

Water Quality at Caddo Lake, Center for Invasive Species Eradication: Final Report

Texas Water Resources Institute TR-468
November 2014



Water Quality at Caddo Lake – Center for Invasive Species Eradication

- Final Report -

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Texas Water Resources Institute Technical Report – 468
November 2014



Funding support for this Center was provided the USDA Natural Resource Conservation Service and Federal funds appropriated by former U.S. Senator Kay Bailey Hutchison

Acknowledgements

The Center for Invasive Species Eradication team would like to thank everyone from individuals to agencies who worked to secure funding for the Center, aided in establishing its facilities at the Caddo Lake National Wildlife Refuge, helped conduct large-scale operation and maintenance activities, assisted in conducting research and weevil releases on the lake and took and continue to take the initiative to fight and work to protect Caddo Lake. Without your efforts, the beauty and reverence of Caddo Lake would not be what it is today.



Spring on Caddo Lake along boat road N

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Introduction

Giant salvinia, a highly invasive aquatic fern native to South America, poses a serious threat to Texas' waters and has done so since its discovery in the state in the 1990s. If left unmanaged, giant salvinia can cause a multitude of impacts including loss of recreational use, reductions in biodiversity, decreases in water quality, and monetary losses due to control costs and lost revenues. To counter this threat, the Center for Invasive Species Eradication (CISE) was created in 2010 with funding support from then U.S. Senator Kay Bailey Hutchison and the USDA Natural Resource Conservation Service (NRCS) to advance the knowledge about this species and treatment options and hopefully enable eradication of this noxious non-native plant species in Texas. Special focus of the Center's efforts was placed on Caddo Lake. It is Texas' only natural lake and has been plagued by giant salvinia since 2006. Levels of the invader present have risen and fallen with changes in weather and lake levels yet the threat it poses remains despite considerable treatment efforts.

To accomplish this goal, the Caddo Lake Giant Salvinia Eradication program was developed and implemented through the Center. Research, demonstrations, educational programs and treatment activities regarding giant salvinia at Caddo Lake were planned and carried out by an integrated team of agencies, universities, special interest groups, local stakeholders and other interested individuals through this program. The Texas A&M AgriLife Extension Service, Texas A&M AgriLife Research and the Texas Water Resources Institute administered and led the effort while working directly and collaborating with the:

- USDA Natural Resource Conservation Service (NRCS)
- USDA Agricultural Research Service (ARS)
- U.S. Army Corps of Engineers (USACE)
- U.S. Fish and Wildlife Service (USFWS)
- Texas Parks and Wildlife Department (TPWD)
- Louisiana Department of Fish and Wildlife (LDFW)
- Louisiana State University, Ag Center (LSU)
- Caddo Lake Institute (CLI)
- Cypress Valley Navigation District (CVND)
- Northeast Texas Municipal Water District
- Greater Caddo Lake Association (GCLA)

Ultimately, the goal of this program was to learn about giant salvinia, evaluate control methods, apply them, and disseminate information regarding their ability to manage and control giant salvinia to audiences ranging from scientists to landowners so public and private entities have readily available, proven methods to fight this invader. Program objectives were numerous and ranged from developing improved understanding of giant salvinia to identifying new treatment methods. Effective delivery of information to a variety of audiences regarding the threat giant salvinia poses and how it can be addressed was also a critical objective. For four years, the program team worked diligently to this end and successfully achieved this goal.

Project Administration

The program was administered by the Texas Water Resources Institute (TWRI), which is a unit of Texas A&M AgriLife Research and Texas A&M AgriLife Extension Service. In this role, TWRI facilitated establishment of the Center for Invasive Species Eradication (the Center) at Texas A&M University. This included carrying out necessary fiscal management and development of the organizational structure. TWRI organized the program team and worked with this team to establish detailed goals and objectives for the Center. TWRI provided program oversight and ensured that progress remained on track, reporting requirements were met and program deliverables were effectively produced. TWRI also worked with NRCS to ensure that program goals and objectives were achieved and that fiscal management met NRCS's needs. Quarterly progress reports were developed that document these tasks and illustrate the progress made throughout the course of the program.

Project Coordination

TWRI facilitated meetings with project personnel to ensure that applied research remained focused on program objectives, maximum collaboration between program participants and external stakeholders was achieved, educational programs were effectively delivered and supported information transfer to the public, and that work was conducted in collaboration with groups actively engaged in controlling giant salvinia. Program products including educational materials and publications were also discussed and planned during these meetings. Meetings conducted primarily focused on coordinating research trials on Caddo Lake with Texas Parks

and Wildlife Department (TPWD) personnel, Cypress Valley Navigation District (CVND) personnel and Caddo Lake Institute (CLI) personnel. TPWD and CVND both conduct large-scale giant salvinia treatment efforts on Caddo Lake and ensuring that research areas were not



‘No Spray Area’ signage on Caddo Lake to reduce the threat of a biological control area being treated chemically

treated was critical. Through these planning meetings and on-lake signage, overlaps in treatment and research were minimized. Salvinia weevil rearing methods, chemical treatment methods and approaches, educational programs, and publications regarding the project’s research findings were all topics of discussion during these coordination meetings.

Throughout the program, the Center’s personnel maintained contact with various agencies and entities. Center personnel began participating in the Inter-Agency Giant Salvinia Control Team, which was formed and organized by U.S. Fish and Wildlife Service (USFWS) as a forum to discuss approaches and current efforts in the fight against giant salvinia. During these meetings, Center personnel discussed activities underway and provided updates regarding biological control research and demonstrations, herbicide treatment trials on the lake and advances in the field on salvinia management. These meetings also provided a direct contact to other entities engaged in giant salvinia control and enabled direct interactions with these people. As a result, trips were made to the LSU Ag Center and the USACE Lewisville Aquatic Environmental Research Facility to discuss facilities and methods for rearing weevils.

Herbicide Treatments and Evaluations

A primary objective of the program was to kill giant salvinia at Caddo Lake. The quickest and commonly used method for this is through herbicide applications. This approach can be quite effective and is an integral tool in the fight to control giant salvinia; however, repeated treatments are often required as complete control is rarely achieved. Costs for herbicide treatments can be significant but vary widely due to differences in both application rates and herbicide costs. Regardless, herbicide treatments are and will remain widely used.

Herbicide Trials

The effectiveness and economics of different herbicide combinations were evaluated through this program. Initially, small-scale trials of commercially available, but relatively newer herbicides with little or no efficacy data for giant salvinia treatment, were evaluated. In these trials, non-ionic surfactants were used in all treatments, and in some cases, a contact herbicide was integrated. Tables 1 and 2 present data from these trials and illustrate the range of efficacies observed in 2011 and 2012 respectively. In the initial trial, treatments were applied to the plant only and percent control and regrowth were recorded 30 days post treatment. During the 2012 trials, the same treatments were applied as the year before, but a submerged treatment approach was also added. Results varied significantly within each trial and also between years and within treatments. Differences in percent control observed between years and within trials could be due to a number of factors; however, weather is a prime suspect causing these differences.

Table 1. Small-scale herbicide trials conducted in 30-gallon tubs in 2011

Product Name	Active Ingredient	Rate/Acre	% Control*	% Regrowth*
Clipper TM	Flumioxazin	6 oz	65 ab	12 b
Clipper TM	Flumioxazin	9 oz	77 ab	7 bc
Clipper TM	Flumioxazin	12 oz	73 ab	7 bc
Reward [®]	Diquat	0.25 gal	73 ab	9 bc
Reward [®]	Diquat	0.5 gal	77 ab	10 bc
Clipper TM + Reward [®]	Flumioxazin + Diquat	6 oz + 0.25 gal	57 b	0 c
ClipperTM + Reward[®]	Flumioxazin + Diquat	6 oz + 0.5 gal	93 a	1 bc
Clipper TM + Reward [®]	Flumioxazin + Diquat	9 oz + 0.25 gal	88 ab	1 bc
Clipper TM + Reward [®]	Flumioxazin + Diquat	9 oz + 0.5 gal	88 ab	0 c
Clipper TM + Reward [®]	Flumioxazin + Diquat	12 oz + 0.25 gal	88 ab	2 bc
Clipper TM + Reward [®]	Flumioxazin + Diquat	12 oz + 0.5 gal	87 ab	0 c
Control			0 c	72

* measured 30 days after treatment; letters denote significant difference groups

Table 2. Small-scale herbicide trials conducted in 30-gallon tubs in 2012

Product Name	Active Ingredient	Rate/Acre	Application Type	% Control*
Clipper™	Flumioxazin	6 oz	Foliar	62
Clipper™	Flumioxazin	9 oz	Foliar	37
Clipper™	Flumioxazin	12 oz	Foliar	70
Clipper™	Flumioxazin	6 oz	Submerged	67
Clipper™	Flumioxazin	9 oz	Submerged	60
Clipper™	Flumioxazin	12 oz	Submerged	63
Reward®	Diquat	0.25 gal	Foliar	32
Reward®	Diquat	0.5 gal	Foliar	30
Reward®	Diquat	0.25 gal	Submerged	13
Reward®	Diquat	0.5 gal	Submerged	12
Clipper™ + Reward®	Flumioxazin + Diquat	6 oz + 0.25 gal	Foliar	37
Clipper™ + Reward®	Flumioxazin + Diquat	6 oz + 0.5 gal	Foliar	50
Clipper™ + Reward®	Flumioxazin + Diquat	6 oz + 0.25 gal	Submerged	73
Clipper™ + Reward®	Flumioxazin + Diquat	6 oz + 0.5 gal	Submerged	88
Clipper™ + Reward®	Flumioxazin + Diquat	9 oz + 0.25 gal	Foliar	48
Clipper™ + Reward®	Flumioxazin + Diquat	9 oz + 0.5 gal	Foliar	53
Clipper™ + Reward®	Flumioxazin + Diquat	9 oz + 0.25 gal	Submerged	77
Clipper™ + Reward®	Flumioxazin + Diquat	9 oz + 0.5 gal	Submerged	85
Clipper™ + Reward®	Flumioxazin + Diquat	12 oz + 0.25 gal	Foliar	70
Clipper™ + Reward®	Flumioxazin + Diquat	12 oz + 0.5 gal	Foliar	62
Clipper™ + Reward®	Flumioxazin + Diquat	12 oz + 0.25 gal	Submerged	82
Clipper™ + Reward®	Flumioxazin + Diquat	12 oz + 0.5 gal	Submerged	85

* measured 30 days after treatment

Utilizing information from the small-scale trials conducted in 2011 and 2012 along with other published efficacy data on other common herbicides, larger treatment trials were conducted on an isolated canal off Caddo Lake.

Treatments were applied in bands across the canal at the rates noted in Table 3 and the percent of salvinia control observed 14, 33 and 70 days after treatment were documented. Costs per acre were also calculated for each applied treatment in this trial. Table 3 presents these data for each treatment combination. Costs range widely



Canal where on-lake chemical trials were conducted

among treatment options as do treatment effectiveness. For example, the product Galleon is applied in the water and is absorbed by the plants roots. It has proven highly effective in small, closed containers and stagnant ponds, but it is not nearly as effective and may be totally

ineffective in larger water bodies where current rapidly disperses the herbicide. As such, the cost effectiveness of each combination cannot be directly compared in all cases and should be carefully considered before selecting the best treatment option. As always, herbicides should be applied in accordance with their label to achieve the published effectiveness levels.

Table 3. On-lake herbicide demonstration results

Treatment Mixture		Concentration	Application Rate	Cost / Acre	% Control for On-Lake		
Trade Name	Active Chemical				14 DAT*	33 DAT*	70 DAT*
Acquamaster	Glyphosate	5.4	3 qt/ac.	\$68.00	90	95	85
Reward	Diquat	2	1 qt/ac.				
AQUAKING	N/A - adjuvant	100	1 qt/ac.				
Thoroughbred	N/A - adjuvant/nonionic surfactant	100	100 fl oz/ac.				
Clipper	Flumioxazin	51	6 oz wt/ac.	\$74.00	85	85	85
Aquathol K	Endothall	4.23	16 oz/ac.				
Agridex	N/A - nonionic surfactant	100	0.5% v/v				
Clipper	Flumioxazin	51	6 oz wt/ac.				
Reward	Diquat	2	1 pt/ac.	\$77.00	85	60	50
Agridex	N/A - nonionic surfactant	100	0.5% v/v				
Clipper	Flumioxazin	51	6 oz wt/ac.				
Reward	Diquat	2	1 qt/ac.				
Agridex	N/A - nonionic surfactant	100	0.5%v/v	\$90.00	90	75	60
Clipper	Flumioxazin	51	6 oz wt/ac.				
Reward	Diquat	2	1 qt/ac.				
Agridex	N/A - nonionic surfactant	100	0.5%v/v				
Clipper	Flumioxazin	51	6 oz wt/ac.	\$78.00	80	98	95
Aquamaster	Glyphosate	5.4	71 fl oz/ac.				
Agridex	N/A - nonionic surfactant	100	0.5%v/v				
Clipper	Flumioxazin	51	6 oz wt/ac.				
Clearcast	Imazamox	1	1 qt/ac.	\$104.00	70	65	65
Agridex	N/A - nonionic surfactant	100	0.5% v/v				
Clipper	Flumioxazin	51	15.68 oz wt/ac.				
Aquamaster	Glyphosate	5.4	71 fl oz/ac.				
Agridex	N/A - nonionic surfactant	100	0.5% v/v	\$169.00	80	98	98
Galleon	Penoxsulam	2	2 fl oz/ac.				
Agridex	N/A - nonionic surfactant	100	0.5% v/v				
Galleon	Penoxsulam	2	5.6 fl oz/ac.				
Agridex	N/A - nonionic surfactant	100	0.5% v/v	\$16.00	45	50	65
Generic	Glyphosate	4	4 qt/ac.				
Thoroughbred	N/A - adjuvant/nonionic surfactant	100	12 fl oz/ac.				
Generic NIS	N/A - nonionic surfactant	100	1 qt/ac.				

* DAT = Days After Treatment

Trials were conducted on and off the lake with initial trials carried out in 30-gallon plastic tubs located near the weevil rearing facility. In this setting, conditions were controlled and salvinia's response to applied treatments was easily observed. Demonstrations of the most effective combinations were subsequently carried out on Caddo Lake in an isolated canal where other treatments were not being conducted.

In isolated areas such as the treatment containers and in small farm ponds, complete control of giant salvinia using herbicides has been observed. Multiple applications are often required to attain complete control even in these smaller water bodies. Effectively treating large lakes is more difficult due to size and accessibility. Repeated treatments on larger water bodies are also warranted as freely floating pieces of giant salvinia are easily missed; using a spray marker minimizes this risk. Caddo Lake is one such water body where effective herbicide treatment will likely never be achieved due to its large and relatively inaccessible backwater areas. Not only is it difficult to get into and treat these areas, but they provide ample opportunity for giant salvinia to hide or be shielded from applied herbicides. Costs often become a concern with herbicide treatments. In smaller areas, chemical and application costs may not be prohibitive; however, on larger water bodies this may not be the case. Despite their effectiveness, herbicides may not be the logical or desired treatment choice due to their costs yet they remain an effective tool for treating giant salvinia that should be considered.

Large-Scale Giant Salvinia Treatment

Following the drought of 2011, giant salvinia coverage in Caddo Lake increased exponentially and several thousand acres of salvinia blanketed the lake by early may. Large-scale giant salvinia treatment was carried out to address this expansion. Through a public bid process, Houston Spraying & Supply, Inc. was hired and commenced spraying on May 7, 2012. Spraying was completed on June 29, 2012 and in total, 348 hours of spraying was completed effectively treating about 700 acres at a cost of \$64,936.80 or an approximate cost of \$93/acre. This included the cost of herbicides and surfactants, application of herbicides, and all personnel and equipment costs incurred by the contractor. Spraying was conducted in concert with other spraying and biological controls being carried out by CVND, TPWD and the CISE program team to prevent duplication of efforts and damage to weevil release areas.



Non-treated giant salvinia mat covered with sedges



Dying giant salvinia and sedges post-chemical treatment

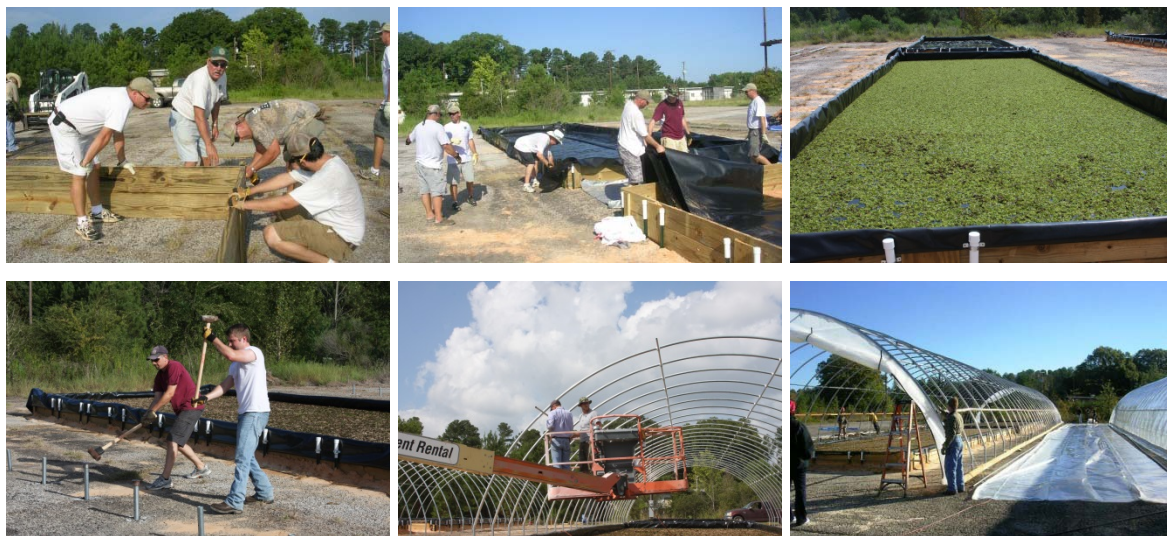
While the treatments were effective, they came at a great cost and only slightly delayed the advance of giant salvinia. This and other spraying campaigns and the resources expended opened the eyes of local residents and stakeholders to the need for a more sustainable approach to controlling giant salvinia.

Biological Treatment and Evaluation

Investigating, demonstrating and building capacity for biological control of giant salvinia was a critical goal of the program from its outset. Through the Center, numerous objectives building toward that goal were implemented and demonstrated great success. These included building capacity to raise weevils at Caddo Lake and investigating weevil effectiveness by determining factors that limit it and evaluating means to increase it.

Constructing a Weevil Rearing Facility

Initially, efforts focused on constructing a weevil rearing facility near Caddo Lake to supply weevils for deployment on the lake and meet the needs for associated research endeavors. Working closely with TPWD on the facility's design and construction, the CISE Team built four 15'x48' shallow tanks enclosed by two cold-frame style greenhouses at the Caddo Lake National Wildlife Refuge. Construction began in August 2010, and tanks were filled with water and salvinia before the end of the month. Greenhouse construction began at the end of September with the frames being completed that month and the skin being installed in early November prior to the first freeze thus allowing weevil populations to overwinter. Construction was a team effort with multiple parties contributing manpower and resources to the effort. CISE and TPWD personnel led the effort and received invaluable support from the Caddo Lake Institute, the Caddo Lake National Wildlife Refuge and local volunteers from around the lake. The construction process, materials and facility design are all discussed in detail in Chapter 4 of the "Guide to Mass Rearing the Salvinia Weevil for Biological Control of Giant Salvinia" (Knutson and Nachtrieb 2012) produced through this program.



Clockwise from top left: CISE, TPWD and local volunteers: constructing weevil rearing tanks, installing pond liners, filled tanks, driving greenhouse legs, erecting greenhouse frames, installing greenhouse skin

Salvinia Weevil Production

Once completed, the focus of the weevil rearing facility was placed on producing as many salvinia weevils as possible. In 2011, the first full year of production, an estimated 100,000 adult weevils were produced and released onto Caddo Lake at four release sites. Estimated production declined in 2012 to 85,400 adults and further decreased to about 42,000 adults in 2013. No weevils were released in 2014 prior to the end of this program. Water quality issues caused the decline in production as the source and quality of water used to fill the tanks changed over time. Initially, an agreement with the local volunteer fire department to haul lake water to the facility on a periodic basis using their tanker truck was in place. When a new emergency services district was established, it absorbed the volunteer fire department and water delivery was no longer an option. Groundwater from the Refuge then became the primary source of water, supplemented with small amounts of lake water. Dissolved solids levels in the well water were higher than the lake water and suppressed the growth of salvinia and subsequently the salvinia weevil in the tanks. Regardless, weevil production was still sufficient to supply weevils for planned research on Caddo Lake. Operational protocols were developed for the facility and are included in Chapter 4 of the “Guide to Mass Rearing the Salvinia Weevil for Biological Control of Giant Salvinia” (Knutson and Nachtrieb 2012).

Salvinia Weevil Research

Researching various aspects of biological control at Caddo Lake was also a primary focus of the program. The salvinia weevil has been used successfully in the U.S. for more than 10 years; however, locations where effective control has been seen are near the coast. Caddo Lake lies in a temperate region that experiences wider temperature extremes, especially freezing temperatures, than coastal locations and thus earlier giant salvinia control attempts using the salvinia weevil at Caddo Lake have yielded limited success. Thus evaluating the cold tolerance of the salvinia weevil received considerable attention. A series of experiments were conducted to achieve this goal and included a chill-coma recovery-time trial, survival time when exposed to 0°C and thermal sensitivity of the weevil regarding egg production.

Cold Tolerance of the Salvinia Weevil

The salvinia weevil's cold tolerance was evaluated using chill coma and survival time experiments conducted in a laboratory setting. In the chill coma test, the time required for

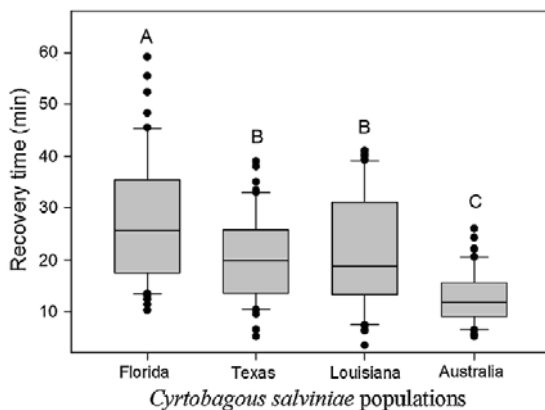


Figure 1. Chill coma recovery time reported by Mukherjee et al. 2014. Differing letters denote statistically significant recovery time differences

weevils to recover from a temperature-induced, coma-like state was measured by subjecting 50 adult weevils from each of four populations from New South Wales, Australia; Houma, LA; Lewisville, TX; and West Palm Beach, FL. Each population was divided into lots of 10 and subjected to exposure at 0°C for 4 hours to induce a chill coma. The time for weevils to recover from this coma and stand up was recorded.

Results indicated that mean recovery times significantly differed between populations with Australian weevils recovering fastest followed by those from Louisiana, Texas and Florida respectively (Figure 1) (Mukherjee et al. 2014).

Salvinia weevil survival time was also evaluated using weevils from the same populations. Five replicates of 10 weevils from each population were subjected to freezing temperatures while on a giant salvinia plant in a cup of water held at 0°C. Treatments of 6, 12, 18, 24, and 36 hours were applied. The number of live salvinia weevils in each cup was counted after 24, 48 and 72 hours. Weevils were considered dead if they could not stand upright. Figure 2

illustrates mean survival rates of the four populations during this experiment and in regard to cold tolerance of each population, yields similar results to the chill coma test (Mukherjee et al. 2014).

Effects of Temperature on Weevil Reproduction

Available literature on reproduction of the salvinia weevil, specifically egg laying, suggests that 19°C is the critical water temperature threshold that the weevil not lay eggs below; however, reported laboratory work suggest that weevils exposed to colder temperatures may lay eggs at lower temperatures. This is important at Caddo Lake, as the average water temperature that triggers egg laying not be met until late spring. Determining if this temperature threshold is different for weevils that have been exposed to lower water temperatures could help in identifying weevils that are able to begin their reproductive cycle earlier in the growing season.

Research conducted utilized weevils collected from Lake B.A. Steinhagen near Jasper, TX and subjected them to temperatures of 17, 19, 21, 23 and 25°C in a laboratory setting. These weevils had been exposed to colder ambient water temperatures while on the lake. Results indicated that this weevil population did not lay eggs below 19°C and only laid an average of 0.5 eggs per female per week at 19°C. At temperatures of 21°C and above, egg production averaged 2.0 eggs per female per week and did not vary significantly between temperature thresholds (Mukherjee et al. 2014).

Weevil Mortality Assessments

Cold tolerance of the salvinia weevil was further evaluated on Caddo Lake using salvinia weevils isolated in floating cages. In each of these cages, 20 adult weevils were placed on living giant salvinia and situated on Caddo Lake.

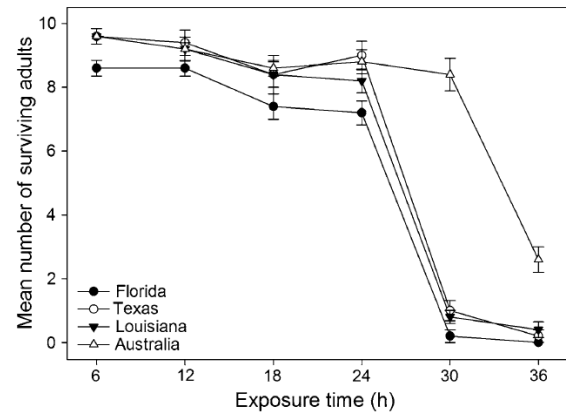


Figure 2. Mean survival of adult salvinia weevils from evaluated populations surviving 72 hours post exposure to 0°C for given durations



Floating weevil cages used in overwintering studies

Identical cages were also placed inside the tanks at the weevil rearing facility. Monitoring began each November and continued until surviving weevils were no longer discovered. Results showed that weevil survival in the cages decreased as the winter progressed. This decrease was more rapid on Caddo Lake than in the more protected greenhouses. Figure 4 illustrates the percentage of weevils surviving during each year's experiment and the stark differences in survival observed between the 2012-2013 and 2013-2014 winters. The first was a very mild winter and the second an abnormally harsh winter. Even with the protection provided at the weevil rearing facility, weevils still succumbed to the colder temperatures.

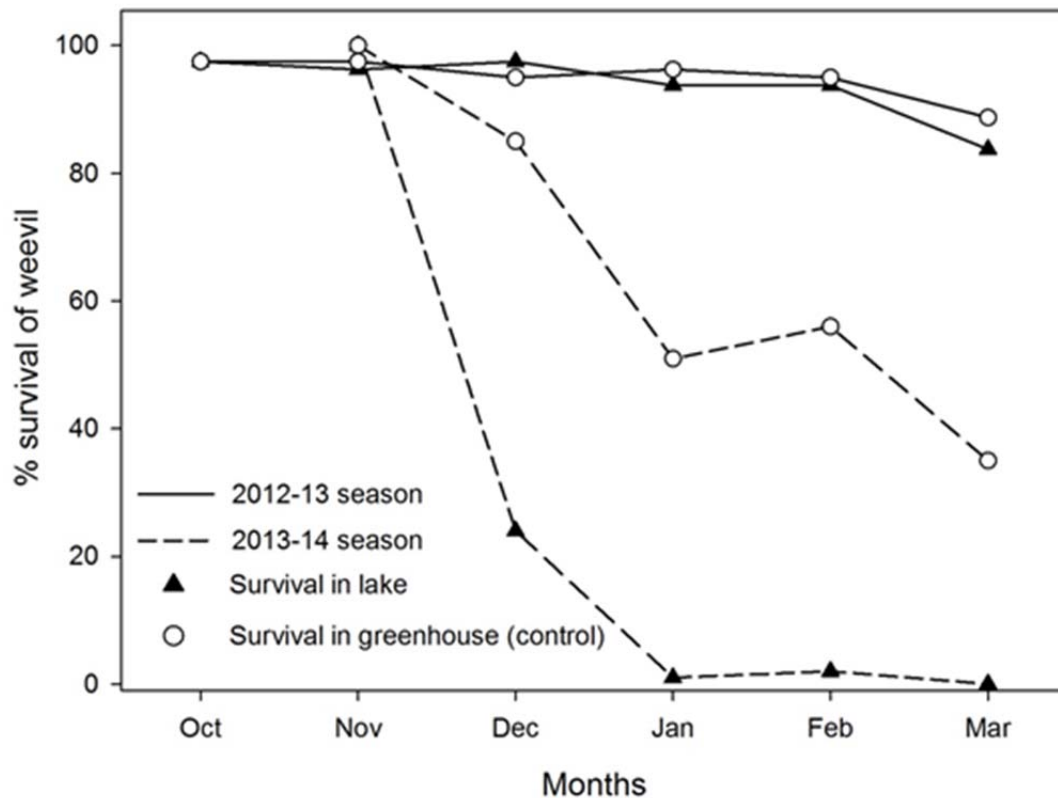
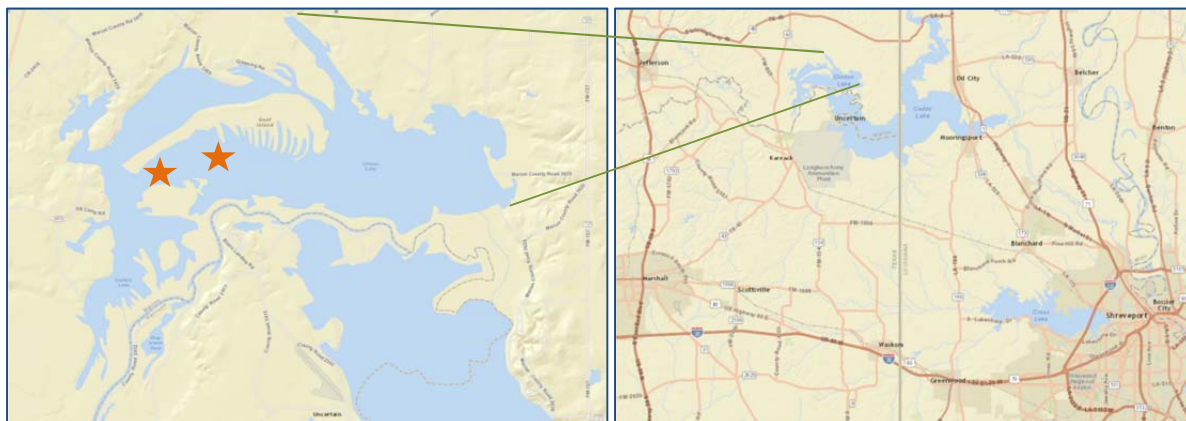


Figure 3. Survival of adult weevils on Caddo Lake and in the weevil rearing greenhouses in the 2012-2013 (mild) and 2013-2014 (harsh) winters

Salvinia Control Following Releases of Salvinia Weevils: 2012 - 2014

As the previously described research proved, cold tolerance is an important factor in the weevil's ability to survive on Caddo Lake. Temperature not only influences survival, but also reproduction and consequently in their ability to control giant salvinia. Laboratory experiments and data from Caddo Lake support these claims. Survival trials conducted and monitored weevil densities in release areas clearly illustrate the response to temperature. The Lone Pine Stretch and Bird Roost areas of Caddo Lake served as the primary weevil release sites during this program and illustrated the effects of favorable weather conditions on released weevil populations and their ability to control giant salvinia.

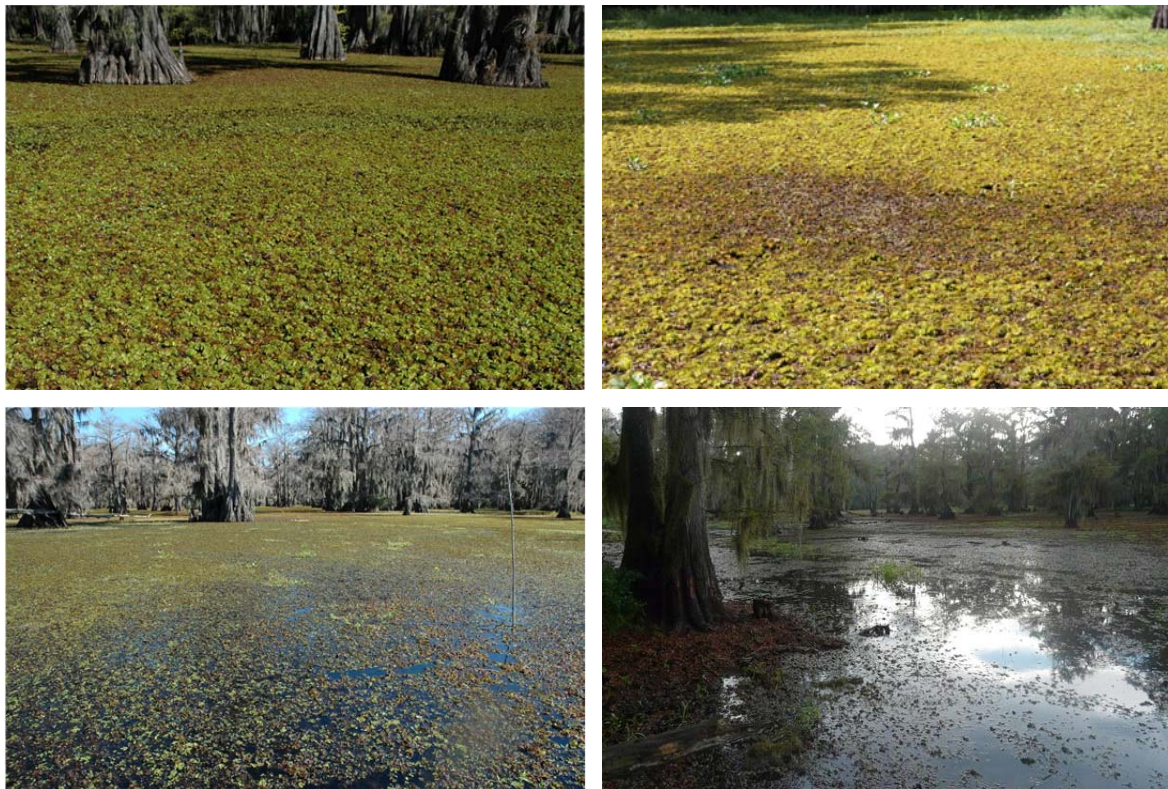


Orange stars denote the Lone Pine Stretch (L) and Bird Roost (R) release sites on Caddo Lake

In May and July 2012, 33,000 adult weevils were released within a 2.5-acre area of a very large salvinia mat in the Bird Roost area of the lake. Monitored weevil densities per kilogram of giant salvinia ranged from 3 to 5 adults from June to August but increased to 22 by September before decreasing to 12 in October when temperatures declined. Further population monitoring through the winter was prevented by low water levels. Weevil numbers were low in the release site in the early spring, suggesting the population was largely lost after the winter; however, reports of possible weevil activity in the area during the fall of 2013 proved otherwise. Upon sampling salvinia at this site in November 2013, a large numbers of weevils (55 weevils per kilogram of salvinia) were found along with severely damaged salvinia when no weevils had been released at this site since July 2012. A survey of this area in late November using GPS points and geographic information systems mapping techniques found that weevils had destroyed

about 10 acres within an extensive salvinia mat infesting the Bird Roost area. The mild winter and normal water levels during the winter of 2012-2013 allowed at least some of the weevils to survive and eventually reach levels high enough during the fall of 2013 to control giant salvinia, thus illustrating the importance of temperature on weevil survival and effectiveness.

In the summer of 2013, 42,000 adult weevils were released in a 0.25-acre area in the Lone Pine Stretch area of the lake. Weevil density and salvinia biomass were monitored each month to track the increase and subsequent decline in numbers and to document the salvinia reduction achieved. In July 2013, weevil densities of 9 adults per kilogram of giant salvinia were present. The population increased each month until November when the density peaked at 45 adults per kilogram. By October 2013, the weevils had reduced giant salvinia biomass in the treated area by 75 percent as compared to the non-treated control area. This reduction yielded areas of open water that encompassed approximately 15 acres. The following winter of 2013-2014 was colder than normal and by January, only 2 weevils per kilogram were found. No surviving weevils were found in subsequent samplings in April and May.



Clockwise from top left: Salvinia mat with no weevils present, early signs of weevil damage to a salvinia mat, damaged salvinia mat in Lone Pine Stretch (August 2013), open water with minimal salvinia present at the Bird Roost site (November 2013) one year after weevil releases

Managing Giant Salvinia Using Salvinia Weevils

Results demonstrate that salvinia weevils can control giant salvinia at Caddo Lake, but releasing large numbers of weevils is needed following cold winters to maintain weevil densities necessary for effective control. In this study, releasing large numbers of adult weevils (15,000/acre) and associated eggs and larvae in a concentrated area (1-2 acres) in May resulted in the establishment of initial populations. Through natural reproduction, the population expanded and controlled salvinia across 10-15 acres each year. Releasing weevils in the spring or early summer provides more time for weevils to reproduce and increase before cooler weather slows reproduction in October. The winter of 2013-2014 demonstrated that cold winters can greatly reduce weevil populations and additional releases are needed to re-establish effective populations.

Cold winters also kill salvinia plants, and observations suggest that salvinia plants protected by an overstory of trees and vegetation can survive freezing weather. Small salvinia plants appear to originate from these sites in the spring and can be carried by high water levels and currents to other parts of the lake. Weevils need salvinia plants for food and shelter during the winter, and these sheltered places provide a site where both the plant and weevil are more likely to survive and persist until spring when salvinia and weevils begin to reproduce. Thus, biological control using weevils is probably most cost-effective when used to target salvinia in these swamps. Weevil populations, either overwintering or resulting from current years releases, can survive and increase in these protected sites to expand their impacts later in the growing season. These swamp areas are also generally inaccessible to spray boats and thus accidental spraying of herbicides on weevil release sites is reduced.

Salvinia management at Caddo Lake will likely continue to consist of biological control using weevils and the use of herbicides to control salvinia infestations along boat roads and around docks, etc. A succession of warm winters will favor biological control while increased herbicidal control will be needed following cold winters until weevil populations are re-established. Results reported by this program demonstrated that weevil populations from Australia are more cold resistant than U.S. populations. Future studies to further evaluate weevils from Australia or from cold regions in Argentina (within the native range of the weevil) are needed to determine if they can be more effective than the current weevil strain. If so, weevils

collected from these populations could be imported into the U.S. for salvinia control in the future. In the interim, this project demonstrated that biological control achieved by the production and release of the salvinia weevil is an effective management practices and along with targeted herbicide applications, can be successfully implemented at Caddo Lake.

Chemical Effects on Weevil Survival

Research findings from weevil density studies led to the need to explore ways to provide a competitive advantage to weevils over giant salvinia. A potential method is to pair biological and herbicide control. If herbicide treatments can be used early in the growing season to allow the density of weevils present to be artificially raised without harming the weevils, then the impacts of the weevils can theoretically be amplified.

The impacts of the chemical and surfactant mixture on weevil survival came into question and were thus evaluated. A mixture of glyphosate (3 qt.), diquat (1 qt.), an adjuvant (1 qt.) and a non-ionic surfactant (20 fl. oz.) is used per acre to treat giant salvinia infestations. The contact toxicity to adult weevils of each herbicide and surfactant was tested alone and as a mixture using eight treatments. In a preliminary trial, eight groups consisting of 10 adult weevils each were isolated and sprayed with one of the treatments. A ninth group of weevils was kept as a control. Results illustrated that weevil mortality rates between the treatment groups and control group were not significantly different, suggesting that little or no impact would be expected to adult weevil survival if they were sprayed. However, further evaluation is needed to vet these findings. Weevil pupae, larvae and eggs were not evaluated in this preliminary trial as they are found inside the plant or below the water surface, and thus would not be directly contacted by the herbicide. However, the eggs and larvae would likely die once the salvinia plant was killed by herbicide.

Assessing Salvinia Weevil Mobility

Weevil mobility from dead or dying giant salvinia to nearby healthy plants was questioned as a result of the previously described chemical effects study. Weevils are not commonly found to have the ability to fly and thus rely on walking as their primary means of travel. Weevils are also known to swim as well, but the distances they can traverse are not clear.

Small-scale evaluations of the weevil's ability to move from a dying to thriving plant were carried out to investigate this question. A mesocosm experiment was conducted to determine if adults can move from herbicide-treated plants to untreated healthy plants. Plastic pools (5' diameter \times 1' depth) were filled with lake water, and salvinia plants were placed inside each pool until a single layer of plants covered 100% of the water surface. The experiment consisted of two treatments, herbicide-treated and a non-treated control, each replicated five times. A diquat herbicide, Reward[®] (0.5 % v/v) mixed with a nonionic surfactant Aqua-King[®] (0.5% v/v), was chosen as it is a contact herbicide and induce rapid salvinia killing. Treatments were applied using a 5-liter hand sprayer applied evenly on the plant surface. A 6-inch layer of plants around the perimeter of the pools was not treated with herbicide to provide a refuge and food source where dispersing adults would congregate for sampling. Fifty *C. salviniae* adults were released at the center of each pool one hour after application of treatments.

The number of weevils dispersing from the center of the pool to the perimeter was estimated by sampling all of the live plants along the perimeter of the pool at weekly intervals. Harvested plants were replaced with fresh plants. Findings from this preliminary trial illustrated that weevils were able to move short distances (up to 2.5 feet) from dying plants to living plants. Since herbicide treatments rarely kill all of the giant salvinia present in a treated area or could be applied in narrow bands, this suggests adults could survive by moving to nearby living plants.

Combining the results of these evaluations suggest that the paired use of biological and chemical controls can work. On-lake demonstrations were planned to demonstrate this approach but were not completed prior to the program's end date, due to the lack of giant salvinia on the lake following the 2013-2014 winter. Thus, the distance that adult salvinia weevils can effectively travel remains unknown and should be further evaluated.

Evaluating Other Treatment Methods

Exploring methods to treat giant salvinia other than biological and herbicidal means was also goal of the program. Initially, use of other means to control giant salvinia in previous studies was investigated to determine their viability on Caddo Lake. Two approaches used in similar settings are lake draw-downs and mechanical harvesting.

Lake Draw-down

In Louisiana, the Department of Fish and Wildlife has used lake draw-downs at Lake Bisteneau for several years. This approach has successfully reduced the amount of giant salvinia present in the lake at the time of the draw-down but has not eliminated the problem. Adverse impacts of this approach seem to outweigh the positive ones. A draw-down effectively empties a lake, thus removing much of its aquatic life and hopefully the invasive plant species too. Theoretically, unwanted plants left in the lake will be stranded and die due to lack of water. Giant salvinia can cope with this scenario, as it can survive indefinitely in moist soil given favorable weather conditions. As a result, the lake must be kept empty for an extended period, or the remaining giant salvinia must be sprayed prior to refilling the lake. The U.S. Army Corps of Engineers has observed similar results at Lake B.A. Steinhagen in southeastern Texas.

At Caddo Lake, a draw-down is not feasible at this time. No water level control device exists on the outfall of the lake, thus the lake's level cannot be purposefully reduced below its typical full level. Even if Caddo's level could be reduced, the vast Bald Cypress forests present in the lake would certainly retain numerous pockets of giant salvinia that would likely remain viable. Additionally, the adverse impacts to the lake's diverse aquatic life community are also viewed as unacceptable by many; however, some positive benefits would occur as well.

Mechanical Removal

Mechanical harvesting is another approach to control aquatic invasive plants. These machines physically remove the plant from water via a conveyor and transport harvested plants to shore where they are unloaded and disposed. While highly effective for rooted plant species, floating plants pose more of a challenge as they can move away from the harvester. Despite this, large volumes of floating species can be harvested. Caddo Lake poses other challenges as its

shallow water depths, numerous stumps and dense Bald Cypress stands may slow or prevent mechanical harvesters from accessing much of the giant salvinia present. Modern machines have features that can overcome many of these obstacles, but the harvester's efficiency is certainly reduced. The major benefit of mechanical harvesting is the immediate removal of targeted vegetation from the treated area.

In a mechanical harvester trial conducted by the Caddo Lake Institute (CLI) in 2009, mechanical harvesting was demonstrated and found to be feasible in portions of Caddo Lake, especially in more open areas with easy access. Based on observed harvesting rates, CLI estimated that the harvester utilized could collect an acre of vegetation in a little as 2.1 hours under ideal conditions. Daily operation and maintenance costs for the harvesting unit demonstrated were estimated at \$350.70 per acre. Compared to other methods, this cost is much higher, making this treatment option less feasible. However, mechanical treatment should not be ruled out based on cost alone. It is an effective treatment option that should be considered depending on the treatment goals and objectives.



Mechanical harvester unloading harvested aquatic vegetation at Caddo Lake (photo courtesy of CLI)

Removal by Hand

In some cases, physical removal of giant salvinia by hand is preferred. Cases where this is especially effective are small water bodies or in isolated area where only a few plants exist. Giant salvinia is often transported from one location to another inadvertently on boats and boat trailers. When a boat is loaded from a water body with salvinia, plants can be trapped between the boat and trailer or simply cling to the trailer. Removing plants prior to the next use of the boat eliminates the threat but is difficult considering that the boat must be lifted off the trailer to ensure that all plants are removed. Failure to completely remove the plants can allow salvinia to float off the trailer the next time it is launched. Giant salvinia can live for an extended period out of water, thus increasing this risk. As such, it is imperative that boats and trailers are cleaned thoroughly to remove any plant matter after loading out of a lake. Several times in recent years, a few plants have been spotted floating near boat ramps at Lake O' the Pines and were quickly removed by lake rangers. If not caught near instantly though, removing these plants by hand becomes practically impossible.

Education and Outreach

Raising awareness about the threat and impacts of giant salvinia and other invasive species was a significant focus of the program. Invasive species are commonly spread due to the actions of humans. In many cases, they are unintentional and result from a general lack of awareness. Through this program, general and targeted information was developed and delivered to audiences via numerous avenues. Existing information developed by other entities was also promoted and distributed to further expand invasive species knowledge at Caddo Lake, in the lake's vicinity, statewide, regionally, nationally and internationally.

Local Signage

The weevil rearing facility at the Caddo Lake National Wildlife Refuge in Karnack provided an excellent opportunity to educate the public about giant salvinia and other aquatic invasive species. The greenhouses are located adjacent to the Refuge Visitor Center and provided ready access to curious visitors. Since Center staff was commonly not present when visitors arrived, signs were developed to provide a general overview of giant salvinia, weevil biology and the weevil rearing facility as well as other invasive species threatening Caddo Lake.

cise.tamu.edu

CENTER FOR INVASIVE SPECIES ERADICATION

cise.tamu.edu

SALVINIA WEEVIL REARING FACILITY


Putting Biological Control to Work to "Eradicate" Giant Salvinia at Caddo Lake



Giant salvinia (*Salvinia molesta*) is a floating aquatic fern native to Brazil that grows rapidly in warm, slow-moving water. It can double its coverage area in as little as 7 days, forming dense mats that block sunlight, degrade water quality and displace native aquatic vegetation, fish and wildlife.



The salvinia weevil is an effective biological control agent for giant salvinia and only feeds on salvinia species, all of which are not native to the United States. Adult weevils are 1.5 to 2.2 mm long and can live up to 250 days with females producing 148 to 383 eggs in their lifetime.




What are we doing here?
Giant salvinia is being grown in four 15 ft x 48 ft tanks and inoculated with adult weevils. When weevil populations reach sufficient numbers (approximately 128 days), infested plant material and weevils will be harvested from these tanks and introduced to control giant salvinia infestations on Caddo Lake and other affected reservoirs.



Salvinia weevils feed on terminal buds of the plant, creating cavities within the plant where females lay their eggs. Larvae emerge from the eggs about 10 days later and begin eating through the terminal buds and rhizomes of the plant ultimately damaging the plant so that it dies, decays and eventually sinks to the lake's bottom.



Giant salvinia is currently present in 11 states threatening all uses of aquatic resources where found. Due to the aggressive growth of giant salvinia, people should be vigilant in their efforts to prevent the spread of this invasive species. Possession of giant salvinia is illegal and punishable by fines of up to \$500 per plant. Report giant salvinia infestations at: texasinvasives.org.



The Center for Invasive Species Eradication was developed through support from Senator Kay Bailey Hutchison and U.S. Department of Agriculture, Natural Resource Conservation Service. The Center is administered by Texas AgriLife Research and the Texas AgriLife Extension Service and managed by the Texas Water Resources Institute. Center collaborators include the Caddo Lake Institute, Caddo National Wildlife Refuge, Texas Parks and Wildlife Department, the U.S. Army Corps of Engineers and the U.S. Fish and Wildlife Service.

Informational sign located between the Caddo Lake National Wildlife Refuge Visitors Center and the CISE Weevil Rearing Facility informing readers of giant salvinia, the salvinia weevil and CISE program efforts

Online Information

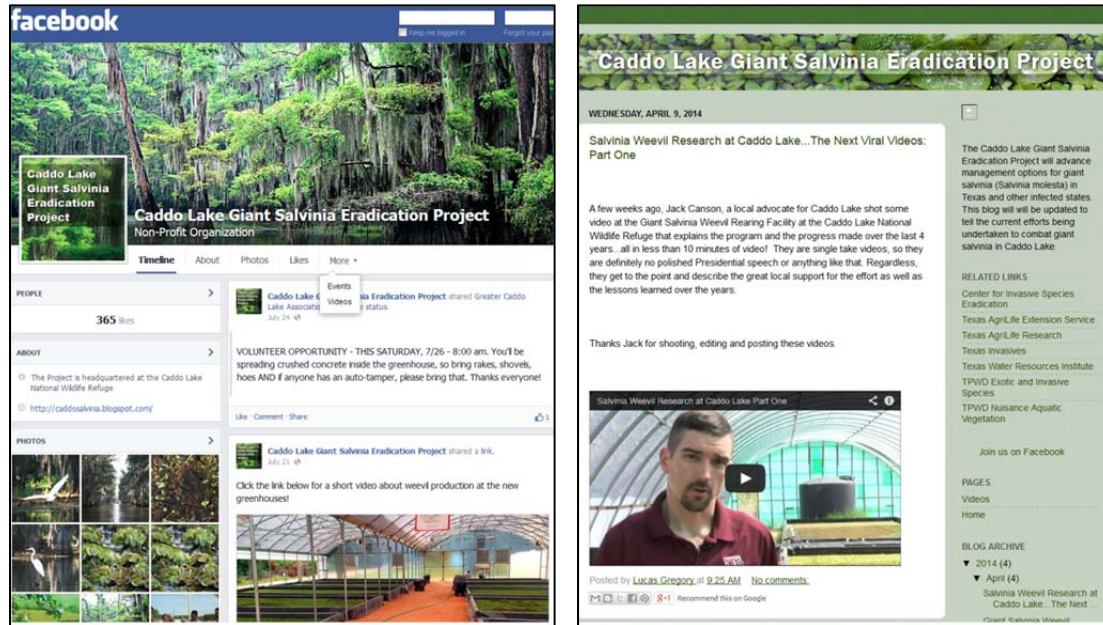
Internet-based resources and platforms were widely used to expand the reach of education and information regarding program activities, giant salvinia, efforts to control giant salvinia at Caddo Lake and other relative information of interest to readers. Initially, a program website was established to not only house program information but to also serve as a platform for the world to access this information. The website houses information on the program, its partners, media mentions regarding the program, educational materials produced, program reports and links to partner websites. During the course of the program, this website was accessed 3,015 times by 2,370 users. The website can be accessed at: <http://cise.tamu.edu/>.

Websites are passive tools to get information to the public and, as such, may not reach the targeted audience as effectively as an active tool. To provide more timely information to the public, the Caddo Lake Giant Salvinia Eradication Project blog and Facebook pages were created. The blog and Facebook pages both went online in January 2011. Blog posts focused on telling the story of activities at the weevil rearing facility, conditions on the lake and efforts to reduce salvinia levels on Caddo. Followers of the blog receive email notifications when new posts are added and can share and comment on each article. The Facebook page provided an even more active platform to reach a wide audience. Photos, meeting notices and links to other online information made up the common content posted to the Facebook page. Essentially everything related to Caddo Lake, giant salvinia and aquatic invasive species was posted and disseminated through this avenue.

These avenues proved especially successful and greatly expanded the reach of information posted on these sites to audiences that would not have been exposed otherwise. In total, the blog generated 23,680 page views and continues to receive traffic; the Facebook page currently has 365 likes and reached 40,948 users. Despite the end of the program, these resources will continue to be updated with appropriate information as it materializes. The blog is available at: <http://caddosalvinia.blogspot.com/> and the Facebook page can be accessed via: <https://www.facebook.com/caddo.salvinia>.

Videos regarding giant salvinia, the threat it poses, mechanisms for transport, treatment strategies, calls for increased awareness of the plant and not to transport it from one site to

another, and research progress were all posted on these outlets and promoted as well. Videos developed by the CISE team and others such as CLI, TPWD and many more were posted. In total, the videos promoted have garnered 23,880 views and counting.



Screen shots of the Caddo Lake Salvinia Eradication Project Facebook page and blog

Printed Resources

Development and dissemination of printed resources also played a critical role in spreading the word about giant salvinia at Caddo Lake and other nearby water bodies. CISE personnel from the Texas A&M AgriLife Extension Service and TWRI worked with CLI, NRCS, TPWD and other agencies to enhance existing and develop new printed education and outreach resources. They focused on proper ways to identify and report giant salvinia infestations, providing a general description of the plant and the threat it poses to water bodies and described treatment strategies that can be used to effectively control the problem. Conveying the importance of prevention was also a critical point made in printed materials. One publication that focused its attention on these issues is a tri-fold brochure entitled “The Pond Destroyers: Common and Giant Salvinia” (TWRI 2012). It was widely distributed near Caddo Lake and across the state and is included in Appendix A.

News releases also served as a significant means of information dispersal locally, regionally, and nationally. Releases were developed by the CISE team covering critical topics, and many local reporters often requested interviews regarding program efforts. News releases are posted on the program website and were linked to via the Facebook page. In total, 12 news articles were developed primarily by the CISE team and are posted on the website. An untold number of additional news releases were also developed by local, regional and national news outlets that include information on giant salvinia and efforts to control it at Caddo and other lakes. These stories were linked to and disseminated via the program's active online platforms.

Presentations, Meetings and Tours

Perhaps the most effective tools, at least for garnering and capturing the attention of local residents, were presentations and discussions held at local meetings. A close-knit community surrounds Caddo Lake, and its residents are deeply connected to this unique natural resource. Research and demonstration findings conveyed paired with their own personal observations of giant salvinia on Caddo Lake greatly influenced their vision for effective giant salvinia control. Initially, presentations focused on describing the CISE program, its goals and objectives, and discussing general salvinia biology and management. As research progressed, results and findings comprised more of the presentation content delivered. Ultimately, this platform evolved into discussions with local groups regarding ways that local resources could be put toward rearing weevils on the shores of Caddo Lake. As a result, the Morley Hudson Weevil Greenhouse was built in Karnack and now supplies weevils to Caddo Lake. Groups commonly met with include the Greater Caddo Lake Association of Texas, local chapters of Texas Master Gardeners and Texas Master Naturalists, the Natural Plant Society, the Dallas Caddo Club, the Shreveport Fly Fishing Club, Cypress Valley Navigation District and others. Local government units such as Commissioner's Courts, City Council Meetings, river authorities and water districts were also visited.

Educational delivery to classes from area schools was also a key avenue of educational delivery. Students from Karnack High School, Marshall High School and the Collins Academy routinely visited the weevil rearing facility to learn about giant salvinia and the salvinia weevil. Presentations on invasive species, control options and related information were routinely provided at the Marshall Independent School District's "Ag Day" and selected classes as well.

Other groups made up of grade-school-aged students were also engaged. Local chapters of the Boy Scouts of America and the Youth Conservation Corps both visited and volunteered at the weevil rearing facility at Caddo Lake National Wildlife Refuge while local 4-H chapters were also visited.

Technical presentations were also made at a number of conferences across the state and nation. In Texas, both poster and oral presentations were given annually at the Texas Aquatic Plant Management Society meetings and the Texas Invasive Plant and Pest Conference. Broad-scale exposure was achieved through presentations at the Annual Aquatic Plant Management Society meetings held in different locales across the U.S. from 2011 to 2014.

Tours of the weevil rearing facility and the giant salvinia infestation at Caddo Lake were often requested and given. Anyone stopping by the Refuge requesting a tour was obliged when CISE Team members were present. Large events such as those associated with visits from elected officials were also held and coordinated with the CISE Team, CLI, TPWD, USFW and other local entities to maximize the effectiveness of the tours given. During these tours goals, objectives, operations and findings of the CISE program were discussed along with giant salvinia's impacts to Caddo Lake. These tours served as a means for participants to see the perils of giant salvinia first hand and realize the impacts it has on Caddo Lake.



From left: U.S. Congressmen John Fleming and Louie Gohmert, U.S. Senator Kay Bailey Hutchison and the CISE Team, then State Representative Elect Chris Paddie, U.S. Congressman Louie Gohmert and Staff touring Caddo Lake and the weevil rearing facility

Peer Reviewed Research Findings

Biological control of giant salvinia is widely used across the globe. A large and diverse scientific community routinely works to advance the science behind biocontrol application. Production of peer-reviewed literature is the best means for distributing information to this community. Through the program, the work on weevil cold tolerance studies was published in the journal *BioControl*. The paper, entitled “Biological control of giant salvinia (*Salvinia molesta*) in a temperate region: cold tolerance and low temperature oviposition of *Cyrtobagous salviniae*,” was authored by Dr. Abhishek Mukherjee and Dr. Allen Knutson of the CISE team as well as Dr. Daniel Hahn from the University of Florida and Dr. Kevin Heinz from Texas A&M University. This paper builds upon the existing body of literature regarding the use of the salvinia weevil in temperate environments. A copy of this paper is included in Appendix A.

A second manuscript regarding overwintering survival of the salvinia weevil is also being developed by Dr. Mukherjee and Dr. Knutson but has yet to be submitted. Once completed, this second document will further add to the scientific contributions achieved through this program.

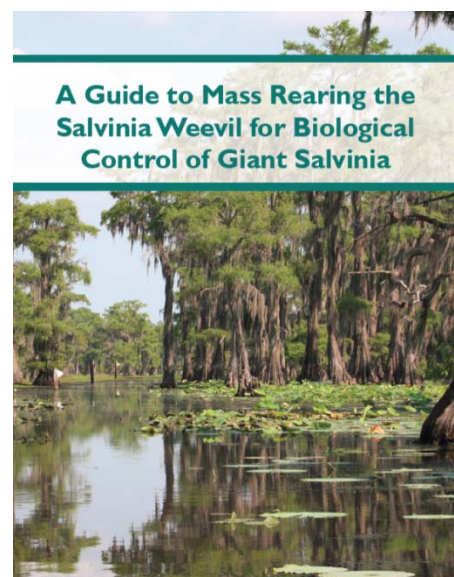
Developing Guidelines

Developing written treatment guidelines that anyone planning giant salvinia treatments can use was also a goal of the CISE program. Using research findings produced through the program, several documents were produced to guide readers through the process of dealing with giant salvinia. Two documents focus on proper identification of giant salvinia and other aquatic vegetation while two others focus on using the salvinia weevil as a biological control.

Proper identification of an invasive species is the first step in selecting the appropriate treatment strategy. To meet this need, “Aquatic Vegetation Identification Cards” (Masser 2013) developed by the Texas A&M AgriLife Extension Service were updated, printed and distributed in the Caddo Lake vicinity and statewide. Extension Specialists continue to distribute this publication at relevant meetings, and it can be found at the Texas A&M AgriLife Extension Bookstore as well. A tri-fold publication mentioned earlier, “The Pond Destroyers: Common and Giant Salvinia” (TWRI 2012), was developed by the CISE Team and NRCS. This resource provides general information on common and giant salvinia, identification information, an overview of the problems they cause, their primary mechanisms of spread, and what to do when an infestation is found. The tri-fold was widely distributed and left at area businesses for patrons to pick up, was distributed statewide and is available on the program website. It is also included in Appendix A.

Weevil Rearing Guide

In association with the establishment of the weevil rearing facility, weevil rearing protocols were developed so that anyone wanting to operate a facility could do so in a similar fashion. Initially, a protocol was developed for the CISE facility only. This was later expanded upon and aggregated with other facility descriptions and operational procedures. Cooperating with multiple agencies and entities, CISE team members led the development of this document, which is titled “A Guide to Mass Rearing the



Salvinia Weevil for Biological Control of Giant Salvinia” (Knutson and Nachtrieb 2012). This document provides detailed descriptions of giant salvinia, salvinia weevil biology, biological control of giant salvinia, water quality, facility design, materials and methods utilized, the procedures implemented at each individual facility reviewed, and costs for establishing and operating each facility. It is available online at:

http://cise.tamu.edu/media/355740/gs_manual_10-15-12.pdf

Biological Control Treatment Description

Furthering the use of the salvinia weevil as a biocontrol agent was also a focus of the program and was accomplished through the development of a ‘how-to’ guide for implementing biological giant salvinia control on small ponds. This document, entitled “Biological Giant Salvinia Management on Small Ponds,” was developed in the style of a NRCS Conservation Practice Standard but is not a NRCS-approved practice. This document provides practical information on the use of salvinia weevils to control giant salvinia in small ponds. Specific information covered includes conditions where the practice applies, application criteria, considerations that should be made and appropriate planning steps that should be carried out prior to acquisition and release of salvinia weevils. It also outlines a step-by-step process for planning use and acquiring, applying and monitoring weevil-treated giant salvinia. This document is available on the program website at: <http://cise.tamu.edu/caddo/publications/> and is included in Appendix A.

Program Conclusions

Collectively, this program produced a number of useful outcomes but further illustrated the gravity of giant salvinia infestations. Biological controls in the form of the salvinia weevil and herbicide trials both proved that giant salvinia can be controlled; however, challenges remain with both approaches. Education and outreach was a focus of this program, and its delivery highlighted the need to further spread the word about the threats of giant salvinia and other invasive species. Prevention of new invasions is the best management strategy; however, if people cannot identify invasive species and understand the threats that they pose, the likelihood that they will take action to prevent the spread of this and other invasive species is minimal.

Biological control was extensively evaluated through this program and shows great promise as an effective alternative to herbicides. Costs, logistics and social concerns can all erode the desirability of herbicide treatments while making the salvinia weevil more attractive. Weevils are not without their faults though. Cold tolerance of the salvinia weevils is currently the main issue hindering their effectiveness. The weevils are a tropical species and have not adapted to the harsh winters that Caddo Lake periodically experiences. When these events occur, they are unlikely to survive. Timeliness is also an issue for weevils. They do not provide the near immediate results that herbicides and mechanical treatments provide but may one day provide sustainable management for giant salvinia. For now, the long-term effectiveness of weevils at Caddo Lake remains in question, but the need to continue supplementing the current weevil population remains.

Herbicide trials, demonstrations and treatments conducted through this project illustrated that a number of chemical combinations can effectively kill giant salvinia. Costs for these treatments range widely though and may make their use impractical for large salvinia infestations. Spraying herbicides is also faced with logistical challenges, especially in lakes like Caddo where dense woody vegetation, underwater hazards and shallow water make spraying near impossible in some areas. As a result, not all giant salvinia can be killed by spraying in this situation thus allowing the problem to persist. Herbicides do have great utility though and remain a viable treatment option that should be considered when planning a treatment.

Potential to integrate treatment options was also discovered through this program and warrants future research to determine its feasibility. Preliminary findings suggest that the commonly used chemicals in herbicide treatments do not adversely affect weevils and thus using the two in concert may give weevils a competitive advantage over giant salvinia and aid in establishing weevil populations within dense giant salvinia mats. Further research is needed though to confirm this and assess its effectiveness.

Ultimately, this program did advance the understanding of giant salvinia and multiple treatment options. No clear solutions to this problem were developed; however, the knowledge gained and conveyed to the public and scientific community continues to pay dividends to Caddo Lake. Local initiative fueled by information transfer spurred the establishment of an additional weevil rearing facility on the shores of Caddo Lake that will provide additional weevils into the future.

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Appendix A: Program Resources Developed



Want to say
"Goodbye!"
to your pond
or lake?

Just let
salvinias in
and don't
manage the
problem.



Then you'll have a major
infestation to deal with.



For information on salvinia control, see
cise.tamu.edu/caddo or
caddosalvinia.blogspot.com.

For assistance in identifying salvinias and for
recommendations for their control, see
aquaplant.tamu.edu.

Salvinias are just one of many invasive species
in Texas. For more information, see
texasinvasives.org.

*Developed by the Texas Water Resources Institute,
through funding from the USDA-Natural
Resources Conservation Service*



EM-109

**THE POND
DESTROYERS**

**COMMON
AND GIANT
SALVINIA**

Learn how you can help stop
the spread of salvinias



What are salvinias?

Salvinias are non-native, floating aquatic ferns. There are two species of salvinia in Texas, common salvinia (*Salvinia minima*) and giant salvinia (*Salvinia molesta*). While giant salvinia has been called the worst or most invasive aquatic plant in the world, common salvinia is also very invasive and problematic. Both salvinias can double in size within a week or less with good summer growing conditions. The salvinias are native to South America and were imported into the United States by the water garden and aquarium industries. Common salvinia was first noted in Texas in 1992 and giant salvinia in 1998. Since then the salvinias have covered tens of thousands of acres of public and private waters in Texas.

Identifying salvinias

Salvinias are relatively small with individual plant leaves from $\frac{1}{3}$ to $\frac{3}{4}$ inches wide for common salvinia and from $\frac{1}{2}$ to $1\frac{1}{2}$ inches wide for giant salvinia. Whole plants are usually 2–8 inches long. Salvinias have a velvety appearance because of the tiny water-repellent hairs that cover the leaf surface. As giant salvinia matures, its leaves fold and compress into chain-like arrangements.



Top: Giant salvinia (note the chain-like appearance)
Bottom: Common salvinia (small leaves and no strong folding)

The problem

New salvinia plants are produced from any small fragment of the stem node, and with their rapid growth they can quickly cover a pond, forming a thick floating mat that prevents sunlight and oxygen from entering the pond.

With no sunlight for other, more beneficial plants to grow and with oxygen from the atmosphere effectively cut-off, the pond water becomes oxygen-depleted, causing loss of habitat for all aquatic plants and animals. Even migrating waterfowl such as ducks and geese do not use ponds covered with salvinias. Nothing eats salvinia, except a few insects. The pond becomes a dead, decaying, lifeless body of water.

The invasion of salvinias

Salvinias get to ponds by travelling on boats and trailers, in bait boxes, and possibly on birds such as herons and egrets, reptiles such as turtles and alligators, and mammals such as nutria and beavers. Salvinias also get into off-channel ponds and can move pond-to-pond during flooding.

The primary mode of salvinia translocation is people moving boats between infested lakes and ponds.



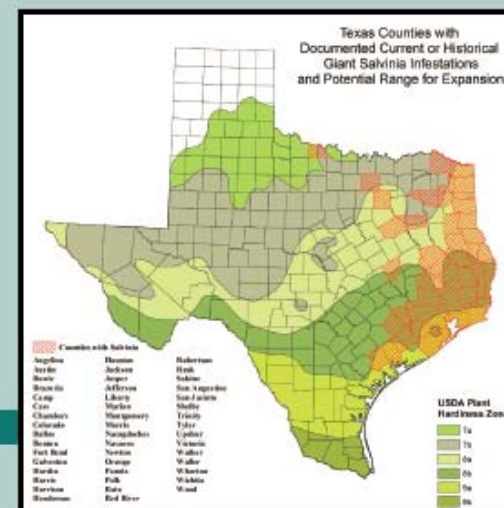
The solution

If our native ecosystems are to be preserved, the spread of salvinias must be stopped and where possible, eradicated.

Cleaning boats, trailers, live wells and bait boxes when leaving a pond or lake infested with salvinia is the first step. Watching for salvinias on ponds and taking action immediately if they are spotted is critical.

Report all infestations to the Texas Parks and Wildlife Department: (409) 384-9965 or giantsalvinia@tpwd.state.tx.us.

Salvinias can be eliminated from small ponds by physically removing them or using registered aquatic herbicides properly. Physically removing salvinia and allowing it to dry or composting it completely will kill it. Salvinia can also be managed biologically with the salvinia weevil.



Biological control of giant salvinia (*Salvinia molesta*) in a temperate region: cold tolerance and low temperature oviposition of *Cyrtobagous salviniae*

Abhishek Mukherjee · Allen Knutson ·
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Received: 9 May 2014 / Accepted: 1 September 2014
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Abstract Success of *Cyrtobagous salviniae* Calder & Sands (Coleoptera: Curculionidae) for biological control of *Salvinia molesta* D. S. Mitchell in temperate regions has been less reliable than in tropical and subtropical regions and this difference is presumed to be due to greater winter mortality. We measured the cold tolerance of *C. salviniae* by comparing chill coma recovery time and survival of adults after exposure to freezing conditions among four geographic populations collected from Florida, Louisiana, Texas and Australia. Effects of winter temperature acclimation on low temperature oviposition also were determined. The Australian population was more cold tolerant than the three US populations. No oviposition by *C. salviniae* was observed at a water temperature of

17 °C and the oviposition rate at 19 °C was less than at 21, 23 and 25 °C. We suggest that introduction of cold tolerant strains of *C. salviniae* could increase its effectiveness in temperate regions of the US.

Keywords *Salvinia molesta* · *Cyrtobagous salviniae* · Cold tolerance · Biological control · Chill coma · Giant salvinia · Curculionidae

Introduction

Salvinia molesta D. S. Mitchell (Salviniaceae) is one of the most important aquatic weeds worldwide. This weed is invasive in the tropical and subtropical regions of Africa, Asia, North and South America and Oceania (Julien et al. 2009). In the United States, *S. molesta*

Handling Editor: John Scott.

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was first detected in 1995 in South Carolina and was later reported from Louisiana and Texas in 1998 (McFarland et al. 2004) and is currently reported from 12 states in the US (EDDMaps 2011; Jacono and Pitman 2001).

The South American weevil *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) has proven to be a highly effective biocontrol agent of *S. molesta* (Room et al. 1981, Julien et al. 2009). In the US, biological control of *S. molesta* began in 1999 with the collection of weevils from *Salvinia minima* Baker (Salviniaceae) in Florida and their release in Louisiana and Texas (Madeira et al. 2006; Tipping and Center 2003). Subsequently, the Brazilian population of *C. salviniae* was imported from Australia and established in western Louisiana and eastern Texas in 2001 (McFarland et al. 2004; Tipping and Center 2003).

While releases of *C. salviniae* have resulted in successful biological control of *S. molesta* in tropical and subtropical regions (Julien et al. 2009), success in temperate regions has been less reliable (Cilliers 1991; Sullivan and Postle 2010). For example, biological control in Australia was not successful south of latitude 34°S (Julien et al. 2009). In the US, efforts to establish *C. salviniae* in the northern areas of Louisiana and Texas (north of latitude 32°N) in 2010–2011 were not successful, presumably due to insect mortality during winter months (Grodowitz 2011; Sanders 2011). Also, efforts to establish *C. salviniae* at Lake Caddo in northeastern Texas (32.71°N, 94.10°W) during 2009–2011 failed and caged populations of *C. salviniae* suffered 100 % mortality during the winter of 2010–2011 (A. Knutson, unpublished data). These observations suggest that exposure to low temperatures may be a limiting factor in establishing *C. salviniae* in temperate areas of Texas and Louisiana. Although recent studies have examined the thermal tolerance of *C. salviniae* (Allen et al. 2012, 2014), a lack of research on the effects of cold temperatures on performance and survival of *C. salviniae* currently limits our ability to predict its potential to establish in temperate regions (van Lenteren et al. 2005). Also, variation in the ability to perform and survive at low temperatures, if present, could be useful in identifying populations or ecotypes of *C. salviniae* better suited for establishment in temperate regions.

In addition to direct effects of cold stress on survival, moderately cold temperatures may constrain

population growth by limiting important life-history characteristics including larval growth or reproduction. Reproduction of *C. salviniae* slows with decreasing water temperature and Forno et al. (1983) reported that no oviposition occurred at a constant 17 °C and only negligible oviposition (mean = 0.019 eggs per female per day) occurred at 19 °C. However, laboratory and field studies suggest that *C. salviniae* adults may begin to oviposit at lower water temperatures following exposure to winter cold temperatures (Sullivan and Postle 2010; Sullivan et al. 2011). These observations suggest a beneficial adaptive response of *C. salviniae* that allows adults to acclimate to cold temperatures and oviposit at lower water temperature than non-acclimatized adults. A lower threshold temperature for oviposition would be important in areas north of latitude 32°N in Louisiana and Texas. In these areas, as observed in Cypress Creek near Karnack, Texas (32.74°N, 94.23°W), water temperatures exceed the lower threshold temperature for oviposition of *C. salviniae* (19 °C) for about six months of the year (United States Geological Service, <http://waterdata.usgs.gov>). We hypothesize that in northern Texas and Louisiana (north of latitude 32°N), biological control of *S. molesta* by *C. salviniae* could be limited due to: (1) increased mortality of overwintering adults and (2) a reduction in the length of time when water temperatures are above the threshold for oviposition.

This study was conducted to address three broad objectives: (1) determine whether several geographically distinct populations of *C. salviniae* differ in their cold hardiness, as assayed by chill coma recovery and recovery after exposure to a freezing event at 0 °C, (2) parameterize the oviposition rates of weevils across a range of ecologically relevant temperatures after acclimation to early winter temperatures and (3) construct GIS interpolation maps of the southeastern US to estimate the numbers of months when temperatures exceed the minimum temperature required for oviposition by *C. salviniae*.

Materials and methods

Cold tolerance of *C. salviniae* populations: origin of populations and insect collection

Adult *C. salviniae* were collected from field populations in New South Wales, Australia and in the US

Table 1 Locations of *C. salviniae* collection and average climatic conditions

Population and collection site	Coordinates	Collection dates	Mean annual air temperature (°C) ^a	Mean air temperature of coldest quarter (°C) ^a	Minimum air temperature of coldest month (°C) ^a	<i>C. salviniae</i> ecotype
Florida: West Palm Beach, Florida, USA	26.652°N 80.335°W	22 Feb, 2012	23.17	18.59	11.74	Florida
Texas: Lewisville, Texas, USA	33.054°N 96.936°W	17 Jan, 2012	18.19	7.85	0.12	Brazilian
Louisiana: Houma, Louisiana, USA	29.551°N 90.781°W	30 Jan, 2012	20.32	13.01	6.45	Brazilian
Australia: Werombi, New South Wales, Australia	33.986°S 150.612°E	19 Apr, 2012	15.85	10.13	2.59	Brazilian

^a Data source: CliMond V1.1 datasets (Kriticos et al. 2011)

from southern Louisiana, south Florida, and from a rearing facility in northeastern Texas (Table 1). These populations are hereafter referred to as the Australia, Louisiana, Florida, and Texas populations, respectively. The Texas population was collected from the weevil rearing facility at the Lewisville Aquatic Ecosystem Research Facility (LAERF), US Army Corps of Engineers at Lewisville, Texas. The Louisiana population was collected from a pond near Houma in southern Louisiana and was established in 2008 by the Louisiana Cooperative Extension Service. The Texas and Louisiana populations originated from the initial establishment of *C. salviniae* by Tipping and Center (2003). However, the Texas population had been maintained in an outdoor rearing facility since 2008 in north Texas (33°N) and subjected to colder winters than the Louisiana population in southern Louisiana (29°N) (Table 1), prompting us to assay each population. The Florida population was collected from *S. minima* at the Loxahatchee National Wildlife Refuge, West Palm Beach, Florida. The Florida population is a distinct ecotype of *C. salviniae* based on molecular differences and its smaller size relative to the Brazilian ecotype that was released in the US and established in Texas and Louisiana (Madeira et al. 2006; Tipping and Center 2003). The population of *C. salviniae* from Australia was collected from a field site near Werombi, New South Wales, Australia, a temperate region of the country. Adult *C. salviniae* were extracted from *S. molesta* using Berlese funnels as described by Boland and Room (1983). Immediately after extraction, insects were kept in plastic containers with fresh *S. molesta* plants and placed inside coolers

to minimize handling stress. All US populations were hand carried to the laboratory. The Australian population was shipped internationally with fresh plants in moistened cloth gunny bags that were placed inside a styrofoam box. The styrofoam box was placed inside another box with cool packs before shipping, thus preventing the insects from coming in direct contact of the cool packs. At the laboratory, insects were immediately placed on host plants and held for three days at room temperature to acclimate before conducting experiments. All cold tolerance experiments were conducted within four days of the US collection dates and seven days of the Australian collection.

Cold tolerance of *C. salviniae* populations: chill coma recovery time

Chill coma recovery time is defined as the time required by an individual insect to recover from a comatose-like state due to the loss of neuromuscular coordination induced by exposure to non-lethal cold temperature (often between 0 and 4 °C) for a specified period of time (David et al. 1998; MacMillan and Sinclair 2011). After exposure to cold, comatose insects are returned to a more benign temperature and the recovery time (time to regain neuromuscular coordination and stand upright) is recorded. Chill coma recovery time is a commonly used assay to determine insect cold tolerance within and between populations and was used in this study as a measure of cold tolerance among the four geographic populations of *C. salviniae* (Ragland and Kingsolver 2008; Sinclair et al. 2011).

Chill coma recovery time was recorded for 50 randomly selected *C. salviniae* adults per population with each insect representing a replicate. Age of the insects was controlled by using only overwintering adult weevils. Age variation in the overwintering population is minimized due to cessation of reproduction in late fall with declining water temperatures. Also, adult females live 3–5 months at 23 °C and longevity is extended during the winter, further stabilizing the age distribution of the overwintering population (Sands et al. 1986).

To allow precise recording of recovery time, each population was observed in five batches each consisting of ten insects. Insect batches were cold treated consecutively and recovery time for all 50 *C. salviniae* adults per population was observed within a day. For each batch, ten adults were placed in a 1.5 ml Eppendorf tube and the tube was placed in ice-water slurry (0 °C) for four hours. Preliminary tests found that 4 h of exposure was sufficient for discriminating recovery times between populations. Immediately after cold treatment, the comatose insects were individually placed ventral side up in a 32 well plastic rearing tray (Bioserv, Frenchtown, NJ, USA), with one insect per well and held at room temperature (25 ± 1 °C). The time (in minutes) required for each insect to recover and stand upright was recorded using a digital stopwatch (precision 0.1 s).

Chill coma recovery times recorded for four populations ($N = 50$) of *C. salviniae* were subjected to ANOVA using PROC GLM (SAS version 9.2, SAS Institute Inc., NC, USA) with a null hypothesis that no variation in recovery time exists between populations. Initial analysis showed no batch effect on chill coma recovery time, and therefore batch was not considered as a factor in the final analysis. Pairwise comparisons of treatment means were performed using the LSMEANS procedure of PROC GLM with Bonferroni-corrected t tests at $\alpha = 0.05$.

Cold tolerance of *C. salviniae* populations:
survival when exposed to 0 °C

The ability to survive exposure to freezing conditions is another important measure of insect cold tolerance (van Lenteren et al. 2005). Because *C. salviniae* is an aquatic herbivore, this species may frequently encounter ice at the surface of the water or on plant

surfaces when temperatures drop in the winter making survival after exposure to ice-forming conditions a potentially important trait. An experiment was conducted to determine the tolerance of *C. salviniae* adults exposed to 0 °C water that had been nucleated with ice for 6, 12, 18, 24, 30 and 36 h, with exposure time as an experimental treatment. Ten adults were randomly chosen from each population and were released on a *S. molesta* plant (a plant is defined as having one apical bud with preceding five ramets) floating in a plastic cup (250 ml) containing 200 ml of reverse osmosis RO filtered water. As explained in the previous section, the potential effect of insect age was controlled by using overwintering adults. A group of ten insects comprised a replicate and each treatment was replicated five times for each population. Before the experiment, groups of insects were kept undisturbed for 24 h at room temperature to allow them to settle on the salvinia plant. Freezing treatments were achieved by placing the cups containing plants, weevils, and water inside an incubator (Percival, Perry, IA, USA, Model E30B) maintained at 0 °C. A temperature data logger (Omega Engineering, Stamford, CT, USA, Model HH506RA) was attached to one representative cup per population to monitor temperature at hourly intervals. The water in each cup was seeded with an ice crystal to hasten freezing. After cold treatment, cups were moved to room temperature and kept undisturbed for 24 h. The cumulative numbers of live insects were recorded after 24, 48 and 72 HAE (defined as hours after exposure to freezing). Insects were considered dead if they could not stand upright when prodded.

The number of surviving weevils per *C. salviniae* population after 72 HAE was subjected to logistic regression analysis using JMP, Version 10 (SAS Institute Inc., Cary, NC, USA). We analyzed 72 HAE data only as these were the longest time period following exposure to freezing treatments and therefore were most conservative, but results for both the 24 and 48 h observations were qualitatively similar. The effects of *C. salviniae* populations, exposure time to 0 °C, and their interaction effects were tested on survival of *C. salviniae* adults. The exposure time to 0 °C necessary to kill 50 % of a *C. salviniae* population, ET_{50} , was calculated for each population using the number of live weevils as the response and the method of inverse prediction.

Thermal sensitivity of oviposition after acclimation to early winter temperatures

The objective of this study was to determine the oviposition rate of overwintering adult *C. salviniae* when held at constant water temperatures of 17, 19, 21, 23 and 25 °C. Adult *C. salviniae* used in this study were collected from a field population in Lake BA Steinhagen, Jasper, Texas (30.88°N, 94.22°W) during December, 2011. This population originated from *C. salviniae* reared at the LAERF rearing facility in Lewisville, TX, USA, released in this lake by the Texas Parks and Wildlife during 2010–2011 and therefore represents the same population as the Texas population used in the cold tolerance studies. The average water temperature in this lake is below the minimum threshold temperature of 19 °C (Forno et al. 1983) for oviposition of *C. salviniae* beginning in early October and the lowest average water temperatures, 11.7 °C \pm 0.25 (mean \pm SE), occurs in this lake during December (USGS Water-Quality Monthly Statistics for the Nation; <http://waterdata.usgs.gov/nwis/qw/>). Thus, weevils at this site had been exposed to water temperatures below 19 °C for two months prior to collection and were therefore thoroughly acclimated to ecologically relevant low temperatures at the time of collection in December.

Adult *C. salviniae* were extracted from infested plants with Berlese funnels and maintained on *S. molesta* in an incubator (Percival, Model E33L Perry, IA, USA) at an average hourly water temperature regime ranging between 10.4 and 13.9 °C and a 12:12 LD photoperiod. This regime was based upon mean hourly water temperature data recorded at Lake BA Steinhagen in the month of December 2011 by a HOBO® temperature logger (Hobo pendant loggers, Onset Computer, Bourne, MA, USA) floating within 1 cm of the water surface within a *S. molesta* mat. The adults were maintained in an incubator for four weeks and then individually extracted from plants by hand and concentrated into a small container with a few *S. molesta* plants and held at 27 °C to observe mating. Once mating was observed, an individual mating pair was transferred to a *S. molesta* plant floating in a transparent plastic cups (250 ml) containing 200 ml of distilled water. A single mating pair represented a replicate and the experiment was conducted with ten replicates and five constant temperatures, 17, 19, 21, 23 and 25 °C (\pm 0.5 °C) under a 12:12 LD photoperiod.

Cups with mating pairs were randomly allocated to each constant temperature. At weekly intervals, mating pairs were individually transferred to a second set of plastic cups each with a floating *S. molesta* plant. Plants were dissected under a microscope and the number of eggs oviposited by the female within each mating pair during the previous week was counted. The experiment was conducted for eight weeks during January to March, 2012.

The effects of time (week), temperature, and their interaction effect on number of eggs oviposited by each *C. salviniae* adult female were subjected to a two way ANOVA using PROC GLM. Pairwise comparisons of treatment means were performed using the LSMEANS procedure of PROC GLM with Bonferroni-corrected *t* tests at $\alpha = 0.05$.

GIS interpolation map of predicted oviposition success

Maps of the annual period when temperatures exceed the minimum temperature required for oviposition by *C. salviniae* were generated using the Inverse Distance Weighted (IDW) interpolation technique available within the ArcGIS Geostatistical Analyst software package (ESRI Inc.) (Diaz et al. 2008). Potential oviposition maps were generated using both water and air temperature data recorded across the southeastern US. Air temperature data were used as these data are available from many sites across the region and were obtained from the climatological database CliMond (Version 1.1, 10 arc-second resolution, 30 years average, 1961–1990, centered on 1975) (Kriticos et al. 2011). We recognize *C. salviniae* is a semi-aquatic insect and predictions generated using water temperature should be more accurate in representing field conditions, although weather station data were successfully used to predict the number of generations completed by the mirid, *Eccritotarsus catarinensis* (Hemiptera: Miridae), a biological control agent of water hyacinth, *Eichhornia crassipes* (Mart.) (Pontederiaceae) (Coetzee 2012). Unfortunately, there are far fewer water temperature data points available by comparison to the available air temperature data. Surface water temperature data were obtained from the USGS Water-Quality Monthly Statistics for the Nation (<http://waterdata.usgs.gov/nwis/qw/>). For each data point, number of months per year when mean average air/water temperatures was above the minimum

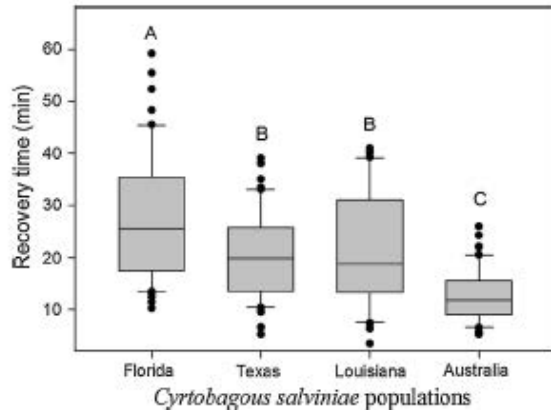


Fig. 1 Box-and-whisker plots show the chill coma recovery time (min.) recorded among *Cyrtobagous salviniae* populations. The middle bar = median, box = inter-quartile range (25th–75th percentile), whiskers (error bars) above and below the box represent the 90th and 10th percentiles and the dots are the outlying points. Bars with the same letters are not statistically different as tested by the LSMEANS statement within PROC GLM with Bonferroni corrected *t* tests at $\alpha = 0.05$

oviposition threshold of 19 °C was determined (threshold temperature selected based on results of oviposition test, see previous section for methods). IDW interpolation was implemented using the months above threshold value. The power optimization option was used to generate optimum power value for each dataset (Diaz et al. 2008). All other parameters were set at their default values.

Results

Cold tolerance of *C. salviniae* populations: chill coma recovery time

All insects survived the four-hour exposure to 0 °C used to induce chill coma. However, there was substantial variation between populations in their chill-coma recovery time ($F_{3,196} = 19.95$, $p < 0.0001$, Fig. 1). Consistent with our expectations for correlations between latitude and cold-resistance, the Australian population appeared to be most cold tolerant with the shortest recovery time (12.73 ± 0.72 min, hereafter representing the mean ± 1 SE, $N = 50$). Chill coma recovery time for the Texas (20.33 ± 1.18 min, $N = 50$) and Louisiana (21.35 ± 1.55 min, $N = 50$)

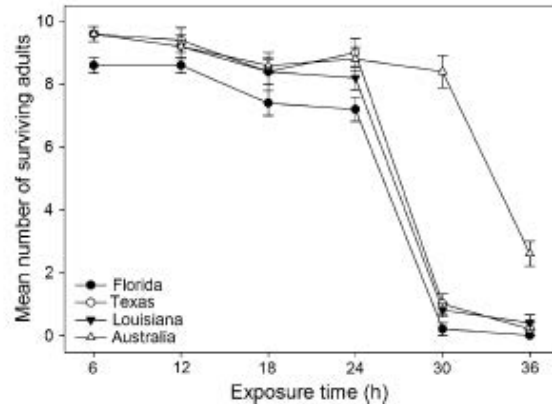


Fig. 2 Mean number (\pm SE, $N = 5$ replications) of adults surviving 72 h following exposure to 0 °C for 6, 12, 18, 24, 18, 24, 30 and 36 h for four geographic populations of *C. salviniae*. The Australian population was the most cold tolerant, while the Florida population was the least tolerant to exposure to 0 °C. Texas and Louisiana populations showed intermediate tolerance to exposure to 0 °C

populations were intermediate and did not differ from each other. Chill coma recovery time for the Florida population (27.54 ± 1.76 min) was the greatest, suggesting it was the least cold tolerant.

Cold tolerance of *C. salviniae* populations: survival when exposed to 0 °C

Most adult *C. salviniae* across all populations survived exposures of 6–12 h to frozen water on plants at 0 °C, but mortality increased substantially between 24 and 36 h of exposure (Fig. 2). Results of regression analysis showed that *C. salviniae* populations ($\chi^2 = 40.99$, $df = 3$, $p < 0.0001$), exposure time to 0 °C ($\chi^2 = 587.08$, $df = 1$, $p < 0.0001$) and their interaction ($\chi^2 = 17.98$, $df = 3$, $p = 0.0004$) have significant effects on insect survival. The Australian population was most cold tolerant with an ET_{50} (exposure time to 0 °C that kills 50 %) = 34.29 h (95 % CI, 31.71–38.09) and the Florida population was least tolerant ($ET_{50} = 22.27$ h, 95 % CI 20.66–23.91) to exposure to 0 °C. The Texas ($ET_{50} = 25.65$ h, 95 % CI 24.30–27.04) and Louisiana ($ET_{50} = 25.06$ h, 95 % CI 23.66–26.50) populations showed intermediate tolerances to exposure to freezing temperature.

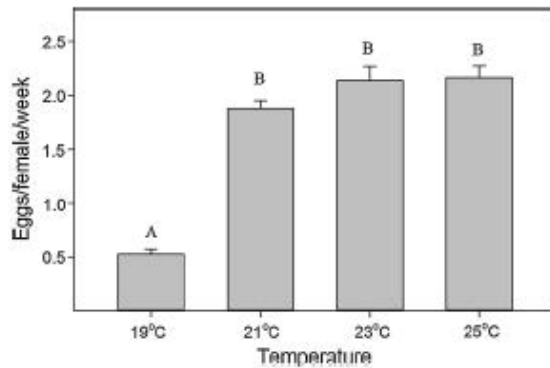
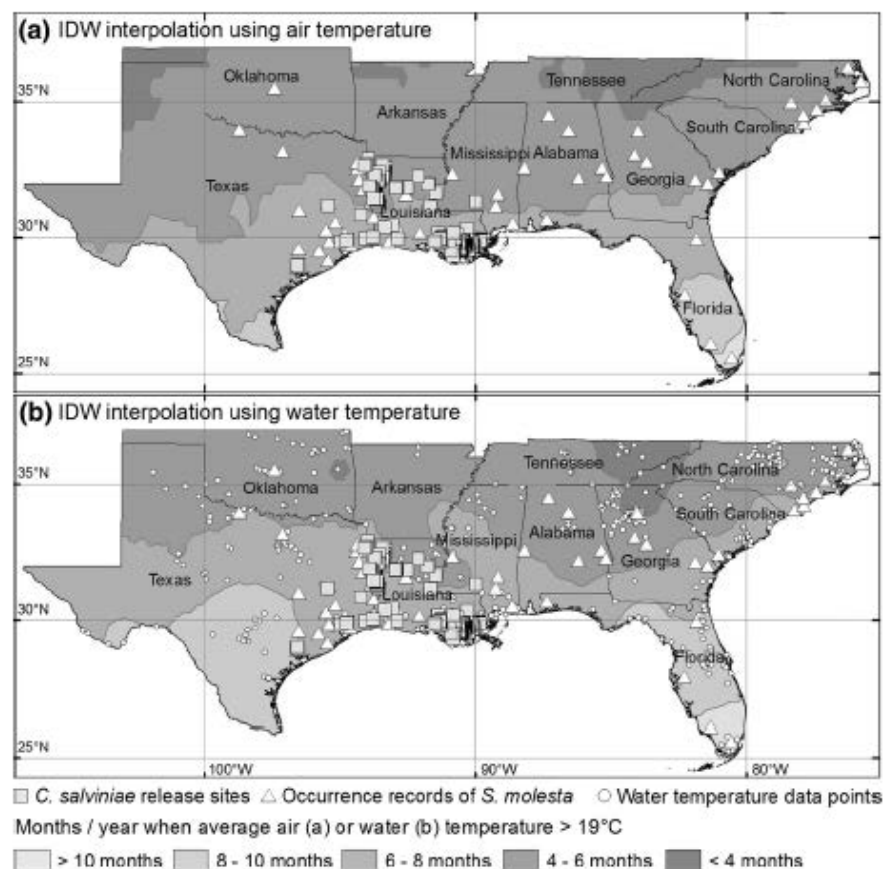


Fig. 3 Average (mean + SE) number of eggs deposited per female per week (averaged over eight weeks) at different constant temperature treatments (x-axis). Bars with the same letters are not statistically different as tested by the LSMEANS statement within PROC GLM with Bonferroni corrected *t* tests at $\alpha = 0.05$

Thermal sensitivity of oviposition after acclimation to early winter temperatures

No eggs were deposited by *C. salviniae* at 17 °C, so this temperature was removed from subsequent analyses. The least number of eggs was oviposited at 19 °C (0.5 ± 0.05 eggs per female per week, $N = 10$, Fig. 3) while oviposition rates observed at temperatures above 21 °C were not different from each other ($F_{3,288} = 55.77$, $p < 0.001$) (Fig. 3). No effects of time (number of weeks ovipositing) ($F_{7,288} = 0.75$, $p < 0.63$) or temperature \times time interactions ($F_{21,288} = 1.03$, $p < 0.43$) were detected on the oviposition rate of *C. salviniae*.

Fig. 4 Potential oviposition map for *Cyrtobagous salviniae* based on average monthly air (a) and water (b) temperature recorded across the southeastern United States. Occurrence records of *Salvinia molesta* are from EDDMaps (<http://www.eddmaps.org>) and release records of *C. salviniae* are from Tipping et al. (2008)



GIS interpolation map of predicted oviposition success

The potential oviposition map based on air temperature showed that in central and south Florida, as well as in the southern tip of Texas, air temperature stays above the threshold oviposition temperature of 19 °C for more than eight months per year (Fig. 4a). In northern Florida, southern parts of Georgia, Alabama and Mississippi as well as in central and southern Louisiana and southeastern Texas, air temperatures stay above the threshold oviposition temperature for 6–8 months (Fig. 4a).

The potential oviposition map based on water temperature indicated that in southern Texas and most parts of Florida water temperature stays above 19 °C for more than eight months per year (Fig. 4b). In contrast, water temperature is suitable for *C. salviniae* oviposition (>19 °C) for only 6–8 months in southern Louisiana, central, eastern and western parts of Texas, southern regions of Arkansas, Mississippi, Alabama, Georgia and South Carolina, as well as parts of northern Florida.

Discussion

For both metrics of low temperature tolerance, chill-coma recovery and exposure to partially frozen water, the Australian population of *C. salviniae* was more cold tolerant than the Texas, Louisiana and Florida populations. For all four *C. salviniae* populations, more than 70 % of the *C. salviniae* adults survived up to 24 h of exposure to 0 °C, including exposure to ice in the water and on the surface of their host plant (Fig. 2). Although the water in each cup was indeed frozen in this assay, we do not know whether the beetles themselves had ice nucleated within their tissues. While we cannot distinguish the relative contributions of injuries due to freezing versus injury due simply to cold exposure (Košťál et al. 2011; Lee and Denlinger 2010), exposure to ice on the host plant and surrounding water, as would occur in a natural habitat, was clearly lethal to most adults beyond 24 h of exposure.

As a sub-aquatic insect, *C. salviniae* spends much of its life under water, avoiding exposure to temperature extremes (Julien et al. 2009). Water temperature data recorded in Texas and Louisiana provided no

evidence of water freezing for more than 24 h (USGS Water-Quality Daily Data for the Nation; <http://waterdata.usgs.gov>), thus we might expect that cold exposure may not limit overwintering survival in *C. salviniae*. However, our assay included only one exposure to freezing temperatures, and at temperate sites *C. salviniae* may experience multiple exposures to low temperature conditions that lead to ice formation in their habitat. A substantial body of work suggests that multiple exposures to sub-lethal stress may compound to produce lethal levels of damage (Marshall and Sinclair 2012), and such studies are needed to determine whether low-temperature stress contributes to winter mortality of *C. salviniae* adults. Further research is also needed to understand the physiological and biochemical traits that confer cold tolerance in the Australian population, such as the potential accumulation of antifreeze proteins, sugars, and polyols. Identifying physiological or biochemical traits conferring cold tolerance may allow rapid screening of *C. salviniae* source populations to identify those that may be most successfully introduced in temperate climates for biological control of *S. molesta*.

Damage to their host-plant, *S. molesta*, due to freezing may also affect the ability of *C. salviniae* to successfully overwinter. *Salvinia molesta* is susceptible to cold temperature and dies when exposed to less than −3 °C for more than 2 h (Whiteman and Room 1991). Grodowitz (2011) reported the cold events during winters of 2009–2010 caused substantial reduction of *S. molesta* mats in northern Louisiana. Dead *S. molesta* plants sink, exposing insects to open water, thus increasing the chance of drowning, predation, exposure to freezing air temperatures, and increased stress from starvation, which could indirectly affect overwintering survival of *C. salviniae* by decreasing organismal condition. Studies directed towards understanding how long *C. salviniae* can survive without host plants or on low-quality, cold-damaged host plants could provide insight regarding overwintering success of this insect.

The greater cold tolerance observed in the Australian population relative to that of the US populations could be a result of greater genetic diversity in the Australian population. The Brazilian population of *C. salviniae* in the US has gone through two founding events, one during initial collection from Brazil in 1980 and another during subsequent collection from Australia and release in the US in 2001. These

founding events could have resulted in the loss of genetic diversity in the US population that could limit adaptation to local climatic conditions.

The results of the oviposition study showed that overwintering *C. salviniae* adults acclimated to the December temperature regime did not oviposit at 17 °C and that the oviposition rate at 19 °C was dramatically less than that when adults were held at 21 °C and greater. These results are consistent with those reported for *C. salviniae* adults that were not acclimated to cold (Forno et al. 1983).

Sullivan et al. (2011) reported successful biological control of giant salvinia by *C. salviniae* at temperate localities in eastern Australia (below latitude 34°S) and Sullivan and Postle (2010) reported that oviposition of *C. salviniae* commenced in this region in the spring when mean water temperature was 17 ± 3 °C. These results coupled with results of our study on cold tolerance of *C. salviniae* collected from Australia suggest that further introduction of cold hardy strains of *C. salviniae* collected from the temperate regions of Australia could increase biocontrol success in north-eastern Texas and Louisiana. Also, exploration in the native range of *C. salviniae* may identify cold-hardy strains adapted to oviposit at cooler water temperatures in the spring. The establishment of more cold-hardy ecotypes could increase the efficacy of *C. salviniae* as a biological control of *S. molesta* in temperate regions and reduce the cost and effort to re-establish populations of *C. salviniae* each spring from mass-rearing facilities.

Acknowledgments This research was supported by grants from the Natural Resource Conservation Service, USDA (USDA NRCS Agreement # 68-7442-10-499). Preliminary cold tolerance experiments were supported by a grant from the US National Science Foundation to DH (NSF-IOS-1051890). The authors thank P. Krauter and M. Lewis (Department of Entomology, Texas A&M University, USA) for their editorial comments on earlier drafts of this manuscript, and Sophia Daniels, Entomology Department, Texas A&M University, USA. The authors are also grateful to Lee Eisenberg, Texas AgriLife Extension; Julie Nachtrieb, Lewisville Aquatic Ecosystem Research Facility, US Army Corps of Engineers; Mathew Purcell, USDA ARS Australian Biological Control Laboratory and Phillip Tipping, Invasive Plant Research Laboratory, USDA-ARS, Ft. Lauderdale for their assistance during this study.

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BIOLOGICAL GIANT SALVINIA MANAGEMENT ON SMALL PONDS

DEFINITION

Management of the aquatic invasive fern, giant salvinia, using the salvinia weevil (*Cyrtobagous salviniae*) on small, privately owned, water bodies

PURPOSE

- Reduce coverage of the giant salvinia on private ponds to manageable levels
- Restore the aquatic habitat and functionality of the treated pond
- Reduce the threat of giant salvinia spreading to nearby or downstream water bodies
- Increase ability of native or desirable aquatic plants to thrive in the treated pond

CONDITIONS WHERE PRACTICE APPLIES

On privately owned ponds currently infested with significant levels of giant salvinia where the landowner or designee has obtained the appropriate permits from the Texas Parks and Wildlife Department for the acquisition, transport and dissemination of salvinia weevil laden giant salvinia. This practice is especially applicable where philosophical reasons or ecological considerations prevent or strongly out-weigh the use of chemical control methods.

This practice is not applicable on public waters. The Texas Parks and Wildlife Department or their designees oversee and manage invasive species on public waters. If invasive species control is needed on public waters, regional Texas Parks and Wildlife Department personnel should be contacted.

CRITERIA

General Criteria Applicable to All Purposes

Biological control of giant salvinia should be applied where complete eradication of the invasion is not practical or possible via chemical, manual, or mechanical control methods. When possible, always completely eradicate this highly invasive species.

Where eradication is not possible, apply biological control to establish a natural equilibrium between the invasive giant salvinia and its natural enemy, the salvinia weevil. This will maintain salvinia levels at low and tolerable levels and provide some salvinia plants necessary to maintain a salvinia weevil population.

Application of weevils should be made as early in the growing season as practical; typically in the first half of April. Treatments should be applied in areas of less dense giant salvinia where plants are actively growing as this is the ideal plant type for the weevil to complete its life cycle.

In areas where the salvinia has formed dense mats, salvinia weevils can/should be applied soon after other control methods are used that temporarily reduce the giant salvinia infestation. Wait until the salvinia begins to regrow after herbicide treatments to ensure the released weevils have plants with new buds on which to feed. Actively growing salvinia increases the ability of the salvinia weevil to expand its population and enhances ultimate control effectiveness.

In all situations, giant salvinia infestations and planned control techniques should be coordinated with Texas Parks and Wildlife Department personnel, Texas A&M AgriLife Extension Service agents and Natural Resource Conservation Service at the local level.

Herbicide usage in concert with biological control must adhere to guidance listed on pesticide labels, Extension Service and other pest management references related to environmental hazards and site-specific application criteria.

CONSIDERATIONS

If complete eradication of giant salvinia is possible via chemical treatment or physical removal, it should be strongly considered.

Salvinia weevils are not widely available in Texas or the rest of the U.S. Only a few facilities produce weevils in Texas and Louisiana and may have available weevils upon request. Texas Parks and Wildlife Department personnel should be able to provide contact information.

Biological giant salvinia control is not rapid. Effective control may require several years and is impacted by many factors including the infestation density, number of weevils stocked, weather conditions, water fertility and predation on the weevil population.

Giant salvinia possession and transport in Texas without proper permission from the Texas Parks and Wildlife Department is a class C misdemeanor punishable by a fine up to \$500. Rapid death and the subsequent decay of giant salvinia may cause low dissolved oxygen

levels in small ponds. An aquatic habitat biologist with AgriLife Extension, NRCS or TPWD should be consulted to plan mitigation strategies for potentially adverse impacts.

PLANS AND SPECIFICATIONS

Site specific plans should be prepared for each infested water body. At a minimum the planning process and plan should include:

1. Notify Texas Parks and Wildlife Department personnel of the giant salvinia infestation by calling (409) 384-9965 or emailing giantsalvinia@tpwd.state.tx.us.
2. Discuss treatment options with TPWD and select the most appropriate method for the given situation.
3. If biological control is chosen, work with TPWD to secure permits to possess and transport weevil infested giant salvinia from its location of origin to the infested pond.
4. Identify a source of salvinia weevils and arrange for their acquisition and transport upon receipt of proper permitting.
5. Define the current size and extent of the problem and plan pre-treatment actions such as thinning the mat with isolated chemical treatments.
6. Define the locations and planned number of weevil release sites and the desired number of weevils to be released at each site.
7. Describe a monitoring plan that notes monitoring increments and the planned approach. At a minimum include photo documentation with scale references to enable control estimates to be made periodically. If possible, physically measure the area using a tape measure or GPS unit.

BIOLOGICAL CONTROL APPLICATION METHOD

1. Following acquisition of required permits, obtain giant salvinia infested with salvinia weevils in a container such a plastic storage container or cooler with a secure lid. The salvinia plants will contain adults, eggs and larvae of the salvinia weevil. The rearing facility should provide an estimate of the number of adult weevils per kilogram of live salvinia plants, as determined by extracting the adults from a known weight of plants. The number of eggs and larvae are difficult to determine and not usually reported. Ensure that plant material is moist but not saturated during transport.
2. Immediately transport the infested salvinia plants to the planned release site while protecting the containers from direct sunlight and high temperatures to prevent weevil mortality. Ensure that containers are well secured to the transport vehicle.
3. Once at the release site, place plants on open water within the salvinia mat; create openings if needed, so that the weevil infested plants directly contact the water.

4. Guidelines on the number of weevils needed to establish a weevil population have not been determined. However, releases should be concentrated within a small area to be sure adults quickly find mates. For small ponds, releasing about 500-600 adults within an area of one square yard at each of 4-5 locations is a good start. A second release 2-3 weeks later can supplement the initial release. Releasing more weevils increases the chances of establishment, the rate of population growth and speed of control.
5. If feasible, mark the release site within the salvinia mat with a stake or other marker. Take post-release photos with a scale reference to document the initial conditions before the biological controls begin working.

OPERATION AND MAINTENANCE

Operation

Once weevils have been released, routinely observe the release site for evidence of plant damage. The salvinia plants at the release site should begin to turn brown 6 - 8 weeks after the weevils are released. This discoloration is a result of weevil larvae feeding within the salvinia plants. These plants will eventually break apart and sink. The area of plant damage should expand as the weevils disperse from the original release site. Take monitoring photos and measurements during these trips as planned.

Ensure that insecticides are not used in close proximity to the release area. If insecticides are planned for use nearby, ensure that any drift will not impact the release site and that any potential runoff carrying residual insecticide will not enter the treated water body.

Keep a log book of activity related to the treatment. Note activities such as release date and quantity of weevils released, inspection dates, estimates of damaged salvinia area, and any additional treatments with herbicides.

Maintenance

During observations, assess the stage of giant salvinia growth and determine if salvinia plants are scattered or if the plants have formed a continuous and dense mat. Plants within a dense mat produce few buds on which the weevil feeds. Thinning the mat with a targeted herbicide will break up the mat, allowing the salvinia to resume growth and provide buds for the weevil population to feed, increase in numbers and eventually reduce the salvinia infestation.

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