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Effects of Nutrients, Salinity, and pH on *Salvinia molesta* (Mitchell) Growth

by Chetta S. Owens and R. Michael Smart

PURPOSE: This study documents effects of macronutrient (nitrogen and phosphorus) concentrations, pH, and salinity on giant salvinia growth.

BACKGROUND: Giant salvinia (*Salvinia molesta* Mitchell) is a floating aquatic fern native to southeastern Brazil. The species is considered invasive and is currently found worldwide in sub-tropical, tropical, and temperate regions. It has been reported in more than 20 countries, including the United States, where it was likely introduced as an aquarium or water garden species (Room et al. 1981; McFarland et al. 2004). Giant salvinia has invaded several freshwater aquatic systems in southern, southwestern, and Gulf coast states of the United States where it has exhibited persistent and sometimes explosive growth (U.S. Geological Survey (USGS) 2004). An aggressive aquatic species under ideal conditions, giant salvinia can completely cover water surfaces and form floating mats up to 1 m thick (Thomas and Room 1986). Dense mats of giant salvinia impede transportation, irrigation, hydroelectric production, flood and mosquito control, and destroy habitats, degrade water quality, and can hinder endeavors such as rice cultivation and fishing (Mitchell 1979; Holm et al. 1977). Despite its potentially overwhelming impacts, several environmental factors including temperature, nutrient availability, salinity, and pH may limit the distribution and survival of giant salvinia within the United States.

Studies have shown that giant salvinia survival and growth are affected independently by water temperature, salinity, nutrients, and pH (Cary and Weerts 1984; McFarland et al. 2004; Owens et al. 2004, 2005). Complete kill of giant salvinia occurs when water temperatures fall to freezing (0° C) for periods of several hours to days depending on ice formation (Owens et al. 2004) thus limiting its distribution in the United States to southern states where low temperatures are not sustained. Although not reported to be tolerant of elevated salinities, giant salvinia has persisted for years in the Colorado River bordering Arizona and California, where conductivity ranges from 950-1400 µhmos (Voichick 2008). Giant salvinia survived but produced significantly less biomass when exposed to salinities of up to 2.5 ppt (measured as 10,000 µhmos conductivity) for a period of 21 days in a controlled study (Owens et al., in preparation; Oliver 1993). Problematic growth of giant salvinia may therefore be limited in systems exhibiting brackish to saline conditions, or that are prone to periods of saltwater intrusion.

Growth of giant salvinia appears to be pH dependent, with optimum pH conditions required for maximum growth ranging from 5-7.5 units (Owens et al. 2005). Reduced growth at higher pH (8 and above) is thought to be due to limited availability of micronutrients such as iron or manganese in the water column. Under high pH conditions, giant salvinia may not be capable of growth that results in problematic coverage (Cary and Weerts 1984; Holm et al. 1977; Gaudet 1973; Mitchell 1979).

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Cary and Weerts (1984) and Gaudet (1973) found that giant salvinia can store concentrations of nutrients (nitrogen and phosphorus) in tissue to sustain growth under stressful conditions. They also found that giant salvinia grows best when the available nitrogen is in the NH_4 form (Cary and Weerts 1984).

Cumulative effects of naturally occurring environmental limitations to giant salvinia growth have not been investigated. The objective of this study was to document effects of macronutrient (nitrogen and phosphorus) concentrations, pH, and salinity on giant salvinia growth. This knowledge may be useful for predicting distributional limits of giant salvinia as well as identifying where giant salvinia may become problematic based on water quality parameters.

MATERIALS AND METHODS: This study was conducted at the U.S. Army Engineer Research and Development Center's Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, TX. Thirty-six treatments were evaluated, including three nutrient ranges, four salinities, and three pH ranges: each treatment was replicated seven times. In May 2007, 252 (19L) containers were each filled with 0.24 L of pond sediment to ensure that micronutrients were available to sustain growth of giant salvinia for the duration of the study (3 weeks) (Smart et al. 1995). Containers were filled to 5 cm from the top with sand-filtered, alum-treated water from nearby Lake Lewisville.

Three nutrient treatments were obtained by addition of ammonium biphosphate ($(NH_{4)2}PO_4$) at three concentrations: high (10mg/L), medium (2mg/L), and low (no amendment). Salinity treatments (measured as specific conductance in µmhos) were amended with NaCl and $NaHCO^3$ to achieve the following concentrations: 0.01 ppt (100 µmhos), 0.035 ppt (350 µmhos), 0.1 ppt (750 µmhos), and 0.25 ppt (1500 µmhos). High pH (9.0+ units) was maintained by adding 50 mg/L $NaCO^3$ to appropriate containers; medium pH (8.5 units) was maintained with no amendment; and low pH (less than 7.0 units) was maintained by adding 0.8L of Canadian peat moss to the water column. Following amendments, one giant salvinia plant with five leaf pairs of similar size was added to each container. Six giant salvinia plants were collected and dried for initial weights (average 0.04 g/DW).

Algal growth was controlled using 100 uL of Aquashade (Applied Biochemist, Germantown, WI) and floating Styrofoam plates to reduce light penetration into the water column. Plates were removed as giant salvinia grew to minimize interference with spread. Percent cover of giant salvinia was visually estimated and recorded on weeks 1, 2, and 3. Plants were collected from each container after three weeks growth, dried to a constant weight at 55° C using a Blue M forced air oven (General Signal, Atlanta, GA), and weighed. Treatment dry weights were analyzed for statistical differences using a three-way analysis of variance (ANOVA). Significant differences between means were ascertained using an LSD post hoc test at $p = 0.05$ level of significance (Table 1). Statistics were performed using Statistica 7.1 (StatSoft, Inc., Tulsa, OK).

RESULTS AND DISCUSSION: Significant interaction ($p= 0.0209$, $F=2.059$) was identified for giant salvinia biomass production (g/DW) among the three tested parameters of nutrients, pH, and salinity (Figure 1). Figure 1 clearly shows that plants grown under low pH conditions (less than 7.0 units) produced greater biomass than other pH treatments, agreeing with findings by Owens et al. (2005) when evaluating the effects of pH alone. The high nutrient treatment (10mg/L ($(NH_{4)2}PO_4$) under low pH conditions significantly outgrew (by approximately two-fold) lower nutrient treatments, regardless of salinity. No differences were found for nutrient treatments between medium (8.5 units) or high pH (9.0+ units) conditions, where in both cases the plants did not exhibit growth during the 3-week study. Although the low pH, high nutrient, low salinity (0.01 ppt, 100 µhmos) treatment was significantly different from other salinity levels, except for this treatment, salinities overall did not significantly impact giant salvinia growth. Room and Gill (1985) found during field surveys that giant salvinia prefers waters with conductivities ranging from 240 to 500 µS/cm.

Table 1
Results of ANOVA tables for biomass. Treatment effects, F values and p values for all statistics.

Treatment Effect	F value	P value
Nutrient	51.857	0.0000
Conductivity	3.091	0.0280
pH	436.226	0.0000
Nutrient* Conductivity	2.043	0.0614
Nutrient*pH	67.172	0.0000
Conductivity*ph	3.840	0.0012
Nutrient*Conductivity*pH	2.059	0.0209

Percent coverage estimates followed the same pattern as giant salvinia biomass (Figure 2). Giant salvinia grown under low pH and high nutrients generally covered 75 to 100 percent of the container by week 3. Plants grown under low pH amended with medium or low nutrient conditions grew to cover only about 20 percent of the container. Giant salvinia plants grown under medium and high pH, regardless of nutrient concentration, covered 20 percent or less of the container by the end of week 3.

Owens et al. (2005) reported the importance of low pH on the growth potential of giant salvinia. Because giant salvinia is a free-floating plant, nutrients must be obtained from the water column via the modified third leaf, which resembles roots. Assuming that major nutrients (nitrogen, phosphorus, potassium) are not growth-limiting factors, increased growth at lower pH may be attributable to the uptake availability of micronutrients such as iron and manganese, which exhibit pH-dependent solubility (Riemer 1984; Wetzel 1983). Without these micronutrients, photosynthesis, chlorophyll synthesis, enzymatic activity, etc. may be affected (Raven et al. 1981).

Although able to tolerate a variety of environmental conditions, this paper supports previous research (Cary and Weerts 1984; Holm et al. 1977; Gaudet 1973; Mitchell 1979) and suggests that certain requirements are necessary for giant salvinia to become problematic, including low pH, low salinity, and the presence of suitable macronutrients. In combination with distribution limitations due to temperature, this information is useful for predicting which aquatic systems in the southern United States would be prone to serious infestation.

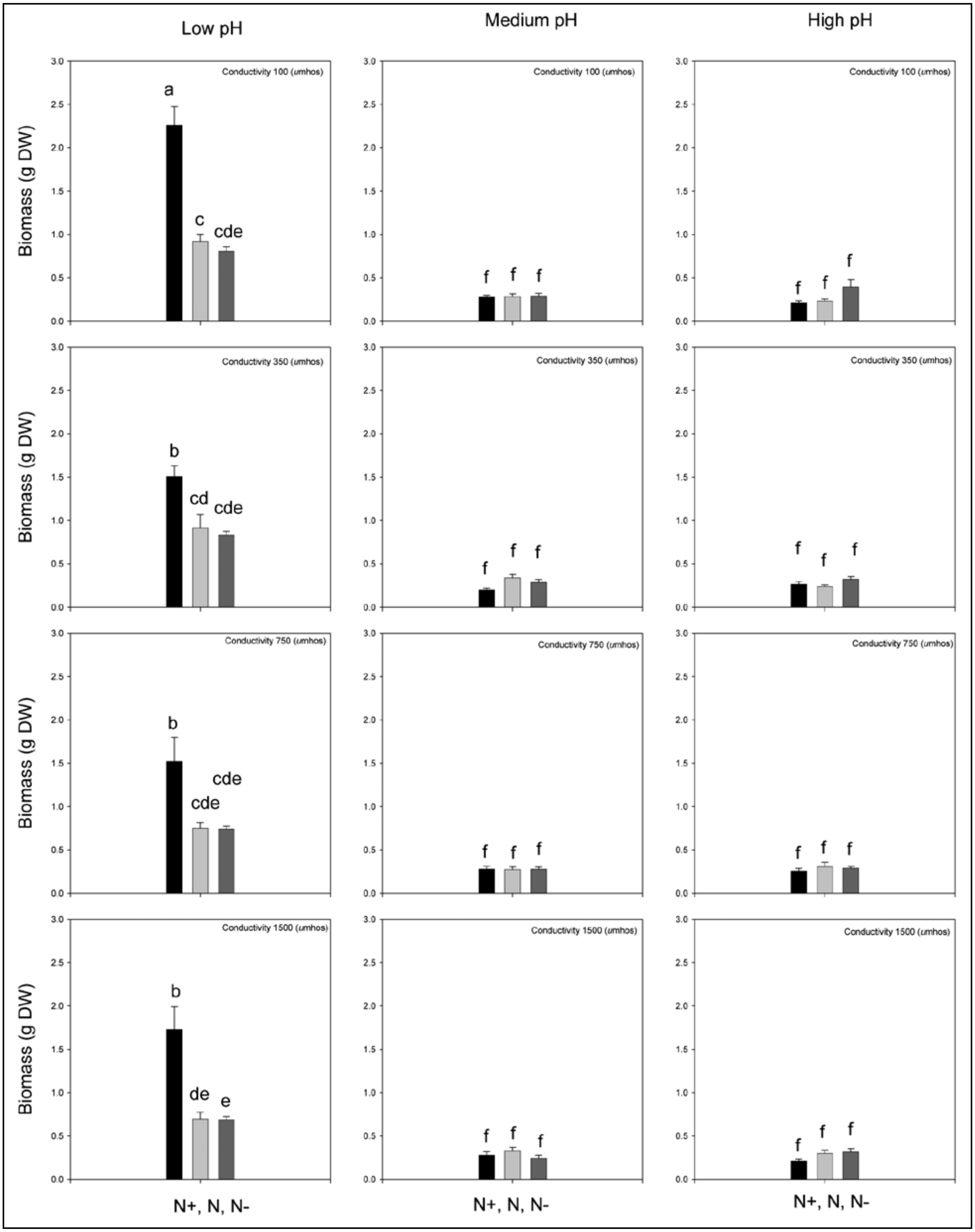

Figure 1. Significant three-way ANOVA interaction (p=0.0209, F=2.059) for salvinia biomass (g/DW) between the three parameters of nutrients, pH, and conductivity. Top column titles are pH levels, bottom is nutrient levels, and conductivity is within rows.

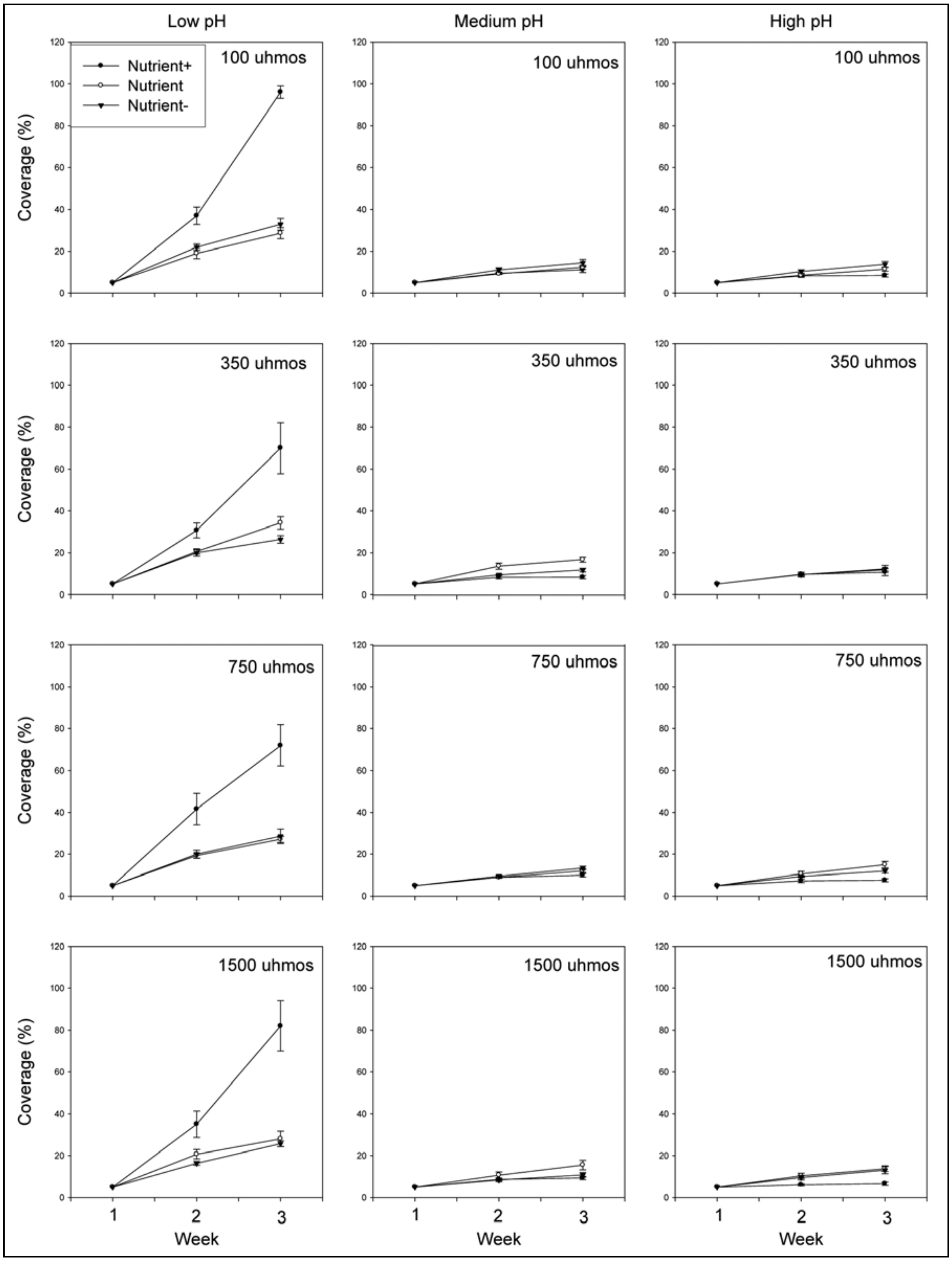

Figure 2. Percent coverage (%) between three parameters of nutrients, pH, and conductivity. Top column titles are pH levels, bottom is nutrient levels, and conductivity is within rows for week 3.

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