Corbicula fluminea INVASION AS A SECONDARY EFFECT OF *Hydrilla verticillata* MANAGEMENT VIA TRIPLOID GRASS CARP (*Ctenopharyngodon idella*)

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A study of Asian clam (*Corbicula fluminea* Müller) colonization in relation to changes in aquatic vegetation community as a result of management of *Hydrilla verticillata* (L. f.) Royle with grass carp was conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF), Lewisville, TX, from April 2015 through October 2016. Percent vegetation cover, *C. fluminea* abundance and water quality metrics (pH, turbidity, conductivity, DO, calcium, chlorophyll a) from 16 experimental subjects were analyzed. Treatments included four replicated grass carp stocking densities; 1-control with no fish stocked $(n = 4)$, 2-low density of 40-43 fish per vegetated ha ($n = 4$), 3-medium density of 72-81 fish per vegetated ha ($n = 4$) and 4-high density of 110-129 fish per vegetated ha $(n = 4)$. Data analysis showed statistical significance in the relation of *C. fluminea* abundance to percent vegetation cover (multiple linear regression, $r^2 =$ 0.820), grass carp stocking densities (two-way analysis of variance, $p = 0.001$) and chlorophyll a (multiple linear regression, $r^2 = 0.339$). Findings of this research indicate the possibility that management of hydrilla had enabled establishment of secondary invasive species.

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CHAPTER 1

INTRODUCTION

Adaptive Management of Aquatic Vegetation

While the U.S. consumption of water has stabilized since 1980, the demand for access to U.S. surface waters has increased (Kenny et al. 2009). Human activities in freshwater systems include real estate, recreation, irrigation, hydropower, potable water, navigation, and efforts to conserve environmental attributes such as fish and wildlife habitat. Aquatic plants are a key component of natural freshwater systems, and resource managers agree that moderate and diverse level aquatic plant assemblages are beneficial for ecosystem functions (Netherland and Schardt 2012). However, an overabundance of invasive non-native aquatic plants threaten the diversity of native species, and otherwise impairs freshwater systems requiring management to conserve waterbody use and functions. Considering this predicament, management strategies should be carefully and situationally selected.

When management strategies are concerned with maximum control and/or eradication of targeted species, beneficial native species can be negatively affected (Simberloff and Stilling 1996, Zavaleta et al. 2001, Bonar et al. 2002, Pipalova 2006). This type of management can lead to more serious ecological problems, including depletion of valuable native plant species, negative secondary effects such as declines in ecosystem services, negative changes in predatorprey and herbivore-plant interactions and trophic cascades (Nichols and Keeney 1973; Zavaleta et al. 2001; Hershner and Havens 2008, Martin and Blossey 2008, Gettys et al. 2009, Schlaepfer et al. 2010 and 2012, Vitule et al. 2012). Such negative effects may open niches for new invasive species infestations or re-infestations of targeted species, cause harmful algal blooms, loss of habitat heterogeneity and degradation of water quality; all of which decrease the value of a water

body as a natural resource (O'Hara 1967, Hansen et al. 1971, McVea and Boyd 1975, Shireman and Smith 1983, Gholson 1984, Taylor et al. 1984, Chilton and Muoneke 1992, Bain 1993, James and Barko 1995, Cory and Myers 2000, Madsen and Owens 2000, Smart et al. 2009). Alternatively, a more beneficial approach may be one that is ecologically-oriented, viewing the ecosystem holistically and giving consideration to consequences of management strategies likely to affect other biological and environmental parameters. General approaches include use of integrated management/restoration tools, substantial monitoring, and then adjustment of strategies to achieve desired results. However, further research on how specific or integrated strategies of invasive species management affect specific ecosystem components is needed to assist resource managers and conservation biologists in decision-making (Dick et al. 2016).

Hydrilla Management with Grass Carp

One such area of need includes the management of the non-native, invasive aquatic plant *Hydrilla verticillata* (L. f.) Royle (hydrilla) (Figure 1.1). Hydrilla infestations can be detrimental to water resources and ecosystem functions and have occurred in the U.S. since the 1960s (Pieterse 1981 and Langeland 1996). A variety of control measures have been developed for the species, however, thus far none have proven to be a long-term solution (Gallagher and Haller 1990). One method that can provide moderately sustained control of hydrilla is the use of triploid *Ctenopharyngodon idella* Valenciennes (grass carp) as a biocontrol agent (Figure 1.2). Grass carp are native to Asia, are one of the largest cyprinid fishes, and were first released for aquatic plant control in the U.S. in 1963. Since then, grass carp populations are found in 45 U.S. states as a result of intentional and unintentional releases (Schofield et al. 2005). Their feeding behaviors

are well documented and appear to have hierarchal preference of aquatic plant species (Pine and Anderson 1991).

Figure 1.1. *Hydrilla verticillata* (L. f.) Royle is a submersed plant native to Africa and Southeast Asia and is major aquatic weed throughout most of the world's warmer climates.

Figure 1.2. The use of triploid grass carp (*Ctenopharyngodon idella* Valenciennes) as a biocontrol for aquatic plants was developed in the 1960s and commonly used for management of hydrilla.

One of these preferred plant species is hydrilla. However, since grass carp are generalist herbivores, under variable conditions grass carp will feed on a variety of aquatic plant species. For example, when grass carp are used for hydrilla management, high density stocking $(>40$ fish per vegetated ha) may result in removal of most, if not all, native vegetation (Hanlon et al. 2000, Bonar et al. 2002, Dibble and Kovalenko 2009, personal communications; Dr. Brent Bellinger City of Austin, Dr. Gary Dick U.S. Corps of Engineers 2015 – Lake Austin, TX and Lake Conroe, TX, respectively). Total removal of the aquatic plant community may consequently open niches for other invaders.

Documented examples have occurred where hydrilla control using grass carp has resulted in increased infestations of *Myriophyllum spicatum* L. (Eurasian watermilfoil), a non-indigenous, invasive species in the U.S. (Fowler and Robson 1978). Grass carp show low preference for Eurasian watermilfoil, with selective removal of hydrilla by the fish providing a competitive advantage to the watermilfoil (Van Dyke et al. 1984). Similarly, evidence of increases in *Limnophila sessiliflora* (Vahl) Blume (Asian marshweed) (a non-indigenous, invasive species in the U.S.) following hydrilla declines has been observed in Lake Seminole, Florida in the presence of grass carp (Spencer and Bowes 1985, personal communications; Dr. Gary Dick US Army Corps of Engineers 2015). These examples imply that management strategies designed to eradicate unwanted vegetation have resulted in replacement of the targeted species with a second undesirable species that will require an alternative control technique. Considering the large number of invasive species now occurring in the U.S., the cycle of management-new infestationmanagement may prove to be never-ending.

Secondary Effects of Managing Nuisance Aquatic Vegetation

Some research has investigated environmental and biological impacts of grass carp application, i.e. impact on macrophytes (Colle et al. 1978, Chilton et al. 1992, Dick et al. 2016), water quality (Shireman and Smith 1983, Bonar et al. 2002, Pipalova 2006), water fowl (McKnight and Hepp 1995, Pipalova 2006, Wittmann et al. 2014) and invertebrates (Kirkağaç and Demir 2006, Kovalenko et al. 2010, Wittmann et al. 2014), however the majority of this research has focused on the field application of grass carp in uncontrolled lakes, reservoirs and streams. Additionally, research on specific unintended secondary effects (i.e. invasion by other nuisance species) of hydrilla management via grass carp is lacking in the literature. This study offers an evaluation of unintended secondary effects of hydrilla management that occurred in an intermediate-scale, replicated experimental investigation. That effect was first observed near the conclusion of a pond study evaluating several stocking densities of grass carp as a means of controlling hydrilla while simultaneously restoring "grass carp resistant" native plant species (Dick et al. 2016). *Corbicula fluminea* Müller (Asian clam), a non-native bi-valve mollusk, were noted to be occurring in some of the study ponds, but not others, indicating the possibility that management of hydrilla had enabled establishment of clam populations in some of the managed ponds (Figure 1.3). Asian clams are a federally listed non-native aquatic species and introductions have consequences to the ecosystem including aquatic vegetation, competition with native species, phytoplankton, zooplankton and higher trophic levels (Phelps 1994, Johnson and McMahon 1998, Strayer 1999, Cherry et al. 2005, Cooper et al. 2005, Sousa et al. 2007). Additionally, Asian clam colonies can impact irrigation, industrial water, and power plant systems, drinking water supplies and alter benthic substrates. Economic costs associated with Asian clams in the U.S. are estimated at 1 billion dollars per year (Pimentel et al. 2005).

Figure 1.3. Asian clams (*Corbicula fluminea* Müller) are a filter feeding bivalve mollusk macroinvertebrate. The species exhibits r-selected characteristics and is capable of producing rapidly reproducing colonies.

Research Objectives

This study investigated Asian clam colonization in response to changes in aquatic vegetation communities densities caused by grass carp. The objective of the research was to document previously unreported secondary effects of hydrilla management in aquatic ecosystems. Results produced from this research will aid resource managers in determining the level of management of hydrilla populations that will minimize unintended consequences.

Hypotheses

- \bullet H_a: Grass carp stocking density has significant effect on Asian clam abundance.
- \bullet H_a: Water depth has significant effect on Asian clam abundance.
- \bullet H_a: Interaction of grass carp stocking density and water depth has significant effect on Asian clam abundance.
- H_a : Grass carp stocking density has significant effect on total percent vegetation cover.
- \bullet H_a: Total percent vegetation has significant effect on Asian clam abundance.
- H_a: Water quality (pH, turbidity, conductivity, DO, calcium, chlorophyll *a*) has significant effect on Asian clam abundance.
- H_a: Water quality (pH, turbidity, conductivity, DO, calcium, chlorophyll *a*) is significantly different between grass carp stocking densities.

Study Area and Site Description

Research was conducted at the Lewisville Aquatic Ecosystem Research Facility (LAERF)

in partnership with the U.S. Army Corps of Engineers (Figure 1.4)

Established in 1990, LAERF was designed to support studies on biology, ecology, and management of aquatic plants. LAERF provides an intermediate scale research environment to bridge the gap between small-scale laboratory studies and large-scale field tests. The facility possess' 53 earthen and 21 lined ponds, 18 flowing water raceways, 3 large outdoor mesocosm facilities, a research greenhouse, and several laboratories to conduct research activities. (Masser and Neisch 2011)

For the original experiment (Dick et al. 2016), eight earthen research ponds were utilized, with volumes ranging from 2,459 to 3,555 m^3 , and mean depths of about 1.0 m (Smart et al 1995). Each pond was equally divided lengthwise by a welded wire fence barrier covered with semi-permeable landscape fabric to prevent movement of grass carp, resulting in 16 individual experimental "subjects" ranging from 0.13–0.16 ha surface area and depths ranging from 0–2 m (Figure 1.5) The ponds were supplied with water from nearby Lake Lewisville and levels were maintained via stand pipe systems.

While Asian clams commonly occurred in inflow piping and drain ditches at the LAERF, they had not established in any of the LAERF ponds over a 25 year period (personal communications; Dr. Gary Dick U.S. Army Corps of Engineers 2015). Prior to the study, ponds supported similar vegetation communities, consisting of primarily hydrilla and volunteer native species, including *Potamogeton nodosus* Poir. (American pondweed), *Najas guadalupensis*

(Spreng.) Magnus (southern naiad), *Chara vulgaris* Linnaeus (muskgrass), *Sagittaria platyphylla* (Engelm.) J.G. Sm. (delta arrowhead), *Eleocharis macrostachya* Britton (slender spikerush) and *Paspalum distichum* L. (jointgrass).

Figure 1.4. Aerial view of U.S. Army Corps of Engineers Lewisville Aquatic Ecosystem Research Facility, Lewisville, Texas (LAERF).

Figure 1.5. Ponds were divided lengthwise with welded wire and semi-permeable landscape fabric fence resulting in two experimental subjects per pond (ponds n=8, experimental subjects n=16). Pictured pond #20, left; control (no grass carp), right; high density (11 grass carp).

CHAPTER 2

MATERIALS AND METHODS

The original grass carp density study was initiated in 2010, with detailed methods and experimental design of that study given in Dick et al. 2016. A summary of those methods are given below.

Grass Carp Stocking

In May 2011, triploid grass carp 25-30 cm in length and averaging 352 g were stocked at four different rates for each of the following experimental treatments: controls (C) with no fish stocked; low density (LD) of 40–43 fish per vegetated ha (approximately 17 per vegetated hectare); medium density (MD) of 72–81 fish per vegetated ha (approximately 31 per vegetated hectare); and high density (HD) of 110–129 fish per vegetated ha (approximately 48 per vegetated hectare) (Appendix A). Treatments were assigned to study ponds in a manner that avoided like-treatments occurring in any pair (treatment pairs were determined and then assigned randomly to split ponds). The study included four replicates of each treatment resulting in 16 experimental subjects.

Native Plant Restoration

Native plant restoration was coupled with hydrilla control to provide an integrated management approach. In June 2012, eleven native aquatic plants were transplanted in each of the study ponds (Table 2.1). Plants selected were those shown to be capable of contributing a persistent pressure occupying similar niches (Smart 1995). Plants were grown from native propagules in commercial nursery pots (Dick et al. 2013). Plantings were made on 1.8 m centers

at two depth tiers. Emergent species were planted at the shallower tier 0.3 m deep, and floatingleaved and submersed species were planted in the shallower tier at 0.6 m depths. Six mature individuals of each species were planted as three pairs at randomly assigned positions along the appropriate depth tier, with one plant of each pair protected from herbivory with a 0.58 m diameter, PVC-coated welded-wire cylinder to exclude grass carp and other large herbivores such as waterfowl and turtles for initial establishment (Figure 2.1)

Table 2.1*.* Eleven native aquatic plant species were transplanted into study ponds with and without herbivore protection to evaluate their suitability for replacing hydrilla being controlled by triploid grass carp.

Growth form	Scientific name	Group Type		
	Heteranthera dubia	Monocot	Perennial forb/herb	
	Potamogeton illinoensis	Monocot	Perennial forb/herb	
Submersed	Potamogeton nodosus	Monocot	Perennial forb/herb	
	Vallisneria americana	Monocot	Perennial forb/herb	
Floating-leaved	Nymphaea odorata	Dicot	Perennial forb/herb	
Emergent	Echinodorus cordifolius	Monocot	Perennial forb/herb	
	Eleocharis quadrangulata	Monocot	Perennial sedge	
	Justicia americana	Dicot	Perennial forb/herb	
	Pontederia cordata	Monocot	Perennial forb/herb	
	Schoenoplectus pungens	Monocot	Perennial sedge	
	<i>Schoenoplectus</i> tabernaemontani	Monocot	Perennial sedge	

Figure 2.1. Native plant restoration was coupled hydrilla control to provide an integrated management approach. Six containerized plants of each species were transplanted into each experimental subject with one individual plant installed with an herbivory protection cage.

Data Collection

Following the conclusion of the original experiment in 2015 (Dick et al. 2016) it was determined that study ponds would be maintained for the purpose of evaluating longer-term effects of grass carp density treatments on vegetation communities. During that time, visual observations of Asian clam colonies were noted to be occurring in some of the ponds but not others that prompted this research. Asian clams were sampled using a cylindrical benthic core sampler in May 2015 and May 2016. The dimensions of the sampler measured 0.30 m diameter by 0.40 m height and were sampled to a sediment depth of 0.15 m. Nine samples were taken from each experimental unit (n=16), 3 samples at each depth (n=3) of 0.30, 0.61, 0.90 m $(n=144)$, and 2 samplings dates (total samples n=288). The sampler was submersed, inserted 0.15 m into benthic substrate and material removed with shovel. Sample material was stored in a five

gallon bucket until processing. Samples were sieved (0.3cm mesh) to collect clams for enumeration.

Vegetation coverage data was collected in October 2015 and October 2016. It was determined that sampling in October would most likely represent annual maturation of the vegetation community in relation to the conclusion of the growing season, thereby reflection conditions when Asian clams were becoming established. The vegetation community was categorized into nine vegetation types/species; bare (vegetation absent), *Chara* sp., emergent planted, emergent volunteer, hydrilla, recruitment, submersed planted, submersed volunteer and *Nymphaea odorata* Aiton (Table 2.3). Percent cover was calculated by geo-referencing vegetation surveys and aerial photography (Google Earth Pro) using Geographic Information System (GIS) software (ArcGIS) (Figure 2.6).

In addition to clam and vegetation sampling, general water quality was obtained from the aforementioned study, Dick et al 2016. Parameters included pH, turbidity, conductivity, DO, calcium, and chlorophyll a. Water quality data was sampled using a Hydrolab Quanta Multiparameter Sonde (Hach Hydromet Loveland, CO) or analyzed in the laboratory following methods described in Clesceri et al. 1995.

Table 2.2. Nine benthic core samples from each pond half / treatment were sieved to achieve Asian clam enumeration. Abundance (clams / $m²$) was determined for treatment and each depth in 2015 and 2016 by averaging Asian clam counts and calculating to square meters. Treatment is the grass carp density in each pond half (C=0, LD=17 per hectare, MD=31 per hectare, and HD=48 per hectare)

Figure 2.2. Vegetation surveys were georeferenced with aerial photography (Google Earth Pro, Google Inc, Mountain View, California) and ArcMap software (ESRI, Redlands, California) to provide illustrations of vegetation cover and area (m^2) of each species / vegetation stand.

Data Analysis

Asian clam abundance (no. clams/m²) was determined and compared for each treatment $(n=4)$, each depth $(n=3)$ and sampling date $(n=2)$. Metrics used to determine and compare vegetation cover were surface area of vegetation (no. $/m²$) and percent vegetation cover (no. vegetation m2 / total pond area m2). Data was analyzed using statistical software SigmaPlot Version 11.0 (Systat Software Inc., San Jose, CA). The following hypotheses are given with their corresponding test to determine the biostatistical significance (α =0.05).

Two-way analysis of variance (ANOVA) was performed for the following hypotheses:

- \bullet H_a: Grass carp stocking density has significant effect on Asian clam abundance.
- H_a : Water depth has significant effect on Asian clam abundance.
- \bullet H_a: Interaction of grass carp stocking density and water depth has significant effect on Asian clam abundance.

Multiple linear regression was used to evaluate these hypotheses:

- \bullet H_a: Grass carp stocking density has significant effect on total percent vegetation cover.
- \bullet H_a: Total percent vegetation has significant effect on Asian clam abundance.
- H_a: Water quality (pH, turbidity, conductivity, DO, calcium, chlorophyll *a*) has significant effect on Asian clam abundance.
- H_a: Water quality (pH, turbidity, conductivity, DO, calcium, chlorophyll *a*) is significantly different between treatments / percent vegetation.

CHAPTER 3

RESULTS

Relationship of Asian Clam Abundance to Grass Carp Density and Depth

A two-way analysis of variance showed significant differences in Asian clam abundance relative to both grass carp density and depth in 2015. Additionally, this test resulted in a slightly significant (p=0.037) interaction of these variables. In 2016, Asian clam abundance was significantly different between grass carp density treatments but not depth. Tables 3.1 and 3.2 provides summaries and results of statistical tests used to reveal these relationships, differences and interaction among grass carp density treatments and depth. Figures 3.1 and 3.2 illustrate these differences in Asian clam abundances, depth, grass carp density and sampling date.

Table 3.1. Summary table of a two-way analysis of variance (ANOVA) comparing Asian clam abundance (no./m²) with grass carp density (treatments were C=0, LD=17 per hectare, MD=31 per hectare, and HD=48 per hectare) and depth (0.90 m, 0.61 m and 0.30 m) (2015).

Source of Variation	DF	SS	MS	F	P
Grass Carp Density	3	24951.6	8317.19	19.172	< 0.001
Depth	◠	3765.83	1882.91	4.34	0.02
Grass Carp Density x Depth	6	6636.78	1106.13	2.55	0.037
Residual	36	15617.9	433.83		
Total	47	50972	1084.51		

Table 3.2. Summary table of a two-way analysis of variance (ANOVA) comparing Asian clam abundance (no./m²) with grass carp density (C=0, LD=17 per hectare, MD=31 per hectare, and HD=48 per hectare) and depth (0.90 m, 0.61 m and 0.30 m) (2016).

Figure 3.1. Asian clam abundance (m^2) between grass carp density and depth in an intermediate scale replicated pond study at LAERF in 2015. Grass carp density treatments were C=0, LD=17 per hectare, MD=31 per hectare, and HD=48 per hectare.

Figure 3.2. Asian clam abundance (m^2) between grass carp density (C=0, LD=17 per hectare, MD=31 per hectare, and HD=48 per hectare) and depth in an intermediate scale replicated pond study at LAERF in 2016.

Relationship of Asian Clam Abundance to Percent Vegetation Cover

Multiple linear regressions were done using dependent variable Asian clam abundance $(m²)$ and independent percent vegetation cover variables. Both models were found to be statistically significant; $r^2=0.312$, $p=0.024$ (2015) and $r^2=0.538$, $p=0.002$ (2016). Table 3.3 and 3.4 provides summaries and results of statistical tests used to reveal the relationship of Asian clam abundance and percent vegetation cover. Figures 3.3 and 3.4 illustrate the relationship of Asian clam abundance and percent vegetation cover.

Table 3.3. Summary table of multiple linear regression model comparing Asian clam abundance and percent vegetation cover (2015).

	DF	SS	MS		D	r^2
Regression		3167.458	3167.458	6.357	0.024	0.312
Residual	14	6975.199	498.229			
Total	15	10142.657	676.177			

Figure 3.3. Multiple linear regression model of Asian clam abundance and percent vegetation cover in 2015. Raw data, 95% regression confidence interval and regression line given, r^2 = 0.312.

	DF	SS	MS			r∠
Regression		16047.6	16047.6	15.134	0.002	0.538
Residual	13	13784.8	1060.37			
Total	14	29832.4	2130.89			

Table 3.4. Summary table of multiple linear regression model comparing Asian clam abundance and percent vegetation cover (2016).

Figure 3.4. Multiple linear regression model of Asian clam abundance and percent vegetation cover in 2016. Raw data, 95% regression confidence interval and regression line given, $r2 =$ 0.538

Relationship of Percent Vegetation Cover and Grass Carp Density

Multiple linear regressions were done using dependent variable percent vegetation cover and independent grass carp density. All models were found to be statistically significant; p<0.001, r^2 =0.759 (2015) and r^2 =0.820 (2016). Table 3.5 and 3.6 provides summaries and results of statistical tests used to reveal the relationship of percent vegetation cover and grass carp density. Figures 3.5 and 3.6 illustrate the relationship of percent vegetation cover and grass carp density.

	DF	SS	MS	F	D	r ₂
Regression		17121.2	17121.2	43.976	< 0.001	0.759
Residual	14	5450.654	389.332			
Total	15	22571.85	1504.79			

Table 3.5. Summary table of multiple linear regression model comparing percent vegetation cover and grass carp density (2015)*.*

Figure 3.5. Multiple linear regression model of Asian clam abundance and percent vegetation cover in 2015. Raw data, 95% regression confidence interval and regression line given, r^2 = 0.759.

Figure 3.6. Multiple linear regression model of Asian clam abundance and percent vegetation cover in 2016. Raw data, 95% regression confidence interval and regression line given, $r^2 =$ 0.820.

Water Quality

Multiple linear regressions were done using dependent variable water quality data (pH, turbidity, conductivity, DO, calcium, chlorophyll *a*) and independent variables grass carp density, percent vegetation and Asian clam abundance. A statistically significance relationship between chlorophyll a and percent vegetation (r^2 = 0.339, p=0.018) was measured. There was no significance among other water quality variables or Asian clam abundances. Table 3.7 provides summaries and results of statistical tests used to reveal the relationship of chlorophyll a and percent vegetation. Figure 3.7 illustrate the relationship of chlorophyll *a* and percent vegetation cover.

Figure 3.7. Multiple linear regression model of chlorophyll a and percent vegetation cover in 2015. Raw data, 95% regression confidence interval and regression line given, $r^2 = 0.339$.

CHAPTER 4

DISCUSSION

It has been widely accepted that aquatic vegetation (native and invasive species), and there management, have a significant effect on ecosystem function goods and services (Carpenter and Lodge 1986, Netherland and Schardt 2012, Schroeder and Fulton 2013). Management of hydrilla can be applied by variety of methods including mechanical removal, herbicide, native plant restoration and biocontrol agents such as grass carp. Depending on the waterbody, the efficacy of these methods may vary over time, emphasizing the importance of adaptive / integrated management techniques. General approaches include integrated management/restoration tools, substantial monitoring, and then adjustment of strategies to improve success. In this study, different levels of grass carp per vegetated hectare as means of controlling hydrilla resulted in a threshold upon which aquatic vegetation (natives and hydrilla) was replaced with bare substrate and habitat suitable for reproducing Asian clam colonies. In the next section, biotic and abiotic interactions that played a role in these dynamics and other implications, are discussed.

Grass Carp Density and Vegetation

The following alternative hypothesis is supported statistically by rejecting the null hypothesis counterpart.

• H_a: Grass carp stocking density has significant effect on total percent vegetation cover in 2015 and 2016.

The relationship of grass carp density to percent vegetation was supported statistically through this experimental investigation. Specifically, medium and high density stocking rates resulted in removal of the targeted hydrilla and the majority of native species. This inverse

proportional relationship yielded approximately 60 – 90 percent bare, unconsolidated benthic substrate in the experimental ponds. Biotic implications of aquatic vegetation removal include changes in predator-prey and herbivore-plant interactions, trophic cascades, cause harmful algal bloom, impacts on macroinvertebrates (Hofstra and Clayton 2014) and open niches for new invasive species or re-infestation of target species. Abiotic implications include nutrient cycling, water quality (discussed in a following section), erosion and human activities such as recreation (Bellinger and Davis 2017), aesthetics (Hofstra and Clayton 2014), economics (Zhang and Boyle 2010) and additional management needs (Richardson 2008). Some of the management strategies (higher density grass carp) used in this experiment failed to produce moderate levels of vegetation cover (i.e. $45 - 55$ %). This indicates the likelihood of variable results when using grass carp for hydrilla management.

Asian Clam Colonization

The following alternative hypotheses were supported statistically by rejecting their null hypothesis counterparts. The two sampling dates provided variation of significance / nonsignificance and tests that were not supported are in parentheses:

- H_a: Grass carp stocking density has significant effect on Asian clam abundance in 2015 and 2016.
- H_a: Water depth has significant effect on Asian clam abundance in 2015 (2016).
- \bullet H_a: Interaction of grass carp stocking density and water depth has significant effect on Asian clam abundance in 2015 (2016).
- \bullet H_a: Total percent vegetation has significant effect on Asian clam abundance in 2015 and 2016.

Results show that Asian clam populations in our experiment were related to percent vegetation cover, grass carp stocking density and colonization depth. Control and low grass carp density treatments exhibited no populations of Asian clams. Asian clams were found in two of the four replicate medium density treatments, LAERF pond 11a (111.85 clams / m^2 , 2016) and 20b (68.14 clams /m², 2016). Populations of Asian clams were found in all high density replicates ranging from 17 to 192 clams $/m²$. Given this circumstance, changes in populations of the Asian clam are most likely caused by alterations of the aquatic habitat related to reduction in the aquatic macrophyte population. Changes in the aquatic community composition may cause major changes in important ecosystem functions that influence the benthic macroinvertebrate community (Levin et al. 2001, Sousa et al. 2011 and Strayer 2012). In this experiment, the elimination of aquatic vegetation caused major changes to food available and substrate characteristics that created a niche for the r-selected species, *C. fluminea*. The Asian clam feeds on particulate organic matter (POC) and is one of the most invasive species in freshwater ecosystems. It has a larval stage that is planktonic for a short period of time and permits wide dispersions to new habitats. Once the juveniles settle onto the substrate the clam has a rapid growth, early sexual maturity, high fecundity. All characteristics that enable rapid population growth in suitable habitats.

When grass carp are introduced to aquatic systems the organic carbon (nutrients), its form (dissolved vs particulate), and how it is cycled through the ecosystem are changed. In plant dominated systems energy is stored in carbon compounds through the process of photosynthesis and dissolved nutrients and elements cycle through the benthic substrate, the water column, the aquatic vegetation and back. The plants provide habitat and food to a benthic community dominated by epiphytic insects. After grass carp are introduced particulate organic carbon, through herbivory and excrement increase the amount of particulate organic carbon in the water. This increase in particulate matter shifts benthic invertebrate community structure from epiphytic

insects to increasing numbers of filter feeding invertebrates, like *C. fluminea*. A second factor is the connection between aquatic plants and the type of benthic substrate available for colonization of the Asian clam. The Asian clam preferentially colonize fine sands (Belanger et al 1985). Vascular aquatic plants and their extensive root systems reduce the availability of open substrates with fine sands and reduce or eliminate habitat suitable for the Asian clam. Colonization was triggered in the benthos of experimental subjects that experienced vegetation removal. Removal of plants did not cause the influx of Asian clams. Direct impacts to the substrate and indirect impacts to food available were more likely restricting the development of population. Asian clam propagules were always present in the system. Removal of plants established a food source (POC) and opened habits which permitted the populations to grow.

Water Quality

The following alternative hypothesis is supported statistically by rejecting the null hypothesis counterparts:

• Ha: Total percent vegetation has significant effect on chlorophyll *^a* (mg/L).

While the water quality data for this study was limited, results showed statistically significant reduction in chlorophyll with decreases in percent vegetation (r^2 = 0.339, p=0.018) This is in contrast to studies by Canfield and his colleagues (2011) who described increasing chlorophyll *a* levels following declines in aquatic macrophytes, a result of the transformation of nutrients in vascular plant form to nutrients released into the system from decomposing and/or biologically processed vegetation. Our investigation showed the opposite relationship with lower levels of chlorophyll *a* in subjects that experience vegetation removal. There are several factors that could have attributed to this effect. One explanation includes the high filtration rates of

bivalves like *Corbicula sp.* by removing suspended organic matter and phytoplankton associated with chlorophyll *a* (Maresaux et al 2016). Another is the symbiotic relationship exhibited with epiphytes and macrophytes (Burholder and Wetzel 1990). Whereas, macrophyte collapse can lead to declines in epiphytic algae and thus reducing chlorophyll.

Difficulties and Potential Enhancements

The timing of initial observations of *C. fluminea* that that led to this research coincided with the conclusion of the previously mentioned study (Dick et al. 2016). Although the original experiment had concluded, the ponds were maintained for *C. fluminea* sampling and vegetation surveys but no continuation of water chemistry analysis. Another difficulty with this project was the massive amount of sample material. Each sample $(n=288)$ consisted of 106 cm³ of benthic substrate material and required constructing an outdoor sample processing station. Given the effort and man power to process these samples, reformatting the sampling regimen may streamline the process. Additional problems occurred when water supply was suspended to one of the ponds resulting loss of water levels to support grass carp, LAERF pond #19, 2016. Subsequently, the fish were moved and data from this pond was not used in the second year analysis. Since the conclusion of this study, alligators in the area have eliminated some grass carp.

Future Research

At this point in invasive species research, a few things are clear and supported by the literature. These include impacts of introductions of non-native species such as changes in food web dynamics (Gallardo et al. 2015), depletion of valuable native species (Dick et al 2016,

Nichols and Keeney 1973), loss of habitat heterogeneity (Theel et al. 2008), degradation of water quality (Cory and Myers 2000) and facilitation of harmful algal blooms (Simberloff 2005), all of which decrease the value of a water body as a natural resource. Additional research has investigated secondary impacts associated implementing invasive species control techniques such as development of resistance / tolerance in chemical control (Koschnick et al. 2006), negative effects on nontarget / bystander species (Pipalova 2006) and declines in ecosystem services (Zavaleta et al. 2006). However, few, if any, have assessed specific integrated / adaptive management strategies that emphasize an ecological perspective and give consideration to other components of ecosystems likely to be affected. This cumulative scientific narrative indicates the potential for management strategies designed to control an unwanted, invasive species resulting in replacement with a second unwanted, invasive species that would require additional management efforts. Considering the large amount of invasive species now occurring in the U.S., identifying and monitoring for potential unintended secondary effects should be top priority when managing waterbodies.

APPENDIX A

GRASS CARP STOCKING DENSITIES AND PERCENT COVER OF DATA VEGETATION

SPECIES / TYPE PER DATE AND TREATMENT

APPENDIX B

RAW COLLECTION DATA FOR *Corbicula fluminea* Müller

APPENDIX C

WATER QUALITY DATA FROMDICK ET AL. 2016

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