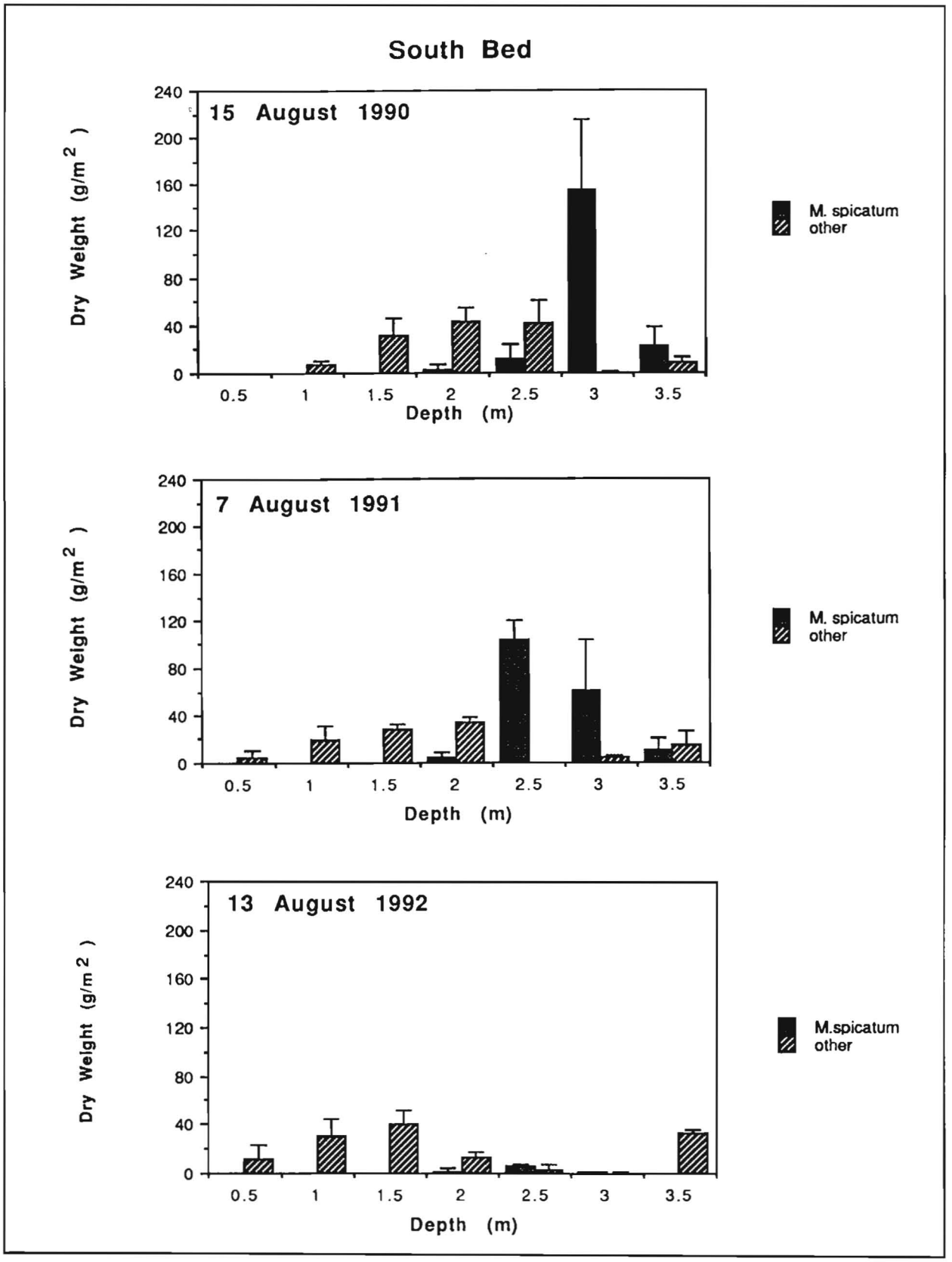

Figure 5. Results of plant transects for the South Bed, 1990-1992. Bars represent mean biomass (± 1 S.E.) of either Eurasian watermilfoil or combined native macrophyte species (=other)

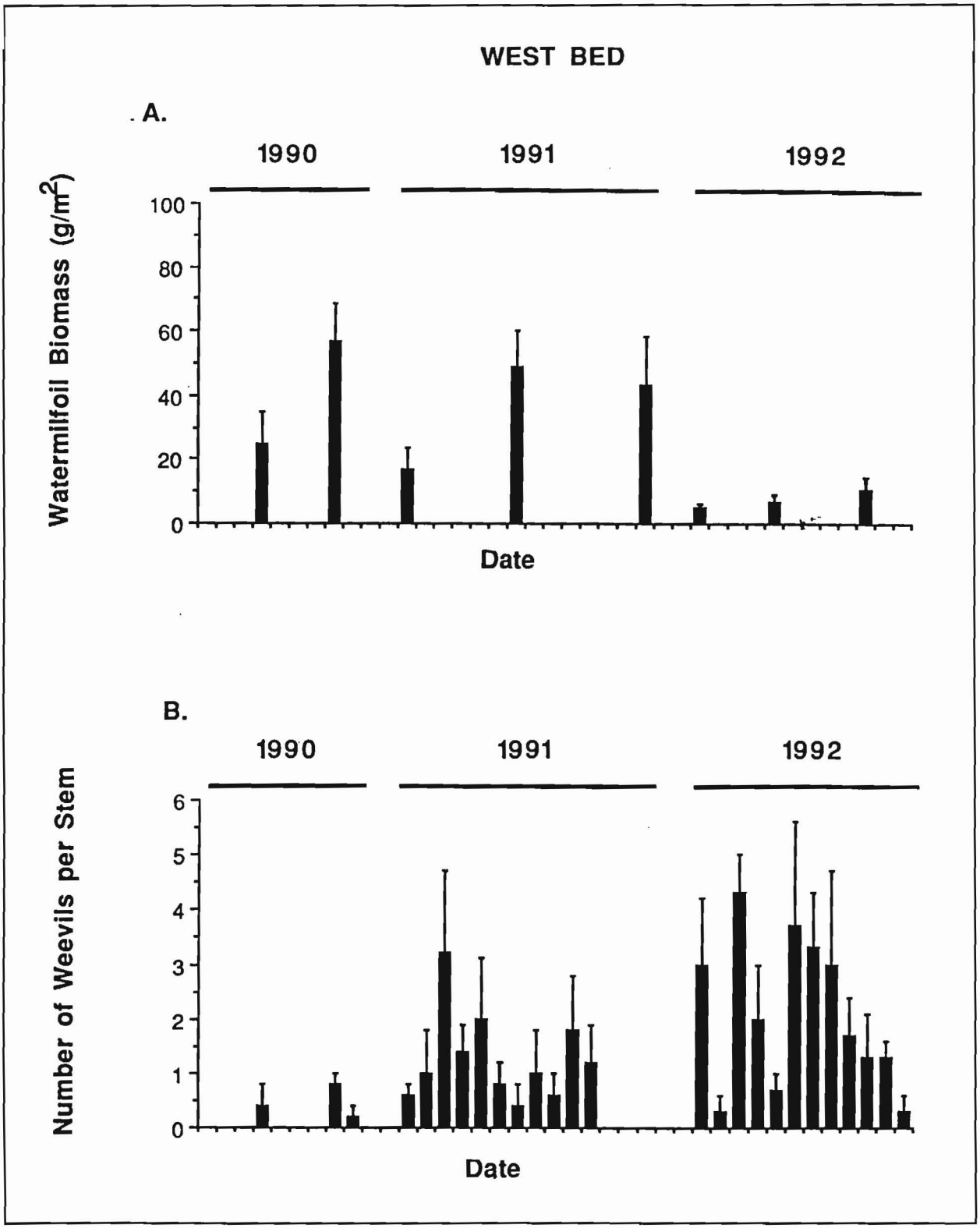

Figure 6. Eurasian watermilfoil and weevil abundance in the West Bed from 1990-1992. A. Watermilfoil biomass (mean ± 1 S.E.). Data are from plant transects. All samples from the 2.0- to 3.0-m depth intervals were used (n = 9 for each date). B. Weevil abundance as mean (± 1 S.E.) number of adults and larvae per stem. Samples collected using the small MIS sampler. N = 5 for all dates in 1990 and 1991. N = 3 for all samples in 1992

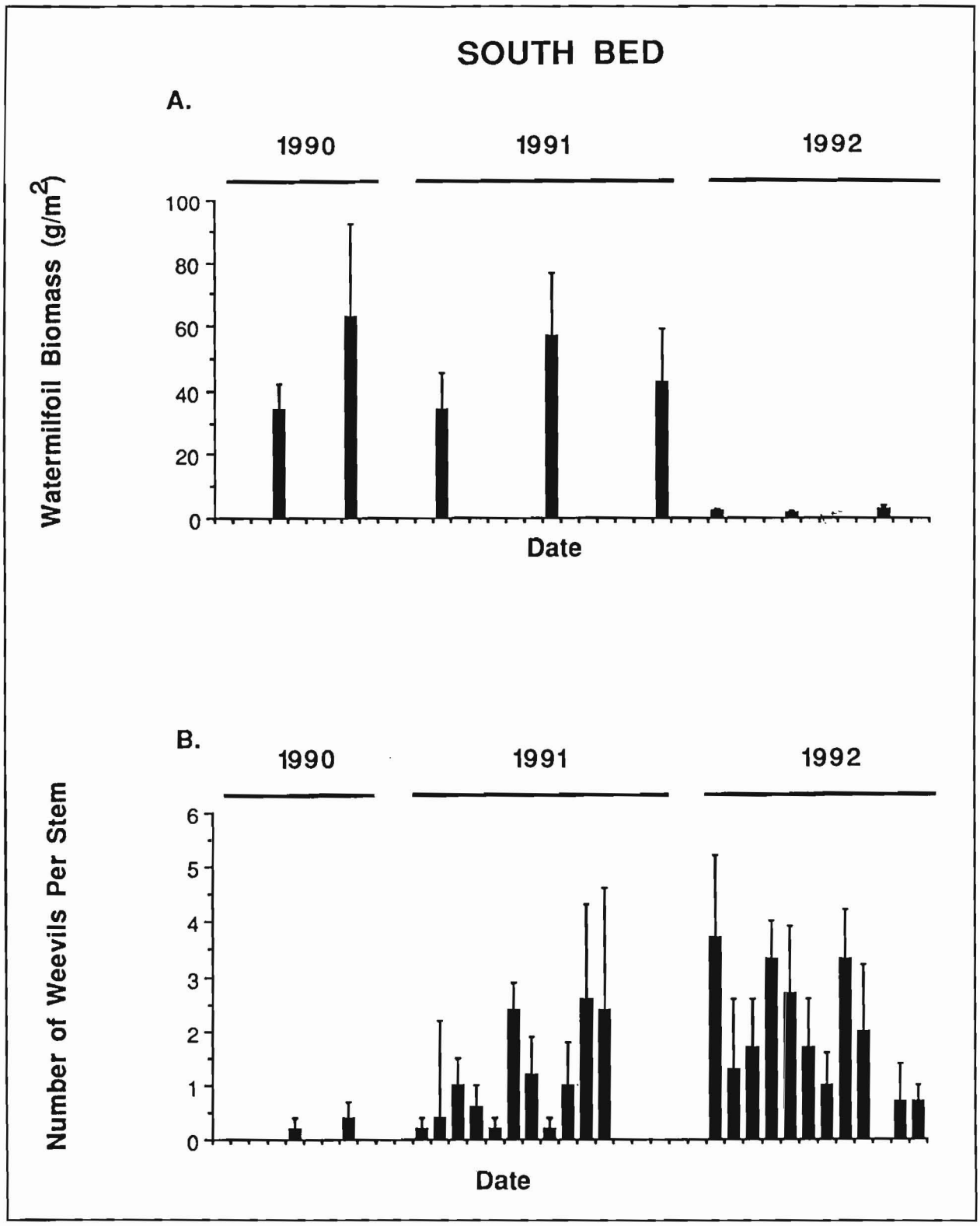

Figure 7. Eurasian watermilfoil and weevil abundance in South Bed from 1990-1992. A. Watermilfoil biomass (mean ± 1 S.E.). Data are from plant transects. All samples from the 2.0- to 3.0-m depth intervals were used (n = 9 for each date). B. Weevil abundance as mean (± 1 S.E.) number of adults and larvae per stem. Samples were collected using small MIS sampler. N = 5 for all dates in 1990 and 1991. N = 3 for all samples in 1992

Table 1
Results of the Analysis for Sediments Collected from Five Sites in Brownington Pond

Variable	Site				
	Natives	South Bed	South Shallow	West Bed	West Shallow
Sediment Extractions					
Exch. NH_4	0.00 ab (0.014)	0.034 b (0.016)	0.056 ab (0.017)	0.134 a (0.035)	0.069 a (0.011)
Exch. K	0.074 a (0.027)	0.050 a (0.017)	0.088 a (0.032)	0.109 a (0.008)	0.075 a (0.017)
Available PO_4	0.147 a (0.013)	1.131 a (0.003)	0.145 a (0.032)	0.173 a (0.016)	0.162 a (0.020)
Total P	0.638 a (0.056)	0.670 a (0.050)	0.535 a (0.109)	0.779 a (0.029)	0.562 a (0.071)
Total N	15.3 a (0.6)	13.5 a (0.9)	12.6 a (2.7)	13.5 a (0.4)	14.0 a (0.4)
Interstitial Water					
NH4-N	2.88 a (0.38)	0.68 b (0.20)	1.17 a (0.22)	3.16 a (0.90)	1.33 ab (0.20)
SRP	0.010 a (0.003)	0.006 a (0.003)	0.006 a (0.004)	0.031 a (0.010)	0.007 a (0.001)
FE	0.24 a (0.13)	0.35 a (0.17)	0.63 a (0.13)	0.45 a (0.04)	0.17 a (0.02)
K	2.09 a (0.64)	1.21 a (0.20)	1.70 a (0.10)	1.69 a (0.16)	2.04 a (0.63)
Sediment Density	0.57 a (0.009)	0.059 a (0.003)	0.070 a (0.015)	0.073 a (0.004)	0.069 a (0.004)
Organic Matter, %	48.28 a (0.41)	41.26 a (1.47)	39.46 a (6.07)	35.46 a (1.05)	41.63 a (0.87)

Note: Values in the table are means (± 1 S.E.). The units for the sediment extraction samples are mg/g; the units for the interstitial water samples are mg/l; the units for sediment density are g/ml. The data were analyzed using an ANOVA, and means for each site were compared using Tukey's HSD test. Treatment means that are significantly different ($p < 0.05$) from one another have different letters next to them.

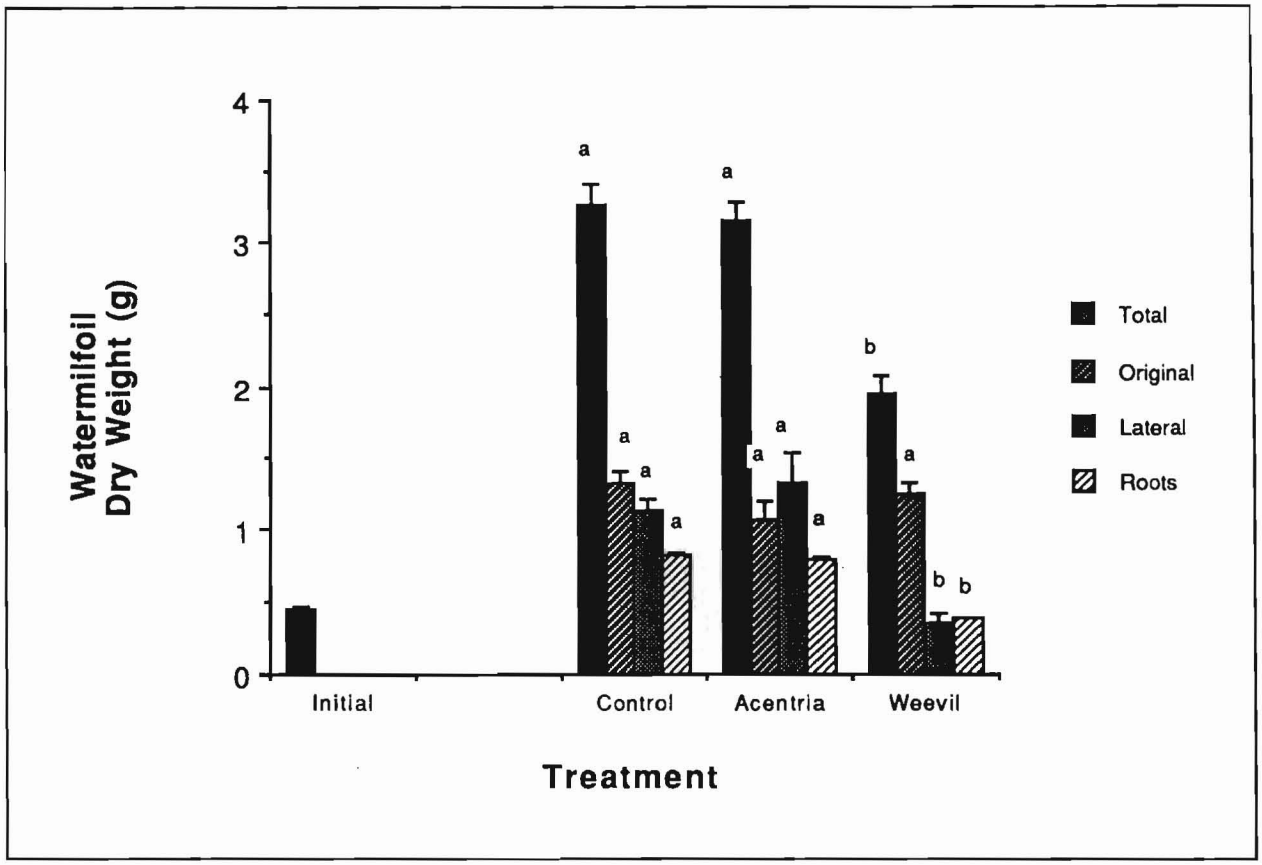

Figure 8. Results of the Brownington Pond enclosure experiment. Data shown include the total watermilfoil biomass per treatment (solid black bars) plus the distribution of that biomass by its components (i.e., original stem biomass, lateral stem biomass, and root biomass). Bars represent mean biomass (± 1 S.E.). Treatments with the same letter are not significantly different

Table 2
The Effect of Weevil and *Acentria* Feeding on Original Stem Height (cm) for the Last 3 Weeks of the Enclosure Experiment Conducted in Brownington Pond in 1992

	Treatment		
Date	Control	*Acentria* Control	Weevil
3 August	94.61 a (3.54)	90.78 a (1.22)	79.29 b (3.26)
10 August	96.11 a (2.47)	90.89 a (1.68)	75.42 b (3.19)
17 August	94.39 a (3.51)	91.17 a (0.44)	70.79 b (3.59)

Note: Values in the table are treatment means (± 1 S.E.). Treatment means that are significantly different ($p < 0.05$) from one another have different letters next to them. N = 6 for all treatments.

damaged versus undamaged plants might have been greater.

Norton Brook

Weevil transects

Neither *E. lecontei* nor watermilfoil plants exhibiting weevil damage were found in preliminary samples in Norton Brook Pond.

Enclosure experiment

Weevil enclosures had significantly less *M. spicatum* biomass compared with control enclosures ($p = 0.007$) and to open water ($p = 0.043$) (Figure 9). There was no significant difference in *M. spicatum* biomass between control enclosures and samples from the surrounding watermilfoil bed. There were

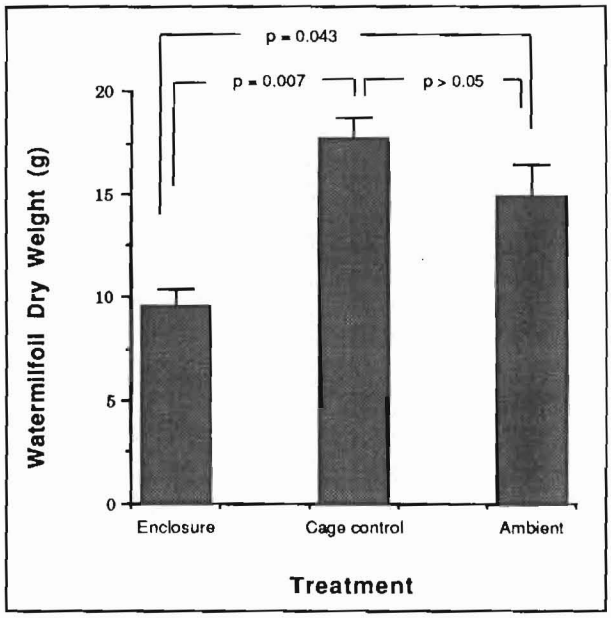

Figure 9. Results of Norton Brook Pond enclosure experiment. Bars represent mean (± 1 S.E.) dry weight of watermilfoil per treatment. Significance level for comparisons betwwen treatments are shown above bars

also visual differences in the position of the plants in the water column between weevil enclosures and control enclosures. In all control enclosures, *M. spicatum* formed a canopy on the surface, as it did in the surrounding bed. In the weevil enclosures, there were no plants at the surface. No plant species other than *M. spicatum* were present in any of the samples.

There were no significant differences in the abundance of macroinvertebrate taxa such as mayflies, caddis flies, mites, and chironomids inside enclosures compared with open water. There were significantly more macrozooplankton (Cladocerans and Copopods) in enclosures compared with open water ($p < 0.02$), however. This is not surprising, as there were no planktivorous fish inside the enclosures. There were no differences in overall taxa richness or organism abundance excluding zooplankton among the enclosures and open water.

An average (± 1 S.E.) of 30 (± 2.64) adults were recovered from the weevil enclosures. Very few weevil eggs and pupae were found in the enclosures. There were six eggs found in one enclosure (five eggs on one meristem and one on another) and one pupa found in another enclosure. No larvae were found.

Mean DO_2 concentrations (± 1 S.E.) at the bottom of the enclosures averaged 8.28 ± 0.37 mg/L except Day 6 when one control enclosure had a DO_2 concentration of 1.18 mg/L and on Day 36 one weevil enclosure had a DO_2 concentration of 1.46 mg/L. On Day 36, DO_2 at the bottom was somewhat lower than under ambient conditions (7.10 ± 1.29 mg/L in enclosures compared with 8.58 mg/L (n = 1) outside enclosures). On Day 36, water temperatures at sediment level and at the surface were 21.5 and 21.9 °C, respectively.

Discussion

The weevil and watermilfoil survey data support the hypothesis that weevils were involved in the observed Brownington Pond watermilfoil declines. In 1990, weevil abundance was at its lowest while that of watermilfoil was high. The summer of 1990 was the growing season subsequent to the first observed watermilfoil decline (see Creed and Sheldon (1991b) for maps illustrating the first Brownington Pond decline). With the marked decline of their major food resource by 1989 (weevils do not appear to feed on any other aquatic macrophyte present in Brownington Pond), it is not surprising that weevil numbers were low in 1990. From 1989 through 1991, the areal extent of the watermilfoil beds increased (see Creed and Sheldon (1991b) and Figure 3). This expansion was also reflected in the permanent grid data. However, the weevil population also began to increase in abundance in 1991, and watermilfoil biomass did not continue to increase over the 1991 growing season as it had in 1990; i.e., peak watermilfoil biomass was in midsummer of 1991 and not late summer as was the case in 1990. Overall, the number of weevils per stem increased through 1991 and were high at the onset of the 1992 growing season. Watermilfoil abundance had declined dramatically by June of 1992. Subsequent to the watermilfoil decline, weevil abundance began to decline by midsummer of 1992. Thus, the peak

abundances of watermilfoil and *E. lecontei* appear to be out of phase with one another. These patterns of abundance are similar to that displayed by simple predator-prey or host-parasitoid models (e.g., Begon and Mortimer 1981; Krebs 1985) and suggest that a similar interaction is occurring between Eurasian watermilfoil and *E. lecontei*. Additional collections over the next 6 to 9 years are needed to verify this cyclic pattern of weevil and watermilfoil abundance.

The results from both enclosure experiments demonstrate that the weevil *E. lecontei* can have a significant negative effect on Eurasian watermilfoil. In both experiments, the primary effect of weevils appears to have been a suppression of watermilfoil growth. In the Brownington Pond experiment, weevils suppressed production of new stems by damaging lateral shoot meristems. The meristem damage observed in this experiment was due to both larval and adult feeding. Weevil attacks on the shoots appear to have had a negative impact on root production. Weevil damage may influence root production, as the removal of stem vascular tissue by weevil larvae may interrupt much of the flow of gases and photosynthate to the root system. Weevil damage to the stem also caused the plants to sink out of the water column. This result with rooted plants confirmed the results of earlier experimental studies (Creed, Sheldon, and Cheek 1992) that demonstrated that weevils could affect the buoyancy of floating watermilfoil fragments. In the Norton Brook Pond experiment, damaged meristems were only found in the weevil treatment. This observation also suggests that weevils suppressed growth in this experiment by damaging meristematic tissue. The bulk of the weevil damage in this experiment was the result of adult feeding; few larvae were found on the plants at the end of the experiment. In conclusion, weevils appear to have two effects on Eurasian watermilfoil: (a) weevils damage existing stems, possibly stressing the plants physiologically as a result of disruption of gas balance and loss of vascular tissue, and (b) weevils inhibit the production of new stem tissue by destroying meristems. These data support the hypothesis that weevils played an important role in the Brownington Pond watermilfoil declines.

Changes in water and sediment chemistry do not appear to have been the primary causes of the Brownington Pond watermilfoil decline. Concentrations of the measured nutrients in the water column displayed essentially no change between 1991 and 1992 or within the 1992 growing season. It is possible that a change in some unmeasured waterborne micronutrient could have caused the decline. However, observations from Brownington Pond suggest that this was not the case. First, watermilfoil did not disappear throughout the pond, which is small and appears to have a well-mixed epilimnion (e.g., temperatures are nearly uniform around the epilimnion of the pond). Second, the Brownington Pond enclosure experiment was conducted adjacent to the site of the former South Bed where the reduction in watermilfoil abundance was greatest between 1991 and 1992. The watermilfoil inside the enclosures readily grew at this site, while little watermilfoil growth was observed immediately surrounding the enclosures. The latter observation suggests that some other factor was preventing the reestablishment of watermilfoil in this area.

Changes in sediment chemistry also do not appear to have been important in producing the decline. Only one sediment variable, the concentration of ammonium, was found to vary significantly among sites. Ammonium concentrations in both the sediment and the interstitial pore water were lowest in the sediments of the former South Bed. These results were the opposite of those of Painter and McCabe (1988), who found that ammonium concentrations were lowest in areas of high watermilfoil abundance. We are not sure why ammonium abundance was lower at the South Bed site. As ammonium is produced by the decomposition of organic matter by heterotrophic bacteria (Wetzel 1983), we would have expected higher sediment concentrations at the South Bed site, as there was a layer of decomposing watermilfoil on the sediment surface for much of the summer. Alternatively, the watermilfoil bed that had previously been

present at this site may have severely depleted sediment ammonium concentrations with the result that watermilfoil was unable to grow here. However, we used sediment from the South Bed in the enclosure experiment. As the experimental watermilfoil grew on this sediment, we do not believe that change in sediment quality was a primary factor in the Brownington Pond decline. The results from Norton Brook Pond also support the hypothesis that herbivory and not changes in sediment quality was primarily responsible for the Brownington Pond decline. The sediment was not disturbed in any way in the Norton Brook Pond experiment. The only difference between treatments was the presence of weevils. While there may be an interaction between nutrient availability and the effect of the weevil on Eurasian watermilfoil (e.g., reduced root production in the presence of weevil herbivory results in reduced sediment nutrient uptake), we do not believe that changes in nutrient availability alone could have produced the Brownington Pond decline. Admittedly, our assertions regarding the effects of sediment nutrients are based on a limited number of samples from a single date. However, our results confirm those of Painter and McCabe (1988), who could find no relationship between sediment quality and the watermilfoil declines observed at the Kawartha Lakes.

The fact that much of the Brownington Pond watermilfoil disappeared during the winter suggests that weevil herbivory stresses the plants in some manner that makes it difficult for watermilfoil to overwinter. There are a number of possible mechanisms that could produce this overwinter decline. For example, weevil damage to stem vascular tissue could prevent movement of nutrients and/or gases from stems to roots (or vice versa) that could physiologically stress the plants (Wetzel 1983). Alternatively, the weevil-damaged plants may be much more susceptible to decomposers than healthy plants. Furthermore, rapidly decomposing plants may alter the chemistry of the surrounding water and/or sediments that could have a negative impact on healthy plants. At present, the reason that the watermilfoil in Brownington Pond declined during the winter remains unknown. However, this observation suggests that winter may be the season when the greatest reduction in watermilfoil biomass occurs. Further research is needed to understand this potentially important effect.

It is unclear why there was apparently so little weevil reproduction in the enclosures at Norton Brook Pond. One possible explanation is that weevil reproduction may have been inhibited by low dissolved oxygen (DO_2) concentrations inside the enclosures. However, on the three dates that we quantified DO_2, the concentrations were usually high and did not differ between enclosures with and without weevils. It is possible that prolonged DO_2 sags occurred inside the enclosures and that this had a negative impact on weevil reproduction. However, there was no significant effect of the enclosures on other macroinvertebrate taxa associated with the watermilfoil. The factor that limited weevil reproduction in Norton Brook Pond needs to be determined if future weevil introductions are to be successful and the weevil population is to be self-sustaining.

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Biocontrol of Waterlettuce (*Pistia stratiotes*) An Annual Report

by
F. Allen Dray, Jr.,[1] *and Ted. D. Center*[2]

Introduction

The floating macrophyte *Pistia stratiotes* L. (waterlettuce) is a nuisance throughout much of the tropics and subtropics. Large impenetrable mats impede irrigation practices, hamper flood-control efforts, block navigational channels, and interfere with the recreational uses of many waterways. These mats are formed when individual plants and their many vegetative offsets intertwine with other rosettes. Although large populations can be controlled by herbicides, the production of seeds (Dray and Center 1989) ensures retreatments are necessary.

Two insects have been used in other countries to manage *P. stratiotes* populations and thereby reduce or eliminate the need for applying herbicides. The first, a weevil (*Neohydronomus affinis* Hustache), has controlled waterlettuce in Australia (Harley et al. 1984), South Africa (Cilliers 1987, 1990, 1991), and Zimbabwe (Chickwenhere and Forno 1991). The second, a moth (*Spodoptera pectinicornis* Hampson), is used in Thailand to prevent destruction of rice paddies (Napompeth 1982). It has also been recommended for use in the Philippines (Bua-ngam 1974) and Indonesia (Mangoendihardjo 1983). A desire to import one or both of these insects for use against waterlettuce in Florida prompted the initiation of a classical biological control program against this weed. Surveys conducted during 1985-86 confirmed that neither of these herbivores was present in Florida (Dray et al. 1988). Quarantine studies later resulted in approval to release these two insect biocontrol agents (Habeck et al. 1989; Thompson and Habeck 1988, 1989) in the United States.

The first field releases of *N. affinis* occurred in April 1987; and by September 1988, persistent populations had become established at several sites (Habeck et al. 1989; Dray et al. 1990). Weevil populations flourished at several, but not all (e.g., Port St. Lucie), release sites. Waterlettuce at Kreamer Island on Lake Okeechobee harbored approximately 45 million *N. affinis* by spring 1989 (Center and Dray 1990). Weevil abundance at Torry Island on Lake Okeechobee reached similar proportions a year later. Waterlettuce populations at both sites were decimated, and plant abundance was reduced by 90 percent relative to preweevil densities. Waterlettuce populations at 45 sites in Florida and 6 sites in Louisiana had become infested with *N. affinis* by the end of 1991. Substantial control had been achieved at five of the Florida sites.

N. affinis populations failed to persist at some sites. At other sites, the weevils never became abundant enough to stress the waterlettuce. These shortcomings strengthened perceptions that the program could benefit from the release of a second biocontrol agent. Consequently, *S. pectinicornis* was imported from Thailand for use against waterlettuce (Habeck et al. 1989; Center and Dray 1990; Grodowitz 1991). Field releases began in December 1990. As we reported last year, however, establishing persistent field populations has proven elusive. This report discusses continued dispersal by *N. affinis* and describes our attempts (December 1990 - December 1992) to establish *S. pectinicornis* on waterlettuce in Florida. Further, it outlines our future plans regarding these two waterlettuce bioagents.

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Culture and Release Techniques

The techniques we employ to culture *S. pectinicornis* are modifications of the methods developed by Dr. Dale Habeck and his assistants during the quarantine testing of this moth in Gainesville. Most commonly, we introduce adult moths into glove boxes containing whole plants and allow them to oviposit for 1 to 5 days (depending upon the condition of the plants). Leaves containing egg masses are then excised from the plants and placed on moist paper towels in plastic bowls (3.4 qt). Eggs are monitored daily, and approximately 5 days after eclosion, larvae are transferred to fresh leaves in large, clear plastic containers. Leaves are replaced as needed. Pupae are removed from these containers and placed on moist filter pads in petri dishes (about 50 pupae/dish). Adults that emerge are introduced into a glove box to continue the cycle.

In a variation of the preceding technique, we introduce a pair of moths into a petri dish containing two or three leaves on moist filter paper. Leaves upon which eggs have been deposited are then handled as described previously. This technique is not as effective as the earlier method, but is preferable for obtaining accurate assessments of oviposition and larval survival. It is more labor intensive, however.

We have attempted to simulate more natural conditions and reduce the labor involved in culturing this insect by releasing adults into screened outdoor tanks (400-gal capacity) or greenhouse tanks (800-gal capacity) containing waterlettuce. One or two generations of moths are allowed to complete development without being disturbed. Then about 75 percent of the infested plants are removed to inoculate new tanks or for release at field sites. Fresh plant material is introduced into the tanks as needed.

Several of the techniques we have examined are capable of producing large numbers of *S. pectinicornis*. We will continue to employ these techniques even as we test additional methods for increasing the productivity of our laboratory colonies, both through attempts to enhance oviposition success and through efforts to improve larvae survival.

This year, we initiated the practice of enclosing segments of the waterlettuce mat with 10-m^2 polyvinyl chloride frames. These frames facilitate locating the exact points within the mat where previous releases were made. This, in turn, enables us to (a) carefully examine during subsequent visits the plants upon which larvae were released, (b) ensure that we are able to flood a small portion of the mat with large numbers of moths, and (c) determine whether larvae are dispersing only within a small area of the mat or throughout the entire mat.

Predation by birds, spiders, and other insects has possibly impeded our attempts to establish persistent populations of *S. pectinicornis*. Thus, we tested the feasibility and utility of releasing moths and larvae into screened cages that would exclude most predators.

Project Status

Spodoptera pectinicornis

We have inoculated 14 field sites with a total of nearly 180,000 *S. pectinicornis* eggs, larvae, and adults over the past 2 years (Figure 1, Table 1). During that period, we have employed three different strategies in our attempts to establish self-perpetuating populations of this moth. The ease with which it could be colonized in the laboratory and reports of its utility for controlling waterlettuce in Thailand initially caused us to believe that establishing field populations of this moth would be a simple matter. We therefore released small numbers of eggs and larvae at several field sites in southern Florida (see Table 1) and then waited for the numbers to build naturally. Unfortunately, subsequent visits to these sites failed to produce any evidence that the moths were persisting at these sites.

The second release strategy we employed consisted of making multiple releases comprising large numbers of insects at few sites

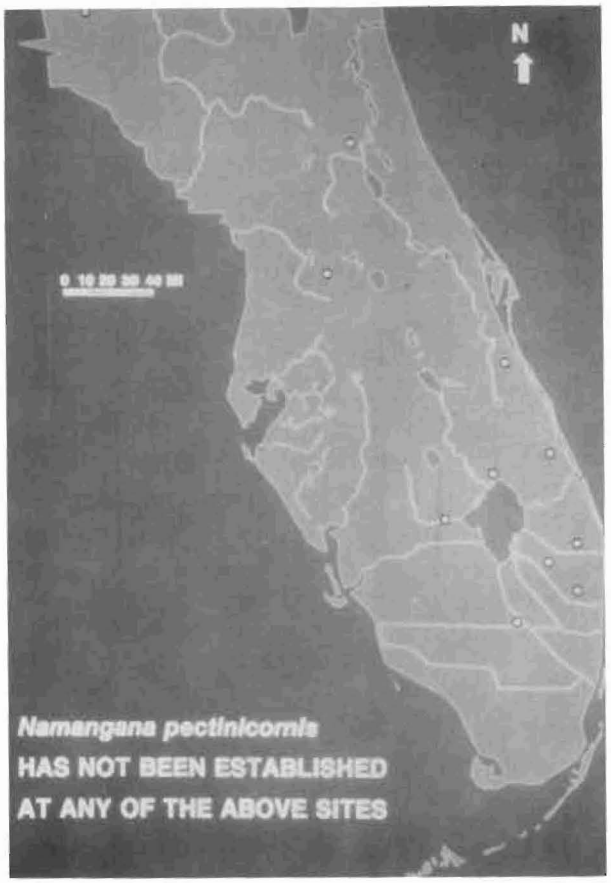

Figure 1. Spodoptera pectinicornis release sites, Decemer 1990-December 1992

(Fisheating Creek, Eagle Bay, and Lake Oklawaha; see also Table 1). Despite the inclusion of precautions similar to ones that have been successful with hydrilla flies (Center, Dray, and Durden 1991), this strategy also seemed ineffective. Consequently, we initiated in late 1991 a highly intensive strategy in which large numbers of *S. pectinicornis* are released several times a week at a single nearby site (Andytown, see Table 1).

Close monitoring of the Andytown site provided much needed feedback concerning the short-term fate of released individuals. For example, we observed that larvae quickly disperse from the leaves on which they were released to plants at the site. A second discovery was that the larvae appear to prefer grazing on the leaf surfaces rather than mining within the leaves. We also noticed that, despite their proclivity for dispersal, *S. pectinicornis* larvae appeared to be very sluggish. These habits heighten the risk of falling prey to birds, spiders, ants, etc., which probably explains why small isolated releases have been unsuccessful.

We had no trouble establishing *S. pectinicornis* populations in large tanks, whether in the greenhouse or outdoors; yet releases at the Andytown site appeared to be ineffective. The moths we were releasing were direct descendants of the moths Dr. Dale Habeck (University of Florida) had initially imported into quarantine for host testing. This suggested to us that perhaps our colonies had become so inbred that they lacked sufficient vigor to persist in the wild. Consequently, Dr. Habeck imported more moths from Thailand in an attempt to improve our colonies' gene pool. We received F2 offspring of these new moths in June and immediately set about releasing a portion of them at Andytown. Subsequent releases came from colonies comprised of this fresh germ plasm.

The results were very encouraging. Within a few weeks, we began to recover a few larvae from plants outside of the release frames. Although this was not the first time we had recovered larvae from the site, it was the first outside the immediate vicinity of plants upon which larvae had been released. We also intermittently began discovering egg masses on plants at the site. This was particularly encouraging because it confirmed that at least some of the larvae were able to complete development and emerge as adults.

Our efforts suffered a major setback in August, however, when Hurricane Andrew struck South Florida. Although the hurricane passed well south of our study site, water levels had been lowered in all of the canals and waterways within the South Florida Water Management District's jurisdiction in anticipation of the storm. Unfortunately, most of the waterlettuce was flushed from our study sites in the process. Visits to the site about a month following the hurricane produced two late instar larvae from what must have been at least the F2 generation. This was a very exciting find because it demonstrated that some *S. pectinicornis* survived

Table 1
***Spodoptera pectinicornis* Release Sites**

Releases				No. Released			
Site	County	No.	Dates	Eggs and Larvae	Pupae	Adults	Plants[1]
Fisheating Creek[2]	Glades	13	12/18/90-12/28/91	22,617	0	0	No
Eagle Bay (Lake Okeechobee)[2]	Okeechobee	12	4/24/91-8/12/91	31,163	30	100	Yes
Lake Oklawaha[2]	Putnam	10	2/13/91-8/30/91	28,100	15	4	No
South Florida Fairgrounds[3]	Palm Beach	1	12/5/90	1,500	0	0	No
Pioneer Park[3]	Palm Beach	1	12/30/90	2,347	0	0	No
Port St. Lucie[3]	St. Lucie	2	12/1/90-12/30/90	3,208	0	0	No
St. John's Marsh[3]	Brevard	2	1/2/91-4/25/91	4,468	0	0	No
Loxahatchee Recreation Area[3]	Broward	1	9/9/91	3,000	0	0	No
Havana Pond[3]	Gadsen	2	9/7/91-9/11/91	1,500	0	0	No
Lake Panasoffkee[3]	Sumter	2	9/25/91-9/30/91	1,200	0	0	No
Andytown[2]	Broward	43	11/6/91-8/17/92	83,306 [5]	1,710	986	Yes
Tallowwood[3]	Broward	1	10/7/92	750	0	0	No
Lake Rousseau[3]	Citrus	1	10/7/92	3,750	0	0	No
Canal Point[4]	Palm Beach	4	8/20/92-10/2/92	5,025	10	0	Yes
Totals		95	12/18/90-10/7/92	191,934	1,765	1,090	

[1] Whole waterlettuce plants (as opposed to individual leaves) infested with unknown numbers of *Spodoptera pectinicornis* larvae and eggs.
[2] Sites where we made multiple releases of large numbers of *Spodoptera pectinicornis*.
[3] Sites where we made few releases of small numbers of *Spodoptera pectinicornis*.
[4] The site where we are currently making intensive releases of *Spodoptera pectinicornis*.
[5] This figure includes estimates of numbers of eggs contained in egg masses.

the storm. Further, it suggested that a self-perpetuating population may have been becoming established prior to the hurricane. Subsequent visits to the site failed to produce evidence that the population was persisting, however.

We established a new release site near Canal Point where we began releasing moths into cages during late August. Visits to the site in September and October produced larvae, pupae, and adults, thereby suggesting that the use of cages was beneficial. Unfortunately, the cages excluded birds, spiders, and flying insects, but not fire ants. Predation by fire ants apparently decimated the fledgling population, because subsequent visits failed to produce additional *Spodoptera*.

Neohydronomus affinis

Although *Neohydronomus affinis* continues to disperse from infested sites to adjacent water bodies (Figure 2), there remain many sites to which the weevils have not yet emigrated. We and our cooperators have thus aided dispersal by inoculating additional sites with weevils collected from Tenoroc State Preserve (Polk County) and Pioneer Park (Palm Beach County). Sites inoculated during 1992 include Alexander Springs (Lake County), Ponce de Leon Springs (Volusia County), Puzzle Lake (Seminole County), Lake Monroe (Seminole County), Orange Lake (Alachua County), the Wekiva River (Seminole County), Havana Pond (Gadsden County), Stick Marsh (Indian River County), St. Johns River Marsh (Brevard County), and the Itchetucknee River (Suwanee County). *Neohydronomus affinis* is dispersing naturally from established sites in Louisiana, where waterlettuce populations at two sites (a pond near Houma and a drainage ditch near Choctaw) declined dramatically in 1992.[1] Also, U.S. Army Engineer Waterways Experiment Station personnel established a weevil population at Lake Dunlop in Texas during 1992.[1]

[1] Personal Communication, 1992, Dr. Michael J. Grodowitz, Entomologist, U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

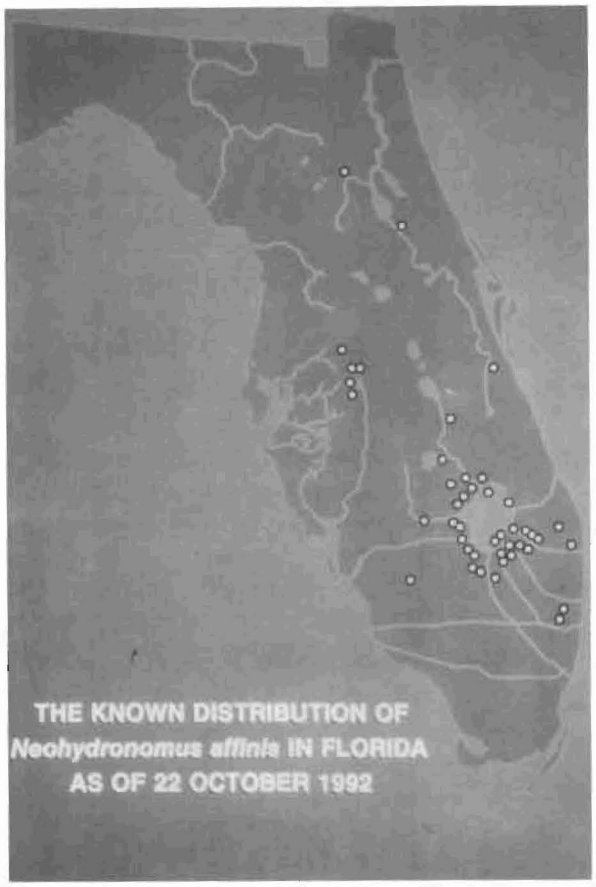

Figure 2. Sites where self-perpetuating Neohydronomus affinis populations have become established in Florida

Future plans

We are now convinced that we have developed an effective strategy for establishing persistent populations of *S. pectinicornis*. This strategy consists of inoculating a single waterlettuce infestation as frequently as possible with as many adult moths as the laboratory colonies at Gainesville and Fort Lauderdale can produce. We will release onto clean (i.e, *Samea multiplicalis*, ants, etc., removed), healthy waterlettuce in a cage. The cage will prevent intrusion by flying predators and by *Samea*. Plants in the cage will be isolated from the surrounding waterlettuce mat to prevent encroachment by spiders and ants. This will be accomplished by removing all plants that are outside of the cage but within an 85-m^2 frame that surrounds the cage. We will begin inoculating a second site only after we are convinced either that (a) the moth population will persist at the Canal Point site or that (b) our efforts there are futile. In the latter case, we may choose to further modify the release strategy.

The coming year will be very similar to 1992 in that few of our resources will be directed towards *N. affinis*. We will, however, continue to monitor dispersal by surveying waterways as opportunity permits. We will also continue cooperating with interested agencies to increase dispersal of this weevil. This will be accomplished primarily by directing operational personnel to waterlettuce sites with large weevil populations when we discover them and by advising these personnel of the most effective techniques for collection and transport of these biocontrols.

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The Allelopathic Ability of Three Species of Aquatic Plants to Inhibit the Growth of *Myriophyllum spicatum*

by
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Introduction

Background

Myriophyllum spicatum (Eurasian watermilfoil) is a noxious aquatic plant introduced into the United States from Eurasia. Plants are often found in lakes, rivers, drainage and irrigation canals, ponds, and streams. Severe infestations of watermilfoil can restrict boat traffic, interfere with fisheries, interfere with waterflow, and displace native species. Watermilfoil is a nuisance submersed aquatic plant that reproduces by fragmentation. Eurasian watermilfoil is found in many southern states and California. Mechanical removal methods tend to increase the spread of watermilfoil because of fragmentation; and while herbicides are used to some extent in various places, there is major concern for the environment and water quality.

Allelopathy refers to the biochemical interactions that take place among plants; however, its effectiveness depends on the addition of a chemical to the environment (Sutton 1986). In general, the term allelopathy refers to the detrimental effects of higher plants of one species (the donor) on the germination, growth, or development of another species (the recipient) (Putnam 1985).

Rice (1974) provided us with a more functional definition of allelopathy as being any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds that escape into the environment. Similarly, Parker (1984) defined allelopathy as the harmful effect of one plant or microorganism on another because of the release of secondary metabolic products into the environment.

Allelopathy may be a potentially important mechanism in the management of undesirable aquatic plants. Because it may provide an inexpensive and more desirable method of control than more conventional methods such as the use of herbicides or mechanical removal, allelopathy may prove to be one of our best methods to control watermilfoil.

This paper reports the results of two experiments: (a) a test tube assay to determine the allelopathic ability of various aquatic plant extracts to inhibit the growth of Eurasian watermilfoil, and (b) addition of organic matter from various aquatic plants to the substrate to determine if similar results could be obtained using rooted plants. Procedures used in this study were modified from Barko and Smart (1983).

Test tube assays were used first because this is a rapid screening method to indicate the allelopathic potential of various candidates. The next logical step in this research effort was to conduct rooted plant studies (tank studies), which represent the next scale up from the test tube assays.

Test 1 species selection

Species selected for analysis (in Test 1) was the result of laboratory bioassays conducted by Elakovich and Wooten (1989). They listed several species of aquatic plants that were potentially allelopathic to Eurasian watermilfoil.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

These species were used in our test tube assays (Table 1).

Table 1
List of Species

Fanwort	*Cabomba caroliniana* Gray var.
Coontail	*Ceratophyllum demersum* L.
Eurasian watermilfoil	*Myriophyllum spicatum*
Watermilfoil	*Myriophyllum heterophyllum* Michx.
Common water nymph	*Najas guadalupensis* (Spreng.)
American lotus	*Nelumbo lutea* (Willd.) Pers.
Fragrant water-lily	*Nymphaea odorata* Aiton
Pickerelweed	*Pontederia lanceolata* Nutt.
Pondweed	*Potamogeton nodosus* poir.
Duck-potato	*Sagittaria lancifolia* L.
Eel-grass	*Vallisneria americana* Michx.

Test 2 species selection

From the results of Test 1, we found that three species, coontail (*Ceratophyllum demersum*), eelgrass (*Vallisneria americana*), and pondweed (*Potamogeton nodosus*) inhibited the growth of Eurasian watermilfoil, thereby being potentially allelopathic to watermilfoil. These three species were selected to be tested in our rooted plant studies.

Methods and Materials

Plant collection

Aquatic plants were field collected from Caddo Lake, LA, and J. D. Murphee Wildlife Refuge, Port Arthur, TX, and transported back to Vicksburg, MS, in ice chests with sufficient ice to prevent plant deterioration. Entire plants were collected whenever possible. Plants were washed to remove dirt and debris. The procedure listed above was conducted for both experiments.

In addition to the procedures listed above, for experiments with organic matter addition, plants were oven-dried at 70 °C, and ground with a Wiley mill to 7 µm.

Watermilfoil stock cultures

Stock cultures of watermilfoil were grown from field-collected specimens and maintained in the greenhouse in 1,150-L fiberglass tanks. Plants were aerated with compressed air and maintained at a constant temperature of 25 °C. Plants were grown in lake sediment and recultured periodically using 11-cm apical tips.

Experiment 1. Test tube assays were conducted in the greenhouse in a circulated water bath at a constant temperature of 25 °C. Two-centimeter apical tips of Eurasian watermilfoil were placed in 60-ml capacity test tubes with 40 ml of artificial lake water media (Smith and Jones 1990). Each tube was randomly selected to receive 1, 3, or 10 ml of one of the various extracts tested. Numbered rubber stoppers with glass tubing inserted (for aeration) were placed in the test tubes and the tubes placed in a circulated water bath. The latex rubber tubing was connected between the glass tubing and a compressed air outlet for proper mixing of chemicals, aeration, providing CO_2, and preventing algal growth. The experiment was allowed to run for 2 weeks, then repeated two additional times.

Extract preparation. Two hundred grams of each of the test plants was cut into small pieces and placed in a Waring commercial blender to which 200 ml of reverse osmosis water was added. This was blended for 5 min on low speed and for 2 min on high speed. Each of the 200-g aliquots were refrigerated for 24 to 72 hr to enhance extraction of the organic compounds. The aliquots were centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge (Beckman J2-21M/E), then filtered through Whatman No. 54, 42, and GF/F filter paper, respectively. The filtrate was frozen until all plants to be tested were processed.

Experiment 2. In greenhouse studies 5 and 20 percent organic matter from *C. demersum*, *P. nodosus*, and *V. americana* were randomly added to 32-oz. plastic cups and 1,248 g of lake sediment (Brown's Lake, MS) and mixed thoroughly. Three 15-cm-long apical tips of Eurasian watermilfoil were placed in the plastic cups to a depth of 10 cm, then overlain with a layer of silica sand to prevent sediment and organic matter from leaching into the water column. The cups were placed in 30-in.-tall (12-L capacity) plexiglass cylinders,

then filled with nutrient solution (modified Barko's medium). The acrylic columns were then placed in 1,150-L capacity fiberglass tanks filled with tap water (as a water bath) and maintained at a constant temperature of 25 °C.

Aeration was supplied to each column through the use of compressed air to prevent algal growth and ensure proper mixing of chemicals in the nutrient solution. The study was allowed to run for a period of 4 weeks, then repeated two additional times for verification.

Data Analyses. The data were subjected to analyses of variance (ANOVA) and the Duncan's Multiple Range Test of the Statistical Analysis System (SAS) to determine if there were significant differences between the controls and test species.

Results and Discussion

In the test tube assays when mean biomass was compared at the 3-ml concentration, all species were significantly different from the controls except *P. nodosus*; however, *V. americana* and *Sagittaria lancifolia* showed the greatest difference between all plants tested.

When mean biomass was compared at the 10-ml concentration, *C. demersum* showed the greatest difference from the controls; however, *V. americana* and *Nymphaea oderata* were also significantly different. In the experiments involving organic matter additions, data analyses from all three experiments showed there were no significant differences between experiments. Therefore, data from all three experiments were combined and analyzed using ANOVA of SAS. Test results showed no significant differences in total biomass with 5-percent concentration of organic matter addition; however, with the 20-percent concentrations, *Ceratophyllum* showed significant differences in biomass when compared with control.

When mean length for the two concentrations of matter additions were compared at the 5-percent concentration, no significant differences were detected; however, at the 20-percent concentration, *Ceratophyllum* and *Vallisneria* were found to be significantly different from the control, while small differences were detected for *Potamogeton*.

Conclusions

Based on the results obtained from these studies (test tube assays and organic matter additions), *C. demersum* and *V. americana* appear to be our two best allelopathic candidates for reducing various submersed aquatic plants including Eurasian watermilfoil biomass. These investigations confirm results of earlier studies conducted at the U.S. Army Engineer Waterways Experimetn Station (Jones and Kees 1991). Field tests with organic matter from *Ceratophyllum* and *Vallisneria* will be our next phase of study. In addition to these two species, we will also take a closer look at *Eleocharis* because it has been reported to be allelopathic to watermilfoil in various literature citations.

Acknowledgments

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Collection Techniques and Growth of Triploid Grass Carp in the Santee Cooper System, South Carolina

by
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Introduction

The Santee Cooper system in South Carolina comprises almost 70,000 ha of impounded water consisting of Lake Marion, Lake Moultrie, and the diversion canal connecting the two lakes. During the last decade, hydrilla *Hydrilla verticillata* became established throughout the upper reaches of Lake Marion (Inabinette 1985). The extent of hydrilla was such that chemical control became impractical. Starting in 1989, triploid grass carp *Ctenopharyngodon idella* were stocked as a potential biological control agent. The stocking rates were 100,000 per year through 1992 with a goal of achieving a stocking density of approximately 50 fish per vegetated hectare. Triploid grass carp, legal for use in South Carolina since 1985, were chosen because of feeding habits similar to diploid grass carp (Wattendorf and Anderson 1984; Sutton 1985; Allen and Wattendorf 1987). They are sterile, less costly, and provide longer control than herbicides.

This article summarizes (a) a grass carp collection technique, (b) length-to-weight relationships of grass carp, (c) information on age distribution, (d) rates of growth, and (e) future research efforts. Development of scientifically sound aging techniques will allow triploid grass carp to be aged in other systems. Survival and growth-rate information will be used to refine the grass carp stocking model developed at the U.S. Army Engineer Waterways Experiment Station (WES).

Methods

Collection techniques

An initial problem was collecting adequate numbers of triploid grass carp using traditional sampling gears. As part of a grass carp monitoring program (Morgan and Killgore 1990), vegetated areas of Lake Marion were extensively electrofished. In addition, the South Carolina Wildlife and Marine Resources Department (SCWMRD) regularly conducted gill-net, cove rotenone, and electrofishing sampling of the Santee Cooper system. Although some grass carp were collected by each of these methods, the numbers collected were inadequate for research needs.

Many triploid grass carp were accidentally harvested during bowfishing tournaments in 1990. As a result, bowfishermen were used in a final attempt to collect triploid grass carp. The collection was organized and permitted by SCWMRD. Bowfishing teams familiar with the reservoirs and who had consistently won local bowfishing tournaments were recruited and paid for their efforts.

The collection efforts took place at night using a boat with five lights powered by a small gasoline-powered generator. Bowfishermen stood on the bow and moved into coves and along shallow flats looking for grass carp. Fish were generally collected in coves and other confined areas as they swam by the

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

boat. Fish had to be quickly identified and shot in depths up to 2 m. Once the fish was shot with a conventional bowfishing arrow, it was played on a line using a reel attached to the front of the bow. After the fish tired, it was reeled near the boat, shot a second time to prevent escape, and then gaffed.

Length-to-weight relationships

Length-to-weight relationships are used to indicate the condition or "plumpness" of a fish population. Another use is to predict weights of fish of a given length determined by backcalculations using scales or otoliths. Total lengths to the nearest millimeter and weights to the nearest gram were collected on 87 triploid grass carp. Measurements were taken on 18 triploid grass carp as they were stocked into Lake Marion in April 1989 and 69 fish collected by bowfishermen in April and May of 1992.

The length-to-weight relationship was computed using a power function (Ricker 1975):

$$\text{Weight} = \text{Intercept} \times \text{Length}^{\text{slope}}$$

The relationship was determined by regressing the log (base 10) of the weight as the dependent variable against the log (base 10) of the length as the independent variable.

Age and growth

Generally, fish are aged by examining annual marks on scales, otoliths (ear bones), or other bony structures. An extensive review of the literature failed to produce usable information dealing with (a) techniques to age grass carp using otoliths or (b) length-to-weight relationships in North American reservoirs.

Age and growth information presented in this paper was obtained by examining scales. The scales appeared to have consistently recognizable annuli except for two samples that contained only regenerated scales. Scales were examined by projecting their image on a microfiche projector. Distances to each annuli and distance to the margin of the projected image were measured using a GP-7 graphbar digitizer and a personal computer. The computer is equipped with a series of basic programs used to measure structures and backcalculate lengths of fish. Backcalculated lengths were estimated using the Fraser-Lee Method (Carlander 1982) which uses the following formula:

$$Li = a + (Lc - a / Sc)\, Si$$

where

- Li = calculated length at age i
- a = intercept of the body scale regression
- Lc = length of the fish at capture
- Sc = diameter or radius of scale at capture
- Si = scale measurement at annulus i

Results

Bowfishing proved to be an efficient and cost-effective technique for collecting triploid grass carp when electrofishing and other collection techniques failed. Sixty-nine triploid grass carp in the Santee Cooper system, SC, were collected during April and May of 1992.

A length-to-weight relationship was developed from fish collected by bowfishermen and fish measured at initial stocking.

The derived relationship was as follows:

$$\text{Weight} = 0.0000027 \times \text{Length}^{3.25}$$

The coefficient of determination (r^2) was 0.99 and indicated a reasonably good fit for a length-to-weight relationship (Figure 1).

During the spring and summer of 1991, attempts were made to determine the age of grass carp collected in gill nets and rotenone samples from Lake Marion, SC, and Lake Guntersville, AL. Whole and sectioned sagittal otoliths were examined, but no annuli were distinguishable. Victor (1982) stated that sagittal otoliths were probably unsuitable

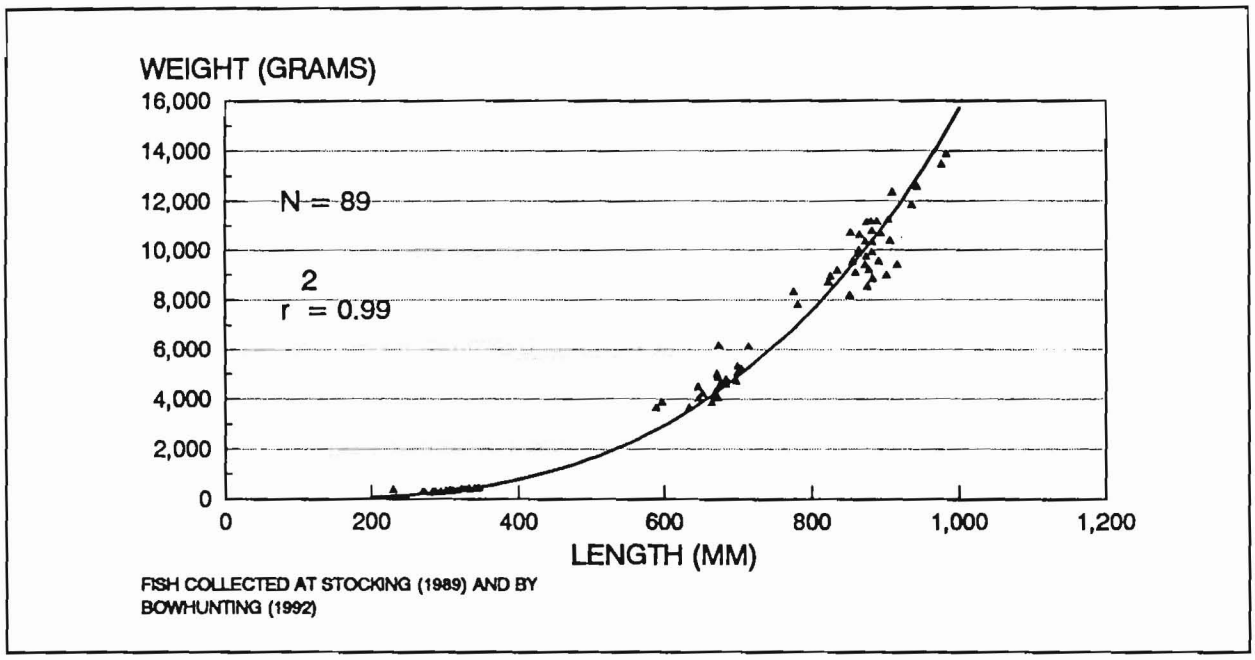

Figure 1. Length-to-weight relationship of triploid grass carp in the Santee Cooper system, South Carolina

for determining age in cyprinids (the same family as the grass carp), but that the utricular otoliths could be used. This pair of otoliths is quite small and hence difficult to locate, even in larger grass carp. Because techniques using otoliths for age estimation are being developed, scales were used for age and growth determinations.

Table 1 lists backcalculated lengths and weight of triploid grass carp collected in the Santee Cooper system in 1992. The greatest growth was from Age 1 to Age 2, where fish grew from an average of 574 to 4,894 g. From Age 2 to Age 3, growth was still substantial to an average weight of 8,294 g, but growth in length was less, about 123 mm. Growth in length from Age 3 to Age 4 was approximately 87 mm, but the growth in weight was approximately the same as Ages 2 to 3. The instantaneous rates of growth (Ricker 1975) were 2.143, 0.528, and 0.328 for Ages 1 to 2, 2 to 3, and 3 to 4, respectively. Table 1 also lists the breakdown of age classes. Age 4 fish were those stocked in 1989 at Age 1. Most of the triploid grass carp were Ages 2 or 3, and no fish stocked in 1992 at Age 1 were collected. Our sample is inadequate to generate age-specific mortality estimates, although oxygen depletions associated with Hurricane Hugo in 1989 may account for the lack of Age 4 fish.

Table 1
Age-Specific Lengths and Weights for Triploid Grass Carp Collected in 1992 from the Santee Cooper System, South Carolina, Using Scales and Backcalculation

Age[1]	No. Collected	Length		Weight	
		mm	inches	g	lb
1	0	361 (7)[2]	14.2	574	1.2
2	23	698 (11)	27.5	4,894	10.8
3	35	821 (13)	32.3	8,294	18.3
4	6	908 (27)	35.7	11,506	25.3

[1] Ages 1 through 4 represent the 1991- to 1988-year classes, respectively.
[2] Values in parentheses are standard errors.

Future Research

Ongoing research efforts will attempt to locate new aging structures and validate the use of scales. Initial results indicate that sagittal otoliths are probably unsatisfactory for age and growth determination. Further, another otolith, the lapillus, has been located and techniques developed for its extraction. Lapilliar

otoliths appear to lay down annuli that match those in spines and offer a promising direction for aging grass carp. Over the next 2 years, we plan to further compare annuli deposited on this otolith with those on scales and spines. Additional efforts will seek to prove these annuli are deposited yearly using marked fish in small ponds. Since very little work has been done in developing aging techniques for grass carp, these efforts should have promising worldwide applications.

Over the next several years, we plan to expand our triploid grass carp collection efforts in the Santee Cooper system with the goal of collections that are large enough to give better estimations of mortality and growth. These results should help refine the grass carp stocking model developed at WES.

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Chemical Control Technology

Overview of Chemical Control Studies

by
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Introduction

The mission of the Chemical Control Technology Team (CCTT) is to develop and evaluate technology that will improve the management of nuisance aquatic plants using chemical herbicides and plant growth regulators (PGRs) in an environmentally compatible and cost-effective manner. In fiscal year (FY) 1992, direct-allotted funds for chemical control research were apportioned among five work units: Herbicide Concentration/Exposure Time Relationships, Herbicide Application Techniques for Flowing Water, Herbicide Delivery Systems, Field Evaluation of Selected Herbicides for New Aquatic Uses, and Plant Growth Regulators for Aquatic Plant Management. A sixth chemical control work unit, Species-Selective Use of Aquatic Herbicides and Plant Growth Regulators, is scheduled for start-up in FY93. In addition, the Coordination of Control Tactics with Phenological Events of Aquatic Plants work unit was under the direction of the CCTT.

Although these work units can function as independent research efforts, they have been carefully designed to operate interactively. This interactive approach allows results obtained from one or more work units to complement results from another or to be used as "building-blocks" for more complex work units. As structured, these work units collectively encourage the development and evaluation of safe and effective chemical formulations and application techniques for the aquatic environment. Consequently, aquatic plant managers are provided with operational tools that minimize chemical dose, while maximizing the control of target plants, reducing the amount of chemicals placed in the environment and the effort and costs associated with aquatic applications.

One important function of the CCTT is to develop working relationships with the chemical industry (primary developers and manufacturers of herbicides and PGRs) and the U.S. Environmental Protection Agency's Pesticide Registration Branch. This cooperation enables CCTT researchers to stay informed of the latest developments in aquatic pesticides and regulations. In addition, interaction with U.S. Army Corps of Engineers Districts and other Federal agencies responsible for aquatic plant management (e.g., Tennessee Valley Authority, U.S. Bureau of Reclamation, and U.S. Department of Agriculture) is necessary to coordinate and focus resources on regional and national problems. Finally, cooperation with state and local agencies and universities is maintained to augment the CCTT's laboratory and field research capabilities.

Interactions among the Chemical Control Technology Area work units are summarized in Figure 1. Selected chemical compounds (unregistered, experimental use permit (EUP), and registered) are studied via a series of laboratory, small-scale, and field evaluations. Once these evaluations have been completed, guidance for the use of experimental use and registered compounds is provided to operational personnel in the form of technical reports and field manuals. In addition, data from these evaluations are used to enhance and develop simulation capabilities. Environmental fate and persistence information obtained on unregistered compounds is eventually provided to the U.S. Environmental Protection Agency (EPA) for use in the aquatic labeling and registration process.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

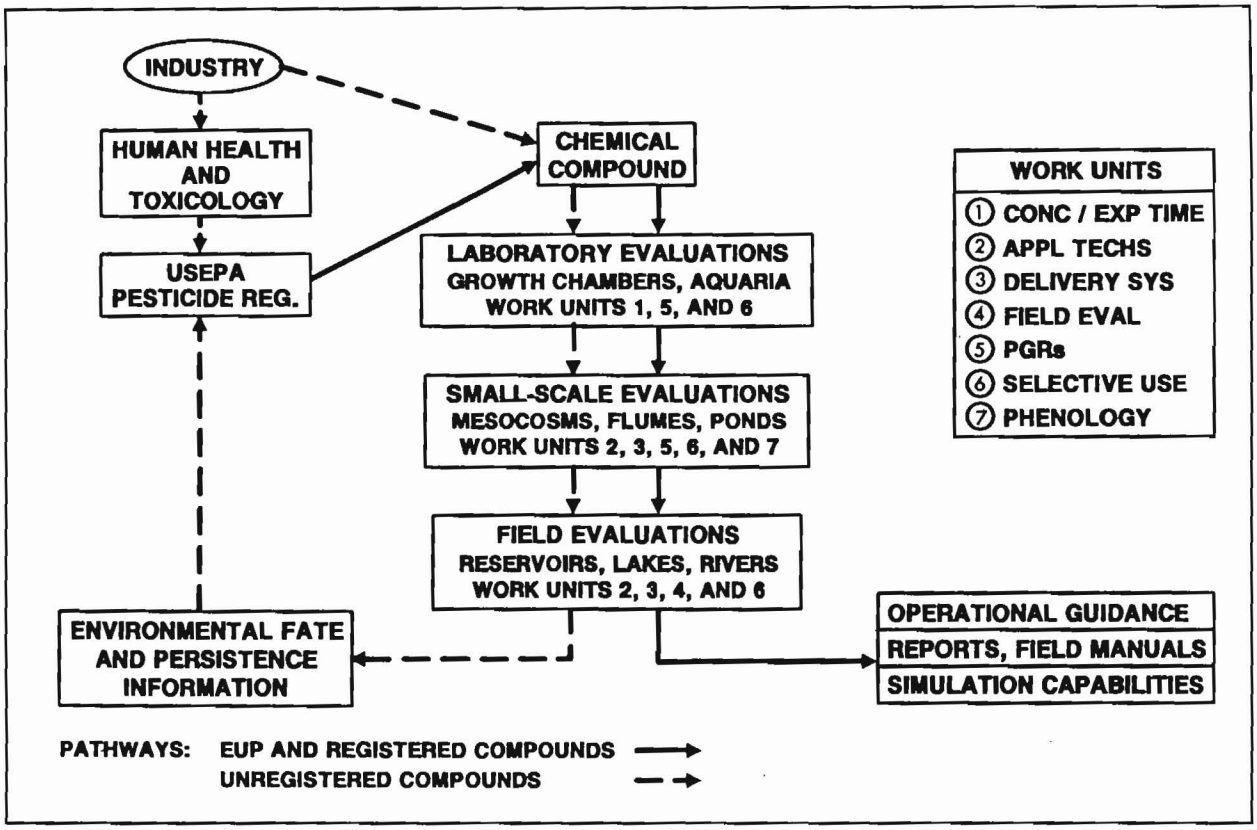

Figure 1. Chemical control technology work units

Chemical control work unit summaries are given below. Detailed updates of each work unit are provided in other articles found in the Chemical Control section of this proceedings.

Herbicide Concentration/Exposure Time Relationships (32352)

Researchers in this area are evaluating herbicides and PGRs under controlled-environment conditions. Major nuisance species (e.g., Eurasian watermilfoil and hydrilla) are treated with various chemicals (e.g., fluridone, diquat, endothall, triclopyr, and copper) over a range of selected doses and contact times. The unique properties of each chemical (i.e., application rate, mode of action, environmental half-life, and species selectivity) require that concentration/exposure time (CET) relationships be developed for each target plant. This information is then employed to develop innovative application techniques, some of which are currently being used on an operational level to control milfoil and hydrilla. In addition, data obtained from this work is being used in the herbicide fate model being developed in the Simulation Technology Area.

Herbicide Application Techniques for Flowing Water (32354)

In this work unit, herbicide application techniques are developed to minimize the amount of active ingredient used and the frequency of treatments while maximizing efficacy against target plants in high water-exchange environments (e.g., rivers, canals, tidal areas, lakes, and reservoirs). Results from the CET work unit provide the pertinent dose/response information required to develop improved and innovative application techniques.

Research in this work unit has focused on understanding water movement and stratification in hydrilla and milfoil stands and on the potential impact of these conditions on herbicide contact time and efficacy. Studies have been conducted within submersed plant stands

using flowmeters and tracer dyes to characterize water exchange. Results from this work are being implemented by operational personnel to select both type and timing of submersed application techniques that will minimize the impacts of water movement in high water exchange environments.

Herbicide Delivery Systems (32437)

This work unit makes use of relationships developed in the CET work unit to improve herbicide delivery to submersed plants by dispensing low doses of an active ingredient over long periods of time. Studies are designed to develop environmentally compatible, controlled-release (CR) carrier systems (e.g., polymers, elastomers, gypsum, and proteins). These CR systems are evaluated for herbicide release rates and plant tissue burden levels, as well as efficacy, in small-scale facilities at the U.S. Army Engineer Waterways Experiment Station (WES). Large-scale verification studies are conducted in outdoor flumes and selected field sites.

Results from this work will be used to improve efficacy of treatments in flowing water systems. This work unit also provides data for herbicide fate and effects models being developed in the Simulation Technology Area.

Field Evaluation of Selected Herbicides for Aquatic Uses (32404)

The most effective application techniques and chemical formulations developed in the work efforts described above are evaluated for efficacy under large-scale field conditions in this work unit. These cooperative studies involve the efforts of chemical companies, Corps Districts, Federal, state, and local agencies, universities, contractors, and WES. In addition, environmental fate and dissipation data collected in these studies are used to prepare field manuals/reports on the use of aquatic herbicides. These data are also available for chemical companies to use in fulfilling EPA requirements for registration of specific herbicide formulations. Results from these field evaluations can aid in changing the registration status, site use, or amendments of residue tolerances for aquatic herbicides.

Plant Growth Regulators for Aquatic Plant Management (32578)

Traditional efforts to control submersed aquatic plants can destroy the standing crop, resulting in widespread plant decomposition and disruption of overall community structure. Total removal of plant biomass may result in fluctuations of nutrient levels, turbidity, and dissolved oxygen; and the concomitant loss of habitat may dramatically impact food-web relationships. PGRs offer the potential of slowing vertical growth rate of nuisance submersed plants, reducing the negative impacts that "topped-out" plants can impose on a water body. Thus, the beneficial qualities provided by underwater vegetation (i.e., invertebrate and fish habitat, waterfowl food, oxygen production, nutrient sinks, and sediment stabilization) can be retained.

Studies designed to evaluate the effectiveness of PGRs on aquatic plants are being conducted in controlled-environment systems at WES. Herbicides that demonstrate PGR potential in the CET work unit are also being evaluated in this work unit. Since PGR efficacy may be quite sensitive to life-cycle events of specific target plants, information obtained from the Phenology of Aquatic Plants work unit will be useful in evaluating growth-regulating effects. The most promising plant growth-regulating compounds will receive additional evaluations in mesocosm, pond, and field situations.

Species-Selective Use of Aquatic Herbicides and Plant Growth Regulators (New)

This new work unit will develop and evaluate species-selective aquatic plant management practices using herbicides and PGRs. While weedy species can be removed using traditional chemical control tactics, these treatments usually impact native species as well.

Using herbicides/PGRs in a species-selective manner can result in the control of target vegetation, while enhancing the growth of desirable or beneficial plants. Allowing these species to grow and flourish can slow the reinvasion of weedy species and provide improved fish and wildlife habitat. In this way, bodies of water plagued with monoculture infestations of exotic plants can be restored to a healthy, diversified, and balanced aquatic community.

Studies at WES will focus on species-selective responses to applications (rate and timing) of various herbicides/PGRs. Once responses of weedy (e.g., hydrilla and milfoil) and various nonweedy species (e.g., pondweeds and wild celery) have been determined, desirable herbicide-resistant plants can be selected for further evaluation. The most promising chemicals will be applied to mixed plant communities in a mesocosm system at the WES Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Results from this work will be used to develop guidance for managing aquatic vegetation using the species-selective approach.

Coordination of Control Tactics with Phenological Events of Aquatic Plants (32441)

Certain factors associated with the survival strategy of plants (e.g., rapid growth, photosynthetic efficiency, effective dispersal, and energy reserves) can enhance a species' ability to attain nuisance population levels. A thorough understanding of a species' survival strategy can be used to identify weak points in its growth cycle, which can then be exploited to improve control of that plant. Once identified, these susceptible periods can be predicted on the basis of growth-cycle events, morphological characteristics, and environmental cues. An easily recognized characteristic or cue will enable field personnel to determine the optimum time for applying appropriate control techniques (chemical, biological, physical, or integrated) by taking advantage of the weak link in the plant's growth cycle to maximize efficacy.

Phenological studies are being conducted on the major aquatic weed species (waterhyacinth, milfoil, hydrilla, and alligatorweed) at the LAERF. Results from these studies will be used in the Flowing Water and PGR work units and will be related to the timing of application techniques based on phenological events associated with the target weed. In addition, phenology information will enhance the use of nonchemical control technologies, as well as contribute to the plant growth modeling effort in the Simulation Technology Area.

Phenology of Aquatic Plants

by
John D. Madsen[1]

Introduction

The primary objective of the phenology work unit is to elucidate potential control points, or "weak spots," in the seasonal cycles of target aquatic plants. This objective is met by examining physiological parameters, particularly carbohydrate storage, and relating these factors to readily observable phenological traits.

The optimal control points are the times at which the plant is low in stored reserves, and thus less likely to recover from control efforts. The primary control point during the seasonal cycle occurs when the plant switches from reliance on stored carbohydrates to plant production of carbohydrates and replenishment of stored reserves during spring regrowth. A secondary control point is any period in which the allocation of carbohydrates to storage organs can be minimized or restricted.

One example of the carbohydrate control point strategy is a study of cattail (*Typha angustifolia* L.) by Linde, Janisch, and Smith (1976) in which they examined the concentration of total nonstructural carbohydrates (TNC) in cattail rhizomes each week from April through July. Carbohydrate concentrations reached a minimum in late June (Figure 1). This point can be described as the point at which carbohydrate use equals carbohydrate production, or the transition from dependence on stored carbohydrates to self-sufficient growth and storage of excess carbohydrates in plant rhizomes. This transition point is the primary control point. By correlating this point with the many phenological parameters they recorded, the investigators determined that the control point coincided with the production of pollen from pollen-bearing flowers (e.g., anthesis). Therefore, management of cattail could be timed to the production of pollen to exploit the primary control point.

Previous research

Previous research by the phenology work unit on waterhyacinth (*Eichhornia crassipes* (Mart.) Solms), both at the U.S. Army Engineer Waterways Experiment Station (WES) (small-scale) and at the Lewisville Aquatic Ecosystem Research Facility (pond scale), has also indicated the utility of examining carbohydrate storage patterns for determining control points (Luu and Getsinger 1990; Madsen, Luu, and Getsinger 1993). Both studies indicated that an early season control strategy was best for reducing waterhyacinth growth. Both studies also found evidence for a secondary control point in late summer and early spring to reduce reallocation of shoot carbohydrates to the stembase. Carbohydrate mass balance also substantiated that maintenance management strategy is effective in reducing plant reinfestation (Luu and Getsinger 1990; Madsen, Luu, and Getsinger 1993). Further studies on waterhyacinth have been performed, but laboratory and data analysis have not been completed.

Current studies

Studies recently initiated include monitoring phenological traits of all target species and sampling of Eurasian watermilfoil (*Myriophyllum spicatum* L.) biomass in ponds at the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

[1] U.S. Army Engineer Waterways Experiment Station, Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

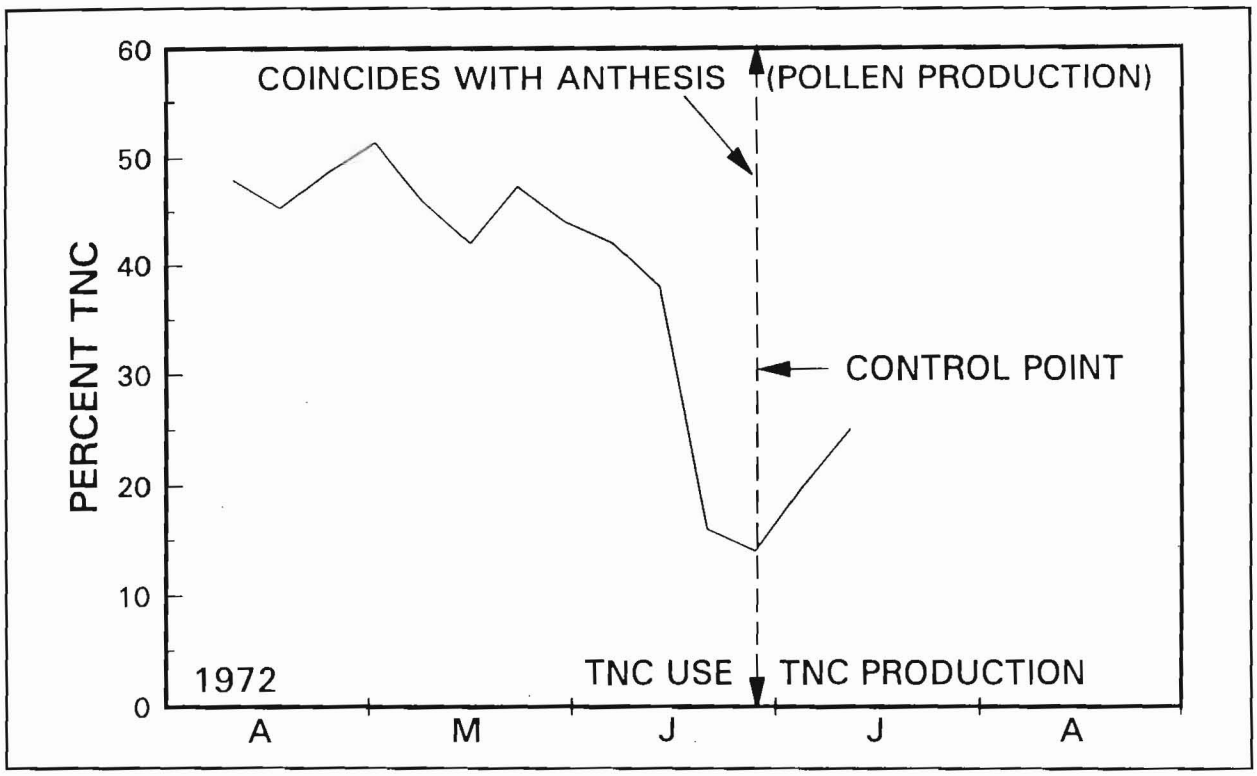

Figure 1. Concentration of total nonstructural carbohydrate (TNC) in cattail rhizomes as percent of dry weight versus sampling date. Data from Linde, Janisch, and Smith (1976)

Methods and Materials

Phenological monitoring

Weekly monitoring of phenological traits for all target species, including Eurasian watermilfoil, was initiated in April 1991. Five ponds dominated by Eurasian watermilfoil were monitored for the presence of stem material, the formation of a surface canopy ("topped-out"), inflorescences, seeds/fruits, and autofragments.

Pond biomass

Eurasian watermilfoil biomass and allocation patterns have been studied since 1991. Eurasian watermilfoil was sampled monthly from Pond 40 during April through December 1991, with twelve 0.1-m^2 samples taken in a stratified-random design. From January 1992 to the present, Ponds 14 and 15 were sampled using a similar design and sample size except that only six samples per pond were taken. Sampling of Ponds 14 and 15 will continue through December 1993.

Biomass samples were sorted into root crown, lower stems, upper stems, autofragments, and inflorescences. Root crowns, basal stems, autofragments, and inflorescences were also counted. Samples were dried and weighed and will be analyzed for tissue nutrients and carbohydrates. For this discussion, data from Ponds 14 and 15 were combined. Air and water temperature were recorded continuously using an Omnidata datalogger, with daily average air and water temperatures calculated.

Results and Discussion

Phenological monitoring

Eurasian watermilfoil, as a perennial herbaceous plant, retains green shoot material throughout the year. However, during the fall and winter, biomass was reduced through senescence. Therefore, plants formed a surface

canopy only during the warm period of the growing season, which corresponded to the time when average temperatures exceeded 20 °C. Most ponds had a surface canopy from May through June and again from late August through November (Figure 2). Canopy formation was somewhat erratic during this period because of an unsynchronous flowering cycle. Flowering can only occur in plants at the surface. After seed set, some senescence was observed that resulted in a slight decline of biomass. Peak flowering periods were from May through June and again from late August through October. Autofragment formation was often associated with this senescence period, although autofragments were also observed at other time periods.

Pond biomass

Total biomass of Eurasian watermilfoil in Pond 40 during 1991 varied from 391 to 733 g m^{-2} (Figure 3). An initial decline in biomass occurred in June, which corresponded to the first flowering period. A second decline occurred in October, following a second flowering period. Much of the loss of biomass in the second event was the result of autofragment formation.

During 1992 in Ponds 14 and 15, biomass began at 430 g m^{-2} and increased to 940 g m^{-2} by May, after which a steep decline in biomass occurred (Figure 3). This corresponded to increased water temperatures in the pond. A second decline began from a late July level of 530 g m^{-2} to a September low of 300 g m^{-2}. This corresponded to a flowering period and the production of autofragments. Many of these autofragments were released from the plant and drifted away from the sampling area.

Biomass allocation patterns were similar for both years, with the exception that a higher percentage of biomass was allocated to autofragments (up to 10 percent) in Pond 40 (Figure 4). The bulk of biomass was found in upper stem material, which corresponds to the

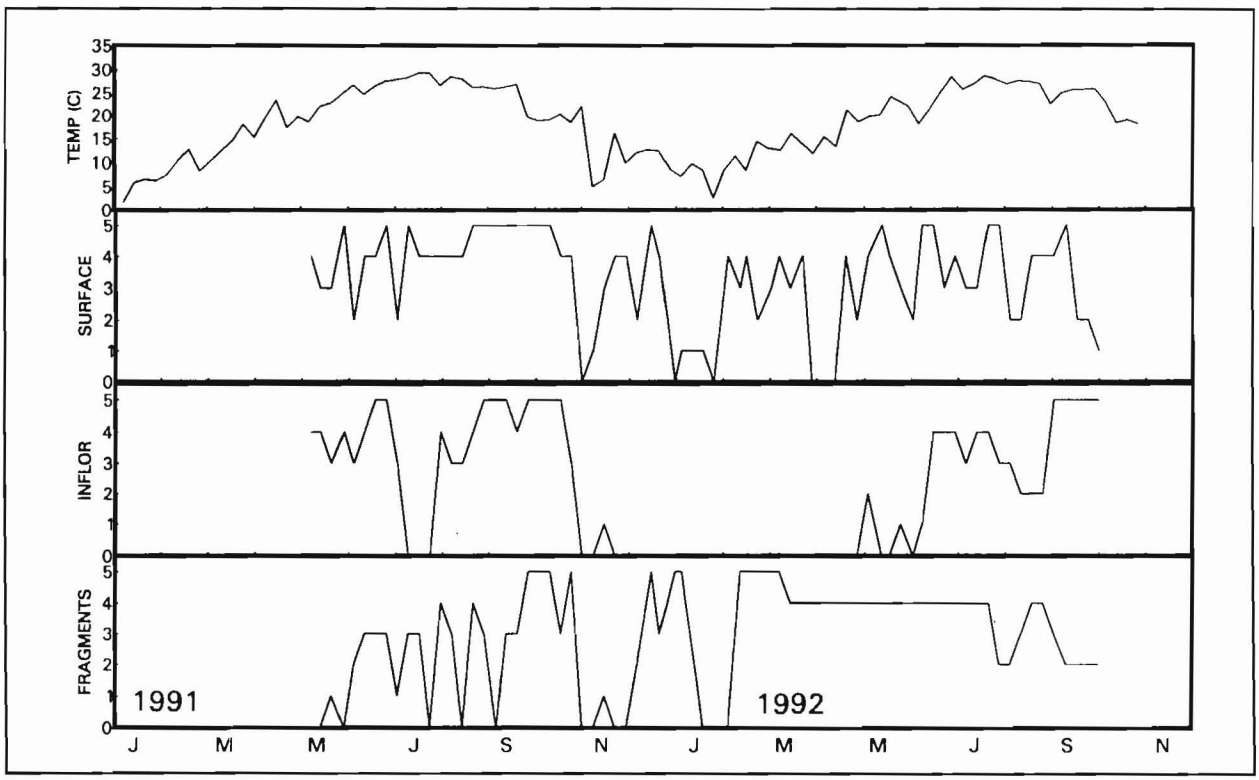

Figure 2. Average daily air temperature at Lewisville, TX, and the number of ponds in which Eurasian watermilfoil grew to the surface, had inflorescences, or had autofragments present at the Lewisville Aquatic Ecosystem Research Facility during 1991 and 1992

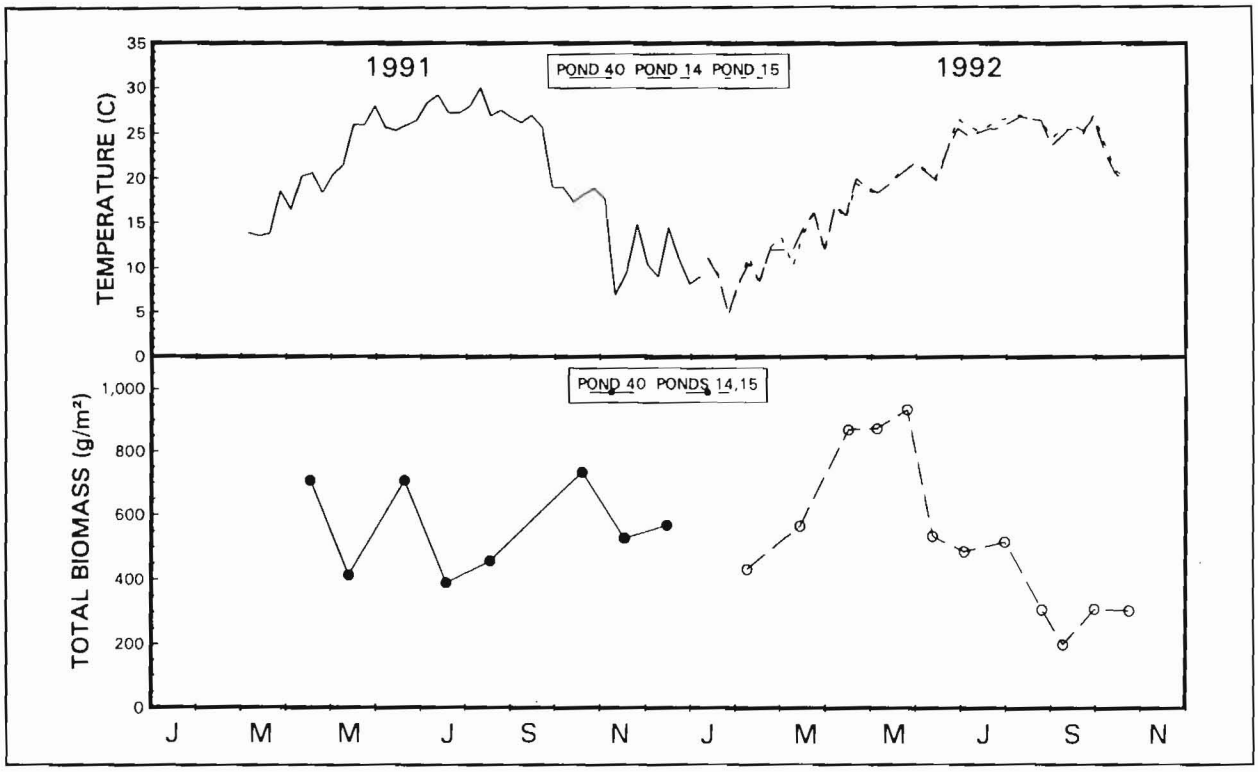

Figure 3. Average daily water temperatures for Ponds 40, 14, and 15 for 1991 and 1992 and total biomass (g m^{-2}) of Eurasian watermilfoil in Ponds 40, 14, and 15 for sampling dates in 1991 and 1992 at Lewisville, TX

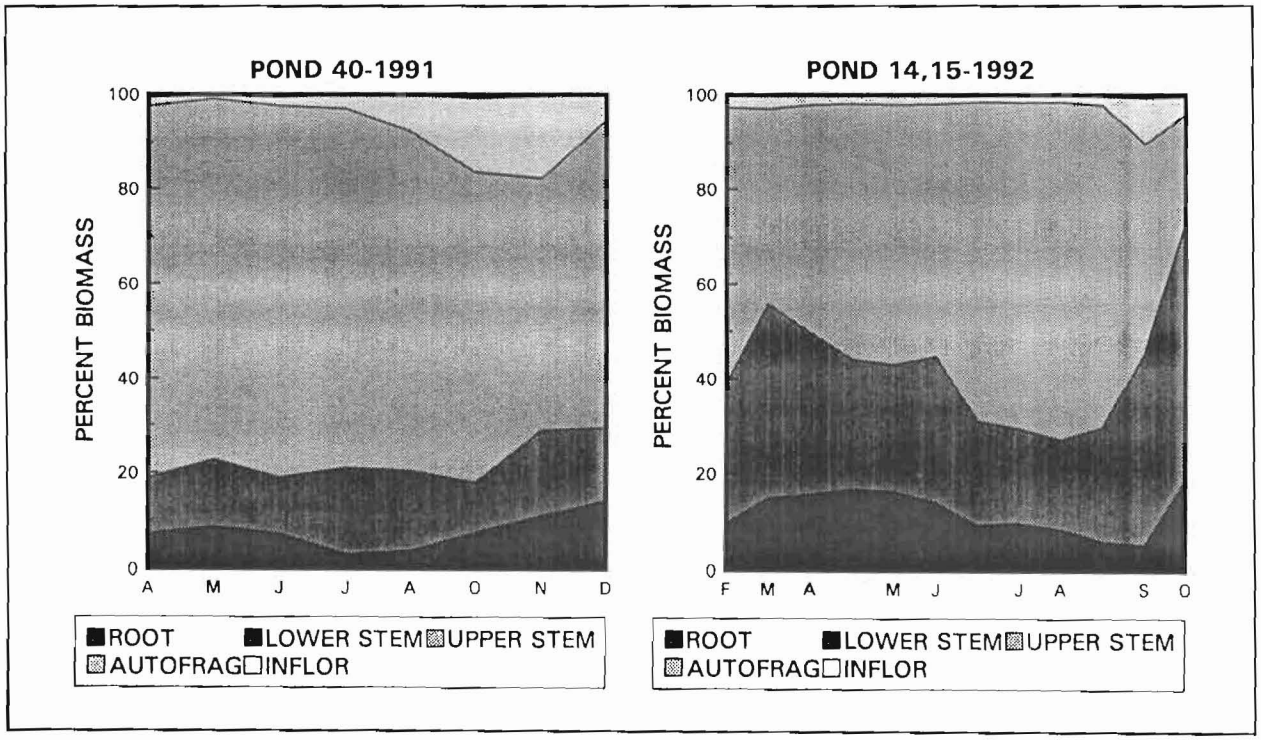

Figure 4. Percent of total biomass of roots, lower stems, upper stems, autofragments, and inflorescences by sampling date for Pond 40 in 1991 and Ponds 14 and 15 in 1992

canopy with leaves. Autofragments were produced entirely from upper canopy material, so autofragment production was at the expense of the canopy. Both years exhibited a peak of autofragment production beginning in late August and continuing until December in the 1991 sampling. Inflorescences never constitute more than 1 percent of biomass.

The density of inflorescences and autofragments exhibited a definite relationship to both seasonal temperatures and physiological processes (Figure 5). During 1991, the first peak in flowering occurred in June when water temperatures first reached 25 °C. After flowering, autofragment formation began to increase. The second flowering peak occurred in October, which corresponded to water temperature decreasing to below 25 °C. Autofragment formation peaked during this period and declined as winter dormancy began. Autofragment densities remained high during 1992 in Ponds 14 and 15, possibly because of nutrient limitation. The first flowering peak occurred in July, followed by a second peak in October. This peak was accompanied by increased autofragment formation. Peak autofragment formation in autumn appears to be typical of many Eurasian watermilfoil populations; but other autofragmentation periods appear related to flowering.

The relationship between flowering and autofragment formation is substantiated by data from Lake George, New York (J. Madsen, unpublished data). Monthly collections of autofragments from set areas in both Huddle Bay and Northwest Bay indicated peak autofragment densities in August and September, after the peak of flowering activity in late June (Figure 6).

These data suggest that flowering activity is typically followed by a period of autofragmentation. Both flowering and autofragmentation may be related to water temperature directly, although certainly through the interrelationship between water temperature and

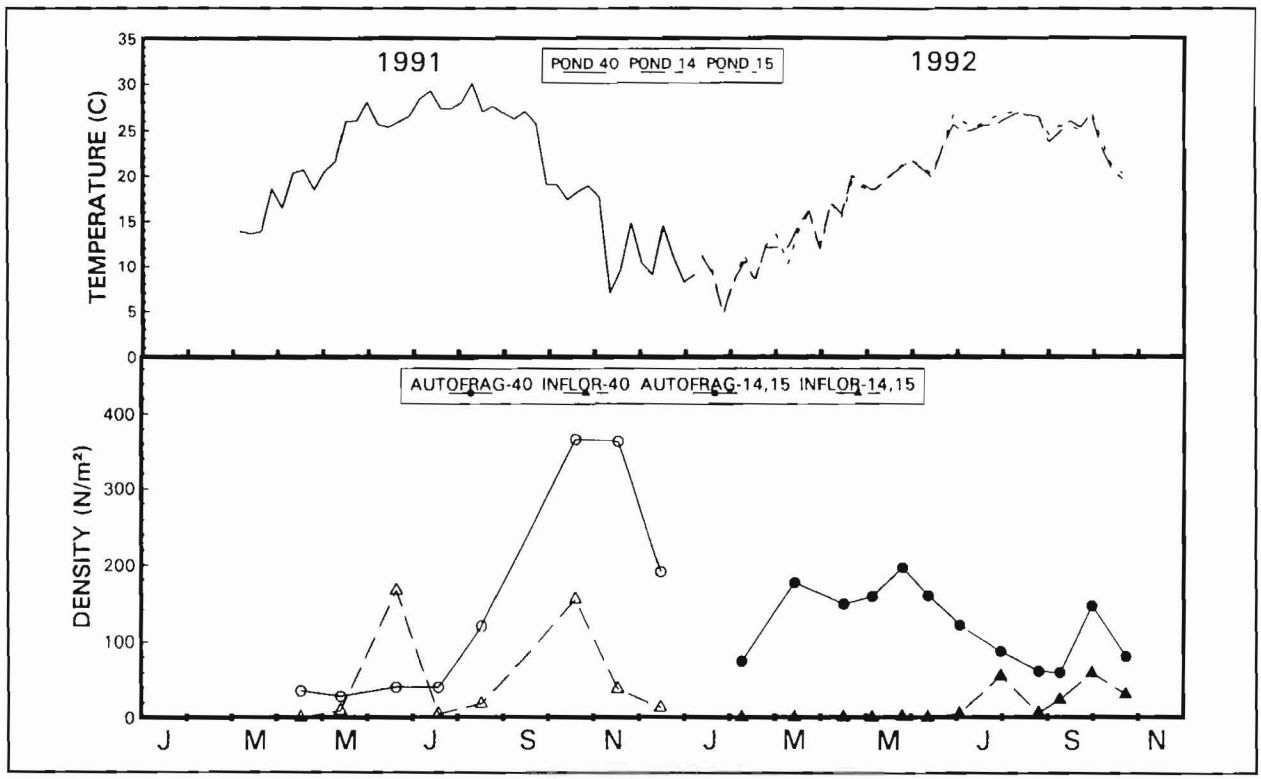

Figure 5. Average daily water temperatures for Ponds 40, 14, and 15 for 1991 and 1992 and the density of autofragments and inflorescences from samples of Eurasian watermilfoil in Ponds 40, 14, and 15 for sampling dates in 1991 and 1992 at Lewisville, TX

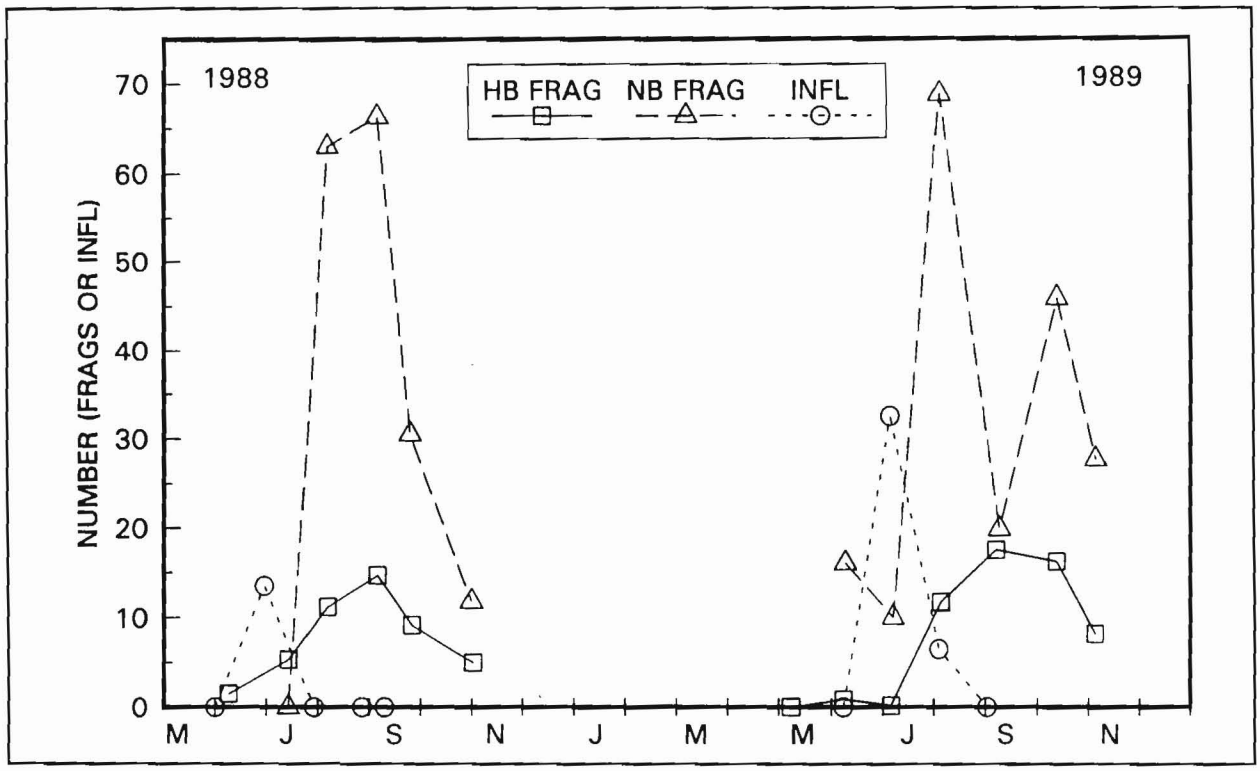

Figure 6. The number of fragments collected from test plots in Huddle Bay and Northwest Bay and the density of inflorescences observed from transects in Northwest Bay during sample dates in 1988 and 1989 at Lake George, New York

the length of time required to form a surface canopy. Also, the "two-peak" biomass curve observed for many Eurasian watermilfoil populations may be related to a length of growing season and water temperatures that allow two flowering periods. The first flowering period induces autofragment formation and senescence of the upper portion of the canopy. Regrowth of a new canopy then allows a second period of flowering, also followed by autofragmentation.

An understanding of autofragmentation and its timing has two important implications for the management of Eurasian watermilfoil. First, if reduction of Eurasian watermilfoil spread is a goal, then control efforts should be timed to prevent autofragmentation. Second, many control techniques encourage the spread of autofragments, which is a concern to some regulatory agencies. For these techniques, such as harvesting or rotovating, control should either be implemented before autofragments are being produced, or other techniques should be utilized after autofragment production has begun. The best way to avoid this time period is to begin control operations before a surface canopy has formed, which has the complementary benefit of avoiding any period of nuisance growth.

New Research

Several new multiyear research efforts were initiated in 1992 involving both Eurasian watermilfoil and hydrilla (*Hydrilla verticillata* (L.f.) Royle) that complement ongoing pond studies (Table 1). The rate of spread study involves monitoring the growth of four Eurasian watermilfoil colonies planted in the middle of a large grid system, examining seasonal rates of spread. The pond pot study examines the growth and allocation patterns of Eurasian watermilfoil and hydrilla planted in large pots submerged in a pond during the spring, summer, autumn, and winter. This study examines the growth and allocation patterns of new colonies, seasonally, and the

Table 1
Multiyear Research on the Phenology of Eurasian Watermilfoil and Hydrilla Initiated in 1992 at the Lewisville Aquatic Ecosystem Research Facility

Study	Eurasian watermilfoil	Hydrilla
Rate of Spread	X	
Pond Pot Study	X	X
Temperature, Growth, and Allocation	X	X
Tuber Bank Dynamics		X
Phenolic Analyses[1]	X	X
Seed Ecology	X	

[1] Analyses to be performed by Drs. Lembi and Nicholson, Purdue University, IN.

overwintering capability of the plants. Temperature, growth, and allocation examines the effect of temperature apart from season on growth rates and allocation patterns of these two species under four controlled temperature conditions between 20 and 35 °C. For the tuber bank dynamics study, containers have been placed in a hydrilla pond, which will be harvested over the next 5 years in the spring and midsummer to examine tuber production rates and tuber dormancy. Samples of Eurasian watermilfoil and hydrilla will be collected for phenolic analyses to determine the seasonal and spatial distribution of secondary compounds, which may be important cues for plant stress or deterrents to biocontrol. These samples will be analyzed by Drs. Carole Lembi and Ralph Nicholson as part of a cooperative research program with Purdue University. Lastly, the seed ecology of waterhyacinth and Eurasian watermilfoil will be examined in growth chamber and greenhouse experiments.

Future Research

Other plans for future research include pond sampling of hydrilla, such as discussed for Eurasian watermilfoil previously, and demonstration projects to indicate direct applications of phenological research to control techniques.

The purpose of these projects is to find specific points in the seasonal cycle of the target nuisance plants for the application of control techniques and to correlate these control points to specific phenological indicators. In addition, important aspects of the life cycle of these target species are being examined to provide more insight for their management.

Acknowledgments

The author wishes to thank the numerous individuals who have assisted with phenological research at both the Lewisville Aquatic Ecosystem Research Facility and the Chemical Control Technology Team, WES, Vicksburg, MS. In particular, the field assistance of Bekah Westover, Chetta Owens, Nathan Standifer, Lovely John, Dian Smith, Melissa Smith, Keith Loyd, Nicole Flint, and Tim Irby at LAERF and the laboratory assistance of Dr. Susan Sprecher, Anne Stewart, Kimberly Deevers, and Jane Brazil at WES is acknowledged.

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Herbicide Concentration/Exposure Time Requirements for Eurasian Watermilfoil and Hydrilla

by
Michael D. Netherland,[1] *Kurt D. Getsinger,*[1] *and E. Glenn Turner*[2]

Introduction

Chemical applications for submersed plant control often result in the rapid dispersion and dilution of herbicide residues from the treatment area (Fox et al. 1991; Getsinger, Fox, and Haller 1992). While rapid residue dissipation is generally considered desirable (from an environmental standpoint), residues that disperse too quickly (via gravity flow, tides, thermal and wind induced circulation patterns, etc.) can result in a lack of plant control because of insufficient exposure to the herbicide. Laboratory studies have shown that efficacy against Eurasian watermilfoil and hydrilla is dependent upon the length of time these plants remain exposed to given concentrations of the herbicide (Green and Westerdahl 1990; Van and Conant 1988; Netherland, Green, and Getsinger 1991; Netherland and Getsinger 1992). Moreover, concentration/exposure time (CET) requirements can vary greatly among herbicides because of the unique properties (rate of application, mode of action, environmental half-life, and species selectivity) of each compound; therefore, CET relationships must be developed for each herbicide and target plant.

Field and laboratory results have shown that maintaining the herbicide fluridone (often at very low levels, <25 µg/L) in the water column over long periods of time (5 to 12 weeks) is critical to achieving plant control (Hall, Westerdahl, and Stewart 1984; Netherland 1992; Getsinger, Fox, and Haller 1992; Fox and Haller 1993). Although efficacy has been linked to sustaining low fluridone concentrations for an extended period of time, these evaluations have only broadly quantified the relationship between dose, exposure time, and efficacy. Therefore, a study was designed to more precisely determine fluridone CET requirements for controlling Eurasian watermilfoil and hydrilla.

In addition, studies have been initiated using combinations of contact herbicides with copper-based compounds to determine their effect on CET requirements. These studies are being conducted to see if additive, synergistic, or antagonistic responses result from the use of various contact herbicide combinations for controlling hydrilla and Eurasian watermilfoil.

Materials and Methods

This study was conducted in a controlled-environment growth chamber (Netherland 1990), with a photosynthetic photon flux density of 520 ± 50 µmoles/m^2/sec at the water surface, a 14L:10D photoperiod, and water temperature of 24 ± 2 °C.

Eurasian watermilfoil (hereafter called milfoil) and hydrilla apical shoots (10 to 15 cm) were planted in 300-ml beakers (four apical shoots per beaker). Ten beakers containing a single target species were placed in 55-L aquaria. Milfoil was allowed to establish for 3 weeks prior to fluridone treatment, while hydrilla was given a 4-week pretreatment period. This ensured the development of healthy shoot and root systems. One beaker was removed from each aquarium immediately prior to herbicide treatment to provide an estimate of pretreatment biomass. Mean pretreatment shoot weights (105 g DW/m^2 for milfoil

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
[2] AScI Corporation, Vicksburg, MS.

and 90 g DW m^2 for hydrilla) approximated spring to early summer field biomass (Grace and Wetzel 1978; Bowes, Holaday, and Haller 1979).

Plants were treated with the commercial formulation of fluridone, Sonar AS, and all treatment concentrations are reported as µg/L (ppb) of the active ingredient fluridone. Treatments included fluridone at concentrations of 12 and 24 µg/L for 30, 60, and 90 days and 48 µg/L for 30 and 60 days. Each treatment was replicated three times and randomly assigned to an aquarium. Because of the length of this study, aquaria were drained and retreated at 30-day intervals to allow for an exchange of fresh water.

Plant response to fluridone treatment was monitored for a 90-day period. Visual assessments were used to characterize initial plant injury, progression of injury symptoms, and initiation of regrowth if any occurred. Apical shoots were sampled at 6, 30, 60, and 90 days and analyzed for chlorophyll. Three beakers were removed from each aquarium at 30, 60, and 90 days, and shoot and root biomass values were measured.

Results and Discussion

Milfoil began to manifest fluridone symptoms by 6 days after treatment (DAT), as indicated by the significant reduction (47 to 74 percent) of total chlorophyll in shoot tips (Table 1). All new shoot growth continued to show fluridone symptoms; however, stem tissue below the active growing points maintained a healthy green appearance. The albescent (bleached) shoot tips became necrotic and decayed (the canopy no longer existed) by 21 DAT. Results of the 30-day harvest indicated that fluridone (at all treatment rates) reduced milfoil shoot biomass by 75 percent (Figure 1). However, data showed that the 24- and 48-µg/L treatments resulted in a 50- to 77-percent greater reduction of chlorophyll than the 12-µg/L treatment (Table 1).

Following the 30-day exposure period, milfoil no longer exposed to fluridone began to recover. Regrowth was rapid and plants had begun to canopy within 12 days. Milfoil recovery from the 48-µg/L treatment was delayed, and some of the early regrowth continued to manifest fluridone symptoms. At the 60-day harvest period, it was difficult to discern plants that had been treated with fluridone from untreated controls. However, it should be noted that shoot biomass recovery was reduced in response to increasing treatment rates (Figure 1). By the 90-day harvest, the 12- and 24-µg/L treatments exceeded shoot and root biomass values of the untreated controls (Figures 1 and 2). Shoot biomass and chlorophyll of the 48-µg/L treatment remained reduced; however, healthy regrowth indicated a complete recovery was likely.

Table 1
Chlorophyll Content of Eurasian Watermilfoil Apical Shoots Sampled at 6, 30, 60, and 90 Days After Initial Fluridone Treatment

Treatment µg/L/day	Chlorophyll Content mg/g fresh weight			
	6 DAT	30 DAT	60 DAT	90 Dat
Control	1.19	1.21	1.05	1.25
12/30	0.64	0.45	0.86	1.31
24/30	0.43	0.23	0.85	1.20
48/30	0.31	0.16	0.72	1.13
12/60	0.62	0.42	0.39	0.96
24/60	0.41	0.21	0.21	0.97
48/60	0.28	0.13	0.04	0.02
12/90	0.59	0.47	0.28	0.09
24/90	0.37	0.19	0.11	0.02

In contrast, milfoil that remained exposed to fluridone (60- and 90-day exposures) continued to decline as new growth was limited to a few albescent shoots. Shoot biomass following a 60-day exposure showed an 87-percent reduction in all treatments compared with the untreated controls (Figure 1).

Milfoil recovery following the 60-day exposure period was not as rapid as the recovery from the 30-day exposure. With the exception of the 12-µg/L treatment, plants remained inactive up to 1 week following removal of the fluridone. However, at the 90-day harvest, shoot biomass of the 12- and 24-µg/L treatments, though 36 to 57 percent less than

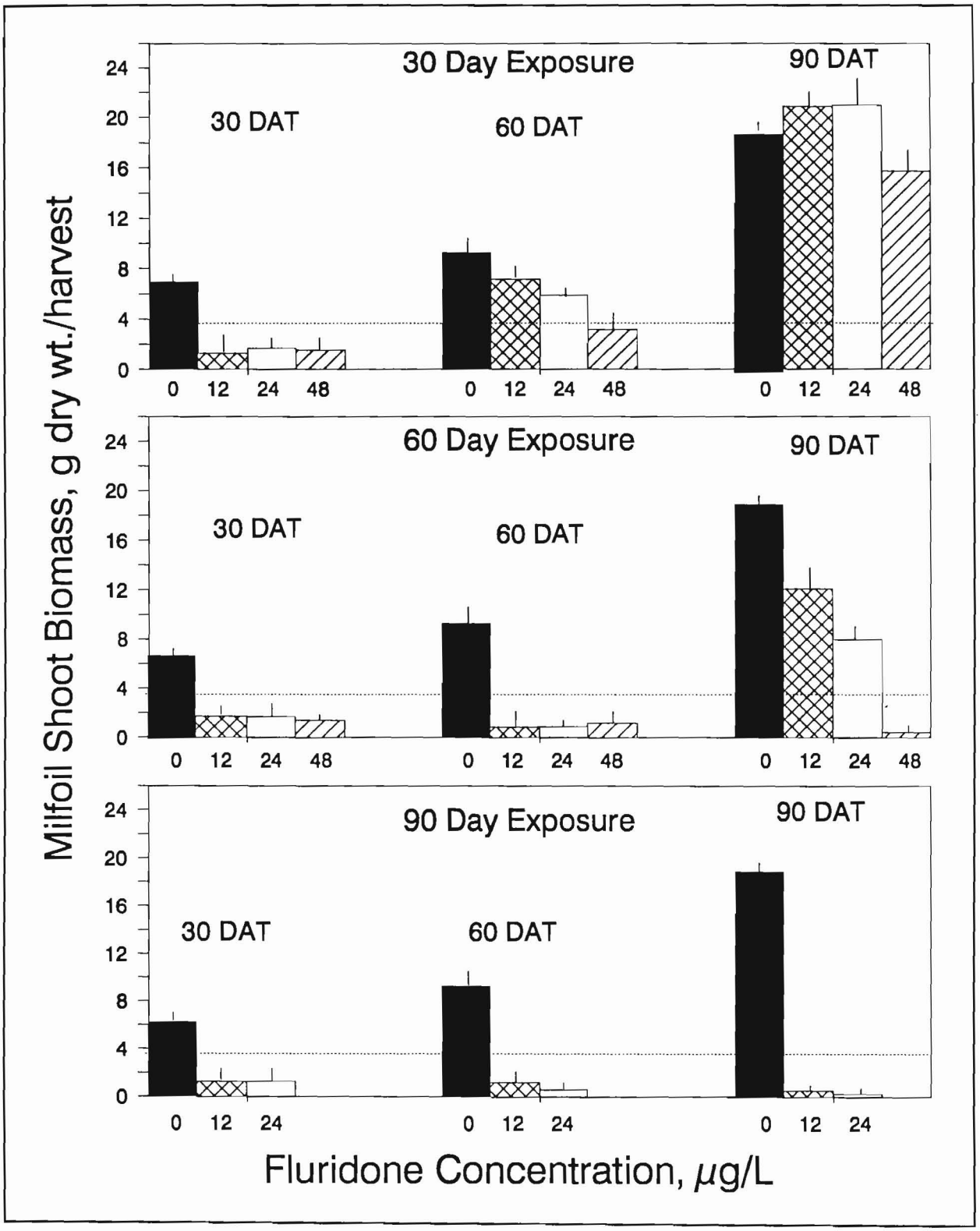

Figure 1. Effects of fluridone on shoot biomass of Eurasian watermilfoil harvested at 30, 60, and 90 days. The dashed line indicates estimated pretreatment biomass. Bars represent the mean of three replicates, and vertical lines represent the standard deviation

untreated plants, had increased dramatically (8- to 12-fold increase in biomass) during the 30-day recovery period. Biomass (Figures 1 and 2) and chlorophyll content (Table 1) of the 48-µg/L treatment continued to decrease during the 30-day recovery period, indicating that milfoil was unlikely to recover following this treatment.

Following 90 days of fluridone exposure, milfoil biomass and chlorophyll were reduced by 90 to 99 percent compared with untreated controls. The fragile condition and extremely reduced biomass of the milfoil following the 90-day exposure indicated that recovery was unlikely, even in the optimal regrowth conditions experienced in the growth chamber.

Table 2
Chlorophyll Content of Hydrilla Apical Shoots Sampled at 6, 30, 60, and 90 Days After Initial Fluridone Treatment

Treatment µg/L/day	Chlorophyll Content mg/g fresh weight			
	6 DAT	30 DAT	60 DAT	90 Dat
Control	1.05	1.01	1.12	1.04
12/30	0.15	0.16	0.97	1.22
24/30	0.13	0.09	1.18	1.06
48/30	0.09	0.09	1.02	0.98
12/60	0.10	0.08	0.19	0.81
24/60	0.09	0.14	0.19	0.92
48/60	0.08	0.10	0.21	1.01
12/90	0.12	0.12	0.14	0.08
24/90	0.07	0.08	0.06	0.04

Immediate regrowth following the removal of fluridone from the water indicates that exposure time was critical for the long-term control of milfoil. The 48-µg/L treatment prevented regrowth following the 60-day exposure, indicating that increased concentration may reduce contact time requirements. Furthermore, the minimum level of fluridone that must be maintained to produce phytotoxicity has not been determined and is likely to require much longer contact times depending on the species and growth stage of the plant.

Exposure of hydrilla to fluridone led to an 85- to 92-percent reduction in chlorophyll by 6 DAT (Table 2). In contrast to milfoil, albescent tissue continued to elongate and maintained its integrity. Following the 30-day exposure period, hydrilla shoot biomass was reduced 70 percent (in all treatments) compared with untreated controls (Figure 3). Chlorophyll remained reduced by 85 to 92 percent, whereas root biomass was reduced only 5 to 18 percent compared with untreated controls (Figure 4).

Removal of fluridone-treated water at 30 days resulted in immediate greening of shoot tips followed by a return of fluridone symptoms. This temporary return of fluridone symptoms remains unexplained, but could be due to fluridone that remained sequestered in the plant tissue. By 12-day recovery, hydrilla again produced healthy green shoots. The 60-day harvest (30 days of recovery) resulted in a 50-percent shoot biomass reduction in the 12- and 24-µg/L treatments, and a 70-percent reduction in the 48-µg/L treatment (Figure 3). Chlorophyll content approached untreated control levels (Table 2), indicating active regrowth. By 60 days of recovery, only the 48-µg/L treatment resulted in reduced shoot biomass (35 percent).

Hydrilla exposed to fluridone for 60 days remained inactive. Shoot biomass was reduced by 70 to 85 percent (Figure 3), whereas root biomass was only reduced by 35 to 50 percent (Figure 4). Following the 60-day drain procedure, hydrilla shoot biomass nearly tripled during the 30-day recovery period (Figure 3). Although biomass was reduced by 35 to 60 percent, healthy regrowth, increased chlorophyll levels, and canopy formation indicated a complete recovery was likely.

Hydrilla exposed to fluridone for 90 days was reduced by 88 percent compared with untreated controls (Figure 3). Stems were flaccid and defoliated; however, the ability of these plants to recover remained unclear.

Results indicated that fluridone exposure time was critical for the sustained control of hydrilla. Although significantly reduced, following a 60-day exposure, hydrilla was able to recover from all fluridone rates tested.

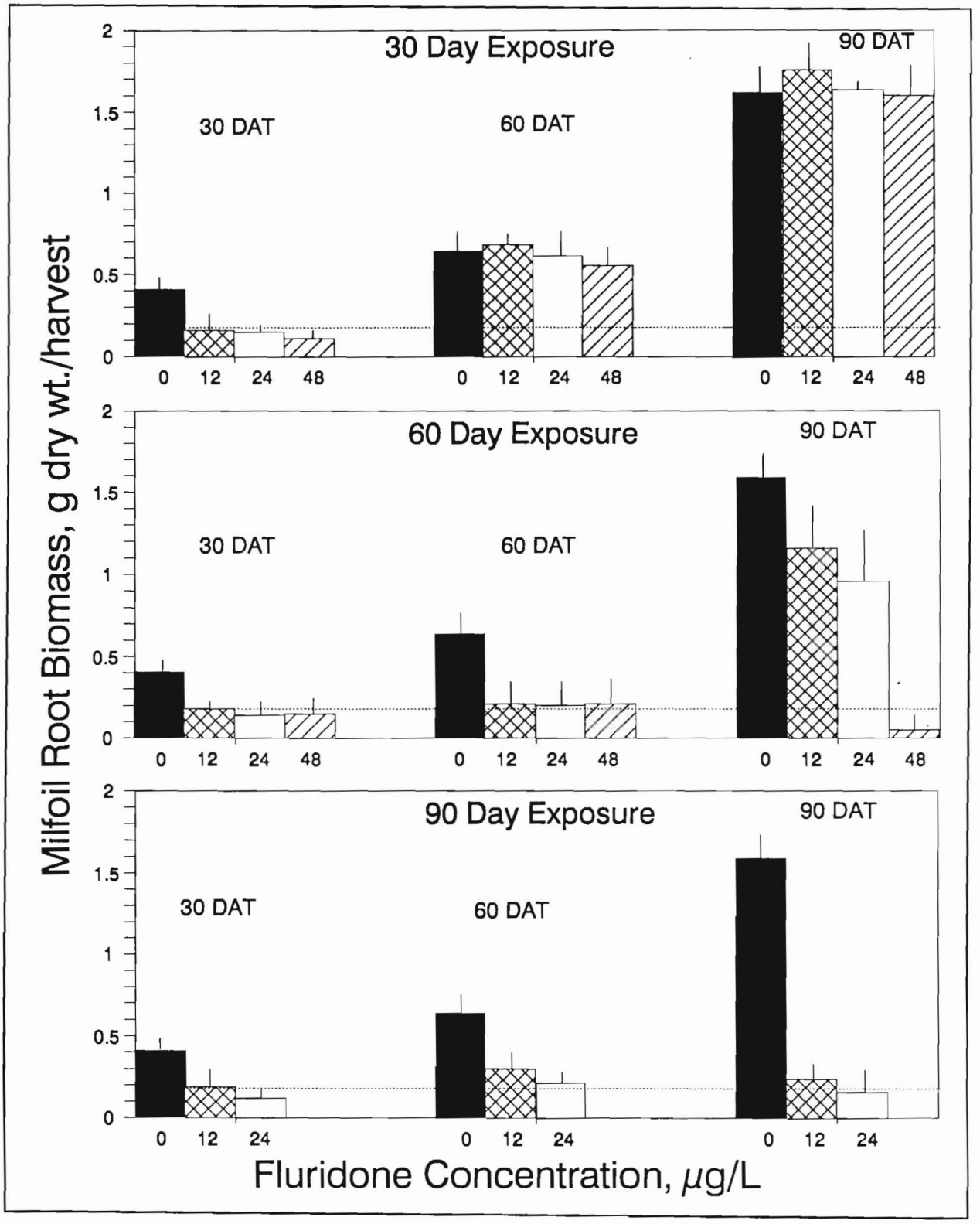

Figure 2. Effects of fluridone on root biomass of Eurasian watermilfoil harvested at 30, 60, and 90 days. The dashed line indicates estimated pretreatment biomass. Bars represent the mean of three replicates, and vertical lines represent the standard deviation

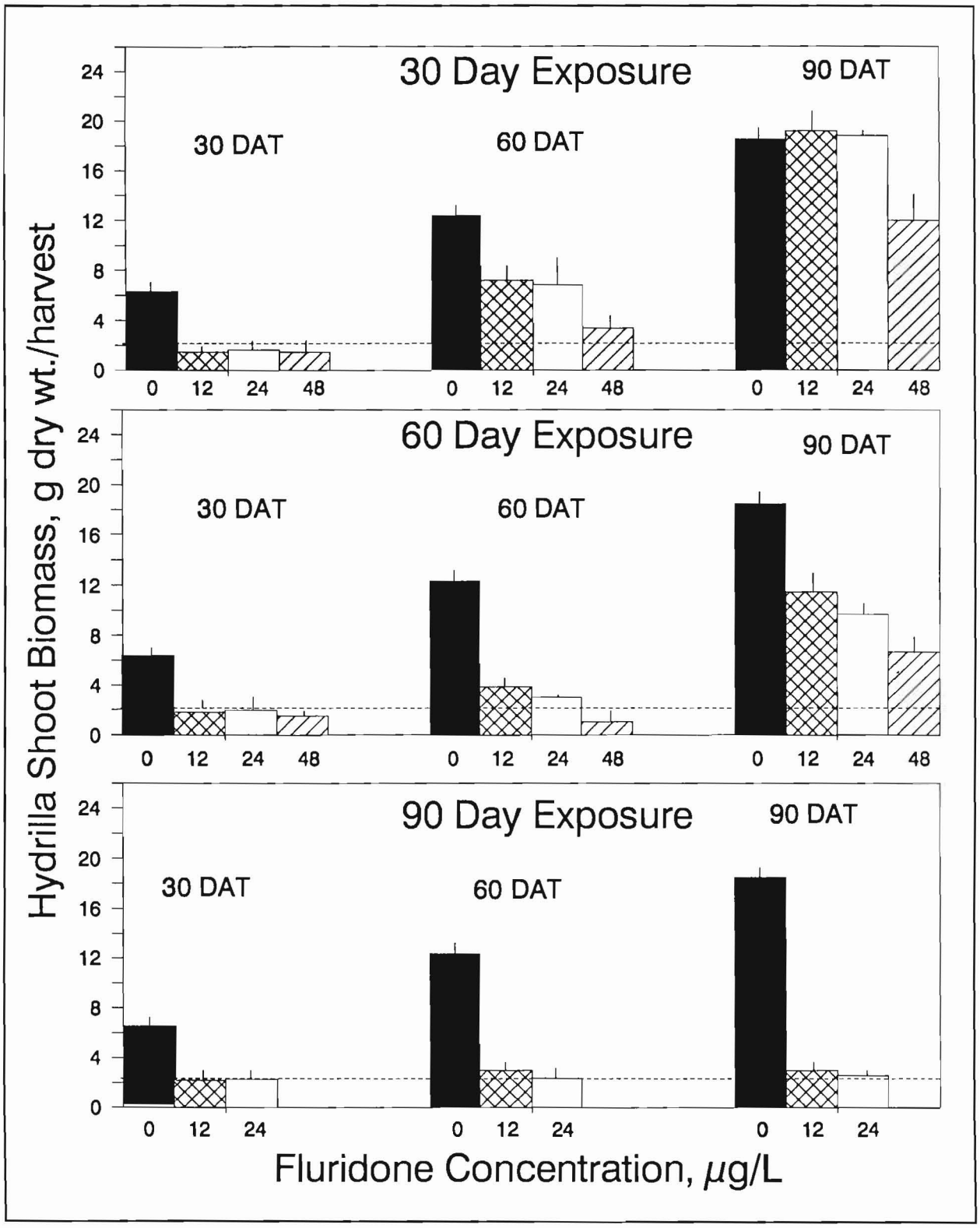

Figure 3. Effects of fluridone on shoot biomass of hydrilla harvested at 30, 60, and 90 days. The dashed line indicates estimated pretreatment biomass. Bars represent the mean of three replicates, and vertical lines represent the standard deviation

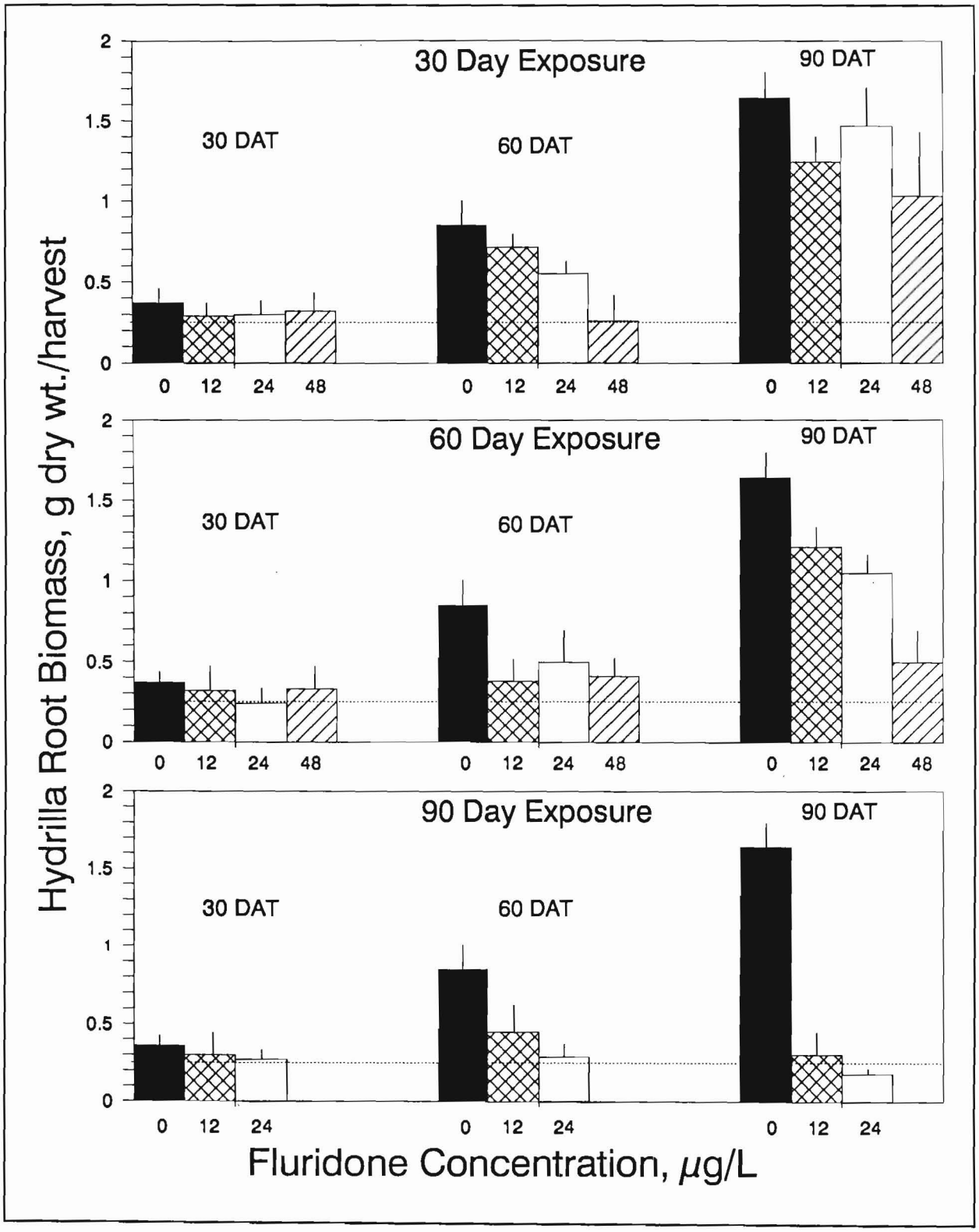

Figure 4. Effects of fluridone on root biomass of hydrilla harvested at 30, 60, and 90 days. The dashed line indicates estimated pretreatment biomass. Bars represent the mean of three replicates, and vertical lines represent the standard deviation

Previous laboratory studies have shown that increasing fluridone rates from 50 to 1,000 µg/L (150 µg/L is the maximum labeled rate) could reduce contact time requirements; however, a 10-fold increase in fluridone concentration often led to only marginal increases in efficacy (Van and Steward 1986; Van and Conant 1988; Spencer and Ksander 1989). Hall, Westerdahl, and Stewart (1984) treated milfoil and hydrilla with fluridone at rates of 10 to 90 µg/L for a 12-week period and achieved a 75- to 90-percent reduction in shoot biomass; yet, increasing the rate of fluridone did not result in a significant difference in shoot mass. This study showed that over a long exposure period, low fluridone rates (10 µg/L) were phytotoxic. Our results indicated that the shorter exposure periods (30 and 60 days) effectively reduced shoot mass, but were ineffective at preventing regrowth (48 µg/L for 60 days did prevent regrowth of milfoil) following removal of fluridone. Since the laboratory offers optimal conditions for plant regrowth following herbicide treatment (e.g., readily available light, stable water quality and temperature, and low mechanical stress), an underestimation of overall efficacy may occur.

Based on information from the laboratory and the field, it is likely that the key to a successful fluridone treatment is in maintaining herbicidally active concentrations for periods exceeding 60 days. Moreover, recent success of sequential applications of fluridone to lotic systems can be explained by the ability to maintain low concentrations (<40 µg/L) over long periods of time (8 to 16 weeks).

Pilot Studies

In contrast to maintaining residues for a long period of time, many applicators have tried using combinations of contact herbicides in an effort to reduce exposure time requirements and increase efficacy. However, the use of combinations can be confusing because of conflicting reports from field applications. Differences in water quality, concentrations and ratios of the herbicides applied, and weed species present at the time of treatment all contribute to confusion concerning the efficacy of various herbicide combinations.

Previous research with combinations focused on the use of copper-based compounds in combination with herbicides such as diquat and endothall (Sutton, Blackburn, and Barlowe 1971; Sutton et al. 1972; Haller and Sutton 1973). Results ranged from synergistic to additive to possible antagonistic effects. Current research at the U.S. Army Engineer Waterways Experiment Station is studying the use of contact herbicides in combination with copper-based compounds and their effect on CET requirements. Preliminary results from a pilot study in which milfoil was treated with endothall, Komeen (copper), or a combination of the two are presented in Figure 5. Results indicate that endothall was most effective when applied alone, and antagonism may have resulted when endothall and Komeen were applied together at fairly high rates. Haller and Sutton (1973) reported that endothall in combination with copper sulfate at low concentrations (0.4 to 2.0 µM) increased endothall-^{14}C uptake by hydrilla, whereas higher copper concentrations (4.0 to 16 µM) inhibited endothall

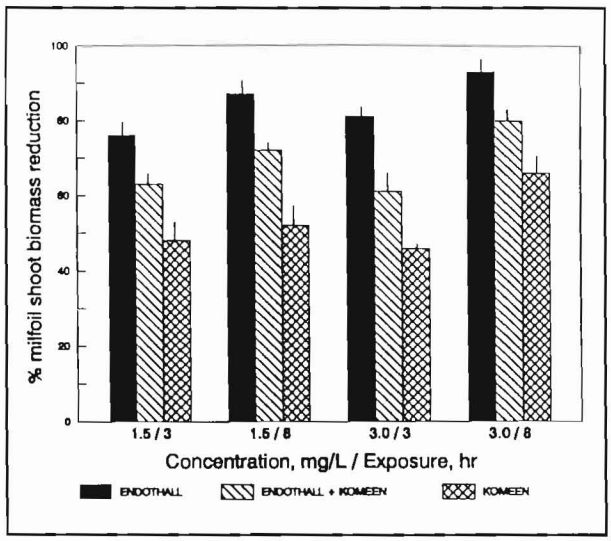

Figure 5. Eurasian watermilfoil shoot biomass reduction in response to endothall, Komeen, or endothall + Komeen treatment. Equal concentrations (mg/L) of endothall and Komeen were used for the combination treatment. Bars represent the mean of three replicates, and vertical lines represent the standard deviation

uptake. Their work suggests that using the higher rates of copper may have an antagonistic effect, whereas, lower rates of copper may enhance the herbicidal effect of endothall.

Future Work

Future work will include studies designed to simulate fluridone field half-lives to test if initial high concentrations followed by varying levels of dissipation can reduce contact time requirements. Research will also focus on the effects of various herbicide combinations (copper, endothall, and diquat) on CET requirements.

Acknowledgments

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Herbicide-Induced Stress in Aquatic Plants

by
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Introduction

An early and reliably quantifiable signal of herbicide effect would have a valuable role in aquatic plant control. A physiological measure of the stress imposed on plants following chemical application could add information to the visual inspection and biomass data used to evaluate control in laboratory studies. In the field, early indication of herbicide efficacy would aid evaluation of treatment and be able to obviate unnecessary reapplication.

Change in enzyme activity is one of the parameters that has been used to monitor physiological disruption in plants, and the enzyme peroxidase (PRX) has been shown to alter as plants respond to externally applied physical and chemical stresses (Cakmak and Marschner 1992; Gaspar et al. 1982). In an evaluation of heavy metal and pesticide contaminants in sediment, Byl and Klaine (1991) found that increasing concentrations from 10 µg/L of the herbicide sulfometuron methyl (Oust) produced a dose-dependent elevation in PRX activity in unrooted apical portions of hydrilla (*Hydrilla verticillata* (L.f.) Royle), and that heavy metal residues in sediments caused a change in the PRX isozymes being expressed. Identifying a syndrome of PRX changes in submersed aquatic plants following chemical control treatment, which could then quantify stress or indicate a lethal dose, would be valuable in studies of concentration/exposure time relationships, plant growth regulator efficacy, and the selective use of herbicides.

Objective

The aim of our preliminary work was to determine effective measurement protocols and to monitor levels of PRX enzyme in hydrilla, Eurasian watermilfoil (*Myriophyllum spicatum* L., hereafter called milfoil), and egeria (*Egeria densa* Planch.), both in untreated material, and following treatment with the herbicides fluridone (Sonar) and endothall (Aquathol K).

Materials and Methods

Material assayed for PRX was taken from rooted milfoil, egeria, or hydrilla plants that had been established from apical portions planted into sediment-filled beakers in 55-L aquaria maintained in controlled-environment chambers. Previously described regimes for establishment, radiation, day length, and fertilization were followed (Netherland, Green, and Getsinger 1991). Approximately 4 weeks after planting, material was treated by adding herbicide to the aquaria, and tissues were analyzed at various days after treatment (DAT). Following the prescribed exposure times, herbicide was flushed from the aquaria and fresh water applied to the plants during recovery periods. Biomass data were collected after PRX sampling ended. Two experiments were conducted: (a) milfoil and hydrilla were exposed to fluridone concentrations of 0, 12, 24, and 48 µg/L for 30 or 60 days, and either tips, leaves, or whole shoots were sampled at 0, 8, 30, and 60 DAT; (b) egeria and hydrilla planted together in the same aquaria were exposed to endothall concentrations of 2 mg/L for 0, 2, 16, and 36 hr, and leaves were sampled over 28 DAT.

Tissues were sorted for analysis. The uppermost 5 cm of growing points were designated as tips; shoots from 5 to 30 cm below apices were used whole or divided into leaves or stems; and roots were analyzed separately.

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For activity analysis, plant parts were harvested immediately before extraction and placed on ice. Tissue was blotted, and a 1.0-, 0.5-, or 0.25-g sample macerated with a chilled mortar and pestle using 0.1 M $NaPO_4$ buffer, pH 6.1 (Wang, Jiao, and Faust 1991), or 0.5 M $CaCl_2$ (Byl and Klaine 1991). The extract was poured into a chilled test tube and centrifuged at 2,000 g for 5 min. The supernatant was removed and the pellet washed with buffer in a repeat centrifugation. Supernatants were then pooled and filtered through one layer of Miracloth (Calbiochem). A ratio of either 1:10 or 1:20 g fresh weight of tissue to total milliliter volume of extraction buffer was maintained. A 200-µl aliquot of supernatant was combined with 2.8 ml of a reaction solution, consisting of either 0.1 M $NaPO_4$, 4 mM guaiacol, and 3 mM H_2O_2 (Wang, Jiao, and Faust 1991) or 5 mM MES, pH 6.0, 80 mM phenol, 44 mM H_2O_2, and 2 mM aminoantipyrine (Byl and Klaine 1991). Absorbance was monitored at 470 nm (guaiacol substrate) or 510 nm (phenol substrate), with readings taken at the end of the 1st and 3rd min after mixing the plant extract and reaction solution together. The rate of activity was reported as change in absorbance per minute per fresh weight of tissue. Three aliquots were reacted from each extract to produce a mean activity per sample. Analysis of variance was run on the data, and treatment means were separated with a Bayesian LSD at the 95-percent confidence level.

For horizontal starch gel electrophoresis, the tank (bridge) buffer used was 0.03 M lithium hydroxide (monohydrate) and 0.19 M boric acid, pH 8.1. The same solution was combined with a buffer of 0.05 M TRIS and 6 mM citric acid, pH 8.4, in a ratio of 1:10, and heated with 10- to 12-percent hydrolyzed starch to produce the gel matrix. Crude plant extract was applied to the solidified gel using paper wicks. The apparatus was placed inside a refrigerator at 4 °C, and a constant current applied to the gel for 4 hr before developing in a 0.1 M Na acetate, pH 5.0, solution containing 4 mM 3-amino-9-ethyl carbazole, 10 percent N,N-dimethyl formamide, and 15 mM H_2O_2.

Results and Discussion

The protocols described here were successful in monitoring activity and isozymes of PRX in the plant species assayed. Tests of the extraction procedure showed that little additional PRX activity, 0.9 percent of the total found, was retrieved by washing the pellet a second time, and the single pellet wash method was used for this work. Since many herbicide compounds have ring structures, those used for treatment were evaluated to ensure that they did not act as PRX substrates when substituted for phenol or guaiacol in the reaction solutions. No herbicide-generated activity was seen.

The different extraction buffers and reaction substrates used produced varying levels of PRX activity from the same plant material (data not presented), suggesting that these protocols extract soluble (PO_4-extracted) and ionically bound ($CaCl_2$-extracted) isozymes that differ in activity and substrate specificity. Use of several buffers and substrates may identify the most diagnostic or informative isozymes of PRX for each herbicide monitored.

Tissue specificity of PRX isozymes in plants is well established (Gaspar et al. 1982). Initial electrophoretic examination of untreated material showed variation in isozyme profiles between leaves and roots in milfoil and among leaves, roots, and tubers in hydrilla (data not presented). Enzyme activity also was seen to vary by tissue in untreated and treated material. The differences among plant parts in untreated milfoil (Figure 1) are consistent with relative levels of PRX found in different tissues of other species and with the negative correlation between PRX and auxin levels known to occur in most plant tissues (Biles and Abeles 1991; Ferrer et al. 1991; Lagrimini 1991). Pretreatment levels of PRX in milfoil and hydrilla leaves in the fluridone experiment were approximately twice that of shoots or of apical portions (data not shown). In the same experiment, a differential increase in enzyme activity by tissue was seen in fluridone-treated hydrilla, where activity in

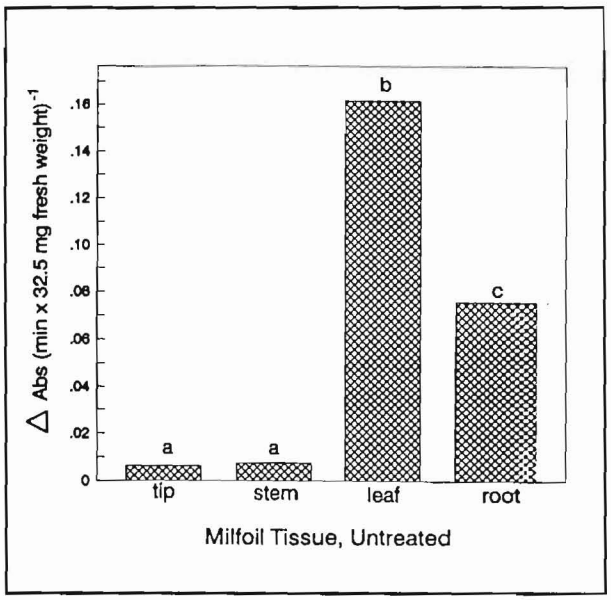

Figure 1. Levels of PRX activity in milfoil tissue taken from untreated aquarium-grown plants, following reaction with guaiacol substrate. Different letters indicate significant differences between tissue means at the 5-percent level according to a Bayesian LSD test

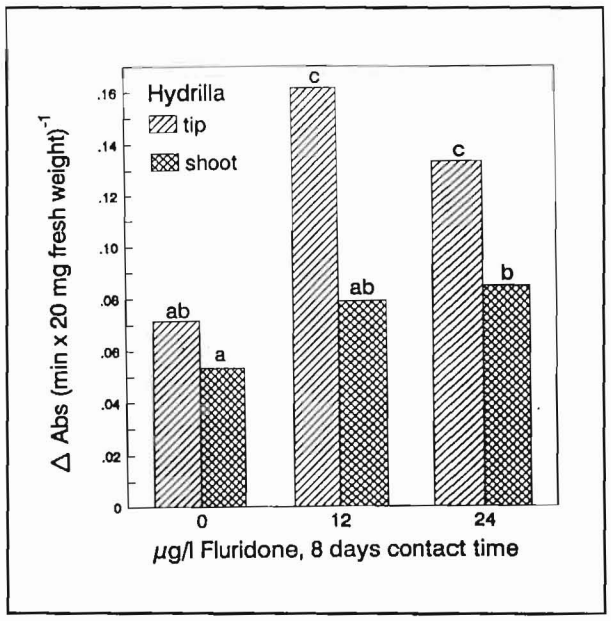

Figure 2. Levels of PRX activity in tips (apical 5 cm) and shoots (stems and leaves below 5 cm) of aquarium-grown hydrilla following 8 days contact time with fluridone at 0, 12, and 24 μg/L. Activity reported following reaction with phenol substrate. Different letters indicate significant differences between t reatment means at the 5-percent level according to a Bayesian LSD test

tips was significantly higher than in treated shoots and in untreated material following 8 days of contact (Figure 2). These results show that samples for PRX analysis need to be defined as to tissue. The nonparallel increase in PRX between fluridone-treated tips and shoots suggests that a localization of stress response may occur. If so, the most informative tissues could be identified for each particular herbicide.

Elevated PRX activity was seen with fluridone application in hydrilla and milfoil (Figure 3). At the end of a 30-day exposure time, milfoil shoots treated with 12, 24, and 48 μg/L were approximately three times higher in activity than reference material, although differences among concentrations were not significant. Shoots from hydrilla treated with 48 μg/L had significantly higher levels than those from plants exposed to lesser concentrations or from untreated material. At 60 DAT, PRX levels in apical tips and shoots of milfoil given 30-day exposure and 30-day recovery did not differ significantly from untreated material, while activity in plants treated continuously for 60 days remained significantly elevated (data not shown). However, strong correlations between increase in PRX and fluridone efficacy, as measured by reduced biomass of hydrilla and milfoil by Netherland, Getsinger, and Turner (in preparation), were not clear from this experiment. The 30-day exposure resulted in reduced shoot biomass by 90 DAT only in hydrilla treated at 48 μg/L, whereas PRX had been elevated at either 8 or 30 DAT in both species (Figures 2 and 3). Milfoil treated for 60 days, which had elevated shoot and tip PRX at that time (data not shown), was reduced in biomass by 90 DAT.

Treatment of egeria and hydrilla with endothall allowed comparison of PRX response between a target and nontarget species. The dipotassium salt of endothall is effective on hydrilla, but produces little control in egeria (Blackburn, Boyer, and Timmer 1971). The 16- and 36-hr treatments used here produced marked reduction in hydrilla biomass by

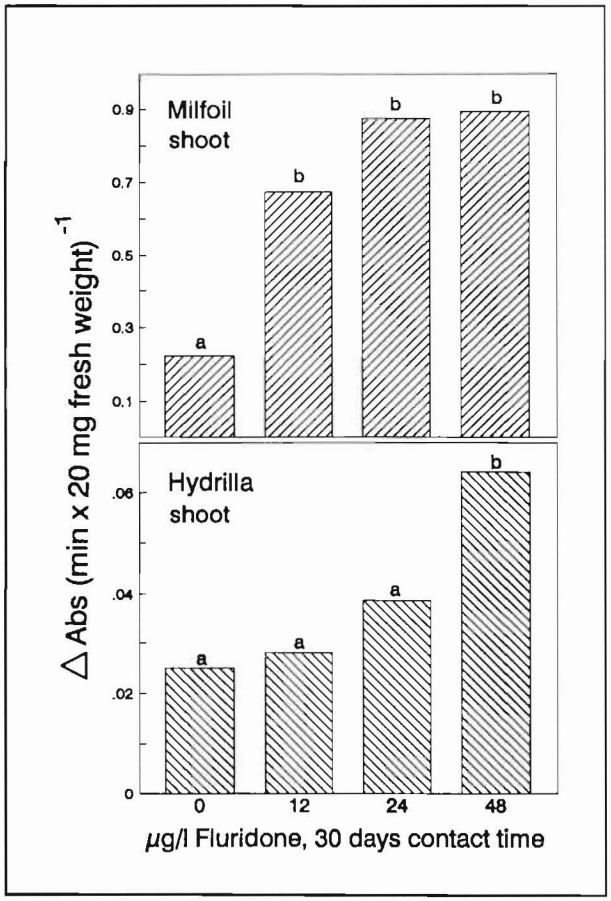

Figure 3. PRX activity in shoots of aquarium-grown milfoil and hydrilla following 30 days contact time with fluridone at 0, 12, 24, and 48 µg/L. Activity reported following reaction with guaiacol (milfoil) or phenol (hydrilla) substrate. Different letters indicate significant differences between treatment means at the 5-percent level according to a Bayesian LSD test

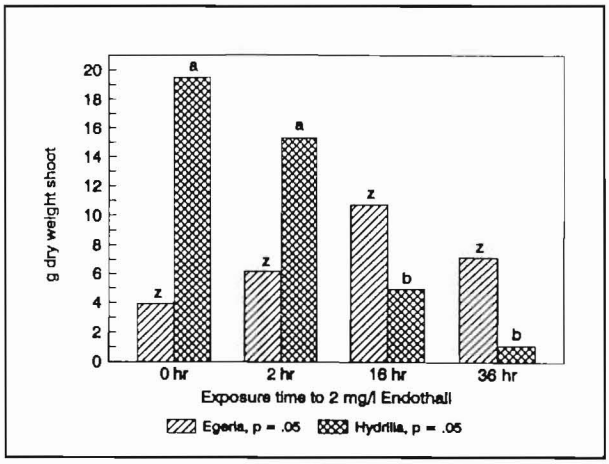

Figure 4. Dry weight biomass at 77 DAT from aquaria-grown egeria and hydrilla following 0-, 2-, 16-, and 36-hr exposures to 2 mg/L endothall. Letters a and b indicate significant differences between treatment means of hydrilla; the letter z indicates no significant differences between treatment means of egeria; both at the 5-percent level according to separate Bayesian LSD tests

77 DAT, but growth of egeria was not significantly altered at that time in any treatment (Figure 4). These biomass indications of herbicide efficacy were reflected in PRX levels. No significant differences were seen among treatments in egeria at any sampling date through 28 DAT. In contrast, hydrilla showed up to a seven-fold increase over pretreatment levels, and over mean levels in egeria (Figure 5). However, untreated hydrilla was seen to exceed pretreatment PRX levels through 8 DAT before returning to its original status, which was similar to that of egeria. Direct relationships between enzyme increase and exposure time were not seen in hydrilla, but PRX activity

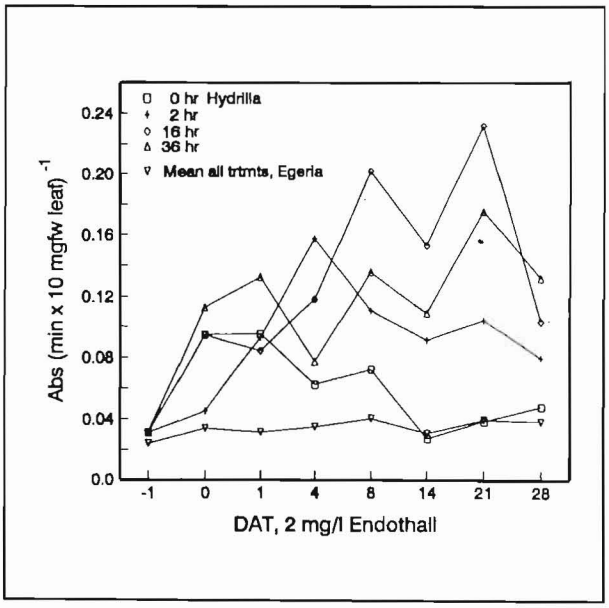

Figure 5. PRX activity to 28 DAT in aquarium-grown egeria and hydrilla following 0-, 2-, 16-, and 36-hr exposures to 2 mg/L endothall. Egeria data points are means of all treatment levels at each sampling date, since these had no significant differences between them at the 5-percent level according to Bayesian LSD tests

in 16- and 36-hr treatments, where exposures reduced biomass, exceeded that of other treatments from 8 DAT on.

The extent of elevation of enzyme with herbicide application seen here indicates that PRX analysis of various tissues may help describe the chronology and severity of treatment stress in aquaria-grown plants and be applicable to mesocosm and field studies. The fact that egeria did not alter in enzyme activity following endothall contact suggests that PRX may be used to evaluate selective herbicide treatment by indicating absence of physiological stress in desirable species during chemical control of a target population.

Future Work

One of the next goals of this work is to reduce variability in enzyme measurement by correlating PRX levels with protein and chlorophyll content of samples and by use of a PRX standard. Another is to verify differential tissue changes in PRX by monitoring multiple plant parts during herbicide treatments. A fuller description of PRX response to selective herbicide effect on target and nontarget species is desirable, beginning with a more detailed examination of endothall-treated egeria and hydrilla. Measurement protocols for other enzymes associated with stress are to be initiated, and isozyme response will be examined electrophoretically. Methods of analyzing fresh or stored material from mesocosm and field studies will also be determined.

Acknowledgments

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Herbicide Application Technique Development for Flowing Water

by
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Introduction

The objective of Work Unit 32354, Herbicide Application Technique Development for Flowing Water, is to identify submersed application techniques that maximize chemical contact time in dynamic systems. This unit provides field data that validate or stimulate laboratory and flume studies designed to determine optimal herbicide concentration/exposure relations for efficient submersed plant control. Studies in this work unit have included investigations of minimal water exchange conditions for application of contact herbicides, such as endothall, to hydrilla-infested tidal canals (Fox, Haller, and Getsinger 1991; Fox and Haller 1992).

Since 1989, studies have concentrated on management strategies for hydrilla-infested rivers in Florida. Previous management regimes in these rivers had used repeated treatments of endothall, diquat, or mechanical harvesting to maintain boat channels. The objective of this research was to provide hydrilla control over larger areas for at least 1 year, with as little impact on native vegetation as possible.

Application of Fluridone to Hydrilla-Infested Rivers

Hydrilla is particularly susceptible to fluridone, so that at low concentrations of 10 to 15 µg/L (over ten times less than the U.S. Environmental Protection Agency's potable water tolerance of 150 µg/L) hydrilla can be selectively removed from other, more tolerant vegetation. Such low concentrations, however, must remain in contact with the hydrilla for several weeks. To achieve such long exposure times in flowing water systems the herbicide must be regularly applied over the necessary contact period. In unregulated rivers, this requires adapting fluridone applications to the prevailing water discharge.

Fluridone was applied to Lake Hell 'n' Blazes on the upper St. Johns River (Haller, Fox, and Shilling 1990) and to Lake Istachatta in the Withlacoochee River. Fluridone treatments were made to these rivers in 1989 and 1990 to 1992, respectively.

Residue Sampling and Summarizing Data

Fluridone residues were regularly collected during and after each of these river treatments to determine whether the objective application rates had been achieved. This sampling program at seven sites on the Withlacoochee River resulted in a comprehensive data set that needed to be summarized in a form that could be related at each sampling site to hydrilla control efficacy. Data from tracking residue concentrations at one site (Figure 1) do not fit the simple pattern of a maximum concentration sustained for a specific period of time as seen in laboratory procedures (Netherland, Getsinger, and Turner 1993). This is particularly complicated if supplementary treatments are made to secondary application sites.

The residue data were summarized for each sampling site by calculating the product of residue concentration and exposure time (e.g., the area under the graph shown in Figure 1). These units were named FEDs (Fluridone Exposure Days) and relate to the concept of "availance"

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described by Hartley and Graham-Bryce (1980) (c.f., degree-frost days used in horticulture). Only concentrations greater than 1 µg/L were considered. The calculations required may be described by the following equation:

$$\sum [C_1 (T_1 - T_0)] - \frac{(T_1 - T_0)(C_1 - C_0)}{2} \ldots$$

$$\ldots [C_n (T_n - T_{n-1})] - \frac{(T_n - T_{n-1})(C_n - C_{n-1})}{2}$$

where:

C_x = fluridone concentration if greater than 1 µg/L at sampling time x

T_x = number of days after start of treatment at sampling time x

n = final sampling time

For example, the data in Figure 1 would have a FED value of 691-µg/L days. Such FEDs could be compared for different sites and treatments, even if their concentration/exposure patterns and timing were quite diverse. These values could also be directly correlated to quantitative data collected on hydrilla control.

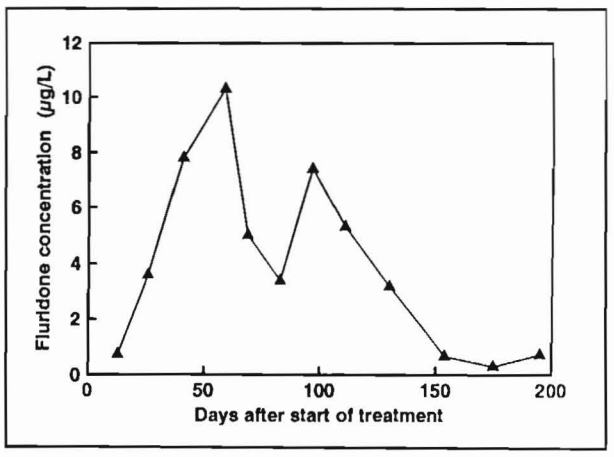

Figure 1. Residue concentrations in Bonnet Lake during and after the 1990 fluridone applications to the Withlacoochee River

Precautions in collection and interpretation of FEDS include the following:

- The collection of replicate residue samples is important, so that sample contamination or errors in residue analysis may be detected and accounted for in calculation of FEDs. Triplicate samples were collected in these studies. A few errors in residue analysis were detected, and the samples were rerun, based on comparisons of these replicates.

- Using a thorough record of all fluridone applications, it is possible to relate changes in residue concentrations at a particular site to primary and secondary applications made upstream. Ideally, samples would be collected every day; but in reality, sample collection must be timed to similar intervals after applications (i.e., do not collect samples immediately before a local application one time and then immediately afterward an application another time).

- The frequency and overall duration of a sampling program must be related to water discharge (e.g., less frequent sample collection but for a longer overall duration under lower discharge conditions). FEDs are underestimated and of limited value if a sampling program has ended before residue concentrations have been reduced to less than 1 µg/L or if residues were detected at too few sampling times prior to complete dissipation.

- The condition of the target plants should be noted prior to fluridone application. Higher FEDs would be needed to control mature, topped-out hydrilla than would be necessary for young, actively growing plants. Such differences may explain why low FEDs (resulting from high discharges at the end of the treatment period) applied to active hydrilla regrowth in the Withlacoochee in 1991 were as effective as high FEDs had been on dense stands of mature plants in 1990.

- Long-term and thorough efficacy data must be obtained to justify such residue sampling programs. Quantitative efficacy data collected at or near residue sampling sites will provide maximum information for use in evaluating the optimal FED

values necessary for hydrilla control. Vegetation sampling programs should also evaluate the impacts of fluridone and hydrilla removal on nontarget species.

Future Work

Monitoring of these types of fluridone applications to rivers will continue in fiscal year 1993. The objective will be to obtain a sufficiently large set of baseline data relating FEDs to control efficacy that will allow predictions of minimum FED values needed to control hydrilla, and what application programs would be needed to obtain such FEDs. The feasibility of making secondary fluridone applications to increase FEDs downstream of significant inflows will be investigated.

The monitoring of hydrilla and native vegetation in the Withlacoochee River will continue so that the long-term influence of these treatments on the river plant communities can be evaluated. Methods will continue to be sought that assess the impact of these treatments on hydrilla tuber and turion populations in the sediment (main source of regrowth).

Acknowledgments

The cooperation in these studies of the Southwest Florida Water Management District, the Jacksonville District, and the staff of Don Shilling's laboratory at the University of Florida has been much appreciated.

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Herbicide Delivery Systems

by
E. Glenn Turner,[1] *Michael D. Netherland,*[2] *David Sisneros,*[3] *and Earl R. Burns*[4]

Introduction

Laboratory herbicide concentration/exposure time (CET) studies have shown that excellent control of submersed aquatic plants can be achieved at low chemical concentrations given sufficient contact time (Netherland and Getsinger 1992; Netherland 1991; Netherland, Green, and Getsinger 1991; Green and Westerdahl 1990). However, maintaining this contact time can become an exceedingly difficult problem in areas of lakes, reservoirs, and rivers with high water exchange. Herbicide controlled-release (CR) technology offers a possible solution to this dilemma. A CR herbicide formulation consists of an active ingredient combined with an inert carrier. The herbicide is slowly released from the matrix into the surrounding water column over an extended period, thus providing a reduced but lethal herbicide concentration for the appropriate amount of time. Ideally, since CET relationships vary for each target plant and herbicide, CR formulations can be designed for specific control situations.

The objectives for this work unit are to develop and evaluate delivery systems that will maximize herbicide contact time against submersed macrophytes in a treatment area. More specifically, the focus this past year has been on the delivery of low levels (less than maximum label rates) of herbicides over extended periods (1 to several days). A few of the potential benefits of this approach include prolonged periods of weed control, reduced cost through the use of less herbicide and fewer applications, and diminished adverse impact on nontarget aquatic organisms.

There were three primary investigative areas within this work unit:

- Flume or large-scale verifications of laboratory-derived CET relationships in which the effectiveness of low herbicide concentrations coupled to long exposure periods against Eurasian watermilfoil were evaluated using mechanical application methods.
- Laboratory evaluations of several experimental and commercially available CR herbicide formulations in which chemical release rates were determined.
- Flume evaluations of experimental triclopyr CR formulations against Eurasian watermilfoil.

Flume Verification of Laboratory CET Principles

Materials and methods

For the flume verifications of laboratory-derived CET relationships, flow-through hydraulic channels located at the Tennessee Valley Authority Aquatic Research Laboratory (TVA-ARL) in Browns Ferry, AL, were utilized. Each of the 12 flumes measures 112 m in length, 4.3 m in width, and is lined with a 50-cm-thick layer of reservoir sediment. Water depths of up to 1.2 m can be attained by stacking weir boards within a set of brackets located at the outlet end of each flume. Water discharge rates generally range from 70 to 73 m^3/hr. For a more detailed description of

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2 U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
3 U.S. Bureau of Reclamation, Denver, CO.
4 Tennessee Valley Authority, Muscle Shoals, AL.

these systems, see Turner, Netherland, and Burns (1992).

Three stands of milfoil (4 by 10 m each) were established in each of four flumes, and stands within the same flume were separated by sections of open water approximately 30 m in length. Each flume was subjected to one of four different treatments (Table 1). In addition, rhodamine WT dye was applied concurrently with all triclopyr treatments. This provided an immediate estimate of triclopyr concentrations within the water columns.

Table 1
Herbicide Treatments

Herbicide	Application Method	Target Rate
Triclopyr	Sequential	1.0 mg/L
Triclopyr	Metered	0.25 mg/L
Triclopyr	Single	2.5 mg/L
Reference	None	0.0 mg/L

The sequential and metered application methods, described below, were designed to provide the plant stands within each flume with successively longer herbicide exposure periods. Thus, Stand 1 would be exposed to the herbicide for the shortest period and Stand 3 for the longest period. In this manner, general correlations between chemical contact time and degree of plant control could be ascertained.

The sequential method consisted of an initial application of the herbicide/dye mix to the entire volume of the flume impacting all three plant stands (Figure 1). After residues were reduced within Stand 2, as estimated by dye concentrations, a second application was conducted along the lower two-thirds of the flume, impacting plant Stands 2 and 3. In a similar manner, when residues were reduced within Stand 3, triclopyr was applied to the lower one-third of the flume, this time impacting only plant Stand 3. All applications were made at target rates of 1.0 mg/L triclopyr and 10 µg/L dye.

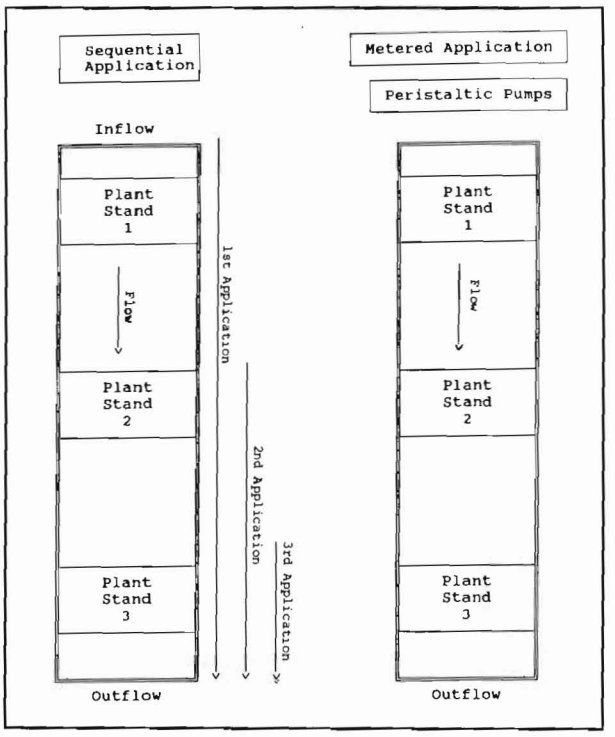

Figure 1. Diagram of sequential and metered herbicide application methods

The metered application method was used to provide a continuous input of chemicals to the flume at target rates of 0.25 mg/L triclopyr and 10 µg/L dye (Figure 1). Peristaltic pumps were initially placed at the inlet end of each flume, delivering chemicals to all three plant stands. After approximately 24 hr, the pumps were repositioned below Stand 1, delivering chemicals only to Stands 2 and 3. After an additional 24 hr, the pumps were placed below Stand 2, thus providing chemicals only to Stand 3. For purposes of comparison, one flume was treated with a conventional single application of triclopyr in which the herbicide was applied to the entire volume of the flume at a target rate of 2.5 mg/L.

Shoot biomass samples were collected from each plant stand 1 day prior to herbicide applications and at 6-weeks posttreatment. Dye concentrations were monitored throughout

each treatment period, and water samples were collected periodically to verify herbicide residues.

Results

Results for triclopyr treatments are presented in Figures 2-5. Although herbicide concentrations were somewhat variable, a general trend was observed within each treatment in which chemical contact time increased moving downstream in each flume from milfoil Stand 1, to Stand 2, to Stand 3. Accordingly, as contact time increased, the degree of plant control increased.

In the single application treatment (Figure 3), no control was observed in Stand 1, where 1.5 mg/L was maintained longer than 1 hr, but less than 5 hr. In Stand 2, where concentrations were above 3.0 mg/L for more than 1 hr and 0.5 mg/L for more than 5 hr, biomass was reduced by 72 percent. In Stand 3, where a concentration of 3.5 mg/L was maintained for more than 1 hr and greater than 1 mg/L for more than 5 hr, complete (100-percent reduction in biomass) control was obtained.

In the sequential treatment (Figure 4), no control was obtained from one application of 1.0 mg/L triclopyr (Stand 1) where concentrations were rapidly reduced below the target rate. In Stand 2, where concentrations were above 0.8 mg/L for more than 3 hr and at least 0.2 mg/L for more than 11 hr, biomass was reduced by 65 percent. In Stand 3, where concentrations of 0.6 mg/L to 1.4 mg/L were maintained for 20 hr, complete control was achieved.

In the metered treatment (Figure 5), low herbicide concentrations (0.05 to 0.37 mg/L) over 28 hr provided no control (Stand 1); whereas in Stand 2, where similar concentrations were maintained for over 50 hr, milfoil biomass was reduced by 40 percent. In Stand 3, where these low concentrations were maintained for more than 92 hr, complete control was obtained.

It is important to note that the lack of control observed in the first plant stands (and to a lesser degree in the second stands) could not be overcome by the single application method. However, the sequential and the metered applications could be modified to achieve complete control throughout the flumes.

Laboratory Evaluations of CR Herbicide Formulations

Materials and methods

Release rate profiles were established for three experimental (2-percent triclopyr granules with either protein or gypsum matrices and a 27-percent endothall granule) and one commercially available (Aquathol K, 10-percent endothall granule) herbicide controlled-release formulations. Predetermined quantities of each formulation were placed in 55-L aquaria at 22 ± 2°C. At the end of each 24-hr period, aquaria were drained twice and refilled with fresh water. For the endothall formulations, water samples were collected at 2, 12, and 24 hr for 3 days. For the triclopyr formulations, samples were collected every 24 hr for 7 days.

Results

Release rates for the endothall formulations are presented in Figure 6. Most of the herbicide release occurred within the first 2 hr. The experimental pellet showed some release following the first 2 hr; however, the concentration was considerably diminished. Thus these pellets are probably more appropriately used for placement of the herbicide within a treatment area rather than for sustained release. If plant efficacy using the 27-percent active ingredient (ai) granule is similar to that provided by the standard 10-percent ai granule, then considerably less inert material will be required for an operational treatment.

Triclopyr release rates are shown in Figure 7. The protein matrix exhibited a somewhat sustained release of triclopyr; however, concentrations were reduced after the first 24 hr. In contrast, although concentrations were lower than targeted release rates, the gypsum matrix showed a more prolonged release of triclopyr over the entire 7-day period. Based on these

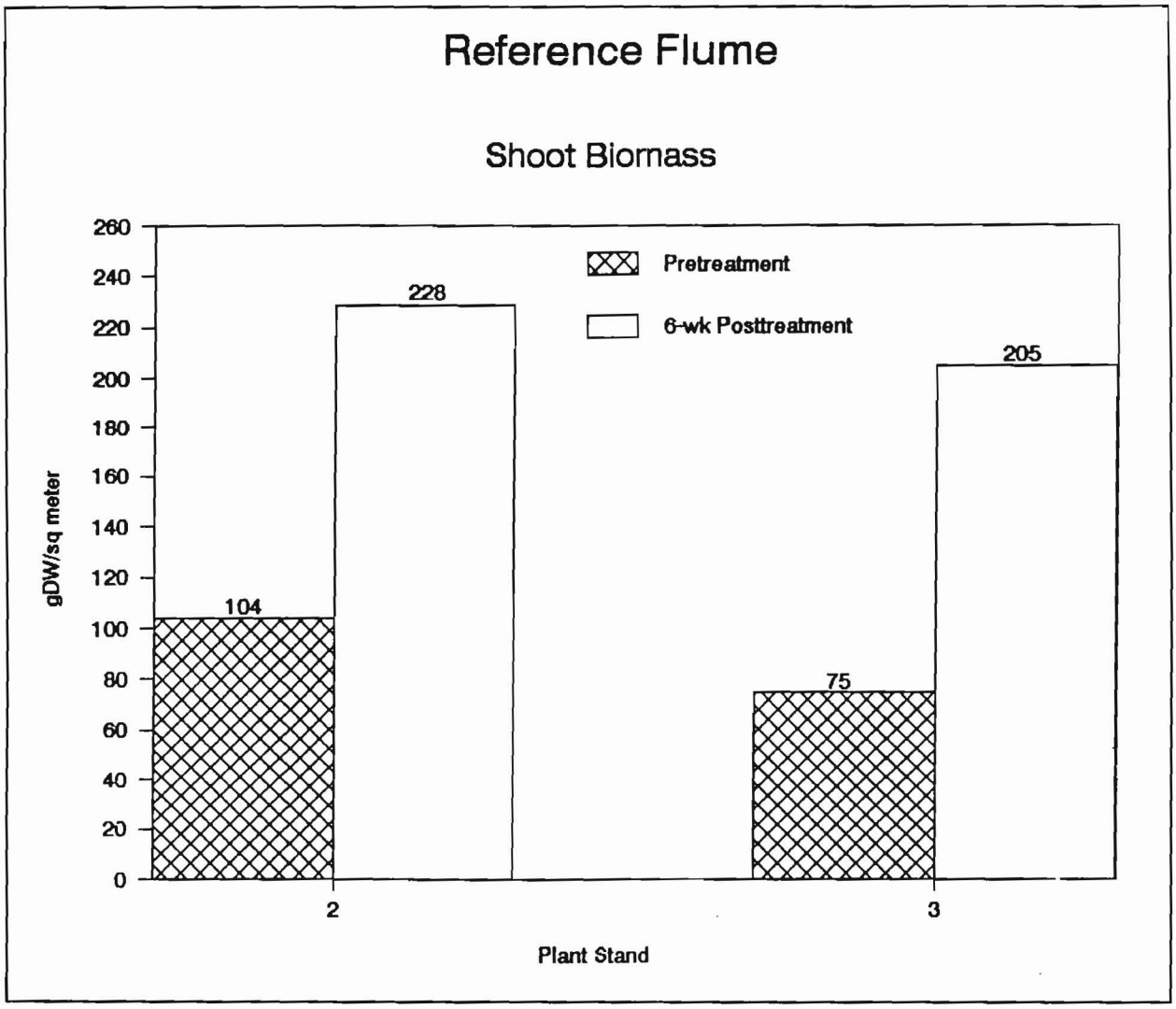

Figure 2. Eurasion watermilfoil shoot biomass in reference flume (samples were collected only from plant Stands 2 and 3)

results triclopyr/gypsum was selected for further CR investigations in scale-up studies.

Flume Evaluations of Controlled-Release Triclopyr Formulations

Materials and methods

For this study, formulations referred to as slow release matrix devices (SRMDs) were utilized. Each SRMD consisted of triclopyr encapsulated within a gypsum matrix, which was then encased within a polyvinyl chloride housing with hardware cloth to either side. Two formulations were utilized, one designed to deliver 0.10 mg/L triclopyr over a period of 5 days and one designed to deliver 0.30 mg/L over 5 days. Two flumes, as previously described, were planted with two stands (4 by 10 m each) of milfoil with a 30-m section of open water between stands. The SRMDs were suspended at mid-depth at the inlet end of each flume for a period of 5 days. Milfoil shoot biomass samples were collected 1 day prior to deployment of the SRMDs and at 6 weeks posttreatment. Water samples were collected at 6 and 12 hr, and every 12 hr thereafter for the 5-day treatment period.

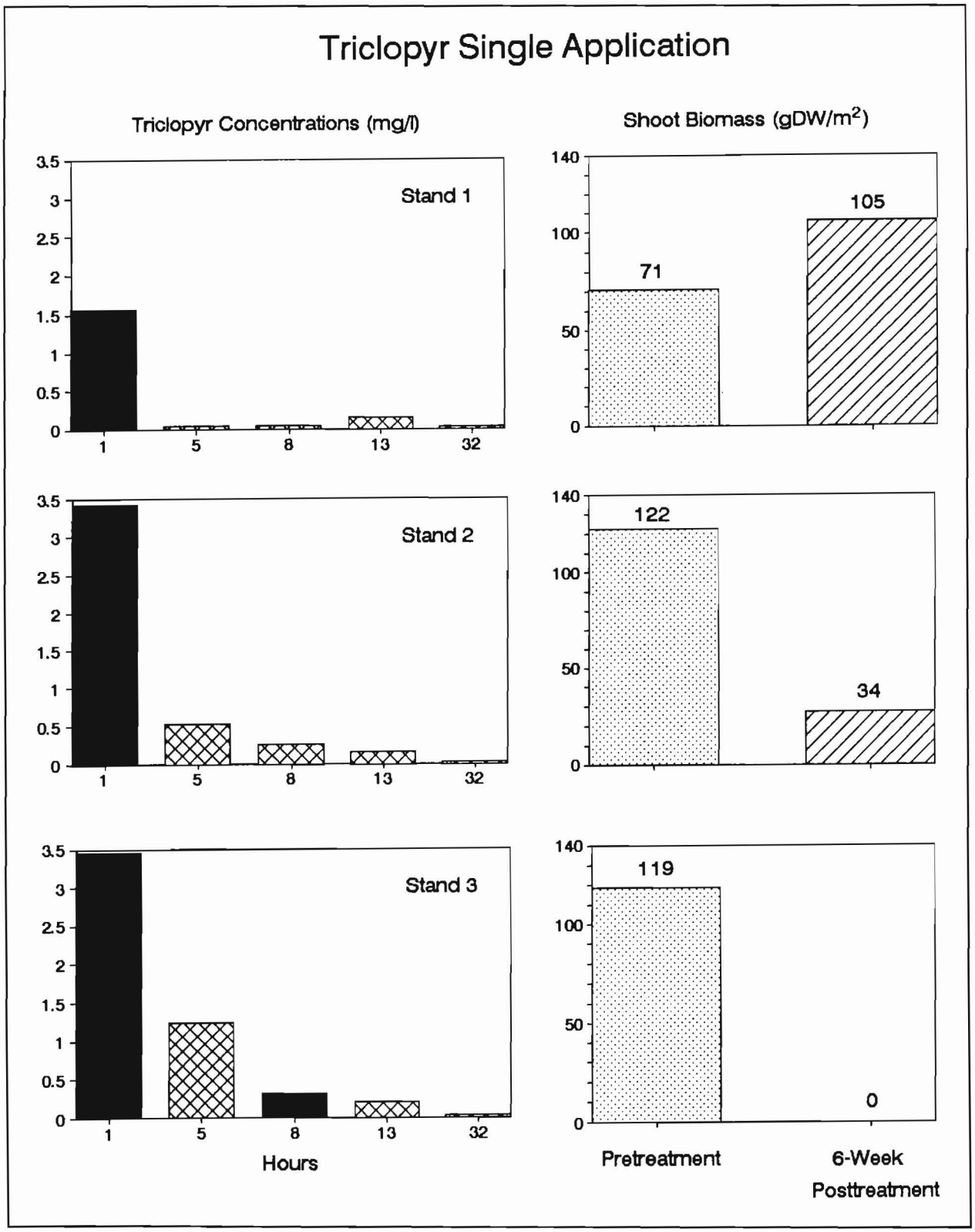

Figure 3. Herbicide concentrations and Eurasian watermilfoil shoot biomass in flume treated with single application of triclopyr. For figures in the left column, filled bars represent actual residue concentrations, and hatched bars represent estimated concentrations based on dye values (R-square for dye/triclopyr correlation = 0.99)

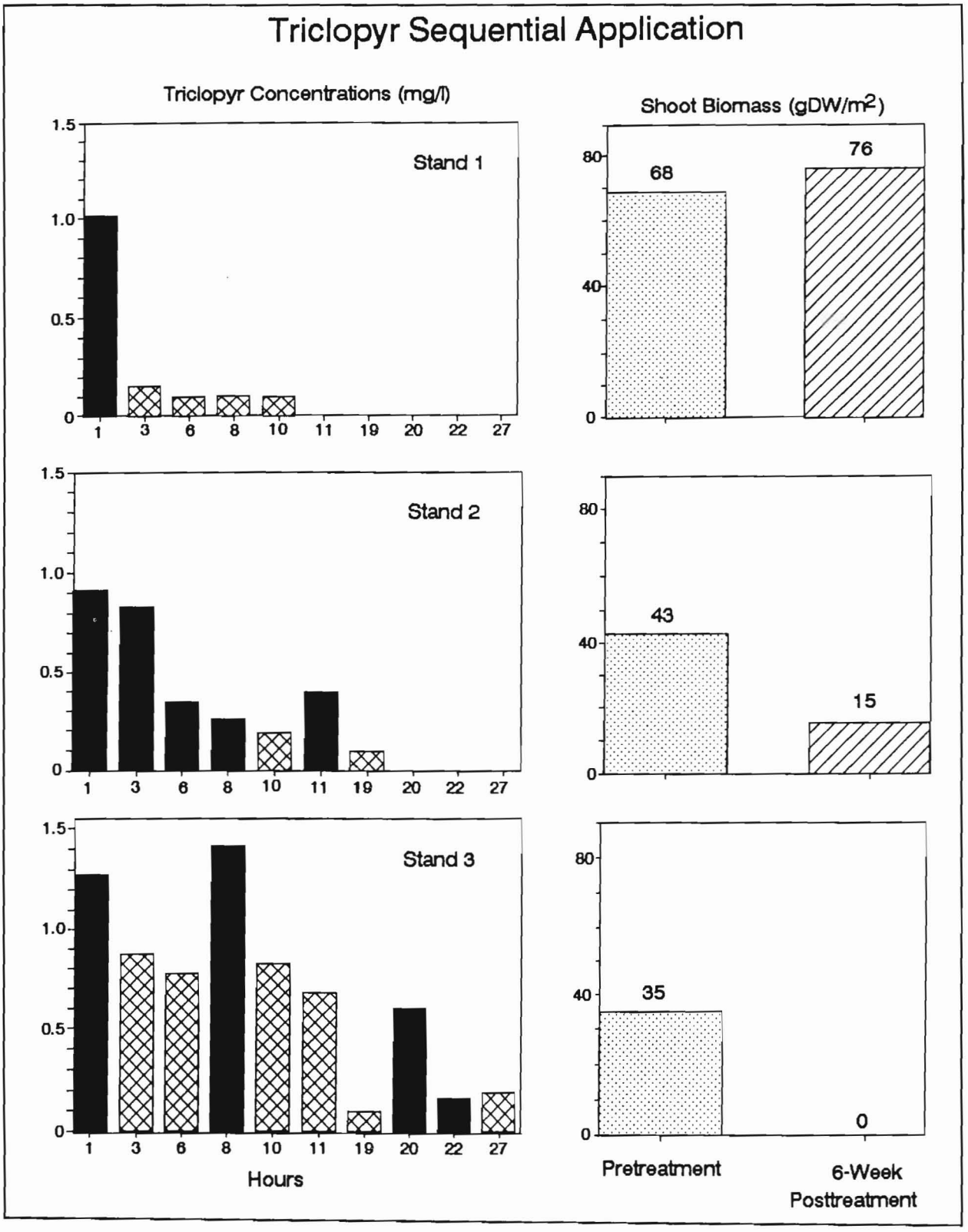

Figure 4. Herbicide concentrations and Eurasian watermilfoil shoot biomass in flume treated with triclopyr using a sequential application method. For figures in the left column, filled bars represent actual residue concentrations, and hatched bars represent estimated concentrations based on dye values (R-square for dye/triclopyr correlation = 0.96)

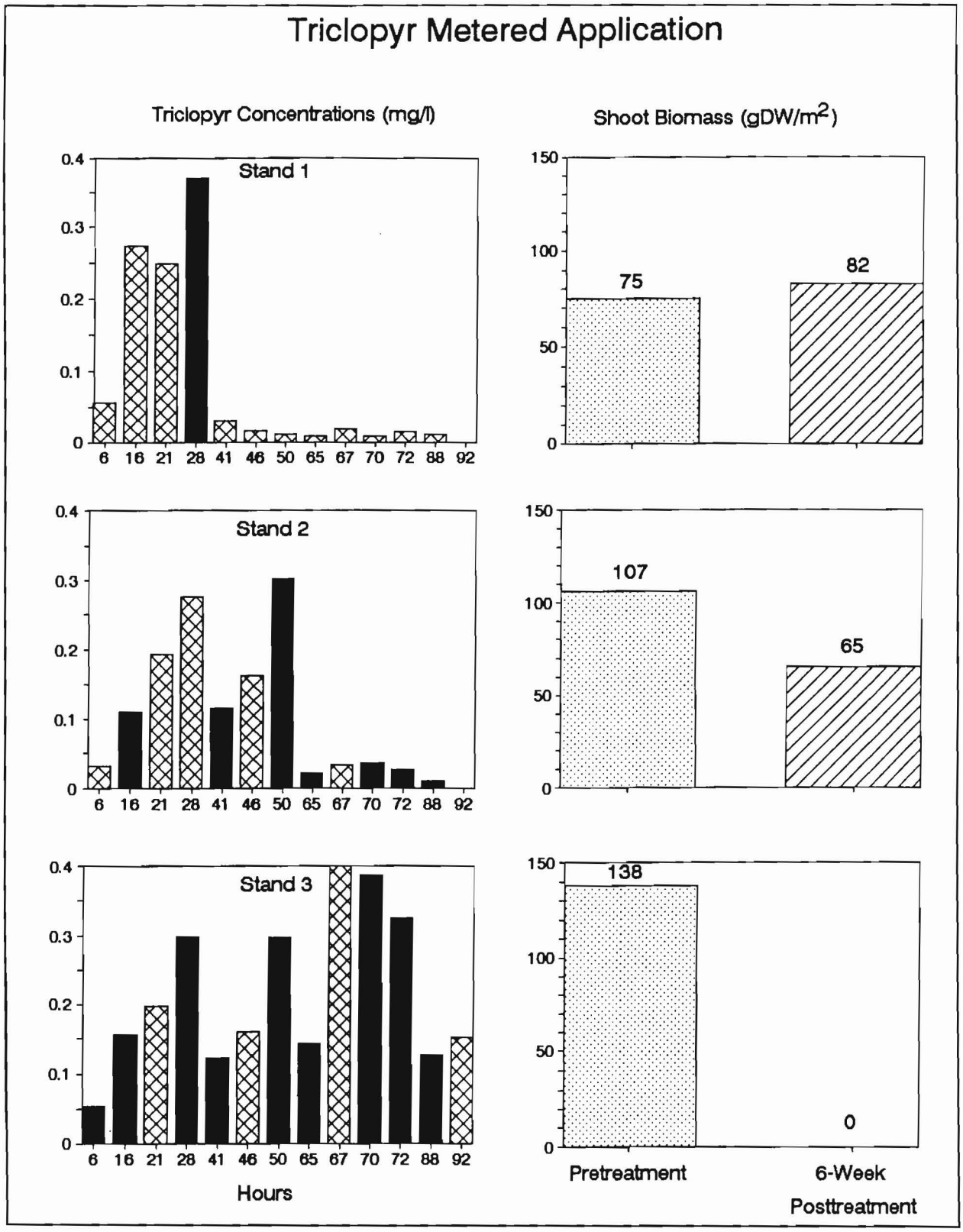

Figure 5. Herbicide concentrations and Eurasian watermilfoil shoot biomass in flume treated with triclopyr using a metered application method. For figures in the left column, filled bars represent actual residue concentrations, and hatched bars represent estimated concentrations based on dye values (R-square for dye/triclopyr correlation = 0.96)

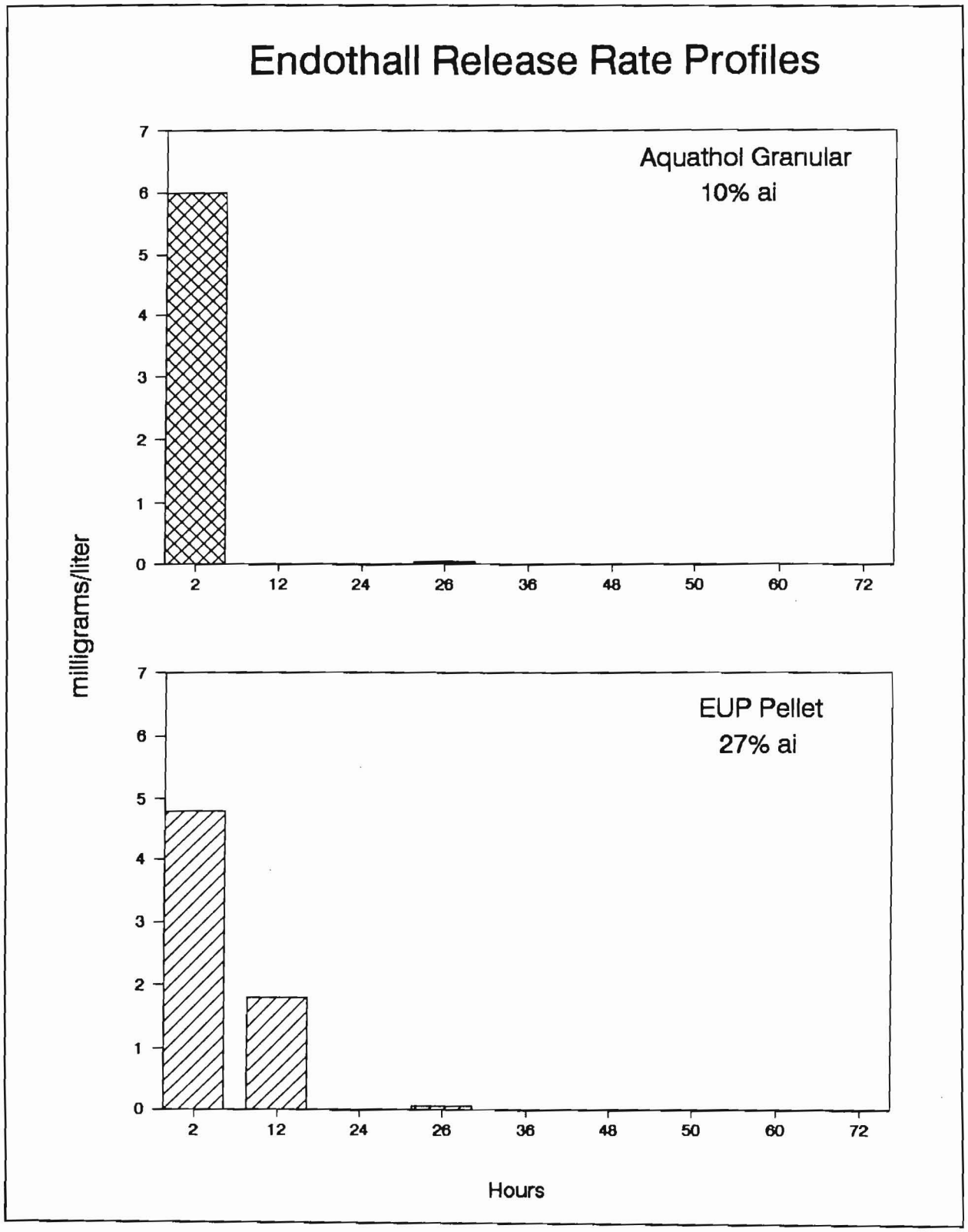

Figure 6. Endothall release rate profiles for Aquathol Granular (top) and an experimental pellet (bottom). Bars represent amount of endothall released between sampling times

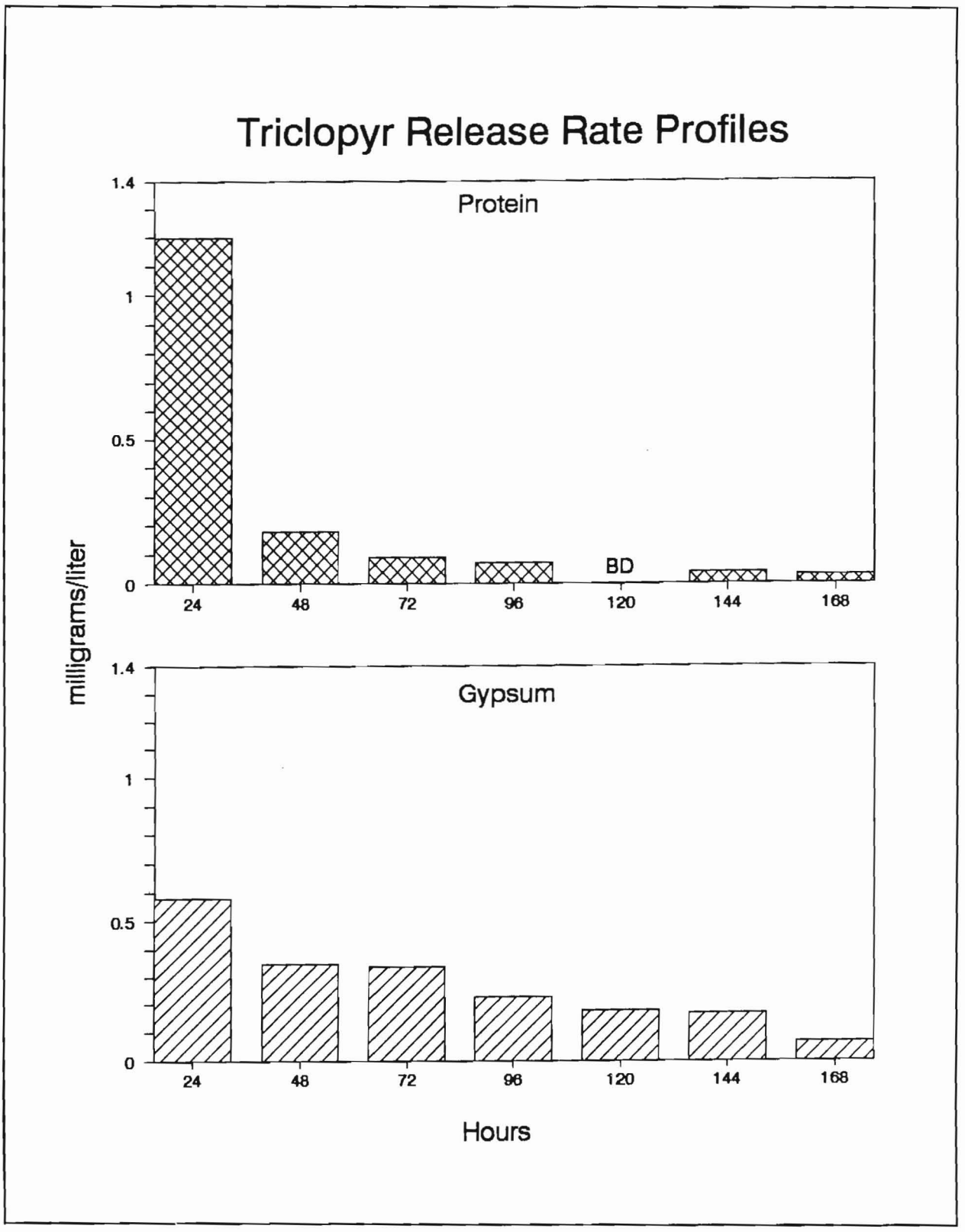

Figure 7. Triclopyr release rate profiles for formulations using a protein matrix (top) and a gypsum matrix (bottom). Bars represent amount of triclopyr released between sampling times

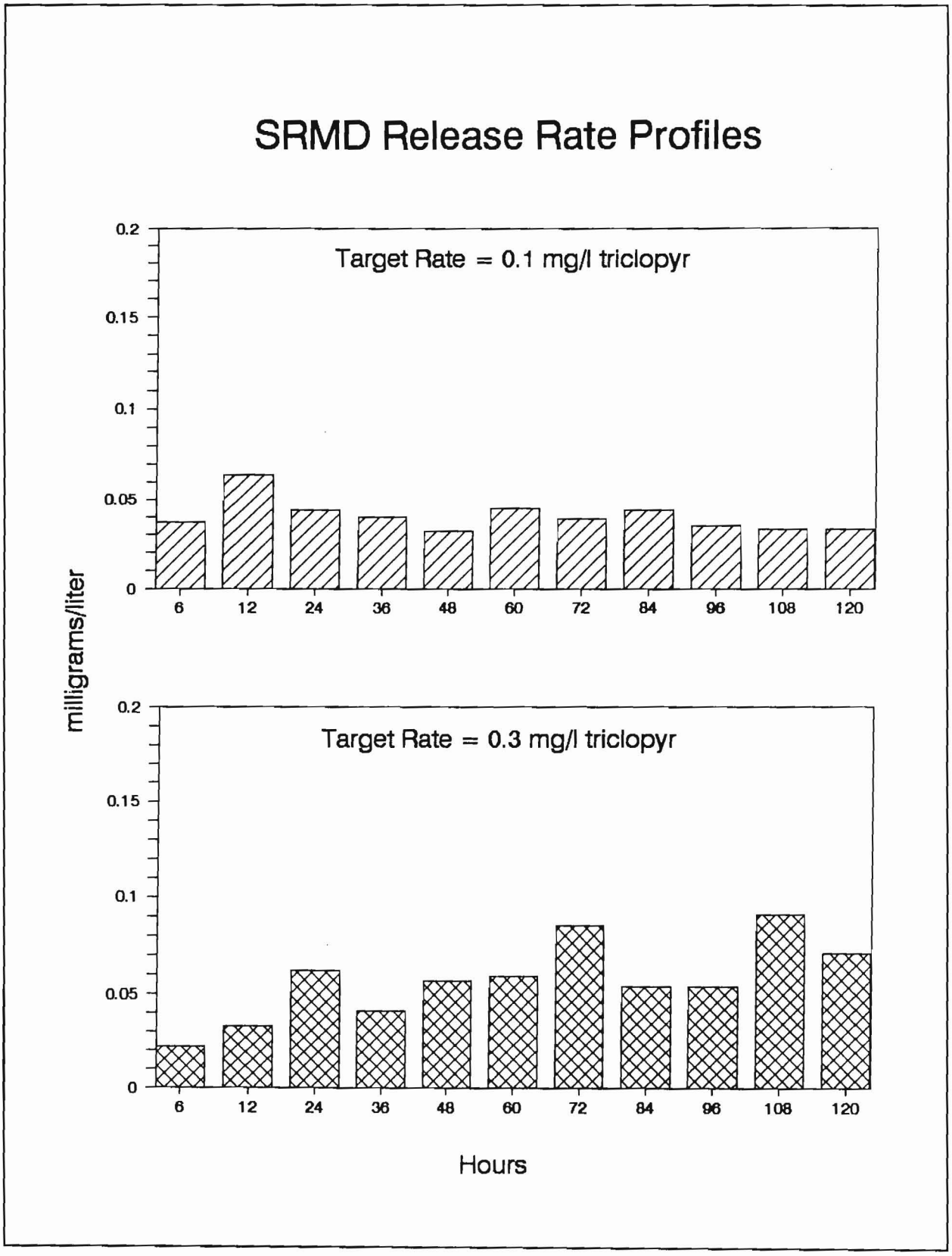

Figure 8. Release rate profiles of triclopyr SRMDs utilized in TVA-ARL flumes

Results

Triclopyr release rates of the SRMDs are shown in Figure 8. Herbicide concentrations were low, and neither target rate was achieved over the 5-day treatment period. Consequently, milfoil plants exhibited initial triclopyr injury symptoms, but there were no significant reductions in shoot biomass. However, within each treatment, the SRMDs showed a well-sustained and quite consistent release rate over the entire 5-day period.

Summary

It has been demonstrated that the laboratory-derived principle in which effective plant control is achieved through low herbicide concentrations coupled to long exposure periods is indeed valid when applied to larger scale flumes that mimic natural systems. As mentioned previously, one approach to accomplishing prolonged herbicide release is the use of controlled-release formulations. Results from laboratory and flume studies indicate that such release can be accomplished utilizing a gypsum matrix. Although herbicide concentrations were low, it is thought that through fairly simple modifications in the gypsum carrier, the release of efficacious herbicide concentrations within targeted areas can be achieved. Further studies are needed to determine exact formulations required.

Overall, results from these studies will aid in the development of herbicide delivery systems and operational guidelines for the implementation of efficacious and environmentally compatible chemical control programs.

Future Work

Plans for future work include continued verification of laboratory-derived CET principles in the TVA-ARL flumes and continued evaluation and development of controlled-release formulations in both the laboratory and flumes. Herbicides scheduled to be evaluated include formulations of fluridone, endothall, and another iteration of the triclopyr SRMDs. Plans also include a preliminary assessment of a starch matrix currently being used in controlled-release formulations of terrestrial herbicides.

Acknowledgments

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Plant Growth Regulator Effects on Four Aquatic Plant Species

by
Linda S. Nelson[1]

Introduction

Recent studies have demonstrated that many plant growth regulators (PGRs) that are active on terrestrial plants are also effective on aquatic plant species (Van 1988; Kane and Gilman 1991; Nelson et al. 1991; Netherland and Lembi 1992). One such compound is flurprimidol ([α-(1-methylethyl)-α-(4-trifluorommethoxy)phenyl]-5 pyrimidinemethanol). Flurprimidol is currently registered by DowElanco for use on turf and ornamentals, but also shows activity on hydrilla (*Hydrilla verticillata* Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Netherland and Lembi 1992; Lembi and Chand 1992). The use of PGRs offers a new dimension to aquatic plant management—allowing plants to remain as a functional part of the aquatic ecosystem by limiting their growth to non-nuisance levels. The many benefits of using PGRs as an aquatic plant management tool have been previously reported (Netherland and Lembi 1992; Klaine and Knowles 1988).

The mode of action of flurprimidol involves a reduction in the biosynthesis of gibberellins, which are endogenous plant hormones responsible for stem elongation. Without gibberellins, stem internode length is reduced, and plants remain short or stunted with little or no phytotoxic effects on other plant functions. In laboratory bioassays, stem elongation of Eurasian watermilfoil (milfoil) and hydrilla was inhibited at very low concentrations (0.75 and 75.0 μg/L, respectively) without affecting photosynthesis, respiration, and chlorophyll content (Netherland and Lembi 1992). In addition to low application rates, flurprimidol dissipates quickly in water and has a low order of acute toxicity to fish and wildlife species (Lily Research Laboratories 1983). Chand and Lembi (1991) showed that under field conditions, approximately 88 percent of applied flurprimidol dissipated within 28 days and that its half-life in water was 6.8 to 8 days. These data suggest that flurprimidol has a low environmental persistence and thus is favorable for use in aquatic systems.

Although all plants produce gibberellins, some are not susceptible to flurprimidol treatment, indicating chemical selectivity. Tolerant plants can metabolize the active ingredient to inactive compounds, whereas susceptible species cannot. Understanding chemical selectivity is necessary in a mixed plant community in which the management objective is to control a target plant species while minimizing effects on nontarget, desirable species. To date, most of the flurprimidol research conducted on aquatic plants has focused on hydrilla and milfoil. Little is known about the effects of flurprimidol on nontarget, native aquatic species.

The objective of this study was to determine the effects of flurprimidol on hydrilla, milfoil, and two nontarget plant species, wild celery (*Vallisneria americana* Michx.), and American pondweed (*Potamogeton nodosus* P.) grown under field conditions in a mesocosm tank system.

Materials and Methods

This experiment was conducted in the mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX. The mesocosm system consists of

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

several large, outdoor tanks that measure 1.4 m tall by 2.6 m in diameter and hold approximately 6,500 L of water. For this study, each tank was divided into into equal areas with a barrier made of netting that allowed water flow between the divided areas but restricted plant growth to each quadrant. Each tank was individually plumbed to regulate water flow as needed and was equipped with airflow for water circulation. A holding pond adjacent to the mesocosm system provided a water source for the tanks. Further description of the mesocosm system is included elsewhere in this publication.

Ten 1-gal plastic pots filled with nutrient-enriched soil were planted with one of the four test species (three plants per pot) and placed in each tank quadrant. Milfoil and hydrilla were propagated from 10-cm apical shoot tips collected from culture ponds at the LAERF. Wild celery and American pondweed (pondweed) were initiated from 3-day-old plantlets germinated from winter buds. Pondweed winter buds were collected from culture ponds at the LAERF, while wild celery winter buds were supplied by Wildlife Nurseries, Oshkosh, WI. All plants were allowed to establish in the mesocosm tanks for 2.5 weeks prior to chemical treatment. Established plants were treated with static exposures of 50 and 100 µg/L flurprimidol on 25 June 1992.

Following treatment, visual ratings of plant appearance and vigor were recorded on a weekly basis. At the conclusion of the experiment, 12 weeks after treatment (WAT), plants were removed from the tanks, and measurements of plant height and shoot and root biomass were recorded. Plant height for each species was measured from the soil surface to the top of the longest leaf or apical shoot tip. For each pot of plants, shoots and roots were separated, washed to remove algae and debris, and dried at 60 °C for 48 hr. Shoot and root biomass are recorded as grams dry weight per pot. Numbers of overwintering propagules (if produced) were also recorded. Plant, water, and sediment samples were collected periodically throughout the study for flurprimidol residue analysis (data not presented).

Additional parameters measured on hydrilla included net photosynthesis, respiration, and chlorophyll content. Photosynthesis and respiration, expressed as oxygen evolution or uptake, were measured at 6 WAT using a digital pH meter equipped with a dissolved oxygen electrode (Selim et al. 1989). Four-centimeter apical plant segments were placed in 300-ml biological oxygen demand bottles filled with fresh culture medium (one plant segment per bottle) at a known dissolved oxygen concentration. The bottles were placed in a circulating water bath in a dark chamber for 4 hr to induce respiration, then removed and measured for dissolved oxygen. Photosynthetic rates were measured in a similar manner but under lighted conditions for a 2-hr incubation period. Total chlorophyll (chlorophyll *a* and *b*) was measured on fresh tissue (4-cm apical segment) at 6 and 12 WAT using a dimethyl sulfoxide extraction method (Hiscox and Israelstam 1979). Total chlorophyll is expressed as mg chlorophyll (gram fresh weight)$^{-1}$.

Treatments were randomly assigned to mesocosm tanks with two replications. Data were analyzed using analysis of variance, and treatment means were separated using the Waller-Duncan *k*-ratio *t* Test at the 0.05 level.

Results

Twelve weeks after treatment, only hydrilla showed significant differences in plant height as a result of flurprimidol application (Figure 1). Compared with untreated hydrilla plants, stem height was reduced by 27 and 57 percent with flurprimidol treatments of 50 and 100 µg/L, respectively. Visual observations recorded throughout the study revealed that hydrilla plants were not only shorter, but formed a very dense mat of vegetation at the bottom of the tank. Instead of growing vertically in the water column as did untreated plants, flurprimidol-treated plants grew laterally producing many stolons with shortened stems. This "carpet-like" growth habit has been observed by other researchers (Netherland and Lembi 1992; Lembi and Chand 1992).

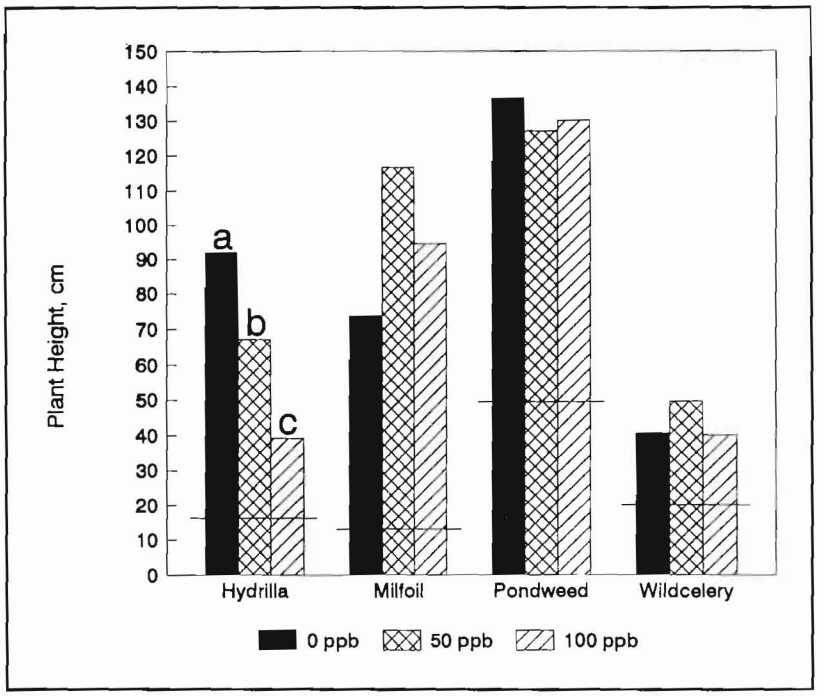

Figure 1. Effect of flurprimidol on plant height of hydrilla, milfoil, pondweed, and wild celery 12 weeks after treatment. Data are means of six observations. Letters denote significant differences at P = 0.05. Horizontal lines across bars represent pretreatment plant heights for each species

Plant height of milfoil, pondweed, and wild celery was not affected by flurprimidol treatment. Results on milfoil contradict results of bioassay tests in which flurprimidol doses as low as 0.75 µg/L reduced main stem lengths by 67 percent (Netherland and Lembi 1992). No measurable effects in our study can be partially attributed to poor milfoil establishment during the pretreatment growth period. Plants were small and were not actively growing at the time of treatment. It is possible that if active plant growth was not occurring, plant uptake of flurprimidol was poor. Furthermore, given the dissipation characteristics of flurprimidol in water (half-life ≈ 7 days), when plants did start to actively grow, levels of flurprimidol may have dissipated below effective concentrations. Studies on plants grown outdoors in sediment-filled barrels also showed that the effective concentration of flurprimidol was considerably higher (200 µg/L) than concentrations required in laboratory bioassay tests (0.75 to 100 µg/L) (Lembi and Chand 1992).

Despite reductions in plant height with hydrilla, there were no differences in shoot and root biomass (Figures 2 and 3). It was not surprising that biomass reductions were not observed with hydrilla after observing the extensive proliferation of stolons that occurred as a result of flurprimidol treatment. Milfoil and wild celery also showed no differences in final shoot and root biomass compared with untreated plants. The only significant differences in biomass were observed with pondweed. At 12 WAT, flurprimidol-treated pondweed averaged a 52-percent increase in shoot biomass and a decrease in root biomass (43-percent reduction with 100 µg/L flurprimidol) compared with untreated plants. The effect of flurprimidol on pondweed can best be described as a delay in plant development. At 12 WAT, untreated plants were undergoing normal senescence, whereas flurprimidol-treated plants were still green, sprouting new growth, and flowering. Thus, when plants were harvested, there was little aboveground tissue to weigh as shoot biomass with untreated plants as compared with treated plants. Higher root biomass measured on untreated plants suggests that these senescent plants were mobilizing aboveground carbohydrates to roots for storage.

Only wild celery and hydrilla had produced overwintering propagules by the end of the experiment. Results indicate that the number of hydrilla tubers was significantly reduced (46 percent less) with treatment of 100 µg/L flurprimidol (Figure 4). Hydrilla treated with 50 µg/L flurprimidol also showed a reduced number of tubers; however, numbers were not statistically different from those of untreated plants. Flurprimidol did not affect winter bud production in wild celery (Figure 5).

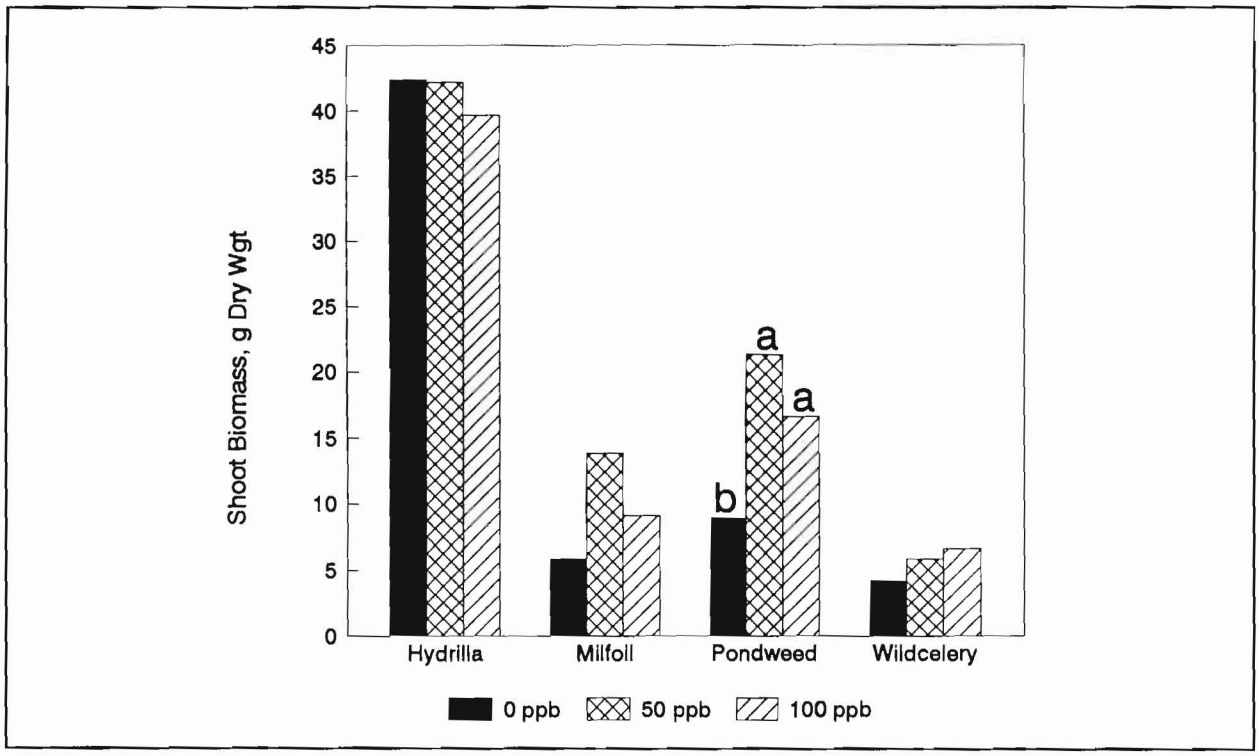

Figure 2. Effect of flurprimidol on shoot biomass of hydrilla, milfoil, pondweed, and wild celery 12 weeks after treatment. Data are means of six observations. Letters denote significant differences at P = 0.05

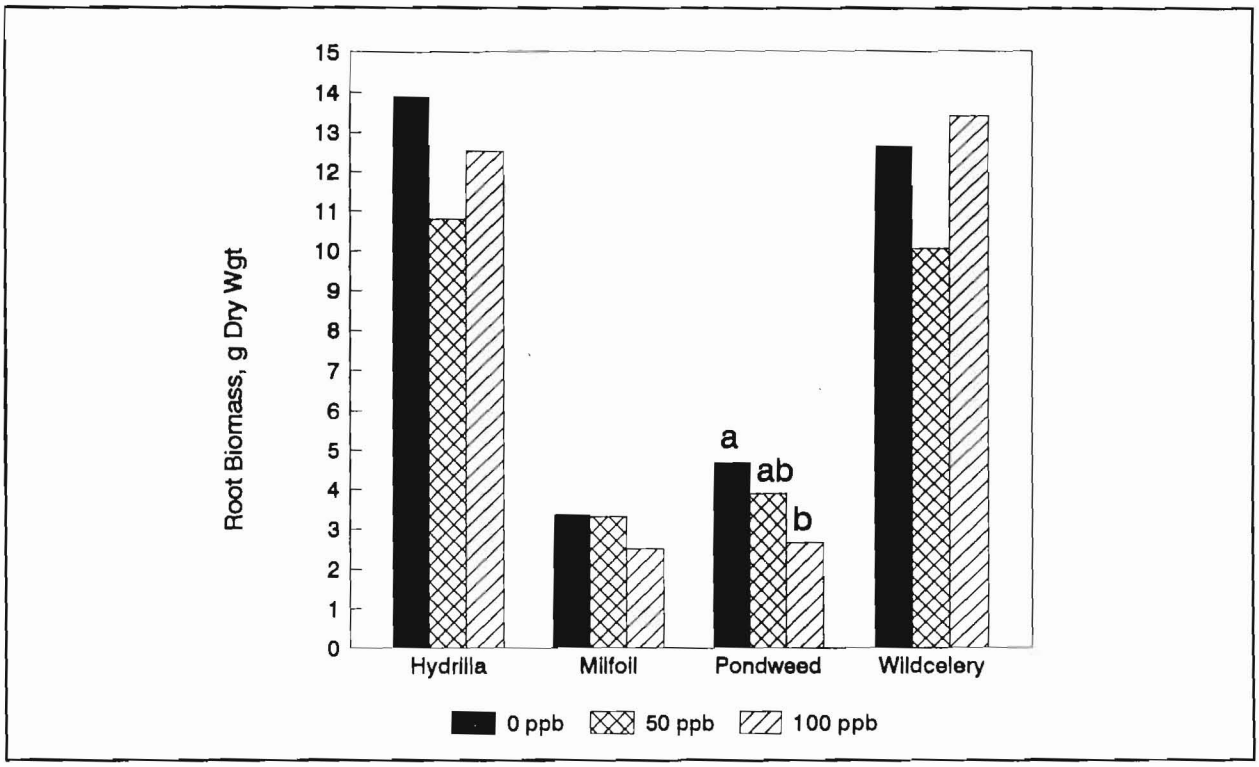

Figure 3. Effect of flurprimidol on root biomass of hydrilla, milfoil, pondweed, and wild celery 12 weeks after treatment. Data are means of six observations. Letters denote significant differences at P = 0.05

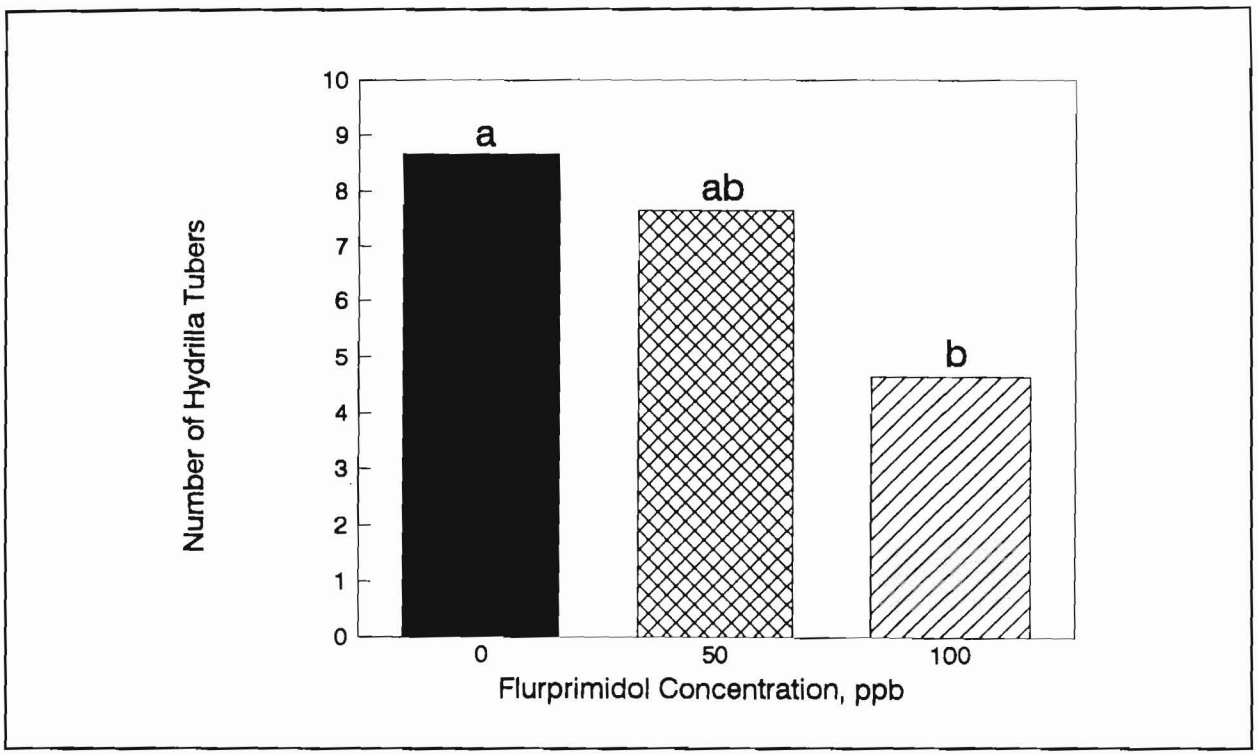

Figure 4. Effect of flurprimidol on tuber production of hydrilla 12 weeks after treatment. Data are means of six observations. Letters denote significant differences at P = 0.05

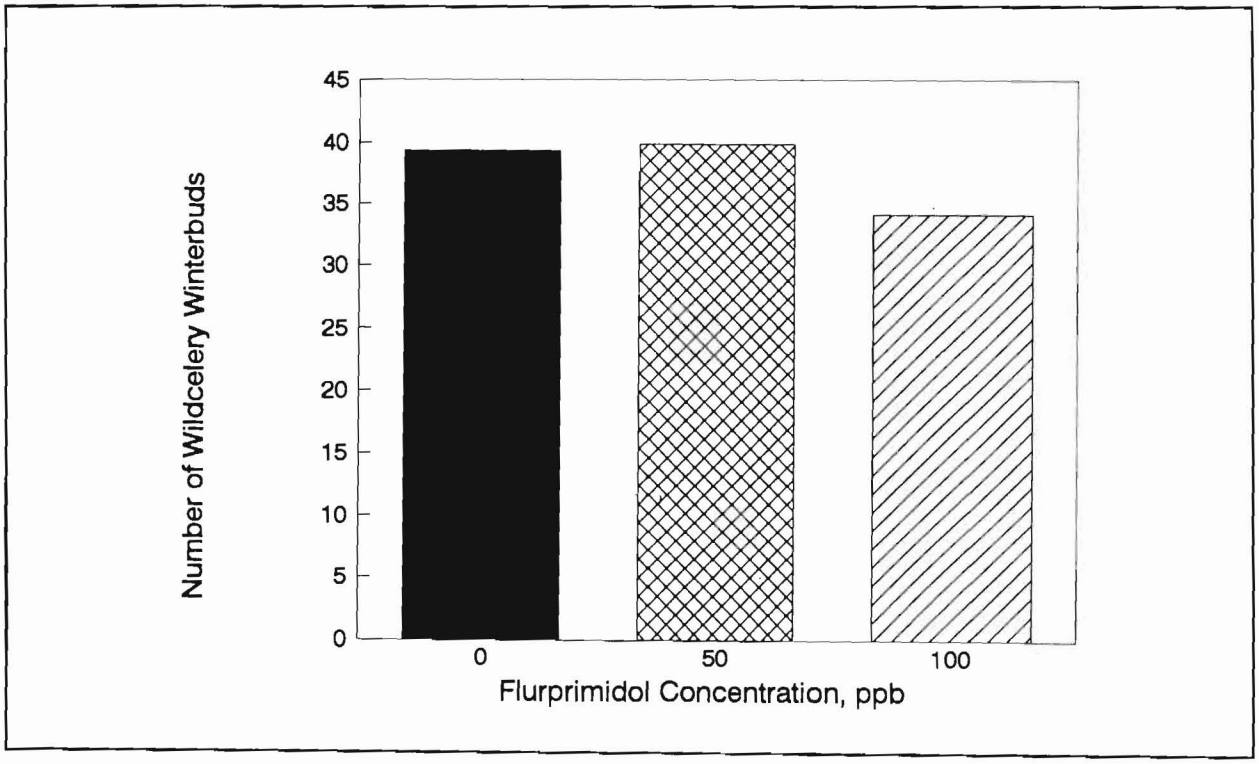

Figure 5. Effect of flurprimidol on winter bud production of wild celery 12 weeks after treatment. Data are means of six observations. Letters denote significant differences at P = 0.05

Flurprimidol-treated hydrilla showed an increase in total chlorophyll per gram of fresh weight from untreated plants (Figure 6). At 6 WAT, plants treated with 100 µg/L flurprimidol had 76 percent more chlorophyll than untreated hydrilla. Chlorophyll levels were also elevated with treatment of 50 µg/L; however, data were not statistically significant. Measured at 12 WAT, differences were still significantly higher, with treated hydrilla averaging 33 percent more chlorophyll. Other researchers have speculated that an increase in chlorophyll, as a result of flurprimidol treatment, can be attributed to a decrease in leaf surface area containing an amount of chlorophyll similar to that in untreated leaves (Netherland and Lembi 1992).

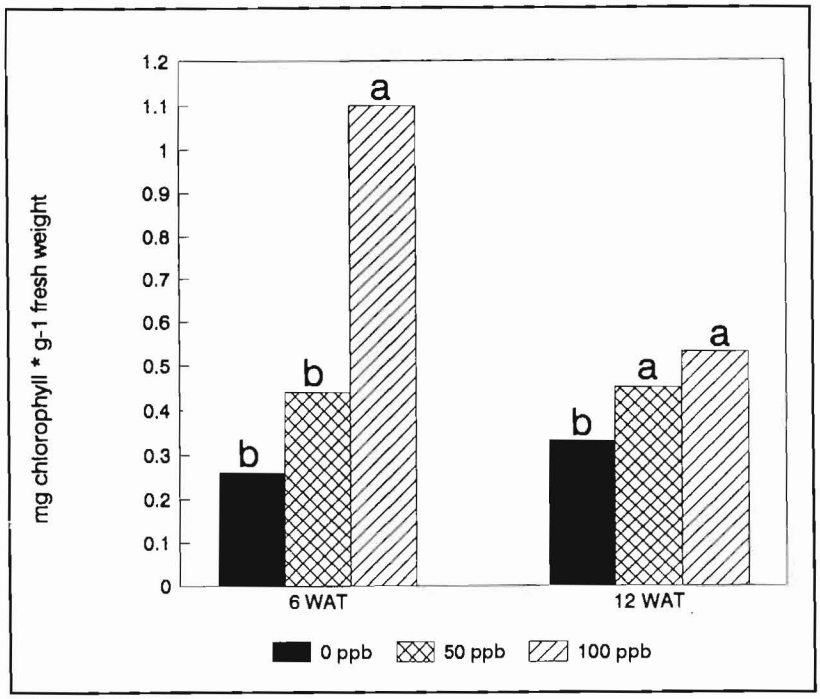

Figure 6. Effect of flurprimidol on chlorophyll content of hydrilla 6 and 12 weeks after treatment. Data are means of three observations. Letters denote significant differences at P = 0.05

Effects on photosynthesis and respiration were also measured on hydrilla at 6 WAT (Figures 7 and 8). Net photosynthesis averaged 58 percent higher with flurprimidol treatment. Respiration rates also increased, but results were not statistically significant. In general, the overall health and physiological competence of shortened hydrilla was not adversely affected by flurprimidol during this study.

In summary, the results of this study have provided us with important information about flurprimidol and its effectiveness on hydrilla, milfoil, wild celery, and pondweed under experimental conditions that more closely mimic a field situation. We observed that flurprimidol is an effective growth inhibitor of hydrilla. Plant height was greatly reduced with no apparent negative effects on physiological processes, which suggests that shortened plants would function as useful components in an aquatic ecosystem. There also was evidence that flurprimidol can inhibit tuber production in hydrilla; however, further studies monitoring flurprimidol effects on tuber production throughout an entire growing season are needed. Results on milfoil were difficult to interpret because of poor plant establishment. Our results suggest that milfoil is not as sensitive to flurprimidol as hydrilla, which is inconsistent with bioassay results reported by Netherland and Lembi (1992). We also observed that flurprimidol had little or no effect on pondweed and wild celery at rates sufficient to inhibit undesirable species, such as hydrilla.

Future Work

Future studies to be conducted under Work Unit 32578, Plant Growth Regulators for Aquatic Plant Management, include the following:

- Continue flurprimidol testing on hydrilla and milfoil to verify dose/exposure time relationships previously established in laboratory studies and to determine whether application techniques, such as sequential applications, can maximize chemical efficacy.

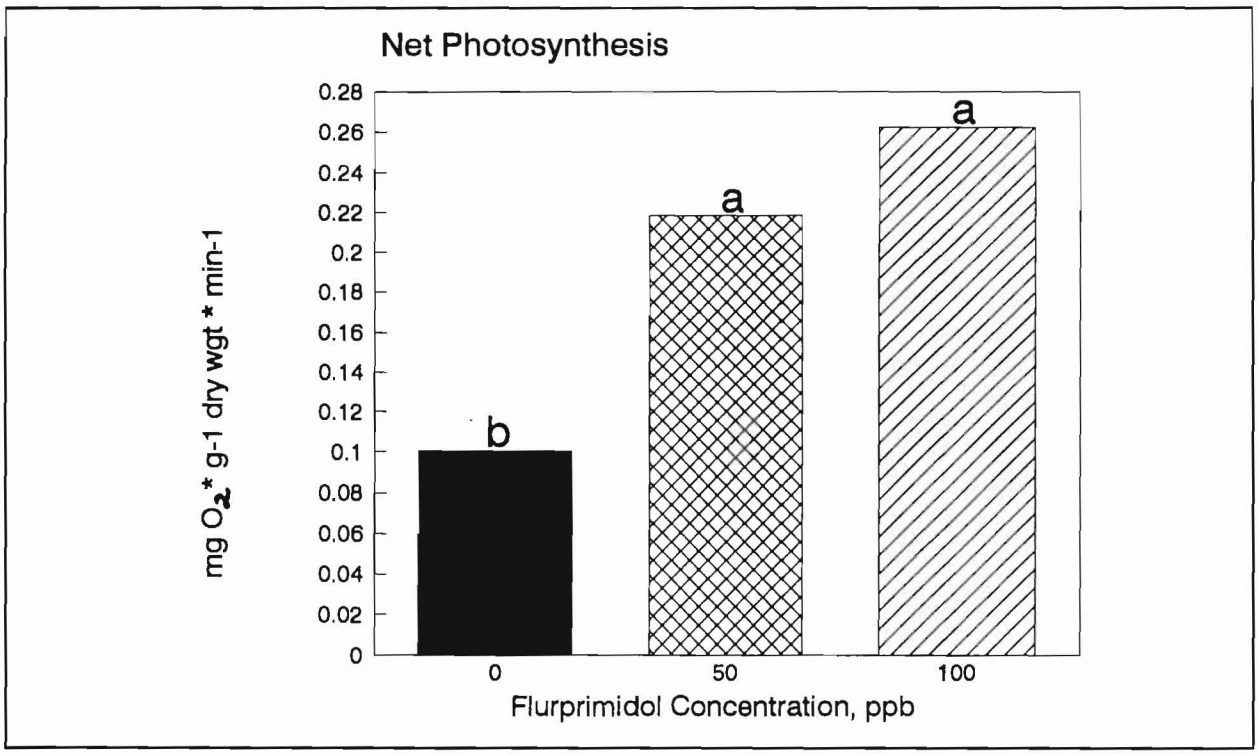

Figure 7. Effect of flurprimidol on net photosynthesis of hydrilla 6 weeks after treatment. Data are means of three observations. Letters denote significant differences at P = 0.05

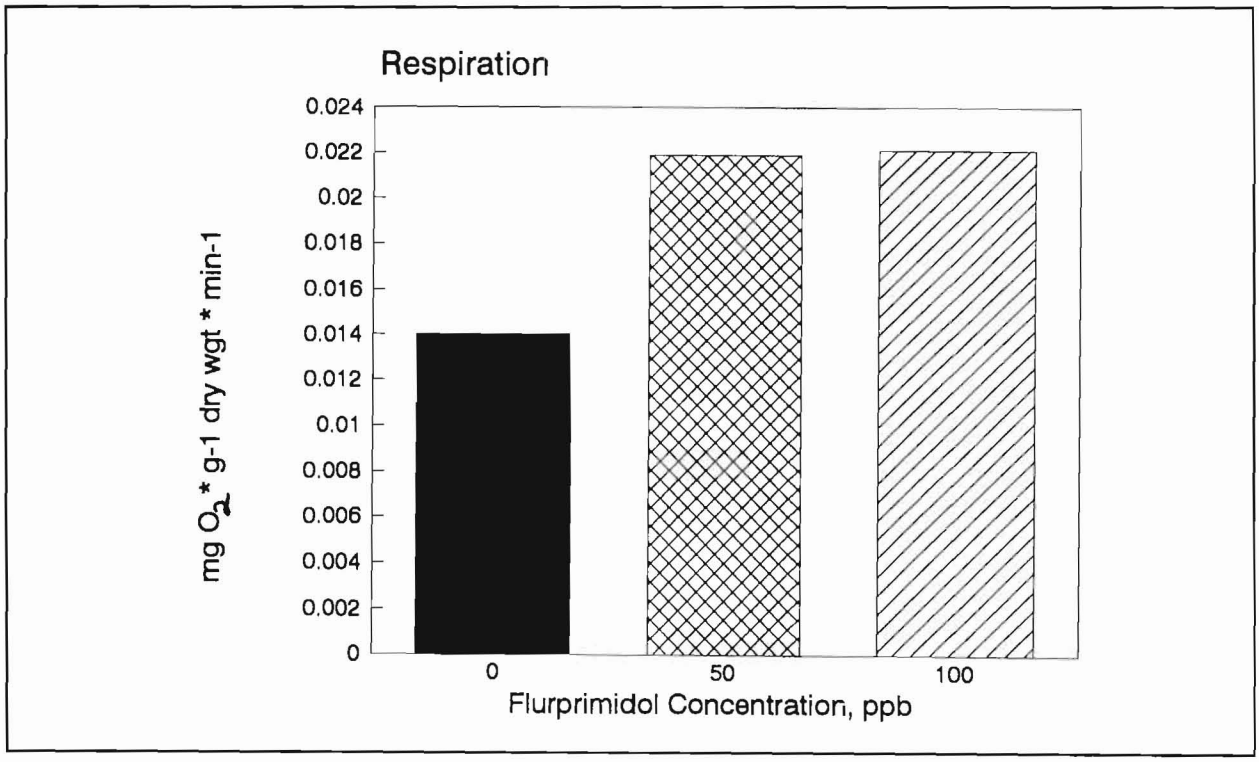

Figure 8. Effect of flurprimidol on respiration of hydrilla 6 weeks after treatment. Data are means of three observations. Letters denote significant differences at P = 0.05

- Further evaluation of flurprimidol effects on hydrilla tuber production. Results from this study indicate that flurprimidol can reduce tuber production. Since overwintering tubers are the main source of hydrilla reinfestation in a water body, reduced tuber formation could significantly affect subsequent populations.

- Initiate studies to further characterize flurprimidol selectivity on aquatic plant species. Results of this study show that flurprimidol is selective, affecting some species and not others. This information will be useful in developing management strategies to control weedy species while minimizing impacts on nontarget, desirable species.

Acknowledgments

The author would like to thank the staff at the LAERF, especially Drs. Gary Dick and Mike Smart, and Mr. Mike Crouch for their valuable assistance in conducting this study.

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Impact of Plant Growth Regulators on Submersed Plant Species and the Environment

by
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Introduction

For the past several years, we have been studying the use of plant growth regulators as a potential tool in aquatic plant management. Rather than kill submersed aquatic plants with herbicides, our goal is to reduce their height. Height-reduction renders the plants "non-weedy" and yet allows them to remain viable and functional in the aquatic environment, i.e., to provide oxygen, habitat, and sediment stabilization.

Much of our research has concentrated on the gibberellin synthesis inhibitors (GSIs). These compounds inhibit the synthesis of the naturally occurring plant hormone gibberellin. Gibberellin is responsible for stem elongation; therefore, the application of GSIs should reduce overall plant height. The three GSIs that we have worked on are flurprimidol, uniconazole, and paclobutrazol.

During the project period 1989-1992, the major objectives of our research were as follows:

a. Test (using a bioassay system) compounds other than gibberellin synthesis inhibitors for growth regulating properties.

b. Determine the response of target species (*Hydrilla verticillata* and *Myriophyllum spicatum*) to flurprimidol dosage and exposure times.

c. Develop procedures to detect flurprimidol in water, plants, and soil.

d. Study dissipation characteristics of flurprimidol in the environment.

e. Determine the effect of flurprimidol on submersed species other than *H. verticillata* and *M. spicatum.*

f. Determine the effect of light on flurprimidol activity.

This report will provide specific information on Objectives *e* and *f*. The results of research conducted on Objectives *a-d* (reported in previous Proceedings) are summarized as follows:

Non-GSI compounds tested using a laboratory bioassay system with *H. verticillata* and *M. spicatum* were bensulfuron methyl, imazapyr, triclopyr, and amidochlor. Of these compounds, bensulfuron methyl and amidochlor showed the greatest potential to reduce plant height. Main stem length was reduced in *M. spicatum* with 6 to 100 ppb (all concentrations in active ingredient) bensulfuron methyl and 250 to 1,000 ppb amidochlor. Some adverse physiological effects were noted with bensulfuron methyl at these concentrations, which may be reflective of the inconsistency observed with this compound in the field. No adverse effects were noted with amidochlor up to 1,000 ppb, and continued experimentation with this compound is suggested. Imazapyr and triclopyr were herbicidal with extremely limited growth regulating properties.

In small scale field tests using 67-L barrels, we found that flurprimidol could significantly reduce main stem length for 28 days in *H. verticillata* treated at 75 ppb and *M. spicatum* at 200 ppb after only a 2-hr exposure (Lembi and Chand 1992). These results suggested that at least one GSI can be taken up relatively

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rapidly under simulated field conditions. We also developed the techniques for extracting and detecting flurprimidol in water, sediment, and submersed plant tissue using gas chromatography (Chand and Lembi 1991). Water, plant tissue, and sediment analyses showed flurprimidol half-lives of approximately 9.1, 9.9, and 178 days, respectively. Although the compound appeared to dissipate rapidly from the plant tissue, minute amounts were still present in height-reduced *M. spicatum* plants 28 days after a 2-hr treatment. The threshold level for effective stem reduction in *M. spicatum* appears to be approximately 3 to 5 ng flurprimidol/gram fresh weight. The latter information may be helpful during field research to determine whether active concentrations of the compound are still present following treatment.

Objectives *e* and *f*, described here in more detail, were undertaken for the following reasons. In Objective *e*, we wanted to know whether GSIs effectively reduce height in target species other than *H. verticillata* and *M. spicatum*. In addition, the effects of these compounds on nontarget, native species must be determined to help evaluate their impact on the total aquatic ecosystem. Objective *f* relates to the effect of low light on GSI activity. It is generally acknowledged that the synthesis and/or activity of gibberellin under low light results in greater rates of stem elongation than under high light. In aquatic situations, submersed plants are often subjected to low light conditions because of turbidity or to light attenuation with increasing depth. The question that must be asked in regard to GSI activity is whether these compounds can overcome the increase in gibberellin synthesis/activity under low light conditions.

Materials and Methods

Survey of submersed species

Metal barrels (67-L capacity) were lined with plastic liners and set in an unshaded outdoor area. Loam soil (free from plant growth regulators, herbicides, and other pesticides) was added to a 10-cm depth in each barrel. Approximately 55 L of well water was added, and the suspended soil was allowed to settle. Submersed plant species were collected from Lakes Wawasee, Shook, and Backwater in Kosciosko County, Indiana. Three stem apices (approximately 7-cm length) of a species, without roots, were planted per barrel. Up to three species were planted per barrel. The plants were allowed to acclimate for at least 10 days prior to flurprimidol treatment. Flurprimidol (50 percent WP, Elanco Products Company, Indianapolis, IN) was applied by diluting the compound in approximately 10 ml of water and then stirring the solution into the barrel, without disturbing the soil, to ensure even dispersal.

Four experiments were initiated during the summer of 1992. In the first two (treatment dates 23 May and 6 June), the barrels were treated with 0, 75, and 200 ppb flurprimidol. Three days after treatment, the water in both control and treated barrels was siphoned off and replaced with fresh, untreated water. The barrels were harvested 4 weeks after the initial treatment. In the third and fourth experiments (treatment dates 26 July and 1 August), the barrels were also treated with 0, 75, and 200 ppb flurprimidol, but the water was not removed. The plants were left in treated water over the 4-week period prior to harvest.

Plant length measurements were taken using a centimeter ruler. Fresh weights were also taken. Each treatment consisted of three replicate barrels. Statistical analysis consisted of analysis of variance and separation of means using the Student-Newman-Keuls test. Significance was set at $P < 0.05$.

Light studies

Six-centimeter stem tip sections from algal-free cultures of *H. verticillata* and *M. spicatum* (Netherland and Lembi 1992) were planted into a loam soil in styrofoam cups (one stem tip per cup). A small amount of a controlled release fertilizer was added to the soil prior to planting. A thin layer of sand was placed over the soil to prevent sediment dispersion when placed in water. Mayonnaise jars (3.5 L)

were filled with Smart and Barko medium (1985), and the cups with plants were placed in the jars (one cup with *H. verticillata* and one cup with *M. spicatum* per jar). A layer of plastic wrap was placed over the mouth of the jar to prevent excessive water loss. The jars were placed on a greenhouse bench. Experiments were conducted in July 1992. Ambient light at mid-day on bright sunny days was 800 to 1,000 $\mu E\ m^{-2}\ sec^{-1}$. One set of jars was placed under ambient light conditions; a second set of jars was placed under nylon shade cloth and cheesecloth to obtain an irradiance of 4 to 18 $\mu E\ m^{-2}\ sec^{-1}$ at mid-day. Light measurements were made with a LI-COR meter with a spherical bulb. Two fans were directed toward the jars to prevent excessively high temperatures. Water temperatures during the course of the experiment averaged 25 °C and never rose above 30 °C.

Since the plants had been growing in culture chambers under relatively low light conditions (400 $\mu E\ m^{-2}\ sec^{-1}$), they were allowed to acclimate to the higher light in the greenhouse in the following way: four layers of cheesecloth were placed over all jars for 2 days; this was followed by one layer of cheesecloth for 1 day. The next day, all plants were treated and placed under the high or low light conditions described above. Treatments were 0, 7.5, 75, and 750 ppb flurprimidol (50 percent WP, Elanco Products Company, Indianapolis). Each light and concentration treatment consisted of three replicates. The experiment was repeated. Both experiments showed the same trends, but only the data from one experiment is reported here. Main stem lengths were measured 7 days after treatment of high light plants and 10 days after treatment of low light plants.

Results

Survey of submersed species

The species that were tested using the 3-day exposures were *Elodea canadensis, Sagittaria graminea* (23 May treatment), *Potamogeton nodosus*, and *Ceratophyllum demersum* (6 June treatment). The plants did not grow very well. In part, this had to be due to the unusually cold, overcast conditions during the early portion of the summer. Untreated control main stem lengths were (mean ± SE) 12.6 ± 1.2 cm for *S. graminea* (leaf length), 11.0 ± 3.0 cm for *P. nodosus*, and 19.1 ± 3.5 cm for *C. demersum*. *Elodea canadensis* initially grew as horizontal runners. Lateral vertical stems on this plant measured only 4.2 ± 0.4 cm.

The plant that showed the most marked response to flurprimidol under these conditions was *P. nodosus* with a stem length reduction of 43 percent at 200 ppb. Even though main stem length was reduced, the plants were "larger" than untreated plants. Individual treated plants had significantly more submersed leaves at 200 ppb (25 versus 11) and more upright stems (8 versus 4). Although not statistically significant, these treated plants had longer rhizomes (70 cm versus 41 cm) and greater fresh weights (5.7 ± 2.1 g versus 2.7 ± 0.9 g) than untreated plants.

Although *E. canadensis* and *S. graminea* plants showed a trend toward length reduction at 75 and 750 ppb, there were no significant differences between treated and untreated plants (data not shown). There was no tendency toward main stem length reduction in *C. demersum*. In fact, the treated plants were slightly longer (21 cm) than control plants (19 cm). However, the number of lateral branches and fresh weights were lower in treated (7.3, 2.5 g, respectively at 200 ppb) than untreated (22, 6.1 g, respectively) plants.

The species that were tested with 4-week exposures were *Vallisneria americana, Heteranthera dubia, Najas flexilis* (26 July treatment), *E. canadensis, C. demersum*, and *Potamogeton foliosus* (1 August treatment). All control plants grew somewhat better than did those earlier in the summer.

The main stem lengths of *H. dubia, N. flexilis, E. canadensis*, and *P. foliosus* were significantly reduced at 75 and 200 $\mu g\ L^{-1}$ (Table 1). Results on main stem lengths of *C. demersum* were similar to those of the short-term exposure tests. Although there

was a tendency toward stem length reduction, the differences between treated and untreated plants were not significant. Unfortunately, we did not measure number of lateral stems or fresh weight of *C. demersum* in this test. The leaf length of *V. americana* also was not reduced at any concentration (Table 1).

Table 1
Main Stem Length of Submersed Plants Treated with Fluprimidol in Outdoor Barrels for 4 Weeks

Plants	Main Stem Length, cm, for Indicated Concentration of Flurprimidol, ppb		
	0	75	200
Vallisneria americana	40.2 (11.4)a	36.5 (0.9)a	33.9 (3.4)a
Heteranthera dubia	34.6 (4.1)a	18.7 (1.1)b	10.6 (4.5)b
Najas flexilis	26.6 (1.7)a	12.2 (2.1)b	13.8 (1.2)b
Elodea canadensis	16.6 (3.4)a	7.8 (0.8)b	7.3 (1.6)a
Ceratophyllum demersum	28.9 (2.6)a	20.1 (4.5)a	20.1 (4.5)a
Potamogeton foliosus	15.6 (0.9)a	5.7 (1.1)b	4.5 (0.4)b

Note: Means (± SE) within a row with same letters are not significantly different at the 0.05 level.

Light studies

Although the number of internodes of low light and high light control (0 ppb) plants was similar (17 to 19 in *M. spicatum*; 30 to 35 in *H. verticillata*), the length of the internodes differed. At high light, mean internode length was 0.53 cm in *M. spicatum* and 0.39 cm in *H. verticillata*. At low light, mean internode length was at least twice as long: 1.2 cm in *M. spicatum* and 1.6 cm in *H. verticillata*. The increase in internode length resulted in control main stem lengths that were 55 and 73 percent longer on low light *M. spicatum* and *H. verticillata* plants, respectively, compared with the high light plants (Figure 1).

Flurprimidol treatment of high light plants did not result in statistically significant differences in main stem length (Figure 1). Although the control plants were slightly longer than the treated plants, the stem length inhibition normally observed in treated plants probably did not have a chance to develop because of the short 7-day exposure times.

Main stem lengths of all treated low light plants of both *M. spicatum* and *H. verticillata* were statistically reduced ($P < 0.05$) compared with the low light control plants (Figure 1). In *M. spicatum*, main stem lengths of treated low light and high light plants did not differ. In *H. verticillata*, treated low light plants were longer than the high light plants treated with the same flurprimidol concentrations but were, as just noted, reduced when compared with the untreated controls.

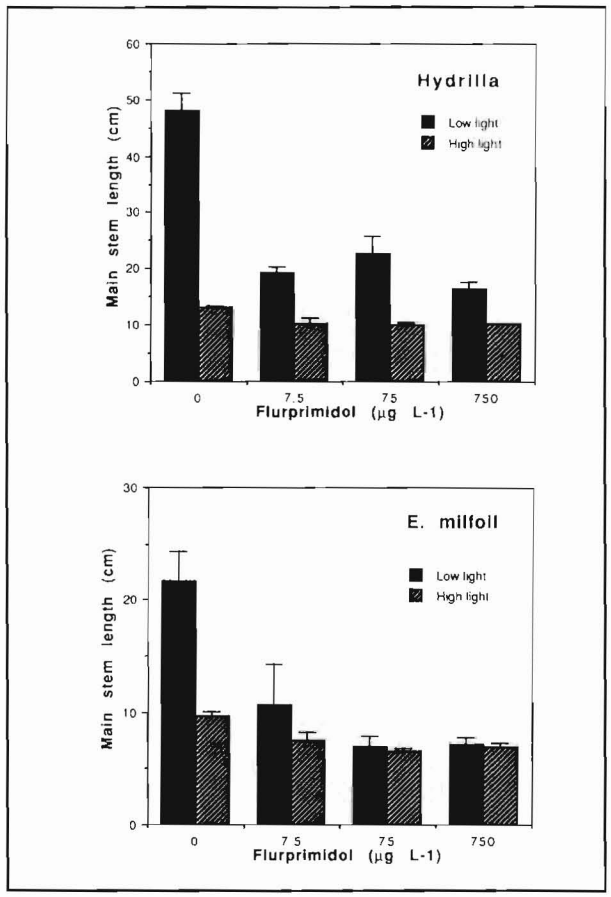

Figure 1. Effect of flurprimidol on main stem lengths of Myriophyllum spicatum and Hydrilla verticillata grown under high light (800 to 1,000 µE m^{-2} sec^{-1}) and low light (8 to 14 µE m^{-2} sec^{-1}) conditions (µg L^{-1} = ppb)

Discussion

Survey of submersed species

Better growing conditions are needed to determine whether short-term exposures will reduce main stem lengths in the species tested as they do in *H. verticillata* and *M. spicatum* (Lembi and Chand 1992). Although the results of these tests, including the 4-week exposure experiments, are preliminary, taken in total, they do suggest that a broad spectrum of aquatic plant species is sensitive to flurprimidol. The only plants that did not seem to be affected were *V. americana* (leaf length) and *C. demersum* (main stem length), although the overall growth of *C. demersum* plants may have been reduced. The minimal effect of flurprimidol on *V. americana* could be advantageous since this plant is considered to be a valuable habitat species.

Light studies

The results indicate that flurprimidol even at a low concentration of 7.5 ppb is, at least initially, effective in reducing main stem elongation under low light conditions in both *M. spicatum* and *H. verticillata*. Although all treatments of *H. verticillata* resulted in stem lengths that were longer than their respective high light treatments and high light controls, the treated plants were still reduced in height compared with the untreated low light controls.

The ability of GSIs to effectively reduce plant height under low light conditions will require further testing using different inhibitor exposure times and concentrations. Short-term exposures to the inhibitor under low light may result in the release of main stem elongation once the inhibitor concentration has decreased below the threshold level required to inhibit the synthesis and/or activity of gibberellin. However, even if some elongation under low light does occur, once the plant reaches the more well-lit portions of the water column, the GSI effect should be more pronounced along the upper stem portions.

Future Research

Sufficient background research has now been conducted on the effects of GSIs on *H. verticillata* and *M. spicatum*. The results, which have been positive to date, suggest scaling up to larger systems such as the mesocosms and ponds at the Lewisville facility. In addition, we would like to study the uptake characteristics of ^{14}C-flurprimidol, using a laboratory/greenhouse system. The results will tell us whether the primary route of entry is from the water to the foliage or from the sediment through the roots. Such information is needed to determine the best delivery system for the compound. Furthermore, uptake of the compound from the sediment suggests the potential for long-term control since flurprimidol has a relatively long residence time in soil. A strictly water/foliage uptake route suggests shorter control periods, particularly in flowing waters.

Acknowledgments

We thank the U.S. Army Engineer Waterways Experiment Station and its personnel for supporting this research. In particular, we thank Drs. Kurt Getsinger and Howard Westerdahl for their encouragement and help during the term of this project.

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Field Evaluation of Triclopyr: One-Year Posttreatment

by
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Introduction

The systemic herbicide triclopyr (3,5,6-trichloro-2-pyridinyloxy-acetic acid) has been used to manage broadleaf weeds in forest, industrial, and other noncrop terrestrial sites for nearly 20 years. Manufactured by DowElanco, the triethylamine salt formulation of the herbicide (Garlon 3A) is currently registered for aquatic sites under a Federal Experimental Use Permit.

Results from evaluations conducted at the U.S. Army Engineer Waterways Experiment Station (WES) have demonstrated that triclopyr can provide excellent control of the submersed plant Eurasian watermilfoil (hereafter called milfoil) at concentrations ranging from 0.25 to 2.5 mg acid equivalent (ae)/L when exposed for periods of from 18 to 72 hr (Netherland and Getsinger 1992). In an effort to verify results from these laboratory studies and to evaluate the efficacy of triclopyr on nontarget submersed plants, WES researchers have been applying triclopyr to milfoil-dominated plant communities in Washington and Alabama. Results from these field studies will be used to provide guidance for the application of triclopyr to aquatic systems.

The objectives of these studies were as follows: (a) determine the dissipation of triclopyr from the application sites, (b) correlate the dissipation of rhodamine WT dye with the dissipation of triclopyr, (c) evaluate the efficacy of triclopyr against milfoil, and (d) evaluate the species-selective properties of triclopyr. This article provides a posttreatment update on some aspects of the triclopyr field study conducted on the Pend Oreille River, WA.

Materials and Methods

Three plots were selected for the triclopyr study in milfoil-dominated communities of the Pend Oreille River (Figures 1 and 2). A tank mix of triclopyr (Garlon 3A) and rhodamine WT dye was applied to a 6-ha river plot (2.5 mg ae/L triclopyr + 10 µg/L dye) and a 4-ha cove plot (50 percent of plot, 2.5 mg ae/L triclopyr + 10 µg/L dye; 50 percent of plot, 1 mg ae/L triclopyr + 4 µg/L dye) in August 1991. A 3-ha river plot, located upstream from the treatment sites, was used as an untreated reference site. These treatment sites and rates were selected based on information obtained from previous water-exchange studies (Getsinger et al. 1991). Detailed descriptions of the study plots, treatment rates, application techniques, and dye, herbicide, and biomass sampling protocols are provided in Getsinger, Turner, and Madsen (1992).

Results

Analysis of posttreatment data showed that triclopyr provided excellent control of milfoil in both the river and cove plots. Milfoil and native species biomass is given in Table 1. Compared with pretreatment levels, milfoil biomass had declined 99 percent in both treated plots 4 weeks after treatment, yet was virtually unchanged in the reference plot. By 52 weeks posttreatment, milfoil biomass had recovered to 28 percent of pretreatment levels in the river plot, but was still extremely low (<1 percent of pretreatment) in the cove plot. Milfoil in the reference plot showed 170 percent greater

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
[2] AScI Corporation, Vicksburg, MS.
[3] Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

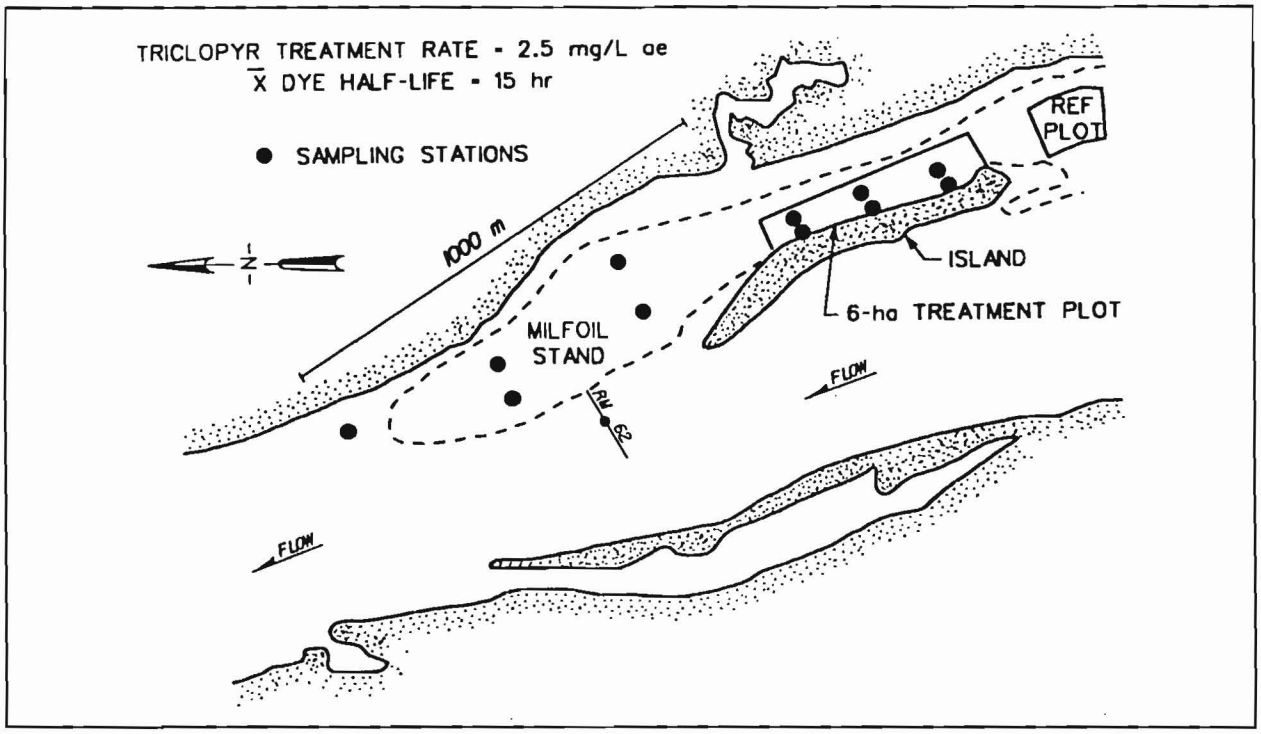

Figure 1. Pend Oreille River dye/triclopyr treatment and reference plots, August 1991

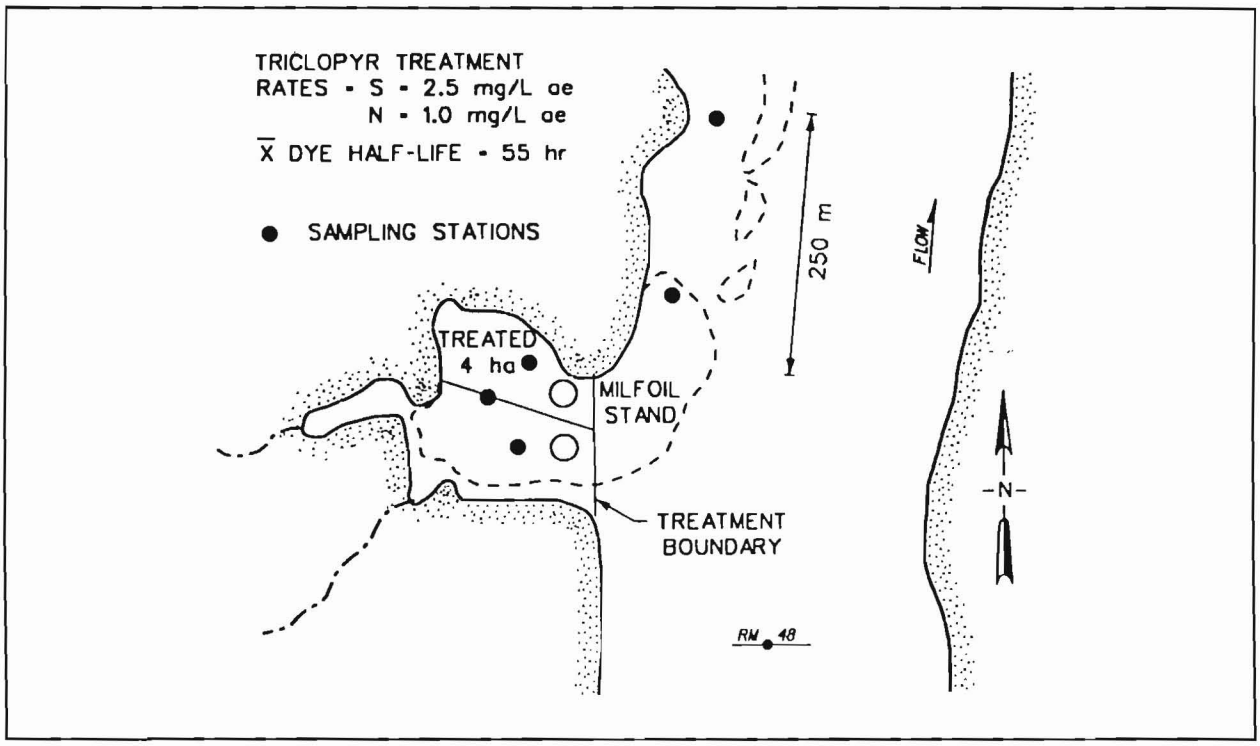

Figure 2. Lost Creek Cove dye/triclopyr treatment plot, August 1991

Table 1
Mean Pretreatment and Posttreatment Plant Biomass Following Triclopyr Applications on the Pend Oreille River, WA, 1991

	Mean Shoot Mass (g DW/m^2)					
	Eurasian Watermilfoil			Native Species		
Plot	Pre	4-wk	52-wk	Pre	4-wk	52-wk
Reference	291	281	498	12	5	17
River	254	3	72	39	18	433
Cove	257	2	2	38	9	200

biomass at 1-year posttreatment compared with the pretreatment biomass measurement.

Although native plant species (elodea, pondweeds, coontail, etc.) had declined slightly at 4 weeks after triclopyr treatment, they showed a 5- to 10-fold increase in biomass in the treated plots by the 52-week sampling period. This release and growth flush of natives was not evident in the untreated reference plot, which was still dominated by milfoil at 1-year posttreatment.

Triclopyr water residues measured at stations downstream from the river and cove plots are presented in Table 2. At the river application site, residues were near or less than the U.S. Environmental Protection Agency potable water tolerance of 0.5 mg/L at 72-hr posttreatment at the 300-m downstream station, and below the tolerance level at 8-hr posttreatment at the 650- and 1,000-m downstream stations. At the cove site, residues were <0.5 mg/L at 8-hr posttreatment at both the 150- and 400-m downstream stations.

Triclopyr dissipation half-lives were 7 hr for the river plot (72-percent milfoil control at 1-yr posttreatment) and 55 hr for the cove plot (99-percent milfoil control at 1-yr posttreatment). This information documents the importance of triclopyr contact time with respect to milfoil efficacy, as demonstrated in laboratory evaluations. The correlation between rhodamine WT and triclopyr dissipation was good in the river plot ($r^2 = 0.80$) and excellent in the cove plot ($r^2 = 0.95$). This

Table 2
Triclopyr Water Residues from Downstream Locations Following Herbicide Application, Pend Oreille River, WA, 1991

Distance Downstream m	Time Posttreatment hr	Triclopyr mg/L
River Plot		
300	8	0.55
	24	1.20
	72	0.57
	168	0.06
650	8	0.07
	24	0.47
	72	0.15
	168	<0.01
1,000	8	0.02
	12	<0.01
Cove Plot		
150	8	0.28
	24	0.02
	48	<0.01
400	8	0.32
	24	0.04
	48	<0.01

correlation suggests that rhodamine WT can effectively simulate the dissipation of triclopyr in water over short periods of time (1 to 7 days).

Summary and Future Work

Results from the Pend Oreille River field study illustrate the effectiveness of triclopyr as a selective tool for the control of milfoil and provide additional insight into concentration/exposure time relationships for the use of that herbicide in flowing-water systems. Researchers at WES will continue to evaluate triclopyr and other promising aquatic herbicides in the field, emphasizing the use of water-exchange information to improve control of nuisance submersed plants.

Acknowledgments

Technical assistance and field support for the Pend Oreille study were provided by the Seattle District, the Libby-Albeni Falls Project Office, the Washington Department of Ecology, the Pend Oreille County Noxious Weed Control Board, Resource Management, and DowElanco.

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Ecology of Aquatic Plants

Ecology of Submersed Aquatic Macrophytes (Overview 1992): Advances, Management Implications, and Related Needs

by
John W. Barko[1]

Introduction

Research within the ecological technology area of the Aquatic Plant Control Research Program (APCRP) is directed towards determining the response of submersed aquatic macrophytes to a wide array of environmental conditions. The role of aquatic macrophytes in affecting environmental conditions is also being addressed. A variety of factors (light, water temperature, nutrition, and sediment composition) interact to determine the productivity, distribution, and species composition of submersed macrophyte communities. In addition, interspecific interactions, including herbivory and competition, are important in structuring aquatic macrophyte communities. Ecological research within the APCRP is designed to address interactions among these factors. Most recent studies focus on mechanisms whereby submersed macrophyte communities influence their environment. This article summarizes results of recent studies, advances in our understanding of the ecology of submersed macrophytes, and management implications and research needs.

Macrophyte Interactions with Sediment Composition

Sediment provides the most important source of supply for nitrogen (N), phosphorus (P), and micronutrients to submersed macrophytes. Thus, it is important to evaluate the effects of macrophytes growth on sediment nutrient availability. Evidence from field studies suggests that rooted submersed macrophytes, even with relatively diminutive root systems, are capable of markedly depleting N and P in sediments. High productivity and biomass turnover of rapidly growing macrophyte species, e.g., *Hydrilla verticillata* and *Myriophyllum spicatum*, can result in high rates of sediment nutrient loss. Thus, even in fertile systems, nutrient uptake by aquatic macrophytes may significantly reduce sediment nutrient availability. In general, it appears that N is depleted from sediments to a much greater extent than P (relative to macrophyte nutritional needs). Thus, N is generally more likely than P to limit macrophyte growth.

Pools of sediment nutrients available for plant uptake are regenerated by (a) sedimentation, which provides new sediment material, (b) biological mixing of sediments, which transports nutrients in and out of the root zone, and (c) mineralization, which transforms nutrients into soluble forms that macrophytes can utilize (Figure 1). Macrophytes can influence the rate and extent of each of these processes to either increase the rate of nutrient resupply or slow it. Once sediment nutrient pools have been depleted, growth limitation will occur unless nutrient regeneration meets or exceeds plant requirements. Thus, nutrient regeneration processes are crucial to long-term maintenance of macrophyte productivity.

As important participants in nutrient regeneration processes, microorganisms and benthic invertebrates make available in newly accreted sediments a variety of elements important to macrophyte nutrition. These microbial and invertebrate-mediated processes need to be examined in attempts to identify novel management approaches that reduce the

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availability of sediment nutrients to submersed macrophytes.

Macrophyte Invasions and Declines

An important characteristic common to weedy aquatic plant species is an ability to aggressively invade new habitats, particularly where these habitats are devoid of potential competitors. Following invasion, these species often spread extremely rapidly, mainly because of very effective and rapid means of dispersal (e.g., autofragmentation in *Myriophyllum spicatum*). Differences in invasion success affected by environmental conditions or plant competition are poorly understood. Thus, it is currently very difficult to predict where and to what extent invading species will reach problem levels. Information on environmental requirements and tolerances of different submersed macrophyte species (obtained to date in the APCRP), in combination with results of ongoing plant competition studies, is being used to provide a better basis for predicting macrophyte invasion success in different regions and in different types of aquatic systems nationwide.

Another characteristic common to weedy aquatic plant species is their tendency to decline after several years of dominance. As with invasions, declines are poorly understood, particularly in mechanistic terms. Possible causes that have been suggested for declines include the following: sediment nutrient depletion, toxin accumulation, shading, parasites and pathogens, climatic fluctuations, macrophyte competition, insect herbivory, and reproductive failure. In reality, it is probable that a variety of these and other factors in combination result in macrophyte declines.

Whatever causes the successful establishments and declines of invading species, some evidence suggests that environmental disturbances associated with macrophyte control may prolong the persistence and/or dominance of the invaders. Thus, recently initiated studies of macrophyte invasions and declines focus attention on the influence of aquatic plant control measures as well as other factors (listed above). Although disturbance appears to favor invasion by aggressive species, it is not clear why some control practices would result in their persistence.

Macrophyte Influences on the Environment

Considerable work in the APCRP has clearly shown marked effects of submersed macrophytes on water quality and habitat. Sedimentation rates are generally much greater in macrophyte beds than in the open water of aquatic systems because of the filtering capacity of submersed vegetation. By reducing turbulence, aquatic macrophytes also serve an important role in sediment stabilization. As suspended sediment is removed from the water column, light penetration increases—providing more light for photosynthesis by submersed macrophytes and their attendant periphyton communities. Both pH and dissolved oxygen are elevated in macrophyte beds during periods of photosynthesis. Elevated values of pH near the sediment surface tend to increase rates of phosphorus release from sediments, which can stimulate algal production. Algal production on macrophyte leaf surfaces provides an excellent source of food for grazing invertebrate communities, which in turn provide a source of

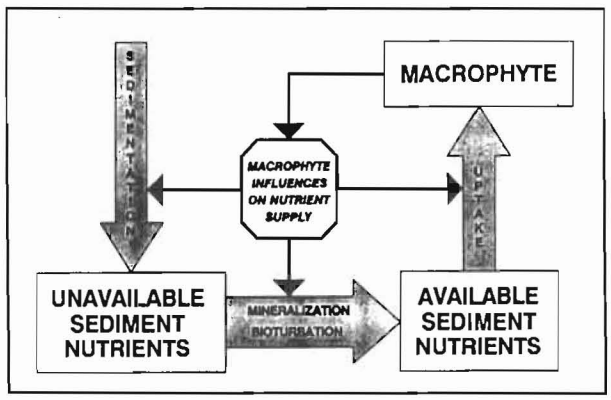

Figure 1. Conceptual model of processes influencing the supply of sediment nutrients to macrophytes

food to fish communities. In a similar manner, detritus from the senescence of macrophytes

contributes to the productivity of benthic invertebrate communities and fish. The productivity of littoral fish communities appears then to be tightly coupled with submersed macrophyte productivity.

Fish communities of aquatic systems are also influenced by the structural complexity of submersed macrophyte beds. These beds provide refugia for smaller fish, and are thus important in predator-prey relationships. The geometry of macrophyte beds varies with plant species composition and abundance. Little is known, however, concerning the exact influences of bed geometry on fish communities. Macrophyte control programs can potentially influence bed geometry as well as biomass distribution in aquatic systems. Thus, it is important to begin considering aquatic plant management within the context of fisheries management.

On a daily basis, shallow littoral regions of aquatic systems heat and cool more rapidly than adjacent open-water regions, partly because of differences in mixed volume. Submersed aquatic macrophytes contribute significantly to the development of resulting thermal gradients, which promote convective hydraulic circulation. Effects of convective hydraulic circulation are potentially far-reaching, since this mechanism operates daily and can account for massive transport of dissolved constituents, including nutrients and herbicides, between littoral and open-water regions of lakes and reservoirs. For example, hydraulic transport affected by convection accounted for about 26 percent of the internal load of phosphorus in Eau Galle Reservoir in Wisconsin. Continuing studies of internal nutrient loading in this reservoir indicate important effects of convective circulation on phytoplankton dynamics. Tennessee Valley Authority-sponsored studies, recently initiated in the much larger and hydrologically more complex Guntersville Reservoir in Alabama, are providing information on the relative importance of convective transport in comparison with other mechanisms of transport, including gravity flow, wind-driven water displacement, and internal seiche activity.

Management Implications and Related Needs

Over geological time, the filling of lake basins drives vegetative change in aquatic systems. However, shorter time scales are more relevant to many important management questions. Invasions of nuisance macrophytes, for example, often have cycles of a decade or so. Short-term compositional changes in aquatic macrophyte communities remain largely unexplained. Macrophyte-sediment interactions have the potential to exert strong influences at both population and community levels. Thus, a better understanding of these complex interactions should be useful in explaining short-term compositional changes, including species invasions and declines.

As described above, the sustained vigor of submersed macrophyte communities requires a balance between nutrient uptake and regeneration. Processes affecting this balance need to be considered explicitly within the context of macrophyte management. Inorganic sedimentation is frequently accelerated by human activities in the watershed; and for unknown reasons, aquatic systems affected by these disturbances are often most susceptible to the invasion and subsequent explosive growth of introduced macrophyte species.

Nitrogen is a key element for the growth of rooted macrophytes. Thus, advances in our understanding of factors regulating sediment N availability are a prerequisite to the development of management approaches based on reductions in sediment nutrient availability. Towards this end, the role of submersed macrophytes in the N economy of aquatic systems needs to be investigated. A variety of physical, chemical, and biological processes (e.g., sedimentation, mineralization, and particulate movement by benthic invertebrates) that potentially contribute to sediment N availability need to be evaluated within the context of macrophyte nutrition.

Laboratory studies need to be continued to improve our understanding of sediment nutrient availability. The feasibility of lessening

nitrogen availability to macrophytes by interfering with naturally occurring chemical and biological processes, thus retarding the growth of nuisance species, needs to be investigated. As an extension of this effort, the possibility of perpetuating reductions in nitrogen availability to nuisance species by interplanting preferred native macrophyte species also needs to be examined.

Studies of hydraulic transport in macrophyte beds are of great value in providing information on rates and volumes of water being exchanged with the open water of aquatic systems. This information needs to be applied in estimating herbicide transport and dilution rates following chemical treatment. Decisions on herbicide type, formulation, concentration, and time/season of application can also benefit. Information on littoral-pelagic nutrient fluxes needs to be expanded in assessing direct effects (i.e., through uptake) and indirect effects (i.e., through reconfigured thermal structure) of macrophyte stands on water quality. Interactions between macrophytes and phytoplankton in aquatic systems need to be examined more fully through consideration of littoral-pelagic hydraulic interactions.

Acknowledgments

Thanks to Drs. David Soballe and Craig Smith for providing helpful reviews of this text. The information highlighted and summarized herein is derived from the efforts of a large number of investigators who have over the years contributed substantially to progress in the Ecological Technology Area in the APCRP.

Effects of Light on *Vallisneria* Growth in Relation to Sediment

by
Anne Kimber[1]

Introduction

Vallisneria americana Michx. has been the dominant submersed aquatic macrophyte in backwaters of the Upper Mississippi River. After the 1988 drought, *Vallisneria* declined in Pools 4 through 19 of the river; and although it is currently abundant in Pool 4, it has not reestablished in most pools. Its loss from Pool 7, Lake Onalaska, has been of particular concern because the lake is a major staging ground for waterfowl during fall migration.

Interactions of several factors probably contributed to the decline; the working hypothesis tested here was that the combination of light limitation during summer algae blooms and high respiratory demands because of record high water temperatures decreased the amount of energy available for tuber production below a critical level.

To determine the potential for restoration of *Vallisneria* to the backwater habitats it earlier occupied, experiments were designed to establish (a) the minimum seasonal light requirements for tuber production, (b) the sensitivity of light requirements to temperature fluctuations, and (c) the effects of sediment fertility on plant responses to light.

The sensitivity of plant growth to sediment fertility under several shading regimes could be used to determine a seasonal light compensation point for tuber production. Below the light compensation point, sediment fertility effects should be negligible; and above the compensation point, they should become increasingly important as limiting factors to growth. Barko, Smart, and McFarland (1991) showed significant interactions between light and sediment fertility on *Vallisneria* growth in a greenhouse study.

Information on the interacting effects of sediment fertility and light availability on growth can be used, both in predicting potential declines and in locating optimal sites for *Vallisneria* restoration.

Methods

Experiments were set up in ponds at the U.S. Fish and Wildlife Service National Fisheries Lab in La Crosse, WI. In late April 1992, sediment was collected from a former *Vallisneria* bed in Lake Onalaska. The sediment was mixed, and part of it was amended 1:4 (sediment:sand) with washed builder's sand to create a low fertility treatment. Tubers were collected from Pool 4 less than 2 weeks before planting and were stored refrigerated in damp paper towels until they were planted on April 26.

Single tubers of approximately equal size (between 1.0 and 1.5 g fresh weight) were planted, one per bucket, in 1.2-L buckets containing 1 L of sediment. The buckets were placed in two concrete ponds, each 4.9 m wide, 9.5 m long and 1.1 m deep. The ponds were partitioned into 12 rows of cells 1 by 1 m top surface area, using black plastic side partitions. Four light treatments were established, using cells open at the top, and black plastic screens rated at 63, 80, and 92 percent shade. A randomized block design was used so that each row (block) had all four light treatments. Within each cell, there were five buckets of tubers in "native sediments" and five buckets

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of tubers in sand-amended sediment to provide 8 light-sediment combinations.

Light (photosynthetically active radiation (PAR)) was measured continuously in each pond using LiCor underwater quantum sensors connected to LiCor loggers. PAR was also measured under each shade cell to determine photon flux density at bucket depth. Temperature was also recorded using thermocouples connected to loggers. Water was circulated in the ponds using two submersible pumps per pond such that turnover occurred every 3 days.

Six replicates of each treatment were harvested every 2 weeks from 15 June to 30 August. In September and October, 12 replicates were harvested once a month. Numbers of leaves, leaf length, biomass of leaves, roots, stolons and tubers, and the numbers of tubers were measured. At four times during the summer, rates of photosynthesis and respiration were measured in leaf segments from all eight treatments using a closed system with oxygen electrodes. Photosynthesis was measured at light intensities from 0 to 800 moles/m^2/sec at 15, 20, 25, and 30 °C.

Results

Data analysis is not complete. Preliminary results from biomass measurements suggest that sediment nutrient limitations become significant at quite low light levels, indicating a very low light compensation point. This is in agreement with other studies of the light requirements for *Vallisneria* such as Titus and Adams (1979). Light availability appeared to affect leaf growth strategies, such as leaf length and the numbers of leaves produced; and for either sediment type, increasing light levels were coupled with increased biomass. Within each light treatment, sediment fertility also affected biomass and overall phenology, especially the timing of tuber initiation and onset of leaf senescence.

Currently, light response curves are being developed from photosynthesis data to determine instantaneous light compensation points. Temperature response curves are being developed from photosynthesis and respiration measurements to determine the sensitivity of light compensation points to temperature changes. Plant tissues and sediments are being analyzed for nutrient concentrations to determine the degree to which nutrient concentrations in low-fertility sediments are reflected in plant tissues grown under varying light availability. Two-way analysis of variance and regression techniques are being used to determine the levels of significance that can be ascribed to light-sediment interactions as measured by growth and tuber production.

Implications for Restoration

From preliminary analyses, it appears that in planning restoration projects, both sediment fertility and light availability will affect project success. For backwaters where long-term monitoring data are available, a geographic information system could be used to define areas where light availability at the sediment surface is above the seasonal light compensation point, and where sediment fertility is unlikely to be limiting.

Acknowledgments

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Sediment Factors Influencing Growth of *Vallisneria*

by
Dwilette G. McFarland[1] *and John W. Barko*[1]

Introduction

Lake Onalaska, WI, is a shallow 7,000-acre lake located in the backwaters of the Upper Mississippi River. Annual surveys of the lake conducted by the U.S. Fish and Wildlife Service (USFWS) show that from at least 1980 to 1987, *Vallisneria americana* Michx. (family Hydrocharitaceae) was by far the dominant aquatic macrophyte species—covering up to about one-half of the lake's total acreage. However, over the following 3 years, USFWS studies indicated that the *Vallisneria* population in Lake Onalaska had declined drastically and by 1990, covered less than 300 acres in small beds scattered about the lake.

Vallisneria americana, also called wild celery, eelgrass, or tape grass, is a native submersed macrophyte (described in Fassett 1975; Godfrey and Wooten 1979) that historically has been an asset to the ecology of Lake Onalaska. For many years, its widespread stands helped to improve water quality by stabilizing mucky sediments and taking up nutrients that potentially support nuisance algal growth (cf., Korschgen and Green 1988; Korschgen 1990; Barko, Gunnison, and Carpenter 1991). Also, *Vallisneria* in Lake Onalaska has long provided an abundance of tubers and rootstocks as forage for migrating ducks, including threatened flocks of canvasbacks and other wildfowl (Korschgen, George, and Green 1988). Because of these and other favorable attributes of the species (cf., Fassett 1975; Chilton 1986; Poe et al. 1986), much recent attention has focused on understanding reasons for the decline of *Vallisneria* and promoting its reestablishment in the lake.

Perhaps the most important event accompanying major losses of *Vallisneria* in Lake Onalaska was a 3-year drought that peaked in 1988. For this reason, certain environmental conditions associated with the drought are thought to have negatively impacted *Vallisneria* growth in the lake. These conditions include the following: (a) reduced light availability because of shading by epiphytic and planktonic algae, (b) increased water temperatures that promoted algal growth and diminished allocations of macrophytic biomass to propagule formation, and (c) depletion of sediment nutrients because of increased uptake rates in the macrophyte beds during an extended period of low flow. Yet, despite the end of the drought in 1990, *Vallisneria* has remained in very low abundance in Lake Onalaska, and in some areas of the lake, has continued to decline.

In this article, we present results of an investigation designed to examine growth of *Vallisneria* on sediments collected from two sites in Lake Onalaska, and on the same sediments with different nutrient amendments. This laboratory investigation was conducted in concert with field efforts (see Rogers and Barko 1993) to determine influences of various in situ conditions on production in *Vallisneria* transplants. Collectively, the results of these studies are intended to promote practices for reestablishing *Vallisneria* in Lake Onalaska and provide insight into possible mechanisms that resulted in its decline.

Methods and Materials

The study was conducted May through July 1992 in a greenhouse facility at the U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS. Eight 1,200-L fiberglass tanks were filled 83 cm deep with the low-alkalinity culture solution described in Smart and Barko

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(1985). One Remcor circulator per tank provided continuous water circulation and temperature control at 25 ± 1 °C. The solution was aerated with humidified air to enhance mixing and air/water CO_2 exchange. Maximum midday photosynthetically active radiation levels inside the tanks averaged 450 $\mu E/m^2/sec$ using a neutral density shade fabric that reduced natural irradiance by approximately 75 percent.

Sediments used in the study were collected from two sites in Lake Onalaska, WI (Figure 1): Site 1, a protected area southeast of Bell Island, and Site 2, an open-water area near the center of the lake. Each sediment was thoroughly mixed as a separate aliquot and divided into four parts. Three parts of each were treated with different nutrient amendments: nitrogen (N), phosphorus (P), and nitrogen and phosphorus in combination (N + P); and the fourth part of each was left as a nonamended control. N in the N-alone and N + P amendments was provided as 0.71 g NH_4Cl/L (wet sediment), and P in the P-alone and N + P amendments, as 0.08 g KH_2PO_4/L. Sediments were placed to a depth of approximately 8 cm in 24.3 by 24.3 by 10 cm deep polyethylene containers. Six replicate containers were assigned per tank, with each tank supporting a single nonamended or amended sediment.

Subsamples of prepared sediments were analyzed for physical and chemical characteristics (Tables 1 and 2). Particle-size distribution (texture) determinations were made using the hydrometer method of Patrick (1958). Sediment moisture content and density were evaluated gravimetrically after oven-drying measured volumes of sediment at 105 °C for approximately 12 hr. Dried samples were placed in a muffle furnace and combusted at 550 °C for estimations of organic matter content from loss of mass following ignition (Allen et al. 1974). Exchangeable ammonium-N was obtained by extraction with 1M NaCl in a cation exchange procedure modified from Bremner (1965). Extractable P was obtained using 0.03 N NH_4F and 0.025 N HCl (after Olsen and Sommers 1982). Both N and P concentrations were analyzed colorimetrically with a Technicon Autoanalyzer II, employing a molydate method for P and a salicylate method for N (American Public Health Association 1985).

Tubers of *Vallisneria americana* were collected from sediments in the vicinity of Lake Onalaska. These propagules were germinated under greenhouse conditions to ensure their viability and uniformity at the initiation of the study. Germinated tubers were planted four per container, with basal ends approximately 3 cm deep in the sediment. Following planting, a thin layer of sand was placed over the sediment surface to minimize physical mixing with the overlying solution.

At the end of a 9-week growth period, *Vallisneria* was clipped at the sand surface, measured for morphological responses, and oven-dried to constant mass at 80 °C. Evaluations of plant growth were based on determinations of aboveground and belowground biomass, rosette (plant) number, and rosette height. As an indication of reproductive potential, tubers and seedpods were counted directly and weighed for separate determinations of tuber and seedpod mass.

Experimental data were analyzed using analysis of variance, multiple range, and t-testing procedures of the Statistical Analysis System (SAS) (SAS 1991). In this report, statements of statistical significance without specific indication of probability level refer to $P < 0.05$.

Results

Sediment composition

The two sediments utilized in this study differed substantially in major compositional characteristics (Table 1). Sediment from Site 1 (the protected site) was predominantly fine textured, with a silt-clay particle fraction exceeding 75 percent. In contrast, sediment from Site 2 (the open-water site) contained a high proportion of coarse-grained sand, and

thus possessed greater bulk density and less moisture than sediment from Site 1. Organic matter content was seven times greater in Site 1 than in Site 2 sediment, but in both cases, fell within the lower range reported for 40 other sediments from North American lakes (Barko and Smart 1986).

Initial concentrations of extractable nutrients in control and treated sediments are presented in Table 2. Comparisons between the two controls showed that sediment from Site 1 had about 40 percent less P, but nearly 80 percent greater N than sediment from Site 2. N additions to these sediments resulted in approximate 6.5-fold and 10-fold increases in exchangeable N concentrations in Site 1 and Site 2 sediments, respectively. P-treated sediments showed relatively minor increases in extractable P. Concentrations of this nutrient were only slightly higher in Site 2 than in Site 1 sediment following P addition.

Growth responses

On the whole, aboveground (AG) production in *Vallisneria* (Figure 2) was strongly affected by treatment ($P = 0.0001$, $F = 33.71$), sediment ($P = 0.0013$, $F = 11.98$), and to a lesser extent, treatment-sediment interaction ($P = 0.0049$, $F = 5.00$). Between Site 1 and Site 2 controls, AG production was approximately 25 percent greater on sediment from Site 1. Relatively low AG biomass was obtained on control sediments and on sediments that received the P addition alone. With N added singly and in combination with P, AG production increased significantly, reaching approximately the same maximum level on both sediments. Additions of N and N + P to Site 1

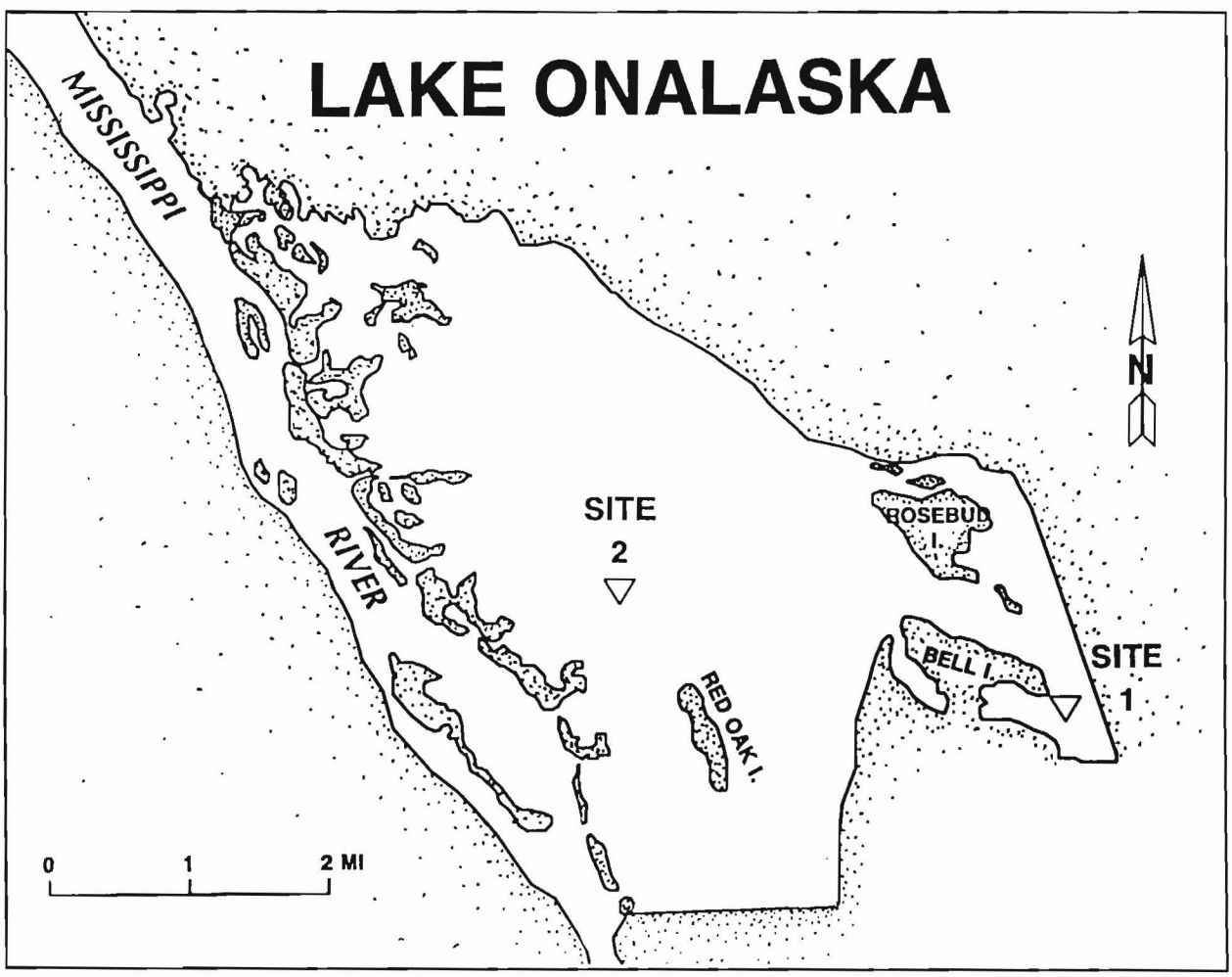

Figure 1. Sampling sites in Lake Onalaska, WI

Table 1
Sediment Physical Characteristics[1]

Parameter	Source	
	Site 1	Site 2
Particle size distribution, %		
Sand (>50 µm diam)	23.35 ± 0.83	79.19 ± 0.83
Silt (2 to 50 µm diam)	45.83 ± 0.83	11.65 ± 1.67
Clay (<2 µm diam)	30.83 ± 0.84	9.16 ± 0.83
Moisture, %	54.33 ± 0.03	21.29 ± 0.03
Organic matter, %	8.97 ± 0.06	1.20 ± 0.01
Dry weight density, g/ml	0.64 ± 0.01	1.54 ± 0.01

[1] Values are means and standard errors based on three replicate determinations.

Table 2
Initial Sediment Nutrient Characteristics[1]

Treatment	Extractable Nutrient, mg/g/Dry Sediment	
	NH_4-N	PO_4-P
S1 Control	.046 ± .007	.065 ± .011
S1 + N	.283 ± .047	.064 ± .009
S1 + P	.060 ± .001	.092 ± .001
S1 + N + P	.338 ± .002	.090 ± .005
S2 Control	.011 ± .001	.107 ± .001
S2 + N	.108 ± .001	.104 ± .001
S2 + P	.011 ± .000	.115 ± .003
S2 + N + P	.115 ± .002	.111 ± .002

[1] Values are means and standard errors based on three replicate determinations. Sediments were collected from Lake Onalaska, WI: S1 = Site 1 (protected area) and S2 = Site 2 (open-water area); control = nonamended sediment; nutrient additions include the following: N = nitrogen, P = phosphorus, and N + P = nitrogen and phosphorus in combination.

sediment promoted a 33-percent increase in AG biomass, while the respective increase on sediment from Site 2 was 50 percent.

Variables significantly affecting overall belowground (BG) biomass in this study (Figure 2) were sediment ($P = 0.0001$, $F = 20.60$) and treatment-sediment interaction ($P = 0.0001$, $F = 12.63$). However, no significant difference in BG biomass was detected on nonamended sediments from the two sites. The addition of N alone to both sediments had essentially no effect on BG production. Interactive effects of treatment and sediment were evidenced in opposing BG responses to additions of N + P and P alone. While BG biomass in *Vallisneria* was stimulated with P and N + P additions to Site 1 sediment, these treatment combinations to Site 2 sediment diminished growth below ground.

Vallisneria produced about the same number of rosettes regardless of nutrient amendment to Site 2 sediment (Figure 3). However, additions of N and N + P to that sediment promoted a near two-fold increase in rosette height (Figure 3). Likewise, additions of N and N + P to Site 1 sediment promoted marked increases in the height of this species, although the number of rosettes in those treatments was significantly reduced. On neither sediment was the height of rosettes affected by the addition of P alone.

Under nonamended conditions, seedpods produced by *Vallisneria* on Site 1 sediment outnumbered those on Site 2 sediment by nearly 10 to 1. N added singly and in combination with P to both sediments significantly increased production of these propagules. In no instance was seed pod production responsive to the addition of P alone. Seedpod mass (Figure 4) was highly and significantly correlated with seedpod number ($r = 0.95$, $P < 0.001$); thus, patterns of these responses to treatment were quite similar.

Tuber number in *Vallisneria* (Figure 5) was affected primarily by treatment ($P = 0.0046$, $F = 5.06$) and treatment-sediment interaction ($P = 0.0001$, $F = 10.03$). On Site 1 sediment, tuber production was maximized in the N + P treatment where the number of tubers produced by *Vallisneria* increased by approximately one-third. With N added separately to Site 2 sediment, tuber production was nearly doubled. P alone added to sediment had no effect on tuber number.

On both sediments, tuber mass (Figure 5) was closely related to tuber number ($r = 0.88$, $P < 0.001$) and to BG biomass ($r = 0.93$, $P < 0.001$). Overall, the contribution of tuber mass to BG biomass ranged from 64 to 71 percent between Site 1 and Site 2 sediments, respectively.

Discussion

The availability of nutrients in sediment has been demonstrated in this and other studies

to be important in influencing growth of submersed macrophytes (Denny 1972; Sand-Jensen and Sondergaard 1979; Bruner and Batterson 1984; Steward 1984; Barko and Smart 1986; Barko et al. 1988; McFarland and Barko 1992). In the present study, *Vallisneria* generally exhibited positive growth responses to N-additions to sediment, resulting in significant increases in biomass, propagule formation, and rosette (plant) height. Positive growth responses of this species to N additions suggest a potential for N limitation of growth on sediments at both study sites. Interestingly, additions of P to sediment appeared overall to have little positive effect on *Vallisneria* growth. Barko, Gunnison, and Carpenter

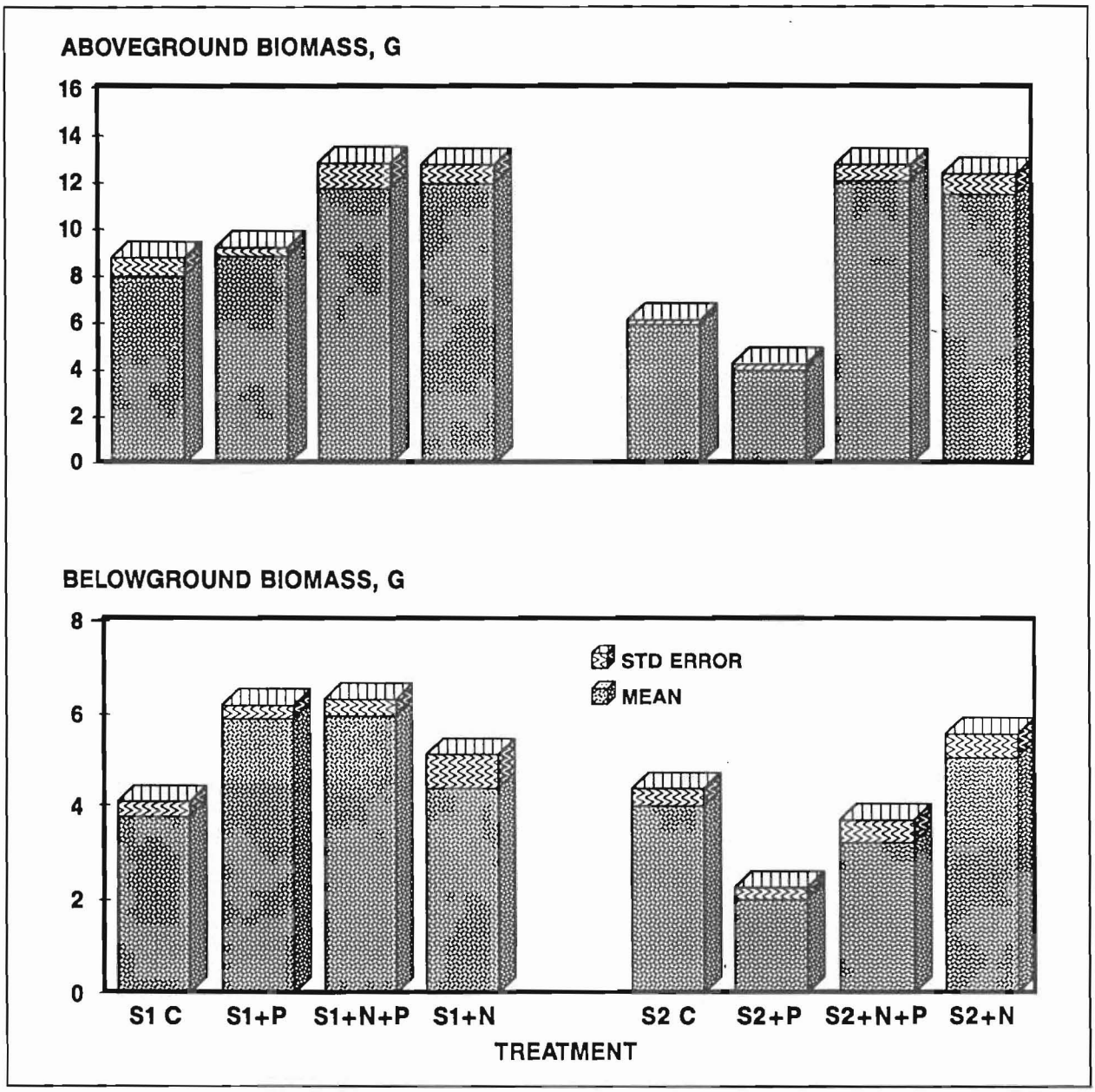

Figure 2. Effects of treatment combinations on aboveground and belowground biomass production in Vallisneria. Values are means and standard errors based on six replicate determinations. Sediments were collected from Lake Onalaska, WI: S1 = Site 1 (protected area) and S2 = Site 2 (open-water area); C = nonamended (control) sediment; nutrient additions include the following: N = nitrogen, P = phosphorus, and N + P = nitrogen and phosphorus in combination

(1991) have suggested that the growth of submersed macrophytes is less frequently limited by P than N because of generally larger pools of extractable P in many sediments. With consideration for the greater demand for N than P for production in *Vallisneria* (Gerloff and Krombholz 1966), it appears that, at least over the short term, growth of *Vallisneria* is more likely to be affected by N than P availability in sediments at the two study sites.

Our analyses of the composition of the two nonamended sediments revealed sediment from Site 2 to be less suitable for growth because of its higher sand content and lower concentration of extractable N than sediment from Site 1 (cf., Barko and Smart 1986). Growth responses of *Vallisneria* in this study essentially mirrored these sediment differences. However, the extent to which biomass might differ because of sediment characteristics under field conditions will no doubt depend on influences of other important environmental variables (e.g., light, temperature, CO_2 availability, and sedimentation rates). Thus, although results of this investigation demonstrated a somewhat greater capacity for Site 1 sediment to support growth, it should be emphasized that assessments of growth potential at the two study sites should consider possible impacts of other environmental variables as well.

Growth responses of *Vallisneria* to treatments in this study have important implications for its survival under different sediment conditions. On N-rich sediment, *Vallisneria* maximized biomass production and plant height while limiting the number of plants produced. Regulation of density under these conditions conveys an advantage to individual plants by reducing potential shading and allowing a greater share of nutrients both from the sediment and water column (Haller and Sutton 1975; Barko, Smart, and McFarland 1991). In contrast, on N-poor sediment, relatively high numbers of plants were sustained at the expense of biomass production and plant elongation. In our laboratory, this growth habit has been observed in other species in addition to *Vallisneria*, and is speculated to enable populations to grow away from unfavorable sediment conditions (McFarland and Barko 1992).

To more accurately assess the potential for nutrient limitation of growth at the two study sites, information on nutrient replenishment in those areas is needed. Typically, nutrients in various forms are transported to lake bottoms by sedimentation (Forsberg 1989; James and Barko 1990), a process which in concert with sediment mixing (e.g., through bioturbation) provides a primary source of nutrients to submersed macrophytes (Barko, Gunnison, and Carpenter 1991). As demonstrated here, nutrient additions (especially N) to sediments from Lake Onalaska increased production in *Vallisneria*. Thus, the extent to which sedimentation contributes to the nutrient economies of the two sites may well determine whether *Vallisneria* growth in those areas is nutrient-limited or not.

Future Studies

It has been widely established that submersed macrophyte species vary in response to sediment nutrient availability (Denny 1972; Bruner and Batterson 1984; Barko and Smart 1981, 1986; Chambers and Kalff 1985; Chambers 1987; McCreary, McFarland, and Barko 1991). Also, various sediment characteristics affecting nutrient supplies in sediment (e.g., texture, density, and organic matter content) have been linked with the distribution and density of submersed macrophyte species in nature (for synthesis, see Barko, Gunnison, and Carpenter (1991)). To date, however, there have been no definitive studies of range of sediment types in Lake Onalaska and the relative responses of different species to these sediment types. Presently, *Myriophyllum spicatum* L., a typically problematic species, is known to occur in small areas of Lake Onalaska. With *Vallisneria* in low abundance in the lake, now appears an opportune time for *Myriophyllum* to spread to areas once occupied by *Vallisneria*. At present, it would be useful to determine areas most vulnerable to invasion by *Myriophyllum* and where in the

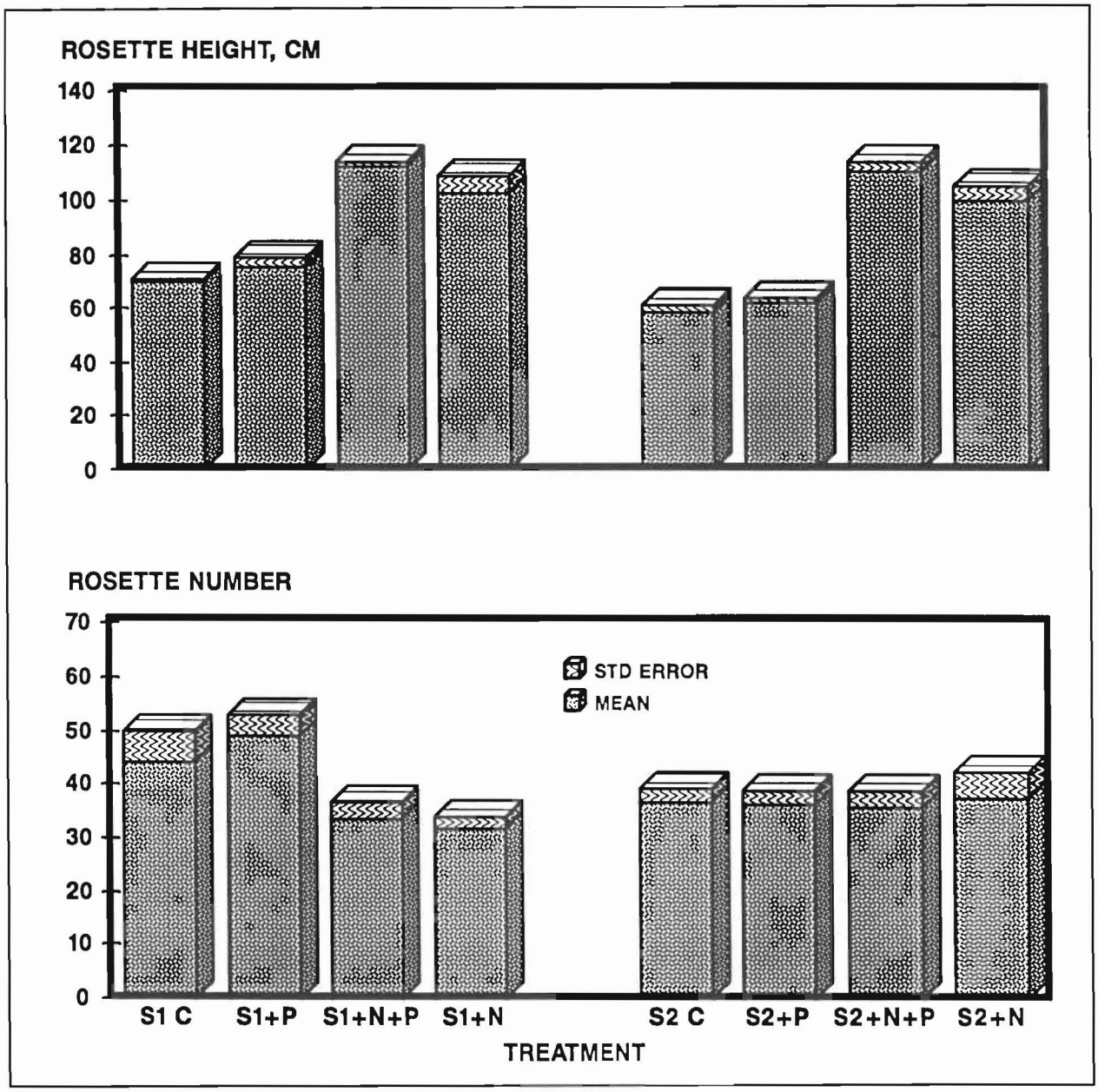

Figure 3. Effects of treatment combinations on rosette (plant) height and number in Vallisneria. Values are means and standard errors based on six replicate determinations. Acronyms same as in Figure 2

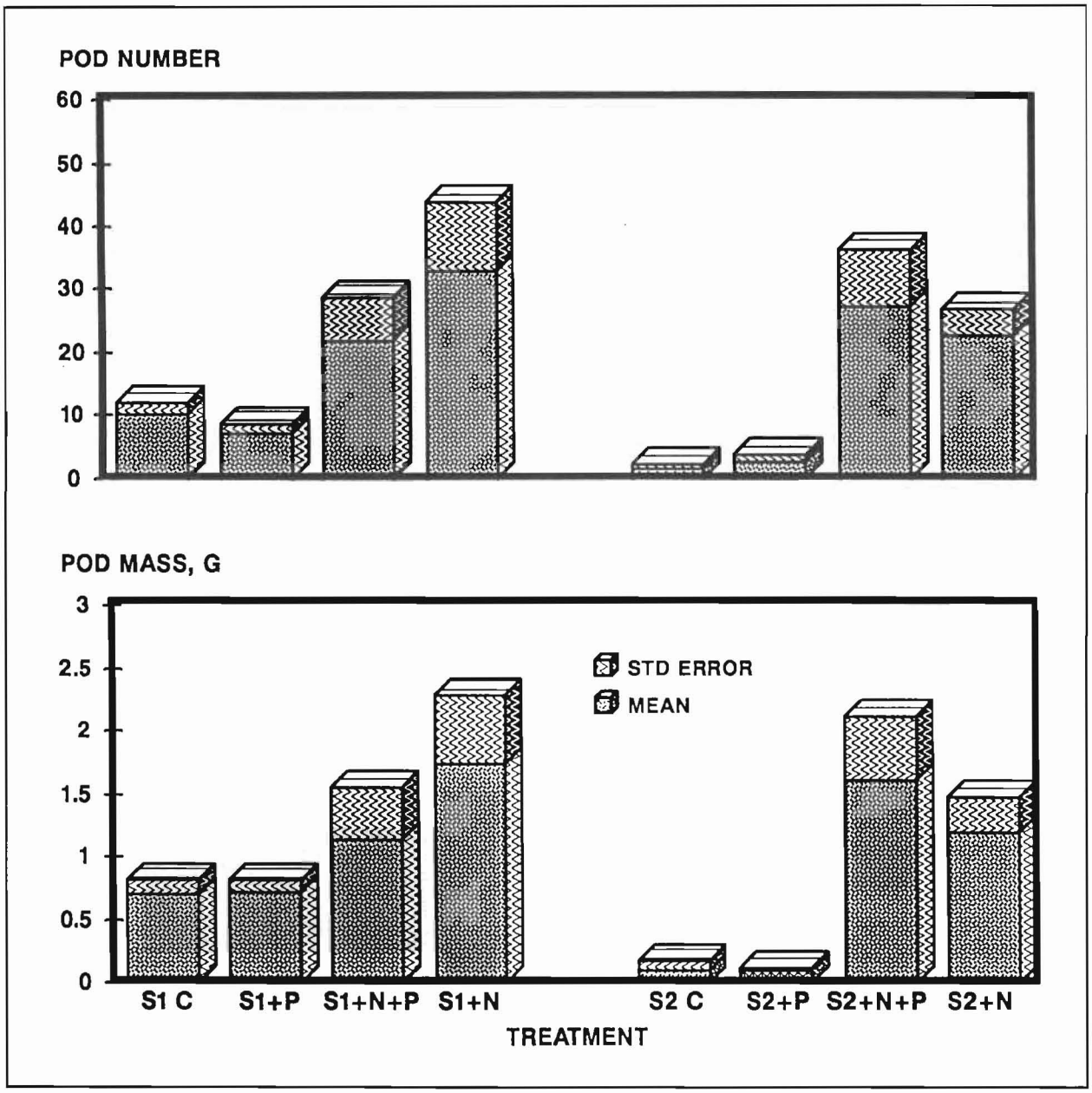

Figure 4. Effects of treatment combinations on the number and mass of seedpods produced by Vallisneria. Values are means and standard errors based on six replicate determinations. Acronyms same as in Figure 2

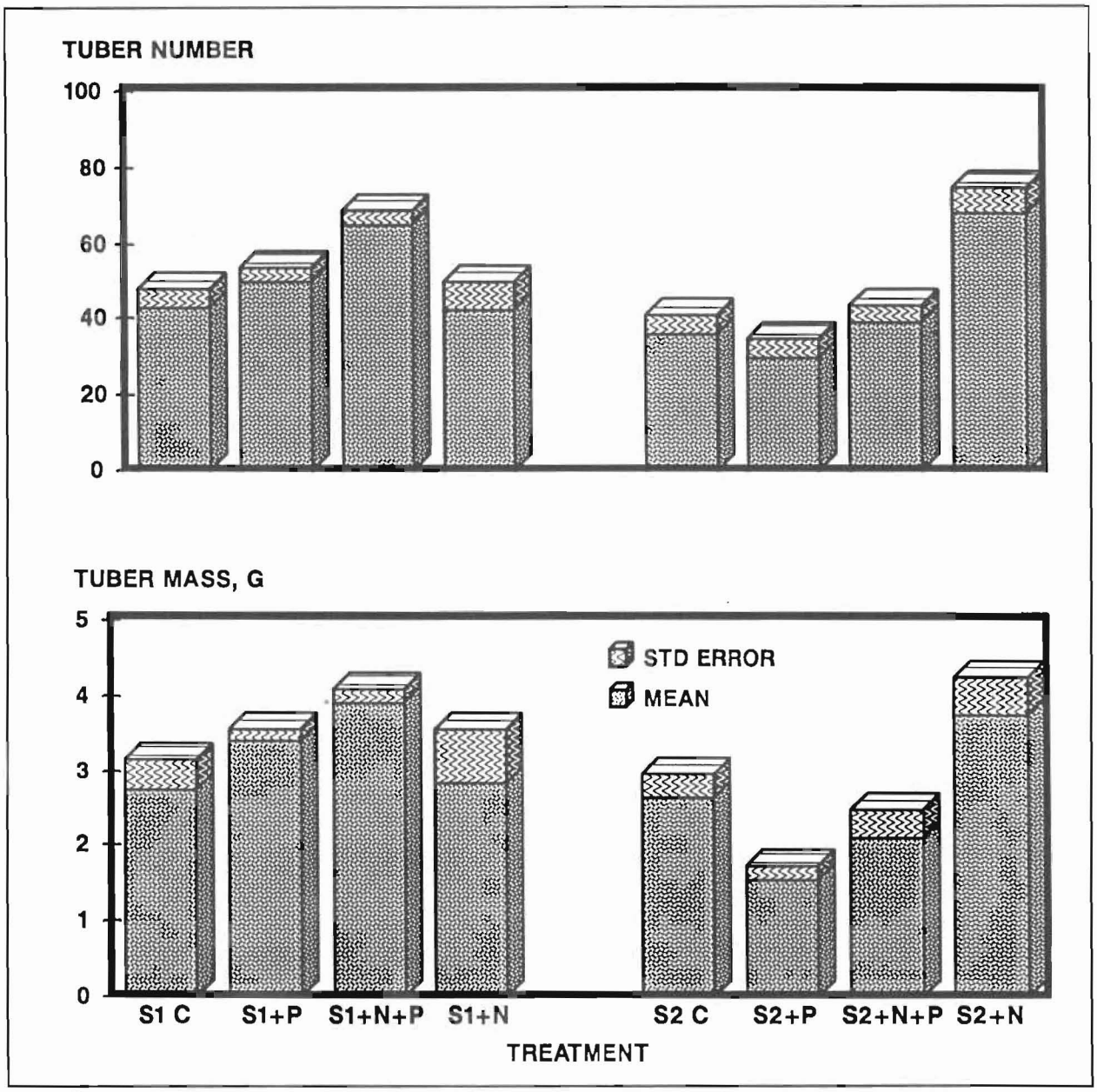

Figure 5. Effects of treatment combinations on the number and mass of tubers produced by Vallisneria. Values are means and standard errors based on six replicate determinations. Acronyms same as in Figure 2

lake preventative measures may be most needed.

Results of this study demonstrated growth responses of *Vallisneria* on sediments from two sites in Lake Onalaska. We suggest that additional studies of this kind be conducted in conjunction with field investigations to determine relative impacts of sediment versus other environmental factors on the growth of this species. We contend that if restoration practices can be tied to site-specific requirements for growth, then the success of *Vallisneria* in Lake Onalaska can be more readily ensured.

Acknowledgments

The authors gratefully acknowledge S. Fox, H. Eakin, W. Dee, F. Boyd, P. Little, and M. Powers for their technical efforts in the conduct of this study. Appreciation is also extended to S. Rogers for assistance in sediment and propagule collection. Technical reviews were provided by H. Eakin and T. Sturgis.

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Field Evaluation of Environmental Factors Affecting *Vallisneria* Growth

by
Sara J. Rogers[1] *and John W. Barko*[2]

Introduction

The pools and backwaters of the Upper Mississippi River (UMR) are physically diverse and biologically productive, providing habitat to hundreds of species of plants and animals. Submersed aquatic macrophyte communities help maintain these habitats by providing food and shelter to a variety of organisms, influencing nutrient dynamics, and stabilizing sediments (Sculthorpe 1967; Wetzel 1983). However, despite the important ecological value of submersed aquatic vegetation, we have little understanding of the influence of anthropogenic and natural stresses on this resource. The submersed aquatic vegetation of the UMR is likely subjected to many disturbances at once, creating complex ecological responses and making observed changes difficult to explain (Lubinski 1991). This inability to understand or anticipate changes in the distribution of submersed aquatic macrophytes was recently demonstrated when declines in *Vallisneria americana* L. (wild celery) became apparent during the late 1980s.

Vallisneria declined in portions of the UMR following a prolonged period of drought. Factors implicated in the decline include light-limiting conditions, high water temperatures, grazing by fish and waterfowl, agricultural herbicides, and low availability of sediment nutrients. However, no one factor or combination of factors has been positively identified as the primary cause for the decline. In addition, little is understood about the potential for *Vallisneria* to return to its former distribution or for exotic species such as *Myriophyllum spicatum* to exploit open niches.

Although the causal factors leading to the *Vallisneria* decline remain unexplained, the potential for this species to become reestablished needs to be explored. In this article, we present results of a field study designed to evaluate whether localized water quality and sediment conditions will currently support *Vallisneria* reestablishment.

Study Location

The study was conducted in Lake Onalaska (Pool 7) of the UMR (Figure 1). The 2,835-ha lake has supported aquatic vegetation since the lake was formed by impoundment in 1937. *Vallisneria americana* L. was reported to be common by 1960; and at its peak in the mid-1980s, this species occupied >3,000 acres (C. Korschgen et al., Northern Prairie Research Center, La Crosse, WI; unpublished manuscript). By 1989, however, less than 300 acres were estimated to remain.[3]

Two sites were selected in regions of the lake where *Vallisneria* had occurred previously (Figure 2). Site 1 (protected), located in the southeastern corner of the lake, was protected from prevailing summer winds by nearby south and southwest shorelines. Surficial sediments consisted of fine-textured materials. Site 2 (unprotected) was located in the west-central portion of the lake >700 m from islands on the west side of the lake and >3,000 m from the north or south shorelines. Surficial sediment at Site 2 consisted mostly of sand. Surficial sediments from each site were analyzed for initial determination of moisture, density, organic matter, NH_4-N, and PO_4-P.

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[1] U.S. Fish and Wildlife Service, Environmental Management Technical Center, Onalaska, WI.
[2] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
[3] Personal Communication, C. Korschgen, Northern Prairie Research Center, La Crosse, WI.

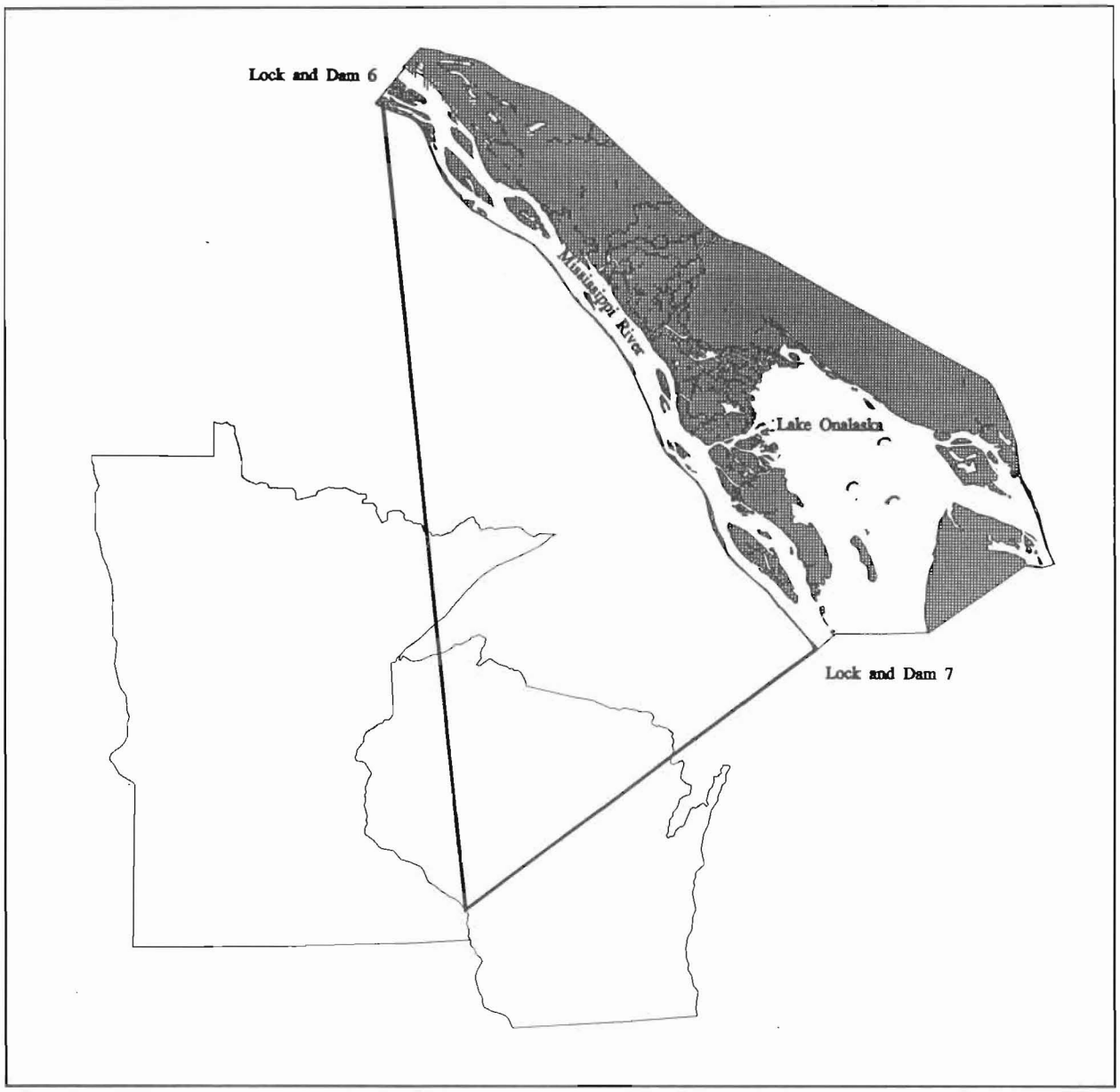

Figure 1. Location of Lake Onalaska in Pool 7, Upper Mississippi River

Vallisneria tubers were collected from Pool 4 during mid-April. A water-pumped dredge was used to disturb the sediments and dislodge the over-wintering tubers. The tubers were stored at approximately 40 °F until planting. In early May, the tubers were planted in eight replicate plots randomly placed within each site. Each plot was delineated by a frame anchored to the substrate with attached legs. A 1-m^2 planting grid divided the plots into 36 15- by 15-cm cells. Two tubers per cell were planted in the substrate using the grid as a temporary guide.

Surface water temperature, Secchi depth, and surface and depth-integrated turbidity were determined approximately weekly. Underwater photosynthetically active radiation (PAR) was measured at each site with a LI-COR quantum sensor (LI-COR, Inc., Lincoln, NE). Integrated water column samples were collected weekly for determinations of seston and chlorophyll-*a* levels.

Plants were harvested in mid-August from four randomly selected plots at each site. Evaluations of plant growth were determined

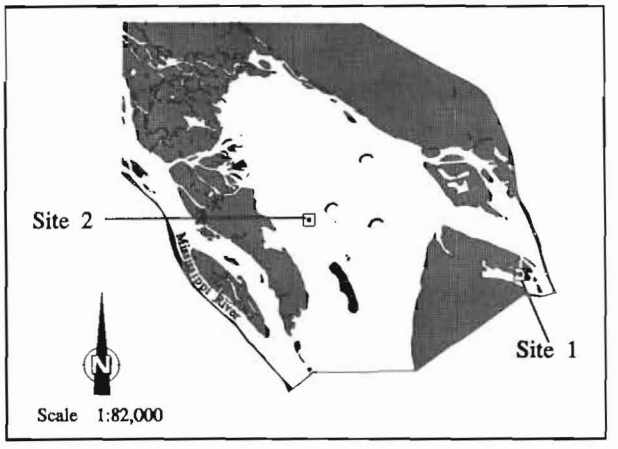

Figure 2. Location of Vallisneria transplant sites in Lake Onalaska

by oven-dry biomass (80 °C), measurements of leaf lengths, number of plants, and the number of flowers per plot.

Sediment cores were taken in September within plots not previously harvested for plant growth determinations. Cores were collected using a 6.5-cm handheld corer equipped with acrylic core liners. In the field, 30 to 40 cm of each core sample were sectioned at 5-cm intervals for nutrient and belowground biomass determinations.

Results

There were no significant differences in aboveground biomass ($p \geq 0.05$) between the two sites (Figure 3). Nor were there significant differences ($p \geq 0.05$) between the two sites in average leaf lengths, maximum leaf lengths (Figure 4), the number of plants that reached leaf lengths >20 cm, or the number of plants that were <20 cm (Figure 5). The number of male flowers and the number of female flowers produced by the plants also revealed no significant differences ($p \geq 0.05$) (Figure 6).

Comparison of initial nutrient concentrations showed that sediment from Site 1 was higher in NH_4-N and lower in PO_4-P ($p < 0.05$). Sediment from Site 1 was significantly higher ($p < 0.05$) in moisture content, and organic matter was finer in texture and was less dense than sediments from Site 2 (Table 1).

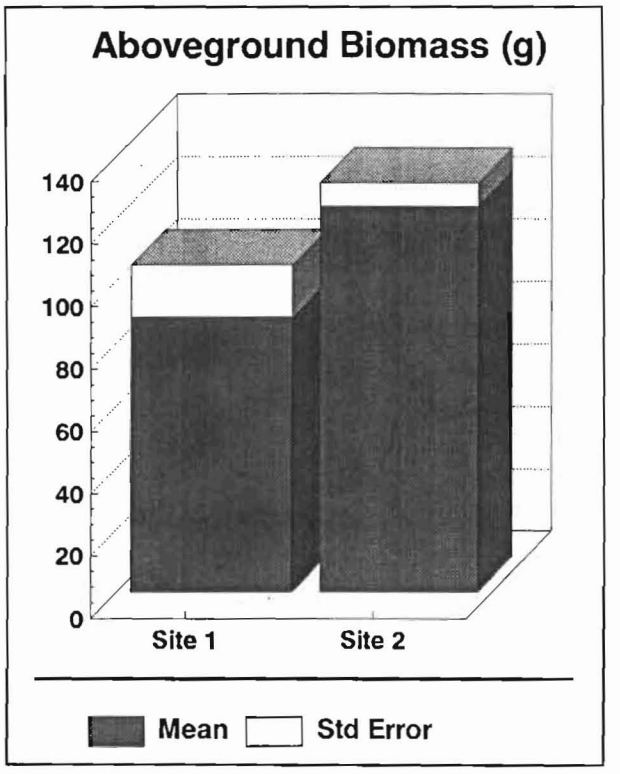

Figure 3. Aboveground biomass production of Vallisneria america L. planted in Lake Onalaska. Values are means (n = 4) with associated standard error bars

Water quality data have not been completely analyzed, but some general trends are evident. Seston and chlorophyll-*a* concentrations were highly variable between sites on the same sampling dates and over time within sites. Turbidity and Secchi depth were also quite variable between sites and over time. In spite of the observed variability in water quality parameters (i.e., seston, chlorophyll-*a*, Secchi, and PAR), 1 percent light depths were not significantly different overall between the two sites (seasonal mean for both sites = 1.5 m).

The sediment cores collected for belowground biomass determinations did not contain enough plant material to quantify the vertical distribution of underground plant structures. However, plant structures found within the cores were distributed primarily within the upper 10 cm for Site 2 and within the upper 20 cm for Site 1.

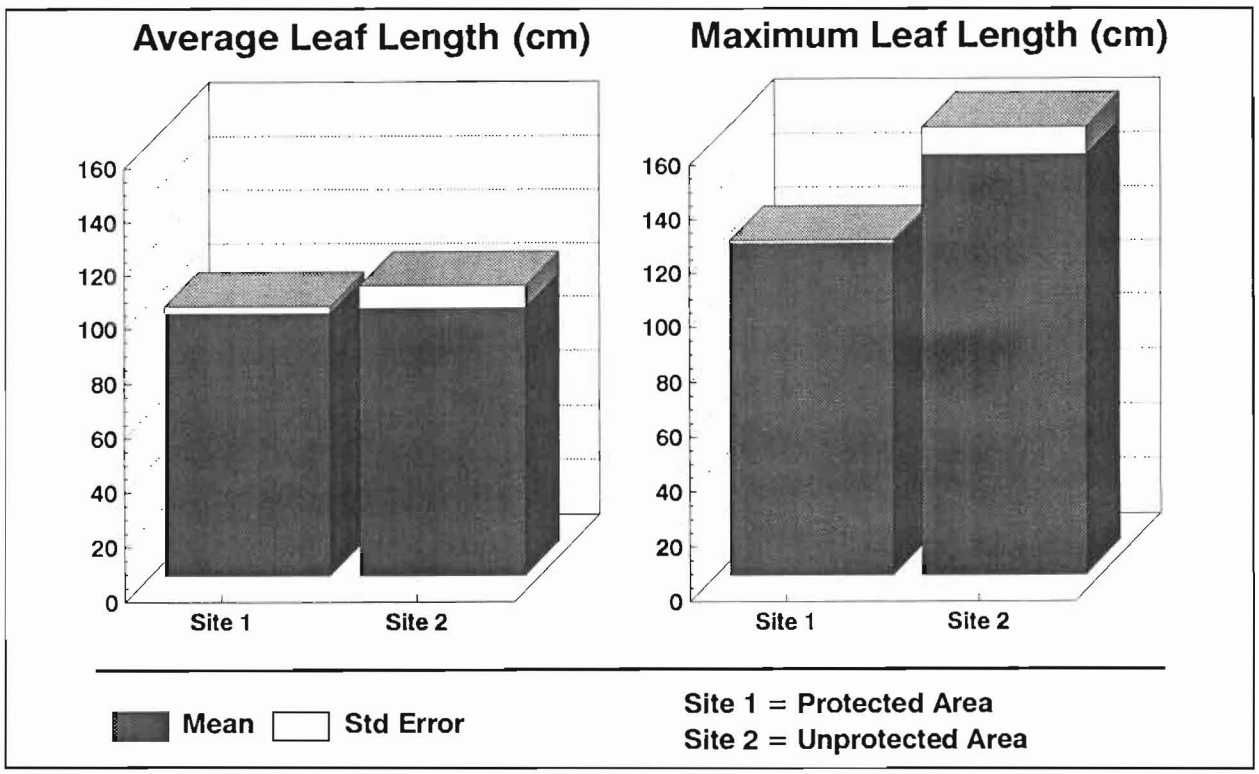

Figure 4. Average and maximum leaf lengths of Vallisneria americana L. Values are means (n = 4) with associated standard error bars

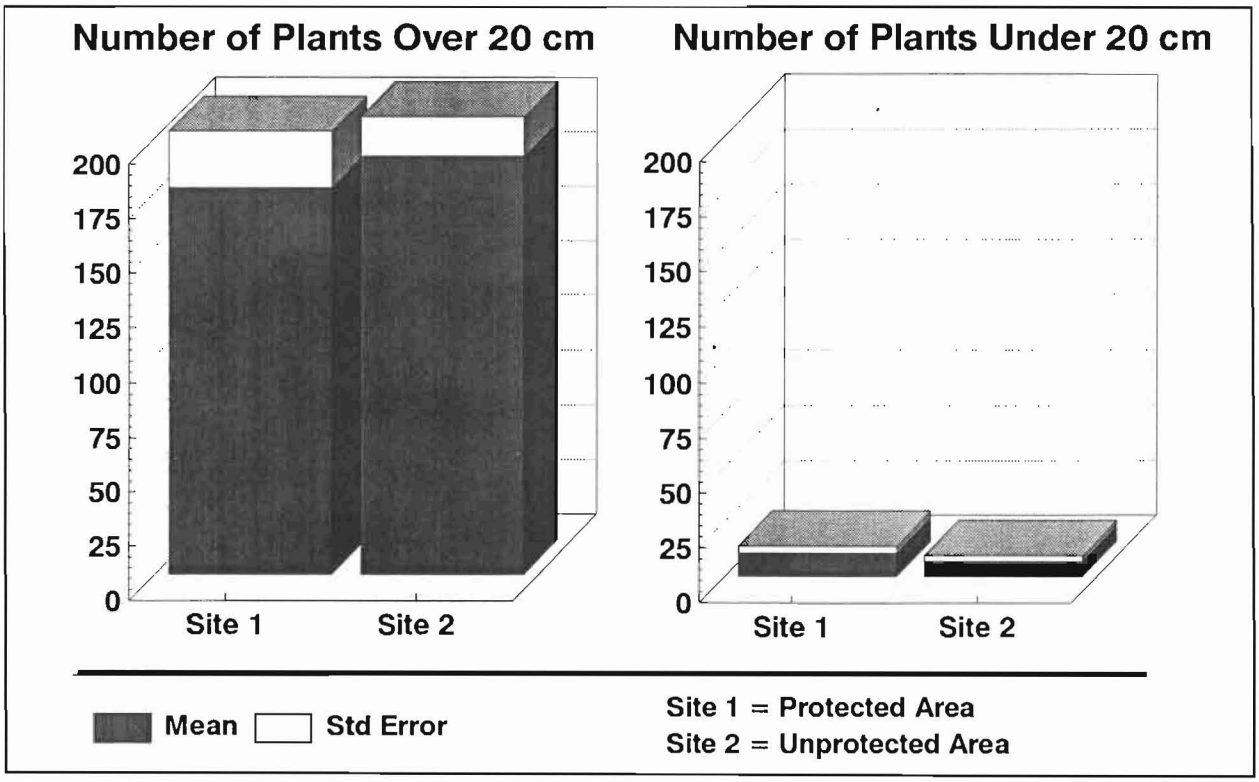

Figure 5. Number of plants produced by Vallisneria americana L. Values are means (n = 4) with associated standard error bars

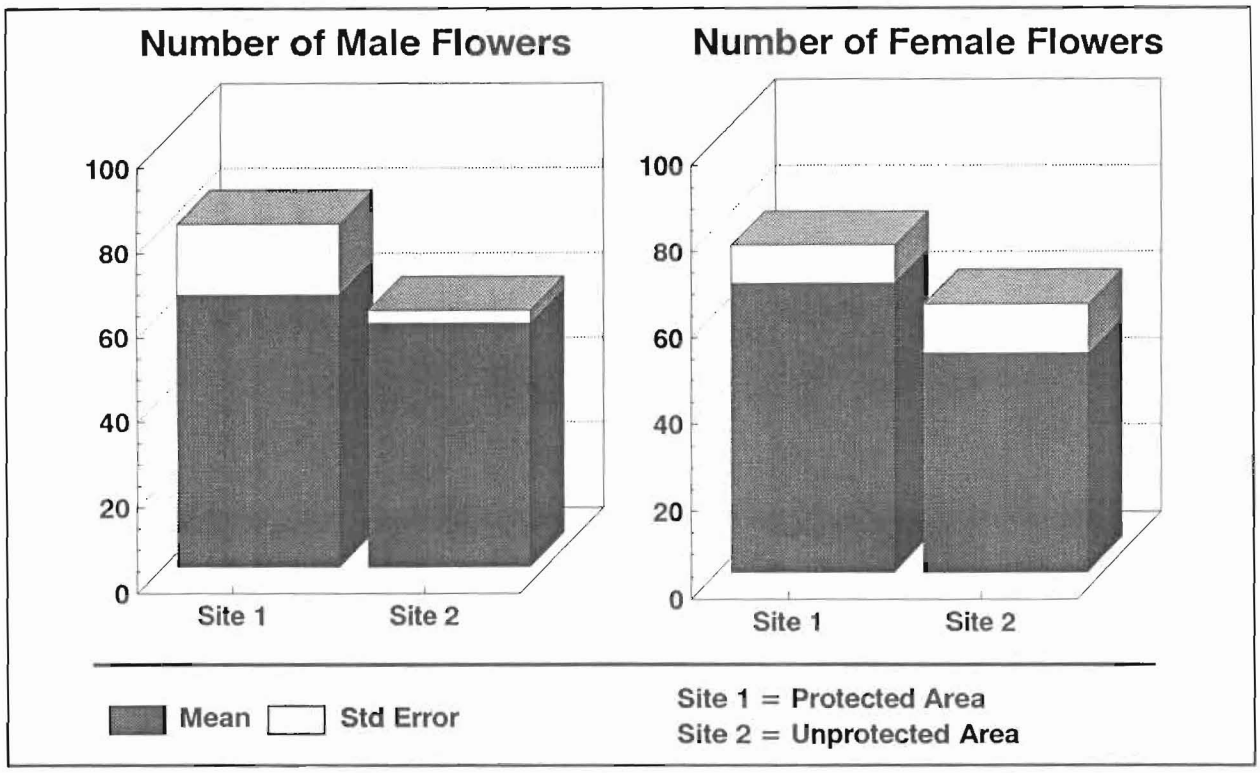

Figure 6. Number of male and female flowers produced by Vallisneria americana L. at transplant sites in Lake Onalaska. Values are means (n = 4) with associated standard error bars

Table 1
Initial Physical and Chemical Characteristics of Sediments from Transplant Sites in Lake Onalaska[1]

Parameter	Site 1	Site 2
Moisture, %	54.33 ± 0.03	21.29 ± 0.03
Dry weight density, g/ml	0.64 ± 0.01	1.54 ± 0.01
Organic matter, %	8.97 ± 0.06	1.20 ± 0.01
Exchangeable nitrogen (NH_4-N) mg/g dry sediment	0.05 ± 0.01	0.01 ± 0.00
Available PO_4-P mg/g dry sediment	0.06 ± 0.01	0.11 ± 0.00
Texture, %		
Sand (>50 μm diam)	23.35 ± 0.83	79.19 ± 0.83
Silt (2 to 50 μm diam)	45.83 ± 0.83	11.65 ± 1.67
Clay (<50 μm diam)	30.83 ± 0.84	09.16 ± 0.83

[1] Values are means ± standard errors based on analyses of three replicate samples.

Discussion

Under 1992 field conditions in Lake Onalaska, *Vallisneria* demonstrated the ability to grow well at a depth of 1 m and to produce flowers and overwintering tubers on two sediments differing greatly in physical and chemical composition. However, in an associated greenhouse study on sediments from the same field study sites, *Vallisneria* growth was demonstrated to be limited by low nitrogen (N) availability (McFarland and Barko 1993). These contrasting results suggest that throughout the 1992 growing season, N may have been provided to *Vallisneria* in the field via sediment transport and accretion. Alternatively, decomposition of plant/algal remains within the sites may have allowed for sustained availability of N to *Vallisneria* in the field. Confirmation of these possibilities as potential determinants of N availability awaits results of tissue N analyses now in progress.

Among a variety of factors influencing the production and distribution of *Vallisneria* in the UMR, the availability of sediment nutrients may be one of the most important (Barko, Gunnison, and Carpenter 1991). Replenishment of nutrients (e.g., nitrogen) via sedimentation may balance sediment nutrient losses because of diffusion or macrophyte uptake (Barko et al. 1988). Thus, accretion, as it may affect N availability in surficial sediments, needs to be considered in evaluating the reestablishment success of *Vallisneria*. Moreover, we hypothesize that during periods of drought, nutrient availability to aquatic macrophytes may be reduced by low river discharge and associated reductions in sediment transport to backwaters of the UMR. Further field investigations are needed to better understand the hydrologic processes involved in sustaining nutrient availability to submersed aquatic macrophytes in the UMR.

Acknowledgments

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Convective Water Exchange During Differential Cooling and Heating: Implications for Nutrient and Herbicide Transport

by
William F. James[1] *and John W. Barko*[1]

Introduction

Recent investigations have shown that convective water exchange is a potentially important mechanism for the horizontal transport of nutrients and other substances between littoral and pelagic zones (Stefan, Horsch, and Barko 1989; James and Barko 1991a,b) and between side embayments and the main basin of reservoirs (Monismith, Imberger, and Morison 1990). Convective exchange is driven primarily by horizontal water temperature (and density) gradients that develop between shallow and deep regions as a result of differences in water volume. During daytime heating, water in shallow regions can heat more rapidly than in deeper regions (i.e., differential heating), resulting in the horizontal movement of shallow water as a surface flow over cooler water in the deep region (Monismith, Imberger, and Morison 1990). During nighttime cooling, the opposite pattern can occur. Water in shallow regions cools more rapidly than in deeper regions (i.e., differential cooling), resulting in horizontal movement of shallow water as an underflow below warmer water in the deep region (Monismith, Imberger, and Morison 1990). These circulation patterns, potentially occurring on a daily basis, can have an important effect on nutrient dynamics and phytoplankton productivity (James and Barko 1991a,b; James and Barko, In Preparation). They also have important implications for aquatic plant management, since convective exchanges can affect both herbicide application rates and the residence time of herbicides.

We examined differential heating and cooling and resultant convective exchange patterns in an embayment (Minky Creek) of Guntersville Reservoir, AL, during September of 1990. The reservoir has experienced relatively high macrophyte biomass levels (dominated by *Myriophyllum spicatum* and *Hydrillia* sp.) during most years, necessitating control via herbicide application and other means. Concerns over the potential transport of aquatic herbicides and soluble nutrients originating from decaying macrophytes to undesirable locations (i.e., swimming and fishing areas) led to an examination of water exchange mechanisms that might result in movement of these substances. Thus, our objectives were to identify convective exchange patterns in this embayment as a potential horizontal transport mechanism and to document the frequency of occurrence of this water exchange mechanism on a daily basis during the month of September. We use vertical and horizontal variations in water temperature to examine differential heating and cooling between shallow and deeper regions and convective exchange patterns that develop as a result of these horizontal water temperature gradients.

Methods

A transect was established along the slope of the basin on the northwestern shoreline of the Minky Creek embayment to examine differential heating and cooling and convective exchange patterns (Figure 1). The transect extended approximately 400 m into the open water. Stations for water temperature monitoring were located at the 1.0- (50 m from shore), 2.0- (125 m from shore), 3.5- (175 m from shore), 5.0- (275 m from shore), and 5.75-m (375 m from shore) depths on the transect.

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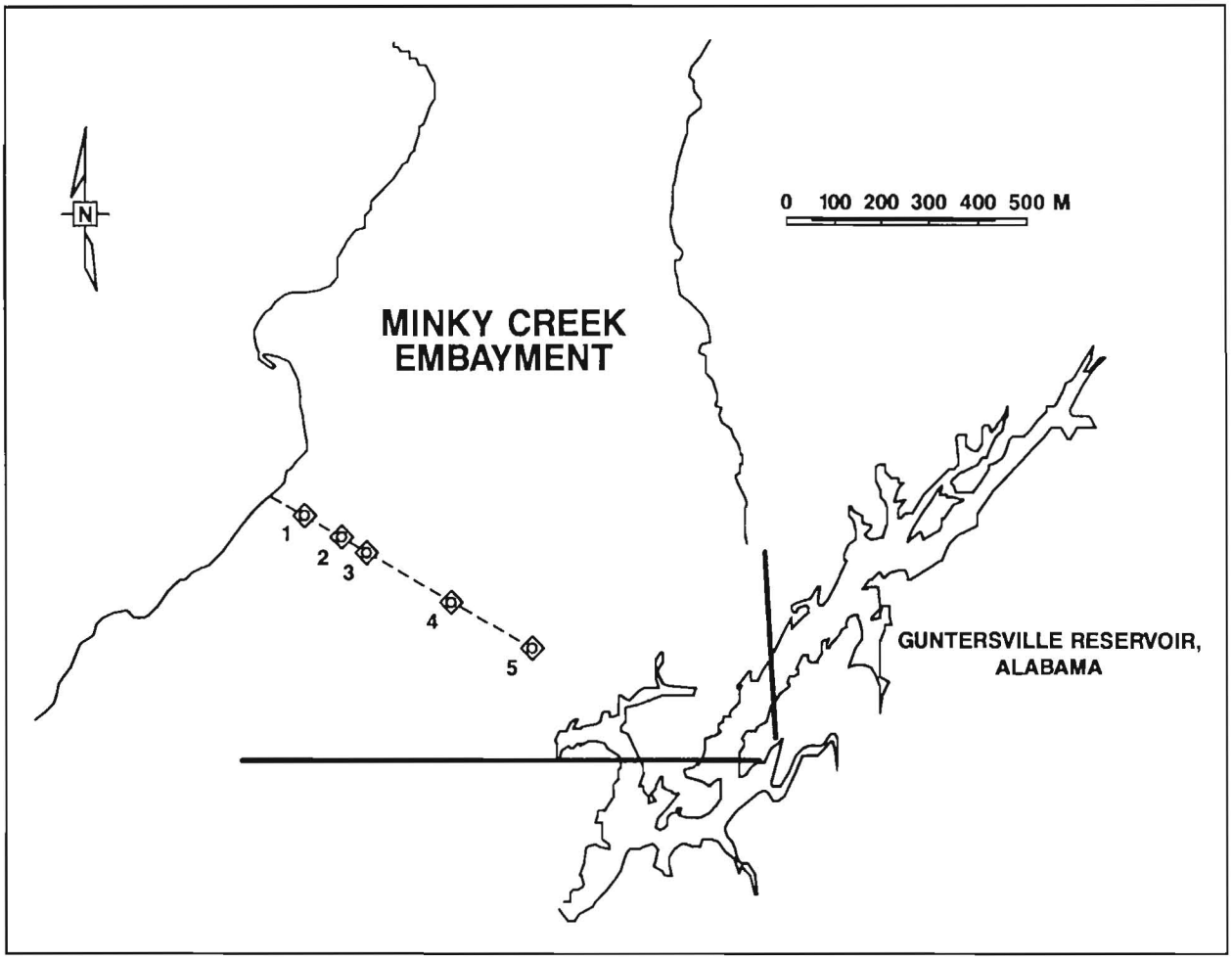

Figure 1. Thermistor station locations

Recording thermister monitors (Omnidata International) were placed on permanently anchored platforms at these stations. Instantaneous air and water temperatures were measured every half hour with thermistor probes, calibrated to the nearest 0.1 °C using a thermometer that was calibrated to a National Bureau of Standards thermometer. Thermistor probes were positioned at 25-cm depth intervals (i.e., from 25 cm below the reservoir surface to near the bottom) at Stations 1-3, and at 50-cm intervals at Stations 4-5. The average of data collected at 2-min intervals was recorded every half hour for wind speed and direction (Omnidata International) at Station 1. A continuous record of air and water temperature and wind speed and direction was collected between 01-24 September 1990.

Results

Differential heating and cooling during September

The surface waters at Stations 1 and 2 generally heated and cooled more rapidly than other stations on a diel basis, suggesting that differential heating and cooling were confined to water column depths ≤2 m. We, therefore, calculated horizontal water temperature gradients using the difference in depth-integrated water temperatures (from the upper 1 m of the water column) between Stations 1 and 5. A positive horizontal water temperature gradient reflected differential heating (i.e., surface water temperature greater at Station 1 than at Station 5), while a negative horizontal water

temperature gradient reflected differential cooling (i.e., surface water temperature less at Station 1 than at Station 5).

In general, positive horizontal water temperature gradients were observed during daytime warming, while negative horizontal water temperature gradients were observed during nighttime cooling (Figure 2). During periods when the water column at Station 1 gained heat, peaks in the negative horizontal water temperature gradient decreased to near zero, while peaks in the positive horizontal water temperature gradient were high, often exceeding 1 °C. During periods of heat loss from the water column, the opposite pattern occurred. Peaks in the positive horizontal water temperature gradient declined to minimal values, while peaks in the negative horizontal water temperature gradient increased in value, as on 09-18 and 22-23 September (Figure 2).

Convective exchange during periods of differential cooling

During the night of 23-24 September, winds were relatively calm (i.e., <1 m/sec) between 2000 and 0230 hours (Figure 3a). Winds then fluctuated between 2 and 3 m/sec from the northeast late in the night (between 0300 and 0500 hours), declined to 1 m/sec at 0600 hours, then increased to >5 m/sec from the east at 1000 hours. Although water temperatures were nearly uniform vertically at all stations during the night, horizontal gradients of decreasing

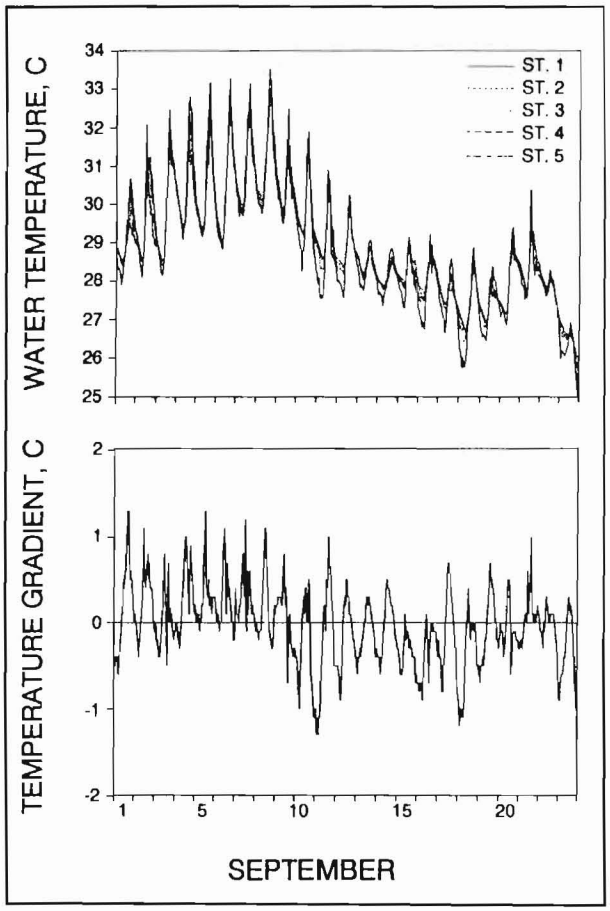

Figure 2. Diel variations in (a) surface water temperatures at Stations 1-5 and (b) horizontal water temperature gradients between Stations 1 and 5

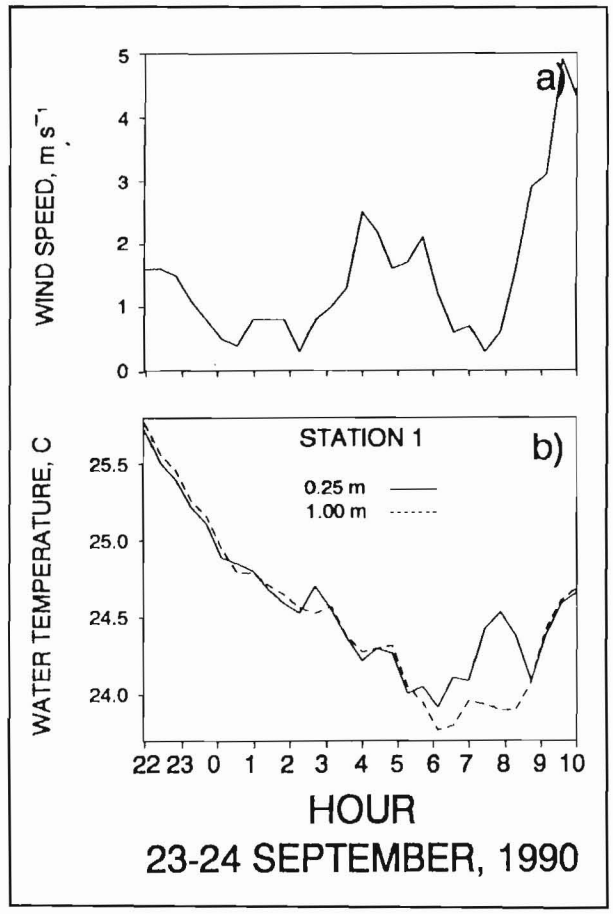

Figure 3. Variations in (a) wind speed and (b) water temperature at Station 1 between 2000 hours on 23 September and 1000 hours on 24 September 1990

water temperature were observed from the open water (i.e., Station 5) to the back of the transect (i.e., Station 1) by 2000 hours (Figure 4). These horizontal water temperature gradients were strong between Stations 2 and 3 at 2000 hours. At 2400 hours, the water column of Stations 1 and 2 had cooled considerably in relation to deeper stations, and cooler water was observed at the bottom of Stations 3 to 5, indicating the development of an underflow current (Figure 4).

As differential cooling continued during the night, water temperature contours slanted strongly downward from Station 2 to Station 4 by 0400 hours, resulting in strong vertical stratification in the bottom waters of Station 3 (Figure 4). Horizontal water temperature gradients also became compressed near the reservoir surface between Stations 1 and 2 at 0400 hours, indicating the occurrence of a return surface flow toward Station 1. At 0800 hours, a return surface flow moving toward Station 1 was clearly evident as surface temperatures at Station 2 increased over those observed at 0400 hours (Figure 4).

Alternating periods of water temperature cooling and heating were observed throughout the night at Station 1 (Figure 3b). Surface and bottom water temperatures at this station cooled steadily from 2000 hours on 23 September to 0100 hours on 24 September. Periodic heating of the water column then occurred at 0130, 0330, 0500, 0600, and 0700 hours on 24 September. These heating periods were subsequently followed by water temperature cooling periods. Water temperature inversions were also observed during these cooling periods, as surface water temperatures were usually cooler than bottom water temperatures. These patterns indicated the movement of

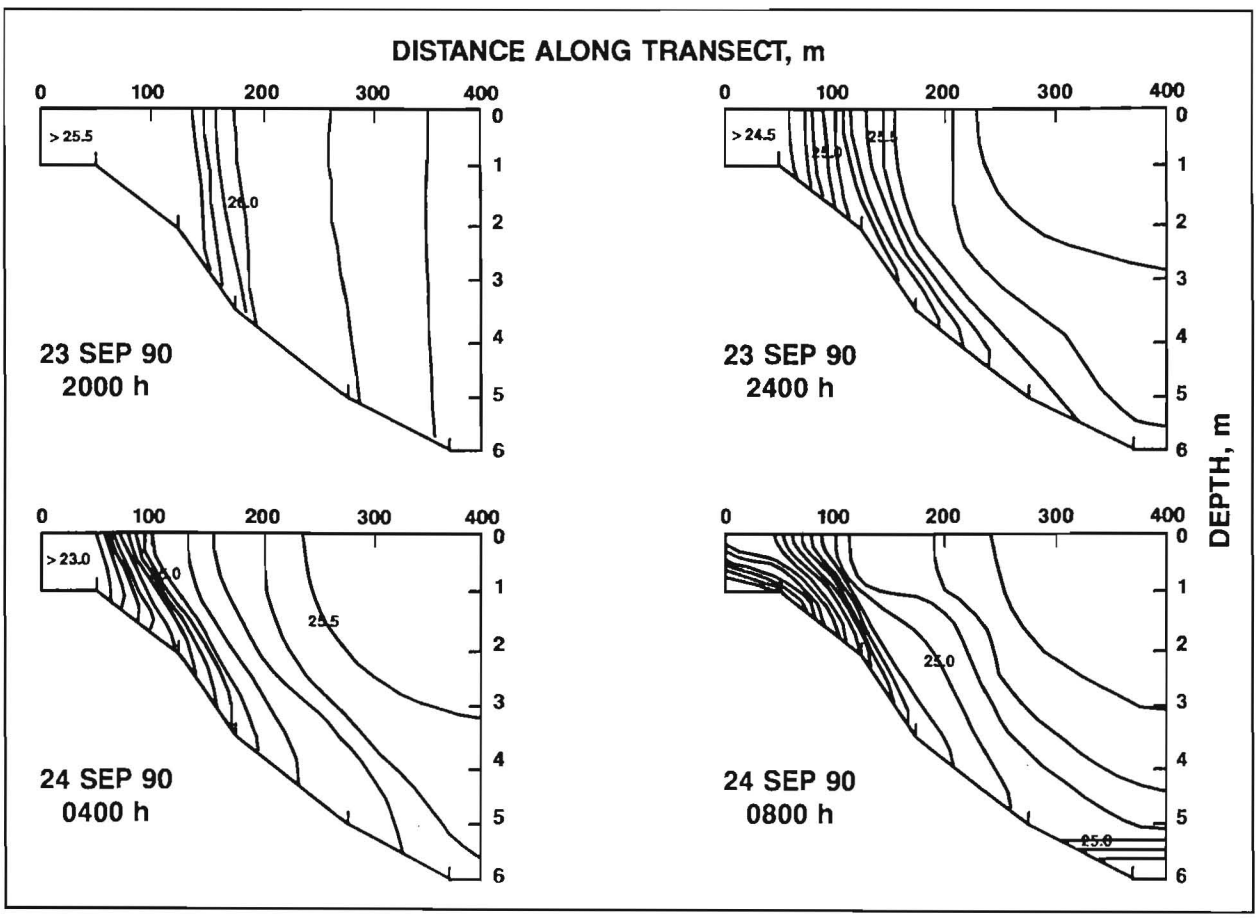

Figure 4. Longitudinal and vertical variations in water temperature (°C) at 2000 and 2400 hours on 23 September and at 0400 and 0800 hours on 24 September 1990

warmer surface water into Station 1, followed by rapid cooling and outward movement toward the open water as an underflow current. This pattern of convective exchange during differential cooling was observed on 13 nights during September (i.e., 54 percent of the measured days in September), generally when the mean negative horizontal water temperature gradient (i.e., during the differential cooling period) exceeded 0.3 °C.

Convective exchange during periods of differential heating

On 07 September, wind speeds fluctuated between 1 and 2.5 m/sec (southwest) throughout much of the afternoon, but were near zero at 1530 hours (Figure 5a). At 1200 hours, the surface waters at Stations 1 and 2 had heated more rapidly than the upper 1.0 m of the water column at Stations 4 and 5 (Figure 6), resulting in pronounced horizontal water temperature gradients. At 1400 hours, a tongue of warm surface water (i.e., represented by the 31.5 °C contour) extended from the back of the transect to about 250 m that was about 0.5 m in vertical expanse (Figure 6). Water temperatures at this time were uniform vertically at Station 1, but a shallow metalimnion (i.e., located between the 0.5- and 1.5-m depths) was observed at other stations. These horizontal and vertical water temperature variations produced a slight upward slant in water temperature contours (i.e., the 29.5 to 31.0 °C contours) from the back of the transect to the open water at 1200 and 1400 hours (Figure 6).

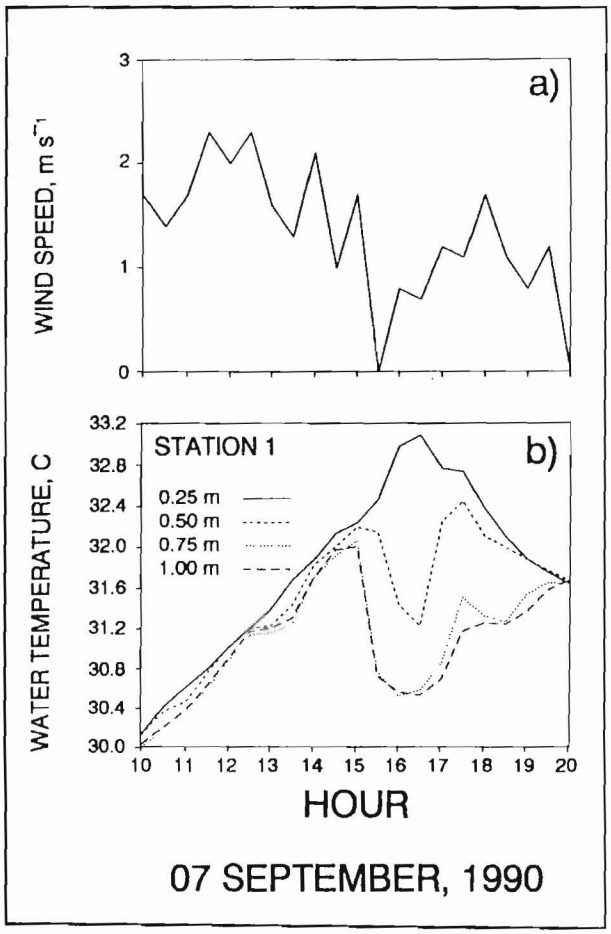

Figure 5. Variations in (a) wind speed and (b) water temperature at Station 1 between 1000 and 2000 hours on 7 September 1990

Movement of warmer surface water toward Station 5 was observed at 1600 hours as water temperatures at Stations 4 and 5 increased in the upper 0.5 m of the water column (Figure 6). Movement of cooler water from the opposite direction into the bottom of Station 1 was also observed at this time as water temperatures below the 0.5-m depth decreased by nearly 1 °C at this station. Water temperature contours were horizontal at 1600 hours, and the 29.0 and 29.5 °C contours moved down nearly 1 m in the water column compared with their positions at 1400 hours, as a result of convective exchange. At 1800 hours, water temperatures at Station 1 began cooling, and horizontal differences in water temperature between shallow and deep stations diminished in the upper 1 m (Figure 6).

Dynamic oscillations in water temperature were observed at Station 1 on 07 September, indicative of convective exchange during differential heating (Figure 5b). Between 1000 and 1500 hours, water temperatures at Station 1 increased steadily and were nearly uniform with depth. Water temperatures below the 0.25-m depth then declined substantially at Station 1 between 1500 and 1630 hours as cooler water flowed into Station 1. Water temperatures at these bottom depths again heated by 1730 hours, then another decrease in water temperature was observed at 1830 hours, suggesting further movement of cool

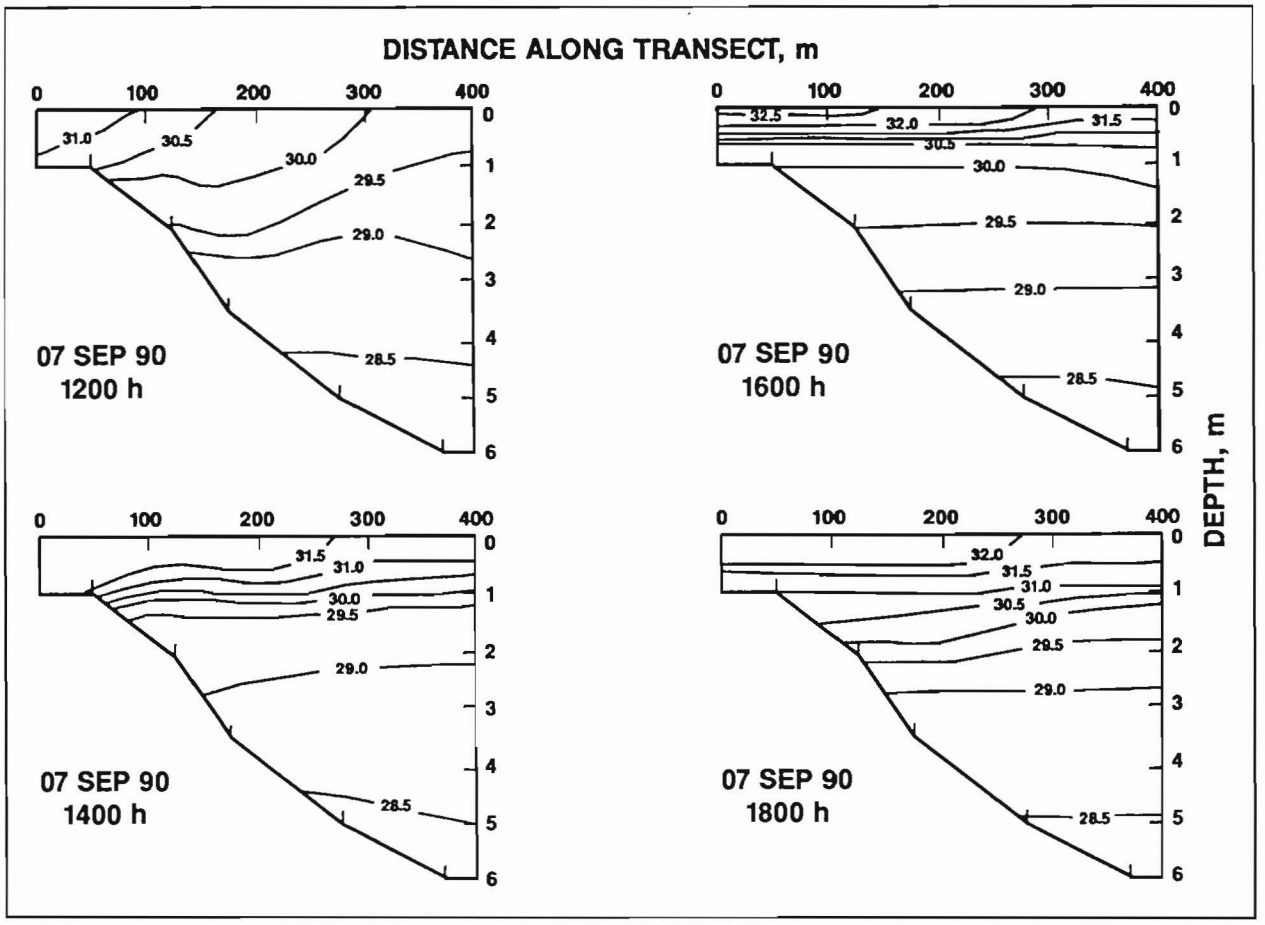

Figure 6. Longitudinal and vertical variations in water temperature (°C) at 1200, 1400, 1600, and 1800 hours on 7 September 1990

water into this station. By 2000 hours, water temperatures were nearly uniform in the water column.

Discussion

During differential heating, convective exchange occurred primarily in the upper 1 to 3 m of the water column because of warm surface flows that developed from a relatively shallow mixed layer. During differential cooling, however, water moved from shallow regions as an underflow current and was replaced by a return current of surface water that then cooled rapidly and, in turn, became an underflow current. Thus, convective exchange was relatively shallow during differential heating phases, whereas water located near the surface during the day potentially moved to the bottom of the embayment at night during differential cooling phases. These fundamentally different convective exchange patterns have important implications for the transport and availability of nutrients to phytoplankton communities located in the euphotic zone (James and Barko 1991a,b). They may also play an important role in regulating the residence time and fate of aquatic herbicides.

We currently do not know the flow velocities during periods of convective exchange under various wind conditions nor other effects wind may have on convective exchange patterns in the Minky Creek embayment. Flow velocities appear to be driven by the intensity of horizontal water temperature gradients. Linden and Simpson (1986) showed experimentally that under calm conditions, convective flows are generated as a result of a sharpening of horizontal water temperature gradients, or frontogenesis, and that turbulence (i.e., wind

mixing) can arrest or reduce convective flow velocities by disrupting these fronts. Thus, flow velocities, which we did not measure, may be strongly affected by wind mixing. In addition, it is often difficult to separate wind-generated circulation patterns from those generated by differential heating and cooling on the basis of water temperature alone (Monismith, Imberger, and Morison 1990).

Convective exchanges during differential heating and cooling, based on variations in water temperature contours, occurred on 39 and 54 percent of the days, respectively, during the study period in September. In general, it appeared that convective exchanges during differential heating periods predominated when the water column at Station 1 gained heat. In contrast, convective exchanges during periods of differential cooling predominated when the water column at Station 1 lost heat. This seasonality in the occurrence and pattern (i.e., differential heating or cooling periods) of convective exchange patterns is of importance when considering the potential transport and fate of soluble nutrients and herbicides.

In particular, macrophytes contain a considerable amount of tissue phosphorus and other nutrients that can become a major source of soluble nutrients to the water column during leaching and decay (Barko and Smart 1980; Carpenter 1980; Landers 1982) after an herbicide treatment. Movement of these soluble nutrients via convective exchange as underflows during the night and overflows during the day may result in considerable nutrient redistribution and potential availability to phytoplankton communities. In addition, herbicide application strategies must consider the potential for movement of herbicides from target areas as a result of both convective exchanges and wind-generated exchanges. Stefan, Horsch, and Barko (1989) have suggested that the residence time of shallow regions can be on the order of hours as a result of convective exchanges. These short residence times need to be considered when evaluating interactions between herbicide concentration and exposure times (Fox et al. 1991; Netherland, Green, and Getsinger 1991; Netherland and Getsinger 1992) for macrophyte control. They may also result in the movement of herbicides to undesirable locations, such as the pelagic zone.

Acknowledgments

D. Brewster and D. Murphy of the Murphy Hill Research Facility are gratefully acknowledged for their participation in deployment of the thermistor platforms. Permission to publish this information was granted by the Chief of Engineers.

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Nutrient Allocation and Uptake Efficiency in *Hydrilla verticillata* and *Potamogeton americanus* in Relation to Supply: Implications for Management

by
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Introduction

Responses of individual macrophyte species to changes in the water column or varying water chemistry parameters have been widely studied (e.g., Barko and Smart 1981; Titus and Stone 1982; Smart 1990; Barko, Smart, and McFarland 1991). While important for light, dissolved carbon, and cations, notably potassium, the water column is not the primary source of nutrition for rooted aquatic macrophytes, which derive most of their nutrition from sediments (Carignan and Kalff 1980; Barko and Smart 1986; Barko et al. 1988). Sediments influence growth both through degree of chemical fertility (Smart and Barko 1985) and characteristics of the physical properties of sediments (Denny 1980).

While the impacts of nutritional factors have been extensively investigated, competitive responses by multispecies assemblages to sediment nutrients have been less well studied. Early studies simply attributed competitive superiority without experimental demonstration of competition as the mechanism (Haller and Sutton 1975; Van, Haller, and Bowes 1978; Sutton, Littell, and Langeland 1980). By assuming that interspecific competitive superiority maintained species dominance, managers may have enacted control technologies that failed to target real weaknesses of nuisance species, at the same time not fully exploiting natural advantages of native populations.

Predicting the outcome of interspecific competition requires knowledge of species monospecific results (McCreary and Carpenter 1987; Smart and Barko 1989; McFarland, Barko, and McCreary 1992) that may be extrapolated to competitive situations in nature. Yet, by far the best evidence for competitive success lies in experiments that examine growth of competitors in monocultures and in mixtures. For example, we have demonstrated the ability of *Potamogeton americanus*, a temperate native pondweed, to successfully outcompete *Hydrilla verticillata* in greenhouse experiments (McCreary, McFarland, and Barko 1991). However, the mechanisms that enable *Potamogeton* to do so are complex and require additional study. Along this line of inquiry, the study reported here examines in greater detail the competitive interactions between *Hydrilla verticillata* and *Potamogeton americanus*.

The objectives of this study were to examine growth of *Hydrilla verticillata*, an exotic nuisance species, and *Potamogeton americanus*, a native North American pondweed, on sediments rich in nitrogen or limited by nitrogen. Nutrient allocation patterns and uptake efficiency were assessed for both species under both sediment treatments. Outcomes were examined in light of typical management practices employed for nuisance species. It is hoped that this study will augment the present body of information available to managers and establish the basis for more holistic approaches to macrophyte control efforts.

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[2] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Methods

Extensive greenhouse experiments were conducted from May to July of 1989 at the U.S. Army Engineer Waterways Experiment Station (WES) Environmental Laboratory in Vicksburg, MS. A complete description of the experimental design and execution can be found in McCreary, McFarland, and Barko (1991). In brief, uniform propagules of *Hydrilla* and *Potamogeton* were planted in containers holding sediments that differed only in that they were either nitrogen-limiting (0.01 ± 0.00 mg N per g dry sediment), or were fertilized to provide a nitrogen surplus (0.21 ± 0.01 mg N per g dry sediment). Sediment phosphorus and potassium were similar between the two sediment treatments. Plants were grown monotypically and in 50:50 mixtures in an ionically balanced culture solution with sufficient potassium but devoid of either nitrogen or phosphorus (for specific composition, see Smart and Barko (1985)).

After approximately 6 weeks, the experiment was harvested, and aboveground and belowground tissues analyzed for morphological characteristics (e.g., shoot number, height, and belowground biomass) and for tissue nitrogen (N), phosphorus (P), and potassium (K). Sediment cores were also assessed for interstitial and exchangeable fractions of sediment N, P, and K.

Data were analyzed on a VAX mainframe computer equipped with Statistical Analysis System (SAS) (SAS Institute 1989). Analysis of variance (ANOVA) was determined where appropriate, and least-squares regressions using the REG procedure (SAS Institute 1988) were performed. Suppression coefficients were calculated for each species to summarize competitive effects in mixtures relative to growth of plants alone (Aarssen 1985). Uptake efficiency was calculated for each tissue type by expressing elemental content as a percentage of tissue type. To obtain elemental costs, uptake efficiency was weighted by the proportionate allocation of biomass to different tissues. Elemental costs thereby reflect allocation patterns to roots and shoots.

Results

Morphological growth characteristics have been reported elsewhere (McCreary, McFarland, and Barko 1991; McFarland, Barko, and McCreary 1992), but indicate that both *Hydrilla* and *Potamogeton* responded most strongly to nitrogen limitation. Further, *Potamogeton* clearly dominated all mixtures, effectively outcompeting *Hydrilla* for sediment nitrogen. However, suppression coefficients indicate that while *Hydrilla* was substantially suppressed by *Potamogeton* during N-limited growth, the degree of suppression declined with sediment N-surplus (Figure 1).

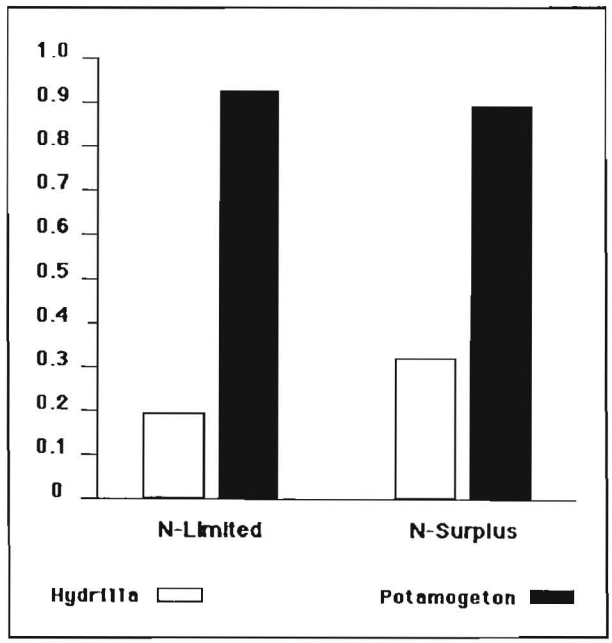

Figure 1. Suppression coefficients (S) for each species by fertility treatment

Sediment nitrogen dynamics were further examined for plants grown with a sediment N-surplus by regressing the total nitrogen content of plant tissues over the nitrogen left behind in sediments following harvest. If all nitrogen removed from sediments had been incorporated into plant biomass, an inverse relationship would be expected, with a declining regression. Regressions of *Hydrilla* tissue nitrogen over both interstitial (Figure 2) and exchangeable (Figure 3) sediment nitrogen fractions significantly demonstrated the predicted

relationship. Regressions for *Potamogeton*, however, showed no relationship between tissue nitrogen and sediment nitrogen either for interstitial (Figure 4) or for exchangeable pools (Figure 5).

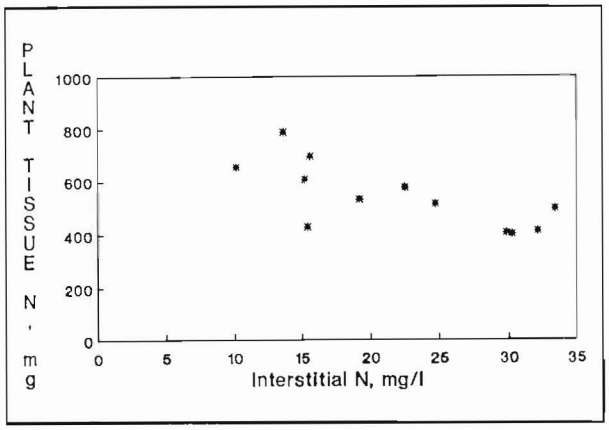

Figure 2. Regression for Hydrilla plant tissue nitrogen as a function of interstitial sediment nitrogen. Regression equation: Y = 789.5119 - 11.3805 X. P = 0.0067, r square = 0.5367

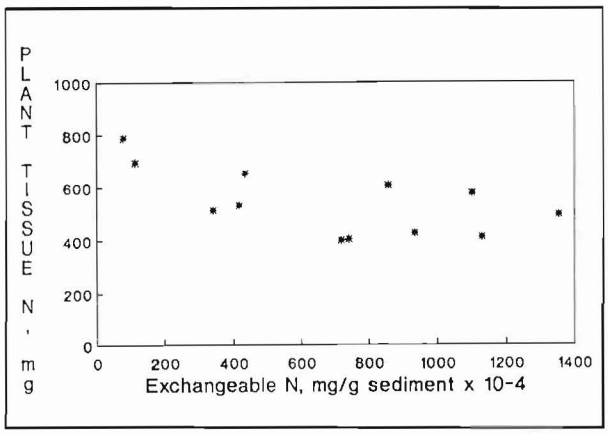

Figure 3. Regression for Hydrilla plant tissue nitrogen as a function of exchangeable sediment nitrogen. Regression equation: Y = 673.0159 - 1923.56 X. P = 0.0284, r square = 0.3956

Nitrogen uptake efficiency under N-limitation reflected allocation patterns for both species in that more biomass was allocated to less nitrogen-"costly" tissues (Table 1). For *Potamogeton*, allocation of biomass was primarily to roots, while *Hydrilla* allocated more biomass to nitrogen-"cheap" shoots. Interestingly, both species incorporated less than half

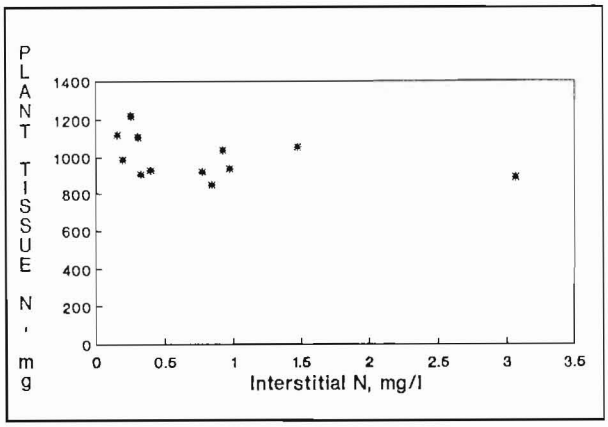

Figure 4. Regression for Potamogeton plant tissue nitrogen as a function of interstitial sediment nitrogen. Regression equation: Y = 1040.7663 - 54.6374 X. P = 0.2071, r square = 0.1539

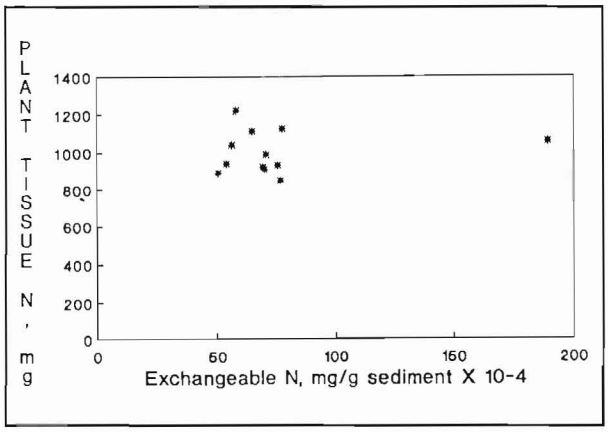

Figure 5. Regression for Potamogeton plant tissue nitrogen as a function of exchangeable sediment nitrogen. Regression equation: Y = 966.4336 + 3979.6268 X. P = 0.6894, r square = 0.0167

Table 1
Uptake Efficiency on Nitrogen-Limited Sediments

	AS % of Biomass	MG N/G	MG P/G	MG K/G
Hydrilla				
Shoot tissue	82	7.168	2.031	20.504
Root tissue	18	9.646	5.038	25.058
Potamogeton				
Shoot tissue	20	9.165	2.725	24.109
Root tissue	80	7.033	4.409	19.103

the phosphorus in shoot tissues as in roots, regardless of biomass allocation patterns (Table 1), while potassium uptake patterns were relatively similar both by tissue and by species.

Under surplus sediment nitrogen conditions, *Hydrilla* allocated even greater biomass to shoots, which had five-fold greater nitrogen uptake (Table 2) compared with N-limited conditions (Table 1). Shoots were nitrogen-"costly" for *Potamogeton* as well, but allocation patterns shifted from those displayed under N-limited growth. Phosphorus uptake in shoot tissues increased relative to N-limited conditions such that roots and shoots of both species incorporated approximately 5.34 mg P/g tissue (Table 2). Uptake of potassium was greater in shoots of both species (Table 2), but particularly so for *Hydrilla*, which doubled shoot potassium compared with N-limited growth (Table 1).

Table 2
Uptake Efficiency on Nitrogen-Surplus Sediments

	AS % of Biomass	MG N/G	MG P/G	MG K/G
Hydrilla				
Shoot tissue	95	37.869	5.684	52.596
Root tissue	5	23.479	5.329	12.859
Potamogeton				
Shoot tissue	86	37.357	5.118	32.931
Root tissue	145	15.529	5.262	21.699

Biomass-weighted elemental costs indicated that nitrogen cost was similar for both species under N-limited conditions, while *Potamogeton* effectively accumulated nearly 25 percent more phosphorus (Figure 6). However, although far more nitrogen was accumulated when it was plentiful in sediments, *Potamogeton* no longer exhibited substantially more P accumulation, and *Hydrilla* had become more effective at K uptake (Figure 7).

Discussion

We have shown that native species such as *Potamogeton* can effectively outcompete inva-

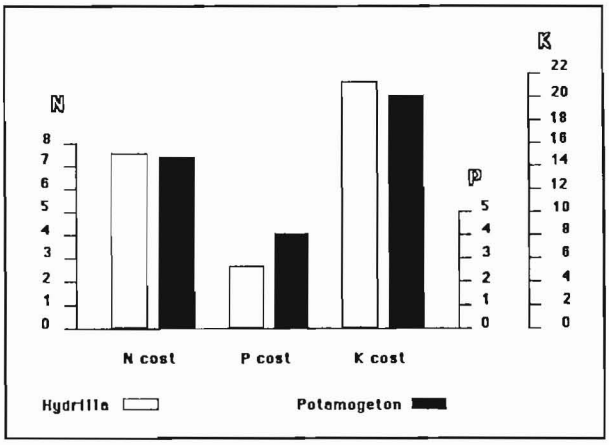

Figure 6. Weighted elemental cost (mg/g plant) under N-limited growth

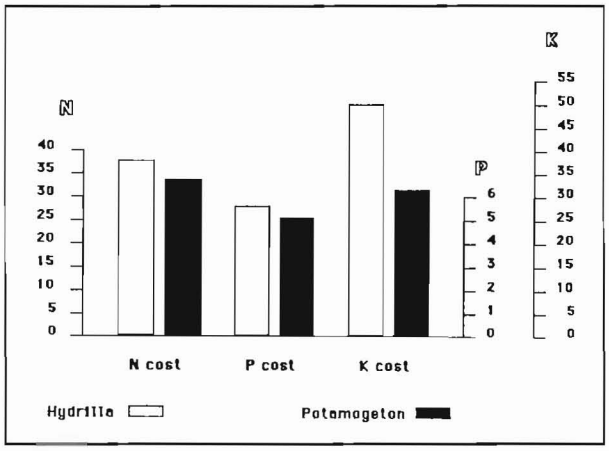

Figure 7. Weighted elemental cost (mg/g plant) under N-surplus growth

sive species such as *Hydrilla*, particularly when sediment nitrogen is limiting. Results from regression analyses raise the possibility of a nitrogen-depleting mechanism in *Potamogeton* that may be absent in *Hydrilla*. For example, roots of *P. perfoliatus* release oxygen to sediments (Kemp and Murray 1986); such oxygen release can substantially reduce rhizosphere sediment redox potentials (Carpenter, Elser, and Olson 1983), resulting in the conversion of ammonium nitrogen to nitrate and, ultimately, nitrogen gas, which may be lost from sediments via the plant lacunar system (Reddy, Patrick, and Lindau 1989). Sediment nitrogen pools may thus be reduced by as much as one-third (Reddy and Patrick 1984). If a similar mechanism functions in

P. americanus, it may permit more effective N-use by this species, as well as prevent N-access to potential competitors by facilitating sediment N-losses. Clearly, this is an area requiring additional study.

The observed decline in competitive superiority of *Potamogeton* over *Hydrilla* with increased sediment fertility may be related to shifts both in allocation patterns and elemental uptake efficiencies. In more fertile sediments, *Hydrilla* simply magnifies its existing pattern of allocation to shoot tissues; *Potamogeton*, however, must effectively switch from a root allocation strategy to one of shoot allocation. This, coupled with differences in elemental uptake efficiency, suggests that under low sediment nitrogen conditions, *Potamogeton* is successful since it more efficiently uses sediment nitrogen, and to a lesser extent, phosphorus. However, under N-surplus conditions, *Potamogeton* no longer enjoys an N-use advantage; it is faced with a need to switch its biomass allocation pattern, and it must contend with *Hydrilla* that, no longer N-limited, comparably uses phosphorus and more effectively utilizes potassium from the water column. The complex sediment N/P dynamics exhibited by *Potamogeton* represent an area for further research.

Recent reviews underscore the importance of fundamental nutrient kinetic knowledge for macrophytes in systems where various management tools are being considered (Barko, Adams, and Clesceri 1986; Smith, Barko, and McFarland 1991). Management technologies may be grouped as physical (drawdown and bottom barriers), mechanical (harvesting and dredging), chemical (herbicides and plant growth regulators), and biological (pathogens, herbivores, and competitors) (Smith, Barko, and McFarland 1991). In light of the differing advantages and disadvantages of isolated management technologies, it becomes apparent that efforts should be directed away from finding any one best control method, and toward an integrative, holistic, middle ground of macrophyte management.

For example, harvesting late in the growing season may reduce nitrogen and phosphorus loading, but if used earlier, can disrupt sediments, increase turbidity, decrease water quality, and increase fragmentation and regrowth of invasive species over natives. Likewise, early season herbicide treatments may facilitate decreased damage to native species, which often reach maximum growth rates more slowly than invasive species. Plant growth regulators have the advantage of disrupting sediment N/P recycling, thereby preventing reentry of sediment nutrients into the system. Introduction of pathogens and, to a lesser extent, herbivores has the advantage of target specificity, but is technology that requires additional evaluation before being readily available with reduced environmental impact.

We believe that evidence indicates successful use of competitors as one part of an integrative management strategy in aquatic systems where conditions warrant. Whether through reintroduction of commercially obtained native species in small plots of littoral zone areas already harvested or simply use of herbicides such as fluridone sufficiently early in the season to exacerbate a native species' competitive advantage, biological tools as part of a multiple technology control program may minimize liabilities of individual techniques while capitalizing on their respective advantages. Such holistic, system-specific management plans appear more reasonable to an increasingly well-educated and demanding public with highly diverse management goals (Sutton 1991). In the long term, they may be less expensive and, therefore, more effective than any one individual management strategy.

Conclusions

In conclusion, we have demonstrated the ability of a native species, *Potamogeton americanus*, to outcompete an invasive species, *Hydrilla verticillata*. We have further provided evidence that such complex biological interactions may represent a viable component of specific management scenarios. We

recommend additional studies to elucidate the mechanisms governing these competitive interactions, as well as to ascertain the contribution biological interactions may make as part of a specific management program.

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Competition Among Introduced and Native Species

by
R. Michael Smart[1]

Introduction

Only a few of the many species of submersed aquatic plants ever cause problems requiring aquatic plant control. Two of the most problematic of these are *Hydrilla verticillata* (*Hydrilla*) and *Myriophyllum spicatum* (Eurasian watermilfoil). These species, as is characteristic of weedy species in general, develop a dense mat, or canopy, at the water surface. This surface mat causes a variety of problems including the following: diminished exchange of gases, water, dissolved substances, and organisms; interference with navigation and other water use; degraded water quality; and reduced habitat diversity.

Many native, nonweedy species exhibit a different growth form. In these species, typified by *Vallisneria americana* (*Vallisneria*), biomass is more uniformly distributed throughout the water column. These species, since they do not develop an extensive surface mat, generally do not cause significant aquatic plant problems. In fact, the presence of nonweedy submersed aquatic plants enhances aquatic habitats, providing both food and shelter for invertebrates and fish.

Our objective as managers of aquatic resources should be not only to control the spread of weedy species, but also to protect, preserve, and promote native, nonweedy aquatic plant communities. By following aquatic weed control operations with the planting of competitive species, we might slow or even prevent the reinvasion of the area by weedy species. This would prolong the effectiveness of the control operation, perhaps resulting in a lower overall cost of management.

The objective of this work unit is to evaluate methods for promoting the establishment and persistence of native, nonweedy species in an attempt to slow the spread of exotic, weedy species. As previously described (Smart 1992), the nature and complexity of competitive interactions require that research in this work unit be conducted on several temporal and spatial scales. Current research includes small-scale, short-term, greenhouse tank studies, as well as large-scale, long-term, pond studies. Future research will also encompass studies conducted at intermediate scales as well as field demonstrations.

Previous research in this work unit has shown that *Vallisneria* can be an effective competitor against *Hydrilla*, particularly when *Vallisneria* is able to fulfill its high sediment nitrogen (N) requirement (Smart 1992). If *Vallisneria* were given an advantage in terms of access to sediment N, its competitive edge might be increased. One way to accomplish this would be to give *Vallisneria* an earlier start to allow it a period of time to accumulate N prior to the introduction of competing *Hydrilla*. In this update, I examine the influence of a period of preemption by *Vallisneria* on competition between it and invading *Hydrilla*. Results of both small- and large-scale studies will be briefly examined, and the future direction of research in this work unit will be discussed.

Small-Scale Studies

Methods

The experimental design included two sets of treatments—one in which both species

[1] U.S. Army Engineer Waterways Experiment Station, Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

were planted at the same time (competition without preemption), and one in which *Vallisneria* is allowed a 4-week period of preemption. Each set included the two species growing alone and in a mixture (both species planted in the same container). In the preemption portion of the experiment, we examined the growth of *Vallisneria* alone and as affected by *Hydrilla* invasion. We also examined the growth of invading *Hydrilla* as affected by the presence or absence of established *Vallisneria*.

The experiment was conducted in 1,200-L fiberglass tanks in a greenhouse facility at the Lewisville Aquatic Ecosystem Research Facility (LAERF). The experiment was conducted at high levels of both inorganic carbon supply (10x ambient CO_2) and high sediment fertility (fresh Lewisville pond sediment). Based on the earlier study (Smart 1992), these conditions were not expected to selectively favor either of the two species. Each tank contained fifty-four 1-L pots planted with two *Hydrilla* apical shoots (15 cm length), two *Vallisneria* plants, or one of each species (competition mixture). An additional two tanks of 54 pots were planted with one *Vallisneria* plant per pot to serve as the preemption treatment and the monospecific reference. After 4 weeks, an additional tank of 54 pots was planted with one *Hydrilla* apical shoot per pot to serve as the *Hydrilla* monospecific reference, and the 54 pots previously planted with one *Vallisneria* plant at the beginning were also planted with one *Hydrilla* apical shoot each (preemption mixture). After 9 weeks, we harvested 12 replicate containers from each treatment for determination of shoot and root biomass.

Results

Hydrilla and *Vallisneria* produced similar quantities of biomass when the two species were grown alone (Figure 1). The level of biomass production was also similar to that attained under similar growing conditions in an earlier study (Smart 1992). When the two species were planted together at the same time (no preemption), *Hydrilla* completely dominated the mixture (Figure 1). This result was surprising since *Vallisneria* had been competitive in the earlier work.

During this experiment, it was apparent that the *Vallisneria* transplants were slow to

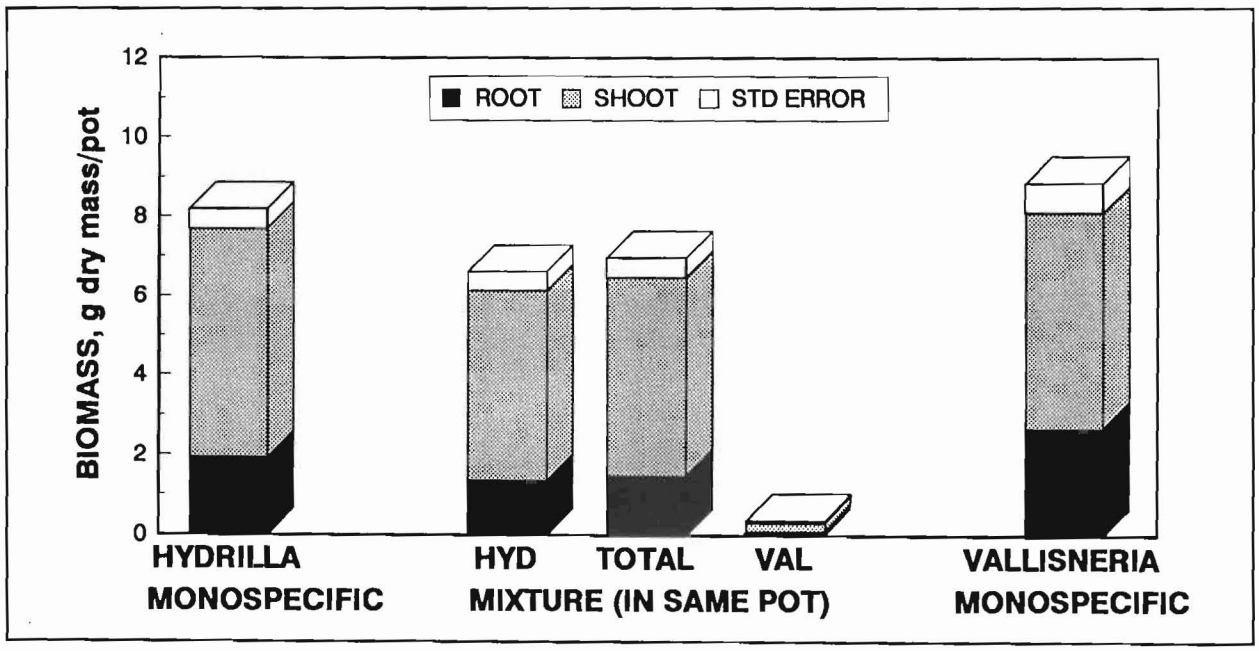

Figure 1. Root, shoot, and total biomass of Hydrilla and Vallisneria grown monospecifically and in mixture in the competition portion of the experiment. Values are means of 12 replications

establish. In the earlier experiment, *Vallisneria* had been transplanted from dormant winter buds rather than transplants. Growth of the *Vallisneria* plants used in the two experiments are compared in Figure 2. In the earlier (U.S. Army Engineer Waterways Experiment Station (WES)) experiment, *Vallisneria* planted as winter buds grew rapidly from the start, whereas the transplants used in the present study produced little growth through the first 4 weeks. Although the plants used in the two experiments achieved equal total biomasses at the end of their respective growth periods, the difference in early growth could account for the differences in competitive ability between the two propagule types. Research with other species of submersed aquatic plants has shown that propagule type can exert a strong influence on subsequent growth (Spencer 1987) and competitive ability (Spencer and Rejmanek 1989).

A 4-week period of preemption by *Vallisneria* increased its competitiveness. The single *Vallisneria* plant used in each monospecific pot produced less total biomass (Figure 3) than did the two plants used in the competition reference (Figure 1). However, the effect of reducing the *Hydrilla* planting density from two to one and delaying planting for 4 weeks was even greater. In the preemption mixture, *Vallisneria* outcompeted *Hydrilla*, indicating that an earlier start allowed it to preempt available resources (presumably sediment N). Later arriving species enter an environment that is not as favorable as it was for the earlier arrivals.

The results of this and earlier greenhouse studies (McCreary, McFarland, and Barko 1991; Smart 1992) indicate that native species can outcompete *Hydrilla*, at least under certain conditions. Larger scale and longer term studies are needed to document the occurrence under more natural "field" conditions.

Large-Scale Studies

The experimental objective of the large-scale study was to determine the abilities of populations of the native species *Vallisneria*

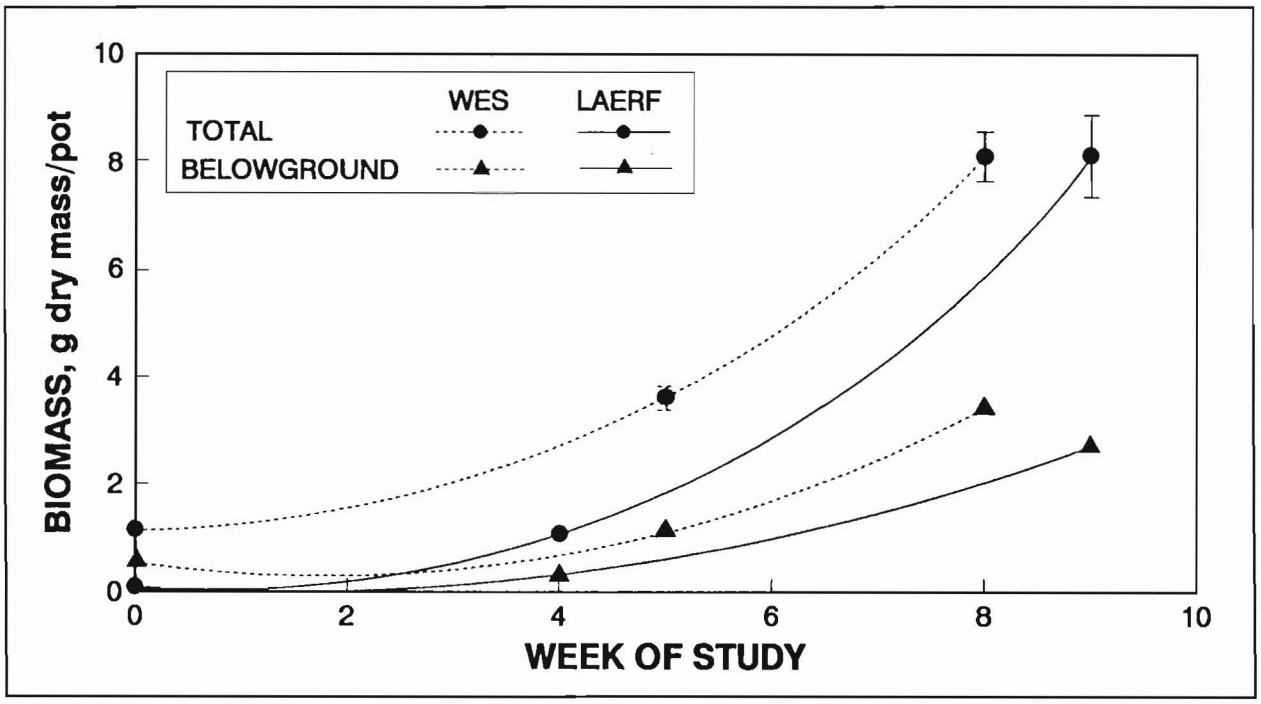

Figure 2. Total and belowground biomass of Vallisneria grown monospecifically under similar environmental conditions in two separate experiments. The WES experiment used winter buds obtained from a commercial collector in Wisconsin, and the LAERF experiment used transplants collected from a reservoir in Texas. Values are means and standard errors based on 10 (WES) or 12 (LAERF) replications

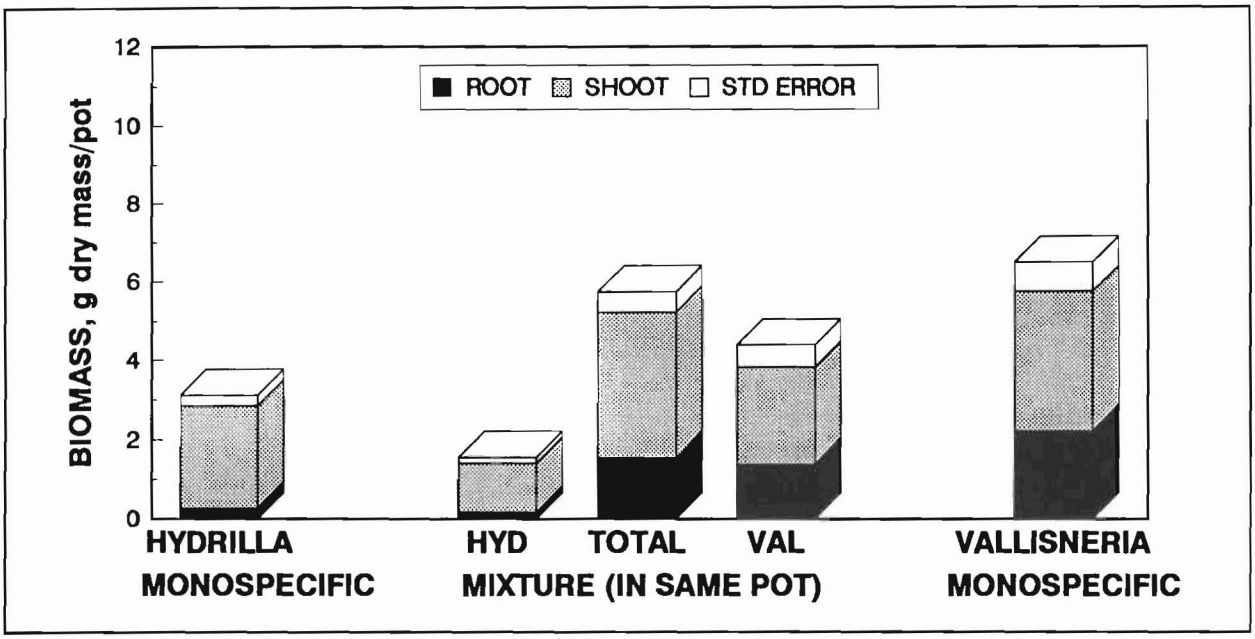

Figure 3. Root, shoot, and total biomass of Hydrilla and Vallisneria grown monospecifically and in mixture in the preemption portion of the experiment. Values are means of 12 replications

and *Potamogeton nodosus* (American pondweed) to resist invasion by *Hydrilla*. The experiment required observation of plant responses within permanent plots over an extended period. A 1-acre pond at the LAERF was selected for the study.

Methods

The plots were laid out as a series of 96 hexagonal cells in a 1-acre pond. Species were assigned to cells in a regular pattern so that each of the three species was surrounded by three cells of each of the other two species. Prior to planting the experiment, the entire pond bottom was rototilled and treated with metam sodium to reduce numbers of spores and seeds of endemic populations of *Chara vulgaris* (*Chara*) and *Najas guadalupensis* (*Najas*), respectively. The metam sodium treatment was also expected to eliminate tubers of *Hydrilla* remaining from the previous study (Smart 1992). The sediment in every other cell of each species was fertilized with nitrogen (ammonium sulfate), which has been shown to potentially limit growth in these ponds and has also been shown, in short-term experiments, to affect the outcome of competition.

Plants of *Vallisneria* and dormant winter buds of *Potamogeton* were planted in their designated cells in June 1991 on 30-cm centers as the pond was being filled. The remaining cells were left unplanted. These cells were to be planted with *Hydrilla* after a 1-year period of preemption by the two native species.

Frequency of occurrence of each species was determined by point-intercept sampling. A series of parallel transects were established in three directions across the pond so that each of the 96 cells was crossed by three transect lines that intersected at the center, forming 60-deg angles. Every 50 cm along the transects, a sampling point was established, and the species of any plants that were touched by a vertical line dropped from this point was recorded. Each cell was sampled at 27 points on each sampling date. Data on each cell were expressed as percent frequency of occurrence for each species. Sampling was conducted in July, August, and September 1992.

Results

In spite of the treatment with metam sodium, we observed growth of all three unwanted

species, *Najas*, *Chara*, and *Hydrilla*. Although the treatment greatly reduced numbers of propagules, the net result was only to delay the growth of the unwanted species. During the summer of 1991, we attempted to control the growth of the *Hydrilla* by hand removal. This proved to be ineffective. In an attempt to slow the spread of *Hydrilla* and provide a period of time for *Vallisneria* and *Potamogeton* to become established, the cells to be planted with *Hydrilla* were covered with a geotextile landscaping fabric in late summer 1991. By this time, *Hydrilla*, as well as *Vallisneria* and *Potamogeton*, had become well established in the pond. *Hydrilla* had invaded much of the unplanted area beyond the cells as well as some of the *Vallisneria* cells. The desired period of preemption was therefore not realized.

The geotextile fabric was removed from the unplanted cells in late spring 1992, and sampling commenced in July.

The greatest diversity of species was found in the "bare/*Hydrilla*" cells (Figure 4). Since these cells had been covered with geotextile to prevent growth, it is not surprising that, initially, they would have the greatest diversity since no species had been allowed to dominate. Between July and September, both *Hydrilla* and *Potamogeton* invaded these cells and increased in percent frequency. *Chara* and *Najas* declined during the sampling period as dominance by *Hydrilla* and *Potamogeton* increased. *Vallisneria* was present at a very low frequency because of the growth of daughter plants from adjacent *Vallisneria* cells.

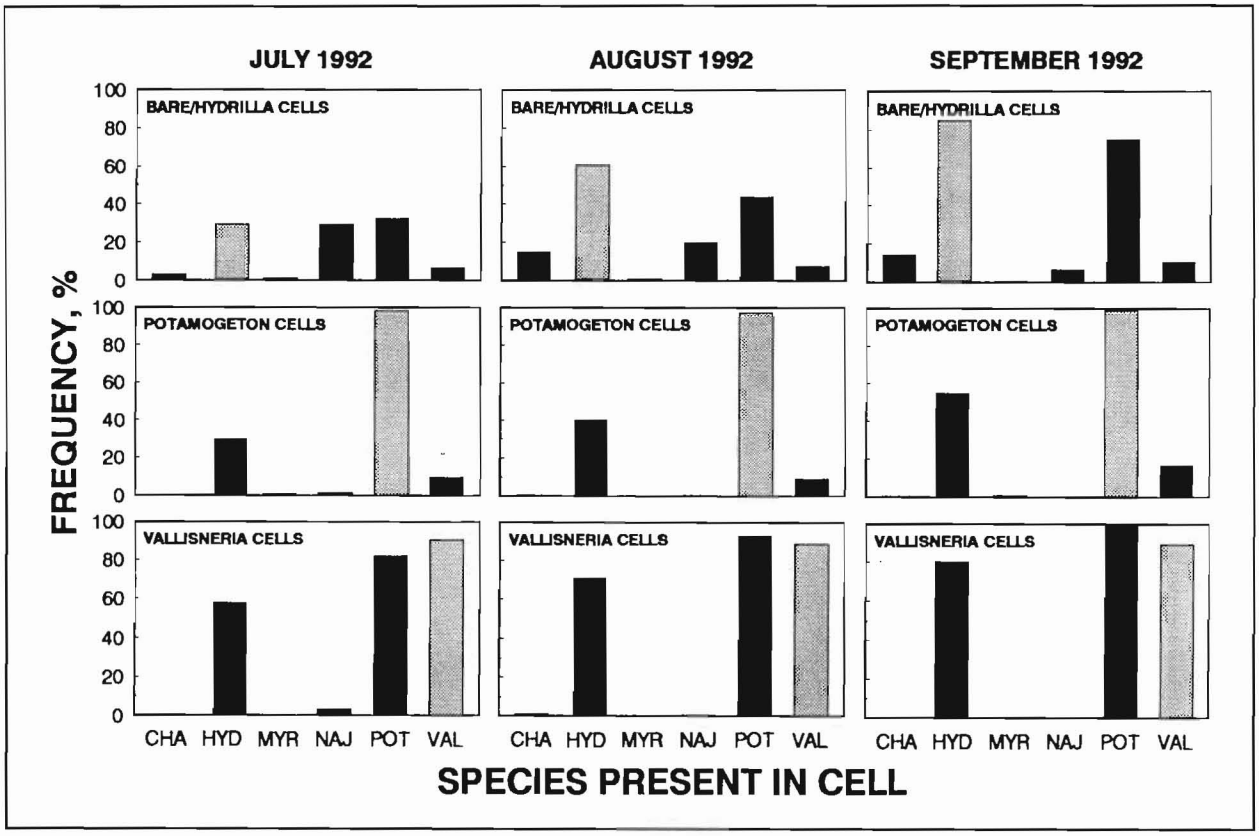

Figure 4. Species composition (percent frequency) of unplanted (Bare/Hydrilla) and planted (Potamogeton or Vallisneria) cells during July, August, and September 1992. Light bars denote planted species. Species abbreviations are as follows: CHA - Chara, HYD - Hydrilla, MYR - Myriophyllum spicatum, NAJ - Najas, POT - Potamogeton, and VAL - Vallisneria

Potamogeton cells were strongly dominated by *Potamogeton* throughout the sampling period. *Hydrilla* did invade these cells, however, and had attained greater than 50-percent frequency by the September sampling.

Vallisneria cells were initially dominated by *Vallisneria*; but by the September sampling, these cells were dominated more by *Potamogeton*. *Hydrilla* had been present in many of the *Vallisneria* cells from the previous season and invaded others during the sampling period. Most of these *Vallisneria* cells contained all three species, with *Potamogeton* and *Hydrilla* in the canopy and *Vallisneria* in the understory. Although its cells were readily invaded by *Hydrilla*, there was no indication that *Vallisneria* was declining. Since *Vallisneria* is generally considered to be more of a higher successional species than *Hydrilla*, it may be that over time it will outcompete the weedy invader.

During the relatively short time period of this investigation, both *Vallisneria* and *Potamogeton* were persistent in the presence of invading *Hydrilla*. Although this finding and those of earlier greenhouse experiments indicate that *Vallisneria* and *Potamogeton* have a high potential competitive ability, longer term monitoring of these experimental populations will be required to determine if this potential can be realized in nature.

Future Research

Small-scale studies

During fiscal year (FY) 1993, we will continue to conduct small-scale, short-term greenhouse studies to obtain additional information on the factors and mechanisms involved in competition between submersed aquatic plants. In addition to providing valuable and needed scientific information, the results of these studies help to direct our longer term efforts.

Intermediate-scale studies

During FY92, we initiated an intermediate-scale, two-season study of competition using containers of plants placed in a pond environment. This study was designed to examine the importance of seasonal events and phenological stage of development on the mechanics of competition. Specifically, we are interested in the relative competitive advantages of different physiological mechanisms of perennation (overwintering), such as the onset and duration of the dormant period and the ability to sequester reserves in dormant tissues. This study also includes a preemption component, and we are considering the relative competitive effects of different periods of preemption.

Large-scale studies

We will continue to monitor the long-term pond study, observing the spread of *Hydrilla* in bare and planted cells. These observations will allow determination of the abilities of *Vallisneria* and *Potamogeton* to resist invasion by *Hydrilla*. Close observation of these experimental populations may reveal important competitive attributes or mechanisms of invasion. These attributes or mechanisms can then be carefully studied under more controlled conditions.

Reservoir-scale studies

We have located adjacent monospecific beds of *Vallisneria* and *Hydrilla* in a reservoir in east Texas. We hope to establish some permanent plots within these naturally occurring populations to monitor any changes in their boundaries or in species composition of the communities. We also hope to initiate a field demonstration employing preemptive establishment of beneficial, native species in a reservoir that is not currently experiencing aquatic weed problems. If this test is successful, this approach may help us delay the occurrence of major aquatic plant problems in new Corps of Engineers' construction projects.

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Competitive Ability of Selected Aquatic Macrophytes in *Lyngbya*-Dominated Littoral Zones of Guntersville Reservoir

by
Robert D. Doyle,[1] *and R. Michael Smart*[1]

Introduction

In recent years, there has been an increasing awareness of the nuisance potential of the filamentous blue-green alga *Lyngbya* in the Southeastern United States (Speziale, Turner, and Dyck 1988; Bowes, Spencer, and Beer 1990). The taxonomy of this organism has recently been clarified by Speziale and Dyck (1992). When well established as a benthic mat, *Lyngbya* fresh weight biomass is reported to be as high as 6 to 7 Kg m^{-2} (Beer, Spencer, and Bowes 1986; Speziale, Turner, and Dyck 1988, 1991); the values are higher than those of most submersed and many emergent macrophyte species. In these densely established areas, *Lyngbya* exists as a monoculture and is a potential competitor to more desirable macrophyte species. Once established, there are currently no generally effective biological or chemical methods for controlling this organism (Dick 1989; Cullimore and McCann 1977; Speziale, Turner, and Dyck 1988). However, preliminary research indicates that cyanophages (Montegue and Phlips 1991), mechanical harvesting (Ritter 1982, cited by Speziale, Turner, and Dyck 1988), grass carp (Zolcynski and Smith 1980), commercial shading compounds (Martin, Martin, and Perez-Cruet 1987), and herbicides (Leland and Carter 1984) are all potential control agents. Also, anecdotal evidence suggests that established macrophyte stands are effective in preventing the establishment of the *Lyngbya* mats (Dick 1989).

Lyngbya's success in becoming established apparently stems from several "opportunistic" traits exhibited by this species such as low light requirements, high temperature optimum, insensitivity to high O_2 concentrations, and the ability to utilize HCO_3 (Speziale, Turner, and Dyck 1988, 1991; Beer et al. 1990). *Lyngbya*'s low light requirement seems especially important. Beer, Spencer, and Bowes (1986) reports light compensation and light saturation levels of only 20 and 150 $\mu E\ m^{-2} \times s^{-1}$, respectively. *Lyngbya* can also exist heterotrophically for up to 1 year in complete darkness.[2] These low light requirements of *Lyngbya* allow it to establish itself and survive long periods at the bottom of macrophyte stands and simply wait for more favorable light conditions provided by some disturbance to the macrophyte community. Dick (1989) provides anecdotal evidence for just such a scenario. *Lyngbya* was present in small quantities in the Crystal River system when the dominant macrophyte *Hydrilla* was wiped out by an unusual saltwater intrusion to the river. In the absence of macrophytes, *Lyngbya* quickly spread throughout the system and soon became a navigational and recreational nuisance.

Lyngbya differs from other filamentous algae primarily in the unusual resilience of the established mats. Unlike most algae, *Lyngbya* is a perennial, with virtually all of the summer biomass accumulation overwintering as a benthic mat (Speziale, Turner, and Dyck 1991). The strength of these mats is derived

[1] U.S. Army Engineer Waterways Experiment Station, Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

[2] Personal Communication, L. A. Dyck, Clemson University.

from the thick, nonliving sheaths that encase the unusually large cells. These sheaths become tangled and bind the filaments together, forming the characteristic mats.

As in other southeastern lakes, *Lyngbya wollei* is perceived to be a major aquatic plant problem in Guntersville Reservoir because of its noxious growth that limits recreational use of the reservoir. In addition, lakeshore property owners object to the strong earthy odors emitted by the mats.

The current research was undertaken for two purposes: first, to continue the evaluation of the establishment and growth of desirable emergent and floating-leaved species of aquatic plants in littoral zones subject to *Lyngbya wollei* infestations, and, second, to examine the potential competition between the *Lyngbya* mats and the vascular aquatic plants for light and sediment nutrients. Our long-term goal is to establish desirable, native species of rooted aquatic plants to compete for sediment nutrients and sunlight. By reducing levels of both of these resources, we hope to reduce the biomass of *Lyngbya* in infested coves and reduce its potential for spreading into currently unaffected areas of the reservoir.

Methods

Experimental design and planting scheme

We selected three emergent and two floating-leaved species for the 1992 studies. Previous research on Guntersville (Smart 1992) has shown that *Eleocharis quadrangulata* (spikerush) and *Scirpus validus* (softstem bulrush) were unsuitable for growth in *Lyngbya*, and these species were eliminated from the experimental design for 1992. *Pontederia cordata* (pickerelweed) was added to the design because of its ability to grow in organic, reducing sediments and the availability of propagules from within the Tennessee River system.

The research presented here was performed at the two *Lyngbya*-dominated sites (Ossa-Win-Tha and Waterfront) and the control site (Boshart Creek) utilized in the previous phase of this research (Smart 1992). Water depths within the 32- by 64-ft exclosures constructed at each site were similar, ranging from 1.7 to 2.5 ft at Ossa-Win-Tha, 1.7 to 2.7 ft at Waterfront, and 1.8 to 3.0 ft at Boshart Creek. Within each exclosure, we established fourteen 4- by 8-ft experimental plots. The experimental design included two replicate plots each of *Justicia americana* (American waterwillow), *Saururus cernuus* (lizard's tail), *Pontederia cordata* (pickerelweed), *Nelumbo lutea* (American lotus), and unplanted controls, and four replicate plots of *Potamogeton nodosus* (American pondweed). Plant species were assigned to blocks along the depth gradients of the exclosures. *Potamogeton* and *Saururus* were assigned to the shallowest depths; *Justicia* and *Pontederia* were assigned to intermediate depths; and *Nelumbo*, *Potamogeton*, and the unplanted controls were assigned to the deepest block.

The plots were planted in March 1992 with plant propagules collected from regional sources. *Justicia* propagules were collected from the reservoir in the fall of 1991 and overwintered in ponds at the Murphy Hill Aquatic Research Facility (MHARF) adjacent to the reservoir. *Saururus* was transplanted as dormant root stock immediately after collection from within the reservoir. *Nelumbo* seeds were collected in the fall of 1991, maintained at room temperature within the laboratory, and planted after 6 hr of acid scarification in concentrated H_2SO_4. *Potamogeton* tubers (winter buds) were collected in February from Cedar Creek Reservoir in Alabama, and kept moist in a refrigerator at 5 °C prior to planting. *Pontederia* was planted from dormant rhizomes collected from Reelfoot Lake, TN, in the fall of 1991 and overwintered buried in sand at the MHARF.

Survival, percent cover, and maximum biomass

The performance of the plants was evaluated every 2 weeks during the growing season by visual inspection from the surface. Parameters

recorded included percent survival and percent surface cover of the planted species and percent surface cover of *Lyngbya*.

In addition to visual observations of survival and percent cover, a single harvest was made at each of the sites in September 1992 to quantify the maximum standing crop biomass within each plot. Three samples (1-ft^2) were taken for each plot; two replicate samples were collected from within the plot and a third from an unplanted area adjacent to the plot. Harvested plants were separated by species and dried to constant weight at 60 °C before weighing. *Lyngbya* biomass, where present, was collected separately from the floating surface mat, from entanglement with macrophyte stems, and from the benthic mat. These collections were summed after weighing to give the total areal biomass of *Lyngbya* within the water column.

Light and nutrient competition

Light attenuation within each plot was measured every 2 weeks during the growing season. Light readings were taken simultaneously from a flat LiCor sensor in air and from a flat, underwater sensor positioned at a known depth within the water column. Multiple depths within each plot were measured; and at each depth, seven separate measurements were taken as the sensor was carefully moved around the plot. This method provided an estimate of both the average light as well as a measure of the variability of light at each depth within the plot.

Finally, we measured profiles of dissolved ammonium and soluble reactive phosphorus (SRP) within the *Pontederia* plots and in adjacent, *Lyngbya*-dominated areas at the Waterfront site. Five sets of measurements were made during the summer of 1992. Each measurement was made by deploying an interstitial water sampler (peeper) containing 12 separate cells. Each cell was filled with nitrogen-sparged, deionized water and covered with a nucleopore membrane (pore = 0.47 μm). For each set of measurements, three peepers were deployed within the *Pontederia* plots and three in the adjacent *Lyngbya* areas to generate a profile that ranged from the overlying water, through the *Lyngbya* mat, through the top organic layer of sediment, and into underlying sandy sediment. After deployment of 10 to 14 days to allow the dissolved nutrients to come to equilibrium concentrations inside and outside each cell, the peepers were retrieved and water collected from each cell. The water samples (18 ml) were acidified with 0.5 ml of 50-percent HCL and shipped on ice to the U.S. Army Engineer Waterways Experiment Station, Lewisville Aquatic Ecosystem Research Facility (WES LAERF) for analysis. Ammonium was measured with an ion-selective electrode and SRP by standard colorimetric procedures.

Results

Survival and percent surface cover

All of the plant species survived well at the Boshart Creek control site, indicating that the transplant methods being employed were acceptable. Particularly impressive was the growth of *Nelumbo*, which by the end of the season, had spread outside the exclosure and covered an area roughly 75 by 75 ft. Within the exclosure, *Justicia* and *Saururus* had about 50-percent survival and 25-percent surface cover; *Potamogeton* and *Pontederia* had 100-percent survival and near 100-percent surface cover throughout most of the season.

The results differed at the *Lyngbya*-dominated sites. *Justicia* and *Saururus* survival and percent cover at these sites was much lower than at the control site, although at least some plants survived until the end of the season within each plot. *Potamogeton* and *Pontederia* both had near 100-percent survival and 100-percent surface cover at the *Lyngbya* sites. *Nelumbo* had excellent survival at the Ossa-Win-Tha site and, by the end of the season, had covered most of the exclosure. At Waterfront, however, *Nelumbo* was completely destroyed by herbivore damage early in the season. We replanted this site, but the seedlings were again consumed by herbivores.

Consequently, we were not able to establish *Nelumbo* at the Waterfront site.

Lyngbya coverage at Ossa-Win-Tha was low in 1992, with maximum surface coverage of only 10 to 30 percent limited largely to the shallower end of the exclosure. At Waterfront, however, *Lyngbya* surface coverage was very high within all of the unvegetated portions of the exclosure after June 1992. Within the unplanted control plots, the *Lyngbya* mats surfaced in June and maintained near 100-percent surface cover during the remainder of the season (Figure 1a). However, the surface mat of *Lyngbya* was effectively excluded from the *Potamogeton* and *Pontederia* plots (Figure 1b, 1c) until October when the macrophyte biomass declined.

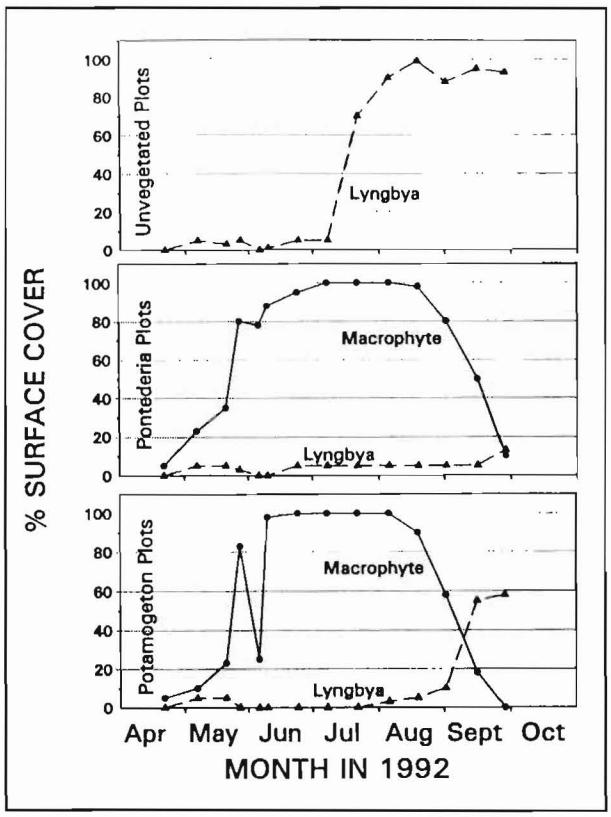

Figure 1. Average percent cover at Waterfront site of Lyngbya and planted macrophytes in unvegetated control plots (top), Pontederia plots (middle), and Potamogeton plots (bottom). N = 2 for each data point

Maximum standing crop biomass

The biomass harvest of September 1992 largely confirmed the conclusions based on visual observations of survival and surface cover. *Justicia* and *Saururus* biomass was low at the two *Lyngbya* sites and averaged only 10 to 40 percent of the biomass at Boshart Creek control site. Biomass of *Potamogeton* and *Pontederia* at both *Lyngbya* sites and *Nelumbo* at the Ossa-Win-Tha site was equal to or greater than that of Boshart Creek.

The biomass harvest also confirmed the visual observation that the macrophytes effectively exclude *Lyngbya* from the planted plots (Figure 2). Within the control plots, *Lyngbya* biomass averaged about 250 g dry mass m^{-2}, while within the vegetated plots, *Lyngbya* biomass was only 50 to 70 g dry mass m^{-2}.

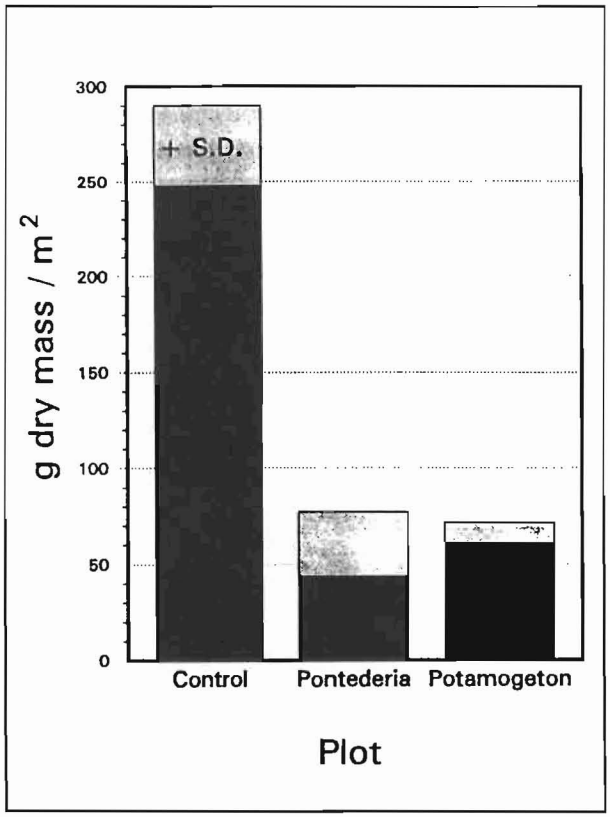

Figure 2. Average (+S.D.) Lyngbya biomass harvested from selected plots at the Waterfront site in September 1992

Light and nutrients

Most of the incident light was attenuated by the emergent or floating leaves of the macrophytes, effectively reducing the light available to the *Lyngbya* mat to only 1 to 5 percent incident light (Figure 3). This shading effect was maintained throughout the season.

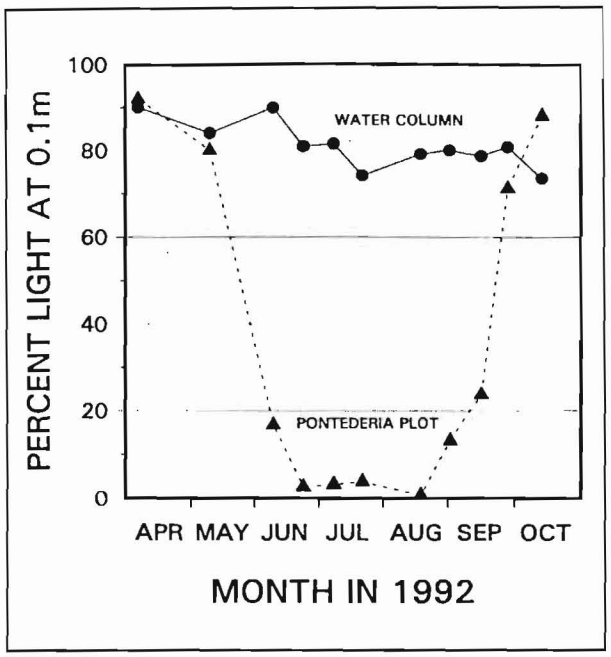

Figure 3. Effect of Pontederia plants on penetration of photosynthetically active radiation (PAR) at the Waterfront site

Ammonium and SRP profiles within the *Pontederia* plots were much different from those within adjacent, unvegetated plots (Figure 4). In the unvegetated plots, ammonium and SRP levels were very high within the *Lyngbya* and organic layer of sediment. Within the *Pontederia* plots, the nutrient levels were much lower, especially for ammonium nitrogen.

Conclusions

Based on the results of the 1991 (Smart 1992) and the 1992 field year, three species have been identified as potentially good competitors with *Lyngbya*. These are *Potamogeton*, *Nelumbo*, and *Pontederia*. Of the other four species tested, spikerush and softstem bulrush were completely incapable of growing in the *Lyngbya*. *Justicia* and *Saururus*, while capable of survival, are not viewed as effective competitors with *Lyngbya*.

Once established, *Potamogeton* and *Pontederia* were able to effectively exclude *Lyngbya* from within the plots. This conclusion is supported by both visual observation of percent surface cover and by biomass harvests during the peak biomass period.

The competitive mechanism that allows the macrophytes to exclude *Lyngbya* from the plots is not completely known. However, data presented here show that the macrophytes are able to reduce the light available to the *Lyngbya* mats to only 1- to 5-percent surface light. Also, *Pontederia* is able to greatly reduce dissolved nutrient concentrations within the sediments and, presumably, the diffusion of nutrients from the sediments into the *Lyngbya* mat. The specific effects of these modifications on the *Lyngbya* mat are unknown as yet, since *Lyngbya* is capable of growth under very low light levels and the *Lyngbya* mat is a potential site of nitrogen fixation.

Future Research

Our research for 1993 will focus on the three species identified as good *Lyngbya* competitors: *Potamogeton*, *Pontederia*, and *Nelumbo*. Our evaluation of these species will shift from the 4- by 8-ft plots to larger (ca. 20- by 30-ft) plots within the exclosures. We will replant *Nelumbo* from seed at the Waterfront site and make supplemental plantings of *Pontederia* at both Waterfront and Ossa-Win-Tha. *Potamogeton* should regrow from dormant winter buds produced at the *Lyngbya* sites. Since this change in scale will require that we vegetate the entire existing exclosures, we plan to add another 32- by 64-ft exclosure to the Waterfront site to act as a *Lyngbya* control.

The *Lyngbya* biomass at Ossa-Win-Tha has declined so dramatically that this site may no longer be suitable for *Lyngbya* research. However, since the cove is being taken over by Eurasian watermilfoil, we will continue

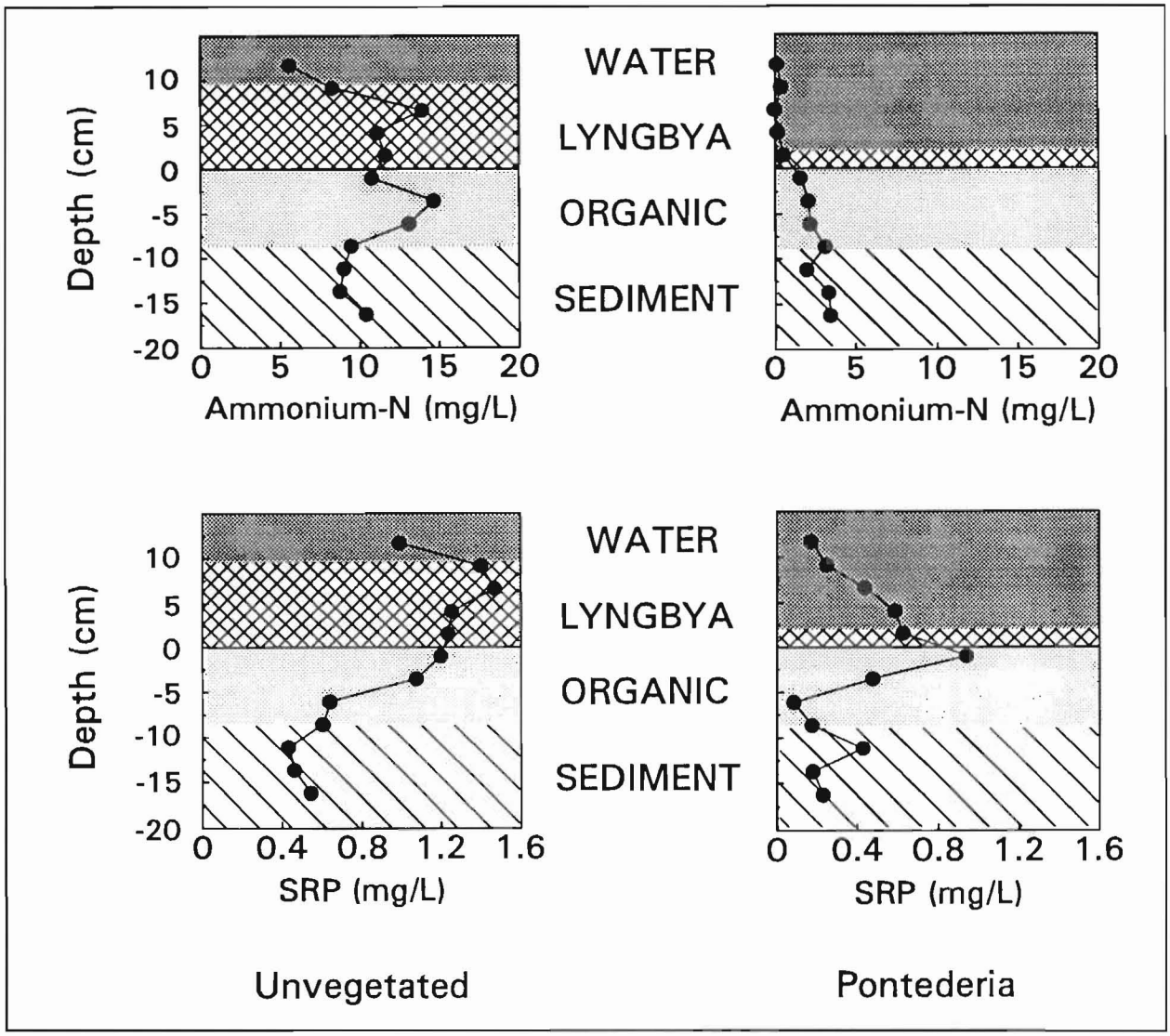

Figure 4. Profiles of ammonium-N and soluble reactive phosphorus (SRP) within the Pontederia plots and within adjacent, unvegetated plots. N = 3 for each data point

monitoring this site, although the focus may change from *Lyngbya* to *Myriophyllum*.

In addition to routine monitoring and maximum biomass harvests, we will continue to quantify the light conditions with the macrophyte stands and to deploy peepers to quantify the dissolved nutrient conditions within the sediments and benthic mats.

Acknowledgments

Numerous people have contributed to this research project. The contributions of the following persons are gratefully acknowledged: Tennessee Valley Authority—Leon Bates, David Brewster, Earl Burns, Mark Dowdey, Stewart Goldsby, Jim Luken, Pete Mangum, Doug Murphy, Wayne Poppe, and David Webb; WES LAERF—David Honnell, Aleida Eubanks, Karen Kuhler, and Cristi Brandon; Clemson University—Larry Dyck and Tim Lee.

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Submersed Macrophyte Invasions and Declines

by
Craig S. Smith[1]

Introduction

Invasions by exotic submersed macrophytes often exhibit a pattern of explosive growth to a relatively stable plateau, followed by a noticeable decline (Figure 1). The maximum abundance and the duration of dominance varies among water bodies because of factors that are at best superficially understood (see Smith and Barko (1990), Smith (1991)). Throughout all phases of the pattern, year-to-year fluctuations in exotic macrophyte abundance may be substantial.

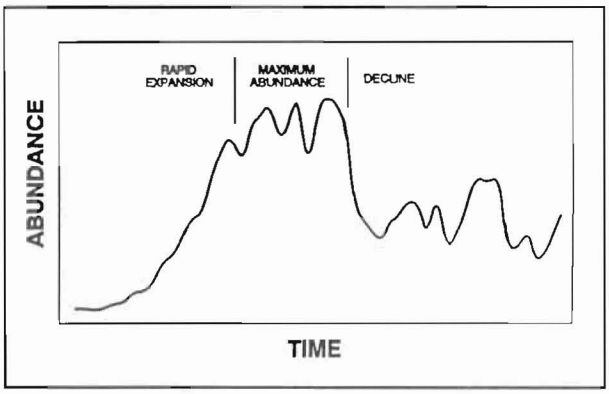

Figure 1. Typical progression of expansion and decline when an exotic macrophyte invades a new location

Fluctuations in submersed plant abundance are evidence of the existence of natural population controls. Once these population controls have been identified, management strategies can be designed to act in concert with them. The goal of the recently initiated research on submersed macrophyte invasions and declines is to identify those factors determining both invasion success and declines and to elucidate associated causal mechanisms.

Invasion and Decline Workshop

A workshop on submersed macrophyte invasions and declines was held in July 1992 in conjunction with the 32nd Annual Aquatic Plant Management Society Meeting and International Symposium on the Biology and Management of Aquatic Plants. Forty-five scientists from 10 countries participated in the workshop. Because the number of participants was large, they were divided into four discussion groups: (a) factors influencing invasions, (b) biotic influences on declines, (c) abiotic influences on declines, and (d) effects of management on invasions or declines. Participants identified a relatively few conclusions concerning invasions and declines and suggested many areas where additional knowledge is needed. Major conclusions of the workshop are summarized below.

Influences on invasion

Influences on invasion differ depending on the scale of analysis. On a continental scale, limits to invasion are determined by climate. On a more local scale, invasions are influenced by the probability of dispersal into a water body and by the match between conditions in the water body and growth requirements of the invading species. Transport of plant fragments by humans is widely believed to be the most important means of dispersal for exotic submersed macrophytes; thus, relative dispersal probabilities probably correlate with factors influencing spread by humans. Few detailed studies have been made of the effects of local site conditions on the rate or success of invasion. Typically, interest in exotic

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species does not develop until after invasion. For most locations, no information exists on preinvasion conditions. Only rarely are observations of the rate and spatial pattern of invasions recorded. As a result, most descriptions of invasion by exotic macrophytes are anecdotal and highly subjective. Systematically collected observations of macrophyte invasions are needed both to identify environmental influences on invasion and to estimate the environmental impact of invasions.

Causes of declines

A variety of possible causes for declines were suggested many years ago (Carpenter 1980), yet little direct evidence that demonstrates the importance of any of them has been collected. Most evidence for specific causal mechanisms is limited to observations that a biotic or abiotic factor seemed elevated or depressed during a decline. Rarely is there a good record of the normal "background" level and variability of the putative cause or an adequate assessment of other potentially important factors.

Abiotic causes have been implicated for widespread declines in submersed vegetation in eastern North America during the late 1980s and early 1990s. Declines in the Potomac, Tennessee, and upper Mississippi Rivers occurred during cool, wet years following droughts. Reduced light availability because of high water levels and high turbidity has been suggested as a possible cause for these declines. Cool spring temperatures may also have contributed, as may reproductive failures caused by the previous, drought-year conditions.

Other declines have been attributed to biotic causes. For example, competition is nearly always implicated in declines of native species following invasion of exotics, despite little evidence of competitive replacement of one submersed macrophyte by another. Herbivores and/or pathogens have been detected in declining populations of submersed macrophytes. The frequency and abundance of these organisms in healthy macrophyte populations is not known, nor has any of them been demonstrated to cause declines in a controlled field experiment. Clearly, field measurements and experiments designed to evaluate the importance of competition, herbivores, and pathogens are badly needed.

Effects of aquatic plant management

Most aquatic plant management is intended to cause the decline of particular target species. A growing body of evidence suggests that other, unintended effects on macrophytes often result from disturbances associated with management. Exotic, invasive macrophyte species typically respond positively to disturbance and often increase following disturbances that produce open habitat for them to invade. Disturbance can also delay or prevent declines by interfering with the mechanisms that cause them. For example, control of exotic macrophyte species might delay plant-induced sediment alterations that reduce growth or prevent herbivore populations from reaching sufficiently high levels to cause decline.

Invasion and Decline Database

Reports from aquatic plant managers around the country and from the published literature are being collected into a database of information on invasions and declines. The database includes information on when and where submersed macrophytes have invaded or declined and ancillary information concerning particular features of these events. Information in the database will be used to characterize invasions and declines and to select field sites for the study of invasions and declines.

Reports of invasions and declines were solicited in a recent Aquatic Plant Control Research Program newsletter (Smith and Barko 1992). Additional forms for reporting invasions and declines are available from the author.

Future Research

In 1993, research will begin to identify environmental factors associated with declines. Sites having increasing, stable, and declining populations of submersed macrophytes will be examined. At each site, environmental conditions and plant status will be evaluated. Environmental measurements will include such variables as turbidity, temperature, sediment composition (e.g., organic content, density, and nutrient content), and the presence and status of other macrophyte species nearby. Plants will be examined for herbivorous insects, pathogens, and dense growths of epiphytes. Plant vigor will be evaluated by measuring in situ rates of physiological processes, such as photosynthesis. Sediments will be collected from selected areas and returned to the laboratory, where they will be bioassayed for their ability to support plant growth. Plants from selected locations will be returned to the greenhouse for growth-rate comparisons under standard conditions.

Locations with long-term records of plant abundance will be examined by mapping spatial patterns of submersed plant invasion and decline. This effort will concentrate on Guntersville Reservoir, where submersed plant distributions have been mapped annually since 1978. Characteristics of areas with fluctuating macrophyte populations will be compared with those of areas supporting stable populations. Areas in which submersed vegetation declined from 1989-1991 will be contrasted with areas in which aquatic vegetation persisted throughout this period.

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Impact of Aquatic Plant Configuration on Fish Distribution and Accuracy of Underwater Observation

by
Eric D. Dibble[1]

Introduction

Investigation of the ecological relationship between fish and aquatic plants is essential to understanding the mechanisms regulating growth, survival, and recruitment in fishes. Aquatic plants are an important component in fish habitat, and aquatic plants offer habitat complexity that serves as important refuge and forage sites for both fish and their prey (Crowder and Cooper 1979). Plant density can decrease growth and condition of large fish by hindering forage efficiency (Betolli et al. 1992), and growth of small fishes can be reduced by limited refuge habitat and low aquatic plant availability (Mittelbach 1981).

Previous research on the control of aquatic plants in aquatic systems has supplied much information on the indirect impact plants have on fish populations, but little is known about how plants directly impact fish at the micro-level. Fish distribution must be accurately measured to access the value of habitat and how fish use aquatic plants. Unfortunately, many of the methods for sampling fish in vegetated habitats are inadequate for gathering these data. Before understanding the mechanism regulating growth and survival in both large and small fishes, direct observations and behavior data is required.

The objectives of this project are to determine plant configuration effects on the distribution and habitat use by fish and to evaluate the accuracy of underwater observations for estimating relative abundance. These data will help determine important distribution patterns of fishes in relation to aquatic plants that affect growth and survival of fishes and supply information to better assess the value of plant habitat.

Methods

Experiments were conducted in ponds (0.25 acre) at the Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX. Two species of submersed plants (*Hydrilla verticillata* and *Potamogeton nodosus*) were planted in three ponds. Each pond contained different spatial configurations of plants: one plant patch (60 by 40 ft), two patches (20 by 50 ft), and four patches (20 by 25 ft) (Figure 1). Plant density was consistent between patches, and water depths were consistent in all three ponds.

Fish were obtained from the Southwest Fish Hatchery, Terrel, TX, and an identical fish assemblage was stocked into each pond. Fish assemblages consisted of 50 juvenile largemouth bass (*Micropterus salmoides*) (50 to 80 mm, total length (TL)), 100 bluegill sunfish (*Lepomis macrochirus*) (25 to 80 mm, TL), 50 orange-spotted sunfish (*Lepomis humilis*) (25 to 80 mm, TL), 20 channel catfish (*Ictalurus punctatus*) (80 to 100 mm, TL), and 200 fathead minnows (*Pimephales promelas*). Twenty fish of each species were obtained and preserved as a reference sample at the time of stocking; and after ponds were drained, all fish were collected.

A diver was used to evaluate the spatial relationship of fish to aquatic plant habitat similar to Dibble (1991). Habitats were defined as open (nonvegetated) and edge (vegetated ecotones). The edge habitats were further characterized by plant species abundance

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

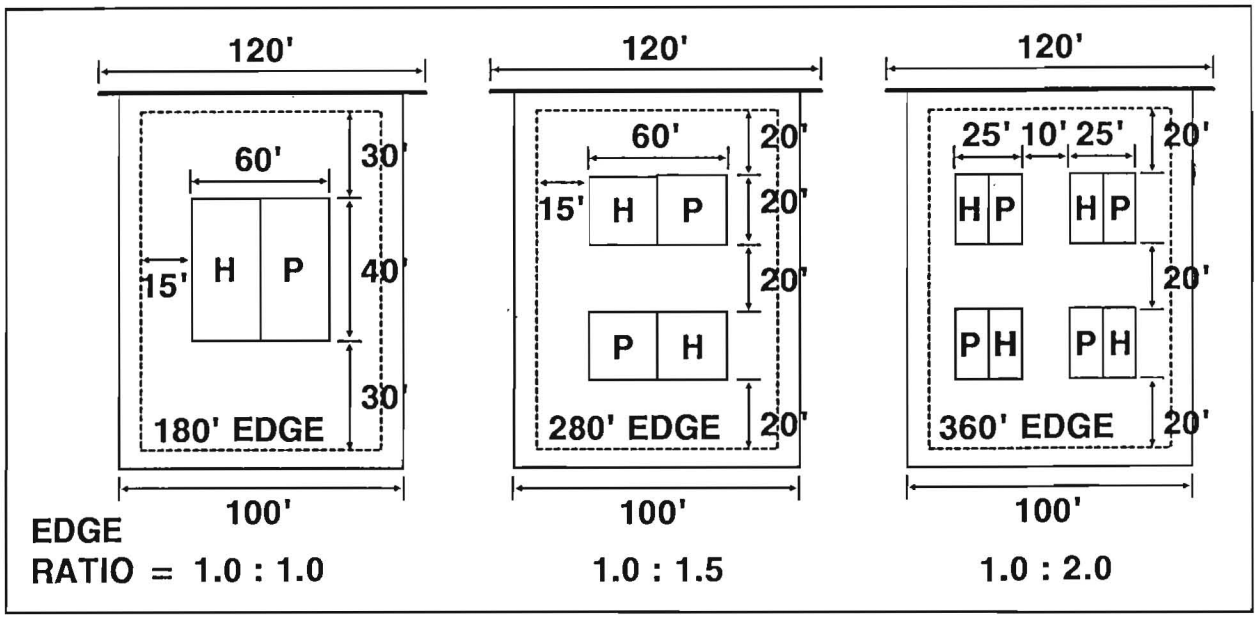

Figure 1. Design of experimental ponds and plant configurations

(80-percent *P. nodosus*, 80-percent *H. verticillata*, and 50-percent mix of each species). Six observation plots were placed according to a stratified random design to assure appropriate sample representation of the different habitats.

Underwater observations were made three times each day starting at 0800, 1200, and 1500 hr during two 3-day sample periods (August 15-18 and September 10-13). Data were recorded on underwater data sheets by the diver. Ten minutes of 15-sec scan intervals were made at each observational plot, and a 5-min acclimation period was allowed at each plot before observations were started. Habitat use was determined by calculating time-budgets from the scan-sample data (Altmann 1974; Poysa 1991).

Results

A total of 1,452 scan samples were taken, approximately 5 hr of underwater observations, and 610 fish observed. Thirty percent of the sample was taken in plots with no vegetation, and the remainder (70 percent) was taken along the edge of the vegetated plots.

Observation data were pooled from all three ponds since no significant difference ($x^2 = 0.62$, df = 2, $P > 0.05$) was noted between the estimated relative abundance of fish in each of the ponds. After the ponds were drained, 388 fish were collected. A significant correlation ($r = 0.89$, $P < 0.001$) was noted between the fish observed and the fish collected (Figure 2). Channel catfish was the only species of fish not observed.

Plant configuration did not affect the microdistribution patterns of fish; no significant differences ($x^2 = 0.24$, df = 2, $P > 0.05$) were noted between microhabitat use by fish in the three ponds. However, the presence of vegetated habitat did influence the amount of time fish used the habitat. A significant difference ($P < 0.001$) was noted between the amount of time fish occupied open and edge habitats (Figure 3). All fish except shad used edge habitat more frequently than open areas with no vegetation. Largemouth bass, bluegill, orange-spotted sunfish, and fathead minnows were observed using edge habitats over 75 percent of the time, whereas shad only used edges 24 percent of the time.

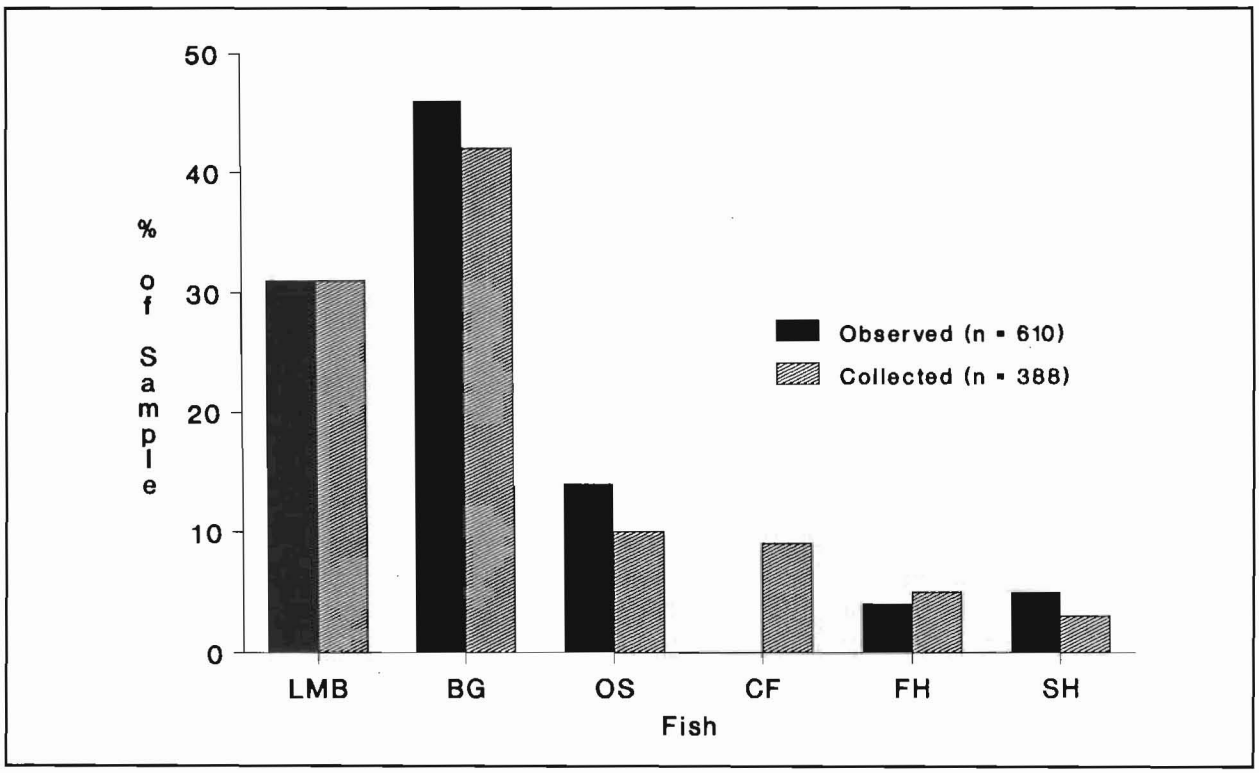

Figure 2. Comparison of fish observerd by divers and the fish collected after experimental ponds were drained

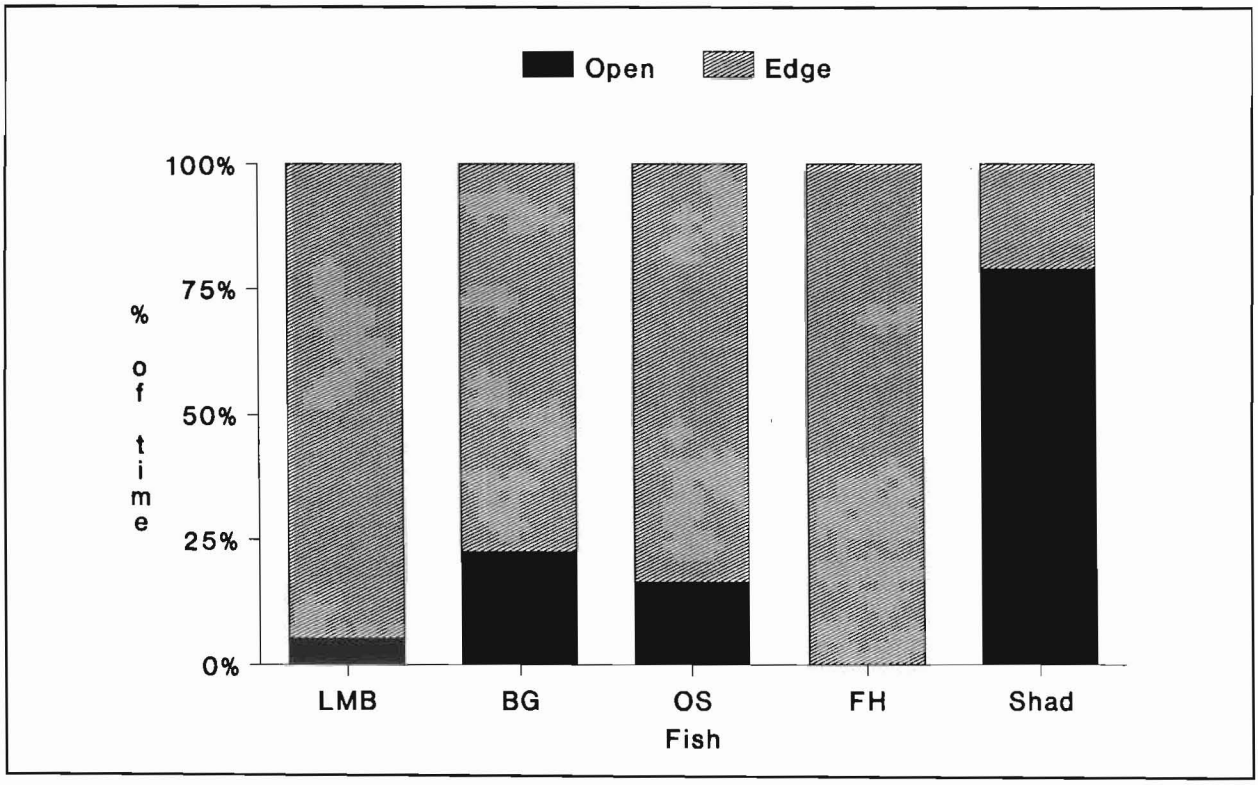

Figure 3. Total time fish spent in open and edge habitats. (LMB = largemouth bass, BG = bluegill sunfish, OS = orange-sided sunfish, FH = fathead minnow, Shad = gizzard shad)

Plant species and abundance also affected fish distribution. Significant differences ($x^2 = 23.8$, df = 2, $P < 0.001$) were noted between the amount of time fish inhabited *Hydrilla*, *Potomogeton*, and mixed patches. Largemouth bass and orange-spotted sunfish used all three habitats approximately the same amount of time (Figure 4). Bluegill and gizzard shad inhabited *Hydrilla* patches less frequently; whereas, fathead minnows spent most of the time there.

Discussion

Except for sampling catfish, the underwater observations were accurate and precise, similar to Northcote and Wilkie (1963), Keast and Harker (1977), and Dibble (1991). Data collected by the diver to determine relative abundance of the centrarchids, shad, and minnows were accurate and represented the actual fish population.

During the day observation periods, catfish apparently used the more dense aquatic plant habitat where diving was inaccessible. Observations were attempted, but no data were collected because plant density was too high and stems hindered visibility. Catfish also tend to be nocturnal in their forage behavior (Robison and Buchanan 1984); since no night observations were made, data were not collected on this species.

Fish spent more time along vegetated habitats and less time in nonvegetated areas. This is in agreement to previous studies that fishes, especially smaller ones, choose to use more complex habitats as sites for refuge and foraging (Crowder and Cooper 1979). Fish also spent much of the time partitioning available habitats in the ponds. Macroinvertebrates and other food items associated with specific plant species may account for this partitioning. Others have demonstrated that specific plant types affect the availability of food items (Gerking 1962; Orth 1977; Virnstein 1977) and that fish partition habitats because of foraging preferences (Werner et al. 1977). Further work is needed on habitat use by fishes and their plant specific food resources.

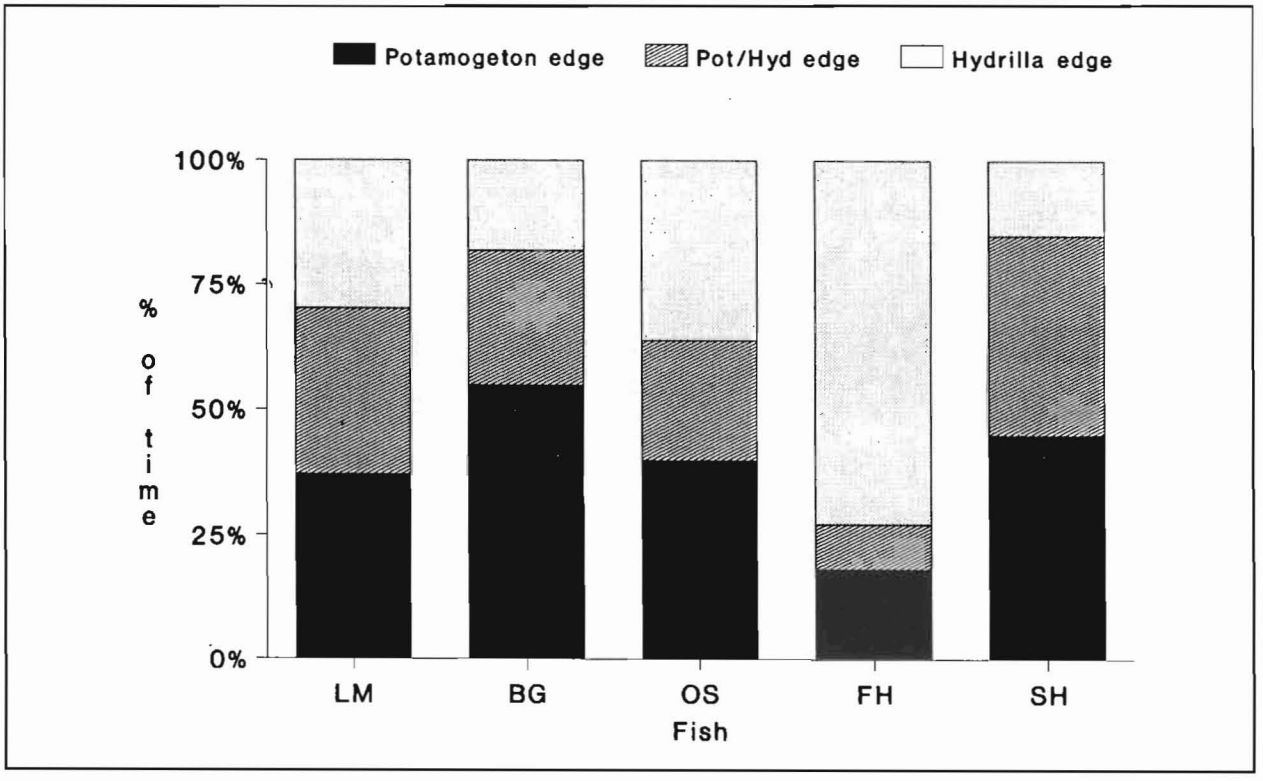

Figure 4. Time partitioning of fish species in plant habitats

plant-edge and open habitats. These results are based on one replicated treatment, and more work is needed to validate these data and to determine the casual mechanisms for the microdistribution of fish in aquatic plant habitats.

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Posters and Demonstrations

Design of a Mesocosm System for Conducting Aquatic Herbicide Evaluations

by
Gary O. Dick,[1] *Kurt D. Getsinger,*[2] *and R. Michael Smart*[1]

Introduction

The Lewisville Aquatic Ecosystem Research Facility (LAERF), located in Lewisville, TX, has recently added a new dimension to its research capabilities in the form of an outdoor mesocosm laboratory. The primary function of this system will be to investigate the use of aquatic herbicides and plant growth regulators at low concentrations to achieve species selective control. The system is designed to complement and expand herbicide concentration versus exposure time studies conducted at the U.S. Army Engineer Waterways Experiment Station.

Design

Tanks

The system consists of 30 fiberglass tanks, each 1.4 m tall and 2.6 m in diameter, with a working volume of approximately 6,500 L. Each tank is molded in one piece with an open top and is finished with an interior light blue, smooth gel-coat. Tanks are arranged in a 5 by 6 configuration with adequate space between them for vehicular access and are set on individual 2.7- by 2.7-m concrete pads for support and stability. Tanks can be independently filled and drained, and barriers may be installed to divide each tank into two or more sections of equal volume.

Reservoir

Water is supplied to the tank facility from a 1,400,000-L synthetic rubber-lined reservoir. Reservoir water quality is adjusted by adding agricultural grade aluminum sulfate (90 kg), which precipitates phosphorus and induces flocculation of organic and clay colloids suspended in the water column. Floc materials are then removed by two sand filters that recirculate reservoir water at a rate of 800 L/min, or about once per day. Alum treatment reduces concentrations of orthophosphates to extremely low levels, thus impeding algae growth in both reservoir and tanks. Full reservoir volume is maintained with gravity-fed, nylon mesh-filtered (100 μm) Lewisville Lake water. A specially designed float valve/standpipe system replenishes water lost because of usage and evaporation, but does not continuously dilute treated reservoir water.

Fill system

An access pipeline associated with the sand filtration system allows operators to divert filtered reservoir water to the tanks. Each tank is supplied water through a 2.54-cm-diam polyvinyl chloride (PVC) fill pipe positioned over the tank's top edge. Flowmeters and flow control needle valves are installed in-line, and flow rates can be regulated to ±0.5 L/min. Maximum flow rate is 56 L/min, and a tank requires about 2 hr to fill. All 30 tanks can be filled simultaneously yet independently in about 5 hr.

Circulation system

Air pressurized by a regenerative blower is supplied to tanks through 1.27-cm-diam PVC pipes and brass valves. An airlift pipe constructed of a 1-m-long, 3.81-cm-diam PVC pipe attached to a flat 30- by 30-cm PVC base

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[2] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

is placed in the center of each filled tank. Intake holes located near the bottom of the airlift pipe allow for water circulation when air is supplied to an airstone positioned inside the pipe. This system produces enough water movement to maintain an evenly mixed water column.

Drain system

Each tank is drained through a 5.08-cm-diam female-threaded fitting molded into the tank's bottom. When filling, a standpipe is installed to plug the drain, compensate for excess water addition (primarily from rainfall), and permit operators to flush tanks without changing water levels. Standpipe construction includes a threaded cap and "T" in its middle to allow half-drawdowns for work in tanks when submersed plants are present and need to be maintained (stocking, partial harvests, etc.). Complete draining is achieved by removing the standpipe. With the exception of the standpipe, all drainage plumbing is underground. Because of the weight of a filled tank (approximately 6,350 kg) and the nature of the soils at the facility, aboveground tank plumbing is attached to belowground plumbing with a rubber "boot," which compensates for shifting of tanks or support pads during wetting and drying cycles without damage to tanks or the plumbing system.

Collection pond

Each tank drainpipe flows into an underground common line, which empties at a lower elevation (about 1.5 m) into a clay-lined collection pond. The pond measures 16 by 16 by 1 m deep, and with a capacity of 225,000 L can receive the entire volume of water held in all 30 tanks (200,000 L). The function of this pond is to hold treated water for detoxification of residual herbicides when deemed necessary.

Wetland cell

The collection pond can be drained through a standpipe into a lower elevation (about 1 m) wetland cell constructed for long-term retention of detoxified water. The wetland cell encompasses approximately 0.2 ha in surface area and is contained by a 1-m-high by 3-m-wide earthen berm. The cell has been populated with flood tolerant species, primarily cattail, by periodic flooding with reservoir water.

Growout pond

A pond adjacent to the mesocosm system has been reconfigured for growout of study plants. The deeper end of the pond bottom was graded to reduce slope and a 15.25- by 18.3-m concrete slab was constructed to serve as a stable surface for setting containers of sediment. The growout pad is designed to hold up to 4,000 each 20-cm-diam by 17-cm-tall plastic sediment pots (volume = 4.7 L). Propagules of test plant species can be planted and the pond flooded to a depth similar to that of the tanks (about 1.25 m). A ramp from the levee crown to the growout pad gives researchers access to the planted pots for transportation to the study tanks.

Sediment prep pad

A 4.9- by 6.1-m concrete pad edged with concrete-filled cinderblocks was constructed for large-scale sediment preparation. Each study is expected to require about 5,000 L of sediment (1,000+ pots), and the pad was designed to hold about 7,500 L of sediment. Generally, sediment is sterilized and fertilized on the pad and then mixed thoroughly with a tractor PTO-driven rototiller. After preparation, sediment is apportioned into pots that are then transported to the growout pad for planting.

Support laboratory

A small metal building erected at the site functions as a working laboratory for processing study harvests and storage of study materials. Freezers, drying ovens, and balances are available in this building for water sample storage and plant drying and weighing.

Function

Plant growth study

A pilot study was conducted on the tank facility during spring and summer of 1992 to test various system functions and ascertain growth of plants in tanks relative to growth in LAERF pond water.

The reservoir was filled with lake water, amended with alum, and sand filtered throughout the study period. Tank standpipes were installed, and tanks were filled to check for plumbing leaks. After inspections and minor repairs, tanks were drained to assess the drainage system. Once satisfied with the performance of all plumbing systems, four tanks were refilled and divided into quarters with 1.2-m-deep 0.64-cm-mesh netting.

Eight 4.7-L plastic pots each of eelgrass (*Vallisneria americana*) and American pondweed (*Potamogeton nodosus*), and 16 pots of Eurasian watermilfoil (*Myriophyllum spicatum*) were placed separately in three tanks. An additional six pots of each species (12 for *M. spicatum*) were placed separately in 1.4-m-diam by 0.25-m-tall tubs set in a pond maintained at a depth of 1.2 m.

After 6 weeks, six pots of pond-grown *M. spicatum* and one quadrant (eight pots) of *M. spicatum* from each planted tank were harvested and dried in a convection oven at 60 °C for 48 hr to ascertain dry biomass. After 12 weeks, all remaining pots were harvested from tanks and pond, then dried and weighed. Growth of the three species was somewhat variable and may have been influenced by small sample sizes (Figures 1-4). However, no species from the pond had outgrown those from the tanks. Duncan's Multiple Range Tests performed on dry biomass of each species indicated no significant differences between tank- and pond-grown plants except for *V. americana*, which had accumulated greater biomass in tanks. These results indicate that growth was not adversely affected by placement in tanks and was, in most cases, similar to growth observed in the pond.

Water quality

Hydrolab data sondes were deployed in the three planted tanks and an unplanted tank periodically during the plant growth study to measure water temperature, pH, and dissolved oxygen for comparison with data taken from ponds (Figures 5-7).

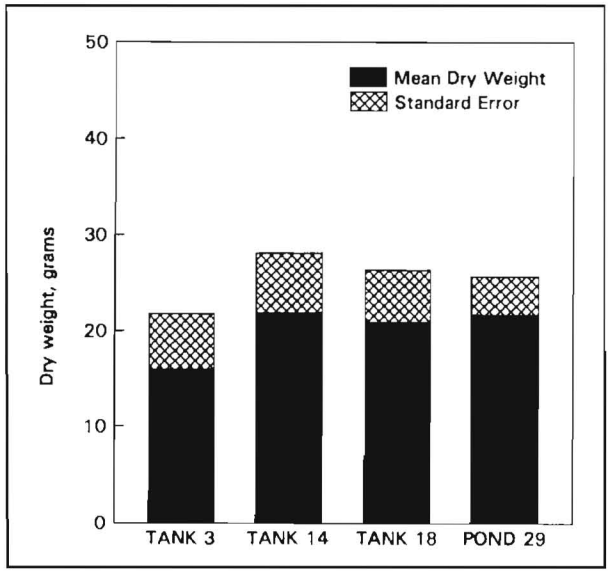

Figure 1. Eurasian watermilfoil (Myriophyllum spicatum) biomass after 6 weeks growth in tanks and pond

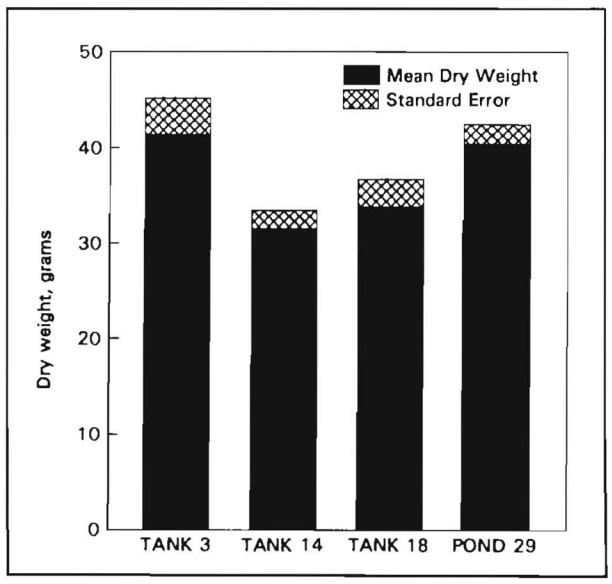

Figure 2. Eurasian watermilfoil (Myriophyllum spicatum) biomass after 12 weeks growth in tanks and pond

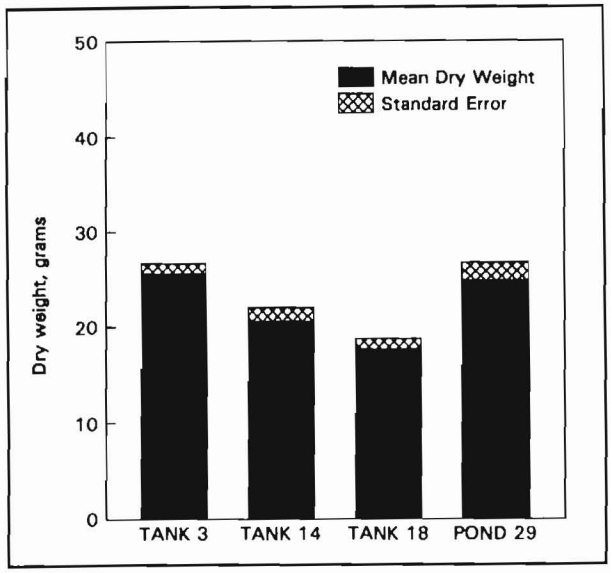

Figure 3. American pondweed (Potamogeton nodosus) biomass after 12 weeks growth in tanks and pond

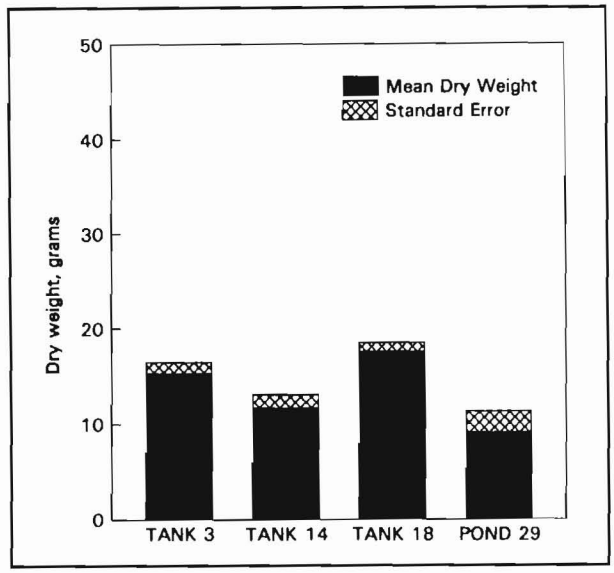

Figure 4. American eelgrass (Vallisneria americana) biomass after 12 weeks growth in tanks and pond

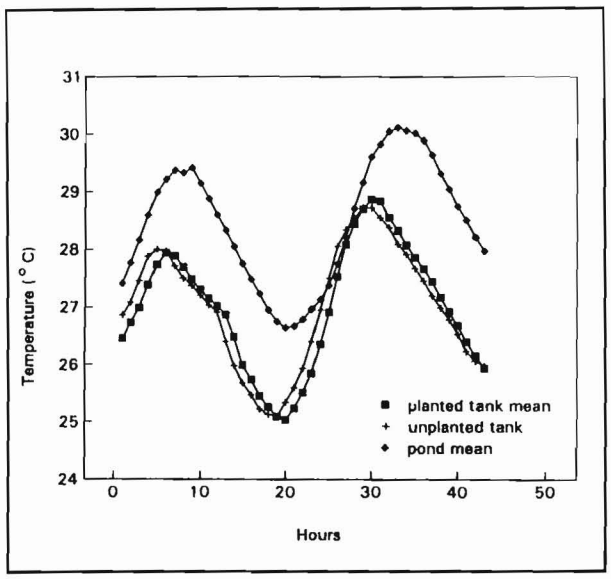

Figure 5. Hourly temperatures in tanks and several ponds during mid-July 1992

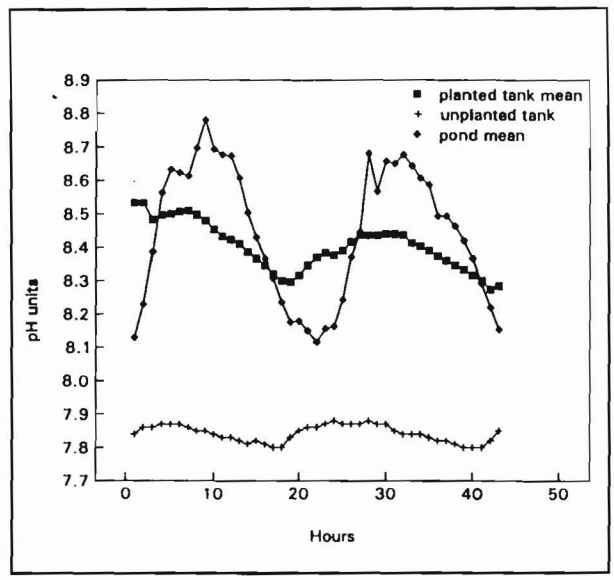

Figure 6. Hourly pH values in tanks and several ponds during mid-July 1992

Diel fluctuations in temperature were similar between tanks and ponds, but tank temperatures averaged about 2 °C lower than ponds. Shifts in pH occurred in ponds, increasing during daylight and decreasing at night, coinciding with periods of photosynthetic and respiratory activity by the plants. Planted tanks also exhibited pH shifts, but these were less pronounced than shifts seen in ponds. The unplanted tank exhibited only minor pH shifts, reflecting the absence of higher plants and minimal algal activity. Dissolved oxygen concentrations were more stable in all tanks than in ponds, probably because of much lower respiratory activity in the artificial systems.

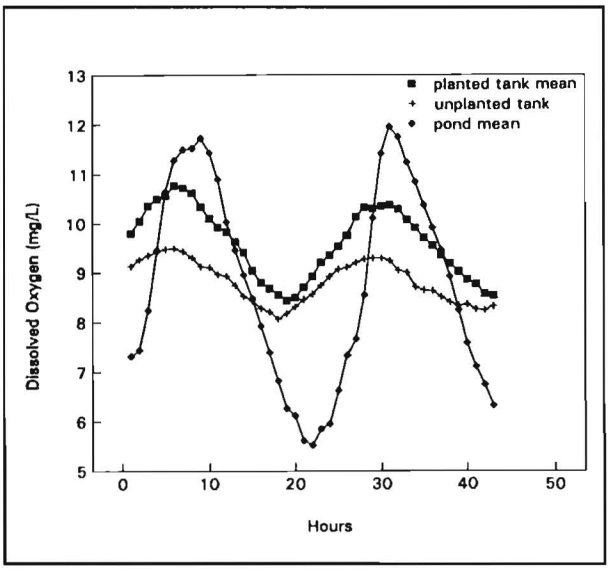

Figure 7. Hourly dissolved oxygen concentrations in tanks and ponds during mid-July 1992

Future Plans

Studies planned for fiscal year 1993 include an evaluation of triclopyr efficacy on a mixed community of submersed macrophytes and flurprimidol concentration versus exposure time effects on *Hydrilla verticillata* and *Myriophyllum spicatum*.

Aquatic Vegetation and Water Quality in Lake Marion, South Carolina

by
M. Scott Robinson,[1] *Jeffrey W. Foltz,*[1] *and James P. Kirk*[2]

Introduction

Dense stands of aquatic vegetation have a significant effect on water quality (Buscemi 1958; Ultsch 1973; Schreiner 1980; Wylie and Jones 1987) and fish habitat (Engel 1988). Dense growth of aquatic vegetation in a reservoir can decrease the amount of vertical mixing that occurs in the water column, decrease lateral flow and mixing, reduce turbulence and aeration, and promote thermal stratification. Buscemi (1958) found dissolved oxygen (DO) to be lower and more sharply stratified under dense beds of *Egeria densa* than in adjacent open water. Water temperatures were higher and DO concentrations lower under extensive surface mats of water hyacinth (*Eichhornia crassipes*) than in open-water areas of experimental ponds (Ultsch 1973; Schreiner 1980). Wylie and Jones (1987) related diel and seasonal changes of DO and pH to the aquatic macrophyte community of a shallow reservoir in southeast Missouri. It is generally recognized that aquatic vegetation, when present in significant amounts, has an important influence on the chemical and physical environments of impoundments.

Lake Marion, SC, is a 44,000-ha impoundment composed of open water, standing submerged trees, and thick cypress swamps. Aquatic vegetation has become a serious problem in upper Lake Marion north of the Interstate 95 (I-95) bridge (Inabinette 1985). The shallow, nutrient-rich upper Lake Marion area is conducive to aquatic plant growth. Many areas of the lake have limited access because of extremely thick vegetation. This has hampered use of the lake by recreational hunters, anglers, and boaters. Lake Marion has a long history of nuisance aquatic vegetation problems, dating back to large-scale alligator weed (*Alternanthera philoxeroides*) infestation in the 1940s (Inabinette 1985). Water quality studies have been completed on both Lake Marion and the Santee Swamp (Inabinette 1985; Harvey, Pickett, and Bates 1987; Bates and Marcus 1989). There have also been assessments of the distribution of aquatic vegetation on Lake Marion (Welch, Fung, and Remillard 1985) and the species of vegetation present in the lake (Inabinette 1985). No studies have attempted to determine the relationship between aquatic vegetation and water quality in Lake Marion, nor have the seasonal changes in species composition of aquatic vegetation been investigated.

Three hundred thousand triploid grass carp (*Ctenopharyngodon idella)* were stocked in upper Lake Marion between 1989 and 1991 for the purpose of vegetation control (South Carolina Aquatic Plant Management Council and South Carolina Water Resources Commission 1989). Current data are needed to assess the impact of this measure on water quality and aquatic vegetation. The objectives of this study were to (a) determine the changes in aquatic vegetation abundance and water quality over the course of 1 year at five different areas in upper Lake Marion; (b) determine the seasonal changes in aquatic vegetation species composition at these five areas; and (c) determine the relationship between water quality and aquatic vegetation abundance in these five areas.

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Study Area

Lake Marion was formed in 1941 by the impoundment of the Santee River (Inabinette 1985). Wateree and Congaree rivers join to form Santee River about 15 km upstream from Lake Marion. Lake Marion is a eutrophic reservoir with an average depth of 4 m and a maximum depth of 23 m (Inabinette 1985).

Upper Lake Marion, the defined study area, is north of the I-95 bridge. This area has standing submerged trees, open shallow flats, and thick stands of bald cypress (*Taxodium distichum*) and water tupelo (*Nyssa aquatica*). Upper Lake Marion has an estimated 4,800 ha of submerged vegetation. Santee Swamp is immediately upstream from upper Lake Marion and covers approximately 6,500 ha. The swamp is anaerobic for most of the year and affects the water quality of upper Lake Marion (Bates and Marcus 1989).

Five sampling areas were selected as representative of different habitat types present in the upper Lake Marion area (Figure 1). Three of the areas, Pack's Flats, Elliot's Flats, and Brown's Lake, were in upper Lake Marion proper. Pack's Flats is a shallow (depth = 1.0 to 2.0 m) open area in the uppermost region of Lake Marion near the Santee Swamp; hence, water quality in Pack's Flats is strongly influenced by the Santee Swamp (Bates and Marcus 1989). Elliot's Flats sampling area is located approximately 3.5 km southeast of Pack's Flats. This area is slightly deeper (depth = 1.5 to 2.5 m) than Pack's Flats and receives water directly from the Santee River via a canal as well as some flow from smaller tributaries and the Santee Swamp. Brown's Lake is located approximately 7.5 km southeast of Elliot's Flats and approximately 1 km below the confluence of Lake Marion and the Santee River channel. It is a relatively deep area (depth 3.5 to 4.5 m) characterized by standing submerged trees. The other two sampling areas, Sparkleberry Lake and Santee River are located in the Santee Swamp and in the Santee River, respectively. Sparkleberry Lake is a small (1-ha), shallow (depth = 1.0 to 1.5 m) lake in the lower end of the Santee Swamp. Santee River sampling area is located in the Santee River channel 9 km above its confluence with Lake Marion. All of these areas experience considerable seasonal changes in water level and flow.

Methods

The five study areas were sampled biweekly for 12 months from January to December 1991. Three parallel transects 60 m long were marked 100 m apart in each area. Aquatic vegetation abundance and primary and secondary vegetation species were recorded at three equidistant positions along each transect for a total of nine readings per area per biweekly sampling period (Figure 1). DO concentrations, water temperature, and conductivity were also recorded at the surface and bottom at each position. Vegetation abundance was recorded as one of five categories for each position. Each category was assigned a numerical value. The categories and their numerical values were as follows: 0 = no vegetation present, 1 = vegetation sparse, 2 = vegetation present but submersed, 3 = vegetation surface coverage less than 50 percent, 4 = vegetation surface coverage greater than 50 percent. Abundance values were summed for each transect resulting in three abundance values per area per sampling period, each with values from 0 to 12. Differences in vegetation abundance among biweekly sampling periods and differences in abundance among study areas were tested in separate one way analysis of variance (ANOVA) tests (Harvey 1982; SAS Institute, Inc. 1985). A t-test on least squares means (Harvey 1982) was also performed to determine which areas differed significantly from other areas.

An aquatic vegetation sample was taken at each position with a rake. Primary vegetation species was the species that comprised the largest proportion of the sample. Secondary vegetation was the species comprising the next largest proportion. Vegetation was identified in the field (Aulbach-Smith and DeKoslowski 1990). Sampling periods were combined into four seasons to examine seasonal differences in primary vegetation species composition.

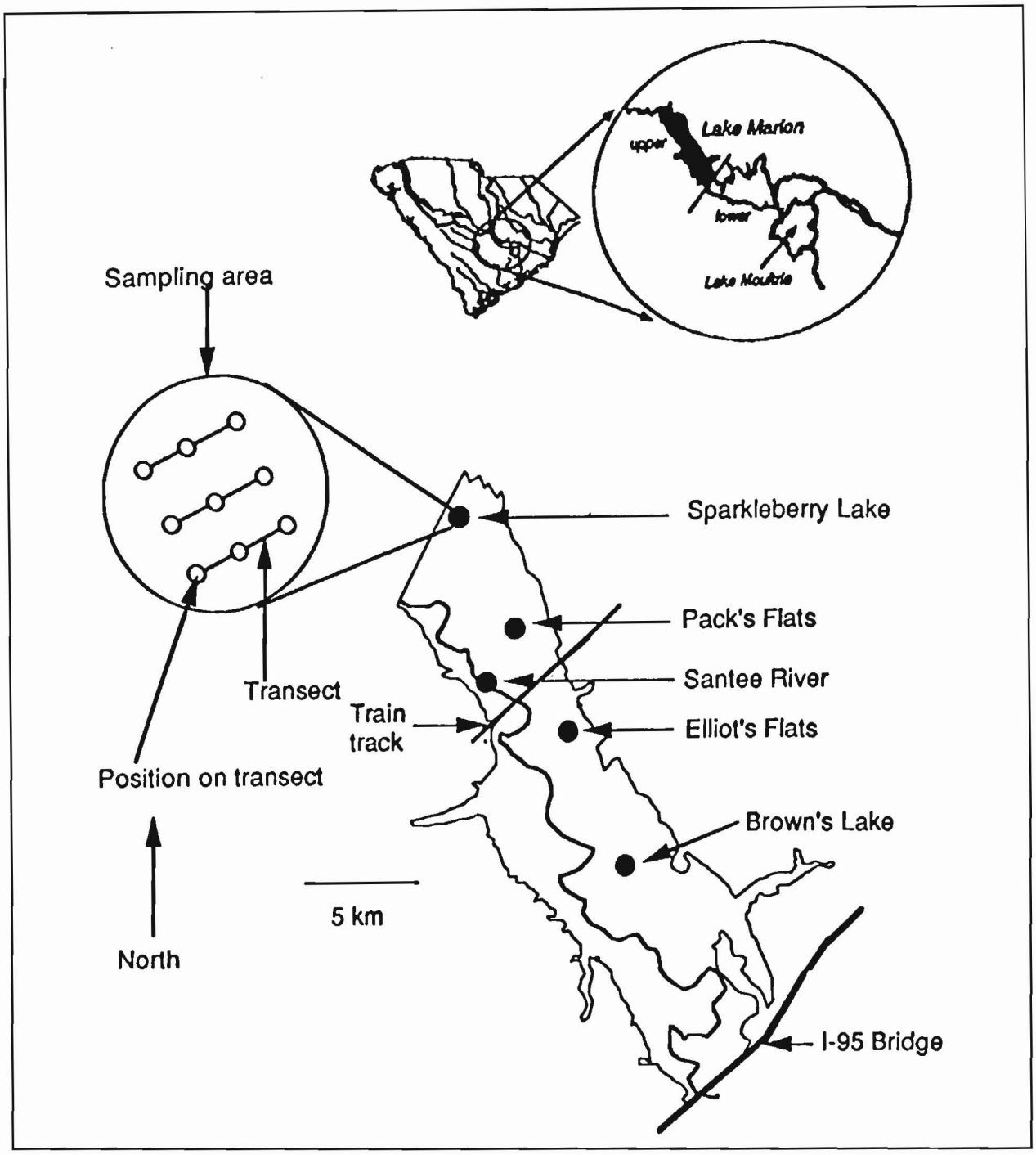

Figure 1. Location of upper Lake Marion on a map of South Carolina (top). Location of study areas in upper Lake Marion and a diagram of transects and positions in an area

The seasons were defined as follows: winter (December 21 - March 20); spring (March 21 - June 20); summer (June 21 - September 20); fall (September 21 - December 20). Two categories of primary vegetation were assigned numerical values: 1 = *Hydrilla* and 0 = other vegetation species. These two categories were used for the purpose of statistical analysis because of the predominance of *Hydrilla* as the primary vegetation species. The mean of the values was calculated for each area during each sampling period. This mean, which was

between 0 and 1, represented the proportion of each area in which *Hydrilla* was the primary species of vegetation. Differences in this proportion among seasons and among areas were then analyzed using ANOVA (SAS Institute, Inc. 1985) and a t-test on least squares means (Harvey 1982). Secondary vegetation species were recorded at each position but were not included in statistical analysis. The Santee River study area was omitted from statistical analysis procedures on vegetation species composition and abundance because of the year round absence of aquatic vegetation in the river channel.

DO concentration (to nearest 0.1 mg/L), water temperature (to nearest 0.1 °C), and conductivity (to nearest µmho/cm) were measured 10 cm below the surface and 10 cm above the bottom at each position. DO and temperature were measured with a Yellow Springs Instruments dissolved oxygen meter (Model 51 B). Conductivity was measured with a Yellow Springs Instruments S-C-T meter (Model 33).

Differences in DO concentrations, water temperature, and conductivity among areas and among sampling periods were analyzed using ANOVA and a t-test on least squares means. Differences between surface and bottom DO concentrations, surface and bottom temperature, and surface and bottom conductivity values were calculated for each area and a t-test performed with the null hypothesis that the difference = 0.

Pearson correlation coefficients were calculated for vegetation abundance and surface and bottom DO concentrations, temperatures, and conductivity values to determine if these parameters were correlated. All error testing was done at alpha = 0.05 level.

Water temperature and DO readings were taken on 17 September (Week 36) at 30-cm intervals from surface to bottom to examine differences in water quality throughout the water column in late summer. Readings were taken at three positions in each area, and the average of the three readings was reported. These readings were taken at all study areas except the Santee River.

Results

Vegetation abundance differed significantly among all study areas ($F = 184.3$, $p \leq 0.0001$) except Brown's Lake and Sparkleberry Lake ($p = 0.77$). Relative abundance rankings (on a scale of 0 to 12) averaged for the year were as follows: Pack's Flats = 9.12, Elliot's Flats = 7.13, Sparkleberry Lake = 2.43, Brown's Lake = 2.27. Relative abundance rankings differed significantly among sampling periods at Elliot's Flats ($F = 7.45$, $p \leq 0.0001$) and Pack's Flats ($F = 18.06$, $p \leq 0.0001$), but not at Brown's Lake ($F = 0.96$, $p = 0.5212$) or Sparkleberry Lake ($F = 1.61$, $p = 0.0822$). Figure 2 illustrates the changes in vegetation abundance over the course of the study. Brown's Lake had very little aquatic vegetation throughout the year, and a surface canopy of vegetation never formed. Sparkleberry Lake had small patches of both submersed and emergent vegetation throughout the year but was never heavily infested. Sparkleberry Lake had slightly higher abundance values in late fall, winter, and early spring when there was little shading from the canopy of cypress and water tupelo trees. In contrast, Pack's Flats and Elliot's Flats were heavily infested with *Hydrilla* and other species of aquatic vegetation throughout the year. In the winter, aquatic vegetation was mostly submersed. As water temperatures and the photoperiod duration increased in spring and summer, vegetation increased, and a dense surface canopy formed at both areas. Elliot's Flats and portions of Pack's Flats were treated with aquatic herbicide by Santee Cooper Public Service Authority (to facilitate recreational access) during Week 18 and Week 29 of the study. Vegetation abundance in these two areas declined in late fall and winter as water temperature and the photoperiod duration decreased.

Vegetation abundance was significantly correlated with the following: surface DO ($R = 0.1977$, $p \leq 0.0001$), bottom DO ($R = -0.2717$, $p \leq 0.0001$), surface temperature ($R = 0.2172$, $p \leq 0.0001$), bottom temperature

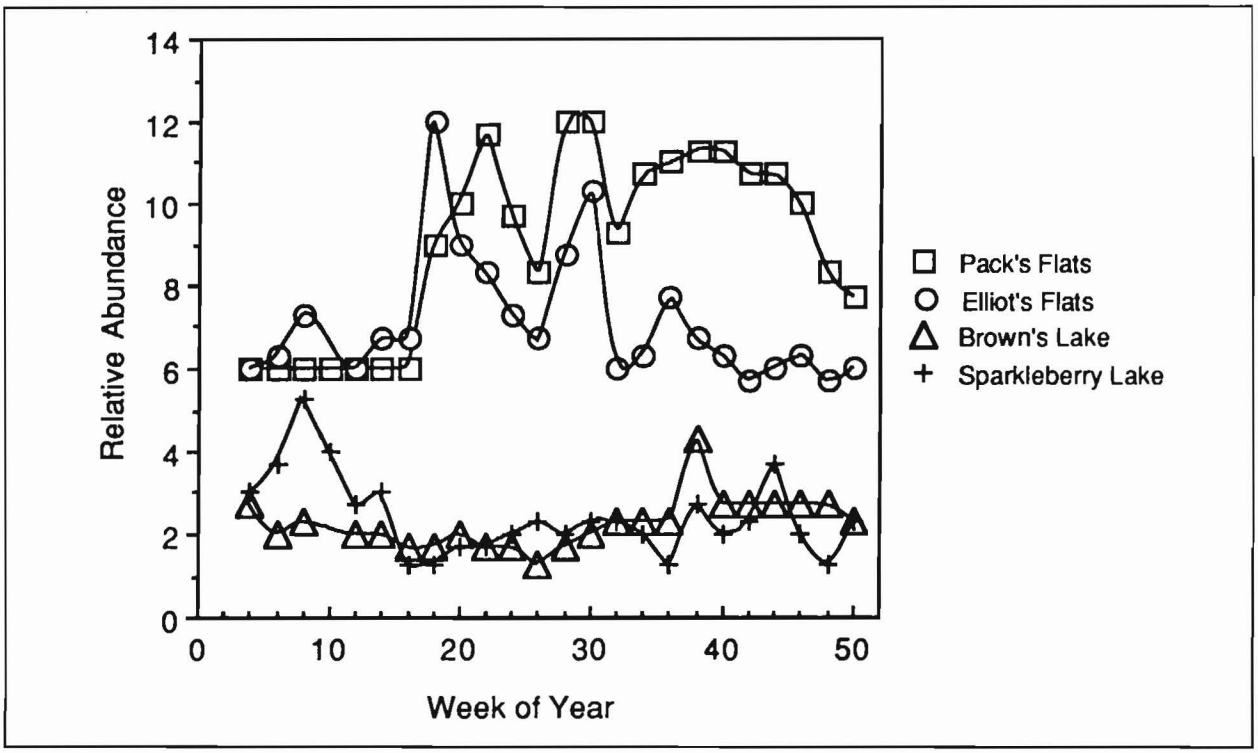

Figure 2. Relative vegetation abundance at four study areas in upper Lake Marion, SC, during 1991

(R = 0.1205, p = 0.0006), surface conductivity (R = 0.2392, p ≤ 0.0001), and bottom conductivity (R = 0.2294, p ≤ 0.0001).

Hydrilla was the primary vegetation for 66 percent of the observations taken during the study (Figure 3). Other vegetation species

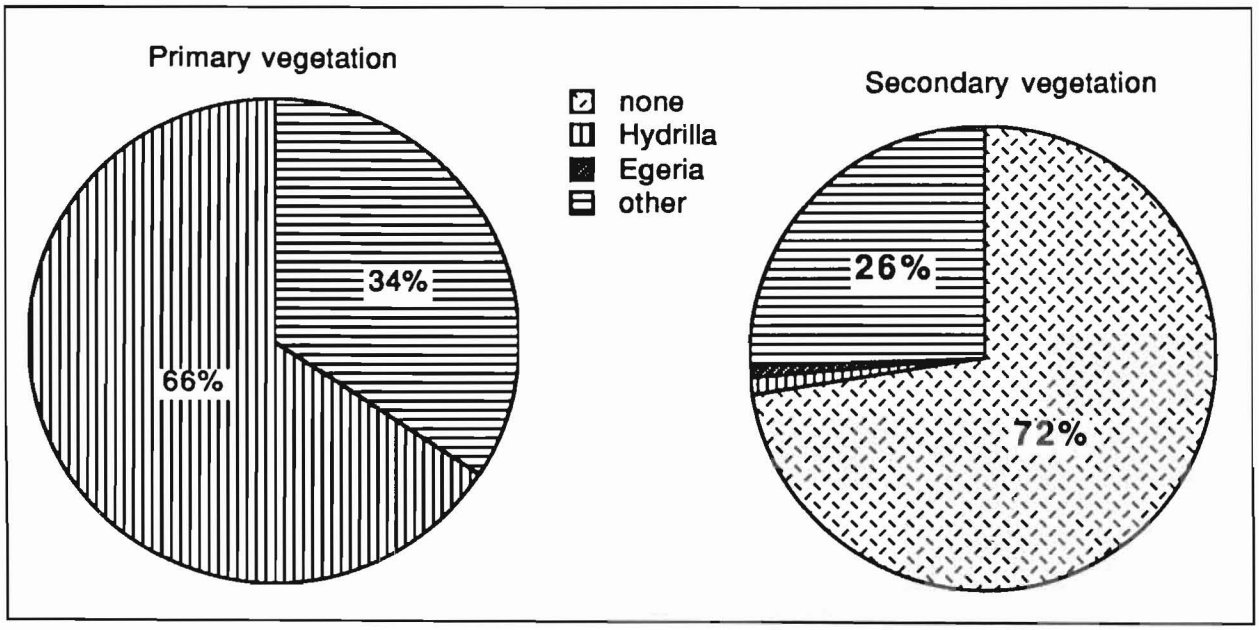

Figure 3. Primary and secondary species of aquatic vegetation at four study areas in upper Lake Marion, SC, during 1991

included water primrose (*Ludwigia uruguayensis*), *Egeria densa*, coontail (*Ceratophyllum demersum*), *Potamogeton* spp., *Najas* spp., and *Bidens* spp. Primary vegetation differed significantly among all study areas ($F = 795.62$, $p \leq 0.0001$) except Pack's Flats and Elliot's Flats ($p = 0.13$). Primary vegetation did not differ significantly among seasons ($F = 1.58$, $p = 0.2003$). No secondary vegetation was present for 72 percent of the observations (Figure 3). *Hydrilla* was the secondary vegetation species for only 1 percent of the observations. Other species of secondary vegetation are listed above.

Dissolved oxygen levels were highest during January (Week 2) and generally decreased throughout the year until late September (Week 38) (Figures 4-8). At this time, DO concentrations began to rise and continued to do so through December (Week 48). Lowest DO concentrations recorded for all areas were during the months of July, August, and early September. Surface DO levels differed significantly from bottom DO levels for all study areas ($t_{\text{Brown's Lake}} = 3.34$, $p \leq 0.0001$; $t_{\text{Elliot's Flats}} = 8.49$, $p \leq 0.0001$; $t_{\text{Pack's Flats}} = 13.00$, $p \leq 0.0001$; $t_{\text{Sparkleberry Lake}} = 9.19$, $p \leq 0.0001$; $t_{\text{Santee River}} = 8.68$, $p \leq 0.0001$).

Surface DO levels differed significantly among all study areas ($F = 80.40$, $p \leq 0.0001$) except Pack's Flats (Figure 4) and Santee River (Figure 5) ($p = 0.11$). Surface DO levels also differed significantly among sampling periods ($F = 99.51$, $p \leq 0.0001$). Elliot's Flats (Figure 6) and Pack's Flats areas exhibited the greatest biweekly fluctuations. Highest mean surface DO concentration recorded was 11.2 mg/L at Pack's Flats on 19 February (Week 6) and 28 May (Week 20) and at Sparkleberry Lake (Figure 7) on 19 March (Week 10). Lowest mean surface DO concentrations recorded were 0.7 and 0.8 mg/L at Sparkleberry Lake on 11 July (Week 26) and 03 September (Week 34), respectively. Surface DO concentration was related to surface temperature and vegetation abundance (R-square = 0.91) (Figure 9). Surface DO concentrations

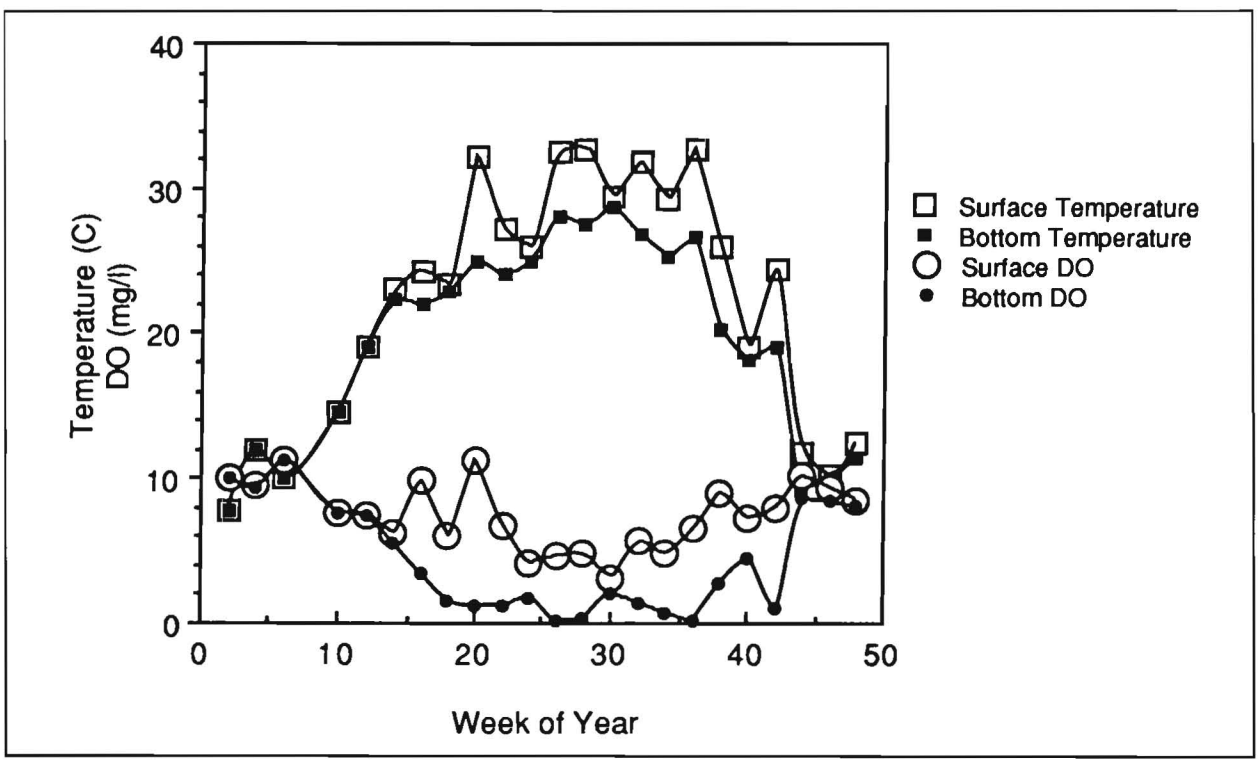

Figure 4. Surface and bottom temperature and dissolved oxygen levels at Pack's Flats, Lake Marion, SC, during 1991

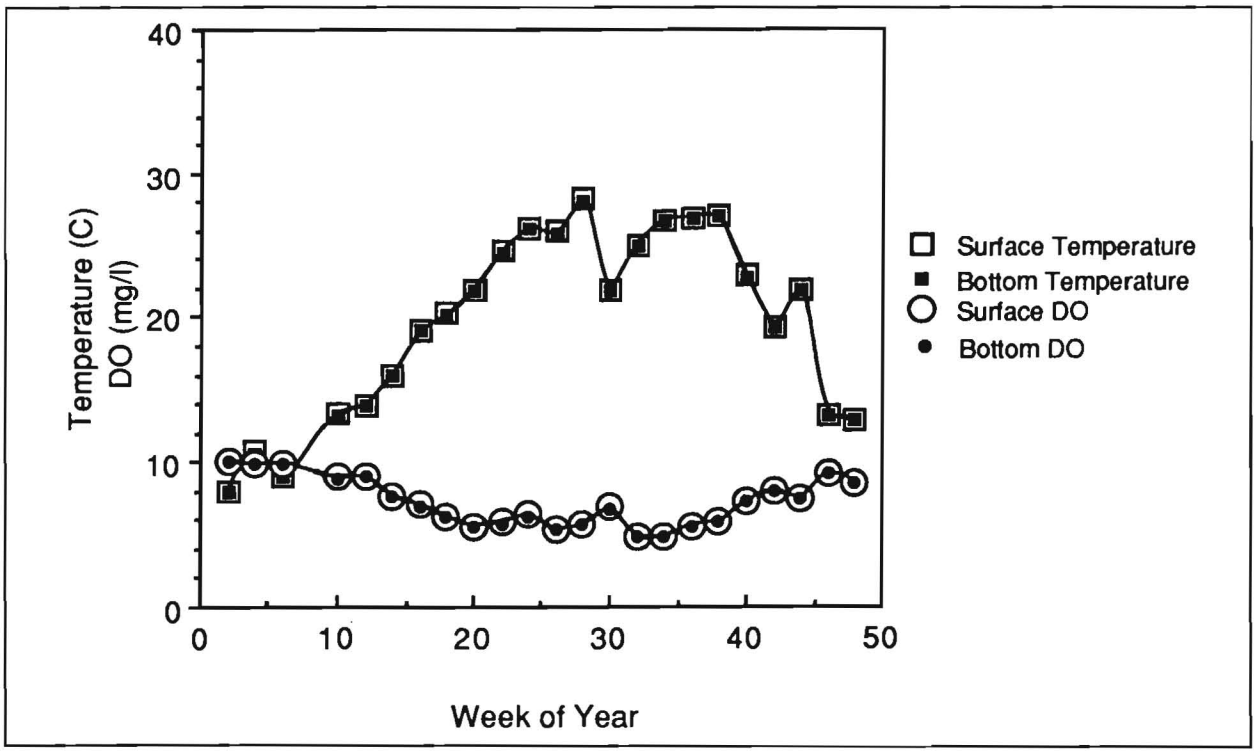

Figure 5. Surface and bottom temperature and dissolved oxygen levels in Santee River, SC, during 1991

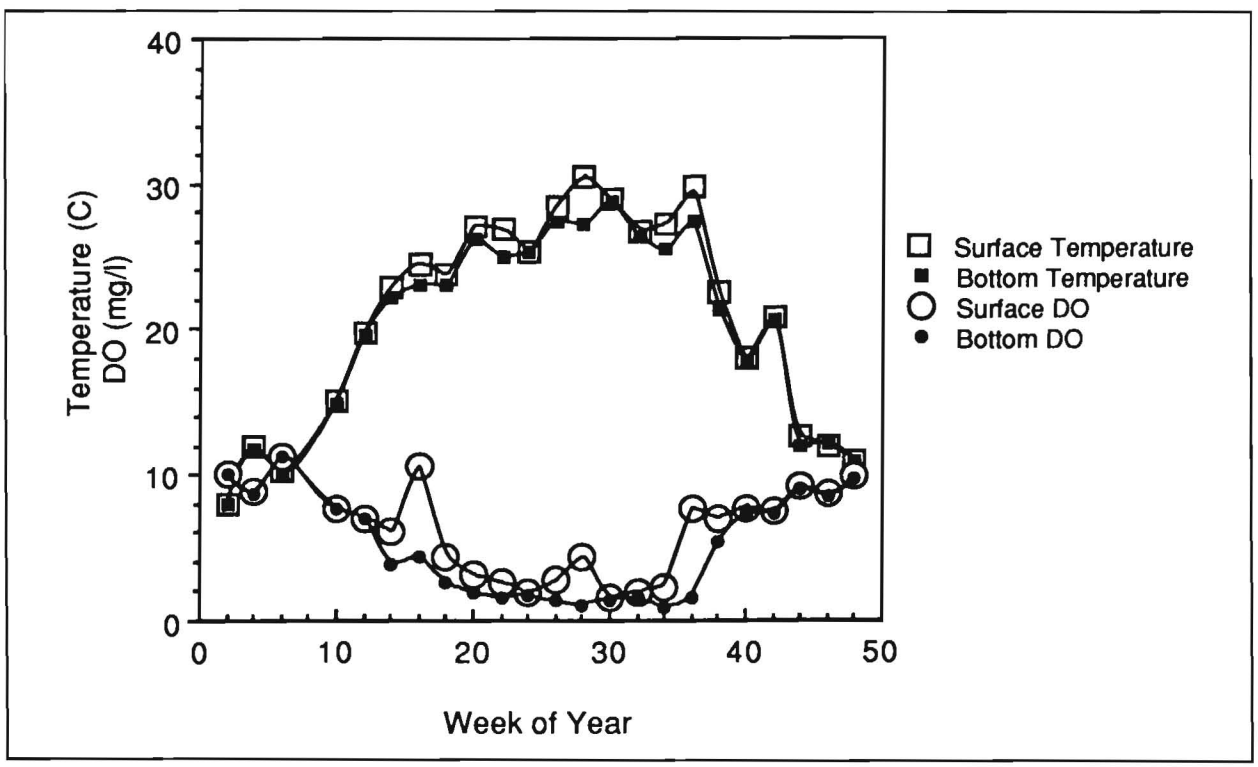

Figure 6. Surface and bottom temperature and dissolved oxygen levels at Elliot's Flats, Lake Marion, SC, during 1991

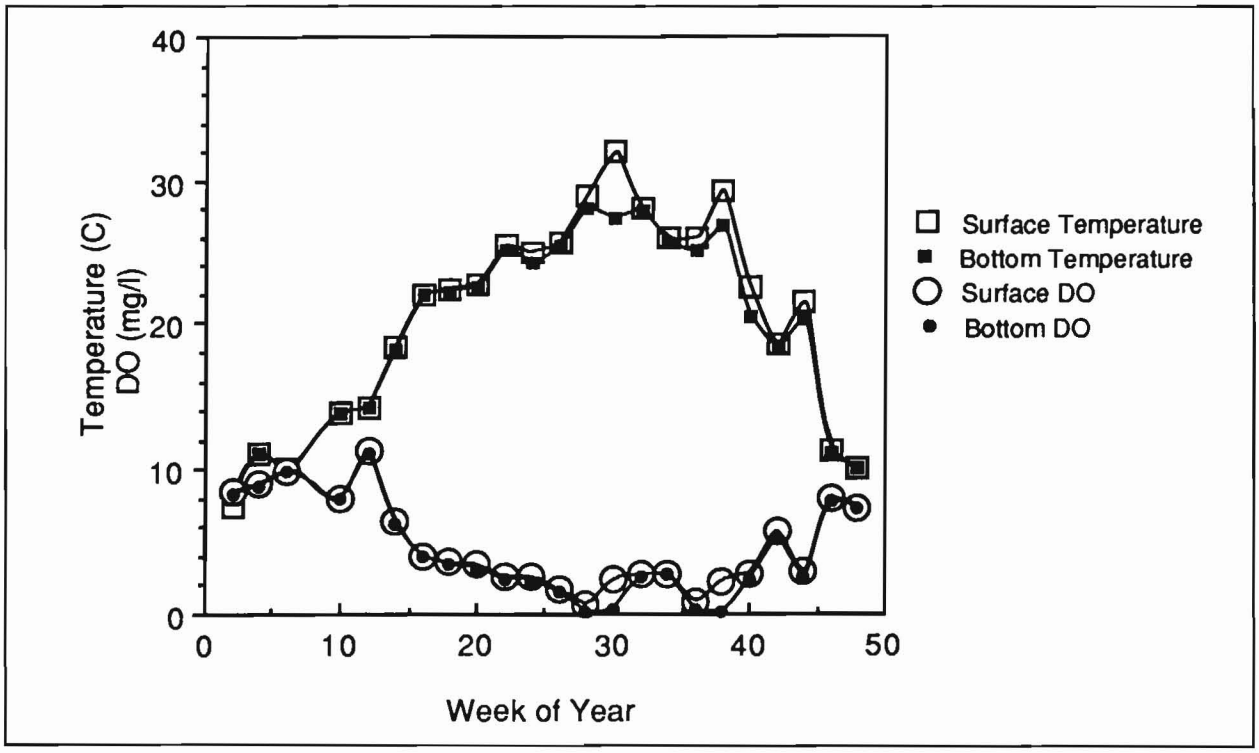

Figure 7. Surface and bottom temperature and dissolved oxygen levels at Sparkleberry Lake, Santee Swamp, SC, during 1991

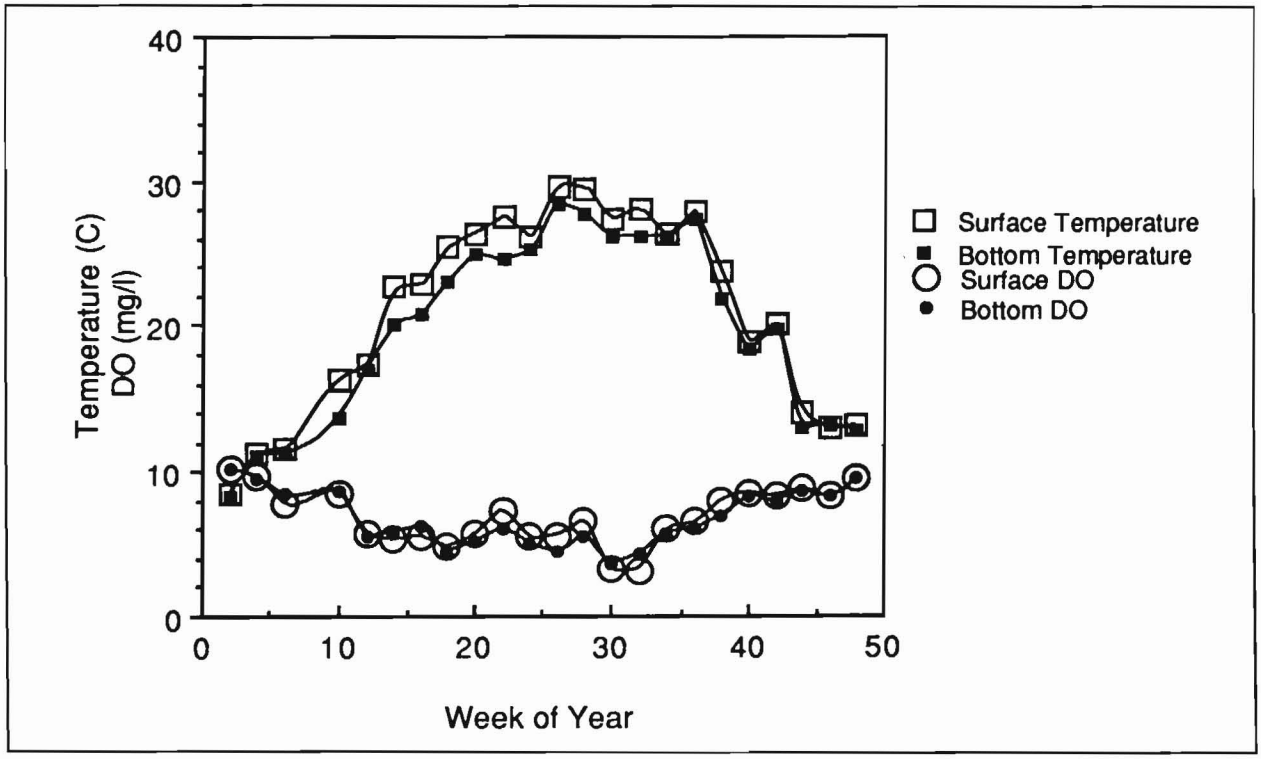

Figure 8. Surface and bottom temperature and dissolved oxygen levels at Brown's Lake, Lake Marion, SC, during 1991

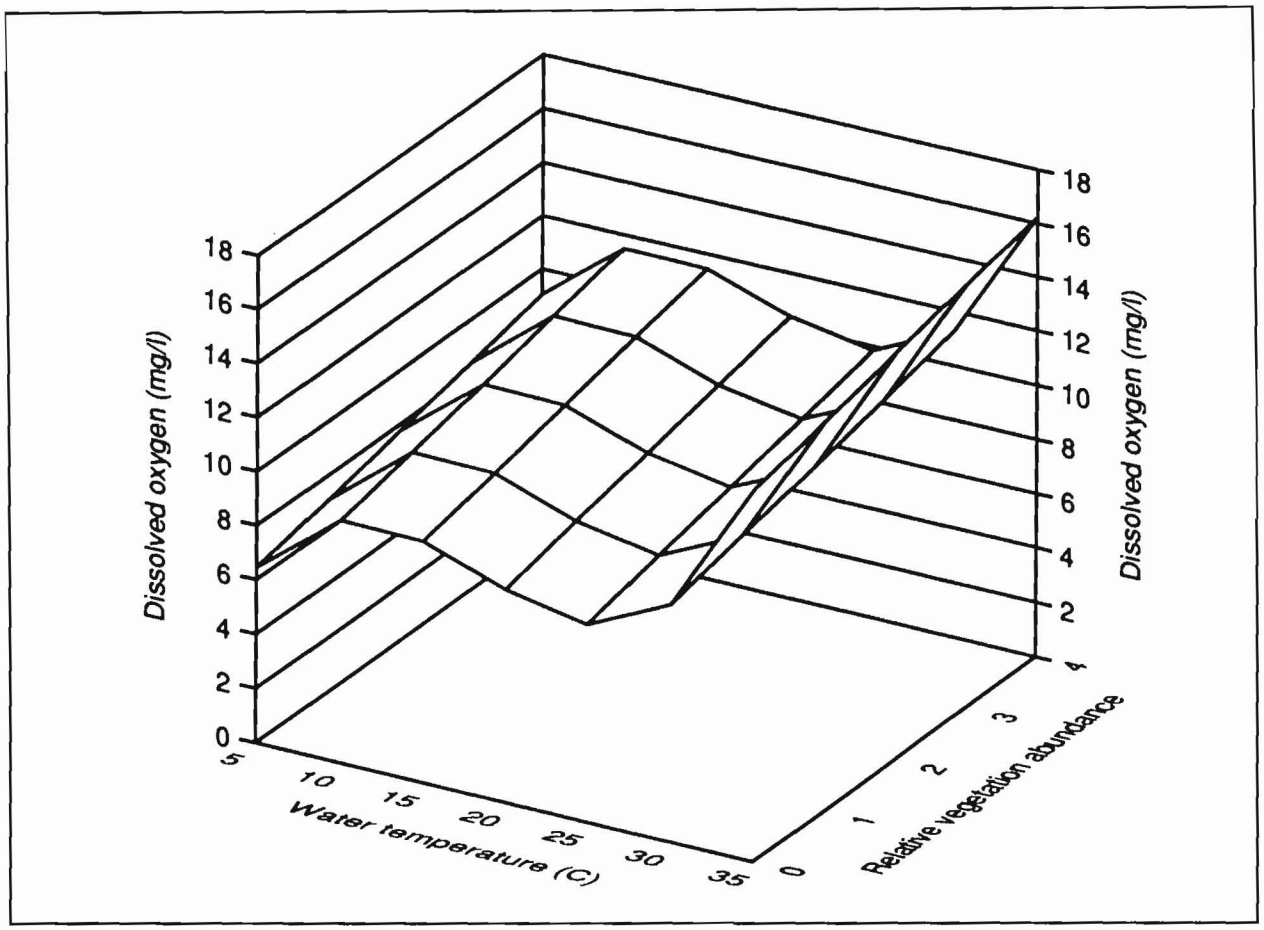

Figure 9. Projected surface dissolved oxygen levels for four study areas in upper Lake Marion, SC, during 1991. Surface DO = 1.786 * Surface temperature + 0.646 * Vegetation abundance - 0.115 * Surface temperature^2 + 0.002 * Surface temperature^3

increased as vegetation abundance increased and were highest at or below 10 °C and above 20 °C.

Bottom DO concentrations were usually lower than those at the surface and did not exhibit as much biweekly fluctuation (Figures 4-8). Bottom DO concentrations differed significantly among all study areas ($F = 240.88$, $p \leq 0.0001$) except Pack's Flats and Sparkleberry Lake ($p = 0.43$) and differed significantly between sampling periods ($F = 200.77$, $p \leq 0.0001$). The highest mean bottom DO level recorded was 11.3 mg/L at Pack's Flats on 19 February (Week 6). The lowest mean bottom DO level recorded was 0.2 mg/L at Sparkleberry Lake on 11 July (Week 26) and 17 September (Week 36) and at Pack's Flats on 18 September (Week 36). Bottom DO was related to bottom temperature and vegetation abundance (R-square = 0.93) (Figure 10). Bottom DO concentrations were relatively high at winter water temperatures and declined with increasing temperature, much like surface DO concentrations. Bottom DO concentrations continued to decline as vegetation abundance and bottom temperature increased.

Biweekly mean surface and bottom water temperatures were lowest during January. Temperatures increased throughout the year to a peak in July, August, and early September (Weeks 26-36 Figures 4-8). After the summer peak, temperatures decreased throughout the remainder of the study period. Surface temperature differed significantly from bottom temperature

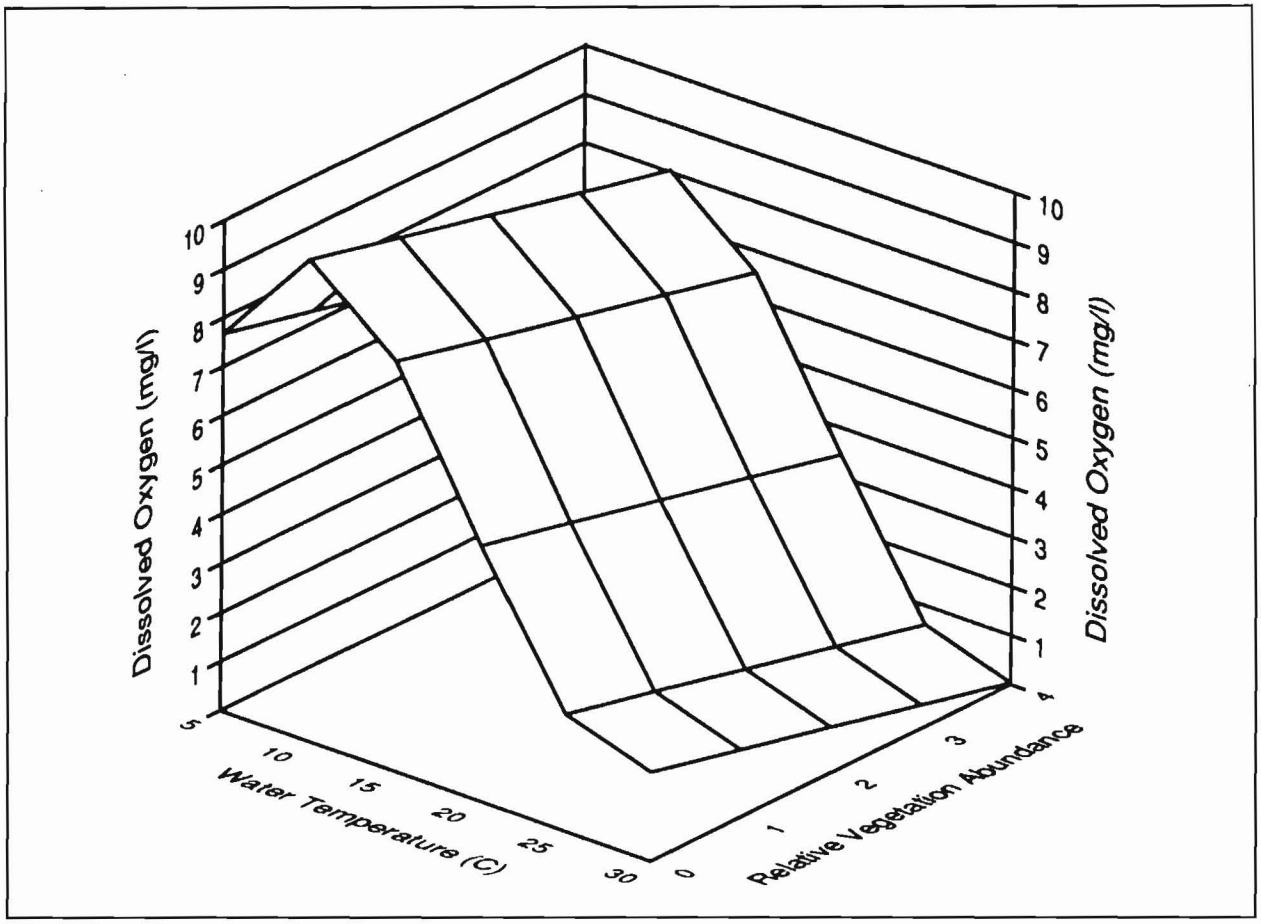

Figure 10. Projected bottom dissolved oxygen levels for four study areas in upper Lake Marion, SC, during 1991. Bottom DO = 2.224 * Bottom temperature - 0.448 * Vegetation abundance - 0.149 * Bottom temperature^2 + 0.003 * Bottom temperature^3

for all areas ($t_{\text{Brown's Lake}} = 14.25$, $p \leq 0.0001$; $t_{\text{Elliot's Flats}} = 11.47$, $p \leq 0.0001$; $t_{\text{Pack's Flats}} = 12.20$, $p \leq 0.0001$; $t_{\text{Sparkleberry Lake}} = 8.04$, $p \leq 0.0001$; $t_{\text{Santee River}} = 6.29$, $p \leq 0.0001$).

Surface temperature differed significantly among all study areas ($F = 81.74$, $p \leq 0.0001$) except Brown's Lake (Figure 8) and Elliot's Flats ($p = 0.27$) and differed significantly among biweekly sampling periods ($F = 966.97$, $p \leq 0.0001$). The highest mean surface temperatures were recorded at Pack's Flats; 32.2 °C on 28 May (Week 20); 32.5 °C on 11 July (Week 26); 32.8 °C on 24 July (Week 28) and 32.7 °C on 18 September (Week 36). Lowest mean surface temperature recorded was 7.4 °C on 22 January (Week 2) at Sparkleberry Lake. Pack's Flats and Santee River usually had the highest and lowest surface temperatures, respectively.

Bottom temperatures were usually lower than surface temperatures. Santee River area usually had the lowest bottom temperatures of all study areas and showed the greatest fluctuations in bottom temperatures. Bottom temperature differed significantly among all study areas ($F = 13.33$, $p < 0.0001$) except Pack's Flats and Sparkleberry Lake ($p = 0.95$) and differed significantly between biweekly sampling periods ($F = 2015.89$, $p < 0.0001$). Highest mean bottom temperatures recorded were 28.8 °C at Pack's Flats and Elliot's Flats on 07 August (Week 30) and 28.4 °C at Brown's Lake on 10 July (Week 22). Lowest mean bottom temperatures recorded were 7.8 °C at Pack's Flats on 22 January (week 2) and 7.9 °C at Sparkleberry Lake on 23 January (Week 2).

Dissolved oxygen profiles and temperature profiles recorded on 17 September (Week 36)

showed the extent to which vegetation abundance influences the DO concentrations and temperature in the water column (Figure 11). Brown's Lake, which had the lowest vegetation abundance values, had uniform DO concentrations (6.5 mg/L at surface, 6.1 mg/L at bottom) throughout the water column. Temperatures at Brown's Lake were also uniform throughout the water column (27.8 °C at surface, 27.6 °C at bottom). In contrast, Pack's Flats, which had the highest vegetation abundance values, exhibited DO and temperature stratification. DO concentration and temperature declined from the surface (9.2 mg/L and 34.5 °C, respectively) to a depth of 30 cm (2.8 mg/L and 28.4 °C) and DO was very low (<1.0 mg/L) at the bottom. Elliot's Flats had relatively high vegetation abundance values and exhibited DO and temperature stratification, although not as extreme as Pack's Flats. Sparkleberry Lake had vegetation abundance values slightly higher than Brown's Lake and exhibited some stratification, but not nearly so much as Pack's Flats or Elliot's Flats.

Conductivity values rose from January through September and declined for the remainder of the year, although Brown's Lake, Elliot's Flats, Pack's Flats, and Sparkleberry Lake did show an upward swing in values for 26-27 November and 09-16 December (Weeks 46 and 48) (Figure 12). The difference between mean surface and bottom conductivity was less than 2 µmhos/cm for all areas and all sampling periods. Thus, surface and bottom conductivity values were averaged in Figure 10. Conductivity values fluctuated more between sampling periods than did temperature or DO. Conductivity differed significantly among study areas (F = 126.18,

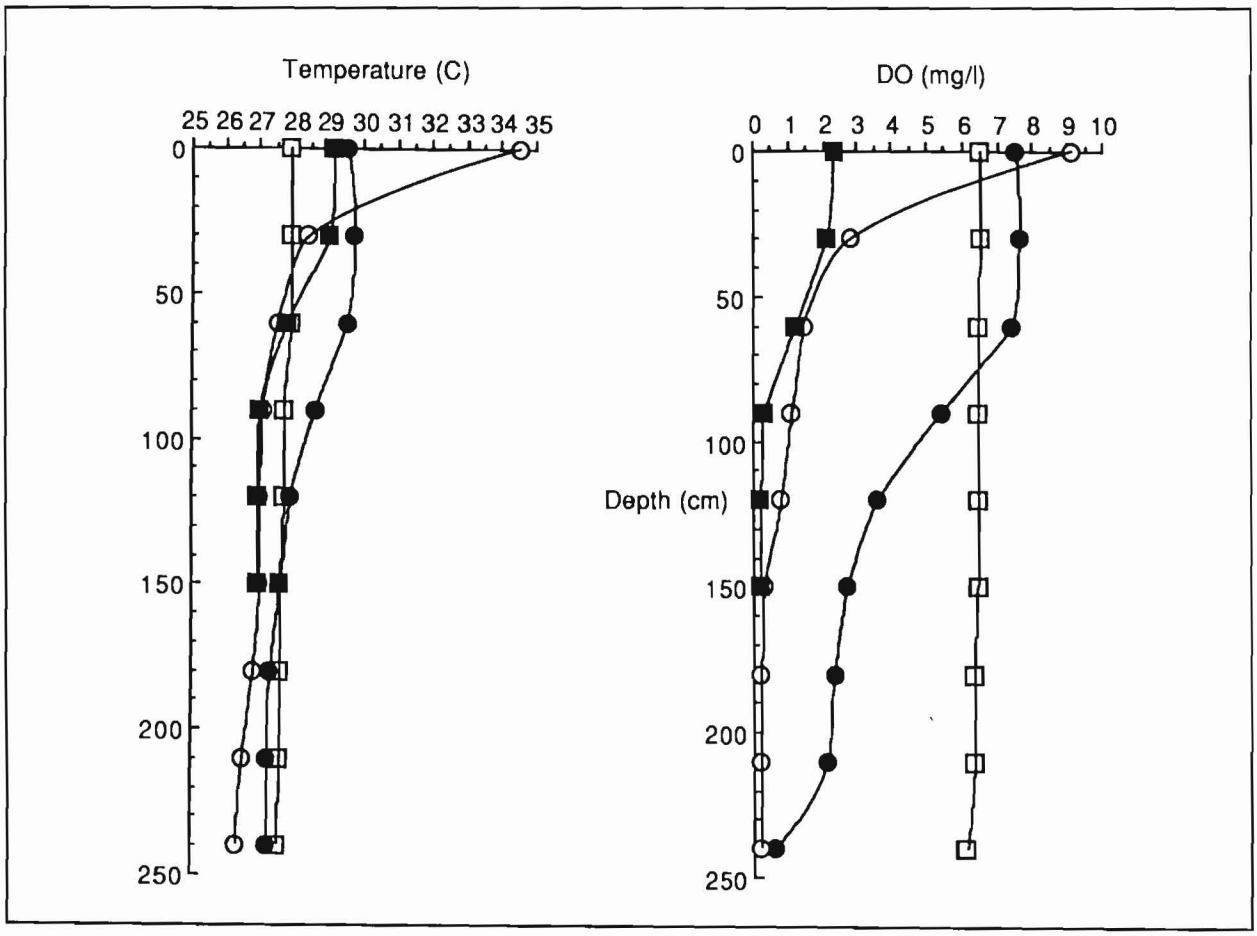

Figure 11. Temperature and dissolved oxygen profiles recorded on 17 September 1991 at four study areas in upper Lake Marion, SC

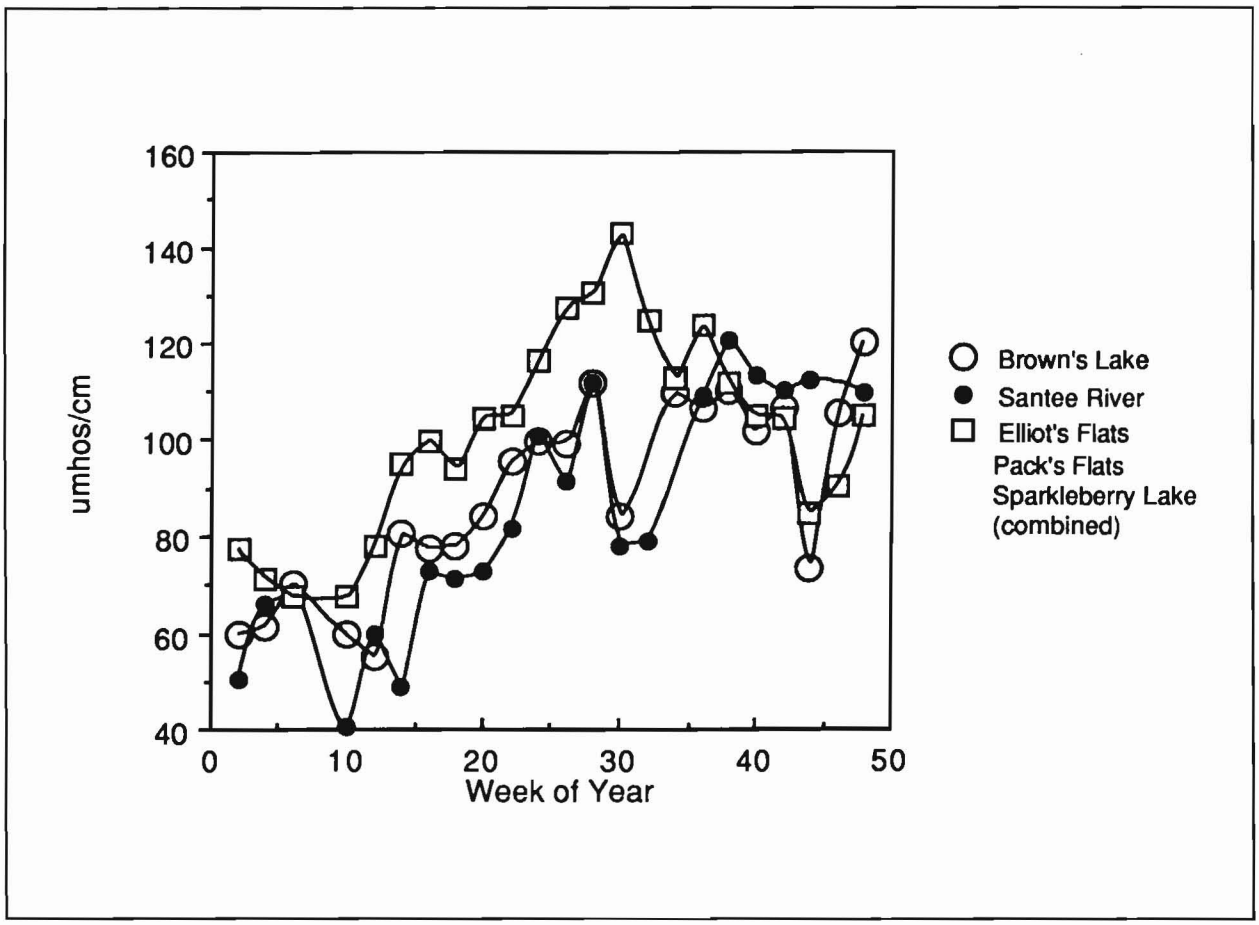

Figure 12. Conductivity values at five study areas in upper Lake Marion, SC, during 1991. Values for Elliot's Flats, Pack's Flats, and Sparkleberry Lake are averaged together

$p < 0.0001$) and sampling periods ($F = 166.45$, $p < 0.0001$). The highest mean conductivity value recorded was 144 µmhos/cm on 11 July (Week 26) and 07 August (Week 30) at Pack's Flats and 151 µmhos/cm on 23 July (Week 28) at Sparkleberry Lake. The lowest mean conductivity value recorded was 40 µmhos/cm on 07 March at Santee River.

Discussion

Aquatic vegetation is a major problem for navigation and recreational activities in Lake Marion north of the I-95 bridge (Inabinette 1985). *Hydrilla* has been the most problematic species in Lake Marion in recent years. *Hydrilla* was the dominant vegetation species present in upper Lake Marion over the course of this study, regardless of season or water temperature. *Hydrilla* appeared to grow faster and form a surface canopy earlier in the year than any other species. This resulted in extensive, dense canopies of *Hydrilla* that shaded out other species of aquatic plants. Profound water quality impacts may occur when aquatic vegetation forms surface canopies over aquatic habitats, as *Hydrilla* did in upper Lake Marion. Buscemi (1958), Ultsch (1973), Schreiner (1980), and Frodge, Thomas, and Pauley (1990) described localized changes in water quality in ponds and lakes with patches of aquatic vegetation. Water quality characteristics in, under, or near plant beds were notably different from water quality characteristics in open-water areas. Physical and chemical characteristics of a vegetated area became more like characteristics of an open-water area when surface canopies of aquatic vegetation were physically removed (Frodge, Thomas, and Pauley 1990). Similar comparisons (between

vegetated and open-water areas) can be made in upper Lake Marion when heavily vegetated areas such as Pack's Flats are contrasted with less infested areas such as Brown's Lake.

Areas of Lake Marion with dense growth of aquatic macrophytes (e.g., Pack's Flats and Elliot's Flats) exhibited distinctly different thermal characteristics from Brown's Lake, Sparkleberry Lake, and Santee River, where vegetation was sparse or absent. However, in winter when plant biomass was relatively low, thermal characteristics were similar for all study areas (Figures 5a-e). Surface temperatures in vegetation canopies were very high (>30 °C) in Pack's Flats and Elliot's Flats during the summer months when canopies became extensive. Bottom temperatures were usually 5 to 10 °C lower than surface temperatures in these areas. In contrast, temperatures were more uniform throughout the water column at Brown's Lake, Sparkleberry Lake, and Santee River, where extensive surface canopies did not form. The extent of thermal stratification was apparently related to vegetation abundance. Thermal stratification was reported present in plant beds and absent in open-water areas by Rorslett, Berge, and Johansen (1986), Wylie and Jones (1987), and Frodge, Thomas, and Pauley (1990).

DO concentrations differed greatly from location to location in Pack's Flats and Elliot's Flats when dense surface canopies were present. This was probably because of limited horizontal mixing through dense growths of aquatic vegetation. DO concentrations as high as 15.0 mg/L were recorded in surface canopies. These supersaturated DO levels were similar to levels reported by Rorslett, Berge, and Johansen (1986), Wylie and Jones (1987), and Frodge, Thomas, and Pauley (1990). Supersaturation is apparently because of photosynthetic activity by surface vegetation. Surface DO concentrations were related to vegetation abundance and water temperature (Figure 9). Winter surface DO levels were relatively low. Even though oxygen solubility is relatively high at winter water temperatures, photosynthetic activity by aquatic macrophytes and phytoplankton is low at winter temperatures and short photoperiod durations. The slight increase in surface DO levels as water temperatures increased from 5 to 10 °C was probably because of increased phytoplankton and macrophyte photosynthesis. In spring and early summer as water temperatures increased from 10 to 25 °C, the increased photosynthetic activity of macrophytes and phytoplankton was probably more than offset by the decreasing solubility of oxygen. As water temperatures increased above 25 °C and relative vegetation abundance increased, photosynthetic activity probably exceeded the effect of decreased oxygen solubility.

Bottom DO levels were lower beneath dense canopies of vegetation. Bottom DO levels were related to vegetation abundance and bottom temperatures (Figure 10). As water temperatures increased, oxygen solubility in water decreased. As vegetation abundance increased, the surface canopy became more dense, allowing less light to reach the bottom and thus decreasing photosynthetic activity at the bottom. Also, as the surface canopy becomes vertically thicker and more dense, some of the bottom layer of the surface canopy will die and fall to the bottom, thus increasing biological oxygen demand at or near the bottom. Subsurface oxygen depletion beneath canopies of aquatic macrophytes has been reported by Buscemi (1958), Wylie and Jones (1987) and Frodge, Thomas, and Pauley (1990).

The water quality conditions present in Elliot's Flats, Pack's Flats, and Sparkleberry Lake during summer present a problem for triploid grass carp. Although grass carp have low oxygen requirements (Opuszynski 1972), conditions present in these areas in the summer is probably not favorable for maximum feeding and growth rates. Chappelear (1990) reported a general downlake movement of triploid grass carp in upper Lake Marion during the summer of 1989. He hypothesized that this movement was due to low DO levels present in the uppermost areas of Lake Marion during the summertime. Triploid grass carp stocked downlake of Pack's Flats during 1990

generally remained in this area, with only a small percentage entering Pack's Flats (Kartalia 1992). Areas such as Pack's Flats, where water quality conditions are most extreme during the summer, do not provide suitable habitat for grass carp in the summer even though there is an abundance of suitable aquatic vegetation available. However, grass carp may enter these areas in fall, winter, and spring when water quality conditions are more favorable because aquatic vegetation is present year round.

There is no general consensus on the impact that reduction of aquatic macrophytes by grass carp has on fish communities (Carpenter and Lodge 1986). Removal of all aquatic vegetation in upper Lake Marion would reduce habitat variability and have an adverse effect on the fish community. However, this study indicates that a reduction of aquatic plant biomass in upper Lake Marion, in addition to improving recreational access, would improve water quality in Lake Marion. As a result of improved water quality, many areas would then be capable of supporting complex fish communities.

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Temperature Study of *Hydrellia pakistanae*

by
Ramona H. Warren[1]

Background

Hydrilla verticillata is a submersed aquatic plant with a high reproductive potential and wide ecological tolerance (Baloch and Sana-Ullah 1973). The plant is located throughout the southern United States, along the east coast, and in southern California (Cofrancesco 1991). This nuisance aquatic plant interferes with water flow and recreational activities in lakes and rivers. It also impedes navigation by clogging waterways.

The main objective of this study was to determine the effect of temperature on the development of the *Hydrellia pakistanae,* an ephydrid fly that feeds on hydrilla. The insect's ability to develop under a range of temperatures needed to be ascertained. Temperature studies allow questions to be answered, such as the following: Do different temperatures affect development at different life stages? Does temperature have an affect on feeding and survival? Questions such as these are important because they allow the best utilization of insects as biocontrol agents in relation to various environmental conditions.

Developmental Studies

Objectives

- Examine developmental rate.
- Determine percent survival.
- Determine leaf damage.

Materials and method

Plant and insects. The experiment was initiated by obtaining the insect *Hydrellia pakistanae* eggs from the U.S. Army Engineer Waterways Experiment Station (WES). The plant material (Hydrilla) was obtained from the WES greenhouse facilities.

Experimental set-up. Nine 15-cm hydrilla sprigs were placed in a petri dish and put into the colony box for oviposition for a 24-hr time period. The eggs were then removed and placed in petri dishes. Each petri dish contained a sheet of black filter paper and 20 individual leaves. A single egg was placed on each leaf. These petri dishes were then placed into an environmental chamber at the desired test temperature ranging from 20 to 35 °C with a 14-hr photophase. The petri dishes were checked daily for hatched eggs.

First instar larvae were placed into fifty 60-ml test tubes (one larva per tube). Each test tube was filled with deionized water and contained a 15-cm sprig of hydrilla. Each tube was covered with nylon organdy and held in place with a rubber band. (Note: The test tubes were placed in the environmental chamber at the same time as the petri dishes so that the environment would be the same for the insect when transferred to the test tube.)

Observations about the following were recorded: development, larval location, damage/day, and percent survival.

The data were analyzed using the Mean Separation Technique of the Statistical Analysis System (SAS), a computer statistical software program. This program produced the means and standard error of the data collected.

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

Results

Shorter developmental rates were associated with higher temperatures (Figure 1). For example, at 27 °C, development from egg to adult took an average of 25 days, whereas, at 20 °C the developmental rate was approximately 40 days. The overall developmental rate from egg to adult for 20, 25, 27, 30, 32.5, and 35 °C were 43, 26, 25, 23, 19 and 22 days, respectively.

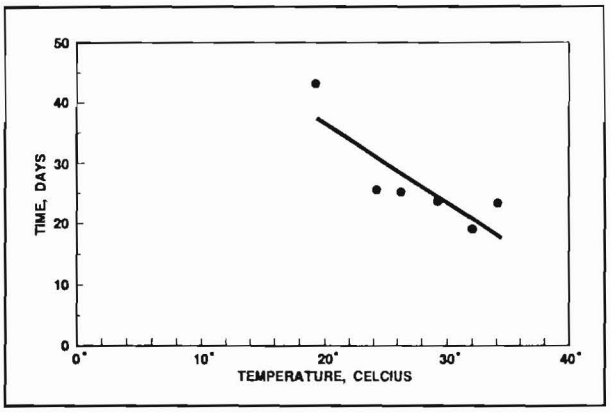

Figure 1. Number of days of overall development from egg to pupa

Generally, faster development was associated with higher temperature. In contrast, feeding rate was apparently inversely proportional to temperature with higher feeding associated with lower temperature (Figures 2 and 3). On the average, 11.3 leaves were damaged at 20 °C and 8.2 leaves at 25 and 27 °C.

Survival rate was not directly correlated with temperature. At 20 °C, the rate of survival was 54 percent; at 25 °C, survival was 87 percent; at 32.5 °C, survival was 75 percent; but at 27 and 35 °C, survival was 40 and 37 percent, respectively (Figures 4*a-f*). We observed that greater mortality occurred in 1st and 2nd instar larvae for all temperatures tested.

Cold Tolerance Studies

Objective

- Determine percent survival at each life stage when exposed to cold temperature.

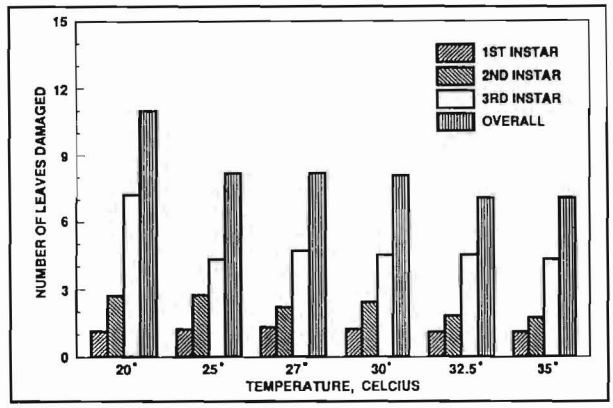

Figure 2. Number of leaves damaged by Hydrellia pakistanae larval stages

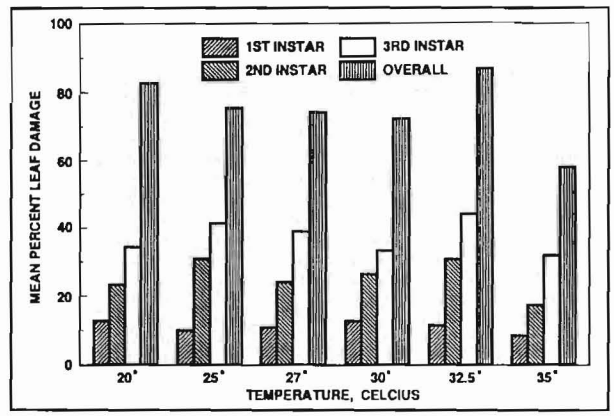

Figure 3. Average percent of leaf damage because of larval attack

Materials and methods

Plant and insects. Plants were obtained from WES greenhouse facilities, and the eggs were obtained from Tennessee Valley Authority facilities.

Experimental set-up. Petri dishes containing eight *hydrilla* sprigs and approximately 200 eggs per dish were set up for each life stage (1st, 2nd, 3rd instar and pupae) and placed in an environmental holding chamber. A temperature of 27 °C was maintained in the holding chamber for rearing and collecting *Hydrellia pakistanae*.

Five replications of twenty 300-ml Mason jars containing hydrilla and 30 insects (collected from holding chamber) were placed in a cold temperature chamber at 4 °C. Replications

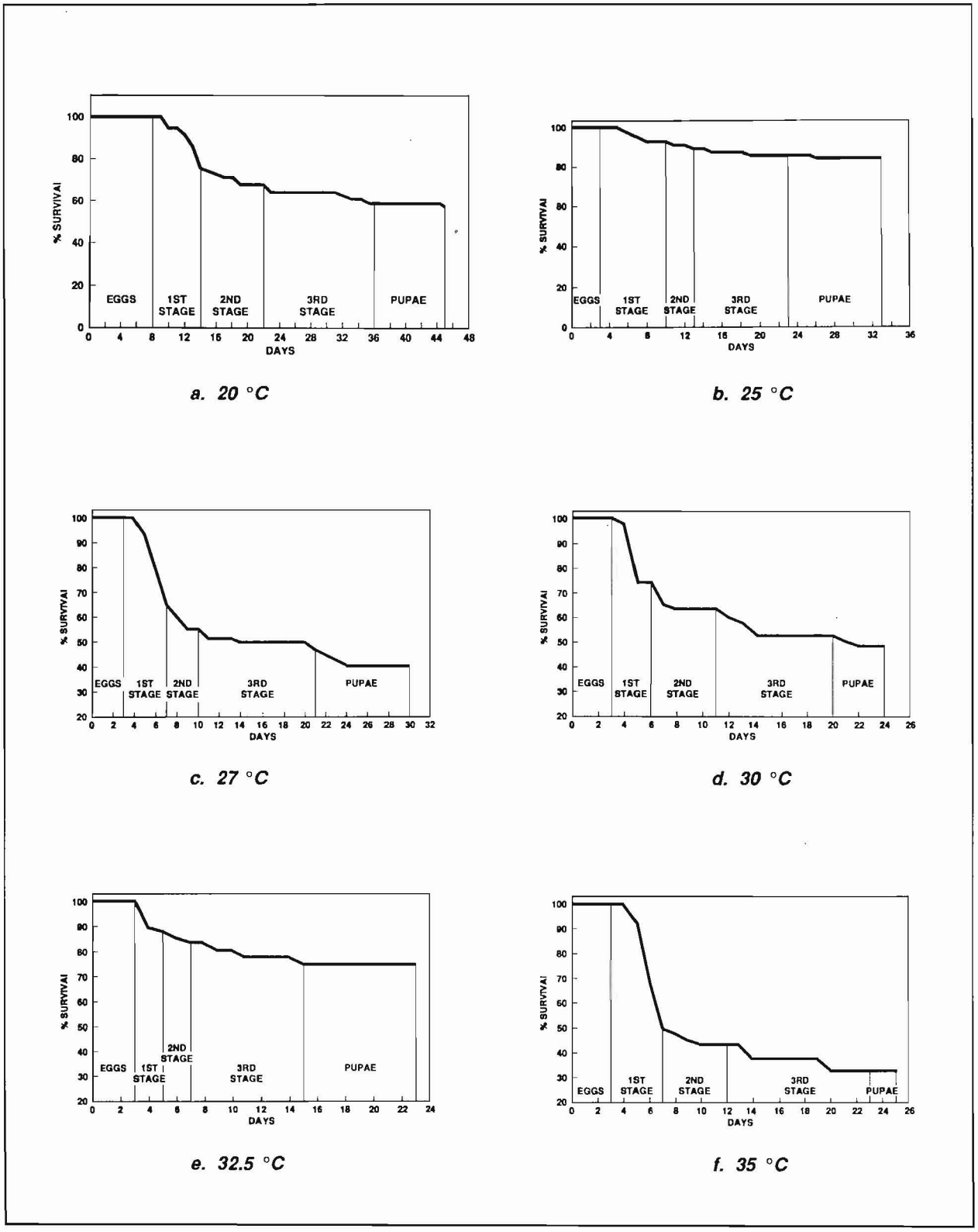

Figure 4. Percent survival from egg to adult for all temperatures tested

remained in the 4 °C chamber for varying exposure times of 1, 2, 4, and 8 weeks.

Zero week served as control, and jars were placed in the holding chamber at 27 °C. At the end of exposure times, jars were then placed in an environmental ramping chamber and the temperature increased from 4 to 27 °C over a 3-day period. Jars were then placed in a 27 °C holding chamber until they reached the adult stage. Emerged adults were collected and placed into labeled vials containing 70 percent ethanol. Data will be collected and percent survival recorded.

Future Work

Present research will continue with cold tolerance studies to determine threshold (high and low) temperatures for developmental rate of *Hydrellia pakistanae*.

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Aquatic and Wetlands Research and Development Support Facility

by
Jan E. Freedman,[1] *LaVon Jeffers,*[1] *and Sandra Young*[1]

The Aquatic and Wetlands Research and Development Support Facility consists of three fiberglass, even-span greenhouses all equipped with evaporative cooling systems and forced-air heaters. Two of the greenhouses are 1,928 sq ft, and the third one is 1,800 sq ft. A greenhouse work building, referred to as a Headhouse, is also included and consists of 1,265 sq ft. An outside tank area has been established about 25 ft from the North Greenhouse, capable of growing aquatic plants in a more natural setting for experimentation. There are also two outside concrete work areas, one next to the South Greenhouse and one behind the Headhouse and North Greenhouse. A parking area is located in front of the Support Facility.

The Support Facility has many capabilities and functions. Propagation and growing aquatic plants are a primary function. Several species of aquatic plants are maintained in the facility including *Hydrilla verticillata*, hydrilla, *Myriophyllym spicatum*, Eurasian watermilfoil, *Pistia stratiotes*, waterlettuce, *Eichhornia crassipes*, waterhyacinth, *Salvinia rotundifolia*, salvinia, *Alternanthera philoxeroides*, alligatorweed, and *Trapa natans*, water chestnut. The Support Facility is used also to rear several species of biocontrol insects. These include two insects that feed on hydrilla, *Hydrellia balciunasi*, the Australian hydrellia fly, and *Hydrellia pakistanae*, the Pakistan hydrellia fly, and the waterlettuce weevil, *Neohydronomus affinis*. The insects are reared both for experimentation and field releases. Other experimentation includes testing plant pathogens as biological controls of hydrilla and Eurasian watermilfoil, and managing aquatic plants with allelopathic and competitive species.

The Support Facility sustains many of the biocontrol work units. Past and present work units studied at the Support Facility include the following:

- Temperate Biocontrol Insects for Eurasian Watermilfoil and Hydrilla
- Biological Control of Hydrilla Using Insects
- Biological Control of Hydrilla Using Plant Pathogens
- Biological Control of Eurasian Watermilfoil Using Plant Pathogens
- Biological Management of Aquatic Plants with Allelopathic and Competitive Species
- Biological Control of Waterlettuce with Insects
- Investigate Impact of Established Herbivorous Aquatic Insects on Eurasian Watermilfoil
- Evaluation of Biocontrol Pathogen for Eurasian Watermilfoil
- Evaluation of Biocontrol Agents for Hydrilla
- Biological Control of *Trapa natans*

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

- Control of Mosquito Populations in Dredge Disposal Areas
- Reduction of Cockroach Habitat in Army Facilities
- Use of Mycorrhiza to Enhance Vegetation Growth
- Biological Control of Waterhyacinth
- Biological Control of Alligatorweed

Future Greenhouse Studies include the following:

- Biological Control of Purple Loosestrife
- Biological Control of *Melaleuca quinquenervia*
- Management of Mosquito Populations in Wetland Habitats

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The 27th Annual Meeting of the U.S. Army Corps of Engineers Aquatic Plant Control Research Program was held in Bellevue, WA, on 16-19 November 1992, to review current research activities and to afford an opportunity for presentation of operational needs. Papers presented at the meeting are included in this report.

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