Limnological responses to active management of the invasive aquatic fern *Salvinia molesta* in Las Curias Reservoir, San Juan, Puerto Rico.

By

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In memory of my grandfather, Joaquín López Avila. For my family. My mother: María M. López Bidot, father: Wilfredo García Colón, My grandmother: Monserrate Bidot Diaz, My Brother: Wilfredo A. García López. Lastly, for my girlfriend, Laura V. González Cruz.

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TABLE OF CONTENTS

List of Figures

Figure **Page**

List of Tables

Table **Page**

ABSTRACT

The anthropogenic deterioration of aquatic ecosystems affects water resources due to agricultural malpractices, pollution due to domestic septic tanks, recreational activities, poor management of watersheds, and others. For instance, introducing invasive aquatic weeds has been recognized as a significant problem in watersheds worldwide due to their ability to create anoxic conditions. This study focuses on the management of the Las Curias Reservoir in San Juan, Puerto Rico since the arrival of the aquatic fern *Salvinia molesta* around 2016. Since December 2019, a community-driven initiative involving state, federal and academic institutions led to the introduction, in December 2019, of the *Cyrtobagous salviniae* to the reservoir. This weevil is considered an effective biological control agent for S. *molesta*. Simultaneously, community members initiated a mechanical removal campaign using an aquatic harvester. Limnological samplings since September 2019 to March 2022 was conducted to measure physicochemical responses in the reservoir in response to the reduction of *S. molesta* cover. In addition, monthly drone flights were conducted to measure changes in aerial plant coverage. Propagation of *S. molesta* was aggravated due to eutrophication after an increase in nutrient-rich sewage discharges from septic tanks after hurricane Maria in 2017. By 2019, the reservoir was completely covered by the fern. It is not until August 2020 that we noticed a considerable change in the reduction of plant cover of 43% (100,462 m2). Upon the reduction of plant coverage, we notably found increases for the period of (2019-2022) in the mean water temperature along the epilimnion of 0.96 °C, 1.37°C in the thermocline, and 1.05 °C in the hypolimnion. Mean dissolved oxygen increased 4.17 mg L-1 along the epilimnion, 0.43 mg L-1 in the

thermocline, and 0.04 mg L^{-1} in the hypolimnion. Crucial nutrients concentrations have also increased during this period. The recovery of the Las Curias ecosystem has been the result of combined biological and mechanical controls, applied effectively for the first time in Puerto Rico.

INTRODUCTION

We are currently facing a global climate crisis with longer and more extreme droughts and more intense rainfall events (Wehner et al., 2017; Gutiérrez et al., 2020; Keellings and Hernandez, 2019). Puerto Rico has not been exempted of this crisis. In 2017, one of the most active Atlantic hurricane seasons in history occurred. The record for consecutive storms was surpassed with two major hurricanes crossing the island: Hurricane Irma (September 7, 2017) and Hurricane Maria (September 20, 2017) (Pasch et al., 2018; Keellings and Hernández, 2019; NOAA, 2018a, 2019). In fact, Hurricane Maria was the most intense tropical cyclone to hit the US territories in the documented history (NOAA 2018a). Under a changing climate, it is critically important to maintain water reserves such as reservoirs and lakes in good conditions to effectively cope with an increase in the frequency of storms and droughts.

Beginning in 2015, Las Curias reservoir was invaded by the exotic aquatic fern *Salvinia molesta* Mitchell 1972 (also known as giant salvinia, Salviniaceae) (Thayer et al. 2018; Martínez, 2016). The plant has been introduced in Puerto Rico via commercial trade and seems to be spreading rapidly to other water bodies in Puerto Rico. The spread of giant salvinia increased especially after Hurricane María in 2017, probably due to eutrophication after an increase in nutrient-rich sewage discharges associated with septic tanks (Lugo et al. 2011; Marrero, 2017; Garcia, 2018; Wahl et al. 2020). Giant salvinia is known as one of the most invasive floating aquatic ferns in the southeastern United States (Luque et al., 2014; Díaz et al., 2015). It is native to southern Brazil, Argentina, and Uruguay and is currently highly invasive in aquatic systems in tropical and subtropical areas around the world do their rapidly fragmentation by asexual reproduction (Forno and Harley, 1979; McFarland et al., 2004). In 2013, giant salvinia was listed among the 100 most harmful invasive alien species in the world by the Species Survival Commission (SSC) of the International Union for Conservation of Nature (IUCN) (Luque et al., 2014). In the United States and territories, this plant is prohibited, and its sale or possession is illegal (Morgan, 2020; Malawi & Virginia, 2017). In Louisiana, the ability to form extensive layers of vegetation in lakes, ponds, reservoirs, and swamps has been well-documented (Díaz et al., 2017; Moshman et al., 2017). The expansion of the giant salvinia in water bodies can limit the prosperity of native species and reduce light penetration and dissolved oxygen below the mats, affecting water quality and ecosystem services, including recreational activities (Díaz et al., 2017). According to McFarland et al (2004), rapid rates of nutrient uptake combined with relatively slow rates of decomposition, enable *S. molesta* to tie up nutrients that could be used by other primary producers that contribute to complex food chains.

Worldwide, lake restoration through aquatic weed control consists of three methods: biological, mechanical, and chemical (USDA, 2001; Jayan and Sathyanathan, 2012). Since December 2019, the local Las Curias community in collaboration with the University of Puerto Rico at Rio Piedras, Louisiana State University, and local and federal agencies, introduced to Las Curias Reservoir the *Cyrtobagous salviniae* Calder & Sands 1985, an effective biological control agent of the giant salvinia. Biological control using the salvinia weevil is considered the most cost-effective method of control of giant salvinia (Sullivan et al., 2011). The salvinia weevil is a host-specific herbivore that has been released in some infested waters in the United States, South Africa, Australia, and Asia (Forno and Harley, 1979). Biological control using the salvinia weevil have resulted in successful biological control of giant salvinia in tropical and subtropical regions (Julien et al., 2009). Simultaneous to the introduction of the salvinia weevil, community members from Las Curias initiated a mechanical removal campaign using an aquatic harvester. Due to its high costs, the effectiveness of the mechanical control is considered a short-term strategy because the ability of salvinia to double its mass in a short time. Therefore, the mechanical control was considered complementary to the biological control. The propagation and establishment of the weevil was expected to have long-term effects on the restoration efforts of the aquatic ecosystem. Anecdotal evidence suggests that during the project period chemical applications of herbicides has been minimal. For this study, up to 13 monthly limnological samplings (September 2019 - March 2022) were considered.

The overarching goal of this study was to monitor the physicochemical, biochemical, and biophysical responses of Las Curias reservoir during the *S. molesta* control project. The specific objectives were to measure changes in biomass coverage, document the ecological impact to the waterbody, and establish a relationship of water quality and plant coverage. Wahl et al. (2020) documented extremely poor water quality conditions under 100% *S. molesta* cover in the reservoir. Therefore, we expected that water quality would improve with reductions in the aerial extent of *S. molesta*. Specifically, we expected an increment in epilimnetic water temperature, dissolved oxygen, pH, and in specific conductance with a reduction in plant coverage. We also hypothesized that less salvinia coverage would increase water transparency and chlorophyll-a. As far as the biological control agent introduced, we expected weevil densities to increase in Las Curias Reservoir thus reducing the aerial extent of giant salvinia. We also expected that the combined effects of the biological and mechanical controls would speed up the reduction in salvinia coverage in the reservoir. Both controls of giant salvinia implemented in Las Curias, involves in situ disposal of the fern via sinking. Thus, we predict that concentrations of nitrogen and phosphorus would increase as salvinia decomposes in the lake bottom.

MATERIALS AND METHODS

Site description

Las Curias Reservoir was built in 1946 by the Puerto Rico Aqueduct and Sewer Authority (PRASA) as the first of the projects to supply drinking water to the San Juan Metropolitan Area (García-Martinó 2000; Torres-Ortiz and Rosa-Castro 2014; DRNA, 2004). Since its construction and through the early 1980's, Las Curias was operated by PRASA as a raw water source to Rio Piedras Aqueduct (Tetra Tech, 2018; DRNA, 2004). Currently, the reservoir is no longer used as an active source of water for public supply but has been used during droughts to supply raw water to rural San Juan. Currently, it is mainly used for recreation and fishing (Wahl et al., 2020). Over the last decade, Las Curias Reservoir has been severely affected by eutrophication. Given its current and potential benefits, its restoration is socially relevant and justifiable.

Las Curias Reservoir has a surface area of 0.192 km2 and is located in the Cupey ward of the municipality of San Juan, Puerto Rico (N18.34169; W66.04828) in the Rio Piedras Watershed (Fig.1) (Wahl et al., 2020). According to the 2020 census, the population of the Cupey ward is 29,790 (3,970.2 people per square mile). The reservoir basin lacks a public sewage collection system, so septic tanks are used for household wastewater disposal. The reservoir has a nominal capacity of 1,120 acres-feet, and a maximum storage of 1,425 acre-feet (Tetra Tech, 2018). At nominal storage, the surface area is 47.5 acres at an approximate elevation of 100m NGVD29. The drainage basin is approximately 2.85 km2 and is mostly rural with evergreen forest (43%), grassland/herbaceous (36%), developed, low intensity (11%), open water (5%), developed open space (4%), and developed, medium intensity (1%) (Tetra Tech, 2018;

DRNA, 2004). Below the dam Las Curias stream flows north for 16 km and discharges into the San Juan Bay Estuary (DRNA, 2004; García-Montiel et al., 2014). Mean annual rainfall in the Rio Piedras watershed is lower in the coast (1,509 mm) and increases in the uplands (1,755 mm at 8 km inland), and the ratio of pan evaporation to rainfall changes in the same way from 1.37 to 0.98 (Lugo et al., 2011). Rainfall varies over the year; with the rainy season running from July to October). The dry season occurs between January to April (Lugo et al., 2011). Mean annual temperature for San Juan and Río Piedras is 25.9°C and 25.7 °C (Lugo et al., 2011).

Salvinia Performance

Based on periodic drone flights, we created mosaics or maps of each aerial image to calculate the coverage of the aquatic fern. The collection of aerial imagery was performed using Unmanned Aerial Systems, UAS or DRONEs. The flight line consisted of 80% photo overlap and 70% flight line overlap. The flight plan includes two flight lines passing over the study area, north-south direction, and west-east direction. The flight altitude guaranteed a ground sample distance, scaled to the local salvinia cover. ArcGIS Pro and Drone2map software's were used to transform drone-captured imagery into 3D imagery products. The changes in coverage represent the monthly changes of the surface of the complete reservoir and not of the sampling stations specifically.

Mat thickness was determined in October 2021 for 15 locations using a plastic tray (0.3 × 0.4 m), with a string, marked in 1 cm increments, tied to the center of the tray. The tray sank below the mat of giant salvinia, then was lifted to measure the thickness of the salvinia layer. The percentage of greenness was estimated visually using the salvinia within the quadrant. The normalized difference vegetative index

(NDVI) was acquired using a handheld Trimble GreenSeeker® (Trimble Agriculture Company, Sunnyvale, CA, USA). The GreenSeeker was attached ~1 m above the mat surface and then swept sideways for ~1 m across the salvinia mat. Feeding damage by weevils (present or absent) and the number of buds with weevil damage were also documented and quantified as part of the plant health description. To characterize these physical conditions of the salvinia, the same techniques used by Wahl et al. (2020) were applied.

Monitoring weevil dispersal

On October 19, 2019, the Cyrtobagous salviniae were transported from Lousiana State University to the University of Puerto Rico-Rio Piedras. In the laboratory, the weevils were transferred to fresh salvinia from Las Curias and within two hours, transported to Las Curias and placed in a protected area of reservoir (initial inoculation site or arm D: Figure 2). Since their introduction, weevil densities and plant damage have been periodically monitored at several locations within the reservoir (Figure 2).

On each monitoring event, about 5 grams of salvinia were collected near each sampling station using a dip net. Weevil damage was documented and quantified as salvinia buds were inspected. salvinia samples were placed in one-gallon resealable plastic bags, transported to the University of Puerto Rico-Rio Piedras, Tropical Limnology Laboratory, and placed in Berlese funnels for 24-48 hours. The wet mass of each salvinia samples was determined prior to transfer to Berlese funnels following the Nachtrieb (2012) method.

Weevil establishment and dispersal was assessed by measuring insect densities initially along arm D and then at the other release sites (Figure 2). In June 2020, samples were collected along arm D in the inoculation area to assess weevil densities. In December 2020, salvinia infested with the weevil was transferred from arm D to the other arms of the reservoir (A, B, C, E). By that time, weevils at arm D had reached the optimal observational density value of weevils (40 individuals per kg of wet salvinia) recommended for transfer to other stations in the reservoir (Rodrigo et al., 2020). The weevil density samplings were carried out between June 6, 2020, December 7 and 20, 2020, May 17 and 21, 2021, October 10, 2021, and February 18, 2022. On October 10, 2021, samples were collected at different points in the reservoir. These data were integrated to the nearest arms. Time series of weevil densities per kilogram of wet salvinia were developed for each monitoring station to assess their rate of dispersal within the reservoir.

Water quality

Two sampling stations were established to measure water quality parameters at Las Curias Reservoir from September 2019 to March 2022 (Figure 2). Using a calibrated Hydrolab MS5 Multiprobe sonde, monthly or bimonthly at least, we measured conductivity (µS/cm), dissolved oxygen (DO), pH (s.u), and temperature (°C) at fixed depths intervals in the water column. Water samples of 250 mL were collected at different depths using a Watermark Horizontal PVC Water Bottle. The samples collected were used for analysis of Chlorophyll-a Total Phosporous (TP), Total Kjeldahl Nitrogen as Nitrogen (TKN-N), Nitrate as Nitrogen (NO3--N), Ammonium as Nitrogen (NH4-N), Ammonia as Nitrogen (NH3-N), and Orthophosphate as Phosphate (PO43-). Ammonia and Phosphate was measured using the Hach SL1000 Portable Parallel Analyzer (PPA). The other nutrients were analyzed in the Agricultural Experimental Station Soil and Water Quality Laboratory in Rio Piedras, P.R. Chlorophyll-a analysis was conducted in water samples filtered through a GF/F filter and extracted following a method adapted from Nusch (1980) and Marker et al. (1980). Chlorophyll-a concentration is used to indirectly estimate the biomass of phytoplankton communities, since it is the main photosynthetic pigment present in algae (Gregor and Marsálek, 2004). To estimate changes in the depth of the photic zone, a Secchi disk was used to measure water transparency.

To evaluate the temporal recovery of the entire lenthic ecosystem of Las Curias and to compare with the historical water quality data available, we assumed a mixed vertical system as in Wahl et al. (2020). And for calculated the average of physico-chemical parameters and nutrient concentrations measured from 2019 to 2022 throughout the water columnwas assumed a mixed vertical system too. To assess the internal dynamics, we also analyzed water quality parameters for each individual water layer (epilimnion, thermocline, hypolimnion). The location of the water layers was determined by observing in situ variations in the water column conductivity on each day of sampling. While dynamic in depth, generally, the epilimnion was in the upper 2 meters, the hypolimnium below 7 meters with a thermocline in between. Changes in Secchi depth and Chlorophyll-a was also used as an index of ecosystem recovery and dissolved oxygen. Linear regression analyzes were used to describe whether the increasing or decreasing trends of the different parameters in the water layers were statistically significant using a confidence value of 95%. Physico-chemical parameters for each sampling station were represented as vertical profiles to analyze temporal changes in the vertical water column at both stations. This allowed to observe seasonal variations from the surface to deeper areas near the substrate.

RESULTS

Plant controls

According to Wahl et al. (2020), community efforts to manually removed salvinia began after April 2016, but soon after Hurricane Maria hit Puerto Rico in September 2017, the fern expanded to the entire lake surface (Wahl et al., 2020). On September 27, 2019, we conducted the first drone flight. Through a State Legislature grant, the community obtained funds to purchase an aquatic harvester which arrived at Las Curias reservoir in October 2019. Mechanical extraction of salvinia began immediately. This was a community-funded initiative. Through neighbor donations, they were able to pay for the harvester operator and diesel. The purchase and maintenance costs of machinery, diesel, and the salary cost of the harvester operator for two years (2020 – 2021) were estimated at $297,000.00. In December 2019, the weevil was introduced, thus, both the mechanical and biological efforts ran mostly simultaneously.

Aerial changes in salvinia coverage have been drastic since 2019 when it was estimated at 100% (176,975 m^2) (Figure. 3 and 4A). In the 2019 and 2020 orthomosaic maps, it was observed that in addition to salvinia, the aquatic grass (*Rhynchospora holoschoenoides*) was also predominant in the arms and forming round islands in open water. On March 5, 2020, mechanical removal efforts focused near the reservoir morning glory spillway. Thus, plant coverage was still high at 83% (146,413 m^2) (Figure 4B). By August 2020, the area near Station 2 was fully covered because of Tropical Storm (July 29, 2020, to July 31, 2020) and because the mechanical removal began to concentrate in open waters. By then, plant coverage was reduced to 57% (100,462 m^2). By October 2020, a large mat of accumulated salvinia was evident near the dam (Figure

4C). For March 2022, a 10.94% (19.36 m^2) of plant coverage was calculated (Figure 3). As part of the mechanical control strategy, the aquatic harvester began to bring salvinia and other aquatic plants from the areas farthest from the spillway. By March 2020, it was noticed that to the area near Station 1 was cleared of salvinia, and the total plant coverage had been reduced to 17% (146,413 m^2). Since January 2021, when the plant cover was reduced by 71% (51,983 m^2) the plant cover has been concentrated in the arms due to the proliferation of *R. holoschoenoides*. Islands of this sedge near the dam were pushed to the bottom of Station E by 2022 as part of the mechanical control strategy (Figure 4D). Figure 5 illustrates the changes and the extent of open water areas near the reservoir dam.

In October 2021, Dr. Rodrigo Diaz had the opportunity to interview the community leader who offered details of the mechanical extraction strategies by the aquatic harvester. In March 2020, intensive mechanical removal efforts began, although they were interrupted by the Covid-19 pandemic. In the interview, the community leader commented that ropes with water jugs were used to hold the salvinia in place as the mechanical removal progressed. A bulldozer was necessary to remove the large piles of plant biomass that included salvinia and other species. The salvinia was piled in and out of the reservoir on mountains using the harvester, causing the salvinia to die and sink into the reservoir. Also, they sank some islands of *R. holoschoenoides* by piling salvinia on top of these grass islands. They used sticks to push the salvinia, placing a pole above and another 30 cm below, and using a wire mesh to increase the recollection area. The salvinia were stacked in sections in such a way that the empty spaces were filled as the wind pushed the salvinia. According to the community leader, the removal process took about 20 months. Wind was also an important factor in moving salvinia towards the spillway changing the mechanical extraction strategies continuously.

In addition to reducing the percentage of plant cover, the thickness of the remaining salvinia mats was also reduced, facilitating navigation and recreational activities such as fishing. Field data from October 10, 2021, show that much of the biomass was reduced throughout the lake. The mean wet weight of giant salvinia extracted from the quadrant (area = 0.0735 m^2) was 165.6g (±113.40, n = 15), and the mean dry weight was 9.3g (±6.87, n = 15). In June 2020, 49 of 189 buds had visible weevil damage or feeding scars identified by “shotgun” hole patterns and browning. Between December 7 and 11 2020, 198 of 1,364 buds had visible weevil damage. In October 2021, 69 out of 150 inspected buds had weevil damage. For October 24, 2021, mean mat thickness was 1.3 cm (±0.88, n = 15), mean percent green was 40% (± 25.57, n = 15), and mean NDVI was 0.51 (± 0.05, n = 15). Compared to the means of the data from September 27, 2019, the thickness of the plant was reduced by 20.7 cm, the percentage of greenness was reduced by 27% and the NDVI by 0.02, indicative of an unhealthy salvinia.

Herbaceous and free-floating plants

Other aquatic plants were documented on October 10, 2021. The *R. holoschoenoides* has been successfully established in different part of the Las Curias reservoir specially in arm E and D during and following the partial eradication of *S. molesta.* It was also observed that salvinia remained only on the edges of this grass. *R. holoschoenoides* is considered common in Puerto Rico and in the Caribbean (DNER et al. 2001). Other plants that are present in the reservoir, but in very low densities, include *Mikania sp*, *Pistia stratiotes*, *Typha sp*, *Hydrocotyle*, and terrestrial grasses. All these plants that persist in addition to salvinia, continue to contribute to the total biomass of floating aquatic plants in Las Curias.

Weevil performance

Periodic monitoring of weevil densities was carried out to quantify its establishment and dispersal in the reservoir (Figure 6). Arm D maintained weevil densities well-above the recommended threshold (40 adults per kg of wet salvinia) from 192 adults per kg of wet salvinia in June 2020 to 166 adults per kg of wet salvinia in October 2021. On October 10, 2021, in arm E, the highest densities of the weevil were reported since 2020, when about 500 individuals (adults + larvae) per kilogram of wet salvinia were quantified. The latest data from station A collected in October 10, 2021 indicate that weevil densities were above the threshold line with 181 adults per kg of wet salvinia. As the density of salvinia in the reservoir was reduced, the stations to be monitored were reduced. In February 2022 we found some patches of salvinia at stations D and C where at station C densities remain above the threshold line with 45 adults per kg of wet salvinia. In the Berlese funnel extractions *Samea multiplicalis* Guenée, 1854, a generalist aquatic plant herbivore moth (Knopf and Habeck 1976), was not present in any of the sampling locations since 2020. In February 2022, densities were below the threshold level (24 per kg of wet salvinia).

Water quality

Data from 13 sampling dates were analyzed from September 2019 to March 2022, except for the conductivity parameter which was 11. Two limnological stations

(Station 1 and 2) were established in the reservoir to determine changes as mechanical removal and biological control are carried out. Mean DO during this period was 1.30 mg L-1 (SD = 2.19, n = 246), mean pH was 6.7 (SD = 0.46, n = 246), mean specific conductivity was 292.80 µS/cm (SD = 47.03, n = 206) and mean temperature was 26.18 °C (SD = 1.63, n = 246) (Table 1). Mean total Kjeldahl Nitrogen mg/L as Nitrogen (TKN-N) was 0.48 mg L-1 (± 0.38, n = 27), mean Total Nitrate mg/ L as Nitrogen (NO3-N) was 0.01 (±0.04, n = 27), mean Ammonium mg/L as Nitrogen (NH4-N) was 0.05 (±0.12, n = 27) mean Total Phosphorus (P) was 0.06 mg L-1 (± 0.05; n = 27), mean Phosphate mg/L as (PO_4^{3-}) was 0.12 mg L-1 (± 0.05, n = 42), mean Ammonia mg/L as Nitrogen (NH3-N) was 0.25 mg L-1 (± 0.39; n = 42), mean Secchi depth (m) was 0.60 (± 0.44, n = 26), and Chlorophyll-a was 3.24 (± 2.57, n = 43) (Table 1).

From 2019 to 2022 there have been notable physico-chemical changes in the water column as the salvinia biomass above the water surface was reduced (Figure 7). For example, at Station 1, located near the reservoir spillway, maximum depth was reduced from 19 m in September 2019 to 8 m in March 2022, suggesting an accumulation of 11 meters of dead salvinia in the bottom. In January 2021, Station 2 still had salvinia cover and other aquatic plants such as *R. holoschoenoides* with small patches of *P. stratiotes*. This station has not had a considerable reduction in its maximum depth, ranging around 8 meters.

For the same period (2019 to 2022), the mean water temperature in the epilimnion increased 0.96 °C. At the thermocline, temperature increased 1.37 °C. Mean temperature also increased during this period at the hypolimnion by 1.05 °C. Mean DO along the epilimnion increased 4.17 mg L-1. Mean DO in the thermocline increased 0.43

mg L-1. In the hypolimnion, DO increased 0.04 mg L -1. Mean pH along the epilimnion increased 1.25 s.u, in the thermocline increased 0.81s.u, and 0.7 s.u. at the hypolimnion. Mean specific conductivity along the epilimnion increased 122 μS/cm, mean conductivity in the thermocline increased 81.12 μS/cm, and 69.59 μS/cm at the hypolimnion. Mean nutrient concentrations in the epilimnion increase 0.26 mg L-1 for TKN-N, 0.27 mg L-1 for NO_3-N, and 0.05 mg L-1 for NH_4-N. TP decreased 0.06 mg L-1. PO_4^{3-} increase 0.03 mg L-1, and NH_3 increase 0.16 mg L-1. Concentrations at the thermocline increase 0.57 mg L-1 for TKN-N, 0.11 mg L-1 for NO_3-N, 0.14 mg L-1 for NH_4-N. Mean TP decreased 0.03 mg L-1. PO_4^{3-} increase 0.09 mg L-1, and NH_3-N increase 0.57 mg L-1, mean Chlorophyll-a along the epilimnion decreased 2.35, and in the thermocline decreased 0.28, and mean Secchi depth increased 1.01 m (Table 1).

There was a significant tendency for increase in the epilimnion for DO, pH, SpCond, NO_3-N, NH_4-N and NH_3-N (Table 2). There was a significant decline in Chl-a in the epilimnion. In the thermocline only the tendency to increase was significant, while in the hypolimnion the tendency to increase DO and pH was significant. During this study, the Secchi depth show a significant tendency to increase.

After the rain event of July 2020, the Secchi depth decreased in both stations. Station 2 did not have considerable changes during these rain events. After the rain events of January and February 2022, the Secchi depth decreased in both stations. The strong thermal stratification observed in Las Curias may be also responsible for the observed lack of dissolved oxygen observed in the deep-water layers of the water column (Gunkel and Casallas, 2002). Dissolved oxygen has been very low since 2019, even near the surface as salvinia was removed, the DO increased considerably in both stations in the epilimnion. In August 2020 and February 2022, it is observed that, in the epilimnion, the DO increased considerably after rain events. Rain events have been important in both stations, increasing oxygen in the epilimnion.

The highest temperatures in the epilimnion (first two meter of the water column) were reported in august 2020 after Tropical Storm Isaías. The thermocline or the plane of maximum rate of decrease of temperature with respect to depth in Las Curias is generally between three to six meters (Figure 7) (Wetzel, 2001). Seasonal weather variations and local environmental conditions can influence the thermocline. Below the thermocline, the hypolimnion layer is characterized by constant temperatures and anoxic conditions (Wetzel, 2001). Through August 2020, Las Curias continued to show very strong thermal stratification with a maximum vertical gradient of 5-6 °C, accentuated during the summer months. Such a large thermal stratification is uncommon in Puerto Rican lakes (Gustavo Martinez, personal communication; Quiñones, 1980). The thermal stratification decreased to 2-3 degrees Celsius in December 2020 and January 2021 as air temperatures dropped. This pattern was repeated during the second winter season. Rain events of January and February 2022 diminished the thermal stratification to 2-6°C. In March 2022, it is observed again that temperatures begin to rise below the epilimnion. Relatively low temperature still in the hypolimnion (~24 ºC) compared with epilimnion temperatures (~27 ºC) in the surface where higher temperatures predominate. According to Horne and Goldman (1994), seasonal changes in precipitation and thermal regimes are important driving factors for water column stratification and mixing patterns in the tropics.

From August 2020 to October 2020 when the mechanical control was at its peak the water became very acidic, especially near the bottom. After Tropical Storm Isaías, the highest levels of acidity were reported in both stations. The water was considerably acidified with pH values less than 6.07 s.u. (Figure 7). It then started to become more alkaline until the present where the January and February 2022 rain events occurred. The rain events of January and February 2022 made the water column very alkaline with pH values from 8.3 in the surface to 7.4 at the bottom. In the cooler months (December through February), the pH tends to become less acidic.

After the mechanical removal efforts, the conductivity began to increase progressively in both stations. The changes are very marked compared to 2019. Both rain events (July 2020, January 2022, and February 2022) decreased the conductivity in the first 4m. After 4m, the conductivity reached its usual value of 300 us/cm. It was observed that during rain events the conductivity tends to increase progressively after ~4m. Since 2019, there are considerable increases in conductivity as we get closer to the substrate of both stations. In March 2022, the conductivity decreased from the surface to the bottom. After the rain event of January and February 2022 the conductivity started to increase again.

DISCUSSION

In the scientific literature it has been documented that biological control of salvinia with *C. Salviniae* is successful in tropical and subtropical areas (Julien et al., 2009). In addition to Las Curias, biological control has been successful in many countries around the world such as South Africa, Senegal, Australia, United States, and others (Martin et al., 2018; Pieterse et al., 2003; Sullivan et al., 2012; Room et al., 1981; Tipping et al., 2008).

Salvinia cover changes had remarkable ecological impacts in Las Curias. Cover changes are associated with biological and mechanical control and rainfall events. Rain events can move salvinia into the discharge zone. If the salvinia layer is very dense, the fragmentation will be greater and will reflect an increase in cover as occurred in September 2020. Mean temperature of the period of 2019 - 2022 was 26.18°C and according to the statistical test the tendency to increase the temperature in this period was not significant. This is favorable for salvinia because since it was introduced the temperatures in the reservoir have not increased or decreased significantly. According to Room and Thomas (1986) a good temperature for *S. molesta* growth is 30°C, but its growth is null below 10°C or above 40°C. Salvinia control was more associated with biological and mechanical controls because ecosystem conditions, especially temperature, continued to be favorable for salvinia except for pH when it became more alkaline during rainy periods. Fortunately, they were also favorable for the salvinia weevil to become established. With an 100% of plant coverture, gas exchange with the atmosphere and the water was practically nil. By 2019 the available oxygen was limited to the first 0.35 m of the water column. After 2019, oxygen started to penetrate deeper.

In the epilimnion, it averages between 2.12 mg/L-1 in 2020 to 2.63 mg/L-1 in 2022. Below the epilimnion, oxygen was non-existent or at very low levels as it was probably being consumed faster than it was being generated via photosynthesis. The increment only was statistically significant in the epilimnion. According to Hutchinson (1957), Wetzel (1983) and Buffle and Stumm (1994), when oxygen in the hypolimnion cannot be replenished as fast as it is consumed, microbial respiration switches to anaerobic pathways and redox stratification develops (i.e., the water column becomes stratified in terms of the distribution and speciation of redox-sensitive elements). We have observed dense black islands of salvinia that have been partially decomposed at the bottom of the reservoir and probably risen to the surface through methane. It is likely that the salvinia is decomposing at the bottom anaerobically, but as it rises to the surface, we can expect its decomposition to continue aerobically. The vertical profiles of both stations do not show considerable mixtures due to the high stratification that remains constant throughout the year. The strong thermal stratification of the Las Curias reservoir during most of the year is not usual in the reservoirs of Puerto Rico. According to Lewis (1973), tropical lakes are more susceptible to the mixing effects of winds and surface cooling by rain. This implies that the stratifications may be more susceptible to disruption. Like Lake Matano in Indonesia and other lakes around the world, the absence of strong seasonal temperature fluctuations precludes seasonal convective overturn as a mechanism to transport oxygen into the deep waters (Crowe et al., 2008). Aquatic organisms such as fish are likely to remain stressed since according to Flores and Carlson (2006) aquatic life becomes stressed when DO falls below 5 mg L-1 and large-fish kills can occur below 2 mg L-1. By removing the salvinia cover, solar radiation penetrated the water column heating the surface water mass, thus increasing its temperature.

Rain events have been shown to be an essential factor for the changes that occur in the water column and stratification. The reduction in pH, increase in temperature and conductivity are also associated with the decomposition of salvinia. However, the rain events of January and February 2022 influenced Las Curias very differently compared to Tropical Storm Isaias. These rain events occurred in the coldest months in Puerto Rico, where temperatures had already dropped and where salvinia decomposition had already begun for more than two years. Statistically, the tendence to increment of temperature were not significant from 2019 to 2022. This could be because the only data for 2022 is highly influenced by the rainfall events that occurred during that period and influenced by inflowing waters (overflow, interflow, and underflow). Fand and Morris (1998) mention that stratification can cause inflowing water to pass through a reservoir as overflow, interflow, or underflow, depending on the relative density of the inflow and the vertical density structure of the impounded water. Warm water will flow through the cooler water as an overflow, intermediate temperature water will flow across the surface of the thermocline, and cold or sediment-laden inflow will flow under the warmer water as a bottom current. Complex vertical profiles have also been documented in the La Plata reservoir in Puerto Rico due to the combination of stratification plus inflow created by the sinking of turbid and oxygenated storm runoff within this tropical reservoir (Fand and Morris, 1998). Also, flow patterns through the reservoir upstream of the outlet are also influenced by the depth at which water is released from the dam. The Las Curias reservoir is stratified most of the year, with anoxia prevalent throughout the hypolimnion. This stimulated the biodegradation rate of organic matter as temperatures also increase because this scenario favors bacterial growth (Zavala et al. 2004). In addition to biological and mechanical control, the alkalinization of water with a pH between 7 and 8 that we have documented in 2022 according to Cary and Weerts (1984) are not favorable conditions for salvinia compared to environments that have a pH ranging from 5 to 6. The increase in DO in the epilimnion is probably because the reservoir has good gas exchange with the atmosphere and that we have phytoplankton communities according to the chl-a trend, which, although they have been reduced, are still present. The conductivity has increased on the surface due to the increase in solar radiation and that the conductivity in the thermocline and hypolimnion may be more influenced by the inner and under flow of rain events. The Secchi depth increased due to the increase in light penetration through the removal of *S.molesta,* and the reduction in Chl-a.

The increase of NO_3^-N in the epilimnion, which is the common form of inorganic nitrogen, usually increases in reservoirs due to increases in runoff from the drainage basin (Wetzel, 2001). According to the Puerto Rico Department of Natural Resources (DRNA, for its Spanish acronym) (2020) and the Pan American Office of Health and Engineering / Pan American Center for Sanitary Engineering and Environmental Sciences (OPSI/CEPIS, for its Spanish acronym), Las Curias reservoir is in a mesotrophic state (P concentration of 0.03 – 0.05 mg/L). Nutrient levels, primary production, and sunlight penetration in mesotrophic lakes are moderate (DRNA, 2020). In addition, a trend analysis based on dissolved oxygen was classified Las Curias as degraded. In addition, Quiros (2003) also mentions that both nitrate (NO_3^-N) and ammonia (NH_4^-N) concentrations are highly variable during lake seasonal mixing events. In Las Curias, the nitrate and ammonia data for 2022 are influenced by the rain events that occurred between January and February 2022 that could have caused some mixing. Total Kjeldahl Nitrogen did not increase although it is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the environmental conditions described (EPA, 1993). P increased to 0.05 mg L-1 in 2020 compared to the mean (0.03 mg L-1) for the 2014 – 2017 period mentioned by Wahl et al., (2020). From 2020 to 2022 it has reduced 0.08 mg L-1 but remains above the levels of the 2014-2017 period, but the increment is not statistically significant. Runoff from pasture and crop lands, atmospheric deposition and stream bank erosion are usually the main sources of the two-thirds of total phosphorus discharges that reach rivers or lakes under normal flow (Taffese et al., 2014). In the case of Las Curias, the lack of dissolved oxygen and the acidification in the deeper zones creates conditions for sedimentary nutrient release, creating a potential internal loading of phosphorus (Hupfer and Lewandowski, 2008; Sondergaard et al., 2001). The tendency of alkalinization in the hypolimnion was statistically considerable but it is probably because the data is highly influenced by 2022 some rainfall events. According to Calvacante et al (2018) phosphate also enters the sediment in inorganic forms, entering the lakes because of erosion or leaching, it can be adsorbed mainly on iron, aluminum, and calcium hydroxides. These inorganic compounds also form in the sediment, where mineralization processes continue to break down organic forms of P (Golterman, 2004). It is likely that due to the accumulation of salvinia and the increase of 0.1 (mg L-1) phosphate (PO_4) from 2017 to 2022, mineralization processes are still breaking down organic forms of P (Golterman, 2004). Although the increase in phosphorus is not currently statistically significant, it could be in the future as salvinia decomposition increases. According to the most recent data of the mean Chlorophyll-a for 2022 we have more Chl-a in the thermocline (3.05 (± 1.75, n = 6)) than in the epilimnion (1.92 (± 1.06, n = 3)). This is probably due to the intense increase in light penetration in the water column after the biomass cover has been reduced, promoting phytoplankton to move further down the water column to achieve the light intensity desirable for photosynthesis. The reduction of Chl-a in the epilimnion could be associated with the decrease in total phosphorus and excess of nutrient. Seeming to have balanced out compared to eutrophic conditions described in 2019.

The removal of salvinia and grasses from the lake surface and it’s in situ disposal in the bottom layers of Las Curias Reservoir is changing the internal cycling of organic matter inducing important water quality changes including high rate of decomposition, sedimentation and, a reduction in depth. The decomposition of all this material has probably caused the proliferation of other aquatic plants such as the *R. holoschoenoides*. The increase in *R. holoschoenoides* may represent the beginning of the terrestrialization in the reservoir due to the decomposition material that this plant can generate. The relatively large biomass represented by aquatic macrophytes proves to be a significant source of lake sedimentation (Lan et al., 2012). Nutrients released from decomposing litter can contribute in the future to the eutrophication of the reservoir (Frodge and Pauley, 1991). Currently it is observed a succession of *R. holoschoenoides* specially in the arms of the reservoir. This grass result challenging to extract mechanically, so it has been decided to push it towards the deepest areas of the arms of the reservoir as part of the reservoir management strategy. Representing an imminent source of organic matter in the future if is eradicated in situ. According to Lan et al., (2012) we can expect that the belt of aquatic plants will then change and finally create shoals in the reservoir and transform it into a marsh as part of terrestrialization process. Throughout the process, the hydrophytes are progressively replaced first by helophytes and then by terrestrial species (Amoros and Henry, 2000; Lan et al., 2012). We have seen that although salvinia can coexist with this grass, its asexual reproduction or fragmentation is limited. This could limit the efficiency and reproduction of the weevil as a biological control agent.

CONCLUSION

This research suggests a number of management measures to continue with the reservoir recuperation and avoid reinfestation of salvinia. Breeding areas for *C. salvinae* need to be established outside the reservoir for potentially future reintroduction. This could be key to assist in future salvinia control programs at other locations in Puerto Rico. Despite its high costs, mechanical control should be limited to extracting the salvinia and removing it from the reservoir. Additionally, weed growth rates are higher than removal rates, limiting the benefits of mechanical control (Coetzee and Hill, 2020). Since we have seen increases in some nutrients, especially TKN, it will be important to know the amounts of fecal coliform and fecal enterococci to determine the magnitude of fecal pollution in the reservoir. In addition to the proliferation of *R. holoschoenoides*, water quality is a priority for the future of the ecosystem. Recent studies have determined that continue understanding the relationship between P and Chl-a is important for making decisions regarding the management of water quality in lakes (Quinlan et al., 2021). Since phosphorus and chlorophyll is considered the primary limiting factors of phytoplankton growth in lakes (Sterner 2008; Xu et al. 2010; Jin et al. 2020). Furthermore, according to Fee (1979) the Total Phosphorus (TP) and its relationship with chlorophyll-a will allow us to understand lake trophic state, algal blooms, and cyanotoxins (Ho et al., 2019; Paerl et al., 2019). This will help to continue understand the trophic state and oxygen availability of the reservoir through the year and after an atmospheric event and how anthropogenic activities continue to affect the Río Piedras watershed. This reservoir is greatly influenced by the local climate, especially rain events. To better understand this ecosystem, it will be necessary to sample once a month. In this way we will have a clearer idea of how this ecosystem responds during frequent rain events and the seasons of the tropic. The effect of salvinia decomposition and management on the future state of this ecosystem cannot be ruled out.

LITERATURE CITED

Amoros, C., Bornette, G., & Henry, C. P. (2000). A vegetation-based method for ecological diagnosis of riverine wetlands. *Environmental Management*, *25*(2).

Anderson, H.R. 1976. Ground water in the San Juan metropolitan area, Puerto Rico. U.S. Geological Survey Water Resources Investigations. Ft. Buchanan, PR: U.S. Department of the Interior, U.S. Geological Survey.

Buffle, J., and W. Stumm. 1994. General chemistry of aquatic systems, p. 1–42. In J. Buffle and R. R. De Vitre [eds.], Chemical and biological regulation of aquatic systems. CRC

Cary, P. R., & Weerts, P. G. (1984). Growth of Salvinia molesta as affected by water temperature and nutrition. III. Nitrogen-phosphorus interactions and effect of pH. *Aquatic Botany*, *19*(1-2), 171-182.

Cavalcante, H., Araújo, F., & Becker, V. (2018). Phosphorus dynamics in the water of tropical semiarid reservoirs in a prolonged drought period. *Acta Limnologica Brasiliensia*, *30*.

Coetzee, J. A., & Hill, M. P. (2020). Salvinia molesta D. Mitch.(Salviniaceae): impact and control. CAB Reviews, 15, 1-11.

Crowe, S. A., and others. 2004. Biogeochemical cycling in Ferich sediments from Lake Matano, Indonesia, p. 1185–1189. 11th International symposium on water-rock interaction. A.A. Balkema.

Department of Natural and Environmental Resources (DRNA, in spanish). 2020 Puerto Rico 305(b)/303(d) Integrated Report

Department of Natural and Environmental Resources (DRNA, in spanish). 2004. Puerto Rico Water Resources Inventory. Water Plan Office, (Departamento de Recursos Naturales y Ambientales)

Department of Natural and Environmental Resources, DOT Federal Highway Administration, Puerto Rico Highway and Transportation Authority, USDA Natural Resources Conservation Service, US Fish and Wildlife Service, and USVI Department of Planning and Natural Resources. 2001. Guide to identify common wetland plants in the Caribbean area: Puerto Rico and the US Virgin Islands. University of Puerto Rico Press. 268 p

Díaz, R., Johnson, S., Mudge, C., Russell, A. (2015). The Biology and Ecology of the Salvinia Weevil: A Biological Control Agent for the Management of Giant Salvinia. Louisiana State University Agricultural Center, Pub. 3474.

Díaz, R., Wahl, C., Moshman, L. (2016). How to Rear Giant Salvinia Weevils in Outdoor Ponds. Louisiana State University Agricultural Center, Pub. 3551.

Díaz, R., Wahl, C., Moshman, L. (2017). How to Release and Monitor Salvinia Weevils for Biological Control of Giant Salvinia in Louisiana. 1-16.

Fan, J., & Morris, G. L. (1998). *Reservoir Sedimentation Handbook: Design and Management of Dams, Reservoirs, and Watersheds for Sustainable Use*. McGraw-Hill.

Fee, E. J. 1979. A relation between lake morphometry and primary productivity and its use in interpreting whole-lake eutrophication experiments. Limnol. Oceanogr. 24:401–416.

Flores D, Carlson JW (2006) Biological control of giant salvinia in East Texas waterways and the impact on dissolved oxygen levels. Journal of Aquatic Plant Management 44: 115–121. http://www.apms.org/japm/vol44/v44p115.pdf

Forno, I.W., Harley, K.L.S. (1979): The occurrence of Salvinia molesta in Brazil. – Aquatic Botany 6: 185-187.

Frodge, J. D., Thomas, G. L., & Pauley, G. B. (1991). Sediment phosphorus loading beneath dense canopies of aquatic macrophytes. Lake and Reservoir Management, 7(1), 61-71.

Garcia LM (2018) The Curias community in San Juan is lost momentum. Metro Puerto Rico, 4 January,https://www.metro.pr/pr/noticias/2018/01/04/se-echa-perder-impulso-la-comunidad-las-curias-sanjuan.html

García-Martinó A (2000) The construction of dams in the face of economic development: Puerto Rico in the 20th century. Dimension CIAPR 4: 7–14

Garcia-Montiel, D. C., Verdejo-Ortiz, J. C., Santiago-Bartolomei, R., Vila-Ruiz, C. P., Santiago, L., & Melendez-Ackerman, E. (2014). Food sources and accessibility and waste disposal patterns across an urban tropical watershed: implications for the flow of materials and energy. Ecology and Society, 19(1).

Gibbons MV, Rosenkranz MG, Gibbons Jr HL, Sytsma MD. Guide for developing integrated aquatic vegetation management in Oregon. Portland (OR): Center for Lakes and Reservoirs, Portland State University; 1999. p. 113.

Golterman, H.L. The chemistry of phosphate and nitrogen compounds in sediments. Klwer Academic Publishers, 2004.

Gregor, J., & Maršálek, B. (2004). Freshwater phytoplankton quantification by chlorophyll a: a comparative study of in vitro, in vivo and in situ methods. Water research, 38(3), 517-522.

Gunkel, G., & Casallas, J. (2002). Limnology of an equatorial high mountain lake—Lago San Pablo, Ecuador: The significance of deep diurnal mixing for lake productivity. Limnologica, 32(1), 33-43.

Gutiérrez-Fonseca, P. E., Ramírez, A., Pringle, C. M., Torres, P. J., McDowell, W. H., Covich, A., ... & Pérez-Reyes, O. (2020). When the rainforest dries: Drought effects on a montane tropical stream ecosystem in Puerto Rico. Freshwater Science, 39(2), 000-000.

Haire, W.J. 1972. Flooding along the Río Piedras in the San Juan area, Puerto Rico. Hydrologic Investigations Map Series No. 1. San Juan, PR: U.S. Department of the Interior, U.S. Geological Survey (WRD).

Ho, J. C., A. M. Michalak, and N. Pahlevan. 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. Nature 574: 667–670.

Holdridge, L.R. 1967. Life zone ecology. San José, Costa Rica:Tropical Science Center.

Howard Morgan, V., 2020, Salvinia minima Baker: U.S. Geological Survey, Nonindigenous AquaticSpeciesDatabase,Gainesville,FL,https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=297, Revision Date: 9/14/2011, Access Date: 12/27/2020

Hupfer, M., & Lewandowski, J. (2008). Oxygen controls the phosphorus release from Lake Sediments–a long-lasting paradigm in limnology. *International Review of Hydrobiology*, *93*(4-5), 415-432.

Hutchinson, G. E. 1957. A treatise on limnology: Geography, physics and chemistry. Wiley

Jayan, P. R., & Sathyanathan, N. (2012). Aquatic weed classification, environmental effects and the management technologies for its effective control in Kerala, India. International Journal of Agricultural and Biological Engineering, 5(1), 76-91.

Jin, Y., R. Yu, Z. Zhang, Q. Zhang, M. Li, Z. Cao, L. Wu, and Y. Hao. 2020. Spatiotemporal variability of phytoplankton functional groups in a shallow eutrophic lake from cold, arid regions. Environ. Monit. Assess. 192: 371.

Julien, M.H., Hill, M.P., Tipping, P.W., 2009. Salvinia molesta DS Mitchell (Salviniaceae). En: Muniappan, R. (Ed.), Control biológico de malezas con artrópodos en los trópicos. Prensa de la Universidad de Cambridge, Cambridge, págs. 378–407.

Keellings, D., & Hernández Ayala, J. J. (2019). Extreme rainfall associated with Hurricane Maria over Puerto Rico and its connections to climate variability and change. Geophysical Research Letters, 46(5), 2964-2973.

Knopf KW, Habeck DH (1976) Life history and biology of Samea multiplicalis. Environmental Entomology 5: 539–542, https://doi.org/10.1093/ee/5.3.539

Lan, Y., Cui, B., You, Z., Li, X., Han, Z., Zhang, Y., & Zhang, Y. (2012). Litter decomposition of six macrophytes in a eutrophic shallow lake (Baiyangdian Lake, China). CLEAN–Soil, Air, Water, 40(10), 1159-1166.

Lewis Jr, W. M. (1973). The thermal regime of Lake Lanao (Philippines) and its theoretical implications for Tropical Lakes 1. *Limnology and Oceanography*, *18*(2), 200-217.

Lugo, A. E., Ramos, O., & Rodriguez, C. (2011). The Río Piedras watershed and its surrounding environment. FS-980. US Department of Agriculture, Forest Service, International Institute of Tropical Forestry. 46 p., 980.

Luque, G. M., Bellard, C., Bertelsmeier, C., Bonnaud, E., Genovesi, P., Simberloff, D., & Courchamp, F. (2014). The 100th of the world's worst invasive alien species. Biological invasions, 16(5), 981-985.

Malawi, M., & Virginia, W. (2017). Salvinia molesta DS Mitch.

Marker, A.F.H., Nusch, EA., Rai, H. , Riemann, B. 1980. The measurement of photosynthetic pigments in freshwaters and standardization of method:e conclusions and recommendations. Arch. Hydrolbiol. Beih, Ergebm Limnol. 14191-106.

Marrero MO (2017) Aquatic Fern seizes Lake Las Curias. El Nuevo Dia. 14 August, https://www. elnuevodia.com/noticias/locales/fotogalerias/helechoacuaticoseapoderadellagolascurias- galeria-2348848/

Martin, G. D., Coetzee, J. A., Weyl, P. S., Parkinson, M. C., & Hill, M. P. (2018). Biological control of Salvinia molesta in South Africa revisited. Biological control, 125, 74-80.

Martínez, E. (2016). Plant Invades Curías Lakes. El Nuevo Día. 8 June, https://www.pressreader.com/puerto-rico/el-nuevodia1/20160608/281994671758628

McFarland, D. G., Nelson, L. S., Grodowitz, M. J., Smart, R. M., & Owens, C. S. (2004). Salvinia molesta DS Mitchell (Giant Salvinia) in the United States: a review of species ecology and approaches to management.

Moshman, L., & Diaz, R. (2017) Winter Management of Salvinia Weevils for Biological Control of Giant Salvinia. Louisiana State University Agricultural Center, Pub. 3620.

Nachtrieb, J.G. 2012. Rearing the Salvinia weevil for biological control of giant Salvinia at the U.S. Army Corps of Engineers Lewisville aquatic ecosystem research facility, pp. 13-24. In A. Knutson and J. Nachtrieb (eds.), A guide to mass rearing the Salvinia weevil for biological control of giant Salvinia. Texas A&M AgriLife Extension Service, SpecialPublication ESP-475. http://cise.tamu.edu/media/355740/gs_manual_10-1512.pdf

Nusch, E.A. 1980. Comparison of different methods for chlorophyll-a and phaeopigment determinations. Arch. Hydrolbiol. Beih., Ergebn. Limnol. 14:14-36.

Paerl, H. W., and others. 2019. Mitigating eutrophication and toxic cyanobacterial blooms in large lakes: The evolution of a dual nutrient (N and P) reduction paradigm. Hydrobiologia.

Pantoja-Agreda, F., Martínez, G. A., Santos-Flores, C., & Sotomayor-Ramírez, D. (2009). Phytoplankton dynamics of the Guajataca reservoir, Puerto Rico. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *30*(7), 1096-1100.

Pasch, R. J., Penny, A. B., & Berg, R. (2018). National Hurricane center tropical cyclone report: Hurricane Maria. TROPICAL CYCLONE REPORT AL152017, National Oceanic And Atmospheric Administration and the National Weather Service, 1-48.

Pease, M.H.; Monroe, W.H. 1977. Geologic map of the San Juan quadrangle, Puerto Rico. U.S. Geological Survey Miscellaneous Investigations Series Map 1-1010. Arlington, VA: U.S. Department of the Interior, U.S. Geological Survey.

Picó, R. 1950. The geographic regions of Puerto Rico. Río Piedras, PR: University of Puerto Rico Press.

Pieterse, A. H., Kettunen, M., Diouf, S., Ndao, I., Sarr, K., Tarvainen, A., ... & Hellsten, S. (2003). Effective biological control of Salvinia molesta in the Senegal River by means of the weevil Cyrtobagous salviniae. AMBIO: A Journal of the Human Environment, 32(7), 458-462.

Quinlan, R., Filazzola, A., Mahdiyan, O., Shuvo, A., Blagrave, K., Ewins, C., ... & Sharma, S. (2021). Relationships of total phosphorus and chlorophyll in lakes worldwide. Limnology and Oceanography, 66(2), 392-404.

Quiñones-Márquez, F. (1980). *Limnology of Lago Loiza, Puerto Rico* (Vol. 79). US Geological Survey.

Quirós, R. (2003). The relationship between nitrate and ammonia concentrations in the pelagic zone of lakes. *Limnetica*, *22*(1-2), 37-50.

Room PM, Harley KLS, Forno TW, Sands DPA (1981) Successful biological control of the floating weed salvinia. Nature 294: 78–80.

Room PM, Thomas PA. Nitrogen, phosphorus and potassium in Salvinia molesta Mitchell in the field: effects of weather, insect damage, fertilizers and age. Aquatic Botany 1986;1:213–32.

Sondergaard, M., Jensen, P. J., & Jeppesen, E. (2001). Retention and internal loading of phosphorus in shallow, eutrophic lakes. TheScientificWorldJournal, 1, 427-442.

Sterner, R. W. 2008. On the phosphorus limitation paradigm for lakes. Int. Rev. Hydrobiol. 93: 433–445.

Sullivan, P. R., Postle, L. A., & Julien, M. (2011). Biological control of Salvinia molesta by Cyrtobagous salviniae in temperate Australia. *Biological Control*, *57*(3), 222-228.

Taffese, T., Tilahun, S. A., & Steenhuis, T. S. (2014). Phosphorus modeling, in lake tana basin, ethiopia. J. Environ. Hum, 2, 47-55.

Tetra Tech (2018) Las Curias reservoir lake watershed characterization. Fairfax, VA, 22 pp

Thayer DD, Pfingsten IA, Jacono CC, Richerson MM, Howard V (2018) Salvinia molesta Mitchell: U.S. Geological Survey, Nonindigenous Aquatic Species Database. https://nas.er. usgs.gov/queries/FactSheet.aspx?speciesID=298 (accessed 12 December 2020)

Tipping PW, Martin MR, Center TD, Davern TM (2008) Suppression of Salvinia molesta Mitchell in Texas and Louisiana by Cyrtobagous salviniae Calder and Sands. Aquatic Botany 88: 196–202.

Torres-Ortiz PA, Roas-Castro R (2014) Rehabilitation of Las Curias dam, an emergency project to prevent piping failure in an old earthen dam, San Juan, Puerto Rico. ASDSO Regional Conference. Montgomery, Alabama, USA, 13 pp

U.S. Census Bureau (2020). *American Community Survey 5-year estimates.* Retrieved from *Census Reporter Profile page for Cupey barrio, San Juan Municipio, PR* http://censusreporter.org/profiles/06000US7212722847-cupey-barrio-san-juan-municipio-pr/

USDA, A. (2001). Demonstration Project: Giant Salvinia-Toledo Bend Reservoir and Surrounding Areas in Louisiana and Eastern Texas. Environmental Assessment.

US Department of Commerce, N. O. A. A. (2020, September 2). *Tropical storm isaias - july 29-31, 2020*. Tropical Storm Isaias - July 29-31, 2020. Retrieved May 3, 2022, from https://www.weather.gov/sju/isaias2020

US EPA. United States Environmental Protection Agency. (1993). Method 351.2, revision 2.0: Determination of total Kjeldahl nitrogen by semi-automated colorimetry.

Wahl, C. F., Diaz, R., & Ortiz-Zayas, J. (2020). Assessing Salvinia molesta impact on environmental conditions in an urban lake: case study of Lago Las Curias, Puerto Rico. Aquatic Invasions, 15.

Wehner, M.F., J.R. Arnold, T. Knutson, K.E. Kunkel, and A.N. LeGrande, 2017: Droughts, floods, and wildfires. In: Climate Science Special Report: Fourth National Climate Assessment, Volume I [Wuebbles, D.J., D.W. Fahey, K.A. Hibbard, D.J. Dokken, B.C. Stewart, and T.K. Maycock (eds.)]. U.S. Global Change Research Program, Washington, DC, USA, pp. 231-256 doi: 10.7930/J0CJ8BNN.

Wetzel, R.G. 2001. Limnology. Lake and River Ecosystems. 3rd. Ed. Academic Press, San Diego, CA, USA

Wetzel, R. G. 1983. Limnology, 2nd ed. CBS College Publishing.

Wetzel, RAG. and Likens, CLE. 1991. Limnological analyses. 2nd Edition. Springer Verlag, New York. 391 p.

Xu, H., H. W. Paerl, B. Qin, G. Zhu, and G. Gaoa. 2010. Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. Limnol. Oceanogr. 55: 420–432.

Zavala ML, Funamizu N, Takakuwa T (2004) Temperature effect on aerobicbiodegradation of feces using sawdust as a matrix. Water Res 38:2406–2416

FIGURE LEGENDS

Figure 1. The Rio Piedras watershed encompasses the Las Curias sub-watershed where the Las Curias Reservoir is located. The map shows the watershed boundaries and the study area of Las Curias Reservoir.

Figure 2. Map of Las Curias Reservoir developed at a scale of 1:1,500 in ArcGIS Desktop. The study sites are the areas where weevil density data were collected (identified with letters) and where water quality data were collected (identified with numbers).

Figure 3. Changes in salvinia cover (%) and total monthly rainfall (mm/month) registered in Las Curias Reservoir since 2019. Rainfall data from July 2019 to November 2021 were estimated from NOAA (https://water.weather.gov/precip/index.php). From December 2021 to March 2022, rainfall was measured with a rain gauge located near the dam.

Figure 4. Maps of changes in the plant biomass coverage in the reservoir from July 2019 to March 2022. The extraction of salvinia has been concentrated in open waters since other aquatic species abound in the arms that require more complex control and extraction methods.

Figure 5. Drone aerial photos that show the drastic change in the plant biomass in the Las Curias reservoir open waters from September 2019 (image above) to October 2021 (image below).

Figure 6. Changes in densities of adult weevils and their larvae. The bars above the gray threshold line (≥ 40 individuals per kg of wet salvinia) indicate that for those months we had good densities of individuals.

Figure 7. Vertical profiles of Temperature (°C), pH (s.u), Dissolved Oxygen mg/L -1 and Conductivity uS/cm at station 1 and 2 in Las Curias Reservoir. The data of 13 samples were evaluated from September 2019 to March 2022, except for the conductivity parameter, which 11 samples were evaluated. Four of the thirteen sampling dates were selected according to drastic changes in depth and in the four limnological parameters.

TABLE LEGENDS

Table 1. Average annual values of physico-chemical parameters measured at Las Curias. The values in parenthesis represent the standard deviation and numbers of observations (n). The means were calculated taking into consideration all available data from Station 1 and Station 2 per year.

Table 2. Temporal trends in various water quality parameters measured in the epilimnion, thermocline, and hypolimnion of the Las Curias reservoir. The means between station 1 and 2 for each water layer from 2019 to 2022 were considered. Trends are significant at the $p < 0.05$ with a confidence level of 95%.

Figure 1

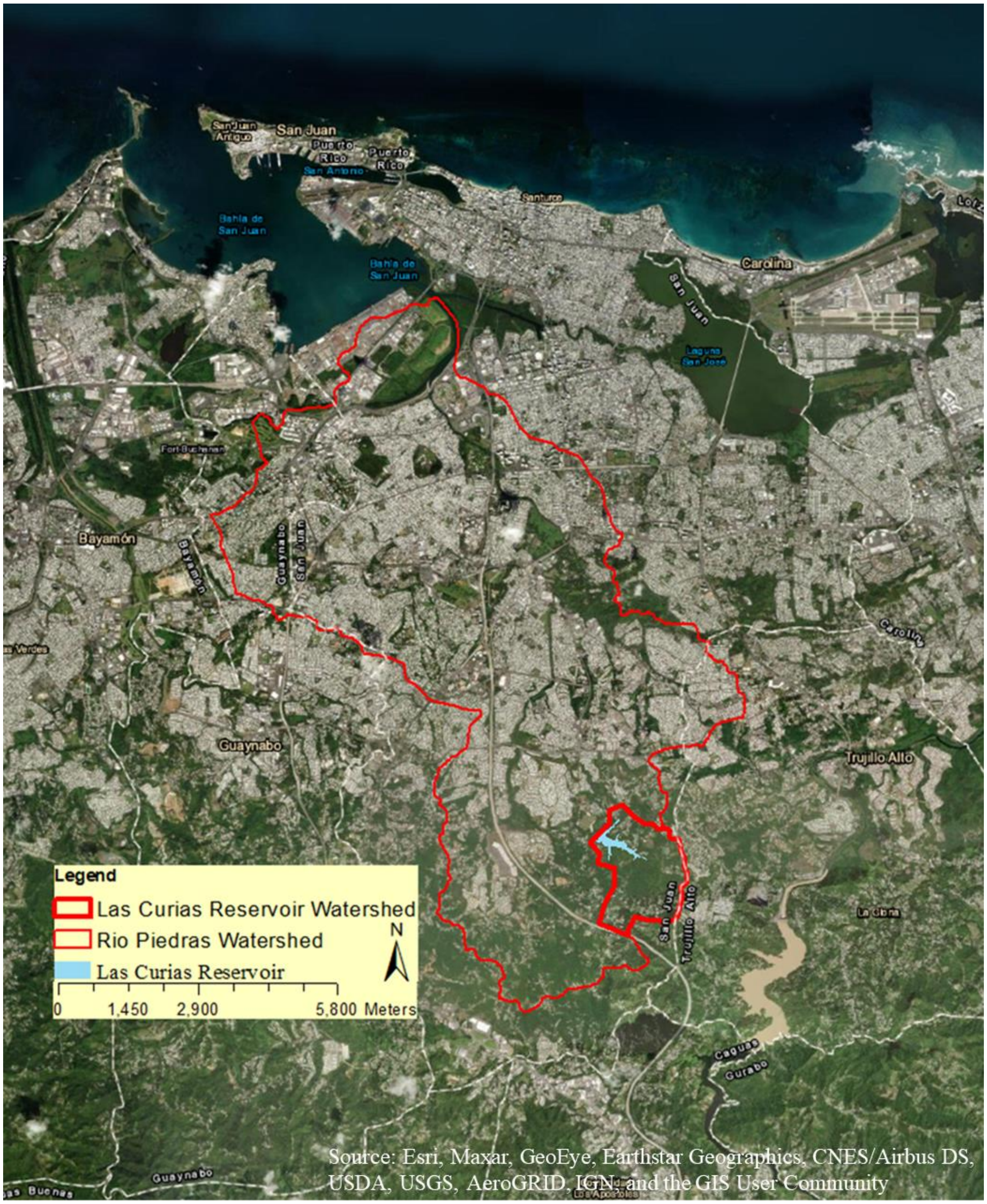

Legend
Las Curias Reservoir Watershed
Rio Piedras Watershed
Las Curias Reservoir
0 1,450 2,900 5,800 Meters
N
Source: Esri, Maxar, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
San Juan
Carolina
Bayamón
Guaynabo
Trujillo Alto

Figure 2

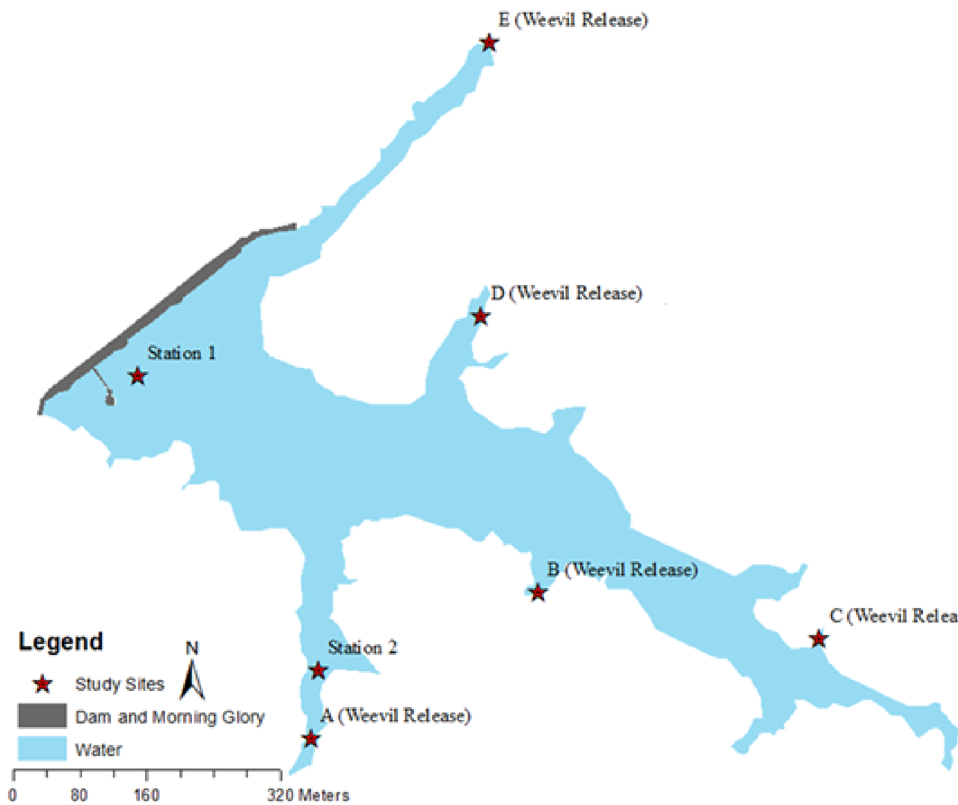
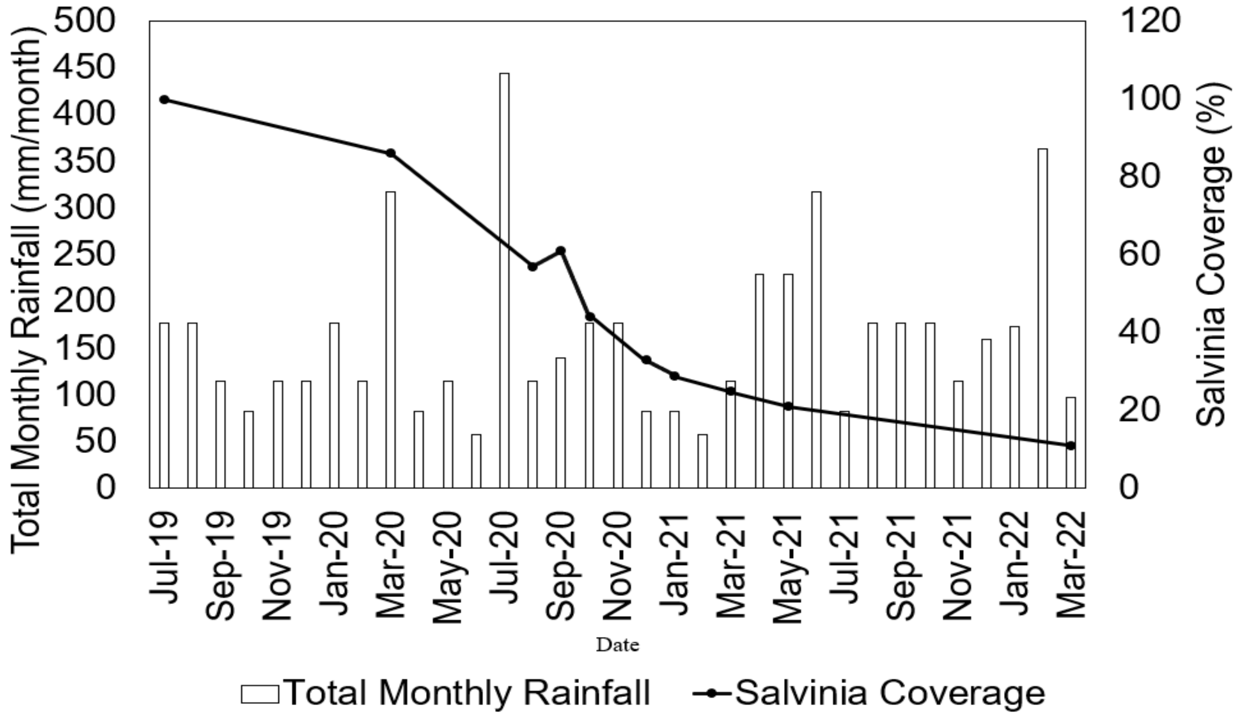
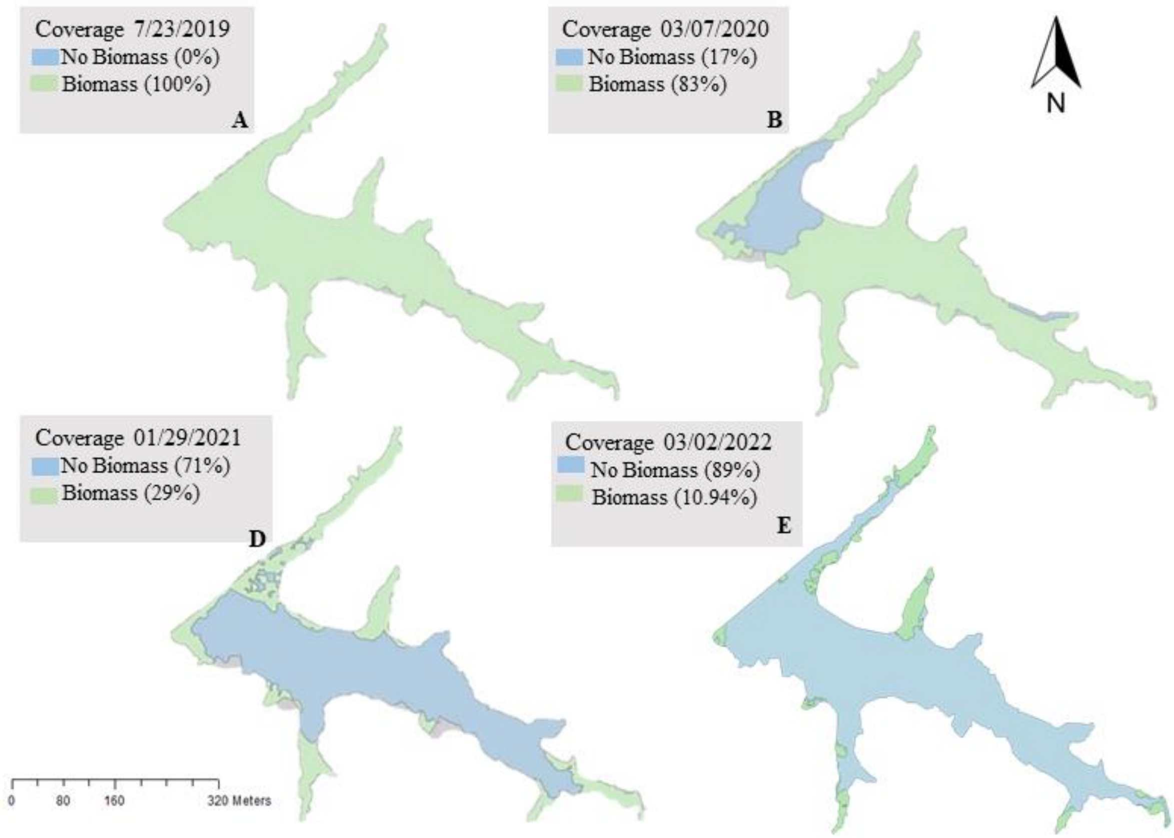

E (Weevil Release)
D (Weevil Release)
Station 1
B (Weevil Release)
C (Weevil Release)
Legend
N
Station 2
Study Sites
Dam and Morning Glory
A (Weevil Release)
Water
0
80
160
320 Meters

Figure 3



Total Monthly Rainfall (mm/month)
Salvinia Coverage (%)
500
450
400
350
300
250
200
150
100
50
0
120
100
80
60
40
20
0
Jul-19
Sep-19
Nov-19
Jan-20
Mar-20
May-20
Jul-20
Sep-20
Nov-20
Jan-21
Mar-21
May-21
Jul-21
Sep-21
Nov-21
Jan-22
Mar-22
Date
Total Monthly Rainfall
Salvinia Coverage

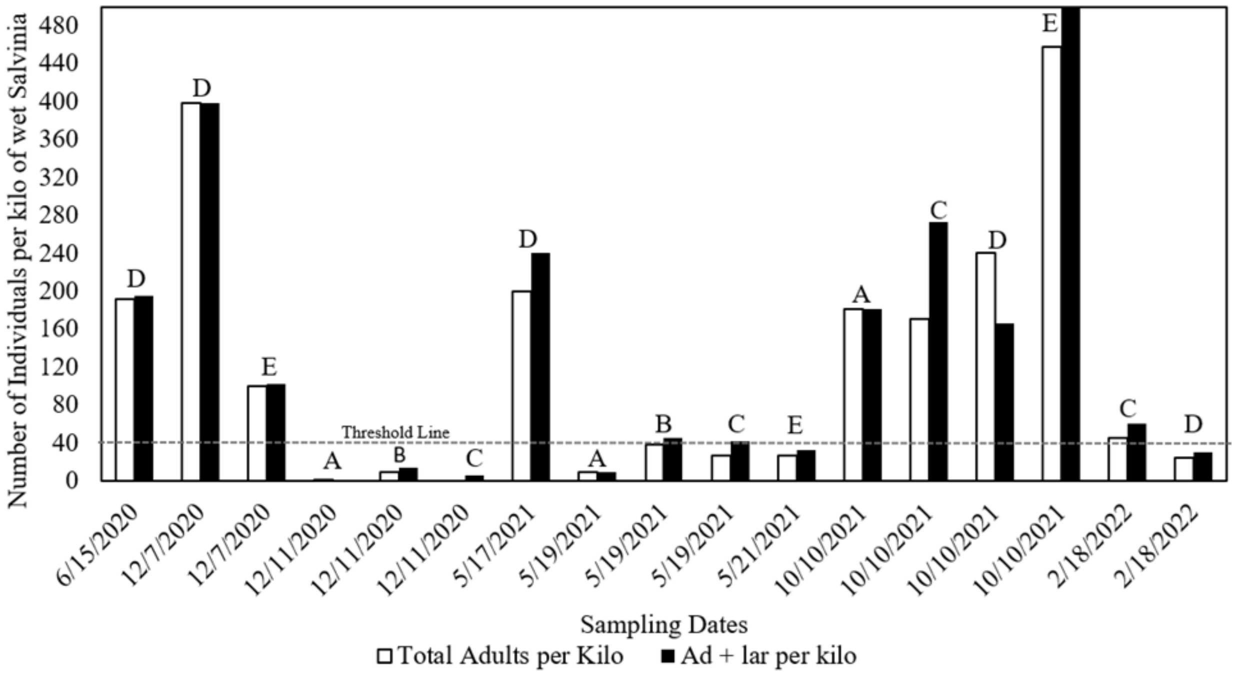
Figure 4



Coverage 7/23/2019
No Biomass (0%)
Biomass (100%)
A
Coverage 03/07/2020
No Biomass (17%)
Biomass (83%)
B
N
Coverage 01/29/2021
No Biomass (71%)
Biomass (29%)
D
Coverage 03/02/2022
No Biomass (89%)
Biomass (10.94%)
E
0
80
160
320 Meters

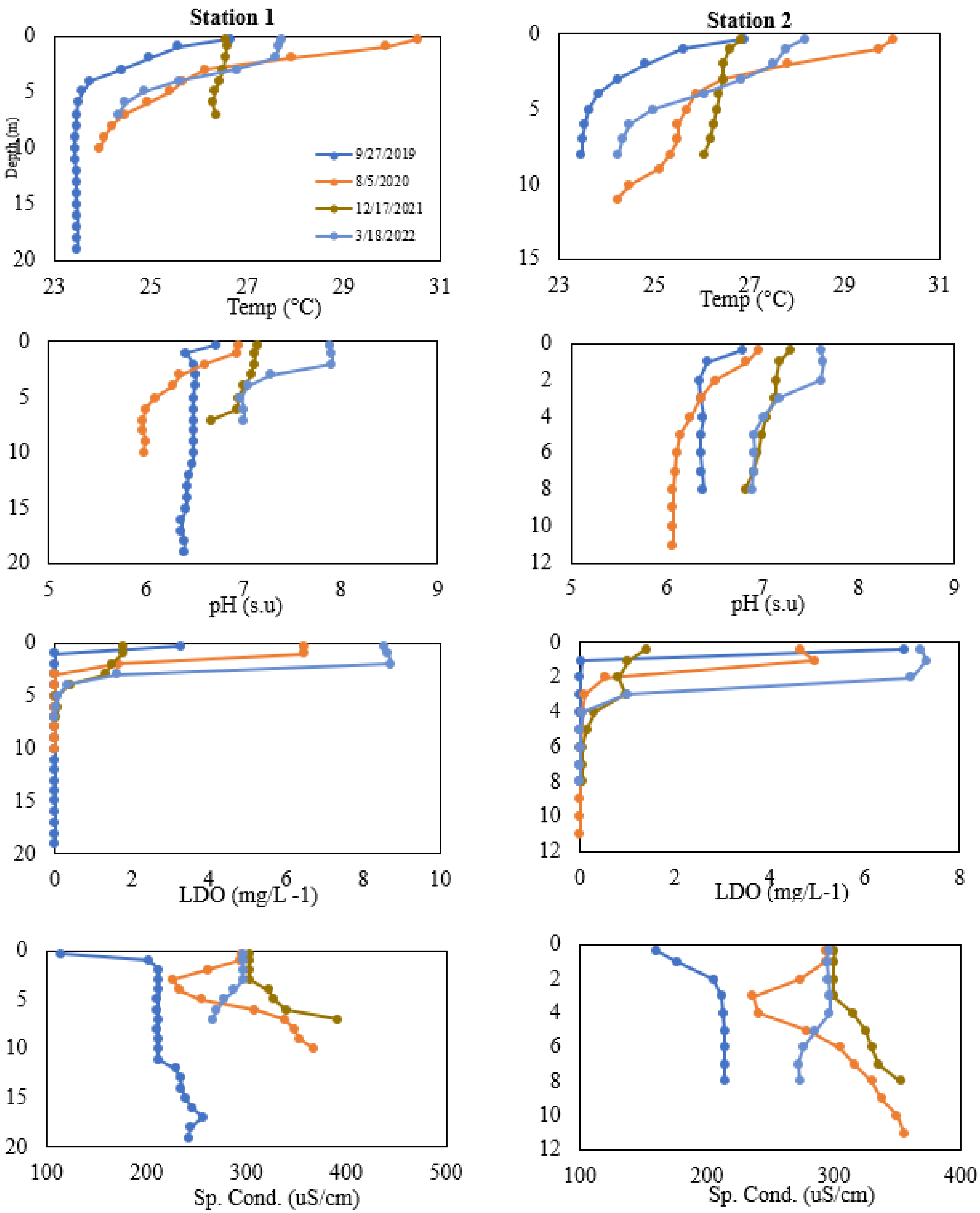
Figure 5

Figure 6



Number of Individuals per kilo of wet Salvinia
480
440
400
360
320
280
240
200
160
120
80
40
0
D
D
E
A
B
C
D
A
B
C
E
A
C
D
E
C
D
Threshold Line
6/15/2020
12/7/2020
12/7/2020
12/11/2020
12/11/2020
12/11/2020
5/17/2021
5/19/2021
5/19/2021
5/19/2021
5/21/2021
10/10/2021
10/10/2021
10/10/2021
10/10/2021
2/18/2022
2/18/2022
Sampling Dates
Total Adults per Kilo
Ad + lar per kilo

Figure 7



Station 1
Station 2
Depth (m)
9/27/2019
8/5/2020
12/17/2021
3/18/2022
Temp (°C)
pH (s.u)
LDO (mg/L-1)
Sp. Cond. (uS/cm)

Table 1

Parameters	2019	2020	2021	2022
DO (mg L-1)	0.35 (± 1.39, n = 29)	1.05 (± 1.84, n = 84)	1.32 (± 1.79, n = 84)	2.28 (± 3.20, n = 49)
pH (s.u)	6.44 (± 0.10, n = 29)	6.40 (± 0.26, n = 84)	6.79 (± 0.27, n = 84)	7.41 (± 0.37, n = 49)
Sp. Cond (μS)	213.28 (± 27.34, n = 29)	307.04 (± 43.17, n = 63)	311 (± 28.84, n = 65)	297.27 (± 30.50, n = 49)
Temp (°C)	24.03 (± 0.98, n = 29)	26.45 (± 1.76, n = 84)	26.99 (± 1.07, n = 84)	25.62 (± 1.14, n = 49)
NH_3-N (mg L-1)	No data	0.04 (± 0.03, n = 14)	0.33 (± 0.47, n = 20)	0.40 (± 0.41, n = 8)
PO_4 (mg L-1)	No data	0.11 (± 0.06, n = 14)	0.11 (± 0.05, n = 20)	0.15 (± 0.04, n = 8)
TKN-N (mg L-1)	No data	0.39 (± 0.35, n = 12)	0.61 (± 0.53, n = 7)	0.65 (± 0.17, n = 2)
Chlorophyll a	No data	4.08 (± 3.5, n = 15)	2.90 (± 2.10, n = 16)	2.62 (± 1.44, n = 12)
Secchi Depth (m)	0.09 (± 0.02, n = 2)	0.25 (± 0.29, n = 8)	0.68 (± 0.23, n = 10)	1.10 (± 0.23, n = 6)

Table 2

Parameter	Epilimnion			
	r^2	n	Trend	p
DO (mg L-1)	0.56	13	Incresaed	0.003
Temp (°C)	0.01	13	--------	0.77
pH (s.u)	0.56	13	Increased	0.003
Sp. Cond (µS)	0.5	11	Increased	0.02
TKN N (mg L-1)	0.01	7	--------	0.22
NO_3-N (mg L-1)	0.29	7	Increased	0.04
NH_4N (mg L-1)	0.61	7	Increased	0.04
NH_3N (mg L-1)	0.35	11	Increased	0.05
TP (mg L-1)	0.3	7	--------	0.2
PO_4^{3-} (mg L-1)	0.01	11	--------	0.82
Chl-a	0.4	11	Decreased	0.04

Parameter	Thermocline			
	r^2	n	trend	p
DO (mg L-1)	0.01	13	--------	0.73
Temp (°C)	0.11	13	--------	0.26
pH (s.u)	0.7	13	Increased	0.003
Sp. Cond (µS)	0.48	11	Increased	0.02

Parameter	Hypolimnion			
	r^2	n	trend	p
DO (mg L-1)	0.32	13	Increased	0.04
Temp (°C)	0.32	13	Increased	0.06
pH (s.u)	0.48	13	Increased	0.01
Sp. Cond (µS)	0.52	11	--------	0.54

Parameter	r^2	n	trend	p
Secchi Depth (m)	0.57	13	Increased	0.03