Research Article

Steven E. Woodley*, Charles Wahl and Rodrigo Diaz

Performance of *Salvinia molesta* (Salviniae: Salviniaceae) and its biological control agent *Cyrtobagous salviniae* (Coleoptera: Curculionidae) in freshwater and saline environments

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Abstract: Giant salvinia (*Salvinia molesta* Mitchell; Salviniaceae) is an invasive aquatic fern that inflicts significant economic and ecological threats when unmanaged. Biological control of giant salvinia using the semi-aquatic weevil, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), has proven successful in tropical and subtropical regions. The study aimed to assess the impact of salinity on *S. molesta* growth and health, as well as *C. salviniae* feeding behavior and density. Laboratory and outdoor mesocosm experiments were conducted at three salinity levels, <1, 5, and 10 ppt, alongside a field survey in southwestern Louisiana. Results indicated that *S. molesta* growth and health declined with increasing salinity, with significant damage observed at 5 ppt and near-total mortality at 10 ppt. *C. salviniae* feeding showed no significant differences across salinity levels in controlled settings, but field data revealed a decrease in weevil density at higher salinities. These findings suggest that while *S. molesta* can tolerate moderate salinity, its biological control via *C. salviniae* is less effective in saline environments. The study underscores the need for adaptive management strategies in coastal regions facing rising salinity due to climate change and sea-level rise.

Keywords: aquatic weed; coastal freshwater wetlands; giant salvinia; salinity intrusion; salvinia weevil

Resumen: Salvinia gigante (*Salvinia molesta* Mitchell; Salviniaceae) es un helecho acuático invasor que causa danos ecológicos y económicos cuando no es controlado. El control biológico de la salvinia gigante utilizando el gorgojo semiacuático, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae), ha demostrado ser exitoso en regiones tropicales y subtropicales. El objetivo de este estudio fué evaluar el impacto de la salinidad en el crecimiento y la daño foliar de *S. molesta*, así como el comportamiento alimentario y la densidad de *C. salviniae*. Se llevaron a cabo experimentos de mesocosmos en laboratorio y al aire libre en tres niveles de salinidad, <1, 5 y 10 ppt, junto con un estudio de campo en el suroeste de Luisiana. Los resultados indicaron que el crecimiento y la salud de *S. molesta* disminuyeron con el aumento de la salinidad, observándose un daño significativo a 5 ppt y una mortalidad casi total a 10 ppt. La alimentación de *C. salviniae* no mostró diferencias significativas entre los niveles de salinidad en entornos controlados, pero los datos de campo revelaron una disminución en la densidad de gorgojos a salinidades más altas. Estos hallazgos sugieren que, si bien *S. molesta* puede tolerar una salinidad moderada, su control biológico a través de *C. salviniae* es menos efectivo en ambientes salinos. El estudio enfatiza la necesidad de estrategias de manejo adaptativas en las regiones costeras que se enfrentan al aumento de la salinidad debido al cambio climático y al aumento del nivel del mar.

Palabras clave: maleza acuática; humedales costeros de agua dulce; salvinia gigante; intrusión de salinidad; gorgojo de la salvinia

1 Introduction

Giant salvinia (*Salvinia molesta* Mitchell, Salviniaceae) is a free-floating aquatic fern native to Brazil (Forno and Harley 1983; Julien et al. 2002; Luque et al. 2014). Over the past 70 years, *S. molesta* has successfully invaded many freshwater habitats in tropical and subtropical regions (ISC 2019; Jacono and Pitman 2001; Luque et al. 2014; McFarland et al.

***Corresponding author: Steven E. Woodley**, Minnesota Department of Natural Resources, Department of Fish and Wildlife, Madelia, MN, 56062, USA, E-mail: steven.woodley@state.mn.us
Charles Wahl, Department of Biology, University of North Carolina Greensboro, Greensboro, NC, 27412, USA, E-mail: CFWAHL@uncg.edu
Rodrigo Diaz, Department of Entomology, University of Louisiana, Baton Rouge, LA, 70803, USA, E-mail: rdiaz@agcenter.lsu.edu

2004; Oliver 1993). *Salvinia molesta* rapidly grows in freshwater habitats (Barrett 1989; Farrell 1978, 1979; Harley and Mitchell 1981; Johnson et al. 2010) due to its ability to reproduce asexually from rhizomes and plant fragments (McFarland et al. 2004; Oliver 1993; Room 1986, 1990). Under favorable conditions, *S. molesta* can double its biomass in less than 3 days (Barrett 1989; Harley and Mitchell 1981; Johnson et al. 2010). When left unmanaged, *S. molesta* can cover an entire waterbody in a dense, floating mat up to 1 m thick, which can negatively affect ecosystem services (Julien et al. 2002; McFarland et al. 2004; Room et al. 1989a; Thomas and Room 1986).

The salvinia weevil (*Cyrtobagous salviniae* Calder and Sands; Coleoptera: Curculionidae) is a successful tool for managing *S. molesta* in tropical and subtropical regions (Cilliers 1991; Room and Kerr 1983; Thomas and Room 1986; Woodley et al. 2023). Native to South America (Calder and Sands 1985; Wibmer and O'Brien 1986), *C. salviniae* was introduced in Louisiana in 2001 (Tipping 2004; Tipping et al. 2008). *C. salviniae* adults average 2.5 mm long and feed on buds and fronds (Johnson et al. 2010; Knutson and Mukherjee 2012). *C. salviniae* females deposit a single egg on *S. molesta* every 2–5 days and then hatch in 9–11 days, depending on temperature (Forno and Harley 1983). Newly hatched larvae feed on external parts of the plant, primarily buds, for 1–2 weeks. The larvae then tunnel into the plant through the petiole and continue to feed, inhibiting *S. molesta* nutrient uptake (Forno and Harley 1983; Sands et al. 1983). After feeding for 17–28 days, larvae pupate and emerge as adults in 10–15 days (Forno and Harley 1983). *C. salviniae* can have two to three generations per year (Forno and Harley 1983; Room et al. 1989b), and an individual female can lay 208–374 eggs in a lifespan (Eisenberg 2011; Jayanth 1989; Sands et al. 1986). *C. salviniae* fecundity and the severe damage caused by the larvae have made it a valuable management tool for *S. molesta* in southern Louisiana (Woodley et al. 2023). Tipping et al. (2008) documented that the biomass of *S. molesta* was reduced by 99 % in coastal Louisiana wetlands 2 years after *C. salvinia* was introduced.

Although *C. salviniae* has been documented to be a valuable management tool, the efficacy of *C. salviniae* in Louisiana's coastal freshwater wetlands may become more variable due to rising global mean sea level (Watson et al. 2015; Yi et al. 2015), subsidence of Louisiana's coastline (Tewari et al. 2019), and enhanced storm activity in the Gulf of Mexico (Dietz et al. 2018) resulting in seawater inundating further inland. This is probably because *S. molesta* can survive in higher salinity environments (Biber 2009; Divakaran et al. 1980; Oliver 1993) than *C. salviniae* (Julien et al. 2002; Thomas and Room 1986). At 3 ppt, *S. molesta* growth rate is reduced by 25 % (Divakaran et al. 1980), but plants can sustain themselves at 5 ppt (Biber 2009). At 10 ppt, *S. molesta* shows signs of severe stress after 10 days (Biber 2009), and after 20 h at 11 ppt, *S. molesta* withers completely (Divakaran et al. 1980). With seawater intruding further inland, a better understanding of how *S. molesta* and *C. salviniae* are affected by salinity is essential for future management decisions. Thus, this study's primary purpose was to examine the effect of salinity on the performance of *S. molesta* and *C. salviniae*. Our specific objectives were: (1) assess *S. molesta* growth (% cover) and health using three salinity levels (<1, 5, and 10 ppt) in the laboratory and outdoor mesocosm settings, (2) measure *C. salviniae* feeding (mm^2) on fronds exposed to the three salinity levels, and (3) evaluate *S. molesta* growth (% cover and mat thickness) and *C. salviniae* densities in the field along a salinity gradient in southwestern Louisiana.

2 Materials and methods

To assess the effects that salinity has on *S. molesta* and *C. salviniae* feeding, we conducted two experiments: a laboratory and an outdoor mesocosm experiment. In addition, we conducted a field survey in August 2020 along a salinity gradient in southwestern Louisiana to determine the performance of *S. molesta* and *C. salviniae*.

2.1 Laboratory experiment

To assess the effects that salinity has on *S. molesta*, three salinity levels (<1, 5, and 10 ppt) were selected to represent salinity concentrations found in freshwater, intermediate, and brackish marshes in Louisiana (He et al. 2022). The water used in the experiments was collected from Louisiana State University's (LSU) Campus Lake (30.409026 °N, 91.172678 °W) to simulate field conditions. To remove debris, the collected water was filtered using an 850 µm sieve (Standard Test Sieve, Fisher Scientific Company, Hampton, New Hampshire, USA). Using a YSI meter (YSI ProDSS, YSI Inc., Yellow Springs, Ohio, USA), we measured the salinity and pH of the filtered lake water, which were <1 ppt and approximately 6.5, respectively. *Salvinia molesta* was collected from a non-heated greenhouse at the LSU Agricultural Center Aquaculture Research Station (30.361176 °N, 91.173783 °W). Plants were healthy (green), in the tertiary growth stage, producing new buds, and did not contain *C. salviniae* or the giant salvinia moth, *Samea multiplicalis* (Guenée; Lepidoptera: Crambidae). Before starting the experiment, plants were acclimated to indoor laboratory conditions for 10 days. The

laboratory setup consisted of a greenhouse enclosure, 206 × 259 × 142 cm (l × w × h), 7.6 m^3, constructed from lumber and clear plastic (HDX Clear 6 mm Plastic Sheeting, Home Depot, Atlanta, Georgia, USA). Inside the enclosure, 54-W fluorescence grow lights (T5 4 ft 4 Tube Fixture, EnviroGro, Petaluma, California, USA) on a 14:10 h (light: dark) hour cycle were suspended 76 cm above the plants. The mean light intensity (Extech Light meter 401025; FLIR Commercial Systems Inc., Nashua, New Hampshire, USA) was 926 ± 23 lux (mean ± SE). Plastic containers (22 × 16 × 10 cm; l × w × h) 1,814 g deep dish (Glad Products Company, Oakland, California, USA) were placed inside the enclosure to contain water and plants. To create suitable growing conditions for *S. molesta*, temperature and humidity were maintained using a heater (AVH10 Whole Room Heater with Auto Climate, Vornado, Andover, Kansas, USA) set at 27 °C, and a humidifier (LV600HH Hybrid Ultrasonic Humidifier, Levoit, Anaheim, California, USA) set at 60 % relative humidity (RH).

Thirty plastic containers, ten replicates per treatment (salinity level), were used to examine the effect of salinity on *S. molesta* over 14 days. Salinity levels of 5 and 10 ppt were attained by mixing approximately 7.5 and 15.0 g, respectively, of Instant Ocean® aquarium salt (Spectrum Brands, Blacksburg, Virginia, USA) into 1,500 mL of the collected water. Before filling individual containers, salinity mixtures were thoroughly mixed and checked using a YSI meter. We also checked the pH of each salinity mixture using a YSI meter. For the <1 ppt treatment, the collected lake water was thoroughly mixed before filling containers. Containers were placed in the constructed greenhouse to condition for 24 h before adding plants. After 24 h, 1.5 mL of liquid fertilizer (Miracle-Gro All Purpose Plant Food (12N-4P-8K), Scotts Miracle-Gro Company, Marysville, Ohio, USA) was added to each container to provide essential nutrients. To confirm that the added fertilizer did not change salinity concentrations, salinity was measured again in each container using a salinity pen (Sper Scientific Large Display Salinity Pen-850036, Sper Scientific, Scottsdale, Arizona, USA). Salinity levels were then measured every 2 days for the duration of the experiment; however, pH levels were not measured. Additional water was added as needed to account for evaporation loss. After adding water, we measured salinity and added appropriate amounts of salt.

Healthy *S. molesta* plants were selected, drained of water for 60 s in a net (Frabill 3049 Wood Baitwell Net, Frabill Inc., Jackson, Wisconsin, USA), and then weighed (Mettler PE 300, Mettler-Toledo LLC, Columbus, Ohio, USA) to 10 g. The weighted plants were then randomly placed into a container.

2.1.1 Impact on plant performance

Salvinia molesta total surface coverage (%) was visually assessed for each container. *Salvinia molesta* health also was evaluated using the following plant damage descriptions: healthy (green), moderately damaged (yellow), and severely damaged (predominantly brown). Coverage assessments were conducted after 1, 7, and 14 days. At the end of the laboratory experiment, three fronds of similar size and shape were collected from five randomly selected containers from each salinity treatment and used in the *C. salviniae* no-choice tests.

2.1.2 Impact on *Cyrtobagous salviniae* feeding

No-choice tests were conducted for the first laboratory experiment to assess *C. salviniae* feeding behavior on *S. molesta* in different salinity levels. Three *S. molesta* fronds of similar size and shape were harvested randomly from five containers from each salinity treatment. Adult *C. salviniae* were collected via Berlese live extractions at the LSU Agricultural Center Reproductive Biology Center in St. Gabriel, Louisiana (30.2723 °N, 91.1045 °W). Once collected, the adults were placed into a container with fresh pond water and *S. molesta*. The containers were kept at room temperature (23 °C) for 3 days. After an 8-h starvation period, one adult weevil was placed in a Petri dish (100 × 15 mm) with two qualitative filter papers (90 mm circle, Whatman 1001-090 Qualitative Filter Papers, Whatman plc, Maidstone, United Kingdom), moistened with 3 mL of pond water, and one *S. molesta* frond. Tests were conducted in a growth chamber at 25 °C with a 14:10 h (light:dark) hour cycle. After 24 h, the feeding level was scored on the presence or absence of feeding. This test was replicated 15 times per salinity level using one adult *C. salviniae* and one *S. molesta* frond from each salinity treatment. Fronds that showed feeding damage were photographed and assessed for the amount of feeding using ImageJ (Rasband 2018).

2.2 Mesocosm experiment

The impact of salinity on *S. molesta* was assessed using two outdoor mesocosms (June and July 2020) at the LSU AgCenter Aquaculture Research Facility (30.3682 °N, 91.1835 °W). The same three salinity levels (<1, 5, 10 ppt) used in the laboratory were tested in the field. The water for the experiments was pumped from a nearby pond (30.3690 °N, 91.1836 °W). The mean pH of the pond water was approximately 7.5. As the pH was in the high range for *S. molesta*, adequate peat moss was added to lower the mean pH to approximately 6.5.

Healthy *S. molesta* was collected from an outdoor mesocosm at the LSU Aquaculture Research Station (30.361176 °N, 91.173783 °W). Because plants were subject to outdoor conditions, we did not acclimate the plants before starting the experiments. The mean daily ambient temperature for the first outdoor mesocosm experiment in June 2020 was 26.6 °C, with a mean minimum and maximum temperature of 21.6 °C and 31.7 °C, respectively. The mean daily ambient temperature for the second outdoor mesocosm experiment in July 2020 was 28.2 °C, with a mean minimum and maximum temperature of 24 °C and 32.4 °C, respectively. Total precipitation for June 2020 was 20.8 cm, and for July 2020, it was 22.4 cm (PRISM Climate Group 2023).

For each experiment, thirty mesocosms (76 L white trash can; Rubbermaid® Brute®, Rubbermaid Commercial Products, USA) were arranged in three rows (10 mesocosms per row) in an open field with full sun for 14 days. Each mesocosm was randomly assigned a salinity level (<1, 5, or 10 ppt) (10 replicates per salinity level). Salinity levels of 5 and 10 ppt were attained by mixing approximately 200 and 400 g, respectively, of aquarium salt (Instant Ocean Sea Salt, Blacksburg, Virginia) into the respective mesocosms filled with about 40 L of water. Salinity concentrations were checked using a YSI meter. No salt was added for the <1 ppt mesocosms.

Before adding plants, approximately 2 tbsp of granulated fertilizer (Miracle-Gro Water Soluble All Purpose Plant Food (12N-4P-8K), Scotts Miracle-Gro Company, Marysville, Ohio, USA) was added to each container to provide the plants with essential nutrients. After thoroughly mixing the fertilizer, salinity was measured in each container. For the duration of the experiment, salinity levels were measured twice per week. Additional water was added as needed to account for evaporation loss. After adding water, we measured salinity and added appropriate amounts of salt.

Healthy *S. molesta* plants were selected and placed in a net for 60 s to remove excess water. Plants were weighed (Scout Pro SP2001; Ohaus Corporation; Parsippany, New Jersey, USA) to approximately 400 g and placed in a randomly selected mesocosm. We chose 400 g as the weight because that amount of plants covered roughly 95 % of the surface in the mesocosm to encourage growth, as *S. molesta* is usually found in a crowded mat in field conditions.

2.2.1 Impact on plant performance

Salvinia molesta total surface coverage (%) was assessed visually for each container. *Salvinia molesta* health was evaluated using the same plant damage descriptions from the laboratory experiments: healthy (green), moderately damaged (yellow), and severely damaged (predominantly brown). Coverage assessments were conducted after 1, 7, and 14 days. As in the laboratory experiments, at the end of the first outdoor field experiment, three fronds of similar size and shape were harvested randomly from five randomly selected containers from each salinity treatment and used in the *C. salviniae* no-choice tests.

2.2.2 Impact on *Cyrtobagous salviniae* feeding

As in the laboratory experiment, no-choice tests were conducted for the first field experiment to assess *C. salviniae* feeding behavior on *S. molesta* in different salinity levels. Three *S. molesta* fronds of similar size and shape were harvested randomly from five mesocosms from each salinity treatment. Adult *C. salviniae* were collected via Berlese live extractions at the LSU Agricultural Reproductive Biology Center in St. Gabriel, Louisiana (30.2723 °N, 91.1045 °W). Following collection, the adults were placed into a container with fresh pond water and *S. molesta*. The container was kept at room temperature (23 °C) for 3 days. After 3 days, adults were starved for 8-h before no-choice tests occurred. After the starvation period, one adult was placed in a Petri dish (100 × 15 mm) with two qualitative filter papers (90 mm circle, Whatman 1001-090 Qualitative Filter Papers, Whatman plc, Maidstone, United Kingdom), moistened with 3 mL of pond water, and one *S. molesta* frond from one of the salinity levels. Tests were conducted in a growth chamber at 25 °C, 14:10 (light:dark) hours cycle. After 24 h, the feeding level was scored on the presence or absence of feeding. This test was replicated 15 times per salinity level using one adult *C. salviniae* and one *S. molesta* frond from each salinity treatment. Fronds that showed feeding damage were photographed and assessed for the amount of feeding using ImageJ (Rasband 2018).

2.3 Field survey

To measure the effect of salinity on *S. molesta* and *C. salviniae*, we sampled along a salinity gradient in coastal freshwater wetlands in southwest Louisiana. The survey was conducted in Burns Wetlands in Cameron Parish, Louisiana, on 31 July 2020. We sampled two wetlands for each salinity environment (freshwater with low salinity and brackish marsh with medium salinity) that closely represented salinity levels in the laboratory and outdoor mesocosm experiments. We surveyed salt marshes with high salinity; however, we could not find enough sampling locations with *S. molesta*. Sites were selected based on accessibility and mean salinity levels reported by

Coastwide Reference Monitoring System (CRMS) stations. Ten points or salvinia patches visually representing the average site conditions (*S. molesta* percent surface coverage and health) were sampled at each site. Mat thickness was measured with a plastic tray (0.3 × 0.4 m), with a string marked in 1 cm increments attached to the center of the tray. By sliding the tray under the salvinia mat and then lifting it, we were able to measure the thickness. The percentage of green salvinia was estimated visually by placing a quadrat (0.35 × 0.21 × 0.17 m (l × w × h); area = 0.01 m^3) in the mat. At each sampling point, pH and salinity levels were measured using a YSI meter approximately 30 cm below the water surface. From each sampling point, a sample of *S. molesta* was collected and brought back to the laboratory to assess biomass and *C. salviniae* densities using Berlese funnels.

2.4 Statistical analyses

For the laboratory and outdoor mesocosm experiments, Gamma distribution generalized linear models (GLMs), with a log link function, were used to examine differences in *S. molesta* total cover among salinity, sampling day, and the interaction of salinity with sampling day. The R package "lme4" (Lenth 2024) was used to develop and run GLMs, and packages "car" (Bates et al. 2015) and "emmeans" (Fox and Weisberg 2019) were used to examine differences among covariates and their interactions. We removed one outlier to meet the model assumptions for analyzing *S. molesta* cover in the outdoor mesocosm experiments. Given a significant result, pairwise comparisons were calculated using estimated means and corrected for multiple comparisons using Tukey's HSD test (α = 0.05). Differences among experiment 1 and 2 for the laboratory and mesocosm studies were compared using gamma-distribution, log-link function, GLMs, and as there was no effect of experiment number for the laboratory (χ^2 = 0.438, *df* = 1, *p* = 0.508) or mesocosm study (χ^2 = 0.224, *df* = 1, *p* = 0.635), the experiment data were merged for analysis for each study. Two-sample *t*-tests were used to assess differences in *C. salviniae* feeding area in the laboratory and mesocosm studies and for differences in *C. salviniae* density, *S. molesta* cover, and mat thickness in the field survey. Due to the limited number of samples from high salinity sites in the field survey, we only examined differences between low and medium salinity sites. We dropped the *C. salviniae* density from the high-salinity environment due to only finding two samples in the field, and one of those samples had a single *C. salviniae*, which inflated the density. Also, no statistical comparisons for *S. molesta* cover or mat thickness were examined in the high salinity environment because only two of the three sampling points had *S. molesta*. All analyses were conducted in RStudio (RStudio Team 2023).

3 Results

3.1 Laboratory experiment

3.1.1 Impact on plant performance

Variation in *S. molesta* percentage cover was explained by the salinity (χ^2 = 260.9, *df* = 2, *p* = <0.0001), sampling day (χ^2 = 115.1, *df* = 2, *p* = <0.0001), and interaction of salinity with day (χ^2 = 71.7, *df* = 4, *p* = <0.0001). *Salvinia molesta* percentage cover significantly differed by day 7 among the three salinity treatments (Table 1). Cover was approximately two times greater in the low salinity treatment than in the medium treatment after 14 days (Table 1) and three times greater than plants in the high salinity treatment after 14 days (Table 1). Cover increased by approximately 10 % in the medium salinity treatment by day 7 and increased by approximately 20 % after 14 days (Figure 1A); however, approximately 30 % of the plants were moderately damaged (yellow), and 20 % were severely damaged (predominantly brown) by day 14. Over the study duration, *S. molesta* cover in the high salinity treatment increased by approximately 5 % (Figure 1A). However, by day 7, more than 50 % of the plants were severely damaged, and >80 % by day 14.

3.1.2 Impact on *Cyrtobagous salviniae* feeding

There were no statistical differences in feeding area between salinity treatments, average = 0.44 ± 0.18 mm^2 (mean ± SE [standard error]); however, observational variation in feeding was evident with feeding damage on *S. molesta* being more apparent in the low salinity treatment, followed by medium then high (Figure 2A).

3.2 Mesocosm experiment

3.2.1 Impact on plant performance

Variation in *S. molesta* cover was explained by the salinity (χ^2 = 268.4, *df* = 2, *p* = <0.0001), sampling day (χ^2 = 512.4, *df* = 2, *p* = <0.0001), and the interaction of salinity with day (χ^2 = 498.1, *df* = 4, *p* = <0.0001). By day 7, *S. molesta* cover significantly differed between the low and high and medium and high salinity treatments (Table 2). By day 14, the percentage cover significantly differed among all the salinity

Table 1: Pairwise differences in *Salvinia molesta* percentage total cover (mean ± SE [standard error]) among the low, medium, and high salinity treatments in the laboratory experiment. Significant differences between treatments are indicated by an asterisk (*) (α = 0.05).

Day	Treatment comparison			Est.[a]	SE	*df*	*t*-value	*p*-value
1	Low	versus	Medium	0.0294	0.0833	171	0.353	1.0000
	Low	versus	High	−0.2088	0.0833	171	−2.505	0.2369
	Medium	versus	High	−0.1793	0.0833	171	−2.152	0.4417
7	Low	versus	Medium	0.5960	0.0833	171	7.153	<0.0001*
	Low	versus	High	−0.9742	0.0833	171	−11.691	<0.0001*
	Medium	versus	High	−0.3782	0.0833	171	−4.539	0.0004*
14	Low	versus	Medium	0.6439	0.0833	171	7.727	<0.0001*
	Low	versus	High	−1.1294	0.0833	171	−13.553	<0.0001*
	Medium	versus	High	−0.4855	0.0833	171	−5.826	<0.0001*

[a]Est. is the difference between estimated marginal means for treatments being compared.

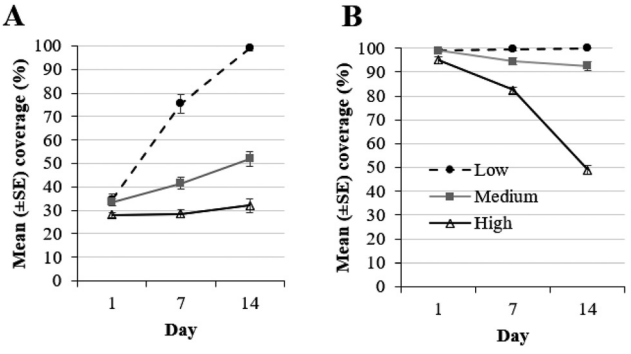

Figure 1: *Salvinia molesta* mean (±SE) total cover (%) in the laboratory (A) and outdoor mesocosm experiment (B) after 1, 7, and 14 days of exposure to low, medium, and high salinity treatments. Variation in *S. molesta* percent cover in the laboratory was explained by the salinity (χ^2 = 260.9, *df* = 2, *p* = <0.0001), sampling day ***(χ^2 = 115.1, *df* = 2, *p* = <0.0001), and interaction of salinity with day (χ^2 = 71.7, *df* = 4, *p* = <0.0001). Similarly, variation in *S. molesta* cover in the outdoor mesocosm experiment was explained by the salinity (χ^2 = 268.4, *df* = 2, *p* = <0.0001), sampling day (χ^2 = 512.4, *df* = 2, *p* = <0.0001), and the interaction of salinity with day (χ^2 = 498.1, *df* = 4, *p* = <0.0001).

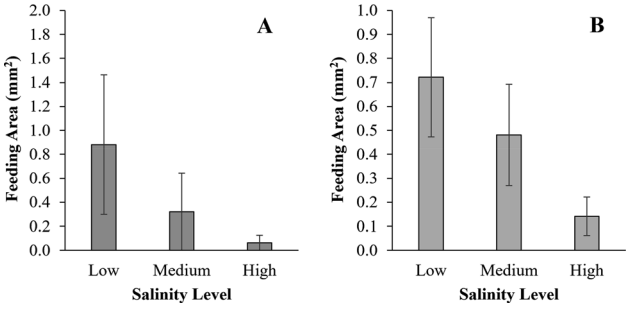

Figure 2: *Cyrtobagous salviniae* feeding (mean ± SE) on fronds from (A) the laboratory and (B) outdoor mesocosm experiment after 14 days of exposure to low, medium, and high salinity treatments. There was no statistical difference in *C. salviniae* feeding on fronds between the salinity levels in the laboratory experiment: low versus high (*t* = 1.9353, *df* = 24, *p*-value = 0.064), medium versus high (*t* = 1.3201, *df* = 24, *p*-value = 0.1992), low versus medium (*t* = 0.73453, *df* = 28, *p*-value = 0.4687). There was also no statistical difference in *C. salviniae* feeding on fronds between the salinity levels in the outdoor mesocosm experiment: low versus high (*t* = 1.1933, *df* = 24, *p*-value = 0.2444), medium versus high (*t* = 0.69939, *df* = 23, *p*-value = 0.4913), low versus medium (*t* = 0.8249, *df* = 27, *p*-value = 0.4167).

treatments (Table 2). *Salvinia molesta* increased cover in the low treatment by >95 % over the study duration (Figure 1B) and was healthy (green) throughout the study. *Salvinia molesta* cover in the medium salinity treatment declined by almost 10 % over the study (Figure 1B), and approximately 75 % of the plants were severely damaged. In the high salinity treatment, *S. molesta* cover decreased by approximately 50 % over the course of the study (Figure 1B), and approximately 90 % of the plants were severely damaged on day 7.

3.2.2 Impact on *Cyrtobagous salviniae* feeding

There were no statistical differences in feeding area between treatments, average = 0.42 ± 0.32 mm^2 (mean ± SE [standard error]); however, as in the laboratory experiment, observational variation in feeding was evident on *S. molesta* fronds as feeding damage was more apparent for the low salinity treatment, followed by medium then high (Figure 2B).

3.3 Field survey

3.3.1 High salinity sites

In the field survey in southwestern Louisiana, we found only small fragments of *S. molesta* at two of the three sampling points in the high-salinity environment. The salinity level at

Table 2: Pairwise differences in *Salvinia molesta* percent total cover (mean ± SE) among the low, medium, and high salinity treatments in the mesocosm experiment. Significant differences between treatments are indicated by an asterisk (*) (α = 0.05).

Day	Treatment comparison			Est.	SE	*df*	*t*-value	*p*-value
1	Low	versus	Medium	0.0000	0.0233	170	0.000	1.0000
	Low	versus	High	0.0412	0.0233	170	1.767	0.7034
	Medium	versus	High	0.0412	0.0233	170	1.767	0.7034
7	Low	versus	Medium	0.0516	0.0233	170	2.209	0.4044
	Low	versus	High	0.1874	0.0233	170	8.029	<0.0001*
	Medium	versus	High	0.1358	0.0233	170	5.820	<0.0001*
14	Low	versus	Medium	0.0780	0.0233	170	3.341	0.0278*
	Low	versus	High	0.6934	0.0236	170	29.321	<0.0001*
	Medium	versus	High	0.6152	0.0236	170	26.023	<0.0001*

[a]Est. is the difference between estimated marginal means for treatments being compared.

the sampling points was 8.7 ± 0.51 ppt (mean ± SE [standard error]) (n = 3), *S. molesta* cover was 6.7 ± 3.33 % (n = 2), and mat thickness was 0.3 ± 0.17 cm (n = 2). The mean number of adult *C. salviniae* per kg of *S. molesta* was 11.4; however, this density is magnified due to only one of the two sampling points with *S. molesta* present having adult *C. salviniae* and the very low *S. molesta* biomass.

3.3.2 Comparison between low and medium salinity sites

Salinity levels in the low and medium salinity wetlands sampled were 0.39 ± 0.01 ppt (mean ± SE [standard error]) (n = 20) and 4.04 ± 0.03 ppt (n = 20), respectively, and significantly differed between the low and medium salinity environments (χ^2 = 144.8, *df* = 2, p = <0.0001). pH levels for the low and medium salinity wetlands were 6.56 ± 0.06 (mean ± SE [standard error]) (n = 20) and 7.10 ± 0.04 (n = 20), respectively, and significantly differed between the low and medium environments (χ^2 = 3162.8, *df* = 2, p = <0.0001).

Salvinia molesta cover in the low-salinity environment and medium-salinity environment was 80.5 ± 0.04 % (mean ± SE [standard error]) (n = 20) and 59.0 ± 0.04 % (n = 20), respectively, and significantly differed (t = 4.09, *df* = 38, p = <0.0001). More specifically, *S. molesta* cover in the low-salinity environments was 20 % greater than in the medium-salinity environments (Figure 3). *Salvinia molesta* mat thickness in the low-salinity environment and medium-salinity environment was 4.8 ± 0.40 cm (mean ± SE [standard error]) (n = 20) and 1.3 ± 0.12 cm (n = 20), respectively, and significantly differed (t = 9.30, *df* = 38, p = <0.0001). Moreover, *S. molesta* mat thickness was also twice as thick in the low salinity environment compared to the medium salinity environment (Figures 3 and 4).

The mean adult *C. salviniae* density (adults per kg) was 12 times greater in the low compared to the medium-salinity

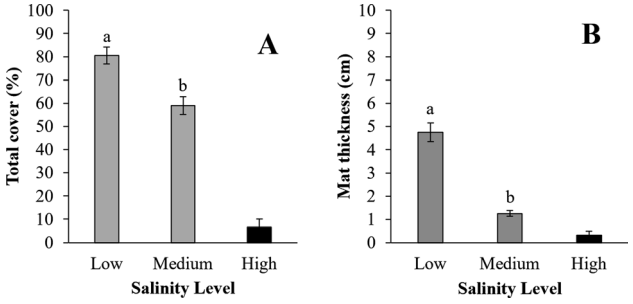

Figure 3: *Salvinia molesta* mean percentage total cover (A) and mat thickness (B) (mean ± SE) in southwestern Louisiana's low, medium, and high salinity environments. *Salvinia molesta* percentage cover (t = 4.09, *df* = 38, p = <0.0001) and mat thickness (t = 9.30, *df* = 38, p = <0.0001) significantly differed between low-salinity and medium-salinity environments. No statistical comparisons were examined with the high salinity environment because only two of the three sampling points had *S. molesta*.

environment, 36.0 ± 6.27 (mean ± SE [standard error]) (n = 20) and 3.8 ± 1.15 (n = 20), respectively, and was significantly different (t = 6.29, *df* = 38, p = <0.0001). The mean larval *C. salviniae* density (larvae per kg) was 2.3 ± 0.87 (mean ± SE [standard error]) (n = 20) and 1.6 ± 1.02 (n = 20), respectively. However, *C. salviniae* larval density did not differ between low and medium-salinity environments (t = 1.91, *df* = 38, p = 0.063).

4 Discussion

Giant salvinia exhibited low tolerance to higher salinity levels in our experiments. Under laboratory conditions, *S. molesta* increased in coverage when subjected to 5 ppt salinity, although ~50 % of the plants showed some impairment (i.e., moderate or severe damage). Under mesocosm conditions, *S. molesta* cover progressively declined, and approximately 75 % of the plants were severely damaged in

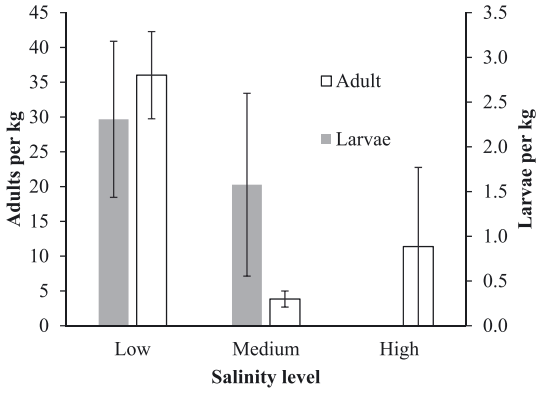

Figure 4: Adult and larval *Cyrtobagous salviniae* densities (mean ± SE) in low, medium, and high salinity environments in southwestern Louisiana. Adult *C. salviniae* densities significantly differed between the low and medium salinity environments (t = 6.29, *df* = 38, *p* = <0.0001). However, *C. salviniae* larval density did not differ between low and medium-salinity environments (*t* = 1.91, *df* = 38, *p* = 0.063). No statistical comparisons were examined with the high salinity environment because only one of the two sampling points with *S. molesta* present had adult *C. salviniae*. No larvae were found in the high-salinity environment.

the 5 ppt salinity treatment. Under field conditions, in sites with high salinity levels (approximately 8 ppt) *S. molesta* was stunted and showed almost no sign of growth. These results are in agreement with previous studies showing the adaptation of *S. molesta* to thrive in freshwater habitats (<1 ppt) (Barrett 1989; Harley and Mitchell 1981; Johnson et al. 2010). Findings from the laboratory and mesocosm experiments and field survey partially support Biber (2009) that *S. molesta* can tolerate 5 ppt salinity. However, our findings support Oliver (1993) in that *S. molesta* is less likely to sustain itself under natural field conditions in brackish wetlands, and its plant tissues and growth are adversely affected when salinity levels reach 4 ppt (Storrs and Julien 1996). Furthermore, at 10 ppt, *S. molesta* cannot sustain itself for prolonged periods (Biber 2009) and likely dies in the field in less than 7 days. We did not measure changes in leaf chemistry or narrow down the salinity level at which damage is evident. However, even at low salinity levels, *Salvinia* undergoes changes in morphology, photosynthetic capacity, proline, total carbon and nitrogen, leaf chemistry, and cation concentrations (Jampeetong and Brix 2009; Moreira et al. 2023).

Our study showed that *C. salviniae* feeding was not affected by salinity levels under laboratory and mesocosm conditions. Although no significant differences in *C. salviniae* feeding were measured among plants subjected to the three salinity treatments, observationally it appeared that feeding decreased with increasing salinity. We recognize that our study only assessed the impact on adult feeding during a short period (hours), and perhaps a longer trial (days) would allow the detection of other impacts, such as larval survival. We could argue that as leaf quality continues to deteriorate due to salinity exposure, it is likely that it becomes less suitable for adult feeding. The observed feeding could reflect an adaptation of adults to feed on green salvinia exposed to elevated salinity, but we do not know how the timing of exposure will result in sublethal effects as observed with other freshwater insects (Herbst et al. 2013). The significant difference in the mean adult *C. salviniae* density between the low (36 adults/kg) and medium (4 adults/kg) salinity environments in the field supports these observational differences. These findings suggest that *C. salviniae* may struggle to survive in saline environments >4 ppt or will disperse to more freshwater environments. However, our findings demonstrate that *C. salviniae* will feed and tolerate salinity environments of <4 ppt. The presence of several adult weevils clinging to tiny clumps of salvinia in the high salinity site in the field might suggest that those clumps drifted from nearby sites with lower salinity. The lack of larvae and poor conditions of those clumps make us speculate that it is unlikely that permanent weevil populations can be established at sites with salinity of 10 ppt or more.

The motivation for this study stems from the increasing salinity intrusion in coastal freshwater wetlands of Louisiana, where biological control of giant salvinia is crucial for maintaining ecosystem services (Tipping et al. 2008; Wahl et al. 2021; Woodley et al. 2023). The poor salinity tolerance observed in our experiments may be due to the salvinia-weevil system evolving in the freshwater wetlands of southern Brazil (Miranda and Schwartsburd 2019). Our findings provide a baseline for *C. salviniae* feeding on *S. molesta* under varying salinity levels and *C. salviniae* densities in natural field conditions. Moreover, our study demonstrates that both *S. molesta* and *C. salviniae* can tolerate salinity levels below 4 ppt. This is critical information given the increasing storm surges from enhanced storm activity in the Gulf of Mexico (Dietz et al. 2018) and the subsidence of Louisiana's coastline (Tewari et al. 2019). With more frequent seawater inundation, our findings suggest that sites should be monitored for at least 14 days following a seawater surge to determine if *S. molesta* is recolonizing. If recolonization occurs, we recommend augmenting the *C. salviniae* population or implementing alternative management techniques (e.g., herbicides, Cozad et al. 2019) to ensure effective control. Therefore, we suggest monitoring weevil populations (Woodley et al. 2023), determining the local population of weevils for reintroduction, or utilizing weevils from mass rearing colonies (Nachtrieb 2014; Wahl and Diaz 2020). To reduce the uncertainty of salinity intrusion in coastal freshwater wetlands, further research is

needed to determine the effect of the timing of elevated salinity exposure on the recovery of biological control services of aquatic weeds.

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References

Barrett, S.C.H. (1989). Waterweed invasions. *Sci. Am.* 261: 90–97.

Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Software* 67: 1–48.

Biber, P.D. (2009). Determining salinity-tolerance of giant salvinia using chlorophyll fluorescence. *Gulf Caribb. Res.* 21: 31–36.

Calder, A.A. and Sands, D.P.A. (1985). A new Brazilian *Cyrtobagous* Hustache (Coleoptera: Curculionidae) introduced into Australia to control salvinia. *Aust. Entomol. Soc.* 24: 57–64.

Cilliers, C.J. (1991). Biological control of water fern, *Salvinia molesta* (Salviniaceae), in South Africa. *Agric. Ecosyst. Environ.* 37: 219–224.

Cozad, L.A., Mudge, C., and Diaz, R. (2019). Integrated management of giant salvinia using herbicides and the salvinia weevil. *J. Aquat. Plant Manag.* 57: 62–68.

Dietz, M.E., Kam-biu, L., and Bianchette, T.A. (2018). Hurricanes as a major driver of coastal erosion in the Mississippi River Delta: a multi-decadal analysis of shoreline retreat rates at Bay Champagne, Louisiana (USA). *Water* 10: 1480.

Divakaran, O., Arunachalam, M., Murugan, T., and Nair, N.B. (1980). Studies on the life cycle and ecology of *Salvinia molesta* Mitchell. *Proc. Plant Sci.* 89: 519–528.

Eisenberg, L.J. (2011). *The reproductive morphology and physiological age-grading of female Cyrtobagous salviniae, the salvinia weevil. Thesis. Entomology Department*. Louisiana State University, Lafayette, Louisiana, pp. 74.

Farrell, T.P. (1978). The spread and control of *Salvinia molesta* in Lake Moondarra, Mt. Isa, Queensland. In: *Proceedings of the first conferences of the council of Australian weed science societies*. Council of Australian Weed Science Societies, Melbourne, Australia, pp. 179–187.

Farrell, T.P. (1979). Control of *Salvinia molesta* and *Hydrilla verticillata* in Lake Moondarra, North West Queensland. In: *Management of aquatic weeds*. Australian Water Resources Council, Department of Natural Resources, Canberra, Australia, pp. 57–71.

Forno, I.W. and Harley, K.L.S. (1983). Distribution, biology, and host specificity of *Cyrtobagous singular* Hustache (Coleoptera: Curculionidae) for the biological control of *Salvinia molesta*. *Bull. Entomol. Res.* 73: 85–95.

Fox, J. and Weisberg, S. (2019). *An R companion to applied regression*, 3rd ed. Sage, Thousand Oaks, California, USA, https://socialsciences.mcmaster.ca/jfox/Books/Companion/.

Harley, K.L.S. and Mitchell, D.S. (1981). The biology of Australian weeds. 6. *Salvinia molesta* D.S. Mitchell. *J. Aust. Inst. Agric. Sci.* 47: 67–76.

He, S., Maiti, K., Swarzenski, C.M., Elsey-Quirk, T., Groseclose, G.N., and Justic, D. (2022). Porewater chemistry of Louisiana marshes with contrasting salinities and its implications for coastal acidification. *Estuar. Coast Shelf Sci.* 268: 107801.

Herbst, D.B., Roberts, S.W., and Medhurst, R.B. (2013). Defining salinity limits on the survival and growth of benthic insects for the conservation management of saline Walker Lake, Nevada, USA. *J. Insect Conserv.* 17: 877–883.

ISC (Invasive Species Compendium) (2019). *Salvinia molesta* (kariba weed), https://www.cabi.org/isc/datasheet/48447 (Accessed 20 November 2019).

Jacono, C. and Pitman, B. (2001). *Salvinia molesta*: around the world in 70 years. *Aquatic Nuisance Species Digest.* 4: 13–16.

Jampeetong, A. and Brix, H. (2009). Effects of NaCl salinity on growth morphology, photosynthesis and proline accumulation of *Salvinia natans*. *Aquat. Bot.* 91: 181–186.

Jayanth, K.P. (1989). Studies on the fecundity and longevity of *Cyrtobagous salviniae*, an effective biocontrol agent of *Salvinia molesta*. *Biol. Control* 3: 62–64.

Johnson, S., Sanders, D., Eisenberg, L., and Whitehead, K. (2010). Fighting the blob: efforts to control giant salvinia. *La. Agric.* 53: 6–9.

Julien, M., Center, T., and Tipping, P. (2002). Floating fern (*Salvinia*). In: Van Driesche, R. (Ed.). *Biological control of invasive plants in the Eastern United States*. United States Department of Agriculture Forest Service Publication, Washington, DC, USA, p. 413.

Knutson, A. and Mukherjee, A. (2012). Giant salvinia, salvinia weevil, and biological control of giant salvinia. In: Knutson, A., and Nachtrieb, J. (Eds.). *A guide to mass rearing the salvinia weevil for biological control of giant salvinia*. Texas A&M AgriLife Extension Service, Special Publication ESP-475, pp. 1–8, http://cise.tamu.edu/media/355740/gs_manual_1015-12.pdf.

Lenth, R. (2024). emmeans: estimated marginal means, aka least-squares means. *R package version 1.10.4*, https://CRAN.R-project.org/package=emmeans.

Luque, G.M., Bellard, C., Bertelsmeier, C., Bonnaud, E., Genovesi, P., Simberloff, D., and Courchamp, F. (2014). The 100th of the world's worst invasive alien species. *Biol. Invasions* 16: 981–985.

McFarland, D.G., Nelson, L.S., Grodowitz, M.J., Smart, R.M., and Owens, C.S. (2004). *Salvinia molesta DS Mitchell (Giant Salvinia) in the United States: a review of species ecology and approaches to management (No. ERDC/EL-SR-04-2)*. Environmental Laboratory – Engineer Research and Development Center, Vicksburg, Mississippi, USA.

Miranda, C.V. and Schwartsburd, P.B. (2019). *Salvinia* (Salviniaceae) in southern and southeastern Brazil—including new taxa, new distribution records, and new morphological characters. *Braz. J. Bot.* 42: 171–188.

Moreira, M.H., They, N.H., Rodrigues, L.R., Alvarenga-Lucius, L., and Pita-Barbosa, A. (2023). Salty freshwater macrophytes: the effects of salinization in freshwater upon non-halophyte aquatic plants. *Sci. Total Environ.* 857: 159–608.

Nachtrieb, J.G. (2014). Mass-rearing *Cyrtobagous salviniae* for biological control of giant salvinia: field release implications. *J. Aquat. Plant Manag.* 52: 22–26.

Oliver, J.D. (1993). A review of the biology of giant salvinia. *J. Aquat. Plant Manag.* 31: 227–231.

PRISM Climate Group (2023). Data explorer: temperature and precipitation monthly values (June and July 2020) for Louisiana state University AgCenter Aquaculture research Facility (30.3682 °N, 91.1835 °W), http://www.prism.oregonstate.edu/explorer/.

Rasband, W.S. (2018). *ImageJ*. U.S. National Institutes of Health, Bethesda, Maryland, USA, Available online: https://imagej.nih.gov/ij/.

Room, P.M. (1990). Ecology of a simple plant-herbivore system: biological control of salvinia. *Trends Ecol. Evol.* 5: 74–79.

Room, P.M. and Kerr, J.D. (1983). Temperatures experienced by the floating week *Salvinia molesta* Mitchell and their prediction from meteorological data. *Aquat. Bot.* 16: 91–103.

Room, P.M. and Thomas, P. (1986). Nitrogen, phosphorus and potassium in *Salvinia molesta* Mitchell in the field: effects of weather, insect damage, fertilizers and age. *Aquat. Bot.* 24: 213–232.

Room, P.M., Gunatilaka, G.A., Shivanathan, P., and Fernando, I.V.S. (1989a). Control of *Salvinia molesta* in Sri Lanka by *Cyrtobagous salviniae*. In: Delfosse, E.S. (Ed.). *Proceedings of 7th international symposium on the biological control of weeds*, 290th ed. Rome, Italy, p. 285.

Room, P.M., Julien, M.H., and Forno, I.W. (1989b). Vigorous plants suffer most from herbivores: latitude, nitrogen and biological control of the weed *Salvinia molesta*. *Oikos* 54: 92–100.

RStudio Team (2023). *RStudio*. Integrated development for R. RStudio, PBC, Boston, Massachusetts, http://www.rstudio.com/.

Sands, D., Schotz, M., and Bourne, A.S. (1983). The feeding characteristics and development of larvae of a salvinia weevil *Cyrtobagous sp*. *Entomol. Exp. Appl.* 34: 291–296.

Sands, D.P.A., Schotz, M., and Bourne, A.S. (1986). A comparative study on the intrinsic rate of increase of *Cyrtobagous singularis* and *C. salviniae* on the water weed *Salvinia molesta*. *Entomol. Exp. Appl.* 42: 231–237.

Storrs, M.J. and Julien, M.H. (1996). *Salvinia: a handbook for the integrated control of Salvinia molesta in Kadadu National Park*. Australian Nature Conservation Agency, Darwin, Australia.

Tewari, S., Manning, F., and Palmer, W. (2019). Sea level rise and land subsidence in coastal Louisiana. Forecasting, mapping and identifcation of at-risk transportation infrastructure. *Proceedings of the 98th annual meeting of transportation research board, Washington, D.C., USA*, pp. 1–19. https://scholarsmine.mst.edu/civarc_enveng_facwork/1540/.

Thomas, P.A. and Room, P.M. (1986). Taxonomy and control of *Salvinia molesta*. *Nature* 320: 581–585.

Tipping, P.W. (2004). Giant salvinia. In: Coombs, E.M., Clark, J.K., Piper, G.L., and Cofrancesco, A.F. (Eds.). *Biological control of invasive plants in the United States*. Oregon State University Press, Corvallis, Oregon, pp. 174–177.

Tipping, P.W., Martin, M.R., Center, T.D., and Davern, T.M. (2008). Suppression of *Salvinia molesta* Mitchell in Texas and Louisiana by *Cyrtobagous salviniae* Calder and Sands. *Aquat. Bot.* 88: 196–202.

Wahl, C.F. and Diaz, R. (2020). Winter and spring conditions determine the production of the salvinia weevil mass rearing programme. *Biocontrol Sci. Technol.* 30: 569–580.

Wahl, C.F., Diaz, R., and Kaller, M. (2021). Nutrients enhance the negative impact of an invasive floating plant on water quality and a submerged macrophyte. *J. Aquat. Plant Manag.* 59: 57–65.

Watson, C.S., White, N.J., Church, J.A., King, M.A., Burgette, R.J., and Legresy, B. (2015). Unabated global mean sea-level rise over the satellite altimeter era. *Nat. Clim. Change* 5: 565–568.

Wibmer, C.J. and O'Brien, C.W. (1986). Checklist of the weevils of South America. *Mem. Am. Entomol. Inst.* 39: 1–563.

Woodley, S.E., Wahl, C.F., Tryforos, A., and Díaz, R. (2023). Biological control of invasive floating fern leads to rapid recovery of ecological functions in coastal freshwater wetlands in Louisiana. *J. Aquat. Plant Manag.* 61: 42–54.

Yi, S., Sun, W., Heki, K., and Qian, A. (2015). An increase in the rate of global mean sea level rise since 2010: increase in sea level rise rate. *Geophys. Res. Lett.* 42: 3998–4006.