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US Army Corps
of Engineers
Waterways Experiment
Station

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Aquatic Plant Control Research Program

Proceedings, 28th Annual Meeting, Aquatic Plant Control Research Program

15-18 November 1993

Baltimore, Maryland

Approved For Public Release; Distribution Is Unlimited

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

Aquatic Plant Control
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Final report

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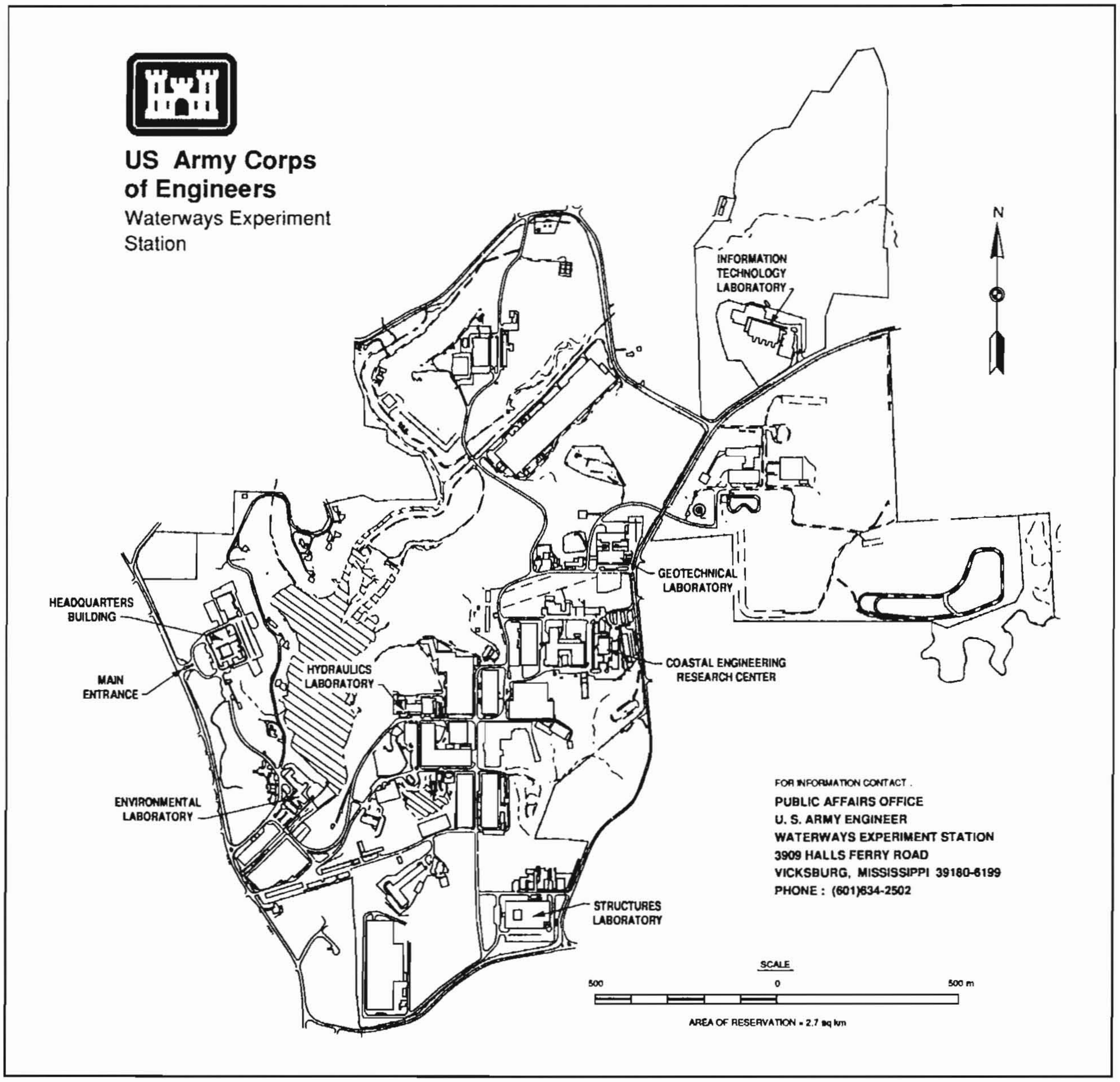

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Contents

Preface

The 28th Annual Meeting of the U.S. Army Corps of Engineers Aquatic Plant Control Research Program (APCRP) was held in Baltimore, MD, on 15-18 November 1993. 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, Manager, ERRAP, EL. Mr. Robert C. Gunkel, Assistant Manager, ERRAP, was responsible for planning the meeting. Dr. John W. Keeley 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, 15 November 1993

1:00 p.m. - 5:00 p.m.	***Registration*** Hotel Lobby
3:00 p.m. - 5:00 p.m.	***Federal Aquatic Plant Management Working Group*** Charles Suite
6:00 p.m. - 7:30 p.m.	***Reception*** Versailles Room

Tuesday, 16 November 1993

7:30 a.m. - 5:00 p.m.	***Registration*** Ballroom Foyer
8:00 a.m. - 5:00 p.m.	***Poster and Demonstration Session*** Calvert Ballroom, Salons A and D
8:00 a.m. - 2:00 p.m.	***General Session*** Calvert Ballroom, Salon C
8:00 a.m.	Call to Order and Announcements * Robert "Bob" C. Gunkel, U.S. Army Engineer Waterways Experiment Station (WES) Vicksburg, Mississippi
8:05 a.m.	Welcome to Baltimore District * Gerald R. Boggs, Acting Deputy District Engineer for Civil Works U.S. Army Engineer (USAE) District Baltimore, Maryland
8:15 a.m.	Comments from the Director of WES * Robert W. Whalin, Director of WES Vicksburg, Mississippi

8:30 a.m.	Current and Future Changes to the APCRP * J. Lewis Decell, Manager, Environmental Resources Research and Assistance Programs, WES Vicksburg, Mississippi
8:45 a.m.	Aquatic Plant Control Operations Support Center (APCOSC) Update * Wayne T. Jipsen, USAE District Jacksonville, Florida
9:00 a.m.	Lewisville Aquatic Ecosystem Research Facility (LAERF) Update (32733) * R. Michael "Mike" Smart, WES-LAERF Lewisville, Texas
9:15 a.m.	Hydroacoustic Measurement and Automated Mapping of Submersed Aquatic Vegetation * Bruce M. Sabol, WES Vicksburg, Mississippi
9:30 a.m.	***Break***
10:00 a.m.	Valuation of Aquatic Plant Alternatives at Lake Guntersville: Preliminary Results from the Recreation Study (32729) * Jim E. Henderson, WES Vicksburg, Mississippi
10:15 a.m.	Collection , Age, and Growth of Triploid Grass Carp: A Status Report (32738) * James P. "Phil" Kirk, WES Vicksburg, Mississippi
10:30 a.m.	The Fish/Plant Technology Area: An Overview * K. Jack Killgore, WES Vicksburg, Mississippi
10:45 a.m.	Guntersville Bass and Grass: Life After 381 * William "Bill" Wrenn, Tennessee Valley Authority (TVA) Muscle Shoals, Alabama

Simulation Technology

Presiding: R. Michael "Mike" Stewart, WES, Vicksburg, Mississippi

11:00 a.m.	An Overview of Current Simulation Technology Research * R. Michael Stewart, WES Vicksburg, Mississippi
11:15 a.m.	Development of an Aquatic Plant Growth Mesocosm System for Conducting Validation Studies for Plant Growth Models (32440) * R. Michael Stewart, WES Vicksburg, Mississippi
11:30 a.m.	***Lunch***

1:00 p.m.	Current Testing of the WES Stocking Rate Model for Guntersville Reservoir and Lake Marion Conditions (32438) * William "Will" A. Boyd, WES Vicksburg, Mississippi
1:15 p.m.	Current Research Towards an Aquatic Herbicide Fate and Effects Model for Submersed Application Techniques (32439) * R. Michael Stewart, WES Vicksburg, Mississippi
1:30 p.m.	The Role of Global Positioning System Technology in Aquatic Plant Control (32506) * Scott Bourne, WES Vicksburg, Mississippi
1:45 p.m.	Incorporation of Simulation Technology into an Aquatic Plant Management System * Richard E. Price, WES Vicksburg, Mississippi
2:00 p.m.	***Adjourn General Session***
2:00 p.m. - 5:00 p.m.	***USAE Division/District Working Session*** Calvert Ballroom, Salon E
2:00 p.m. - 5:00 p.m.	***Joint Agency Guntersville Project Meeting*** Calvert Ballroom, Salon B

Wednesday, 17 November 1993

8:00 a.m. - 3:00 p.m.	***Poster and Demonstration Session*** Calvert Ballroom, Salons A and D
8:00 a.m. - 3:00 p.m.	***General Session*** Calvert Ballroom, Salon C

Ecological Technology

Presiding: John W. Barko, WES, Vicksburg, Mississippi

8:00 a.m.	Overview of Ecological Studies * John W. Barko, WES Vicksburg, Mississippi
8:15 a.m.	Interrelationships Between Sediment Composition and Water Quality Conditions Affecting the Reestablishment of *Vallisneria* (32351, 32805) * Sara J. Rogers, U.S. Fish and Wildlife Service Onalaska, Wisconsin
8:30 a.m.	Consequences of Drought for the Native Species *Vallisneria americana* (32805) * Ann Kimber, Iowa State University Ames, Iowa

8:45 a.m.	Effects of High Temperature on Growth and Propagule Formation in Submersed Aquatic Macrophytes (32351) * Dwilette G. McFarland, WES Vicksburg, Mississippi
9:00 a.m.	Initial Evaluation of Submersed Macrophyte Decline Sites (32805) * Craig S. Smith, WES Vicksburg, Mississippi
9:15 a.m.	A Spatial Approach to Understanding Invasions and Declines (32805) * M. Rose Kress, WES Vicksburg, Mississippi
9:30 a.m.	***Break***
10:00 a.m.	Aquatic Plant Competition Studies (32577) * R. Michael Smart, WES-LAERF Lewisville, Texas
10:15 a.m.	Guntersville Reservoir Plant Competition Studies (32736) * Robert D. Doyle, WES-LAERF Lewisville, Texas
10:30 a.m.	Dynamic Patterns in the Development of Convective Circulation in the Littoral Zone of Eau Galle Reservoir (32405) * William "Bill" F. James, WES Eau Galle Limnological Laboratory Spring Valley, Wisconsin
10:45 a.m.	Hydraulic Exchange Processes in the Littoral Zone: Initiation of Numerical Modeling (32405) * Michael "Mike" Schneider, WES Vicksburg, Mississippi

Biological Technology

Presiding: Alfred "Al" F. Cofrancesco, WES, Vicksburg, Mississippi

11:00 a.m.	Overview of Biological Technology * Alfred F. Cofrancesco, WES Vicksburg, Mississippi
11:15 a.m.	Overseas Research (32730) * Chris A. Bennett, University of Florida Gainesville, Florida
11:30 a.m.	***Lunch***
1:00 p.m.	Quarantine Research (32730) * Gary R. Buckingham, U.S. Department of Agriculture (USDA) Gainesville, Florida

1:15	Release and Establishment of Hydrilla Biocontrol Insects (31799) * Michael "Mike" J. Grodowitz, WES Vicksburg, Mississippi * Ted D. Center, USDA Fort Lauderdale, Florida
1:45 p.m.	Release and Establishment of Insect Biological Control Agents of Pistia (32406) * F. Allen Dray, University of Florida Fort Lauderdale, Florida
2:00 p.m.	Pathogen Biological Control Agents of Eurasian Watermilfoil (32202, 32735) * Judy F. Shearer, WES Vicksburg, Mississippi
2:15 p.m.	Pathogen Biological Control Studies of Hydrilla (32200) * Judy F. Shearer, WES Vicksburg, Mississippi
2:30 p.m.	Allelopathy (32408) * Harvey L. Jones, WES Vicksburg, Mississippi
2:45 p.m.	Trapa Biological Control Research * Alfred "Al" F. Cofrancesco, WES Vicksburg, Mississippi
3:00 p.m.	***Adjourn General Session***
3:30 p.m. - 10:00 p.m.	***National Aquarium Tour and Dinner***
3:30 p.m. - 4:30 p.m.	***Shuttle Buses to the National Aquarium***
7:00 p.m.	***Dinner and Cash Bar in Marine Mammal Pavilion***
9:00 p.m. - 10.00 p.m.	***Shuttle Buses Return to Radisson Hotel***

Thursday, 18 November 1993

8:00 a.m. - 12:00 noon	***General Session*** Calvert Ballroom, Salon C

Chemical Technology
Presiding: Kurt D. Getsinger, WES, Vicksburg, Mississippi

8:00 a.m.	Overview of Chemical Control Studies * Kurt D. Getsinger, WES Vicksburg, Mississippi

8:15 a.m.	2,4-D Update * Donald L. Page, Industry Task Force II Belhaven, North Carolina
8:30 a.m.	Herbicide Concentration/Exposure Time Studies (32352) * Michael "Mike" D. Netherland, WES Vicksburg, Mississippi
8:45 a.m.	Herbicide Delivery Systems (32437) * Michael "Mike" D. Netherland, WES Vicksburg, Mississippi
9:00 a.m.	Herbicide Plant Tissue Burden Relationships (32437) * John H. Rodgers, University of Mississippi Oxford, Mississippi
9:15 a.m.	Field Evaluation of Herbicides in Guntersville Reservoir * Earl R. Burns, TVA Muscle Shoals, Alabama
9:30 a.m.	***Break***
10:00 a.m.	Herbicide Application Techniques for Flowing Water (32354) * Alison M. Fox, University of Florida Gainesville, Florida
10:15 a.m.	Triclopyr Field Evaluation: Application and Water Residues (32404) * Kurt D. Getsinger, WES Vicksburg, Mississippi
10:30 a.m.	Triclopyr Field Evaluation: Target/Non-Target Efficacy (32404) * John D. Madsen, WES-LAERF Lewisville, Texas
10:45 a.m.	Species-Selective Use of Herbicides/PGRs (32841) * Kurt D. Getsinger, WES Vicksburg, Mississippi
11:00 a.m.	Monitoring Herbicide Induced Stress in Submersed Plants (32352) * Susan L. Sprecher, WES Vicksburg, Mississippi
11:15 a.m.	Evaluation of Plant Growth Regulators (32578) * Linda S. Nelson, WES Vicksburg, Mississippi
11:30 a.m.	Phenology of Aquatic Plants: Eurasian Watermilfoil (32441) * John D. Madsen, WES-LAERF Lewisville, Texas
11:45 a.m.	Report on Tuesday's Division/District Working Session * Wayne T. Jipsen, USAE District Jacksonville, Florida
12:00 noon	***Adjourn 28th Annual Meeting***

1:00 p.m. - 4:00 p.m. **FY95 Civil Works R&D Program Review**
(Corps of Engineers Representatives Only)
Calvert Ballroom, Salon E

Posters And Demonstrations

Comparisons of Stocking Rate Model Results for Guntersville Reservoir and Lake Marion Stocking Efforts (32438)
* R. Michael Stewart, WES, Vicksburg, Mississippi

HERBICIDE Simulation Model (32439)
* R. Michael Stewart, WES, Vicksburg, Mississippi

Geographic Information System Technology in the Field Office (32506)
* Scott Bourne, WES, Vicksburg, Mississippi

Estimation of Water Movement in Submersed Aquatic Macrophyte Beds by Gypsum Dissolution (32405)
* Harry "Butch" L. Eakin, WES, Vicksburg, Mississippi

Biological Control Course for Jacksonville District
* Michael J. Grodowitz, WES, Vicksburg, Mississippi

Impact of Cold Temperatures on *Hydrellia* Larvae (32734)
* Ramona H. Warren, WES, Vicksburg, Mississippi

New Biocontrol Agents
* Jan E. Freedman, WES, Vicksburg, Mississippi

Fish Utilization of Aquatic Plants: A Framework for Research
* K. Jack Killgore, WES, Vicksburg, Mississippi

Hydrilla Defense Mechanisms: Gene Induction of Phenolic Compound Biosynthesis
* Stewart L. Kees, Vicksburg, Mississippi

New APCRP Educational Tools: Videotapes and Videodiscs
* Victor Ramey, University of Florida, Gainesville, Florida

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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, videos, 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 28th Annual Meeting held in Baltimore, MD, 15-18 November 1993.

Current and Future Changes in the Aquatic Plant Control Research Program

by

J. L. Decell[1]

Over the past 19 years, there have been many changes to the Corps Aquatic Plant Control Research Program (APCRP). While all of these have not been major changes, they have been significant because they were made to respond to changing trends in both the science of our work and in the need for more improved methodologies for Operations, who were being responsive to changing requirements in the field. While I want to concentrate on the potential for changes in the current and future program, I want to first reflect on a few of these past changes.

During the late 1960s and early 1970s, the emphasis was on the control of alligatorweed and waterhyacinths. The primary methods being employed were herbicides and mechanical devices. Biological agents were being released on alligatorweed, and there were a few insects in quarantine for waterhyacinths; but this technology area was in its infancy, and there was no indication of success for biocontrol. We placed great faith in our premise that if our understanding of the natural systems was correct, then biocontrol should work and be our long-term method of control.

why?

Alligatorweed populations began to reduce throughout the release regions, and we had a success on our hands. Unfortunately, our predecessors had not anticipated an operational success. We found ourselves with no way to capitalize and repeat the success for waterhyacinths. It was this experience that caused the first change in the APCRP. We began to focus on the long-term aspects of all of the control methods. We became a proponent of the way that we conducted our research, and not a proponent of any particular method of control. We were thus able to gain success with waterhyacinth insects and had one major advantage. We could explain why and how effects took place and why variations were not surprising.

?

We also became committed to do our research so thoroughly that we would never have to do it again. That is not entirely possible, but it is an objective that has merit. I can personally testify, in that we only built one CO_2 laser.

We also began to develop models and simulations. It not only provided us with some research tools and a method for extrapolating results, but, equally important, it gave us the capability to conduct "what if?" evaluations. At this time, we also initiated studies on the ecology of the plants and began for the first time, to gain a knowledge of our "enemy."

About this same time, the Jacksonville District began to focus on a long-term approach for reducing the waterhyacinths on the St. Johns River to a maintenance level using herbicides. It took a couple of years, but they were successful; it was a prelude to the researchers and the operations people working together in the field. It was also a prelude to the formation of the Aquatic Plant Control Operations Support Center. From that time on, almost every research unit in the APCRP has a clearly identified operational objective before the research is initiated.

As the populations of alligatorweed and waterhyacinths were reaching maintenance levels throughout the Southeastern United States, it was obvious that the submersed plants hydrilla and milfoil were rapidly expanding. It was equally obvious that we had

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

not been conducting a commensurate level of research on these plants. The next change was to shift the emphasis of research to the submersed species. Shortly after that, we focused our research on these plants in a flowing water environment. The rationale was that if we could successfully apply technology in a dynamic environment, we could repeat that in a "static" environment; but the converse was not true.

Our simulation capabilities allowed us to forego costly field evaluations of mechanical devices, and we essentially ended all research on mechanical harvesters.

Through this process, we now had a research program that consisted of long-term management technology (biocontrol), methods for herbicide use that reflected a long-term objective, and studies devoted to the understanding of the plants role in the environment, both positive and negative.

One of our more current changes is reflected in the establishment of the Lewisville Aquatic Ecosystem Research Facility (LAERF). This facility provides a midscale level research facility that ensures a much greater degree of successful field applications for developed technologies. Prior to the establishment of the LAERF, we were forced to conduct very costly, large-scale field evaluations. Today, when we take a technology to the field, it is essentially an operational application. We are simply collecting data to quantify what we already knew was true!

Another current change has been the study of convective hydraulic circulation, which proves that for aquatic plant control, there is no such condition as "static." This knowledge is explaining former apparent anomalies in herbicide treatment results, and is beginning to be incorporated into herbicide treatment techniques that make them more and more environmentally compatible.

A significant current change has been the initiation of research to develop the relationships between aquatic plants and fish. An additional change was a Congressional add-on for work to be conducted with the University of Miami. This not only provides an opportunity to broaden the technical scope of the APCRP, but to bring technical disciplines, formerly not accessible, to the management of aquatic plants as a component of the aquatic ecosystem.

The last major current change is the establishment of the Corps' Center for Aquatic Plant Research and Technology at the U.S. Army Engineer Waterways Experiment Station. This center has the mission to coordinate all Federal aquatic plant research and act as a clearing house for information, technology, and interactions with regulatory agencies.

In the future, we will have to take an even broader view of aquatic plant management than we have begun to do recently. Recently, I challenged the scientists of the APCRP to consider the fact that aquatic plants are a major component of an aquatic ecosystem. The fact that they are causing a problem should not be the primary consideration in scoping the research or in developing a tactic to manage the plant population. The primary considerations should be the role of the plant in the aquatic ecosystem, the interactions existing between the other components, and the relationships between these components.

My reason for this challenge is simple. If we recognize that we are managing a significant component of an aquatic ecosystem, then we will not conduct the same research in the future as we have conducted in the past. Even if some of it is the same type research, we will not conduct it in the same way. Our research and our operational actions must begin to reflect the knowledge that we are managing habitat. It is not enough to simply determine what effect our management actions have on other components and organisms; we must initially recognize that we are manipulating a part of the habitat, and we must know how to do that in a compatible manner.

Major areas of interest and research in the future will be as follows:

- Relationships between fish and aquatic plants.
- Economic valuation of aquatic plant control programs—worth to the user.
- Cause and effects between freshwater ecosystems and estuarine systems.
- Partnerships with regulatory agencies so they have a better understanding of our programs and objectives.
- Better partnerships with other Federal agencies for sharing technology.
- Better partnerships with user/interest groups to accommodate their objectives into existing programs and thereby change the programs.
- More proactive efforts to educate the public and the interest groups regarding the true nature of our business—not public information efforts—education!
- Increased investments in scholarship programs as an investment in the future of aquatic plant management.

The future, based on current trends, can only hold the promise of having to do better with less—not necessarily doing more, but taking a longer range view of objectives, prioritizing on that basis, and then doing less numbers of things with increased quality for a long-term dividend. There will still be "brush fires" to fight, but it should be the easy exception—not the way we routinely do business.

Whether or not you think you must make the shift to "habitat management" because you believe that there is a "new environmental movement," or "the old movement is continuing," is not really the point. The problem levels of aquatic plants occur in a ecosystem and are habitat to organisms. If we propose to alter that, to meet the requirements of the users of that system, then the recognition that we are managing habitat is simply doing the right thing right!

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 of the APCOSC and relationships with other Corps aquatic plant control (APC) programs as outlined in Engineer Regulation 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) 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 1993 (FY93), the Center responded to 145 requests for assistance. Figure 1 indicates the types of information requested; Figure 2 provides a breakdown regarding source of information requests.

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 curly leaf pondweed in Reelfoot

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

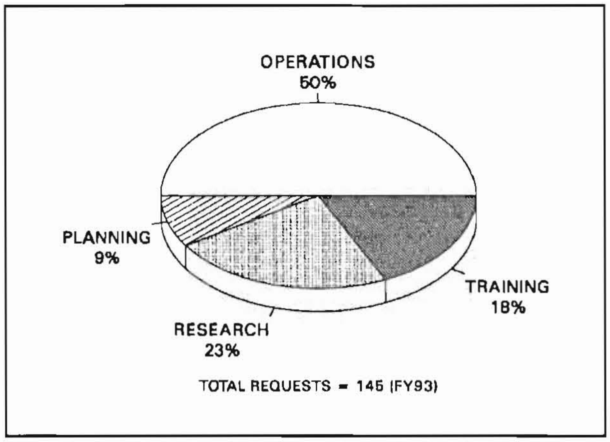

Figure 1. Types of information requested

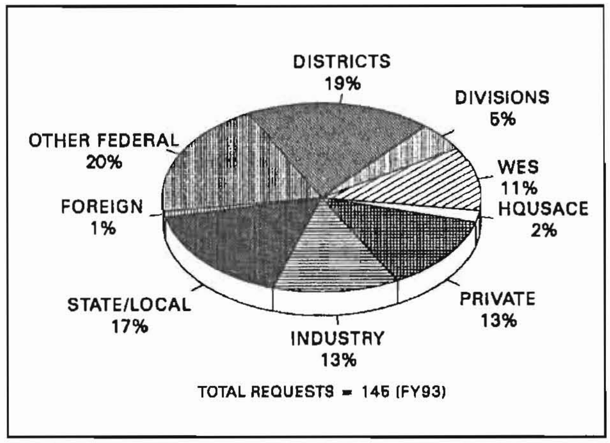

Figure 2. Sources of requests

Lake, participation in hydrilla management workshops at Lake Seminole (Mobile District), John H. Kerr Reservoir (Wilmington District), and the Lake Marion/Moultrie/St. Stevens Power Plant complex (Charleston District), and a visit to Puerto Rico to evaluate water quality problems associated with floating vegetation. The annual collection and shipment of alligatorweed flea beetles was canceled in FY93 because of a lack of insects in the donor areas. This activity will be resumed in FY94 if insect populations are adequate.

The Center conducted and/or participated in a number of training activities which included the following: (a) development of a session for the Jacksonville University seminar on aquatic plant management; (b) teaching a section on "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"; (d) providing a history and overview of the Corps of Engineers role in the biological control of alligatorweed during an East Coast (Florida) applicators workshop; (e) presenting an aquatic plant management workshop at the South Atlantic Division's Ranger Conference; and (f) providing airboat training for a number of Corps employees and visiting representatives of the Mexican government. In addition to these formal training activities, Center staff also participated in a variety of activities at the annual meetings of the Aquatic Plant Management Society (APMS), the Mid-South APMS, and the Florida APMS.

Annual Report of 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 provide intermediate-scale research environments to support studies of the biology, ecology, and control of aquatic plants. The LAERF is primarily funded by research projects of the resident staff and from fees charged to users of the ponds. The facility also receives partial funding (currently about 10 percent of the operating budget) directly from the APCRP to assist in its renovation and development.

The objective of this article is to provide an update on the continuing renovation, development, and operation of the LAERF.

Research Environments

The importance of scale in the study of submersed aquatic plants has recently been emphasized (Farmer and Adams 1989). Critical processes occur at different hierarchical levels from the individual plant (physiology and phenology), through plant populations (phenology), communities (competition), and ecosystems (plant succession and fish-plant interactions). Understanding the role of submersed aquatic plants in aquatic ecosystems under the Corps' stewardship and developing appropriate long-term management strategies for these reservoirs and waterways will require study of aquatic plants at each of these hierarchical levels.

The important uses of various scales of research are described in Table 1. Physiological processes such as photosynthesis and nutrient uptake are best studied in small-scale, closely controlled laboratory or growth chamber systems. However, determination of the effects of these physiological processes on the growth or phenology of aquatic plant populations requires longer term study in larger systems such as greenhouse tanks, mesocosms, or ponds. Community interactions such as plant competition, fish-plant interactions, and the effects of biological or chemical control on the species composition of aquatic plant communities can also be appropriately studied in mesocosms or ponds. Slower processes such as plant succession or the recolonization and recovery of plant communities following widespread disturbance or management operations are perhaps best studied in whole ponds or lakes. Assessing the impacts of exotic species on aquatic ecosytems also requires long-term observation and analysis of ponds, lakes, or reservoirs.

Small-scale studies provide rapid and inexpensive results, are easy to manipulate, can be replicated, and allow for maximum control and sensitivity. Large-scale studies are slow (perhaps lasting several years), difficult to control, expensive to conduct, often unreplicated, and less sensitive, and their results may be complicated by uncontrolled factors. However, since long-term results are not always predictable based on the behavior of smaller, simpler systems, large-scale studies or tests of management strategies are the only "true" experimental tests of developing technology.

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

Table 1
Importance of Different Spatial and Temporal Scales in Aquatic Plant Research

Scales	Hierarchial Level	Research Environment	Considerations
Small-scale Short-term	Plant, tissue, cell	Laboratory Growth chamber	Easy to manipulate Closely controlled environmental conditions Rapid screening Focus research
	Population	Raceways Greenhouse tanks	
Midscale Seasonal	Population	Mesocosms Contained pond populations	Easy to manipulate Moderately controlled environmental conditions Relatively rapid results Physical size adds "realism"
	Community	Mesocosms Contained pond communities	
Large-scale Long-term	Community	Ponds Lake/reservoir exclosures	Difficult to manipulate Many uncontrolled environmental conditions Requires long-term observation Only "true" test of technology
	Ecosystem	Ponds Lakes Reservoirs Waterways	

Since these larger tests are so slow and expensive, it is very important that the factors affecting the outcome of management actions be clearly identified and studied prior to large-scale testing. An intermediate-scale environment employing mesocosms or artificially contained populations or communities in ponds provides a near-optimum research environment with acceptable environmental control and adequate replication, at a scale that allows an appropriate degree of realism without too great a sacrifice in time or research funds.

In addition to ponds, the LAERF also includes other smaller scale research environments (Table 2) that facilitate research on plants, populations, or communities and allow us to optimize the use of the ponds for larger and longer term studies. The characteristics of these different facilities and the research conducted in them are documented in an APCRP bulletin (Smart and Decell 1994).

During fiscal year 1993 (FY93) the temperature-controlled greenhouse tanks were used in several short-term (5 to 9 weeks duration) studies to provide information on the competitive abilities of beneficial native plants and on the regrowth of exotic species under different light and temperature conditions. These tanks were also used to culture plant material during the winter and early spring months when plant populations in the culture ponds were dormant.

A shallow-water tank system was added in FY93 to support studies of the life cycle or phenology of aquatic plants. This system consists of twenty-four 2,000-L capacity fiberglass tanks measuring 1.8 m in diameter and 0.75 m in depth. The tanks are supplied with water from the Chemical Control Mesocosm System (CCMS) water-supply pond. In addition to supporting studies of the phenology of exotic aquatic plants, the system was used in Environmental Protection Agency (EPA) sponsored studies of the interactive effects of adverse sediment and water composition on the establishment and growth of aquatic and wetland plants.

Flowing-water raceways were in use throughout the year for culturing/holding aquatic plants and animals as well as holding sensitive aquatic plants during the winter. Many laboratory, greenhouse, and mesocosm studies require plant material during the winter or early spring, prior to the onset of active growth in the culture ponds. To conduct aquatic plant research throughout the year and to initiate mesocosm studies in the early spring, it is necessary to maintain needed species in greenhouse/raceway cultures during periods when they would be unavailable from the culture ponds.

During FY93, the CCMS was expanded from 22 to 30 mesocosm tanks to meet the high demand for this system. The system was

Table 2 Experimental Systems Available at the Lewisville Aquatic Ecosystem Research Facility			
Experimental System	**Physical Size (volume, surface area, depth)**	**Replicability (No. of Units)**	**Factors Controlled**
Greenhouse tanks	1,200 L, 1.35 m^2, 0.86 m	20	Temperature, Light, Water depth, Sediment composition, Water composition, Species composition, Inorganic carbon supply, Other biotic components
Shallow-water tank system	2,000 L, 2.65 m^2, 0.75 m	24	Light, Water depth, Sediment composition, Water composition, Species compostion, Other biotic components
Flowing-water raceways	3,600 L, 6.0 m^2, 0.60 m	18	Light, Water depth, Sediment composition, Water flow, Species composition, Other biotic components
Chemical Control Mesocosm System	6,500 L, 5.3 m^2, 1.25 m	30	Light, Water depth, Sediment composition, Water composition, Species composition, Other biotic components
Variable Depth and Light Tank Facility	14,000 L, 4.7 m^2, 1.0-3.0 m	18	Light, Water depth, Sediment composition, Water composition, Species composition, Other biotic components
Ponds	2,000,000 - 3,500,000 L, 2,100 - 3,200 m^2, average 1.1 m	18-12	Water depth, Water composition? Species composition?

used to investigate the use of species-selective herbicides to manage mixed aquatic plant communities dominated by exotic species and in studies of the efficacy of plant growth regulators to eliminate canopy formation in exotic species. In addition to the tanks, the CCMS also includes a lined water-supply pond, growout pond, and a small laboratory. During FY93, the usable area of the growout pond was doubled to accommodate the increased need to produce plants for use in the CCMS and other experimental systems. Also in FY93, we expanded our sediment preparation area, and we added plant processing facilities to expedite data collection.

The Variable Depth and Light Tank Facility (VDLTF) became operational in FY93 and was used to study the regrowth of exotic species under different light and temperature conditions. This system currently consists of nine 14,000-L capacity, fiberglass tanks measuring 2.5 m in diameter and 3 m in depth. The tanks are equipped with parabolic diffuser covers to disperse sunlight and to prevent shadowing by the tank sidewalls, which are black to minimize reflected light. These design features were necessary to mimic the light climate occurring in natural aquatic systems. The VDLTF shares both the water-supply pond and growout pond with the CCMS.

The LAERF ponds are an integrally important research environment—intermediate in size between the laboratory/greenhouse and large, multipurpose Corps' reservoirs. We are continuing to renovate the ponds based on demand and availability of funds. During FY93, 26 of the ponds were used for research, 5 ponds were used to culture plants used in research, and 19 additional ponds were available for use.

In addition to the ponds, Lewisville Lake, located immediately adjacent to the LAERF, and Lake Ray Roberts, 5 miles[1] up the Elm Fork Trinity River from Lewisville Lake, provide additional opportunities for large-scale research. Both of these Corps reservoirs have recently been invaded by hydrilla. Although it is indeed unfortunate that hydrilla has become well established in Lake Ray Roberts, the close proximity of these lakes to the LAERF does provide opportunities for close study and observation of the mechanisms and rates of hydrilla expansion. Identification of important environmental factors contributing to hydrilla expansion and assessment of the efficacy of various management operations could provide important information needed to improve our ability to manage the growth of this troublesome exotic species in these and other Corps reservoirs.

An onsite analytical laboratory continues to provide analytical support to many of the APCRP research projects conducted at the LAERF. We are also continuing an environmental and water quality monitoring program to obtain basic information on the environmental conditions and water chemistry of the ponds, tanks, and mesocosms.

In FY93, improvements were made to our plant processing laboratory, and we began work on a plant physiology laboratory. Improvements included a covered, outdoor root-washing station and additional oven space for drying the large volumes of plant material collected during major harvests. The physiology laboratory will house instrumentation and equipment that will enable us to study important physiological processes such as photosynthesis and nutrient uptake.

Research

All major technology areas of the APCRP are benefitting from research conducted at the LAERF. Many of these research projects are described in other sections of this report. During FY93, biological control was represented by studies of the efficacy of a microbial pathogen for control of hydrilla. Chemical control studies included an evaluation of the efficacy of a plant growth regulator to prevent canopy formation in exotic, weedy species and a preliminary study on the use of a low-level herbicide application to achieve species-selective control in a mixed aquatic plant community dominated by an exotic species. Several pond and tank studies were conducted to provide information on the phenology (or life cycle) of waterhyacinth and Eurasian watermilfoil. These studies should lead to advances in the area of applications technology. Simulation technology benefited from the collection of data in both greenhouse tanks and the VDLTF on hydrilla tuber sprouting and regrowth of hydrilla and Eurasian watermilfoil from root crowns and apical stem fragments under different low light conditions. Within the ecology area, studies were conducted to examine fish-plant interactions, including changes in fish growth and behavior in relation to the species of submersed plant cover. Additional studies within the ecology area included both pond and greenhouse studies of competitive interactions among introduced weedy species and beneficial native species.

In addition to the APCRP-sponsored research, the facility is also supporting the Corps' Wetlands Research Program. In FY93, LAERF ponds were used in a collaborative effort between the U.S. Army Engineer Waterways Experiment Station (WES) and Texas A&M University to evaluate moist soil management strategies for waterfowl habitat enhancement. The LAERF was also involved in a mesocosm and field study of nonpoint source pollution abatement in artificial wetlands, and a WES Hydraulics Laboratory study of the effects of wetland vegetation on water flow and sedimentation.

The LAERF also provides research on aquatic plants and wetlands for other Federal agencies provided that this research complements ongoing APCRP efforts. During the

[1] A table of factors for converting non-SI units of measurement to SI units is presented on page xxiii.

past 4 years, LAERF greenhouse tanks and shallow-water outdoor tanks have been used to provide data needed for an EPA Clean Lakes Program Demonstration Project concerned with reestablishing submersed aquatic and emergent wetland vegetation to improve water quality and provide aquatic habitat in chronically polluted Onondaga Lake, New York. During FY93, LAERF scientists conducted an EPA-sponsored study of experimental techniques for establishing aquatic and wetland vegetation at an artificial wetland development site on Lake Ray Roberts, Texas. During FY93, the LAERF was also used to conduct tank and pond studies to provide data needed to determine the potential impacts of herbivorous turtles on the reestablishment of submersed aquatic vegetation in Guntersville Reservoir. This research, which will continue in FY94, is funded by Tennessee Valley Authority.

Future Plans

Renovation and development of the LAERF will continue during FY94. The successful operation of the VDLTF has prompted the expansion of this facility from 9 to 18 tanks to meet anticipated demand. During FY94, we are adding a neutral-density shade covering to the CCMS to moderate high light levels and high summer temperatures. The laboratories will continue to be equipped based on need and availability of funds.

During FY94, we anticipate an additional increase in activity associated with APCRP research conducted at the facility. We anticipate relocating an additional government employee to LAERF to assist in the conduct of expanded CCMS studies. Several additional contractors will also be sought to maximize the utilization of (and potential benefits provided by) the excellent research facilities now available at the LAERF.

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: Ken Howell, Larry Prestien, and Kay Glassie of the Elm Fork Project Office; Michael Crouch, Gary Dick, Robert Doyle, David Holland, David Honnell, Chris Houtchens, John Madsen, Kimberly Mauermann, Rita Pierson, Joe Snow, and Stephen Wood of LAERF; Kurt Getsinger, Debra Katzenmeyer, Richard Price, and Mike Stewart of WES.

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Hydroacoustic Measurement and Automated Mapping of Submersed Aquatic Vegetation

by

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Introduction

Available methods for mapping the distribution of aquatic vegetation rely on detecting plants at or near the water surface. Consequently, vegetation surveys are often not started until the plants are well developed and have begun to reach the water's surface. When surveys are conducted, the observed distribution of plants visible at the surface tend to underrepresent the actual distribution of the plants.

Hydroacoustic methods have been used experimentally to detect submerged vegetation (Maceina and Shireman 1980; Maceina et al. 1984; Thomas et al. 1984; Duarte 1987; Thomas et al. 1990). The susceptibility of underwater plants to acoustic detection suggests that effective submersed aquatic vegetation surveys can be performed earlier with acoustics than with traditional methods. In addition, it suggests that the spatial extent of vegetation can be potentially described more completely with acoustical methods than with traditional methods. These two advantages, earlier detection and better spatial delineation, make it possible to provide more lead time for planning plant control activities and may make it possible to provide more environmentally compatible and effective aquatic vegetation control.

Acoustic signal processing used to extract data from the echo returns of plants can be accomplished in real time to produce a digital data stream suitable for distribution mapping. When combined with geographic coordinate data from the global positioning systems (GPS) and the geographic mapping capabilities of geographic information systems (GIS), a complete aquatic plant survey package can provide accurate and complete mapping of aquatic plants.

The purpose of this project is to integrate existing hydroacoustic, GPS, and GIS technologies into a single package suitable for accurately mapping the spatial extent of submersed aquatic vegetation during early growth stages when plants are not yet detectable at the surface. The project has four major tasks (Figure 1). First, a hydroacoustic (HA) task involves identifying a suitable acoustic system for detection and discrimination of aquatic vegetation. This entails identifying sonar system characteristics appropriate for plant detection and developing signal processing software for detecting and discriminating aquatic plants. Second, accurate real-time position data are acquired with GPS equipment. Third, the position data are combined with acoustic data into a single data stream. This task involves using commercially available software for real-time consolidation of acoustic and positioning data. Finally, a mapping task involves developing an overall package for mapping and display of aquatic vegetation.

In this report, we summarize progress made this past year toward accomplishing these four tasks. We also report on results from experimental collections of acoustic data from aquatic plants in Lake Guntersville, AL. The data were collected (a) to identify echo levels from aquatic plants at various stages in their development; (b) to determine how echo levels vary with acoustic transmission frequency and transducer beam width; (c) to investigate our ability to detect sparse, early-growth vegetation; (d) to determine the

[1] U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
[2] Biosonics, Inc., Seattle, WA.

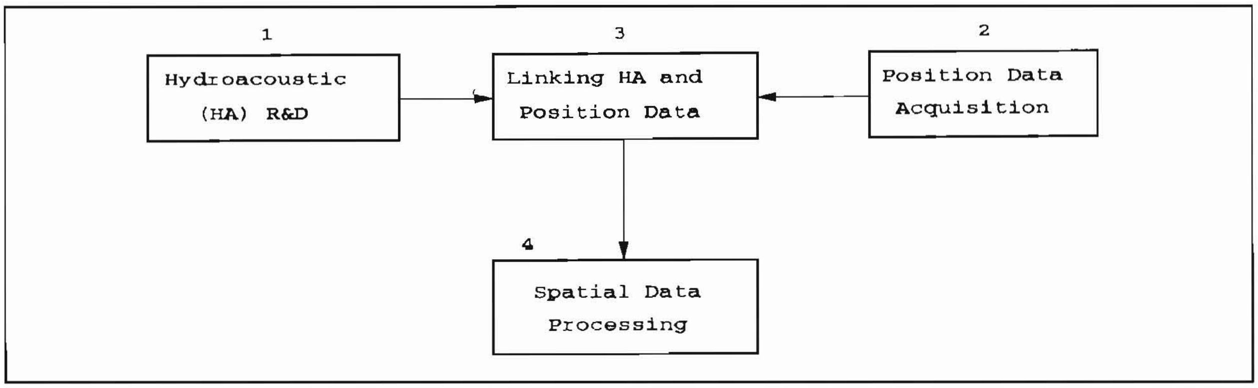

Figure 1. Tasks within project

near-surface performance of acoustic detection; and (e) to evaluate the bottom detection capabilities of acoustic systems in the presence of sound attenuation caused by dense vegetation.

Methodology

Field testing was performed during 21-26 June 1993 at Guntersville Reservoir in northeastern Alabama. Tests were conducted around the plant exclosures near the Chisenhall Ramp, located approximately 5 km downstream of Comer Bridge (Highway 35) at Scottsboro (Figure 2). Depths ranged from 4 m in the middle of the secondary channel to less than a meter northeast of the exclosures. At the time of testing, aquatic vegetation, primarily Eurasian watermilfoil (*Myriophyllum spicatum*), was topped out to depths of about a meter and was present at lesser density to a maximum depth of about 2.5 m. Reservoir elevation during this time was approximately 594.5 ft msl.

Implementation tasks (2-4 in Figure 1) were performed separate from the HA task (1). Once procedures for tasks 2-4 were established, emphasis could be placed on the HA research task. When completed, the HA system could be substituted for the echo sounder initially used in tasks 2-4. Procedures used in the various tests are described below.

Positioning, data linkage, and spatial processing

Table 1 lists equipment and software employed. The GPS systems use only coarse acquisition code (C/A code), which is available to civilian users. One of the two systems is set up at a known land position (survey benchmark) and programmed to radio transmit position correction data (difference between benchmark location and C/A code GPS position estimate). This transmission is picked up by the other radio-equipped GPS in the mobile unit (16-ft johnboat). The GPS position estimate of the mobile unit is then altered by the transmitted correction. This mode of operation,

Table 1
Equipment and Software Used for Tasks 2-4

Component	Description
GPS	2 – Trimble 4000 SST GPS receivers 2 – Trimble GPS antennas
Radio	2 – Motorola model M 208 (40-watt UHF radio) 1 – 3-m UHF fiberglass whip antenna (base station) 1 – 1-m UHF magnetic mount antenna (mobile unit, boat)
Modem	1 – BDLC base Modem 1 – MRM mobile modem
Fathometer	1 – SDH-13A sounder with Odom Digitrace (DT3MS) 1 – 200 kHz, 10-deg transducer
Computer	1 – Sharp PC-5500 with black and white monitor (286 laptop, for field use)
Software	1– GeoLink XDS[1] v. 2.0 with NMEA format 1– PC ARC/INFO[2] v. 3.4D Plus 1– PC ARC/EDIT v. 3.4D Plus 1– PC ARC/TIN v. 2.2D

[1] GeoResearch, Inc., Billings, MT.
[2] All PC ARC/products from Environmental Systems Research Institute, Redlands, CA.

referred to as real-time differentially corrected GPS (RDGPS), cancels out the effects of selective availability and reduces other error sources so that average error is about 2.4 m (Logsdon 1992). This error level was judged acceptable for the project.

The laptop personal computer (PC) aboard the boat ran the GeoLink XDS software and was connected to the GPS unit and an echo sounder with LCD decimal display via two RS-232 serial ports. The GeoLink program read the corrected position data from the GPS, merged it with digital output from the echo sounder (which reported the depth of the first strong echo return), and stored this 1-Hz output data stream on the hard disk. The program also provided navigation graphics to aid in conducting the survey.

After completion of the field data collection, the stored data were converted to an ARC/INFO ASCII format using the GeoLink Editor. These files were then imported into PC ARC/INFO using the GENERATE command.

Testing consisted of trying various positions of the GPS base station, relative to the mobile unit survey area, until the mobile unit received uninterrupted radio signals from the base station, which collected data from the same set of GPS satellites as the mobile unit. Once this was accomplished, various transect patterns were run in the vicinity of the exclosures. ARC/INFO software was then used to generate maps.

Hydroacoustic measurements and plant sampling

Experimental data collections were made 21-26 June 1993 in Lake Guntersville, AL, near the Chisenhall Ramp located approximately 5 km below the Comer Bridge (Highway 35) at Scottsboro, AL. All data were collected from four permanent transects for which end points were identified by buoys (Figure 2). Eurasian watermilfoil was the most commonly found nuisance plant species and appeared to dominate the survey area in occurrence and plant volume. Three transects (AB, CD, EF), approximately 90 m in length, paralleled the bottom contour at nearly constant bottom depths of 1.5, 1.9, and 2.5 m, respectively. Abundance of milfoil was greatest along the 1.5-m contour. Here, plants extended from the bottom to approximately 0.5 m below the surface. Individual plants or clumps of plants were spaced every 2 to 4 m. Each plant or clump of plants intercepted an area of 0.2 to 0.5 m^2. Considerable branching was evident in these plants. Those plants along the 1.9-m contour exhibited nearly the same level of development, but were spaced at somewhat wider intervals and exhibited somewhat less branching. Along the 2.5-m transect, plants were sparsely distributed single stems that extended no more than 0.5 m from the bottom. A fourth transect (GH), approximately 270 m in length, extended across the depth gradient and encompassed depths from 4 m to <1 m (Figure 2). This transect contained the entire range of plant development from sparse single stems in the deeper water to dense "topped" vegetation mats in water less than 1 m deep.

Hydroacoustic data for different acoustic frequencies and transducer beam widths were collected by repetitively surveying the four transects from a pontoon boat. Data collections were made simultaneously with an array of scientific-grade calibrated measurement equipment. This equipment, listed in Table 2, is the same type used by Thomas et al. (1984). For the present study, the sounders were calibrated at the lowest preamplifier settings available in each sounder commensurate with the large reflectance of watermilfoil demonstrated by Thomas et al. (1984). With this instrumentation, as many as four different acoustic configurations were operated simultaneously. Switching among the different available options on different surveys allowed the user to obtain data on seven different transmission configurations. These consisted of 38 kHz, 10-deg beam width; 120 kHz, 10-deg beam width; 200 kHz, 6-deg beam width; and 420 kHz with beam widths of 2, 4, 6, and 10 deg. Downward-looking acoustic transducers were attached to a rigid plate that was suspended with cables through the deck to just below the surface of the water. All seven

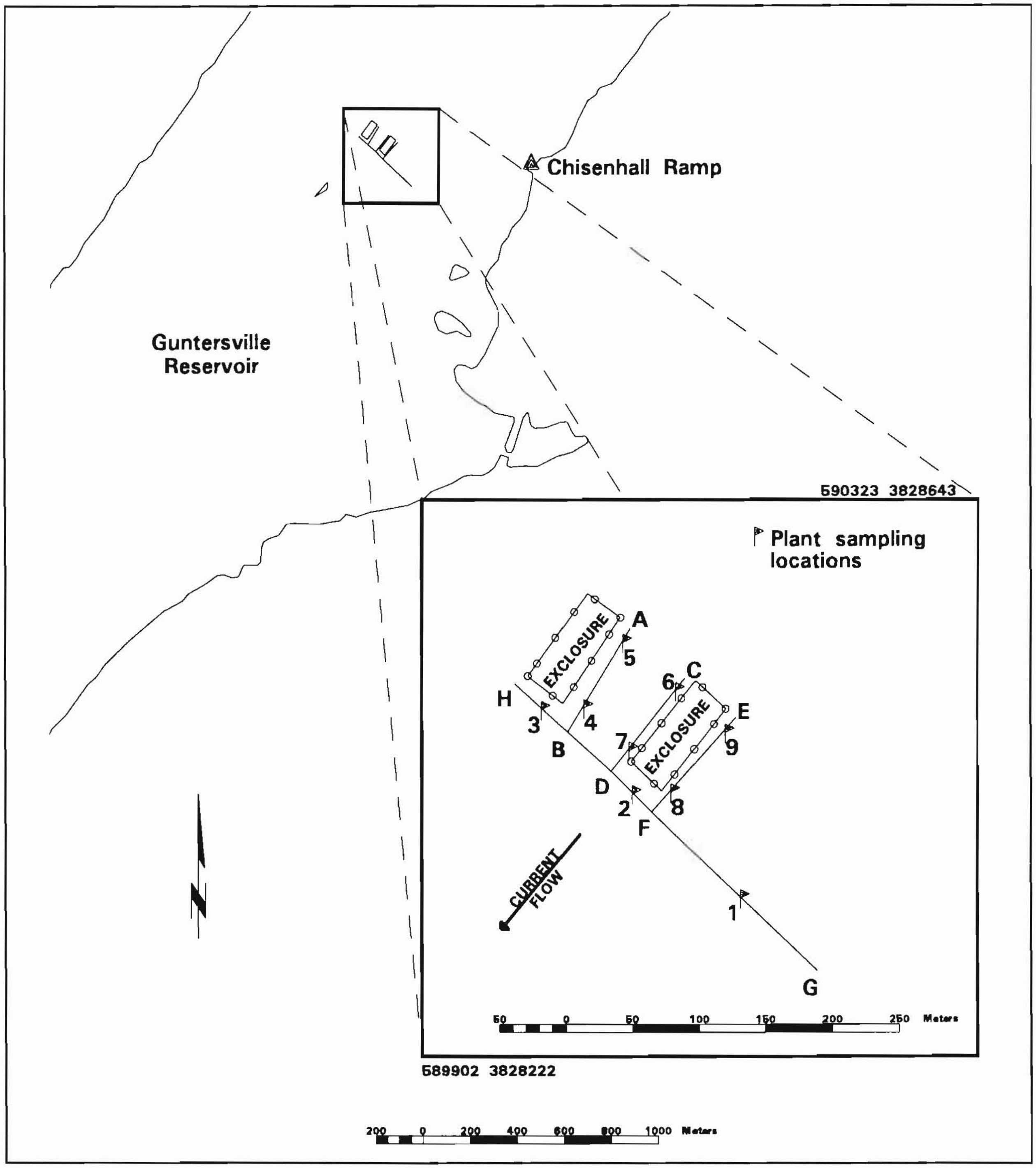

Figure 2. Site location map with Zone 16 UTM coordinates indicated in enlarged area

transducers were mounted along a central axis in line with the direction of boat movement. Thus, all transducers that were operated at the same time sampled the same volume of water as the boat traversed each transect. A low light video camera with a VHS recorder was also mounted on the axial center line of the plate in a downward-looking orientation. This provided a simultaneous visual record of plant conditions.

Hydroacoustic data were recorded on digital tape. From the recorded data, backscattering from plant biovolume was summarized in

Table 2
Equipment Used as Part of Hydroacoustic Measurements

Component	Description
Sounders	1 – Biosonics[1] 102 sounder, 120 and 420 kHz 1 – Biosonics 102 sounder, 200 and 420 kHz 1 – Biosonics 101 sounder, 38 kHz
Transducers	1 – 38 kHz, 10-deg beam width 1 – 120 kHz, 10-deg beam width 1 – 200 kHz, 6-deg beam width 1 – 420 kHz, 2-deg beam width 1 – 420 kHz, 4-deg beam width 1 – 420 kHz, 6-deg beam width 1 – 420 kHz, 10-deg beam width
Recording and Monitoring equipment	2 – Oscilloscopes 3 – Biosonics 171 Recording Interface 3 – Sony DAT Recorder, DTC-10000 1 – volt meter
Underwater camera	1 – low light underwater video camera, Outland Technologoy model UWC-300, with light source and monitor 1 – VCR recorder
Power source	1 – gasoline-powered generator 2,500-W Honda

[1] All hydroacoustic instrumentation leased from Biosonics, Inc., Seattle, WA.

0.1-m range classes by voltage-squared echo integration. Results were used to characterize volume backscattering levels for plants of different growth stages and for comparison of backscattering levels associated with different frequencies and beam widths.

Near the end of the acoustic survey period, plant samples were taken at nine quadrat stations identified along the four survey transects (numbered flags in Figure 2). At each station, all plants in three randomly placed 1-m quadrat samples were collected by divers. Plant material rooted inside each quadrat was cut off at the bottom and brought to the surface one plant or one clump at a time. The number of stems and the total length of each clump were recorded; then plants were cut into 25-cm lengths from the base.

For each length, plants were sorted by species, the number of stems and leaf whorls were counted, and both wet and dry weights were taken. Samples of stem cross sections were also taken and mounted on laboratory slides for microscopic measurement of the dimensions of the lacunal air space present in stems.

Current Findings

Preliminary procedure development for RDGPS usage, data linkage, and spatial data analysis were completed. Data necessary to address preliminary HA and phenomenology questions were also collected, and analysis is currently underway. A brief summary of the current results for each task is described below.

Positioning, data linkage, and spatial processing

Radio linkage and comparable satellite visibility proved to be the critical factors in successful RDGPS operation. Several candidate base stations proved unworkable because of either radio interference (high voltage transmission lines) or terrain/vegetation shielding of certain satellites from the base station. The final base station location, Chisenhall Ramp, was within a kilometer of the mobile unit, and the GPS antenna was elevated 5 m to avoid vegetation shielding. The range between base and mobile stations could be increased by using a more powerful radio. Terrain and vegetation shielding will always be an important concern, particularly given the mountainous terrain along the southeastern shore of Guntersville Reservoir. As RDGPS technology improves and permanent radio-transmitting base stations are established, costs and complexity of this task will decrease significantly.

No particular problems were encountered either in linking the data streams with the GeoLink software or in entering and processing this stream with ARC/INFO software. The field operation, however, would be improved with the use of a laptop PC with increased processing power, more RAM and hard drive capacity, a mouse, and color VGA display. Also, some additional research will be necessary to determine the optimum transect pattern needed to accurately describe the spatial distribution of submersed vegetation.

Hydroacoustic measurements

Nine repetitions of the transect series were run for various system configurations. Figure 3 illustrates a graphic example, called an echogram, of the signal generated by one transducer (420 kHz, 6-deg beam width) for a portion of the CD transect. The vertical axis represents distance (meters) from the transducer face, which was about 10 cm below the water surface. The horizontal axis represents output report number, starting at buoy C and proceeding towards buoy D at a rate of 10 outputs per second. The boat speed was approximately 1.2 m/sec, resulting in an average distance of 12 cm horizontal distance between reports. Each report consists of the squared voltage[1] per unit volume, corrected for 1-way geometric spread (20 Log (range) correction), within each 10-cm depth increment. Higher return values are represented by darker shades. Note also that for this particular transducer, no data are reported in the top five depth increments. This corresponds to the acoustic near-field in which the pulse wave front is not yet formed and from which reliable data cannot be extracted. This distance varies as a function of frequency and beam width and is discussed later. The sediment interface in this echogram occurs at vertical distance of approximately 1.3 m and is represented by the dark band centered at 1.7 m. Plants are represented by the intermittent dark spikes rising to 0.5 m vertical distance.

Figure 3 presents several general features of the data. Plant occurrence and resulting echo intensity are highly variable spatially. Also, sediment echo intensity is relatively constant, although it was variable along other transects, particularly GH. Plant signatures can be quite high, and under these conditions, the bottom may not be evident at all. To illustrate the overall effect of plants, echo

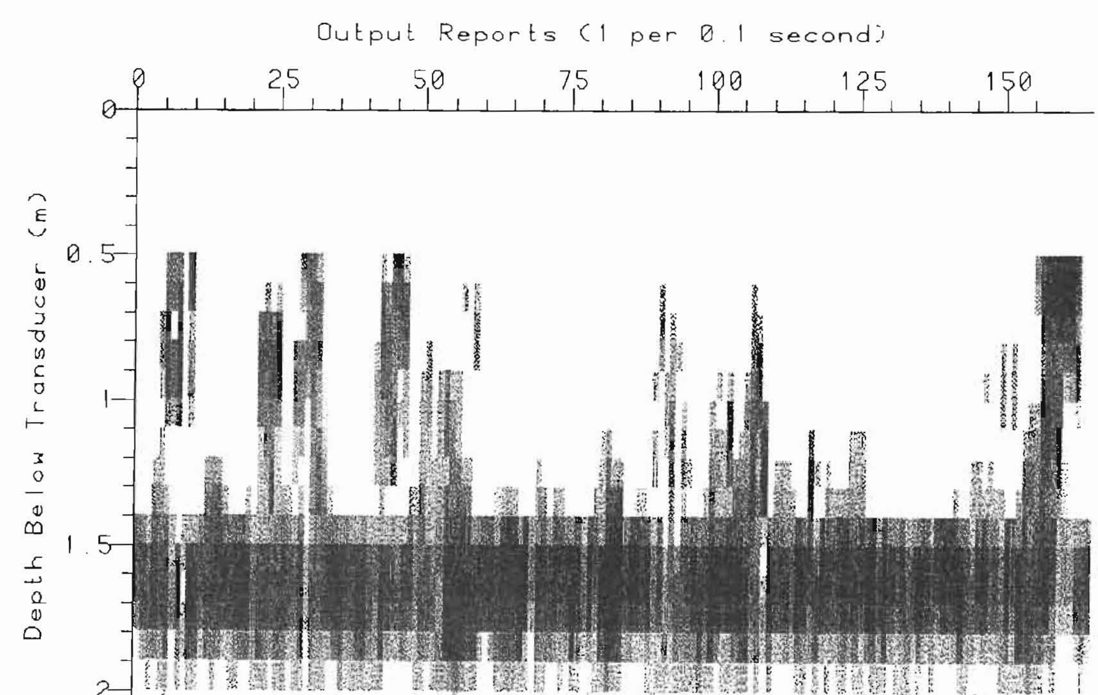

Figure 3. Display of digital signal from 420-kHz, 6-deg beam width transducer along transect CD at 1.2 m/sec

[1] Voltage squared is proportional to intensity of the acoustic echo.

integration (which averages the squared voltage returns within each depth layer) was performed for the entire CD transect using the same transducer. Integration was performed on two data groupings—all pings (dark shaded bars) and only those with plants present (light shaded bars)(Figure 4). In the all ping case, the overall effect of plants is relatively minor—the bottom is the most prominent feature. This data grouping accounts for patchiness of the plants. The plants only case illustrates that when plants are present (indicated by an above-threshold signal above the bottom), their signatures may be stronger than the bottom return. Bottom and sub-bottom layer echoes may be stronger when plant roots are present in the sediment.

To satisfy project requirements, several transducer parameters are of particular importance. The system should have a large dynamic range to accommodate the full range of echo strengths from small echoes generated by single stems to large echoes generated by dense mats of vegetation. To achieve the desired operating depth range, the acoustic near field distance must be no more than 0.5 m. Range resolution must be sufficiently fine to be able resolve the plant tops, the structure within the plant canopy, and the bottom. A frequency must be selected based on its having high sensitivity to sparse vegetation, acceptably low ambient noise levels, and an acceptable near-field.

Achieving a short acoustic near-field is probably the overriding consideration. The transition distance at which the near-field ends is usually considered to occur somewhere between D^2/λ and $2D^2/\lambda$, where λ is the wavelength and D is the diameter of the radiating face of a circular transducer. The beam width has a nonlinear inverse relationship with D^2. Short near-field distances are achieved using high frequency and wide beam widths. Table 3 contains estimated near-field distances for the transducers used. Only the 420-kHz transducers with 6- and 10-deg beam width meet the 0.5-m requirement and provide much data under the shallow test conditions. Another

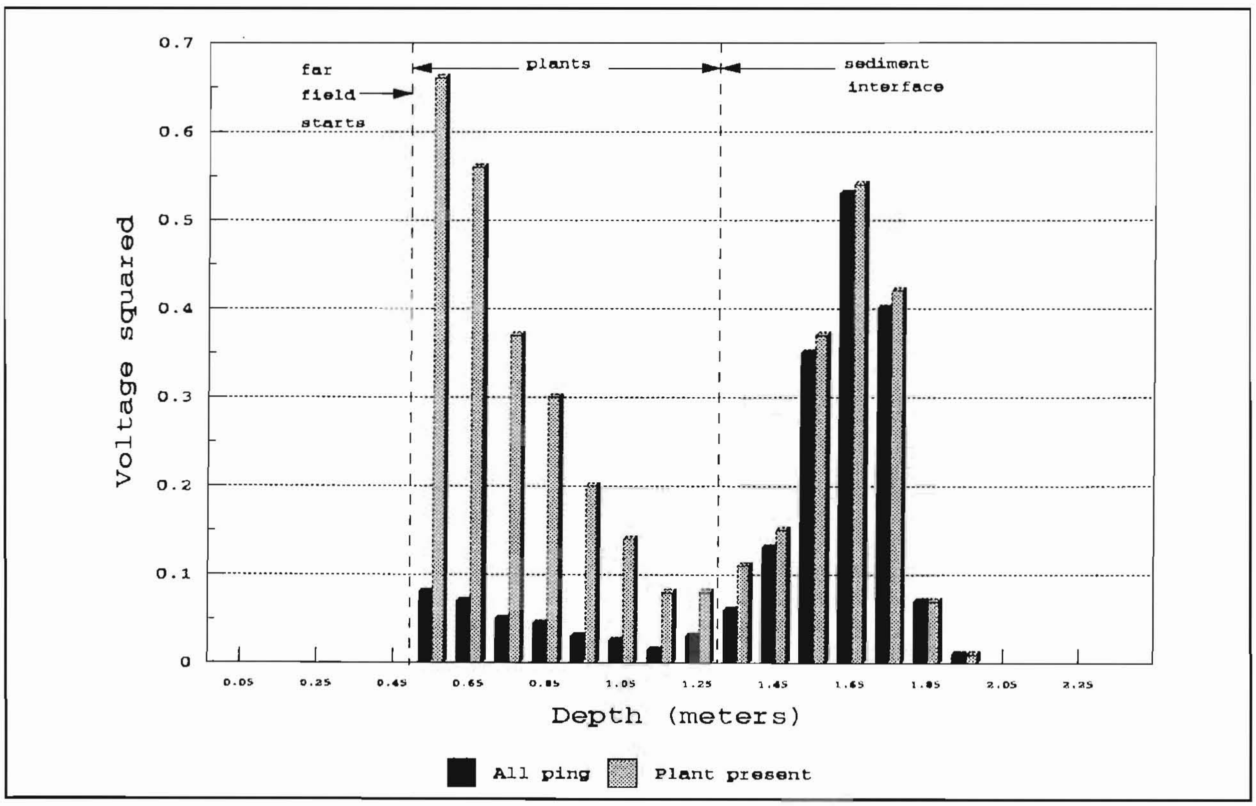

Figure 4. Echo integration of 420-kHz, 6-deg transducer along transect CD

Table 3 Acoustic Near-field Distance for Transducers Tested		
Frequency kHz	Beam width deg	Near-field Range, m
38	10	1.34
120	10	0.64
200	6	1.07
420	2	2.24
420	4	0.72
420	6	0.41
420	10	0.15

consideration to achieving short measurement distances is ringing. This is an artifact of using a single transducer to transmit and receive; it results from the transducer's resonating after the signal pulse ends. Both wide beam 420-kHz transducers exhibited marked increases in noise levels at ranges less than 0.5 m. This evidence of ringing suggests that it may not be possible to obtain measurements at less than 0.5-m distance with a single transducer.

Frequency comparison analyses were performed along transect FE, which represents the best example of sparse, short vegetation. Calibration-adjusted echo-integration results (V^2/m^3), averaged over three replicates, showed that backscatter from vegetation increases with frequency. The average increase is 1.5 times from 120 to 200 kHz and 12.3 times from 200 to 420 kHz. Although this is less than the $(f_2/f_1)^4$ increase predicted by scattering theory (Urick 1983), it is much greater than the zero increase, which would be attributable to surface reflectance effects. This indicates that the phenomena is primarily caused by volume scattering (i.e., by objects much smaller than the wavelength, such as fine leaf structure and bubbles within the plants). Ambient noise measurements, removed from the effects of close-range ringing, are all relatively low (well below those of returns from sparse plants) and exhibit the expected increase with frequency.

Range resolution is a measure of the minimum distance at which two separate objects can be resolved in the signal. It is approximately $c\tau/2$, where c is the speed of sound in water (around 1,470 m/sec) and τ is the signal pulse width in seconds. To achieve a range resolution of 0.1 m, τ should be no more than 0.14 milliseconds (m/sec). The slow rise of the return signal when the bottom is encountered (Figure 4) results from using too large a τ (0.4 m/sec). A fine range resolution would potentially provide more detailed information on vegetation and the bottom. A τ around 0.1 msec would probably be suitable.

These preliminary analyses suggest that the transducer should be high frequency, high dynamic range, relatively wide beam width, and short signal pulse width. The higher frequency (about 420 kHz) achieves a better sensitivity to sparse vegetation and minimizes the acoustic near-field distance. The wide beam width (≥6 deg) also minimizes acoustic near-field distance; it results in sampling a larger volume, which should increase sensitivity to vegetation. The short signal pulse width (about 0.1 msec) should achieve the range resolution adequate to distinguish slight differences in canopy height and structure and to determine the depth of the bottom more accurately.

Several analyses tasks are ongoing. Physical plant measurements, obtained by diver sampling, will be correlated with HA signatures in an attempt to understand the relationship between the two. Additionally, signal processing algorithms necessary to detect vegetation and the bottom and to extract vegetation attributes will be developed from the measured HA signal data. Apparently, it will be necessary to use multiple ping reports to extract vegetation and bottom information. Using such a scheme would output one plant and bottom attribute report for every 10 or 20 ping reports. This output rate is expected to yield adequate spatial resolution for vegetation mapping.

Fiscal Year 1994 Plans

We have identified the range of system configuration parameter values necessary to achieve our detection goal. We are currently in the process of comparing these with commercially available HA systems. By the summer, we will have obtained a suitable system and will have integrated it with first-generation processing algorithms. Testing with this new system will be performed at Guntersville Reservoir, and the entire system (HA/RDGPS/data linkage/GIS) will be tested.

Acknowledgment

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Valuation of Aquatic Plant Alternatives at Lake Guntersville: Preliminary Results from the Recreation Study

by
Jim E. Henderson[1]

Introduction

Preliminary results from the Lake Guntersville recreation valuation study have been completed and are presented here. Beginning in 1990, data were collected on existing recreation use, expenditures, and valuation and use under different aquatic plant control alternatives. These data were collected in a series of both onsite and mail-back surveys of Lake Guntersville recreationists. Data collection and analyses were contracted with the Environmental Resources Assessment Group (ERAG), comprised of the Department of Agricultural and Applied Economics, University of Georgia, and the U.S. Department of Agriculture Forest Service, Southeastern Forest Experiment Station, Athens, GA.

There are three types of economic evaluations of interest in aquatic plant control; that is, there are three basic questions that should be answered when choosing one alternative over another. These are as follows:

- Control Alternative Evaluation—What are the economic benefits associated with each alternative?
- Economic Impact Evaluation—What are the impacts on production of goods and services, income, and job associated with the different alternatives?
- User Group Evaluation—Are there differences between recreation user groups in terms of impacts on and preferences for aquatic plants?

Control Alternative Evaluation

Evaluation of plant control alternatives requires knowing the recreation use and economic benefits associated with each of the alternatives under consideration. Calculation of economic benefits requires knowing the costs associated with use of the reservoir. The cost information was collected through an expenditure survey of recreation users. Willingness-to-pay (WTP) for recreation use under different alternatives was collected through use of a contingent valuation method (CVM) survey.

The CVM survey presented five different plant control alternatives, asking recreation users to state how many times they would use the reservoir under the different plant control conditions, summarized in Table 1. As it turned out, three of the alternatives were from recent years so the respondents should have not have trouble visualizing the plant conditions.

Table 1
Plant Control Alternatives—Lake Guntersville

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

For each of the five alternatives, historic aerial photography was used to develop artist

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

depictions of these six different recreation settings or environments:

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

To elicit valuation responses for each alternative, the CVM questionnaire asked whether the user would be willing to pay a specified increase in costs to use the reservoir under the alternative's plant conditions. The increases in costs ranged from $10 to $4,500 per year.

If the respondent answered affirmatively to the increase in costs, he was asked to estimate the number of trips he would take under the increased cost conditions. In this way, the use (number of trips) and WTP data were collected for each of the alternatives. Economic benefits are then calculated by taking the WTP information and subtracting what it costs to use the reservoir, i.e., the expenditure survey data.

The CVM survey was developed after a series of public meetings to elicit input on aquatic plants, aquatic plant management, and recreation use. Based on the public input and historic use patterns, it appears there was a difference in preferences for aquatic plants depending on the type of recreation use. That is, the fishers at the meetings believed the plants are beneficial as fish habitat, whereas nonfishers believed the plants interfere with recreation, e.g., clogging boat propellers. Based on the input from the fishers, one would expect willingness-to-pay values for fishermen to increase as the amount and distribution of plants increased. That is, if fishers believe "the more the better" as far as plants, then WTP was highest for the Minimum Control Alternative and lowest for Alternative D. The opposite should be true for nonfishers.

The results of the CVM benefit evaluation are presented in Table 2, showing preliminary benefit estimates for anglers and nonanglers. The annual economic value of the five alternatives is presented for the fishers and nonfishers groups. The estimates are for annual per visitor benefit values. The benefit estimates in the table were estimated using a CVM valuation model developed from the CVM survey data. Variables in the model included bid/costs; i.e., amount of the increase in cost to use the lake, income of the recreator, age of the respondent, availability of lake substitutes or alternatives to using Lake Guntersville, and whether the respondent owned or rented a lake residence.

Table 2
Mean Net Economic Value Per Visitor (willingness-to-pay)

Management Scenario	Mean Net Economic Value Per Visitor	
	Fishers, $	Nonfishers, $
Minimum	790.47	-166.29
Alternative A	778.46	593.16
Alternative B	503.77	1,087.47
Alternative C	9.43	1,218.75
Alternative D	-639.87	1,168.31

Examination of Table 2 shows that, indeed, WTP values for the fishers increase with the amount of aquatic plants, ranging from a high of $790 for the Minimum Alternative to a low of $639 for Alternative D. The negative estimates mean that the user would have to be compensated to still use the lake under that alternative's plant conditions. For the nonfishers, Alternative C has the highest mean value rather than Alternative D, the "No Plants" alternative. This could be possibly explained by the fact that the nonfishers, while preferring lessor amounts of plants, still recognize that there is a need for some level of aquatic vegetation for the ecology of the lake (Environmental Resources Assessment Group (ERAG) 1993).

Based on the WTP values for fishers and nonfishers, if an individual had to select the most desirable alternative, the choice would be different depending on whether one fished or not. The alternative that produces the highest benefits may not be either of the group alternatives' highest WTP values. While WTP values may be higher for one group, the amount or proportion of group use may come into play in determining the alternative that produces the highest benefits. This is the case at Lake Guntersville. Decision makers using this information need to look not only at the mean WTP values for the groups, but also at the proportion of use the groups represent. Table 3 shows that one-third of the recreation visits are made by fishers, while two-thirds of the visits are nonfishers.

Table 3
Recreation Visits Use at Developed Recreation Sites, Lake Guntersville, Fishers and Nonfishers

User Group	Total Onsite Rec Visits	% of Total Visits
All Users	2,844,718	100
Fishers	976,508	34
Nonfishers	1,868,209	66

For comparison of expenditures, Table 4 shows that on the average, per person expenditures are about 9 percent higher for nonfishers than fishers. The two groups exhibit differing expenditure and WTP values and have different proportions of use. Because of the these differences, the alternative that produces the highest net benefits is Alternative B (Table 5), rather that Minimum (fishers) or Alternative C (nonfishers)(ERAG 1993).

When considering the $140 million benefit estimates produced under Alternative B, it should be emphasized that these benefits are based on the difference between expenditures and the WTP values that are given by respondents. The valuation is a response to a hypothetical, though realistic, set of aquatic plant and recreation conditions. The benefits accrue to the individual, and no money actually changes hands. Thus, one relying on the CVM estimate of benefits for selecting one alternative over others should be cognizant of the nature of the benefits, individual rather than money in the treasury, and the variables included in the model. Each of the variables included in the CVM valuation model has been used to predict visitation with differing degrees of importance (Stoll et al. 1991).

Economic Impact Evaluation

Economic impact evaluation involves determining the change in demand for and production of goods and services. Because the alternatives result in different visitation, meaning spending is different for the alternatives, the economic impacts resulting from the alternatives must be compared and evaluated just as the different levels of economic benefits discussed above. Recreators at Lake Guntersville spend money for food and supplies at home, perhaps each time before they come to the lake, and then purchase things at the lake, from a cold drink or gas for the boat to a week's stay at a campground. Additionally, there are annual (e.g., fishing license) and one-time or infrequent expenditures for durable goods, such as a new boat or motor.

Recreators to Lake Guntersville expend money for food, lodging, vehicular costs, and other goods and services. Increased use of the lake results in increased demand, producing increases in production of goods and services. The initial purchase of say food or bait from a lakeside store results in increased sales from the suppliers, thus resulting in a ripple or multiplier effect of the purchases. Multiplier effects cause increases in production through many economic sectors, resulting in increased gross output, income, and employment.

To estimate the economic impacts, data were collected in a mail-back expenditure survey. The survey elicited expenditure data on the range of goods, licenses, and services associated with recreation at Lake Guntersville. Respondents were asked to state what expenditures were incurred during the last year and where the expenditures took place. Boaters may have bought all their vehicle and boat fuel at home before leaving, having no effect on economies around Guntersville, or waited until they got to the lake—thus the import of knowing where the expenditures take place.

Table 4
Mean Trip Expenses of the 11-County Impact Region (per person, per trip, 1990 dollars)

	Expenditure Category, $					
User Type	**Lodging**	**Food**	**Transportation**	**Activites**	**Miscellaneous**	**Total**
Fisher	52.88	43.21	77.27	4.85	6.90	185.11
Nonfisher	8.33	58.90	67.30	4.95	22.12	161.60

Table 5
Grand Aggregate Net Economic Value (aggregate willingness-to-pay)

Management Scenario	Grand Aggregate Net Economic Value, $
Minimum	38,245,439.36
Alternative A	111,302,593.50
Alternative B	140,387,601.96
Alternative C	119,136,342.01
Alternative D	69,543,182.23

To estimate the economic impacts from the alternatives, the IMPLAN economic impact model developed by the U.S. Forest Service was used. IMPLAN is an input-output model that uses expenditures for goods and services in one economic sector to estimate changes in output, income, and employment for all economic sectors affected by the new expenditure, i.e., the multiplier effect (Probst 1988). Summaries of gross output, income, and employment for the alternatives under consideration are in Tables 6, 7, and 8 respectively.

Table 6
Total Gross Output Because of Recreational Spending, 11-County Impact Area, 1990 Dollars

	Total Gross Output – Economic Impact, million $				
User Type	**Minimum Management Alternative**	**Management Alternative A**	**Management Alternative B**	**Management Alternative C**	**Management Alternative D**
Fisher	279.45	313.96	242.94	165.94	80.98
Nonfisher	163.39	273.97	337.12	356.74	334.26
Total	442.84	587.93	580.06	522.68	415.24

Table 7
Total Income Output Because of Recreational Spending, 11-County Impact Area, 1990 Dollars

	Total Income – Economic Impact, million $				
User Type	**Minimum Management Alternative**	**Management Alternative A**	**Management Alternative B**	**Management Alternative C**	**Management Alternative D**
Fisher	152.46	171.30	132.55	90.54	44.18
Nonfisher	89.57	150.19	184.81	195.57	183.24
Total	242.03	321.49	317.36	286.11	227.42

Table 8
Total Employment Because of Recreational Spending, 11-County Impact Area

	Total Employment – Total Number of Jobs				
User Type	**Minimum Management Alternative**	**Management Alternative A**	**Management Alternative B**	**Management Alternative C**	**Management Alternative D**
Fisher	6,750.25	7,584.01	5,868.39	4,008.46	1,956.16
Nonfisher	4,120.54	6,909.49	8.502.00	8,996.82	8,429.96
Total	10,870.79	14,493.50	14,370.39	13,005.28	10,386.12

Increases in output, income, and employment are highest for Alternative A, followed closely by Alternative B. These data indicate the economic impacts resulting from choice of either Alternative A or Alternative B would be almost the same. There is a 1-percent difference between the economic impacts of Alternative A and B, a 10-percent difference between Alternatives B and C, and a 24-percent difference between the Minimum Alternative and Alternative A. The plant conditions represented by Alternatives A and B should be considered before accepting them or recommending one of them as the chosen alternative. Alternative A is the 1988 conditions, the historic high for aquatic plant infestation. While fishers did indicate high use and valuation under Alternative A, it may be that Alternative A's plant conditions may not actually be acceptable overall; these conditions prompted Congressional action. Comparing economic impacts, Alternative B, the 1989 conditions provide virtually the same economic impacts with improved plant conditions.

User Group Evaluation

An important objective of the recreation study is to obtain perceptual information on aquatic plants and aquatic plant management programs. It sometimes seems that the only thing known about public perceptions is expressed by vocal groups pursuing their seemingly single-minded goals. The implementation of the recreation-use surveys allowed for the collection of probably the largest sample of data that elicited public perceptions on aquatic plants and aquatic plant management.

A series of questions were asked about respondents' perceptions of plant conditions at Lake Guntersville. The data were collected during 1990-1991, when the plant populations were at the lowest point, about 8,000 acres or 10 percent of the reservoir; the responses may be reflective of these more ideal conditions. While a number of preference/perception questions were asked, the two most important are as follows:

- How do aquatic plants affect recreation users, and do aquatic plants affect different recreation activities differently?
- Compared to existing conditions, do recreation users want more or less aquatic plants, and are there differences between different user groups?

Impacts of aquatic plants

Respondents to the surveys were asked to describe the impact of aquatic plants on their recreation, summarized in Table 9. Few respondents said the plants were bothersome, and the large majority said the plants were not harmful or a help. The differences in user groups should be noted. For the bank fishers, about half said the plants did not affect their recreation, with a fifth responding "Helps" and a quarter finding them bothersome at least part of the time. There is a difference in perceptions between bank fishers and boat fishers, with almost half of the latter saying the plants are beneficial (Helps), 36 percent saying no effect, and only 17 percent of the boat fishers finding the plants bothersome. The boating group is composed of pleasure boaters, sightseers, and waterskiers that boat but did not identify their primary activity as fishing. Almost three quarters of this group said there was no effect on boating, with just over a fifth responding as bothersome.

Table 9
Impact of Aquatic Plants on Recreation Activities

Main Activity[1]	Helps, %	Does Not Affect Activity,%	Bothersome Sometimes, %	Bothersome Most of the Time, %
Bank fishing	20.1	54.2	15.3	10.4
Boat fishing	47.1	36.0	9.9	7.1
Boating	4.1	74.4	11.9	9.6
Shore-based recreation	7.2	86.4	4.0	2.4
All Visitors	26.9	58.1	8.7	6.2

[1] Chi-square = 451.1; $p < 0.0001$.

Preferences for amounts of aquatic plants

Preferences for the amount of plants people would like to see were asked as in terms of more, the same, or less than presently exists at the 8,000 acres of 1991 (Table 10). The importance of examining the user groups rather than just the combined results can be shown by looking at the All Visitors category. There are generally equal percentages across the responses of As Much as Possible, More, Same, and Less Than Presently Exists. There are differences in preferences between groups that are hidden by combining all responses.

For the bank fishers, 35 percent are satisfied with 1990 conditions, an equal number prefer a lessor amount of plants or none at all, with 28 percent preferring more than 1991 conditions. The boat fishers show higher preferences for more plants compared with the bank fishers or any of the other groups. A third of the boat fishers want more than presently exists, and another 29 percent desire as much as possible. A fifth of the boat fishers are satisfied with the existing conditions, compared with 35 percent of the bank fishers. The Boating group shows the strongest preference for less plant coverage. Although 29 percent were satisfied with present conditions, 69 percent wanted less than presently exists of none at all. Only 1.4 percent of the pleasure boaters and water-skiers wanted more plants than existed with 1990 conditions. Of the day users, the Shore-based recreation group, about half want fewer plants than existed in 1990 (less or none at all). About a fifth wanted the same as presently exists or as much as possible. Again, the differences between groups point out the importance of not relying entirely on the combined responses, which can, as in this case, hide real differences between groups.

How to Use the Recreation Valuation Information

Data on the value and economic impact of different plant control alternatives can be used to make decisions on the level of control to incorporate in yearly work plans. Differences between recreation user groups can be evaluated in terms of proportion of each group's use and differences in valuation of plant control. Economic impacts associated with each alternative can be compared with economic benefits produced by other project purposes that are affected by aquatic plant control (Figure 1). For lakes like Lake Guntersville where there is such a high presence of bass and other sportfishing, the impact on and value of recreation can appear to be the only or most important consideration. For decision making, recreation must be considered and its benefits traded off with other economic values that are produced by operation of reservoirs and waterways. In some cases, maximizing recreation benefits may mean interfering with other authorized project purposes. This demonstrates the import of determining value of recreation differences between recreation user groups.

Table 10
Amount of Aquatic Plant Coverage They Would Like to See

Main Activity[1]	As Much as Possible, %	More Than Presently Exists,%	Same as Presently Exists, %	Less Than Presently Exists, %	None at All, %
Bank fishing	11.1	17.3	35.8	24.7	11.1
Boat fishing	28.9	33.1	20.3	13.6	4.1
Boating	0.0	1.4	29.6	42.3	26.8
Shore-based recreation	20.9	9.1	22.7	36.4	10.9
All Visitors	23.9	26.0	22.8	19.8	7.5

Note : Only those visitors who said aquatic plants have some effect on their recreational activity responded to this question.
[1] Chi-square = 174.6; $p < 0.0001$.

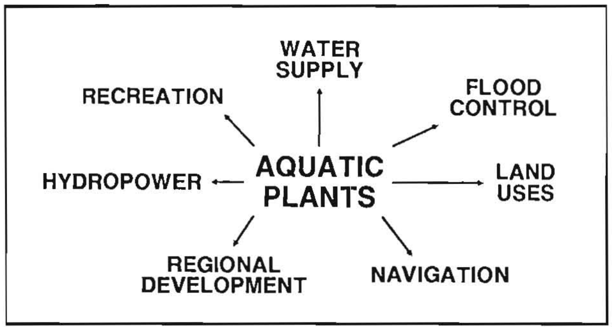

Figure 1. Economic values affected or impacted by aquatic plants and aquatic plant control

Summary

The Lake Guntersville recreation study is the largest effort to collect data on the public perception and valuation of aquatic plant control for recreation. The data on valuation of each alternative plant control plan, economic impact, and public perception can be incorporated in planning for future plant control programs at Lake Guntersville and throughout the Tennessee Valley. Though it may be inappropriate to apply the economic benefit and impact values at lakes other than Lake Guntersville, these data are the first data for large multipurpose reservoirs. The magnitude of the economic benefits and economic impacts associated with plant control for recreation provides a measure of the value of aquatic plant control.

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Grass Carp Collection, Aging, and Growth in Large Water Bodies—A Status Report

by

James P. Kirk,[1] *James V. Morrow, Jr.,*[1] *and K. Jack Killgore*[1]

Introduction

In recent years, triploid and diploid grass carp (*Ctenopharyngodon idella*) have been stocked in reservoirs and lakes to control nuisance aquatic vegetation such as hydrilla (*Hydrilla verticillata*). Triploid grass carp have value as a biocontrol agent because they are sterile, can provide cheaper and longer control than herbicides, and do not present an application hazard (Allen and Wattendorf 1987). Potential disadvantages of triploid grass carp are as follows: (a) the fish have dietary preferences and may not control target vegetation (Leslie et al. 1987), (b) they may emigrate out of areas targeted for vegetation control (Stanley, Miley, and Sutton 1978), and (c) stocking rates need further refinement to meet management objectives (Kirk 1992). Scientists at the U.S. Army Engineer Waterways Experiment Station developed a grass carp stocking model (Boyd and Stewart 1992), but need objective data to refine the model for different regions and types of vegetation.

This report summarizes efforts during 1993 to obtain basic biological information needed to refine the stocking model. Little useful scientific literature existed concerning collection or aging of grass carp. Our tasks were, in order, to develop cost-efficient collection techniques, adequately sample systems, develop length-to-weight relationships necessary for use in backcalculation, and develop methods to age the fish. As these techniques are developed and information is gathered, estimates of growth, mortality, and standing stocks of grass carp in large water bodies will be made available to refine the stocking model.

Methods

Grass carp were collected from two major reservoir systems, Lake Guntersville, AL, and the Santee Cooper reservoirs (Lakes Marion and Moultrie) in South Carolina. Lake Guntersville is a 68,000-acre reservoir in northern Alabama managed by the Tennessee Valley Authority. A total of 118,400 diploid and triploid grass carp were stocked between 1988 and 1990 to control hydrilla.[2] The Santee Cooper lakes total approximately 160,000 acres and have a major hydrilla infestation. Lake Marion was stocked with 100,000 triploid grass carp per year from 1989 through 1992 to control hydrilla. An additional 50,000 triploid grass carp were stocked into Lake Moultrie during 1993 to control spreading colonies of hydrilla.

Grass carp collection techniques, length-to-weight relationships, and preliminary estimates of growth and age were evaluated in 1992 (Kirk et al. 1993). Techniques to age grass carp by examining scales were discussed in the same article. Scales have been used for aging fish since 1898 (Dahl 1910); however, scales sometimes exhibit false annuli (annual age marks) or have missing annuli. For this reason, we used both scales and otoliths (ear bones) to age fish (Jearld 1983). Preliminary studies were performed during 1993 to find other aging structures and to validate aging structures.

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

[2] Personal Communication, 1991, David Webb, Tennessee Valley Authority, Muscle Shoals, AL.

Results

Our initial efforts using classical fisheries techniques such as electrofishing, netting, or rotenone were unsuccessful in collecting sufficient numbers of grass carp (Kirk et al. 1993). Skilled bowfishermen proved an effective, cost-efficient method to collect grass carp. A total of 125 triploid grass carp were collected during May through August 1993 in Lakes Moultrie and Marion. While these fish have not yet been aged, the size of some fish suggests that Age I were collected for the first time. Substantially less effort was expended on Lake Guntersville where tournament bowfishermen were not recruited in time to make a concerted collection effort. However, 43 grass carp were shot during a bowfishing tournament in April 1993 and provided initial estimates of age and growth.

The length-to-weight relationship for grass carp from Santee Cooper collected during 1993 has not yet been determined. The length weight relationship for fish collected on Lake Guntersville follows:

$$\text{Weight} = 0.0000045 \times \text{Length}^{3.163}$$

After calculating the length-to-weight relationship, it was possible to backcalculate lengths and weights for grass carp from Lake Guntersville using scales and otoliths from the 43 fish. The following age-specific sizes were calculated for grass carp of unknown ploidy collected in Lake Guntersville: Age I was 311 mm and 339 g, Age II was 596 mm and 2,699 g, Age III was 746 mm and 5,491 g, Age IV was 845 mm and 8,144 g, and Age V was 899 mm and 9,907 g for total length and weight, respectively. Table 1 compares age-specific sizes for the Santee Cooper reservoirs and Lake Guntersville for grass carp collected during 1992 and 1993, respectively. Grass carp in the Santee Cooper reservoir appear to be growing much more rapidly.

Sagittal otoliths of cyprinids often show no clear marks and are not suitable for age determination. When aging cyprinids, the lapillus (utricular otoliths) should be used (Victor and Brothers 1982). The lapillus appears to lay down annuli and should be a suitable aging structure to compare with scales. Figures 1 and 2 show all three otoliths and a sectioned lapillus, respectively. Almost complete

Table 1
Age-Specific Lengths and Weights of Grass Carp from Lake Guntersville, Alabama, and the Santee Cooper Reservoirs, South Carolina

	Lake Guntersville		Santee Cooper	
	Length mm	Weight g	Length mm	Weight g
Age I	311	339	361	547
Age II	596	2,699	698	4,894
Age III	746	5,491	821	8,294
Age IV	845	8,144	908	11,506
Age V	899	9,907	n/a	n/a

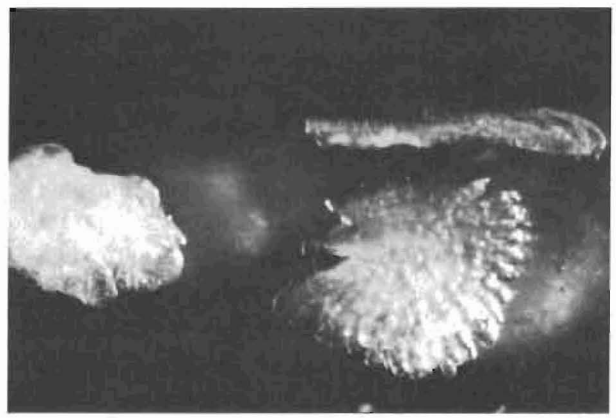

Figure 1. View of grass carp otoliths (left most is the lapillus; right top is the asteriscus; and right bottom is the sagittus)

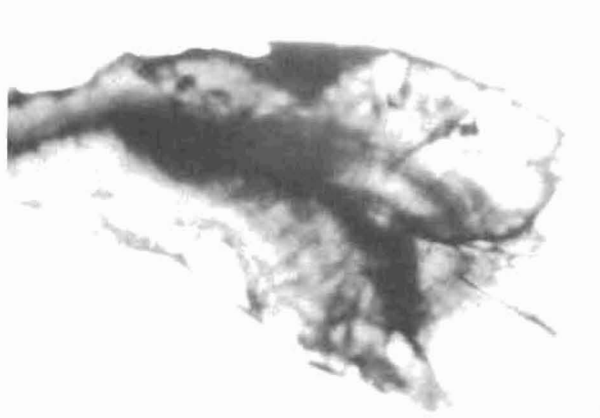

Figure 2. Sectioned utricular otolith (the lapillus), showing annuli

agreement existed in ages determined from sectioned otoliths and scales from fish collected from Lake Guntersville. Additionally, sectioned pectoral fins also appear to have potential as an aging structure.

Discussion

Skilled bowfishing is an effective method of collecting grass carp in large water bodies. We intend to expand our collection efforts and increase sample sizes in both systems in 1994. Instead of relying on grass carp collected at bowfishing tournaments, we will attempt to contract with individual bowfishermen and collect between 100 and 200 fish during 1994.

Initial estimates of growth strongly suggest that grass carp in the Santee Cooper reservoirs are growing at a much faster rate than in Lake Guntersville. This may be related to the availability of preferred foods. Some fish collected from Lake Guntersville in early April 1993 appeared to be eating filamentous algae and other less preferred foods (Sutton and Vandiver 1986; Leslie et al. 1987). Hydrilla, a preferred food of grass carp is almost entirely gone from Lake Guntersville, while extensive stands of Eurasian watermilfoil (*Myriophyllum spicatum*) are not being utilized by grass carp. Hydrilla in the Santee Cooper system is being controlled in upper Lake Marion, but is rapidly expanding into other parts of the system, especially Lake Moultrie. Thus, a preferred food is not limiting in this system.

Scales and utricular otoliths need to be validated as aging structures for grass carp. Preliminary results suggest these structures are suitable for aging grass carp, and research is ongoing to validate these aging structures. There was high agreement between sectioned utricular otoliths and scales; the backcalculated length of Age I fish was very close to the length of known Age I fish stocked into the Santee Cooper reservoirs and Lake Guntersville.

Future efforts will focus in several directions. Scales and otoliths need to be validated. Fish collected from the Santee Cooper system in 1993 and fish to be collected from both systems in 1994 need to be aged to generate growth and morality estimates. This information can be incorporated into a Ricker Table (Ricker 1975) to provide estimates of numbers, biomass of grass carp by age class, and total grass carp biomass. The primary relationship used to derive these estimates follows:

$$N_t = N_0 e^{-zt}$$

This relationship states the number at time (t) is equal to the initial number (0) raised to the instantaneous rate of total mortality (z) times the number of years (t) (Ricker 1975).

Triploid grass carp have potential to control aquatic vegetation in large water bodies. However, their use must not result in overstocking or understocking. Basic biological data are needed to refine parameters used in the stocking model and achieve appropriate stocking densities. In that regard, our future estimates of mortality, growth, and biomass should improve the stocking strategies and better allow managers to predict reductions in aquatic vegetation.

Acknowledgments

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A Habitat-Based Approach for Studying Fish-Plant Interactions

by
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Introduction

Aquatic plants form distinct habitats within a water body that can be identified through remote sensing or direct observation. This attribute provides a practical means of evaluating the importance of structurally complex habitats to species composition and size of fish. Mapping the distribution of aquatic plants is a commonly used management technique. Aquatic plant maps are used to prioritize plant control locations, evaluate efficacy of the control program, and predict colonization of new sites within a water body. These maps also indicate repeatable, homogenous landscape units that when properly recognized, can be rated as poor to excellent fish habitat.

Habitat assessment of aquatic plants is important because plant abundance, distribution, and configuration are altered as a result of plant control operations. Thus, identification and value of these plants are important in preserving optimal fish habitat. A variety of approaches have been used to estimate and characterize standing crops of submersed plants (Killgore, Dibble, and Hoover 1993); however, little has been done to quantify aquatic plants at an appropriate scale. The value of fish habitat has been assessed largely by aquatic plant abundance at a macrolevel (i.e., lakes, streams, and reservoirs) because there is little information on specific characteristics of plants that optimize fish habitat. For example, plant biomass and density have been positively correlated with fish abundance (Durocher, Provine, and Kraai 1984; Maceina and Shireman 1985; Maceina et al. 1991); yet the mechanisms that directly affect fishes in these habitats are missed by measurements that are indirect and merely allude to factors in aquatic plants that affect fish growth and survival.

A combination of appropriately scaled plant measurements and direct observations of fish behavior in these plant habitats is essential before the mechanisms that govern growth and survival in fish are more thoroughly understood. Reasons why fishes use and benefit from particular plant habitats can be determined if (a) appropriate plant morphology (i.e., spatial complexity, shading properties, and architecture) is defined, and (b) the effects of these morphological characteristics on behavioral responses (i.e., foraging efficiency and predator avoidance) that impact growth and survival in fishes are quantified.

Here, we present a hierarchal approach to classify plant habitats: regional, system, and local (Figure 1). Regional and system criteria are evaluated on a macrolevel, while local classification criteria are on a microlevel or proximate level. Parameters for these criteria and their use in calculating habitat indices to bridge microlevel and macrolevel assessments are discussed.

Macrolevel Classification

Regional criteria

Regional classification criteria include latitudinal effects on growing season, geographic extent of plant distribution, and fish zoogeography (Table 1). Regional patterns of plant growth are at least partially a result of temperature extremes. Northern latitudes have shorter growing seasons where plants usually

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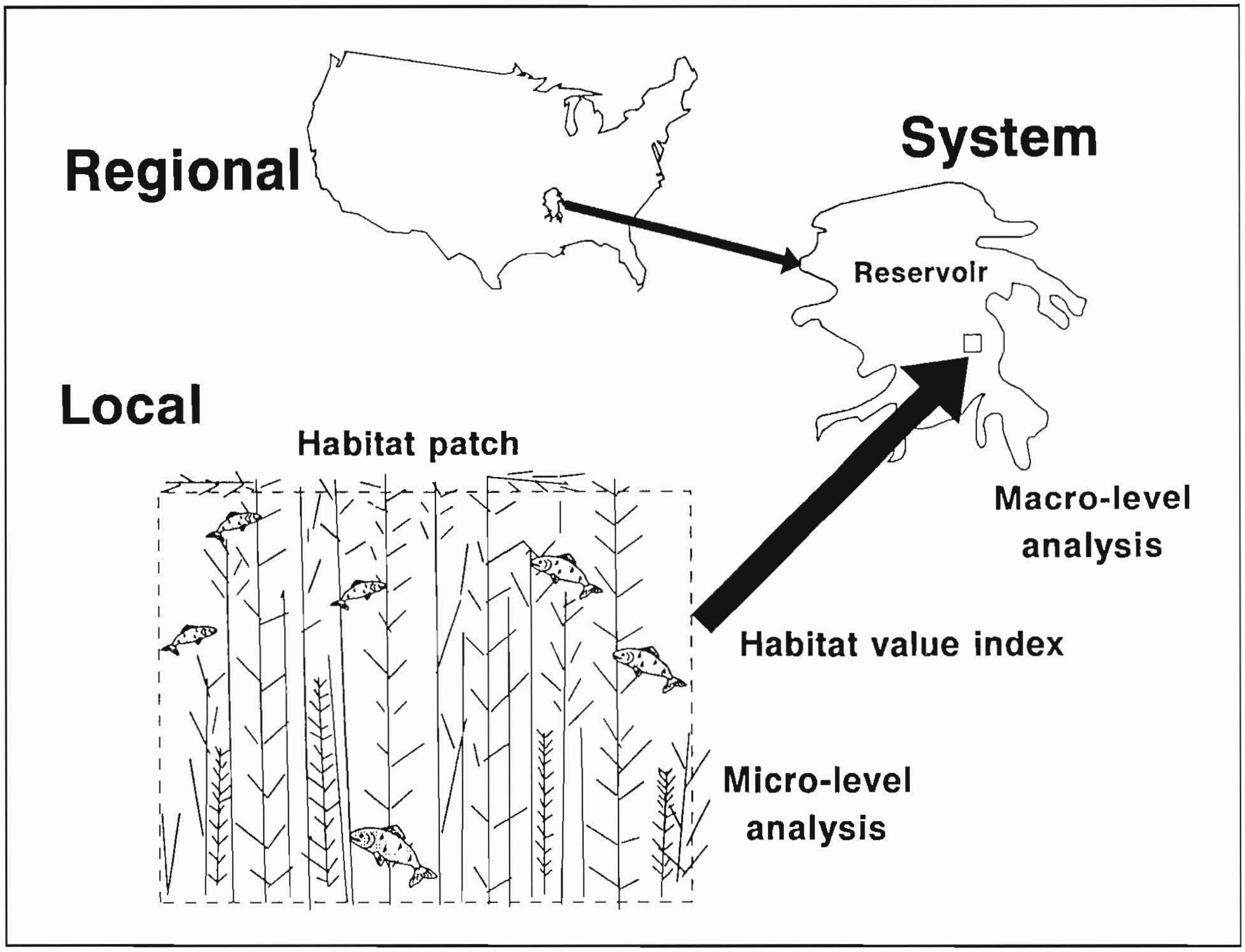

Figure 1. Illustration of a hierarchal approach in the classification of plant habitats at a regional, systems, and local level

Table 1
Three Levels for Investigating Relationships Between Fish and Aquatic Plants and Criteria Used to Differentiate Scale

	Scope of Measurement	
Criteria	**Plants**	**Fishes**
	Regional Level	
Latitude Drainage	Species extension Climatic factors Seasonal periods	Community Species distribution
	Systems Level	
Lakes Streams Reservoirs	Patch abundance and configuration Species composition Patch type: floating, submersed, emergent	Population dynamics Assemblage Recruitment Nest sites/spawning
	Local Level	
Patch dynamics Fish and plant interactions Predator risk	Spatial complexity Invertebrate abundance and distribution Shading effects	Behavioral response: foraging and predator avoidance

senesce during the fall. Growing season extends year round in southern latitudes, and senescence is minimal. The delineation between north-south boundaries is not discrete, but forms a continuous variation in latitudinal zonation of aquatic plant communities across the United States (Muenscher 1967)(Figure 2). Although the importance of plant senescence to fish composition is poorly understood, temporal changes in plant abundance can influence abundance and growth of phytophilic fishes such as largemouth bass (Bettoli, Noble, and Betsill 1992; Hinkle 1986; Smith and Crumption 1977).

Aquatic plants are distributed across the United States. However, the extent of aquatic plant distribution in the arid Southwest and Rocky Mountain regions of the United States is restricted relative to other regions. Fish zoogeography also follows similar patterns. Regions with high species density occur in the southeastern United States, while the fauna is generally depauperate in areas west of the 98th meridian of longitude (McAllister et al. 1986). The assumption is that vegetated habitats in areas of high species density have a higher incidence of phytophilic fishes that compete for food and space. In these areas, aquatic plants are an important component of the ecosystem and can be managed to improve fish habitat.

Overlaying plant distribution with species density suggests three broad regions in the United States that have either different plant growth patterns, different fish species assemblages, or a combination of both: Southeastern, Northeastern, and Pacific Northwest (Figure 2). Synoptic studies on fish-plant interactions in each of these regions would ensure a broad geographic scope.

System criteria

A system classification considers within-water body characteristics of the plant bed. Plant communities are often a mixture of

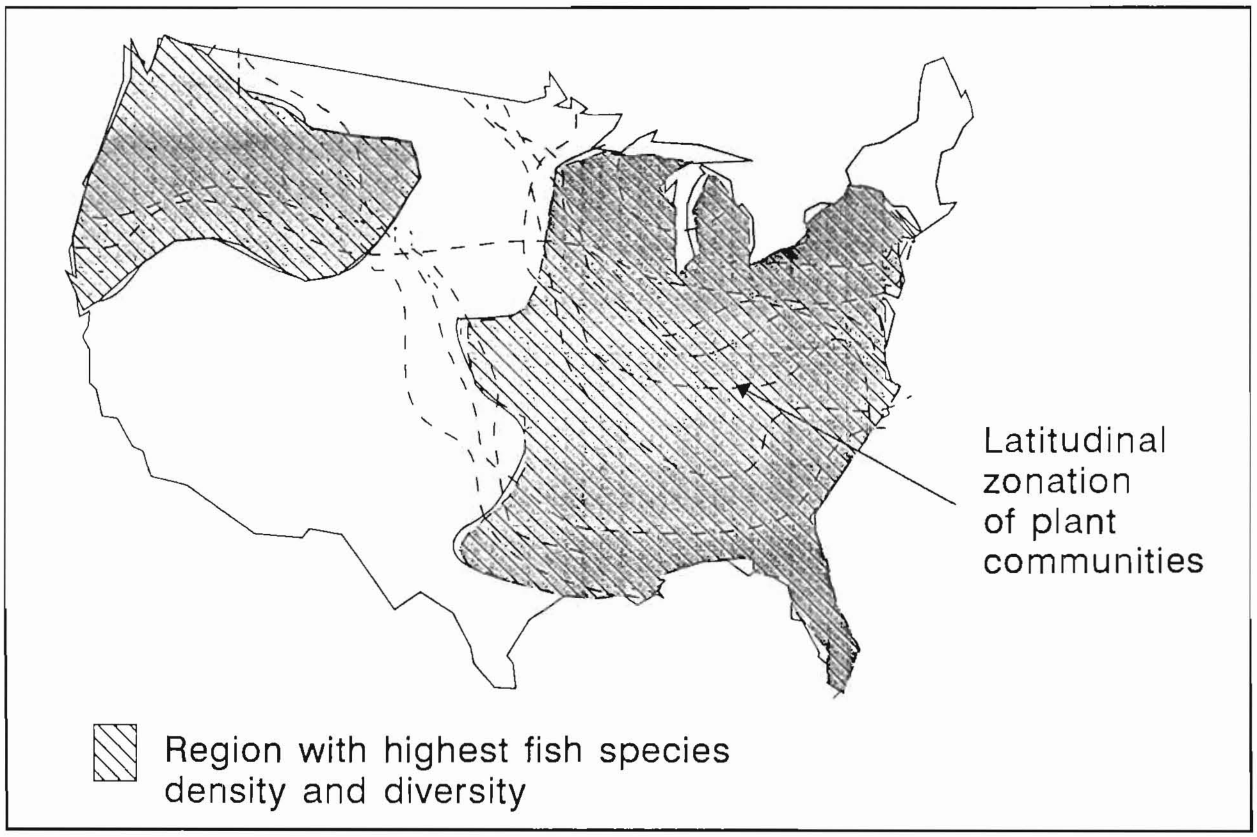

Figure 2. Geograpical delineation of regional zones for synoptic studies on fish-plant interactions

emergent, floating, and submersed species. The density and aerial coverage of these plants are conventionally used to characterize plant beds. However, the morphology and hydrology of the aquatic system can influence plant growth patterns resulting in distinct vegetated habitats or patches. For example, shallow water bodies can be totally covered by aquatic plants, while water bodies with deep pelagic zones have plants growing only in the shallower littoral areas.

A map of aquatic plant distribution supplemented by ground truth data can reveal different types of habitats. Several examples are (a) unvegetated zone between the shoreline and plant bed, (b) large expanse of monospecific plants forming a canopy on the water surface, and (c) clumped or patchy distribution of plants.

In addition, dispersion of individual patches can be easily delineated. Dispersion characterizes the spacing of patches with respect to one another. The general spacing patterns formed are clumped, uniform, and random. A coefficient of dispersion (i.e., ratio of the variance to the mean) can be used to determine if the dispersion is clumped (greater than 1.0) or uniform (less than 1.0) (Pielou 1977). A truly random (Poisson) distribution has a coefficient of dispersion of 1 (Brown and Downhower 1988). Once patches are recognized as discrete landscape units, they can be described according to physical, chemical, and biotic variables.

Microlevel Habitat Assessment

Microlevel assessments of plant habitats are currently being conducted in experiment ponds at the U.S. Army Corps of Engineers Louisville Aquatic Ecosystem Research Facility, Lewisville, TX (LAERF). Habitat assessment includes direct underwater observations of foraging and predator avoidance behaviors by fishes and measurements of the shading properties, spatial complexity, and invertebrate abundance in aquatic plants. Based on these criteria, each plant species is ranked by a value index similar to suitability indices developed for other systems (Williamson and Nelson 1985; Stuber, Gebhart, and Maughan 1982) that can be linked to and incorporated as an integral part of a larger scaled assessment.

Habitat criteria at the microlevel

Structural characteristics unique to aquatic plants create spatial complexity in aquatic habitats which are important to growth and survival of freshwater fishes (Engel 1985; Crowder and Cooper 1982). Spatial complexity in these habitats deters predation by altering the outcome of predator-prey interactions (Savino and Stein 1982; Johnson, Beaumier, and Lynch 1988) and serves as critical refuge sites for smaller fishes (Werner et al. 1977; Mittelbach 1981). Availability of spatial complexity in aquatic habitats is important for successful spawning by adult fishes (Mesing and Wicker 1986), and it increases survival and recruitment of juvenile fishes (Aggus and Elliott 1975; Miranda, Shelton, and Bryce 1984). Growth rates of young fishes increase in these habitats (Mittelbach 1981) because plant leaves and stems offer a habitat rich in food resources for fish by providing attachment substrate and protection for many microinvertebrates (Pardue 1973; Gilinsky 1984; Keast 1984).

In addition to these structural benefits provided by aquatic plants for fishes, the shade that is created by plant structure also attract and may benefit fish in these habitats (Johnson 1993; Helfman 1981). It is hypothesized that shaded areas available to fishes are important for visual acuity to improve both vigilance and foraging behaviors (Diehl 1988; Lynch and Johnson 1989). Thus, availability of aquatic plant species that provide appropriate shade may increase both foraging efficiency and predator avoidance by offering microhabitat that improves detection of food items and predators, which ultimately increases growth and survival.

Measurements to determine spatial complexity

Measurements of habitat spatial complexity used here are similar to those described by Dibble (1993) and Lillie and Budd (1992) and reflect both horizontal and vertical interstice size and abundance present in the aquatic plant. Plant complexity is determined by the sum of horizontal and vertical interstices ratios (I_{hv}). The greater this value, the higher the patch spatial complexity. Ratios (I_i) represented the number of interstices intercepted per meter (r_i) and the mean size (cm) of each interstice (x_i), and was calculated as:

$$I_i = r_i + x_i$$

$$\text{Spatial complexity} = (I_{hv})$$

A line intercept method for determining the abundance of interstices in plants is used to determine the size and abundance of plant interstices (gaps among stems and leaves) along a horizontal and vertical axis (Bonham 1989). Horizontal and vertical measurements of each plant are collected at low, mid, and top strata of the macrophyte. Replications of aquatic plants species are used to accurately validate difference in individual plant morphology.

Measurements to determining shading effect

In addition to these measurements, light transparency is measured in different plant habitats to determine differences in shading effects. Light data are treated as relative measurements among plant types and represent percentage of light transmitted from the surface. Combined with complexity data, light data are used to determine differences in plant architecture. We do not make the assumption that light transparency is dependent on spatial complexity as defined herein. Light transparency may decrease in patches with low spatial complexity where a habitat contains a plant with a canopy. For example, a plant habitat that exhibits a low value of vertical complexity (I_v) and low vertical light transparency (L_t) describes an architecture containing few numbers of horizontal stems or leaves, thus large vertical interstices, and a canopy forming a light block (Figure 3). Plant habitat containing high vertical and horizontal complexity and low light transparency suggest a complex habitat with many small interstices and many leaves or stems that block light from the substrate to the surface of the patch (Figure 3).

Measurements of behavior

The effects of aquatic plant morphology on fish behavior are not well understood. This is largely due to the lack of data classifying plant characteristics and inappropriate fish behavioral data to adequately assess plant criteria important to fishes. Many of the recent attempts to demonstrate the relationship between fish and plant habitat have come from data collected from large-scale lake studies measuring effects of plant removal on fishes (Bettoli, Morris, and Nobel 1991; Maceina et al. 1991; Colle, Cailteux, and Shireman 1989).

Assuming that aquatic plant characteristics facilitate specific behaviors, i.e., foraging, predator avoidance, and choosing spawning sites that impact growth and survival in fishes, it is important to quantify these responses to determine how aquatic plants impact fishes. Not only are unique differences in plant morphology important to fish, but they may be important for different reasons. For example, shading effects of aquatic plants may be more important for one fish avoiding predators than the overall spatial complexity the plant offers, whereas the foraging efficiency of another fish species may improve when plant spatial complexity increases and light transparency decreases.

In the experiments conducted at LAERF, behavioral responses by fishes are directly observed and recorded as video data to determine plant morphology effects on fishes. Videography is a successful method for collecting accurate fish behavioral data in the field (Collins and Hinch 1993). Video cameras contained in waterproof housing are placed

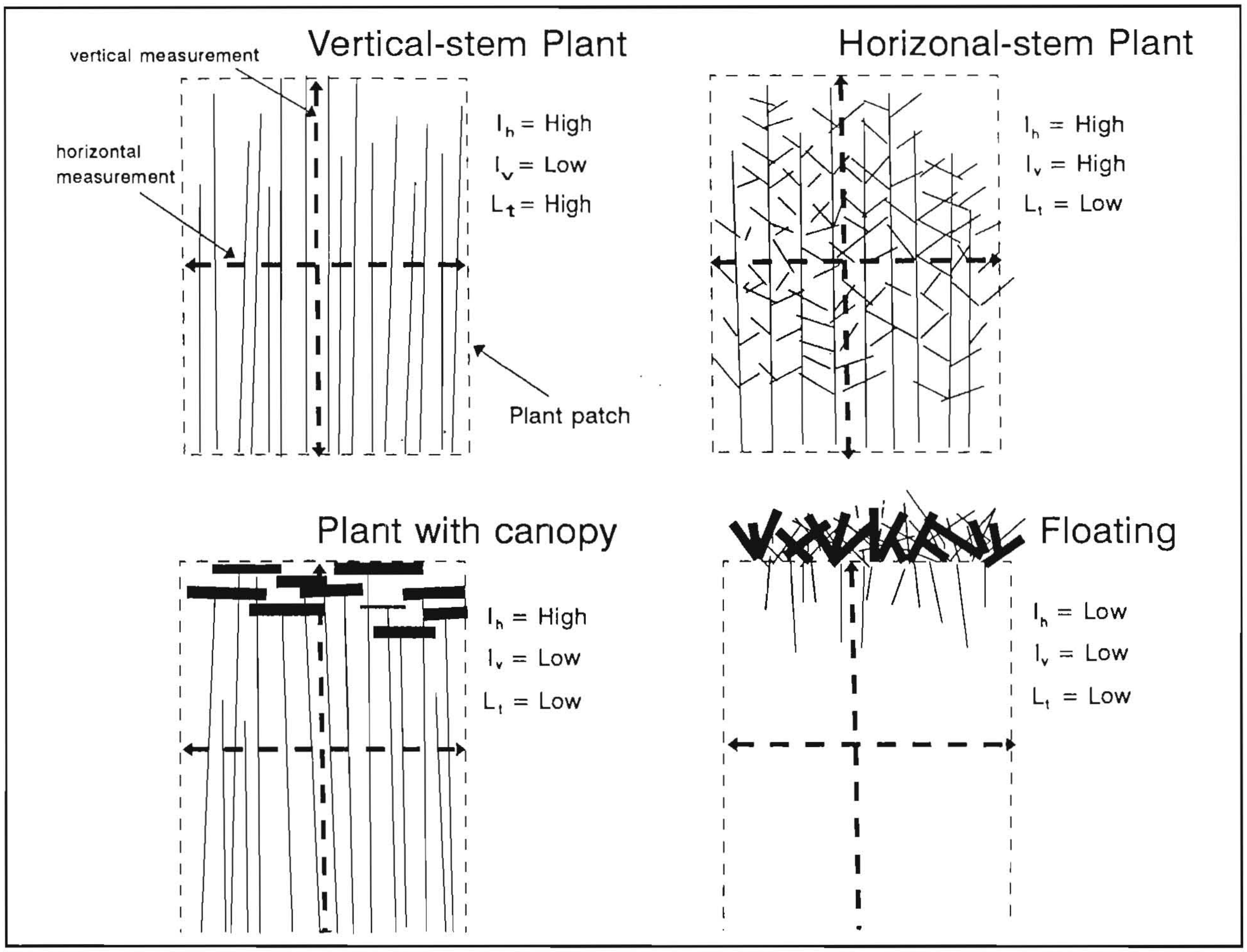

Figure 3. Example of the within-patch measurements taken to determine differences in aquatic plant complexity and light transparency (I_h = number of interstices transacted on a horizontal axis, I_v = number of interstices transacted on a vertical axis, L_t = percent of light transmitted from the surface)

underwater adjacent to aquatic plant habitats cultured in enclosures in experimental ponds (Figure 4). These plant cultures and pond enclosures enable plant and fish species manipulations to more accurately test plant effects on fishes. Unbiased and appropriate scaled behavioral data are collected in the field and returned to the laboratory where they are analyzed as focal animal or scan samples (Altmann 1974; Poysa 1991). Behavioral data are organized in ethograms and grouped as specific behavioral categories (i.e., predator avoidance and foraging)(Martin and Bateson 1986). Correlation between time budgets and frequencies of these behavioral groupings and aquatic plant criteria are used to evaluate effects that aquatic plants have on foraging efficiency and predator risks.

Relation of Habitat Classification to Plant-Fish Research

A habitat classification provides descriptive information on physicochemical attributes that can be related to composition of fish assemblages. If assemblage structure can be predicted simply by recognizing distinct landscape units, then structural complexity of plant beds can be managed to improve or create fish habitat. However, high variation in species composition among habitats often confounds development of predictive relationships. This variation can be explained from behavior studies and related back to predetermined habitats. Adjustments can be made as necessary.

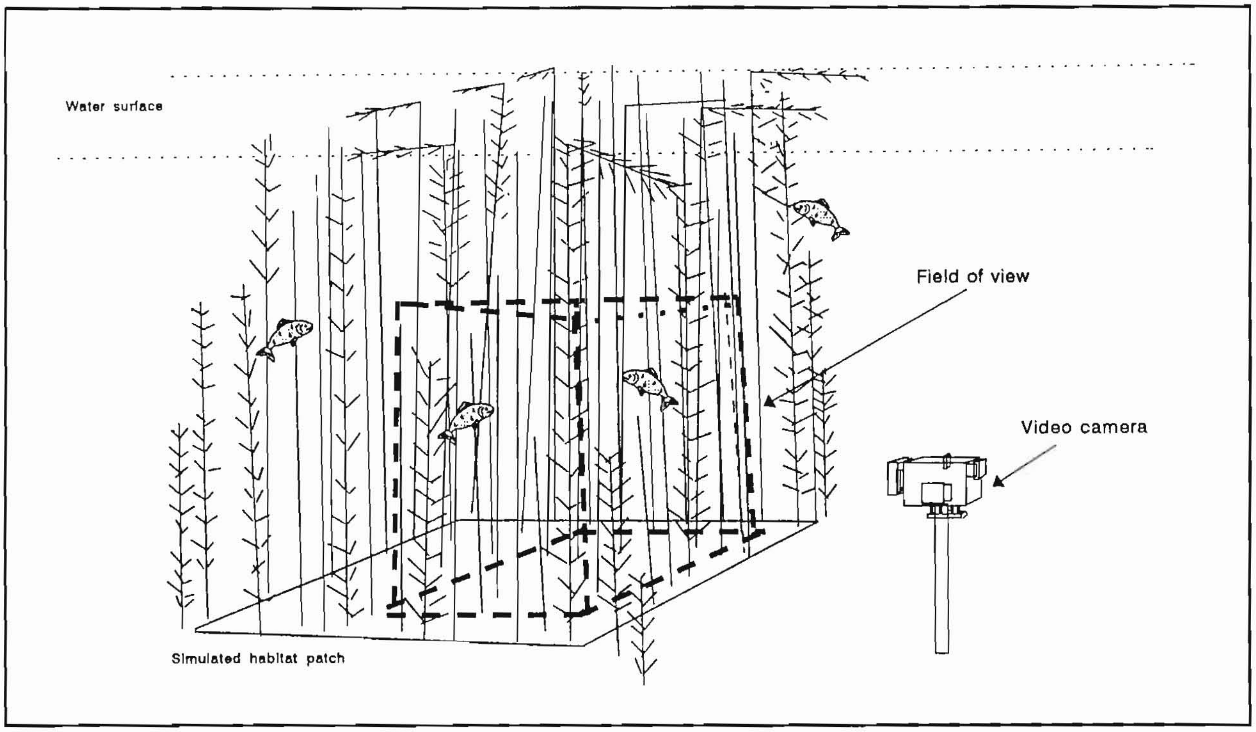

Figure 4. Experimental design for collecting behavioral data within simulated aquatic plant patches

A habitat-based approach is an essential element in evaluating the value of aquatic plants as fish habitat. Quantification of aquatic plant characteristics and behavioral responses by fish at a microlevel furthers the understanding of how aquatic plants enhance fish habitat. More importantly, this information supplies criteria to classify plant characteristics important to fishes. These criteria then can be used to rate plants and develop individual plant indices that can link information collected at the microlevel to larger scaled and more applicable macrolevel assessments.

Future research, however, is required before the benefits and value of these habitats are fully identified and before value indices can be applied. More experiments are planned to further test the importance of plant characteristics and their value to fishes.

Acknowledgments

We would like to express appreciation to M. Smart, G. Dick, and M. Crouch and other personnel that have worked on this project at the Lewisville Aquatic Ecosystem Research Facility. In addition, the authors would like to thank J. Hoover, S. Herrel, E. Nelson, and K. Gerdes at the U.S. Army Engineer Waterways Experiment Station for their expertise in the laboratory.

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Guntersville Bass and Grass: Life After 381

by

William B. Wrenn,[1] D. R. Lowery,[1] and M. J. Maceina[2]

In many areas, there continues to be an on-going conflict between aquatic plant control or management and largemouth bass anglers. Usually the underlying issue from the anglers' standpoint is that a relatively high level (e.g., >30 percent) of areal plant coverage is best for bass fishing and the bass. Although it seems that this perception cannot be refuted in some cases, the limited data on this subject has been derived from various types of water bodies, frequently resulting in an "apples versus oranges" comparison. In a recent survey of 60 Florida lakes, Canfield and Hoyer (1992) found no relation between the standing crop of harvestable largemouth bass and the percent area covered with aquatic macrophytes. However, they suggested that, considering the total fish population, a moderate macrophyte coverage of 15 percent seems to preclude the probability of any adverse fisheries problems.

This is an interim report from the fisheries assessment part of the Joint Agency Guntersville Project (JAGP), which began in 1990. The primary objective is to provide an overview of the size-structure of the largemouth bass population in Guntersville Reservoir (Alabama) for the period 1983 to 1993, along with some of the creel survey results. While the overall objective of the JAGP fisheries component is to assess changes in the resident fish community relative to the various aquatic plant control methods tested for this large-scale demonstration, emphasis was directed to the sport fishery and the well-publicized largemouth bass fishery in particular. During the first 4 years of this study, there was no restriction on the size of bass that could be harvested. Near the close of the fourth year of the creel survey (October 1993), a 15-in. or 381-mm minimum length limit was installed. Therefore, this overview actually examines the past, present, and future relative to the 381-mm size restriction and aquatic plant coverage.

Methods

Size-structure of the largemouth bass population was examined by use of relative stock density (RSD), a length-categorization system of the proportions of the sizes of bass in a given population that can be ranked according to a management objective (Gabelhouse 1984). The management objective most applicable to Guntersville is moderate density of largemouth bass (one of several species of equal importance in a balanced community). The statistic P-RSD, the proportion of preferred size, ≥381 mm total length (TL), corresponds to the new minimum size limit for bass on Guntersville Reservoir. Whereas, PSD or proportional stock density applies to bass 203 to 304 mm in length, and M-RSD applies to memorable-size bass, ≥508 mm.

Largemouth bass were collected by electrofishing at 10 stations in autumn during 1990-1993. Also, catch-depletion population estimates, using electrofishing, were conducted in the spring in 1992 and 1993 (Maceina, Wrenn, and Lowery, manuscript submitted to *North American Journal of Fisheries Management*).

A roving creel survey, based on nonrandom probability sampling design, was conducted during 1990 through 1993. (Survey design and analysis provided by Fisheries Information Management Systems, Auburn, AL). The survey was stratified temporally and spatially and included 24,000 ha of the

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total reservoir area of 28,000 ha. The components summarized for this report are largemouth bass catch rates (number/hour), fishing pressure (hours), and bass harvest. Aquatic plant coverage was determined from the aerial photographic surveys conducted each October.

No comparable creel surveys or electrofishing sampling were conducted during 1983 to 1989, the period of highest aquatic plant coverage; however, in conjunction with a test-demonstration of the use of grass carp (Webb et al. 1989), a large-scale rotenone sample (11 ha) was collected from the Town Creek embayment in 1983, 1986, and 1988, which provided a large sample of bass to compute relative stock density.

Results

Based on the creel survey results since 1990, angling success for largemouth bass on Guntersville Reservoir declined in relation to the decrease in areal coverage of aquatic plants, although high catch rates and harvest were recorded in 1990. Submersed aquatic macrophyte coverage peaked in 1988, 29 percent, and dropped to about 7 percent in 1991 (Figure 1). The dramatic decrease in aquatic plant coverage was due primarily to changes in environmental conditions rather than from plant control activities. Total catch rate for bass, which includes those caught-and-released plus those harvested, declined from 0.66 bass/hr in 1990 to 0.43/hr in 1992 (Tables 1-3). Analysis of all 1993 creel data has not been completed, but preliminary results indicate they are similar to 1992. More than 230,000 bass were harvested in 1990, but harvest had declined about 70 percent by 1992. This decline was influenced by not only catch rate but also by angling pressure (hours of targeted fishing effort for largemouth bass). Compared with the peak of 960,000 hr in 1990, angling pressure for largemouth bass declined by more than 40 percent in creel zones A and B, and by 20 percent in zone C by 1992. Although there are several possible causes for this decline in bass angling effort, including the decline of plants, this discussion is beyond the scope of this report.

Despite the obvious decline in bass fishing success after 1990, results of concurrent sampling of the bass population did not show any significant changes in the numbers of bass in Guntersville Reservoir. However, at the end of 1991, it became readily apparent that standard (1.3-ha coves) rotenone surveys were

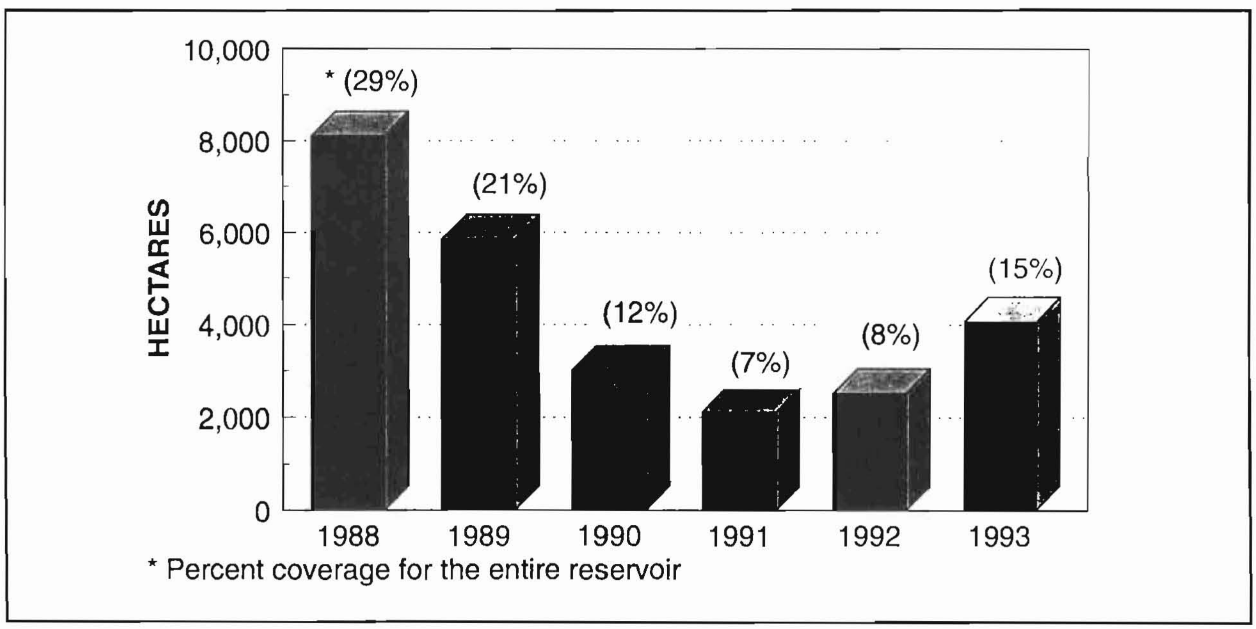

Figure 1. Percent areal coverage of aquatic plants in Guntersville Reservoir, 28,000-ha main stem impoundment, Tennessee River

Table 1
Guntersville Reservoir Largemouth Bass Harvest – 1990

	Creel Areas			
	A	B	C	Total
Harvested/hour	0.17	0.19	0.19	0.18
Caught and released/hour	0.47	0.49	0.46	0.48
Number harvested	86,105	94,272	51,175	231,552
Plant coverage, %	4	9	30	12

Table 2
Guntersville Reservoir Largemouth Bass Harvest – 1991

	Creel Areas			
	A	B	C	Total
Harvested/hour	0.13	0.15	0.14	0.14
Caught and released/hour	0.42	0.47	0.39	0.43
Number harvested	61,366	61,826	43,909	167,341
Plant coverage, %	0.2	6	24	7

Table 3
Guntersville Reservoir Largemouth Bass Harvest – 1992

	Creel Areas			
	A	B	C	Total
Harvested/hour	0.05	0.06	0.07	0.06
Caught and released/hour	0.37	0.35	0.39	0.37
Number harvested	22,089	21,925	22,560	66,584
Plant coverage, %	0.2	8	25	8

providing a conservative estimate of the number of harvest-size bass (>250 mm TL) or that the harvest of bass was exceedingly high, >60 percent of the population. Subsequent spring electrofishing, using the stock-depletion method to determine standing stocks in coves, indicated that it was the former. Standing stock estimates of harvest-size bass were 19 kg/ha and 33 kg/ha in 1992 and 1993, respectively—comparable with the standing stock estimates derived from the large rotenone samples in 1983 (36 kg/ha), in 1986 (31 kg/ha), and in 1988 (35 kg/ha).

Comparison of the RSD index values for the bass population since 1990 with those of the 1980s indicates that the size-structure of the bass population is currently within the established or acceptable limits, whereas, the size-structure in the 1980s was not (Table 4). For example, the size-structure of the bass population in 1983-1988 reflected an imbalance toward stock-size fish (<304 mm TL) and too few in the P-RSD category (Figures 2-4). Without belaboring the issue regarding whether or not the three large rotenone samples during the 1980s provided a representative sample of bass for the entire reservoir, the important point is that the current size-structure is judged to be in excellent condition relative to the imposition of a 381-mm size limit. Also, it should be recognized that the number of bass available for harvest without a size-limit regulation will always be higher than that available for harvest with a 381-mm length limit regardless of the condition of the size-structure (Figure 5).

Although these results do not facilitate a direct prediction of optimum plant coverage for largemouth bass, even for Guntersville Reservoir, they do provide a procedure to closely monitor two of the variables that have been documented to negatively affect growth of largemouth bass: excessive plant cover and density of bass. In the past, these two

Table 4
Largemouth Bass Relative Stock Density, Guntersville Reservoir (P-RSD > 381 mm), 1983-1993

	Method	PSD	P-RSD	M-RSD
1983	LR	40	8	1
1986	LR	30	7	0
1988	LR	16	7	1
1990	AEF	48	21	7
1991	AEF	43	18	4
1992	SEF	33	21	8
	AEF	53	25	8
1993	SEF	63	26	7
ACC. Range		40-70	10-40	0-10

Note: LR = large rotenone samples; AER = autumn electrofishing; and SEF = spring electrofishing.

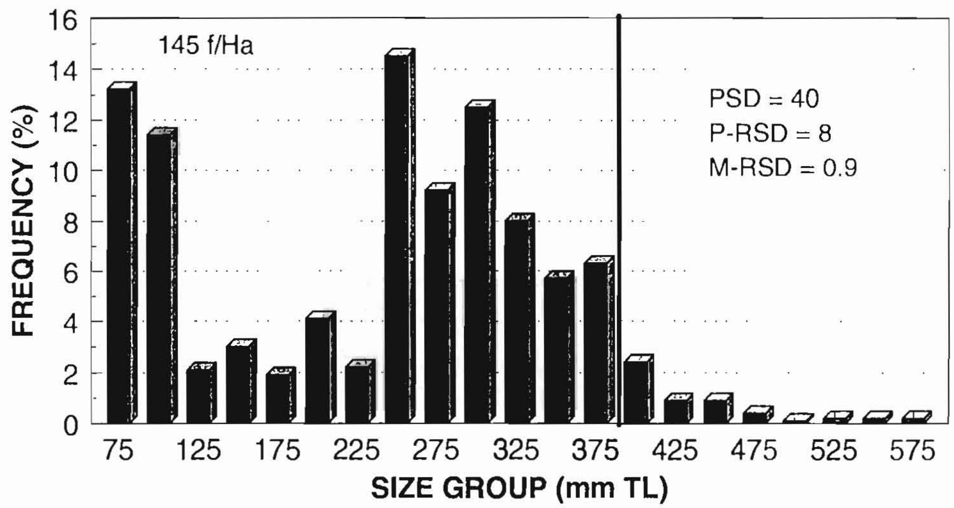

Figure 2. Length frequency and size-structure of largemouth bass in Guntersville Reservoir, in an 11-ha rotenone sample, 1983

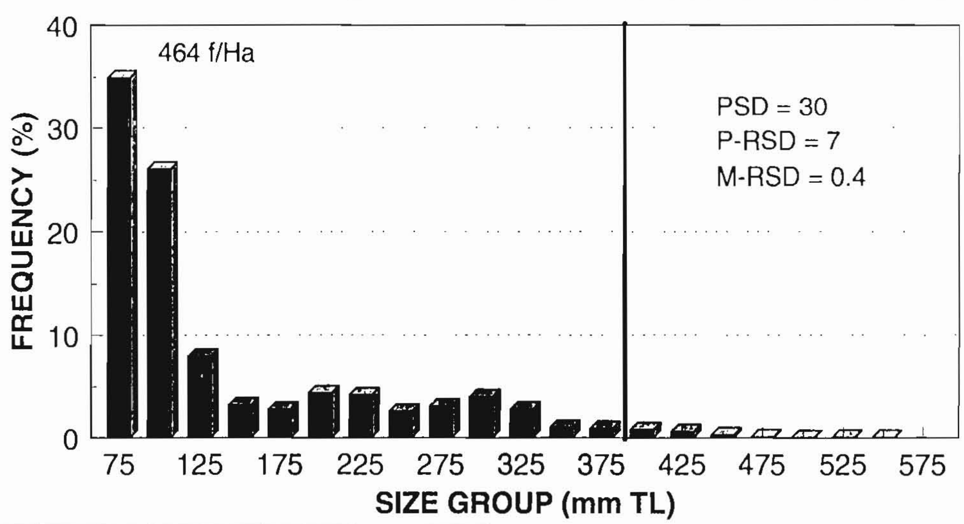

Figure 3. Length frequency and size-structure of largemouth bass in Guntersville Reservoir, in an 11-ha rotenone sample, 1986

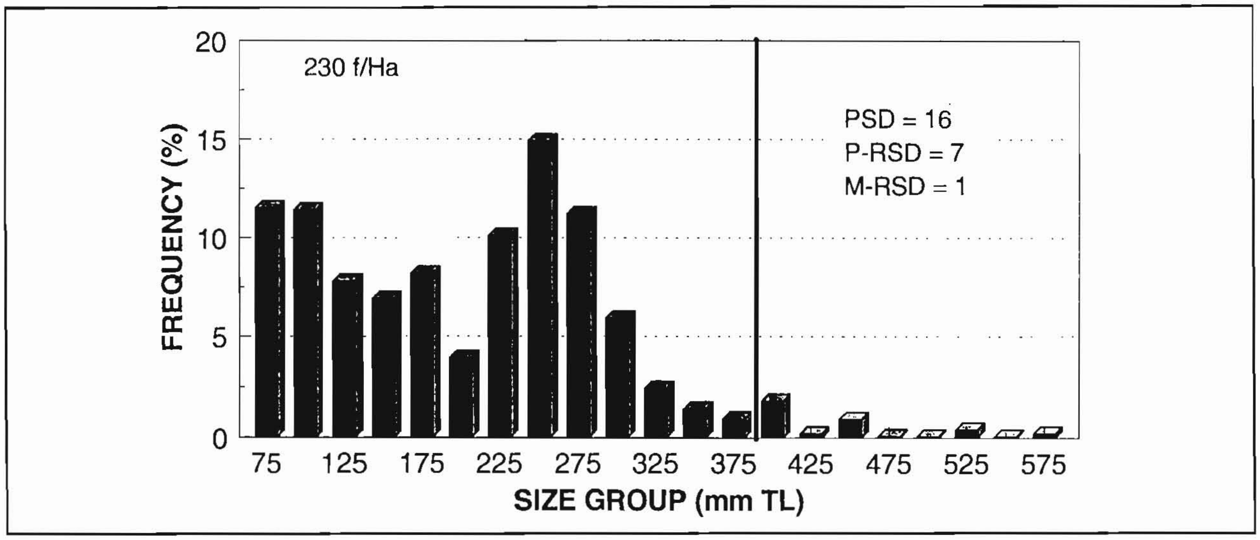

Figure 4. Length frequency and size-structure of largemouth bass in Guntersville Reservoir, in an 11-ha rotenone sample, 1988

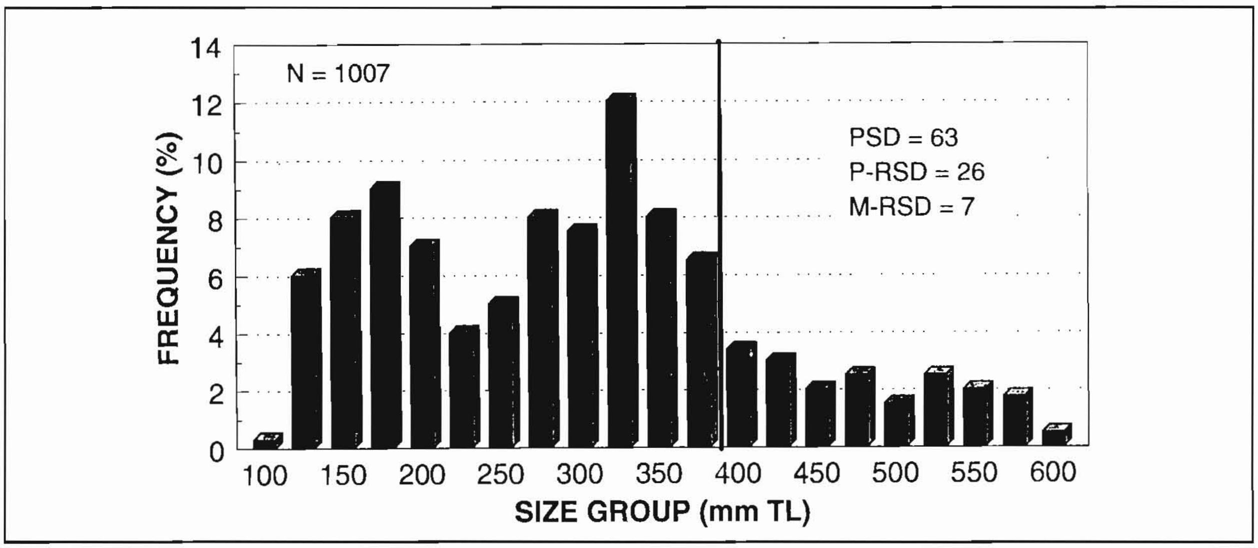

Figure 5. Length frequency and size-structure of largemouth bass in Guntersville Reservoir, electrofishing catch-depletion samples, 1993

variables appear to have operated in concert to affect bass growth in Guntersville. As recently as 1990, age-growth analyses by the Alabama Game and Fish Department indicated slower than normal growth for bass less than 4 years old in this reservoir.[1] Similar growth effects have been reported by Bettoli et al. (1992) and by our work in the Town Creek embayment (Webb et al. 1989). Growth analysis of the spring 1993 electrofishing sample showed normal to good growth for age-groups less than 4 years. If slow growth returns as a result of whatever cause, the immediate effect would likely be stock-piling of sublegal size bass, which can be monitored rapidly by use of a size-structure index as examined in this report.

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[1] Personal Communication, 1993, W. C. Reeves, Alabama Game and Fish Department.

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

by
Wayne T. Jipsen[1]

The seventh annual Operations Breakout Session was held 16 November 1993 during the Aquatic Plant Control Research Program (APCRP) Review. Representatives from Headquarters, 1 Division Office, 11 District and Project Offices, and the U.S. Army Engineer Waterways Experiment Station (WES) attended. Other attendees included Federal, state, local, university, and industry representatives. A total of 39 people participated.

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

Re-registration updates were provided for copper, diquat, endothal, fluridone, and 2,4-D. In-progress reviews were also provided on the initial aquatic registration process for both imazapyr and triclopyr.

District and state personnel discussed the ongoing grass carp programs on Lake Marion, SC, and Lake Istokpoga, FL. Other topics of discussion included new approaches in state funding sources for aquatic plant control, continued spread of exotic species, and funding availability in the O&M program for management efforts on Corps lakes.

Reports were given on the APC Program Review Document (PEG) and the spring meeting of the Field Review Group (FRG). The PEG, which has been utilized on a trial basis within the South Atlantic Division, is expected to be ready for Corps-wide use in fiscal year 1994. The FRG met in the spring of 1993 to review the overall field of simulation technology and its applicability to the APC Program.

Participants in the Operations Breakout Session were provided with demonstrations on two educational tools that are being developed within the aquatic plant management field. WES researchers demonstrated a biological control training program developed for the Aquatic Plant Control Operations Support Center (APCOSC). This computer program, as well as a slide show and course materials, will be available from the APCOSC in mid-1994. Staff members of the University of Florida's Center for Aquatic Plants provided an overview of video disk technology and a demonstration of a video disk application of the APCRP's publication "Aquatic Plant Identification and Herbicide Use Guide."

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

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

Simulation Technology

An Overview of Simulation Technology in the Aquatic Plant Control Research Program

by
R. Michael Stewart[1]

Technology Area Objectives

The objective of this technology area is to develop personal computer (PC)-based simulation procedures for estimating the growth and control of nuisance aquatic plant species under different sets of environmental and operational conditions. The purpose for development of these simulation procedures is to provide systematic evaluation procedures that promote transfer of technology developed under the Aquatic Plant Control Research Program (APCRP) to agencies and private firms who utilize this technology for aquatic plant management.

Development of aquatic plant control simulation procedures also accomplishes two important intermediate functions prior to release of the software packages as technology transfer tools. These intermediate functions are (a) synthesis of information and (b) testing and evaluation of information. In regards to these intermediate functions, the simulation development process provides an added mechanism for testing information/relationships developed under the other APCRP technology areas: Aquatic Plant Ecology Technology, Chemical Control Technology, Biological Control Technology, and Applications Technology.

Because simulation procedures are being developed for each of the major APCRP technology areas, separate work units have been developed for Plant Growth Simulations (Work Unit 32440), Chemical Control Simulations (Work Unit 32439), and Biological Control Simulations (Work Unit 32438). The simulation procedures are designed to allow consideration of the effects of generalized site conditions on growth of the target plant and on the effectiveness of control techniques. To facilitate development and execution of the simulations, a separate work unit for Aquatic Plant Database Development (Work Unit 32521) was added to the overall technology area for designing and compiling environmental databases compatible with the simulation procedures.

A summary of the development activities underway in each of the four Simulation Technology work units is provided herein and in the following four papers of this proceedings. Beginning in fiscal year 1994 (FY94), development activities under this technology area will be expanded to facilitate development of an overall Aquatic Plant Control Evaluation System (APCES). Price, Smith, and Stewart (1994) provide an introductory summary of the APCES.

Overview of Tasks Areas

Plant growth simulations

Plant growth simulation models for waterhyacinth, hydrilla, and Eurasian watermilfoil are being developed under this work unit. These simulation procedures provide a systematic, PC-based tool for estimating the rate and level of growth these plant species will attain under specific environmental conditions. Environmental conditions that can be considered in these simulations are water depth, water clarity, temperature, irradiance, and initial plant biomass levels. Outputs from these simulations help aquatic plant managers determine when to apply control techniques and also serve as

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

a reference or baseline for evaluating the effectiveness of control applications by providing estimates that represent an untreated control.

Current work (Stewart and Monteleone 1993; Stewart 1994a) is developing validation data sets for testing the accuracy of algorithms in first-generation simulations for hydrilla and Eurasian watermilfoil. Main relationships being tested concern regrowth of these plant species under light-limiting conditions. To support this work, a deep water plant growth mesocosm system (Stewart 1994a) has been developed at the Lewisville Aquatic Ecosystem Research Facility (LAERF). Data collected from this mesocosm system will allow testing of relationships for growth initiation, shoot elongation, biomass production, and photosynthesis and respiration balances under controlled-light conditions.

Biological control simulations

Simulation procedures to evaluate interactions among biological control agents and aquatic plants are being developed under this work unit. In an interactive but modular framework, these procedures generate simulation outputs useful in determining the likely effects of environmental conditions on growth of a target plant infestation that is being impacted by biocontrol agent herbivory. Modules in these procedures include a plant growth module, an insect population dynamics module, and an herbivory module that provides a basis for considering the biocontrol agent and host plant interactions.

Development of biological control simulation procedures has been limited by the lack of development of required relationships on the individual biological control agent species by Biological Control Technology studies. To date, simulation procedures have been developed for white amur (Boyd and Stewart 1992), a herbivorous fish introduced for control of submersed plant species, and for *Neochetina* weevils (Stewart and Boyd 1992), herbivorous insects that were introduced for the control of waterhyacinth. Though numerous other insect biocontrol agents have been introduced into the United States for control of other exotic plant species, sufficient studies that quantify the effects of these agents on their host plants have not been conducted to support simulation development. To date, model development efforts for these other insect species have been limited to generalized, temperature-based population dynamics modules (Boyd and Stewart 1993).

Development activities (Boyd and Stewart 1994) being continued for the AMUR/STOCK simulation procedure are based on studies (Kirk et al. 1993; Kirk, Morrow, and Killgore 1994) on growth of triploid white amur in large, mixed plant community reservoirs in southeastern states. Additionally, the existing AMUR/STOCK software package is being converted to a WINDOWS application tool for release during the end of FY94.

Chemical control simulations

Effective treatment of an aquatic plant infestation with herbicides requires maintaining a critical concentration (dose) of the active ingredient in contact with the target plants for a required exposure time. Following application, aqueous concentrations of the active ingredient depend on the amount of active ingredient applied, the formulation release rate, and various processes that determine the fate of the herbicide in the particular aquatic environment. Mortality relationships for the different combinations of target plants and herbicide concentration/exposure time levels are being developed by Chemical Control Technology investigations. These relationships, along with relationships representing the major fate processes affecting herbicide active ingredient concentrations in aqueous environments, are incorporated in the HERBICIDE simulation procedure.

Stewart (1992) describes the functionality of the first-generation version of HERBICIDE for chemical control techniques of submersed plant infestations. Example applications of HERBICIDE utilization for determining the effects of site conditions on postapplication aqueous concentrations of a herbicide are given in Stewart (1994b). Scheduled work for FY94 includes completing the conversion of the existing simulation procedure into a WINDOWS application tool, with release of the converted software package scheduled toward the end of the fiscal year.

Aquatic plant databases

The purpose of this work unit is to employ and demonstrate state-of-the-art database techniques to support development and execution of simulation procedures described herein. A large portion of this work involves compiling climatic data needed for initializing the software packages for different water bodies and geographic regions (Kress and Causey 1992; Kress and Holt 1993). Recent studies (Bourne, Kress, and Berry 1994) have investigated use of Global Positioning Systems (GPS) for compiling data sets for initializing and evaluating simulation procedures, as well as for other applications by aquatic plant managers. As described by Price, Smith, and Stewart (1994), tools and procedures developed under this work unit will also be key components of the APCES package.

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Investigations of Plant Growth Under Low Light Conditions for Testing Submersed Plant Simulation Models

by
R. Michael Stewart[1]

Introduction

Background

The proper use of aquatic plant control techniques in an overall aquatic plant management plan should consider natural variability in aquatic plant growth patterns. In natural water bodies, both the rate and peak level of growth may vary spatially and annually because of differences in water depth, temperature, light availability, hydrological conditions, sediment characteristics, and other environmental factors. This natural variability, if not considered, often makes it difficult to determine the posttreatment effectiveness of control measures. Additionally, failure to adequately consider natural plant growth variability when selecting control techniques with long-term effect times (e.g., grass carp stockings) may result in attaining undesired levels of control.

Under the Simulation Technology area of the Aquatic Plant Control Research Program, personal computer (PC)-based simulation procedures are being developed to predict aquatic plant growth patterns under different sets of environmental conditions. Types of environmental conditions that can be considered are water depth, temperature, water clarity, irradiance levels, and initial plant biomass. Information obtained from the simulations will help aquatic plant managers consider variability in plant growth patterns and help them make more informed aquatic plant management decisions.

Simulation procedures developed for *Hydrilla verticillata* and *Myriophyllum spicatum* (Wooten and Stewart 1991) are currently under beta testing prior to release. Testing has included comparison of simulation outputs for plant biomass with estimates derived from 1990 through 1992 field measurements made at Guntersville Reservoir, AL. Results of these comparison tests indicate a need to conduct further verification studies under more controlled conditions for relationships that determine plant growth under low light conditions.

A preliminary investigation of hydrilla and milfoil growth under light conditions below 10-percent full sunlight was presented by Stewart and Monteleone (1993). Their results demonstrated growth inhibition (i.e., both rates and peak levels) at light levels below 5 percent surface levels. However, direct application of their results to natural low light conditions is limited because the studies were conducted within shallow (0.86 m) tanks with essentially uniform light levels (i.e., no depth-induced light gradient). In natural waters, submersed plants encounter a light gradient that decreases with depth because of absorption (Kirk 1983). Obviously, growth of submersed plants such as hydrilla and milfoil under low light is enhanced by their tendency to elongate, a response that often positions the upper portions of their shoots within more favorable light conditions. Survival of the plant, however, is dependent upon eventually achieving an overall positive photosynthesis and respiration balance, which is determined by the sum of these processes over the entire shoot length.

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

Objectives and scope

Further testing of submersed plant growth model relationships for low light conditions requires consideration of growth responses within depth-induced light gradients. The objectives of fiscal year 1993 (FY93) efforts were to develop a mesocosm facility at the Lewisville Aquatic Ecosystem Research Facility (LAERF) for conducting these type studies. This included a preliminary study to evaluate regrowth of milfoil and hydrilla under low light conditions established in the mesocosm tanks. The following briefly describes the low light mesocosm system and summarizes initial plant growth studies conducted therein during FY93.

Methods

Mesocosm facility description

The low light mesocosm facility consists of replicated fiberglass tanks, each 3.0 m tall and 2.4 m in diameter, with a working volume of approximately 14,000 L. The inside walls of the tanks are covered with a flat black gel coating to reduce light scattering. Additionally, the top of each tank is fitted with standard, commercially available reflective louver and diffuser panels to equalize light distribution, which otherwise would be skewed because of shadowing from the tank sides. Tank water is pumped through sand filters from a 1,400,000-L supply pond established for the adjacent chemical control mesocosm system (Dick, Getsinger, and Smart 1993). Water in the supply pond is treated with alum to reduce phosphorous and suspended material. After filling, tank water is circulated by an airlift mechanism to prevent stratification. Smaller sand filters attached to pairs of adjacent tanks provide routine filtration of tank water to limit algae growth during a study.

Test conditions

Tests conducted during FY93 measured growth of milfoil apical tips and hydrilla apical tips and tubers under two light conditions. Both conditions were established by placing one layer of highly reflective, hexabolic-patterned louvers and two layers of standard fluorescent lighting diffusers over the tank openings. Light at the water surface was reduced to approximately 40 percent outside-tank irradiance levels, with further reduction occurring with depth in the water column because of attenuation. Differences in light treatments were established by setting two planting depths within the tanks. A deep water, low light treatment was established by placing test plant pots on the bottom (i.e., 2.5 m water depth) of the tank. The shallow water, high light treatment was established by placing test plant pots on a false bottom of the tanks constructed at a water depth of 1.5 m. The treatment light gradient encountered during growth in the low light treatment ranged from 10 to 40 percent (i.e., depth to surface) of surface irradiance levels. Under the high light treatment, the established gradient ranged from 20 to 40 percent of surface levels.

Each treatment included three replicate tanks. Within each tank, 20 pots for each of the three types of regrowth structure were randomly positioned at the respective treatment depth. Plant growth was estimated using both nondestructive and destructive sampling techniques. Nondestructive techniques included weekly measurements of shoot length and number of branches. Destructive sampling was conducted when shoot length for the majority of pots of a given structure and treatment reached the water surface. At this time, pots for that structure were removed and measurements were made of primary shoot length, branch number, branch origination height along primary shoot length, branch length, aboveground mass (in 0.5-m depth increments), and belowground mass. Selected tissues were also sampled for determining chlorophyll and carbohydrate levels.

Preparation of test plant material

Apical tips. Apical tip sections for both hydrilla and milfoil were collected from culture ponds at the LAERF on May 19, 1993. Tips were cut to 10-cm lengths and "rooted" into nutrient-enriched, sterilized sediments within 0.86-L plastic pots. "Rooting" was such that the top 4 cm of the tip remained above the sediment, which was then covered with silicon granules to limit nutrient leaching into the water column. Pots were then placed at their assigned positions within the tanks.

Hydrilla tubers. Hydrilla tubers were collected from hydrilla culture ponds at the LAERF during winter months. Tubers were washed in a weak bleach solution, placed on moistened paper toweling in a covered plastic container, and placed in an environmental chamber at 20 °C under constant light. Tubers were weighed after sprouting, and tubers with weights ranging from 0.30 to 0.40 g were wrapped in moistened paper toweling and stored until planting in a dark refrigerator. Tubers were planted individually in pots such that the tip of the sprouted shoot was at the sediment surface. Silicon granules were layered over the sediment prior to pot placement at assigned positions within the tanks.

Results

Nondestructive sampling

Plant growth measurements estimated by nondestructive, weekly sampling included shoot height and branch number. Under the high light treatment, shoot height (Figure 1a) for both hydrilla tubers and apical tips increased more rapidly than for milfoil apical tips. Shoots from both of the hydrilla growth structures reached the surface after only 3-weeks growth, whereas shoot growth from milfoil apical tips required 6 weeks to reach the surface. Branch production (Figure 1b) under high light was consistently greatest for hydrilla tubers and lowest for milfoil apical tips. For the low light treatment, shoot height reached the surface by week 4 for hydrilla tubers and between weeks 4 and 5 for hydrilla apicals (Figure 2a). Shoot height for milfoil apicals was less than 1.0 m after week 6 and did not reach the surface until week 19. As in the high light treatment, branch production (Figure 2b) was greatest for hydrilla tubers and lowest for milfoil apicals under the low light treatment.

Destructive sampling

Destructive sampling was conducted to determine differences in shoot structure development by the different plants under the two light treatments. This sampling was conducted at the time the developing shoots for a given plant/light treatment reached the surface. As illustrated in Figure 3a, similar amounts of aboveground mass were produced by hydrilla tubers and apical tips under the high and low light treatments, even though the periods of growth to reach the surface had been approximately 1 week longer in the low light treatment. Milfoil shoots, however, generated significantly more mass before reaching the surface under the low light treatment than under the high light treatment. Further, milfoil shoots generated significantly more mass than hydrilla shoots under both light treatments. Hydrilla tubers generated the greatest total shoot length (Figure 3b) under both treatments than either hydrilla or milfoil apicals.

These results indicate that under the conditions represented by the two light and depth treatments, hydrilla apical tips and tubers were capable of elongating and reaching the surface faster than milfoil apical tips. Under treatment conditions, hydrilla exhibited a smaller mass requirement for both vertical growth (mass:depth) (Figure 4a) and total shoot production (mass:length) (Figure 4b). The rapid elongation rate by hydrilla significantly accounts for its competitive dominance over milfoil under lower light conditions. Had the hydrilla shoots not been taken out of the tanks at the time they reached the surface, the developing shoots would have undoubtedly formed a canopy and produced significantly more mass than was measured at the time of sampling.

Recommendations for FY94 Studies

During FY94, the remainder of the data analyses for this study will be completed.

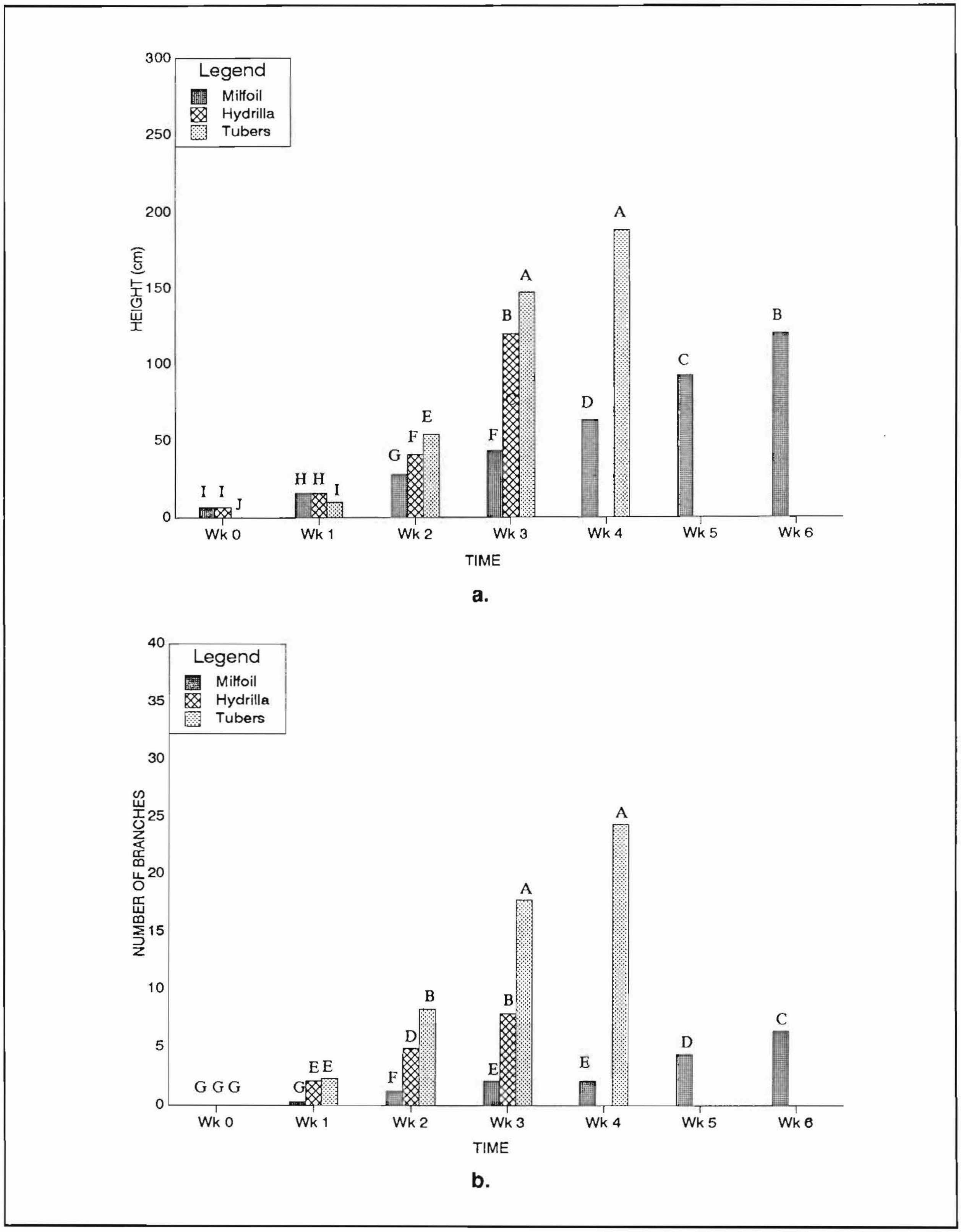

Figure 1. Weekly growth measurements of shoots from milfoil and hydrilla apical tips and from hydrilla tubers under the high light treatment. Measurements are shoot length (a) and number of branches (b). Bars with the same letters are statistically similar (P = 0.05)

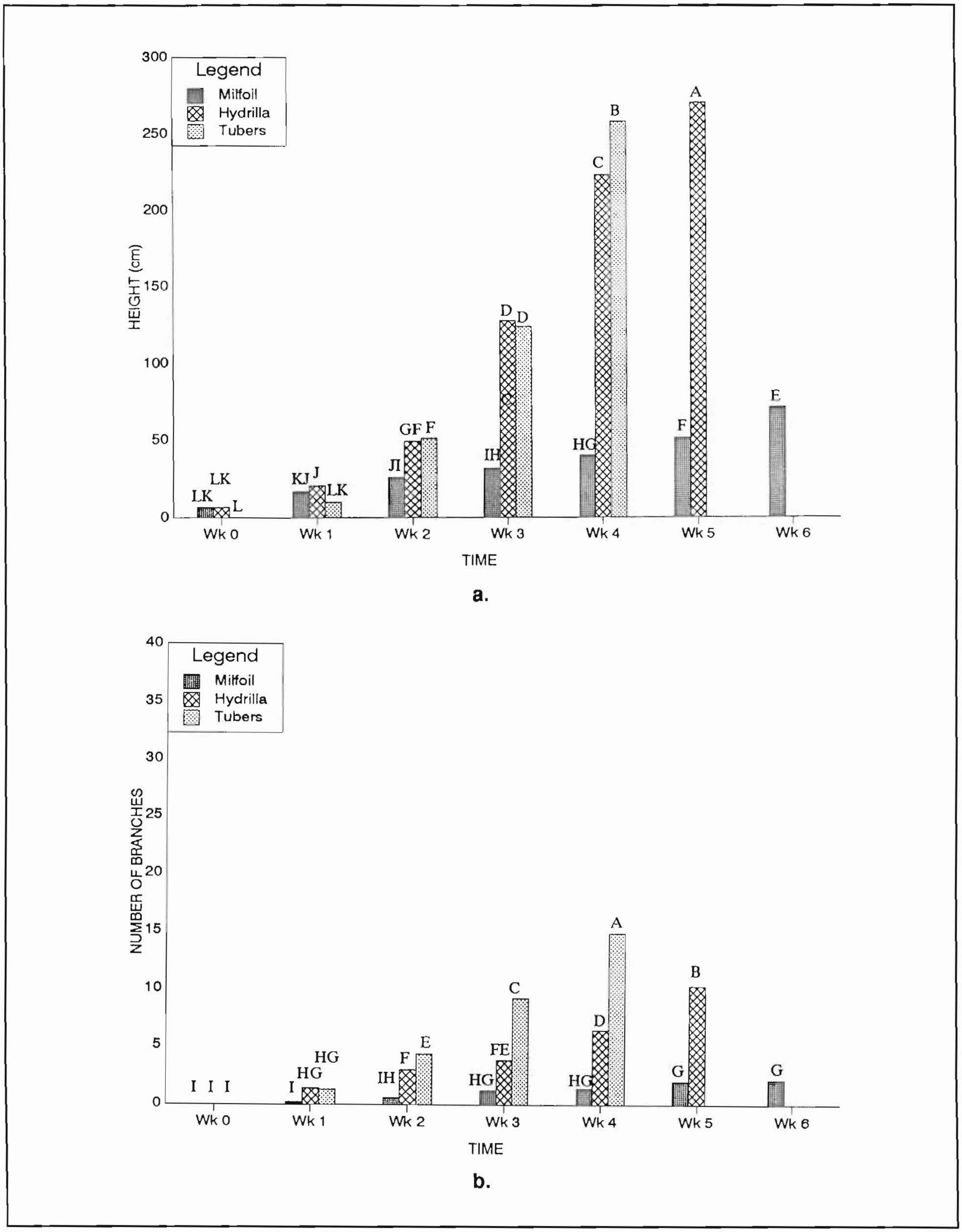

Figure 2. Weekly growth measurements of shoots from milfoil and hydrilla apical tips and from hydrilla tubers under the low light treatment. Measurements are shoot length (a) and number of branches (b). Bars with the same letters are statistically similar (P = 0.05)

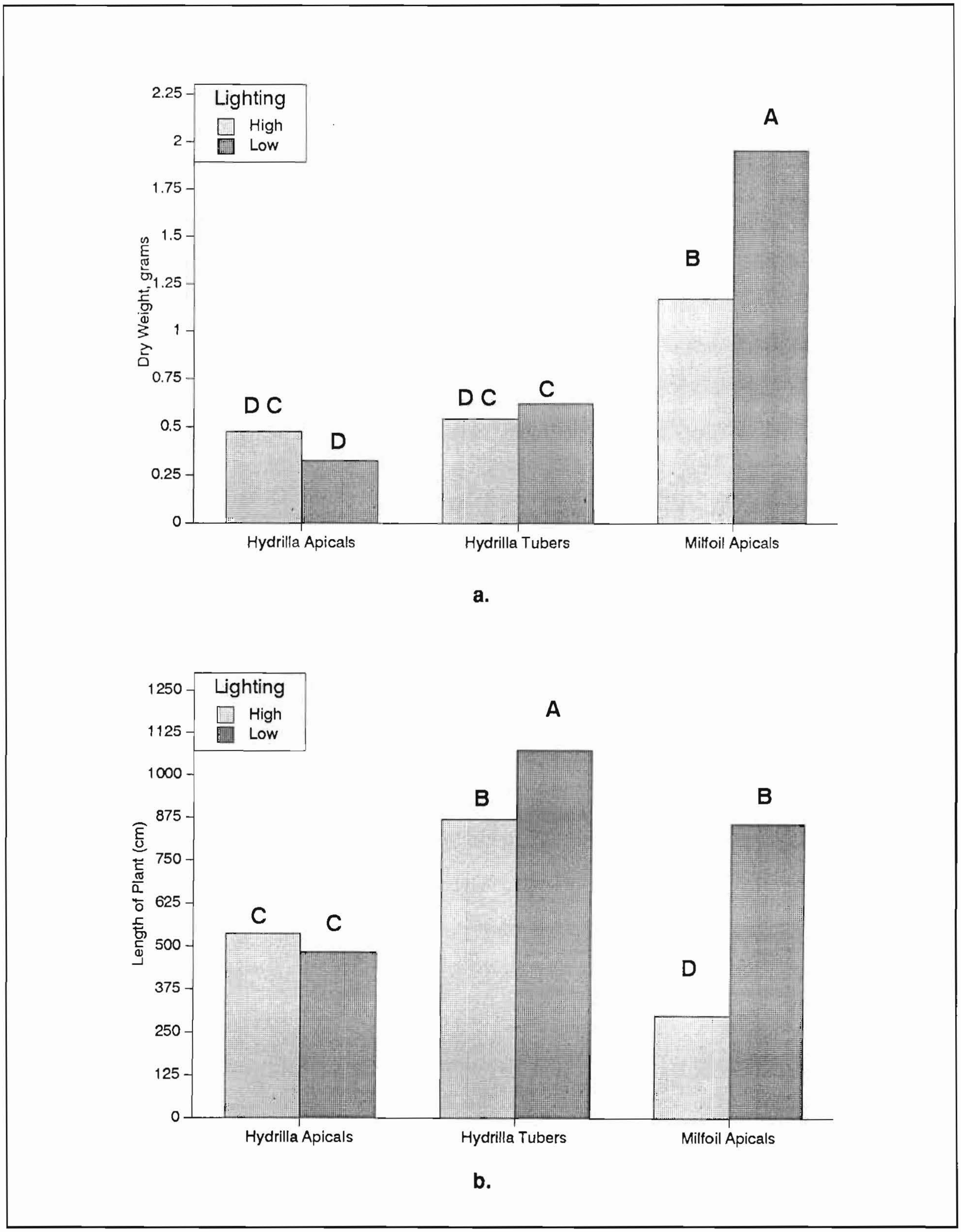

Figure 3. Growth measurements of shoots from milfoil and hydrilla apical tips and from hydrilla tubers after reaching the surface under the high and low light treatments. Measurements are aboveground mass (a) and total shoot length (b). Bars with the same letters are statistically similar (P = 0.05)

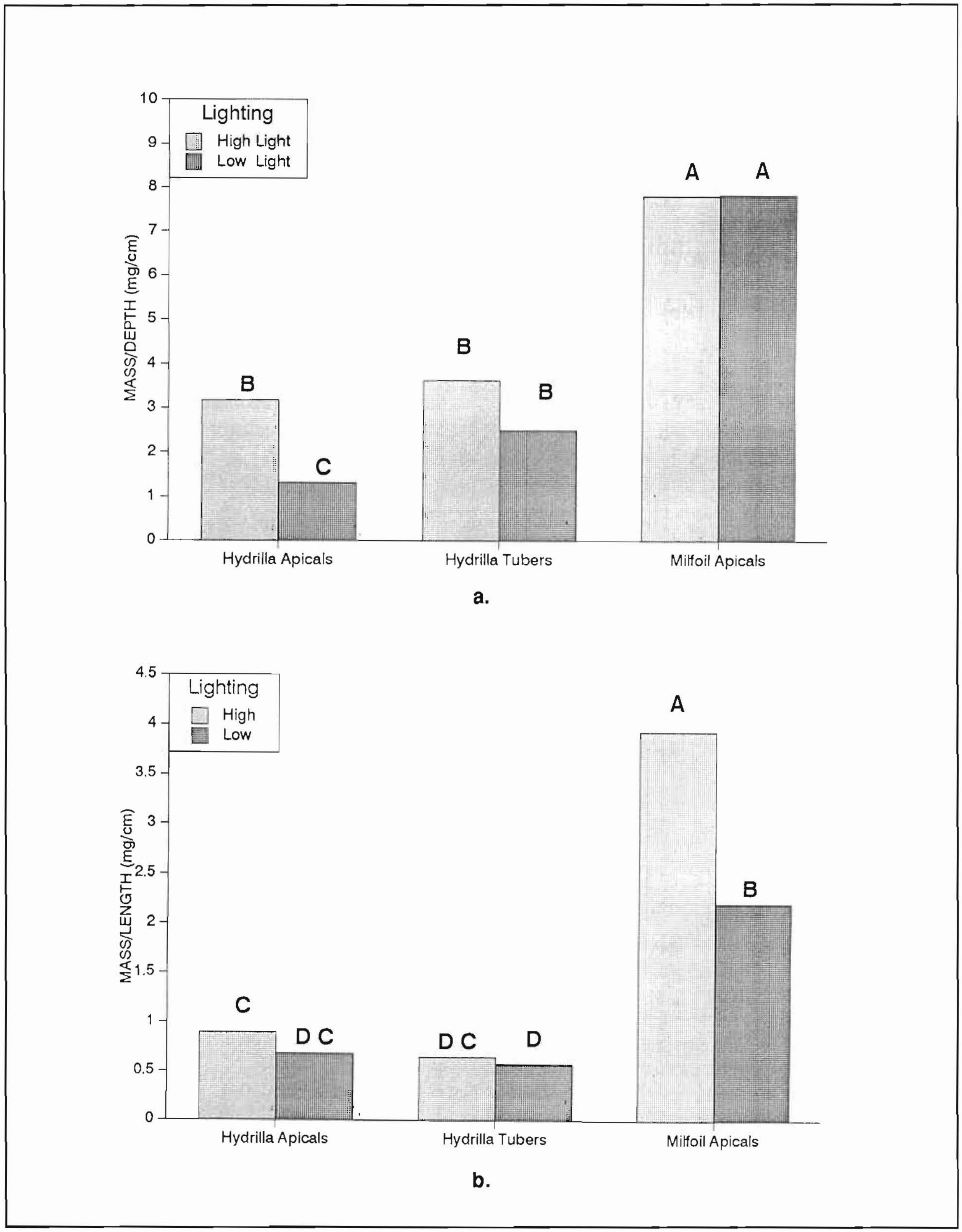

Figure 4. Growth measurement ratios of shoots from milfoil and hydrilla apical tips and from hydrilla tubers after reaching the surface under the high and low light treatments. Ratios are mass:depth (a) and mass:total shoot length (b). Bars with the same letters are statistically similar (P = 0.05)

Resulting findings will be used to test shoot elongation and mass production relationships used in the HYDRILLA and MILFOIL plant growth simulation models. Additionally, development of the deep tank mesocosm facility will continue, with priority given to designing techniques for establishing more realistic light gradients.

Acknowledgments

This work was supported extensively by the LAERF researchers and staff. Thanks especially to Drs. Michael Smart and Gary Dick for technical assistance during development of the deep tank facility and for advice in designing the FY93 studies. Ms. Susan Monteleone was responsible for daily monitoring of the studies, sample processing, and data compilation. The majority of the sampling was conducted by Mr. Joe Snow. Destructive sampling events required processing large numbers of samples and were supported by the majority of the other LAERF staff members.

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Comparison of AMUR/STOCK Simulation Outputs with Observations from Guntersville Reservoir and Lake Marion

by

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

In an effort to achieve long-term control of hydrilla *(Hydrilla verticillata)*, triploid white amur were stocked at Lake Marion, SC (Roach, Inabinet, and Tuten 1993), and Guntersville Reservoir, AL (Bates 1990). This report gives a description of white amur stockings made at the two reservoirs, provides example simulation outputs that illustrate how AMUR/STOCK is used to predict stocking effects, and evaluates possible causes for differences observed in stocked fish growth at the two reservoirs.

Triploid White Amur Stockings at Lake Marion and Guntersville Reservoir

In 1989, Santee Cooper entered into a joint funding agreement with the South Carolina Aquatic Plant Management Council, the South Carolina Water Resources Commission, and the United States Army Corps of Engineers (USACE) to stock white amur in upper Lake Marion. The target plant species was hydrilla. In the summer of 1989, the Santee Cooper lakes supported approximately 15,000 acres of hydrilla, and by the fall of 1990, this coverage had expanded to a peak of 32,000 acres. The stocking effort originally called for the release of 100,000 fish per year for 3 years. Because of the widespread fish kills experienced in Lake Marion following Hurricane Hugo in 1989, however, the stocking program was extended into a fourth year. This incremental stocking of 100,000 white amur per year from 1989 through 1992 into a 170,000-acre lake system represents a total of 400,000 triploid white amur.

Similarly, the Tennessee Valley Authority (TVA) was designated as lead agency for a joint TVA/USACE project in which TVA, with support provided by the U.S. Army Engineer Waterways Experiment Station, as well as the Nashville District, would develop a plan for reducing excessive vegetation on Guntersville Reservoir.

The initial phase, planned and implemented by TVA, began with stocking 100,000 triploid white amur into the 68,000-acre impoundment during April-June 1990. The target plant was hydrilla, which had infested approximately 3,000 acres in 1988.

Application of AMUR/STOCK

The AMUR/STOCK model was developed to provide users with a systematic evaluation tool for answering "What if" questions regarding the results of various white amur stocking scenarios. The model has been applied primarily to Guntersville Reservoir (see Boyd and Stewart 1990, 1991, 1992) to predict the level of control that will result from the 1990 white amur stockings by TVA.

Because Guntersville Reservoir supported a mixed plant community prior to stockings, assumptions had to be made for proportional feeding rates on the different plant species. Assumed feeding proportions were based on two factors: (a) the feeding preferences of white amur for the plant species included in the scenario and (b) the availability of each plant species (Boyd and Stewart 1992). For Guntersville Reservoir simulations, it was

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assumed that the fish would first feed on the "preferred" plant species (i.e., hydrilla and annuals); and that after these were controlled, they would begin to feed on "nonpreferred" plant species (i.e., Eurasian watermilfoil). Example simulation outputs based on these feeding assumptions and on 1989 Guntersville Reservoir aquatic plant infestation levels are presented in Figure 1.

Comparison of Fish Weights for Lake Marion and Guntersville Reservoir

Initial comparisons of fish weights by age class for Lake Marion and Guntersville Reservoir are shown in Figure 2. Weights for the same age-class fish were consistently higher at Lake Marion than at Guntersville Reservoir. At age class 4, the fish at Lake Marion were more than 3.3 kg larger than at Guntersville Reservoir. Kirk, Morrow, and Killgore (1994) provide additional detailed information on derivation of these fish weight estimates. It is known that water temperature directly affects fish feeding and thus indirectly affects fish weight. The lower fish feeding threshold temperature for triploid white amur is 11 °C. As can be seen in Figure 3, average monthly water temperatures at Lake Marion are generally higher than at Guntersville Reservoir. There are no real significant differences in the two water bodies; however, simulations discussed above for Guntersville Reservoir that were based on these lower water temperatures generated fish size estimates higher than estimates from field measurements (Figure 4). Additionally, fish size estimates generated by Lake Marion simulations compared well with field estimates (Figure 5). Thus, other factor(s) must be contributing to the differences found in fish weights at the two reservoirs.

Because of the large differences between the simulated and calculated white amur weights for Guntersville Reservoir, a review of the observed aquatic macrophyte coverages during the poststocking years (see Figure 6) was made to investigate other possible causes for the low growth rates. The review revealed that while hydrilla and the annuals have been controlled, the Eurasian watermilfoil has continued to expand in coverage, thus indicating that white amur have had little if any impact on Eurasian watermilfoil. Therefore, additional

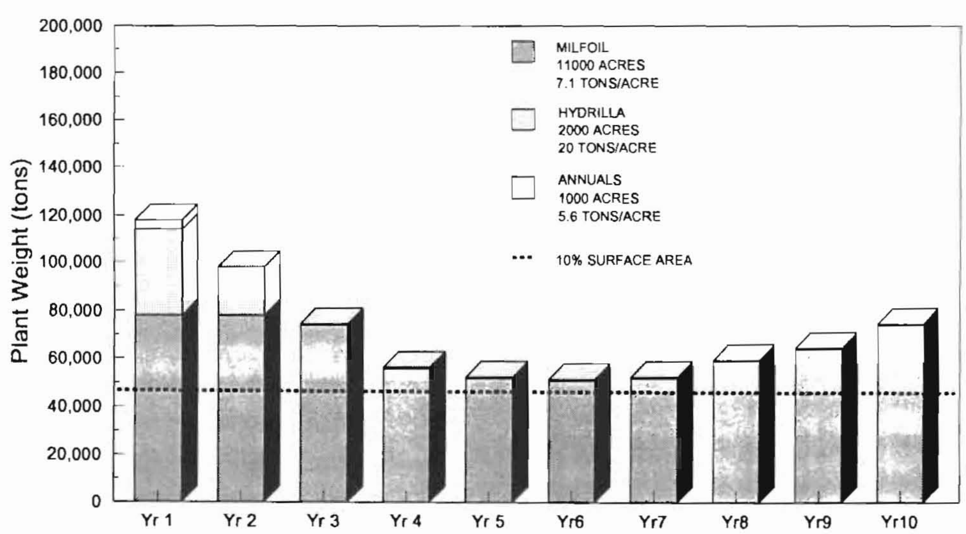

Figure 1. Standing crop resulting from three stockings in Year 1 of a 10-year simulation: 35,000-May, 50,000-June, and 15,000-July. Initial fish size was 0.75 lb/fish, and plant infestation levels were based on 1989 Guntersville Reservoir conditions

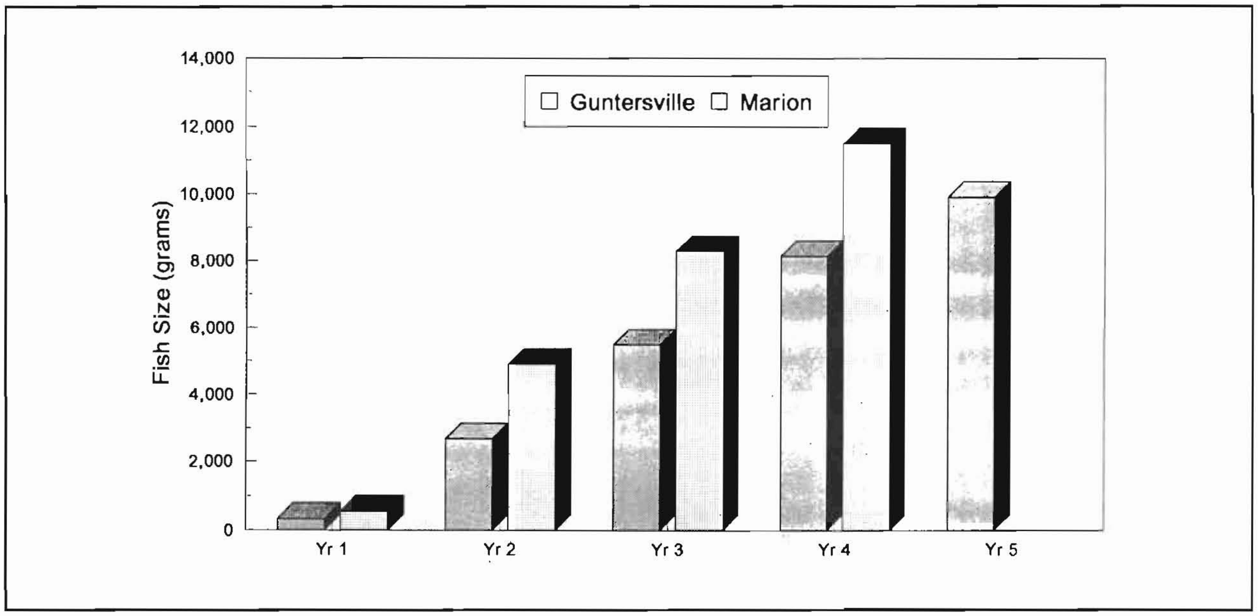

Figure 2. Comparison of age and growth of triploid white amur stocked at Lake Marion, SC, and Guntersville Reservoir, AL

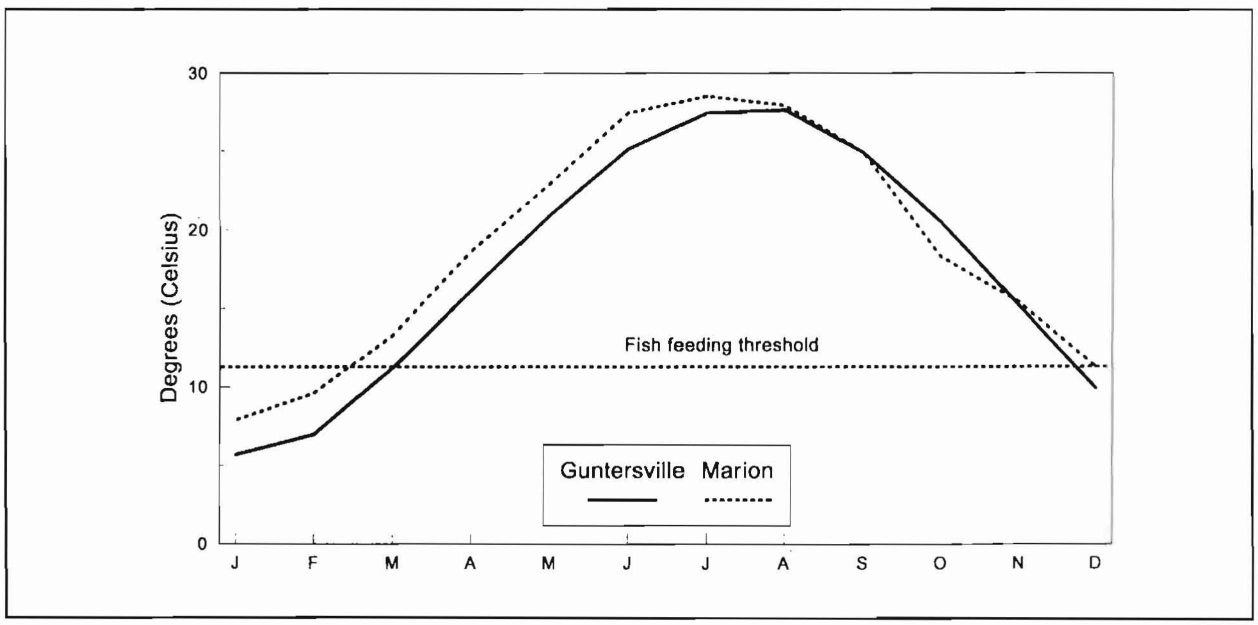

Figure 3. Average monthly water temperatures for Lake Marion, SC, and Guntersville Reservoir, AL

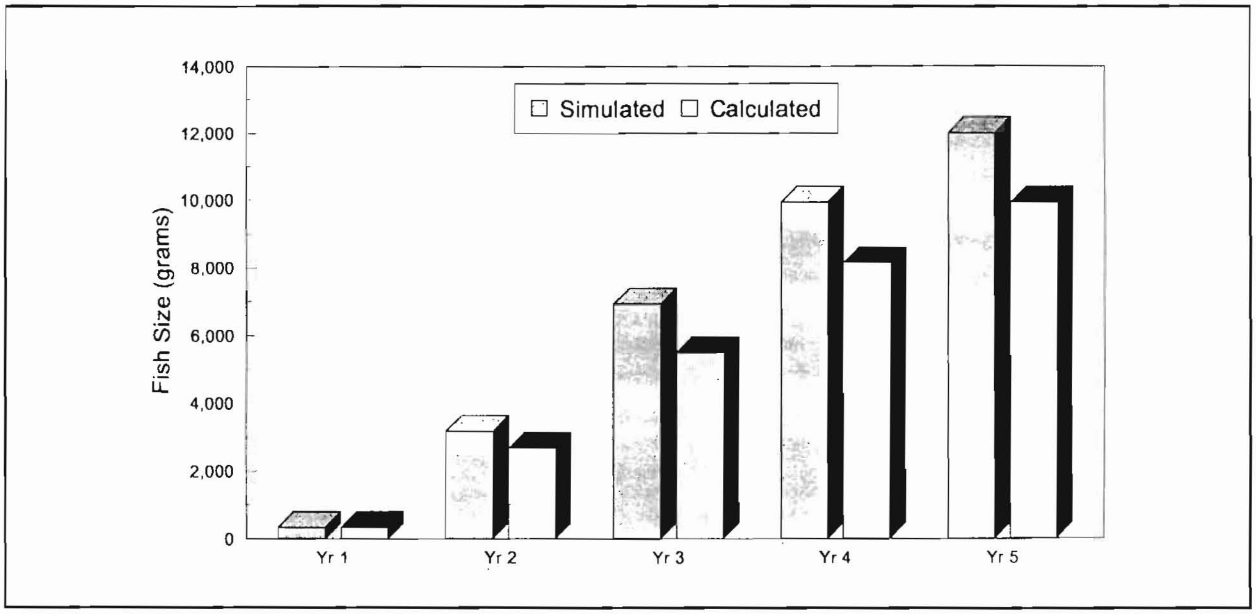

Figure 4. Comparison of age and growth of triploid white amur stocked at Guntersville Reservoir, AL. Simulations assume the fish feed on Eurasian watermilfoil

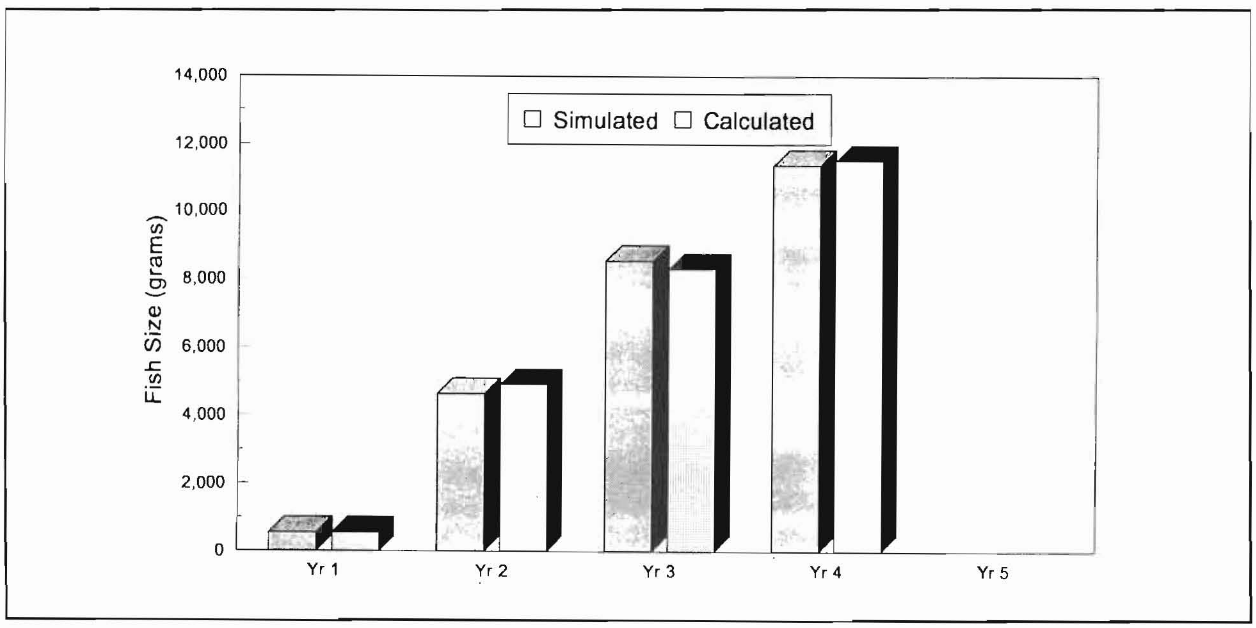

Figure 5. Comparison of age and growth of triploid white amur at Lake Marion, SC

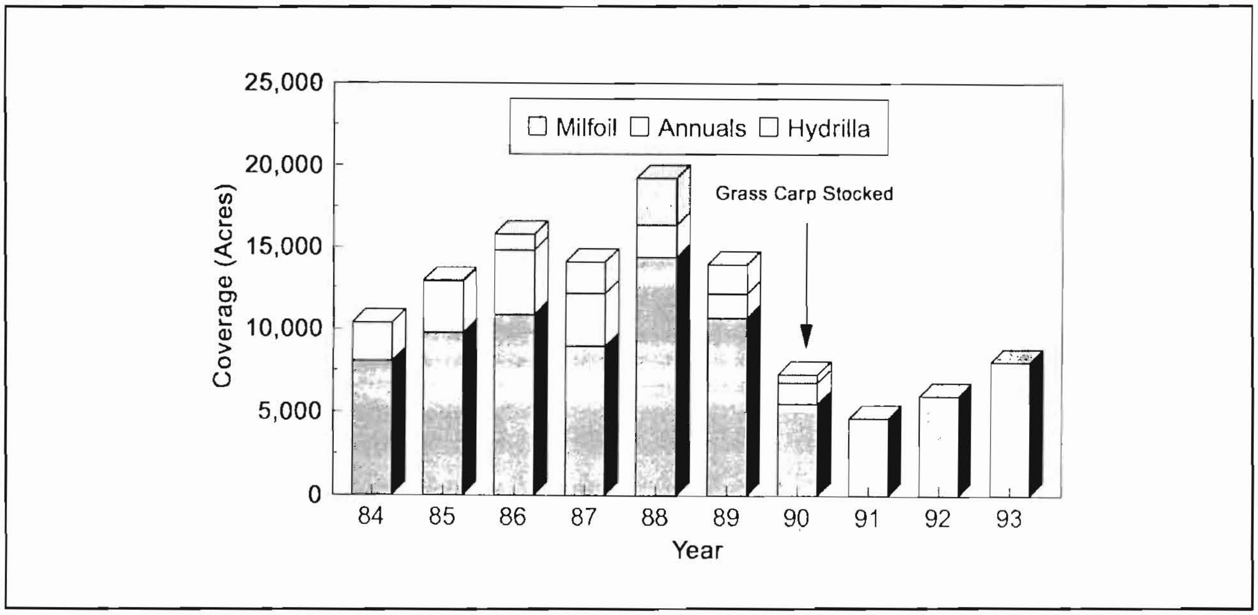

Figure 6. Observed aquatic macrophyte coverage at Guntersville Reservoir, AL, from 1984-1993

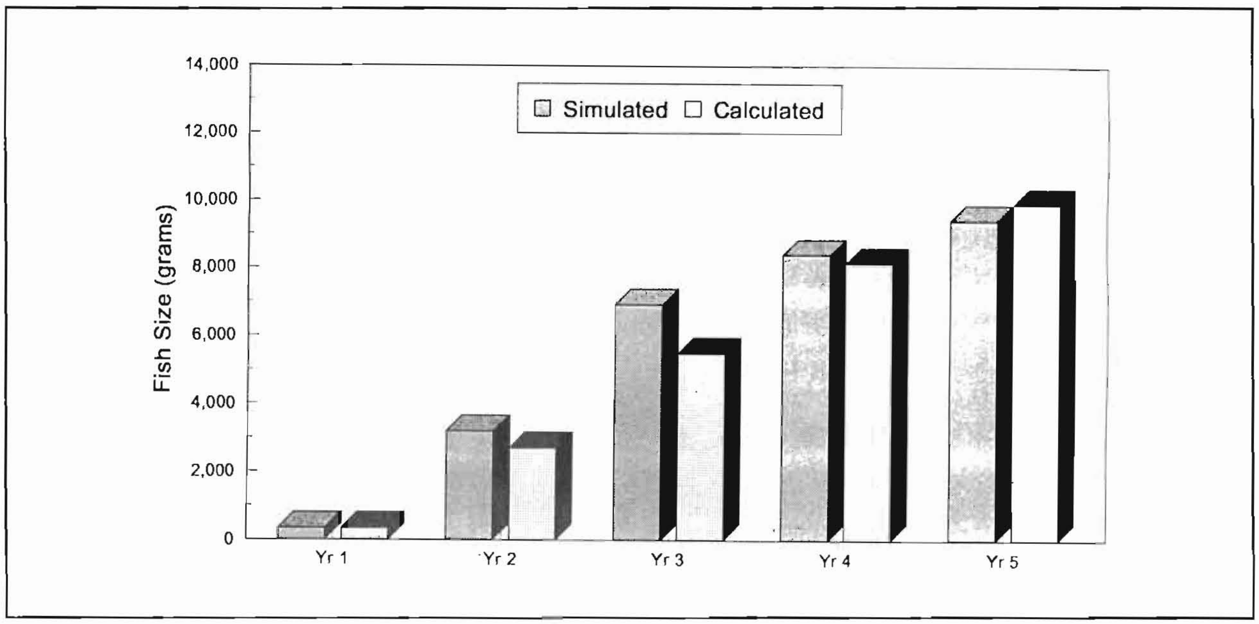

Figure 7. Comparison of age and growth of triploid white amur stocked at Guntersville Reservoir, AL. Simulations assume the fish do not feed on Eurasian watermilfoil

AMUR/STOCK simulations were run under the assumptions that after hydrilla and the annuals were controlled, the fish would not maintain their maximal consumption rates by feeding heavily on milfoil. Based on these assumptions, consumption rates were lower and simulated fish weights were much closer to calculated fish weights (Figure 7).

Future Work

Fish mortality may vary significantly from one system to another, especially when extreme circumstances occur, such as the widespread fish kills experienced at Lake Marion in 1989 as a result of Hurricane Hugo. In an effort to allow the user to account for excessive losses, or if the user has an annual percentage value for fish mortality, AMUR/STOCK is designed to accept these user-input annual fish mortality rates. It is realized that most users will not have access to fish mortality rates; therefore, AMUR/STOCK sets a default fish mortality percentage that is a function of both the fish size and age. Further validation, however, is needed to validate these fish mortality rates and relationships.

As collections of triploid white amur continue at both Lake Marion and Guntersville Reservoir, more information will become available on relationships between fish age-classes and fish weights. This information will be useful in further validating fish growth relationships already used in AMUR/STOCK.

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HERBICIDE Simulation Model for Evaluating Fate Processes Effects

by
R. Michael Stewart[1]

Description of HERBICIDE

Model overview

The HERBICIDE simulation model currently under development is a decision support software package (Rodgers, Clifford, and Stewart 1991; Stewart 1993) that generates information useful for determining the effectiveness of aquatic herbicide application techniques for particular control requirements and site conditions. The structure of the model allows estimations or predictions for (a) the postapplication fate of a herbicide formulation active ingredient and (b) the level of target plant control resulting from the attained exposure. Recent work has focused on testing and demonstrating the applicability of the overall simulation. Example applications are briefly discussed herein.

Fate considerations

Significant reductions to initial concentrations of herbicide active ingredients following their release into aquatic systems are effected through the action of various fate processes. The level of the reductions is dependent upon the nature of the herbicide formulation (e.g., liquid, slow-release matrix), the chemical properties of the active ingredient, and the site conditions (Westerdahl and Getsinger 1988). Fate processes can be classified as either transfer processes or transformation processes (Reinert and Rodgers 1987). Transfer processes considered by HERBICIDE simulations result in partitioning of the active ingredient into the water, the target plant tissues, and the sediments. Within these partitions, herbicide levels are continually reduced through the collective action of various transformation or degradation processes. Ultimate rates and levels of partitioning and degradation are determined in the simulation through user initialization of the parameters listed in Table 1.

In still or slow-moving waters, herbicides are often applied as liquid formulations, and the applications result in immediate release of the active ingredient fraction. Though partitioning into sediments and biological (e.g., target plants) compartments contributes to overall aqueous concentration reductions, degradation processes often account for the majority of the reductions in low water exchange systems. Degradation rates are typically estimated as half-life decay rates. For most active ingredients, degradation is a summation of multiple processes, and cumulative rates vary depending on site conditions (e.g., water temperature and turbidity). For this reason, degradation rates are typically reported as a range.

As the water exchange rate at the application site increases, dilution becomes the most significant process effecting herbicide reductions in the treatment area. Applicators must consider the effects of water exchange in order to obtain proper plant exposure in the treatment area. Research for development of better herbicide formulations and application techniques for systems with moderate to high water exchange rates is currently being conducted by Chemical Control Technology's Herbicide Delivery System work unit (Turner et al. 1993).

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

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

Demonstration of HERBICIDE Use

Objectives and assumptions

The objectives of the following sections is to demonstrate the use of HERBICIDE for evaluating the level of reductions that selected fate processes can have on postapplication herbicide concentrations. For the demonstration, simulations were initialized for three categories of fate conditions. Category 1 and 2 simulations considered the effects of different degradation and water exchange rates, respectively, following application of a liquid herbicide formulation. Category 3 simulations considered effects of different water exchange rates following application of a slow-release herbicide formulation.

Outputs from individual simulations are then compared on the basis of concentration and exposure time combinations required to achieve an effective level of aquatic plant control. The theoretical concentration and exposure time relationship illustrated in Figure 1 was developed strictly for this demonstration purpose and should not be confused with empirically determined relationships reported by Chemical Control Technology's Concentration/ Exposure Time work unit (Netherland, Getsinger, and Turner 1993). We assumed under this relationship that simulations that provide aqueous concentrations of 2.5 mg/L or greater for at least 12 hr, 1.0 mg/L or greater for 1 or more days, 0.5 mg/L or more for 2 or more days, or 0.25 mg/L or greater for 3 or more days would provide effective control. To provide a basis for further comparison of formulation types (i.e., liquid versus slow-release formulation), we assumed that label restrictions prohibited water concentrations from exceeding 2.5 mg/L.

All of the simulations were initialized for an application rate of 25 kg of active ingredient. The application site considered was 1 ha in area with an average depth of 1 m. The initial aqueous concentration for all simulations considering liquid formulations (i.e., Category 1 and 2 simulations), therefore, was 2.5 mg/L.

Category 1 simulations: Degradation rate comparisons

Category 1 simulations were initialized to evaluate the effects of half-life degradation rates of 0.5, 1.0, 2.0, and 4.0 days on herbicide aqueous concentrations. Outputs of these four simulations are illustrated in Figure 2. Because of degradation, herbicide concentrations in all four simulations fell below 2.5 mg/L prior to the 12-hr exposure period that our assumptions required for effective control. The simulations representing the longer three degradation rates indicate that effective control would be achieved. In each of these simulations, concentrations greater than 1.0 mg/L were maintained longer than 1 day. In the simulation for the 0.5-day half-life degradation rate, however, concentrations consistently fell below the critical level before the required exposure time, indicating that control would not be

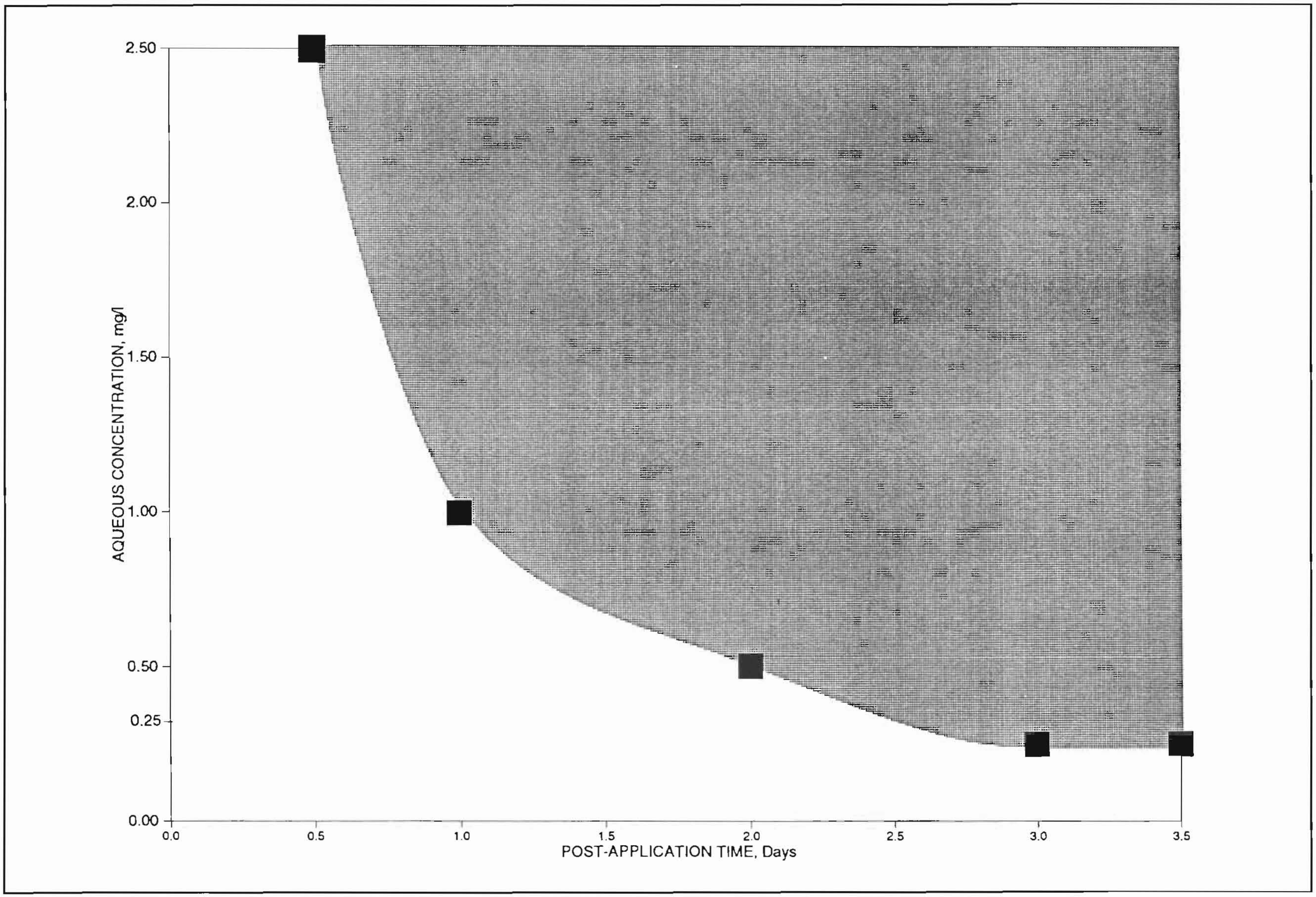

Figure 1. Herbicide concentration and exposure time relationship used for estimating plant control effectiveness in this demonstration. The zone of effective concentration and exposure time combinations are indicated by shading

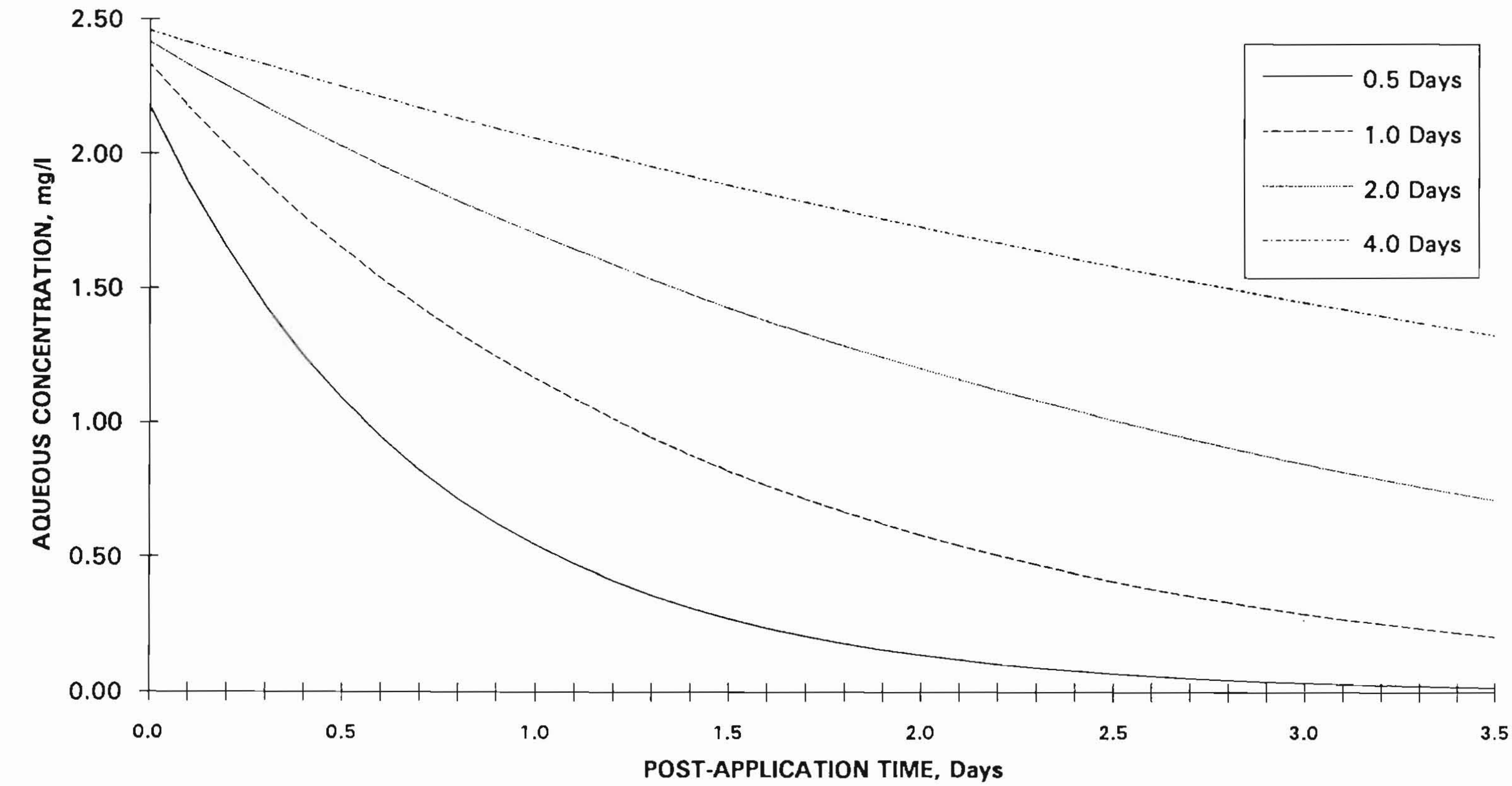

Figure 2. Water concentrations plotted over time for the four Scenario 1 simulations. Degradation rates are defined in the legend

achieved. For effective control to be attained under this degradation rate, the application rate would need to be augmented, either by adding more herbicide initially or by making additional applications on later dates. The former of these would be prohibited by our assumed label restriction on maximum water concentration. The latter may be prohibited by increased operational costs (e.g., man-hours and equipment) associated with a repeat application.

Category 2 simulations: Water exchange rate comparisons

Under real application conditions, water exchange is often responsible for failure to obtain exposures adequate for effective control. Under this scenario, simulations were initialized for application of a liquid herbicide formulation into sites with water exchange rates of 0.5, 1.0, 2.0, and 4.0 exchanges per day. Outputs shown in Figure 3 indicate that concentration and exposure time combinations needed for effective control would only be attained under the slowest water exchange rate.

Category 3 simulations: Formulation release rate considerations

Current research on development of slow-release formulations is offering promise for improving herbicide application effectiveness under moderate to high water exchange conditions. In comparison with liquid formulations, slow release formulations provide gradual release of the active ingredient over time, thereby extending the exposure period attained by a single application. To demonstrate how the HERBICIDE model can be used to evaluate this aspect of slow-release formulations, simulations were initialized for application of a 4-day half-life release rate formulation under water exchange rates of 0.5, 1.0, 2.0, and 4.0 exchanges per day. Outputs of these simulations are shown in Figure 4. In comparison to Category 2 simulations (Figure 3), peak concentrations were reduced but maintained for a longer period of time by the slow-release formulation, even though the same amount of herbicide was applied. In Figure 4, concentration and exposure time combinations required to effect control were attained under the 0.5 and 1.0 per day water exchange rates. As with Scenario 2 simulations discussed above, effective control was not indicated in Figure 4 for the higher two water exchange rates. However, since the peak concentrations were reduced, initial application rates for these simulations could have been increased sufficiently to provide required herbicide levels without exceeding the 2.5-mg/L maximum label concentration.

Summary

The HERBICIDE simulation model is being developed as a decision support software package that generates information useful for determining the effectiveness of aquatic herbicide application techniques for particular control requirements and site conditions. This paper briefly describes the use of the software package for accomplishing these objectives. For herbicide applications where degradation and water exchange rates can be accurately estimated, outputs of the type presented herein from HERBICIDE, coupled with information from established concentration/exposure time mortality relationships, will help provide guidance for selecting the proper application technique for effective aquatic plant control.

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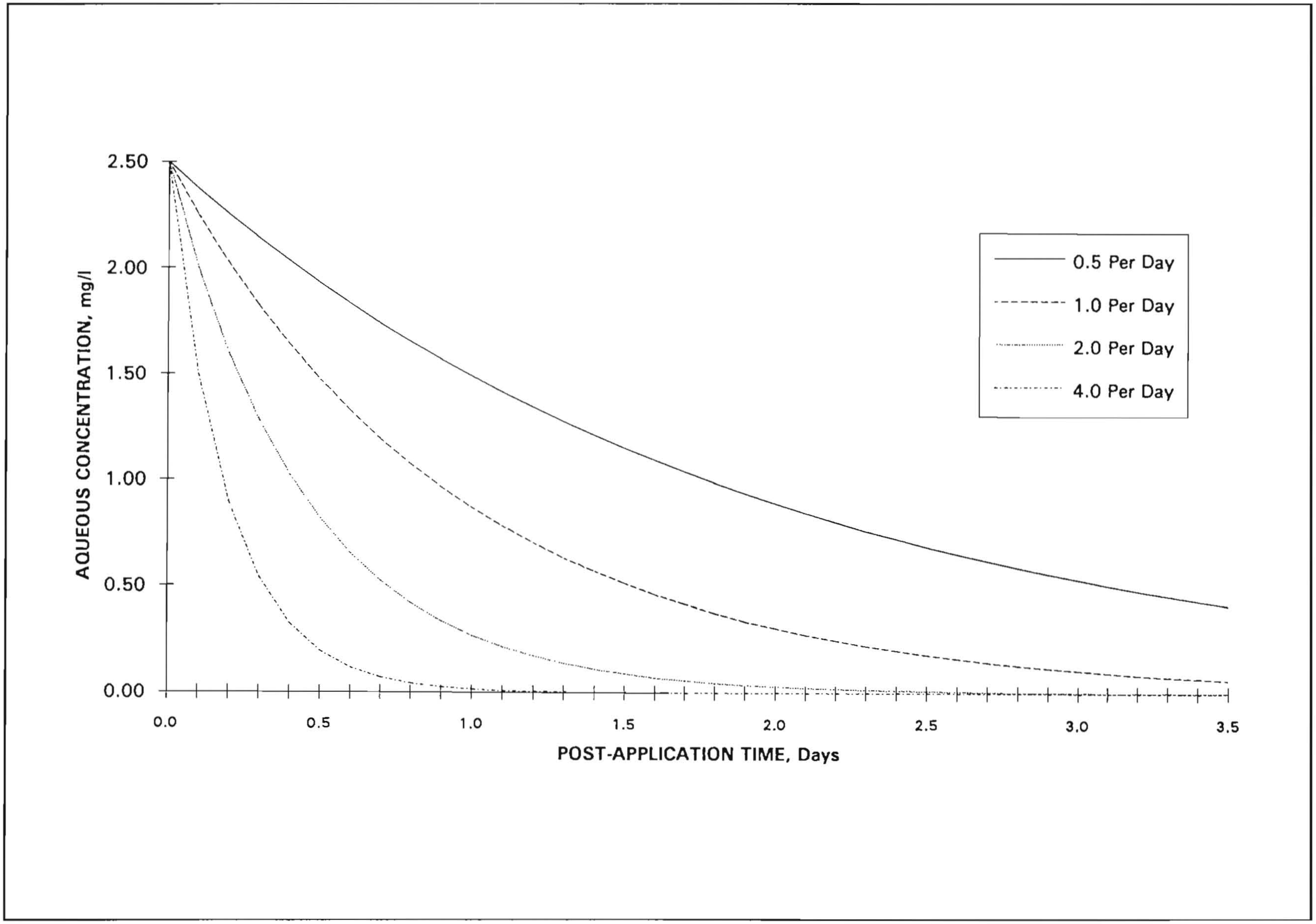

Figure 3. Water concentrations plotted over time for the four Scenario 2 simulations. Water exchange rates are defined in the legend

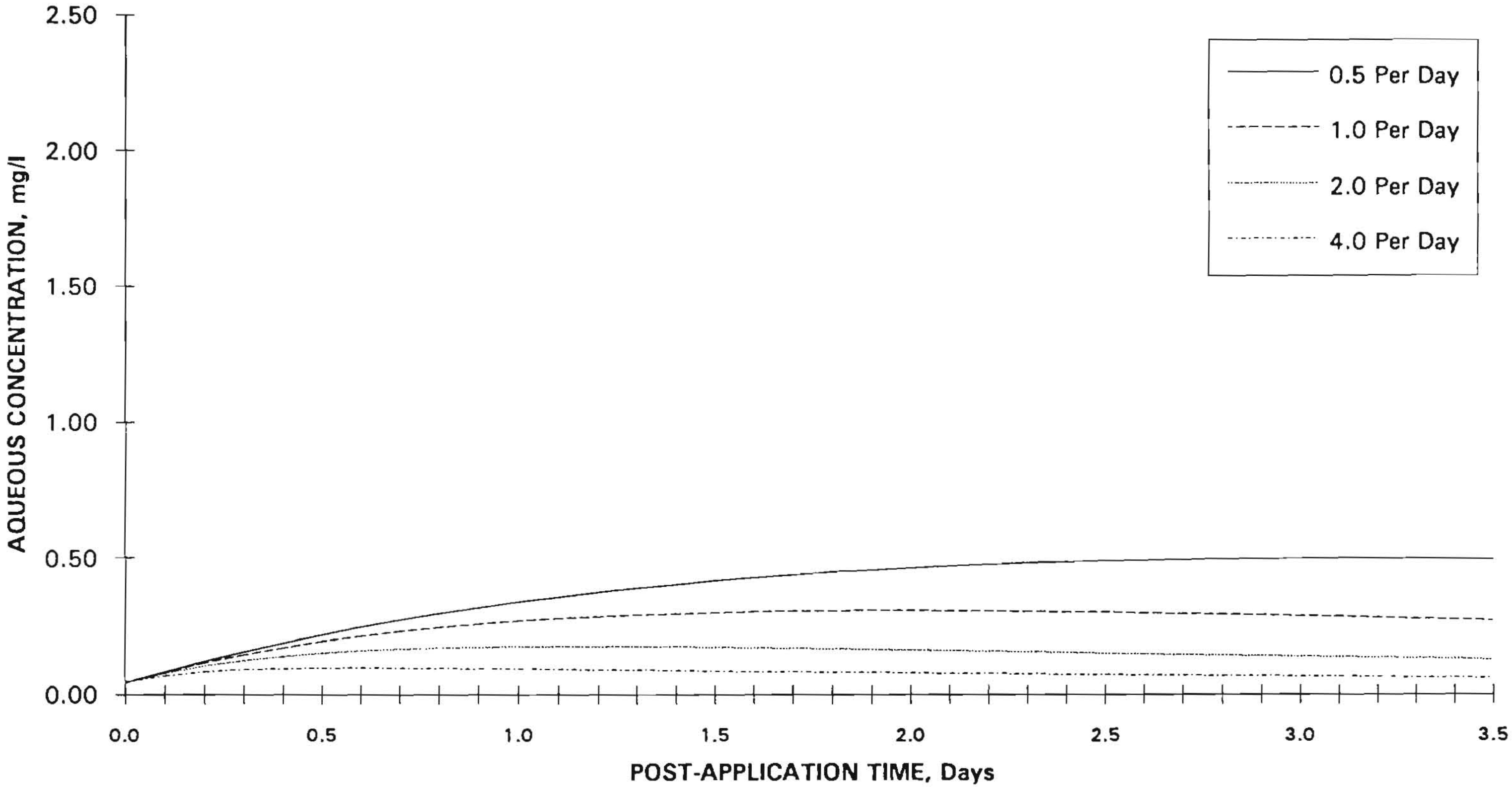

Figure 4. Water concentrations plotted over time for the four Scenario 3 simulations. Water exchange rates are defined in the legend

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The Role of Global Positioning System Technology in Aquatic Plant Control

by
Scott Bourne,[1] *M. Rose Kress,*[1] *and Tommy Berry*[1]

Photo interpretation of aerial photography and visual inspection of aquatic plants by resource managers have been the primary methods of estimating the extent of aquatic plant infestations. While both of these methods provide an acceptable technique for mapping aquatic plants, they have limitations. The acquisition and interpretation of aerial photography is accurate but time-consuming and expensive. In many cases, months are needed to obtain the photography, delineate the plant types, and determine plant acreage. Experienced aquatic plant managers can conduct visual inspections of aquatic plant beds and make acreage estimates. Realistic control program budget requests and contract specifications for chemical treatment applications depend directly on the accuracy of the infestation acreage estimates.

Global positioning system (GPS) technology provides project managers with the capability to survey and map aquatic plant infestations accurately, timely, and at a relatively low cost. The aquatic plant manager can use GPS technology for (a) initial plant mapping, (b) documentation of the locations and extent of chemical applications, and (c) for follow-up (repetitive) mapping of treated areas for monitoring the effectiveness of the treatment.

The basic components of a GPS system are (a) the GPS satellite constellation, (b) a base station receiver (optional), (c) one or more mobile receivers, and (d) software. The satellites are maintained and operated by the U.S. Government. They transmit coded signals that are collected by ground GPS receivers. The geographic (xyz) coordinates of the ground receiver are calculated by the software. These geographic coordinates are the basic output from GPS and are the cornerstone of a fast, easy, low cost, and accurate mapping capability.

The satellite constellation contains 26 operational satellites (at the time of this report). These satellites provide 24-hr continuous, all-weather geographic positioning. An optional, stationary receiver (base station) is needed to achieve the highest positional accuracy. A base station, if used, is always positioned over a known control point (benchmark). The improved positional accuracy is achieved with differential correction of GPS data. This correction is based upon the differences between the known geographic coordinates (xyz) of the base station and the calculated ranges from the satellite to the base station. Without a base station, horizontal positional accuracy (xy coordinates) is 15 to 30 m. With a base station, horizontal positional accuracy improves to 2 to 5 m.

The GPS software is used in planning the data collection process and in postprocessing the collected data. The planning software uses an almanac transmitted by the satellites and captured by the GPS receiver. This almanac provides information about the satellite locations and is used to determine optimum data collection times. The postprocessing software is used to download data from the data logger to a personal computer, perform calibrations, differential corrections, and xyz coordinate calculations, and export the GPS data for transfer to other data analysis softwares.

To effectively use GPS technology for aquatic plant control programs, special techniques and operational procedures were established. Four of these techniques are discussed below: (a) placement of GPS equipment on

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the boat, (b) optimum boat maneuvering, (c) data format and attribute assignment, and (d) export of GPS data to other software packages.

Equipment placement is an important factor in how well the GPS equipment operates and should not interfere with operation of the boat. Equipment consisted of the GPS compact dome antenna, GPS receiver and system battery, data logger, barcode wand, and barcode notebook. The antenna for the GPS unit was positioned on the boat where the signals received from the satellites were not obstructed by the operator/passengers or any objects on the boat. The best place determined to mount the antenna on an airboat was the cage that encloses the engine and propeller (Figure 1). Special care was taken to ensure that the antenna and antenna cable were secured properly. The GPS receiver and system battery were placed under the seat of the operator, out of the way, but convenient enough so the battery could be changed easily. The data logger, notebook of barcodes, and the scanning wand were placed on a removable platform mounted to the side of the boat operator. This platform allowed the operator to operate the boat without having to physically hold the GPS equipment.

To map aquatic plant stands, the airboat was navigated along the plant/water or plant/plant interface line. Figure 2 shows examples

Figure 1. GPS antennas are securely mounted on the wire cage of the airboat. To the right, the base station antenna is mounted on a tripod and elevated above building roof

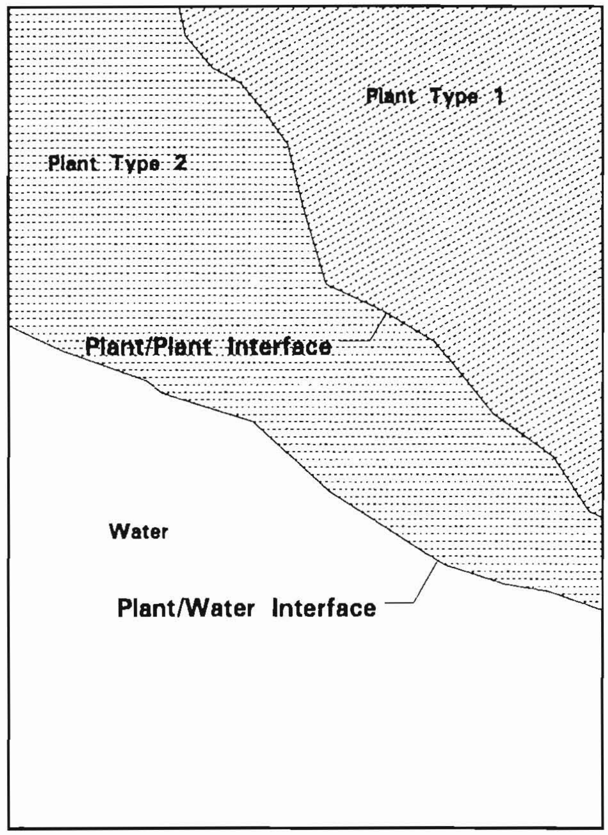

Figure 2. Boundary or interface line between plant beds and/or open water are mapped as line features with GPS

of plant/water and plant/plant interface lines. The speed of the boat and the rate of data collection was determined so that enough GPS points were recorded to adequately define the interface lines and at the same time not use a large amount of disk space on the data logger. A point recorded by the GPS unit and the attributes stored with that point occupy roughly 60 bytes of storage. If the airboat is traveling at a speed of 10 miles/hour and the GPS unit is collecting data every second, the amount of disk space needed to store points collected over a mile is 21,600 bytes. Table 1 shows the rate of data collection in relationship to the speed of the boat and how many points will be recorded each mile traveled. If separate aquatic plant beds are to be mapped and are far apart (>1 mile), the operator should turn the GPS unit off to save disk space on the data logger. The maneuvering space and the shape of the interface are key factors in selecting a proper data collection rate. Long,

Table 1
Boat Speed and GPS Data Collection Rate per Mile Traveled

Points per Minute	Boat Speed, mph				
	5	10	15	20	25
60	720	360	240	180	144
20	240	120	80	60	72
12	144	72	48	36	29
6	72	36	24	18	14
4	48	24	16	12	10

straight interface lines can be accurately defined with only a few data points. A curved or complex shaped interface requires more points for accurate delineation of the plant bed. The data collection rate is specified as the number of seconds between signal capture.

GPS data can be collected as points, lines, or polygons (enclosed areas) depending on the user's need. The best GPS data collection format for mapping aquatic plant beds for control operations was line features. Descriptive information (attributes) was assigned to the line features during active data collection with the GPS unit. For aquatic plant mapping, each interface line was assigned two attributes. The two attributes described what was on the left side and right side of the boat (line) (e.g., left side = hydrilla, right side = water). Barcode technology was used to encode and store descriptive attributes with the GPS signals. A sheet of barcodes, each representing an aquatic plant species, was carried on the boat. The operator passed the barcode wand over the appropriate barcode symbol to identify the plant type (or water) present on each side of the interface. When the plant type changed, a new barcode was selected and scanned.

For mapping, plotting, data inventory, and analysis, the corrected GPS data were transferred to a geographic information system (GIS). Figure 3 is a GIS plot of GPS data collected using a mobile receiver and base station while tracking a hydrilla treatment (sonar) application boat. The GPS data presented in Figure 3 show the path taken by the boat during application and reflects the areas of hydrilla that received some level of direct treatment. Later, this data file will be important documentation for evaluating the effectiveness of the chemical application. Two types of information used by aquatic plant managers are length of the plant/water interface line and the acreage of the plant bed. A special program was written by the U.S. Army Engineer Waterways Experiment Station to assist in transfer of GPS data to the GIS and the direct retrieval of this information from the GIS. The program assists the user to reference the GPS line features to the shoreline as defined in the GIS database, identify and edit individual plant polygons, determine the acreage of plant beds, and calculate interface lengths.

Summary

GPS technology is being used as an effective tool in aquatic plant control operations and management. With this technology, the aquatic plant manager is able to map plant distributions, transfer the data to a GIS, and accurately determine plant acreage. Also, once the data have been transferred into the GIS, the aquatic plant manager is able to generate hard copy output of aquatic plant distribution maps and characteristics. These plant distribution maps assist managers in planning and coordinating treatment applications. GPS and GIS technologies are also used to document locations and extent of chemical applications. This documentation is valuable to managers for assessing long-term treatment effectiveness and scheduling repeat applications. GPS technologies are being used by aquatic plant managers to make better decisions based on accurate information about plant distributions and chemical applications.

Acknowledgment

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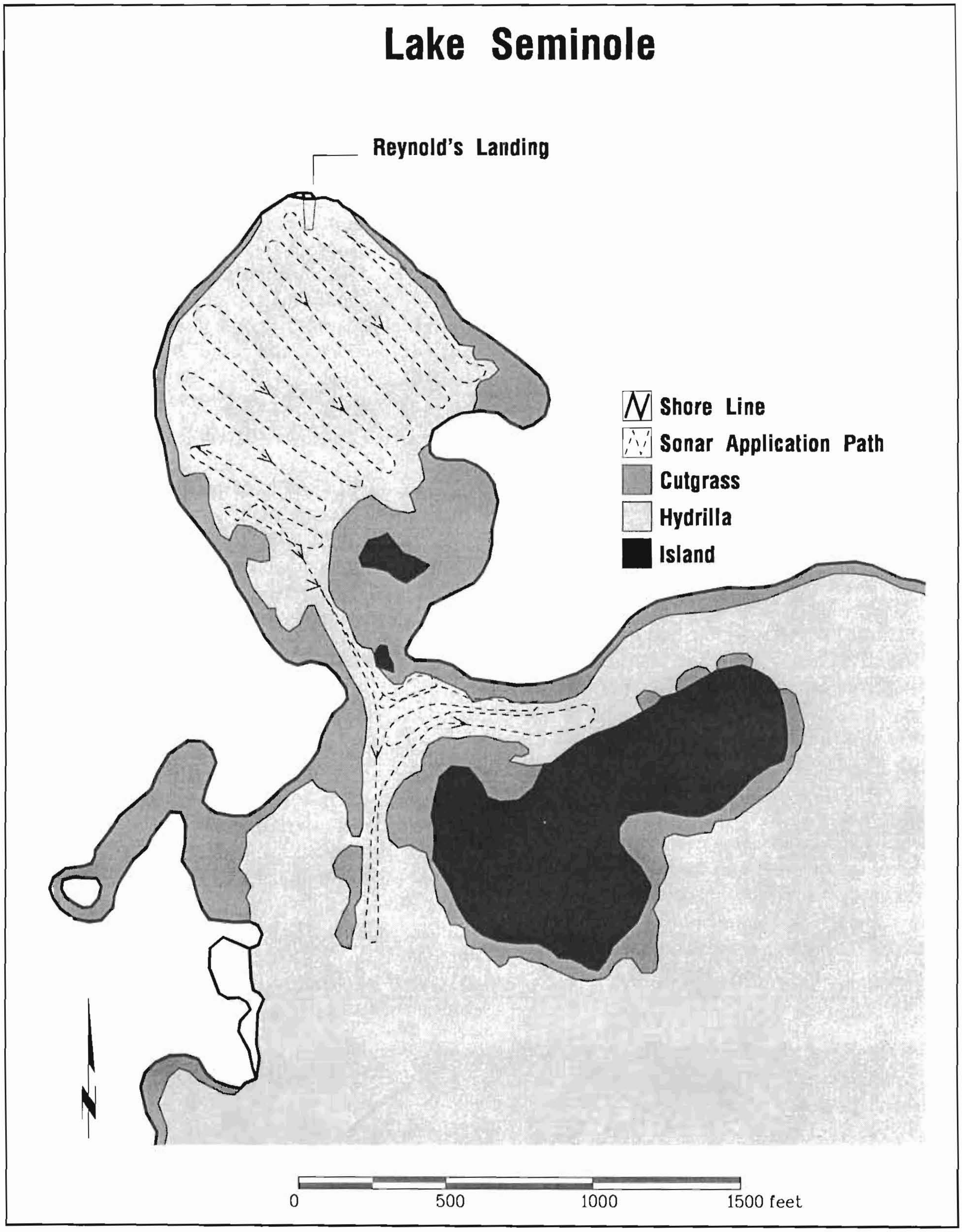

Figure 3. GIS plot of GPS data collected while tracking a chemical treatment application boat. The dashed line is path taken by application boat and thus area of direct treatment

Incorporation of Simulation Technology into an Aquatic Plant Management System

by
Richard E. Price,[1] *Craig Smith,*[1] *and R. Michael Stewart*[1]

Background on Simulation Technology Area

The Simulation Technology area was established to research and develop simulation techniques for evaluation of the effects of various aquatic plant control techniques on plant populations. The technology area had its beginnings in the late 70s with evaluation of numerical modeling approaches for use in aquatic plant control. At the 15th annual Aquatic Plant Control Research Program (APCRP) meeting in 1980, Wolsinski (1981) reported on a workshop held at the U.S. Army Engineer Waterways Experiment Station (WES) to evaluate the use of models to predict effects of various management and control strategies. A variety of mechanistic model approaches were identified to evaluate short-term effects such as chemical control techniques and long-term effects from biocontrol techniques. However, the extensive input data sets required for operation of these models were an unattractive feature for aquatic plant managers.

At the 18th annual APCRP meeting in Raleigh, NC, in 1984, Sabol (1984) presented a paper on conceptual development of methods for determining effectiveness of control techniques under the Mechanical Control Technology Development area. The idea of using simple empirical simulation techniques with minimal input data was extended to include the prediction of degree of control and effectiveness of a given technique.

By 1986, the need to integrate control techniques with management objectives was recognized. At the 20th annual APCRP meeting, Hart and Getsinger (1986) presented an assessment concept for integrated management of aquatic plants. This aquatic plant management strategy included definition of management objectives, information on control methods and effects of methods, operational constraints, and economic considerations. Defining management goals, selecting appropriate control techniques, identifying operational constraints, minimizing costs, and documenting results were key components of this strategy. This management-oriented control approach added additional evaluation requirements to those previously included in a "control only" oriented approach.

Although the need for inclusion of management considerations and integration of control approaches was recognized, research did not expand beyond development of simulation models. At the 21st annual APCRP meeting, West (1987) presented a procedure for development of aquatic plant control simulation models. He reported on a chemical control model that included dispersion of the chemical (herbicide) as a function of time and chemical half-life. Development of simulations for biological control of waterhyacinths using the Neochetina weevils was also reported. The following year, Stewart (1988) reported on simulation technology development, including plant growth simulations along with existing chemical and biocontrol simulation models. At this point, development of simulation procedures for all control techniques was underway with the ultimate goal of providing aquatic plant managers with individual evaluation procedures for each control technique.

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This approach has been effective for the development of simple process-oriented simulations that have been utilized by a number of resource managers. At the 25th annual APCRP meeting, Stewart (1991) reported on historical development of simulation procedures indicating that models developed for mechanical harvester evaluation (HARVEST) and stocking of white amur (AMUR/STOCK) have been well received with broad distribution within the Corps of Engineers, other government agencies, and private businesses. An additional effort was included in the simulation technology area involving development of aquatic plant databases to support simulation models. The following year, Stewart (1992) added evaluation and technology transfer as important functions for the development of simulation models.

The simulation technology area currently consists of four work units: plant growth models for stand-alone evaluations and for inclusion into control evaluation systems, chemical control simulations to evaluate impacts of chemical control techniques on aquatic plant infestations, biocontrol simulations to evaluate impacts of biological control techniques on aquatic plant infestations, and aquatic plant database development for use with computer simulation technologies.

Simulation Technology for Aquatic Plant Management

The need for more comprehensive plant management programs has also been recognized by aquatic plant managers. In December 1992, the Aquatic Plant Control Program Evaluation Guidance Task Force completed the Aquatic Plant Management Self-Evaluation Document describing a procedure for aquatic plant managers to assess the efficiency and effectiveness of their programs. In 1993, this Aquatic Plant Management Self-Evaluation was implemented by the South Atlantic Division on a trial basis to evaluate its use within aquatic plant management programs. A major feature of this guidance was the provision for consideration of ecological factors, current aquatic plant control technology, recent developments, and technology transfer. Although the procedure is implemented as a checklist for the manager to complete several times a year, a variety of requirements and responsibilities are listed. An aquatic plant management plan is the first requirement, including management philosophy and objectives. Other requirements include annual surveys to include mapping and assessment of problems (target species, quantities, and location) and assessment of previous control effectiveness. Technical knowledge requirements also include responsibility for reference publications and materials on control techniques.

The approach in the Simulation Technology Area has been to develop single-purpose models of plant growth and individual control techniques. These models have been nonspatial, emphasizing detailed processes and developed parallel with other APCRP technology areas (chemical control, biocontrol, and plant ecology). An aquatic plant manager may implement individual models to provide simulation information on specific chemical (herbicide) application rates to control certain aquatic plants, such as a stand of watermilfoil or develop costs associated with the use of a mechanical harvester such as the costs to control waterhyacinth in a lake. The plant manager must then assess the impacts of the control technique on both the target and nontarget plants through his personal experience, literature, and experience of others working in the field.

The single-purpose modeling approach has been very useful in the development of simulation models, but it assumes the aquatic plant manager must have the expertise, data, and experience to implement the models and interpret the output relative to his management objectives. From the background of the simulation technology area, the need for more comprehensive integration of simulation technology with plant management programs has been recognized. With a management-driven system, the aquatic plant manager could input his/her management philosophy, goals, and objectives into the Aquatic Plant Control Evaluation

System that would formulate criteria for evaluation of potential management strategies and provide guidance on a given approach for control of aquatic plants. Knowledge-based modules would organize management objectives and criteria in the database for use of assessment and simulation modules. Links between modules would allow sharing of data and display routines for consistent comparison of results from data analyses and simulation routines. With additional knowledge-based modules, the system could be operated to determine the advantages and disadvantages of a given control technique. Regulatory criteria associated with the use of some techniques could be incorporated into knowledge-based modules for rapid evaluation of effects of management approaches.

There now exists the need for integration of simulation modules with aquatic plant and control technique databases, evaluation procedures, and field techniques to provide the manager with a comprehensive aquatic plant management system. This comprehensive management system is described below.

Overview of the Aquatic Plant Management System

The Aquatic Plant Management System is a personal computer-based system for development of aquatic plant management objectives, determination of sampling and monitoring procedures, aquatic plant database operations and simulation, evaluation of control techniques, and assessment of management success. This system of knowledge-based modules, databases, and simulation modules will allow the user to taxonomically identify aquatic plants, assess the current situation relative to resource utilization requirements, determine sampling/monitoring needs, maintain an aquatic plant database and simple display routines, and conduct simulations of aquatic plant populations relative to aquatic plant control activities.

An important aspect of this system is the flexibility for the user to interact with individual modules of the system directly. Through a windows-based approach, the manager may conduct simulations or database operations without interacting with the other modules. This would allow the user to conduct an evaluation of a chemical control application on a stand of milfoil. If the user wishes to determine the best control approach consistent with his management objectives, the system will conduct simulations with all control approaches requested and rank them according to user-defined evaluation criteria, such as costs, longevity, effectiveness, or potential impacts on nontarget species. In a windows environment, criteria for aquatic plant management will be available to other modules, as well as data from the database. This will also allow consistent update of routines as new information is developed or routines are revised to reflect changes in the technology.

The system will be composed of five major components or modules, which are described in the following sections. Although the modules are linked for integration of technologies, the user may access any of the five modules directly to address specific concerns.

Management Strategy Planner

This module is the primary module for description of aquatic plant setting and linking of management objectives with other modules. Within this module, a knowledge-based routine will evaluate current setting or situation through specific questions to the user; it will then identify management objectives and record management goals, in a qualitative manner, and ask for criteria for evaluation of management objectives. This will result in a referral to other modules for further evaluation or a recommendation of no further action. If further action is needed, it will then determine the need for data collection or monitoring programs and proceed to the database module to establish database formats.

An assessment module will be included that is a knowledge-based tool incorporating output from the situation module and databases for referral to a simulation technique. This module will evaluate changes in spatial and temporal scales and determine which simulation/model is most appropriate. Criteria from the situation module is used to predict success from the application of a given control method. This module also allows the user to determine the mode of operation for simulation/ models, either directly through specification of which simulations/models to implement or allowing the system to determine the best simulation/model approach for the given situation. This module also provides user information on control approach for cost, longevity, degree of success, implementation information, and secondary impacts of a given technique.

Field Techniques Toolbox

The Field Techniques Tool Box includes a knowledge-based system for identification of proper field sample techniques based on initial criteria and management objectives defined by the user. It will then define the type and frequency of samples, provide a statistical design, and provide guidance on quality control of field and laboratory analytical techniques. Knowledge-based systems developed for plant identification keys are also included in this package.

This module requires site data and descriptions, vegetation data, aquatic plant concerns, objectives and information from the management strategy planner, and any information on ongoing sampling, monitoring, or treatment programs. It will provide appropriate sample techniques (such as the need for global positioning systems, remote sensing, or field surveys) or design and provide guidance on monitoring frequency and location.

Aquatic Plant Database

The Aquatic Plant Database module will provide data storage for the aquatic plant manager. It will include simple data analysis routines for simple statistical analysis and a display package for simple plotting of data. This will allow the user to input plant survey data and compare graphically or statistically against data sets from previous years or other reservoirs with similar situations. As geographical information systems (GIS) for aquatic plant management develop, links to external GIS packages will be included. Databases from experimental and demonstration programs conducted by WES and other agencies will be available for inclusion at the users discretion.

Data analysis routines will include simple statistical analysis (means, range, regression, and significance tests) and plot capability (scatter plots, bar graphs, etc.).

Simulation/Model Toolbox

This module consists of a user interface for simulation models as well as routines for model access to data from the database module. Models of water flow, plant growth, chemical control, white amur, insect, mechanical approaches, and plant succession reside as submodules in this module. Initially, output from the simulations will be displayed in numerical or graphical form. As GIS techniques develop, links to a GIS through the database module will allow spacial considerations.

This toolbox is subdivided into three major areas: growth models, control technique models, and macrophyte effects models. The growth models will simulate growth and expansion of macrophyte beds in the absence of control techniques. The control techniques models will simulate the effects of control techniques on the target plants and the degree of control. Macrophyte effects models will predict the effects of control activities on fish/invertebrate habitat and economic considerations.

Control Techniques Database

This module consolidates narrative and numerical data on chemicals for control application, information on mechanical harvesters, information and data on biocontrol organisms, status of various control techniques, case histories of actual field applications and simulations, and a literature reference database with abstracts. Using a knowledge-base approach, it will provide the user with technology and case histories on operational approaches, effectiveness of control techniques, regulatory information, compatibility of data with management approaches, and costs of various techniques. This module would allow the user to prepare justifications for control approaches.

Summary

The Simulation Technology Area in the APCRP has developed a number of single purpose simulation/model tools to evaluate aquatic plant control strategies. The need now exists for incorporation of the Simulation Technology Area into a broader based Aquatic Plant Control Evaluation System. This simulation/model system would include modules for evaluation of control strategies, sample techniques, plant database and references, as well as simulation/model routines. With the development of this system, aquatic plant managers would have a single simulation/model system for evaluation of control approaches using user defined criteria and objectives.

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Ecology of Aquatic Plants

Overview of Advances in Aquatic Macrophyte Ecology

by
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Introduction

Research within the ecological technology area of the Aquatic Plant Control Research Program (APCRP) is directed largely towards determining the response of submersed aquatic macrophytes to environmental conditions. A variety of environmental factors including light, water temperature, nutrients, and sediment composition interact in affecting the productivity, distribution, and species composition of submersed macrophyte communities. Complex interactions among these factors and submersed macrophyte growth are currently being addressed. Additional efforts focus on mechanisms whereby submersed macrophyte communities influence hydrodynamic conditions. Virtually all of the knowledge accumulated to date, based on ecological investigations in the APCRP, is being focused on newly initiated investigations of factors contributing to invasions and declines of submersed macrophyte populations.

The purpose of this article is to provide an overview of recent advances in aquatic macrophyte ecology, based on research activities in the APCRP. This article highlights results of current research activities reported in greater detail elsewhere in this volume.

Light, Temperature, and Competition

Light is important in determining macrophyte morphology and distribution (with latitude, season, and depth), thereby influencing productivity and species composition as well. Differences in the morphological and/or physiological adaptability of submersed macrophyte species to various conditions of irradiance may account for the greater competitive ability of some species compared with others in aquatic systems. In this connection, species capable of concentrating photoreceptive biomass at or near the water surface in low-irradiance environments are able to competitively displace species possessing relatively prostrate growth forms (Smart 1994). For example, *Vallisneria americana* appears to be disadvantaged in aquatic systems characterized by low water clarity, because of its limited elongation potential and high light requirements for photosynthesis (Kimber 1994). Conversely, *Egeria densa, Hydrilla verticillata, Myriophyllum spicatum*, and *Potamogeton americanus* possess the ability to form a foliar canopy at the water surface and, thus, have access to a high light environment.

Most submersed macrophyte species demonstrate increased growth with increasing water temperature up to about 28 °C. Lower temperatures effectively diminish the growth capacity of most submersed macrophytes. However, at temperatures greater than 28 °C, production of reproductive structures (e.g., tubers) can be diminished significantly (McFarland and Barko 1994). Considering the distribution of submersed macrophytes in North America, differences in water temperature regimes in combination with basic differences in life cycle probably account for some of the variations in the latitudinal range of macrophyte species.

The potential for aquatic systems to support excessive submersed macrophyte growth generally increases from north to south in the United States because of increasingly favorable temperatures. However, a notable exception to this generalization is *Myriophyllum spicatum*, which initiates growth at relatively

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cool water temperatures. For species capable of accessing the water surface, conditions there of both relatively high light and high temperature provide an optimal environment for growth even early and late in the growing season. For this reason, macrophyte species that effectively concentrate biomass at the water surface (e.g., *Myriophyllum spicatum* and *Hydrilla verticillata*) are potentially more productive than other species restricted to lower positions in the water column.

Nutrition and Sediment Composition

From research conducted in this laboratory and elsewhere, it is now generally accepted that rooted submersed macrophytes obtain nitrogen, phosphorus, and micronutrients primarily by direct uptake from sediments (Table 1). The role of sediment as a direct source of these elements for submersed macrophytes is ecologically significant, since their availability is normally very low in the open water of aquatic systems. Considering the usual abundance and conservative nature of other major elements in the open water of most aquatic systems, it is unlikely that low concentrations of these directly limit growth of submersed macrophytes.

Table 1
Primary Sources of Nutrient Uptake by Submersed Aquatic Macrophytes

Nutrient(s)	Source
Nitrogen	Sediment
Phosphorus	Sediment
Iron	Sediment
Manganese	Sediment
Micronutrients	Sediment
Calcium	Open water
Magnesium	Open water
Sodium	Open water
Potassium	Open water
Sulfate	Open water
Chloride	Open water

Sediment composition affects nutrition and thereby has a pronounced influence on the growth of submersed macrophytes. In general, growth is relatively poor on both highly organic sediments and on sands compared with fine-textured inorganic sediments. Poor growth on sands is caused by low sediment fertility and on organic sediments by low sediment density. High concentrations of organic matter in sediments negatively affect the growth of submersed macrophytes by reducing the availability of essential nutrients (most notably N and P) as a function of low sediment density.

Mechanisms of growth regulation on sand and organic sediments are similar, since both involve nutrition. The physical and chemical characteristics of bottom sediment, which influence macrophyte nutrition, are clearly affected by sedimentation patterns. Thus macrophyte nutritional relationships need to be addressed through studies of sedimentation.

At moderate rates of sedimentation, sediment deposits can provide a nutritionally favorable environment for the growth of submersed macrophytes. This process refurbishes nutrients lost because of root uptake or diffusional processes and provides new substratum potentially available for macrophyte expansion. As demonstrated in APCRP investigations conducted both on Eau Galle Reservoir and the Potomac River (see articles by James and Barko, past APCRP Proceedings), macrophyte communities themselves can significantly influence both the rate and nature of sedimentation.

Convective Hydraulic Circulation

On a daily basis, shallow nearshore regions of aquatic systems typically heat and cool more rapidly than deep open-water regions, primarily because of differences in mixed volume (James and Barko 1994; Schneider1994). The presence of submersed macrophytes in shallow regions contributes to the development of thermal gradients in both the vertical and lateral planes, since foliage near the water surface converts solar irradiance to heat. Thermal gradients give rise to density gradients that promote hydraulic circulation.

Implications of hydraulic circulation driven by convection are potentially far-reaching, since dissolved constituents can be moved with water. Dissolved constituents may include nutrients, contaminants, or herbicides. In the case of nutrients, hydraulic transport from the littoral zone can contribute significantly to pelagic nutrient budgets. With herbicides, information on the periodicity of hydraulic transport can be of enormous value in maximizing both the efficiency and effectiveness of treatment applications (see articles in Chemical Control Technology section, this volume).

In Eau Galle Reservoir in Wisconsin and Guntersville Reservoir in Alabama, dye studies have been conducted for several years in combination with close-interval thermal monitoring in an attempt to evaluate the seasonal dynamics of convective circulation. Because of the eutrophic nature of these impoundments, studies have focused primarily on phosphorus transport. However, the results obtained from these reservoir studies apply to all dissolved constituents. These results indicate the potential significance of shallow water macrophyte beds in affecting chemical budgets in aquatic systems.

Macrophyte Invasions and Declines

Declines of submersed aquatic macrophyte communities, involving a variety of different species, have been reported worldwide. For example, *Vallisneria americana* declined rather abruptly in several pools of the Upper Mississippi River following a prolonged period of drought in the late 1980s . Notably, this particular decline was paralleled by declines of other species in other major river systems of the United States. The contemporaneous nature of these declines suggests possible climatic effects, perhaps involving reproductive failure. However, the exact reasons for submersed aquatic macrophyte declines following the drought remain unknown. Factors proposed as contributing to declines are many: reduced irradiance at leaf surfaces, nutrient depletion, parasites and pathogens, toxin accumulation, damage by fish, insect herbivory, climatic fluctuations, competition, and others.

Invasions by exotic species have been associated with declines of native species. Thus, invasions and declines may in some cases be interconnected events. In general, factors contributing to invasion success are essentially unknown. However, in some instances invasions appear to be linked with environmental disturbances. Although the factors or suites of factors (biotic or abiotic) that actually control submersed aquatic macrophyte invasions or declines are not clear, many opinions have been forwarded. Through efforts in the APCRP, these opinions have been documented recently for North America and are currently the subject of detailed regional investigations. Studies have been initiated to better identify environmental factors associated with naturally occurring invasions and declines and to evaluate the potential for manipulating natural processes to either minimize invasion success or hasten natural declines (Smith 1994).

Acknowledgments

Thanks to Dr. 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 of the APCRP.

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Interrelationships Between Sediment Composition and Water Quality Conditions Affecting the Reestablishment of *Vallisneria*

by
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Introduction

The Upper Mississippi River, with its extensive shallow backwaters, side channels, and lake-like impoundments, supports several species of submersed aquatic macrophytes. However, declines in this important resource, notably *Vallisneria americana* Michaux, occurred in Lake Onalaska (Pool 7) and other portions of the river between 1988 and 1991. Although evidence is generally lacking to explain the factors involved in the declines, a 3-year drought occurred during the same time period. This concomitant dry spell was characterized by near record-low rainfalls that caused unusually low water levels (Riebsame, Changnon, and Karl 1991). Consequently, drought-related conditions have been suspected as leading factors influencing the declines.

Although the drought ended in 1989, *Vallisneria* has not returned to its former abundance in Lake Onalaska. Because of the ecological importance of *Vallisneria* in this portion of the river (Korschgen and Green 1988), the potential for this species to become reestablished needs to be evaluated.

In an effort to determine if *Vallisneria* could grow in areas where it had previously declined, we planted tubers of *Vallisneria americana* into selected areas of Lake Onalaska in 1992. The study was designed in close association with a greenhouse study designed to specifically evaluate whether water quality and sediments in Lake Onalaska would support *Vallisneria* reestablishment (McFarland and Barko 1993). Conclusions from the field study are presented in this article.

Study Location

The study was conducted in Lake Onalaska (Pool 7) of the Upper Mississippi River System (UMRS) (Figure 1). The 2,835-ha lake is shallow (mean depth = 1.3 m) and has supported aquatic vegetation since it was formed by impoundment in 1937 (Green 1960). *Vallisneria* was reported to be common by 1960 (Green 1960), and at its peak in the mid-1980s, this species dominated the >1,200 ha of submersed vegetation within the lake.[3] Following the drought in 1989, however, <121 ha of submersed vegetation were estimated to remain.[4]

Methods

Two sites were selected in regions of the lake where *Vallisneria* had occurred previously (Figure 1). Site 1 (protected), located in the southeastern corner of the lake, was protected from prevailing summer winds by nearby south and southwest shorelines. 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 sediments at Site 1 consisted of predominately fine-textured materials and at Site 2, mostly sand (see McFarland and Barko (1993) for sediment data). Depths at both sites averaged 1 m.

1 U.S. Fish and Wildlife Service, Environmental Management Technical Center, Onalaska, WI.
2 U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS.
3 Unpublished Manuscript, C. Korschgen et al., Northern Prairie Research Center, La Crosse, WI.
4 Personal Communication, 1989, C. Korschgen, Northern Prairie Research Center, La Crosse, WI.

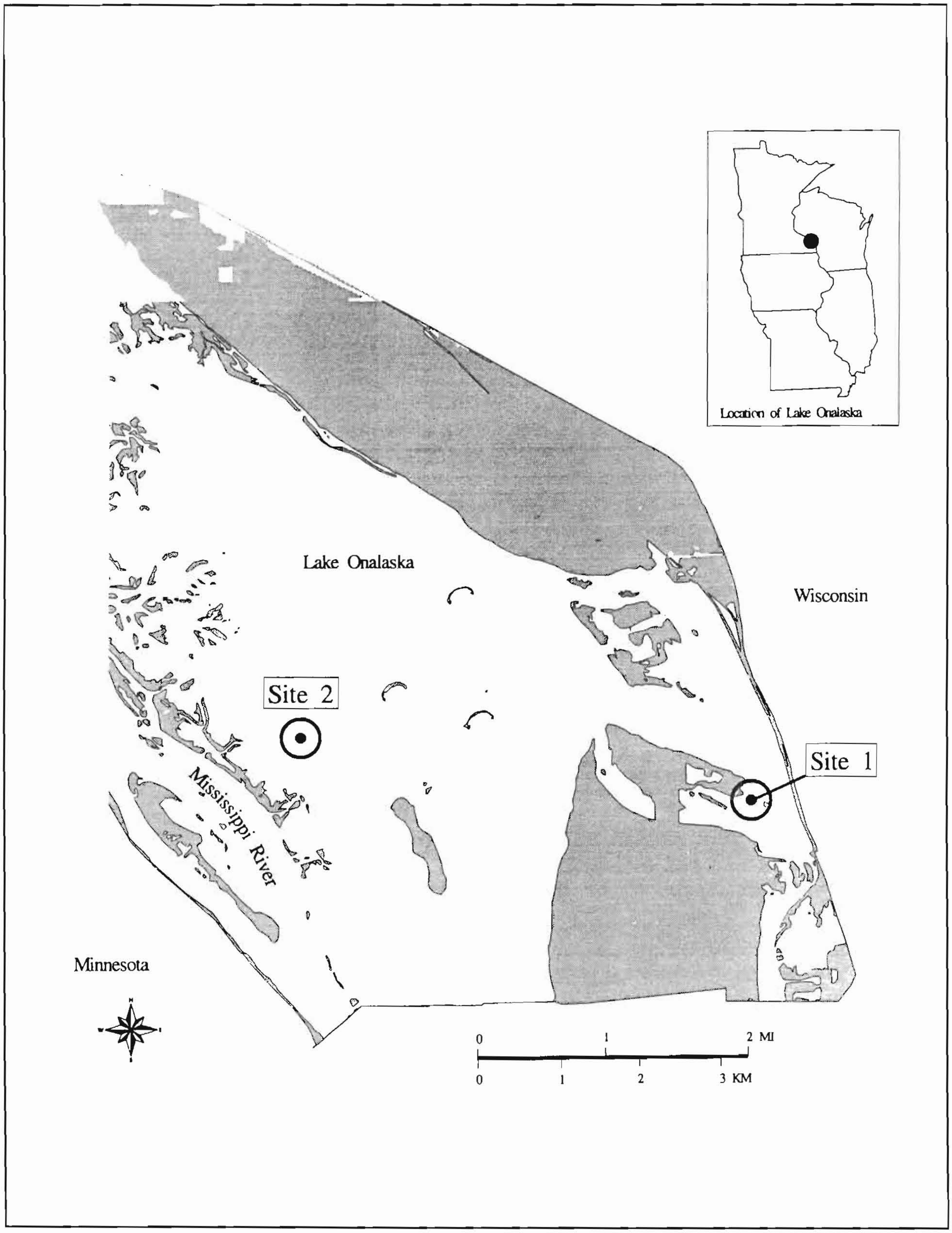


Figure 1. Location of transplant sites in Lake Onalaska, 1992

Vallisneria tubers were collected from Pool 4 of the UMRS during mid-April of 1992. A water-pumped dredge was used to dislodge the overwintering tubers. Tubers were stored at approximately 4 °C until planting. In early May, the tubers were planted about 5 cm deep by a scuba diver in eight 1-m^2 plots selected randomly at each site. Each plot was delineated by a frame placed on the sediment surface and anchored with attached legs. A 1-m^2 planting grid divided the plots into thirty-six 15- by 15-cm cells. Two tubers were planted per cell in the sediment using the grid as a temporary guide.

Surface water temperature and Secchi depth were determined approximately weekly. Light attenuation was determined at 10-cm intervals through the water column by simultaneous measurement of underwater photosynthetically active radiation with LI-COR quantum sensors (LI-COR, Inc., Lincoln, NE). Measurements were based on an average of five readings. Attenuation coefficients were calculated using the Lambert-Beer equation:

$$I_z = I_o e^{-kz}$$

where

I_o = average light intensity at a given depth

I_z = average irradiance at depth z 0.15 m below

k = vertical attenuation coefficient (m^{-1}).

The 10-percent light depth (Z_{10}) was calculated from the attenuation coefficient (Z_{10} = 2.3/k).

Depth-integrated samples of the water column were collected weekly for seston and chlorophyll-*a* determinations. Water collected with a polyvinyl chloride pipe lowered vertically through the water column was mixed in a bucket and subsampled for analyses. Samples for chlorophyll-*a* were filtered using glass-fiber filters (type A/E 47-mm diam). Chlorophyll-*a* and phaeophytin concentrations were determined spectrophotometrically following acetone extraction (APHA et al. 1985). Samples for seston concentrations were vacuum-filtered through tared 47-mm glass-fiber filters, dried at 105 °C, and reweighed to obtain total seston mass. Organic fractions of seston mass were determined following combustion at 550 °C in a muffle furnace for 24 hr using a modification of procedures of Allen et al. (1974).

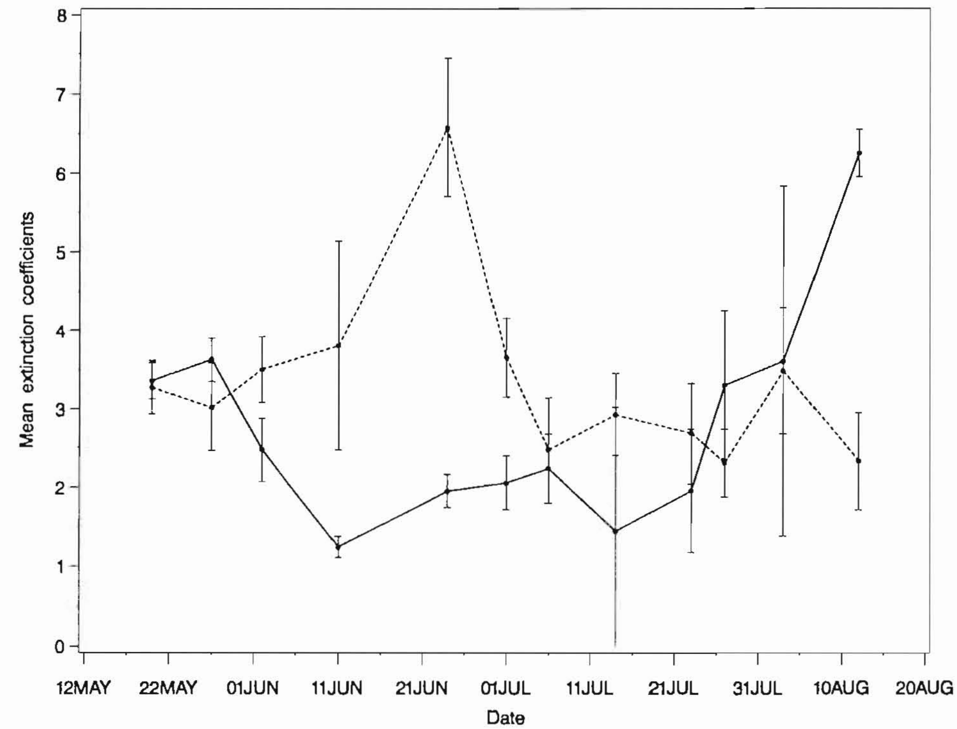
Vallisneria was harvested by hand cutting at the sediment surface in mid-August from four randomly selected plots at each site. The plants were rinsed of loose epiphytic materials, measured for morphological characteristics, and then oven-dried (80 °C) to a constant mass. Plant growth was evaluated through determinations of oven-dry biomass, leaf lengths, number of rosettes, and the number of flowers per plot. Leaf length measurements included average leaf length and maximum leaf length per plot.

Data were analyzed using the Statistical Analysis System (Raleigh, NC). Nonparametric (Wilcoxon Rank Sum) tests were used for comparison of plant growth and water quality data between the two sites. Statistical significance is reported at the 5-percent probability level.

Results

Water quality

Seasonal means for attenuation coefficients were not significantly different between the two sites over the entire period, averaging 3.5 at Site 1 and 3.1 at Site 2. However, for most of June and July, attenuation coefficients were significantly lower, and 10-percent light depths were deeper at Site 1 (Figure 2). Chlorophyll-*a* and seston concentrations were similar at both sites throughout the season except for the month of June, when concentrations at Site 2 were frequently higher, reflecting onset of phytoplankton blooms within the main body of the lake (Figure 3). Additionally, seston concentrations were higher at Site 2 when strong winds (>15 mph) were blowing from the west or northwest. Seston and chlorophyll-*a* concentrations at Site 2 and chlorophyll-*a*



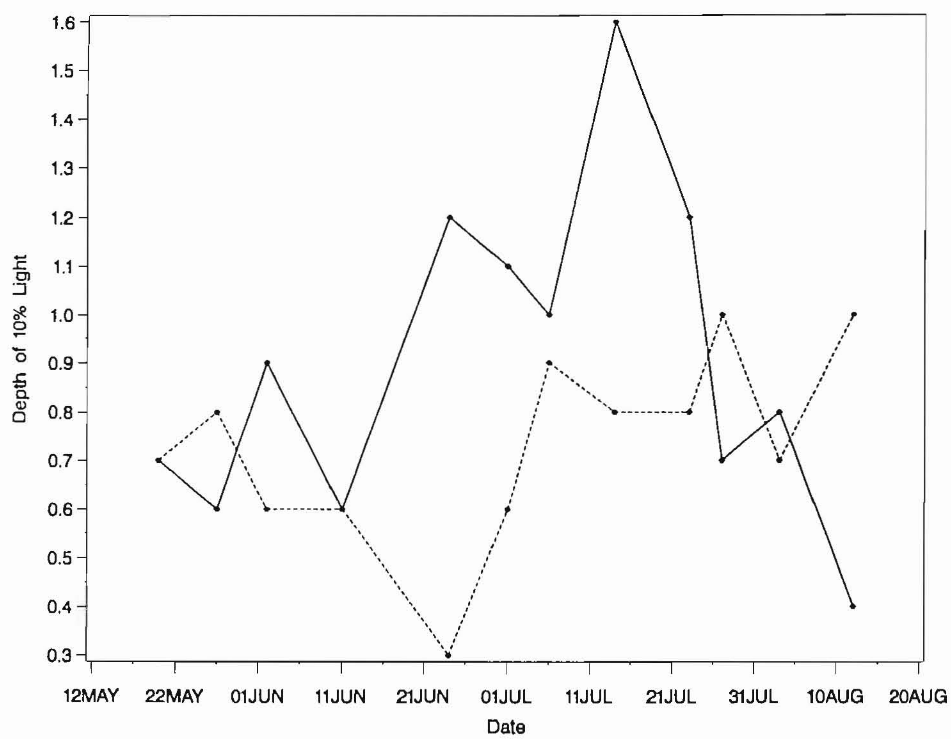


Figure 2. Mean light attenuation coefficients and 10-percent depth of light penetration at Site 1 and Site 2, Lake Onalaska, 1992 (Site 1 —— Site 2 – – –)

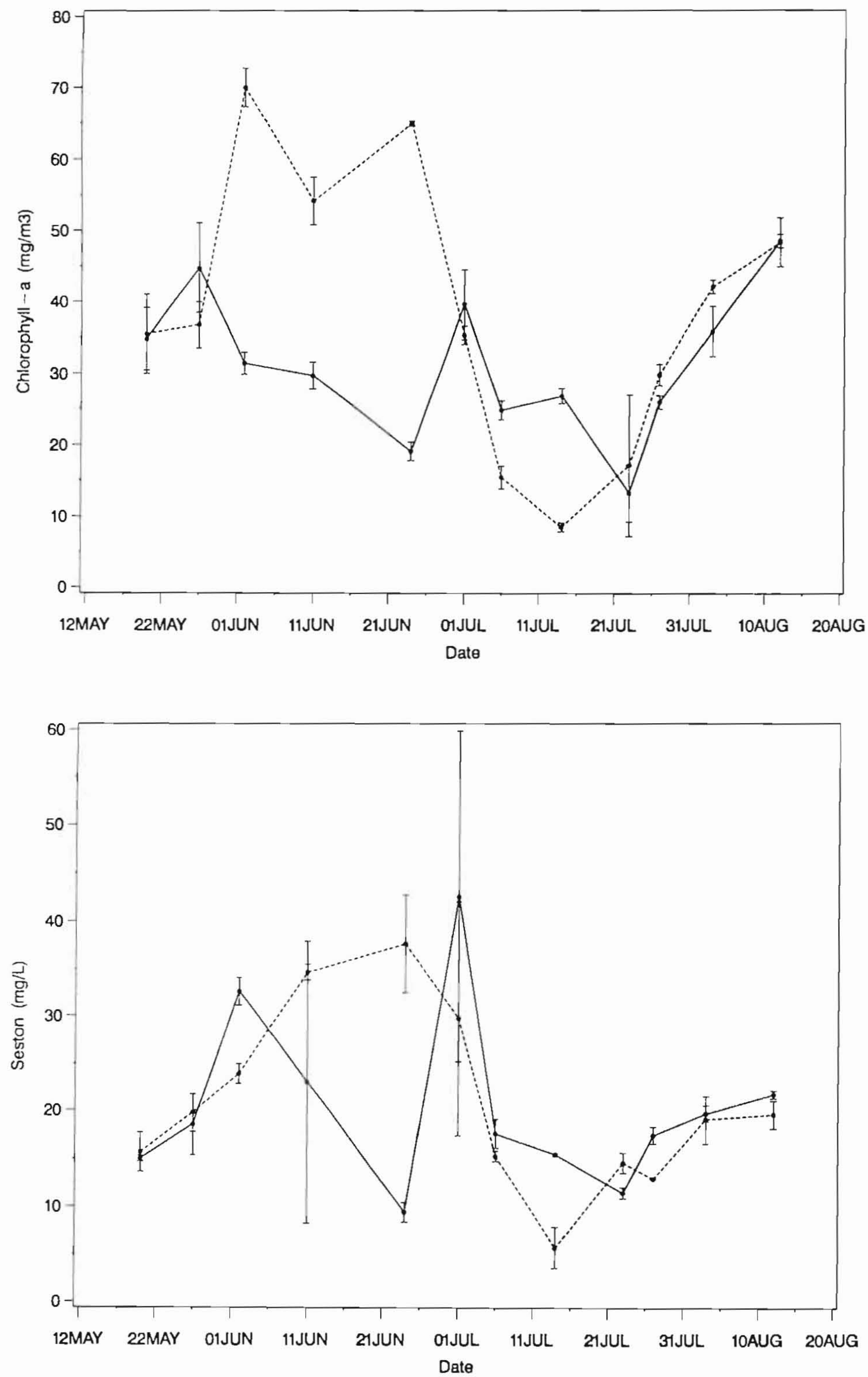


Figure 3. Mean chlorophyll-a and seston concentrations at Site 1 and Site 2 in Lake Onalaska, 1992 (Site 1 —— Site 2 - - -)

concentrations at Site 1 were positively correlated with light attenuation coefficients and negatively correlated with 10-percent light depths (Table 1).

Table 1
Pearson Correlations Among Light Measurements, Chlorophyll-*a*, and Seston at Sites 1 and 2, Lake Onalaska, 1992

Light Measurement	Chlorophyll-*a*		Seston	
	Site 1	Site 2	Site 1	Site 2
Attenuation coefficient	0.672 (0.016)	0.594 (0.041)	n.s.	0.755 (0.004)
10% light depth	-0.622 (0.010)	-0.589 (0.043)	n.s.	-0.760 (0.004)

Note: Propability levels are shown in parentheses.

Secchi depths were somewhat greater at Site 1, with a seasonal average of 53 cm compared with 45 cm at Site 2 (Figure 4). Secchi depths were negatively correlated with seston ($r = -0.661$; $p = 0.019$) and chlorophyll-*a* ($r = -0.623$ $p = 0.030$) at Site 2, but were not correlated with either of these factors at Site 1. Temperatures ranged from 16 to 27 °C during the study, averaging 21.7 °C at Site 1 and 20.9 °C at Site 2 (Figure 4). Seasonal means for temperatures were not significantly different between the two sites.

Plant growth

There was no significant difference in aboveground biomass between the two sites, although average biomass at Site 1 was somewhat more variable than biomass at Site 2. No significant differences were found between the two sites in the number of plants that reached leaf lengths >20 cm or the number of plants <20 cm. While average leaf lengths were similar between the two sites, leaf lengths were more variable at Site 2, ranging from 80 to 121 cm compared with 89 to 103 cm at Site 1. Maximum leaf lengths of *Vallisneria* were significantly different between the two sites, averaging about 26 percent longer at Site 2 than Site 1 (Table 2).

At both sites, *Vallisneria* plants >20 cm in length outnumbered plants <20 cm long by at least 12 to 1. About 20 percent of the plants >20 cm produced flowers. There was no significant difference in the production of male or female flowers between the sites (Table 2).

Table 2
Morphological Measurements of *Vallisneria americana* Michaux Plants from Transplant Sites in Lake Onalaska, 1992

Growth Characteristics	Site 1	Site 2
Aboveground biomass, g	88.2 (16.7)	123.4 (7.7)
Number of plants >20 cm	177.2 (26.1)	199.7 (18.3)
Number of plants <20 cm	10.7 (3.1)	7.0 (2.7)
Average leaf length, cm	96.0 (2.8)	98.0 (8.5)
Maximum leaf length, cm	121.5 (1.1)	154.0 (10.4)
Number of male flowers	64.0 (17.0)	59.2 (3.1)
Number of female flowers	66.5 (9.1)	50.5 (11.3)

Note: Means are given with standard errors in parentheses.

Discussion

Many factors affect the productivity of submersed macrophytes; among the most important are light, water temperature, sediment composition, and the availability of sediment nutrients (Barko and Smart 1986). In this field study, the growth response of *Vallisneria* to 1992 conditions in Lake Onalaska indicates suitable temperature and sediment conditions, adequate light, and sustained availability of inorganic nutrients during the growing season. Moreover, differences in ambient light and sediment conditions at the two sites did not affect most aspects of *Vallisneria* growth.

The only significant difference in plant growth between the two sites was in maximum leaf length, which was greater at Site 2. We suggest that greater maximum leaf length at Site 2 was most likely a response by *Vallisneria* to differences in ambient light levels between the two sites, particularly during June and July when 10-percent light depths were significantly less at Site 2 than at Site 1. This suggestion is supported by results reported for *Vallisneria* in a laboratory experiment by Barko, Hardin, and Matthews (1982). In that investigation, shoot length of *Vallisneria*

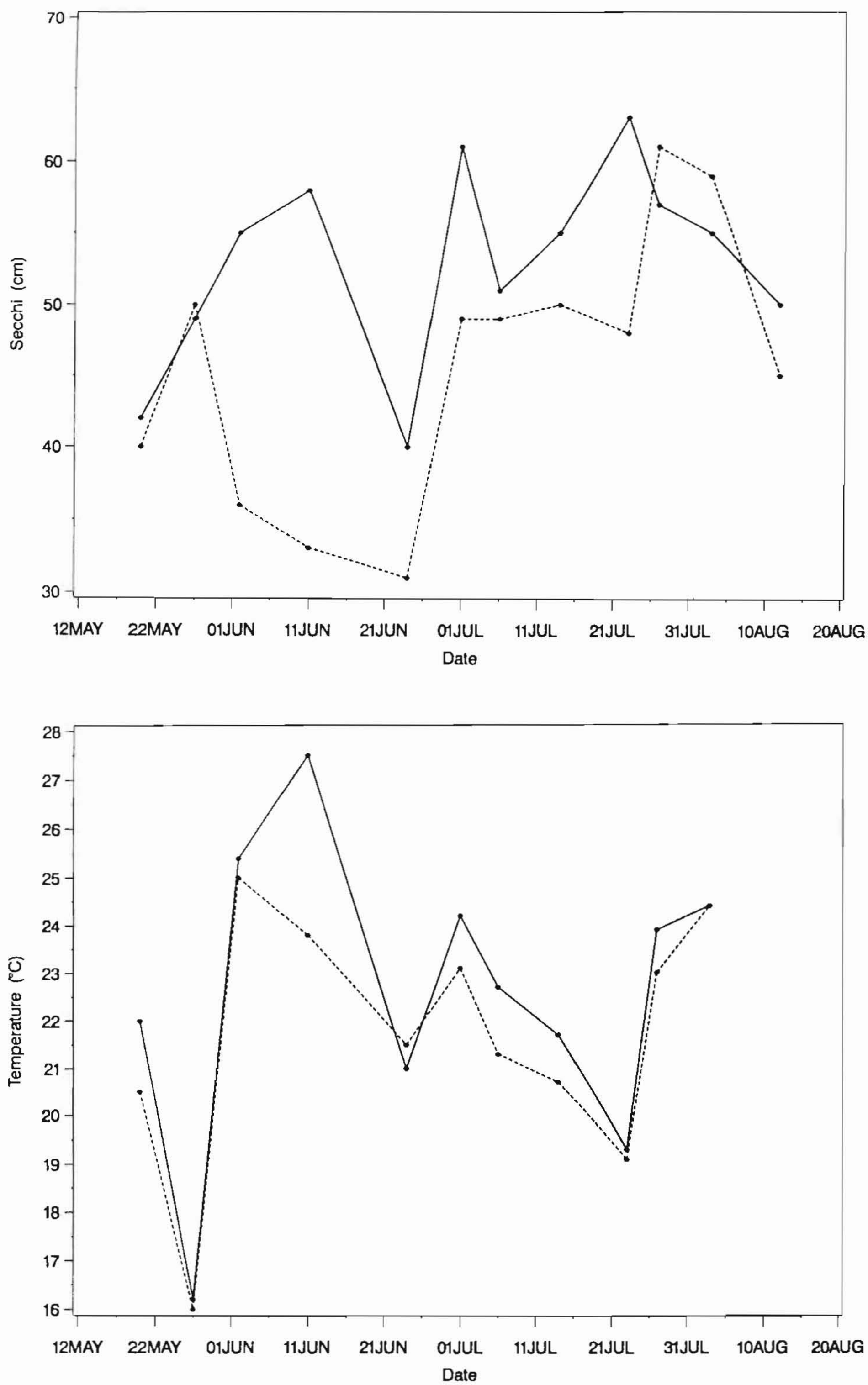


Figure 4. Secchi disk transparency and water temperature at Site 1 and Site 2 in Lake Onalaska, 1992 (Site 1 —— Site 2 - - -)

increased with decreasing irradiance at temperatures between 20 and 32 °C. Similar morphological responses have been described for other macrophytes (Barko and Smart 1981). Goldsborough and Kemp (1988) suggest that even small increases in leaf lengths may yield increases in available light, especially in turbid systems. The ability to increase leaf length to adjust to diminished light availability is likely critical to the survival of *Vallisneria* in the shallow, turbid waters of the UMRS. In addition, morphological adaptability would increase the potential for *Vallisneria* to expand its range into a variety of habitats with differing light conditions.

In an associated greenhouse study on sediments from the same field study sites, *Vallisneria* growth was shown to be limited by low N availability (McFarland and Barko 1993). These contrasting results suggest that throughout the 1992 growing season, N may have been provided to *Vallisneria* in situ through processes such as sediment transport and accretion. Replenishment of N via sedimentation may balance sediment nutrient losses because of diffusion or macrophyte uptake (Barko et al. 1988). Thus, accretion of N as it affects N availability in surficial sediments needs to be considered in evaluating the reestablishment success of *Vallisneria*. Accordingly, we hypothesize that during periods of drought, as in 1987-1989 for example, nutrient availability to aquatic macrophytes may be reduced by low river discharge and associated reductions in sediment (and N) transport to backwaters of the UMRS.

Several laboratory studies have clearly indicated that macrophytes can obtain N exclusively from sediments (Barko and Smart 1981; Huebert and Gorham 1983). Thus, in lacustrine systems where the concentration of ammonium-N in sediment is usually greater than in the water (e.g., Nichols and Keeney 1976), sediments are likely the primary source of N for submersed aquatic macrophytes (Barko and Smart 1986). This may also be true in river systems; Chambers et al. (1989) showed that in the South Saskatchewan River, *Potamogeton crispus* obtained most of its nutrients through the roots. However, Barko, Gunnison, and Carpenter (1991) suggest this generalization may not apply to enriched riverine systems where ammonium-N concentrations in the water are high.

Two other potential sources of nutrients need to also be considered. Although we know little about groundwater flow into Lake Onalaska, nutrient uptake from groundwater by roots of *Vallisneria* could have occurred if groundwater nutrient concentrations were higher than in sediment pore water. Alternatively, decomposition of plant/algal remains within the sites may have allowed for sustained nutrient availability to *Vallisneria* in the field. The extent to which these processes individually or in combination may have contributed to the favorable growth of *Vallisneria* within Lake Onalaska observed experimentally in 1992 requires further investigative attention.

Acknowledgments

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Consequences of Drought for the Native Species *Vallisneria americana* from the Upper Mississippi River

by
Anne Kimber[1]

Introduction

In recent decades, *Vallisneria* beds have declined in the Yahara Lakes (Lind and Cottam 1969), the Detroit River (Hunt 1963; Schloesser and Manny 1990), the Chesapeake Bay and Potomac River (Bayley et al. 1978; Haramis and Carter 1983; Carter, Paschal, and Bartow 1985), the Illinois River (Mills, Starrett, and Bellrose 1966), and in the Upper Mississippi River (Serie, Trauger, and Sharp 1983). Declines have been associated with increased nutrient and sediment loads from surrounding watersheds (Schloesser and Manny 1990) and competition from other submersed macrophyte species, especially *Myriophyllum spicatum* (Titus and Adams 1979).

The recent decline of *Vallisneria americana* from shallow backwaters of the Upper Mississippi River occurred after a drought in 1988 during which record low flows, long hydraulic residence times, high water temperatures (27 to 29 °C), high turbidity, low dissolved oxygen, and low light availability were recorded in backwaters (NOAA 1988; Wisconsin Department of Natural Resources, unpublished data; Sullivan 1991). Few observations of plant beds were recorded during the summer of 1988; however, poor light penetration caused by algal blooms, both planktonic and filamentous, may have been exacerbated by long hydraulic residence times (Sullivan 1991). In years following the drought, light penetration has been low because of increased algal blooms and high concentrations of suspended sediment (Sullivan 1991). *Vallisneria* has been slow to reestablish in areas where it formerly grew.

The loss of *Vallisneria* in backwaters has resulted in increased turbidity because of wind and navigation-generated resuspension of unconsolidated sediment. There has been continuing interest in restoring *Vallisneria* to this system and especially to Lake Onalaska, Pool 7. The reason for this in part is that in recent years, up to 75 percent of the global canvasback (*Aythya vallisineria*) population has staged on Pool 7 during fall migration (Korschgen, George, and Green 1987).

High temperature stimulates growth in high light levels in greenhouse studies (Barko, Hardin, and Matthews 1982; Madsen and Adams 1989). Titus and Adams (1979) estimated the optimum temperature for light-saturated photosynthesis to be 32.5 °C for *Vallisneria americana*. However, high water temperatures have been implicated in seagrass declines (Bulthuis 1987), and net photosynthesis declines with increasing temperature have been recorded for seagrasses (Bulthuis 1983a, b; Wetzel and Penhale 1983; Marsh, Dennison, and Alberte 1986).

This study was designed in part to examine the effects of high temperature on respiration and net photosynthesis of *Vallisneria* plants grown in four light environments and on high and low fertility sediments in each light treatment. The hypothesis was that net photosynthesis would decline significantly with increasing temperatures in the range of light levels normally available to plants in the Upper Mississippi backwaters.

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Methods

Plants were grown in two concrete ponds (16 by 31 m) at the National Biological Survey Fisheries Research Laboratory (Kimber 1994).

Vallisneria tubers were harvested 2 weeks before planting, from Pool 4 of the Upper Mississippi River, Lake Pepin. Single tubers (1 to 1.5 g fresh weight) were planted April 26 into 5-qt plastic buckets containing 1 L of either lake or sand-amended lake sediment. Lake sediment (67-percent sand) was collected from Pool 7, Lake Onalaska, WI, from a former *Vallisneria* bed. The sand-amended sediment treatment (91-percent sand) was created by adding four parts washed builder's sand to one part sediment.

The experimental design was a split-plot, with two levels of sediment type within each of four levels of light. In three of these, black plastic shade screen was stapled to frames to reduce light levels at the base of a plant by 65, 78, and 93 percent. A fourth treatment had no shade cloth. In the ponds, five buckets of each sediment type were placed in each of 48 shade cells (24 cells in each pond), which were arranged in rows (randomized blocks) of four shade treatments each. Each cell (1 by 1 m) was isolated from the next by black plastic side walls that extended down to approximately 20 cm from the bottom of the ponds to allow water flow among cells and decrease water temperature differences among light treatments.

Two LiCor underwater quantum sensors were installed in each pond. Two terrestrial LiCor quantum sensors were used to measure surface photosynthetic photon flux density (PPFD). Sensors were connected to a LiCor datalogger, and integrated measurements of terrestrial and underwater PPFD were recorded hourly.

For photosynthesis measurements, plants harvested were transported to Ames, IA. Dark respiration was measured at the beginning and end of each run. Light was provided by halogen lamps shaded with neutral density filters (cheese cloth); light levels were established at 10, 30, 60, 100, and 800 μmoles/m^2/s PPFD; these were calibrated for each chamber with a LiCor quantum sensor. Chamber temperature was maintained by a refrigerating/heating water bath at temperatures ranging from 15 to 35 °C (± 0.5 °C). Rates were measured on three or more replicates of each of the eight light-sediment treatment combinations.

The contributions of light, sediment, and temperature treatment effects to respiration, compensation point, and net photosynthesis were determined by split-plot analysis of variance.

Results

The mean instantaneous PPFD in each shade treatment ranged from 210 μmoles/m^2/s in the 25-percent treatment to 72 μmoles/m^2/s in the 9-percent treatment, 45 μmoles/m^2/s in the 5-percent treatment, and 14 μmoles/m^2/s in the 2-percent treatment (Kimber 1994). Pond water temperatures ranged from 13 °C (30 September) to 23 °C (24 August) and were similar to those reported for Pool 8 (Wisconsin Department of Natural Resources (DNR) Long-Term Resource Monitoring Program, unpublished data for 1992).

Increasing water temperature from 15 to 35 °C increased dark respiration rates significantly (Figure 1). Respiration rates were significantly lower in plants grown in low light treatments regardless of temperature. Light compensation points (Figure 2) increased significantly with increasing temperature and were higher for plants grown in sand. Increasing the temperature from 15 to 35 °C also affected the rate of net photosynthesis, but the magnitude and direction (positive or negative) of the effect depended upon the experimental light level at which photosynthesis was measured. At light levels (Figure 3) of 100 μmoles/m^2/s or less, higher water temperatures decreased net rates of photosynthesis so that the highest rates of photosynthesis occurred at 15 °C. Conversely, at the highest light level measured, 800 μmoles/m^2/s, the

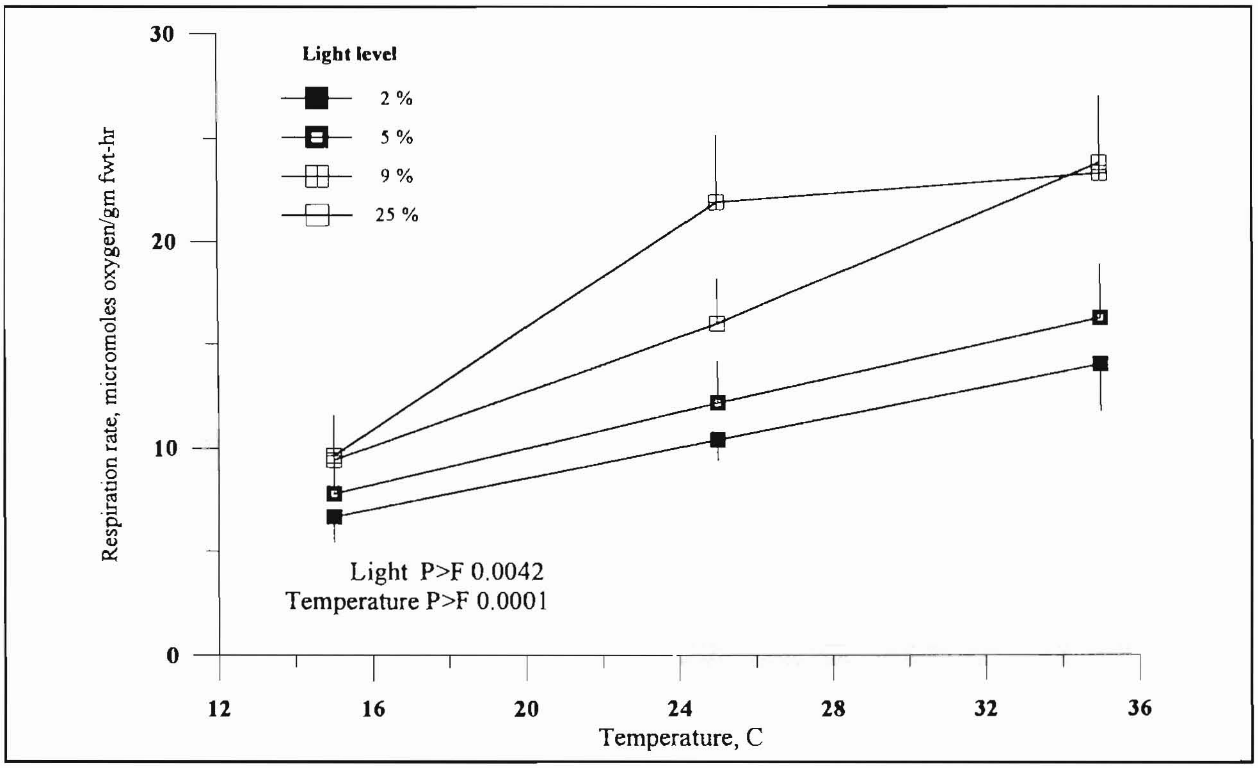

Figure 1. Effect of temperature on respiration rate

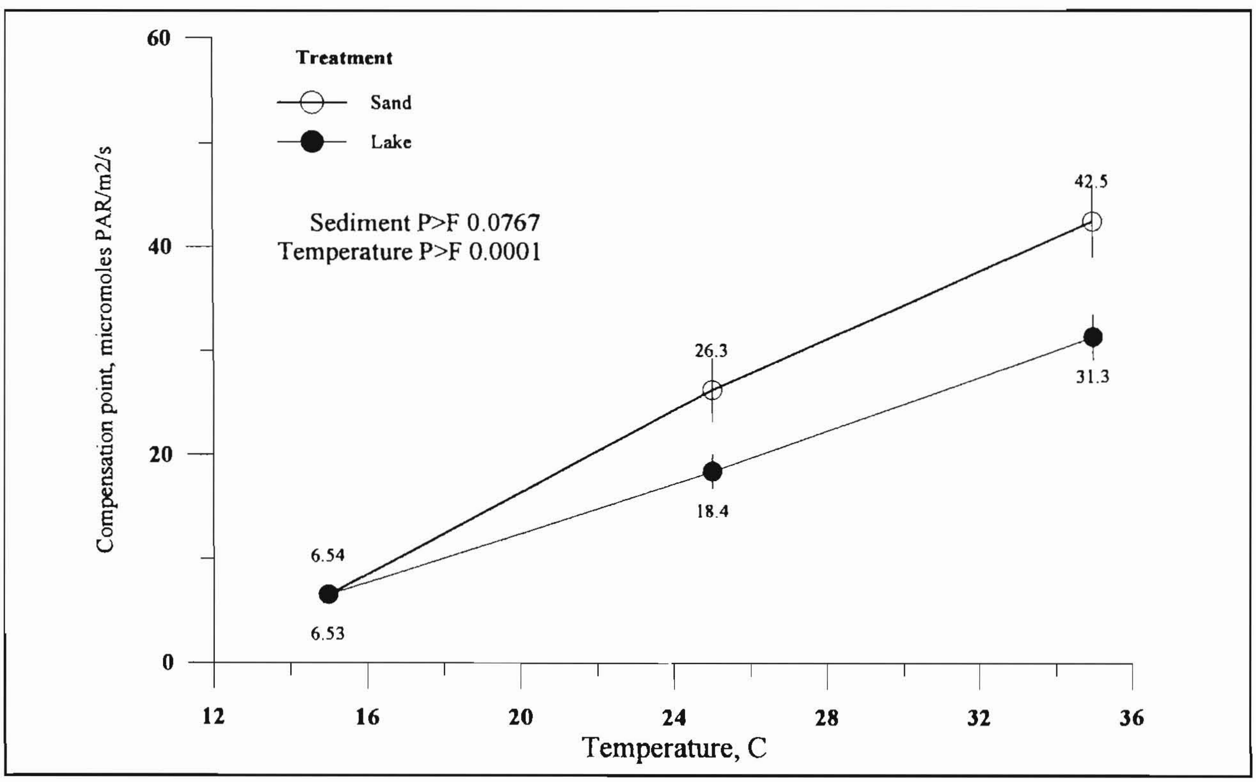

Figure 2. Effect of temperature on light compensation point

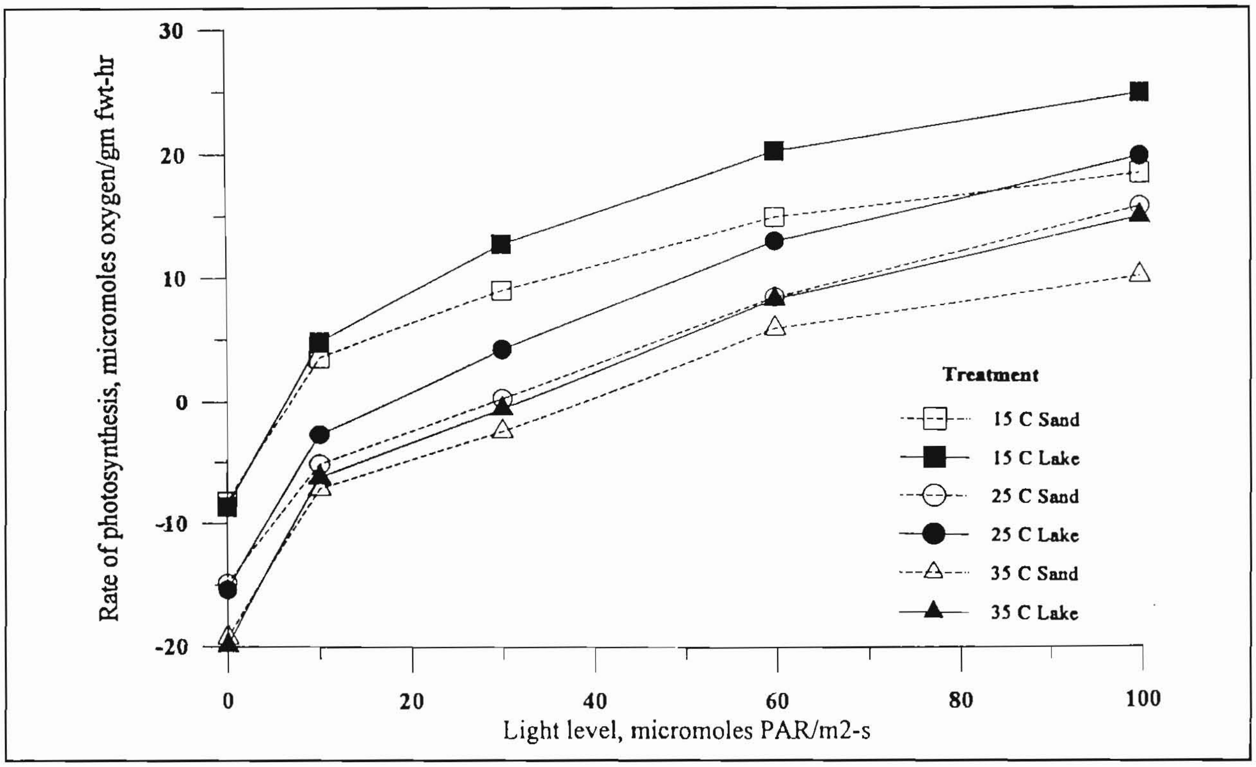

Figure 3. Effects of low light level, temperature, and sediment type on net photosynthesis

pattern was reversed, and net photosynthetic rate was highest at 35 °C (Figure 4). The crossover point where increasing temperature appeared to increase was where net photosynthesis occurred at light levels greater than 400 µmoles/m^2/s. At light levels above the compensation point, net photosynthesis was consistently lower on a gram fresh weight basis in plants grown on the sand-amended sediment.

Discussion

From measurements of photosynthesis, it appears that at low light levels, temperature increases, especially those in late summer, would decrease rather than increase net photosynthesis, and consequently decrease growth in addition to increasing whole-plant dark respiration. Similar late summer declines in plant growth and net photosynthesis with increasing summer temperature have been described for seagrasses by Wetzel and Penhale (1983), Bulthuis (1983b), and Marsh, Dennison, and Alberte (1986). For *Vallisneria*, temperature increases may be expected to increase net photosynthesis only at high light levels that may not occur in turbid backwaters. *Vallisneria* photosynthesis is also limited by growth form in comparison with plants which concentrate leaves at the water surface (Titus and Adams 1979; Goldsborough and Kemp 1988). A temperature optimum of 32.5 °C for light-saturated photosynthesis by *Vallisneria* has been reported by Titus and Adams (1979); however, the optimum temperature for shaded plants would be lower and dependent on the light available for photosynthesis (Bulthuis 1983a).

Plant respiration rates increased with increasing temperature in these experiments. Respiration was also affected by light level and sediment type especially at very high water temperatures; plants in shaded treatments had lower respiration rates as did those grown in the lower nutrient sand sediment. Lower respiration rates may have indicated lower contributions of growth respiration in slower growing plants (Hutchinson 1975).

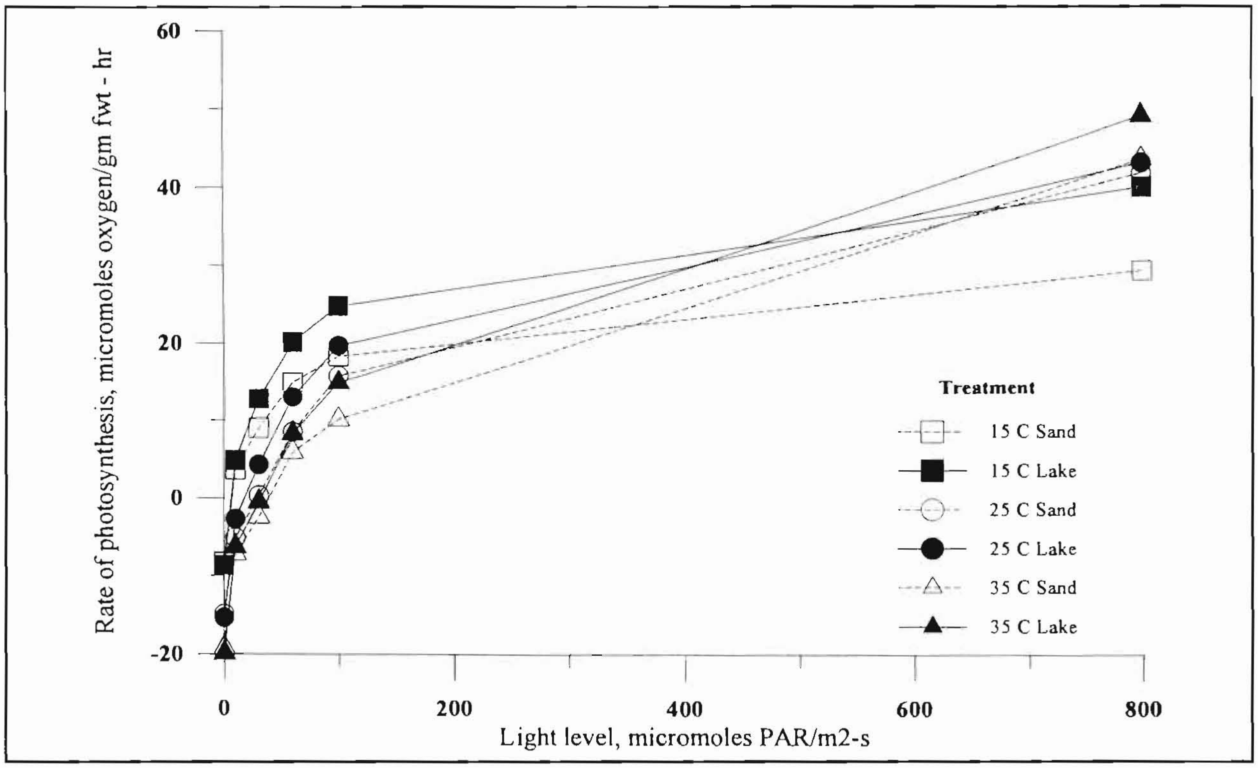

Figure 4. Effect of light level, temperature, and sediment type on net photosynthesis

Some species of *Potamogeton* can adapt to low light levels by increasing photosynthetic efficiency (Spence and Chrystal 1970a, b; Hutchinson 1975).

It appears from other measurements made in late July and August that plant respiration and net photosynthesis may be more sensitive to temperature increases later in the growing season (Kimber 1994). The decrease in net photosynthesis because of the combined effects of high temperature and decreased light may have resulted in lower tuber production in the drought year.

Acknowledgments

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Influences of High Temperature on Growth and Propagule Formation in Monoecious Hydrilla

by
Dwilette G. McFarland[1] *and John W. Barko*[1]

Introduction

Past investigations to expand understanding of physiological ecology of submersed aquatic vegetation have predominately examined effects of environmental factors (e.g., light, temperature, sediment, and water chemistry) on various aspects of growth. Thus far, numerous studies of submersed macrophytes have shown temperature to be a major factor influencing production and morphological development (Barko and Smart 1981; Barko, Hardin, and Matthews 1982), photosynthetic rates (Titus and Adams 1979; Barko and Smart 1981), oxygen consumption (Anderson 1969), sexual and asexual propagule germination (Teltscherova and Hejny 1973; Haller, Miller, and Garrard 1976; Miller, Garrard, and Haller 1976; Steward and Van 1987), and duration of growth cycle (Anderson 1969; Young 1974; Grace and Tilly 1976; Barko and Smart 1981). For many submersed macrophyte species, high temperatures (within the range of 28 to 32 °C) promote biomass production, with accompanying increases in shoot number and length (for synthesis, see Barko, Adams, and Cleseri (1986)). Changes in species composition of submersed macrophyte communities due to alterations in thermal regimes have also been reported (Anderson 1969; Allen and Gorham 1973), indicating temperature to be important in affecting interactions among coexisting species (Barko, Adams, and Cleseri 1986).

Interestingly, however, little research of submersed macrophytes has addressed the role of temperature in regulating vegetative propagule formation. Typically, submersed macrophytes generate vegetative propagules of various types, e.g., regenerative fragments, tubers, turions, stolons, rhizomes, and root crowns. Among these, tubers and turions are most important in facilitating population regrowth (Weber 1973; Basiouny, Haller, and Garrard 1978; Sastroutomo 1982). In temperate species, production of tubers and turions usually begins under short photoperiods of autumn (Spencer and Anderson 1987). Van, Haller, and Garrard (1978) found that under a 10-hr photoperiod, tuberization in dioecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) increased with increased temperature to 33 °C. Weber and Nooden (1976) found that (under short-day conditions) turions in *Myriophyllum verticillatum* L. can be induced at 15 °C and lower, but not at 20 °C. From a management perspective, effects of temperature on propagule formation are of particular interest due to possible impacts on the survival of submersed macrophytes from one growing season to the next. Additionally, knowledge of how propagule initiation is affected by temperature and other conditions may be valuable in allowing timely application of control methods or suggests more effective means of reducing the numbers of propagules produced.

The research presented here is an extension of previous work described in McFarland and Barko (1987) wherein growth of monoecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) was examined over a range of temperatures from 16 to 32 °C. In the present study, we provide further information on the growth of this biotype, but with greater emphasis on its tuber and turion production. The study was specifically designed to determine how warm-water temperatures extending into autumn may affect production of these two propagule types. The results are contrasted over three growth periods (up to 16 weeks) to allow

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ample time for propagule formation and assessments of response over an extended period of time.

Methods and Materials

The investigation began the last week in July 1992 in a greenhouse facility at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. Low alkalinity culture solution was prepared in 1,200-L white fiberglass tanks according to Smart and Barko (1985). Solution temperatures were maintained at specified levels (±1 °C) using Remcor circulators plumbed singly to each tank. The solution was aerated with humidified air to enhance mixing and air/water CO_2 exchange. Neutral density shade fabric affixed to the roof of the greenhouse reduced natural irradiance by approximately 75 percent.

Nine tanks were positioned in three groups of three tanks each. Temperature was assigned to each group at one of three levels: 25, 30, and 35 °C. One tank per temperature level was harvested at 8, 12, and 16 weeks. Temperature/growth-period treatment combinations (assigned separately by tank) were replicated six times; each replicate consisted of a single 24.3- by 24.3-cm by 10.0-cm-deep sediment container, planted with four 15-cm-long apical cuttings of monoecious hydrilla. At 4-week intervals, one-third of the solution in each tank was replaced with freshly prepared culture solution. Water temperatures were monitored three times daily and minor thermostat adjustments made as necessary.

Surficial sediment dredged from Brown's Lake, WES, was used as the rooting medium in this study. The sediment was fertilized with ammonium chloride (0.6 g per liter of wet sediment) and thoroughly mixed in a large (>70-gal) capacity mortar mixer. Physical and chemical characteristics of this sediment following N amendment are summarized in Table 1. N-amended sediment was placed to a depth of about 8 cm in each planting container. After planting, a thin layer of washed silica sand was placed over the sediment surface to prevent physical mixing with the culture solution.

Table 1
Sediment Characteristics[1]

Texture, %	
Fine particles (<50 µm diam)	90.00 ± 0.00
Coarse particles (>50 µm diam)	10.00 ± 0.00
Moisture, %	42.12 ± 0.16
Organic matter, %	5.63 ± 0.12
Dry weight density, g/ml	0.87 ± 0.01
Extractable nutrients, mg/g	
Nitrogen	0.16 ± 0.01
Phosphorus	0.14 ± 0.01
Potassium	0.12 ± 0.00

[1] Values are means and standard errors based on three replicate sediment samples.

Monoecious hydrilla (*Hydrilla verticillata* (L.f.) Royle) used in the study was clipped from apices of a WES continuous greenhouse stock. The clippings were evenly spaced (four/container) with basal ends buried approximately 4 cm in the sediment. At the end of each growth period, hydrilla was clipped at the sediment surface and oven dried to constant mass (at 80 °C). Evaluations of growth were based on separate determinations of shoot (aboveground) and root (belowground) biomass, which were subsequently used in calculations of total biomass and root-to-shoot biomass ratio. Tubers and turions were counted directly and weighed for separate determinations of tuber and turion biomass.

Data were analyzed using analysis of variance (ANOVA) and post-ANOVA capabilities of the Statistical Analysis System (SAS) (SAS Institute, Inc. 1991). Hereafter, statements of statistical significance without specific indication of probability level refer to $P < 0.05$.

Results

Results of two-way ANOVA (Table 2) indicate the relative significance of independent and interactive effects of temperature and growth period on biomass and propagule production in hydrilla. In all cases, independent

Table 2
Two-Way ANOVA for Biomass and Propagule Production in Monoecious *Hydrilla verticillata* (L.f.) Royle Relative to Temperature and Growth Period

Response	Source[1]	F Value	P
Total biomass	Pd	35.03	0.0001
	Temp	11.50	0.0001
	Pd * Temp	2.27	0.0804
Root-to-shoot biomass ratio	Pd	56.03	0.0001
	Temp	150.46	0.0001
	Pd * Temp	7.11	0.0003
Tuber number	Pd	99.74	0.0001
	Temp	143.09	0.0001
	Pd * Temp	8.91	0.0001
Tuber mass	Pd	130.68	0.0001
	Temp	213.98	0.0001
	Pd * Temp	19.57	0.0001
Turion number	Pd	15.07	0.0001
	Temp	66.40	0.0001
	Pd * Temp	14.37	0.0001
Turion mass	Pd	16.70	0.0001
	Temp	65.81	0.0001
	Pd * Temp	16.38	0.0001

[1] Abbreviations: Pd = growth period; Temp = temperature; Pd * Temp = growth period/temperature interaction.

effects of temperature and growth period were highly significant and explained greater treatment-related variance than did the interaction terms. However, interactions between these variables did occur and significantly affected the magnitude of most measured responses (see below).

On the whole, total biomass in hydrilla was more responsive to growth period than to temperature in this study (Figure 1). At 8 weeks, total biomass did not differ significantly among temperature treatments. Peak biomass production occurred by 12 weeks both in the 25 and 30 °C treatments, and was significantly greater than in the 35 °C treatment. Beyond 12 weeks, variations in total biomass due to temperature were less pronounced because of modest increases in production at 30 and 35 °C.

Ratios of root-to-shoot biomass in hydrilla were generally greatest at 25 °C and declined with increased temperature to 35 °C (Figure 1). In 25 and 30 °C treatments, these ratios increased significantly with increased growth period up to 16 weeks; peak ratios in the 35 °C treatment were reached by 12 weeks.

Tuber production (i.e., tuber number and tuber mass) also increased over time and was consistently least in hydrilla grown at 35 °C (Figure 2). At 8 weeks, the number of tubers produced at 30 °C was significantly less than at 25 °C. This initial lag in the 30 °C treatment was overcome by 12 weeks when tuber number was similar between 25 and 30 °C treatments. The differential in tuber mass was likewise diminished between 25 and 30 °C treatments over time. However, by 16 weeks, the number and mass of tubers at 30 °C were threefold and sixfold greater than the respective tuber responses at 35 °C.

Turion production (i.e., turion number and mass) was primarily affected by temperature treatment. No turions were produced at 35 °C, and the number and mass of those obtained at 30 °C were markedly low (Figure 3). Most turions were produced in the 25 °C treatment, with peak production of these propagules occurring between 12 and 16 weeks.

Discussion

Results of this study clearly showed growth period to strongly influence biomass and propagule (i.e., tuber and turion) formation in monoecious hydrilla. However, in most cases, temperature appeared to have the greatest overall effect. Notably, the interaction between temperature and growth period was apparent with respect to propagule formation, which within the limits of this investigation, generally decreased with increasing temperature but increased over time. While these findings support those of others indicating the importance of temperature in affecting responses of monoecious hydrilla (McFarland and Barko 1987; Steward and Van 1987; Sutton, Van, and Portier 1992), they further suggest that assessments of its propagation potential consider duration of exposure to high temperature conditions.

In the present study, high temperature levels affected propagule formation to a greater extent than total biomass production. These results are in general agreement with those of past studies of this biotype (McFarland and

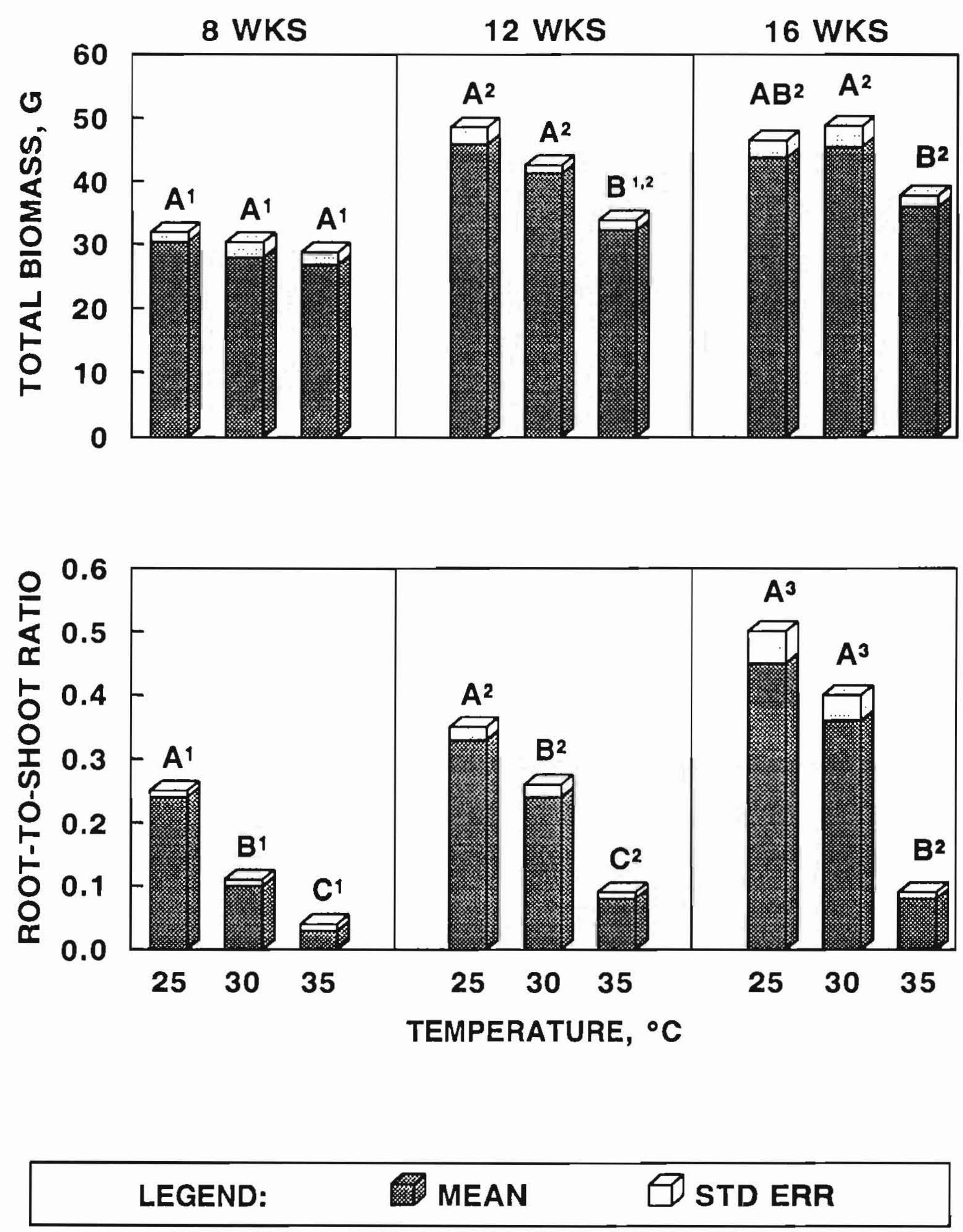

Figure 1. Effects of temperature and growth period on total biomass (top frame) and root-to-shoot ratio (bottom frame) in monoecious hydrilla. Within each growth period, uppercase letters denote results of comparisons made across temperature. For each temperature, superscripts denote results of comparisons made across growth period. Bars sharing the same letter or superscript do not differ significantly from each other. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

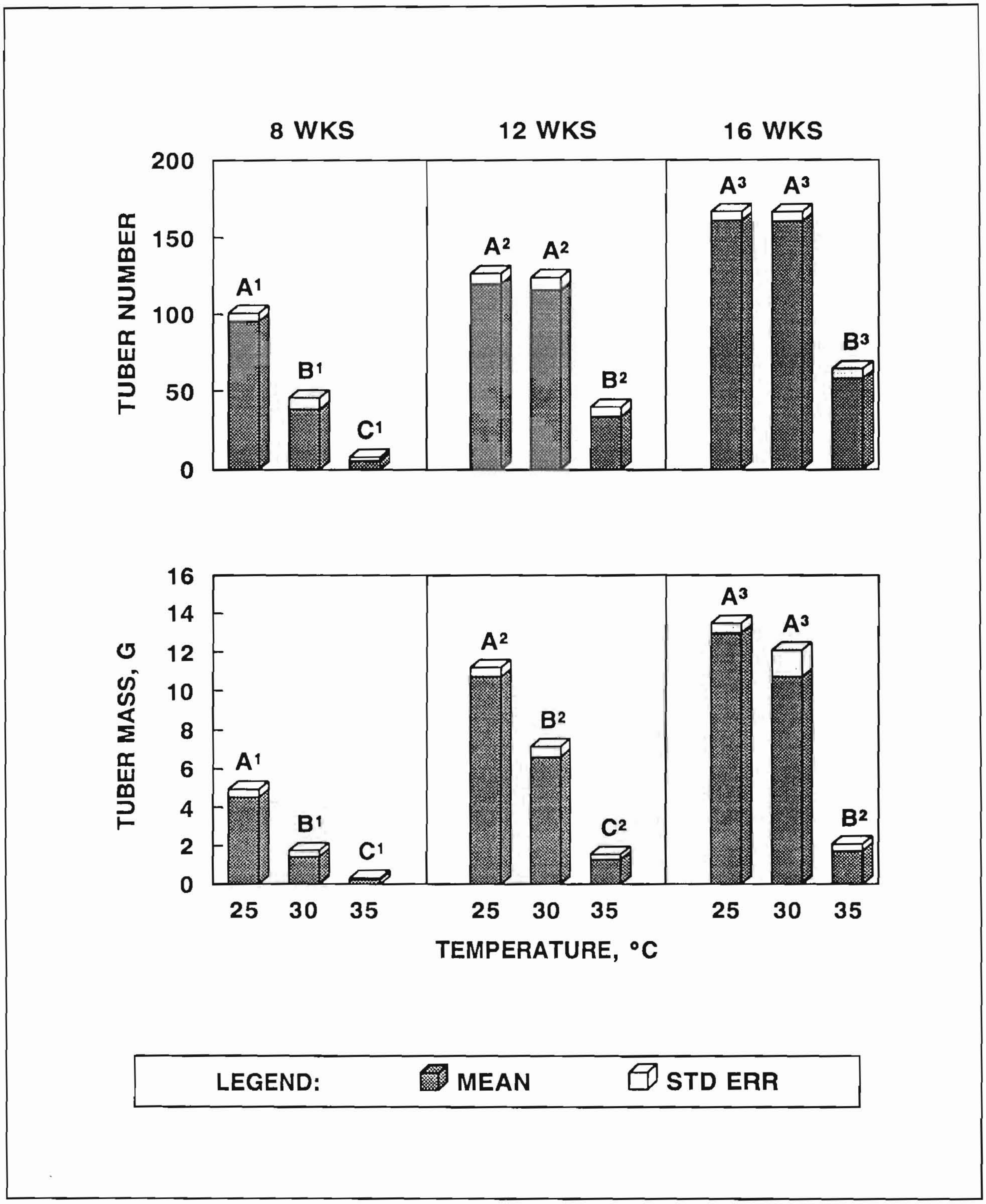

Figure 2. Effects of temperature and growth period on tuber number (top frame) and tuber mass (bottom frame) in monoecious hydrilla. Within each growth period, uppercase letters denote results of comparisons made across temperature. For each temperature, superscripts denote results of comparisons made across growth period. Bars sharing the same letter or superscript do not differ significantly from each other. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

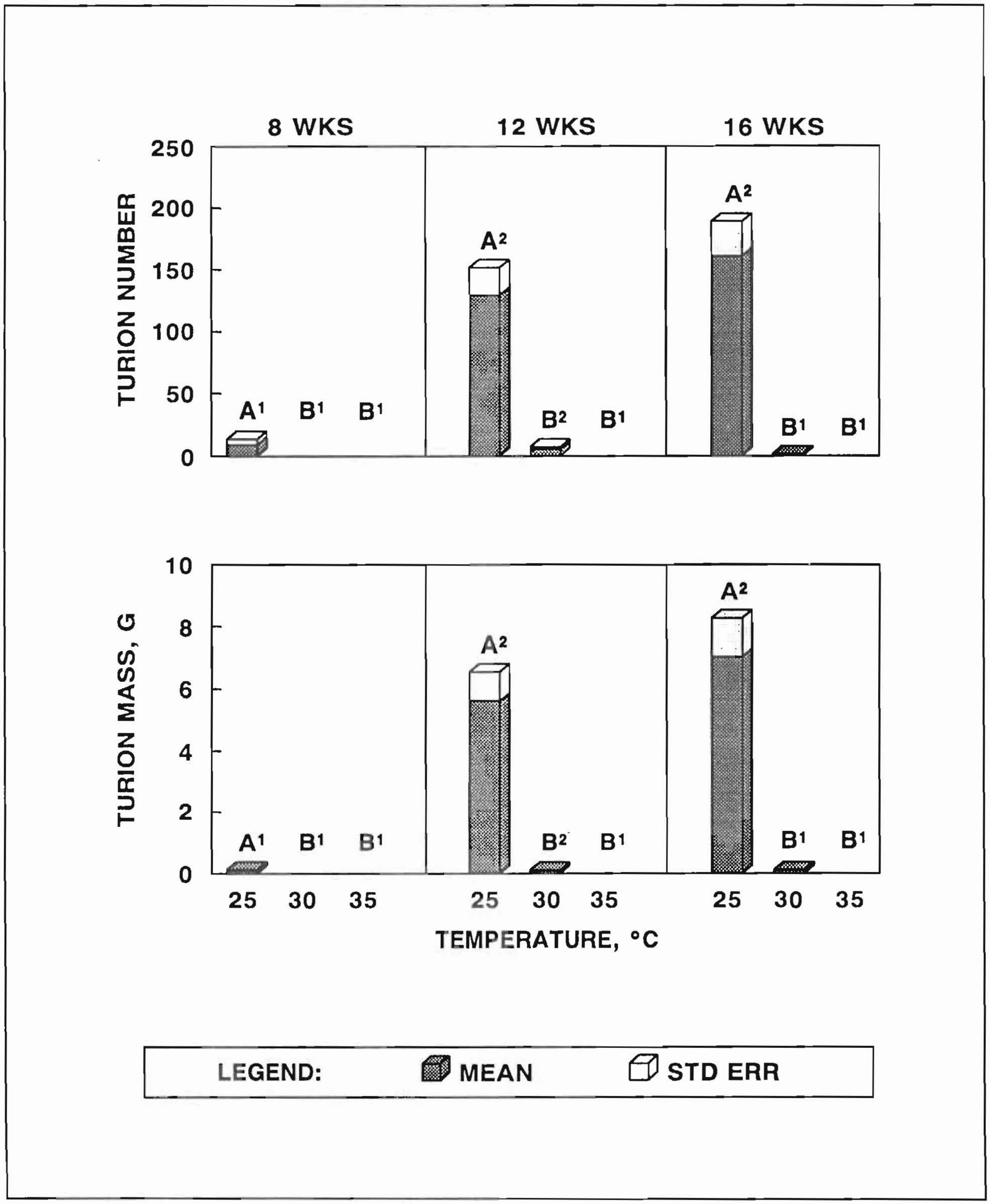

Figure 3. Effects of temperature and growth period on turion number (top frame) and turion mass (bottom frame) in monoecious hydrilla. Within each growth period, uppercase letters denote results of comparisons made across temperature. For each temperature, superscripts denote results of comparisons made across growth period. Bars sharing the same letter or superscript do not differ significantly from each other. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

Barko 1987) wherein tuber production declined dramatically, but total biomass varied far less within a high temperature range (from 28 to 32 °C). As demonstrated here, the production of turions in hydrilla was also strongly restricted at 30 °C and above. The sharp reductions in propagule formation exhibited in this study indicate the existence of a high temperature threshold, approximately between 30 and 35 °C for tubers and 25 and 30 °C for turions, which severely restricts propagule growth.

Mean mass per tuber, calculated by dividing total tuber mass by total tuber number in this study, revealed that by 12 weeks, increases in temperature (from 25 to 35 °C) significantly reduced individual tuber mass (unpublished data). Although these results are preliminary, they provide an initial indication that high temperature exposure can affect tuber size. At present, influences of tuber mass on the success of hydrilla are not well known. However, for *Potamogeton pectinatus* L., germination and initial growth rate have been shown to be positively related to tuber fresh weight (Spencer 1986). Differences in individual tuber mass affected by temperature may also influence these processes in hydrilla as well. Further research of the mass-related performance of vegetative propagules of hydrilla would be useful in predicting recruitment and postgermination vigor of this species under field conditions.

Responses of monoecious hydrilla in this investigation and elsewhere have significant implications for its survival under different thermal regimes. Here, both tuber and turion production were stimulated at the lowest temperature level (25 °C). Moreover, McFarland and Barko (1987) and Steward and Van (1987) demonstrated substantial production and germination of monoecious hydrilla tubers at 15 to 16 °C. To date, however, equivalent information regarding turions of this biotype is lacking. Efficient reproduction at moderate to low temperatures provides an advantage to monoecious hydrilla in many northern localities (Steward and Van 1987). Conversely, diminished propagule production as observed in this study implies an impaired ability of this biotype to withstand long-term exposure to high temperatures, as might occur in some southern localities or during extended periods of drought.

Recommendations

Understanding the relative influences of environmental variables and their interactions on growth of submersed macrophytes is fundamental to assessments of their potential proliferation in various aquatic systems. With these considerations, we suggest that future studies of submersed macrophytes examine effects of temperature and other important environmental variables on propagule production, survival, and early stages of growth. This information would enhance our ability to predict changes in population density and promote the development of strategies for regulating the number and success of propagules produced.

Acknowledgments

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Initial Evaluation of Submersed Macrophyte Decline Sites

by
Craig S. Smith[1]

Introduction

Populations of exotic submersed macrophytes often exhibit a pattern of explosive growth followed after 10 to 15 years by a noticeable decline (Carpenter 1980; Smith and Barko 1990). Declines in submersed plant abundance are evidence of the operation of natural population controls. The controlling factors producing declines are at best superficially understood (Smith and Barko 1990; Smith 1991). Once these population controls have been identified, management strategies can be designed to act in concert with them. The goal of research on submersed macrophyte invasions and declines is to identify those factors influencing invasions and declines and to elucidate associated causal mechanisms.

This paper reports preliminary results of studies designed to identify environmental factors associated with declines. Sites having increasing, stable, and declining populations of *Myriophyllum spicatum* (Eurasian watermilfoil) were examined. At each site, environmental conditions and plant cover were evaluated. Sediments were collected and returned to the laboratory, where they were bioassayed for their ability to support plant growth. This research was coordinated with the collection of samples to determine whether the *M. spicatum* plants from the sample sites harbored fungal pathogens or herbivores conducted by the U.S. Army Engineer Waterways Experiment Station (WES) Biomanagement Team members Judy Shearer and Michael Grodowitz. Their results will be presented elsewhere.

Comparison of Sites with Healthy, Declined, and Recovering *M. spicatum* Populations

Sites where *M. spicatum* populations had remained constant, declined, or declined and recovered were examined to determine conditions that may have contributed to differences in the ability to support submersed macrophyte growth. Sites for initial studies were located primarily in Tennessee Valley Authority (TVA) reservoirs, where long-term records of plant cover are available from aerial photography, and in reservoirs on the adjacent Cumberland River, where *M. spicatum* populations are still expanding (Figure 1).

The vegetation cover history of sites was determined from aerial photographs taken in 1987 or 1988, 1990, and 1992. Sites were selected to represent a range of environments (e.g., overbank and embayment) and management histories. Sites were taken from locations where *M. spicatum* beds had been sufficiently large or were close enough to distinct landmarks so that it was possible to be reasonably sure of being within the former bed limits when collecting samples in the field. Compass bearings to two to four recognizable landmarks were recorded at sample locations.

Basic environmental data were recorded for each sample site. These included a Secchi disk measurement, observations of plant cover, and information concerning any recent aquatic plant management. *Myriophyllum spicatum* cover was recorded in semiquantitative categories ranging from absent to very dense.

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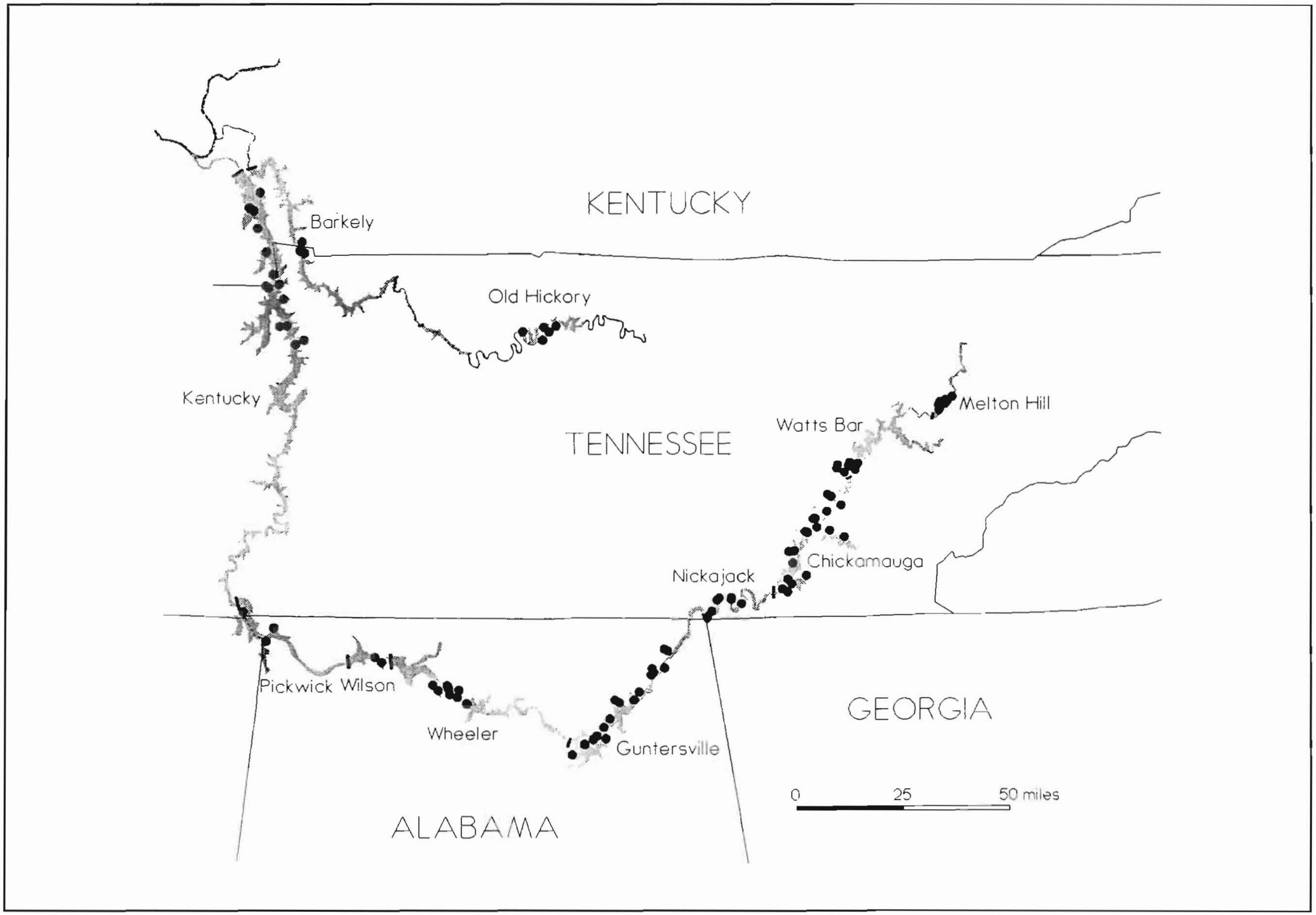

Figure 1. Sample locations for initial studies of healthy and declining Myriophyllum spicatum populations

Presence and species composition of any other submersed vegetation was noted. Recent management of aquatic plants in the area was determined from TVA herbicide application records. The type of herbicide used, the year of the most recent application, and the approximate frequency of application were noted.

Sediments from the sample sites were collected and bioassayed for their ability to support *M. spicatum* growth. Sediment cores were collected in 10-cm-diam polyvinyl chloride tubes 28 cm long. Core tubes were filled with intact cores of sediment by inserting the core tube vertically into the sediments except where dense or rocky sediments made it impossible to insert the corer completely. In these cases, additional surficial sediments were added to the top of the core to fill the tube.

Cores were planted with four 15-cm sprigs of *M. spicatum* from greenhouse stock cultures. Additional core tubes were filled with sediments from Browns Lake, on the WES grounds, and planted to provide a measure of growth on a known reference sediment. After planting, each core was inserted into the base of an individual acrylic column that was then filled with growth medium (Smart and Barko 1985). Columns were placed in a growth room, where they were maintained at 25 ± 1°C and illuminated to approximately 400 µmol quanta m^{-2} sec^{-1} of photosynthetically active radiation for 14 hr/day. After 6 weeks of growth, aboveground parts of plants were harvested, dried to a constant weight at 80 °C, and weighed to determine the final biomass. Sediments from the bioassays are currently being analyzed to determine bulk density, particle size, and organic content.

Results of the bioassays completed to date are shown in Figure 2. Sediments differed considerably in their ability to grow *M. spicatum*. Approximately 70 percent of the sediments produced growth that was within the expected range for growth on the reference sediments, while the remainder supported less growth than the reference sediments. All sediments tested supported some plant growth.

Once bioassays of sediments from all of the sample sites have been completed, the results will be analyzed to determine whether sediments from sites where *M. spicatum* has declined support less plant growth than those from sites where declines have not occurred.

Attempts to Establish *M. spicatum* in Former Decline Sites

On June 30 and July 1, 1993, *M. spicatum* was planted at five sites in Chickamauga Reservoir. Each site was located in one of the bioassay sample locations (see above) in an area formerly vegetated by *M. spicatum*. At each location a 2- by 2-m plot was planted with *M. spicatum* sprigs spaced 0.25 m apart. Sprigs were approximately 1 m long and were planted by inserting the lower third of the stem into the sediments. Half of each plot was covered with 1- by 1-m exclosures of wire fencing 1 m high by placing two exclosures over diagonally opposed quarters of the plot.

By mid-August, all of the plants had disappeared from nonexclosed portions of plots. Plants were still present in the exclosures. Monitoring of these sites will continue into 1994 to determine whether the plants in the exclosures become established.

Future Research

Future studies will add additional sites to the sample base described in this paper. Natural lakes having *M. spicatum* populations that vary in age and vigor will be emphasized. Anomalous sites (e.g., sites that have environmental characteristics apparently suitable for growth of submersed vegetation but are unvegetated) will be selected from the existing sample locations and examined in greater detail. Additional transplant experiments will be conducted in locations where *M. spicatum* has declined. These transplant experiments will be designed to identify factors that influence the success of plantings and to determine whether exclosures are protecting planted fragments from herbivory or simply helping to anchor them in place.

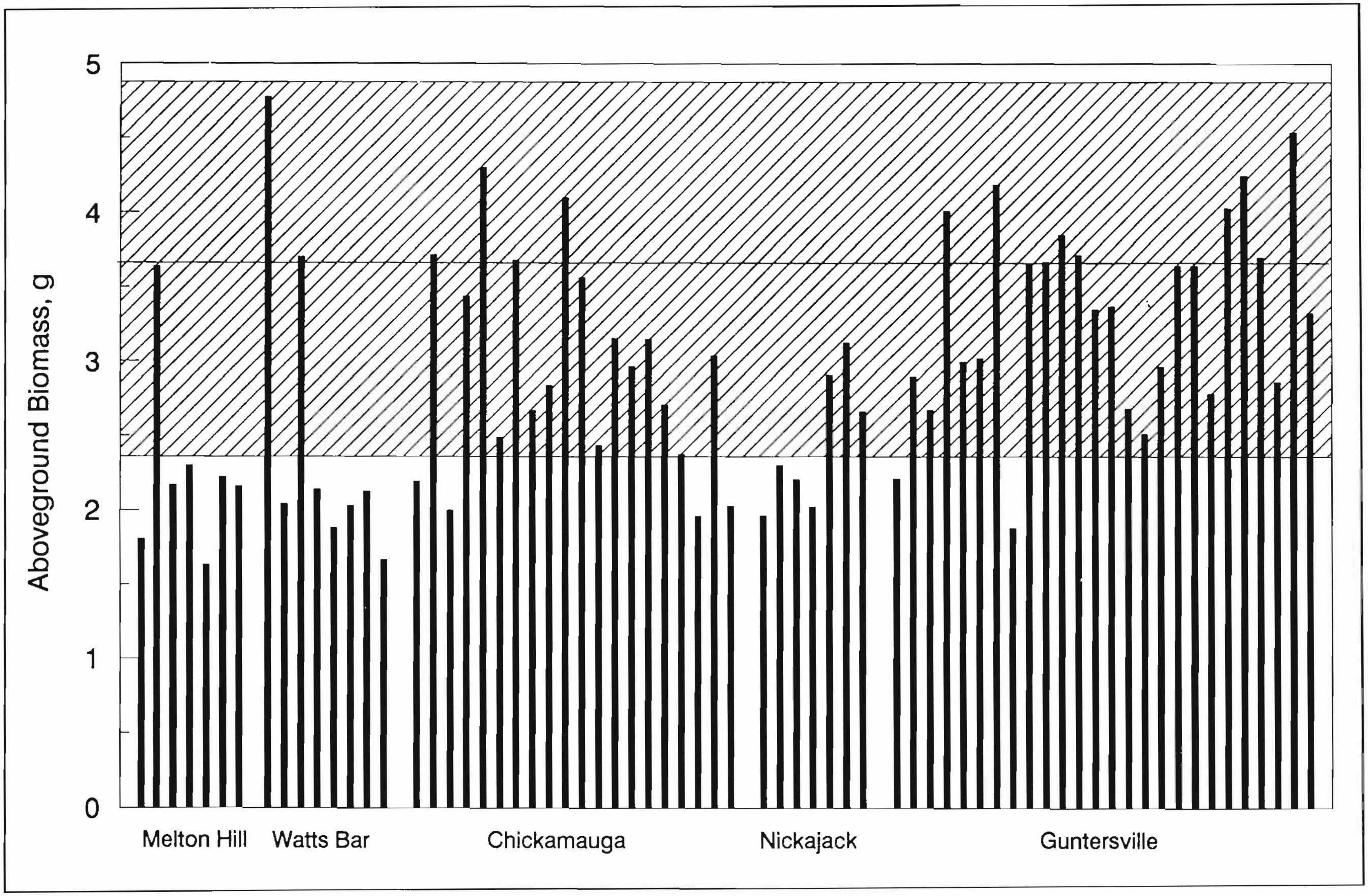

Figure 2. Growth of Myriophyllum spicatum plants on sediments from five TVA reservoirs. Each bar represents the biomass produced on an individual sediment sample from the indicated reservoir. The shaded region indicates the 95-percent confidence zone for the growth of plants on reference sediment from Browns Lake

Acknowledgments

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Spatial Patterns of Aquatic Plant Invasions and Declines

by
M. Rose Kress[1]

Within waterways, aquatic plant invasions and declines are influenced by an array of environmental factors such as nutrient availability, sedimentation rates, and even major weather events. The complex interaction of environmental factors over space and time result in dynamic patterns of aquatic plant invasions and declines that are difficult to measure, characterize, interpret, or predict. In this report, an approach to analyzing plant distribution patterns is presented. The basic approach is to compile a digital database of multidate aquatic plant distributions and analyze changes in the patterns using spatial data analysis routines in a geographic information system (GIS).

Two subareas of Guntersville Reservoir were chosen for analysis. Multiple date aquatic plant interpretations were available for both sites. Figure 1 shows the locations of the two sites, Brown's Creek and Connor's Island. Four maps (different dates) of aquatic plant distributions were analyzed in this study. All interpretations were based on aerial photography of the same scale, type, and season (Table 1) and were performed by personnel at Tennessee Valley Authority (TVA).

The original photography was similar for each date, but the base materials (Table 1) from which the digital database was developed were different. The 1978 data layer was developed from individual 9- by 9-in. Mylar overlays with the original interpretations hand drawn. For the 1984 and 1988 data, TVA personnel had manually transferred the aquatic plant interpretations onto paper copies of the navigation chart, and these were used as base materials. The 1989 aquatic plant interpretation was available from TVA in a digital format and was used directly.

Table 1
Information Sources for Digital Data Files

Date	Photography Type	Scale	Base Materials
1978 Oct 4	Color	1:7200	9- by 9-in. Mylar overlays
1984 Oct 17	Color	1:7200	Navigation chart
1988 Oct 17	Color	1:7200	Navigation chart
1989 Oct 24	Color	1:7200	Digital data file

Water depths, based on navigation charts, were included in the database. These navigation charts depict elevation of the lake bottom using a 5-ft contour interval. These data were digitized and converted to water depths assuming a constant 595-ft MSL pool elevation and retaining a 5-ft class interval. Figure 2 shows a portion of the water depth data layer at Brown's Creek developed in this manner.

Once a digital database was in place, characterization and analysis was possible using spatial data analysis tools in the GIS. The geometric properties of a water body, as characterized by water depth distribution, will affect aquatic plant invasions and declines. Table 2 compares the surface area versus water depth distribution for the two sites. A larger percent of the Brown's Creek area has water depths <15 ft. Nuisance plant infestations are more likely in shallow water.

A total of nine plant species were identified from the aerial photography. Table 3 lists the species and the abbreviations used. These nine species were mapped as occurring in 36 different associations during the four

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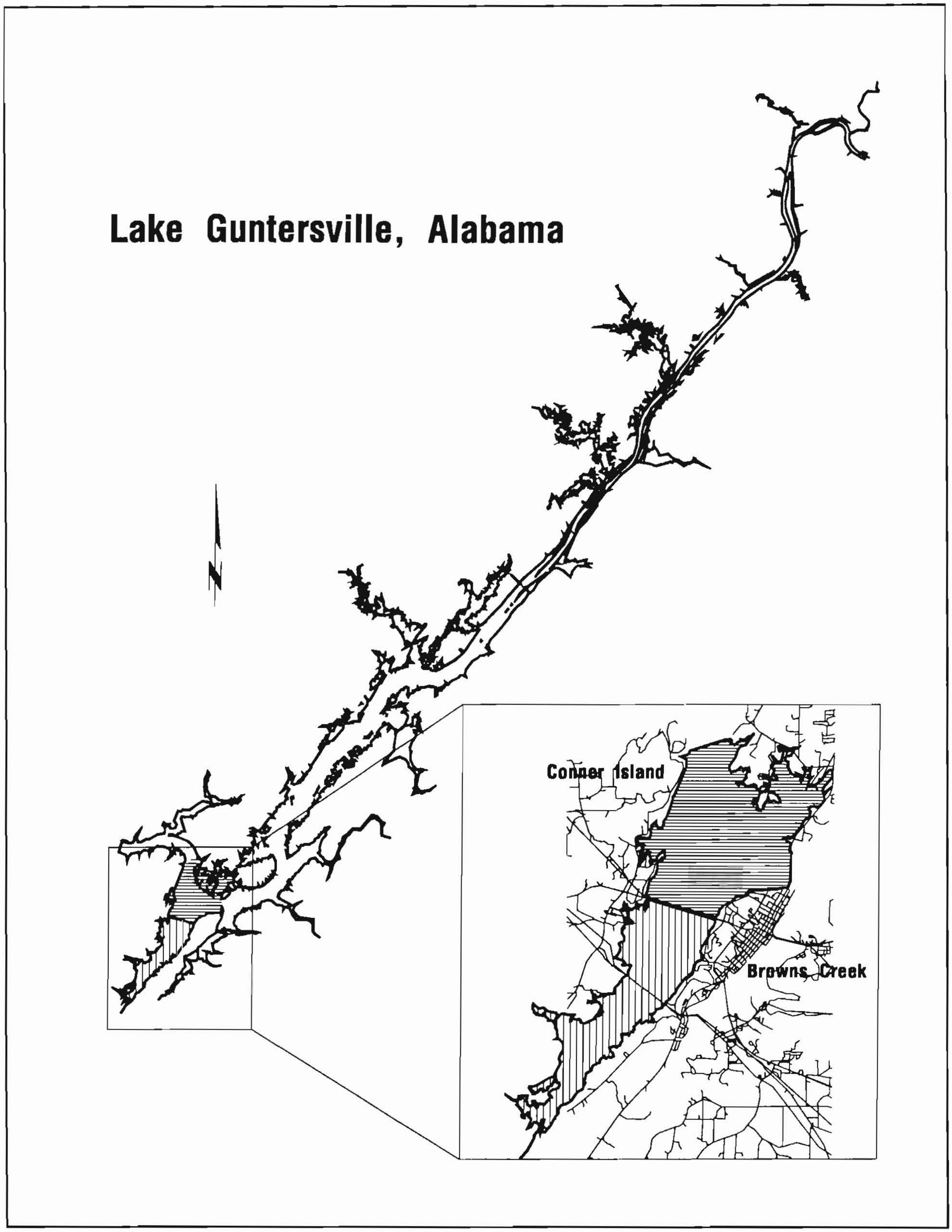

Figure 1. Location of study sites for the spatial analysis of invasions and declines

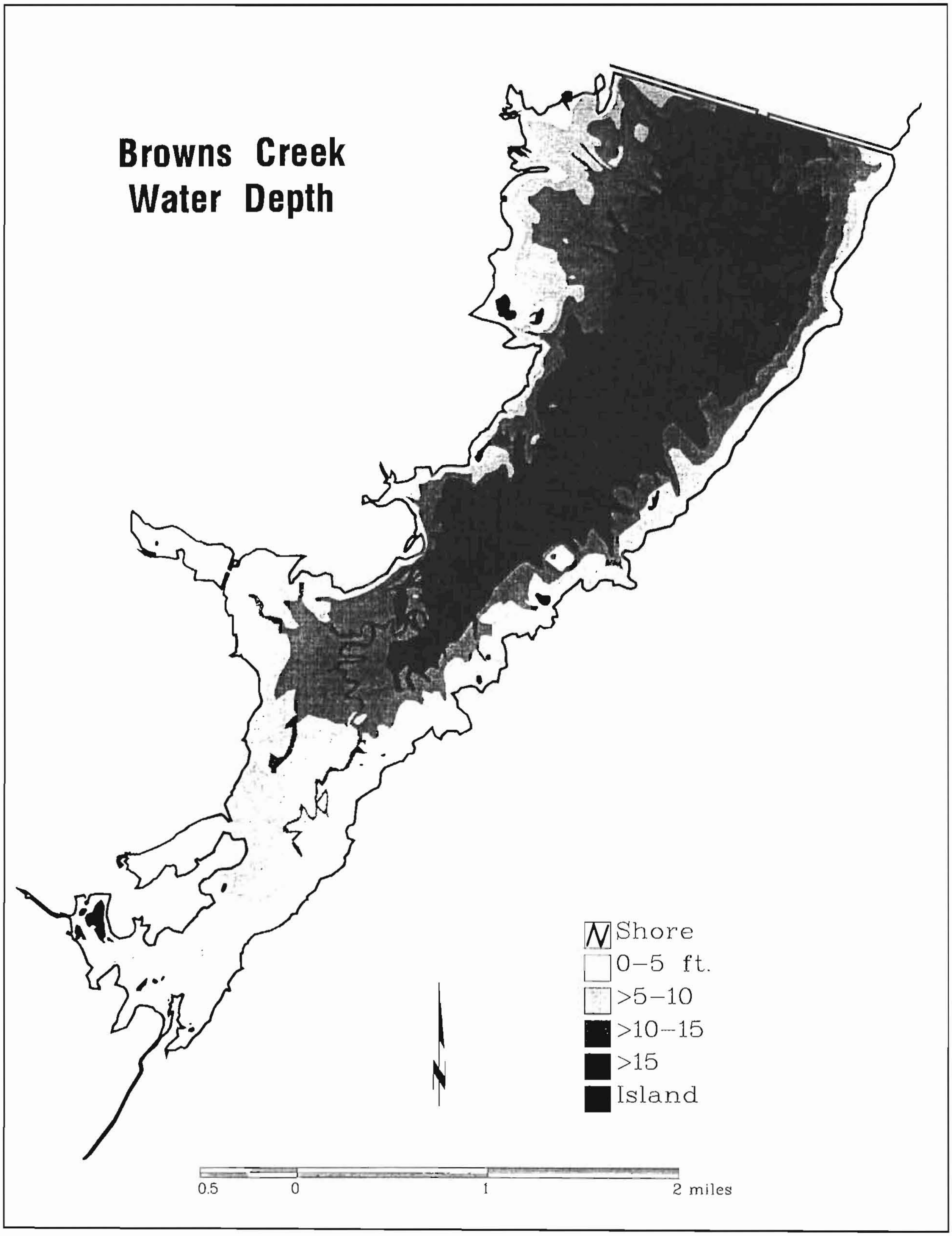

Figure 2. Distribution of water depth in the Brown's Creek site as determined from navigation chart 501

Table 2
Surface Area Versus Water Depth for the Two Sites

Water Depth, ft	Area, acres	
	Brown's Creek	Connor's Island
0-5	748	499
>5-10	872	705
>10-15	870	886
>15	1,551	3,721
Total	4,040	5,812

Table 3
Plant Species Identified at Both Sites and All 4 Years

Hydrilla verticillata	(HV)
Cabomba caroliniana	(CC)
Myriophyllum spicatum	(MS)
Potomogeton nodosus	(PN)
Najas minor	(NM)
Filamentous algea	(FA)
Chara sp.	(CH)
Ceratophyllum demersum	(CD)
Najas guadalupensis	(NG)

time intervals. Table 4 lists these associations by site and year. Table 4 illustrates one of the difficulties in developing a spatial or temporal characterization for even these small areas. There was a wide disparity in apparent detail and complexity of plant mapping between sites and years. These differences were probably real. However, similar disparities can result from differences in the interpreter's skill or the quality of the photographs.

The total acres of aquatic plants mapped at each site was calculated and is presented in Table 5. These data show an increase in plant acreage at both sites between 1978 and 1984 and again between 1984 and 1988. A substantial decline in total acreage occurred between 1988 and 1989. To illustrate the spatial patterns associated with these changes, the behavior of *myriophyllum* was examined in more detail.

Table 4
Aquatic Plant Associations as Photo-Identified for Each Year at Each Site

Connor's Island				Brown's Creek			
1978	1984	1988	1989	1978	1984	1988	1989
Allmix	CD	CD/MS	CH	MS	CH	CC	CC
MS	CD/CH	CH	CH/MS/HV	MS/NM	CH/FA	CC/FA	MS
MS/NM	CH	CH/HV	FA	MS/PN	FA	CD	MS/CD
NM	CH/NM	HV	FA/MS	NM	MS	CH	
NM/MS	FA	HV/MS	HV	NM/MS	MS/NM	CH/MS	
PN	FA/CH	MS	HV/MS		MS/PN	CH/NM	
	MS	MS/CD	MS		NG/FA	FA	
	MS/CD	MS/HV	MS/CH		NM	FA/MS	
	MS/NM	MS/NG	MS/HV		NM/CD	HV	
	MS/PN	MS/NM	MS/HV/CD		NM/FA	HV/MS	
	NG	MS/PN	PN/NM		NM/MS	MS	
	NG/CH	NM			NM/NG	MS/CD	
	NM	NM/FA			PN/MS	MS/FA	
	NM/CH					MS/HV	
	NM/MS					MS/NG	
	NM/NG					MS/NM	
	PN					NG/MS	
	PN/NM					NM	
						NM/FA	
						PN	

Table 5
Total Acreage of Aquatic Plants Identified by Site and Year

Year	Area of Aquatic Plants, acres	
	Brown's Creek	Connor's Island
1978	179	491
1984	451	1,128
1988	1,497	1,787
1989	602	1,039

In Table 6, the total aquatic plant infestation in Brown's Creek is separated into those areas with *myriophyllum* present and those areas where *myriophyllum* was absent. Included in the *myriophyllum* category (Table 6) are all associations of *myriophyllum* with other plant types as well as pure stands of *myriophyllum*. In 1984, about 25 percent of the aquatic plant-infested area was free of

Table 6
Extent of *Myriophyllum* Infestation at Brown's Creek (acres)

	1978	1984	1988	1989
MS	137	320	1,431	601
Other	42	137	70	1
Total	179	457	1,500	602

myriophyllum. In 1988, only 5 percent of the infested area was without *myriophyllum*; in 1989, all the aquatic plant-infested area contained *myriophyllum*.

Similar data for Connor's Island are given in Table 7. The same basic pattern is present with one exception. After the observed increase and decline, there were still areas of aquatic plants that had not been invaded by *myriophyllum*.

Table 7
Extent of *Myriophyllum* Infestation at Connor's Island (acres)

	1978	1984	1988	1989
MS	448	1,037	1,768	952
Other	43	91	19	87
Total	491	1,128	1,787	1,039

The total area invaded by *myriophyllum* between 1984 and 1988 at Brown's Creek is presented in Table 8. The values (Table 8) represent only those areas in which *myriophyllum* appeared between the 2 years. This information was extracted from the database by formulating a query to search for those locations that contained *myriophyllum* in 1988 and not in 1984. All sites meeting this criteria were considered to have been invaded by *myriophyllum*. Figure 3 depicts these areas of *myriophyllum* invasion between 1984 and 1988 at Brown's Creek. Once these areas were identified, the composition prior to the invasion (e.g., the 1984 composition) could be determined. The greatest expansion of *myriophyllum* was into open-water areas, with smaller areas of existing plant communities being invaded (see Table 8).

Results of the analysis of areas where *myriophyllum* disappeared between 1988 and 1989 are presented in Table 9. These areas were isolated by formulating a query to search

Table 8
Antecedent Composition of Areas Invaded by MS Between 1984 and 1988

1984		1988	
Composition	Acres	Compostion	Acres
Open water	1,068	MS	918
NM	26	MS/NM	92
CH/FA	17	MS/CD	72
NM/FA	12	FA/MS	25
CH	10	MS/HV	9
NM/CD	3	MS/NG	8
FA	2	MS/CH	8
		MS/FA	2
		CH/MS	2
		NG/MS	2

Table 9
Resulting Composition of Areas in Which MS Disappeared Between 1988 and 1989

1988		1989	
Composition	Acres	Compostion	Acres
MS	685	Open water	890
MS/NM	87		
MS/CD	62		
FA/MS	25		
MS/HV	16		
MS/CH	5		
MS/NG	3		

for those locations mapped as *myriophyllum* in 1988 and not in 1989. The locations meeting the criteria were identified, and their composition after the decline was determined. Virtually all the areas showing a decline in *myriophyllum* were designated as open water. This indicates that in Brown's Creek, the decline was not due to the loss of *myriophyllum* from plant associations, but a loss of entire plant populations. In Figure 4, the areas with a decline in *myriophyllum* are indicated.

It was important to identify those areas where *myriophyllum* was present during both years. These areas are shown in Figure 5 and were not affected by the large decline between 1988 and 1989.

The ability to analyze the spatial patterns of aquatic plant species in terms of invasions and declines provides insight into the dynamics of aquatic plant populations. The primary constraints to this approach are finding sites (water bodies) that have experienced substantial invasions or declines and also have sufficient aerial photographic coverage.

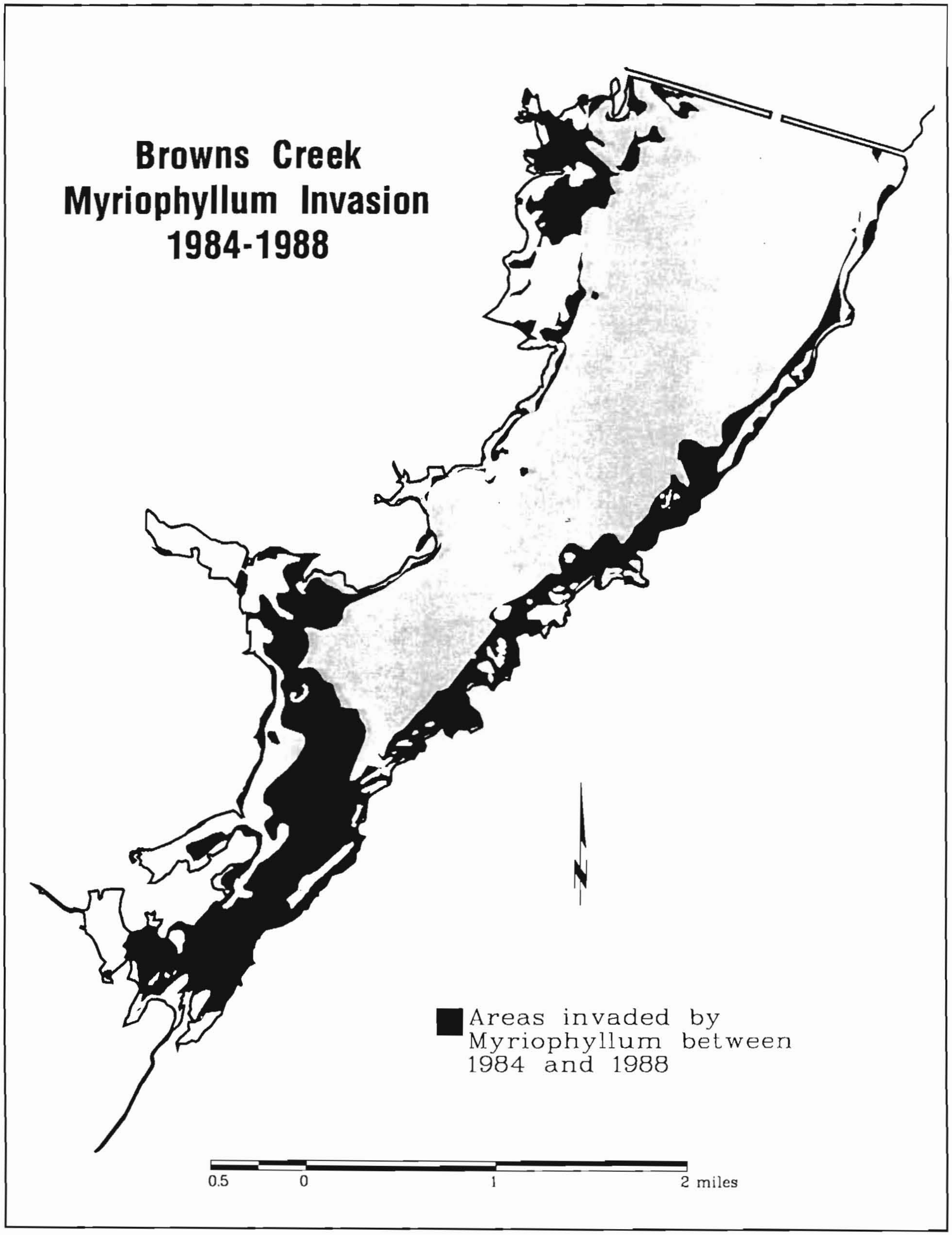

Figure 3. Invasion of myriophyllum between 1984 and 1988 in Brown's Creek

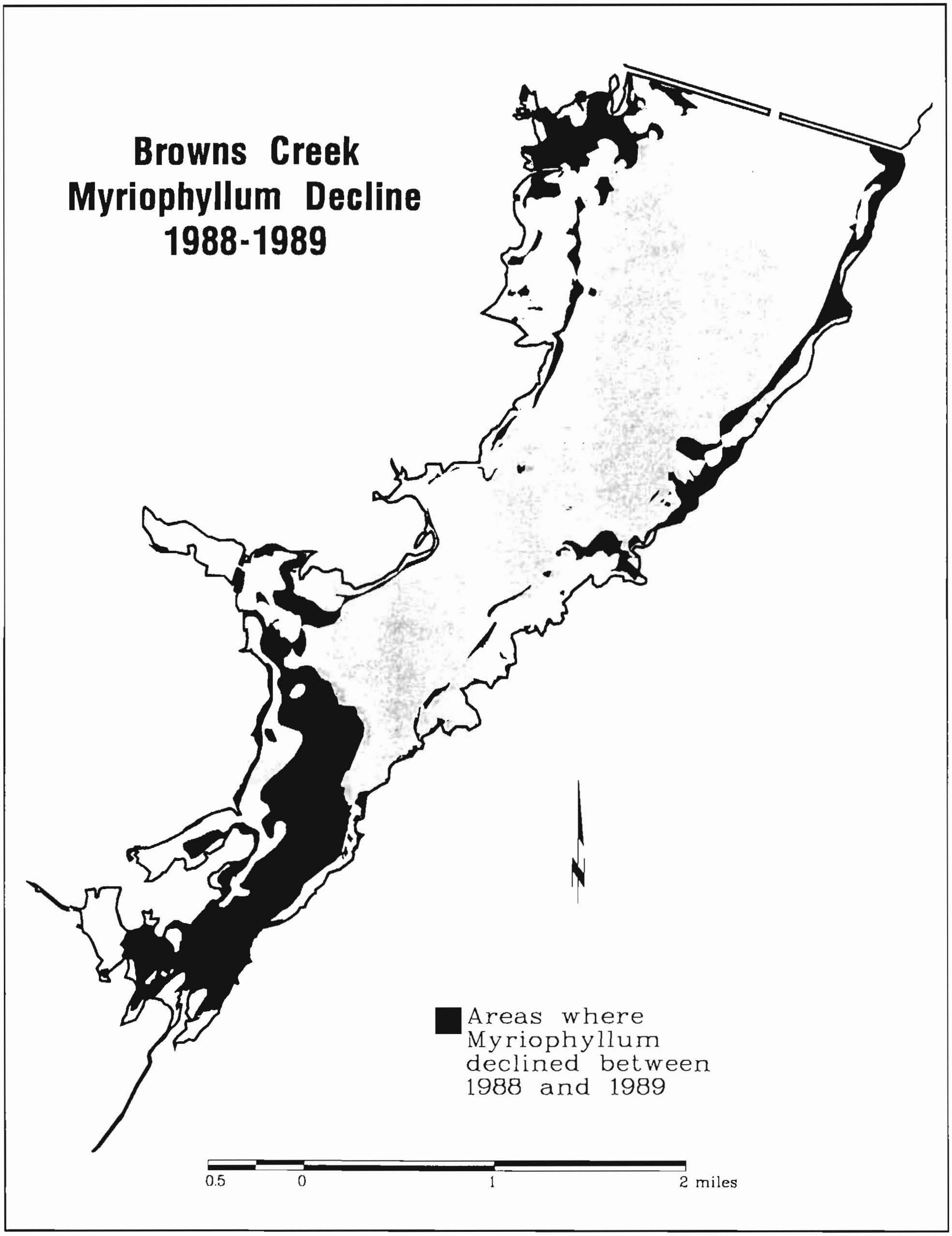

Figure 4. Decline of myriophyllum between 1988 and 1989 in Brown's Creek

monitoring in the vegetated littoral zone (Stations 1 to 6) were positioned at 7-m intervals along the transect (Figure 1). Stations in the pelagic zone (Stations 7 and 8) were located at the 2.5-m (65 m from shore) and the 3.5-m (108 m from shore) depths on the transect. Thermistors (OmniData International), which had been precalibrated to the nearest 0.1 °C, were placed at 25-cm-depth intervals at Stations 1 to 7 and at 25- to 50-cm-depth intervals at Station 8. A weather station was established at Station 3 for measurement of net solar radiation, wind speed and direction, air temperature, and relative humidity. Probes for monitoring weather conditions were positioned on a pole approximately 2 m above the reservoir's surface. Instantaneous values for water and air temperature, wind speed and direction, net solar radiation, and relative humidity were collected at 5-min intervals from 07 to 21 August 1991.

The heat budget model, described by Stefan, Horsch, and Barko (1989), was used to estimate rates of convective exchange (Q) between the littoral and pelagic zones. In general, the model estimates the flux of heat between two adjacent, well-mixed cells located on a littoral slope (Figure 2). Since $h_2 > h_1$, the water temperature in Cell 1 will be less than the water temperature in Cell 2 ($T_1 < T_2$) during cooling periods (i.e., differential cooling). Under these unstable thermal conditions, exchange flows will develop naturally that can be described by changes in water temperature. However, rates of convective exchange determined by this method of calculation should be regarded as only an estimate because the model assumes that the cells are well mixed during convective exchange. However, a weak two-layered exchange system can actually develop (i.e., James and Barko 1991a).

For the model, the littoral zone of the southwest bay in Eau Galle Reservoir was conceptually divided into two adjacent cells, each having a particular geometry and thermal

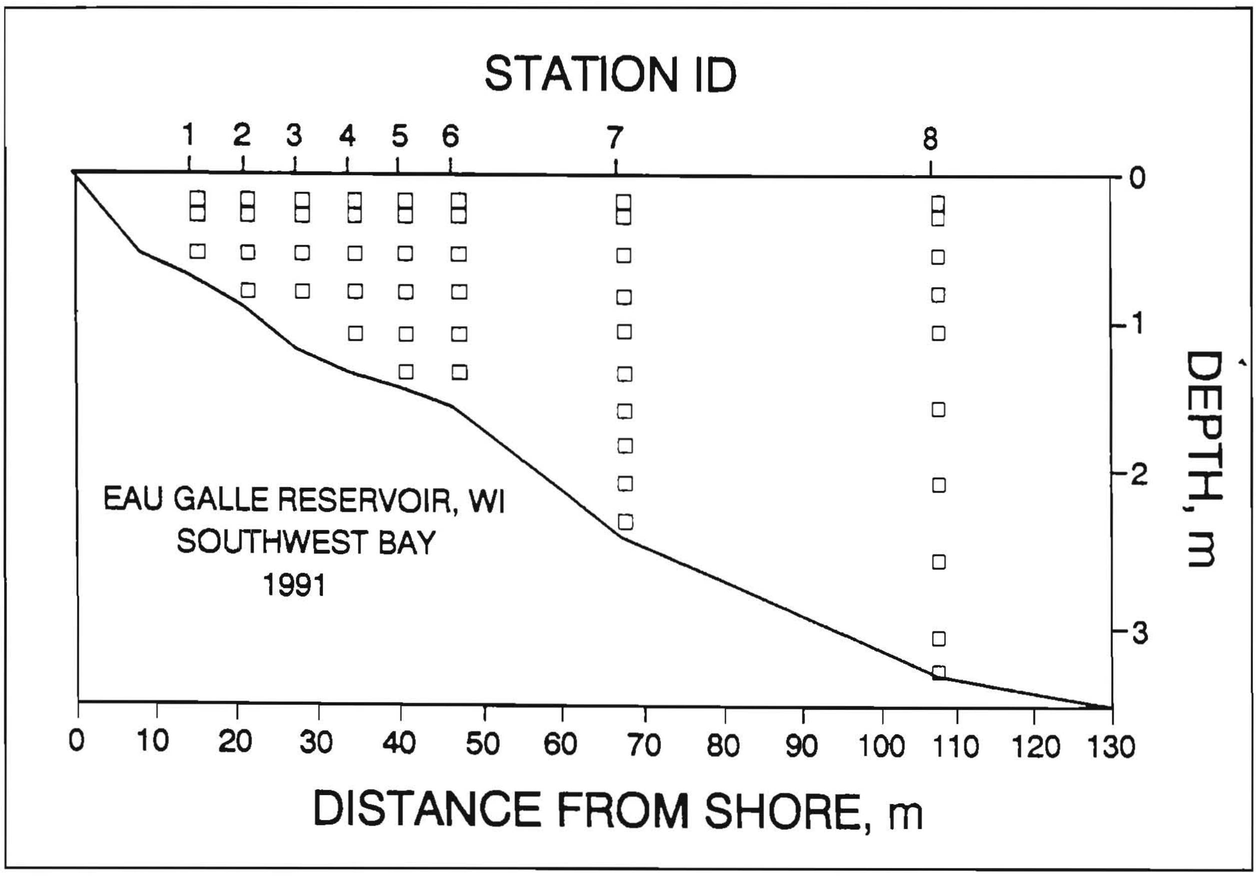

Figure 1. Thermistor locations in the southwest embayment of Eau Galle Reservoir

$$Q = ((A2\ V1\ dT1/dt) - (A1\ V2\ dT2/dt))/((T2 - T1)(A1 + A2))$$

Q = Convective Exchange Rate, $m^3/m\ s$
A = Surface Area, m^2/m
V = Volume, m^3/m (Ah)
T = Temperature, C

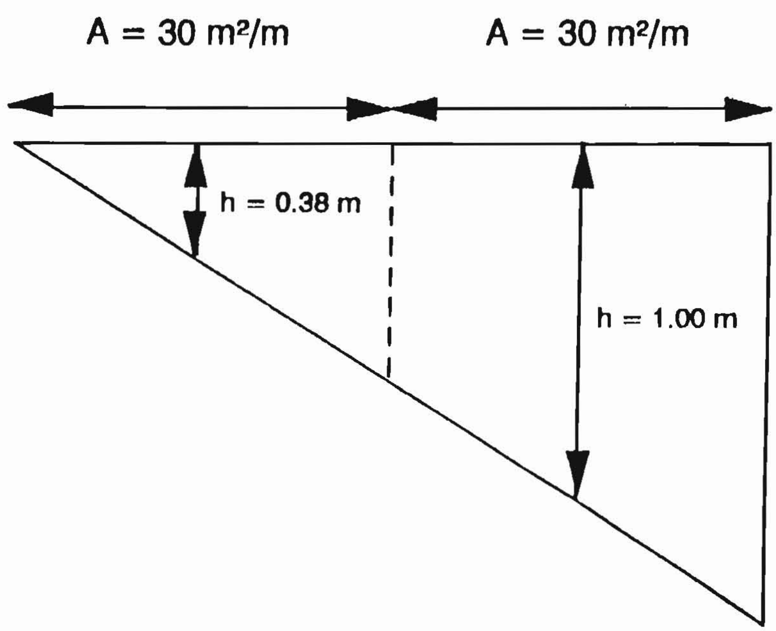

Figure 2. Heat budget equation for estimating convective exchanges during differential cooling and conceptual division of the littoral zone into two adjacent cells

regime (Figure 2). Water temperature profiles from Station 1, located nearest the shoreline, and Station 5, located near the littoral-pelagic interface, were used to represent Cells 1 and 2, respectively. Rates of convective exchange between Stations 1 and 5 were estimated at 5-min intervals throughout the month of August during periods of differential cooling (i.e., at night). We assumed that differential cooling was occurring when vertically integrated water temperatures at Station 1 were less than those at Station 5.

Results and Discussion

An example of weather conditions giving rise to differential cooling and convective exchange occurred during the night of 18-19 August. During this period, air temperatures cooled from a maximum of 20.6 °C at 1300 hours on 18 August to 9.4 °C at 0500 hours on 19 August (Figure 3a). The potential for convective exchange was great, as surface water temperatures cooled much more rapidly at Station 1 than at Station 5 during the night on these dates, resulting in a 1.3 °C differential between the two stations by 0600 hours on 19 August (Figure 3b). With the exception of some minor breezes, wind speeds were zero from 2000 hours on 18 August to 0800 hours on 19 August (Figure 3c). Wind-generated water movement was, thus, probably minor in the embayment during that particular night.

By 0200 hours on 19 August, strong horizontal water temperature gradients had developed between Stations 1 and 5 in the littoral zone as a result of differential cooling (Figure 4a). In the pelagic zone, water temperatures were uniform in the upper 2 m of the water column and stratified vertically below this depth. Movement of cooler littoral water as an underflow toward the pelagic zone was suggested by the strong downward slant of the 22.2 °C contour line at 0200 hours (Figure 4a). Intrusion of the underflow current into the pelagic water column at about the 2-m depth was suggested by the position of the 21.4 °C at 0800 hours (Figure 4b). A return surface flow moving from the pelagic zone toward the littoral zone was clearly evident at 0800 hours, as water temperature contours (ranging from 21.4 to 19.4 °C) in the surface waters slanted toward Station 1 (Figure 4b).

Detailed movement of the underflow down the littoral slope during convective exchange was observed from a time series of vertical water temperature profiles at Stations 1 and 5 during the night of 18-19 August (Figure 5). During the early stages of water column cooling, water temperatures at Station 1 became uniform vertically and cooled steadily between 2000 and 2300 hours (Figure 5). As the night progressed, however, intrusion of cooler water was observed at the bottom of Station 1 at about 2230 hours, as an apparent result of an underflow current that originated upstream of Station 1. The underflow reached Station 5, located 28 m down the transect, about 4 hr later, as suggested by the more rapid cooling of the bottom waters than the surface waters at this station at about 0230 hours (Figure 5). We estimate a flow velocity of 2 mm/s or 7 m/hr, based on this information. From earlier research (James and Barko 1991a, b), we found that the vertical expanse of underflows generated during convective exchanges is ~ 0.25 m. Assuming a similar vertical expanse for the underflow that developed during 18-19 August, we estimate an areal convective exchange rate of 0.0005 m^3/m s.

A mean areal convective exchange rate of about 0.0006 m^3/m s (Figure 6) was calculated from the heat budget model of Stefan, Horsch, and Barko (1989) for the time period 0000-1000 hours on 19 August, which was similar to the areal convective exchange rate estimated from the examination of the time series of vertical water temperature profiles at Stations 1 and 5 for the same date (Figure 5). In general, the heat budget model estimated relatively high areal convective exchange rates during the onset of differential cooling on 19 August (0000 to 0200 hours; Figure 6). Areal convective exchange rates (i.e., at 5-min intervals) then declined to < 0.001 m^3/m s by 0330 hours and exhibited minor fluctuation until 1000 hours on 19 August.

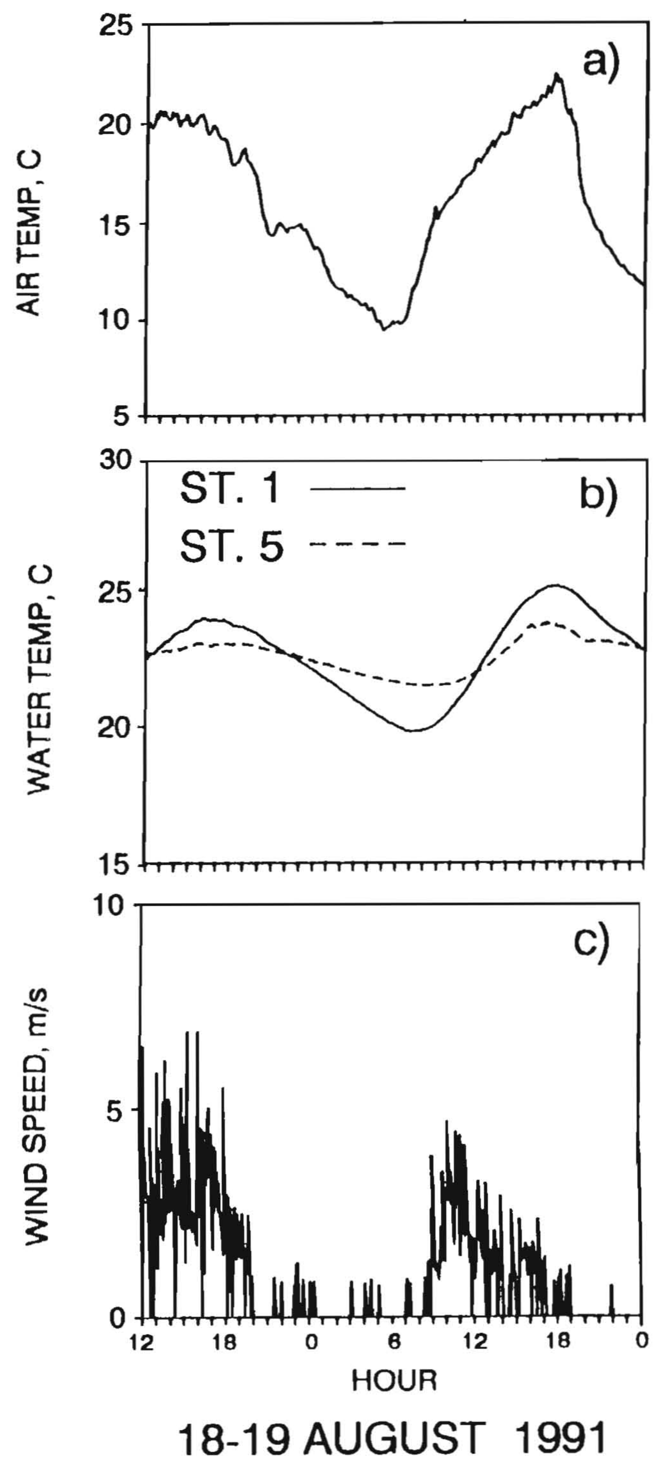

Figure 3. Variations in (a) air temperature, (b) surface water temperature gradients at Stations 1 and 5, and (c) wind velocity between 1200 hours on 18 August and 2400 hours on 19 August 1991

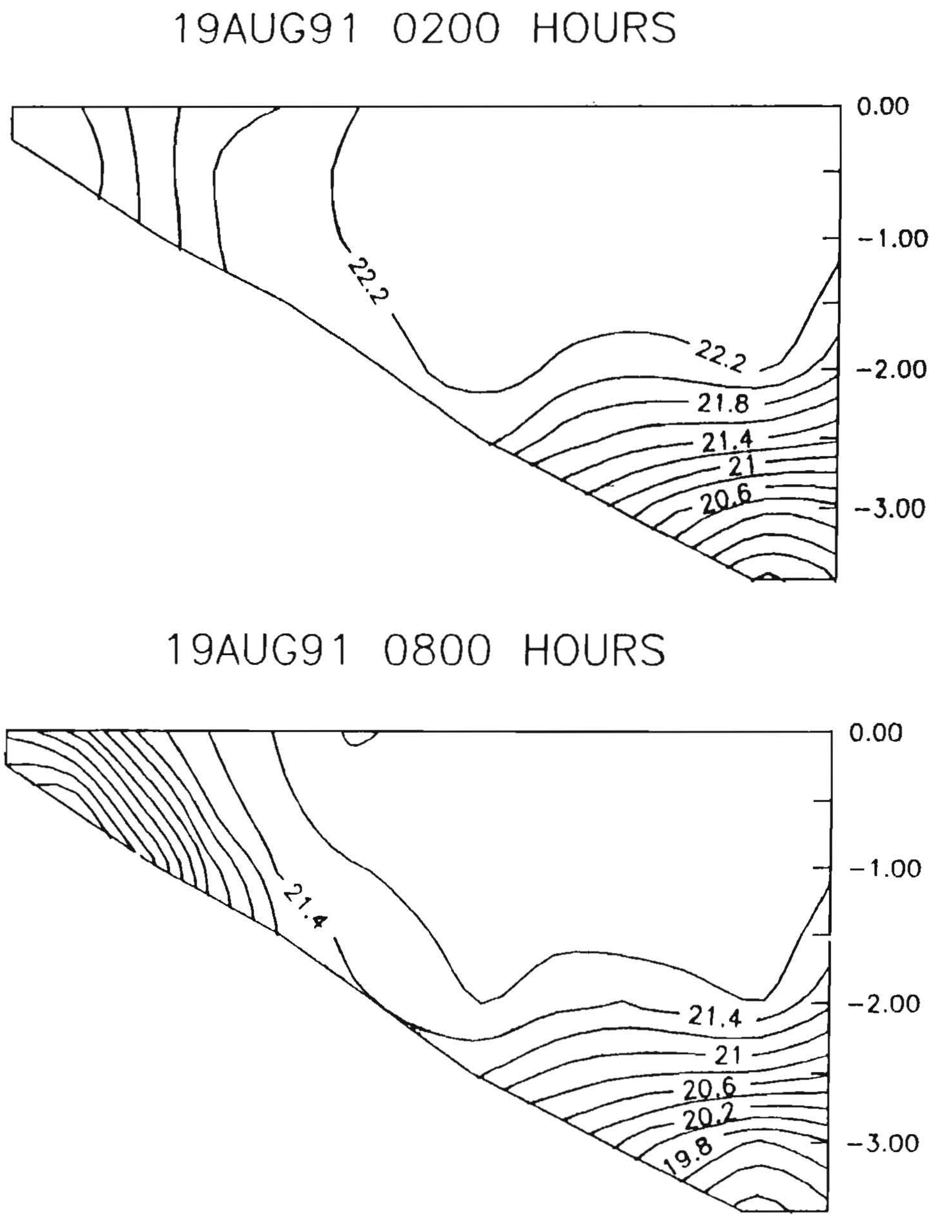

Figure 4. Longitudinal and vertical variations in water temperature (°C) at 0200 hours and 0800 hours on 19 August 1991

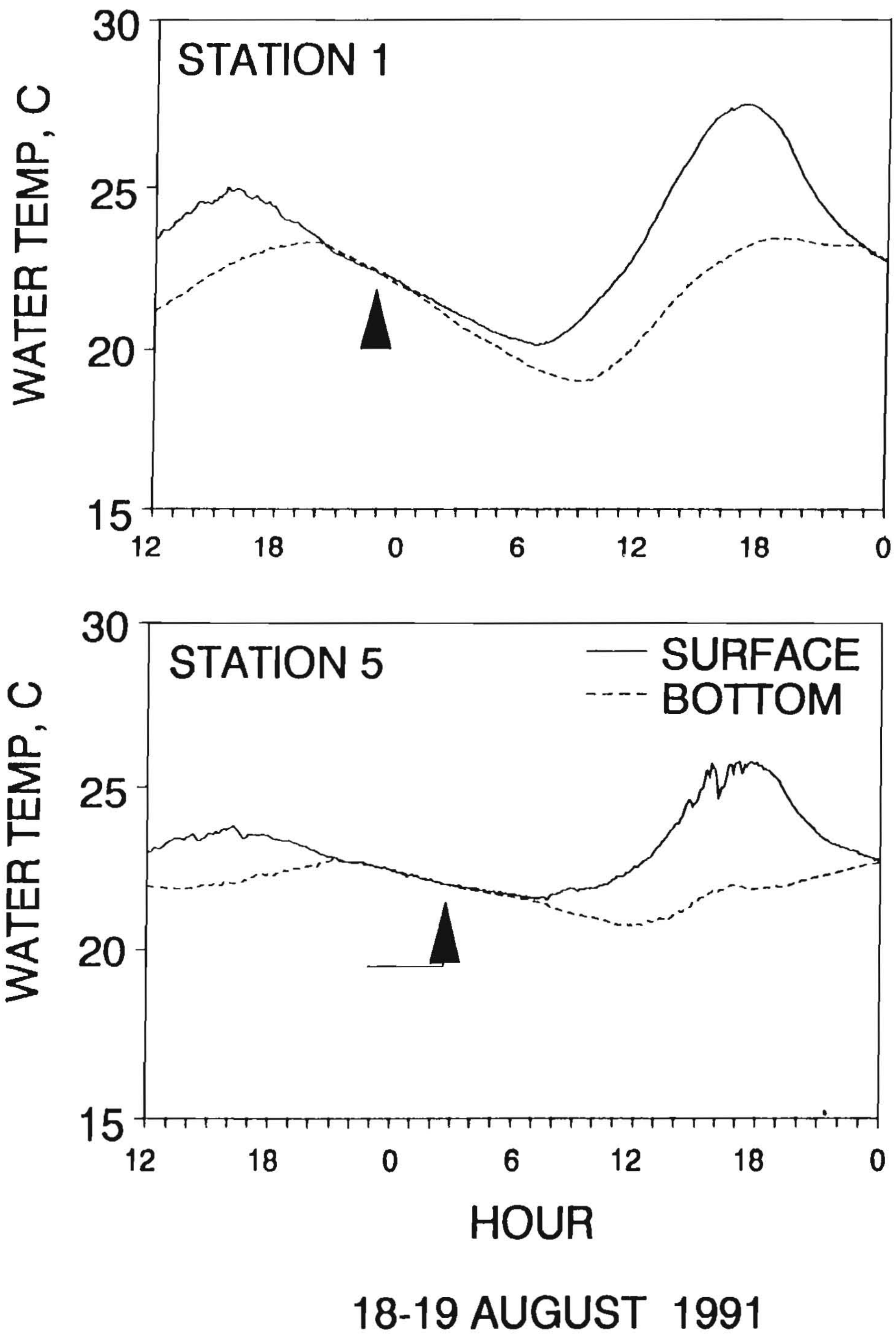

Figure 5. Variations in water temperature at Station 1 (upper) and Station 5 (lower) on 18-19 August 1991

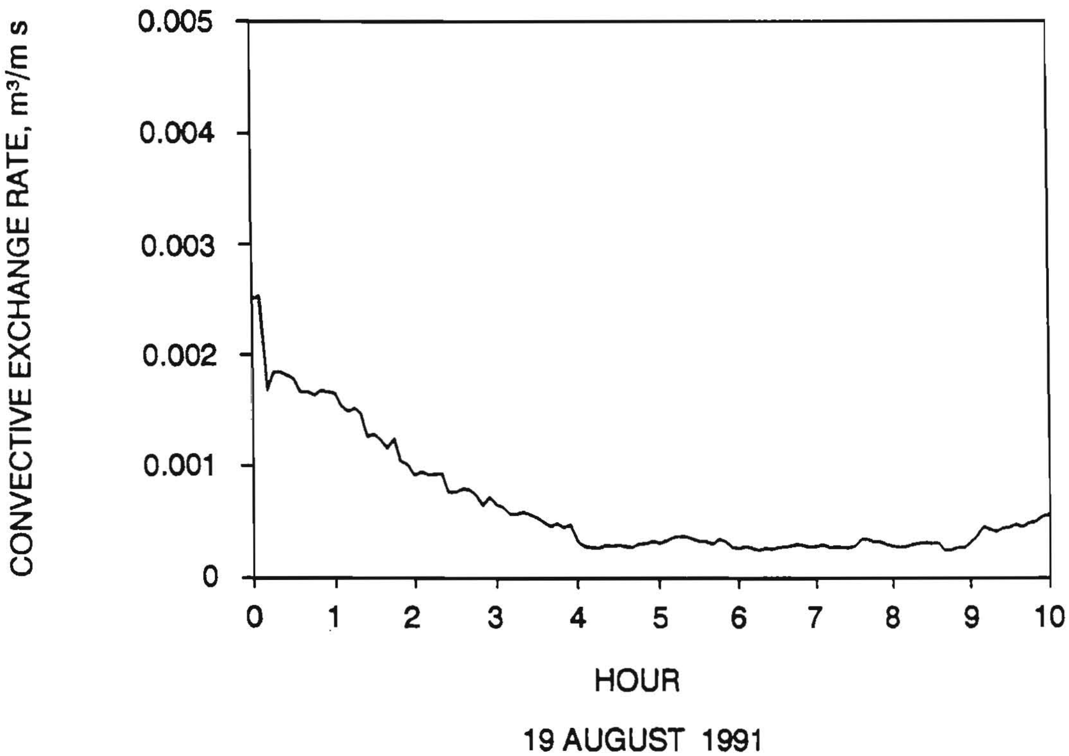

Figure 6. Variations in areal convective exchange rates between 0000 and 1000 hours on 19 August 1991. Rates were smoothed by determining a running average over a 1-hr period at 5-min intervals

We used the heat budget model to estimate mean areal convective exchange rates during periods of differential cooling for nearly the entire month of August (7 to 21 August). Differential cooling occurred on every night of the study, and mean areal convective exchange rates ranged from 0.00047 m^3/m s on 15 August to 0.00088 m^3/m s on 13 August (Figure 7). A grand mean convective exchange rate of 0.00066 m^3/m s was determined for the month of August 1991, which was comparable with the grand mean convective exchange rate of 0.0005 m^3/m s estimated for the summer of 1989 (June - August) using Rhodamine WT dye movement as a surrogate for water exchange (James and Barko 1991b). The similarity in rates between years suggest that the heat budget model provides a good estimate of convective exchange rates for this reservoir.

Wind velocities were essentially zero throughout the nighttime periods during the study, with the exception of the nights of 07 and 26 August 1991 (Figure 8). Thus, convective exchanges were probably the dominant means of horizontal transport in this embayment at night during the month of August. We estimate a theoretical hydraulic residence time of water in the littoral zone of this embayment (i.e., ~ 10,000 m^3) of only about 1 day during these periods of differential cooling and convective exchange, which is much more rapid than the reservoir-wide theoretical residence time of >30 days (James and Barko 1991a, b).

The results of this study have several implications for aquatic plant management. First, convective exchanges need to be considered as a potentially very important mechanism for the horizontal transport of solutes between littoral and pelagic zones. Even in the complete absence of wind, the residence time for water in the southwest embayment of Eau Galle

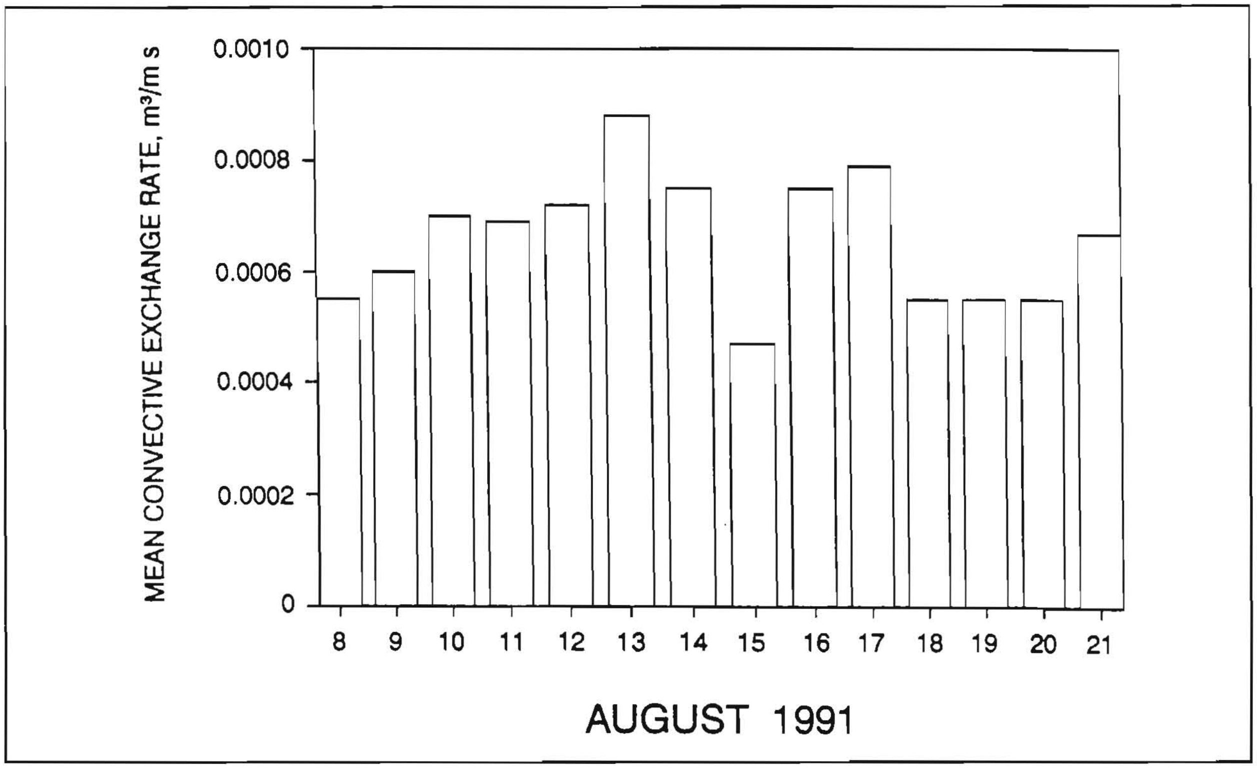

Figure 7. Mean areal convective exchange rate during periods of differential cooling for the period 08 to 21 August 1991

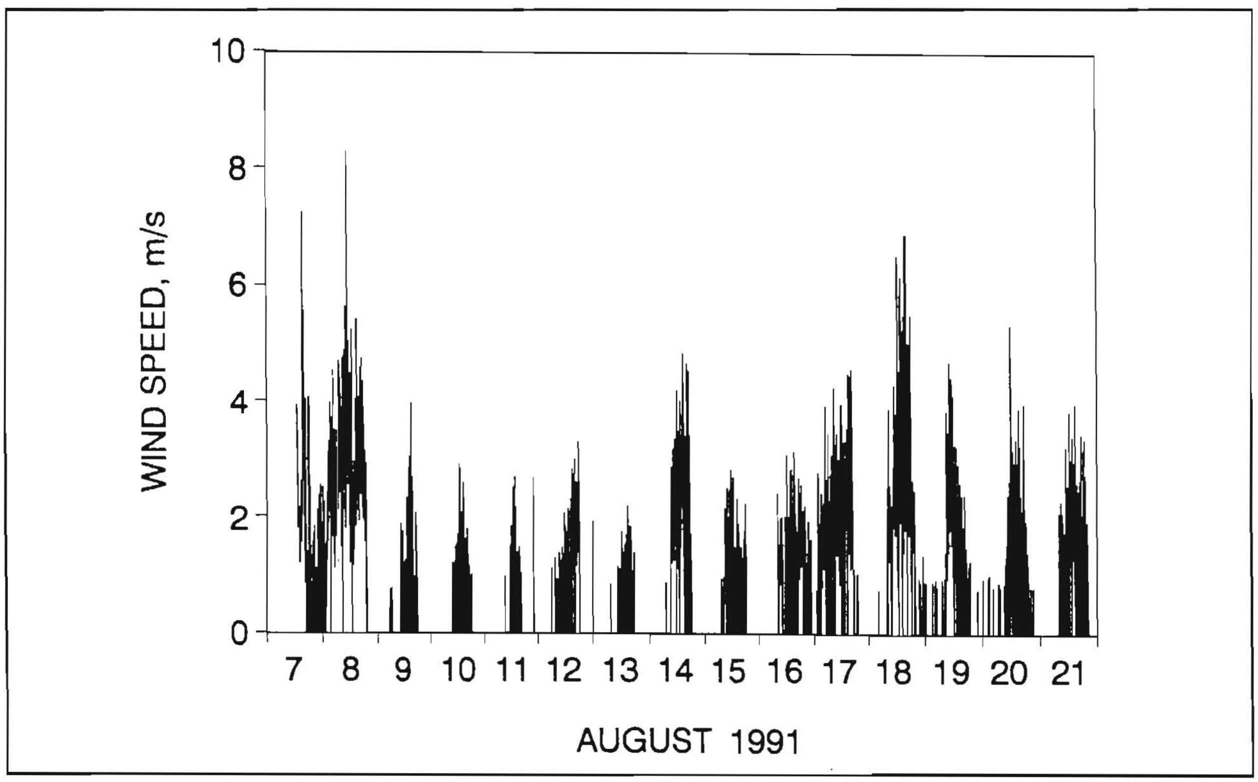

Figure 8. Variations in wind velocity from 08 through 21 August 1991

Reservoir is low because of convective exchanges. Thus, herbicide application strategies must consider these short residence times when evaluating interactions between herbicide concentration and exposure times (Netherland, Green, and Getsinger 1991) and the movement of herbicides from target areas to open-water sites via convective as well as wind-generated water exchange. Second, horizontal movement of soluble nutrients originating from decaying macrophytes to the pelagic zone via convective exchanges may stimulate undesirable algal blooms after a herbicide treatment.

Acknowledgments

We gratefully acknowledge H. Stefan of the University of Minnesota and M. Schneider of the U.S. Army Engineer Waterways Experiment Station for their discussions on convective exchange processes and L. Albrightson, R. Charbonneau, S. Dixen, D. Dressel, A. Niccum, and E. Zimmer for participation in the study.

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Initial Modeling of Hydraulic Exchange Processes

by
Michael L. Schneider[1]

Introduction

The exchange of water between the littoral and pelagic regions of a water body can significantly affect the transport of dissolved constituents. This exchange of mass can have a significant effect on nutrient exchange and the effectiveness of waterborne herbicides used for aquatic plant control. There are several processes that can sustain the circulation of water between the littoral zone and open water. The differential heating and cooling of lakes through the water surface produces horizontal convective exchange flow. A two-dimensional mechanistic numerical model is presented that estimates the horizontal exchange flow driven by a surface heat flux. This model was applied to Minky Creek, a sidearm in Guntersville Lake, AL, and the circulation patterns were calculated for a 24-hr period.

Background

Water exchange from the littoral zone can be initiated by advective currents generated by inflows or outflows, wind, or a surface heat flux. The initiation of internal water movement and water surface fluctuations associated with peaking hydropower releases or tributary inflows can trigger secondary water exchange within the littoral zone. A small change in the water surface elevation in a side embayment can result in a large exchange of water because of the stage-storage relationships of most embayments.

Winds can generate surface and internal currents and waves that can influence the exchange of water in the littoral zone. Surface drift currents can be generated in the direction of the wind in open water. Near the shoreline, the path of water movement becomes much more complex. The magnitude of surface currents can reach about 3 percent of the wind speed, depending upon the fetch, wind speed, direction, duration, and degree of sheltering. Wind-generated short period water waves create turbulence and result in vertical mixing of the water column. Internal waves and seiches can also propagate along the thermocline, resulting in frequent fluctuations in temperature and water movement in the littoral zone. Horizontal density gradients can be generated by the mixing associated with a differentially applied wind field, resulting in the development of a density-driven current.

The variation in depth common in sidearms and embayments of reservoirs can result in differential heating and cooling, which induces horizontal water movement and exchange. The shallower regions in the littoral zone will cool faster than the deeper open water, resulting in an unstable horizontal density gradient. This density instability drives the cold water out of the littoral zone as an underflow moving along the floor of the basin. A surface current returning warmer water to the littoral zone will also develop. If the cold water underflow reaches deeper water, the density structure may force the current to propagate into intermediate depths of similar density. Convection mixing is penetrative, resulting in a thick surface layer and horizontal water exchange that can continue well after the nighttime cooling stops (Monismith, Imberger, and Morison 1990).

A similar but opposite circulation pattern can develop during daytime heating. In this case, the littoral zone will warm faster and flow over the water in deeper reaches. The

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heating of the water enhances stratification, restricts turbulence, and therefore limits the surface current to a very thin layer that is sensitive to wind effects. Differential heating can also be generated by differences in the vertical penetration of light caused by aquatic plants or suspended sediment.

Approach

The focus of this study was to develop a better understanding of circulation patterns in the littoral zone as a result of differential heating and cooling. A numerical model was used to simulate the two-dimensional time-dependent flow and temperature fields in a sidearm subject to heating and cooling. The model was used to approximate the conditions in the Minky Creek embayment located in Guntersville Lake, AL. The simulations of the temperature field were compared with field observations for a 24-hr period during late May 1991.

Numerical Model

There have been several attempts to model the convective circulation in sidearms of lakes. Brocard and Harleman (1980) developed a model of flow in a sidearm of a cooling lake by determining the steady-state integral solution of the conservation equations of mass, momentum, and energy for a two-layer fluid.

Horsch and Stefan (1988) used a numerical model to estimate the flow in a triangular enclosure subject to a fixed heat loss at the water surface. Stefan, Horsch, and Barko (1989) developed a "cells-in-series" model to estimate the exchange of water from the littoral zone in response to surface cooling. Farrow and Patterson (1993) used both asymptotic and numerical solutions of the governing equations to study the diurnal response of a sidearm to heating and cooling. Most of these models have been used for process description or comparison with simple laboratory experiments. In this study, a numerical model was used to simulate the flow field in Minky Creek embayment located in Guntersville Lake, AL, during a 24-hr period in May 1991.

The flow in a sidearm, subject to arbitrary stratification and surface momentum and energy flux, was approximated by the incompressible two-dimensional equations of motion solved in a Cartesian coordinate system. The two-dimensional approximation assumes the lateral component of water exchange to be small relative to the circulation along the major axis of the region. The coupled system of governing equations includes the continuity, vertical and longitudinal momentum, and energy equations (Paktankar 1980). These equations are solved to obtain the time-dependent velocity, pressure, and temperature fields. The variation in the density field was small, such that the Boussinesq approximation could be applied.

A finite volume method was used to discretise the governing equations on a rectangular domain with 34 cells in the y- (vertical) direction and 46 cells in the x- (longitudinal) direction. The model reproduced the topography along the major axis of Minky Creek embayment, with the depth varying from 0.16 m at the headwaters of the embayment to almost 6.0 m at the entrance. A unit width was applied throughout the domain. A hybrid scheme was used to calculate the flux associated with both the convective and diffusive terms. The SIMPLER algorithm (Paktankar 1980) was used for solving the system of equations, with careful attention given to mass conservation. All simulations were performed with a eddy viscosity of 10^{-3} kg m/sec and a Schimdt number of 1000.

The driving mechanisms for the calculated exchange of water in this embayment were the applied surface fluxes for momentum and energy. The model used measured meteorologic data to estimate these surfaces fluxes. The surface flux of momentum, sensible heat, and latent heat are computed from bulk aerodynamic formulae. The absorption of incoming shortwave radiation was assumed to follow Beer's Law. The flux of long-wave radiation was governed by the law of Stefan-Boltzmann. Henderson-Sellers (1984) presents a summary of the empirical representation of surface fluxes used by the model.

The water surface was assumed to remain fixed (rigid-lid), with no mass exchange through any of the flow domain boundaries. The initial velocity field was assumed to be at rest, and the solution marched forward in time. The initial thermal conditions throughout Minky Creek embayment were estimated through linear interpolation between the five stations located along the major axis.

Study Area

Guntersville Reservoir is a hydropower and flood control impoundment located on the Tennessee River in northeastern Alabama operated by the Tennessee Valley Authority. Minky Creek embayment is a shallow embayment located near Town Creek about 16 miles upstream of Guntersville Dam. The plan-view form of Minky Creek embayment is nearly V-shaped, with the major axis roughly 2,000 m long and the mouth of the creek 1,000 m wide. The maximum depth in Minky Creek of 6 m was located at the mouth of the embayment. The bottom slope along the major axis averages almost 0.0036 m/m in the northern half of the embayment and 0.0024 m/m in the southern half. During the study period, there was no submersed vegetation in the Minky Creek embayment and the flow in Minky Creek insignificant.

Water temperatures and meteorologic conditions were monitored in Minky Creek embayment as described in Smith, James, and Barko (1993). A total of eight water temperature stations were located throughout the embayment monitoring vertical temperature profiles every half-hour. Wind velocity and direction were measured every half-hour at the mouth of the embayment and at the shallowest station near the entrance of Minky Creek. The air temperature, relative humidity, and irradiance were measured every half-hour at an interior station. Strong temperature gradients along the longitudinal axis of Minky Creek embayment were frequently observed, indicating the presence of differential heating and cooling. However, temperature data along the lateral axis demonstrated little variation in thermal properties. This observation supports the model's representation of the embayment as two-dimensional.

Results

The water exchange in the Minky Creek embayment for May 28-29, 1991, was simulated by the numerical model. This date was chosen to correspond with the detailed analysis of temperatures and meteorologic conditions presented in Smith, James, and Barko (1993). The initial thermal conditions for the numerical simulations corresponded to observations at 8 p.m. on May 28, 1991, when surface water temperatures near the headwaters of Minky Creek embayment (28.1 °C) were almost 1.8 °C warmer than conditions near the entrance. The warmer temperatures in the shallower portions of Minky Creek were the result of differential heating the previous day. The thermocline was located at about 3 m, resulting in weak stratification throughout the shallower half of the embayment. During the next 5-hr, air temperatures remained around 23 °C; the speed and direction wind were variable, averaging around 1.6 mph. A weak surface current developed in response to the horizontal gradient in density directed toward the entrance of the embayment. In response, a return flow developed, transporting colder water from deeper portions of Minky Creek embayment into the shallower portions. The surface temperatures continued to cool at a constant rate during this period. The calculated circulation pattern during this period is shown in Figure 1a.

During the remainder of the night, the circulation in the littoral zone was dominated by vertical mixing driven by surface cooling. The air temperature continued to decrease, reaching a minimum air temperature of 21.2 °C just before dawn. Surface cooling results in a density inversion than breaks apart as a plunging negatively buoyant plume. The cold surface water is replaced by buoyant warm water and the process repeated. The temperatures in the littoral zone cooled off at a greater rate than in deeper water because of the volummetric differences. The differential cooling during this period resulted in a slight longitudinal

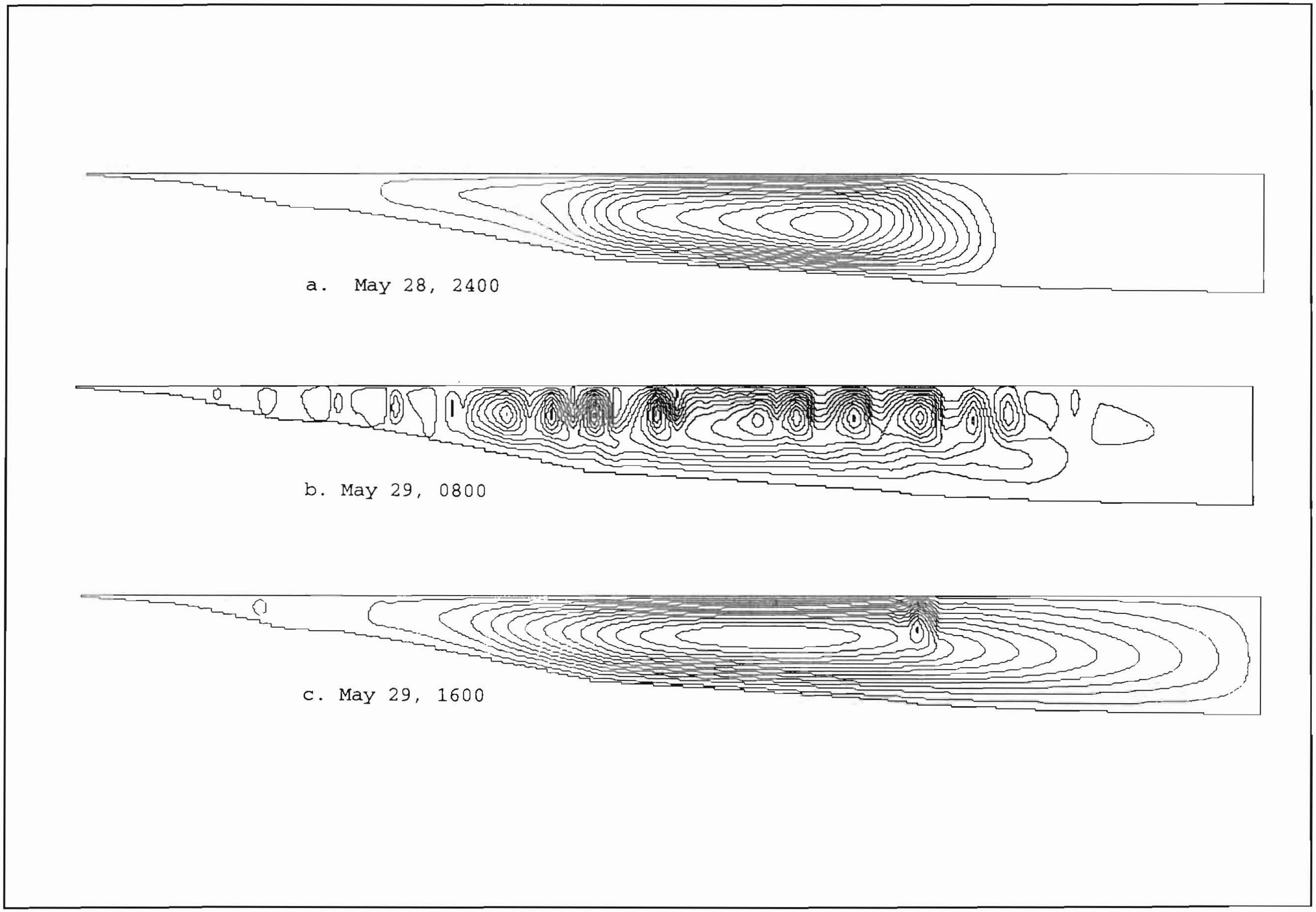

Figure 1. Calculated circuation patterns in Minky Creek embayment

density gradient. However, this gradient was not strong enough to overcome the vertical circulation pattern. Most of the transport of mass during this period was limited to small circulation cells scaling with the depth of the embayment (Figure 1b).

The circulation patterns in the embayment were dominated by differential heating and wind-driven currents after daybreak. The radiant heating resulted in stable thermal profiles throughout the embayment, resulting in the dissipation of large-scale vertical convective mixing events. The wind speed increases significantly during the first several hours of daylight. By the early afternoon, a density-driven surface current directed from the littoral zone into open water had developed (Figure 1c). A steady wind directed along the major axis of the embayment towards the shallow end acted against the surface current and slowed the arrival of warmer water to the entrance of the embayment.

The calculated thermal conditions in the littoral zone and near the mouth of the embayment were compared with observed conditions throughout the 24-hr period. The model closely reproduced the diurnal variation of temperature in the littoral zone at a depth of 0.5 m as shown in Figure 2. The model closely simulated the 2.5 °C cooling during the nighttime hours. The rapid warming during the midday hours on May 29 was also closely reproduced. The model slightly overpredicted the amount of warming during the late afternoon on the May 29.

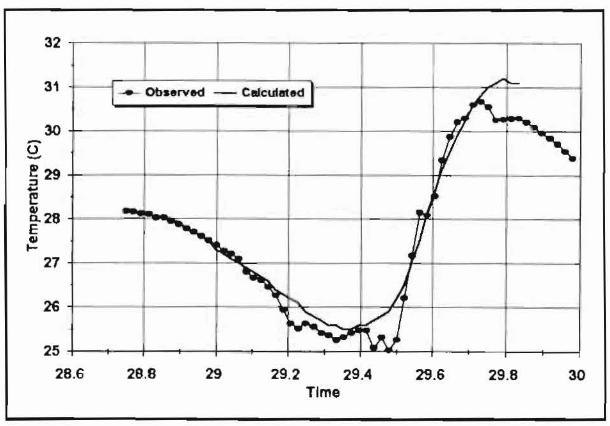

Figure 2. Observed and calculated temperatures in the headwaters of Minky Creek embayment

The observed temperature patterns at the mouth of Minky Creek embayment were also reasonably reproduced by the model as shown in Figure 3. The amount of cooling during the night was less than 0.5 °C. The mixed layer depth extended beyond the depth of 2 m. The surface temperature in the littoral zone was only slightly cooler than at the mouth of the sidearm during the night, providing little opportunity for a horizontal exchange flow to develop. The model slightly overpredicted the warming in the surface layer. The thermal characteristics at a depth of 2 m remained nearly constant throughout the simulation period. The variability of observed temperatures at a depth of 4 m was greater than the other two locations and probably reflects the propagation of internal waves generated in the main body of the lake.

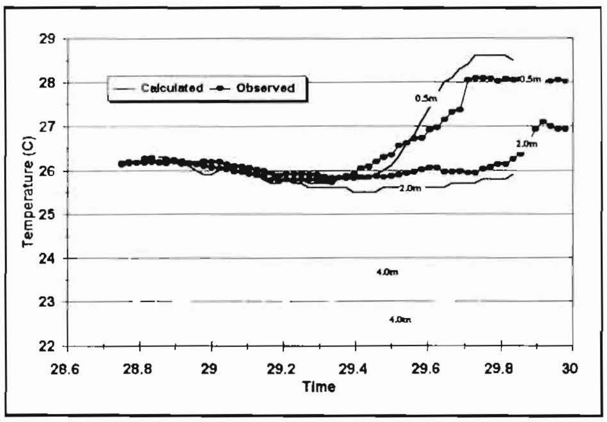

Figure 3. Calculated and observed temperature at the entrance to Minky Creek embayment

Conclusions

The detailed circulation and thermal patterns in Minky Creek embayment on May 28-29 were studied by applying a two-dimensional numerical model. The circulation patterns during this period were dominated by differential heating and wind. The warm atmospheric conditions limited the amount of cooling during the nighttime hours. Convective circulation developed shortly after midnight and became most intense at daybreak. These circulation patterns mainly contributed to the vertical exchange of water. The rapid heating of the embayment resulted in a density-driven surface current directed into deeper water. The afternoon winds opposed this surface current slowing the arrival of warmer surface water at the entrance to the Minky Creek embayment. This approach can be used to help characterize the processes resulting in the exchange of water from the littoral zone.

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Estimation of Water Movement in Submersed Aquatic Macrophyte Beds by Gypsum Dissolution

by
Harry L. Eakin[1]

Introduction

The capability to estimate flow rates within submersed aquatic vegetation (SAV) beds is of fundamental importance to understanding how SAV influences such ecologically important processes as water circulation, sedimentation, and nutrient or contaminant transport in lotic and lentic environments. Most contemporary methods of flow rate determinations use metering devices having either mechanical or electrical sensors. Nevertheless, the resolution of these types of devices has proven insufficient in the relatively quiescent low-energy environments of SAV beds (Getsinger, Green, and Westerdahl 1990).

Methods of flow rate measurement in SAV beds, other than metering devices, have included tracking water parcels labeled with fluorescent dye (Getsinger, Green, and Westerdahl 1990; Fox et al. 1991), flowmeters indexed to the dissolution of NaCl tablets (Madsen and Warncke 1983), and also dissolution of gypsum cylinders (Petticrew and Kalff 1991, 1992). Yet, there appear to be significant disadvantages associated with fluorescent dye tracking and NaCl flowmeters. Dye tracking is expensive, requiring costly equipment and instrumentation, technical labor support, and supplies (e.g., Rhodamine WT dye). NaCl flowmeters were developed primarily for microscale use around individual plant shoots and leaves in examinations of plant metabolism. Flow measurement by dissolution of gypsum cylinders, however, appears to provide versatility and reliability at minimal expense, considerations important to most ecological investigations.

The attributes of flow measurement by gypsum cylinder dissolution important to ecologists may also benefit aquatic plant managers involved in aquatic herbicide applications. It is generally known that herbicide efficacy in aquatic environments is largely dependent upon three factors: the type of herbicide, the concentration of the effective compound, and time of contact with the targeted plant. The latter two factors are directly influenced by flow rate. Consequently, to achieve maximum efficiency in aquatic herbicide applications, managers must be knowledgeable of the rate of water movements within targeted plant stands. Determining water movements by using gypsum cylinders may provide aquatic plant managers with a means to achieve efficient herbicide applications with much less effort and expense.

Petticrew and Kalff (1991) established gypsum flux, i.e., weight loss per unit time, relationships for use in north-temperate regions. However, the ability to extrapolate these relationships to higher water temperatures and velocities (e.g., >25 °C and >7 cm/sec, respectively) cannot be assumed. The objectives of this study were to develop gypsum flux relationships for elevated water temperatures and velocities and conduct field testing of the method for practicality in a warmer climatic region, specifically the Southeastern United States.

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

Materials and Methods

Cylinder preparation

Cylinders were constructed, using calcined gypsum ($CaSO_4 \cdot \frac{1}{2}H_2O$) commercially available as #1 Moulding Plaster from United States Gypsum, Chicago, IL, by mixing 100 g gypsum with 70 mL distilled water and pouring mixture into cylindrical polyvinyl chloride (PVC) molds (4.0-cm-diam by 10.2-cm-height). Each mold was slotted lengthwise to facilitate removal of the gypsum after curing. Molds were readied for the gypsum mixture by tightening screw-type hose clamps placed near each end to sufficiently close the lengthwise slot, then sealing one end with a #9 rubber stopper. Immediately after the gypsum mixture was poured into each mold, a 10-cm carriage-type bolt, threaded about 5 cm through a hole centered on a 5.1- by 5.1- by 0.64-cm Plexiglas plate, was inserted head down into the mixture at the open end. The molds, containing the gypsum mixture, were positioned vertically and allowed to stand for 2 hr. After removal from the molds, each gypsum cylinder was dried for 48 hr at about 39 °C, not to exceed 40 °C, and weighed (±0.0005 g). Following each calibration or field exposure, the cylinders were dried and weighed as before. Gypsum weight loss was determined by difference.

Relying on the relationship between gypsum weight loss per unit surface area per unit time and percent weight loss per unit time established for similarly constructed gypsum cylinders[1] (Figure 1), all flux relationships presented herein are expressed simply as percent weight loss.

Cylinder calibration

Flux relationships were determined at differing water velocities, temperatures, and exposure times under laboratory conditions at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS. Triplicate cylinders were exposed to water velocities of 0, 5, and 15 cm/sec and water temperatures of 10, 22.5, and 35 °C for periods of either 24, 48, 72, or 96 hr. All static, i.e., 0 velocity, calibration runs were performed in a 40-L aquarium, while calibration runs at 5 and 15 cm/sec were performed in a Living-Stream oval recirculating flume (Frigid Units, Inc., Toledo, OH). The flume channel was about 15 m (center line of channel) in length by 32 cm wide and 30 cm deep. Constant water velocities were maintained by an electric boat motor. Turbulent flow produced by the propeller was lessened by a series of baffles establishing a homogenous flow within the channel prior to reaching the gypsum cylinders. To ensure constancy of flow, water velocities were monitored with a Marsh-McBirney Model 2000 portable velocity meter equipped with an electromagnetic sensor positioned near the cylinders at the center of the channel and middepth. Water temperatures in both calibration vessels were maintained to ±1 °C of each targeted temperature by Remcor temperature controller/ circulators. To prevent possible reductions in dissolution of gypsum during calibration runs, water from each vessel was drained between runs to ensure concentrations did not exceed 15-percent saturation.

Field testing

In situ estimations of flow rates were conducted in Mud Creek Cove, Lake Guntersville, AL, during August 1993. Gypsum cylinders were deployed at six sites in and about a dense *Myriophyllum spicatum* bed (Figure 2). Sites 1 and 2 were located entirely within the topped-out plant bed. Sites 3, 5, and 6 were located in a transitional zone between the topped-out plant bed and the open water. Within the transitional zone, SAV was present, although sparse and below the water surface. Site 4 was totally within the open water having no SAV.

A deployment apparatus, consisting of a 4-cm ID by 3-m length of PVC pipe, was designed to place cylinders at selected depths in

[1] Unpublished Data, 1993, Robert F. Gaugash, National Biological Survey, Environmental Management Technical Center, Onalaska, WI.

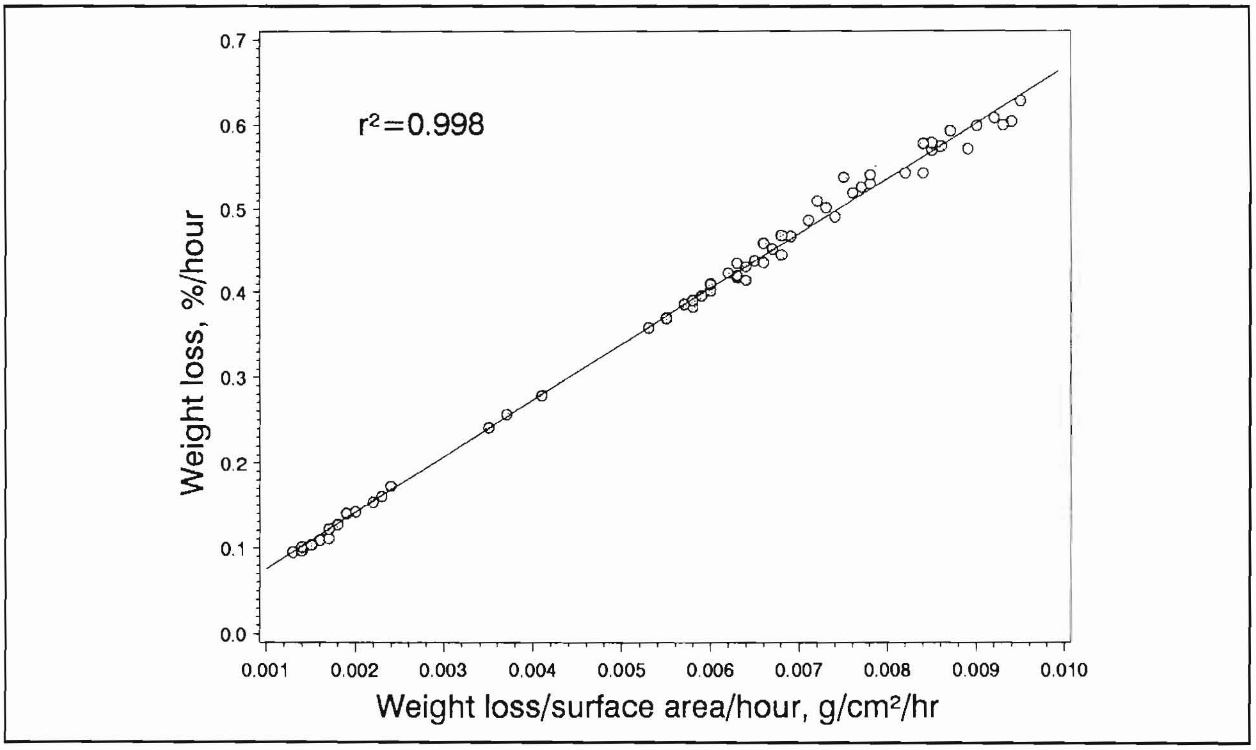

Figure 1. Relationship between gypsum weight loss, grams/square centimeter/hour, and gypsum weight loss, percent/hour

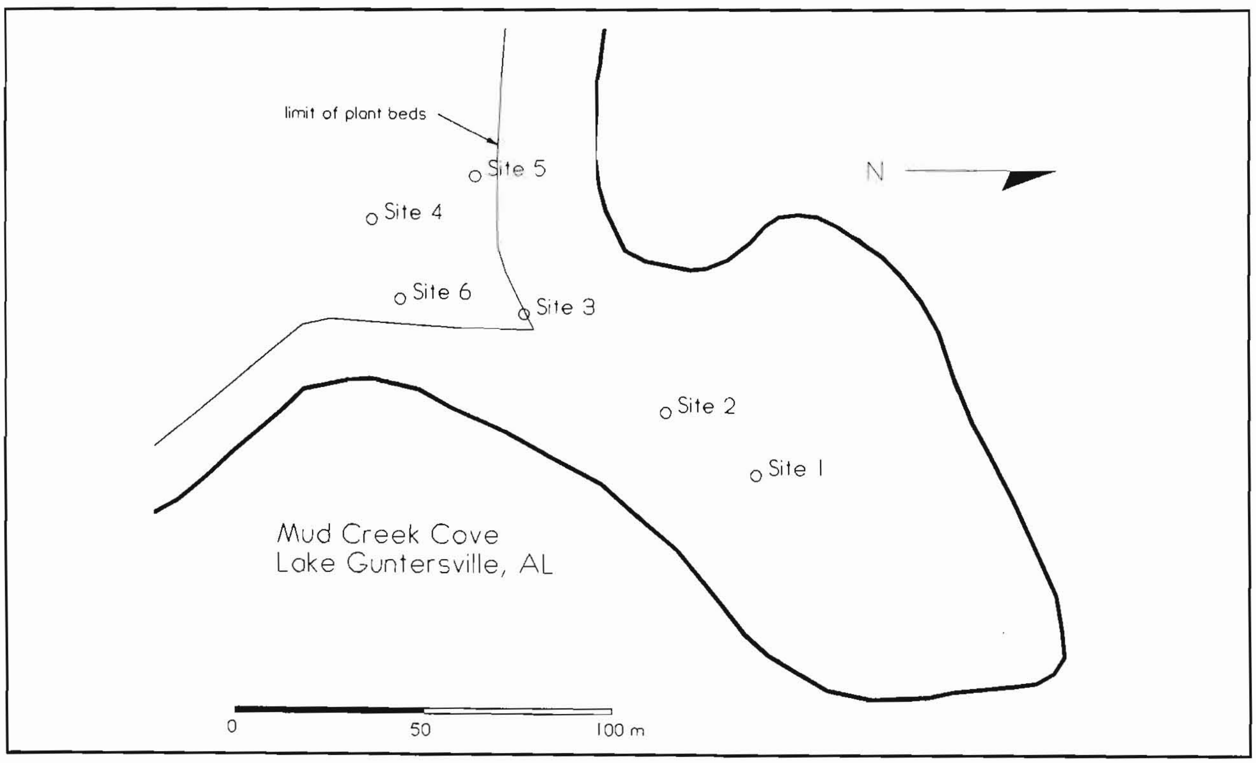

Figure 2. Gypsum cylinder deployment sites in Mud Creek Cove, Lake Guntersville, AL, during August 1993

relation to the water column by means of adjustable brackets. Each cylinder was separated by an angle of approximately 120 deg. The retrievable apparatus was lowered into position over a 2.8-cm-OD steel pipe previously driven vertically into the sediment at each site. Cylinders were positioned 25 cm above the sediment and 25 cm below the water surface at each site. Water depths were different for each site, ranging from 0.85 m at Site 1 to 1.75 m at Site 4. Cylinders remained in place for about 55 hr. Upon retrieval, each cylinder was carefully placed in a resealable plastic bag, stored in packaging material to prevent breakage or chipping, then transported to WES for processing.

Results and Discussion

Gypsum flux, expressed as percent weight loss, increased linearly with exposure time for each flow rate examined (Figures 3, 4, and 5). Regression analyses indicated highly significant relationships between flux and exposure time (minimum $r^2 > 0.96$, $p = 0.01$). These results are in general agreement with flux relationships established by Petticrew and Kalff (1991), although the cylinder dimensions, flow rates, temperatures, and method of calculating flux, i.e., weight loss (percent/hour) versus (grams/square centimeter/hour), were different for this study.

Clearly, flux-flow relationships are linear for given temperatures (Figure 6). However, the ability to predict flows using gypsum flux data for any temperature requires the quantification of the effects of temperature on gypsum cylinder dissolution. This can be accomplished using the general equation of flux-flow relationships established by Petticrew and Kalff (1991)

$$u = (F - A_T) / B_T$$

where the predicted value of flow (u) is dependent on the contribution of zero flow (A_T) and nonzero flow (B_T) at a given temperature T.

Derivation of A_T is dependent upon the slope and intercept of flux-temperature relationships at zero flow. Linear regression analysis of mean values of flux for temperatures 10, 22.5, and 35 °C were used to determine

$$A_T = 1.64 \times 10^{-3} + 3.13 \times 10^{-2} \times T$$

$$r^2 = 0.99, n = 9$$

Whereas, the slopes of each nonzero flux-flow relationship were used to determine B_T. Flux-flow relationships for each nonzero flow were analyzed by linear regression against temperature. The resultant slopes of each flux-flow relationship were then analyzed by linear regression temperature to determine the slope and intercept used to determine

$$B_T = 0.87 \times 10^{-3} + 1.22 \times 10^{-3} \times T$$

Thus, if both the temperature and gypsum flux is known for zero and nonzero flows, predicted flows can be easily calculated using the equations described above.

In situ estimations of flow integrated over 55 hr were found to be somewhat variable within the Mud Creek Cove study sites. Flows at Sites 1 and 2 were estimated at 0.18 cm/sec and 0.21 cm/sec, respectively. Estimated flow at Site 6 was 0.85 cm/sec and was the highest of all sites measured. Flows at Sites 3, 4, and 5 were estimated at 0.27, 0.56, 0.29 cm/sec, respectively. Flow estimates by gypsum dissolution were generally less than those determined during fluorescent dye tracking experiments within Mud Creek Cove in the summer of 1993 (unpublished data).

Gypsum cylinders can provide an inexpensive tool for making flow estimates in various aquatic environments. However, further study is needed in both lotic and lentic environments for comparisons with existing flow measurement techniques. Continued emphasis should be placed on measurements within low-energy environments such as macrophyte beds.

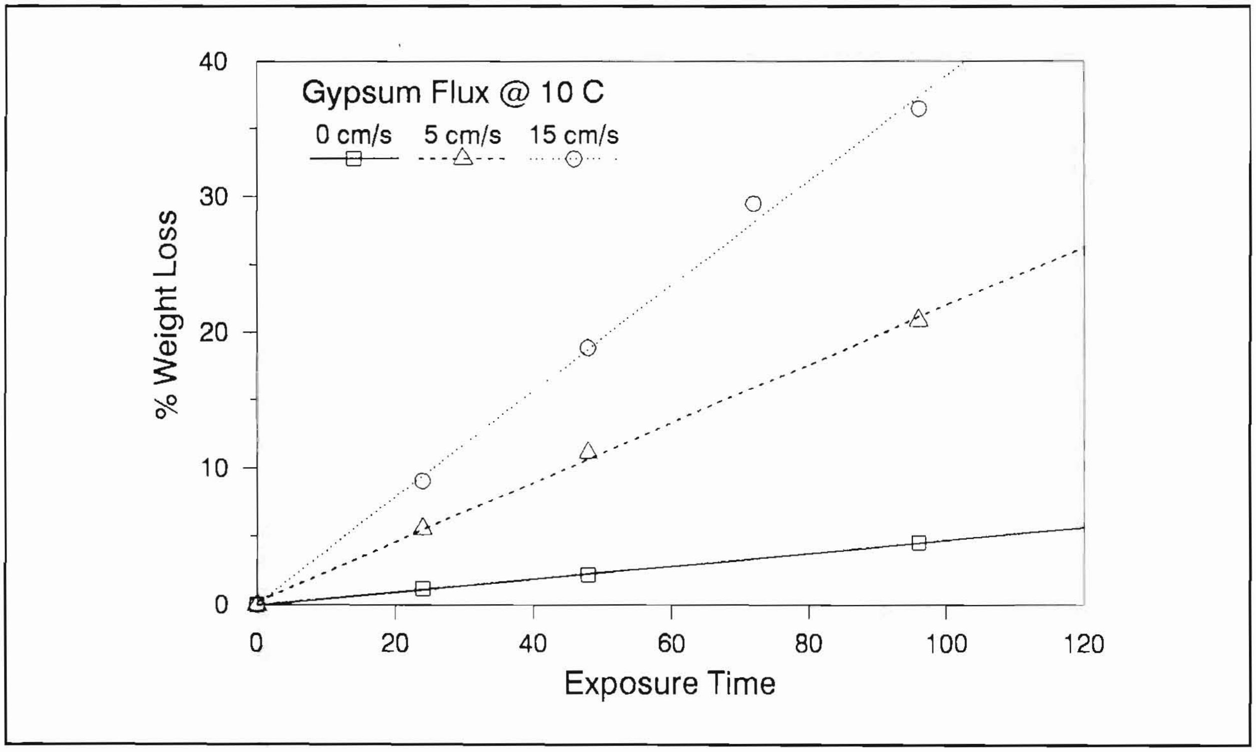

Figure 3. Gypsum flux relationships for 0-, 5-, and 15-cm/sec flows at 10 °C. Each datum represents mean flux (n = 3)

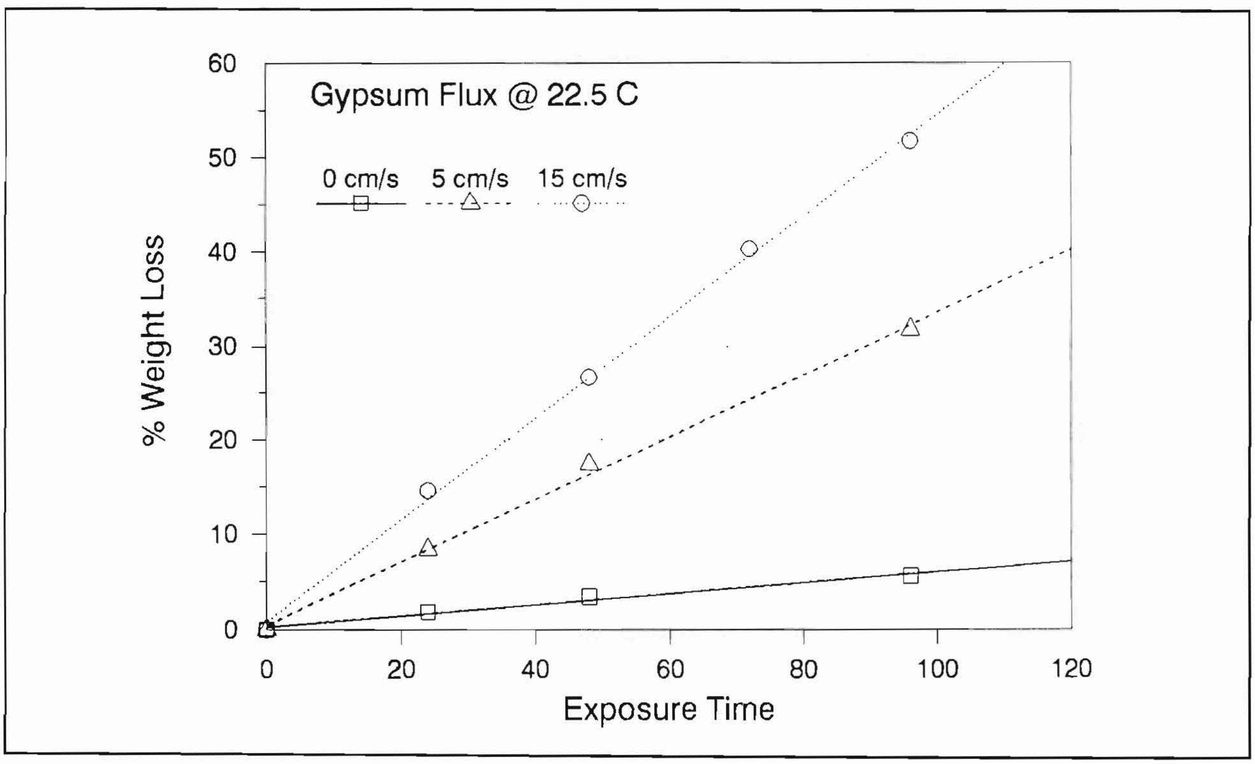

Figure 4. Gypsum flux relationships for 0-, 5-, and 15-cm/sec flows at 22.5 °C. Each datum represents mean flux (n = 3)

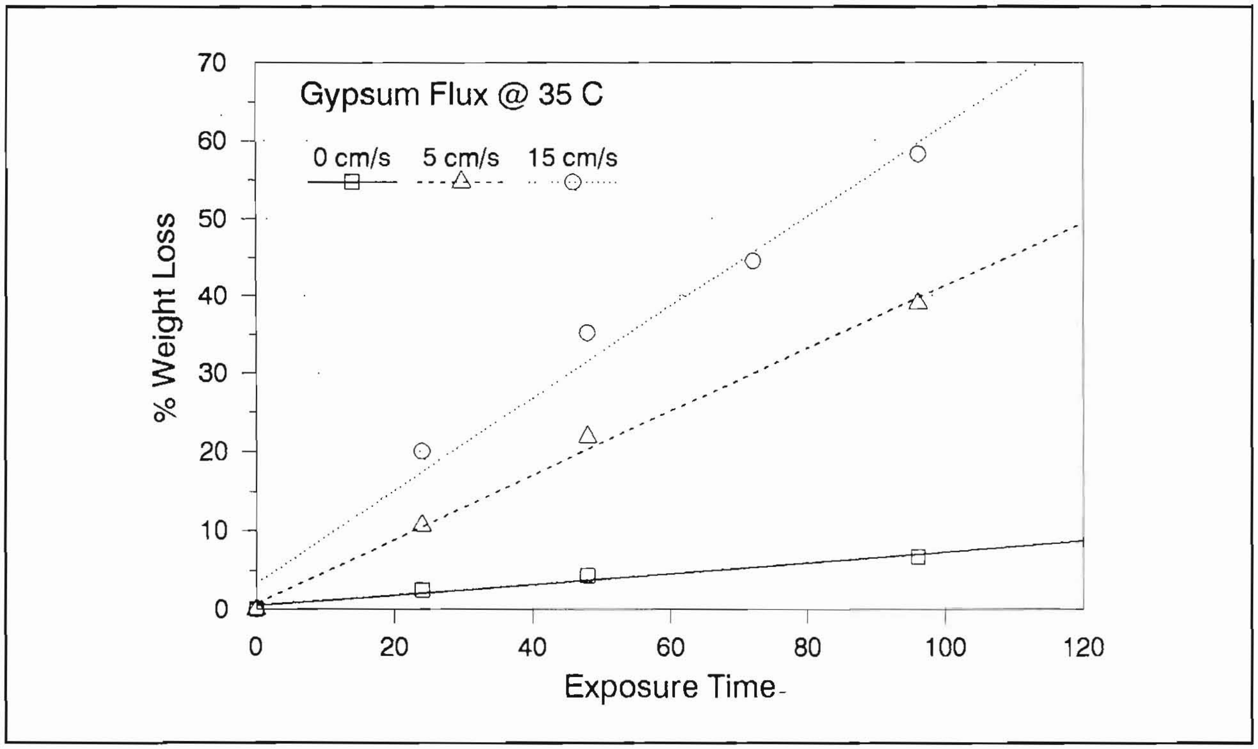

Figure 5. Gypsum flux relationships for 0-, 5-, and 15-cm/sec flows at 35 °C. Each datum represents mean flux (n = 3)

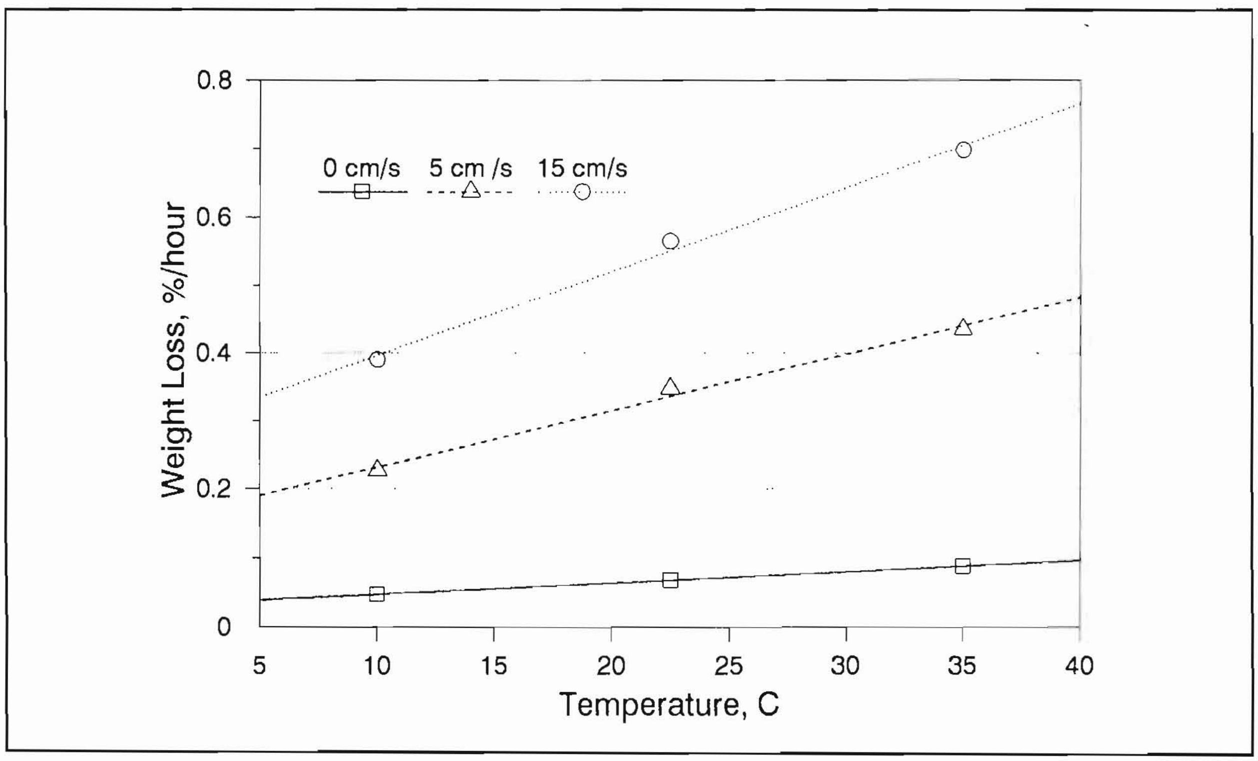

Figure 6. Gypsum flux relationships for 0-, 5-, and 15-cm/sec flows and temperatures of 10, 22.5, and 35 °C. Each datum represents mean flux (n = 9)

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Biological Control Technology

Biological Control Technology Overview—Fiscal Year 1993

by
Alfred F. Cofrancesco, Jr.[1]

In the area of biological control technology, there were eight direct allotted projects and six reimbursable projects conducted during fiscal year 1993. The details of each direct allotted and reimbursable work unit will be presented by the Principal Investigators.

The research that will be reported has been conducted at research facilities and field sites in the United States and a number of foreign countries. I will not cover each work unit, but will give a general overview of the goal of biological control. In general, biological control is the introduction by man of parasites, predators, and/or pathogenic microorganisms to suppress populations of plant or animal pests. Many exotic organisms that are introduced into a new habitat expand rapidly and occupy all available resources. Although populations of these organisms often fluctuate seasonally, they often expand and occupy the maximum-carrying capacity of the system (Figure 1). Biological control agents are used to attempt to reduce these populations to below a problem state (Figure 2).

At the onset of joint Corps of Engineers (CE) and U.S. Department of Agriculture (USDA) research activities 35 years ago, there was skepticism about the ability of biocontrol agents to impact aquatic vegetation, particularly submersed aquatic plant problems. As we developed potential agents, we realized that these agents are not really tools, but they are resources that are subject to abiotic and biotic factors that need to be managed.

Biological agents can be influenced by climate, weather, geographic barriers, and other abiotic factors. These influences need to be considered when utilizing the agents. In addition, biotic factors, such as predation and various forms of competition also regulate how potential agents perform.

To date, we have had numerous successes using biological control agents to manage aquatic plants. The best example is the management of alligatorweed in the United States.

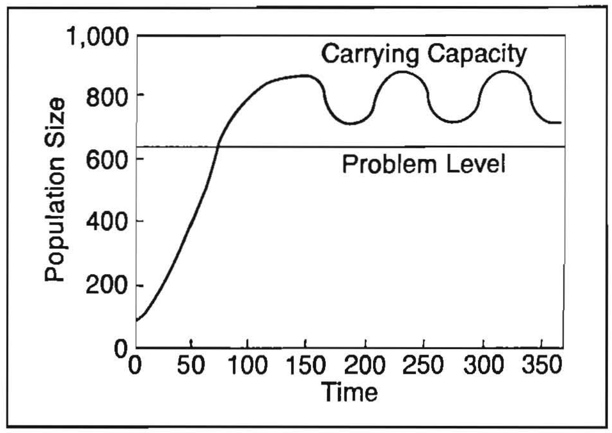

Figure 1. Logistic growth curve of exotic organisms before use of biocontrol agent

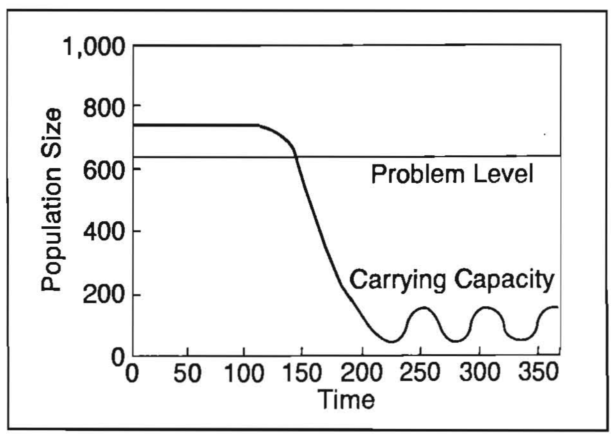

Figure 2. Logistic growth curve of exotic organisms after use of biocontrol agent

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

In 1959, alligatorweed was the most extensive aquatic plant problem (Coulson 1977). By 1980, none of the states surveyed considered it a major problem and depended almost totally on the three agents that were released for management (Cofrancesco 1988). In particular, the flea beetle (*Agasicles hygrophila*) exerts extensive impact on the aquatic growth form of alligatorweed in short periods of time (Figure 3). Another example of impact from biological control agents occurs with waterhyacinth. Agents were first introduced into Louisiana in 1974. The release, dispersal, and buildup of the agents took years; however, unprecedented textbook reduction in the total acres of waterhyacinth infestation has been reported (Figure 4). Dramatic declines have also been documented from insect impact on waterhyacinth in Texas.

The research that has been conducted also presents information on how to manage biological control agents. In general, some agents can be released with minimal management efforts, and they perform well (Cofrancesco 1988). However, these agents can be impacted by biotic or abiotic factors and their expected

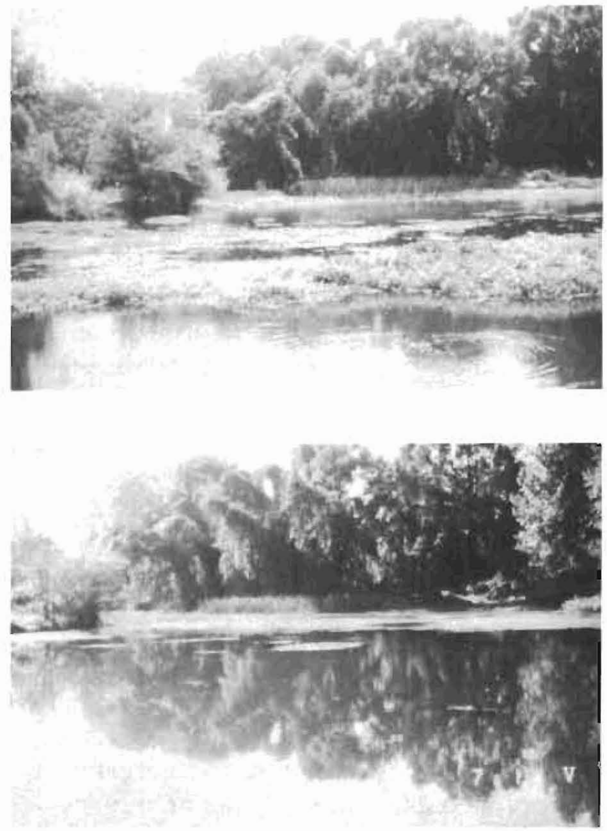

Figure 3. Before and after introduction of Agasicles hygrophila on alligatorweed

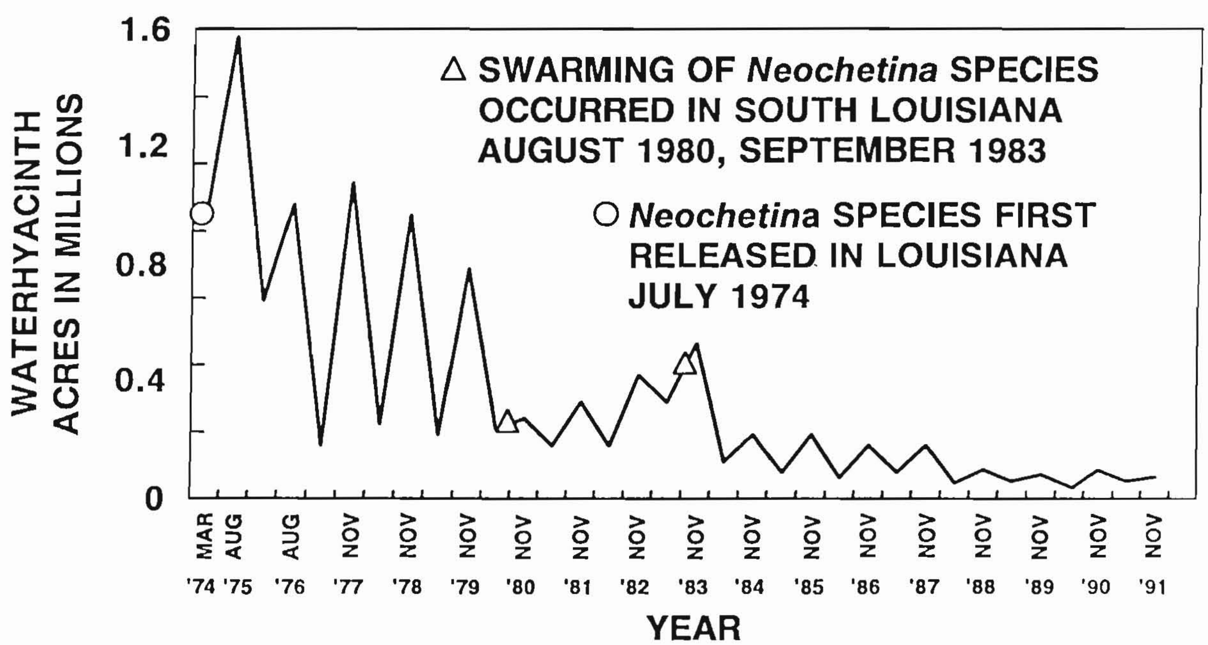

Figure 4. Total waterhyacinth acreage in Louisiana

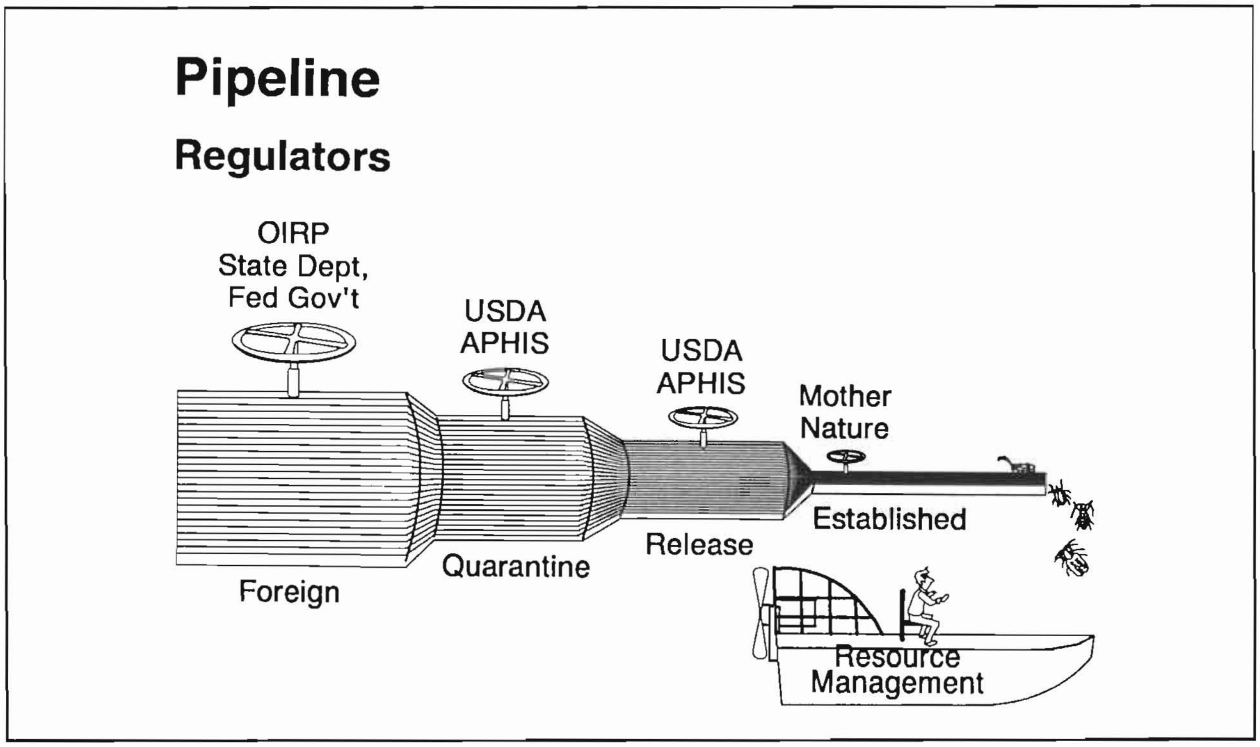

Figure 5. Pipeline of biocontrol agents

impact altered. If managers are unaware of these conditions and requirements of the agents, their lack of impact may be attributed to the agent and not the outside factors influencing the agent.

Operations personnel need to be more active in the management of their resources. To accomplish this, they need to have the latest information available about the agent. We prepared a four-step system called "KISS": Knowledge/Information, Integrate, Survey, Supplement. By addressing these factors for agents that a manager has available, he can enhance their performance.

At the present time, there have been 12 agents released through the joint research efforts of the CE and USDA for aquatic plant management in the United States. There are also many agents being evaluated in quarantine and overseas laboratories. These research activities add extensive amounts of information to an already voluminous library of information.

A computer-based information management system is being developed that will contain important information on all aquatic plant biocontrol agents and detail how to supplement and integrate the agents into an overall program. In addition, the system will present information on native plants and organisms that are often found associated with native and exotic plants. By effectively utilizing this system, resource managers can obtain the most impact possible from the biocontrol agents that have successfully passed through the pipeline (Figure 5).

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Overseas Research

by
Christine A. Bennett[1]

In 1988, the United States Department of Agriculture/Agricultural Research Service (USDA/ARS) entered into a cooperative project with the People's Republic of China for surveys and research on insects that attack hydrilla, *Hydrilla verticillata* (L. f.) Royle, and Eurasian watermilfoil, *Myriophyllum spicatum* L., in temperate climates. Thus the nuisance aquatic plant project at the Sino-American Biocontrol Laboratory (SABCL) was created. The laboratory is located at the Chinese Academy of Agricultural Science in Beijing, China. Dr. Lu Qing Guang is Director of SABCL. Three rooms in the basement of the Biological Control Laboratory are reserved for SABCL. Other projects at SABCL include biocontrol of terrestrial nuisance plants and insect pests. In 1992, a newly graduated entomologist, Mr. Chen Zhi Qun, was hired specifically for our nuisance aquatic plant project. Before this, we had almost yearly changes in SABCL personnel assigned to our project. Mr. Chen assists us in the field and continues the research after we leave. He traveled to the United States this winter and spent 3 months in our laboratory learning to identify aquatic plants and how to collect, preserve, and rear aquatic insects associated with these plants.

Dr. Joe Balciunas, USDA/ARS, Townsville, Australia, began surveys in China in 1989 (Balciunas 1990) and continued in 1990 (Balciunas 1991) and 1991 (Buckingham 1992) with help from Dr. Gary Buckingham, USDA/ARS, Gainesville, FL. In 1992, the surveys and research were conducted by Dr. Buckingham and me (Buckingham 1993).

Insects found in these surveys on hydrilla included two species of leaf-mining flies, one of which was released from quarantine in 1991 (*Hydrellia pakistanae* Deonier). The other fly, *Hydrellia sarahae* Deonier, is currently being studied in quarantine. Also found was a tip-boring midge, a caterpillar that feeds on the leaves, but is not specific to hydrilla, and the grub-like larvae of a donaciine leaf beetle that feeds on the roots (Buckingham 1993).

The most important potential biocontrol agents found on Eurasian watermilfoil were a flower-feeding *Phytobius* weevil, a stem-boring *Bagous* weevil, a tip-boring midge tentatively identified as the same one in British Colombia, and root-feeding larvae of a second donaciine leaf beetle.

In 1993, Willey Durden, USDA/ARS, Fort Lauderdale, FL, and I traveled independently to China to continue the research. Our goals this year were (a) to find and collect a *Bagous* weevil believed to be associated with hydrilla, (b) to collect the stem-boring *Bagous* on milfoil and to study its field biology and host range, (c) to collect pupae or adults of the unidentified donaciine leaf beetle larvae on hydrilla roots and study its field host range, and (d) to collect specimens of the larvae, pupae, and adults of the tip-boring midges for positive identification.

Willey Durden arrived in Beijing at the end of May. He spent the first week sampling hydrilla sites in the Beijing area. At many sites, hydrilla was present, but not at the surface probably because of the time of year. Plants were collected and placed in Berlese funnels at the SABCL laboratory. No insects were recovered.

[1] University of Florida, Institute of Food and Agriculture Service, Department of Entomology and Nematology, Gainesville, FL.

Willey and Mr. Chen then traveled to south China to survey sites in Hunan Province for hydrilla and a *Bagous* weevil that cooperators of Dr. Balciunas had found associated with hydrilla in 1989. The first site was northeast of Yueyang near the small town of Yunix. Two sites on two different reed farms near Dongting Lake, China's second largest, were sites previously visited by Dr. Balciunas. Another large lake near Wuhan was also surveyed. At most of the sites, however, hydrilla was present but had not reached the surface. Plants were collected and searched, but no damage or insects were found. Insect damage was found on plants collected at the two sites near Dongting Lake, but no insects were recovered. Fly larvae and midge damage were found on hydrilla collected from a lake near Xinghe Village.

Willey and Mr. Chen then flew to Shenyang, a large city in the northeastern province of Liaoning. Three sites around the city were surveyed for hydrilla, watermilfoil, and pondweeds. Hydrilla in all the sites was sparse, and no damage or insects were found. At one site, Hun Pond, an orange and black beetle larva was found on Eurasian watermilfoil by a Chinese colleague. Insects were collected by Mr. Chen from pondweeds at two different sites.

I arrived in Beijing July 22. Mr. Chen and I spent the rest of the week collecting adults of the leaf-mining fly *Hydrellia pakistanae* at various sites around Beijing. Flies were collected from leaves of floating vegetation or from a piece of white styrofoam placed on top of the plants by using a small vacuum made from a modified flashlight (Figure 1). The flies were identified and sexed in the laboratory with a microscope. Females were then placed in jars with hydrilla to obtain eggs. Larvae were to be hand-carried back to Gainesville to add new germplasm to our colony.

Our travel plans included trips to Harbin in Heilongjiang Province and Shenyang in Liaoning Province. Before we left, we spent 1 day packing all our equipment for the trip. Not only did we have to pack and carry our field-collecting equipment, including heavy, bulky waders, but because our hotel room was our laboratory, we also had to carry all materials for rearing the insects, dissecting equipment, and even our own microscope (Figure 2).

We arrived in Harbin July 26 and were met at the airport by our host, Mr. Fu, from the Plant Protection Institute. He arranged our schedule in Harbin and provided us with a car and driver during our stay. The next day, we visited a small pond in the area where Dr. Balciunas had found *Bagous* larvae in 1991 in association with hydrilla. We found only a few sprigs of hydrilla and no insects. Our hosts told us the weather had been cooler and wetter than normal, and this may have contributed to the lack of hydrilla at this site.

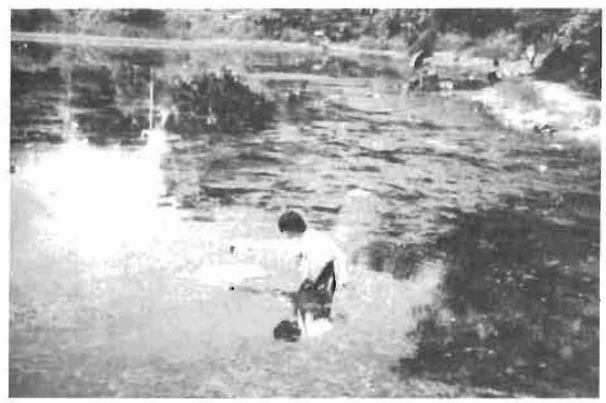

Figure 1. Mr. Chen Zhi Qun collecting Hydrellia pakistanae August 1, Beijing

Figure 2. Example of field equipment and supplies carried to China for surveys of nuisance aquatic plants (clothing and personal items not shown)

Mr. Fu arranged for a driver and for Mr. Yang, Director of Weed Control for the Plant Protection Institute, to accompany us to Shi-er-li-Pao marsh near Wo-li-tun, a small town 150 km west of Harbin, where in 1992, Dr. Buckingham had found *Bagous* sp. on Eurasian watermilfoil (Buckingham 1993). We collected at two different sites in the marsh. The first site was the site visited by Dr. Buckingham in 1992. The milfoil was very patchy here and in deep water. We were able to rent a small boat from the fisherman who lived at this site. We poled ourselves around from patch to patch collecting milfoil stems and weevils. Larvae fed inside the stems and adults on the stems below the waterline. Small areas along the stem damaged by larval boring caused the stem to become transparent. The insects were found by inspecting the stems while holding them toward the light. Larvae, pupae, and teneral adults could be found in these transparent areas.

Milfoil flowers in this area were heavily attacked by the flower-feeding weevil *Phytobius* sp. The majority of flower stalks were black from heavy feeding. The stems of these plants were waterlogged and not healthy. The second site was about a mile from the first site, but still on the same canal system. The water was not as deep, and there was a larger milfoil mat. We were able to collect here in waders. *Myriophyllum verticillatum* L. and *M. spicatum* were present at both sites, and both insects were associated with both plants. More work is needed to study the field host range of both insects.

The adult *Bagous* is a small mottled weevil. We collected 9 adults, 31 larvae, and 20 pupae at the two sites. The adults were held in small plastic vials and fed *M. spicatum* for the remainder of our trip. Larvae and pupae were left in the stems, and the leaves were removed. The leaves were removed because they would rot, which would cause the whole stem to break down and kill the insects. The stems were then wrapped in dry newspaper and put in a plastic bag closed with a rubber band. The bundles were checked periodically for condition and adult emergence.

The flower-feeding *Phytobius* is a grayish or brownish weevil that blends in well with the seeds. The adults feed on the flowers and seeds. The larvae bore into the flower stalks. We collected 54 adults, 1 larva, and 15 cocoons at the two sites, and I brought 45 adults home to quarantine. The one larva and cocoons were handled in the same manner as the *Bagous*. Even though we had collected the *Phytobius* in previous years, it had not been imported to quarantine because its biology appeared similar to the native *Phytobius*. Our discovery of the extensive damage done by this species prompted the importation.

The insects collected at both sites represent work done over a 2-day period by two people working 6 hr in the field and 4 to 5 hr in the hotel searching through milfoil stems. This reflects the small populations in the field and how much work it takes to bring these insects home.

At the second site, we found donaciine leaf beetle larvae attached to the roots of the milfoil (Figure 3). Examination of the roots under the microscope confirmed feeding. Beetles in this group are elongate and slender. They are dark colored and metallic, usually black, greenish, or coppery. These beetles are seldom seen far from water. The eggs are laid on the underside of aquatic plants, and the larvae feed and pupate on the roots. The larvae were collected and taken back to Beijing where they were placed on cut stems and roots in

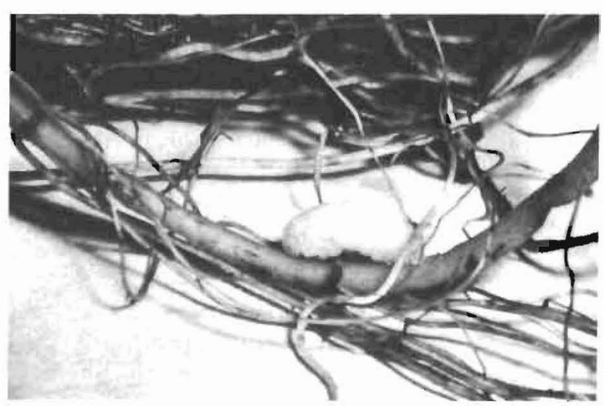

Figure 3. Grub-like larva of the unidentified donaciine leaf beetle feeding on the roots of Eurasian watermilfoil

aerated water. I left these insects in Beijing; the last I heard, they were still alive.

We left Harbin and flew to Shenyang August 3. Our host was Professor Quan Guang-qing from the Shenyang Agricultural University. He and his colleagues provided us with transportation to the various sites and helped us collect. In Shenyang, we visited sites along a drainage canal where Dr. Buckingham in 1992 had collected larvae of an unidentified donaciine leaf beetle attached to the hydrilla roots (Buckingham 1993). This is a different species from the one collected on milfoil at Shi-er-li-pao. Collecting here was difficult because the water level in the canal was very high, as it had been raining for 10 days and was still raining. We only collected three small donaciine larvae from the lower stems of hydrilla, which we took back to Beijing and placed on hydrilla planted in a jar. The jar was then placed in the greenhouse. It will be next spring before we know if the larvae survived and adults emerge.

Future Plans

Future plans call for more travel in China. We will continue to look during our travels for the *Bagous* sp. associated with hydrilla that Dr. Balciunas reported. The field biologies and host ranges of the milfoil stem-boring *Bagous* sp. and the flower-feeding *Phytobius* sp. need to be investigated. Adults of the donaciine leaf beetle feeding on milfoil and the species collected on roots and stems of hydrilla need to be collected and identified. When identified, the field host range and biology of both insects need to be studied. Additional field studies are needed on the two different species of tip-boring midges that attack hydrilla and milfoil. We also hope that Mr. Chen Zhi Qun, SABCL, can travel to Gainesville again this year for more training.

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Quarantine Research

by
Gary R. Buckingham[1]

Introduction

Our quarantine program for the introduction and safety testing of nuisance aquatic plant biocontrol agents is interlocked not only with the U.S. Army Engineer Waterways Experiment Station (WES), but also with the Department of Entomology and Nematology, University of Florida, which provides technical support personnel, and with the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, which provides the facilities and other support. Ms. Barbara L. Dawicke, MS in entomology, joined us this year through a Specific Cooperative Agreement with Dr. Dale Habeck, University of Florida. Barbara worked on all projects, but primarily on melaleuca (Figure 1). Christine Bennett continues to work with the submersed nuisance aquatic plants. Two student assistants, Rob Lowen and Billy Talton, left our program this year, but Yuvora Nong and Jason Etchart remained and were joined by Karen Stonaker. Our University of Florida agreements allow us to increase manpower rapidly in response to program needs, but limited space in the shared quarantine facility dampens the increases.

We continued studies on hydrilla, *Hydrilla verticillata* (L. f.) Royle, this year; but unlike in past years, it was not the principal focus of the quarantine research. The foreign surveys mentioned by Chris Bennett had hydrilla insects as targets, but no new species were shipped to quarantine. Eurasian watermilfoil, *Myriophyllum spicatum* L., insects from China and melaleuca, *Melaleuca quinquenervia* (Cav.) S. T. Blake, insects from Australia occupied most of our time.

Figure 1. Barbara Dawicke entering quarantine with a shipment of melaleuca insects from Australia

Hydrilla Leaf-Mining Flies

Chinese *Hydrellia pakistanae* Deonier flies from the quarantine colony were sent to WES for laboratory colonization. New germplasm was collected by Chris Bennett in Beijing and colonized in quarantine. The *Hydrellia* n. sp. CH 1 or n. sp. silver-face mentioned in previous reports was described this year by Dr. Dick Deonier as *Hydrellia sarahae* Deonier (Deonier 1993). Two subspecies were described: *H. sarahae sarahae* Deonier from China and *H. sarahae laticapsula*

[1] U.S. Department of Agriculture, Agricultural Research Service, Biological Control Laboratory, Gainesville, FL.

Deonier from India. Our research with these two subspecies was summarized by Chris Bennett in the 1992 Aquatic Plant Control Research Program (APCRP) proceedings (Bennett 1993). Only minor studies of larval development at different temperatures were conducted this year. Dr. Deonier determined that flies collected by me in Korea in 1991 and in Japan in 1992 from hydrilla are an undescribed species, *Hydrellia* n. sp., which is similar to *H. sarahae*.

Australian Hydrilla Moths

Two species of aquatic pyralid moths that attack hydrilla in flowing water in Australia were illustrated by Chris Bennett in the 1992 APCRP proceedings (Bennett 1993). *Nymphula eromenalis* Snellen died during the F1 generation. However, it fed heavily on *Potamogeton* spp. along with hydrilla. *Aulacodes siennata* Warren died during the F3 generation. Larvae of this species were about twice as large as our native *Parapoynx* caterpillars and highly destructive to hydrilla. Unlike *Parapoynx* caterpillars, they did not live in portable cases but webbed plants together forming shelters from which they exited to feed. Hydrilla webbed together in a rearing pan remained in the pan even when the pan was turned on its side. The shelters should help the larvae remain in place during periods of strong water current.

Larval development was highly asynchronous with adults emerging, while their siblings were still very small. This greatly hindered laboratory colonization and experimentation. Small larval numbers prevented extensive host range tests, but larvae developed on *Egeria densa* Planch., *Elodea canadensis* Michaux., *Najas guadalupensis* (Spreng.) Magnus, *Myriophyllum spicatum*, and *Potamogeton nodosus* C. and S. in addition to hydrilla. Our host range data are scant, but when combined with the field observations of Dr. Dale Habeck and Dr. Joe Balciunas, they indicate that this species is not sufficiently specific for a biocontrol program. We have no plans for additional importations.

Florida Hydrilla Midges

Two species of midges, *Cricotopus sylvestris*-group sp. and *Dicrotendipes* sp. A, were mentioned by Chris Bennett in the 1992 APCRP proceedings as being collected from hydrilla tips at Crystal River, FL (Bennett 1993). Although hydrilla populations were severely reduced by a strong winter storm, midge larvae were still present during this past summer. Two small samples yielded 13 percent (n = 100) and 73 percent (n = 48) of the apical and lateral buds damaged.

Watermilfoil Stem Weevil

This Chinese weevil, *Bagous* n. sp., was illustrated by Chris Bennett in the 1992 APCRP proceedings (Bennett 1993). Larvae bore and pupated in the stems of *Myriophyllum spicatum*. F3 weevils stopped laying eggs, but a few lived through the winter. Chris Bennett and Chen Zhi Qun collected additional adults at the site near Harbin in July, and Chen Zhi Qun collected more in September. The July adults produced F2 adults, but all died in a temperature cabinet malfunction. The September adults produced a few F2 adults before all stopped ovipositing in January. They are now being exposed to simulated autumn-winter conditions in hopes of breaking their diapause, or arrested development. Unfortunately, we have been unable to produce enough adults to study the biology and host range. Field populations have been light, so we are unable to judge the damage potential.

Watermilfoil Flower Weevil

A second Chinese weevil, *Phytobius* sp., attacks the flower stalks of *M. spicatum* at most of the sites (Figure 2). Adults eat flowers and holes in the stems. Eggs are laid in feeding scars on the stem of the flower stalk and larvae bore into the stems. Some larvae feed in the flower buds and flowers. Pupae are formed in cocoons in the stems. Preliminary biology and host range studies were conducted in 1990 and 1991 by Ms. Jiang Hua at the Sino-American Biological Control Laboratory

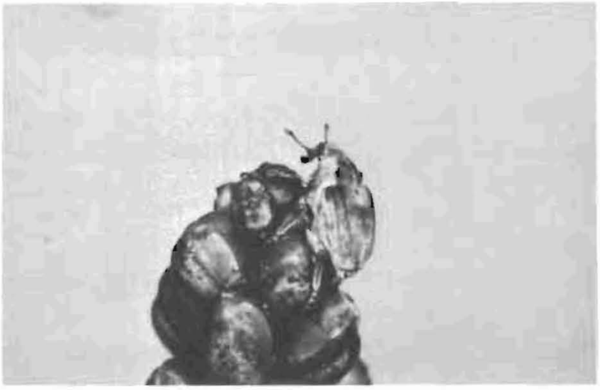

Figure 2. Watermilfoil flower weevil Phytobius sp. from China on flowers in quarantine

in Beijing. However, because of the similarity of the life cycle to life cycles of native weevils already on milfoil in North America, this species was not imported into quarantine. While collecting stem weevils this year near Harbin, Chris Bennett and Chen Zhi Qun noticed that plants attacked by the flower weevil appeared to lose bouyancy and sink in a manner similar to that reported by Creed, Sheldon, and Cheek (1992) for a native stem weevil in the Northeastern United States. We decided to import this species for testing because of that damage and because this species should be more closely adapted to Eurasian watermilfoil than are the native weevils.

Our biology studies are continuing, and thus the data are preliminary. Unfortunately, mifoil has almost stopped flowering for the season, which will curtail our studies. Pairs of weevils provided with two flower stalks daily ate 8 percent of female flowers and 17 percent of male flowers. The number of flowers eaten was about the same as the percentage. Females have deposited 9 eggs/day and 337 total eggs (means), but final means should be larger. The mean development time from egg to adult was 13 days.

We were able to conduct preliminary host range tests only with adults and only with nonflowering portions of plants. There was no feeding on *Potamogeton illinoensis* Morong and *Polygonum punctatum* Ell. Feeding was minor on *Myriophyllum heterophyllum* Michaux and *M. aquaticum* (Velloso) Verdc., moderate on *M. sibiricum* Komarov, and heavy on *M. spicatum*. Our observations in P. R. China and the host records of the known milfoil phytobiine weevils suggest that this species will be specific to milfoils.

Melaleuca Sawfly

Melaleuca, *Melaleuca quinquenervia*, is a large Australian tree that infests the Florida Everglades and other areas of south Florida. The estimated rate of spread is 50+ acres per day. Saplings form dense thickets that should be as susceptible to insect or pathogen damage as a cornfield or a grassland. Large trees might require a different complex of control agents from saplings, but that should be no problem since Joe Balciunas' surveys in Australia have identified hundreds of insects associated with melaleuca. Thorough pathogen surveys are still needed, however.

Two insect species, a weevil and a sawfly, have been imported into quarantine. Both were illustrated by Chris Bennett in the 1992 APCRP proceedings (Bennett 1993). The sawfly, *Lophyrotoma zonalis* Rohwer, is a nonstinging primitive wasp. Larvae feed on older leaves often defoliating large trees in Australia. Young larvae are gregarious and do not do well when separated from their siblings. This behavior will make eventual host range testing difficult; but in the field, it will prevent individual larvae from wandering away from melaleuca to neighboring plants.

We received three shipments of larvae/prepupae from which we obtained adults. Females laid eggs; larvae were produced; all F1 progeny were males. We are unsure if lack of female progeny is because parent females did not mate or because female larvae died because of host plant effects. Dr. Balciunas believes that unmated sawflies do produce female progeny. We plan companion tests in Australia and Florida to determine if we have mating or plant problems. As of early November, written permission had not been received from the Animal Plant Health Inspection Service/U.S. Department of Agriculture for Dr. Balciunas to send additional sawflies for host range testing, although the Technical Advisory Group has approved the research.

Melaleuca Weevil

Most of our research this past year has been with the Australian weevil *Oxyops vitiosa* Pascoe. Adults and larvae attack the young tip growth in contrast to the older leaves preferred by the sawfly. Adult weevils eat holes in the leaves and stems, and larvae scrape the upper and lower surfaces of the leaves. Older larvae will move to older leaves if young leaves are no longer available. Eggs are laid on the tips of young leaves and are covered with a hardened layer of black excrement. Pupae are formed in fragile cocoons in the soil. Dr. Balciunas and Matthew Purcell are studying the laboratory biology in Australia. We are conducting host range tests in quarantine.

Our host range tests have been (a) multichoice adult feeding screening tests, (b) no-choice adult feeding tests, and (c) oviposition/larval development tests. We used the screening tests to determine which plants warranted more intensive testing. Adult weevils were exposed to individual bouquets of multiple plant species without hydrilla for 3 days. The bouquets were then replaced and hydrilla was added. Estimates of feeding with and without hydrilla were thus obtained. Ninety-five plant species were tested. They included species that are in the melaleuca family Myrtaceae (27 species), in related families, in related superorders, important cultivated plants, and common south Florida natives. Three Florida threatened or endangered species were included. Fifty-seven of the species were not damaged. With the other 38 species, none of the damage estimates exceeded 50 percent, and most were less than 25 percent of the melaleuca control.

Nineteen plant species were chosen for the no-choice adult feeding tests in which weevils were exposed to each test species with separate melaleuca controls. Sixteen of the species were eaten significantly less than melaleuca. The three plant species not significantly different from melaleuca were *Callistemon citrinus* (Curtis) Stapf. (an Australian bottlebrush, Myrtaceae), *Myrica cerifera* L. (native wax myrtle, Myricaceae), and *Psidium Guajava* L. (guava, Myrtaceae).

Oviposition and subsequent larval development were tested with *C. citrinus*, *M. cerifera*, and *P. Guajava*. In one test, no eggs were placed on *M. cerifera* and *P. Guajava*, 8 on *C. citrinus*, and 41 on melaleuca. Four larvae reached maturity on *C. citrinus* and 17 on melaleuca. In a second test, no eggs were placed on *M. cerifera* and *C. citrinus*, 8 on *P. Guajava*, and 42 on melaleuca. No neonates survived on *P. Guajava* compared with 17 on melaleuca. Both *C. citrinus* and *P. guajava* are found in Australia, but the weevil has never been reported on them.

Future Plans

Studies will be terminated with the leafmining flies unless field researchers show interest in the *Hydrellia* n. sp. from Korea and Japan. Chinese watermilfoil flower weevils will be collected in summer 1994 for additional biology studies and for host range studies. Our attempt to colonize the Chinese watermilfoil stem weevil will continue, and additional weevils will be collected in summer 1994. Research on the melaleuca sawfly and the melaleuca weevil will be continued, but not as part of the APCRP.

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Release and Establishment of Insect Biocontrol Agents for the Management of Hydrilla

by

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Introduction

Hydrilla (*H. verticillata*) is a submersed, caulescent vascular hydrophyte that is widely distributed in the Old World. Cook and Luond (1982) suggested that its center of origin lies in tropical Asia. Its distribution extends nearly contiguously from Iran and Afghanistan through Pakistan, India, and Southeast Asia, then northward along coastal China to the Soviet Union, Japan, and Korea, and southward through the Indo-Pacific region to coastal regions of Australia. Disjunct populations occur in the central African Rift Valley lakes, in northeastern Europe (Poland and Lithuania), and in Ireland. An extirpated population existed until about 1945 in England (Cook and Luond 1982).

Numerous extralimital introductions of hydrilla have occurred. Cook and Luond (1982) indicated that populations in Mozambique, the Ivory Coast, the Canary Isles, New Zealand, Austria, the United States, Panama, and Jamaica were recently introduced. Pieterse (1981) suggested that it was introduced to Fiji, and Swarbrick, Finlayson, and Cauldwell (1982) considered it adventive on Mauritius. Severe, troublesome infestations exist mainly in New World localities.

The first New World introduction of hydrilla occurred during the 1950s in Florida (Schmitz et al. 1991). It rapidly dispersed. It was a serious nuisance plant throughout central and south Florida by 1961 when it was found at Lake Seminole on the Florida-Georgia border (Haller 1978). It then spread to neighboring states in the Southeast and reached Iowa by 1972 (Cook and Luond 1982), Louisiana by 1973 (Wherry 1974), Texas by 1974 (Guerra 1976), and California by 1976 (Sonder 1979). Steward et al. (1984) reported a new monoecious biotype from the Potomac River near Washington, DC, during 1982. Hydrilla reached North Carolina by 1979 (Langeland and Schiller 1983) and Delaware by 1981 (Haller 1982). This range expansion has since continued, so new occurrences of hydrilla anywhere in North America would not be unexpected.

History of the Hydrilla Biological Control Project

The successful deployment of nonnative insects for alligatorweed and waterhyacinth control in the United States during the 1960s and 1970s demonstrated the effectiveness of the introduction (i.e., "neoclassical") approach towards biological control technology in aquatic ecosystems. This encouraged development of biological control programs designed to address other aquatic plant problems. *Hydrilla verticillata* L. infested 40,000 to 60,000 acres in Florida alone during the period between 1982 to 1989 (Schardt and Schmitz 1989) and was rapidly expanding into northern regions. Expensive herbicidal control methods were only temporarily effective. Partial treatment with herbicides of an infestation at one lake (Lake Istokpoga) during 1989, for example,

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cost $1.2 million, an amount equivalent to 20 percent of Florida's total statewide nuisance aquatic plant control budget for that year. Clearly, widespread herbicidal control of hydrilla exceeded the resources of most public agencies, and alternatives were desperately needed. As a result, when the U.S. Department of Agriculture and the U.S. Army Corps of Engineers considered collaborative projects for biological control of new target species, *H. verticillata* was positioned as the top priority by both agencies.

Worldwide faunal inventories resulted in the selection of candidate hydrilla biological control agents from tropical and subtropical regions (Balciunas 1982, 1983, 1984, 1985; Balciunas and Dray 1985; Baloch and Sana-Ullah 1974; Baloch, Sana-Ullah, and Ghani 1980). The two first insect species selected were from India, a tuber-feeding weevil (*Bagous affinis*) and a leaf-mining fly (*Hydrellia pakistanae*). These had originally been discovered in Pakistan during the early 1970s as part of an excess foreign currency (PL-480) project (Baloch and Sana-Ullah 1974; Baloch, Sana-Ullah, and Ghani 1980). Host range tests conducted in the Gainesville, Florida, quarantine facility proved that both of these species thrived only on hydrilla (Buckingham 1988a,b, 1989, 1990). Both were released in Florida during 1987 (Center 1989, 1990). *Bagous affinis* failed to establish because of its need for dry conditions, such as a distinct dry season or a prolonged lake drawdown, both atypical occurrences in the Southeast (Bennett and Buckingham 1991; Baloch, Sana-Ullah, and Ghani 1980). Conversely, *H. pakistanae* populations did establish throughout the region.

Faunal surveys were succeeded in Australia by more specific studies. Six candidate Australian species were eventually recognized (Balciunas 1987; Balciunas and Center 1988; Balciunas et al. 1989). Two of these, the leaf-mining fly *Hydrellia balciunasi* and the stem-boring weevil *Bagous hydrillae*, were later imported to the Gainesville, FL, quarantine facility for supplemental testing (Balciunas 1987; Balciunas and Center 1988; Buckingham 1990). Both proved acceptable for field use and were released in 1989 and 1991, respectively. Additionally, four stream-dwelling species of moths were found. These were of interest for their perceived potential to control hydrilla in flowing-water systems, a habitat in which other control measures were particularly ineffective. One species, *Strepsinoma repititalis* (now *Margarosticha repititalis?*), was eliminated from further consideration when it was found that its diet was not restricted to hydrilla (Balciunas et al. 1989). A second species, *Nymphula dicentra*, may be conspecific with *Parapoynx diminutalis*, an Asian species already present in the United States, so studies were deferred until its taxonomic status could be ascertained. *Aulacodes siennata* (now *Theila siennata*?) seemed a promising candidate, but the Australian project terminated before evaluations could be completed. A fourth species, *Nymphula eromenalis* (now *Ambia ptolycusalis*?), also had not been evaluated prior to cessation of the project. Dr. Dale Habeck later spent a few months in Australia during 1992 to study these species further. As a result, *A. siennata* was imported into the quarantine facility for host-specificity testing. However, the feeding range exhibited under laboratory conditions proved too broad to risk field release, so it has been dropped from consideration (see Buckingham 1994).

Additional research is continuing in China to study biological control agents that might be useful in temperate regions. As a result, a temperate strain of *H. pakistanae* from northern China was released in 1992. Also, the silver-faced *Hydrellia* species, now named *Hydrellia sarahae*, was imported to United States quarantine, and preliminary testing has begun (see Buckingham 1994). Also, midges similar to the African species presumed to be the causal agent of severe tip damage and stunting of hydrilla plants in Lake Tanganyika (Pemberton 1980) have been found in the Far East (see Buckingham 1994). Additional searches for a stem-boring weevil earlier reported from central China proved fruitless. This bagoine species, unlike the similar *B. hydrillae*, purportedly completes its entire developmental cycle under submersed conditions and would therefore probably be a superior biological control agent. A donaciine beetle was also found associated with hydrilla. Dr. Buckingham observed larvae of these chrysomelid beetles feeding within the root zone of hydrilla beds. The resultant damage caused the plants to appear stressed and generally unhealthy.

Objectives

The domestic portion of the field work conducted during fiscal year 1993 (FY93) emphasized the evaluation of established biological control agents (particularly *H. pakistanae*), continuation of endeavors aimed at colonizing new agents (*B. hydrillae*), or evaluations of the success of previous attempts (*H. balciunasi*). These aspects of the project are the subject of this report.

Hydrellia pakistanae and *H. balciunasi*

Hydrellia pakistanae and *H. balciunasi* are small (about 3 mm) leaf-mining flies that were introduced into the United States from India and Australia, respectively, for the management of hydrilla. *Hydrellia pakistanae* was first released into Lake Patrick, a small south-central Florida lake in 1987 (Table 1). The first releases of *H. balciunasi* occurred in 1989 in a small pond located on the Orangebrook Golf Course in southern Florida and at Lake Patrick in central Florida. Since then, over 4 million *H. pakistanae* and >200,000 *H. balciunasi* have been released in many areas of the southeastern United States.

Biology

The biology and life histories of both these species are remarkably similar. Adults tend to reside on or near infestations of hydrilla. They can be readily found during the day crawling, hopping, and flying on emergent hydrilla as well as other adjacent aquatic vegetation. The adults of the introduced species are small and lack any readily observable features, making them highly difficult to separate from each other as well as from the commonly collected native *Hydrellia*, *H. bilobifera* and *H. discursa*. Because of a lack of distinguishing characters, identifications are typically based on the external genitalia, relative size of the abdomen compared with the thorax, as well as the presence or absence of lobes on the abdomen (Buckingham and Okrah 1993).

Females lay their eggs (or oviposit) on many emergent plant species located within or near stands of hydrilla (Buckingham, Okrah, and Thomas 1989, Figure 1). Females have a relatively high reproductive potential, being capable of ovipositing up to 200 eggs during their active reproductive period.

The larvae emerge from the eggs and subsequently crawl into the water along plant stems and leaves in search of hydrilla. The larvae enter hydrilla leaves by boring through the leaf cuticle, where they feed and develop within the internal leaf tissues (Figure 2). In the process of feeding, the larvae remove most of the internal leaf tissues. After larval feeding, the leaves appear almost entirely clear. During the larval feeding periods, upwards of 12 leaves can be attacked and destroyed.

Table 1
Total Releases for *Hydrellia pakistanae*, Both the Pakistan and Chinese Strain, and for *H. balciunasi* Since the First Release of *H. pakistanae* in 1987

Species	Number of States	States	Number of Release Sites	Number Released	Date of First Release
H. pakistanae (India strain)	5	FL,GA, AL, LA, TX	27	4,178,223	1987
H. pakistanae (Chinese strain)	3	AL, LA, TX	3	35,800	1993
H. balciunasi	2	FL, TX	9	208,045	1989
Total	5		39	4,422,068	

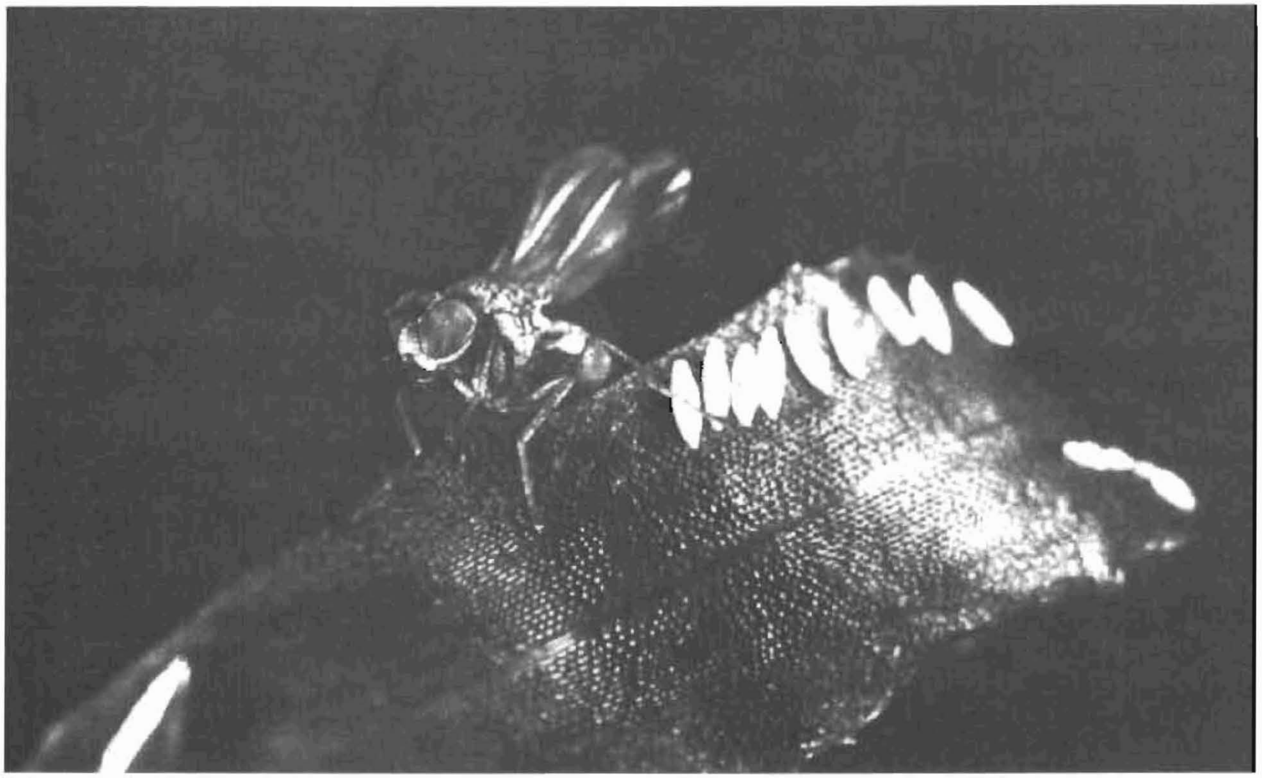


Figure 1. Adult H. pakistanae on hydrilla leaf immediately following oviposition (photo by W. Durden)

Figure 2. H. pakistanae larva within hydrilla leaf

At the time of pupation, the larvae pierce the stem with specialized needle-like hooks located on the posterior portion of their bodies. The larvae apparently pierce the stem to allow the pupa to obtain oxygen from internal air spaces. The larva pupate within the larval cuticle, which forms a protective covering over the pupa (Figure 3). This protective cover formed from the last larval cuticle is known as the puparium. After pupation, the remaining leaf cuticles often fall off, and the revealed puparium closely resembles an axial leaf bud.

The adult emerges within the puparium and "pops" the top of the puparium off using an inflatable, eversible sac, the ptilinum, which is located at the front of the head. The adult, housed within a small bubble of air, emerges from the puparium (Figure 4). The adult may remain attached to the puparium within the air bubble for extended periods. The adult detaches the bubble from the puparium and subsequently floats to the surface. The total process of adult emergence and escape may take upwards of 1 hr.

Developmental time from egg to adult varies proportionally with temperature. Total time at 25 °C is approximately 16 to 21 days. With the relatively short developmental periods and high egg production, large damaging field populations can potentially be formed relatively rapidly.

Releases

Since the first release of *H. pakistanae* in 1987, large advancements have been made in the total releases for this species. For example, >4 million individuals have been released in four southeastern states at 27 different locations. Currently, additional releases are being made of a more northern *H. pakistanae* strain collected from Bejing, China (Table 1). Lower but significant numbers of *H. balciunasi* have been released in two states and nine locations for a total of greater than 200,000 individuals.

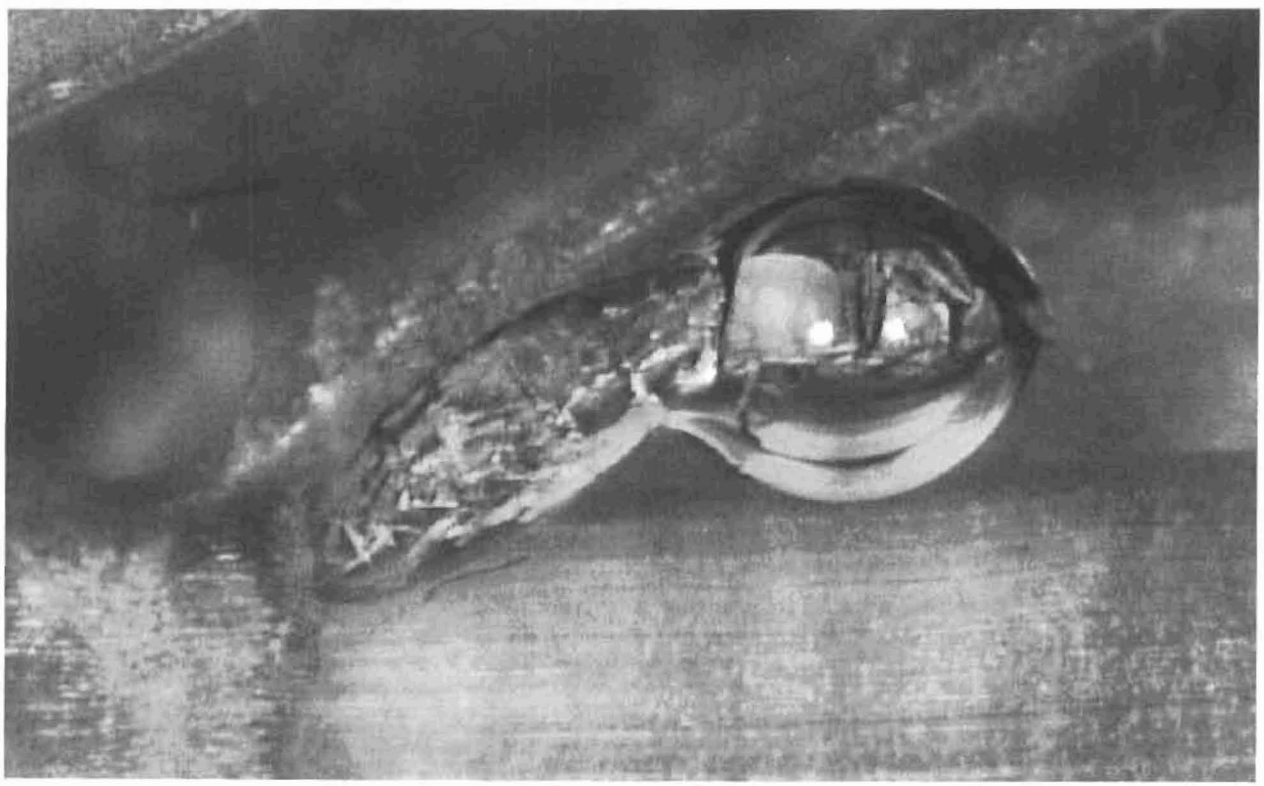
A large proportion of these individuals were released from mass rearing facilities housed at three major Federal agencies including the U.S. Department of Agriculture, Agricultural Research and Education Service, Aquatic Plant Management Laboratory, Fort Lauderdale, FL; the U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS; and pond facilities at the Tennessee Valley Authority Reservation, Muscle Shoals, AL. While mass rearing has proven to be a viable method for rearing and releasing large numbers of *Hydrellia*, more competitive and better adapted individuals can usually be obtained directly from field populations.

Toward this end, two additional methods of collecting and releasing individuals have been attempted with varying degrees of success. The first is the field collection of adults from already established sites. This method has great potential for the collection of large numbers. For example, at sites in Muscle Shoals, AL, >50,000 adults were vacuumed from floating styrofoam platforms in less than 8 hr. At other sites in south Florida, similar numbers were collected in relatively short periods of time.

How these adults are released varies with the new site and/or time of year. The adults can either be released directly onto another site, or they can be held in large containers and the females allowed to oviposit on hydrilla. The resulting infested hydrilla sprigs are held in large Petri dishes until the immatures reach late 2nd or 3rd instars (about 2 weeks at 27 °C), when the infested plant material can be introduced into new field sites. With a higher number of sites across the country containing large populations of *Hydrellia* (especially *H. pakistanae*), this method is proving to be more cost efficient than mass rearing techniques.

Another collection method that is proving to be effective for obtaining large numbers of field-collected *Hydrellia* is the use of small fish hatchery ponds located on the Tennessee Valley Authority Reservation in Muscle Shoals, AL, to acquire individuals. In this case, *H. pakistanae* larvae were introduced into one pond during summer of 1992. By summer's end, large populations of *H. pakistanae* had

Figure 3. H. pakistanae pupa



Figture 4. Newly emerged adult H. pakistanae housed within air bubble

developed throughout the many ponds found at the facility. In December 1992 and January 1993, two-thirds of a one-tenth-acre pond planted with hydrilla was hand harvested using waterproof hedge cutters. The resulting plant material was placed into plastic garbage cans and transported to sites on Lake Seminole, GA, and Lake Boeuf, LA. Based on estimates from stem counts, over 1.5 million *H. pakistanae* individuals were released at these two sites. While we have yet to confirm establishment at these sites, the immatures apparently survived the harvest and transportation process since stem counts made after the releases revealed mortalities of <5 percent. This method may prove to be highly important for future large-scale releases.

Establishment and distribution

For *H. pakistanae*, successful establishments have been high. Currently, *H. pakistanae* is apparently permanently established throughout the entire peninsula of Florida from the extreme southern portions to as far north as Gainesville, FL. This indicates that *H. pakistanae* populations are flourishing, and their range is expanding rapidly with little outside help by way of supplementary large-scale releases. In addition, populations of *H. pakistanae* are surviving and expanding in the more northern range of hydrilla. For example, small but persistent populations have been observed in certain sections of Lake Seminole, FL. The most northern established population is found in Muscle Shoals, AL, where high population levels of *H. pakistanae* have occurred since 1992. *H. pakistanae* is occasionally collected as far west as Sheldon Reservoir near Houston, TX, where it was accidentally released as a contaminant with releases of *H. balciunasi*. *H. pakistanae* has also been released in considerable numbers in southern Louisiana on Lake Boeuf. While consistent collections of *H. pakistanae* were observed in the past on Lake Boeuf, recent collections do not reveal the presence of *H. pakistanae*. We are planning to continue to survey the southern Louisiana region for the presence or absence of *H. pakistanae*.

H. balciunasi is not as widespread as that observed for *H. pakistanae*. While the number and distribution of releases for *H. balciunasi* is considerably less than attempted for *H. pakistanae* (Table 1), releases have been more than adequate in many of the areas. However, the only permanently established population of *H. balciunasi* in the United States occurs in Sheldon Reservoir near Houston, TX, where a consistent but low population has been found since 1992.

Reasons why *H. balciunasi* has not established at more locations are not clear. For example, with numerous release attempts in southern Florida, no established populations can be found. At many Florida sites, establishment appears to be successful at first, but subsequent sampling reveals no *H. balciunasi*. In many of these sites, relative high numbers of *H. pakistanae* are found instead. Such preliminary information may indicate some sort of competitive exclusion between the species, but more information is needed. Similarly, releases of H. balciunasi have been accomplished at several sites in the south Texas area; but, to date, no confirmed reports of establishment have been reported except at Sheldon Reservoir. We are currently continuing our release efforts with *H. balciunasi* at several Texas locations, including the Huntsville State Park site and on Choke Canyon Reservoir, where releases were made as recently as fall of 1993 and winter of 1993/1994, respectively.

Impacts

There is increasing evidence, both qualitative and quantitative, that indicates that the introduced *Hydrellia* sp. cause significant damage to hydrilla infestations. For example, qualitative observations on lakes and ponds since 1987 have indicated that 75 percent had significant declines in the status of the hydrilla infestation after the introduction of *H. pakistanae*. An excellent example is a pond on Orangebrook Golf Course near Fort Lauderdale, FL, where a year after establishment of *H. pakistanae*, a majority of the hydrilla was declining. This decline was correlated with qualitative observations of increases in *H. pakistanae* population levels. Similar declines after the introduction of *H. pakistanae* have been observed at sites in Louisiana and Alabama. For 28 sites that were observed periodically since the initial introductions of *H. pakistanae,* 39 percent had significant reductions in hydrilla after confirmed establishment and 35 percent had declines even though establishment was weak or not confirmed. In a majority of cases, the introduction of *H. pakistanae* is apparently having significant impact on the hydrilla. However, simple qualitative observations are not sufficient to critically examine the impact of *Hydrellia* on hydrilla infestations. More frequent observations taken more quantitatively are needed for adequately examining the effects of *Hydrellia* on hydrilla infestations.

Toward this goal, we are developing quantitative methods for accessing field population levels and associated direct damage of both *H. pakistanae* and *H. balciunasi.* While a variety of methods have been utilized, our most successful techniques use actual counts of immatures and damage obtained from sections of stems pulled at random from our sites. In most cases, we try to examine at least forty to fifty 20- to 30-cm-stem sections for each site where the weight, stem length, number of whorls, leaf estimates, immatures, and damaged leaves are determined. From this data, we are able to estimate mean number of immatures per kilogram of wet plant weight as well as the direct damage caused by the larvae; i.e., percent of leaves damaged.

Figure 5 presents changes in the mean number of immatures per kilogram of wet plant weight and percent damaged leaves through time for *H. pakistanae* populations at three nearly adjacent ponds located in Muscle Shoals, AL. Note how population levels in all three of the ponds decline almost linearly during the late fall and winter, with minimal numbers occurring during April through early June. Populations recover dramatically in late spring when the plants begin growing more actively, and by midsummer to late summer, highest populations are found. The means appear to track the overall changes in population level fairly accurately, as indicated by highly similar trends in mean values through time for all three ponds. Differences appear to be in the magnitude of the populations only. The accuracy of this sampling method is further strengthened by the fact that there is a high positive correlation ($p < 0.0001$, $r^2 = 0.76$) between the observed numbers of immatures and the actual damage, especially during periods of active population growth (Figure 6). In other words, the damage that is found on the stem is apparently caused by the observed immatures. Unfortunately, the variation, i.e., the difference between the means and actual sample values, is relatively high. This is indicated by examining the means and variance components for both number of immatures/kilogram and percent damaged leaves for Pond 20 for the 1993 growing season (Figures 7 and 8). Note how the variance is larger when mean values become higher. Such high variation makes it much more difficult to determine differences in population size or damage.

Similar quantitative estimates have also been taken at Sheldon Reservoir, the only known location where permanently established populations of *H. balciunasi* occur. We find that there is a low but relatively consistent number of immatures (about 1,000 immatures/kilogram) and associated damage (about 5 percent) during the active growing season for *H. balciunasi* populations (Figure 9). Similar to the *H. pakistanae* populations found in the ponds located at Muscle Shoals, AL, population numbers and associated damage are minimal during the period between April and early June and higher during that period of time when the plants are actively growing.

As indicated previously, it is not known why this population has not expanded significantly since October 1992, when establishment was confirmed. Experience with *H. pakistanae* indicates that after initial establishment, populations enlarge and expand rapidly. More information is needed to understand the lack of establishment and population expansion for this species.

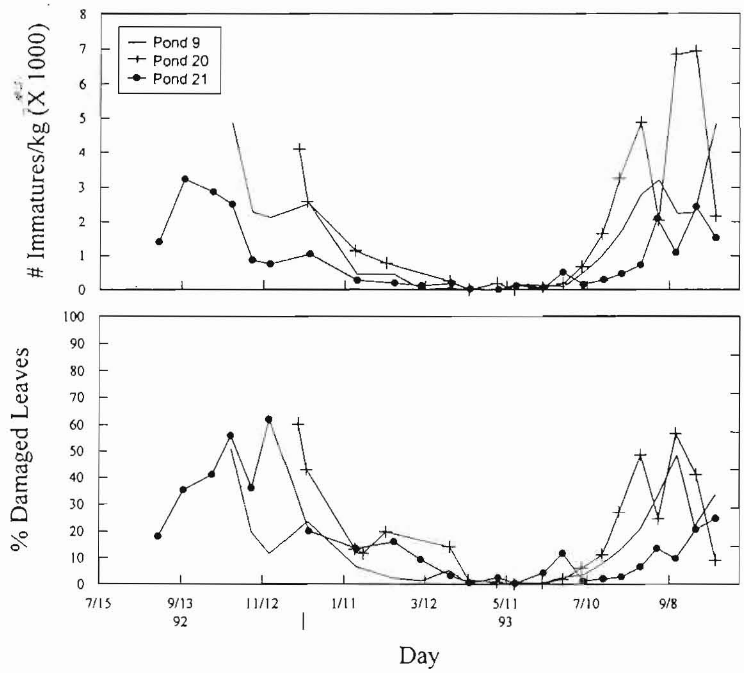


Figure 5. Total number of immatures per kilogram of dry plant weight and percent damaged leaves through time for established populations of H. pakistanae in three ponds located on the Tennessee Valley Authority Reservation in Muscle Shoals, AL

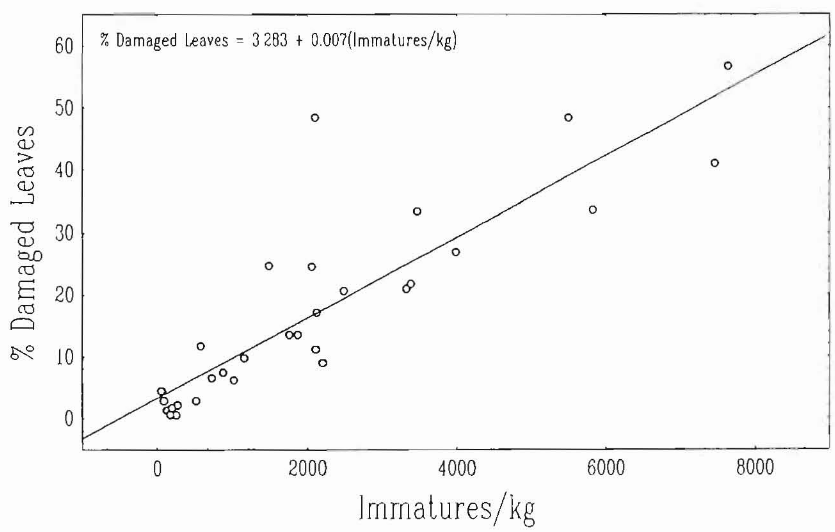


Figure 6. Correlation between the percent damaged leaves and numbers of immatures per kilogram of wet plant weight for the period from June 1993 through October 1993 for established populations of H. pakistanae in three ponds located on the Tennessee Valley Authority Reservation in Muscle Shoals, AL

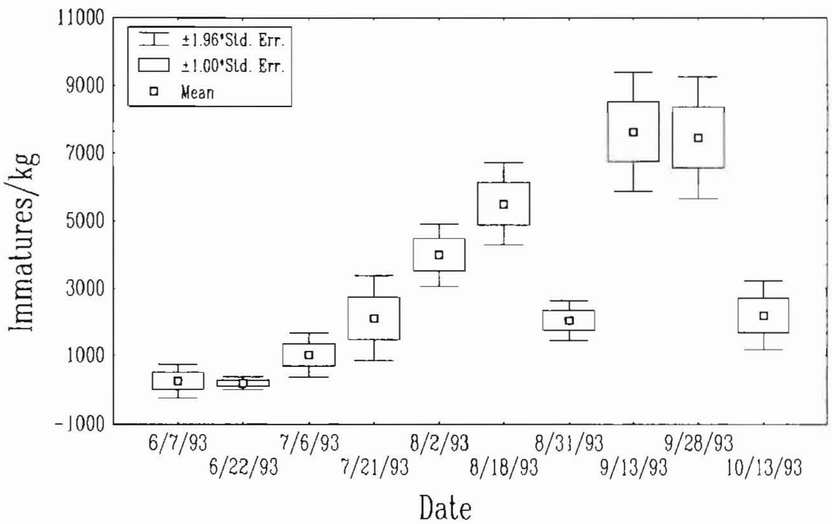


Figure 7. Means, one standard error, and two standard errors of the mean for number of immatures per kilogram of wet plant weight for established populations of H. pakistanae in three ponds located on the Tennessee Valley Authority Reservation in Muscle Shoals, AL

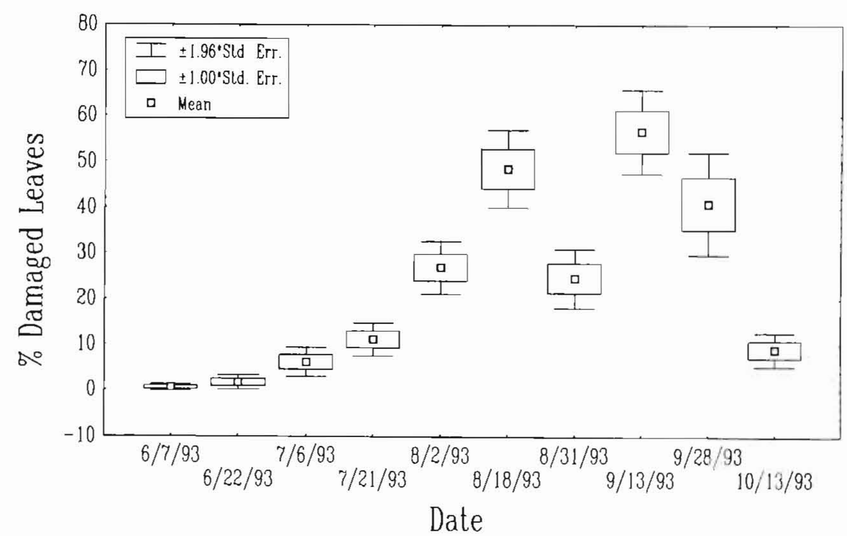


Figure 8. Means, one standard error, and two standard errors of the mean for percent damaged leaves for established populations of H. pakistanae in three ponds located on the Tennessee Valley Authority Reservation in Muscle Shoals, AL

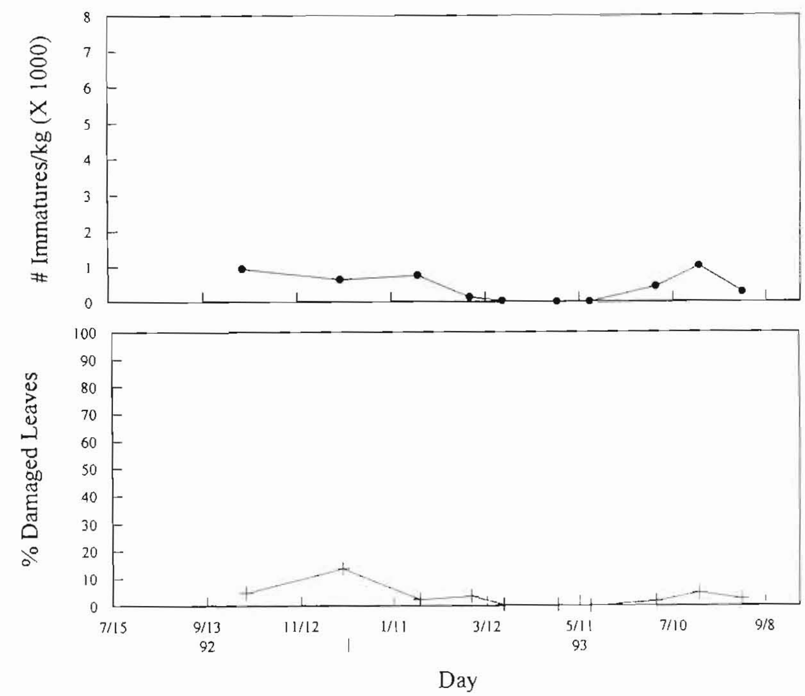


Figure 9. Total number of immatures per kilogram of wet plant weight and percent damaged leaves through time for established populations of H. balciunasi on Sheldon Reservoir, TX

Bagous hydrillae O'Brien and Askevold

The genus *Bagous* includes 182 species distributed throughout most of the world excluding South America and Antarctica (O'Brien and Askevold 1992). *Bagous hydrillae* is a small (1.8- to 3.0-mm), gray weevil bearing two conspicuous white spots on the posterior portions of the wing covers (elytra). The species is native to Australia, where it is easily extracted from hydrilla fragments that wash up on the shorelines of various water bodies. We first released this insect in southern Florida on 8 March 1991 at Lake Osborne in Palm Beach County (Center 1992). We subsequently released 218,827 weevils between then and the end of 1993, 42 percent (90,880) of which were released during this reporting period (FY93). Approximately 74 percent of the total have been released in Florida, 20 percent in Georgia (Lake Seminole), and the remainder in Alabama and Texas.

Biology

The Australian hydrilla stem weevil, previously referred to as *Bagous* new species, finally received a name in 1992, *Bagous hydrillae* O'Brien and Askevold. The life cycle and biology of *Bagous hydrillae* O'Brien in Australia were described by Balciunas and Purcell (1991). The small weevils feed on leaves and stems of the hydrilla plant. This feeding behavior creates shot-like punctures in the leaves and distinct notches in the stems. The female chews a hole in the stem tissue, often near a leaf node, in which to embed an egg. The eggs are always laid individually (as opposed to in masses). Each female lays about three eggs per day and up to 300 eggs (average 100) during her lifetime. The adults live 3 months or more. The eggs hatch in about 2 days. The larvae burrow within the stems, creating long galleries. These galleries become necrotic, eventually causing the stem around the damaged area to appear blackened externally. The stems weaken at these points and at the notches caused by adult feeding. According to Balciunas and Purcell (1991), this causes the plants to break apart and the fragments to float to shore. Some of these fragments carry larvae. The fully grown larvae purportedly pupate within the stranded hydrilla or in the soil directly beneath to consummate their developmental cycle. The larval developmental period is brief, requiring only about 6 to 8 days at 25 °C. The adults emerge from pupation in about 4 days. Newly emerged females do not lay eggs immediately, but require a 3- to 10-day preovipositional period while their ovaries fully mature. Hence, the total generation time required for an egg to transform into a mature egg-laying female is only 2 to 3 weeks.

Postrelease observations in Florida suggest the possibility that the life history of *B. hydrillae* may be significantly different from the one proposed by Balciunas and Purcell (1991). We have identified at least four possible life history scenarios or paradigms for *B. hydrillae* based on observations from releases made in Florida. These include the following: (a) perhaps the entire developmental process takes place entirely on the shoreline in stranded hydrilla and never involves submersed hydrilla at all; (b) perhaps the developmental sequence occurs entirely in submersed hydrilla and never necessitates the transition to the shoreline conditions; (c) perhaps they develop partially in submersed hydrilla and then complete development when dry season conditions cause dewatering of the site; and (d) perhaps, as described by Balciunas and Purcell (1991), they develop partially in submersed conditions and then drift to the shore to complete development. The following are descriptions of releases attempted in Florida at various sites that appear to suggest differences in the life history of *B. hydrillae* from that proposed by Balciunas and Purcell (1991). It is extremely important that the life history be understood before releases and establishment techniques can be developed.

The Ferncrest site. We reasoned, on the basis of the developmental cycle proposed by Balciunas and Purcell (1991), that if we caged numerous adults on totally submersed hydrilla with no means for fragments to drift out or larvae to escape, then these fragments should remain in the cage, be easily recovered, and contain larvae waiting to become stranded on shore. Furthermore, if the upper stratum or plant canopy (presumably the source of the drifting fragments) is preferentially attacked as indicated by the same authors, then most of the mature larvae should occur in this stratum. We constructed a cylindrical cage (Figure 10) in which to test the likelihood of these events. The cage was constructed of a rolled cylinder of 0.5-in. (1.27-cm) 12-gauge mesh hardware cloth covered with a fine mesh (0.4-mm) polychiffon fabric. The dimensions of the cage were 53 cm diam by 129 cm height. A second piece of fabric was placed over the top of the cylinder and secured with an elastic (bungy) cord that encircled the upper portion of the cylinder. The site chosen for this test was a small pond (designated the Ferncrest site) designed for highway stormwater catchment. A portion of the existing hydrilla bed was gathered up into a clump while the cylinder was worked down over it. The cage was

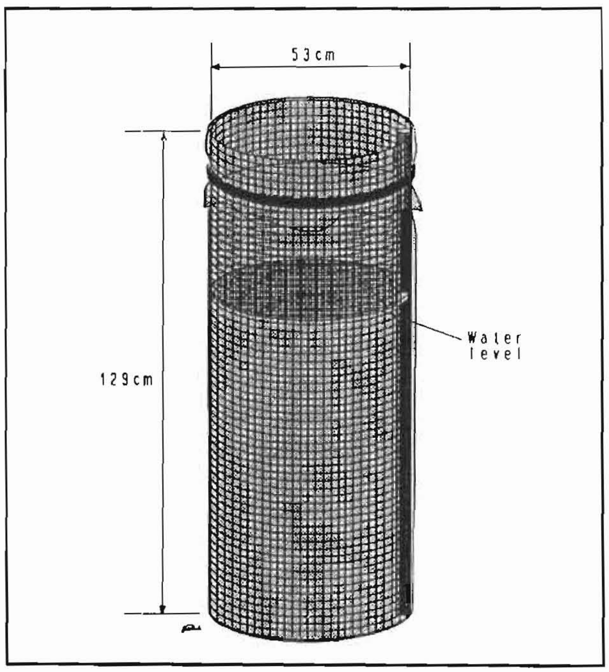


Figure 10. Cylindrical cage into which B. hydrillae were released at field sites in Florida

then tied to an 8-ft metal fence post (anchored into the hydrosoil) to hold it upright. On 25 November 1992, 1,000 weevils, all of which previously had been marked with paint, were released into the cage. Two weeks later, the hydrilla was removed as an upper canopy stratum (surface down to 25 cm) and a subcanopy stratum (25 to 125 cm below the surface). The hydrilla from each stratum was placed in a Berlese funnel.

Berlese funnels are commonly used in entomological research to extract insects from plant tissue. They consist of a metal cylinder mounted above a funnel with a grate placed in between (Figure 11). Light bulbs in the lid provide enough heat to slowly dry the plant material. This drying process forces the insects out of the plant tissue over a period of several days, through the grating, into a container of alcohol attached at the bottom of the funnel.

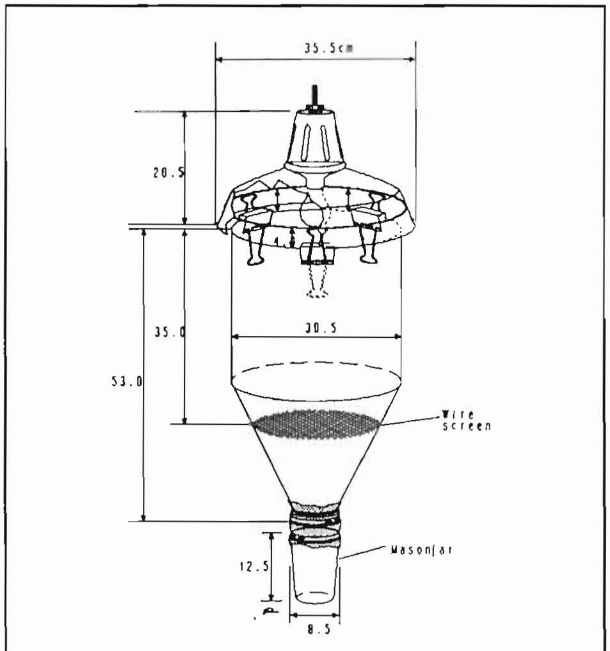


Figure 11. Berlese funnel used to extract insects from plant samples

The stem lengths of the plant material harvested from the cage totalled 4,594 cm, 74 percent of which was in the upper stratum. Berlese extractions yielded 57 larvae (about 60 cm of stem per larva) and 222 marked adults in the upper stratum and 634 larvae (about 2 cm of stem per larva) and 95 marked adults in the lower stratum. About 70 percent of the adults were in the upper stratum, which was consistent with the distribution of stem tissue. However, 92 percent of the larvae were in the lower stratum on 26 percent of the stem tissue. No unmarked adults were found, indicating that a second generation had not been produced. The short time interval would have precluded this from happening, so we could not conclude that it was not possible. The distribution of the larvae suggested that they do not reside in the upper canopy, as would be expected if they were waiting to drift ashore in detached fragments. Furthermore, no fragments were evident in the cage. The presence of larvae deep within the water column suggested that perhaps they burrow downwards towards the soil in preparation for ensuing dry conditions, and that perhaps they consummate development in the dewatered hydrosoil after the water body dries out (Paradigm 3). The fact that so many larvae developed to late instars under submersed conditions diminishes the likelihood that development takes place totally and solely on the shoreline (Paradigm 1). It does not rule out the possibility that complete development can occur on the shoreline, however, because rearing procedures routinely simulate this condition.

A second study was conducted at this site in which the weevils were allowed to remain in the cage for a longer period of time. An identical cylindrical cage was placed at the site on 4 January 1993, and the hydrilla was harvested on 11 March, 66 days later. The column was divided into three strata (surface to 0.26 m, 0.27 to 0.57 m, and 0.58 to 0.81 m), and a dredge pump was used to extract the uppermost layer of hydrosoil. In this study, the hydrilla was dried and weighed after the Berlese extractions were completed. The cage was initially inoculated with 1,067 marked weevils. The quality of the hydrilla in the cage was poor at the end of the 66-day period, and only two of the weevils were recovered (<1 percent as compared with 30 percent after

15 days in the previous study). The hydrilla biomass (total 443 g) seemed to present a dual-layered profile within the cage with the most of it (43 percent) in the lower stratum, a nearly equal amount (38 percent) in the upper stratum, and very little in the middle layer (19 percent). Only 19 larvae were extracted from the plant material. The data were too scant to assess the vertical distribution of larvae within the water column, but numbers seemed consistent with the distribution of biomass. Most (53 percent) were in the upper stratum with somewhat fewer (37 percent) in the lower stratum. One larva was found in the hydrosoil.

A third study initiated at this site employed the use of a rectangular cage designed to simulate a shoreline condition (Figures 12 and 13). The cage, which measured 122 cm high, 122 cm wide, and 244 cm long, was constructed of a wood frame with a solid plywood bottom that covered only two-thirds of the bottom opening. The remaining portion at one end of the bottom remained uncovered. The upper part of the cage was covered with the poly-chiffon netting described above. The cage was placed at the water's edge with the open portion in the water and positioned so that the naturally occurring hydrilla bed was exposed within the cage and accessible through the opening. The wooden portion of the bottom rested on shore. Clean, builder's sand (41 kg) was poured onto the wooden bottom and was graded towards the water to simulate a gently sloping shoreline. We released 1,000 marked weevils in the cage on 4 December 1992. A generous quantity of fresh hydrilla was periodically placed in the cage to supplement the natural beds and to provide an artificial strandline. The hydrilla in the cage was removed on 6 January 1993, 33 days later, and the sand was sieved. The plant material was sorted according to its location within the cage (offshore bed, transition zone, and strandline) and the total stem length in each area was measured (0.33, 1,188, and 568 m, respectively). A combination of Berlese extractions and visual inspections yielded 58 larvae, 8 pupae, and 127 adults. The presence of pupae and one unmarked adult indicated that they were completing development under these conditions. Larval counts were relatively evenly distributed with 28, 33, and 38 percent in the three respective areas, but most of the adults were found on the shoreline (77, 20, and 3 percent, respectively). Larval intensity differed in the three areas with 2, 62, and 26 cm of stem available per larva in the three respective areas. These data do not reflect natural circumstances, however, because the cage was continually replenished with fresh hydrilla. Seven of the eight pupae were found floating in the water after all of the hydrilla was removed. It was impossible to ascertain the original locations of these pupae because of the disorder caused by the harvesting process, but they might have fallen from the submersed plant material.

The Lakeview site. A second study with cages was initiated at a pond in northern Broward County, FL, which we designated the Lakeview site. Three cages were placed at the site on 11 June 1993, and 1,000 adults (unmarked) were placed in each cage. Each cage was set up as described above and was initially placed about 20 ft apart at a water depth of about 60 to 70 cm (about half of the cage height) on the lee side of the pond. All obstructions between the cage and the shoreline that might have impeded drifting fragments were removed from a triangular area with the cage at the apex of the triangle. Three weeks after release of the weevils, the cages were removed to allow any floating fragments contained within to drift towards the shore. Each week during the following 9 weeks (until 26 August 1994), three additional cages were set up in an identical manner in a different, but nearby, part of the pond. Two exceptions occurred. On 25 June, four new cages were established, and on 29 July, only one new cage was established. Because the cages were removed after 3 weeks, nine cages were usually in place at any given time. The cages were periodically sampled in order to ensure that larvae had been produced. On 25 June, 14 days after setting up the first three cages, some of the hydrilla was removed and subjected to Berlese extraction. A total of 276 adults and 58 larvae were recovered.

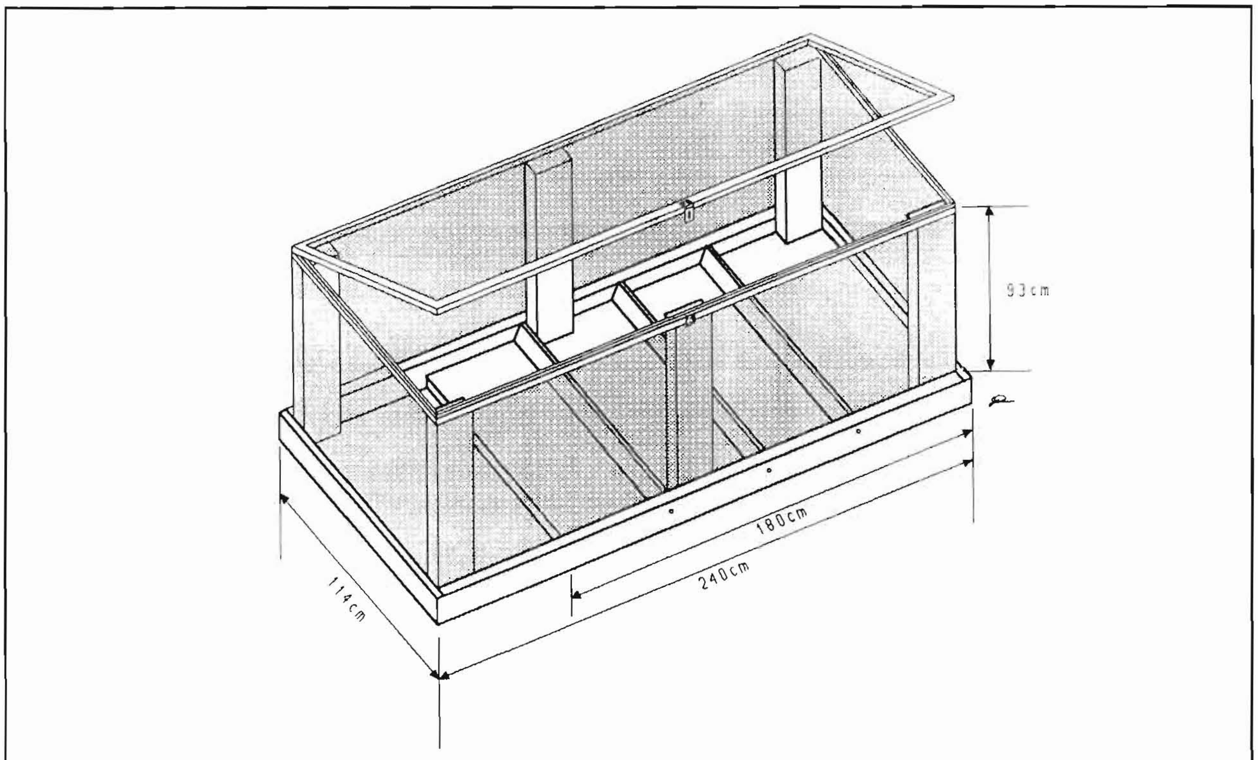


Figure 12. Three-dimensional view of rectangular cage designed to simulate natural shoreline for releases of B. hydrillae at Ferncrest site near Davie, FL

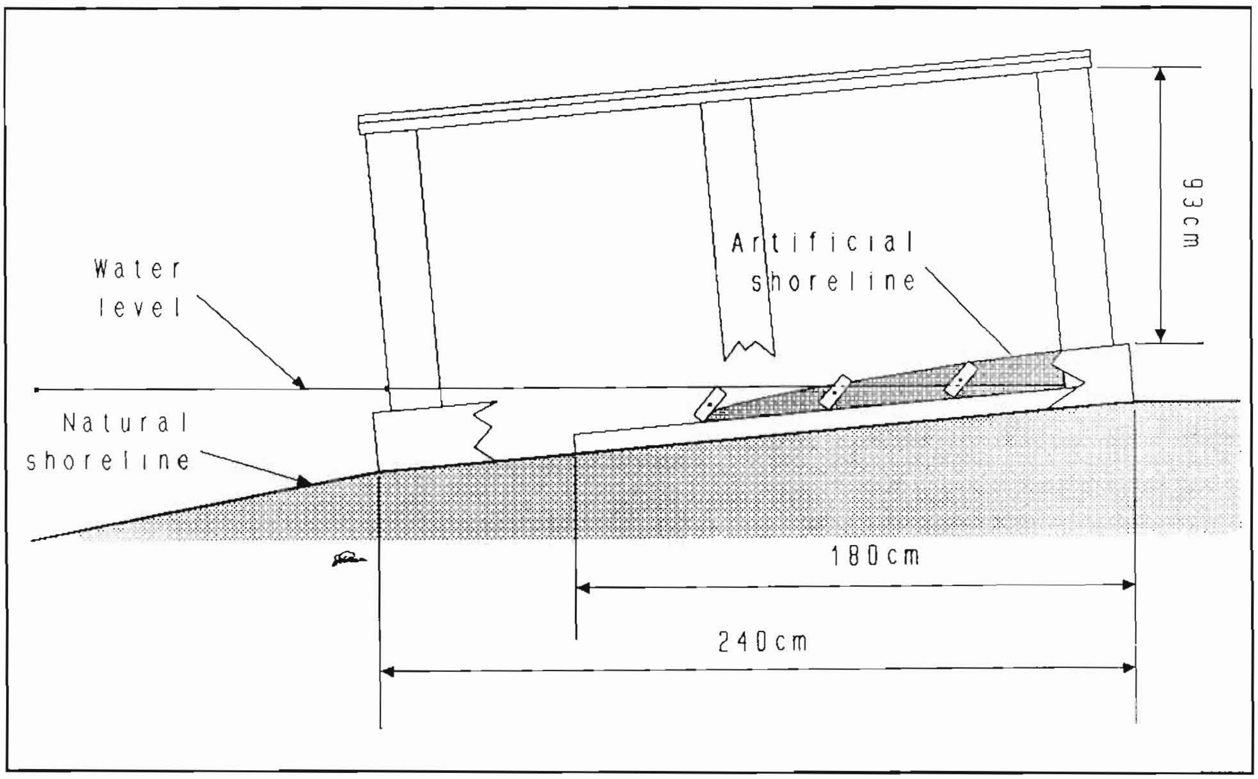


Figure 13. Side view of rectangular cage designed to simulate natural shoreline for releases of B. hydrillae at Ferncrest site near Davie, FL

The presence of so many larvae provided ample evidence that the weevils were reproducing.

After removal of the first cages and during all later site visits, the cleared areas between the release point and the shore were examined for drifting fragments. Also, the shoreline was checked for stranded fragments. On 16 July, fragments were collected that were found in the water between the shore and positions of cage previously set up on 18 June and removed on 9 July. Only a single adult was recovered. Hydrilla collected from the shoreline on 26 August yielded one adult weevil. Unusual numbers of fragments were never found, neither in the water nor on the shore, and larvae were never extracted from them. This provided further evidence of the intractability of the Australian developmental scenario (Paradigm 4).

On 23 and 29 July, hydrilla fragments were collected from the vicinity of the cages that were set up on 11 and 18 June. These were subject to Berlese extraction, and three larvae were collected from near a cage established on 11 June.

Nine adults were extracted from plant material collected on 23 July from within a cage that had been set up on 2 July. Interestingly, three of these were teneral (reddish and soft), indicating that they had recently emerged from pupation. This suggested that they had completed development within the cage, possibly in submerged conditions. We cannot, however, discount the possibility that they had been placed in the Berlese as late-instars that then pupated and emerged before the plant material had completely dried. The pupal stadium is brief, so it is not unfeasible that this could have happened.

The hydrilla beds began to deteriorate during August. In particular, the rooted lower stratum had broken loose from the hydrosoil, and a strand line was beginning to form. Hydrilla strands, collected from the shore on 19 August, yielded only one adult after Berlese extraction. We removed all of the hydrilla from one of the cages that had been set up 34 days earlier (16 July). The plant matter was separated into three strata as it was harvested. These were the top 17 cm, the section 18 cm from the surface down to 34 cm, and from 34 cm down to 5 cm below the hydrosoil. Only one larva was extracted, which was located in the upper stratum. Twenty-six adults were extracted from the upper stratum, none in the middle, and 14 in the lower stratum. It was unusual to find so many adults in the deeper portion, but these may have washed off the upper layer during handling. Very little plant material was present in the lower portion of the water column (173 g in the top 17 cm, 62 g in the middle 17 cm, and 61 g dry weight in the lower 36 cm).

By 2 November, the hydrilla beds were in terrible shape. They had broken completely free of the substrate and were freely floating within the pond. As a result, a large portion of the pond was open, whereas before it had been nearly completely occupied by hydrilla. The floating mass of hydrilla, which had drifted towards the lee side of the lake, was close to shore, and portions had formed strand lines. Hydrilla was collected from five distinct locations: (a) the contents of one cage that had been set up on 19 August (75 days earlier); (b) a remaining bit of offshore bed; (c) floating fragments; (d) floating uprooted beds; and (e) the onshore strand line. A total of 2 adults and 26 larvae were extracted from the plants in the cage, and 5 adults and 35 larvae were extracted from the strand line collections. Neither larvae nor adults were present in the other samples. This was one of the few examples where a population of this insect was found on the strand line, but the presence of the strand line was due to a general deterioration of the hydrilla beds, not to the fragmenting of stems by the insects. Hence, this cannot be construed as supporting evidence for the Australian model.

The Gainesville site. We later encountered additional evidence that the weevils might develop in completely aquatic conditions. We released 1,500 weevils in an identical cylindrical cage at a site near Gainesville, Alachua Co., FL. This cage was situated entirely in

Table 2
Numbers and Stages of *Bagous hydrillae* Released in Florida and Georgia During 1991-1993

County (State)	Site	Dates Released	Number of Releases	Numbers Released[1] Eggs	Larvae	Pupae	Adults	Infested Material[2]
Broward (FL)	Ferncrest	11/25/92-5/13/93	5	0	0	0	3,967	no
	Research Station	8/12/93-9/14/93	3	0	0	0	8,000	no
	Lakeview	6/11/93-8/26/93	10	0	0	0	29,000	yes
	Orangebrook Golf Club	7/15/91-12/17/93	14	0	0	0	44,544	yes
Brevard (FL)	Bulldozer Canal	1/29/92-10/29/93	7	0	0	0	35,415	yes
Decatur (GA)	River Junction Landing	7/9/92-11/5/92	6	0	0	0	43,754	yes
Palm Beach (FL)	Lake Osborne	3/26/91-6/28/91	3	0	23	0	1,082	yes
	West Jupiter	5/18/92-11/19/93	6	0	0	0	22,975	yes
Seminole (GA)	Reynold's Landing	5/2/92	1	0	0	0	1,000	no
Denton (TX)	Lake Ray Roberts	—	1	0	0	0	3,000	no
Alachua (FL)	Gainesville Pond	8/25/93-10/20/93	3	0	0	0	14,400	yes
Colbert (AL)	Muscle Shoals	—	2	0	0	0	6,800	yes
(TX)	Choke Canyon	—	1	0	0	0	2,800	?
Sumpter (FL)	Lake Panasoffkee	9/25/91-12/17/91	3	0	0	0	1,578	yes
	Totals	**3/26/91-12/17/93**	**65**	**0**	**23**	**0**	**218,315**	

[1] All immatures released were infesting hydrilla sprigs.
[2] We sometimes collected weevils from our colonies several days in advance of the actual release. Fresh hydrilla sprigs provided these insects with food and shelter prior to and during transport to the release site. This plant material, infested with whatever eggs the weevils had produced in the interim, was released along with the adult weevils.

the water and enclosed a portion of the hydrilla bed that existed naturally at the site. The weevils were placed in the cage on 25 August 1993. On 12 October 1993, 48 days later, the plant material within the cage was removed and divided into the top, middle, or bottom strata (surface down to 25, 25 to 75, and below 75 cm, respectively). The three strata contained 29, 4, and 0 adults and 229, 45, and 1 larvae, respectively. The adults had not been marked prior to release, so we could not readily ascertain whether a second generation had been produced; but six appeared to be newly emerged (soft cuticle, reddish coloration). A male and a female were dissected, and both seemed young (soft cuticle, soft wings, and high fat content). The female was, in fact, nulliparous with no follicular differentiation of the ovarioles, and her spermatheca was empty. This indicated that she had emerged recently and could not have been one of the weevils initially placed in the cage. Hence, this constitutes evidence that perhaps this insect can complete development in totally submersed conditions. Unfortunately, however, because of the short pupal stadium, we cannot rule out the possibility that this individual had been collected as a mature larva that pupated and emerged from the plant material after it had been placed in the Berlese funnel. It is also noteworthy that, in contrast to the Ferncrest study, most of the larvae in this experiment occurred in the upper stratum. Although a few detached stem fragments were present, the number was not unusual, and they did not seem to be associated with weevil feeding damage.

The Palm Bay site. A population of *B. hydrillae* was discovered at this site in September 1993. This was the first site at which we felt that this species might be established. Significant numbers of larvae and adults were extracted from hydrilla samples that were collected a year and a half after the weevils were last released at the site (see Establishment section, below). On 3 December 1993, we sampled the hydrilla present at the site (Bulldozer Canal) from three distinct zones: (a) stranded material that was relatively dry, (b) submersed hydrilla in the shallowest portions of the beds nearest the shoreline, and (c) in the deepest part (about 1 m deep) of the canal. Five samples were taken in each zone. A total of 3 adults and 11 larvae were extracted from the stranded material, 0 adults and 9 larvae from the shallow beds, and nothing from the deeper beds. We repeated this a month later (6 January 1994), but deleted the samples from the deeper beds. The shoreline samples yielded 14 adults and 3 larvae, whereas the shallow beds yielded only one larva. In January, the shallowest beds were separated from the shore by a region of uninfested water, whereas during the previous month, the shallow beds were adjacent to the shoreline. Hence, at this site, it appeared that the weevil infested the shoreline and near-shore portions of the hydrilla beds and not the deeper water portions. Perhaps the portions that are most susceptible to becoming stranded, particularly as water levels fluctuate, are favored by the weevils.

Releases

The productivity of the *B. hydrillae* cultures maintained in Fort Lauderdale increased by at least one order of magnitude in 1992-93 relative to 1991-92. Improved productivity was due to streamlining of the rearing process, which reduced handling time and allowed for the maintenance of more colonies. As a result, we released about 91,000 weevils this year, increasing the total numbers released to over 218,000 (Table 2).

Weevils released prior to 1992 had been the progeny of insects originally brought into United States quarantine in 1987. Because of this long-term laboratory rearing, we were concerned that inbreeding might have reduced vitality of the weevil cultures. As a precaution against this, we imported new stock from Australia during March 1992. We terminated laboratory colonies containing the older stock after new colonies began producing sufficient numbers of weevils for release.

Lake Seminole (Georgia) received nearly 20 percent of the *B. hydrillae* released (Table 2). Our strategy was to inundate the site with large numbers of weevils over a relatively short (July-October 1992) period. Consequently, we conducted seven releases at this site, averaging about 6,400 weevils apiece. We were encouraged by surveys conducted between releases that yielded damaged plants and adult weevils. Unfortunately, flooding raised water levels to the point that the hydrilla no longer reached the water surface and might, therefore, have been unavailable to the weevils. Flood waters also scattered fragments and inundated the sandy shorelines needed for pupation. However, we cannot rule out the possibility that nascent populations have become established elsewhere on the lake by way of the scattered, larvae-infested fragments. The project at Lake Seminole was terminated just before we made the last releases there, so the results of these efforts were never fully evaluated.

About half of the weevils were released in south Florida in an attempt to establish nursery colonies. These nursery colonies, after they are established, serve as a source of insects for distribution to other areas. This ability to field collect weevils allows for discontinuation or reduction of expensive and labor-intensive laboratory rearing and so is quite important. It also allows for more emphasis on research and less on insect production, and so is eagerly sought. This is why such a large portion of the total production was devoted to this effort.

A few of the weevils (17 percent) were released in central Florida (Brevard and Sumpter counties) and northern Florida (7 percent). Towards the end of the reporting period, we had also begun supplying weevils for release at Tennessee Valley Authority facilities and in Texas. Hence, as our ability to produce weevils has increased, we have supported release efforts over a progressively broader geographic area.

Establishment

Visual examinations of the Palm Bay site in mid-September 1993 produced five adult weevils, one of which appeared to be *B. hydrillae*. Hydrilla samples collected during the same trip produced about 1,500 adults and nearly 600 larvae during 30 days in Berlese funnels. Weevil abundance in these samples was surprisingly high and has not been duplicated during sampling on later dates. *B. hydrillae* has a generation time of only 2 to 3 weeks (see Biology section above). Thus, it is conceivable that weevil colonies developed in the Berlese funnels from a small number originally infesting the Palm Bay material. The possibility that the large number of weevils represented a seasonal population peak is equally plausible, however.

These recoveries represent the first concrete evidence that the Australian stem weevil is established in Florida. No weevils had been released at the site for 17 months. Thus, the population had successfully survived winter 1992-93. Also, the hydrilla population at the site had been uprooted and washed downstream during flooding in June 1992, so the weevils had survived this catastrophe. These adults potentially represented the 25th generation.

Weevils recovered at the Lakeview site on 2 November 1993 indicated that a population had persisted for 68 days. This was a promising sign of establishment, but we had previously recovered insects after similar periods at sites where populations failed to develop. The numbers recovered, however, were unusually high and indicate that the population might be viable. We will continue watching this site to verify establishment.

Future plans

We feel we have come far in terms of the release and establishment of the leaf-mining flies used for the management of hydrilla, especially *H. pakistanae*. However, more research is needed before these organisms can be used operationally for the management of hydrilla. The main thrust of our future research in the use of the leaf-mining flies is to determine the nature of their impact to hydrilla across large geographical regions. This will involve additional quantitative sampling from field-established populations from differing regions of the country. We are also planning more controlled experimentation where both the plant's and agent's environmental regimes are controlled in an effort to separate the effects of the fly feeding from that of changes in environmental conditions. In addition, we are planning to continue our releases of *H. balciunasi* in southern Texas in the hope that a larger number of field sites can be established. Finally, we will continue our releases of the Chinese strain of *H. pakistanae* in the more northern hydrilla ranges. It is believed that this strain may afford more cold hardiness since it originates from a more temperate region of China.

We also feel that populations of the hydrilla stem weevil *B. hydrillae* are now established. However, this establishment is tentative in that the weevils seem to be doing well at only one site. We intend to continue efforts to establish additional populations at other sites, particularly in other states. We also hope to make a final determination on the ability of this insect to impact offshore hydrilla beds. This determination will not be possible, however, until thriving populations are available for study. If this insect is restricted to shoreline or drawdown conditions, it may not be a useful biocontrol agent, or different strategies for its use may be needed.

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Release and Establishment of Insect Biological Control Agents of Pistia

by
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Pistia stratiotes L. (waterlettuce) grows in slow-moving waters throughout much of the tropics and subtropics (Sculthorpe 1967). Rapid vegetative growth and copious seed production allow this aquatic macrophyte to achieve nuisance levels in many of these regions (Bua-Ngam 1974; Mangoendihardjo 1983). In Florida, for example, large impenetrable mats impede irrigation practices, hamper flood control efforts, block navigational channels, and interfere with the recreational uses of many waterways (Habeck et al. 1987; Thompson and Habeck 1989). Thus, a South American weevil, *Neohydronomus affinis* Hustache, was released in 1987 as a biological control for use against waterlettuce in Florida (Dray et al. 1990; Dray and Center 1992). This weevil has been associated with waterlettuce declines on several waterways, but has not been universally effective (Dray and Center 1992). Failure of this biocontrol agent to establish at some sites (Dray and Center 1992) reinforced the idea that an effective biocontrol program against waterlettuce would require the introduction of several (rather than single) agents.

The moth *Spodoptera pectinicornis* Hampson is native throughout much of tropical Asia (Habeck et al. 1988, 1989; Dray and Center 1993). Larvae severely damage waterlettuce leaves, but will also feed on other vegetative portions of the plant (Suasa-Ard 1976; Suasa-Ard and Napompeth 1982). Consequently, Bua-ngam (1974), Suasa-Ard (1976), and Mangoendihardjo (1983) all recommended this insect as a biological control against waterlettuce. In Thailand, augmentation of natural *S. pectinicornis* populations with cultured insects helps prevent seedling destruction by waterlettuce that invade rice paddies (Napompeth 1982). Augmentative releases have also been used to eliminate *P. stratiotes* populations that threatened Thai hydroelectric operations (Napompeth 1982). The success of these programs suggested that *S. pectinicornis* was an ideal candidate for use in Florida. Host screening studies demonstrated that this insect specializes on waterlettuce (Habeck et al. 1988, 1989; Center and Dray 1990). As a result, it was approved in 1990 for release in the United States (Grodowitz 1991; Dray and Center 1993).

We began releasing *S. pectinicornis* in Florida during December 1990 (Grodowitz 1991; Dray and Center 1993). Establishing persistent field colonies of this insect has proven more difficult than first imagined, however. Consequently, the strategies that we have employed in our attempts have evolved during the past 3 years. This report documents this evolution and describes the current status of the project.

Culture Techniques

The techniques we employ to culture *S. pectinicornis* are modifications of methods developed by Dr. Dale Habeck (University of Florida, Institute of Food and Agricultural Sciences, Department of Entomology and Nematology) and his assistants during the quarantine testing of this moth. Most commonly, we introduce adult moths into clear plastic bowls (2.8-L, 20-cm-diam) containing

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waterlettuce leaves on moist paper towels. Females oviposit for 1 to 5 days (depending upon the condition of the leaves). Eggs are monitored daily. Approximately 5 days after eclosion, infested leaves are placed on wire screens (13-mm mesh) over clear plastic shoe boxes (4.5-L, 32- by 18- by 10-cm). These containers are filled with small (<15-cm-diam), intact waterlettuce plants from which roots and senescing leaves have been excised. Larvae migrate out of the decaying material on the screen and fall onto the fresh plant material below. Immatures are transferred via this technique to fresh plant material every 3 days. Pupae are removed from the containers and placed on moist filter pads in large (153-mm-diam, 44-mm-high) Petri dishes (about 50 pupae/dish). Adults that emerge are released at field sites or introduced into a plastic bowl to continue the cycle.

We also attempt to simulate more natural conditions and, thereby, reduce the labor involved in culturing this insect. Adults are released 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.

Release Strategies

We have attempted over the past 3 years to establish self-perpetuating populations of *S. pectinicornis* in Florida. With each attempt, our knowledge has improved regarding factors that impair the establishment process. Consequently, the strategies employed in conducting these releases have gradually evolved from a simple, "hands-off" approach to a very involved process whereby we attempt to control as many mitigating factors as possible. In total, we have applied four distinct release strategies to inoculate 16 field sites with nearly 223,000 *S. pectinicornis* eggs, larvae, and adults.

Broadcast release strategy

Dr. Habeck found during host screening studies (Habeck et al. 1989) that maintaining laboratory colonies of this moth was a simple matter. Larvae fed voraciously, so much so that providing sufficient waterlettuce became quite a chore. Adults emerged routinely, and though emergences were asynchronous, females appeared to find mates and oviposit without difficulty. These factors convinced us that establishing *S. pectinicornis* populations at field sites would be a simple matter. Thus, during initial efforts, we employed a strategy of releasing small numbers of eggs and larvae at many sites and then waited for the numbers to build naturally.

About 42,000 *S. pectinicornis* were released at nine sites using this broadcast release strategy (Table 1). Unfortunately, follow-up visits failed to produce evidence that the moths were persisting at any of these sites. Plant samples recovered from the sites held no egg masses, larvae, or pupae. Adults were never collected nor observed. Feeding damage that was clearly attributable to this insect was rare. Consequently, we concluded that we needed to revise our approach to establishing persistent field colonies.

Composite release strategy

Our inability to establish persistent *S. pectinicornis* populations utilizing the broadcast release strategy (also known as the "Johnny Appleseed" approach) suggested that we needed to inoculate sites with larger numbers of insects. There were limitations to the numbers of *S. pectinicornis* that the laboratory could produce within a given time frame, however. This factor, coupled with the requirement for more insects per site, served to reduce the number of sites inoculated under the new strategy. Thus, we initiated the composite release strategy whereby we conducted several releases of large numbers of insects at a few sites. In addition, routine visits to release sites for monitoring purposes seemed advisable.

Table 1
Florida Waterways Inoculated with *Spodoptera pectinicornis* Employing Broadcast Release Strategy

County	Site	No. Releases	Dates	No. Released: Eggs and Larvae	Pupae	Adults	Plants[1]
Brevard	St. John's Marsh	2	1/2/91-4/25/91	4,468	0	0	No
Broward	Loxahatchee Recreation Area	1	9/9/91	3,000	0	0	No
	Tallowwood	1	10/7/92	750	0	0	No
Citrus	Lake Rousseau	4	7/9/92-10/7/92	14,750	0	0	No
Gadsen	Havana Pond	2	9/7/91-9/11/91	1,500	0	0	No
Palm Beach	Pioneer Park	5	12/30/90-10/4/93	11,771	2	44	No
	South Florida Fairgrounds	1	12/5/90	1,500	0	0	No
St. Lucie	Port St. Lucie	2	12/1/90-12/30/90	3,208	0	0	No
Sumpter	Lake Panasoffkee	2	9/25/91-9/30/91	1,200	0	0	No
	Totals	**20**	**12/1/90-10/4/93**	**42,147**	**2**	**44**	

[1] Whole waterlettuce plants (as opposed to individual leaves) infested with unknown numbers of *Spodoptera pectinicornis* larvae and eggs.

This strategy was applied during 1991 to three Florida waterways (Table 2). Initial results were favorable. Examinations of release areas produced feeding damage attributable to *S. pectinicornis*. In addition, larvae and pupae were occasionally recovered, though never in large numbers, from plants at these sites. We released only larvae at these sites. Thus, the presence of pupae indicated released insects were feeding and continuing to develop. Unfortunately, flood waters washed the waterlettuce from one of these sites. Recoveries of insects waned at the remaining two sites, suggesting further modification of our approach was needed.

Although the composite release strategy produced disappointing results regarding *S. pectinicornis* population establishment, it provided several useful lessons. First, our inability to consistently recover immature stages, even a few days after the release of thousands, suggested that establishment required greater numbers of insects than we were applying to each site. This, in turn, implied that we needed to concentrate our efforts at a single, nearby site. Such an approach would permit us to access the site as frequently as necessary for releases and monitoring. Second, shifting of the plant mats at two of our sites made locating the specific plants that we inoculated

Table 2
Florida Waterways Inoculated with *Spodoptera pectinicornis* Employing Composite Release Strategy

County	Site	No. Releases	Dates	No. Released: Eggs and Larvae	Pupae	Adults	Plants[1]
Glades	Fisheating Creek	13	12/18/90-12/28/91	22,617	0	0	No
Okeechobee	Eagle Bay (Lake Okeechobee)	12	4/24/91-8/12/91	31,163	30	100	Yes
Putnam	Lake Oklawaha	10	2/13/91-8/30/91	28,100	15	4	No
	Totals	**35**	**12/18/90-12/28/91**	**81,880**	**45**	**104**	

[1] Whole waterlettuce plants (as opposed to individual leaves) infested with unknown numbers of *Spodoptera pectinicornis* larvae and eggs.

or released very difficult. This demonstrated the importance of clearly delimiting, and perhaps trying to restrict the movement of, the area of the mat in which releases occurred. Third, the absence of eggs on plants in the release areas suggested that adult moths were dispersing after emergence rather than remaining in the area to mate and oviposit. Alternatively, adults emerging in the absence of few or no other adults may have fallen victim to predation prior to locating a mate. Finally, the sluggish behavior of the larvae suggested that insects released at field sites may be suffering extremely high mortality because of predation. We suspected this lack of vitality was genetically based because we were releasing descendants of moths imported into quarantine during 1987. This suggested that our colonies had become so adapted to laboratory conditions that the insects they produced lacked sufficient vigor to persist in the wild.

Inundative release strategy

In an attempt to resolve these problems, we adopted an approach called the inundative release strategy. This scheme required conducting frequent releases of large numbers of *S. pectinicornis* at a single site. Also, Dr. Habeck imported more moths from Thailand to improve the available gene pool, thereby overcoming any problems related to the inbreeding that occurs during long-term laboratory colonization. We received F_2 offspring of these new moths in June 1992 and immediately set about releasing a portion of them at field sites. Subsequent releases came from colonies comprised of this fresh germplasm.

We released about 86,000 *S. pectinicornis* utilizing the inundative release strategy (Table 3). Releases averaged nearly 2,000 insects per week. Flooding the site with insects served to improve the probability that some of the released insects would survive to complete development. This, in turn, meant that adults should emerge in greater numbers and more frequently. Consequently, females would be more likely to locate a mate and oviposit in the immediate vicinity of their emergence. A 10-m^2 floating polyvinyl chloride (PVC) frame surrounded waterlettuce plants inoculated with the insects. The frame restricted intermixing of infested plants with other plants at the site, thereby aiding our monitoring efforts.

Acquisition of the fresh germplasm provided immediate benefits; larvae from the new colonies were clearly more active than those from the older colonies. Within a few weeks, we began to recover larvae from plants both inside and outside the release frames. Further, we began to occasionally recover plants containing *S. pectinicornis* egg masses at the site. This latter observation was particularly important because it presented the first concrete evidence that released individuals were completing development, emerging as adults, and ovipositing to produce an F_1 generation.

Hurricane Andrew struck South Florida in August 1992. Water level manipulations by the South Florida Water Management District in anticipation of this storm resulted in the loss of most of the waterlettuce at the Andytown study site. A posthurricane visit to the site in September produced two late instar larvae

Table 3
Florida Waterways Inoculated with *Spodoptera pectinicornis* Employing Inundative Release Strategy

County	Site	No. Releases	Dates	No. Released: Eggs and Larvae	No. Released: Pupae	No. Released: Adults	Plants[1]
Broward	Andytown	43	11/6/91-8/17/92	83,306[2]	1,710	986	Yes
Palm Beach	Canal Point	4	8/20/92-10/2/92	5,025	10	0	Yes
	Totals	**47**	**11/6/91-10/7/92**	**88,331**	**1,720**	**986**	

[1] Whole waterlettuce plants (as opposed to individual leaves) infested with unknown numbers of *Spodoptera pectinicornis* larvae and eggs.
[2] This figure includes estimates of numbers of eggs contained in egg masses.

from the few plants that remained. About 6 weeks had passed since the last release at the site. Thus, these larvae had to represent an F_1 and possibly even an F_2 generation. Subsequent visits to the site failed to produce evidence that the population was persisting, however.

Results from Andytown were very encouraging. The infusion of fresh germplasm combined with application of the inundative release strategy seemed to have provided for the development of a nascent *S. pectinicornis* population at Andytown prior to the hurricane. Our observations had suggested, however, that releasing the insects into cages might improve mating success of emerging females and concentrate oviposition onto a small patch of the waterlettuce mat. Thus, we began releasing into cages at a site near Canal Point.

We inoculated a floating cage (1.2-m-long by 0.9-m-wide by 0.5-m-high) covered with nylon screen (1.85-mm-mesh) at this site with about 5,000 larvae during late August (Table 3). A second cage was inoculated with a small number of pupae during September (Table 3). Examinations of plants within the cages produced *S. pectinicornis* larvae, pupae, and adults, but also larval *Samea multiplicalis* (a native moth). As *S. multiplicalis* densities increased within the cages, the nascent *Spodoptera* populations declined. Finally, fire ants invaded the release cages during late September after which we were unable to recover any *S. pectinicornis.*

Modified inundative release strategy

Further refinement of the release strategy was clearly needed. Releasing into cages was effective at concentrating the *S. pectinicornis*, but we needed to prevent invasion by *S. multiplicalis* and fire ants. Also, it appeared that releasing adults or pupae, rather than larvae and eggs, allowed females to choose optimal oviposition sites. This, in turn, would enhance colony development. Finally, *S. pectinicornis* colonies established readily and decimated waterlettuce in outdoor aquaria at our laboratory. Failure of field releases to produce similar results led us to suspect that poor plant quality may be inhibiting field colonization. Thus, we modified the inundative release strategy to address these problems.

The modified inundative release strategy employed frequent releases of *S. pectinicornis* onto high-quality, insect-free plants in a cage isolated from surrounding vegetation. Laboratory-cultured waterlettuce were treated with Malathion to eliminate *S. multiplicalis* and other insects. Treated plants remained isolated for at least 3 weeks. This permitted leaves present during treatment to senesce and slough off. These plants were then placed into floating PVC cages (3.05- by 3.05-m) covered with nylon screening (1.85-mm-mesh) (Figure 1). The cages contained floating chlorine dispensers adapted to distribute Osmocote (a 4-month, resin-coated, controlled-release fertilizer with 14N:14P:14K) among the waterlettuce roots in an effort to maintain high plant quality on site. Each float contained 500 g of fertilizer, and each cage held four floats. These cages were enclosed by a second floating PVC frame (9.35- by 9.35-m), and vegetation was removed from the area between the two frames (Figure 1). This action effectively created a "moat" around the cages that inhibited fire ants, spiders, and other crawling predators from accessing the caged plants.

We began releasing *S. pectinicornis* at Port Mayaca during January 1993 (Table 4). Our strategy was to release adults, thereby allowing the females as natural a choice of oviposition sites as possible. We inoculated two cages at Port Mayaca with about 2,600 adults between January and May 1993 (Table 4). Plants in the first cage became stressed during March, when Cage 1 held about 23.5 *S. pectinicornis*/square meter (Table 5). In late May, the plants from both cages were inserted into the surrounding waterlettuce mat. Plants from Cage 1 exhibited extensive feeding damage at this time, while those from Cage 2 showed only mild damage. Waterlettuce samples collected from the cages prior to the release failed to produce any *S. pectinicornis.* However, plants from Cage 1 became heavily

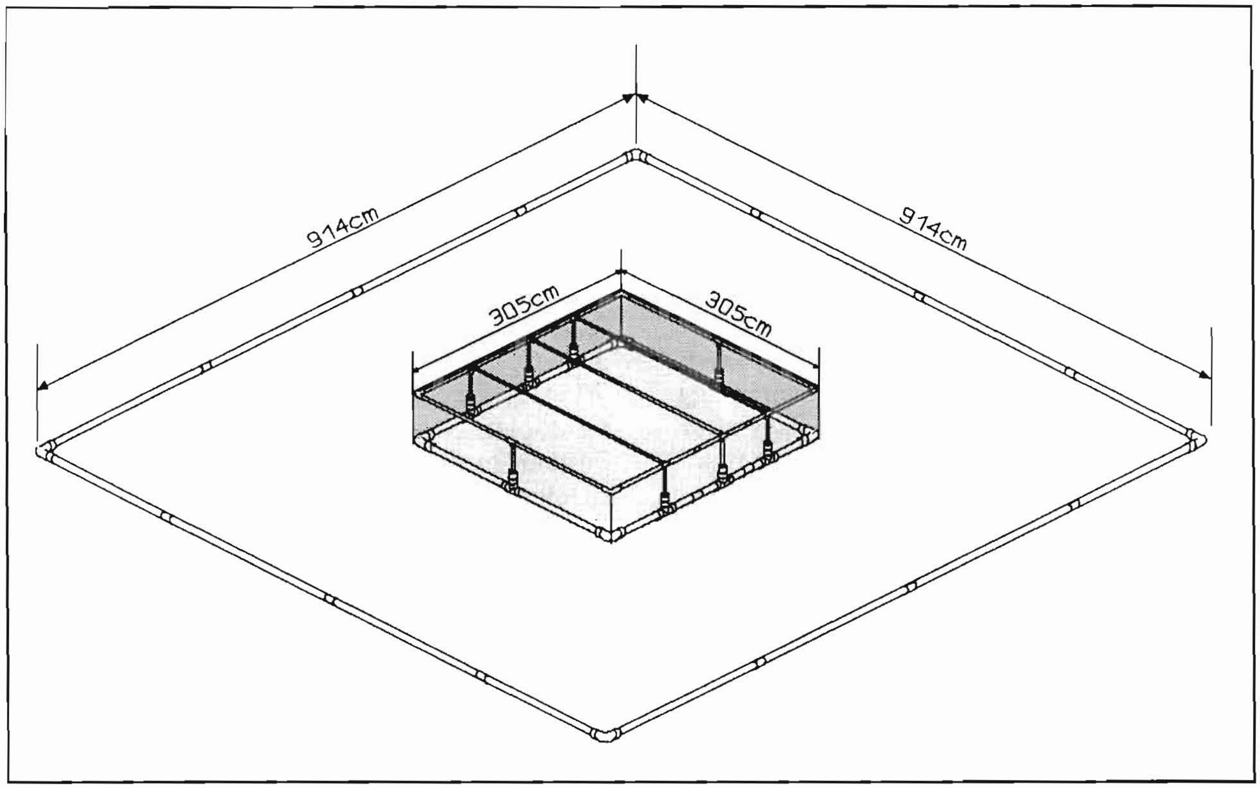

Figure 1. Cage design used with the modified inundative release strategy employed in attempts to establish persistent Spodoptera pectinicornis populations in southern Florida

Table 4
Florida Waterways Inoculated with *Spodoptera pectinicornis* Employing Modified Inundative Release Strategy

County	Site	No. Releases	Dates	No. Released: Eggs and Larvae	No. Released: Pupae	No. Released: Adults	Plants[1]
Broward	Andytown	3	7/30/93-8/18/93	5,350	0	0	No
Palm Beach	Port Mayaca	24	1/11/93-4/28/93	75	23	2,609	No
	Totals	**25**	**1/11/93-8/18/93**	**5,425**	**23**	**2,609**	

[1] Whole waterlettuce plants (as opposed to individual leaves) infested with unknown numbers of *Spodoptera pectinicornis* larvae and eggs.

Table 5
***Spodoptera pectinicornis* Recovered from Waterlettuce in Cage 1 at Port Mayaca, Florida**

Date	No. Plants Examined	*S. pectinicornis*: Larvae	Pupae	Adults
2/19/93	10	13	0	0
2/26/93	14	57	1	0
3/3/93	15	64	9	0
3/19/93	8	21	18	1
4/15/93	10	41	0	0
5/12/93	7	0	0	0

infested with fire ants prior to processing, so the absence of *S. pectinicornis* may represent predation. An examination in mid-June of 30 plants from the infested mat produced similar results.

Failure to recover *S. pectinicornis* from the infested mat was disappointing. However, 3 months of intensive *S. pectinicornis* feeding pressure had devastated plants in Cage 1, leaving very little plant material to support the insect population. Insect density in Cage 2 appeared low. Thus, we may have released only a few *S. pectinicornis*. Also, the waterlettuce in the mat surrounding the cages appeared to be poor quality relative to plants within the cages.

We were encouraged by results from the Port Mayaca study despite the disappointing final outcome. A large *S. pectinicornis* population developed within one of the cages, and population density remained high for at least 60 days (Table 5). Further, we recovered egg masses from Cage 1 as much as 21 days after release of adults. Adult longevity ranges from 3 to 6 days (Suasa-Ard and Napompeth 1982), so these eggs represented progeny of F_1 adults produced onsite. Consequently, we felt that the modified inundation release strategy was effective. However, infested plant material needed to be transferred from the cages to the surrounding waterlettuce mat after only a few weeks rather than waiting several months.

We returned to the Andytown site in July 1993 to apply the modified inundative release strategy on waterlettuce that had reinvaded the site after the hurricane. Producing large numbers of adult *S. pectinicornis* had proven to be very labor intensive, so we decided to inoculate a single cage at Andytown with larvae (Table 4). Infested waterlettuce were removed from the cage weekly and placed within the outer frame. The infested material initially was replaced by clean plants (as described above for Port Mayaca), but eventually replaced by plants from the surrounding mat.

Examinations of the site conducted on 2 November 1993, 76 days after the final release, produced larvae, pupae, and adults. Larvae were recovered up to 15 m away from the outer frame. Subsequent examinations at 114, 132, and 140 days following the last release always produced at least egg masses outside the cage. Live pupae were recovered from within the cage 140 days following the last release. Suasa-Ard and Napompeth (1982) reported complete development required from 21 to 35 days. Thus, eggs and pupae recovered 140 days after the last release represent at least the F_4 and perhaps as much as the F_7 generation. These data suggest that a self-perpetuating *S. pectinicornis* population has become established at the Andytown site.

Future Plans

We will continue monitoring the Andytown release site during fiscal year 1994. Although *S. pectinicornis* appears to be established at the site, population density is extremely low. Whether the incipient population will survive the cooler winter months remains to be seen. Further, mere survival is insufficient. If this species is to be an effective biocontrol of waterlettuce, numbers must increase and additional sites must become infested from the Andytown locus.

Anecdotal evidence from Thailand suggests this moth may be slow to disperse. If so, we will need to augment the natural spread of this insect. Also, we plan to infest other waterlettuce sites using the modified inundative release strategy beginning in Spring 1994. Thus, we will continue to maintain the laboratory colony of *S. pectinicornis*.

Preliminary surveys of the waterlettuce fauna in Mexico, conducted during October 1993, produced adult and larval weevils in abundance. The larvae were very large and appeared to be devastating the waterlettuce populations they infested. The adults were later identified by Dr. Charles O'Brien (Florida Agricultural and Mechanical University, Tallahassee, FL) as *Neohydronomus elegans* O'Brien and Wibmer, but there is some question regarding whether the larvae are of the same species. We plan to repeat this survey during 1994 and hope to collect additional larvae that can be reared to adults for identification.

About a dozen weevil species from Central and South America, including *N. elegans*, are known only from *P. stratiotes*. Studies with the closely related *N. affinis* suggest that determining the host range of *N. elegans* should require very little effort. In fact, several of the weevils found on waterlettuce may be specialists. Thus, we would like to gather sufficient data through the surveys to support requests to import one or more of these weevils into quarantine for host range studies. Should they prove to be the specialists that they appear, we would like to release them against waterlettuce in Florida over the next few years.

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A Historical Perspective of Biocontrol of the Submersed Macrophytes *Myriophyllum spicatum* and *Hydrilla verticillata* Using Plant Pathogens

by
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Introduction

Biocontrol of the submersed aquatic plants *Myriophyllum spicatum* (Eurasian watermilfoil) and *Hydrilla verticillata* (Hydrilla) using plant pathogens has to date focused exclusively on the use of endemic fungal organisms. Two of the most promising candidates have been strains of the fungus *Mycoleptodiscus terrestris* (Gerd.) Ostazeski (Mt) isolated from plant material of Eurasian watermilfoil collected in Massachusetts and hydrilla in Texas. An inundative approach rather than a classical approach has been used in the testing and development of the pathogens for biocontrol purposes.

Eurasian Watermilfoil

The watermilfoil pathogen was isolated in the late 1970s from necrotic milfoil plant tissue collected from a pond in western Massachusetts (Gunner 1983). That the organism had promise as a biocontrol agent was demonstrated in small-scale laboratory tests. Milfoil plants grown in small jars exhibited 100-percent mortality within 24 days following treatment with Mt (Gunner 1983). Subsequent laboratory tests produced similar results (Gunner, Limpa-Amara, and Weilerstein 1988). When testing was upscaled to 1.6-m^2 pools planted with milfoil, biomass harvested from control pools was found to be significantly greater than biomass harvested from Mt-treated pools (Gunner, Limpa-Amara, and Weilerstein 1988).

Small-scale field testing of Mt was initiated in the mid-1980s at Stockbridge Bowl, a milfoil-infested pond located in Stockbridge, MA. Two-weeks postinoculation, leaf necrosis and chlorotic stems were diagnostic of Mt-treated plants (Gunner, Limpa-Amara, and Weilerstein 1988). Little new growth was observed as compared with untreated plants. At the termination of the experiment, no significant reductions in aboveground biomass were found between treated and untreated plots (Gunner, Limpa-Amara, and Weilerstein 1988). The following year, a successful field test at the same location produced a 16-fold reduction in stem-leaf biomass and a 10-fold reduction in root biomass (Gunner 1987).

Encouraged by results of laboratory, pool, and pond tests, development of a mycoherbicide with Mt as the active ingredient was undertaken by researchers at EcoScience, Inc., in the 1980s. Production of formulated Mt was a two-stage process whereby the fungus was first grown in large fermenters to produce a mycelial mat and then incorporated into a calcium alginate medium. The resulting pellet-shaped formulation was subjected to laboratory testing at EcoScience and at the U.S. Army Engineer Waterways Experiment Station with promising results. Within a temperature range of 20 to 28 °C, a significant reduction in aboveground biomass was achieved when pellets were applied to milfoil grown in tall cylinders (Shearer 1992).

Results from the first small-scale field test of the formulation in milfoil-planted ponds at the Lewisville Aquatic Ecosystem Research Facility in July 1990 were disappointing. It was suggested that elevated water temperatures in excess of 30 °C during the test period suppressed a disease epidemic because it retarded

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growth and reduced virulence of the fungus (Smith and Winfield 1991). The spheroidal-shaped formulation also showed deficiencies in that water movement in the plant beds dislodged the pellets from the milfoil plants resulting in most of the inoculum settling to the sediment surface and rendering it useless.

A major modification was made to the shape of the formulation in 1991. The mycoherbicide was redesigned to be exuded into strings to improve retention rate in the plant canopy. A formulation field test at the Lewisville facility confirmed that strings had a better capacity to become entangled in the milfoil vegetation than pellets.

An experimental use permit to allow field testing of Mt as formulated strings, Aqua-Fyte, in designated sites was approved by the U.S. Environmental Protection Agency in December 1991. In July 1992, Aqua-Fyte was tested in field plots set up on a 62-acre pond adjacent to Guntersville Reservoir, Guntersville, AL. One-month postapplication of the mycoherbicide, no significant differences were found in wet weights of aboveground biomass samples from treated versus untreated plots (Shearer 1992).

Inconsistencies in performance of formulated Mt between laboratory and field trials dictated the need to reexamine the fungus for its capacity to incite a disease epidemic on watermilfoil. The inability of the mycoherbicide to perform in the field might be attributed to the pathogen, to the formulation, or to any number of biotic or abiotic factors. However, because the only previously reported successful field test using Mt as a potential biocontrol agent for milfoil (Stockbridge Bowl) had used fungal mycelium as the inoculum rather than a formulated product, the effectiveness of the fungus alone needed to be reassessed. Following a series of laboratory tests confirming that Mt applied as fungal mycelium to milfoil plants produced disease and plant mortality, another field test was initiated June 1993 at the Guntersville, AL, pond site. Mt grown in large industrial fermenters was applied as a mycelial matrix to milfoil plots. One-month postapplication, no significant differences were found in wet weights of aboveground biomass samples from treated versus untreated plots.

Poor results from field trials utilizing the Massachusetts strain of Mt emphasized the need to reassess endemic pathogens as control agents for milfoil. Comparison of the effectiveness of the Massachusetts isolate versus Mt isolates from different geographical regions as well as other endemic pathogens of milfoil need to be evaluated in laboratory and greenhouse studies. Pathogenic isolates will be screened in the laboratory and rated for pathogenicity and virulence. The best performing candidates will undergo further testing for field effectiveness in small research plots set up in milfoil-planted ponds at the Lewisville Aquatic Ecosystem Research Facility.

Hydrilla

The hydrilla pathogen was first isolated from hydrilla growing in Lake Houston, TX (Joye 1990). Initially identified as *Macrophomina phaseolina* (Tassi) Goid., it was later confirmed by B. C. Sutton at the International Commonwealth Institute to be *Mycoleptodiscus terrestris*. Subsequent plating of diseased hydrilla stem tissue collected at Lake Sheldon, TX, yielded additional isolates of Mt equally pathogenic on hydrilla.

Testing of the Texas isolates of Mt in laboratory and greenhouse studies have shown the potential of the pathogen for controlling hydrilla. Joye (1990) reported a 95- to 99-percent reduction in biomass of treated versus untreated hydrilla grown in cylinders 150 cm long and 13.75 cm in diameter. Similar results were obtained with the Sheldon Reservoir isolates of Mt (Shearer, unpublished).

Field studies in which a mixture of fungal mycelium and microsclerotia were applied to hydrilla in small enclosures (1- by 1- by 2-m high) were successful in reducing aboveground biomass of hydrilla by 61.3 and 58.0 percent for 1988 and 1989 tests, respectively (Joye 1990). Disease symptoms on plants in the field were similar to those reported from greenhouse-grown hydrilla. Disease progression began as leaf chlorosis and advanced to tissue degradation within 10 days. High dose rates brought about complete disintegration of plant tissues within 21 days.

Field tests of the hydrilla pathogen applied to pond-grown hydrilla at the Lewisville Aquatic Ecosystem Research Facility produced diagnostic disease symptoms on the host plant. Mt significantly reduced aboveground biomass of hydrilla in three of the four test plots 1 month postapplication compared with untreated plots.

Although Mt-treated hydrilla became diseased, plants appeared to recover and regrow. This phenomenon was most likely caused by effective colony-forming unit rates 200 times less than applied in the 1988 and 1989 field tests. The actively growing hydrilla was able to overcome the effects of disease induced by the fungus. To obtain control, a threshold inoculum level must be applied. Rates below the threshold level will not produce a disease epidemic, although disease symptoms may appear on the target plant.

Future research with the hydrilla pathogen will focus on development of a formulation designed specifically for use on submersed aquatic vegetation. Poor contact time and retention rates of pathogenic organisms on aquatic host plants have severely limited the success of formulations in the past. A determination of threshold levels of pathogen inoculum must be determined to consistently achieve good control in the field. Finally, host specificity studies that address disease-producing ability of the fungus on terrestrial and nontarget aquatic plants need to be completed before any large-scale testing of the pathogen can begin.

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Allelopathic Ability of Various Aquatic Plants to Inhibit the Growth of *Hydrilla verticillata* L.f. Royle and *Myriophyllum spicatum* L.

by
Harvey L. Jones[1]

Introduction

The term allelopathy was first coined by Molisch in 1937. 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). Specifically, allelopathy refers to the biochemical interactions that take place among plants, but its effectiveness depends on the addition of a chemical to the environment (Sutton 1986a). Rice (1974) provided us with a more functional definition 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.

Background

Hydrilla verticillata (L. fil.) Royle (common name hydrilla) is a noxious aquatic plant introduced into the United States from Africa through the aquarium industry and sold under the name "oxygen plant" or "star vine." Hydrilla has long branching stems that often fragment and form large floating mats (Tarver et al. 1980) and can grow in water depths up to 15 m.

Two reproductive structures that enable hydrilla to withstand extremely harsh weather conditions are turions or winter buds (dense clusters of apical leaves that are produced in the leaf axils, green and ovoid-conical shaped buds) and bulb-like hibernacula, commonly but incorrectly called tubers (which are formed at the ends of stolons buried in the substratum).

Plants are found in lakes, rivers, drainage and irrigation canals, ponds, and streams. Severe infestations of hydrilla can restrict boat traffic and interfere with fisheries and waterflow.

Importance of allelopathy

Allelopathy may be a potentially important mechanism in controlling undesirable nuisance aquatic plant problems. Studies have shown that some plant have the capability of eradicating other species in the same area; however, most of these studies have been conducted using terrestrial plants or plants located in the littoral zone of aquatic habitats.

Objectives

There were two objectives for conducting this research. The first was to conduct experiments (test tube assays) to determine potential candidates of aquatic plants that would reduce the growth, reproduction, and or distribution of two nuisance aquatic hydrophytes *Hydrilla verticillata* (hydrilla) and *Myriophyllum spicatum* (Eurasian watermilfoil). The second objective of this area of research was to test the three aquatic plant species that were most inhibitory in the test tube assays using rooted plants. Test tube bioassays were used as a first step in determining allelopathic potential because it is a rapid screening technique to

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indicate allelopathic potential. Through laboratory bioassays, Elakovich and Wooten (1989a) listed several aquatic plants that have the allelopathic potential to reduce or inhibit the growth of other species.

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 keep the plants from deteriorating. Entire plants were collected whenever possible. Plants were washed to remove dirt and debris and allowed to drip to remove excess water.

Test species selection

Species selected for analysis were based on reports of potentially allelopathic hydrophytes pertinent literature reviews and Elakovich and Wooten (1989a, 1989b). In the study reported herein, the species selected were examined for their allelopathic potential to impact the growth of the target species *Hydrilla verticillata*. A list of selected species is given in Table 1.

Stock hydrilla cultures

Axenic cultures were grown from hydrilla tubers and turions obtained from greenhouse-grown plants. Cultures were grown in three 8-L capacity nalgene cylinders in 6 L of ALW media (artificial lake water). The cylinders each contained 25 to 35 tubers and were aerated with compressed air. The plants were grown for approximately 3 weeks, then clipped to 11 cm from the apical tips and recultured.

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 RO (reverse osmosis) water was added and 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 rpms 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 1. One 2-cm-long apical tip explant of *Hydrilla verticillata* (axenic cultures) was placed in each of the eighty 90-ml capacity test tubes with 50 ml of ALW media. Experimental cultures were randomly selected to receive 10 ml of one of the test plant extracts (Table 1). Test tubes were numerically arranged in test tube racks with a vacant space on each side. Numbered aeration plugs (Figure 1) were inserted in the test tubes and compressed air supplied to each tube. Five replicates of each test plant extract were used in this study in addition to ten controls each of which contained an additional 10 ml of ALW media in lieu of test plant extracts.

Table 1
Plant Species and Plant Parts Used to Prepare Extract for the Hydrilla Bioassays

Common Name	Scientific Name	Plant Part
Fanwort	*Cabomba caroliniana* Gray	1
Coontail	*Ceratophyllum demersum* L.	1
Parrot feather	*Myriophyllum aquaticum* (Vell.) Verdc.	1
Eurasion watermilfoil	*Myriophyllum spicatum* L.	1
Common water nymph	*Najas guadalupensis* Spreng.	1
American lotus	*Nelumbo lutea* (Willd.) Pers..	3
American lotus	*Nelumbo lutea* (Willd.) Pers.	2
Fragrant waterlily	*Numphaea odorata* Aiton	2
Pickerelweed	*Pontederia lanceolata* Nutt.	2
Pondweed	*Potamogeton nodosus* Poir.	3
Pondweed	*Potamogeton nodosus* Poir.	2
Duck-potato	*Sagittaria lancifolia* L.	2
Duck-potato	*Sagittaria lancifolia* L.	3
Eel-grass	*Vallisneria americana* Michx.	1

Note: 1 = Entire plant used for extraction.
2 = Stems and leaves used for extraction.
3 = Roots used for extraction.

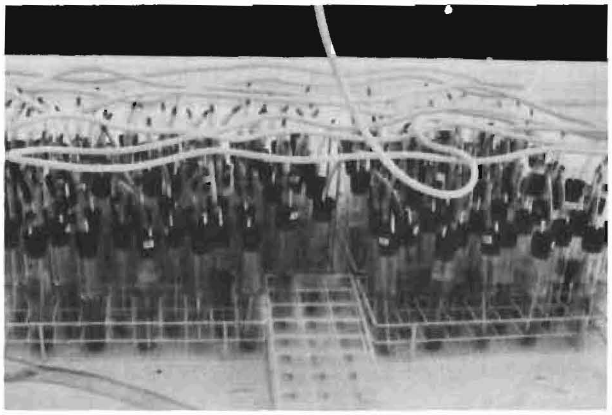

Figure 1. Experimental set up of test tube assay showing plant extracts and Hydrilla verticillata

Plants were grown for 14 days before harvesting. Plant length and health status were recorded; then plants were placed in aluminum foil, dried at 70 °C for 7 days, and biomass weight recorded.

The same procedures were repeated using *Myriophyllum spicatum* as the target species.

Experiment Two. In greenhouse studies, 5- or 20-percent organic matter from *Ceratophyllum demersum*, *Potamogeton nodosus*, and *Vallisneria americana* were 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 hydrilla were placed in the plastic cups to a depth of 10 cm, then overlaid 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 and then filled with nutrient solution (modified Barko's media). The acrylic columns were then placed in 1,150-L capacity fiber glass tanks filled with tap water (as a water bath) (Figure 2) and maintained at a constant temperature of 25 °C. Controls were planted using the same procedures as above except no additional organic matter was placed in the sediment. 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.

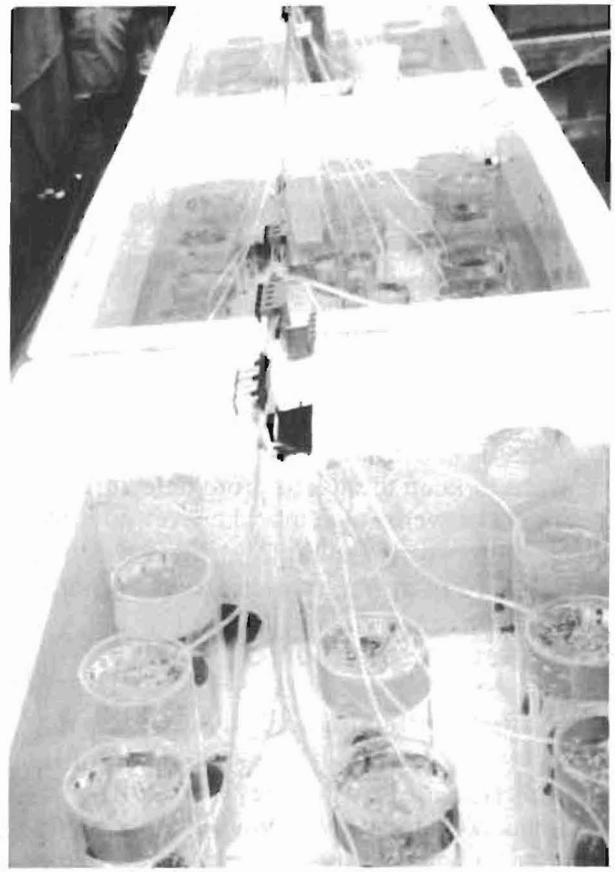

Figure 2. Experimental set up of rooted plant study showing plants grown in fiberglass tanks with organic matter addition

Data analyses

Each study was repeated three times, and all data were subjected to analyses of variance (ANOVA) and the Duncan's Multiple Range Test in the Statistical Analysis System (SAS) to determine significant differences.

The same procedures were repeated using *Myriophyllum spicatum* as the target species.

Results

In the test tube assays with hydrilla as the target species (Figure 3), all extracts were significantly different from the controls; however, *Vallisneria americana* and *Najas guadalupensis* appeared to increase hydrilla biomass. *Potamogeton nodosus* extract failed to significantly increase or decrease hydrilla biomass;

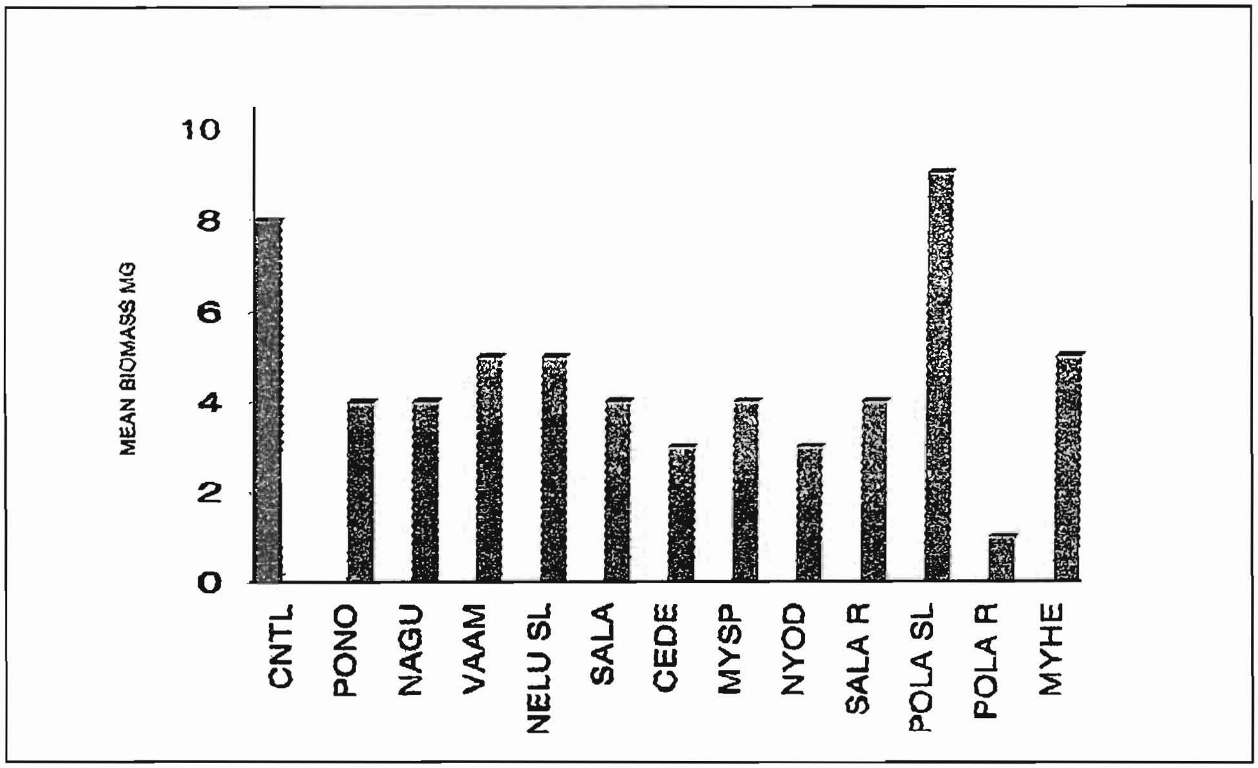

Figure 3. Allelopathic ability of various aquatic plant extracts to inhibit the growth of Hydrilla verticillata at the 10-ml concentration

Ceratophyllum demersum showed the greatest reduction in hydrilla biomass.

In *Myriophyllum spicatum* test tube assays when mean biomass was compared at the 10-ml concentration, *Ceratophyllum demersum* showed the greatest difference from the controls (Figure 4); however, *Vallisneria 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 for the hydrilla studies. 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 (Figure 5); however, with the 20-percent concentrations, *Ceratophyllum* showed significant differences in biomass when compared with control.

At the 20-percent concentration *Ceratophyllum* and *Vallisneria* were found to be significantly different from the control (Figure 6), while small differences were detected for *Potamogeton.* Mean length data were recorded but not used because mean length is not a true indicator of allelopathic activity. Some plants did not grow in length but formed branches, while others were not healthy and, yet, they grew in length; therefore, these data were not included in the analyses.

Results from tank studies showed that *Potamogeton nodosus* and *Ceratophyllum demersum* did not significantly reduce *Myriophyllum spicatum* biomass with 5-percent organic matter (Figure 7); however, *Vallisneria americana* did. When compared with controls at 20-percent organic matter additions, all species were significantly different from the controls. *Myriophyllum spicatum* biomass was stimulated beyond that of the controls by *Vallisneria americana* and *Potamogeton nodosus* at 5-percent organic matter; although

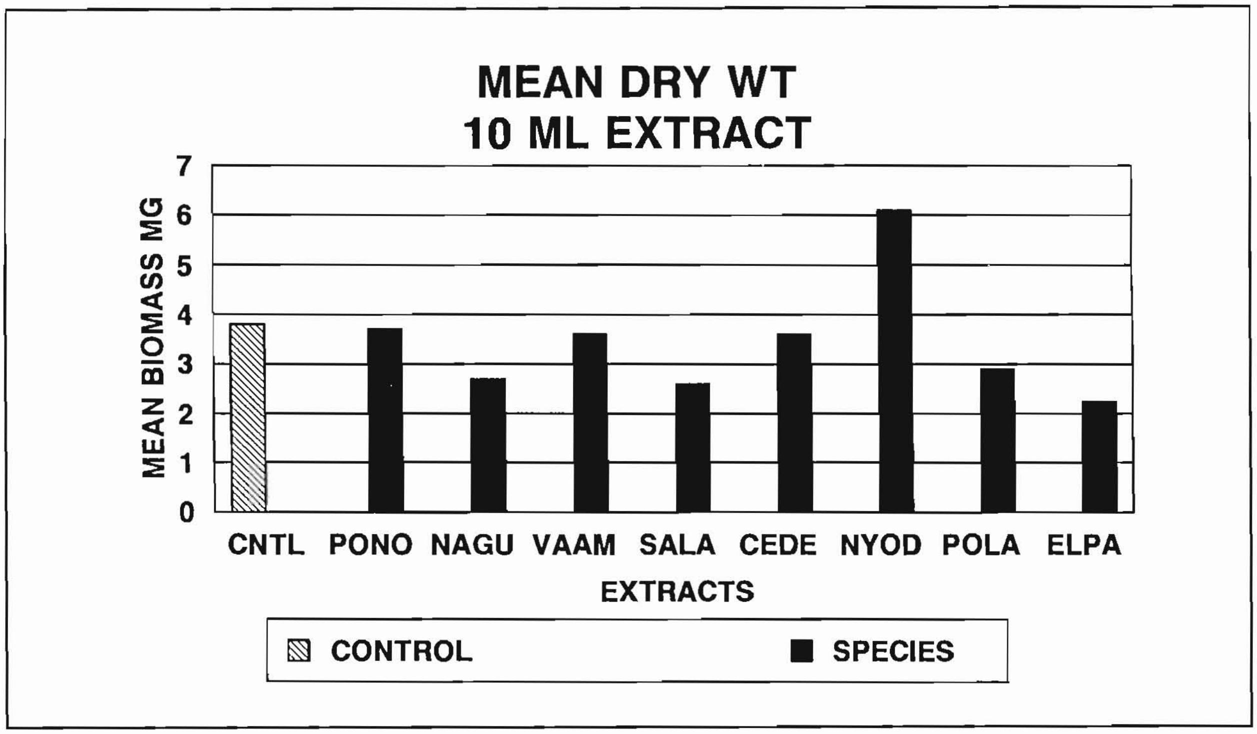

Figure 4. Allelopathic ability of various aquatic plant extracts to inhibit the growth of Myriophyllum spicatum at the 10-ml concentration

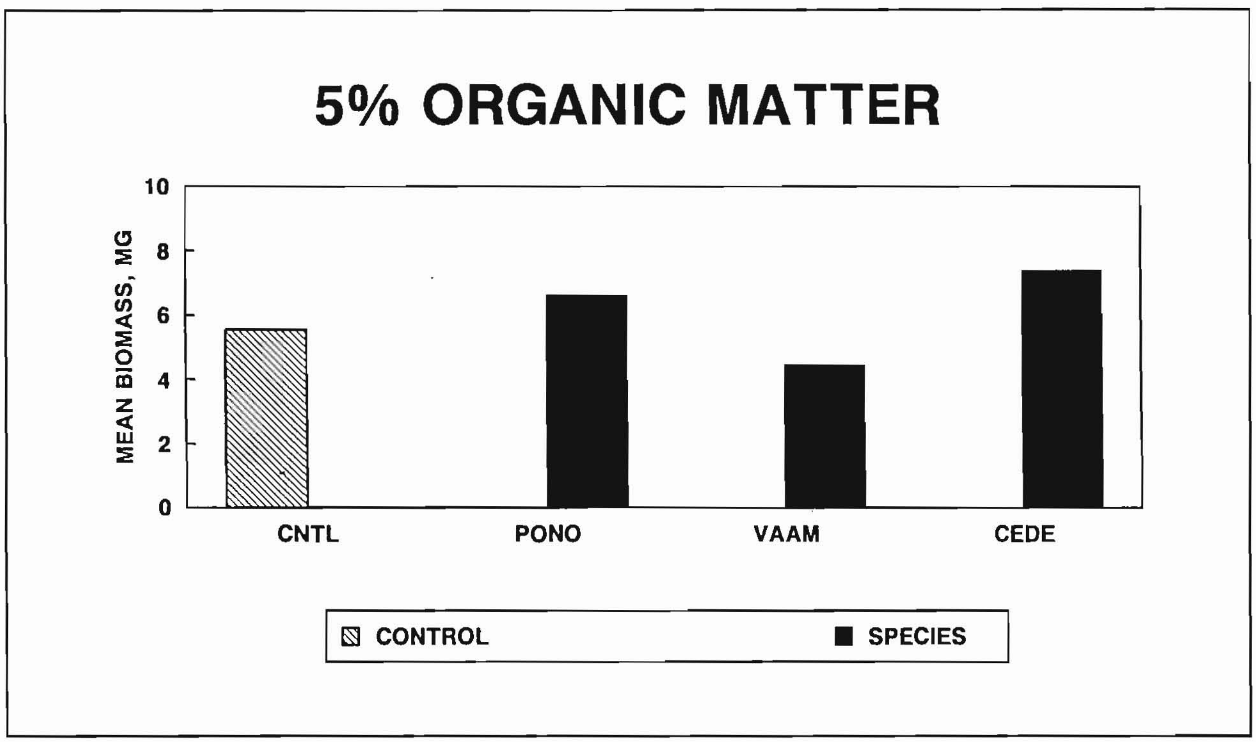

Figure 5. Allelopathic influence of organic matter additions (5 percent dry weight) on the above-ground biomass of Hydrilla verticillata (values are the means of seven replicates)

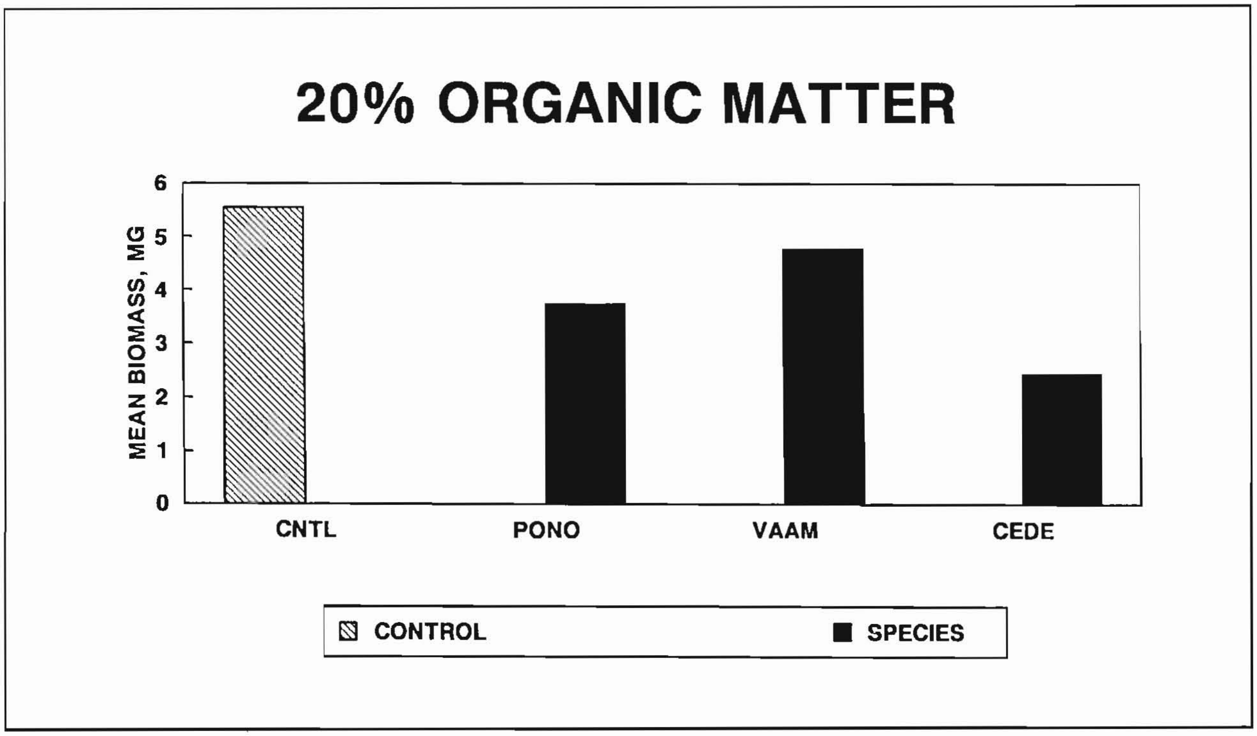

Figure 6. Allelopathic influence of organic matter additions (20 percent dry weight) on the above-ground biomass of Hydrilla verticillata (values are the means of seven replicates)

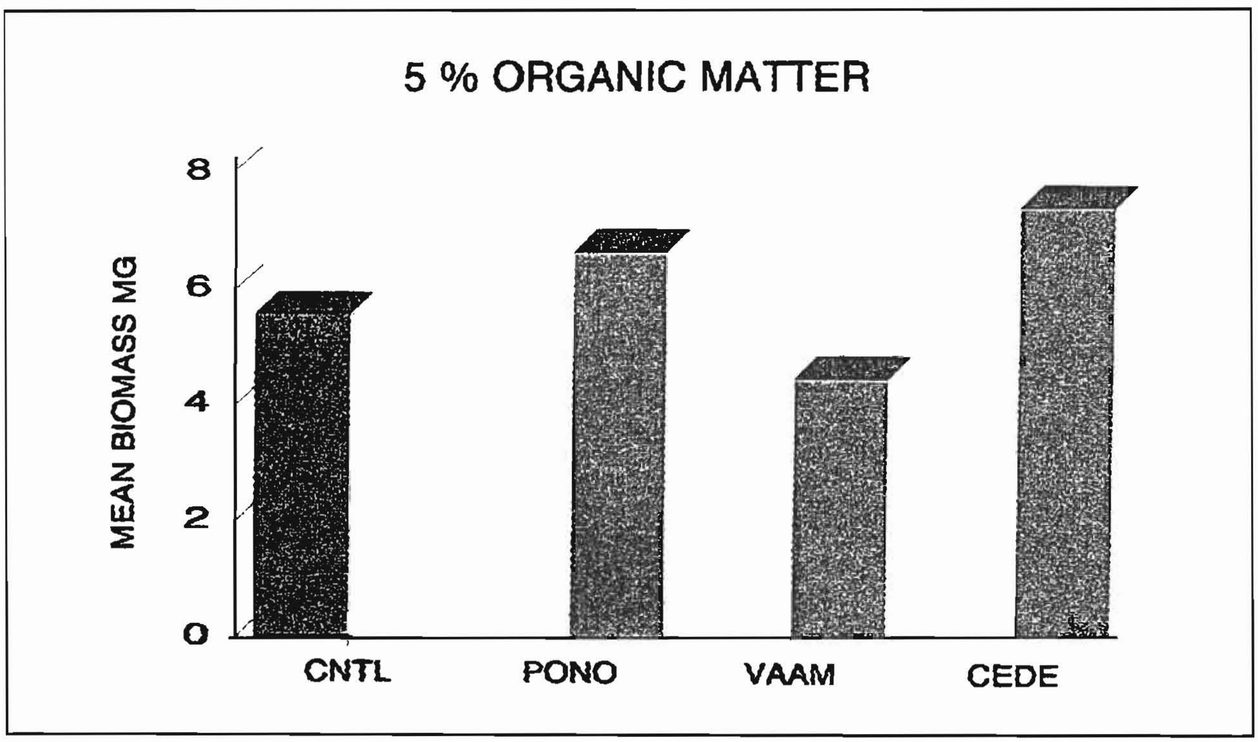

Figure 7. Allelopathic influence of organic matter additions (5 percent dry weight) on the above-ground biomass of Myriophyllyum spicatum (values are the means of seven replicates)

mean biomass was reduced by *Ceratophyllum demersum*, it was not significant. With 20-percent organic matter addition (Figure 8), only *Ceratophyllum* was significantly different from the control; although *Vallisneria americana* reduced the mean biomass of myriophyllum, it was not significant.

Conclusion

The type of organic matter present in sediments can greatly influence the growth of aquatic plants. Aquatic plants modify the underlying sediments by passively collecting organic matter from elsewhere and by their own production (Wetzel 1979; Carpenter 1981). According to our preliminary data, we have found *Ceratophyllum* to be our best allelopathic potential candidate for hydrilla. Kulshreshta and Gopal (1983) investigated the allelopathic nature of hydrilla toward *Ceratophyllum* and found that hydrilla inhibited the growth of *ceratophyllum* when grown in tanks together. In our study, we used plant extracts. There are other species that warrant additional attention because they appeared to be slightly allelopathic during various phases of our study or in literature reviews. These species include Southern naiad (*Najas guadalupensis* Spreng.) Magnus, and dwarf spikerush (*Eleocharis coloradoensis* Britt.) because it was found to be allelopathic to several aquatic species in various literature reviews. Sutton (1986b) found that when hydrilla was grown in established stands of spikerush, hydrilla biomass production was decreased by 85 to 90 percent when compared with hydrilla plants grown alone. These data show that allelopathy is a possibility in controlling undesirable aquatic plants; however, additional studies are necessary. Field testing of some or all of the species that were successful in the laboratory and greenhouse must be completed before a final decision can be made concerning allelopathy as a practical tool in the control of undesirable aquatic plants.

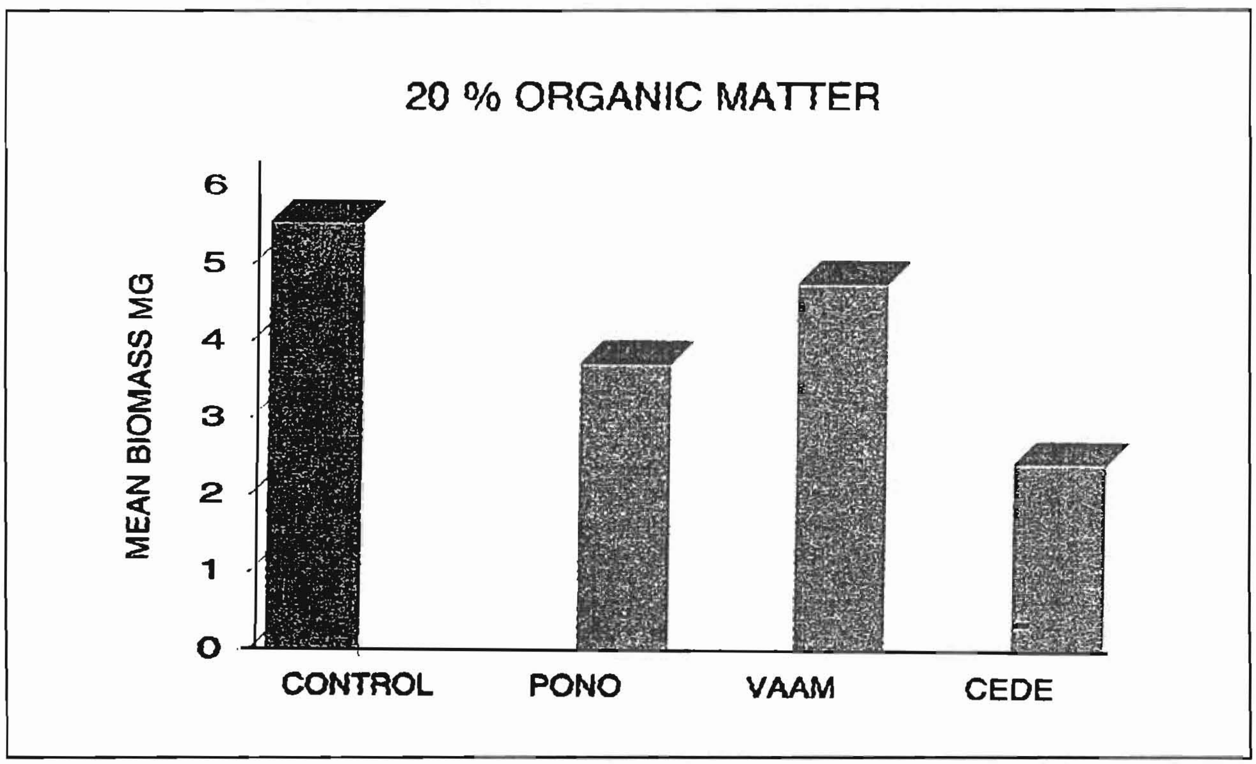

Figure 8. Allelopathic influence of organic matter additions (20 percent dry weight) on aboveground biomass of Myriophyllum spicatum (values are the means of seven replicates)

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Natural Enemies of *Trapa* spp. in Northeast Asia

by
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Introduction

Water caltrop or water chestnut (*Trapa natans* L.) is an introduced annual plant with floating rosettes and stems rooted in the hydrosoil. *Trapa natans* was discovered growing in New York State in 1884 and has become a problem in canals, lakes, and quieter areas of rivers and other fresh waters in many places in the Northeastern United States and in Maryland and Virginia (Madsen 1993). Water caltrop infestations limit navigation and recreation and displace more valued water plants (Bickley and Cory 1955; Bogucki, Gruendling, and Madden 1980).

Trapa natans belongs to the Trapaceae, a family whose natural distribution is in the Old World (Cook et al. 1974). Because of the morphological variation in *Trapa* species, there is little agreement about the number of species in the genus. Various classification schemes have listed from 1 to 30 species of *Trapa* in the world (Cook 1978). The four-horned nut-bearing plants, which are usually called *T. natans*, range from Western Europe to Northeast Asia (Sculthorpe 1967; Voroshilov 1982). *Trapa* species are cultivated in China for their edible nut-like fruit. The unrelated water chestnut or Chinese water chestnut that is sold in cans and in American Chinese restaurants is the tuber of *Eleocharis dulcis* (Burm. f.) Trin. ex Henschel (Cyperaceae).

Because of the success of biological control in managing some important waterweeds (Room et al. 1981; Center, Cofrancesco, and Balciunas 1989) and the high costs of other methods of aquatic weed control, it is worthwhile to examine the possibilities of using biological control for water caltrop control. This plant is a good candidate for biological control since it is an introduced species that has no native relatives that could limit the selection of potential natural enemies. Any agent with a family-level host plant specificity could be used. The plant's annual habit and single meristem in each rosette may make it particularly vulnerable to natural enemy attack.

Materials and Methods

Northeast Asia was selected as the initial survey region because of the occurrence of published accounts and preliminary observations indicating that *T. natans*, other *T.* spp., and some natural enemies were present in the region in areas with similar climates to the infested regions of North America. Northeast Asia was also selected because of the presence of the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) - Asian Parasite Laboratory in Seoul, South Korea, and the allied Sino-American Biological Control Laboratory in Beijing, China, through which the research could be done. Herbarium specimen localities, regional floras, and specialists guided the process of selecting survey areas to examine for *Trapa* populations, which were located by searching shallow, quiet bodies of water. All of the *Trapa* populations examined were wild, except some in the Nanjing area that were cultivated. The surveys were made in 1992 and 1993.

Results and Discussion

Figure 1 shows the survey region and the areas where *Trapa* populations were examined. The areas in the south have warm temperate

[1] U.S. Department of Agriculture, Agricultural Research Service, Asian Parasite Laboratory, Seoul, South Korea; Current Location: U.S. Department of Agriculture, Agricultural Research Service, Aquatic Plant Management Laboratory, Fort Lauderdale, FL.

Figure 1. Trapa survey areas in Northeast Asia

climates with mild winters, while the areas in the far north have cold temperate climates usually with cool summers. All the areas have moist climates except for Chichihaerh in China, which is a dry grassland zone.

A complex of *Trapa* spp. occurred in the region. *Trapa japonica* Flerov. was the only species observed in Hokkaido and in the northern areas of South Korea. *Trapa natans* occurred in the other areas often with *T. japonica*, along with a variety of other species and forms. The natural enemies should be considered to be associated with *Trapa* spp. rather than a single species, since most natural enemies appeared to occur on all of the *Trapa* spp. and because of the impossibility of identifying host plants not in fruit, as well as the uncertainty of *Trapa* taxonomy. Table 1 shows the natural enemies found on the surveys.

Insects

Homoptera. The polyphagous *Rhopalosiphum nymphaeae* (L.) was common in the region on many aquatic plants. The leafhopper *Macrosteles purpurata* Kuoh and Lu was mainly observed in China and at Hinkanski in Russia. It reached high densities at some sites and probably transmitted a virus observed to infect *Trapa* species where the leafhopper was abundant. The leafhopper feeds on unrelated water plants (Lu, Zhu, and Liu 1988).

Lepidoptera. At least three Nymphulinine Pyralidae were observed to attack *Trapa* species in the region. They were most abundant in Korea and Nanjing in China where they could be damaging. *Nymphula interruptalis* (Pryer) was the most damaging because of its feeding on and killing of the central bud late

Table 1
Natural Enemies of *Trapa* Species in Northeast Asia

Insect Species	Feeding Site	Host Range
Aphididae (Homoptera)		
Rhopalosiphum nymphaeae (L)	leaves	polyphagous
Cicadellidae (Homoptera)		
Macrosteles purpurata Kuoh et Lu	leaves	polyphagous
Curculionidae (Coleoptera)		
Nanophyes japonica Roelofs	petiole-floats	stenophagous
Nanophyes sp.	leaf blades and petiole-floats	stenophagous
Chrysomelidae (Coleoptera)		
Galerucella birmanica Jacoby (=*G. nipponensis* Laboissiera)	leaves	oligophagous
Pyralidae (Lepidoptera)		
Paraponyx vittalis (Bremer)	leaves	polyphagous
Nymphula interruptalis (Pryer)	leaves and buds	polyphagous
Nymphula responsalis (Walker) (=*N. turbata* Butler)	leaves	polyphagous
Noctuidae (Lepidoptera)		
Spodoptera litura Fab.	leaves	polyphagous
Chironomidae (Diptera)		
Chironomus spp.	petiole floats	filter feeder
Unknown spp.	leaves and buds	oligophagous?
Disease Species	**Feeding Site**	**Host Range**
Fungi Imperfecti	leaves	broad
Cercospora sp.	whole plant	broad
Sclerotium rolfai Sacc.	whole plant	broad
Botrytis cinerea Pers. et Fr.	whole plant	?
Unknown virus		

in the season. It and the related *N. responalis* (Walker) are recorded from many aquatic plants (Shin, Pak, and Nam 1983). *Paraponyx vittalis* (Bremer), also at times common, is a pest of rice (Japanese Plant Protection Association 1980). *Spodoptera litura* Fab., an important agricultural pest, was observed to feed on the leaves of cultivated *Trapa* plants in Nanjing, China. An unidentified, small leaf miner was observed in some leaves of the plants at Harbin in China.

Coleoptera. The leaf beetle *Galercella nipponensis* Laboissiera (= *G. birmanica* Jacoby) was observed to be abundant except in Hokkaido and Slavanika in Russia. It can be very damaging to water caltrop, causing entire mats to be defoliated. It is a well-known pest of cultivated *Trapa* in China and India (Lu et al. 1984; Khatib 1934). The beetle is recorded from other unrelated water plants, including *Brasenia schreberi* J.F. Gmel. in the Nymphaceae (Hayashi, Morimoto, and Kimoto 1984). It appeared to be using a floating *Polygonum* sp. as a host plant in northern China.

Nanophyes. Two *Nanophyes* weevils were observed to attack *Trapa* species. *Nanophyes japonicus* Roelofs was abundant in central Japan and in the Nanjing area of China. This weevil lays its eggs in the floating leaf petioles of the plants. The larvae feed within and pupate in the spongy petioles. The leaf blades having attacked petioles appeared to be normal, suggesting that this weevil has a limited ability to injure the plants. The other *Nanophyes*, as yet an unidentified species, was observed in the Harbin area of China and at Hinkanski in Russia. This weevil lays an egg into the central vein of the upper leaf blade. The hatching larva mines the central vein downward into the petiole float, where it finishes feeding and pupates. Only one larva was observed per leaf; and though almost all of the leaves of some plants were attacked, the plants appeared to be normal and healthy. The developmental periods of both of the weevils is about the same as the life span of a leaf. The adults do a modest amount of feeding on the upper sides of the leaf blades. These *Nanophyes* are the most specialized *Trapa* feeders seen in the region.

Diptera. Chironomid larvae were commonly associated with the plants. A *Chironomus* species was frequently seen in the petiole floats. The larvae were in tunnels that had two openings to the outside. These are assumed to be filter feeders just using the floats as a place to live. Other chironomid larvae were found between the overlapping leaves of the central bud, where they ate narrow channels in the unexpanded leaves. The damage was magnified when the leaves expanded. These midges proved difficult to rear and remain unidentified. They were most abundant on Hokkaido, where most of the leaves of the plants experienced some damage. A few chironomid larvae were also found inside a couple of fruit of *T. japonica* in Korea. These were reared but are not yet determined. They were so rarely seen that they may well be filter feeders incidently boring into a few *Trapa* fruit.

Diseases

No diseases causing much damage were observed. The broad spectrum pathogens on Table 1 are pests of crops. Little is known about the virus that produced a dwarfing of the rosettes, reducing the leaves to tiny spoon-shaped structures.

Future Directions

Although some damaging agents were found in the surveys of Northeast Asia, no strong biological control candidates were identified. Since most of the geographic range of *Trapa natans* has yet to be examined, more survey work is recommended. Northern India and southern Europe are two areas of interest. Both areas have *Bagous* weevils recorded from *Trapa*.[1] Weevils in this genus are narrow specialists of aquatic plants; that can be damaging to their hosts.

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Chemical Control Technology

Overview of Chemical Control Technology Development

by
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Introduction

The mission of the Chemical Control Technology Development area is to develop technology that will improve the management of nuisance aquatic plants using herbicides and plant growth regulators (PGRs) in an environmentally compatible and cost-effective manner. In fiscal year 1993, direct allotted funds for chemical control research were apportioned among six work units:

a. Herbicide Concentration/Exposure Time Relationships.

b. Herbicide Application Techniques for Flowing Water.

c. Herbicide Delivery Systems.

d. Field Evaluation of Selected Herbicides.

e. Plant Growth Regulators for Aquatic Plant Management.

f. Species-Selective Use of Aquatic Herbicides and Plant Growth Regulators.

In addition, a seventh work unit, Coordination of Control Tactics with Phenological Events of Aquatic Plants, was under the direction of the Chemical Control Technology Development area.

Although these work units can function as independent research efforts, they have been carefully designed to operate interactively as well. Selected chemical compounds (herbicides and/or PGRs) are evaluated in a series of laboratory, mesocosm, and field studies (Figure 1). 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 and reducing the amount of chemicals placed in the environment and the effort and costs associated with aquatic applications.

Chemical control researchers develop working relationships with the chemical industry and the U.S. Environmental Protection Agency (USEPA) Office of Pesticide Programs. This cooperation enables researchers to stay informed of the latest development in aquatic pesticides and regulations. In addition, interaction with U.S. Army Corps of Engineers (CE) Districts and other Federal agencies (e.g., Tennessee Valley Authority, U.S. Bureau of Reclamation, and U.S. Department of

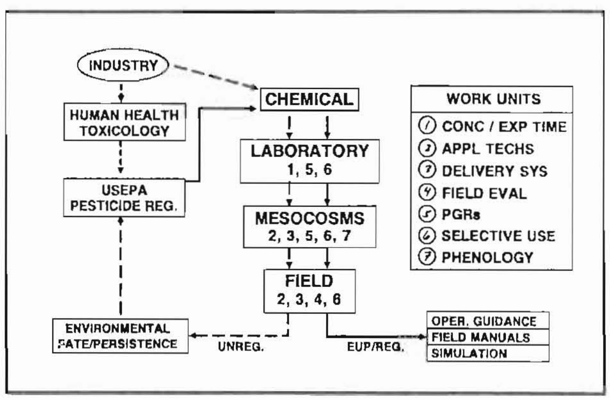

Figure 1. Chemical Control Technology Development area work unit interactions

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

Agriculture) is maintained to coordinate and focus resources on regional and national aquatic plant management issues. Finally, cooperation with state and local agencies and universities is maintained to augment laboratory and field research capabilities.

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

Herbicide Concentration/Exposure Time Relationships (32352)

Investigators are evaluating herbicides and PGRs under controlled-environment conditions at the U.S. Army Engineer Waterways Experiment Station. Major nuisance species, such as Eurasian watermilfoil and hydrilla, are treated with registered or experimental use permit (EPU) chemicals over a range of selected doses and contact times. To date, the compounds 2,4-D, triclopyr, endothall, fluridone, diquat, copper, and bensulfuron methyl have been evaluated. The unique properties of each chemical (i.e., application rate, mode of action, half-life, and selectivity) require that concentration/exposure time (CET) relationships be developed for each target plant. This information is then employed to develop innovative application techniques.

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 zones, lakes, and reservoirs). Results from the CET work unit provide the pertinent dose/response information required to develop improved and innovative application techniques.

Research 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. Using technology developed in this work unit, several successful submersed treatments in flowing water systems have been demonstrated around the Nation including (a) Crystal River, FL - endothall versus hydrilla; (b) St. Johns and Withlacoochee rivers, FL - fluridone versus hydrilla; (c) Pend Oreille River, WA - triclopyr versus milfoil; (d) Long Lake, WA - fluridone versus milfoil; and (e) Foster Creek, SC - fluridone versus hydrilla.

Herbicide Delivery Systems (32437)

In this effort, relationships developed in the CET work unit are used to design systems that deliver low doses of herbicides over extended periods of time. Studies are designed to develop environmentally compatible controlled-release (CR) carriers (e.g., polymers, gypsum, elastomers, and proteins). These CR carriers are evaluated for herbicide release rates and plant tissue burden levels, as well as efficacy, in small-scale systems. Large-scale verification studies are conducted in outdoor flumes and selected field sites. Results from this work are used to improve efficacy of treatments in flowing water systems.

Field Evaluation of Selected Herbicides for Aquatic Use (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. Cooperators on these studies include chemical companies, CE Districts, Federal, state and local agencies, universities, and contractors. Environmental fate and dissipation data collected in these studies are used to prepare reports on the use of aquatic herbicides and are available to support USEPA requirements for registration of specific herbicide formulations. Results from these field evaluations verify results from laboratory-scale and mesocosm-scale studies and can aid in modifying the registration status of aquatic herbicides.

Plant Growth Regulators for Aquatic Plant Management (32578)

Plant growth regulators offer the potential of slowing the vertical growth rate of nuisance submersed plants, reducing the negative impacts that "topped-out" vegetation can impose on a water body. Thus, the beneficial qualities provided by underwater vegetation (e.g., fish habitat, waterfowl food, oxygen production, nutrient sink, and sediment stabilization) can be retained.

Compounds that demonstrate PGR potential are being evaluated in this work unit, including bensulfuron methyl, flurprimidol, paclobutrazol, and uniconazole. Since PGR efficacy is sensitive to life-cycle events of target plants, information obtained from the Phenology work unit will be useful in evaluating growth-regulating effects. The most promising PGRs are being evaluated in mesocosms and ponds.

Species-Selective Use of Aquatic Herbicides and PGRs (32841)

In this work unit, species-selective aquatic plant management practices using herbicides and PGRs are being developed and evaluated. While weedy species can be removed using traditional chemical control tactics, these treatments can also impact native species. Using chemicals in a species-selective manner can result in the control of target vegetation while enhancing the growth of desirable/beneficial plants. Allowing these species to flourish can slow the reinvasion of weedy species and provide improved fish and wildlife habitat. In this way, water bodies plagued with monoculture infestations of exotic plants can be restored to a healthy, diversified, and balanced aquatic community.

Studies are focusing on species-selective responses to applications (rate and timing) of various herbicides/PGRs. As responses to weedy and various nonweedy species are determined, desirable herbicide-resistant plants can be selected for further evaluation. The most promising chemicals (e.g., triclopyr, fluridone, endothall, and diquat) are being applied to mixed plant communities in a mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Results from this effort will be used to provide guidance for managing aquatic vegetation using the species-selective approach.

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

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 recognizable characteristic or cue will enable field personnel to determine the optimum time for applying appropriate chemical (or other) control techniques by taking advantage of the weak link in the plant's growth cycle to maximize efficacy.

Phenological studies are being conducted on Eurasian watermilfoil, hydrilla, and waterhyacinth at the LAERF. Results from these studies are being used in the Flowing Water, Species-Selectivity, and PGR work units and are related to the timing of application techniques based on phenological events associated with target and nontarget plants. In addition, phenology information is contributing to the plant growth modeling effort in the Simulation Technology area of the Aquatic Plant Research Control Program.

2,4-D Reregistration Update

by
Donald L. Page[1]

The year 1993 was critical in the long history of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). The sustained availability of the compound was threatened by the high cost of reregistration, Task Force difficulty in meeting reregistration study deadlines, and continued negative attention resulting from earlier publication of certain National Cancer Institute (NCI) epidemiological studies.

However, a number of positive developments helped strengthen the weight of the scientific evidence supporting 2,4-D. These included publication of additional data pertinent to 2,4-D from the NCI Iowa/Minnesota study, two studies questioning the validity of proxy interviews used in the NCI research, and an independent scientific review on 2,4-D commissioned by the Environmental Protection Agency (EPA). These developments and a strong commitment from industry have helped ensure the continued availability of the widely used herbicide.

Available commercially since 1948, 2,4-D is believed to be the most widely used herbicide in the world and, according to the EPA, the third most widely used in the United States. Almost all the 2,4-D currently used in the United States is applied in mixture with other herbicides to reduce the total cost of application; increase the spectrum of weed control; or help avoid the development of weed resistance, an increasing problem with many of the newer herbicides. Conservation tillage programs, which require economical weed control to be viable and often are mandated by the Government, recently have further increased grower use of 2,4-D.

2,4-D is one of about 1,100 pesticides undergoing reregistration in the United States, and its manufacturers face significant costs to comply with reregistration requirements. According to the EPA, some 35,000 research studies will be required for all these compounds to be reregistered. If 2,4-D reregistration costs are typical, the total cost of reregistering the 1,100 compounds will exceed $3 billion. As a result, fewer than 450 of these pesticides actually will be reregistered.

The first 2,4-D Task Force was formed to meet the study requirements of EPA's 1981 data call-in (DCI) on 2,4-D acid. By the time the 1988 DCI (reregistration standard) was issued on the acid, ester, and amine salts of 2,4-D, that Task Force already had spent $4 million. Task Force II, formed to meet the 1988 DCI, currently estimates additional study costs at about $20 million. Individual members are investing an additional $5 million to reregister proprietary or specialized ester and salt formulations not under the purview of the Task Force.

Meeting study protocols for the 1988 DCI required the development of new, state-of-the-art technologies, including a method of analysis (MOA) many times more sensitive than existing methods, before the studies could begin. The Task Force encountered significant technical problems in devising the new MOA, spending several years and more than $1 million before perfecting an adequate method. In the meantime, many of the required studies fell behind EPA's schedule for completion—a situation exacerbated by the Task Force's failure to keep EPA apprised of the extent of its technical problems.

As a consequence, in June 1992, EPA threatened to issue a notice of intention to suspend. To keep 2,4-D on the market, the Task

[1] Executive Director, Industry Task Force 11 on 2,4-D Research Data, Belhaven, NC.

Force reached an agreement with the Agency to initiate an exposure reduction program, pending completion of the required studies. This program involves significant label changes, including some reduced application rates; new safety procedures for mixing, handling, and applying 2,4-D; and the use of protective clothing and improved personal hygiene practices. The exposure reduction program also requires the Task Force to implement an extensive user education campaign.

2,4-D is the most thoroughly researched herbicide with more than 90 pertinent epidemiological studies published to date. However, two NCI studies (Hoar et al. 1992; Zahm et al. 1990) published in recent years suggest a link between 2,4-D and a rare form of cancer, non-Hodgkin's lymphoma (NHL). The first study, conducted in Kansas (Hoar et al. 1992), dealt with herbicides in general. Survey participants were asked no questions specific to 2,4-D use. However, in interpreting the findings, both the investigators and the news media made the assumption that herbicide use and 2,4-D use were synonymous. That study had a statistically significant positive finding based on seven cases of NHL in a group of farmers alleged to have applied herbicides 21 days a year or more.

Following publication of the Kansas study, EPA decided to convene a panel of experts to review both the Kansas study and two other NCI studies then in progress. The panel's findings would help the Agency determine whether 2,4-D should be placed in special review.

One of these additional NCI studies, conducted with farmers in Nebraska, did collect information specific to 2,4-D use (Zahm et al. 1990). The study found a nonstatistically significant positive result based on only three cases of NHL, also among a group of farmers who purportedly applied the herbicide 21 or more days a year. The other NCI study (Cantor et al. 1992), involving farmers in Iowa and Minnesota, did not initially survey duration or frequency of 2,4-D use in particular, but based risk estimates on respondents claiming "ever" to have used 2,4-D. It showed an odds ratio of 1.2, a nonstatistically significant outcome. In an effort to strengthen the study, NCI agreed with EPA to reinterview some of the subjects, asking whether and how often they used 2,4-D. When the study was published, however, NCI did not include the reinterview data. A Task Force request for information about the data was declined; it was obtained later through the Freedom of Information Act.

An analysis of the Iowa/Minnesota reinterview data, which contained more subjects reporting exposure to 2,4-D than the two previous NCI studies combined, showed no association between the use of 2,4-D and NHL. The Task Force reported the finding to the EPA independent review committee, known as the Scientific Advisory Panel/Scientific Advisory Board (SAP/SAB) Special Joint Committee, and NCI subsequently published the data (Cantor 1993).

The NCI studies were based on a population of cancer cases, usually obtained from state cancer registries, plus a control group drawn from random telephone sampling. Both groups were asked to complete questionnaires about their farming practices, including pesticide exposure, over a period of 40 years or more. Since many of the cancer cases had died before the studies began, proxy or next-of-kin respondents answered the questionnaires on behalf of the deceased. Unfortunately, no prior studies had determined whether the use of proxy respondents yields reliable or valid results in pesticide exposure research.

NCI later conducted a data validity study that found 80 percent or better agreement between self-respondents and their next-of-kin, but the study was conducted among the control population, rather than the cancer cases. A subsequent study by the U.S. Centers for Disease Control and Prevention (Boyle 1991) in contrast showed less than 50-percent agreement between cancer-case self-respondents and their proxies. The authors wrote, "The poor quality of surrogate (i.e., next-of-kin) information calls into question its use in epidemiological studies." A study recently completed at the University of Minnesota School of Public Health (Johnson et al. 1993) similarly concluded, "The findings indicate that pesticide data provided by proxy respondents will not necessarily result in the same estimate of risk and/or lead to the same conclusions as data provided by self-respondents."

Perhaps the most significant positive development for 2,4-D in 1993 involved the EPA SAB/SAP Special Joint Committee, comprised of 11 independent scientists selected by EPA, six of them epidemiologists. On April 2, 1993, at a public meeting in Arlington, VA, the panel reached the following conclusions (EPA 1993):

a. 2,4-D has no known mechanism for carcinogenicity.

b. Based on the toxicology, 2,4-D is an "improbable" carcinogen.

c. The human studies (epidemiology) provide only "weak" evidence of carcinogenicity.

The committee's written report will be available sometime in 1994.

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Herbicide Concentration and Exposure Time Requirements for the Control of Eurasian Watermilfoil and Hydrilla

by
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Introduction

Laboratory studies have shown that herbicide efficacy is dependent upon the concentration and length of time a target plant remains exposed to a selected herbicide (Van and Conant 1988; Green and Westerdahl 1990; Netherland, Green, and Getsinger 1991; Nelson and Netherland 1993; Netherland and Getsinger 1992; Netherland, Getsinger, and Turner 1993). In the field, herbicide residues are subject to rapid dilution and dispersion (via gravity flow and thermal and wind-induced circulation patterns) from the treatment area (Fox et al. 1991; Getsinger, Green, and Westerdahl 1990). Moreover, concentration/exposure time (CET) requirements can vary greatly among herbicides because of the unique properties (mode of action, rate of application, environmental half-life, and species selectivity) of each compound; therefore, CET relationships must be developed for each herbicide against target and nontarget species.

Several laboratory and field studies have shown that maintaining a long exposure (6 to 15 weeks) to the herbicide fluridone (Sonar) is the key to achieving control of hydrilla (Hall, Westerdahl, and Stewart 1984; Netherland 1992; Getsinger 1993; Netherland, Getsinger, and Turner 1993; Fox and Haller 1993). These studies have focused on maintaining constant low levels of fluridone (10 to 40 ppb) for an extended period of time. While this strategy has proved to be successful, potential methods for reducing exposure requirements or application rates are being investigated. Studies to assess the efficacy of high initial fluridone levels with a range of half-lives were conducted to determine if exposure requirements can be reduced. In addition, research has focused on determining the minimum concentrations of fluridone that influence growth and physiological parameters of hydrilla. Preliminary results of these studies will be presented in this report.

Laboratory studies were also initiated with the contact herbicides diquat and chelated copper (Komeen) versus Eurasian watermilfoil (hereafter called milfoil). Preliminary data will be presented and discussed along with the problems encountered in using diquat in laboratory-scale experiments.

Materials and Methods

Studies were conducted in a controlled-environment growth chamber with a light intensity (PPFD) of 570 ± 75 µmoles/m^2/sec at the water surface, a 14L:10D photoperiod, and water temperature of 23 ± 2 °C (Netherland 1990). Four hydrilla or milfoil apical shoots were planted in sediment-filled 300-ml beakers. Ten beakers containing a single target species were placed in 55-L aquaria, and plants were given a pretreatment growth period. All treatments were replicated three times and randomly assigned to a test aquarium.

Fluridone dissipation studies

Following a 21-day pretreatment growth period, hydrilla was treated with fluridone (Sonar AS) and rhodamine WT dye at concentrations and half-lives ($t^{1/2}$) summarized in Figure 1. Based on the initial treatment rate and half-life, regression equations were used

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

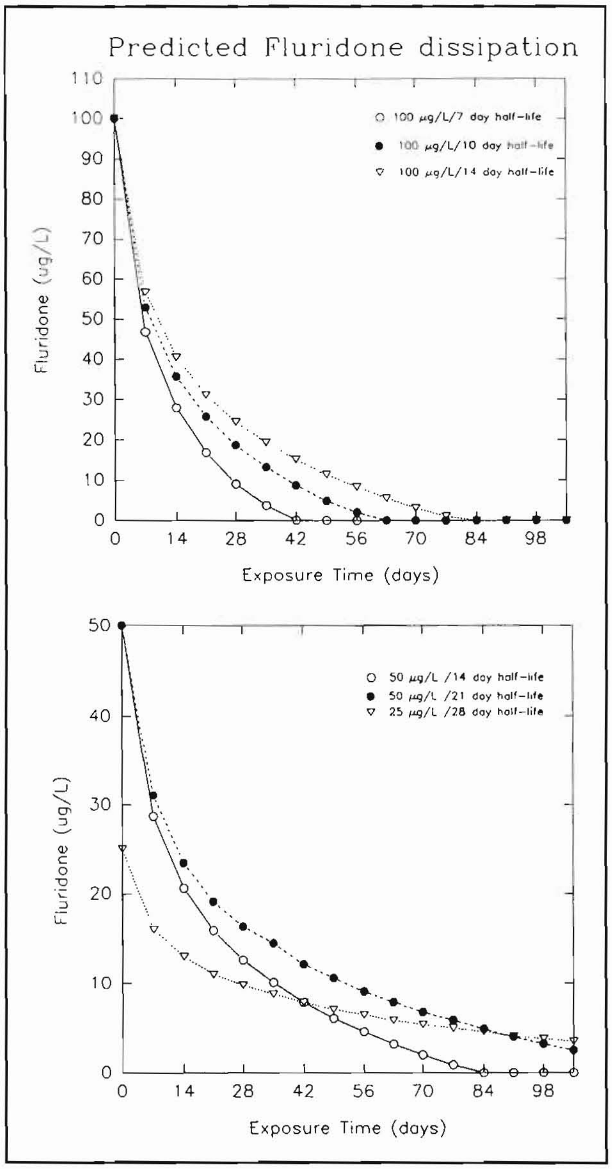

Figure 1. Weekly predicted fluridone dissipation based on an initial treatment rate and half-life

to determine the daily target concentration of fluridone for each treatment. These calculations were used to determine the volume of fluridone/dye-treated water to be removed and replaced with untreated water on a daily basis. Dye values (10 percent of the fluridone levels) were measured daily to confirm that the water removal and replacement scheme provided the predicted fluridone concentrations. During the course of the study, dye values never varied more than ±3 percent of the predicted concentrations. Moreover, analyses of selected fluridone samples indicated that fluridone and dye values were highly correlated ($r^2 = 0.98$).

Plant response to fluridone treatment was monitored for a 105-day period. Net photosynthesis (PHS) and total chlorophyll of apical shoots (four per aquarium) were measured at 7-, 28-, 60-, 77-, 90-, and 105-day posttreatment. Three beakers were harvested from each aquarium at 28, 77, and 108 days, and shoot and root biomass values were measured.

Fluridone threshold concentrations

Hydrilla was given a 7-day pretreatment growth period and treated with fluridone at concentrations of 0.1, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, and 25 µg/L to determine threshold concentrations for growth inhibition and selected physiological parameters. Exposures remained static for the duration of the study. Net PHS and total chlorophyll of apical shoots were measured at 7, 14, 28, 42, and 60 days. Four beakers were removed from each aquarium at 28 and 60 days, and shoot and root biomass values were measured.

Komeen and diquat versus milfoil

Milfoil was given a 21-day pretreatment growth period and treated with Komeen at concentrations of 1.0 and 3.0 mg/L for 1-, 3-, and 12-hr exposure periods and diquat at concentrations of 0.1, 0.25 and 0.5 mg/L for 1-, 2-, and 6-hr exposure periods. Following the exposure period, aquaria were drained and refilled three times to remove herbicide residues. Injury symptoms were visually monitored, and milfoil was harvested at 35 days following Komeen exposure and 21-day posttreatment following diquat exposure.

Results and Discussion

Fluridone dissipation

Hydrilla began to manifest fluridone symptoms within days of the treatment. Differences in injury symptoms (bleached apical tips) were not distinguishable between treatment rates with the exception of the 5-µg/L treatment (initial injury was not as severe). Results of the 28-day harvest showed that fluridone reduced hydrilla shoot biomass by 30 to 56 percent (Figure 2). Net PHS measurements at 28-day posttreatment showed that only the 5-µg/L treatment maintained positive 0_2 production at the apical tip (Figure 2). However, this still represented a 90-percent reduction in PHS compared with the untreated plants.

By the 77-day harvest, two treatments (100 µg/L/7days and 100 µg/L/10 days $t^{1/2}$) had significantly increased in biomass from the 28-day harvest (Figure 2). Based on predicted dissipation (and dye readings), these treatments reached 0-µg/L fluridone at 40 and 60 days, respectively. Visual observations indicated that shoot recovery began within days after fluridone concentrations reached 0. All other treatments still contained active concentrations of fluridone and continued to suppress healthy regrowth. Shoot biomass values were reduced from 82 to 94 percent compared with untreated controls. Net PHS measurements showed a marked recovery in treatments that contained no fluridone, whereas, treatments with active fluridone concentrations continued to result in negative photosynthetic rates at the apical tip (Figure 2).

At the final harvest, only three treatments still contained active fluridone concentrations (5 µg/L, 50 µg/L/21 days, and 25 µg/L/28 days $t^{1/2}$). These treatments continued to suppress healthy regrowth and showed a reduction in shoot biomass (89 to 95 percent) and negative PHS rates (Figure 2). Following a 14-day half-life (approximately 80 days of exposure), both biomass and net PHS continued to lag behind untreated controls; however, increased biomass and positive O_2 production indicated healthy regrowth was occurring.

In summary, the initial high fluridone rates did not significantly reduce exposure time requirements. However, it should be noted that as fluridone dissipated to very low levels (between 0.5 and 4 µg/L), plant growth continued to remain completely suppressed. This suggests that following initial fluridone injury, hydrilla recovery may be prevented by

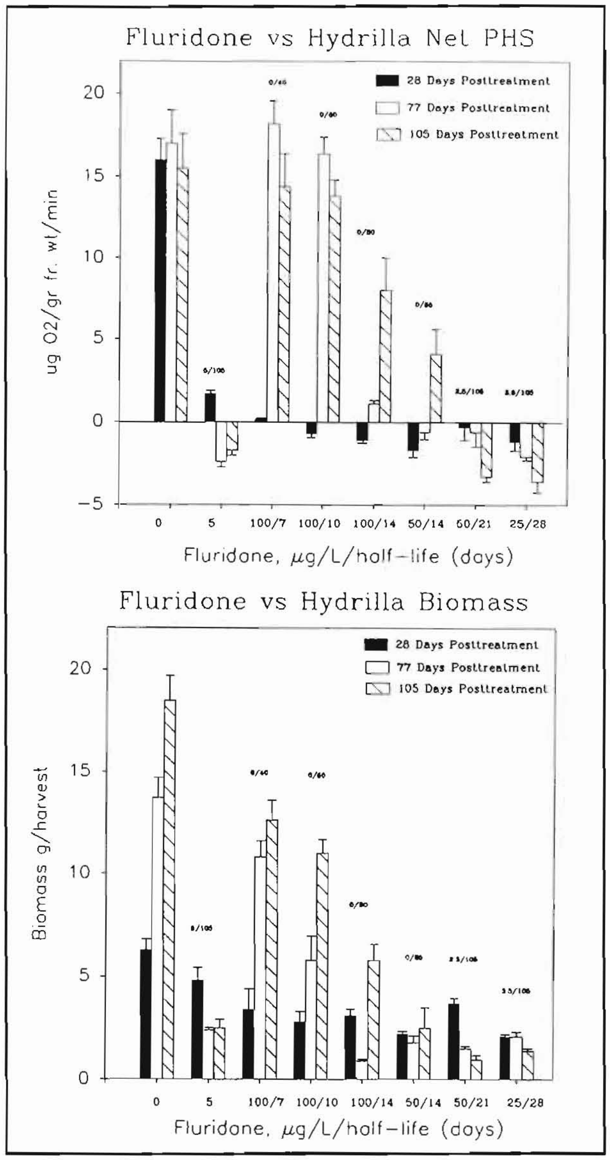

Figure 2. Hydrilla net photosynthesis (PHS) and biomass at 28-, 77-, and 105-day posttreatment following exposure to various treatment rate/half-life scenarios (Numbers above the bars indicate either the time (days) at which fluridone concentrations reached 0 or the concentration at 105-day posttreatment. Error bars represent one standard deviation from the mean)

extremely low concentrations of fluridone. The static 5-µg/L treatment eventually provided a better level of hydrilla control than high treatment rates followed by a 7-, 10-, or 14-day half-life. This laboratory data support treatment strategies designed to maintain low concentrations of fluridone for extended periods of time.

Fluridone threshold concentrations

Preliminary results indicate that at 30-day posttreatment, shoot biomass was not significantly affected by fluridone concentrations <2 μg/L, and net PHS was not significantly affected at concentrations <1 μg/L (Figure 3). Visual injury symptoms were noted at 10 days with concentrations of 2 to 25 μg/L; however, the 25-μg/L treatment showed much greater injury symptoms throughout the course of the study than the other treatments. By 60-day posttreatment, shoot biomass was significantly reduced (53 to 96 percent) by concentrations >1 μg/L, and net PHS was significantly reduced by concentrations >0.75 μg/L (Figure 3). Results show that fluridone is capable of inhibiting young, actively growing hydrilla at concentrations as low as 1 μg/L. While this data does not suggest that treatment rates should be adjusted this low, it does indicate that as fluridone dissipates in the field (because of dispersion, dilution, photolysis, etc.), it is likely to actively suppress hydrilla regrowth and recovery at extremely low rates.

Komeen and diquat versus milfoil

Milfoil injury symptoms were noted within hours following Komeen treatment at all rates and exposures tested. Injury symptoms were manifested on milfoil leaves, but were not evident on the stems. Following the initial browning of the leaves, regrowth from lateral buds and injured apical tips began to occur within 1-week posttreatment. Although the 3 ppm treatments resulted in significant biomass reductions versus untreated milfoil, plants were able to recover from these treatments. This is evidenced by the fact that the final biomass of these treatments was greater than the pretreatment biomass (Figure 4). Treatments at 1 ppm produced only temporary effects that resulted in no significant decrease in biomass versus untreated controls (Figure 4). Results suggest that milfoil may be somewhat tolerant of Komeen and other copper based herbicides. Although exposure periods were fairly short, Komeen at three times the label rate did not provide milfoil control.

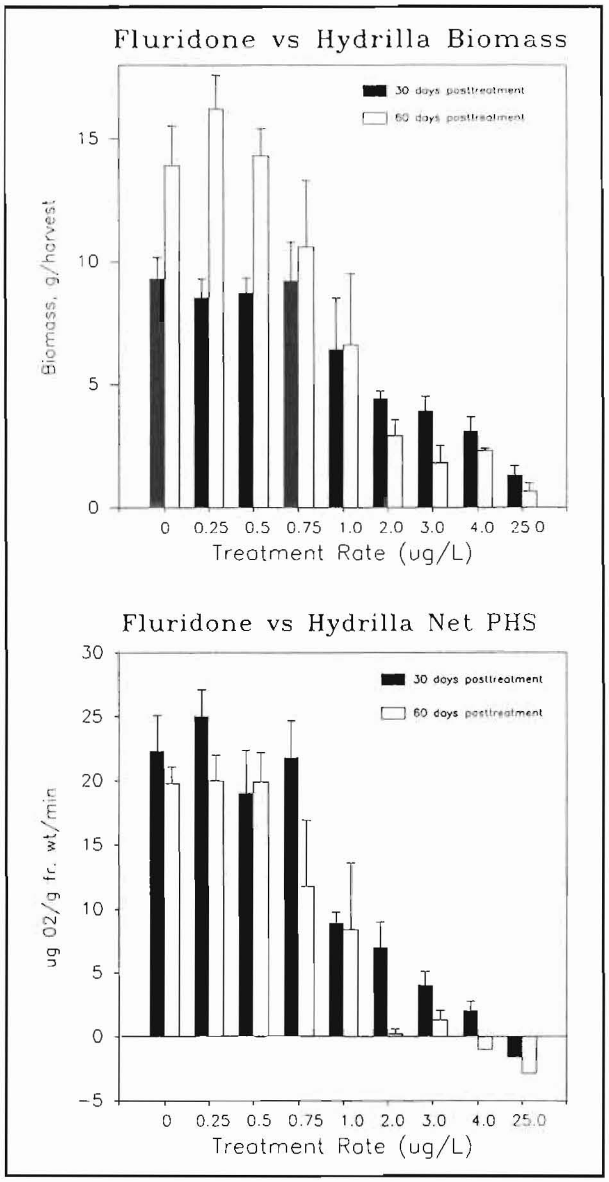

Figure 3. Hydrilla biomass and net photosynthesis (PHS) at 30- and 60-day posttreatment following static exposures to low levels of fluridone (Error bars represent one standard deviation from the mean)

Following diquat treatment, milfoil showed initial injury symptoms at all rates and exposures tested. Moreover, within 1-week posttreatment all diquat treatments resulted in severe injury characterized by leaf and stem browning and lack of stem buoyancy. By 2-week posttreatment, milfoil had disintegrated to the point where no harvestable material remained in the aquaria. At the 3-week harvest,

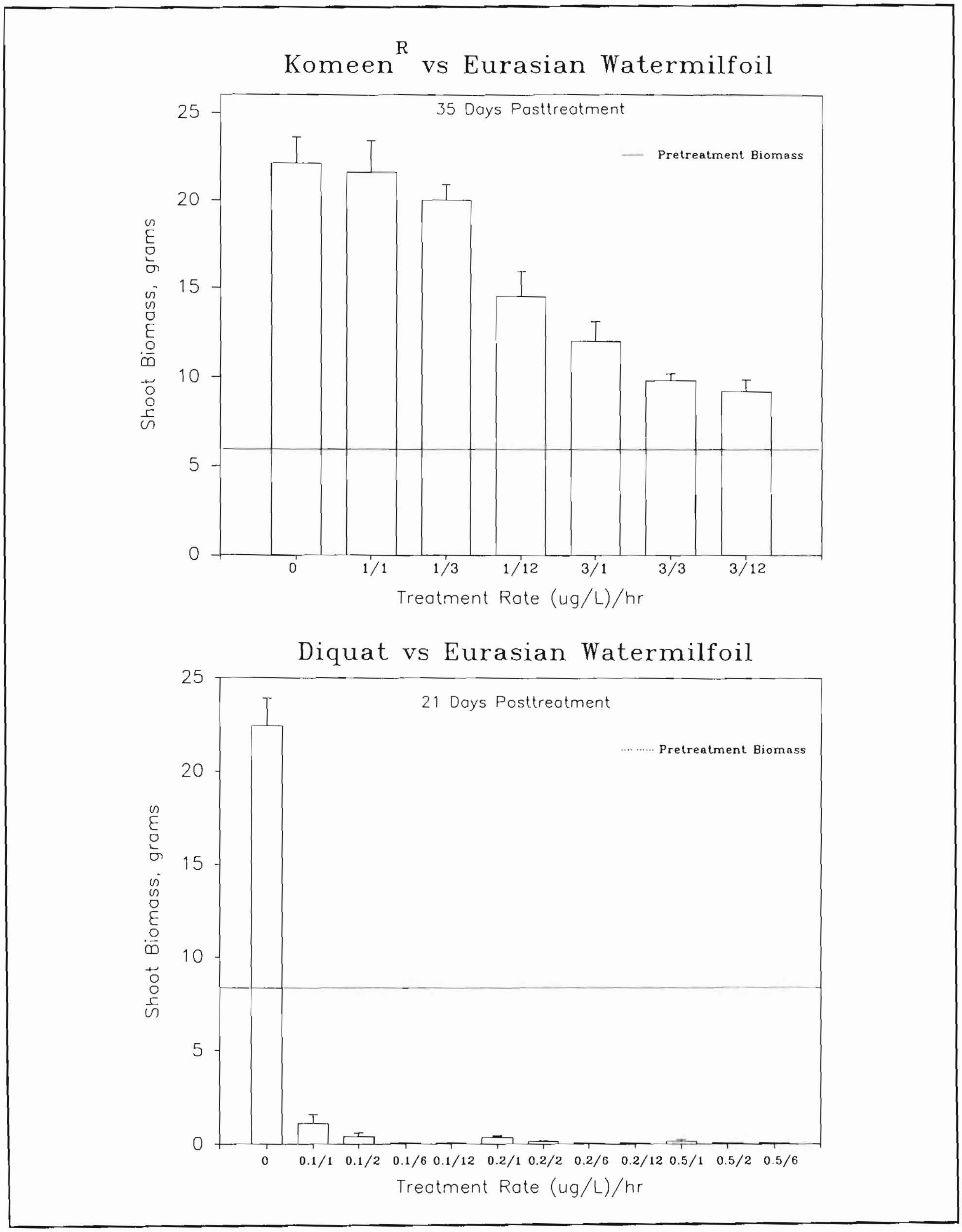

Figure 4. Eurasian watermilfoil biomass following exposure to various rates and exposures of Komeen and diquat herbicide (Error bars represent one standard deviation from the mean)

a few green tips emerged from the sediment of aquaria that had been treated at 0.1 and 0.2 ppm for 1 and 2 hr (Figure 4). All other treatments remained completely plant free.

The excellent efficacy achieved with diquat (at low rates and short exposures) in the laboratory should be viewed with some degree of caution. Diquat is a cation that is rapidly and strongly bound to negatively charged particles such as clays (Weed Science Society of America (WSSA) 1992). Once diquat is bound to these materials, it becomes herbicidally inactive. In the field, suspended sediments and particulate materials covering the plants likely account for an immediate loss of herbicidally active diquat. In contrast, our current experimental system is designed to minimize any sediment (clay) or turbidity in the water column. Moreover, the plants are generally free of particulate material. This combination of factors results in minimal diquat loss during the exposure period. Residue analyses indicated that only 5 to 12 percent of the diquat was lost during the exposure period. In addition, our experimental system also ensures rapid and thorough mixing of the herbicide. This results in all plant tissue (exposed to the water) receiving an equal exposure to the diquat.

In summary, results show that milfoil is extremely sensitive to diquat at very low rates (0.1 ppm) and short exposures (1 hr). However, notable differences exist in the field characteristics of diquat (rapid binding and loss of herbicidal activity, uneven mixing) and the laboratory characteristics of diquat (minimal residue loss, rapid thorough mixing). Therefore, using laboratory-derived data for predicting field efficacy of diquat is not appropriate at this time.

Future Work

Future work will include studies to determine minimum threshold concentrations of fluridone versus milfoil. In addition, CET relationships will be determined for selected nontarget species versus fluridone. Modifications to the current experimental system may be attempted to accommodate further diquat and copper testing on hydrilla and milfoil.

Acknowledgments

The author would like to thank Michael Bull, Charles Mayfield, Anne Stewart, Susan Sprecher, and Kurt Getsinger for technical assistance. The cooperation of DowElanco, Griffin Chemical, and Valent Chemical for providing herbicides for these studies is also appreciated.

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Flume Evaluations of a Gypsum Controlled-Release Matrix as a Potential Herbicide Delivery System

by
Michael D. Netherland[1] *and David Sisneros*[2]

Introduction

Laboratory studies have shown that excellent control of submersed aquatic plants can be obtained at low chemical concentrations if given sufficient exposure periods (Green and Westerdahl 1990; Netherland, Green, and Getsinger 1991; Netherland and Getsinger 1992; Netherland, Getsinger, and Turner 1993). However, in areas of high water exchange, maintaining herbicidally active residues within the treatment area can be exceedingly difficult (even at high initial treatment rates). Each aquatic site has its own unique water exchange patterns, and herbicide residues are often rapidly reduced by flow-, thermal-, tidal-, and wind-generated circulation patterns (Fox, Haller, and Getsinger 1990; Fox et al. 1991; Getsinger, Green, and Westerdahl 1990). As a result, widespread inconsistencies in submersed plant control can often be attributed to the high degree of variability in herbicide dispersion following conventional applications. Recognition of this problem has stimulated research into methods of extending herbicide contact time in the water column (especially in areas of high water exchange). Developing methods to deliver herbicides to submersed plants in a more efficient manner offers the potential of using less active ingredient (ai), and at the same time, achieving equal or greater efficacy. This process ultimately translates into lower treatment costs and better environmental compatibility.

One approach for extending herbicide contact time is to develop a controlled-release (CR) carrier or matrix as a delivery system. A gypsum-based (CaSO4) matrix was identified in laboratory screening tests conducted at the U.S. Army Engineer Waterways Experiment Station as a potential candidate for larger scale (mesocosm/field) testing. Mesocosm-scale pilot studies were conducted in a series of hydraulic flumes located at the Tennessee Valley Authority (TVA) Aquatic Research Laboratory near Athens, AL. Results of these studies showed that the matrices maintained a steady concentration of the herbicide triclopyr in a high-flow environment (three to four water exchanges per day) over a 7-day period (Turner et al. 1993). Although release rates were consistent, triclopyr residues were much lower than target concentrations, resulting in very poor control of the target species Eurasian watermilfoil (hereafter called milfoil). The sustained release properties of the gypsum matrices in these pilot studies warranted further flume testing. Therefore, the objectives of the current study were to determine if gypsum matrices (modified to increase loading rates and target concentrations) could maintain consistent herbicide release over time in a large flowing water outdoor system and to determine the efficacy of these treatments on Eurasian watermilfoil (and nontarget species) in a flowing-water environment.

Materials and Methods

Six flow-through hydraulic channels measuring 112 m in length, 4.3 m in width, and 1.3 m in depth (plus a 50-cm layer of reservoir sediment) were utilized for gypsum CR testing. Flume volume was measured at approximately 570,000 L, and water discharge rates ranged from 0.021 to 0.025 m^3 per second (1.8 to 2.1 million L per day). Therefore,

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[2] U.S. Bureau of Reclamation, Environmental Sciences Section, Denver, CO.

complete water volume exchange occurred 3.2 to 3.7 times per day in each flume. These systems are described in detail in Turner et al. (1993).

In May 1993, two milfoil stands measuring 4.3 by 12 m were established in each flume. Stand 1 was located 65 m below the water inlet and Stand 2 was located 103 m below the water inlet of each flume. In addition to milfoil, mixed stands of naiads (*Najas spp.)*, pondweeds (*Potamogeton spp.)*, coontail (*Ceratophyllum sp.)*, and chara were present throughout the flumes. Milfoil was established for a 6-week pretreatment growth period at which point pretreatment biomass samples were obtained.

Gypsum matrices were formulated with triclopyr (Garlon 3A) to contain either a 12- or 18-percent ai loading rate. Triclopyr CR matrices were designed to achieve target concentrations of 300 (Flumes 1 and 2) and 500 µg/L (Flumes 4 and 9) for 120 hr. In addition, matrices were formulated with endothall (Aquathol K) to contain 14-percent ai. The endothall CR matrix was designed to deliver 500 µg/L for 96 hr. The matrix design for flume evaluations was based on a prototype used by Sisneros (1991) for dye release studies in the Pend Oreille River. Matrices were formulated by Acugran, Inc., Minneapolis, MN, and are depicted in Figure 1.

On June 27, 1993, 11 matrices were suspended near the water inlet of each of five flumes at middepth in the water column. At 84-hr posttreatment, the catwalks to which triclopyr matrices were attached were moved below plant Stand 1. Therefore, milfoil in Stand 1 received only an 84-hr exposure, while plants in Stand 2 remained exposed to triclopyr for 120 hr. Water samples were collected at 12-hr intervals, and triclopyr residues were analyzed onsite (by high performance liquid chromatography) within 12 hr of collection. Endothall samples were placed in a freezer and remained frozen until analysis.

Visual observations of plant injury were recorded for 7 days. At 8-week posttreatment, milfoil and nontarget biomass were sampled to determine treatment efficacy.

Results and Discussion

Triclopyr release rates of the two gypsum CR formulations are shown in Figure 2. Data were combined from two replicate flumes for each target rate. Results showed triclopyr

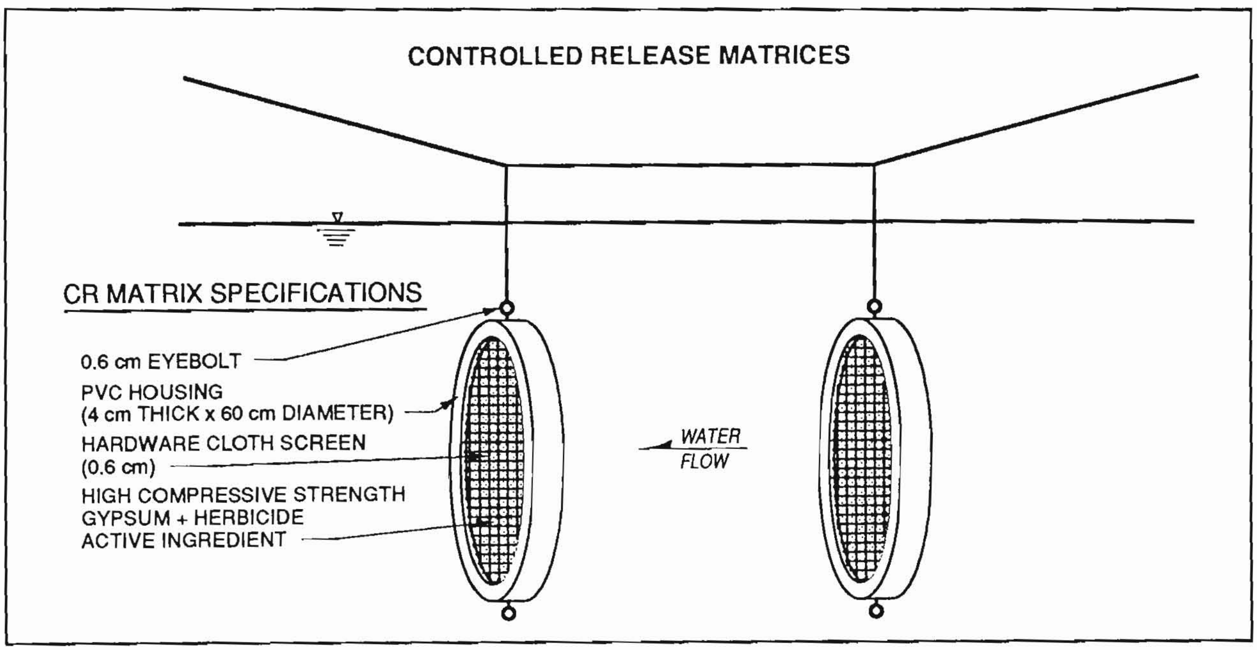

Figure 1. Diagram of prototype CR matrix used for flume testing

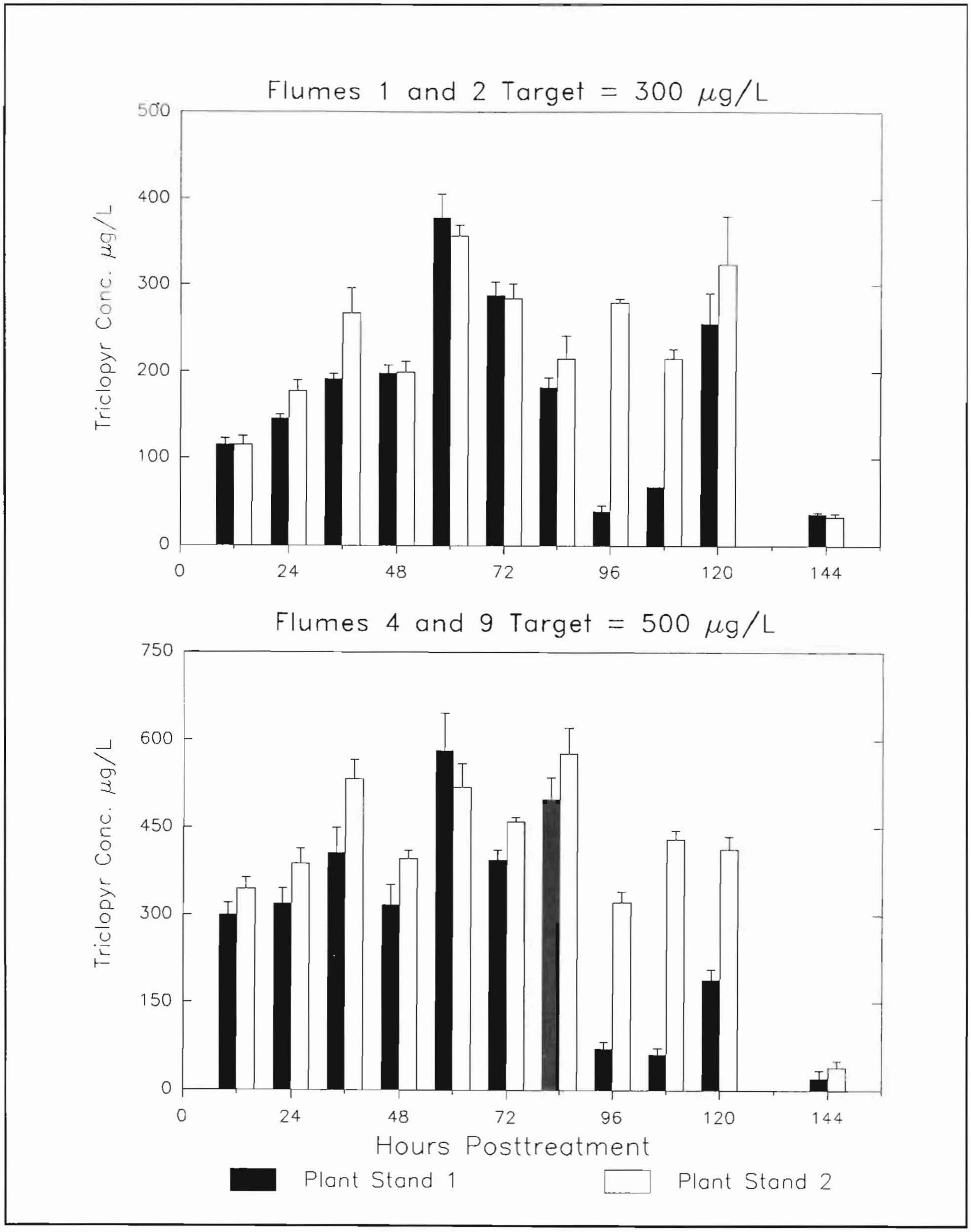

Figure 2. Aqueous triclopyr concentrations in hydraulic flumes following deployment of a 12-percent ai matrix targeted to deliver 300 µg/L and a 17-percent ai matrix targeted to deliver 500 µg/L for an 84- (plant Stand 1) and 120-hr (plant Stand 2) exposure period (Bars represent the average of four replicates taken from two flumes, and vertical lines represent one standard error of the mean)

release was fairly consistent and in the range of the target concentrations (300 and 500 µg/L) throughout most of the 84- and 120-hr exposure periods. Movement of the matrices at 84 hr resulted in a sharp drop in triclopyr residues in plant Stand 1 at the 96-hr sampling period (Figure 2).

At 115-hr posttreatment, the large pumps that supply water and flow to the flumes failed. In response, matrices were removed from the flumes (at 117 hr) to prevent accumulation of triclopyr. Despite removing the matrices, analyses showed that triclopyr remained in the flume and began to move back into plant Stand 1 by 120 hr. This reexposure of Stand 1 prevented an efficacy comparison between the two exposure periods. Residue analyses showed that although triclopyr residues had increased at 120 hr, concentrations had markedly decreased by 144 hr (likely because of the large volume of dilution water remaining in the flume).

Within hours following matrix deployment, triclopyr injury symptoms were noted on milfoil. No injury symptoms were noted for any of the other plant species. Pretreatment and posttreatment biomass results are presented in Figure 3. In addition to milfoil, *Najas spp.* biomass was included as an indicator of triclopyr selectivity. Results show that in the untreated flume, milfoil continued to increase in biomass during the 8-week posttreatment period, whereas, *Najas spp.* biomass remained suppressed. In contrast, both triclopyr treatments resulted in near 100-percent milfoil control in plant Stands 1 and 2 at 8-week posttreatment. Moreover, *Najas spp.* biomass increased significantly (within the plant stands) following triclopyr treatment.

Calculations indicated that liquid static treatments of 1,500 and 3,000 µg/L required a total of 0.85 and 1.43 kg of triclopyr per flume, whereas CR target rates of 300 and 500 µg/L were determined to require 0.6 and 1.0 kg of triclopyr per flume per day. Consequently, a total of 3.0 and 5.0 kg of triclopyr were required over the 5-day exposure period. Although it appears that these CR treatments resulted in greater herbicide use, one must consider the potential area of treatment. For example, if the length of the flumes were increased from 100 to 1,000 m, the static treatments would also need to increase tenfold from 0.85 and 1.43 to 8.5 and 14.3 kg of triclopyr per flume. CR treatments were based on flow rates; therefore, although the treatment area increased tenfold, the total amount of triclopyr required (3.0 and 5.0 kg) remained constant over the 5-day exposure period.

The flumes demonstrate that treatment of small areas in which plants are growing in high-flow environments could require more initial herbicide loading (kilograms/hectare) for a CR matrix than for a conventional application. In addition, treatment of small areas with CR formulations (especially long-term release matrices) could result in significant off-target movement and nontarget injury. However, as water-exchange rates decrease or treatment area increases, the advantages of CR delivery systems (less ai per acre treated) become manifest. Treatment sites will need to be carefully picked to avoid these potential problems.

In addition to the triclopyr matrices, one endothall formulation targeted to achieve 500 µg/L for a 96-hr exposure was evaluated. Results of endothall release rates are presented in Figure 4. Data showed that endothall release was close to target concentrations and remained quite consistent over the 96-hr exposure. Although some quality control problems existed with these matrices, release rates achieved were consistent.

Following matrix deployment, damage was noted at the apical tips of the milfoil by 36-hr posttreatment. Injury symptoms were not as obvious as triclopyr symptoms, however, by 7-day posttreatment, both milfoil and naiad stems were discolored and waterlogged.

Biomass results of the endothall matrix treatment are presented in Figure 5. Data are presented for plant Stand 1 only, as milfoil never became established in the second plant

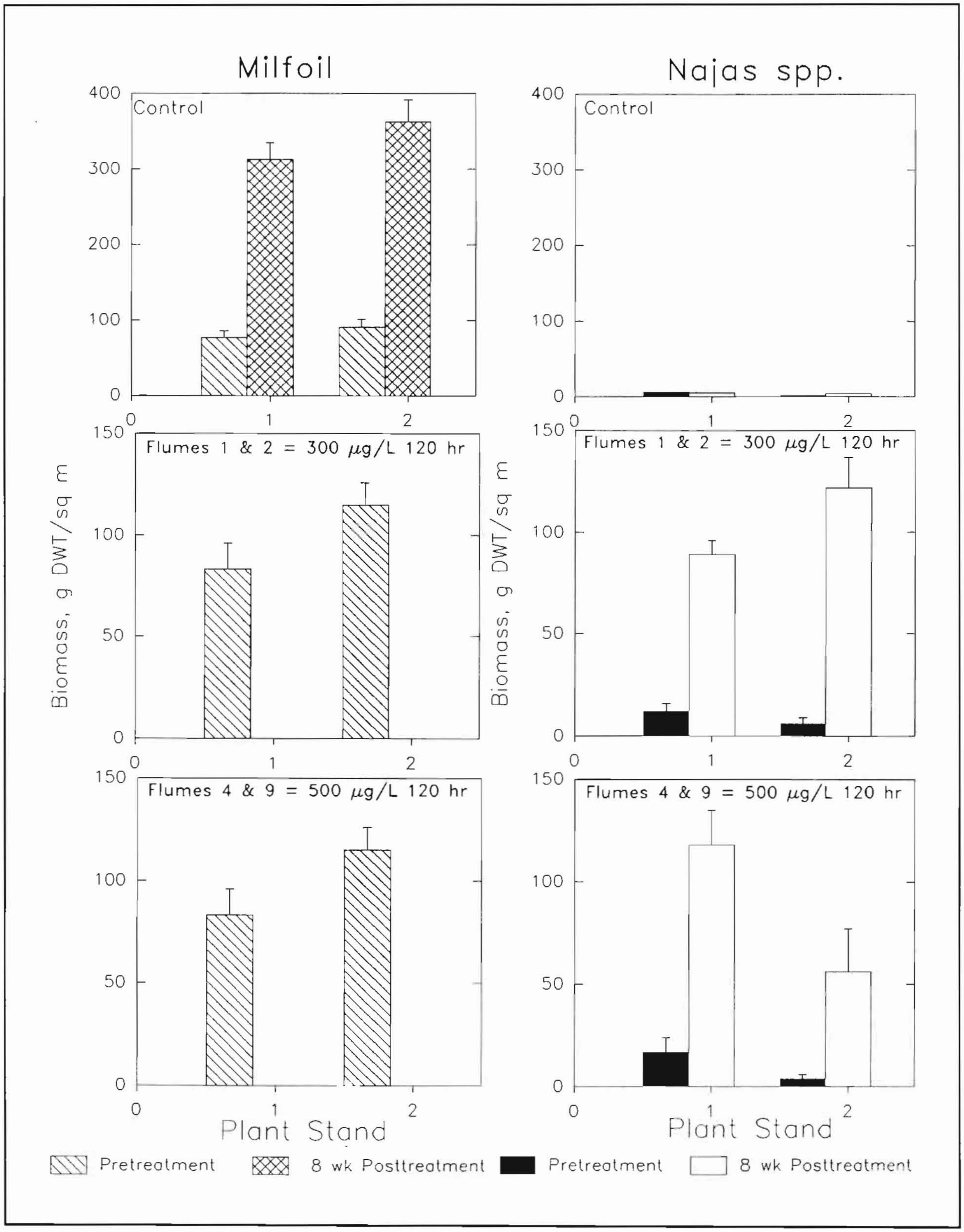

Figure 3. Pretreatment and posttreatment Eurasian watermilfoil and Najas spp. biomass in two plant stands following treatment with triclopyr CR matrices formulated to deliver 0, 300, and 500 µg/L for a 120-hr exposure period (Bars represent the average of eight replicates, and vertical lines represent one standard error of the mean)

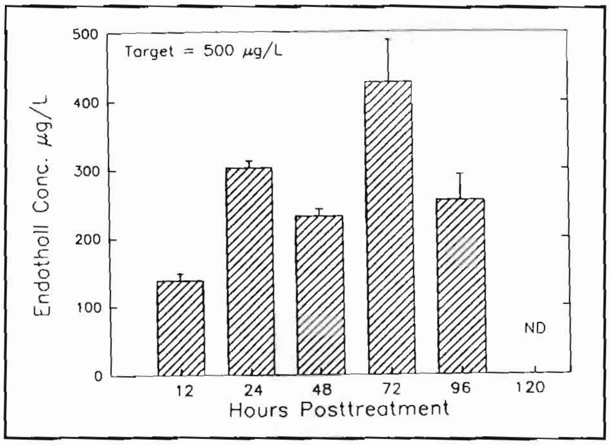

Figure 4. Aqueous endothall concentrations in a hydraulic flume following deployment of a 14-percent ai matrix targeted to deliver 500 µg/L for a 96-hr exposure period (Bars represent the average of three replicates, and vertical lines represent one standard error of the mean. ND denotes a sample that was nondetectable)

stand. Unlike triclopyr, endothall is considered to be nonselective, and therefore control of most submersed species would be expected. Results showed that endothall was highly effective at controlling both milfoil and naiads following 96 hr of exposure. Complete submersed plant control was observed at 8-week posttreatment throughout the flume.

Results of these studies suggest that the ability to deliver low herbicide concentrations over several days can improve efficacy in areas where moderate to high water exchange (4 to 12-hr half-life) reduces the effectiveness of conventional herbicide applications. The gypsum CR matrices provided sustained triclopyr release and excellent control of milfoil. Matrix design and quality control of matrices still require much improvement; however, current or slightly modified designs may be applicable in linear flow irrigation and drainage canals.

One important aspect of the use of CR technology that has not been mentioned is the ability to deliver herbicides at rates below established potable water tolerance levels. Delivery of herbicides in this manner could

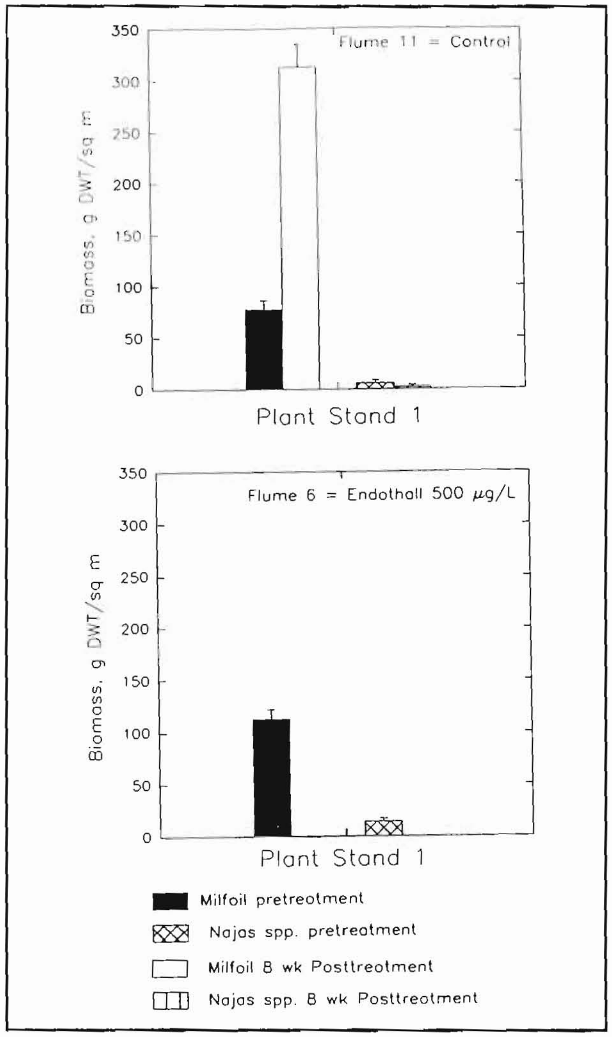

Figure 5. Pretreatment and posttreatment Eurasian watermilfoil and Najas spp. biomass in two plant stands following treatment with an endothall CR matrix formulated to deliver 0 and 500 µg/L for a 96-hr exposure period (Bars represent the average of four replicates, and vertical lines represent one standard error of the mean)

greatly reduce the restrictions placed on water use following treatment for submersed plant control.

Future Work

Plans for future work include continued TVA flume evaluation of gypsum CR and new supersorbent polymer matrices with the ai endothall. In addition, a field site will be selected to test the release and efficacy (versus hydrilla) of the new supersorbent polymer endothall formulation. Plans also include selection of irrigation or drainage canals as potential sites for field testing the gypsum CR matrices.

Acknowledgments

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Triclopyr Concentrations in Eurasian Watermilfoil: Uptake Under Differing Exposure Scenarios

by

John H. Rodgers, Jr.,[1] Arthur W. Dunn,[1] and Alan B. Jones[2]

Introduction

Development of new herbicide formulations for aquatic plant control is necessary to ensure that the most environmentally sound and effective chemistry is available for use by the Corps of Engineers, as well as state and local agencies. The most efficient approaches for development of new herbicide formulations are apparent when we understand the mechanisms and kinetics by which herbicides are absorbed and transported to active sites within target plant tissues and cause mortality in target plant populations. Toward that end, HERBICIDE version 3.0 (Rodgers, Clifford, and Stewart 1991) was developed to couple the processes regulating the persistence and fate of herbicides applied to aquatic plants with the anticipated responses of plant populations. Through this modeling effort, it was apparent that at least two major factors may influence herbicide efficacy: (a) the initial concentration of the herbicide applied, and (b) the dissipation rate of the herbicide in an aquatic system. In nonequilibrium conditions such as flowing waters, data are needed to understand the relationships between plant uptake rates and control efficacy (Cassidy and Rodgers 1988; Clifford, Rodgers, and Stewart 1990).

With existing formulations, many systemic herbicides will not have sufficient contact time with the target plants to be effective in these flowing-water situations (Getsinger, Sisneros, and Turner 1993). To provide a tool for use in these lotic systems, recent research has focused on controlled-release (CR) formulations that increase herbicide contact time with the target plants while minimizing the exposure concentration and risks to nontarget species. CR formulations may (a) provide long-term control, (b) minimize residual pesticide in aquatic environments, (c) maintain the concentration of pesticides in close proximity to the target plants, (d) increase the efficacy of the pesticide by protecting it from environmental degradation processes, and (e) decrease application costs by decreasing the number of applications required (Harris, Norris, and Post 1973; Trimnell et al. 1982; Riggle and Penner 1991).

For susceptible plants, each specific herbicide has unique concentration and exposure time requirements to obtain efficacious control (Clifford, Rodgers, and Stewart 1990; Netherland 1991). An expected trend is that as the duration of exposure to the herbicide is increased, lower concentrations of herbicide will be needed to achieve control of the target plant (Netherland 1991).

Knowledge of internal herbicide tissue burden of target plants will be valuable for development of CR matrices. Recent research has shown that for many aquatic herbicides, only a small portion of the herbicide in the water is taken up by the plant (Haller and Sutton 1973; Reinert et al. 1985; Van and Steward 1986). These results have indicated that plant uptake rates for some herbicides are slow, and extending the contact time should improve the efficacy of these herbicides (Netherland 1991). Use of CR formulations may be particularly suitable for systemic herbicides such as triclopyr.

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Triclopyr (3,5,6-trichloro-2-pyridinyloxy-acetic acid) has been used for over 15 years to control broad-leaved weeds in forestry, industrial, and other noncrop terrestrial sites (Solomon et al. 1988; Getsinger, Turner, and Madsen 1992). Manufactured by DowElanco, triclopyr is registered for aquatic sites under a Federal experimental use permit (EUP). The U.S. Environmental Protection Agency is currently considering triclopyr for full aquatic registration.

This study was conducted to better understand relationships between the exposure-response of Eurasian watermilfoil to triclopyr and uptake of triclopyr to critical plant tissue burdens that would ultimately control the growth of this plant. In this study, triclopyr concentrations in Eurasian watermilfoil under differing exposure scenarios were evaluated. The specific objectives of this study were to evaluate triclopyr uptake following (a) a gypsum-based CR matrix treatment in flowing water, (b) a liquid application in flowing water, and (c) liquid applications in static water. Under these varying exposure scenarios, we wanted to evaluate relationships between triclopyr concentrations in the water and concentrations in plant tissue.

Materials and Methods

Study site

Research was conducted at Tennessee Valley Authority (TVA) Aquatic Research Laboratory (ARL) located at Brown's Ferry, AL. At this facility, we used eight flow-through hydraulic channels. Seven channels were used for triclopyr treatments, and one served as an untreated control. Each channel measured 112 m in length, 4.3 m in width, and contained a 50-cm-thick layer of reservoir sediment (Figure 1). Water for each flow-through channel was pumped from Wheeler Reservoir, and water depths of up to 1.2 m were maintained in each flume.

Establishment of Eurasian watermilfoil stands

One or two stands of Eurasian watermilfoil (4 by 10 m) were established in each flume, depending on the specific treatment for that flume (Figure 2). This was accomplished by draining each flume and then planting four to six freshly harvested Eurasian watermilfoil apical tips, 20 to 30 cm in length on 30-cm centers and allowing the plants to grow in flowing water for 6 weeks.

Triclopyr treatments

Three different triclopyr treatment scenarios were utilized: (a) CR matrices in flowing water, (b) a liquid application in flowing water, and (c) liquid application in static water.

CR matrices consisted of a plastic housing and a matrix core of calcium sulfate (gypsum). The plastic housing was constructed from 0.48-cm-thick, polyvinyl chloride pipe that was 4 cm wide by 60 cm in diam. Eye screws (0.56-cm) were attached to the housing at selected locations to support the matrices in the water column. In addition, 0.6-cm hardware screen was placed over both sides of the matrices to prevent the matrix material from escaping. The matrices contained sufficient triclopyr based on calculations to load the appropriate concentration throughout the exposure period (Figure 2).

Four flumes were utilized for the slow release matrix device (SRMD) treatments. Each of these flumes contained two plant stands. Two flumes contained matrices designed to deliver 0.3 mg/L of triclopyr and the other two flumes contained matrices designed to deliver 0.5 mg/L of triclopyr. All matrices were initially suspended within the water column near the inflow end of each flume, thus delivering the herbicide to both the upstream and downstream plant stands. Following 3.5 days of exposure, the matrices

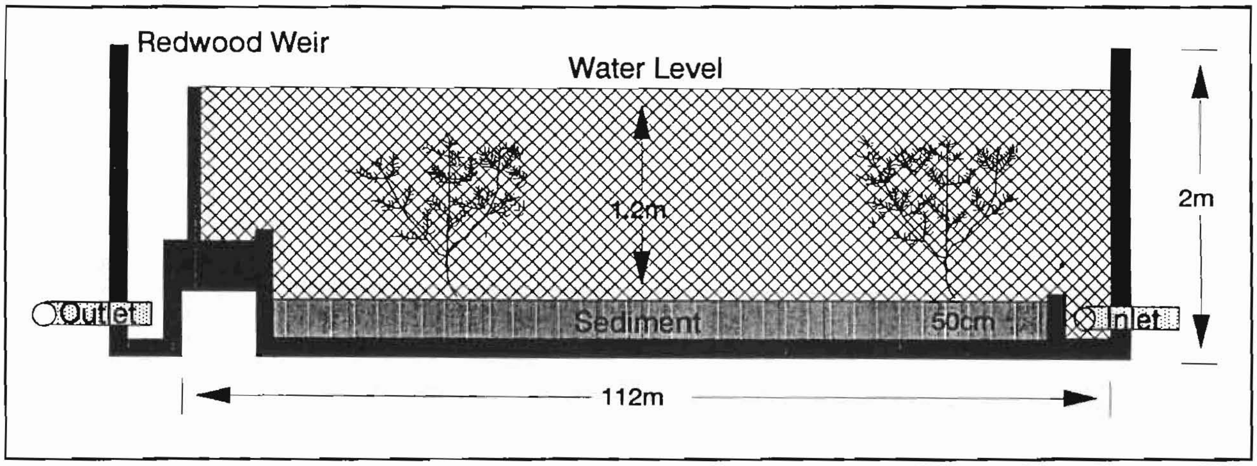

Figure 1. Diagram of TVA ARL flume

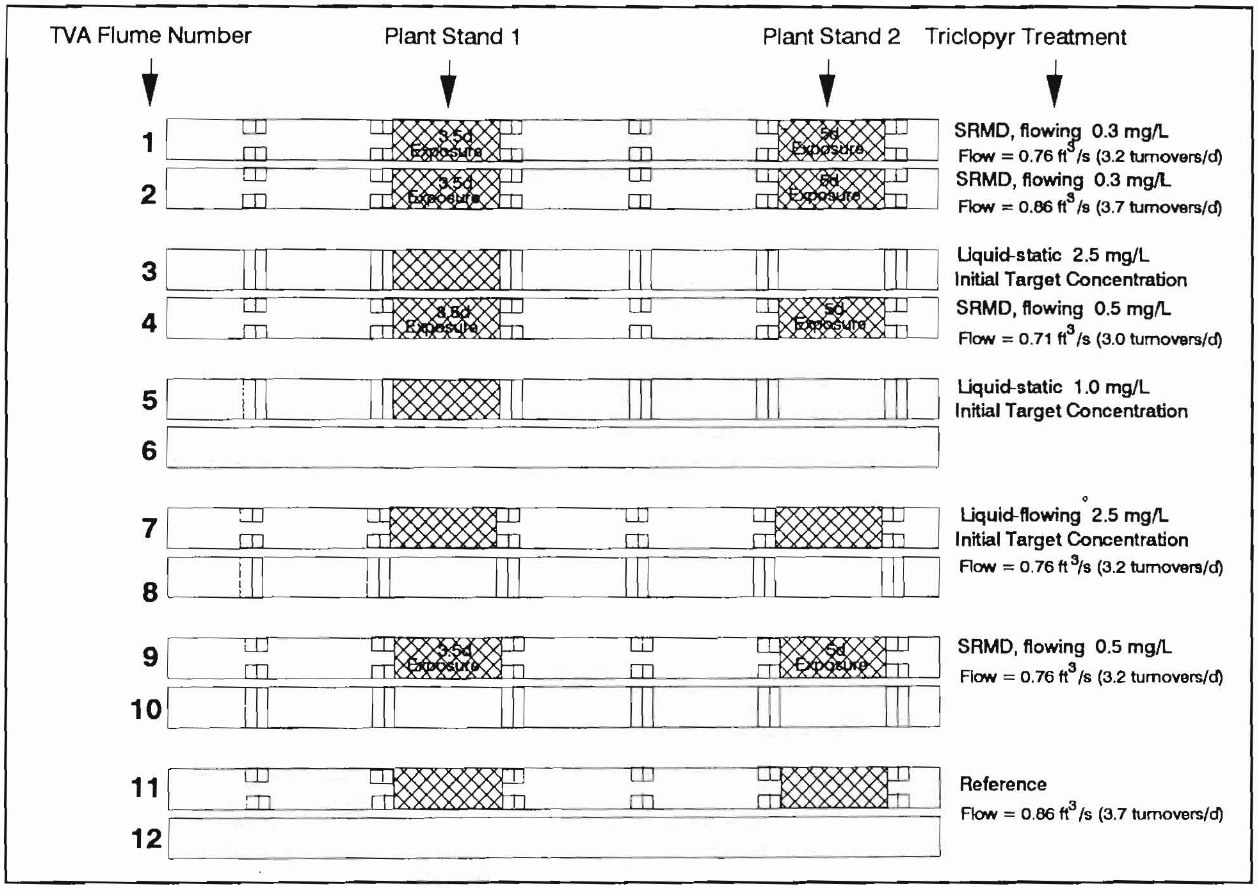

Figure 2. Experimental design of triclopyr/Eurasian watermilfoil uptake study under differing concentration/exposure scenarios

were repositioned below the first (upstream) plant stand and delivered the herbicide only to the second or downstream plant stand. Matrices were left in place for 1.5 additional days. Thus, the 0.3- and 0.5-mg/L treatments were designed to deliver triclopyr to Stand 1 for 84 hr and Stand 2 for 120 hr (Figure 2).

An additional flume containing two plant stands was used to evaluate milfoil uptake following a liquid triclopyr application in flowing water. Liquid triclopyr was applied to the entire volume of the channel at a rate calculated to achieve 2.5 mg/L.

To evaluate milfoil uptake rates under static conditions, two flumes containing one plant stand were utilized for liquid applications of triclopyr. Liquid triclopyr was applied to achieve rates of 2.5 and 1.5 mg/L, respectively. The designated static exposure period for these two flumes was 48 hr. In all cases, treatments were initiated on 26 June 1993.

Plant sampling

Tissue samples (shoots) of Eurasian watermilfoil were collected in triplicate (provided plant tissue was available) from each plant stand 1 day prior to triclopyr treatments and at intervals posttreatment to monitor uptake and depuration. Water samples were collected at the upper and lower ends of each plant stand every 12 hr throughout each exposure period. Biomass samples were collected to determine treatment efficacy. Collection and analyses of water and plant biomass samples were accomplished by U.S. Army Engineer Waterways Experiment Station and Bureau of Reclamation personnel.

Plant samples for triclopyr analysis were collected from the flumes with a plant rake while standing on a portable catwalk. Plant tissue was washed to remove algae and debris and wrapped in aluminum foil. These plant samples were stored in a Ziploc freezer bag at -10 °C until analysis.

Analysis of plant tissue for triclopyr

The plant samples were analyzed for triclopyr according to a DowElanco protocol (ACR 77.4). The protocol was modified by including 2,4-D acid as an internal standard. Triclopyr was extracted from dried powdered plant material using 0.3N barium hydroxide: methanol (1:2). The mixture was filtered, the filter washed with methanol, and the filtrate reduced in volume under nitrogen. The extract was then washed with ether and the ether phase discarded. The aqueous phase was acidified with 1N H_2SO_4 and extracted with ether. The ether phase was transferred and evaporated to dryness under nitrogen, and the residue was dissolved in 0.3N barium hydroxide: methanol (1:2). After an additional extraction into ether, the triclopyr was derivatized using tetramethylammonium hydroxide:dimethyl sulfoxide (1:40) and iodomethane. The derivatized triclopyr was partitioned into hexane. The methylated derivatives of triclopyr and 2,4-D acid were injected into a Hewlett Packard gas chromatograph (HP5890) filled with a 60-m DB-5 capillary column, which was operated isothermally at 170 °C. Detection of the derivatized herbicide was accomplished using an electron capture detector (ECD).

Results and Discussion

One of the basic premises of aquatic toxicology, as applied to aquatic plant management, is that to understand responses, accurate information regarding exposure of target plants to herbicides is required (Clifford, Rodgers, and Stewart 1990). The targeted concentrations and exposures of triclopyr were chosen based upon previous laboratory experimentation (Netherland and Getsinger 1992) and anticipated degradation rates within the flumes used for this experiment (Figure 2). There is an inverse relationship between the targeted concentration and the duration of exposure. For example, as the concentration of triclopyr that is targeted in the flowing situations decreases, the duration of exposure should increase with similar efficacy in terms of controlling the Eurasian watermilfoil. However, because of similar exposure periods under static conditions, we would expect greater efficacy with increased treatment concentrations. Plant tissue concentrations make sense only in terms of the triclopyr exposure concentrations. As noted in the results and discussion below, the exposure scenarios were complicated by failure of the pump delivering water to all of the flumes. The time interval when there was no flow was approximately 115- to 240-hr posttreatment. After

240-hr posttreatment, the pump was repaired, and water flow was restored.

Plant tissue concentrations in CR matrix exposures

In the exposures involving CR matrices, triclopyr concentrations were measured through time in water and Eurasian watermilfoil. The targeted concentrations of triclopyr were 0.3 and 0.5 mg/L with targeted exposure durations of 3.5 and 5 days.

Triclopyr concentrations in the water were 0.1 mg/L 12 hr after the matrix designed to maintain 0.3 mg/L was introduced into two flumes (Figure 3). After 84-hr posttreatment, the concentration had doubled to 0.2 mg/L. Residues dissipated rapidly after removal of matrices from the upstream portion of the flumes at 84 hr; however, 0.275-mg/L triclopyr was observed in the upstream water column at 120-hr posttreatment. This reexposure of the upstream plant stand to triclopyr was likely caused by the failure of the pumps during this time interval. Desorption or depuration of triclopyr from plant tissue or sediments may have also contributed to this reexposure.

Concentrations of triclopyr in plant tissue under this scenario were maximum (6.5 mg/kg) by 48-hr posttreatment (Figure 3). After the matrices were moved downstream, concentrations of triclopyr in plant tissue declined to 4.0 mg/kg at 120 hr. When flow in these flumes was restored after 240-hr posttreatment, concentrations of triclopyr in Eurasian watermilfoil declined to 0.22 mg/kg at 360-hr posttreatment. Plant tissue collected at 360-hr posttreatment was severely injured, and the decrease in concentration of triclopyr could be a function of depuration as well as leakage because of cell injury or degradation because of microbes colonizing the plant detritus.

Downstream in the same flumes, the plant stands were exposed to matrices designed to release triclopyr for 5 days. The aqueous concentrations of triclopyr in this exposure scenario ranged from 0.1 to 0.2 mg/L (Figure 4). Based upon designed release rates, approximately 66 percent of the targeted concentration of 0.3 mg/L was achieved. The highest aqueous concentration of 0.2 mg/L was achieved after 48 hr and maintained through 120-hr posttreatment. At 144-hr posttreatment, aqueous residues measured approximately 0.065 mg/L. This rapid decrease was likely due to the large volume of untreated dilution water in the flume, following movement of the matrices to the downstream portion of the flume. After 240-hr posttreatment, the concentration of triclopyr in the water column declined to nondetectable levels.

Concentrations of triclopyr in plant tissue reflected concentrations of triclopyr in the water (Figure 4). Tissue residues were maximal at 48-hr posttreatment (3.3 mg/kg) (Figure 4). The concentrations of triclopyr in plant tissue declined steadily throughout the remainder of the sampling, up to 360 hr when a concentration of 0.46 mg/kg was noted. Again, the plant tissue exhibited signs of severe injury; thus depuration and leakage of triclopyr probably played a role in decline of tissue concentrations at that point.

Matrices were also designed to release 0.5 mg/L with exposure durations of 3.5 and 5 days, (Figures 5 and 6). The exposure duration of 3.5 days was accomplished by moving the matrices below plant Stand 1 to a downstream area of the flumes. Aqueous concentrations of triclopyr in this situation ranged from 0.29 mg/L at 48 hr to 0.06 mg/L at 144 hr (Figure 5). About 80 percent of the targeted concentration of 0.5 mg/L was achieved in this situation. Because of pump failure, flow in these flumes was not restored until 240-hr posttreatment. The triclopyr concentration in the water decreased rapidly from 120 to 144 hr because of the large volume of upstream dilution. After resumption of flow at 240 hr, triclopyr concentrations in the water column were nondetectable.

Triclopyr tissue residues were maximal 24 hr after exposure (16.1 mg/kg) following the 3.5-day exposure (Figure 5). The concentrations of triclopyr in plant tissue dissipated rapidly after 240 hr. A similar pattern was

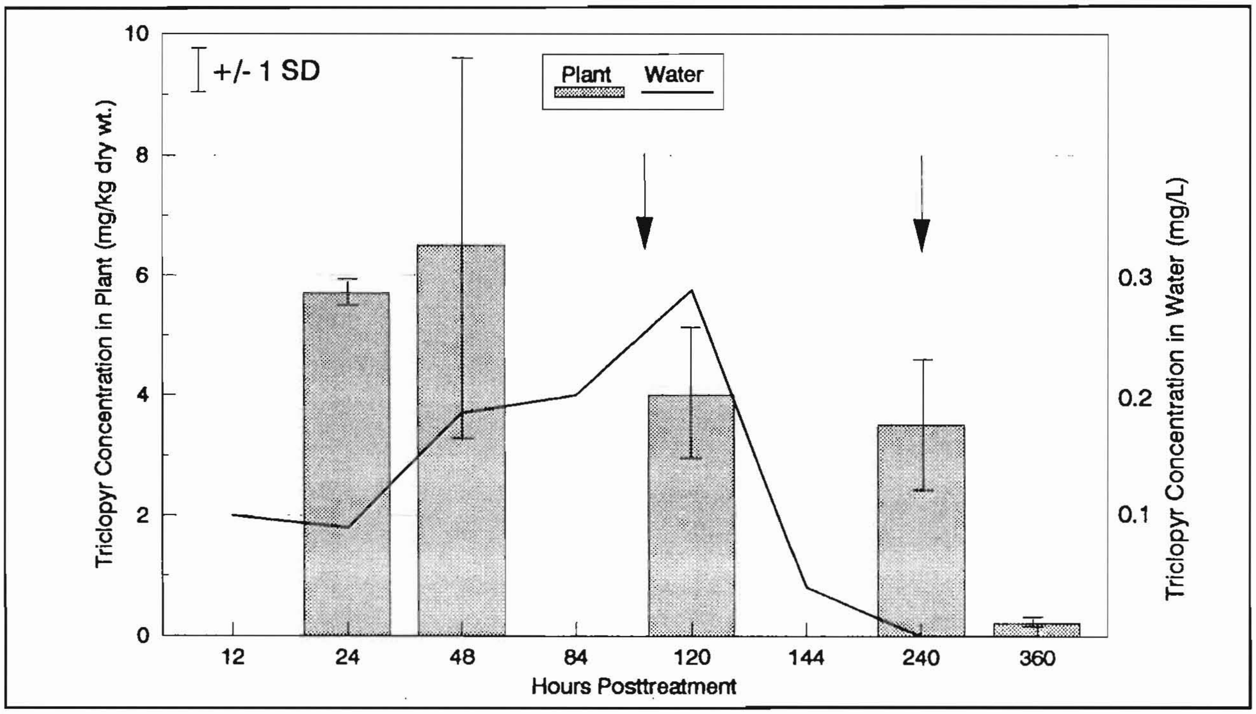

Figure 3. Triclopyr concentrations in water and Eurasian watermilfoil (Target concentration = 0.3 mg/L SRMD, exposure duration = 3.5 days, water flow = 0.81 ft³/s. Arrows note time interval of no blow because of pump failure)

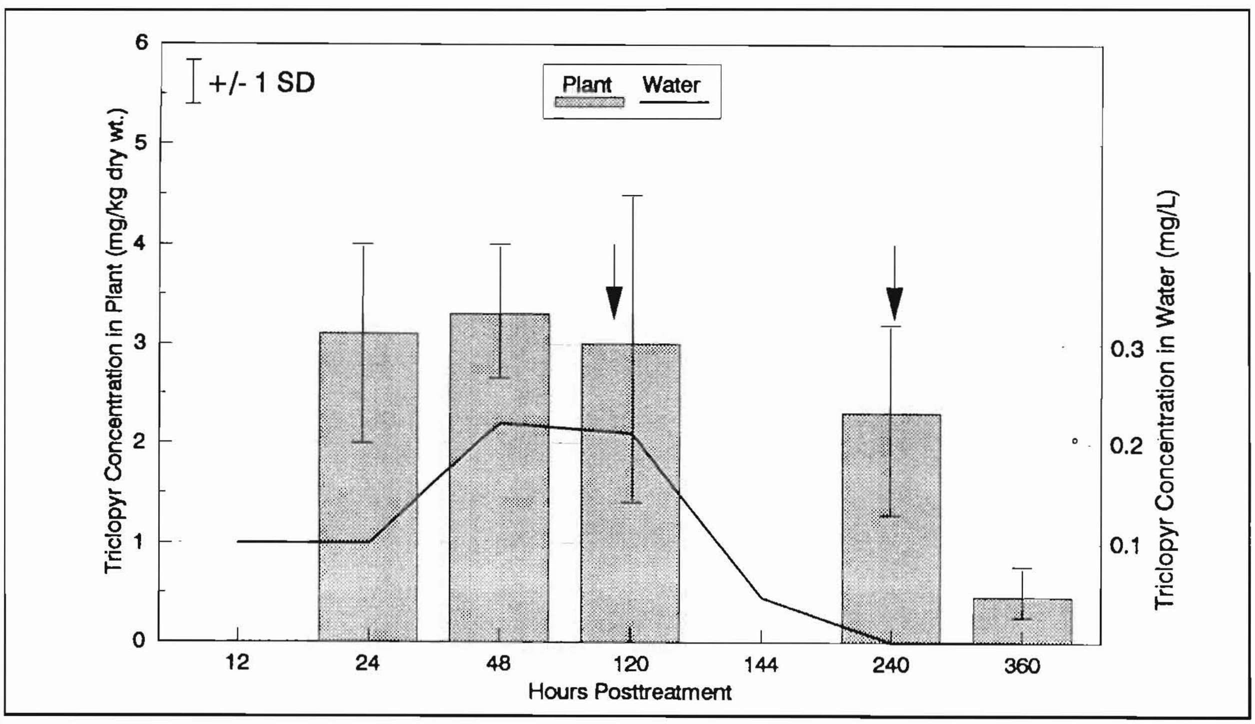

Figure 4. Triclopyr concentrations in water and Eurasian watermilfoil (Target concentration = 0.3 mg/L SRMD, exposure duration = 5 days, water flow = 0.81 ft³/s. Arrows note time interval of no blow because of pump failure)

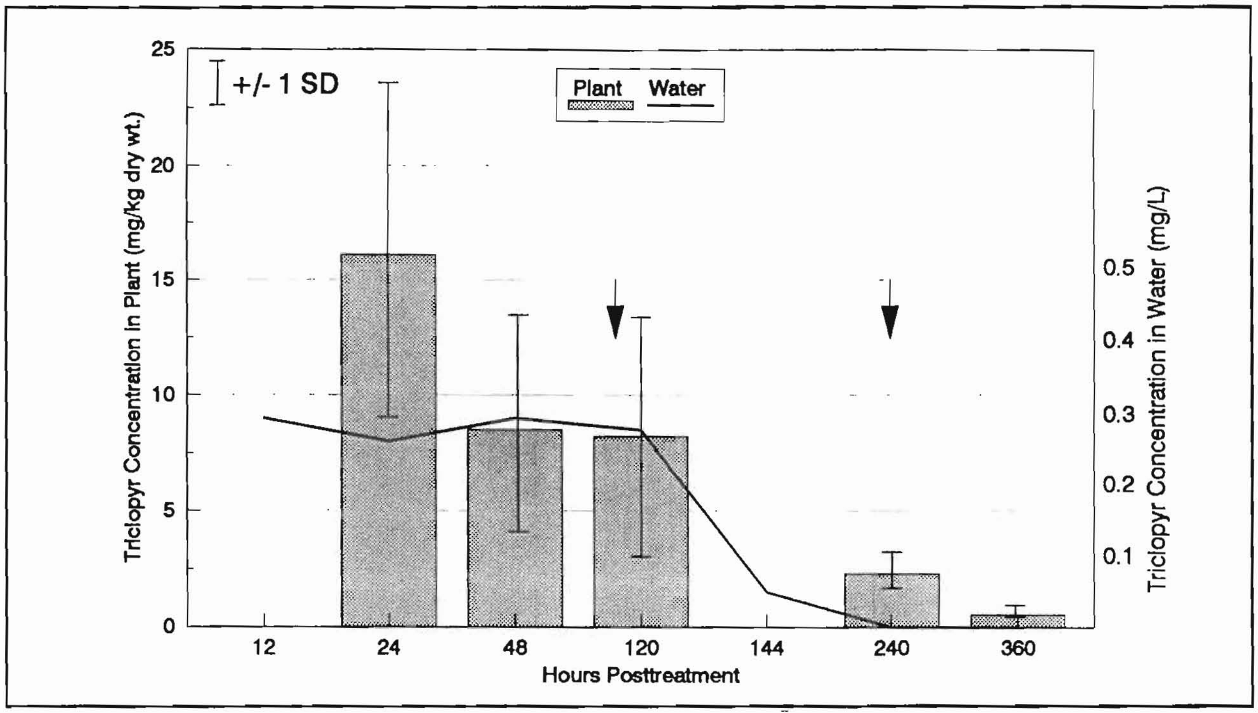

Figure 5. Triclopyr concentrations in water and Eurasian watermilfoil (Target concentration = 0.5 mg/L SRMD, exposure duration = 3.5 days, water flow = 0.74 ft³/s. Arrows note time interval of no blow because of pump failure)

observed with the 5-day exposure duration (Figure 6). Concentrations of triclopyr in the water were maximal at 120 hr (0.3 mg/L). This is 60 percent of the targeted concentration. In plant tissue, the observed concentrations of triclopyr (9.4 mg/kg) were maximal at 48-hr posttreatment, but declined to 4.6 mg/kg by 120-hr posttreatment (Figure 6). After removal of the matrices at 120-hr posttreatment, concentrations of triclopyr in plant tissues remained steady until flow was resumed at 240-hr posttreatment.

Liquid application, flowing water

The targeted concentration of 2.5 mg/L was exceeded slightly (3.3 mg/L) 2 hr after the liquid was applied, likely because of unequal mixing of the water column (Figure 7). Triclopyr dissipated at a predictable rate with seven water turnovers at 48 hr. Triclopyr concentrations were reduced 90 percent by 24 hr and were below detection by 48 hr. Although water flow was lost at 115-hr, the exposure scenario had been completed well prior to the pump failure. Maximum concentrations of triclopyr in plant tissue approached 18.2 mg/kg (on a dry weight basis) by 24-hr posttreatment (Figure 7). The concentration in plant tissue decreased sharply with the decline in aqueous concentration of triclopyr. The depuration of triclopyr from plant tissue is relatively rapid under this scenario. However, triclopyr concentrations in plant tissue (2.0 and 0.9 mg/kg) remained detectable by 240- and 360-hr posttreatment. Injury to the plant tissue was readily apparent.

Liquid application, static water

Triclopyr concentrations in static water were targeted at 1.5 and 2.5 mg/L using liquid applications. Pumps were shut off to produce a static water situation for 48 hr. Pumps were enabled at 48-hr posttreatment, and the water flow was resumed. In the flume receiving the 1.5 mg/L treatment, triclopyr concentrations were maximal 2-hr posttreatment when a concentration in the water column of 1.8 mg/L was observed (Figure 8). Triclopyr had degraded (likely by photolysis) approximately 50 percent in the water column by 48-hr posttreatment,

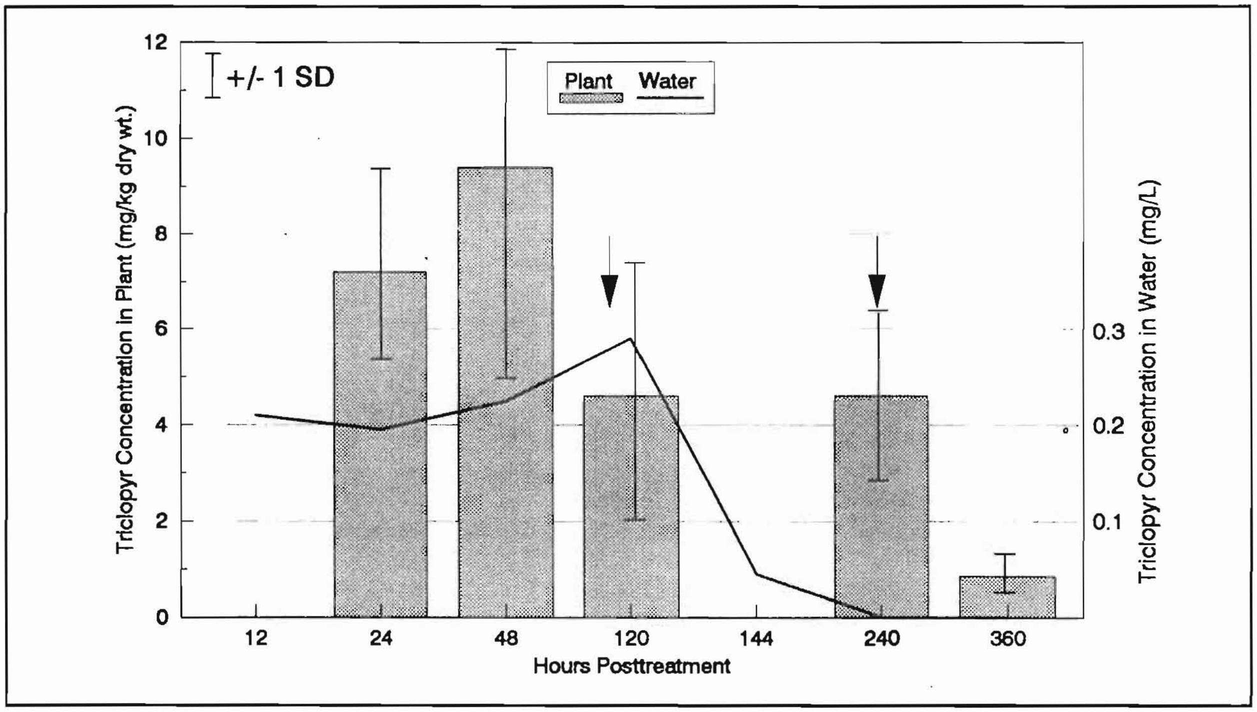

Figure 6. Triclopyr concentrations in water and Eurasian watermilfoil (Target concentration = 0.5 mg/L SRMD, exposure duration = 5 days, water flow = 0.74 ft³/s. Arrows note time interval of no blow because of pump failure)

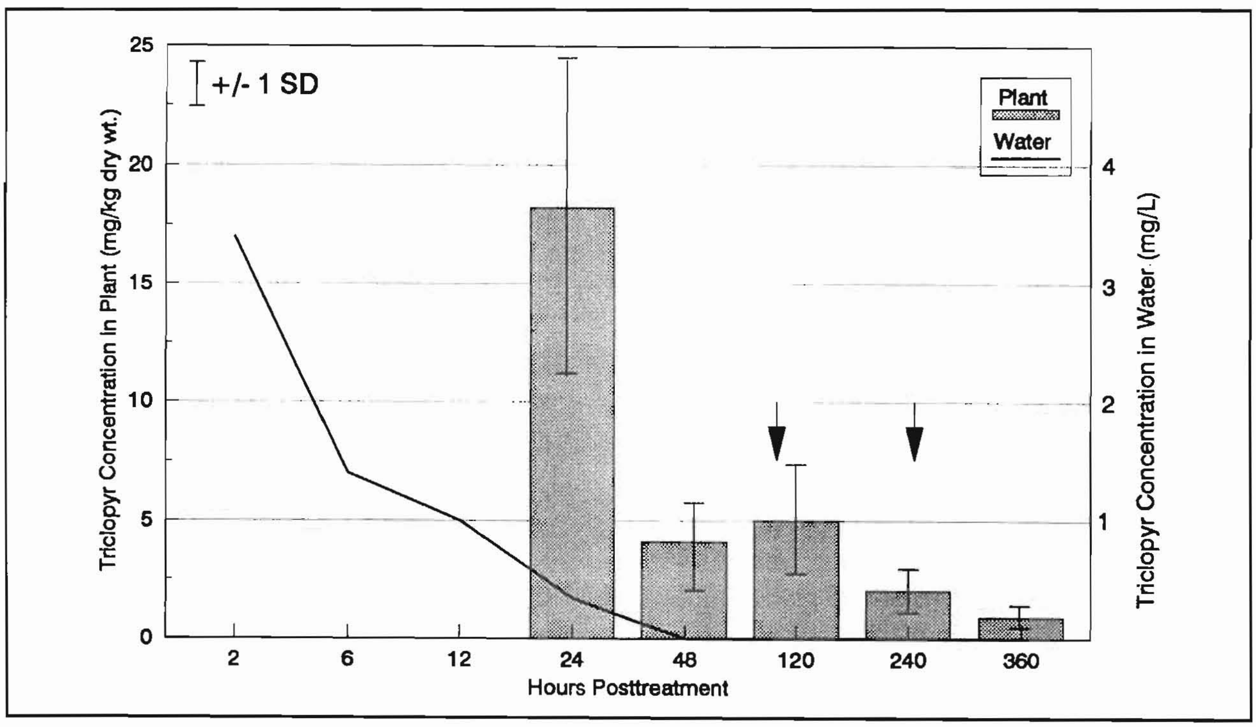

Figure 7. Triclopyr concentrations in water and Eurasian watermilfoil (Initial target concentration = 2.5 mg/L liquid application, flowing water. Water flow = 0.76 ft³/s. Arrows note time interval of no blow because of pump failure)

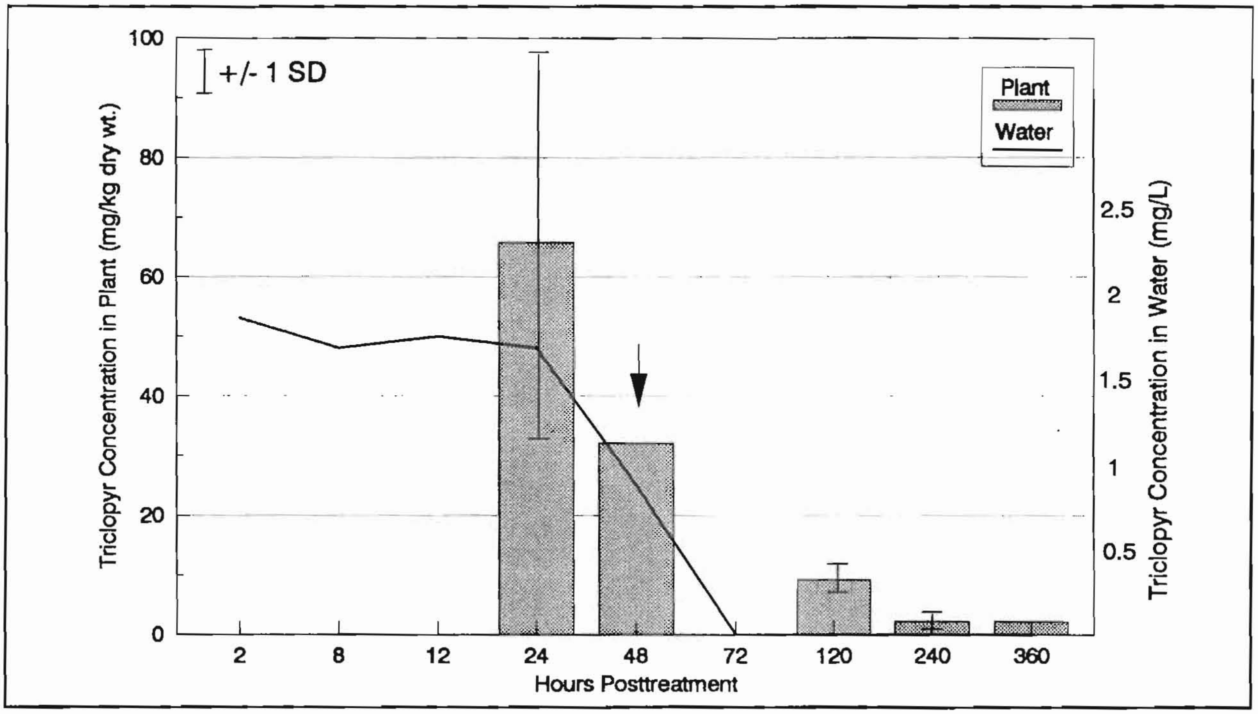

Figure 8. Triclopyr concentrations in water and Eurasian watermilfoil (Initial target concentration = 1.5 mg/L liquid application, static water. Arrow denotes resumed flow after 48 hr)

and following return of water flow was undetectable at 72-hr posttreatment. Triclopyr plant tissue concentrations were maximal 24 hr after treatment (65.7 mg/kg) (Figure 8).

In the other static water exposure scenario, the target concentration was 2.5 mg/L, and the flow was resumed after 48 hr to flush the system. The maximum triclopyr concentration observed in the water column was 2.9 mg/L at 12-hr posttreatment (Figure 9). Triclopyr concentrations declined rapidly after 24-hr posttreatment with 1.5 mg/L observed at 48 hr and nondetectable levels at 72-hr posttreatment. The triclopyr concentration in Eurasian watermilfoil tissue at 24-hr posttreatment was 113 mg/kg on dry weight basis (Figure 9). By 48-hr posttreatment, plant tissue concentrations had declined to 66.8 mg/kg; by 120-hr posttreatment, plant tissue levels declined to 18 mg/kg.

Plant tissue concentrations relative to triclopyr exposures

Plant tissue concentrations on the order of 15 to 30 times the aqueous exposure were observed in flumes treated with the 0.3-mg/L matrices, indicating that milfoil tissue levels may have been limited by the rate of release of triclopyr from these matrices. Further evidence of this situation is provided by the flumes treated with 0.5-mg/L SRMDs where plant tissue concentrations on the order of 40 to 100 times the aqueous exposure were observed. In aqueous exposures of triclopyr using liquid applications, plant tissue concentration factors ranged from 28 to 105 times greater than the aqueous concentrations of triclopyr. In the liquid application of 2.5 mg/L in flowing water, a maximum concentration ratio of 105 was observed. In the liquid applications in static water, a maximum concentration factor of 42 was observed in the 2.5-mg/L treatment. The maximum concentration factor observed in the 1.5-mg/L treatment was 28.

Green et al. (1989) observed Eurasian watermilfoil bioconcentration factors of only 5 (concentration of triclopyr in plant tissue: concentration of triclopyr in water) for triclopyr treatments in Lake Seminole, Georgia. However, concentrations of triclopyr in plant tissues and water declined rapidly following treatment.

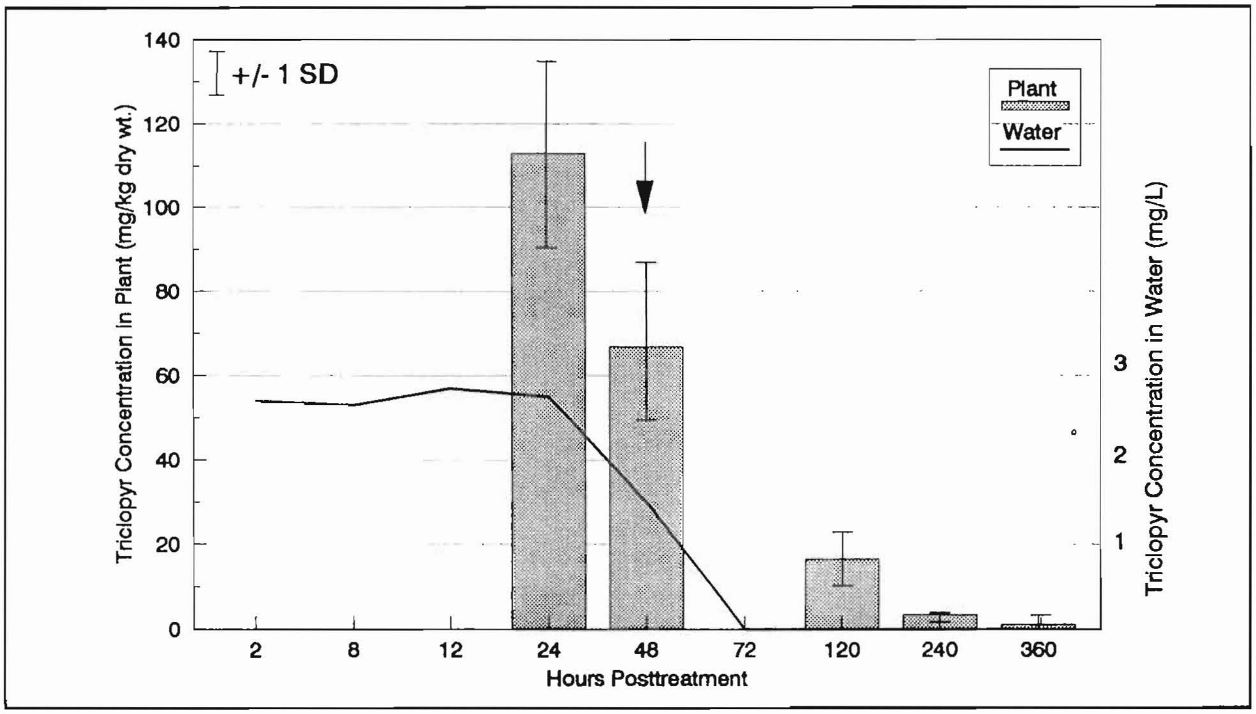

Figure 9. Triclopyr concentrations in water and Eurasian watermilfoil (Initial target concentration = 2.5 mg/L liquid application, static water. Arrow denotes resumed flow after 48 hr)

The key factor for obtaining plant control is achieving the critical tissue burden required to effectively control the growth of Eurasian watermilfoil. In all cases in this study, the plants were severely injured. Based upon the results of this study, the minimum critical tissue burdens would be on the order of 8 to 10 mg/kg dry weight of triclopyr for this species. This critical tissue concentration can be achieved in a variety of ways. Clearly, the CR matrices offer advantages in terms of reducing opportunities for exposures of nontarget organisms. Critical tissue burdens of herbicides under realistic exposure scenarios will provide useful information to guide development of those and other control release matrices.

Acknowledgments

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Summary of Field Evaluations of Herbicides on Guntersville Reservoir

by
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Field evaluations of herbicides on Guntersville Reservoir under the Joint Agency Guntersville Project (JAGP) have focused on three areas: (a) to verify under field conditions herbicide concentration/exposure time relationships developed in laboratory and flume studies, (b) to monitor herbicide residues and nontarget effects from operational use of aquatic herbicides, and (c) to determine if aquatic herbicides affect largemouth bass behavior. This report presents a summary of these studies.

Field Verification of Herbicide Concentration/Exposure Time Relationships

Laboratory and flume studies have shown that excellent watermilfoil control can be achieved with the herbicide triclopyr at concentrations from 0.5 to 2.5 mg/L depending upon exposure times (Netherland and Getsinger 1992; Turner et al. 1993). In 1991, two sites on Guntersville Reservoir in Northeast Alabama were selected for treatment with 0.5 and 1.0 mg/L triclopyr (Getsinger, Turner, and Madsen 1992). In these studies, rhodamine WT dye was included in the spray mix to provide a means of rapidly estimating herbicide dissipation from treated areas. Dye dissipation was compared with herbicide residues of water samples collected from the treated areas.

Results from this study revealed that the half-life of dye was less than 2.0 hr in the plot treated with 0.5 mg/L and had completely dissipated within 2.0 hr from the plot treated with 1.0 mg/L triclopyr. Laboratory results showed a positive correlation between dye and herbicide dissipation.

Milfoil control 30 days after treatment was approximately 60 percent in the plot treated with 0.5 mg/L triclopyr. No control was observed in the plot treated with 1.0 mg/L triclopyr, although milfoil did show visual symptoms of herbicide effects 1 week after treatment. In 1992, a single plot on Guntersville Reservoir was treated with 2.5 mg/L of triclopyr. Excellent (100-percent) watermilfoil control was achieved 30 days after treatment. These studies showed that exposure times with half-lives greater than 2 hr would be necessary for effective milfoil control at concentrations of triclopyr below 1.0 mg/L and confirmed results from previous laboratory and flume studies (Netherland and Getsinger 1992; Turner et al. 1993).

In another study, two sites on Guntersville Reservoir were pretreated with dye to predict exposure times that could be expected from field application of herbicides. In Site 1, the dye half-life was 12.8 hr and in Site 2, 5.9 hr. Following dye characterization, both sites were treated with Aquathol K to provide an initial concentration of 2.5 mg/L dipotassium salt of endothall. Watermilfoil control 30 days after treatment was 100 percent in Site 1 and 75 percent in Site 2. These results verified laboratory studies (Netherland, Green, and Getsinger 1991) and demonstrated the importance of exposure time on efficacy of field applications of herbicides.

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Residue Monitoring and Nontarget Effects

The purpose of this study was to generate site-specific data on herbicide dissipation from treated areas; residue accumulations in sediments, plants, water, fish, and mollusks; and acute toxicity to fish and mollusks confined to treatment sites. This study was contracted to the University of Mississippi Biology Department. Results of the first-year study have been reported in Rodgers, Dunn, and Robinson (1992).

In this study, fish and mollusk were confined to cages in sites treated with the following herbicides: 2,4-D DMA salt (Weedar 64), dipotassium endothall (Aquathol K), fluridone (Sonar SP), and a combination of diquat dibromide (diquat) and chelated copper (Komeen). Prior to treatment and at various intervals after treatment, fish and mollusks were examined for mortality. Samples of fish, mollusk, sediment, water, and plants were collected for residue analysis.

No mortality was observed to fish or mollusk in any of the treatments. Residue analysis showed that herbicides followed expected rapid dissipation rates from treated water. Except for the diquat and copper treatment, there was no long-term accumulation in sediment or in fish, mollusk, or plant tissue. Copper concentrations were above background levels in fish 48 hr after treatment and in sediment and mollusk tissue throughout the 29-day sampling period. Low levels of diquat were detected in sediments 29 days after treatment.

Herbicide Treatment and Largemouth Bass Behavior

To address the concern of many bass anglers, studies were initiated to determine if application of aquatic herbicides caused fish to move from or stop feeding in treated sites. The first phase of this study involved the use of radiotelemetry to track movement of largemouth bass following treatment of aquatic plants with 2,4-D DMA salt (Weedar 64) and a combination of diquat dibiomide (diquat) and copper-ethylenediamine complex (Komeen). Results of this study showed no evidence that localized herbicide applications changed the abundance, size structure, condition, or movement of largemouth bass (Bain and Boltz 1992).

The second phase of the study was conducted to determine if largemouth bass stopped feeding or feeding declined after herbicide treatment. This effort involved fishing treated and untreated areas before and after treatment. In 1991, the study was conducted by Tennessee Valley Authority (TVA) fisheries biologists and, in 1992, by TVA fisheries biologists and local experienced anglers. Herbicides used in the study were the DMA salt of 2,4-D (Weedar 64) and dipotassium salt of endothall (Aquathol K). A water treatment was also included in the study. Each herbicide plot and water plot were compared with control plots where no treatment or airboat disturbance occurred.

Results of this study showed that there was no effect of herbicide or water treatment on catch rates or size of largemouth bass. Post-treatment largemouth bass captured ranged from 113 g to 3.4 kg. The largest fish was caught in a plot treated with 2,4-D 11 min after treatment.

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Herbicide Application Techniques in Flowing Water

by

Alison M. Fox[1] *and William T. Haller*[1]

Introduction

There has been close cooperation between the Center for Aquatic Plants, University of Florida, U.S. Army Engineer Waterways Experiment Station (WES), and the Jacksonville District since 1987 working on Herbicide Application Technique Development for Flowing Water, Work Unit 32354. Since the 1993 Annual Meeting of the Aquatic Plant Control Research Program was held in Baltimore, relatively close to where studies under this work unit were carried out in 1989, it seemed appropriate to review what progress had been made since then.

Potomac River Studies

In September 1989, rhodamine WT dye was applied to four plots on the Potomac River near the Woodrow Wilson Bridge in Washington, DC (Getsinger et al. 1991). The half-life of dye was estimated for each plot with the movement and dilution of dye being influenced by river flow, tidal fluxes, and stands of monoecious hydrilla. Half-lives did not vary greatly between the plots (ranging from 7.2 to 11.4 hr), despite some being in apparently sheltered coves and marinas and others being in hydrilla stands in the open river.

At this time, there was not much information available directly relating dye half-lives to herbicide efficacy, so conclusions regarding which herbicides might be appropriate, should chemical control of these hydrilla stands be considered, had to be derived from known exposure periods needed for typical application rates of each herbicide. Thus, for diquat at 1 mg/L, an exposure period of 24 hr was needed; for endothall at 3 mg/L, at least 12-hr exposure was necessary; and fluridone at 0.05 mg/L required several weeks exposure. These values, however, were for continuous exposure to a constant concentration, not taking into account a steady dilution (as indicated by the dye half-lives). Also, the dye is fairly resistant to adsorption and degradation over these time periods and conditions, factors which could only be assumed to be similar for fluridone. Thus, contact herbicides diquat, endothall, and copper would be expected to have even shorter half-lives in these plots than the dye.

Progress Since the Potomac River Study

Data collected under various environmental conditions in tidal canals in Crystal River, Florida, indicated that type of tide (spring or neap) and vegetation density did not influence dye half-lives (Fox, Haller, and Getsinger 1991). The relative temperatures of water in the canal and that issuing from nearby springs were very influential, resulting in almost 10-fold differences in dye half-lives at different times of year. While there are not likely to be springs influencing the target section of the Potomac River, such results indicate that further studies comparing water temperature and conductivity/density relations within the river channel might reveal circumstances under which water retention times (dye half-lives) might be prolonged (or shortened) in these plots.

Laboratory studies of concentration/exposure time (CET) relations for hydrilla and Eurasian watermilfoil have been conducted at WES using fluridone, endothall, and triclopyr (milfoil only), and are in progress, or planned, for various combinations of the contact herbicides diquat, copper, and endothall (e.g., see

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Netherland, Getsinger, and Turner 1993). These types of data have greatly improved the prediction of weed control when applied to sites in which a given concentration of herbicide is retained for a known period of time. However, such studies alone cannot directly relate dye half-lives to herbicide retention and activity.

Field studies in which rhodamine WT was added concurrently to herbicide applications on hydrilla (dioecious) have been conducted for diquat, endothall, and fluridone, and from these treatments, ratios of dye and herbicide half-lives could be estimated. Diquat applied to three plots in Orange Lake in Florida and sampled for up to 168 hr after treatment had half-lives in the plot surprisingly similar to those of the dye (Table 1). Reductions in dye concentration resulted from dilution and movement out of the plot, but diquat residues were more likely to have been adsorbed or degraded as indicated by nonsignificant residue concentrations outside the treated plots (Langeland et al., in preparation). There is not much field or laboratory data on the minimum concentration/half-lives needed for diquat to be effective, a subject needing more research.

Table 1
Summary of Half-Lives from Concurrent Applications of Herbicides and Rhodamine WT to Hydrilla

Herbicide	Site	Herbicide Half-Lives,hr	Dye Half-Lives, hr	Ratio Herb/Dye Half-Lives	Maximum Sample Time, hr
Diquat	Orange Lake[1]				
	South plot	25	33	0.8	168
	Middle plot	39	50	0.8	168
	North plot	25	36	0.7	168
Endothall	Crystal River[2]				
	Canal A	13	75	0.2	141
	Canal B	17	83	0.2	94
	Canal D	7	30	0.2	94
	Lake Washington[3]				
	South plot	4	6	0.7	30
	North plot	15	29	0.5	121
Fluridone	Crystal River[4]				
	Canal	128	114	1.1	339
	Lake Hell 'n' Blazes[4]	9	9	1.0	93

[1] Langeland et al., in preparation.
[2] Fox, Haller, and Getsinger (1993).
[3] Fox and Haller (1990).
[4] Fox, Haller, and Shilling (1991).

Concurrent applications of endothall and rhodamine WT in tidal canals at Crystal River, Florida, indicated that endothall had significantly shorter half-lives than the dye when collected over a period of up to 4 to 6 days (Table 1). Dye and endothall half-lives were not significantly different when applied to plots in Lake Washington, Florida (Table 1). While this was not particularly surprising in the south plot with a dye half-life of only 6.1 hr, the half-life estimations in the north plot were complicated by an increase in concentrations of both chemicals at the sampling stations within the first 24 hr. Hydrilla was controlled in both of these plots, although there was rapid regrowth in the south plot. These data indicate that half-lives of endothall applied to the Potomac plots might be sufficient for some hydrilla control, but with only a single application, regrowth might be expected to be quite rapid.

Fluridone and dye half-lives were equal after concurrent applications in Crystal River and Lake Hell 'n' Blazes, Florida, when sampled for up to 10 days after application (Table 1). The dye half-lives in the Potomac River plots were much too short for single applications of fluridone to be effective in controlling hydrilla, but several other breakthroughs in herbicide technology in flowing water since 1989 were of even greater relevance to this type of site.

The dye half-life in Lake Hell 'n' Blazes was within the range of values found in the Potomac River, but effective hydrilla control was achieved using fluridone in this lake and for 16 km (10 miles) downstream in the St. Johns River in 1989. Fluridone AS formulation was applied to the lake twice a week for a period of 10 weeks at a rate (estimated from the inflowing river discharge) to result in a constant concentration of 15 μg/L fluridone (Haller, Fox, and Shilling 1990). Such types of treatment were repeated annually in the Withlacoochee River, Florida, from 1990 to 1992 where applications of fluridone three times a week were estimated to result in concentrations of 10 to 12 μg/L, and hydrilla controlled in 400 ha (1,000 acres) of downstream river/lakes.

These applications of liquid formulations of fluridone to treat whole reaches of river assume that the whole river discharge can be treated. In a river as large as the Potomac with annual discharges estimated to be 323 m^3/sec (11,400 cfs) (Carter, Rybicki, and Turtora 1991), this would be prohibitively expensive ($11 millon to maintain 10 μg/L for 10 weeks). However, this type of treatment might be applied to coves or just the part of the cross section of a river where hydrilla beds occur. The use of controlled-release matrices to deliver fluridone at several points downstream over a limited width of a river (Netherland 1992; Sisneros 1992) may have greatest potential for treatment of hydrilla beds within large river channels.

Future Work

A considerable amount of information has been gained concerning herbicide treatment of submersed vegetation in flowing water since the Potomac River dye study in 1989. These improvements in our knowledge of herbicide technology in flowing water is allowing the development of site-specific recommendations.

The CET relations of contact herbicide combinations that might be effective at lower concentrations or shorter exposure times than the single compounds will be relevant. Estimating half-life relationships between rhodamine WT and copper will complete these data for the four herbicides available for use on hydrilla. Further improvement of controlled-release matrices could be of great importance for use of fluridone in such sites.

After successful fluridone applications in the Withlacoochee River over 3 successive years, it was found that the treatment could be delayed for a year (1992) because the hydrilla had not returned to problematic quantities. Future work with the use of fluridone on hydrilla is being focused on two areas: the impact of such repeated treatments on (a) nontarget vegetation and (b) on hydrilla propagule (particularly tuber) populations in the substrate. Since dioecious hydrilla only produces tubers in the fall and winter, there is the potential to deplete the "tuber bank" in the substrate if annual treatments are planned to kill plants germinating in the spring and to prevent tuber formation (possibly using low doses of fluridone) in the fall/winter.

Tuber sampling in the Withlacoochee River and selected lakes is planned to examine this treatment objective. However, if such a strategy were successful in eliminating or severely repressing hydrilla, it might not be applicable for control of monoecious hydrilla, which produces tubers throughout the year (Sutton, Van, and Portier 1992).

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Field Evaluation of Triclopyr: Two-Year Posttreatment

by
Kurt D. Getsinger,[1] *E. Glenn Turner,*[2] *and John D. Madsen*[3]

Introduction

Manufactured by DowElanco, the triethylamine salt formulation of the herbicide triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) is currently registered (as Garlon 3A) for use in aquatic sites under a Federal experimental use permit. Results from previous laboratory work have demonstrated that triclopyr can provide excellent control of Eurasian watermilfoil (hereafter called milfoil) at aqueous concentrations ranging from 0.25- to 2.5-mg acid equivalent (ae)/L when that target plant is exposed for periods of from 18 to 72 hr (Netherland and Getsinger 1992). In an effort to verify results of laboratory work and to evaluate efficacy of triclopyr on nontarget plant species, U.S. Army Engineer Waterways Experiment Station (WES) researchers have been applying triclopyr to milfoil-dominated plant communities in various locations around the Nation. Results from these field studies will be used to provide guidance for the application of triclopyr to aquatic systems, once full registration of the product has been issued.

The objectives of the field studies are to (a) determine the dissipation of triclopyr from application sites, (b) correlate the dissipation of rhodamine WT (a tracer dye) with the dissipation of triclopyr, (c) evaluate the efficacy of triclopyr against milfoil, and (d) evaluate the species-selective properties of triclopyr. This report provides a 2-year posttreatment update on some aspects of a triclopyr field study conducted on the Pend Oreille River, Washington.

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 plot. These treatment sites and rates were selected based on information obtained from previous water-exchange studies on the river (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 through 1-year posttreatment and good control through 2-year posttreatment in both the river and cove plots. Biomass of milfoil and native submersed species are shown 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 1-year posttreatment, milfoil biomass had recovered to 28 percent of pretreatment levels

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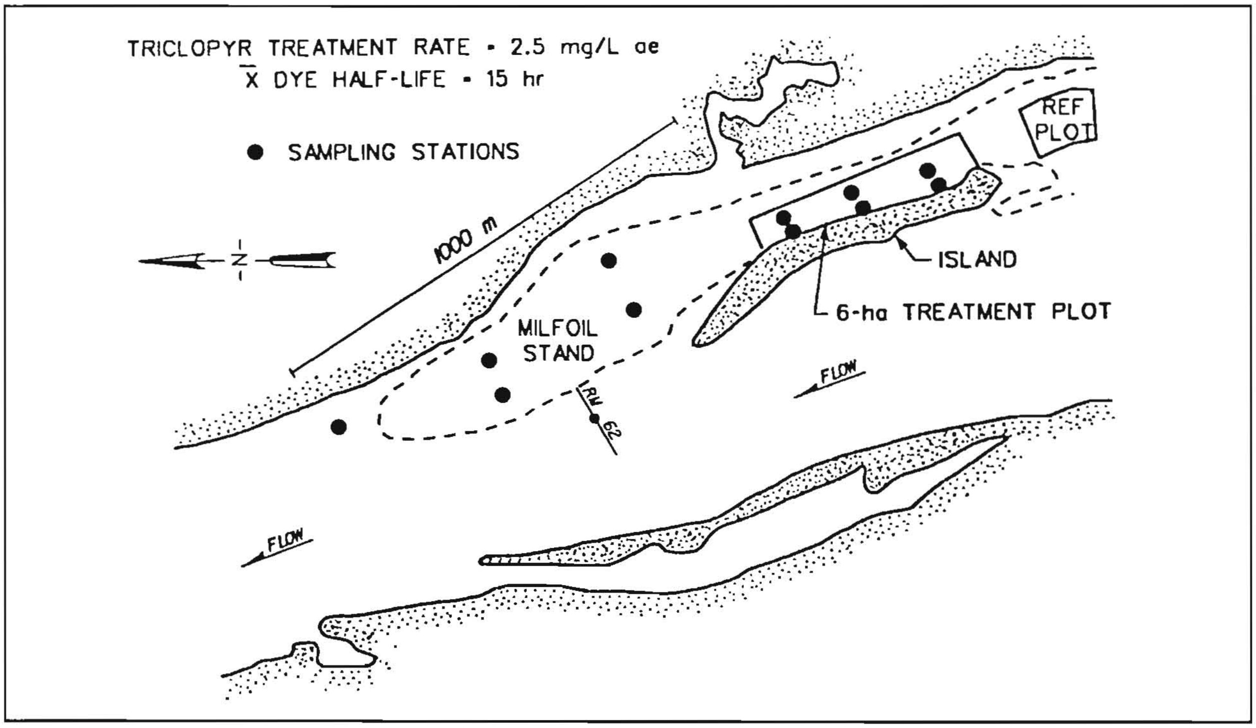

Figure 1. Pend Oreille River dye/triclopyr treatment and reference plots, August 1991

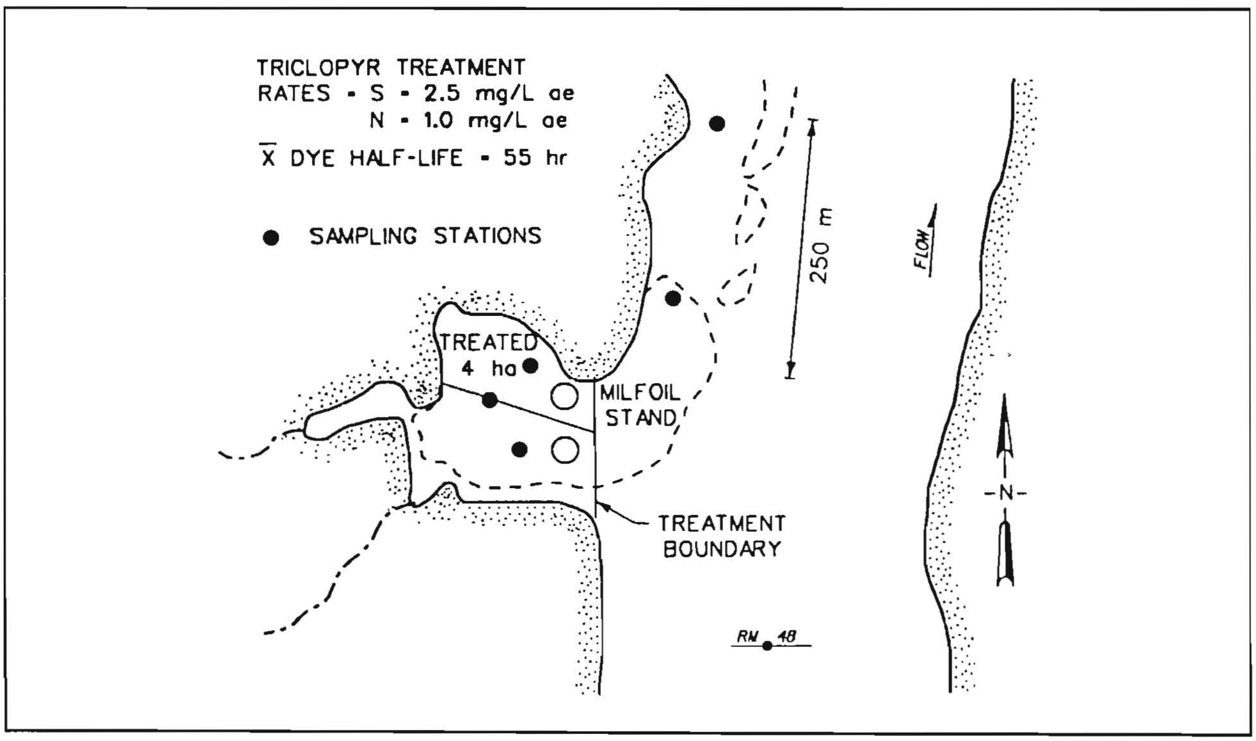

Figure 2. Lost Creek Cove dye/triclopyr treatment plot, August 1991

Table 1
Pretreatment and Posttreatment Plant Biomass Following Triclopyr Applications on the Pend Oreille River, Washington, 1991

	Mean Shoot Mass (g DW/m^2)							
	Eurasian Watermilfoil				Native Plants			
Plot	Pre	4-wk	1-yr	2-yr	Pre	4-wk	1-yr	2-yr
Reference	290	280	498	232	16	7	17	76
River	254	3	72	111	39	18	435	191
Cover	257	2	2	136	38	9	200	189

in the river plot, but was still extremely low (<1 percent of pretreatment) in the cove plot. Milfoil in the reference plot showed a 170-percent greater biomass 1-year posttreatment compared with the pretreatment biomass measurement. At 2-year posttreatment, milfoil biomass had recovered to 44 and 53 percent of pretreatment levels in the river and cove plots, respectively. Milfoil biomass in the reference plot had declined from the previous year, but was still 80 percent of the pretreatment level.

Although native plant species (e.g., elodea, pondweeds, and coontail) had declined slightly at 4-week posttreatment, they showed a 5- to 10-fold increase in biomass in the treated plots by 1-year posttreatment and still had nearly five times as much mass as the milfoil by 2-year posttreatment. This release and growth flush of native plants did not occur in the untreated reference plot, which was still highly dominated by milfoil at 2-year posttreatment.

Triclopyr water residues measured at stations downstream from the cove and river plots were below the established potable water tolerance of 0.5-mg ae/L by 8-hr posttreatment at 150 to 650 m downstream (Getsinger, Turner, and Madsen 1993). Triclopyr dissipation half-lives were 7 hr for the river plot (72-percent milfoil control at 1-year posttreatment) and 55 hr for the cove plot (99-percent milfoil control at 1-year posttreatment). This information verifies the importance of triclopyr contact time with respect to milfoil efficacy, as demonstrated in laboratory evaluations. The correlation between rhodamine WT triclopyr dissipation was good to excellent, ranging from $r^2 = 0.80$ in the river plot to $r^2 = 0.90$ in the cove plot (Turner, Getsinger, and Netherland, in preparation). This 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. WES researchers will continue to evaluate triclopyr and other promising aquatic herbicides in the field, emphasizing the use of water-exchange information to improve the 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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Response of Native Vegetation to an Application of Triclopyr

by
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Introduction

Eurasian watermilfoil (*Myriophyllum spicatum* L.), a nonnative submersed species introduced to this country during the 1940s, creates a dense surface canopy that obstructs navigation, water flow, and recreational use of infested water bodies. Dense canopies of Eurasian watermilfoil also affect aquatic environments by obstructing light, suppressing the growth of native plant species, and creating a monospecific community with correspondingly lower diversity of other aquatic organisms (Madsen, Hartleb, and Boylen 1991; Madsen et al. 1991).

Therefore, herbicides can be utilized to increase biodiversity by selectively controlling the nonnative invasive plants, releasing native plant species from suppression, and restoring the native plant community. Our study documents the use of the herbicide triclopyr (Garlon 3A) to selectively control Eurasian watermilfoil and monitor the response of the native plant community from before treatment to 2 years after treatment.

Materials and Methods

Study site

The Pend Oreille River, located in northeastern Washington State, has had Eurasian watermilfoil since the early 1980s. We selected a site with low water velocities (river) and a site with essentially static water conditions (cove) for treatments. The river site had both a treatment and untreated reference plot; the cove site had no untreated reference plot. More information on the study site and treatment is given in the article by Getsinger, Turner, and Madsen (1994) and in previous reports (Getsinger, Turner, and Madsen 1992a, b; 1993).

Methods

At each plot, four transects were established to quantify vegetation. At each transect, three biomass samples were collected by a SCUBA diver from stratified-random locations using a 0.1-m^2 quadrat, for a total of 12 biomass samples per plot. Samples were sorted to species, separated into roots and shoots, and dried at 50 °C. Biomass samples were collected before treatment and 4 weeks, 1 year, and 2 years after treatment. Transects were also used to quantify the distribution and diversity of aquatic plants. Each 100-m transect was divided into 1-m intervals, and the species present under each 1-m interval were recorded by a diver. The transects were examined before treatment and 1 and 2 years after treatment.

Results and Discussion

Species present

A total of 17 submersed plant species were encountered during the 3-year study: 2 were nonnative (exotic) species; 15 were native species; 12 were monocots; and 5 were dicots (Table 1). Summarizing frequency of occurrence from all intervals on the transects,

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Table 1
Plant Species Observed Along Transects During the 3-Year Pend Oreille River Study

Species	M or D	N or E	Frequency %
Ceratophyllum demersum	D	N	23
Elodea canadensis	M	N	36
Heteranthera dubia	M	N	5
Myriophyllum sibiricum	D	N	1
Myriophyllum spicatum	D	E	71
Myriophyllum verticillatum	D	N	1
Potamogeton crispus	M	E	22
Potamogeton illinoensi	M	N	0
Potamogeton nodosus	M	N	2
Potamogeton obtusifolius	M	N	5
Potamogeton pectinatus	M	N	6
Potamogeton perfoliatus	M	N	2
Potamogeton praelongus	M	N	1
Potamogeton pusillus	M	N	3
Potamogeton vaseyii	M	N	3
Potamogeton zosteriformis	M	N	35
Ranunculus longirostris	D	N	16

Note: Monocot or dicot (M or D), native or exotic (N or E), and percent frequency summarized for all transects is indicated.

the most common species was Eurasian watermilfoil (71 percent), followed by the native species *Elodea canadensis* (36 percent) and *Potamogeton zosteriformis* (35 percent).

Biomass

Eurasian watermilfoil biomass in the river reference plot maintained relatively constant levels with the exception of higher biomass during the first year after treatment (Figure 1). In contrast, Eurasian watermilfoil biomass was considerably reduced in both the river and cove treatment plots up to 2-year posttreatment. Biomass at 4-week posttreatment was 1 percent of pretreatment levels at both treatment sites. One-year posttreatment, Eurasian watermilfoil biomass at the river treatment plot was 28 percent of pretreatment and 1 percent of pretreatment at the cove plot. Eurasian watermilfoil biomass was still significantly lower than pretreatment levels at both treatment plots 2 years after treatment.

Native plant biomass levels responded to the removal of Eurasian watermilfoil. At the reference plot, native plant biomass remained mostly unchanged, with a slight increase 2-year posttreatment. Native plant biomass

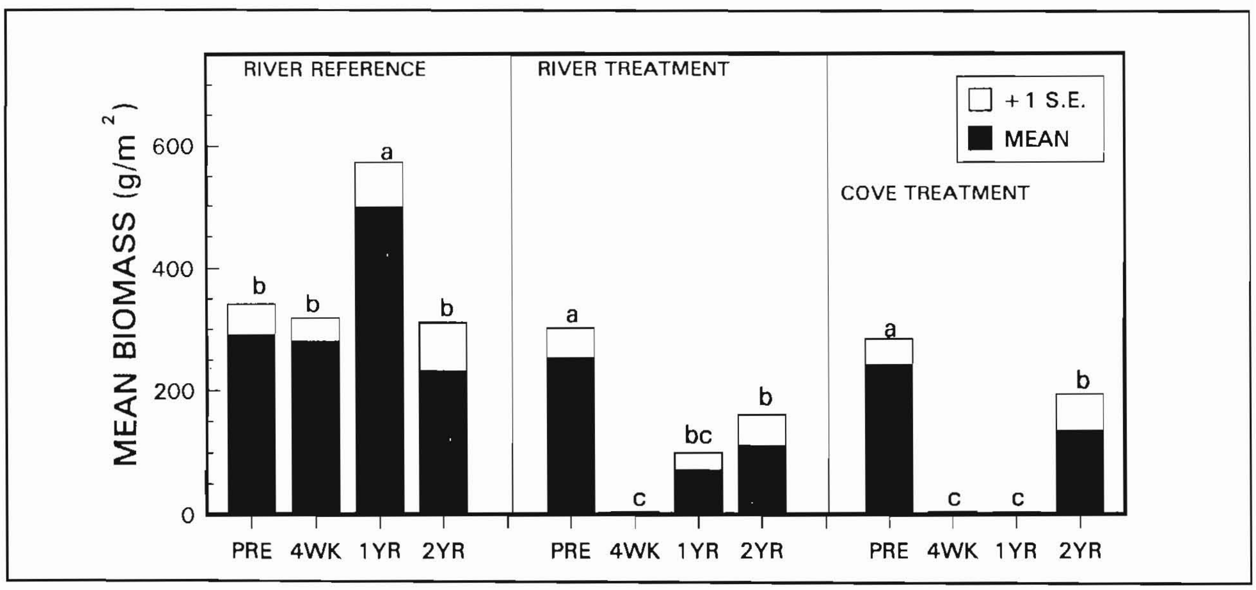

Figure 1. Eurasian watermilfoil biomass (g m^{-2} dry weight) at three study plots in the Pend Oreille River (Letters indicate significant difference at the p = 0.05 level using analysis of variance least significant difference(ANOVA LSD))

remained low 4 weeks after treatment, in part because of the lateness of the season, but increased dramatically at both treatment plots 1-year posttreatment (Figure 2). Native plant biomass remained higher at the cove plot 2-year posttreatment, but was not significantly higher at the river treatment plot at this time. Removal of Eurasian watermilfoil biomass resulted in significantly higher abundance of native plants up to 2 years after treatment with triclopyr.

Species diversity (transect data)

The diversity measure used in this study was average number of species per transect interval or average species richness. If all species are included, all three plots were at approximately two species per interval before treatment (Figure 3, left). Species richness remained low in the river reference plot 1-year posttreatment, but increased to over 2.5 at 2-year posttreatment because of the increased distribution of *P. crispus*. Average species richness increased to over three species per interval in both treatment plots 2-year posttreatment. If only native species are considered, all three plots were at approximately one native species per interval before treatment, and the river reference plot remained near this level throughout the study (Figure 3, right). Species richness of native species increased to over two species per interval, more than doubling the diversity of native species in both treatment plots. The higher diversity continued to be observed in both treatment plots 2-year posttreatment.

Conclusion

Herbicides can be used to selectively remove target species. In the case of nonnative species, which reduce the diversity of native species such as Eurasian watermilfoil, herbicides can be utilized to restore the native plant community and increase community diversity. When the invasion of exotic species has been recent, such as the case with Eurasian watermilfoil into the Pend Oreille River, sufficient propagules of the native species remain in either the seed bank or nearby areas to recolonize the community without human intervention. In the case of communities with longer invasion histories, seed banks and refugias of native plants may not be available; native species might have to be revegetated to restore the native plant community.

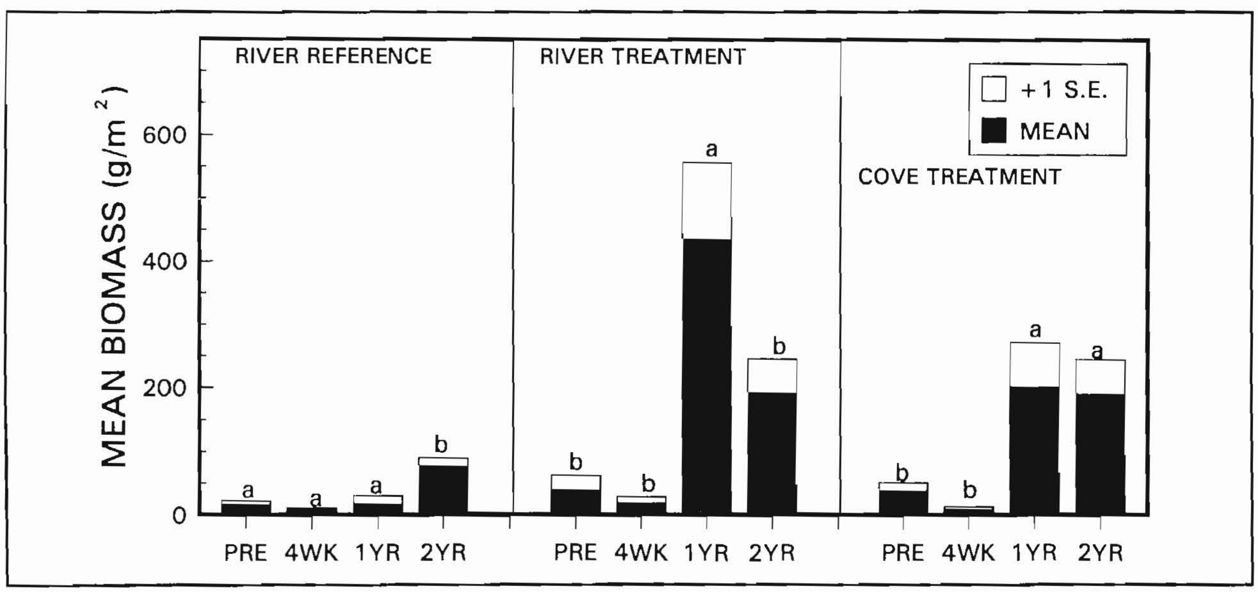

Figure 2. Native submersed plant biomass (g m^{-2} dry weight) at three study plots in the Pend Oreille River (Letters indicate significant difference at the p = 0.05 level using ANOVA LSD)

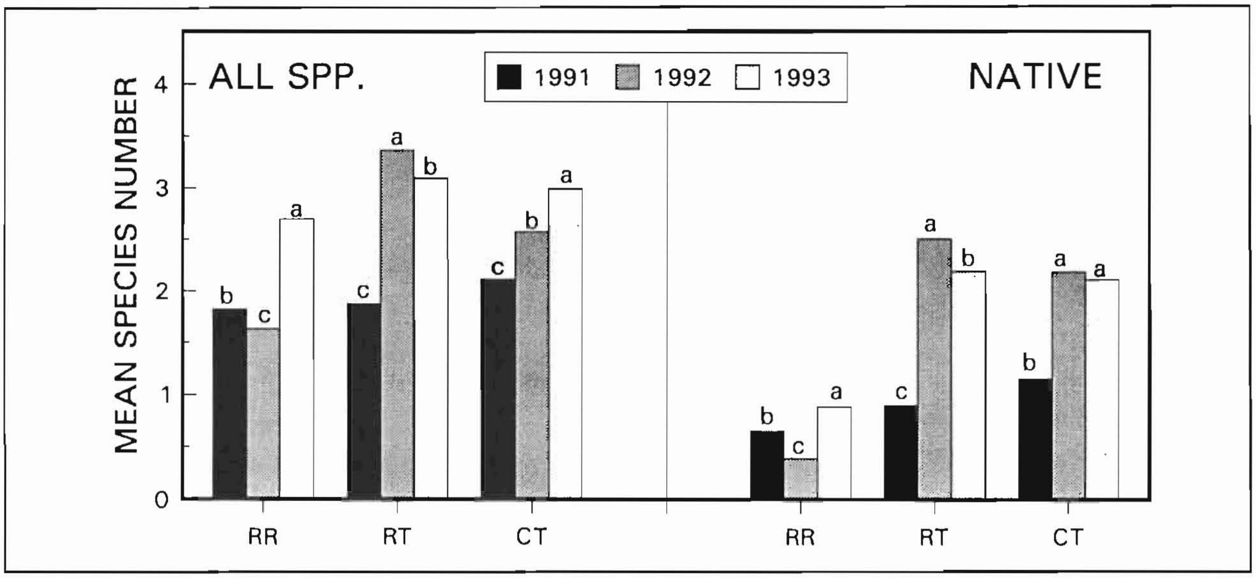

Figure 3. Average number of species per transect interval at three study plots in the Pend Oreille River over 3 study years (Left, all species; right, native species only. RR, river reference; RT, river treatment; CT, cove treatment. Letters indicate significant difference at the p = 0 .05 level using ANOVA LSD)

Acknowledgments

Logistical support was provided by Washington State Department of Ecology, U.S. Army Engineer District, Seattle, and Corps of Engineers Albeni Falls Project, Priest River, ID. SCUBA dive team included Joel Everett, Mike Netherland, and Dr. R. Michael Smart. Technical assistance was provided by Linda Nelson and Dr. Susan Sprecher.

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Species-Selective Use of Aquatic Herbicides and Plant Growth Regulators

by

Kurt D. Getsinger[1] and R. Michael Smart[2]

Introduction

Weedy species such as hydrilla and Eurasian watermilfoil spread throughout large water bodies often displacing desirable native plants such as *vallisneria*, pondweeds, and elodea. While weedy species can be removed using traditional chemical control tactics, these treatments can impact native species as well. If the impact to nontarget vegetation is severe, the weedy species can quickly reinvade and dominate the plant community.

Using herbicides and plant growth regulators (PGRs) in a species-selective manner can result in the control of target exotic vegetation while enhancing the growth of desirable native plants. Allowing the beneficial species to grow and flourish can provide a diverse aquatic plant community that may slow the reinvasion of weedy species and provide improved fish and wildlife habitat. In this way, water bodies plagued with monoculture infestations of exotic plants can be restored to healthy ecosystems.

Objective and Approach

The objective of this new work unit is to develop and evaluate species-selective aquatic plant management practices using herbicides and PGRs. Mesocosm studies will be conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Details of the 30-tank mesocosm system are presented in Dick, Getsinger, and Smart (1993). Mixed plant communities (comprised of exotic weeds such as hydrilla and Eurasian watermilfoil and desirable native species) will be established in the LAERF mesocosm system and treated with selected aquatic herbicides and/or PGRs. These studies will focus on the rates and timing of application with respect to the phenology of target and nontarget plants and will determine the species-selective response to the herbicides/PGRs. Chemicals and application rates used in the mesocosm selectivity evaluations will be chosen based on results from laboratory concentration/exposure time studies conducted against target and nontarget plants. Results from mesocosm studies will be subsequently verified in ponds at the LAERF or at selected field locations.

Dye Validation of Mesocosm Flow-Through Mode

One design feature of the LAERF mesocosm system is the ability to operate the tanks in a continuous flow-through mode. This capability allows researchers to simulate field dissipation of herbicides from a treated plot and to determine the impact of that water-exchange dilution on herbicide efficacy. Fifteen mesocosm tanks were used to validate design water-exchange half-lives of 6, 12, and 24 hr. Tanks were filled with reservoir water (1,700 L) and treated with rhodamine WT dye to achieve an aqueous concentration of 20 μg/L. Once complete mixing of dye-treated water was confirmed (approximately 15 min), flowmeters were set to deliver selected water-exchange rates. Dye concentrations were measured using a Turner Designs fluorometer for up to 7-day posttreatment. Actual water-exchange half-lives differed only slightly from the

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design target half-lives (Table 1), demonstrating that simulated field water-exchange rates can be achieved in the mesocosm system.

Table 1
Dye Validation of Mesocosm System in Flow-Through Mode

Design Half-life, hr	Actual Half-life, hr	r^2	Rep
6	5.6	98.9	3
12	13.1	97.3	6
24	22.6	99.5	6

Future Work

Future work in the mesocosm system includes triclopyr selectivity studies focusing on northern and midlatitude Eurasian watermilfoil-dominated plant communities. In addition, fluridone selectivity studies will be conducted in the system targeting hydrilla, Eurasian watermilfoil, and appropriate native plant species.

Acknowledgments

Technical assistance for the dye validation studies were provided by Gary Dick, Mike Crouch, Mike Netherland, and Glenn Turner.

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Monitoring Herbicide Stress in Submersed Plants

by
Susan L. Sprecher[1]

Introduction

The ability to quantify herbicide injury or effect on aquatic plants more precisely than by visual inspection alone is desirable in chemical control research and field operations. Low levels of certain herbicides may affect target populations without producing immediate visible changes, while in selective treatments, physiological evaluation provides a method to researchers and applicators for quantifying any short-term stress on nontarget plants, verifying their escape from permanent injury, or documenting off-target herbicide movement. During the past 2 years the Chemical Control Technology Team (CCTT) at the U.S. Army Engineer Waterways Experiment Station (WES) has investigated physiological characters that give some indication of herbicide effect in aquatic plants. Changes in peroxidase enzyme activity (PRX) were found to be associated with certain herbicide treatments and to differentiate between affected and unaffected species in some cases. For example, in Eurasian watermilfoil (*Myriophyllum spicatum* L.: milfoil), triclopyr treatment produced an increase in PRX not seen in control material (Figure 1). An elevation in PRX was seen in hydrilla treated with labeled rates of the dipotassium salt of endothall, whereas egeria (*E. densa* Planch), which is uncontrolled by this formulation, did not increase PRX activity (Figure 2).

However, PRX changes are associated with general metabolic stress in plants, rather than direct response to herbicides. The desirability of identifying specific responses led the CCTT in 1993 to invite a group of interested researchers to review methods for evaluating aquatic herbicide effects. A technical meeting on "Physiological Assessment of

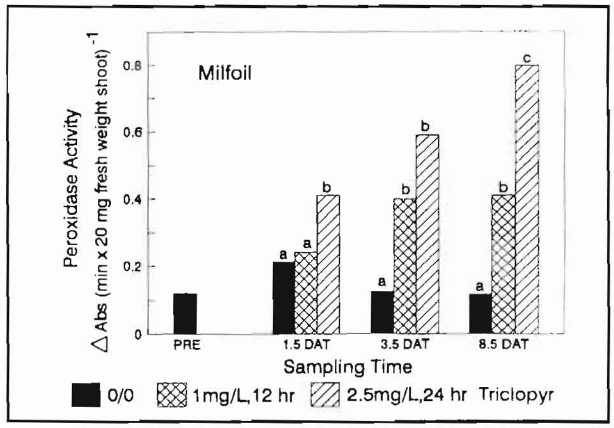

Figure 1. Effect of triclopyr on PRX activity in milfoil treated with 1 mg/L for 12 hr, 2.5 mg/L for 24 hr, and an untreated control, measured at pretreatment and 1.5, 3.5, and 8.5 days after treatment (Bars represent the means of three replicates; letters indicate significant differences between treatments on the same date,as measured by a t-test, p ≤ 0.05)

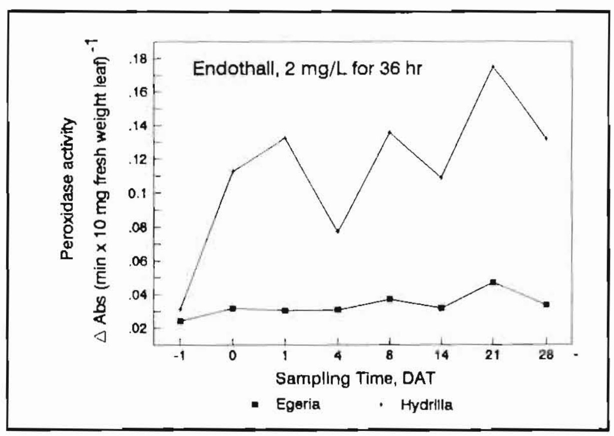

Figure 2. Effect of the dipotassium salt formulation of endothall on PRX activity in egeria and hydrilla treated with 2 mg/L for 36 hr, measured at pretreatment, immediately after treatment, and through 28-day posttreatment (Graph points represent mean deviation of three replications)

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

Herbicide Stress in Aquatic Plants" was held in June and convened weed physiologists and aquatic plant researchers from various universities, the U.S. Geological Survey, the U.S. Department of Agriculture Plant Laboratories in Fort Lauderdale, Davis, and Stoneville, and WES and the WES Lewisville Aquatic Ecosystems Research Facility.

Suitable stress assessment methods were discussed, and suggestions for their use in monitoring herbicide effect were outlined. It was pointed out that the optimal strategy in assessing change in vegetation status is to measure growth along with other metabolic processes and to use multiple regression analysis of data to derive correlations among them, with the goal of identifying the most efficiently predictive subset of characters. Any assessment technique becomes more informative as it is correlated to change in other significant parameters. The physiological parameters suggested for measuring treatment-induced changes were categorized as (a) those that monitor general metabolic stress in plants, and (b) those that are linked to the mode of action of the herbicide in question (Table 1). Both types may be used to assess physiological change following herbicide treatment. Guidelines for choosing assessment methods and suggested measurement parameters for aquatic herbicides are summarized below.

- Reduction in growth or change in growth habit is the most basic and easily measured effect of herbicide treatment and is the most important parameter with which to correlate physiological changes. Comparisons may be made of biomass, stem length, enumeration of apical shoots or branches, total nonstructural carbohydrates, or other growth parameters affected by the herbicide. With fluridone treatment, for example, production of achlorophyllous (bleached) apices depletes carbohydrate reserves until death occurs. Here, correlations between growth measures (number of new shoots, change in photosynthetic reserves, etc.) and metabolites such as oxygen consumption have the potential to indicate at what point the plant cannot recover following dissipation of this slow-acting compound.

- Since an increase from an initial zero or negligible level of a compound is easier to measure than a small change in a preexisting substance, a metabolite that is induced or accumulates to high levels because of herbicide treatment is a useful indicator of effect. In the case of the growth regulator-type herbicides triclopyr and 2,4-D, a 100-percent increase in ethylene and ethane production occurs soon after treatment. The magnitude of this parameter is in contrast to the decrease of approximately 20 percent in chlorophyll concentration seen later, and makes it preferable for monitoring impact from these herbicides.

- Physiological and developmental changes in young tissue can obscure definitive measurements of some processes, and it is advisable to include assays of mature portions of the plant. Although developing tissue may be more immediately and directly affected by a compound, such changes may not reflect significant damage. The example given was of fluridone effect, where emerging tissue is bleached because of lack of carotenoid formation, but older tissue remains viable and may support recovery once the herbicide is removed. Assays of other parameters that are affected by this herbicide (oxygen consumption/evolution, phytoene, and carbohydrate content) are preferably done on mature, chlorophyllous tissue.

- Whenever possible, use "canned" or prepackaged assays. They provide convenience, standardization, and repeatability, particularly in the field. In assessment of triclopyr and 2,4-D effect, where protein concentration is expected to increase, commercially available assays for total proteins using the Bradford method (Bio-Rad; Bradford 1976) provide standards and dye reagent with an easily-used protocol.

- A parameter that changes because of herbicidal disruption of a metabolic pathway may provide a diagnostic or quantitative assessment of treatment effect. Dose-related responses based on accumulation or expenditure of a specific compound avoid physiological variations caused by environmental change. In fluridone-treated plants, phytoene accumulation is correlated to dose, and this effect is diagnostic for exposure. With the copper herbicides and the triazines, which directly inhibit the functioning of Photosystem II, the rapid increase in chlorophyll *a* fluorescence because of loss of electron transport function is a diagnostic character (Miles 1990).

Table 1
Physiological Parameters for Assessing Herbicide Treatment-Induced Changes in Aquatic Vegetation

Indicators of General Metabolic Stress	Indicators Related to Mode of Action of Herbicide	
Biomass/Growth	*a*-ketobutyrate derivates	Bensulfuron methyl
Carbon exchange	Branch chain amino acids	Bensulfuron methyl
Chlorophyll content	Carotenoids	Fluridone
Chlorophyll *a* fluorescence	Chlorophyll content	Fluridone
Conductivity/Cell leakage	Chlorophyll *a* fluorescence	Triazines Glyphosate
Oxygen evolution/uptake	Cytochrome f	Fluridone
Protein/Enzyme levels	Ethylene	Triclopyr
	Nucleic acids	Bensulfuron methyl Triclopyr
	Phenylaline ammonium lyase	Glyphosate
	pH effects	Triclopyr
	Phytoene	Fluridone
	Protein/Enzyme levels	Triclopyr
	Shikimate/Benzoate	Glyphosate

- However, the best rapid assay for treatment effect may not be the one that measures a parameter directly affected by the herbicide's mode of action. With glyphosate, shikimate acid and benzoate levels increase as their metabolism is affected by the compound's action. However, significant change in chlorophyll *a* fluorescence occurs rapidly after treatment becaue of an indirect effect.[1] Fluorescence is easily measured, in contrast to shikimate response, and is therefore a parameter of choice in monitoring effects of this herbicide.

Assessing Selective Herbicide Effects

Herbicide effects on growth and PRX were compared in target and nontarget species in a test carried out at WES during 1993.

Materials and Methods

Triclopyr treatments of 1 mg/L for 12 hr, 2.5 mg/L for 24 hr, and an untreated control were applied in three replications to rooted plants of the monocots elodea (*Elodea canadensis*), sago pondweed (*Potamogeton pectinatus*), and wild celery (*Vallisneria americana*), and to the target dicot milfoil (*Myriophyllum spicatum*) established from apical tips or tubers and growing separately in 55-L aquaria in a controlled-environment chamber under conditions previously described (Netherland, Green, and Getsinger 1991).

[1] Personal Communicaion, 1993, S. Duke, Director, U.S. Department of Agriculture Southern Weed Science Laboratory, Stoneville, MS.

PRX was measured before treatment in all species and in milfoil at 1.5, 3.5 , 8.5, and 35 days after treatment (DAT). In elodea, sago pondweed, and wild celery, measurements were taken at 1.5, 8.5, and 35 DAT. Three 0.5-g fresh weight samples of shoot (milfoil, elodea, and sago pondweed) or leaf (wild celery) tissue were taken from each aquarium, macerated in 5-ml 0.1 M Na_2PO_4 buffer, pH 6.1, and centrifuged. Two hundred-microliter aliquots of the supernatant were reacted with 4mM guaiacol. The reaction was monitored spectrophotometrically at 470 nm, and activity was reported as change in absorbance per minute per 20 mg fresh weight (Sprecher, Stewart, and Brazil 1993). Replication means were used in analysis of variance and t-tests to separate treatment effects. Total dry weight measurements were made pretreatment and 35 DAT.

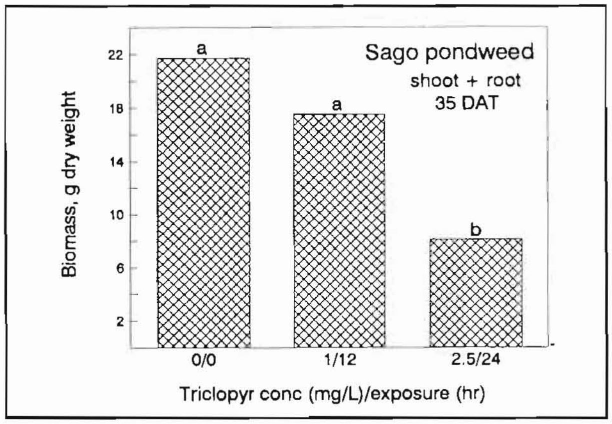

Figure 3. Effect of triclopyr on dry weight biomass of sago pondweed treated with 1 mg/L for 12 hr and 2.5 mg/L for 24 hr, at 35 DAT (Bars represent the means of three replicates; letters indicate significant differences between treatments as measured by a t-test, $p \leq 0.05$)

Results

Various treatment effects were seen on biomass of target and nontarget species before final harvest at 35 DAT. Milfoil tissue was completely destroyed in treated tanks by 10 DAT, and no regrowth occurred at either treatment level by 35 DAT. No decline or loss of condition was seen in elodea or wild celery plants following treatment, and differences in biomass found among treatments within these species at 35 DAT were not significant (data not shown). Sago pondweed treated at 1 mg/L for 12 hr was unaffected by the herbicide; but by 20 DAT, plants treated at 2.5 mg/L for 24 hr were visibly reduced in vigor, with less chlorophyll, some necrotic tissue, and reduced growth. Although these plants were still living at final harvest, this treatment had significantly less biomass at 35 DAT than the others (Figure 3).

PRX response reflected the selective effect of the herbicide. In milfoil, as mentioned above, PRX had increased above pretreatment and control in the higher treatment by 1.5 DAT and was elevated in both treatments by 3.5 DAT; by 8.5 DAT, the two treatments were significantly different from each other and the control (Figure 1). However, in untreated plants, PRX did not vary significantly from pretreatment levels (mean = 0.1532 ± 0.022 s.e.).

In elodea and wild celery, triclopyr treatment did not significantly alter PRX levels from those of untreated controls at 1.5, 8.5, and 35 DAT (data not shown). In sago pondweed, PRX was unaffected through 8.5 DAT. However, by 35 DAT, activity in plants treated with 2.5 mg/L for 24 hr had declined (significant at $p \leq 0.06$) (Figure 4).

Discussion

Change in PRX was correlated with triclopyr dose in the target dicot milfoil and with the lack of herbicidal effect seen in the nontarget monocot species elodea and wild celery. In sago pondweed, PRX was unaffected in the short term, and in the long term at the lower treatment level, mirroring the visible condition of the plants. This suggests that PRX can be used as a physiological monitor for lack of triclopyr-induced stress on the nontarget species tested here and may be applicable to other nontarget plants also.

Since sago pondweed was not adversely affected at a triclopyr rate able to control milfoil

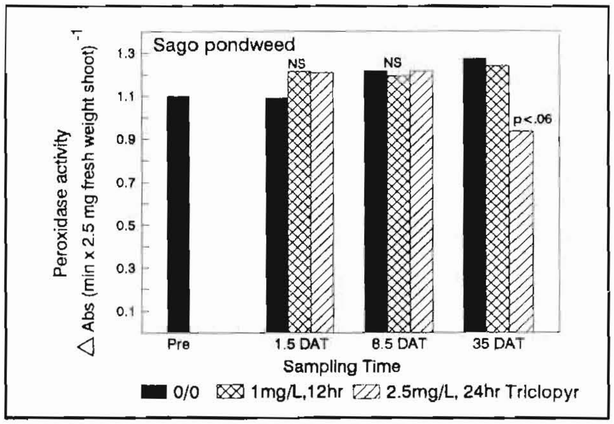

Figure 4. Effect of triclopyr on PRX activity in sago pondweed treated with 1 mg/L for 12 hr, 2.5 mg/L for 24 hr, and an untreated control, measured at pretreatment and 1.5, 8.5, and 35 DAT (Bars represent the means of three replicates, and lack of significance among treatments sampled at the same date and tested with a t-test, $p \leq 0.05$ are marked NS except where noted)

(1 mg/L for 12 hr), it is expected that this treatment level can be used selectively where sago is desirable. The PRX decrease noted in the higher treatment at 35 DAT may be a reaction to long-term metabolic stress, ie., qualitatively different from the initial null reaction to herbicide treatment. Here, measurement of other parameters associated with triclopyr effect may have indicated how deterioration occurred.

Parameters suggested by researchers at the WES technical meeting as being most diagnostic for the growth regulator herbicides are listed in Table 2. Change in total protein, oxygen consumption, conductivity, and pH effects can be measured with current resources in the CCTT laboratory, and these are expected to be part of triclopyr evaluations in the future.

Future Work

We plan to evaluate suitable assessment methods and incorporate them into laboratory, mesocosm, and field tests of selective herbicide effect, allowing improved monitoring of effect of herbicides on target and nontarget plants in aquatic ecosystems.

Table 2
Expected Physiological Changes in Aquatic Vegetation Treated with the Growth Regulator-Type Herbicides 2,4-D and Triclopyr

Ethylene	Early, dose-related increase; sensitive to treatment
Nucleic acids and proteins	Increase within approximately 24 hr, unique response to this type of herbicide
Oxygen consumption	Increases initially
Conductivity	Increases on both tolerant and target species
pH effects	Caused by proton and conductivity changes
Tissue burden	

Acknowledgments

The author would like to thank Anne Stewart, Jane Brazil, and Kimberly Deevers for technical assistance. The cooperation of DowElanco in providing experimental use permit herbicide for this study is appreciated.

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Mesocosm Evaluation of Flurprimidol on Four Submersed Species

by
Linda S. Nelson[1]

Introduction

In addition to herbicides, the Chemical Control Technology Team evaluates other chemical means with which to manage nuisance aquatic plants. Plant growth regulators (PGRs) are synthetically produced compounds (as are herbicides) that act to temporarily control or alter some aspect of growth and development, such as suppressing plant height. PGRs may offer a new approach to aquatic plant management, allowing vegetation to remain as part of the aquatic ecosystem by limiting growth to nonnuisance levels. In most aquatic systems, maintaining some degree of vegetative cover is important for habitat structure, sediment stabilization, oxygen production, and community diversity. Herbicide treatments often eliminate vegetation in the area of treatment, and therefore the benefits of plant cover are not realized.

The overall objective of Work Unit 32578, Plant Growth Regulators for Aquatic Plant Management, is to evaluate the effects of PGRs on the growth and reproduction of aquatic plant species and to determine the utility of these compounds as an alternate management strategy. Of the 10 or more growth regulating compounds assayed for use on aquatic plants, flurprimidol is one of the most effective. Flurprimidol is a gibberellin synthesis inhibitor currently registered for turf and horticultural applications and is effective on nuisance aquatic plants such as hydrilla (*Hydrilla verticillata* Royle) and Eurasian watermilfoil (*Myriophyllum spicatum* L., hereafter called milfoil) (Netherland and Lembi 1992; Lembi and Chand 1992; Nelson 1993). Flurprimidol also shows selectivity among aquatic plants species. Studies have documented that desirable, nontarget species such as wild celery (*Vallisneria americana* Michx.) and coontail (*Ceratophyllum demersum* L.) were not affected by flurprimidol at rates sufficient to inhibit hydrilla (Lembi and Chand-Goyal 1994; Nelson 1993). Thus the potential exists to regulate the growth of noxious species without affecting native species.

The objectives of this study were (a) to further identify the effects of flurprimidol on nonnative and native aquatic plant species grown under field-like conditions and (b)to compare the effectiveness of single versus split applications of flurprimidol.

Materials and Methods

This experiment was conducted in a mesocosm system at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX. The mesocosm system consists of large, outdoor tanks that measure 1.4 m tall by 2.6 m in diam and hold approximately 6,500 L of water. For this study, each tank was divided into four equal areas with netting that allowed water flow between the divided areas, but restricted plant growth to each tank quadrant. Each tank was individually plumbed to regulate water flow as needed and was equipped with air flow for water circulation. A holding pond adjacent to the mesocosm system provided a water source for the tanks. Further description of the mesocosm system can be found in Dick, Getsinger, and Smart (1993).

Ten 1-gal plastic pots filled with nutrient-enriched soil were planted with one of the

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

four test species (four plants per pot) and placed in each tank quadrant. Test species included milfoil, hydrilla, elodea (*Elodea canadensis* Rich.), and American pondweed (*Potamogeton nodosus* P., hereafter called pondweed). Milfoil, hydrilla, and elodea were propagated from 10-cm apical shoot tips and planted 4 to 5 cm into the soil. Milfoil and elodea were collected from culture ponds at the LAERF; hydrilla was supplied by Suwannee Laboratories, Lake City, FL. Pondweed was initiated from tubers collected at the LAERF. All plants were allowed to establish in the mesocosm tanks for 2 weeks prior to flurprimidol treatment. Established plants were treated on 6 May 1993 with static exposures of 100, 100 X 2 (split treatment with a second dose applied on 17 June, 6 weeks after the initial dose), and 200 µg/L flurprimidol. Results of residue analyses from the 1992 flurprimidol-mesocosm study showed that under similar experimental conditions, flurprimidol had a half-life in water of approximately 7 to 10 days (unpublished data). Therefore, it was estimated for this study that most (95 percent) of the initial dose of 100 µg/L flurprimidol would have dissipated by the time the second dose was applied. Prior to the initial treatment, one pot of each plant species was removed from each mesocosm tank, and pretreatment shoot length and total plant (shoot and root) biomass were measured.

Following treatment, visual ratings of plant appearance and vigor were recorded on a weekly basis. Six weeks after treatment (WAT), four pots of each plant species were removed from each mesocosm tank and measured for shoot length and shoot and root biomass. Shoot length 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 g dry weight per pot. At the conclusion of the experiment, 12 WAT, the remaining pots were removed from the tanks and were subjected to the same measuring and harvesting procedures as above. Water samples were collected periodically throughout the study for flurprimidol residue analysis (data not presented).

Treatments were randomly assigned to mesocosm tanks with three 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 and Discussion

All species showed reduced shoot lengths 6 WAT (Figure 1). Hydrilla was most sensitive to flurprimidol with all treatment rates showing the same response. Compared with untreated hydrilla, shoot lengths of treated plants were 73 percent shorter. Milfoil and elodea also showed no significant differences between flurprimidol treatments. Compared with untreated plants, reductions in shoot length averaged 51 percent for milfoil and 39 percent for elodea. Only pondweed showed a treatment rate response. Flurprimidol at 100 µg/L (both 100 and 100 X 2 treatments are the same at 6 WAT) reduced shoot lengths by 54 percent, whereas doubling the treatment rate (200 µg/L) decreased lengths by 73 percent.

Plant biomass was also measured at 6 WAT. Hydrilla, pondweed, and milfoil produced approximately 50 percent less shoot biomass when treated with flurprimidol (Figure 1). There were no differences between treatment rates. Elodea shoot biomass was not affected by flurprimidol. It was observed during the harvest that elodea growth varied greatly among pots within the same mesocosm tank, some pots having no plant material at all. Sparse growth was attributed to poor plant establishment and contributed to the fact that statistical differences in biomass were not observed. Milfoil plants also did not establish well. Although a treatment response was noted at this time, milfoil shoot biomass was low, even in untreated tanks. Milfoil was also difficult to establish in previous mesocosm studies (Nelson 1993). It is likely that poor establishment of milfoil and elodea was due to high water temperatures (35 °C) and high light conditions (low water turbidity) that are

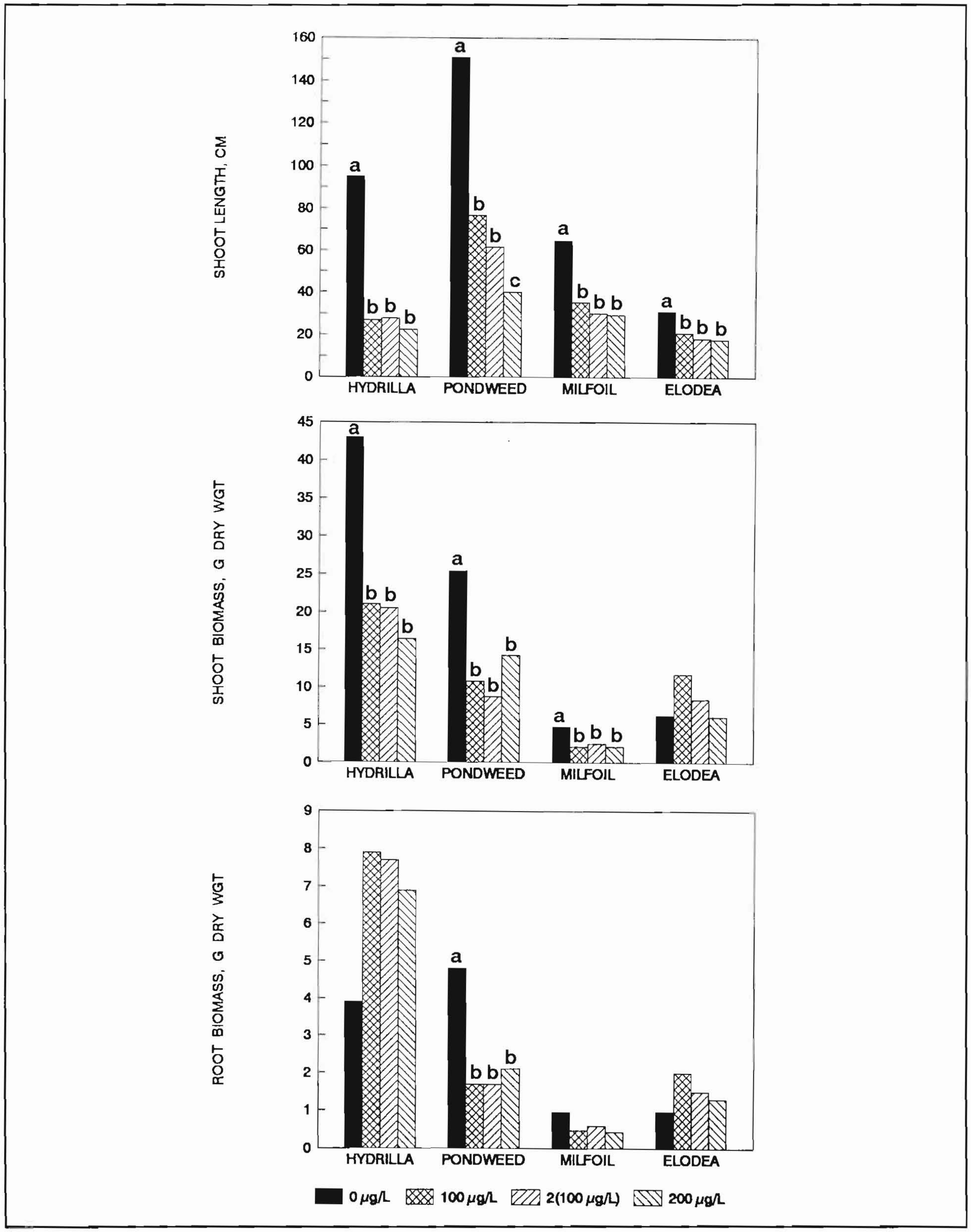

Figure 1. Effect of flurprimidol on shoot length, shoot biomass, and root biomass of hydrilla, pondweed, milfoil, and elodea 6 weeks after treatment. Means (for each species) with the same letter are not significantly different at the P = 0.05 level. Average pretreatment shoot length (cm) for each species was hydrilla = 17.5; pondweed = 17.5; milfoil = 20.2; elodea = 15.8

encountered in the mesocosm tanks. Because of poor growth and continued deterioration of remaining biomass, no further data were collected on these two species after 6 WAT.

Effects on root biomass were observed only with pondweed (Figure 1). All flurprimidol treatments showed the same response, with root biomass reductions averaging 63 percent of that of untreated plants. Root biomass data for the other plant species were extremely variable, again, probably because of inconsistent plant establishment.

Following the 6-week harvest, the second dose of 100 µg/L flurprimidol (100 X 2 µg/L) was applied. Data collected from the final harvest (12 WAT) showed that applying a second dose of flurprimidol resulted in the greatest growth regulator response. Compared with untreated plants, hydrilla and pondweed subjected to a second flurprimidol application had shoot lengths that measured 69 and 34 percent shorter, respectively (Figure 2). Plants exposed to a one-time application of 100 and 200 µg/L flurprimidol also exhibited reduced shoot lengths; however, statistically, treatments were not different. This suggests that a lower rate of flurprimidol (100 µg/L) is adequate to inhibit plant growth, but that a longer exposure time, as experienced with the 2(100 µg/L) treatment, will prolong effects on shoot length.

Although a second dose of flurprimidol enhanced inhibitory effects on hydrilla shoot length, it did not affect final shoot biomass. Hydrilla shoot biomass was the same (50 percent) for all flurprimidol treatments at 12 WAT (Figure 2). In contrast, a differential response among flurprimidol treatments was noted with pondweed. Compared with untreated plants, shoot biomass was 86 percent less with the 2(100 µg/L) treatment and averaged 60 percent less with the 100- and 200-µg/L flurprimidol treatments.

Morphological differences between treated and untreated plants were visually noted throughout the study. Hydrilla treated with flurprimidol was green and healthy looking and was extremely dense with an extensive proliferation of stolons. This vertical or stoloniferous growth habit has been observed by other researchers (Netherland and Lembi 1992; Lembi and Chand 1992; Nelson 1993). Visual differences in plant appearance were also noted for pondweed. Although shoot biomass was not separated, it appeared that treated pondweed had more submersed leaf tissue and less surface or floating leaf tissue than untreated plants. Estimates of percent canopy cover showed that untreated pondweed developed a canopy of floating leaves that covered 90 to 95 percent of the water surface by the end of the study, but plants treated with two applications of 100 µg/L flurprimidol had very few floating leaves, resulting in a canopy cover of only 5 percent. Pondweed treated with 100 and 200 µg/L flurprimidol had surface canopies of 80 and 70 percent, respectively. Lembi and Chand-Goyal (1994) also reported that *Potamogeton nodosus* treated with flurprimidol (200 µg/L) had more submersed leaves than untreated plants.

By the end of the study, pondweed still showed reduced root growth as a result of flurprimidol treatment (Figure 2). Inhibitory effects observed at 6 WAT had dissipated with the 100 and 200 µg/L flurprimidol-treated plants, but persisted on plants treated with a second dose of 100 µg/L flurprimidol. In fact, there was little change in root biomass from 6 to 12 WAT with plants subjected to second flurprimidol application. Hydrilla showed no differences in root biomass at 12 WAT.

In summary, results of this study are in agreement with those of other researchers in that growth of hydrilla and pondweed can be regulated with flurprimidol treatment (Lembi and Chand-Goyal 1994; Netherland and Lembi 1992). Better growing conditions are needed in the mesocosm system to determine the extent of growth regulation on elodea and milfoil; however, other studies have shown that both of these species are sensitive to flurprimidol as well (Lembi and Chand-Goyal 1994; Lembi and Chand 1992; Netherland and Lembi 1992). For future mesocosm studies,

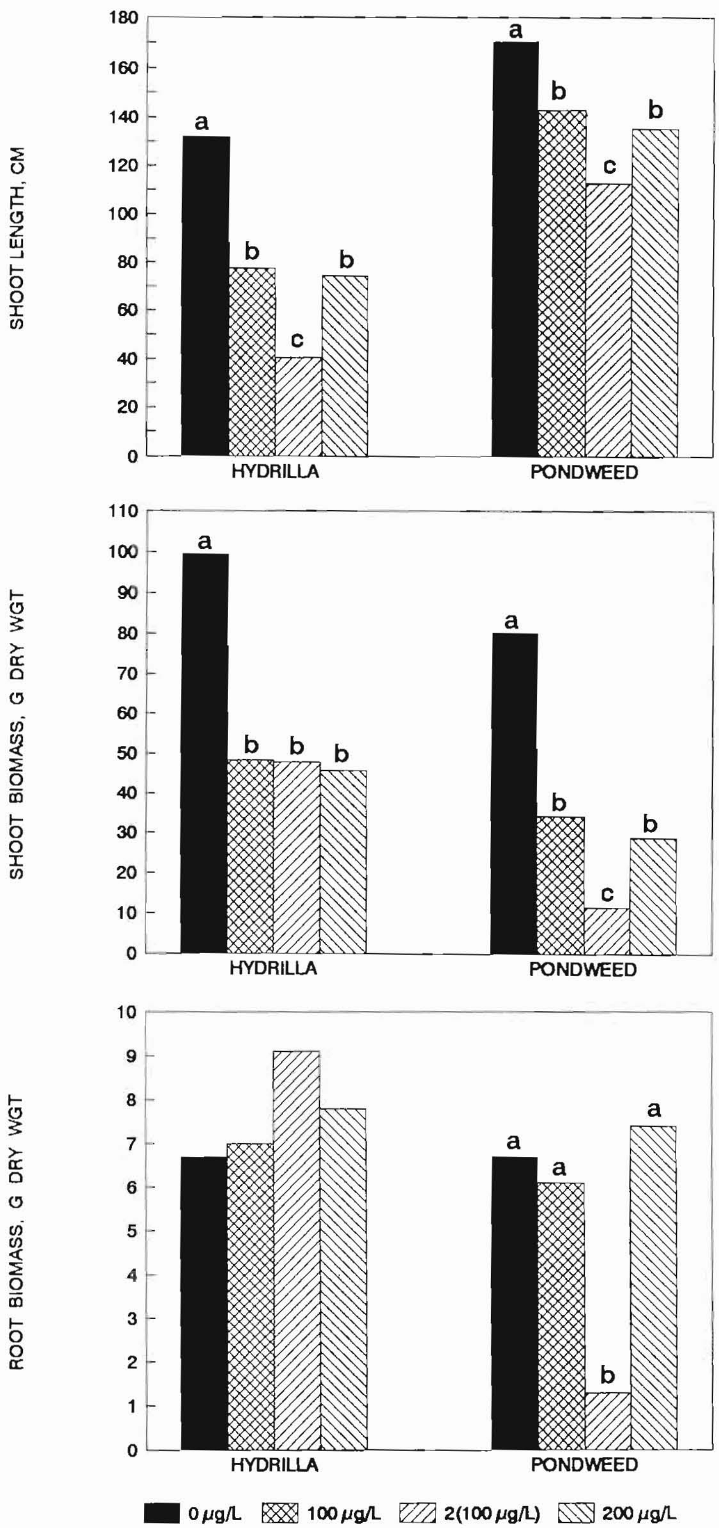

Figure 2. Effect of flurprimidol on shoot length, shoot biomass, and root biomass of hydrilla and pondweed 12 weeks after treatment. Means (for each species) with the same letter are not significantly different at the P = 0.05 level

a shade cloth covering (25 percent) will be used to reduce high water temperatures, thereby encouraging better establishment of temperature-sensitive species.

Applying a split treatment of 100 µg/L flurprimidol (100 X 2 µg/L) was more effective for reducing plant height of hydrilla and pondweed than a one-time dose of either 100 or 200 µg/L. The data also showed that for both species, there was no advantage to doubling the treatment rate in a one-time application situation. Initially, pondweed showed an increased response to increasing flurprimidol concentration; but by the end of the study, there were no statistical differences between the 100- and 200-µg/L treatments.

For the duration of this study (12 weeks), the most effective treatment (100 µg/L applied twice) reduced plant height of hydrilla by 69 percent and aboveground biomass by 50 percent. Reductions of this magnitude would greatly decrease the weediness of hydrilla, in that plants would remain well below the water surface. In situations where mechanical harvesting is utilized to remove plant biomass at the water surface, using a PGR such as flurprimidol would produce similar effects but with greater efficiency, less plant fragmentation, and less disturbance to the water body. Wherever maintaining a vegetative component is important for community structure, PGRs would be effective management tools.

Future Work

The effectiveness and feasibility of using PGRs in a field situation must be determined. Since laboratory and mesocosm studies have identified flurprimidol as an excellent and consistent growth inhibitor of hydrilla and milfoil, larger scale field demonstrations (LAERF pond studies) with this compound are warranted. Data on efficacy and chemical dissipation under field conditions and effects on hydrilla tuber production and nontarget organisms will be collected. Additional growth chamber and/or mesocosm studies will be conducted to identify and compare the efficacy of flurprimidol on both hydrilla biotypes and to identify how the effects on nontarget aquatic plant species relate to interspecies competition. Laboratory studies will also continue to evaluate new PGR compounds for use in aquatic systems as they become available.

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Phenology of Aquatic Plants

by
John D. Madsen[1]

Introduction

The goal of phenological studies on aquatic plants is to determine points in the growing cycle of target nuisance species during which control tactics would be optimal, based on the storage of carbohydrates (Madsen 1993a,b). These "weak points" would then be correlated to observable morphological indicators, such as flowering or fruit formation, to allow the resource manager to easily exploit the weak point. Weak points might include times at which the maximum amount of storage carbohydrates are used during spring regrowth, called primary weak points, or timing applications that would prevent reallocation of carbohydrates to overwintering storage structures, which is a secondary weak point.

During the past year, research concentrated on determining weak points for the nonnative species Eurasian watermilfoil (*Myriophyllum spicatum*). Research included sampling two pond populations to elucidate carbohydrate storage patterns in this species and compare carbohydrate storage patterns noted in Texas populations with those found from literature sources in northern populations.

Methods

Research was performed at the Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. During 1991, one pond population was sampled monthly, with 12 samples taken each month. During 1992, six samples were taken from each of two ponds, but the data will be combined in this presentation. Samples were sorted into root crowns, lower stems, upper stems, autofragments, and inflorescences and dried at 50 °C to constant weight. After grinding to pass a 1-mm grid, the samples were analyzed for total nonstructural carbohydrates at the Chemical Control Technology Team laboratory at the U.S. Army Engineer Waterways Experiment Station (WES), Vicksburg, MS, following procedures described previously (Madsen, Luu, and Getsinger 1993).

Carbohydrate Content of Eurasian Watermilfoil

Carbohydrates are primarily stored in the root crown and lower shoots of Eurasian watermilfoil. Carbohydrate storage in roots and lower stems approached 20 percent of dry weight in the winter for both populations (Figure 1). Primary weak points were observed in July of 1991 and in April of 1992. The earlier weak point would be more typical of a southern population such as Texas, but weak points can vary considerably from year to year and location to location (Table 1). Secondary weak points were observed in October of both 1991 and 1992 (Figure 1). Reallocation of carbohydrates to storage areas appears to increase as days shorten, even though water temperature is still within optimal growth conditions for Eurasian watermilfoil. By graphing the timing of weak points as presented in Table 1, it is apparent that northern populations exhibit only primary weak points, and these are evenly distributed from May through July (Figure 2). The timing of primary weak points in Texas is split between April and July, but the secondary weak points are more uniformly distributed in October. This might suggest that fall treatments of Eurasian watermilfoil in southern populations, such as the Tennessee Valley Authority reservoirs, could be employed to take advantage of a secondary weak point.

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

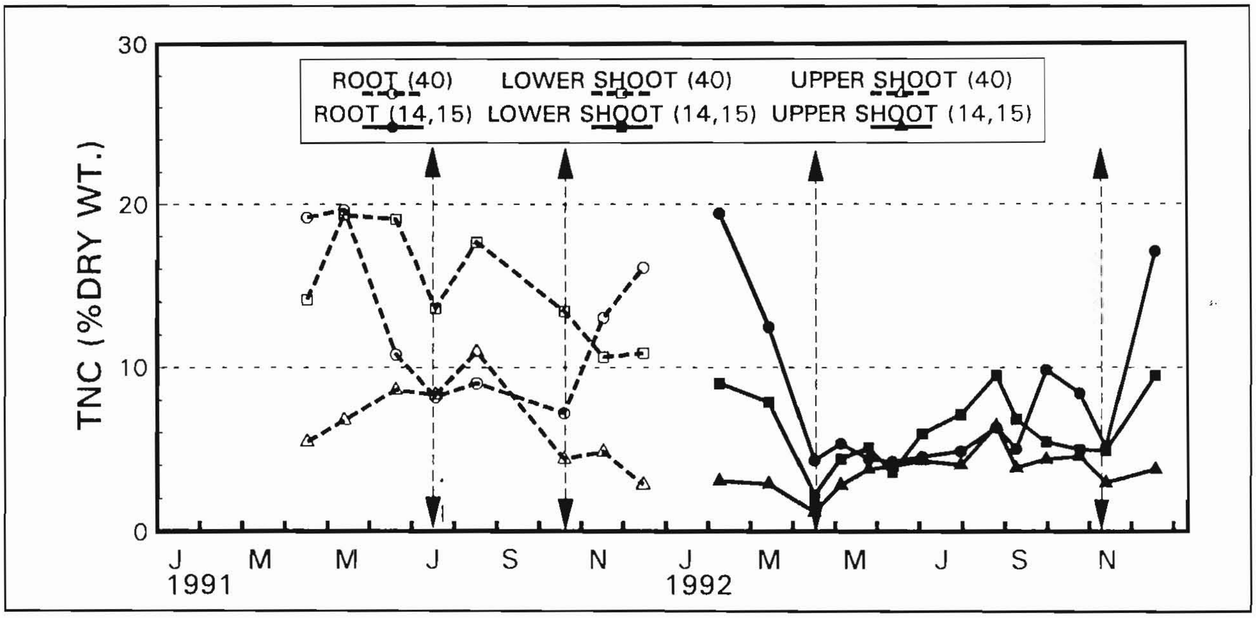

Figure 1. Total nonstructural carbohydrate concentrations (as percent of dry weight) in the upper shoot, lower shoot, and root tissue of Eurasian watermilfoil from pond populations in Lewisville, TX

Table 1
Phenological Weak Points in Seasonal Growth Cycle of Eurasian Watermilfoil Populations as Determined by Minima in Annual Carbohydrate Storage

Lake	State/ Province	Date of Weak Point	Citation
Buckhorn Lake	ON	06-15-79	Painter 1988
		06-15-80	
		06-15-81	
		05-30-82	
		07-15-83	
		07-15-84	
Lewisville Ponds	TX	07-15-91	Madsen 1993a
		10-15-91	
		04-15-92	This Study
		10-30-92	
Lake Washington	WA	07-30-80	Perkins and Sytsma 1987
		05-30-81	
Lake Wingra	WI	07-15-74	Titus and Adams 1979
		05-30-74	

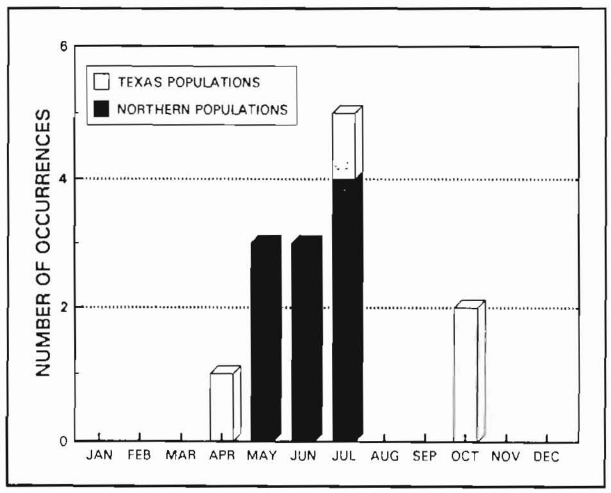

Figure 2. Frequency distribution of weak point timing by month of the year for Eurasian watermilfoil populations in northern areas and in Texas (Data from Table 1)

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The 28th Annual Meeting of the U.S. Army Corps of Engineers Aquatic Plant Control Research Program was held in Baltimore, MD, on 15-18 November 1993, 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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