Biological Control of Giant Salvinia in East Texas Waterways and the Impact on Dissolved Oxygen Levels

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ABSTRACT

Over 651,000 larvae, pupae, and adult salvinia weevils (*Cyrtobagous salviniae* Calder and Sands) were released in late 2001 at five sites in East Texas for biological control of giant salvinia (*Salvinia molesta* Mitchell). The agent quickly established and populations of salvinia were reduced to less than ten percent of the original infestation at four of the sites within nine months. After plant coverage was reduced by sustained weevil feeding, mean dissolved oxygen (DO) levels increased >2.5 fold just nine months after insect release. DO averaged only 1.7 mg/l before release and 4.3 mg/l after plants were removed by sustained weevil feeding. This result was a significant increase in mean DO of 2.6 mg/l at all the sites where biological control strategies were effective.

Key words: Cyrtobagous salviniae, Salvinia molesta, aquatic weed, biocontrol, fern.

INTRODUCTION

Giant salvinia is a rapidly proliferating, floating, aquatic fern native to southern Brazil that has spread to many countries including Australia, New Guinea, New Zealand, South Africa and the United States (Forno et al. 1983, Nelson 1984, Barrett 1989, Chilton 1998). In the US, giant salvinia has been detected in 12 states and has been well established in Texas since 1998 (Jacono 1999). Giant salvinia develops dense mats that interfere with rice cultivation, clog fishing nets, and disrupt access to water for humans, livestock, and wildlife (Mitchell and Gopal 1991, Creigh 1991). It damages aquatic ecosystems by overgrowing and replacing native plants that provide food and habitat for native animals and waterfowl (Mitchell 1978, Mitchell and Gopal 1991).

Giant salvinia has a rapid growth rate and can form dense mats up to one meter deep across the water surface (Holm et al. 1977, Oliver 1993). These mats can severely reduce the oxygen supply to the water column by limiting gas exchange between the atmosphere and the water. Dissolved oxygen (DO) production from photosynthesis is also limited due to minimal light penetration to phytoplankton and submersed aquatic plants. More importantly, minimal light penetration decreases oxygen concentration to the detriment of fish and other aquatic species (Cook 1990, Mitchell and Gopal 1991, Holm et al. 1977, Oliver 1993). When plant masses die, decomposition further decreases dissolved oxygen levels (Kannan 1979).

When DO levels drop below 5.0 mg/l, aquatic life becomes stressed and oxygen levels that drop below 2.0 mg/l can result in large fish kills (Water Quality Assessments 1996). According to the Texas Commission on Environmental Quality (2002), average DO levels range from 3.0 to 5.5 mg/l year-round in East Texas waterways. Oxygen levels of 2.0 mg/l or lower are common in small lakes and ponds covered with giant salvinia in Texas (Helton and Chilton 2001). The impact of herbicides on giant salvinia can cause rapid declines on DO levels because organic material requires oxygen for decomposition.

An important herbivore of giant salvinia is a small curculionid which has been introduced to at least 13 countries (Julien and Griffiths 1998). This biocontrol agent is recognized as the leading and most often used control strategy for management of giant salvinia in most areas of the world due to its highly effective nature (Flores and Wendel 2001). Other than a few areas in Australia, giant salvinia has been reduced to acceptable levels in 12 of 15 countries where the natural enemy was introduced for control of the weed (Julien and Griffiths 1998). The salvinia weevil is so host specific that when the giant salvinia population is reduced, the weevil will often starve to death instead of switching host plants. This has been evident in all areas of introduction (Forno et al. 1983, Thomas and Room 1986) with the exception of one case where feeding occurred on *Pistia stratiotes* as documented by Forno et al. (1983).

Room et al. (1981) report that a few thousand weevils released in Australia in 1980 increased to more than 100 million individuals. In this case, time for establishment and control was measured in terms of months instead of years, as is the typical case with most insect biological control studies (Coombs et al. 2005). Biological control strategies are highly cost-effective since the impact is realized for years without reintroduction of the natural enemy. The USDA-APHIS-PPQ-CPHST-Pest Detection, Diagnostics, and Management Laboratory (PDDML) in Mission, TX has mass produced over two million salvinia weevils for field release since 2001 (Wood 2005).

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Herein, we describe events that led to the establishment of the salvinia weevil at several release sites in East Texas. The numbers of insects released and the conditions and time under which establishment occurred are reported. The impact of biological control of giant salvinia on dissolved oxygen is statistically analyzed.

MATERIALS AND METHODS

This study was initiated in the Beaumont/Port Arthur region of East Texas in August 2001 on six private waterways (McKey, Wheeler, Doucet, Ortolon, Cable, and Lyons) which ranged from 0.5 to 2 hectares in size and 1 to 3 meters in depth. These sites were selected because they were heavily infested with giant salvinia and water flow was minimal. The McKey site served as the control site for this study. Five visits to the sites were made from August to November 2001 before any insects were released and six subsequent visits were made from August to December 2002 after control had been attained. Visits to the study sites were made every three weeks to monitor insect establishment, assess biological control impact, and monitor dissolved oxygen levels.

All insects and their host-plant material used for this study were reared in forty 2,365 liter tanks at the PDDML. Infested plants were sampled and insects per tank estimated. The contents from each tank were strained and packaged into 114 liter square-shaped plastic containers with lids and transported to the study sites. In November 2001, the biological control agents were released into five of the six study sites (Table 1). Releases were made in a floating square made of PVC pipe-tubing (1-m² × 5-cm diameter). A global positioning system (GPS) unit was used to record points of release. Subsequent releases were made based on insect availability and final releases were made in January 2003.

Insect population densities were sampled by collecting two giant salvinia samples from each location. Densities were based on insects per plant (Forno 1987). Samples were collected 10 to 20 m away from the point of release with the use of a 21.6-cm diameter strainer (9 by 9 cells per cm²). All insect stages of the weevil are attached or embedded in the plant reducing the possibility of being lost through the strainer. The strainer was held below the mat of giant salvinia and in a vertical upward motion was pulled up with the sample of plant material. Each sample of plant material was then put into a 7.5-liter re-sealable plastic freezer bag. All the samples were labeled and placed into an ice chest for preservation. The ice chest was then transported to the PDDML where storage and examination of the plant material occurred. At the laboratory, the bags of sampled material were removed from the ice chest and placed into a walk-in-freezer where the temperature was a constant 0°C. Each bagged sample was removed from the freezer within 24 to 48 hours for insect enumeration.

Five random giant salvinia plants were removed from each bagged sample and placed on the dissecting table. Each leaf stem was removed and split down the keel when examining for eggs or larvae of the salvinia weevil. After all the stems were removed and bisected, the third submerged leaf commonly mistaken for a root was scanned for any life stages. The rhizome was also searched with the aid of a scalpel. A scalpel was used to bisect the rhizome allowing us to observe weevil larvae or eggs. The adults were easily detected on the plant while the leaves, stems and rhizomes are being removed. All numbers of eggs, larvae, pupae, and adults were quantified during the process. The entire process was repeated for each bagged sample after which all plant material was autoclaved (121°C, 20 psi, 30 minutes) and discarded.

A visual estimate of percent surface coverage was recorded at the release sites according to methods employed by Room et al. (1984). Salvinia infestation level was expressed on a 0 to 100% scale where 0% equals no salvinia and 100% equals waterway completely covered. Salvinia mat thickness was not measured. In weed biological control, equilibrium between the herbivore and plant is maintained at greatly reduced densities over a long period never achieving complete eradication (i.e., 0% surface coverage). Thus, in this study a rating of 10% coverage or less was regarded as an acceptable level.

Observations on dissolved oxygen were recorded in the field using a Horiba Water Quality Checker U-10™ (Horiba Instruments Incorporated, Irvine, CA). Water measurements were recorded at the points of release at all study sites before releases were made and each time that samples were collected thereafter. Depths where samples were made ranged from 0.5 to 1.5 m. A GPS unit was used to ensure the location of the sampling points. Statistical analyses were conducted for both treatment and environmental effects. Data was analyzed using a one-way repeated measures analysis of variance (ANOVA) to test for DO differences among locations and for a DO difference resulting from biological control of giant salvinia with the weevils. The locations that showed effective biological control were analyzed separately for significant differences. Correlation analyses were conducted to determine the relationship between dissolved oxygen levels and the percentage of the waterways covered by salvinia (i.e., percent surface coverage).

RESULTS AND DISCUSSION

Over 651,000 larvae, pupae, and adult forms of the salvinia weevil were released and assessed in five Texas waterways between November 2001 and January 2003 (Table 1). Forty-three days after initial releases, larvae and adults were recovered in giant salvinia samples collected 10 to 20 m from the release point (Figure 1).

Salvinia weevil populations reduced weed infestations to less than ten percent of the original sizes at four of the five study sites (Table 1) within nine months. These findings are very similar to those reported by Julien and Storrs (1993) where successful control of the weed resulted in under eight months at certain sites in Kakadu National Park. The lowest mean weevil density found at all sites was 0.5 insects/five plants (Figure 1). This density occurred in the first sample collected at Cable's site after releases were made. All densities at all locations remained much higher than this level throughout the entire study. No significant correlations were observed between insect density and percent surface coverage. According to Forno (1987), equilibrium (i.e., a stabilized plant population density) is established at less than one insect per 20 plants and the insects will control the growth of salvinia; however, weevil densities were much higher at all study sites.

TABLE 1. DETAILS OF RELEASES AND RECOVERIES OF THE SALVINIA WEEVIL IN EAST TEXAS WATERWAYS INFESTED WITH GIANT SALVINIA AND WATER PARAMETERS OBSERVED.

Site	Control	Wheeler	Doucet	Ortolon	Cable	Lyons
Date releases began	**	11/07/01	11/07/01	11/07/01	11/07/01	11/07/01
Percent surface coverage at time of release	100%	100%	100%	100%	100%	100%
Dispersal confirmed	**	12/19/01	12/19/01	12/19/01	12/19/01	12/19/01
Date releases ended	**	01/14/03	06/12/02	06/12/02	06/12/02	06/12/02
Total number of insects released	0	198,759	156,441	33,518	66,006	196,412
Number of releases	0	17	17	5	10	16
Minimum salinity observed (mg/L)	0	0	0	0	0	0
Maximum salinity observed (mg/L)	0.01	0.10	0.02	0.19	0.10	0.02
Minimum temperature observed (°C)	10	10	10	10	10	10
Maximum temperature observed (°C)	31	28	32	30	28	29
Minimum pH observed	5	4.4	3.5	3.3	4.5	4.7
Maximum pH observed	9.3	8.8	8.3	8.4	8.8	8.1
Percent surface coverage at end of study	100%	80%	10%	1%	5%	1%
Date acceptable control achieved	*	**	08/07/02	08/07/02	08/07/02	07/17/02

*No insects released.
**Percent surface coverage was less than 10% in 2005.
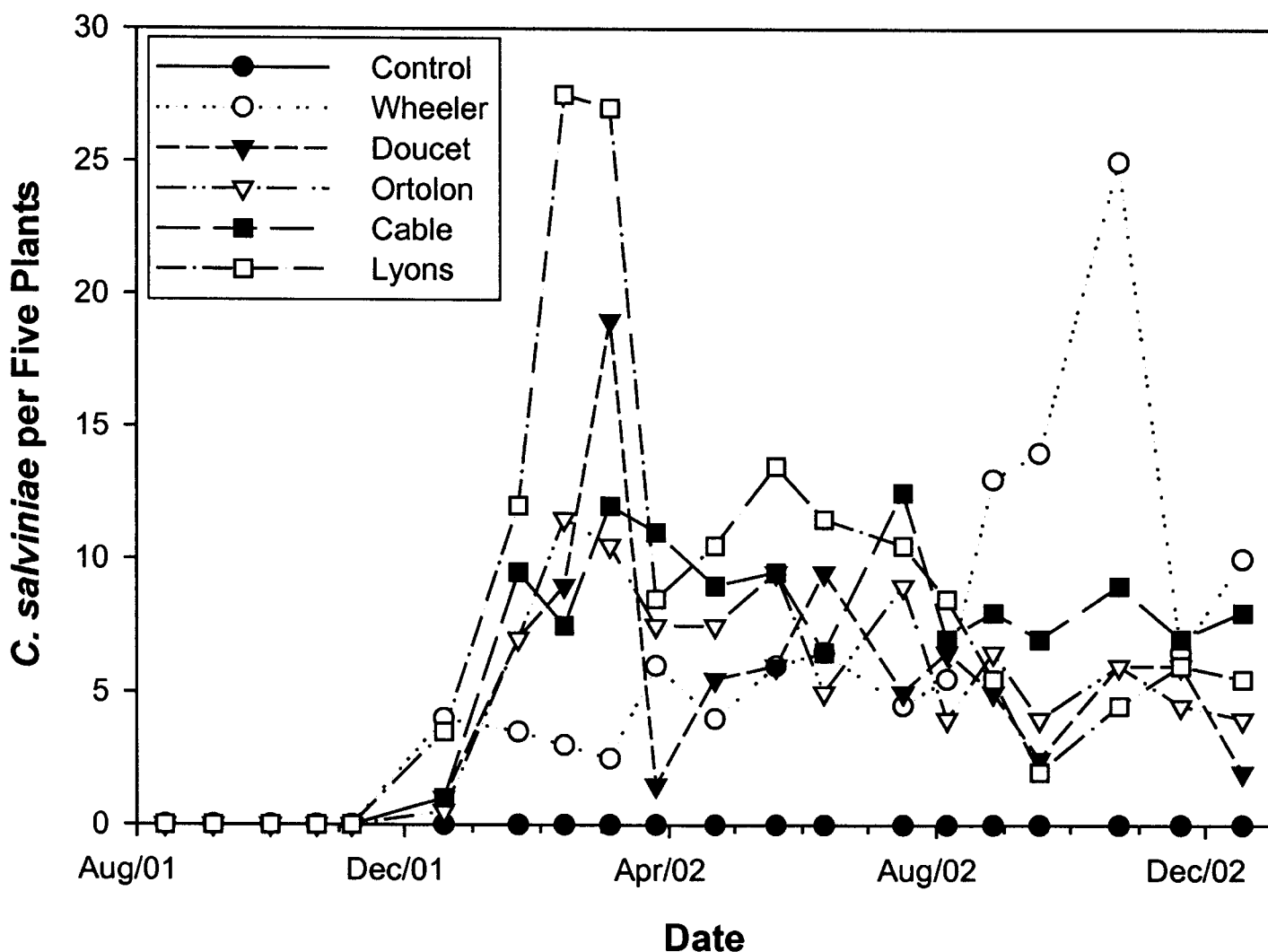

Figure 1. Mean *C. salviniae* (larvae, pupae, and adult forms) per five plants recovered in samples at all sites. First releases were made on November 2001.

After initial releases, feeding damage by the salvinia weevil was visually observed up to 10 m from the floating square at all of the release sites. Damage became apparent through a color gradient displayed by the giant salvinia plants across the waterway as reported in other studies (Pieterse et al. 2003). Severely damaged plants had predominantly dark brown foliage with very few green fronds; moderately damaged plants had an integrated mixture of brown and green foliage; and healthy plants appear green, but have a few brown fronds within the canopy (Everitt et al. 2005). After plants were severely damaged, they subsequently sink to the bottom (Pieterse et al. 2003, Sands and Schotz 1985, Forno et al. 1983).

By August 2002, weevil populations had caused over 90% damage to the salvinia at four of the release sites (Doucet, Ortolon, Cable, and Lyons). At this time, plant buoyancy was compromised and the majority of the mat subsequently sank. Decrease in coverage at Wheeler's site was minimal at this time

exhibiting about 80% coverage. However, as of 2005, the coverage had significantly decreased to less than 10%. It is unknown why Wheeler's site responded slower than the other sites, yet these results were very similar to the results documented by Julien and Griffiths (1998), where it took 1 to 5 years to control giant salvinia in the African nation of Botswana. Julien and Storrs (1993) indicated that when heavily damaged mats of salvinia were very tightly packed, they were less likely to sink and more difficult to control. We suspect this to be the case at Wheeler's site. The efficiency of the salvinia weevil to control giant salvinia exceeded all expectations, especially in the relatively short time period when control was achieved. By January 2003, it was concluded that giant salvinia was no longer a problem at four of the six waterways being studied in Texas.

No significant difference (p = 0.106) in dissolved oxygen levels among the study sites were noted before the introduction of the salvinia weevils (Figure 2). DO at a majority of the
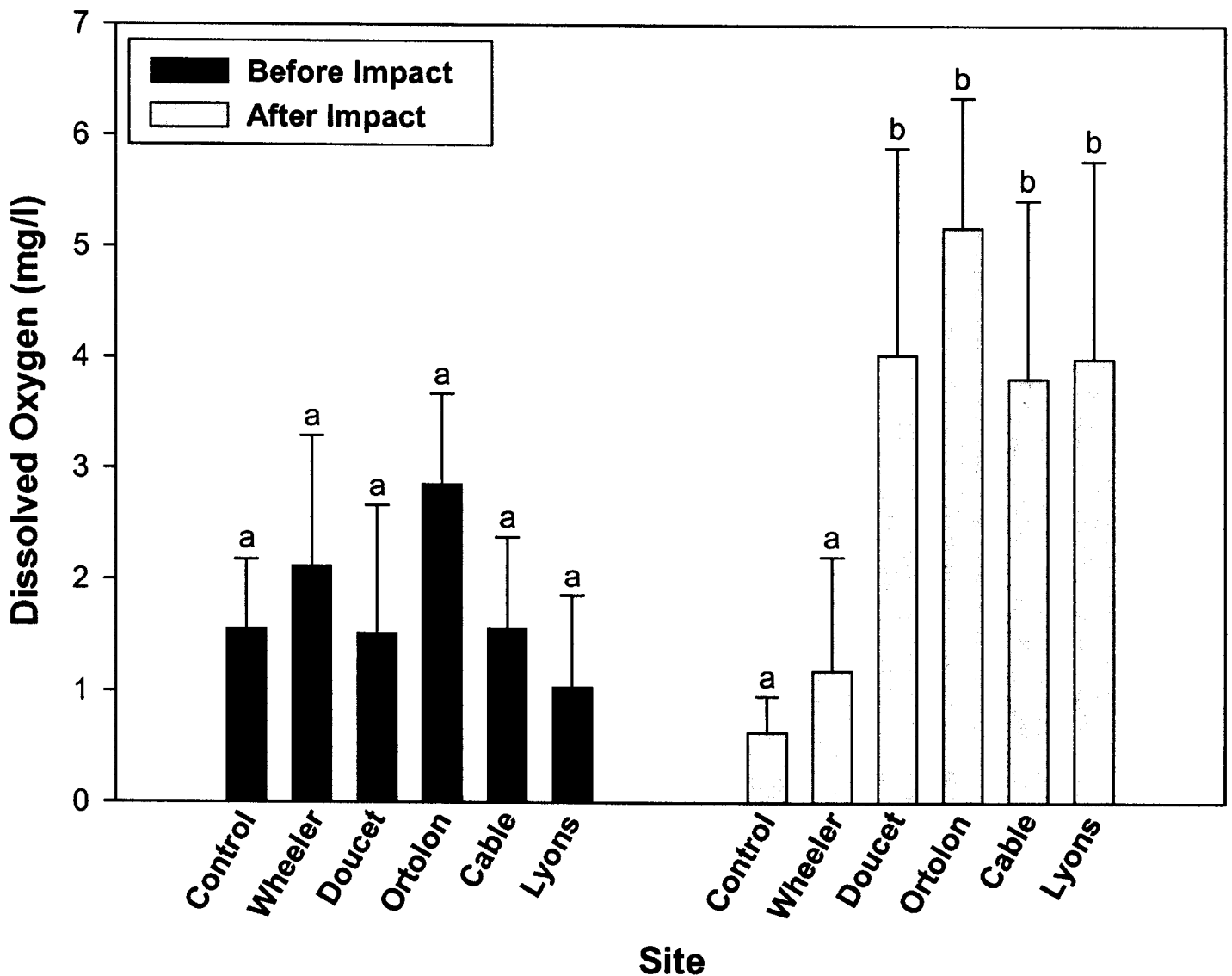

Figure 2. Mean dissolved oxygen levels (± s.e.m.) before introducing the biological control agents indicated that a significant difference (P = 0.106) was not observed among the sites. After biological control was achieved nine months later, a significant difference (P < 0.001) in dissolved oxygen levels was observed among the sites. Means followed by the same letter are not significantly different (P < 0.05).
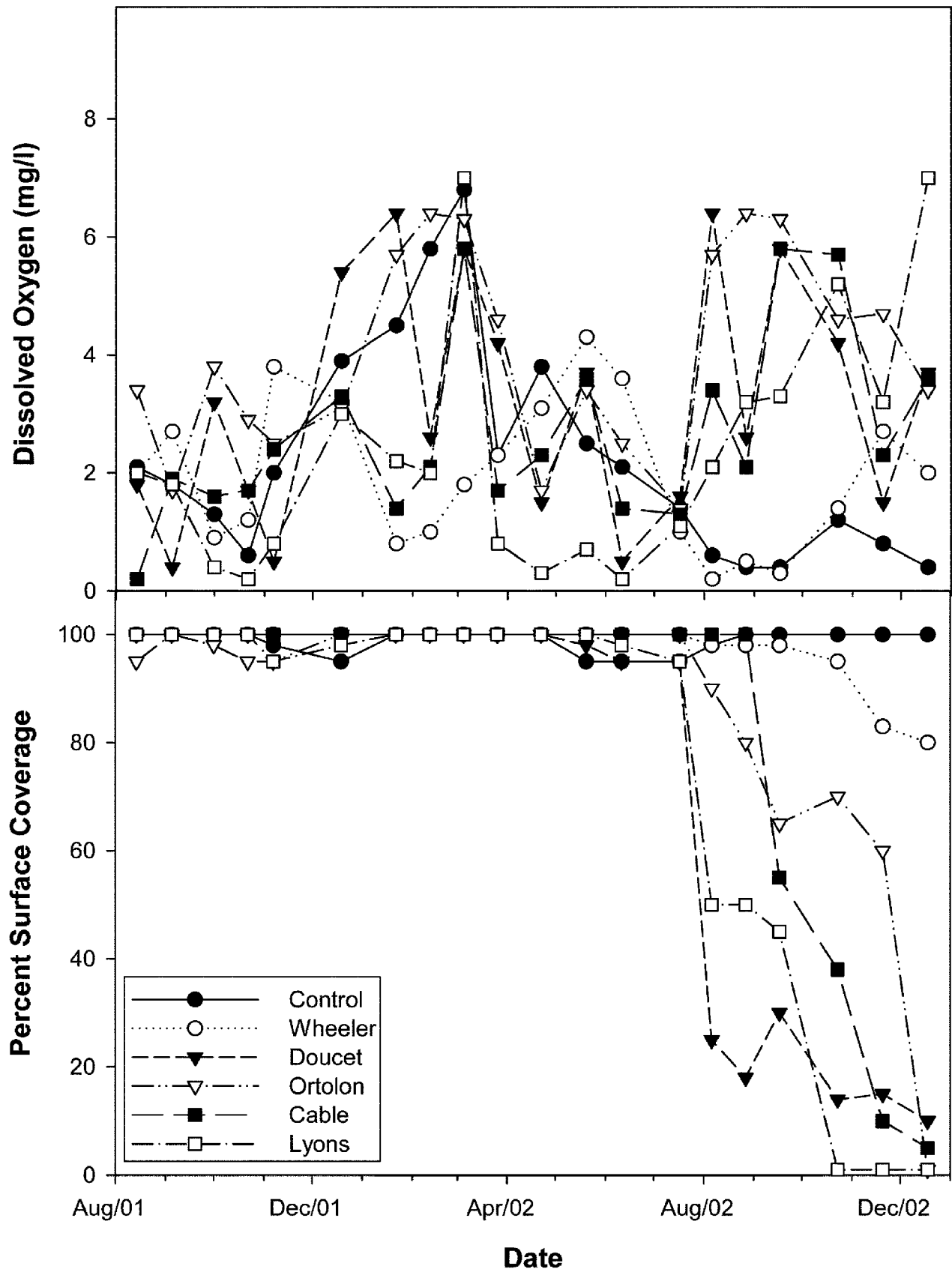
Figure 3. Mean dissolved oxygen levels and changes in percent surface coverage by giant salvinia over time at all study sites. Insects were introduced in November 2001 at five release sites.

sites before weevil introduction were low ranging from 1.5 to 2.8 mg/l. However, after plant coverage was reduced by sustained weevil feeding, significant differences (P < 0.001) in dissolved oxygen levels were noted in four of the five study sites including Doucet, Ortolon, Cable, and Lyons. Pairwise multiple comparisons procedures using Tukey's Test showed that the DO levels at the control and Wheeler's site were not significantly different from each other (P < 0.05); yet, were significantly different from DO levels among the other sites. These results indicate that the biological control agents had a significant impact on the plant populations which contributed to the increases in dissolved oxygen. The comparison of mean DO levels for McKey's site, which was our control site, showed a significant difference (t = -3.2, df = 9, P = 0.011). Mean DO level in the control decreased from 1.56 ± 0.28 mg/l at the beginning of our study to 0.63 ± 0.32 mg/l by the end of our study where giant salvinia continued to thoroughly infest the site. This decline in DO level may be the result of an increase in plant surface coverage. At Wheeler's site, the mean difference in DO level before and after treatment was not significant at the P = 0.05 level where 80% surface coverage was reported in August 2002. However, the biological control agents impacted the plants decreasing surface coverage which significantly increased DO levels at the Doucet (t = 2.62, df = 9, P = 0.028), Ortolon (t = 3.75, df = 9, P = 0.005), Cable (t = 2.83, df = 9, P = 0.020), and Lyons (t = 3.41, df = 9, P = 0.008) sites. When percent surface coverage reached 10% or less at these four sites, mean DO levels ranged from 3.8 to 5.2 mg/l.

Figure 3 shows mean DO levels and changes in percent surface coverage at all six study sites over time. When mean DO level at each percent surface coverage value was regressed as a function of percent surface coverage, the regression equation was y = -0.02x + 4.08, r = -0.67, n = 21 (Figure 4). The negative correlation coefficient (p < 0.001) indicates that there is an inverse relationship between DO level and percent surface coverage. As the biological control agent reduces surface coverage, an increase in DO occurs. Dissolved oxygen levels decline with increasing plant coverage.

Data was pooled for all sites where impact was indicated to show an overall increase in DO (Figure 5). The results showed a mean DO of 1.7 mg/l before treatment (n = 20) and a mean DO of 4.3 mg/l after nine months when biological control was achieved (n = 24). This result was a significant increase (t = 5.93, df = 42, P = <0.001) in DO of 2.6 mg/l. These findings indicate that biological control strategies for reducing plant surface coverage are compatible with DO requirements in East Texas waterways (TCEQ 2002). In addition to the federal noxious weed population being reduced, the aquatic environment may benefit from the increase in DO levels.

The salvinia weevil became quickly established in East Texas after its introduction and continued to spread naturally. The results of this study indicated that the weevil's impact was not the same at all sites. Most sites showed significant damage to the weed within months; yet, at one site the impact took years. A better understanding of the relationship between the weed and weevil is necessary in order to determine the differences in length of time to achieve control. The salvinia weevil is a highly successful biological control
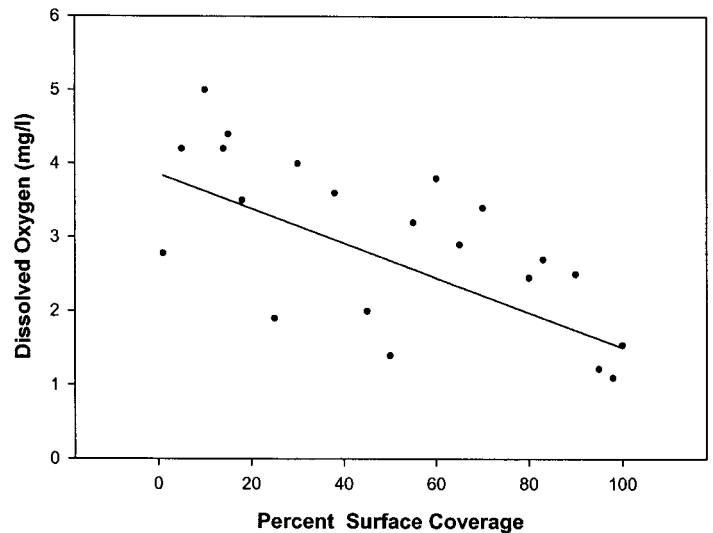

Figure 4. Relationship between dissolved oxygen level as a function of percent surface coverage. The regression equation was y = -0.02x + 4.08, r = -0.67, n = 21. The negative correlation coefficient (p < 0.001) indicates that there is an inverse relationship between DO level and percent surface coverage. Dissolved oxygen level declines with increasing plant coverage.

agent for controlling giant salvinia and positively impacts dissolved oxygen levels in the waterways due to the gradual decomposition of plant material.
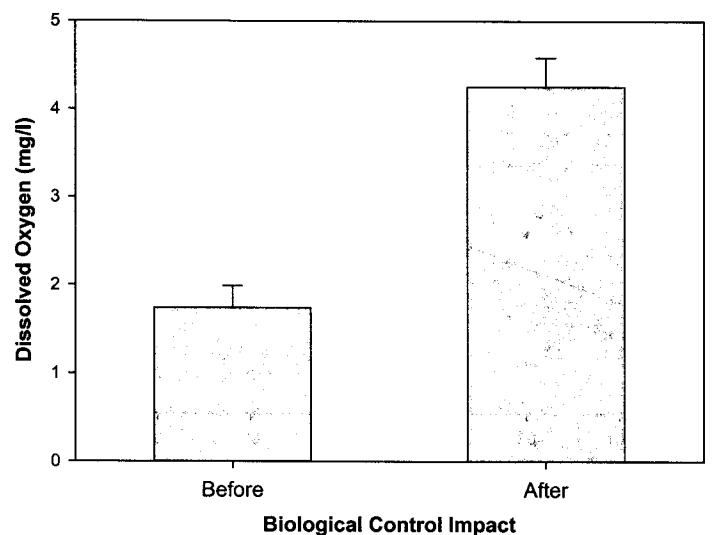
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Figure 5. Mean dissolved oxygen level (± s.e.m.) before releasing the biological control agents was 1.7 ± 0.2 mg/l (n = 20; August to November 2001). After acceptable control was achieved nine months later, the mean dissolved oxygen level increased to 4.3 ± 0.3 mg/l (n = 24; August to December 2002). This was a significant increase of 2.6 mg/l.

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