

# ABSTRACTS

THIRTY-THIRD  
ANNUAL MEETING

**THE AQUATIC  
PLANT MANAGEMENT  
SOCIETY, INC.**



AND

THE FIFTEENTH  
ANNUAL MEETING

**THE SOUTH CAROLINA  
AQUATIC PLANT  
MANAGEMENT  
SOCIETY, INC.**



OMNI HOTEL  
CHARLESTON, SOUTH CAROLINA  
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Sunday through Wednesday

Aquatic Plant Management Problems and Solutions  
in South Carolina

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South Carolina is faced with a variety of aquatic plant problems, most of which are caused by non-native species such as hydrilla, Brazilian elodea, and water hyacinths. Annual expenditures for aquatic plant control operations have increased steadily as hydrilla populations continue to expand. However, total expenditures are expected to decline as long-term results are realized from biological control agents. Successful management of the State's aquatic plant "Challenges" has required flexibility, innovation, and public support.

Aquatic Weed Infestations Impacting the  
St. Stephens Hydroelectric Powerhouse

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The St. Stephen Hydroelectric Plant is an 84 megawatt plant located near the northeast corner of Lake Moultrie, SC. Since 1990, plant operations have been affected by aquatic plants; primarily hydrilla and Brazilian elodea. Integrated approaches to mitigate these problems have included chemical, mechanical, and biological controls with varying degrees of success. Several innovative, mechanical approaches have been utilized to remove material from the face of the powerhouse while a permanent cleaning system is designed and installed. These efforts have resulted in some insights concerning the management and handling of plant materials on the face of a large water control structure.

Aquatic Plant Control Operations on the Santee Cooper Lakes

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The Santee Cooper lakes, consisting of Lake Marion and Lake Houltrie, are large run-of-the-river reservoirs located in the Coastal Plains of south central South Carolina. Impounded in 1941, the lakes have supported nuisance levels of aquatic macrophytes since 1943. Since its discovery in Lake Marion in 1982, *Hydrilla verticillata* became the dominant submersed plant species in the system, currently infesting some 34,000 of the lakes' 172,000 acres.

The South Carolina Public Service Authority (Santee Cooper), a state owned electric utility, is responsible for managing and maintaining all aspects of the lakes, including aquatic macrophyte control. While numerous control technologies have been and continue to be evaluated Santee Cooper has historically relied on large scale herbicide applications to control problem growths of aquatic plants. Since the discovery of Hydrilla in the lakes, annual expenditures for herbicide applications have risen from \$50,000 to \$1.7 million. During 1993, some 7,000 acres of Hydrilla will be treated by both aerial and ground based equipment.

Grass Carp Movements and Habitats in Lake Marion, S. C., 1989-1991

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Movements and habitat selection of triploid grass carp and water quality characteristics prior to vegetation control were studied in upper Lake Marion, S. C., from 1989 to 1991. Lake Marion is a 44,000 ha impoundment located in S. C.'s coastal plains and has a well-known history of nuisance vegetation, primarily Hydrilla. Radio-frequency transmitters were surgically implanted in 51 grass carp in 1989/90 and 42 fish in 1990/91. Core use and home range areas of the 1989/90 fish averaged 49 km<sup>2</sup> and 165 km<sup>2</sup>, respectively. Core use and home range of the 1990/91 released fish averaged only 9 km<sup>2</sup> and 49 km<sup>2</sup>. Similarly, movements averaged 0.29 km/day in the 1989/90 study compared to 0.10 km/day in 1990/91. Smaller core use and home range areas and lower movement rates during the second study were attributed to more favorable water quality characteristics resulting from superior release sites. Fish demonstrated that they initially wander following release. Their movement rates declined curvilinearly from 0.30 km/day to 0.02 km/day at 200 days after release. Typical habitats used by grass carp were shallow flats (2-3 m deep) with stands of Hydrilla that covered about 50% of the water surface. Dissolved oxygen averaged 8 mg/l at the surface and only 1 mg/l at the bottom. About 2200 water samples were taken in order to relate aquatic plant relative abundance to water quality during 1991. Samples sites with dense stands of vegetation demonstrated higher water temperatures, greater surface to bottom thermal and dissolved oxygen gradients and lower bottom dissolved oxygen concentrations than sites with lower aquatic plant abundances.

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

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In Lakes Marion and Moultrie, sixty-nine triploid grass carp (*Ctenopharyngodon idella*) were readily collected during May-June 1992 by bow fishing when conventional collecting techniques failed. Scales from these fish exhibited recognizable annuli, but lapillar otoliths and pectoral spines also showed potential as aging structures. The weight to length relationship derived from fish measured at stocking and collection was:  $\text{weight} = 0.0000027 \text{length}^3 \cdot 25$  with an  $r^2 = 0.99$ . Back calculations of total length and weight provided the following estimates of fish size: Age 1) 361 mm, 0.6 kg; Age 2) 698 mm, 4.9 kg; Age 3) 821 mm, 8.3 kg; Age 4) 908 mm, 11.5 kg. Future research will: 1) attempt to increase collection sample size to improve estimates of mortality and 2) refine aging techniques by validating the use of otoliths and scales.

Preliminary Evidence for Overlap Between Field Populations  
of Monoecious and Dioecious Hydrilla

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Comparisons were made among monoecious (Lake Gaston), dioecious (Florida), and suspected dioecious hydrilla from Wilmington, NC, and Lake Gaston, NC/VA. The suspected dioecious plants were similar both morphologically and reproductively to Florida dioecious plants. Non-denaturing electrophoresis of tuber extracts indicated that both monoecious and dioecious biotypes are present in Lake Gaston. This is the first non-anecdotal evidence for the existence of overlap between monoecious and dioecious hydrilla populations in the United States.

Distribution and Physicochemical Properties of  
Lake Gaston Hydrosols

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A field study was initiated in the spring of 1992 to map the hydrosols of Lake Gaston NC/VA. USDA - SCS soil maps, completed prior to lake construction, were used to select sampling areas. Hydrosols were collected from 1- and 2-meter depths at 175 locations. These samples were analyzed for particle size distribution, hardness, and nutrient content. Results of analyses showed 8 soil textural classes and a wide range of hydrosol hardness and nutrient values.

Influence of Varying Lake Gaston Hydrosols  
on Monoecious Hydrilla Growth

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Monoecious hydrilla was grown on six different Lake Gaston hydrosols in concrete aquaria during the summer of 1992. Over a 24-week experiment, 4-week interval harvests showed luxuriant growth on silt loam and loam with minimal growth on sandy clay loam. Intermediate growths occurred on the other 3 hydrosols. Maximum shoot lengths and biomass allocation was observed at 16 weeks. Tuber production began within 4 weeks, but turions were not formed until final harvest.

Dynamics of *Lyngbya* Infestations in Southeastern Reservoirs

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How should one manage reservoirs that are infested with the mat-forming cyanobacterium, *Lyngbya*? This question has prompted an investigation of the dynamics of infestations in order to determine how biomass accumulates, how it is dispersed, and when it is biologically active.

Infestations occur in two principal forms: Resident Infestations, comprised of broad expanses of continuous mats (biomass 10-20 kg m<sup>-2</sup>FW), usually located within sheltered coves; and Transient Infestations, located along shorelines within more exposed sites, and comprised of fragmented mat and masses of 1-2 cm diameter spheres (biomass up to 40 kg m<sup>-2</sup>FW). Resident populations are the more productive of the infestations and are the source of much of the biomass within transient sites. The dynamic aspects of mat movement from resident and transient sites together with a slow rate of decomposition leads to significant accumulation. Because of the net gains and losses associated with these populations, it is proposed that management-treatments should be directed toward resident populations, even though transient sites may represent a greater accumulation of algal material.

Revegetation Strategies After the Cleanup of Lost Lake, A  
Contaminated Carolina Bay at the Savannah River Site  
Aiken, SC

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A Carolina Bay cleanup and restoration project was started in 1989 at the U. S. Department of Energy Savannah River Site near Aiken, SC. The primary goals of the project were the following: (1) clean up a contaminated Carolina Bay by draining and excavation, (2) establish new wetland vegetation, and (3) monitor the success of revegetation. Six treatment zones and two control zones were established in 1991 and consisted of combinations of disking, topsoiling, fertilizing, liming, and planting of approximately 10,000 units of native vegetation.

Monitoring efforts started in the fall of 1991 and included measurements within each of the 8 treatment zones of percent cover, density, and percent survival of wetland plants found in 280, 2 x 2 m plots; 56, 3 x 10 m plots; 56, 5 x 10 m plots; and 40, 10 x 10 m plots. Of the 8 species planted, three were the most successful (*Panicum hemitomon*, *Eleocharis* sp., and *Pontederia cordata*). Successful naturally invading species included *Eleocharis acicularis*, *Eupatorium* sp., *Typha latifolia*, *Polygonum* sp., *Panicum dichotomiflorum*, and *Setaria* sp.

Strategies for successful revegetation of a Carolina Bay are suggested and include consideration of stockpiling seed bank and controlling hydrology.

There Is Good Reason Bass Anglers Appreciate Aquatic Vegetation

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Anglers, particularly those fishing for largemouth black bass have an almost mystical reverence for aquatic vegetation. The black bass is far and away this nation's most popular and widely distributed game fish. It has an almost tactile affinity throughout its life for abundant cover. This cover can be provided by a variety of structural diversity, often in the form of logs, overhanging vegetation, rocky ledges and outcrops, etc. However, most lakes have limited structural diversity and most reservoirs have been impounded long enough that wooden structure has decayed and sedimentation has covered most other structural habitat. Thus, one can understand the growing roll played by aquatic vegetation in providing the major source of black bass structural diversity and cover.

Bass Anglers Sportsman Society (B.A.S.S., Inc.) membership numbers in excess of 550,000. It is estimated nationally there are 30 million black bass fishermen. In spite of the numerous gamefish species available, at least half of the estimated 60 million U.S. anglers spend most angling trips for black bass. The predominance of substantial amateur and professional tournament fishing activity is concentrated on black bass. Collectively there is an almost incomprehensible number of angler hours, days and trips, many at professional or semi-pro levels, spent in pursuit of black bass. With this level of shared interest, incentive, experience and "how to" media coverage, it is not surprising bass anglers are fairly well informed and demanding about bass habitat and fishing preferences. They become vocal advocates and well informed amateur scientists supporting favorable fish habitat, particularly the all-important structural diversity aquatic vegetation provides for the fish -- and the fisherman.

Management of Aquatic Plants on Lake Guntersville, Alabama:  
Implications for Largemouth Bass

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During the past 15 years, areal coverage of submersed macrophytes fluctuated between 7 and 29% on Lake Guntersville, a 28,000 hectare mainstream impoundment of the Tennessee River. Conflicting recreational activities dictates that aquatic plant management must be conducted. Currently, an excellent largemouth bass, *Micropterus salmoides*, fishery exists. Harvest rates averaged 6 kg/hectare, and catch rates were 0.6 fish/h which were about twice the national average for public impoundments. Management agencies partly attribute this quality fishery to the presence of submersed plants. A catch depletion technique estimated a harvestable standing crop of 19 kg/hectare indicating a moderate exploitation rate of about 25%. Angler catch rates of largemouth bass did not vary between vegetated and unvegetated regions or over time even though macrophyte coverage varied four-fold between 1986 and 1991. However, average weight of tournament fish varied inversely ( $r = -0.78$ ) with plant coverage and angler effort to catch a memorable fish ( $> 2.27$  kg) increased ( $r = 0.74$ ) at higher plant abundances. Production of age-0 fish was an order of magnitude greater in habitats that contained plants, but by age-1, size- and density-dependent mortality resulted in only a two-fold difference in age-1 population density. Thus, vegetated habitats provided some advantage to largemouth bass recruitment to age 1.

Effects of Aquatic Macrophytes on Chickamauga  
Reservoir Fish Communities, 1970-90

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Expansion of submersed aquatic macrophytes in Chickamauga Reservoir, Tennessee, had a dramatic effect on the resident fish community between 1970 and 1990, according to cove rotenone surveys. Midwater insectivorous fish species (golden shiner, warmouth, bluegill, redear sunfish, brook silverside, yellow bass, black crappie, and yellow perch) became more abundant as aquatic vegetation increased. The forage fish base shifted from shad to small sunfish during periods of heavy vegetation, and numbers of largemouth bass increased. In contrast, open-water, benthic insectivorous/omnivorous species (carp, smallmouth buffalo, spotted sucker, channel catfish, and freshwater drum) declined as their feeding habitat in shallow, silted overbanks became colonized by vegetation. Decline of piscivorous adult white crappie is attributed to several factors, most of which are directly related to macrophyte increases. In the 1960's and 1970's Eurasian watermilfoil was the dominant species of submersed aquatic vegetation; but during the 1980's, spinyleaf naiad became more common in Chickamauga Reservoir. The shift in macrophyte dominance affected abundance of several fish species.

Aquatic Macrophytes and Their Relation to Fish Populations  
of Florida Lakes

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The water quality, aquatic macrophyte community, and fish population of 60 Florida lakes were sampled over a five year period from 1986 to 1990. Lake trophic status was positively related to total and harvestable fish weight when the fish populations were collected with rotenone sampling (Kg/ha), experimental gillnets (kg/net/24 hr), and electrofishing (kg/hr). To account for the overriding effect of lake trophic status, a ratio of fish biomass (estimated with rotenone sampling) to adjusted chlorophyll a (a value of measured chlorophyll a plus the chlorophyll a that would be predicted from the nutrients incorporated in macrophyte and periphyton tissue) was calculated for examining the relation between aquatic macrophytes and fish populations. Locally weighted regression analysis revealed a parabolic trend line between total and harvestable fish biomass to adjusted chlorophyll a ratios (g fish/g chlorophyll a) and the percent volume infested (PVI) with aquatic macrophytes. The trend line suggests that total and harvestable fish biomass per unit of adjusted chlorophyll a is maximized at intermediate levels of aquatic macrophytes (PVI values between 20% and 40%) and are depressed at extreme aquatic macrophyte levels (PVI values < 15% and > 85%). Although these findings are consistent with other published studies and suggest that some intermediate amount of aquatic vegetation will maximize fish populations, some lakes in this survey with extreme PVI values < 15% and > 85% had high fish biomass to chlorophyll a ratios. Thus, these data suggest that only a potential for depressed fish populations exists at both low and high levels of aquatic macrophytes.

Response of a Reservoir Ecosystem to  
Aquatic Vegetation Removal

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University, Alabama; and Richard L. Noble, North Carolina  
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Over 3,600 hectares of aquatic vegetation in Lake Conroe, Texas, were eliminated one year after 270,000 grass carp were stocked in 1981-1982 and substantial changes in the fish community were documented. Biomass of numerous species, particularly sunfishes, in cove rotenone samples declined after vegetation removal. Largemouth bass density, but not biomass, declined and growth rates increased. Biomass of several species, particularly channel catfish, increased. Threadfin shad density increased and faster growth rates of crappie were documented. Seining revealed that cyprinodont populations collapsed following vegetation removal. Large year-classes were produced by two Morone species following vegetation removal. Although offshore abundance of white crappie declined, the gill net catch of black crappie remained unchanged. The original largemouth bass crappie-hybrid striped bass fishery was replaced by a channel catfish white bass-hybrid striped bass-largemouth bass-black crappie fishery after vegetation removal. The response of several species was predictable, but mechanisms governing the dynamics of some species were unclear. Weed management using grass carp in multiple-use reservoirs should proceed with the knowledge that user groups and different components of the ecosystem will respond in different and often unpredictable ways.

Fish Productivity and Aquatic Vegetation in  
Selected Duke Power Company Reservoirs

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Aquatic vegetation can be an important factor in the success of reservoir fish communities. How important aquatic vegetation is in the production of reservoir fish has been debated heavily, particularly between multiple use reservoir managers and anglers. The purpose of this paper is to compare indicators of reservoir productivity (fish standing stock, sportfish harvest) in reservoirs without significant aquatic vegetation coverage to similar data from reservoirs where aquatic vegetation coverages have been considered significant. Reservoirs in the Duke Power system have

aquatic vegetation coverages that have been historically less than 1% of the total surface area. Empirical modeling based on summer chlorophyll a values indicates that Duke Power Company reservoirs are producing fish standing stocks and sportfish harvests that are reasonable for the productivity of the body of water. Comparison to reservoirs where aquatic vegetation coverages range up to 30% of the total surface area indicates that most of the reservoirs fall within the range of values predicted by chlorophyll a concentrations. These data analyses provided no evidence that fish standing stocks and sportfish harvest were enhanced by the presence of aquatic vegetation.

Relationship of Submerged Aquatic Plants to Fish Production  
and Lake Trophic Status in Florida

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Research findings will be presented from Florida lakes relative to their biological health, fish production and sport fisheries development. As lake trophic state advances, fisheries production becomes more critically tied to the submerged aquatic plant community. Failure to carefully manage these plant communities can result in the collapse of sport fisheries and a tremendous economic loss to the local economy. Case histories will be presented showing worst case and best case scenarios from Florida waters.

The Effects of 2,4-D on Largemouth Bass Feeding Behavior

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A tank study to determine if largemouth bass (*Micropterus salmoides*) can detect low levels of herbicide on prey species was completed at the Center for Aquatic Plants in Gainesville, Florida. This study consisted of three phases. Initial observations were made of each of the bass feeding on a single golden shiner. These shiners were held for 90 minutes in aerated coolers containing 1 to 50 ppm of 2,4-D. The second phase consisted of using three bluegill in each tank, marked with pelvic fin clips and treated with one ppm 2,4-D, 50 ppm 2,4-D, and a control fish that had no 2,4-D. The results were analyzed with Friedman's ranked sum test and the presence of a treatment effect was not evident ( $X^2=0.1428$ , d.f.=13,  $p \leq 0.05$ ). The final aspect of this project was to determine if the presence of 2,4-D in the water would alter feeding activity of bass. In these trials, bass were fed crayfish after the tank level of 2,4-D had been raised to 50 ppm. In all trials, bass fed before and after the addition of 2,4-D.

The feeding behavior of the bass was not effected by any additions of 2,4-D to the prey or surrounding water even at 50 ppm. This level is at least 50 to 100 times higher than that found in the environment after spraying.

Alabama Power's Reservoir Habitat Enhancement Program

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In August 1992, Alabama Power Company and Bass Anglers Sportsman Society (B.A.S.S.) signed a "Memorandum of Understanding" to maintain and enhance the warm water sport fisheries resources on Company hydro electric projects. The first projects initiated in 1993 were the installation of Christmas trees from the Company's Recycling Program as fisheries habitat in selected reservoirs; the establishment of native vegetation in selected areas of one reservoir for demonstration purposes; and the seeding of drawdown zones of selected reservoirs with annual terrestrial plants for fisheries and wildlife benefits. Based on this two year pilot project, the program will be expanded to other Company reservoirs. A brief overview will be presented on these projects.

Fish-Plant Interactions:  
Research by the U.S. Army Engineer Waterways Experiment Station

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Control of aquatic plants is rarely free from controversy. Aquatic plants that are a nuisance to one faction of water users may be beneficial to another faction. This dichotomy is particularly evident when fish are concerned. Much of the controversy arises because there are still questions on the amount of aquatic plants necessary to support a viable sport fishery. Biologists from the U.S. Army Engineer Waterways Experiment Station have conducted field surveys of vegetation-associated fish communities in the southeast: Potomac River, VA; Lake Marion, SC; Lake Seminole, GA; Lake Guntersville, AL; and Lewisville Experimental Pond Facility, TX. These studies indicate that higher fish abundance, diversity, reproduction, diet shifts, and growth are associated with intermediate densities of plants. However, "intermediate densities" is a subjective term and recent evidence suggests that both spatial distribution and relative abundance of plants must be considered in plant management strategies. Artificially structured plant beds (i.e., boat lanes) and associated fishes strongly suggest that plant beds could be engineered for increased fishery value. Infrastructure of a new "technology area" for synoptic studies of fish-plant interactions has been developed under the auspice of the Corps of Engineers Aquatic Plant Control Research Program. This technology area will

develop causal relationships between the structure of plant beds and specific responses of the fish community and verify protocols for enhancement of fish habitats coincident with control of plants.

#### Control of *Hydrilla* Utilizing Triploid Grass Carp and Herbicides While Maintaining Littoral Vegetation

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and Eric Cotsenmoyer, Lake County Mosquito and  
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The purpose of this study was to evaluate the use of triploid grass carp used in conjunction with aquatic herbicides to control hydrilla without loss of shoreline vegetation. The study was conducted in five central Florida lakes. Since grass carp undergo a reduction in their feeding rate with an increase in size, large fish were used to lessen their impact on shoreline plants. Stocking rates of 5 fish per acre were sufficient to control hydrilla after the use of aquatic herbicides. Shoreline vegetation was not significantly affected. Even during the third year, hydrilla control continues to be complete. Enclosures placed in the lakes to exclude the grass carp demonstrated that hydrilla control from the aquatic herbicides only lasted from 6 to 10 months.

#### Grass Carp Herbivory of Eurasian Watermilfoil and Several Native Aquatic Plants on a Large Reservoir in Alabama

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Effects of grass carp (*Ctenopharyngodon idella*) herbivory on Eurasian watermilfoil (*Myriophyllum spicatum*) (hereafter referred to as milfoil) and native aquatic plants were investigated at Guntersville Reservoir, Alabama, in 1992. Herbivory of milfoil was assessed by placing 5 1 m<sup>2</sup> welded wire enclosures in each of 4 areas dominated by milfoil (n=20). Herbivory of native aquatic vegetation was assessed by placing 4 1 m<sup>2</sup> welded wire enclosures in each of 3 areas that were dominated by *Chara* spp., *Potamogeton* spp., and *Najas* spp. in 1990 but were replaced by open water in 1991 (n=12). Enclosures were placed on 4 April, 1992, and sampled from 6-8 August, 1992. A control (grazed) plot randomly located 5 m from each enclosure was sampled similar to enclosures.

In milfoil areas, vegetative biomass was not different ( $P > 0.05$ ) in grazed and enclosure treatments, suggesting no herbivory. However, in the native vegetation areas, overall vegetative biomass was greater ( $P < 0.0001$ ) in enclosures. Biomass of *Chara* spp., *M. spicatum*, *N. guadalupensis*, and *P. pusillus* was greater ( $P < 0.03$ ) in enclosures.

Although other studies have quantified grass carp food preference in relatively small confined impoundments, results from this study suggest that grass carp preference for species other than *M. spicatum* is sufficient to effectively eliminate native plants in whole areas, even on a reservoir as large (27, 479 ha) as Guntersville Reservoir. Such reduction of native vegetation may diminish habitat quantity and quality for the >40,000 ducks and coots that use Guntersville Reservoir during the winter.

#### Giant Cutgrass - Perfect Revegetation Tool or Perfect Menace?

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Giant cutgrass is a tall, emergent grass species native to the southeastern United States. It is able to colonize and rapidly form dense stands in shallow freshwater sites by means of seed dispersal, rhizome growth, and vegetative shoot production from flowering stems. This latter propagative mechanism, which allows robust new plants to be grown from stem fragments in 4 to 8 weeks, has made giant cutgrass an attractive tool for revegetation projects, especially in sites to be protected from shoreline erosion. Results from studies examining the influences of environmental factors, such as water depth and exposure to wind and wave action, on rates of stand expansion will be discussed in relation to some less desirable consequences of planting giant cutgrass in highly erosive sites, areas that become more sheltered, or where water depth is reduced.

#### Physiology of Hydrilla Reproduction: Role of Abscisic Acid

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The plant hormone abscisic acid (ABA) has been shown to regulate tuber and turion formation in hydrilla (*Hydrilla verticillata* L.f. Royle) and vegetative reproduction in other several species. The actual role of ABA is unknown, but elevated levels of ABA have been associated with tuber and turion formation. Therefore, factors that affect ABA levels (chemical inhibitors, environment) should have profound impact on turion formation and development. Studies were conducted to develop a procedure for quantitating ABA in hydrilla in order to evaluate the role of ABA in hydrilla tuber and turion formation.

## Tissue Culture Propagation Of Wetland Plants: *Pontederia Cordata*

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Many wetland plant species used for aquascaping and wetland revegetation projects are collected from donor wetland sites for planting elsewhere. Increased demand for wetland plants has lead to over-collection and subsequent environmental damage to these donor sites. Micropropagation techniques could provide ecologically sound alternatives to field collection and allow for production of under utilized wetland species that are slow growing or difficult to propagate using conventional methods. Cultures of *Pontederia cordata* L. (Pickerelweed) were established *in vitro* from surfaced sterilized rhizome buds cultured on agar-solidified full strength Linsmaier and Skoog mineral salts and organics (LS) supplemented with 2.0  $\mu\text{M}$  benzyladenine (BA) and 0.5  $\mu\text{M}$  indole-3-acetic acid (IAA) and 87.6 mM sucrose. Maximum shoot proliferation (19 shoots/explant) occurred from single node explants cultured for 28 days on agar-solidified LS medium supplemented with 20  $\mu\text{M}$  BA and 1.0  $\mu\text{M}$  IAA. Maximum acclimatization and survival (100%) of microcuttings *ex vitro* occurred when 1.0 cm microcuttings were pre-rooted *in vitro* in agar-solidified LS medium without growth regulators. The post-transplant field performance of *in vitro* produced plants was evaluated through several growing seasons. The micropropagation protocol developed provides an efficient propagation system to meet increasing market demand for this species.

## *In Vitro* Shoot Regeneration In *Hygrophila Polysperma*

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*Hygrophila polysperma* (Nees) T. Anderson (Hygro), is an exotic amphibious angiosperm with a high potential for vegetative reproduction. Native to India, this species was first introduced into Florida in the early 1950s and by 1990 had become naturalized and problematic in canals, rivers and lakes. The capacity of this species to regenerate from tissues with pre-existing buds (shoot tips and nodal segments) and via adventitious shoot formation from tissues without pre-existing buds (internode and leaf segments) was evaluated following *in vitro* culture in the absence and presence of the cytokinins (0 - 25  $\mu\text{M}$ ) benzyladenine, zeatin or (2 isopentenyl)adenine and the auxin (1.0  $\mu\text{M}$ )  $\alpha$ -naphthaleneacetic acid (NAA). Maximum shoot regeneration occurred from shoot tips cultured in liquid medium consisting of full-strength Murashige & Skoog mineral salts (MS) supplemented with 3.0% sucrose for 28 days. Culture shaking (100 rpm) did not significantly enhance shoot production. Regeneration from nodal segments was enhanced in medium supplemented with cytokinins. Internode segments developed adventitious shoots when cultured on agar-solidified MS without growth regulator supplementation. Adventitious shoot development was further enhanced on internode and leaf blade segments cultured in the presence of a cytokinin and 1.0  $\mu\text{M}$  NAA. The implications of these results to the spread of *Hygrophila in situ* will be discussed.

## Selective Control of Eurasian Watermilfoil Using Triclopyr

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Eurasian watermilfoil-dominated communities were treated with the herbicide triclopyr in the Pend Oreille River, WA, in July, 1991. Triclopyr (Garlon 3A formulation) was applied at a 2.5 ppm rate to a 15-acre riverine plot, and at a 1 to 2.5 ppm rate to a 10-acre cove. A simultaneous application of rhodamine WT dye was applied to both sites at a rate of 10 ppb. The effectiveness of triclopyr on milfoil was good to excellent in both treated plots. By 1-year posttreatment milfoil biomass had increased by 70% in the untreated reference plot, yet had decreased 72% in the riverine plot and 99% in the cove plot. One year posttreatment native plant (e.g. elodea, pondweeds, etc.) recovery was excellent in both treatment sites, but still remained suppressed in the untreated reference. Native plant biomass comprised only 3% of total plant mass in the reference, but accounted for 86 and 99% of total plant mass in the riverine and cove plots, respectively. Triclopyr water residues were below detection (<0.01 ppm) 3200 ft downstream from the riverine plot by 12 hr posttreatment, and 1330 ft downstream from the cove plot at 48 hr posttreatment. Good to excellent correlations ( $r^2 = 0.80$  and  $0.95$ ) were observed between the dissipation of triclopyr and rhodamine WT.

## Response of Native Vegetation to an Application of Triclopyr

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Environmental impacts of exotic plants on native vegetation are often neglected as a consideration of aquatic plant management. Eurasian watermilfoil-dominated communities were treated with the herbicide triclopyr in the Pend Oreille River, WA, during July, 1991. The species composition of two treatment and one reference plots were evaluated before treatment and one year posttreatment using four line intercept transects per plot, with each transect having 100 1-m intervals. Eurasian watermilfoil percent cover in the riverine treatment plot decreased from 94% before treatment to 54% one year posttreatment, while the reference plot maintained nearly 100% cover. The cove treatment plot decreased from 89% pretreatment to 25% one year posttreatment. Native plant species abundance in the riverine plot averaged 0.90 species per interval pretreatment, and increased to 2.50 species per interval posttreatment. The riverine reference decreased slightly, from 0.65 native species per interval pretreatment to 0.39 native species per interval posttreatment. The cove



treatment plot increased from 1.15 native species per interval to 2.18 species per interval one year posttreatment. The near-monospecific dominance of Eurasian watermilfoil suppressed the native plant community; management of Eurasian watermilfoil resulted in release and growth of native plants, which may in turn delay the reinfestation and dominance of Eurasian watermilfoil in these communities.

#### Use of Triclopyr Herbicide to Re-vegetate Eurasian Milfoil Infested Portions of the Columbia River System

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Triclopyr is currently proposed for an aquatic registration by DowElanco and is being used in the field under a Federal Experimental Use Permit (EUP). This product has shown excellent activity on the exotic aquatic weed Eurasian Milfoil in the laboratory and in field trials conducted by the US Army Corps of Engineers Aquatic Plant Control Research Program. This herbicide has selectivity toward Eurasian Milfoil while most native aquatic plant species are tolerant of it. Corps researchers have also determined that this product has a low concentration/exposure time requirement and can function in some flowing water situations. These characteristics should provide users with a tool to remove the noxious milfoil plants from infested areas and allow natives to reclaim these treated zones.

This project looked at this potential technique in sections of the Columbia River. A number of parks along the river have been developed to mitigate for loss of recreational opportunities caused by dam construction. These sites are heavily impacted by Eurasian Milfoil. In most cases, there are understories of native aquatic plants in the pondweed family. The goal of this project was to evaluate the response of the plant communities to Triclopyr treatments.

The project team followed methodology developed by the Chemical Control Technologies Team at the Corp's Waterways Experiment Station to evaluate the herbicides impact on target and non-target vegetation. Pre-treatment biomass and species diversity data were collected from three Triclopyr sites, one Sonar treatment site, and one reference site. Next florescent dye was applied to obtain information on potential movement of the herbicide out of the plot and to estimate contact/exposure time. The sites were then treated with the two herbicides. Plots of 5, 5, 15, and 15 acres were treated. Six week post treatment biomass and one year post treatment biomass and species diversity data were collected.

The results of the six week post treatment survey was promising. Good to excellent Eurasian Milfoil control was evident in the Triclopyr plots. The Sonar Plot plants showed signs of injury. Biomass data indicated a major reduction in plant density in these plots. The Triclopyr plots also showed little damage to non-target plant species during underwater survey. The one-year post-treatment data will be collected just before the APMS meeting this summer and presented in this paper.

#### Monitoring Herbicide Impact on Non-Target Plants Using Peroxidase Analysis

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The use of 2 ppm of endothall active ingredient, applied for 2-, 16- and 36-hr contact times as a dipotassium salt, was found to increase peroxidase (PRX) enzyme activity levels for up to 28 DAT in aquarium-grown hydrilla (*Hydrilla verticillata* (L.f.) Royle), a species which is controlled by this herbicide in the field. These treatments did not change PRX levels in egeria (*Egeria densa* Planch.), on which this formulation of endothall is not effective. Dry weight measurements at 77 DAT showed that 36-hr contact time controlled hydrilla, but that none of the treatments significantly affected egeria biomass. Using starch gel electrophoresis, isozymes were examined in various tissues of treated plants to monitor herbicide-induced changes in expression of PRX. The use of PRX analysis for physiological monitoring of herbicide selectivity is considered.

#### Pre-emption As a Factor in Competition Between *Vallisneria americana* and *Hydrilla verticillata*

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Competition between the native species, *Vallisneria americana*, and the introduced weedy species, *Hydrilla verticillata*, was evaluated in a greenhouse tank experiment. Two types of competitive situations were considered: direct competition, in which the 2 species were transplanted simultaneously into bare sediment, and pre-emption, in which *Vallisneria* was planted 4 weeks prior to the invading *Hydrilla*. The growth of each species was evaluated relative to its growth under similar environmental conditions without the competing species. Under the conditions evaluated (high CO<sub>2</sub> supply and high sediment fertility), *Hydrilla* outcompeted *Vallisneria* when the 2 species were growing in direct competition. *Hydrilla's* competitive advantage was associated with its rapid initial growth rate. *Vallisneria* transplants were much slower to become established and suffered in competition with *Hydrilla*. A period of pre-emption by *Vallisneria* greatly reduced the growth of invading *Hydrilla* and lessened *Hydrilla's* competitive advantage. *Vallisneria*, by virtue of its ability to sequester nutrients from sediment, should be able to outcompete *Hydrilla* over the long term.

## The Effects of Fluridone and 2,4-D on Southern Wild Rice

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There is much public concern over the effect of various herbicides on non-target native vegetation. Southern wild rice (*Zizania aquatica* L.) is one of the native plants which could be affected by herbicide use in Florida. Southern wild rice, growing in 4" pots, was treated at the floating leaf stage with fluridone at 0, 2, 4, 8, 16, and 32 µg/l and with 2,4-D at 0, 200, 400, 800, 1600, and 3200 µg/l. Average plant dry weight was found to be significantly different from the control at the 8, 16, and 32 µg/l rates of fluridone and at the 400, 800, 1600, and 3200 µg/l rates of 2,4-D.

## Effects of Treated Kraft Liquor on *Hydrilla verticillata* (Royle)

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The kraft process is one of three chemical processes that convert wood chips to fibers using chemical solutions to dissolve the lignin. The kraft process uses sodium hydroxide and sodium sulfide mixtures as pulping agents, and a product is so-called "black liquor," which is treated to regenerate starting materials. Our research looks at the treatment of black liquor to serve as a shading substance for the management of submersed aquatic plants. Black liquor was diluted, treated with concentrated sulfuric acid, and thoroughly aerated to give a product low in trace metals, herbicides, and dioxins. The treated black liquor inhibits the growth of hydrilla in a concentration dependent manner, and we are examining the comparative effects of shading and chemical action on this plant.

## Impact of Hydrilla Life Cycle on the Mode-of-Action of Fluridone

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Responses of hydrilla to fluridone can change depending on the size and stage of development. Hydrilla produces large amounts of shoot tissue very rapidly during vegetative growth, which occurs from spring to summer. During this period, hydrilla shoot tissue is very susceptible to fluridone because of a high demand for new pigments which are used in photosynthesis. Fluridone prevents this pigment formation and therefore growth. Hydrilla reproduces by producing tubers during the fall when shoot growth is extremely slow. Even though hydrilla is growing reproductively, demand for pigments is low because tubers do not contain these pigments and shoot growth is minimal. Consequently, shoot tissue is less susceptible to fluridone even though pigment production is blocked. However, these pigments are also used to produce ABA, a plant hormone thought to be involved in hydrilla tuber formation. Therefore, fall fluridone treatments could be used to block reproductive growth even though vegetative growth responses to herbicide treatment would be minimal. Research results dealing with testing these ideas will be presented.

## A Physiological Process Model for Aquatic Plant Growth

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A physiological process model for aquatic plant growth was developed to predict the response of aquatic plant growth to global climate change and the associated potential for geographic shifts of aquatic vegetation distribution. The model includes three major parts: leaf photosynthesis, gas exchange between atmosphere and the water-plant system, and biomass production. Photosynthesis was modelled mechanistically on photosynthesis biochemistry level. The possible use of HCO<sub>3</sub> in addition to CO<sub>2</sub> and shift from C3 to C4-like photosynthesis were addressed explicitly in model structure. A diffusion model was developed for CO<sub>2</sub> and O<sub>2</sub> exchange between atmosphere and the water-plant system which provided the concentrations of CO<sub>2</sub>, HCO<sub>3</sub> and O<sub>2</sub> in water as the input of photosynthesis submodel. The production of leaves, stems, roots and reproductive organs were simulated. The model was calibrated and validated using leaf photosynthesis data and plant growth data of *Hydrilla* at different levels of CO<sub>2</sub> and temperature in growth chambers. The photosynthetic responses to temperature, light, and CO<sub>2</sub> were measured using a LI-6251 infrared CO<sub>2</sub> gas analyzer incorporated into an open system. We are applying the model to predict or assess the possible impacts of global climate change on *Hydrilla* growth and distribution at different water bodies.

## Washington Lakes Benefit From Caring Lake Residents

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Lake residents around Long Lake in Washington State were faced with a noxious aquatic plant problem. Eurasian milfoil was found in the lake in 1987. Washington lake property owners have faced

milfoil before, but this infestation was the first in the South Puget Sound Region and posed a threat to other lakes in the area. The lake residents understood the threat this plant posed, so they mobilized to protect the resource.

The first step was to form an aquatic weed options committee. This group was made up of 25 members from the Long Lake watershed. They met every other week for six months. Invited speakers from industry discussed aquatic plant control methods. Other presentations were given from people with experience with all aspects of lake and aquatic plant management. State and county regulatory agencies worked with the committee to develop a program that would succeed through the regulatory process.

Next, the committee performed outreach to the lake community and educated their neighbors about the control options available to target the Eurasian milfoil problem. The focus of this effort was to build a consensus of property owners to the alternatives the group felt would solve the problem. The preferred alternative was to implement a five year integrated aquatic plant management program using the aquatic herbicide SONAR as an initial treatment and selected underwater observation, plant removal, and other non-chemical methods in successive years to rid the lake of milfoil.

This program developed by the Long Lake Options Committee started in January of 1989 and took until the summer of 1991 to implement. During this period, the Committee presented recommendations to the County, helped with the development of an Environmental Impact Statement for the project, and convinced the citizens around the lake to form a taxing district to fund the five year program.

This program became a successful model for other lake associations in Washington solving similar problems. The Long Lake Residents recognized government would not solve the problem. The lake residents took the responsibility to develop a program that met their needs and raised most of the funding by implementing a taxing district. This kind of interaction with community and government captured the eye of governmental agencies. They responded with both support and supplemental funding.

#### Distribution and Abundance of Eurasian Watermilfoil, *Myriophyllum spicatum*, in the Peoples Republic of China

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*Myriophyllum spicatum*, which is native to Europe and Asia, has become one of North America's worst aquatic weeds. Its geographic range is still expanding, and Minnesota was recently added to the long list of states which are infested by this weed. Knowledge of the endemic distribution of this submersed macrophyte is desirable, especially when initiating a classical biological control research program for this pest. While the geographic area of China is vast, the distribution of aquatic plants there is poorly known, especially by westerners. As part of an ongoing effort to locate biological control agents for *M. spicatum* in China, we have examined herbarium specimens of this plant at numerous Chinese institutions. We also attempted to collect it at many aquatic sites in a dozen of China's 30 provinces. We report on the results of our distribution studies and personal collections of this aquatic plant in China. We also discuss the implications this distributional information has on the future spread of *M. spicatum* in North America.

#### Aquatic Weed Problems and Programs in Mexico

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Central and Northern Mexico generally have a very dry climate and seasonal rainfall. Consequently, Mexico relies upon large numbers of reservoirs and irrigation systems for providing water for agriculture, industry and municipalities. Hydrilla is a problem in several irrigation projects in Northern Mexico and the grass carp is being used on an experimental/operational basis. In Central Mexico, water hyacinth covers 18% of the 250,000-acre Lake Chapala in Jalisco State near Guadalajara. Control and research programs with glyphosate and 2,4-D are being undertaken in several reservoirs and a Joint Mexican-American Workshop is being planned for the Fall of 1993.

#### Regulatory Status of AQUATHOL Aquatic Herbicides

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Attempts by Atochem to make much needed changes to the federal Aquathol labels during the past five years have been delayed by the 1989 USEPA Reregistration Data Call In.

Prior to 1989, endothal and its salts had not been through any formal regulatory review in at least ten years. As a result, the supporting data base was outdated when the Data Call In was issued. It was eventually concluded that only six of the 75-100 studies on file at that time could be upgraded to meet the Agency's new study guidelines. When considering the number of studies required and resources needed to complete these studies, it has been almost like developing a new active ingredient.

Without guideline studies, the Agency did not approve most of the label amendments proposed for Aquathol in 1990. We now have completed or are conducting almost 150 new studies to support Aquathol which includes several proposed amendments to the federal label.

Proposed amendments include: (1) Additional treatment sites (submitted 4/93); (2) remove skull and crossbones; (3) add specific wording for potable water; and (4) reduce or eliminate fishing, swimming, and irrigation restrictions.

## Sediment Factors Influencing the Growth of *Vallisneria*

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Variations in the growth of *Vallisneria spiralis* Michx were examined on sediments collected from two sites in Lake Okauchesa, Wisconsin, and on the same sediments with different nutrient amendments. Growth was generally enhanced in treatments where N was added, resulting in significant increases in plant height and biomass and tuber and seed pod production. Relatively inhibited responses of this species on nonamended sediments suggest a potential for N-limitation of growth at the two sites. P alone added to each sediment overall had little if any positive effect on measured responses. It appears that at least over the short term growth of *Vallisneria* at the two study sites may be affected more so by levels of N than P availability in sediment.

## Comparative Regrowth Studies of Two Exotic Macrophytes Under Experimental Low Light Levels

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*Hydrilla verticillata* root crowns cultured in outdoor raceways at the Lewisville Aquatic Ecosystem Research Facility in Lewisville, Texas, were exposed to uniform light levels approximating 10, 5, 1, and 0 percent maximum ambient levels at 30 deg C. Shoots from individual root crowns were sampled at 2, 5, and 9 weeks and measured for: a) number of shoots, b) shoot length, c) meristem density, d) shoot biomass, and e) root biomass. Shoot regrowth was delayed in the 5 percent treatment in comparison to the 10 percent treatment. At both lower light levels, regrowth was significantly reduced in terms of parameters measuring both individual and overall shoot growth. Follow-up studies during 1993 using large circular tanks (2.4 m diameter X 2.5 m depth) with established experimental light/depth gradients will provide information on comparative regrowth rates of *H. verticillata* and *Myriophyllum spicatum* under realistic, low light conditions.

## Estimating the Abundance of Underground Propagules of Aquatic Plants

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Even though subterranean propagules of submersed aquatic plants are commonly sampled using coring devices, there is little information concerning the number of samples required to achieve reliable estimates of propagule abundance or the efficiency of different sized corers. We compiled data on the mean ( $M$ ) and variance ( $s^2$ ) of propagule abundance for 5 types of aquatic plants growing in a variety of habitats. The data set consisted of 379 values for  $M$  and  $s^2$ , based on 4942 individual samples. Regression analyses relating  $\log s^2$  to  $\log M$  yielded an equation which was solved for the number of samples ( $N$ ) required to achieve a given degree of precision as measured by the standard error ( $SE$ ). For precise estimates ( $SE = 20\%$  of  $M$ ), larger values of  $N$  (25-200) were required when propagule density is low ( $< 200 \text{ m}^{-2}$ ). Between 200 and 1000 propagules  $\text{m}^{-2}$ , 8-25 samples were required. For  $SE$  that is 30% of  $M$ ,  $N$  decreased by about 50%. Sampler area did not affect estimates of  $N$ . The time required to collect and process samples collected with either a 5-, 10-, or 15-cm corer was approximately 7, 10, or 13 minutes, respectively. This information can be used in studies designed to measure changes in the abundance of underground propagules.

## GIS/GPS Support to Aquatic Plant Chemical Control Operation

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Procedures for integrating geographic information system (GIS) and global positioning system (GPS) technologies to support the planning, execution and long-term monitoring of aquatic plant chemical control programs are being developed and tested by the U.S. Army Corps of Engineers. These technologies provide local resource managers in-house tools to quickly gather accurate plant distribution and acreage information; produce scaled maps of target treatment areas for use by contract personnel; automate treatment history records and evaluate the effectiveness of control strategies. These procedures are being jointly tested by the U.S. Army Engineer Waterways Experiment Station and the Lake Seminole Resource Management Office, Chattahoochee, FL, Mobile District, U.S. Army Corps of Engineers. The GPS, mounted on an airboat, is used to delineate aquatic plant infestations in the reservoir. The collected GPS data are transferred to the GIS. The GIS is used to generate plant distribution maps, calculate acres of plants and linear distances of plant-water interface. This information is used by the manager to identify and select areas to be treated and to calculate the volume of herbicides required. Data generated and stored using these procedures can also be used to evaluate the effectiveness of previous treatments.

Feasibility of Eradicating Catclaw Mimosa  
(*MIMOSA PIGRA* L.) in Florida

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Catclaw mimosa (*Mimosa pigra* L.), a native of Central America, is naturalized in Florida. Plants were first recorded in Florida in 1953 and now are in six or more fairly widespread locations. Although large populations are not yet present, the plant poses a threat to the Everglades wetland ecosystem. Adventitious roots and corky tissue develop on stems of flooded plants and may allow catclaw mimosa plants to colonize wetland areas at the expense of native plants. Eradication is essential to prevent further spread of catclaw mimosa in Florida. Catclaw mimosa causes major problems in several countries where it has been introduced. The plants are very adaptable and will grow under a variety of soil types and environmental conditions. Foliar or cut-stump applications of herbicides will provide effective control of catclaw mimosa plants. Seedlings may be removed by pulling. Based on ecological studies and herbicide trials, and the number of known infestations, a two-person team working full time surveying and spraying every site once a month should be adequate to eradicate catclaw mimosa in Florida. A year-round once-a-month schedule is required since catclaw mimosa plants flower year round, and only about 5 weeks are required from flowering to seed set. Surveying and spraying will need to continue until the seed bank is depleted, approximately 10 years.

Aquatic Plant Management in Puerto Rico

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Since 1982, the U.S. Army Corps of Engineers Jacksonville District has conducted a cooperative Aquatic Plant Control Program with the Commonwealth of Puerto Rico. This program differs in some ways from management programs in the U.S. and presents some interesting and unique challenges. This presentation details program objectives, technology and opportunities.

The Effects of 2,4-D on the Movements and  
Spawning Behavior of Largemouth Bass

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Anglers often oppose the use of aquatic herbicides in the control of aquatic vegetation. They believe that the use of herbicides reduces fish feeding activity and that fish flee from or avoid herbicide-treated areas. The objective of this research was to determine if the aerial application of 2,4-D affects the distribution and movement of largemouth bass.

Twenty-four largemouth bass were implanted with internal radio transmitters and tracked for six months in Bivens Arm Lake and Lake Bryant, Florida (twelve in each lake). Their home ranges and daily movement patterns were delineated by repeated observations of the individual fish. After stable home ranges were observed, the number of fish remaining in each lake at the time of spraying was divided equally between the treatment group consisting of fish sprayed with the legal limit of 2,4-D (4 lb/A) and a control group sprayed with only lake water. All applications were made from an airboat. Fish movements were then recorded for six consecutive days immediately following spraying. The movements will be compared and tested for differences between groups. Preliminary analyses show equal number and distances moved by fish in the two groups indicating no treatment effect during any of the four treatment periods, including the prespawn, spawning (2), and post-spawn period.

Influence of Fluridone in Irrigation Water on Azaleas

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Azaleas grown in a sand-bark mixture were irrigated three times weekly for four weeks with solutions containing fluridone. Symptoms did not appear until approximately three weeks after treatment began, even at the highest rate (2 mg/l). Evaluations continued for eight weeks after ceasing treatment. Plants cut back at this time and allowed to regrow showed immediate signs of recovery, except at 1 or 2 mg/l. No damage occurred on plants treated below 0.25 mg/l.

Progress in Locating Insects in Australia for Controlling  
*Melaleuca quinquenervia*, Florida's Worst Wetland Weed

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Since its introduction into the U.S.A. at the beginning of this century, melaleuca (*Melaleuca quinquenervia*) has become naturalized in Florida, and has since become its most troublesome weed. Vast portions of Florida's wetlands, along with many other habitats, are being replaced by this tree. Since 1986, we have been investigating in Australia, the original home of *M. quinquenervis*, for insects which might be useful in controlling this pest in Florida. An Australian weevil and a sawfly are now in quarantine in Gainesville, Florida, completing their final tests for safety. We report on the two insects, as well as other potential melaleuca biocontrol agents being studied in Australia.

Removal of Exotic/Nuisance Aquatic Species at  
Orlando International Airport

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The Greater Orlando Aviation Authority entered into a contract in the fall of 1992 for the removal of exotic/nuisance aquatic species growing in lake/pond/canal systems around and throughout the airport boundaries.

The exotic nuisance species consisted of approximately 300 acres of water hyacinth, water lettuce, primrose-willow, cattails, alligator weed and torpedo grass. In January, 1993, Aquatics Unlimited was called in to perform the mechanical removal of the above mentioned species along with approximately 30 acres of floating mud islands and tussocks that were present.

Aquatics Unlimited dispatched five pieces of water borne equipment including four aquatic harvesters and one Aquamog for the removal the aquatic species from the water systems. Approximately 500,000 pounds of exotic plants were removed on a daily basis.

A final report on the project will be presented including total acres of vegetation removed, the total cubic yards and tons of biomass removed and their implications on the systems, hauling and disposal methods utilized, and new equipment innovations utilized to complete the project. The scheduled completion date for the project is June 1, 1993.