

Final Report Executive Summary
ACES Project Summary: Part 1 Final Technical Report

Period Covered by the Report: 8/01/01 – 12/31/03
Date of Report: 12/31/03
EPA Agreement Number: R827072-01-1
Title: Influence of Invasive Plant Species in Determining Diversity of Aquatic Vegetation in the Mobile-Tensaw Delta
Investigators: Dr. Timothy Sherman and Dr. Anne Boettcher
Institution: University of South Alabama
Research Category: Small Grant for Exploratory Research
Project Period: 08/01/01-12/31/03

Background and Objectives:

Introduction of non-indigenous species (NIS) is recognized as one of the leading causes of loss in biodiversity, second only to habitat loss (Walker and Steffen 1997, Wilcove *et al.* 1998). However, the identification of factors that lead to the establishment and persistence of these invaders has remained elusive (Lodge *et al.* 1998). The Mobile-Tensaw River Delta, an area rich in species diversity, has not escaped the advance of invasive species. Previous studies have shown that, in certain areas, non-indigenous aquatic plants can dominate in terms of both frequency and biomass (Nelson 1999). However, the impacts of these species on native plant assemblages and on plant-animal interactions in this system have not been examined previously. This work and a companion study entitled, “Role of Invasive species in Shaping Plant-Animal Interactions in the Mobile Delta” were designed to evaluate the role of introduced plant species in shaping plant-animal interactions and plant-plant interactions in an effort to elucidate the environmental impacts that NIS may have on the Delta system. The studies focused on NIS currently present in the system, with study sites located in waters surrounding Gravine Island, Baldwin County, AL. The primary objectives of the studies were to:

1. Develop a bio-inventory of native and non-indigenous aquatic plant species in the waters surrounding Gravine Island.
2. Gather data on physiological and growth parameters of dominant native and introduced aquatic plants.

The information that we present here is very similar to that presented in our report for EPA R827072-02, because this is an expansion of that study

Material and Methods:

Study Site

Gravine Island, a natural island in the Tensaw River, is primarily lowland forest and is dominated by a spoilbank at its northern end. Gravine Creek and a small and large cove lie on the western side of the island. Preliminary vegetation analysis of Gravine Island was carried out as part of a larger project investigating the population ecology of the Alabama redbelly turtle (*Pseudemys albamensis*) (Nelson 1999). Sampling sites for this study centered on the creek and cove areas, and sampling was carried out from April-June 1999. Sampling for the proposed work included areas surveyed as part of the redbelly turtle project (Nelson 1999), and additional sites along the southern and eastern shores of Gravine Island.

Sampling of plants

Sampling trips were made monthly from August 2000 – August 2002. Twenty meter transects were used to quantify plant species located in each of the ten sites. Two lengths of 10-foot PVC pipe were driven into the ground at the end of each transect to mark the ends of the transect. The transect method consisted of identifying the type of vegetation that was in contact with the 20-meter transect tape every 25-cm. This method allowed us to quantify plant diversity and relative abundance. Sites were located using hand held global positioning units (GPS).

Physical parameters

Light and temperature were measured at the study sites every 15 minutes via Onset Data Loggers (Onset Computer Corp., Pocasset, MA). The light meters were calibrated against a submersible radiometric light meter (Heinz Walz GmbH, Effeltrich, Germany). Dissolved oxygen concentration (YSI 55 oxygen electrode), salinity (Fisher Salinity refractometer with automatic temperature compensation), and pH (IQ Scientific Instruments, Inc., San Diego, CA, IQ151 handheld pH/mV/temperature meter) were determined monthly at each location. Average current velocities were measured at the edge and center of each study site (10 minute period, 0.6 x depth, Flow Wand, Edutech Technologies Corp. Gibson Landing, BC, Canada). Values were below detection limits and so further measurements were discontinued. Dissolved nutrients also were monitored monthly. Samples of the water column were taken (n=2/site), filtered (Whatman GF/C), and ammonium, nitrate, nitrate/nitrite, and phosphate concentrations were determined with a Skalar SANplus autoanalyzer using standard protocols (Parsens *et al.* 1984). This unit was calibrated using six-point standards of known value. Additionally, samples of known value were placed into the testing regime at 10 to 15 sample intervals to ascertain calibration of the unit during the analytical run.

Nutrient abundance and uptake

In addition to nutrient abundance in the water column, it is important to determine plant capacity to uptake those nutrients. Pursuant this, we conducted a series of simple uptake experiments in the field and in the lab under ambient field conditions of light and temperature. In these uptake assays, uptake analyses were conducted by the batch method described by Pederson (1994). Replicate samples of key species (n=3) were placed in chambers containing physiologically relevant nutrient concentrations [i.e. 1 μM , to 50 μM (Pennock *et al.* 1994)] and water samples were taken at time 0, 10, 20, and 30 min. Equal wet weights of samples were immersed in 200 ml of filtered water in 500 ml bottles with air bubbled through using an air stone attached to an aquarium pump. Water samples were analyzed for ammonium, nitrate and nitrate+nitrite (as described above). Bottles were maintained at ambient light and temperature for the incubation period. These uptake experiments helped to determine if substantial differences exist among the key macrophyte species in ability to scavenge water column nutrients.

In the water column of estuarine systems, the most readily available form of nitrogen is normally nitrate (Ogilvie *et al.* 1997, Page *et al.* 1995). To follow assimilation capacity of nitrate, we monitored activity of the enzyme nitrate reductase (NR). This enzyme is recognized as the key regulatory enzyme of the nitrate reduction pathway (Campbell 1988, Hoff *et al.* 1992, Solomonson and Barber 1990). Submerged tissues of two dominant native (*Vallisneria americana* and *Najas guadalupensis*) and an invasive (*Hydrilla verticillata*) harvested about 3 to 4 hours after the onset of the light period, as NR activity has been shown to be maximal after exposure to light for this period (Gao *et al.* 1992, Lillo 1994). *In situ* NR assays were used to

assess NR activity in all experimental regimes. Approximately 0.2 g was taken from each plant sample and weight was recorded. Tissue was placed into a 30 ml screw cap test tube and 5 ml of assay mix (100mM K_2HPO_4 pH 7.5, 2% propanol, 30 mM KNO_3). One set of tubes was placed in a 30°C water bath for 1 hr then transferred to an 80°C, while the other set of tubes was placed immediately in an 80°C water bath. After the samples had cooled to room temperature, 0.5 ml was extracted and placed into a cuvette. To the cuvette, 0.5ml of 1% sulfanilamide and 0.5 ml of 0.1% N- (naphthyl)-ethylenediamine were added. After 10 minutes, the absorbance was taken at 540nm.

Results and Discussion:

Data for the aquatic plant and macroinvertebrate bio-inventories have been collected and are being logged into the recently-created SQL database. The database will allow for rapid analyses of plant and animal abundance, diversity, and distribution. Preliminary analyses of plant distribution and abundance reveal several patterns. As expected, plant abundance follows seasonal changes in temperature, with peak abundance occurring during the summer season, decreasing with decreasing temperatures. During the first year of sampling the most common NIS were *Alternanthera philoxeroides* (alligator weed), *Eichhornia crassipes* (water hyacinth), and *Hydrilla verticillata* (Hydrilla). Interestingly, during the second year and third summer of sampling, *H. verticillata* was the dominant NIS and *E. crassipes* was rarely detected. There were similar shifts in native species abundance. During the first year, *Zizaniopsis miliacea* (cut grass), *Potamogeton nodosus* (longleaf pondweed), and *Najas guadalupensis* (bushy pondweed) were dominant species at specific sites, but there was no common pattern across sites. However, during the second year and third summer, the dominant natives across sites were *Z. miliacea*, *N. guadalupensis*, and *Ceratophyllum demersum* (coontail).

Key native and invasive species were monitored for their abilities to take up nitrate or ammonium from the water column. That work showed no clear difference between invasive and native species that would help to explain the ability of invasives to dominate a system. However, it should be noted that this was a small-scale exploratory study with low power and so further comparisons are warranted.

Ammonium uptake was common under field conditions, however, nitrate uptake was rarely observed. To address this anomaly, lab experiments were performed on native (*Vallisneria americana* and *Najas guadalupensis*) and a key invasive (*Hydrilla verticillata*) species to examine Nitrate Reductase (NR) is the enzyme responsible for catalyzing the first step in nitrate assimilation. NR is considered the limiting step in the nitrate assimilation pathway. While NR regulation is fairly well understood in terrestrial vascular plants, considerably less is known about the control of activity of NR in aquatic macrophytes. Our results revealed no enzyme activity for *H. verticillata* when grown in the presence of nitrate, a condition that normally induces NR activity in other aquatic plants. Under similar conditions, both native species possess low, but detectable activity. The enzyme activity in all of these species is much lower than that found in terrestrial vascular plants. It is possible the ammonium derived from photorespiration is responsible for this low activity. Experiments were conducted under conditions, which should decrease photorespiration. This work suggests that that this may be partially responsible for the low NR activity found in this species.

If so, more rigorous uptake experiments under controlled conditions of fluid velocity and nutrient concentration (Davis and Minshall 1999, Koch 1994) will be conducted in subsequent work.

Literature Cited

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- Nelson, D.H. 1999. Population ecology of the Alabama red belly turtle (*Pseudemys alabamensis*)—vegetation, diet, clutch size. *Final report to Department of Conservation and Natural Resources*, December 1999.
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Changes in Scope or Objectives:

This is a continuation of a grant entitled, "Role of Invasive Species in Shaping Plant-Animal Interactions in the Mobile Delta" to A. Boettcher, T. Sherman, and J. Valentine was funded during 2000 cycle. This grant provided funding for a second year of plant sampling, which was completed during the summer of 2002. Based on preliminary work, it was determined that the photosynthetic parameters (PAM measurements) were not useful approach for comparative physiological analyses.

Publications/Presentations:

- Penton, A., J. Valentine, J. McClintock, C. Amsler, T. Sherman, and A. Boettcher. 2002. Role of invasive species in shaping plant-animal interactions in the delta. Oral presentation, ACES Scientific Advisory Committee Meeting. Dauphin Island Sea Lab, Dauphin Island, AL. May 2002.
- Fairley, S. and T. D. Sherman 2001 "Regulation of Nitrate Reductase Activity by Environmental Factors." Poster presentation at the Second Annual Tuskegee Undergraduate Research Conference, held on November 18, 2001. Ms. Fairley won second place in poster presentation.
- Fairley, S. and T. D. Sherman 2001 "Regulation of Nitrate Reductase Activity by Environmental Factors." Poster presentation the Annual Biomedical Research Conference for Minority Students(ABRCMS) was held in Orlando on October 29 - November 4, 2001.