

POTENTIAL THREATS OF THE EXOTIC APPLE SNAIL *POMACEA INSULARUM* TO
AQUATIC ECOSYSTEMS IN GEORGIA AND FLORIDA

by

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(Under the Direction of Susan Bennett Wilde)

ABSTRACT

The Island apple snail, *Pomacea insularum* is a freshwater gastropod in the family Ampullaridae. It was introduced into the United States via the aquarium trade approximately 30 years ago and now has established reproducing populations in at least seven southeastern states. It is a highly invasive species with high rates of reproduction and consumption of native aquatic vegetation. A survey of reported Georgia populations confirmed that there are at least ten individual occurrences of exotic apple snails in the state, and that they have not reached their equilibrium distribution. We also investigated the ability of *P. insularum* to harbor a cyanotoxin that may be detrimental to its avian predator in Florida, the endangered Florida snail kite (*Rostrhamus sociabilis*). The invasive *P. insularum* transferred the undescribed cyanotoxin associated with Avian Vacuolar Myelinopathy to domestic chickens in a laboratory feeding study.

INDEX WORDS: apple snail, *Pomacea insularum*, invasive species, Avian Vacuolar Myelinopathy, AVM, Florida snail kite, *Rostrhamus sociabilis*, *Hydrilla verticillata*

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TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vii
LIST OF FIGURES	viii
 CHAPTER	
1 INTRODUCTION AND LITERATURE REVIEW	1
Invasive Apple Snails	1
Objectives and Justification	5
References	6
2 THE DISTRIBUTION OF THE EXOTIC APPLE SNAIL <i>POMACEA INSULARUM</i> IN GEORGIA	9
Abstract	10
Introduction	10
Methods	14
Results and Individual Site Assessments	17
Discussion	30
Conclusions	35
References	39
3 ALTERNATE FOOD-CHAIN TRANSFER OF THE BIOTOXIN LINKED TO AVIAN VACUOLAR MYELINOPATHY (AVM)	43

Abstract	44
Introduction	45
Methods.....	48
Results.....	52
Discussion	54
References	59
4 CONCLUSIONS.....	62
References.....	65

LIST OF TABLES

	Page
Table 2.1: Experimentally determined incipient physiological tolerance limits for adult and juvenile <i>Pomacea insularum</i> collected in Texas	14
Table 2.2: Summer 2011 field sampling locations	15
Table 2.3: Summer 2011 field survey sites and current status of IAS populations	19
Table 2.4: Water quality measurements for all sites visited during 2011 IAS survey.....	29
Table 2.5: Monitoring recommendations based on results of 2011 IAS survey.....	37
Table 2.6: Aquatic plant community assessment data from 2011 surveys	38
Table 3.1: Groups and diets of chickens in laboratory feeding trials	51
Table 3.2: Results of histological analysis of brain section slides from feeding trials.....	53

LIST OF FIGURES

	Page
Figure 2.1: Current U.S. distribution of <i>Pomacea insularum</i>	12
Figure 2.2: Adult <i>P. insularum</i> laying eggs	13
Figure 2.3: 2011 confirmed IAS populations in southern Georgia.....	18
Figure 2.4: IAS egg masses on cattails at St. Mary’s Aquatic Center Pond.....	20
Figure 2.5: Fire ant predation of IAS eggs at St. Mary’s Aquatic Center Pond	21
Figure 2.6: Japanese mystery snail (<i>Cipangopaludina japonica</i>) shells collected at the Savannah River at Jackson, SC in April 2012.....	24
Figure 2.7: IAS egg mass on the main channel of the Ochlockonee River above Thomasville....	26
Figure 2.8: Lake Seminole IAS sighting locations	28
Figure 2.9: Retaining wall in front of a residence on the southern side of the Flint River Arm of Lake Seminole covered with IAS egg masses on September 9, 2011	28
Figure 2.10: USDA Plant Hardiness zones for Georgia	31
Figure 3.1: (a) Light micrograph of optic tectum and optic lobe (40x) of chicken fed control snail material. (b) Light micrograph of optic tectum and optic lobe (40x) of chicken fed treatment snail material.....	53
Figure 3.2: (a) Light micrograph of cerebellum (40x) of chicken fed control snail material. (b) Light micrograph of cerebellum (40x) of chicken fed treatment snail material	53
Figure 3.3: Potential route of AVM toxin transfer from cyanobacterial colonies to snail kites....	54

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Invasive Apple Snails

Many species of freshwater mollusks introduced outside of their native range become invasive, causing environmental and economic damage to natural ecosystems (Naylor 1996, Pimentel et al. 2000, Matsuzaki et al. 2009, Kirsch and Dzialowski 2012). Exotic invasive mollusks have been linked to whole-ecosystem regime shifts, declines in native species and the transmission of disease (Carlsson et al. 2004, Okamura and Feist 2011, Vrtílek and Reichard 2012). The invasions of some species, i.e. the zebra mussel (*Dreissena polymorpha*), Asian clam (*Corbicula fluminea*), and the New Zealand mudsnail (*Potamopyrgus antipodarum*) (Strayer 1999, Brenneis et al. 2011), have documented detrimental effects on freshwater systems in the United States. Non-native mollusk species that are more recently introduced to the U.S. or restricted to a smaller geographic range such as apple snails (*Pomacea* spp.) and mystery snails (*Cipangopaludina* spp.) are less studied, but may be emerging threats to aquatic ecosystems (Bury et al. 2007, Rawlings et al. 2007, Solomon et al. 2010). Non-native apple snails of the genus *Pomacea* have recently increased their range in North America, and new research efforts are investigating their current and future impacts on freshwater systems.

The island apple snail (*Pomacea insularum*) is native to the Río de la Plata river system in Uruguay and Argentina, and was introduced to the U.S. via aquarium releases at least 20 years ago (Thompson 1985, Howells et al. 2006, Rawlings et al. 2007). Although it has been established in the U.S. for some time, research efforts are now increasing because of its recent

range extension, threat to agriculture and competition with native gastropods such as the Florida apple snail (*P. paludosa*). A related species, the channeled or golden apple snail (*P. canaliculata*), is listed as one of the “100 World’s Worst Invasive Species” and has had serious ecological and economic impacts in rice-growing regions of Southeast Asia and China since its intentional introduction as a potential food source in 1980 (Naylor 1996, Lowe et al. 2000, Carlsson et al. 2004). Due to taxonomic confusion surrounding the family Ampullariidae, channeled apple snails first collected in Florida and Texas in 1978 and 1989, respectively, were identified as *P. canaliculata*, but a genetic study confirmed that southeastern populations of channeled apple snails are in fact *P. insularum* (Thompson 1985, Howells et al. 2006, Rawlings et al. 2007). However, more recent research suggests that *P. canaliculata* is also present in Florida (Bernatis 2008). The presence of both species in the southeast is cause for immediate concern considering the alarming invasion history of *P. canaliculata*. The island apple snail (*P. insularum*), hereafter IAS, is now widespread in Florida and established in at least six other southeastern states (USGS 2012). The rapid spread of this species is receiving attention from researchers and government agencies across the southeastern U.S., due to its voracious appetite for aquatic plants, potential to affect native apple snail populations and ability to harbor parasites and cyanotoxins.

Island apple snails are herbivorous and prefer native North American aquatic vegetation, although they consume both native and exotic plants heavily (Burlakova et al. 2009, Baker et al. 2010). This species also has alarmingly high fecundity: it is estimated that one adult female can produce over 250 viable hatchlings in one reproductive season (April – October in the southeast) at an *average* rate of egg production (~2000 eggs/clutch) and hatching efficiency (~70%) (Barnes et al. 2008). Their appetite and rate of reproduction enable them to remove all aquatic

vegetation from a system shortly after their introduction. Overgrazing by exotic *Pomacea* in shallow lakes and wetlands can cause a shift from a system with good water clarity and plentiful aquatic macrophytes to one of turbid water and frequent algae blooms (Carlsson et al. 2004, Richardson 2007). An herbivore-mediated shift from one stable state to another could result in a fundamental loss of ecosystem integrity and function in wetlands (Scheffer et al. 2001, Carlsson et al. 2004).

The range of the IAS in Florida overlaps with the native Florida apple snail, *P. paludosa*. There is growing concern that the expansion of exotic apple snails in Florida could negatively affect populations of native apple snails (Conner et al. 2008). Compared to the IAS, the Florida apple snail is smaller in size and lays larger eggs in much smaller clutches of only 20 to 30 eggs (Turner 1996, Darby et al. 1997). *P. paludosa* shows a similar preference for aquatic vegetation, but consumes it at a much lower rate than the IAS, suggesting that exotic apple snails may be able to out compete natives for food (Morrison and Hay 2010). Additionally, juvenile Florida apple snails had reduced rates of growth and survival in the presence of IAS in a lab experiment, further evidence that IAS may reduce native snail populations (Conner et al. 2008). Finally, *P. paludosa* is the primary source of food for the Florida snail kite, *Rostrhamus sociabilis plumbeus*, a federally endangered raptor (Sykes 1987). Snail kites frequently prey on IAS where natives are unavailable, but juvenile kites have difficulty handling the larger snails. Consequently, there is an increased handling time for kites feeding on IAS, which can result in a net energy loss (Darby et al. 2007, Cattau et al. 2010).

Another concern associated with the invasion of exotic apple snails is their potential to harbor parasites and cyanobacterial toxins and to transmit human diseases. *Pomacea canaliculata* is a known host of the rat lungworm parasite, *Angiostrongylus cantonensis*, which

has caused outbreaks of eosinophilic meningitis in people who consumed raw or undercooked snails in the Pacific Islands, Southeast Asia and recently China (Yen et al. 1990, Lv et al. 2009). *A. cantonensis* has been found in *P. insularum* specimens collected in New Orleans, Louisiana, but no incidence of human disease associated with *P. insularum* has been reported (Teem and Gutierrez 2008). Many species of freshwater mollusks bioaccumulate cyanobacterial toxins (Negri and Jones 1995, Chen et al. 2004) and there is evidence that Pomacean snails may be able to confer cyanotoxins in freshwater food webs. A study in Mexico identified cylindrospermopsin and paralytic shellfish toxins in the tissues of native Pomacean snails (Berry and Lind 2010). In addition, some species of potentially toxic cyanobacteria grow as epiphytes on aquatic vegetation (Rai et al. 2000, Mohamed and Al Shehri 2010), the preferred food of exotic apple snails. Recently, an undescribed species of epiphytic cyanobacterium was documented on submerged aquatic vegetation in Lake Tohopekaliga, Florida (hereafter Lake Toho) (Wilde, unpubl. data). This species is associated with a neurologic disease that affects bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*) on reservoirs in the southeastern Piedmont called Avian Vacuolar Myelinopathy (AVM) (Wilde et al. 2005, Wiley et al. 2009). If exotic apple snails at Lake Toho can transfer this cyanotoxin to their avian predators, specifically the Florida snail kite, they may pose an additional threat to Florida freshwater ecosystems.

Objectives and Justification

The goal of my research was to document the spread and impacts of the exotic apple snail *P. insularum* in freshwater ecosystems in Georgia and Florida. My specific objectives were to:

1. Document the current distribution of established IAS populations in Georgia.
2. Determine if sensitive habitats in Georgia are vulnerable to invasion by IAS.
3. Determine if IAS, feeding on aquatic vegetation with the specific cyanobacterial epiphyte, can transfer the undescribed cyanotoxin associated with AVM to their avian predators.
4. Evaluate the risk to wild birds, including the endangered Florida snail kite, posed by a novel toxin-transmission pathway.

Although IAS have been established in the U.S. for at least 20 years, there are few published studies on their life history and effects on aquatic habitats. The rapid spread and devastating effects that *P. canaliculata* has had on wetlands in Southeast Asia make the presence of *P. insularum* in the U.S. particularly worrisome. The discovery of exotic apple snail populations in four new states in the past five years warrants further research on their biology and potential to affect sensitive ecosystems in the southeastern U.S.

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CHAPTER 2

THE DISTRIBUTION OF THE EXOTIC APPLE SNAIL *POMACEA INSULARUM* IN
GEORGIA¹

¹ Robertson, S.M., R.S. Haynie, J.E. Byers and S.B. Wilde. To be submitted to *Southeastern Naturalist*

ABSTRACT

The Island Apple Snail, *Pomacea insularum* was introduced to the southeastern United States in the last 25 years from South America via the aquarium pet trade. Its high fecundity and voracious appetite for aquatic plants has allowed it to spread rapidly in Florida, where it is affecting freshwater ecosystems by overgrazing and outcompeting native snail species. A reproducing population of *P. insularum* in Blackshear, Georgia was reported to the Georgia Department of Natural Resources in 2005, and more sightings have been reported across southern Georgia since then. We surveyed locations where *P. insularum* populations have been reported over the past seven years and documented the current distribution of *P. insularum* in Georgia. We collected water quality data at each site and compared it to known tolerance limits for *P. insularum* and other freshwater gastropods. *Pomacea insularum* is established in at least nine locations in Georgia and has the potential for further spread. Although it has probably not reached the northernmost limit of its possible range in Georgia, its distribution will most likely be limited by cold winter temperatures.

INTRODUCTION

Exotic apple snails of the genus *Pomacea* have existed in the United States for at least 50 years and are still expanding their range (Rawlings et al. 2007). Members of this genus are large freshwater gastropods in the family Ampullariidae and are endemic to humid, tropical and subtropical regions of South and Central America, and the Caribbean (Cowie and Silvana 2003). The native range of one species, *P. paludosa*, extends into Florida and Georgia in the U.S. (Thompson 1985). The common name “apple snail,” is given to several Ampullariid genera, and refers to their large, globular shell shape that is sometimes greenish in color, resembling an apple

(Cowie and Silvana 2003). Several members of the family Ampullariidae are popular aquarium pets, and have been introduced outside of their native ranges in many areas from aquarium releases (Perera and Walls 1996, Cowie 2002).

Although the taxonomy of Pomacean snails is not wholly resolved, recent research has confirmed the presence of four exotic species of *Pomacea* in the continental U.S. (Rawlings et al. 2007). Native to Brazil, Argentina and Uruguay, the Island Apple Snail (IAS) *Pomacea insularum*, has invaded freshwater systems in the southeastern U.S. (Rawlings et al. 2007). Exotic Pomacean snails were first reported from Florida in 1978 and identified as *P. canaliculata*, a very morphologically similar species to *P. insularum* that is present in Hawaii, California and Arizona (Thompson 1985, Rawlings et al. 2007). As of 2007, *P. insularum* populations were confirmed in only Florida, Texas and Georgia, but were recently reported in four additional southeastern states: South Carolina, Alabama, Mississippi and Louisiana (Figure 2.1) (Rawlings et al. 2007, USGS 2012).

Several of the general biology and life history traits of Pomacean snails have made them successful invaders of novel habitats. In their native range, *Pomacea* spp. have adapted to wetlands and rivers that dry periodically by employing an amphibious life strategy. They possess both a gill for aquatic respiration and a siphon and lung for aerial respiration and can aestivate in dry conditions for long periods, allowing them to survive in a variety of aquatic environments (Cowie 2002). Some *Pomacea* spp. are highly fecund and can lay hundreds to thousands of eggs in a single clutch deposited on emergent vegetation or fixed structures above the water line (Figure 2.2). *Pomacea canaliculata* lays an average of around 200 eggs per clutch, and *P. insularum* can lay up to >4000 eggs per clutch (Cowie 2002, Teo 2004, Barnes et al. 2008).



Figure 2.1: Current U.S. distribution of *Pomacea insularum*. (USGS Nonindigenous Aquatic Species Database, 2012). Arizona record is a probable misidentification of *P. canaliculata* (Rawlings et al. 2007).

Invasive Pomacean snails can also survive by feeding on a wide variety of both submerged and emergent aquatic plant species, with no preference for native versus exotic invasive aquatic plants (Burlakova et al. 2009b, Baker et al. 2010, Burks et al. 2011).

Invasive *Pomacea* can have profound effects on aquatic ecosystems by overgrazing food resources and competing with native snails. They are voracious herbivores and can rapidly eliminate both native (*Arum*, *Sagittaria*, *Ceratophyllum*, *Panicum*) and nonindigenous (*Hydrilla*, *Egeria*, *Myriophyllum*) aquatic macrophytes from a system shortly after they are introduced (Burlakova et al. 2009a, Morrison and Hay 2011). Since *P. insularum* does not feed preferentially on nonindigenous aquatic macrophytes, an infestation may significantly affect native plant species (Gettys et al. 2008, Burlakova et al. 2009b, Baker et al. 2010). Depletion of

aquatic macrophytes by a novel herbivore can increase available water-column nutrients and cause a system-level shift to turbid water and phytoplankton dominance, resulting in a loss of ecosystem services (Cowie 2002, Carlsson et al. 2004a, Richardson 2007). Additionally, the presence of *P. insularum* in Florida could negatively affect the survival of already declining populations of *P. paludosa*. Reduced growth rates of the native Florida apple snail *P. paludosa* have been observed when high densities of *P. insularum* adults are present in laboratory experiments (Conner et al. 2008).



Figure 2.2: Adult *P. insularum* laying eggs.

A breeding population of *P. insularum* was first discovered in Blackshear, Georgia in 2005 and at that time probably represented the northernmost introduction of exotic apple snails in the U.S. (Chad Sexton, pers. comm.). Only one study has since been published that documented IAS in Georgia (Rawlings et al. 2007) although there are anecdotal reports of IAS populations throughout the southern portion of the state. The recent spread of IAS to neighboring states has resource managers in Georgia concerned that IAS may invade sensitive aquatic habitats such as the Okefenokee National Wildlife Refuge. Several environmental factors influence the distribution of aquatic species. Colder water temperatures are considered the most important factor in limiting the spread of invasive apple snails, and they cannot survive freezing ($<0^{\circ}\text{C}$) (Martin et al. 2001, Ramakrishnan 2007, Matsukura et al. 2009). Water pH, calcium concentration and salinity may also limit the spread of invasive apple snails in the southeast. Calcium concentrations below $\sim 5 \text{ mg l}^{-1}$ in freshwater may inhibit growth, shell hardness and hatchling success in freshwater gastropods and the native *Pomacea paludosa* (Lodge et al. 1987,

Madsen 1987, Watson and Ormerod 2004, Ramakrishnan 2007, Glass and Darby 2009). A Texas study reports the physiological tolerance limits of *P. insularum* adults in the laboratory, where whole sample mortality occurred within 28 days or less (Ramakrishnan 2007, Table 2.1). The objectives of this study were to update information on the current range of IAS in Georgia; collect habitat data in systems that host IAS populations, and use this information to predict further spread within Georgia. We conducted a field survey during the summer of 2011 of known and reported IAS locations and collected water quality and plant community data at each site. We also compared the salinity, pH and temperature limits reported in Ramakrishnan (2007) with *in situ* measurements where IAS is found in Georgia.

Table 2.1: Experimentally determined incipient physiological tolerance limits for adult and juvenile *Pomacea insularum* collected in Texas (from Ramakrishnan 2007). For salinity and pH the ranges of values bracket the median lethal values at 28 days exposure (LD₅₀/28). Temperature limits were statistically calculated from experimental data to yield the temperatures at which 99% mortality occurred in 28 days (LT_{p99}).

Parameter	Lower Limit	Upper Limit
Salinity Tolerance	0.0 ‰	6.8 ‰
pH Tolerance	4.0	10.5
Temperature Tolerance	<15.23°C (59.4°F)	>36.6°C (97.9°F)

METHODS

GIS mapping

Field-derived GPS coordinates were recorded during the summer 2011 field surveys (see methods in *Field survey*) and imported into ArcMap 10 (ESRI 2011). The GPS coordinates were overlaid on maps of Georgia counties and HUC-8 watersheds obtained from a University of Georgia, Warnell School of Forestry and Natural Resources database and the United States Geological Survey. Watersheds where IAS were found in 2011 were identified as “vulnerable watersheds.”

Field survey

The detection probability of this species is highest during egg laying because adults may be seen leaving the water to deposit conspicuous bright-pink eggs on vertical substrate just above the water's surface (Neck and Schultz 1992, Barnes et al. 2008). We conducted field surveys in June to September 2011 to coincide with the adults' emergence and egg laying. During each site assessment we documented the overall conditions of the site and noted IAS presence, measured water quality parameters, and surveyed the aquatic plant community. We performed identical site assessments, described below, at each water body we visited.

Table 2.2: Summer 2011 field sampling locations.

Locale	Water body	Lat/Long	County	Drainage	Year Discovered
St. Simons ¹	Golf course ponds	31.150783 -81.401633	Glynn	Cumberland	2005
St. Mary's ¹	Durango Mill Pond	30.733467 -81.545333	Camden	St. Mary's	2007
St. Mary's ¹	Aquatic Center Pond	30.73881 -81.55098	Camden	St. Mary's	2007
Blackshear ¹	Ponds off Hwy 84 adjacent to Alabama River	31.315233 -82.225583	Pierce	Satilla	2005
Alma ¹	Lake Lure	31.553217 -82.482350	Bacon	Satilla	2007
Jackson, SC ¹	Adjacent to Savannah River	33.275983 -81.833583	Aiken, SC	Middle Savannah	2008
Quitman ¹	Sunset Lake	30.780733 -83.5734	Brooks	Withlacoochee	2007
Albany ³	Retention pond, canal	31.591327 -84.21703	Dougherty	Flint	2010
Thomasville ³	Ochlockonee River mainstem	30.9244 -83.99967	Thomas	Ochlockonee	2010
Lake Seminole ²	Flint River Arm	30.76604 -84.75134	Decatur	Flint	2008

¹(Benson 2012), ²Jack Wingate, ³Dean Barber (GADNR), personal communication

Study sites

We surveyed a total of ten sites that were known or suspected to contain IAS (Table 2.2). Distribution data was collected from the USGS, Nonindigenous Aquatic Species database (USGS 2012) and combined with GADNR data and anecdotal reports made to GADNR and

UGA by local citizens. All sites were surveyed between June and September 2011. A return trip was made to the Jackson, SC Savannah River location in April 2012.

Water quality parameters

Upon arrival at each site, the area was visually surveyed for the presence of IAS and/or egg masses. The overall site and any IAS presence was photo documented and GPS locations were recorded. Water temperature, pH, dissolved oxygen and salinity were measured once (subsurface) at the edge of the water body using a hand-held Hach-Hydromet Quanta multimeter (Hach Company, Loveland, CO). Total hardness (as mg/L CaCO₃) was measured once in each water body using a LaMotte Smart2 colorimeter (LaMotte, Chestertown, MD). Total calcium hardness was converted to calcium concentration (mg/L Ca²⁺). Turbidity was measured using a Hach 2100Q portable turbidimeter. Depth-integrated samples (500 mL) were taken from each site for phytoplankton abundance (Chlorophyll-*a*). Specifically, phytoplankton abundance water samples were collected in triplicate, filtered using a hand vacuum pump over a glass fiber filter, and immediately wrapped in aluminum foil and placed on ice. Filters were stored at -20°C until analysis. Chlorophyll-*a* was extracted from the filter sample with methanol and measured by mass spectrophotometer. Due to a freezer failure in the lab, we are unable to report chlorophyll-*a* measurements for the Albany, Quitman, Ochlockonee River and Lake Seminole surveys. We considered salinity, pH and temperature measurements within the limits reported by Ramakrishnan (2007) (Table 2.1) and Martin et. al (in review) and calcium concentrations for freshwater gastropods reported in the literature (≥ 5 mg/L Ca²⁺) to be “within normal limits” for IAS. Any measurements that fell outside of these limits are noted in the results section for each site.

Aquatic plant community assessment

Presence data was collected for aquatic macrophytes (including submersed, floating, and emergent) and littoral/riparian zone plants at each site. We used a line intercept sampling method and deployed two 50-m transects in a stratified random design around the site (Madsen 1999). At 2-m intervals along each transect, the observer counted presence for all aquatic and littoral/riparian zone plant species, and apple snail egg masses within a one-meter radius of the observer. Plants were identified to genus and to species when possible. For any unknown plants, voucher specimens were collected and identified using a dichotomous key (Radford et al. 1968).

RESULTS AND INDIVIDUAL SITE ASSESSMENTS

*Current distribution of *P. insularum* in Georgia*

We created a GIS-based map of the known *P. insularum* locations in Georgia, and identified watersheds that are vulnerable to further invasions (Figure 2.3). A watershed was considered “vulnerable” if IAS were discovered in a waterbody within that watershed. No IAS were discovered in the Spring Creek or Chattahoochee River watersheds, but these were marked as vulnerable due to the presence of IAS in the Flint River arm of Lake Seminole. During our summer 2011 surveys, we discovered breeding IAS populations (indicated by the presence of egg masses) at nine out of ten locations surveyed (Table 2.3; the two St. Mary’s populations were considered separate locations). We documented established IAS populations in seven small ponds or impoundment systems, two river channels and one large reservoir. We were able to confirm several anecdotal reports of IAS in recently reported locations, including drainage ponds in Albany and in the Flint River Arm of Lake Seminole. No IAS or egg masses were detected in the Savannah River at Jackson, SC, where suspected IAS shells had been reported in 2008.

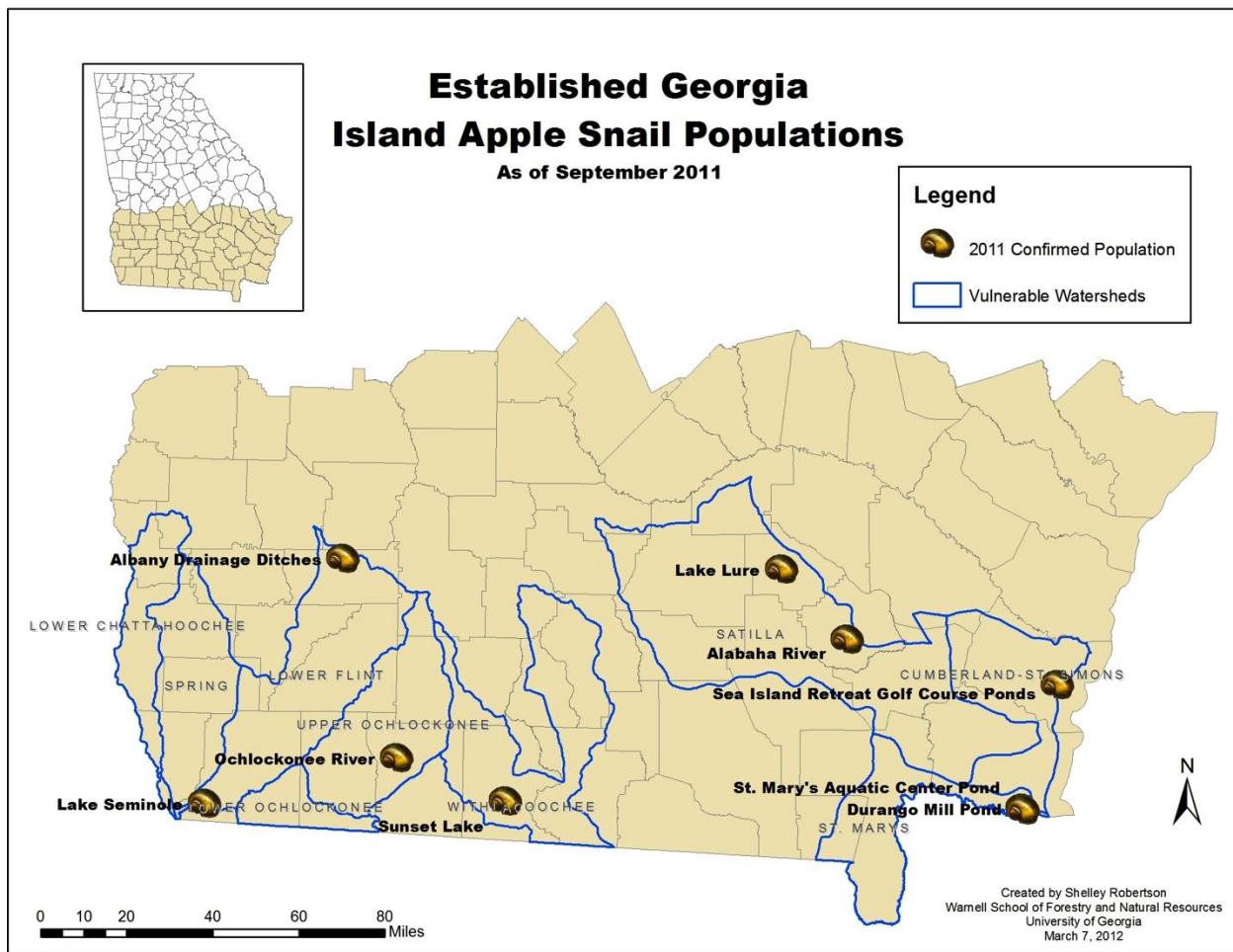


Figure 2.3: 2011 confirmed IAS populations in southern Georgia

Currently, breeding populations of IAS inhabit at least six major river drainages in eight counties in southern Georgia. We discovered egg masses in the main channels of the Alabaha (Satilla drainage), the Flint (Lake Seminole) and the Ochlockonee Rivers.

Table 2.3: Summer 2011 field survey sites and current status of IAS populations

Locale	Water body	Lat/Long	County	Drainage	2011 status
St. Simons	Golf course ponds	31.150783 -81.401633	Glynn	Cumberland	Established
St. Mary's	Durango Mill Pond	30.733467 -81.545333	Camden	St. Mary's	Established
St. Mary's	Aquatic Center Pond	30.73881 -81.55098	Camden	St. Mary's	Established
Blackshear	Alabaha River mainstem	31.315267 -82.225183	Pierce	Satilla	Established
Blackshear	Ponds off Hwy 84 adjacent to Alabaha River	31.315233 -82.225583	Pierce	Satilla	Not detected
Alma	Lake Lure	31.553217 -82.482350	Bacon	Satilla	Established
Jackson, SC	Adjacent to Savannah River	33.275983 -81.833583	Aiken, SC	Middle Savannah	Not detected
Quitman	Sunset Lake	30.780733 -83.5734	Brooks	Withlacoochee	Established
Albany	Retention pond, canal	31.591327 -84.21703	Dougherty	Flint	Established
Thomasville	Ochlockonee River mainstem	30.9244 -83.99967	Thomas	Ochlockonee	Established
Lake Seminole	Flint River Arm	30.76604 -84.75134	Decatur	Flint	Established

1. St. Simons

We detected established IAS populations in two ponds and two water hazard ditches on the Sea Island Retreat Golf Course. These populations were first reported in 2005, and we documented egg masses in four connected water bodies on the course. No egg masses or IAS were detected in the other water hazards on the golf course. Water temperature and pH were within the tolerance range reported by Ramakrishnan (2007) for *P. insularum*. Location 1 had a salinity of 7.6 psu, which exceeds the upper limit of this species reported by Ramakrishnan (2007) (Table 2.1), but is below the reported tolerance limit of juvenile IAS from Mobile, Alabama (<10 psu, Martin & Valentine, in review). At location 1 (pond at 16th hole), 41 egg masses were counted in one 50-m transect.

The Sea Island Retreat Golf Course sites have maintained an established IAS population for at least six years. There have been no reports of IAS beyond the Sea Island Retreat course but

there are several golf courses nearby with water hazards that should be investigated. We did not have permission to access the other courses the day of our survey. Dispersal of snails from these sites will most likely be limited, due to the high salinity of water surrounding St. Simons Island, and will probably not pose a threat to sensitive freshwater bodies. Golf course ponds are often susceptible to algal blooms due to their usual lack of littoral zone vegetation and nutrient inputs from fertilizers used on the greens (Lewitus et al. 2008). Although no algal blooms were detected during our survey, the apple snail grazing of any aquatic plants may increase the susceptibility of the ponds to algae blooms.

2. & 3. St. Mary's

A well-documented population of IAS exists in a detention pond adjacent to the St. Mary's Aquatic Center on Highway 40 just northeast of downtown St. Mary's. This population was first documented in 2007, and has been visited frequently by both UGA and DNR personnel for research collections.



Figure 2.4: IAS egg masses on cattails at St. Mary's Aquatic Center Pond.

We observed abundant adults and egg masses on the date of our survey. We discovered one egg mass in an ephemeral flooded wetland area in the woods approximately 50 meters from the edge of the pond. Empty IAS shells were discovered in the same area until the woods changed to pine forest approximately 65 meters from the pond. The pond was devoid of aquatic macrophytes with the exception of large patches of a species of the macroalga *Chara*, on which snails appeared to be feeding. We recorded 105 egg masses in one 50-m transect that included a

large stand of cattails (Figure 2.4). Fire ants were observed feeding on IAS eggs in the stand of cattails (Figure 2.5).



Figure 2.5: Fire ant predation of IAS eggs at St. Mary's Aquatic Center Pond

IAS did not appear to have spread from this site via the drainage ditches or ephemerally flooded wetlands that surround the pond. Surrounding water bodies are most likely not at risk of an IAS invasion, due to high salinity approaching the St. Mary's River. Although an algae bloom was not detected, this pond has potential to develop algae blooms due to lack of aquatic vegetation and lack of littoral zone vegetation. Since cattails were largely the only substrate available for egg deposition, fire ant predation of snail eggs may slightly suppress the snail population during periods of low water when ants can

access the cattails.

The detention pond adjacent to the site of the former Durango Paper Mill has supported a population of IAS since at least 2007. Upon our visit, we observed numerous empty shells, egg masses, and live adults and juveniles. The pond drains to nearby roadside ditches. We observed empty apple snail shells in a roadside ditch several blocks south and west of the pond. The salinity in the ditch where the last apple snail shell was observed was 27 psu, and increased to 33 psu two blocks south of the last shell. High salinity should prevent IAS from becoming established in any of the nearby roadside ditches. No other populations were discovered outside of the Aquatic Center Pond and the Durango Mill Pond. Other small ponds in St. Mary's may be

vulnerable to invasion by invasive IAS, but the high salinity of tidal creeks approaching the St. Mary's River will most likely prevent establishment of a permanent population.

4. Blackshear

We were unable to survey the 3 private ponds where the initial Satilla watershed IAS introduction occurred; neither the property owner nor caretaker could be reached prior to our survey. This population may have been unintentionally introduced with water hyacinth plants brought to the pond (*Eichhornia crassipes*) (Chad Sexton, pers. comm.). An established population of IAS was also documented downstream from the initial infestation in a small pond adjacent to the Alabaha River and Highway 84 northeast of Blackshear in 2005. However, we were unable to discover evidence of IAS in the pond during our visit. We measured a calcium concentration of 3.6 mg/L and a chlorophyll-*a* concentration of 66.93 ($\mu\text{g l}^{-1}$) in the pond (Table 2.4). No submerged vegetation was apparent in the pond. A short distance (~40m) from the pond is the Alabaha River, where we documented two IAS egg masses on cypress knees in the river ~50 m downstream of the Highway 84 bridge crossing. We measured a pH of 6.5 and a calcium concentration of 3.6 mg/L in the mainstem of the Alabaha (Table 2.4).

The apple snail population in the Blackshear pond may have recently crashed due to overcrowding or unavailability of food. The high chlorophyll-*a* concentration in the pond is indicative of an algal bloom, which could have resulted from over grazing of macrophytes by apple snails. Populations of established invasive species have been known to decline or crash periodically, but rarely disappear (Daniel and Leah 2004). Apple snails were probably able to invade the nearby Alabaha River during a flood. Ca^{2+} here was lower than the 5 mg/L that has been reported as the minimum requirement for freshwater gastropod shell formation and maintenance (Nduku and Harrison 1976, Dussart and Kay 1980, Lodge et al. 1987). One study

on the native Florida apple snail reports that pH may be the more important limiting factor in apple snail distribution and that snails will only be affected below a pH of 6.5 (Glass and Darby 2009). Regardless of less than ideal water quality, IAS are established in the Alabama. Low pH and calcium concentrations may prevent IAS from reaching dense numbers in the Alabama and Satilla, but sloughs and small ponds within the Satilla watershed with sufficient pH are vulnerable to invasion, especially during flooding events.

5. Alma

Lake Lure, a 24-ha reservoir in a residential neighborhood in Alma, has hosted an established population of IAS since at least 2005. Although the infestation of IAS here has been very dense in the past (there have been reports to DNR from residents of snails and egg masses covering their docks), we observed only two egg masses and no adults or empty shells. Calcium concentration in Lake Lure was low, measuring 5.6 mg/L. All other water quality parameters were within normal limits (Table 2.4). The water appeared turbid and floating planktonic algae was visible. The lake was devoid of aquatic vegetation.

The Lake Lure IAS population may have naturally declined due to overcrowding or lack of available food, but does not appear to have completely disappeared and could rebound in the future. Populations of invasive exotic species often experience dramatic collapse, but rarely disappear (Crooks and Soule 1999). If the previously dense population of IAS is responsible for the lack of aquatic vegetation, the IAS population may rebound once the aquatic vegetation community has recovered, following a boom-and-bust cycle that has been observed for invasive species (Simberloff and Gibbons 2004). The calcium concentration was near the lower limit of what is reported as required for freshwater gastropods (5.6 mg/L), but has not affected IAS here

in the past. Lake Lure is on a tributary to Little Hurricane Creek, a tributary the Alabama River and the possible source of the Blackshear IAS population.

6. Jackson, SC

We were unable to verify the Savannah River IAS population during two separate field surveys. We surveyed approximately 12 river miles downstream

of the boat ramp by boat in July 2011 and again in April 2012. The initial report, of

numerous shells (but no live snails or eggs) was made by a USFWS malacologist and therefore had been considered highly reliable (L. Zimmerman, pers. comm.). We did discover shells and live specimens of another large aquatic snail (Figure 2.6) during our second visit in April 2012.

This discovery is most likely a new record of the Japanese mystery snail, *Cipangopaludina japonica*, which is known from several locations in North and South Carolina (Dillon Jr et al. 2006). None of the measured water quality parameters are outside the reported tolerance range of apple snails (Table 2.4). There are many small sloughs that extend from the river in this area with little to no flow and abundant emergent aquatic vegetation such as wild rice, alligator weed and smartweed (Table 2.6).

Although we did not detect any IAS during our survey, this area has a shallow, swamp like habitat with abundant aquatic vegetation ideal for apple snail oviposition and feeding. It is possible that a population of IAS occurred there at one time, but winter temperatures in this area could have prevented the persistence of the population.



Figure 2.6: Japanese mystery snail (*Cipangopaludina japonica*) shells collected at the Savannah River at Jackson, SC in April 2012

7. Quitman

The Sunset Lake IAS population was reported to USGS in 2007. IAS egg masses and shells were present on the date of our survey in September 2011. Sunset Lake is a small pond in a residential neighborhood with little to no littoral vegetation. Water quality parameters were within the reported tolerance limits of apple snails (Table 2.4). Residents reported that the Sunset Lake dam failed approximately two years prior to our visit and was eventually repaired. Below the Sunset Lake dam, ditches eventually connect to Negro Branch, which flows into Withlacoochee Creek. We surveyed Negro Branch at Bethlehem Church Road south of Sunset Lake, which was dry at that time, but found no evidence of apple snails. We also surveyed a nearby pond with suitable IAS habitat at the Quitman Country Club and found no evidence of IAS.

If IAS are not already present in the Withlacoochee watershed in Florida, they could easily spread there from the Sunset Lake population. Further survey of the Quitman area may reveal new IAS populations that could have become established as a result of the dam failure at Sunset Lake.

8. Albany

An IAS sighting in drainage ditches near the city of Albany was reported to DNR by a resident in the area in April 2010. We surveyed this area in September 2011 and observed IAS egg masses and shells in three drainage ponds connected by ditches behind a residential neighborhood. Our survey was preceded by a large rain event, and many egg masses on the edge of the ponds were underwater which may have affected our count of egg masses in the survey transects (Table 2.6). In the largest and southernmost of the 3 ponds, we observed only one IAS shell and no egg masses. Residences and woods with some ephemerally flooded wetlands

surround the pond, but we did not observe evidence of apple snails outside of the ponds and ditches. All water quality parameters were within the reported tolerance limits of apple snails.

To our knowledge, this population is the newest population of IAS in Georgia. Although the Albany drainage ponds are not directly connected to any other water bodies, a flood event could easily spread apple snails to surrounding water bodies, including the Flint River. Water quality in this area is not likely to limit the spread of apple snails. Their presence in the Flint River arm of Lake Seminole (see below) and this new introduction in Albany may make the entire Flint River basin vulnerable to apple snail invasion.

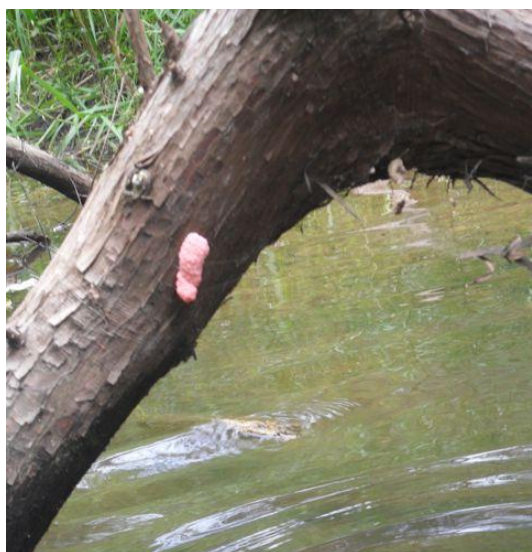


Figure 2.7: IAS egg mass on the main channel of the Ochlockonee River above Thomasville.

9. Thomasville

An apple snail sighting was reported to DNR in the Ochlockonee River at Highway 200 north of Thomasville in 2010. We surveyed a large pond area adjacent to the main channel by kayak, and eventually discovered a total of four IAS egg masses: one in the main channel of the river and three on an island in a slough outside of the main channel (Figure 2.7). Water quality measurements were within the reported tolerance limits for apple snails (Table 2.4).

We did not discover a dense infestation of IAS in the Ochlockonee River, but they are established within the mainstem of the river. The river is mostly shallow and slow flowing, with plenty of substrate for snail oviposition. Although there was no evidence of aquatic plants in the mainstem of the river, oxbow ponds off of the mainstem contained large macrophyte populations. Although they may not reach high densities in the river, wetlands and oxbow lakes

connected to the mainstem Ochlockonee could support IAS populations and be a source of possible dispersal to other areas of the watershed.

10. Lake Seminole

In 2008, USGS received reports of apple snails in Lake Seminole at Wingate's Lodge (30.7671°, -84.7365°). We surveyed the Flint River arm of the lake by airboat on September 9, 2011. We observed very dense infestations of snails at several locations on the south side of this section of the lake (Figure 2.8). Snails appeared to have infested almost every shallow area in between these two locations. In several small shallow coves, hundreds of live adult snails were seen grazing on abundant submerged aquatic plants, such as coontail (*Ceratophyllum demersum*) and hydrilla (*Hydrilla verticillata*). At one location, we counted 375 egg masses in a 50-meter section along a retaining wall (Figure 2.9). Water quality measurements were within the reported tolerance limits of apple snails.

Although Lake Seminole supports a very dense infestation of IAS, it also supports populations of many types of exotic invasive aquatic vegetation and the mat-forming filamentous cyanobacterium, *Lyngbya wollei*. Submerged vegetation remains very dense, even in areas where grazing by IAS was evident. We spoke with a few residents on the lake, who were aware that the IAS were exotic and invasive, but seemed more concerned with aquatic plant and *Lyngbya* infestations than apple snails. Although apple snails may not be significantly altering water or habitat quality in Lake Seminole, their presence there is cause for concern due to their ability to disperse into the Spring Creek and Chattahoochee River watersheds. IAS are already present in most of the Florida watersheds south of the Georgia populations, but snails may easily be dispersed north into unaffected Georgia watersheds. We only surveyed the Flint River arm of

Lake Seminole, and a full survey of the lake would be necessary to determine how far the IAS may have already spread.

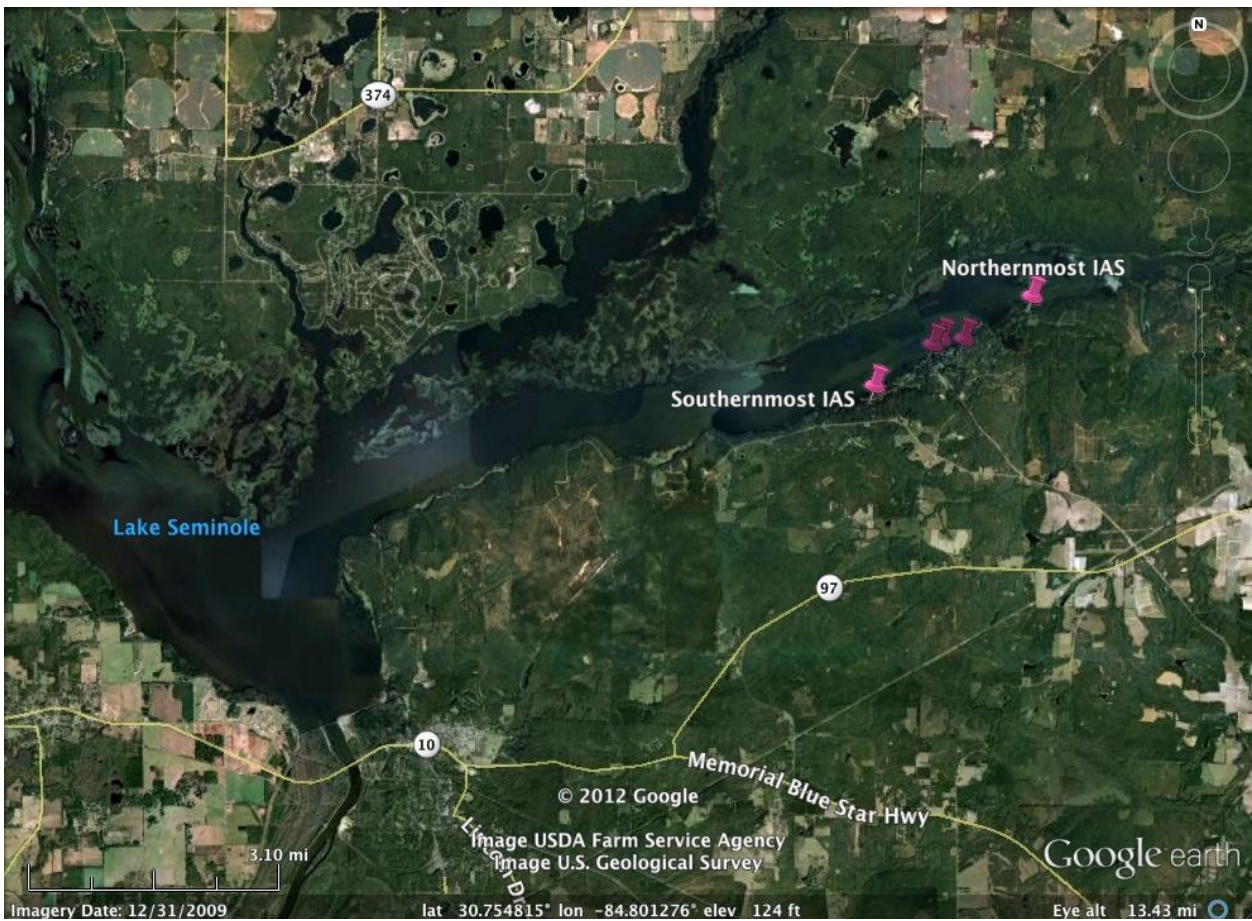


Figure 2.8: Lake Seminole IAS sighting locations. Pink pins denote IAS adult or eggmass sightings. Northern and Southern most sightings are marked.



Figure 2.9: Retaining wall in front of a residence on the southern side of the Flint River Arm of Lake Seminole covered with IAS egg masses on September 9, 2011 (30.77449°, -84.7374°).

Table 2.4: Water quality measurements for all sites visited during 2011 IAS survey. pH, salinity, and temperature measurements within the limits reported by Ramakrishnan (2007) (Table 2.1) and calcium conc. for freshwater gastropods reported in the literature (≥ 5 mg/L Ca^{2+}) to be “within normal limits” for IAS. Any measurements that fell outside of these limits are highlighted in gray.

Locale	Water body	Lat/Long	Snail Presence	Temp (°C)	DO (mg/L)	pH	Salinity (psu)	Turbidity (NTU)	Calcium (mg/L Ca^{2+})	Chl- <i>a</i> ($\mu\text{g l}^{-1}$)
St. Simons	Golf course pond 1	31.150783°, -81.401633°	Egg masses, Shells	31.4	5.2	7.6	7.6	9.0	39	0
	Golf course pond 2	31.160150°, -81.407733°	Egg masses, Adults	28.4	2.2	7.0	1.1	24.3	139	3.74
	Golf course pond 3	31.154567°, -81.403050°	Egg masses	33.3	7.5	8.4	0.2	16.3	33	24.21
St. Mary's	Aquatic Center Detention pond	30.738810°, -81.550980°	Egg masses, Shells, adults	30.1	8.05	8.7	0.38	3.21	41	3.74
	Durango Mill Pond	30.733467°, -81.545°	Egg masses, Shells, adults, juveniles	30.6	6.3	8.6	0.28	12.6	76	17.44
Blackshear	Ponds off Hwy 84 adjacent to Alabaha River	31.315233°, -82.225583°	None detected	30.7	9.42	7.9	0.07	15.9	3.6	66.93
	Alabaha River Mainstem	31.315267°, -82.225183°	Egg masses	27.1	8.02	6.5	0.05	6.35	3.6	Not measured
Alma	Lake Lure	31.553217°, -82.482350°	Egg masses	31.3	7.2	7.8	0.04	6.14	5.6	5.07
Jackson, SC	Adjacent to Savannah River	33.275983, -81.833583	None detected	27.5	8.2	7.4	0.05	13.2	23	9.88
Albany	Retention pond, canal	31.591327°, -84.21703°	Shells, egg masses	25	2.45	7.5	0.09	8.1	40	Not measured
Quitman	Sunset Lake	30.780733°, -83.5734°	Egg masses, Shells	27.9	7.0	8.2	0.04	9.45	12.4	Not measured
Thomasville	Ochlockonee River mainstem	30.9244°, -83.99967°	Egg masses	25.7	8.1	7.9	0.08	31.4	15.6	Not measured
Lake Seminole	Flint River arm 1	30.76604°, -84.75134°	Egg masses, adults	23.4	4.0	7.5	0.06	1.62	20	Not measured
	Flint River arm 2	30.77449°, -84.73269°	Egg masses, adults	23.7	4.6	7.8	0.11	4.2	28	Not measured
	Flint River arm 3	30.7821°, -84.71842°	Egg masses, adults	24.0	4.7	7.4	0.07	2.02	Not measured	Not measured

DISCUSSION

With the exception of the Jackson, SC location, all previously reported IAS populations still persisted at the time of our surveys. These included several populations that have persisted since at 2005, for at least six years. We confirmed the anecdotal reports of IAS at Albany and Lake Seminole and documented egg masses in the mainstem of the Alabama River above Blackshear, which had not been previously reported. As of 2007, the St. Simons, St. Mary's, and Blackshear populations were the only known IAS populations (Rawlings et al. 2007). IAS have become established in at least five additional locations within the past four years.

Temperature is likely the most important factor in limiting the spread IAS since they reportedly cannot survive freezing ($<0^{\circ}\text{C}$) (Martin et al. 2001, Ramakrishnan 2007, Matsukura et al. 2009). The *in vivo* chronic lower thermal tolerance limit of *P. insularum* is 15.23°C , with 100% sample mortality occurring in 25 days. However, it took eight days to reach 100% sample mortality when the water temperature was held at 2°C (Table 2.1, Ramakrishnan 2007). The acute lower thermal tolerance limit is therefore probably much lower than 15.23°C and is likely an important factor in determining their potential distribution in the southeastern U.S. (Karatayev et al. 2009). In Albany, the northernmost IAS population we discovered, the average low temperature in January is $\sim 2^{\circ}\text{C}$, while the average January high temperature is $\sim 16^{\circ}\text{C}$ (National Oceanic and Atmospheric Association 2012). To estimate temperature zones that IAS may survive in, we used the USDA's Plant Hardiness Zones, which are based on average annual extreme minimum air temperatures. The USDA zones were updated in 2012 to account for rising average air temperatures due to climate change. Climate change may alter the distribution of invasive species by allowing temperature-sensitive species such as IAS to expand their range (Hellmann et al. 2008). In the southeastern U.S., IAS have become established through Zone 8b

(USGS 2012). Zone 8b in Georgia extends further north than the current range of IAS and includes the entire coastline (Figure 2.10). This suggests that IAS may be able to overwinter further north than their current distribution, and more northern water bodies may be

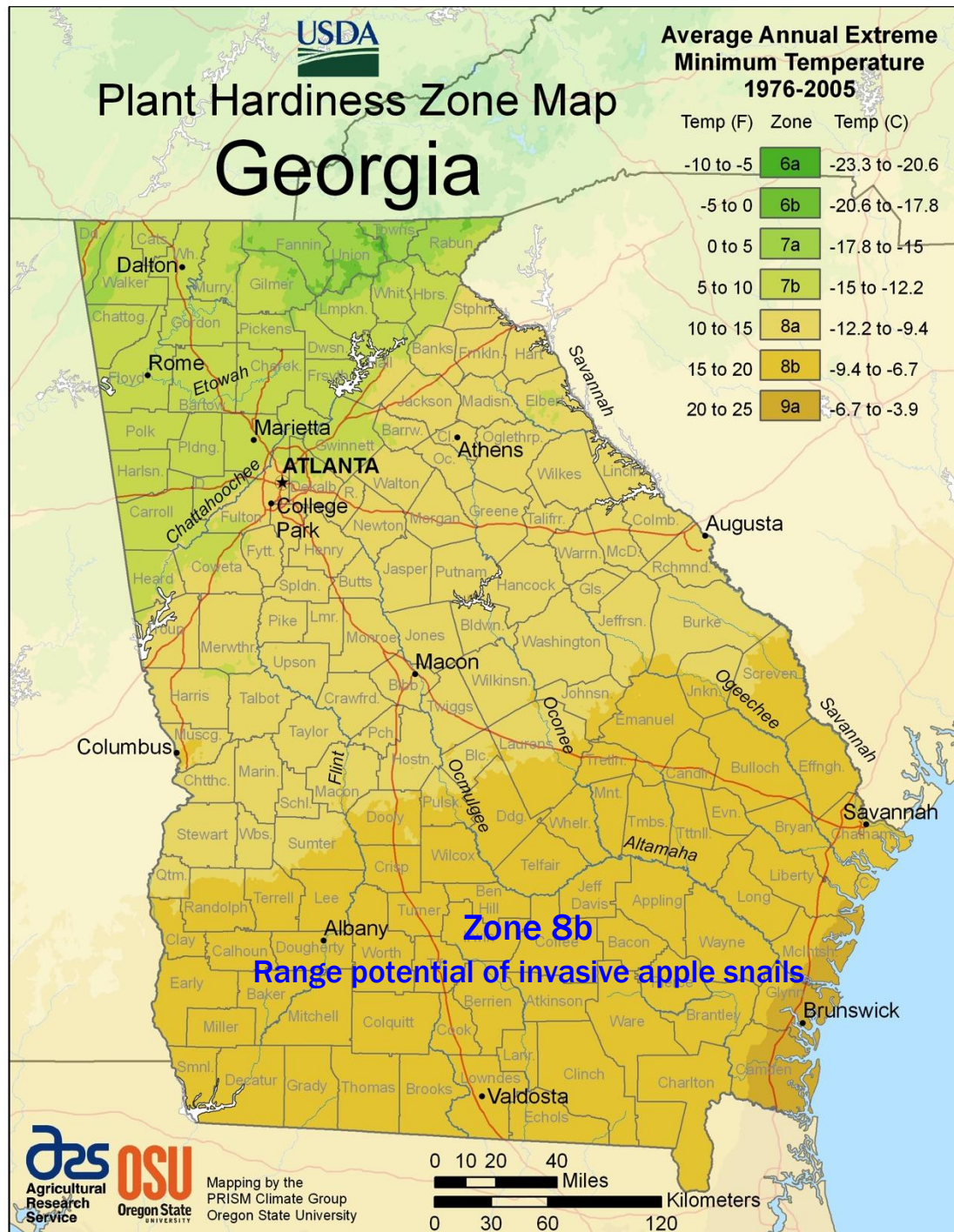


Figure 2.10: USDA Plant Hardiness zones for Georgia.

vulnerable to invasion. Disturbingly, increased cold hardiness has been observed in *P. canaliculata* specimens acclimated to colder temperatures in the lab, possibly allowing them to expand their range (Wada and Matsukura 2011). However, it is unlikely that IAS will become established in northern Georgia where winter temperatures frequently drop below freezing.

We documented IAS egg masses in one pond at the St. Simons golf course where salinity measured 0.8 ‰ above the tolerance limit reported for this species by Ramakrishnan (2007). However, *P. insularum* tolerated salinities above that limit (6.8 ‰) for a short time, and 100% mortality occurred at 13.6 ‰ (Table 2.1, Ramakrishnan 2007). A Mobile, AL study on IAS saw 50% survival of juveniles at 10 and 15 psu, but did not measure growth of juveniles at 15 psu, suggesting that IAS can tolerate fluctuating salinity of tidal creeks (Martin & Valentine, in review). Although high salinity is expected to limit the spread of IAS, they have successfully invaded coastal wetlands in Alabama, and may affect wetland plant communities in lower salinity estuaries surrounding St. Simons Island and St. Mary's (Martin et. al, in review).

Studies on calcium requirements for freshwater gastropods are limited, and there is only one study for species of the genus *Pomacea* (Lodge et al. 1987, Madsen 1987, Watson and Ormerod 2004, Glass and Darby 2009). These studies report that freshwater gastropods require at least 5 mg/L of calcium measured as Ca^{2+} for shell formation, and that damage or death occurs around 2-5 mg/L (Lodge et al. 1987, Madsen 1987, Watson and Ormerod 2004). IAS occur in two water bodies where calcium concentrations were near or below this limit: the Alabama River (3.6 mg/L) and Lake Lure (5.6 mg/L). Since we only documented egg masses in the Alabama River, it is possible that mature adults that dispersed from other locations are able to survive in the river but that the calcium concentration is not sufficient for juveniles to form and maintain shells. However, the presence of egg-laying adults in the Alabama increases the vulnerability of

surrounding water bodies with sufficient calcium and pH to an IAS invasion. One study on the native Florida apple snail *P. paludosa* suggests that pH may be the more important factor in limiting apple snail distribution, and that snails will only be negatively affected below a pH of 6.5 (Glass and Darby 2009). However, Ramakrishnan (2007) did not document any mortality in 28 days in snails exposed to a pH of 4.0. Although the pH in blackwater rivers like the Satilla and Alabaha can often be <6, IAS may survive in the Satilla until salinity increases closer to the mouth of the river.

In their current range in southern Georgia, where cold temperatures and high salinity do not limit their distribution, IAS may have the potential to become established in any water body with sufficient pH and food supply. Their current distribution in Georgia, however, probably does not reflect habitat requirements, but simply the rate of introduction and spread. At least four new documentations of IAS have occurred in the past five years; whether these occurred via independent introductions or dispersal events is unknown. Most introductions of IAS in the U.S. are believed to be the result of aquarium releases, but there is likely some mechanism that is resulting in dispersal and secondary introductions of apple snails (Howells et al. 2006). There are reports of juvenile snails being dispersed from plant nurseries along with ornamental aquatic plants (Howells et. al 2006, R. Haynie, pers. comm). Further complicating eradication efforts, IAS have been observed filling their body cavities with air and floating downstream after pesticide applications (Martin et. al, in review), possibly allowing them to invade downstream habitats. In addition, IAS can survive emersion for up to 308 days, and may be able to be inadvertently dispersed by recreational boaters or migratory birds (Buchan and Padilla 1999, Ramakrishnan 2007).

Dense IAS infestations can have significant impacts on wetland plant communities due to grazing activities (Carlsson et al. 2004b, Fang et al. 2010). This raises concern for unique wetland habitats in southern Georgia such as the Okefenokee Swamp. The pH in the Okefenokee is frequently <4.0, which is the lethal lower limit for *P. insularum*. In addition, gastropods of any kind are very rarely found in the Okefenokee (Kratzer and Batzer 2007), and it is therefore unlikely that IAS could be successful in becoming established there. Isolated populations of vulnerable or rare species such as the Bluenose shiner (*Pteronotropis welaka*) in Spring Creek that depend on aquatic plant communities could be negatively affected if IAS invaded their unique habitats (Madsen 1999, Albanese et al. 2007, Rosenfeld and Jones 2010). The dense population of IAS that we documented in Lake Seminole and the population in Albany are of particular concern. Both populations are located in the Flint River basin, which has an estimated 412,000 total acres of wetlands (Georgia River Network 2012). Much of the Lower Flint River Basin lies over the Floridan Aquifer, which seeps groundwater into dozens of natural springs along the river. These springs remain a constant temperature of around 68°F year round, and could provide a refuge from cold temperatures for IAS if they expanded their range in the Flint Basin (Hicks and Opsahl 2002). The presence of IAS in Lake Seminole also makes the Chattahoochee River basin susceptible to invasion.

Once IAS become established in a water body, they are very difficult to remove. The only chemicals that are effective molluscicides labeled for aquatic use in the U.S. are copper sulfate and chelated copper. As of 2011, a special local need permit was issued in Georgia for use of a copper carbonate product from SePro Corporation, Natrix™. This product is available and labeled “for use to control invasive/exotic aquatic mussels, snails oysters, or clams.” We know of no reports of elimination of IAS using copper compounds, but some reduction has been

accomplished in Mobile, Alabama and in Tallahassee, Florida (Martin et. al, in review; J. Van Dyke, pers. comm). Hand-picking of egg masses and snails achieved temporary suppression of IAS in the St. John's River in Florida (C. Bedard, pers. comm.). Trapping and removal of IAS has also been successful at controlling IAS in small ponds in Florida (J. Van Dyke, pers. comm.) There is also extensive research on possible biological control methods of the related species *P. canaliculata* from rice paddies in southeast Asia (Dai et al. 2011, Massaguni and Latip 2011).

CONCLUSIONS

Future monitoring should be continued around all sites we visited during these surveys, but in recognition of time and resource constraints, priority should be given to locations based on water body type, density of snails and relative risk to nearby sensitive habitat (Table 2.4). Due to the ideal potential habitat for IAS within the Flint River watershed, the Lake Seminole and Albany areas should be given the highest priority for continued monitoring. The Spring Creek and Chattahoochee River sections of Lake Seminole should be surveyed during the next egg-laying season (approx. May-September) to discover if IAS have extended their range beyond the Flint River arm. The Ochlockonee River area should also be given priority for continued monitoring, since this is a recently discovered population, and it is unknown how far IAS may have spread within the watershed in Georgia. IAS are already present in the Lower Ochlockonee river watershed near Tallahassee, Florida (USGS 2012). Managers should consider treatment or removal of IAS from small ponds such as those in St. Mary's, Quitman, Blackshear and Albany to prevent further spread of this species from those sites. Public awareness of the presence of IAS in Georgia could also help to prevent further spread and identify new infestations. Posting of signs or brochures with pictures of IAS and contact information for the public notify Georgia

DNR or USGS's Nonindigenous Aquatic Species database would involve the public in identification of any new infestations of IAS.

Table 2.5 presents a ranking of our priority for monitoring for each location visited during the 2011 surveys. A ranking of "1" is the highest priority monitoring site. Each site received a ranking of very high, high, medium or low for the relative density of IAS that was observed and of the habitat suitability at each site based on water quality and aquatic plant community assessment results (Tables 2.4, 2.6).

Table 2.5: Monitoring recommendations based on results of 2011 IAS survey

Site	St. Simons golf course	St. Mary's Aquatic Ctr	St. Mary's paper mill	Alabaha River	Lake Lure, Alma	Savannah River	Albany	Sunset Lake, Quitman	Ochlockonee River	Lake Seminole
River Basin	Cumberland- St. Simons	St. Mary's	St. Mary's	Satilla	Satilla	Savannah	Flint	Withlacoochee	Ochlockonee	Flint
Water body type	Ponds	Pond	Pond	River channel	Small impoundment	River Channel	Ponds, ditches	Pond	River Channel	Large Reservoir
Total egg masses counted	54	113	32	2	1	0	1	2	4	194
Relative Density	High	High	High	Low	Low	None detected	Medium	Medium	Low	Very high
Habitat Suitability	High	High	High	Low	Medium	High	High	High	Medium	High
Monitoring Priority Rank	9	8	8	5	6	4	2	7	3	1

Table 2.6: Aquatic plant community assessment data from 2011 surveys. Presence data was collected for aquatic macrophytes (including submersed, floating, and emergent) and littoral zone plants in two 50-m line transects at each site. At 2-m intervals along each transect, the observer counted presence for all aquatic and riparian zone plant species, and apple snail egg masses within a one-meter radius of the observer.

Site	St. Simons golf course	St. Mary's Aquatic Ctr pond	St. Mary's paper mill pond	Blackshear Pond	Lake Lure, Alma
Total egg masses counted	54	113	32	0	1
Number of species	15	9	5	9	8
Submerged	<i>Naiad sp.</i>				<i>Naiad sp.</i>
Floating	<i>Azolla caroliniana</i>				
Shoreline and wetland	<i>Alternanthera philoxeroides</i> <i>Hydrocotyle umbellata</i> <i>Pontederia cordata</i> <i>Panicum repens</i> <i>Polygonum densiflorum</i> <i>Carex sp.</i> <i>Leersia sp.</i> <i>Scirpus sp.</i> <i>Micranthemum umbrosum</i> <i>Cyperus sp.</i> <i>Eleocharis sp.</i> <i>Althaea officinalis</i>	<i>Hydrocotyle umbellata</i> <i>Typha latifolia</i> <i>Alternanthera philoxeroides</i> <i>Panicum repens</i> <i>Leersia sp.</i> <i>Cyperus sp.</i> <i>Carex sp.</i> <i>Scirpus sp.</i>	<i>Typha latifolia</i> <i>Hydrocotyle umbellata</i> <i>Eleocharis sp.</i> <i>Salix caroliniana</i> <i>Morella cerifera</i>	<i>Alternanthera philoxeroides</i> <i>Micranthemum umbrosum</i> <i>Taxodium distichum</i> <i>Polygonum densiflorum</i> <i>Cephalanthus occidentalis</i> <i>Scirpus sp.</i> <i>Eleocharis sp.</i> <i>Carex sp.</i>	<i>Hydrocotyle umbellata</i> <i>Alternanthera philoxeroides</i> <i>Typha latifolia</i> <i>Panicum hemitomon</i> <i>Eleocharis sp.</i> <i>Polygonum densiflorum</i> <i>Scirpus sp.</i>
Algae	<i>Pithophora sp.</i>	<i>Chara sp.</i>		<i>Lyngbya sp.</i>	
Site	Albany drainage ponds	Sunset Lake, Quitman	Ochlockonee River	Lake Seminole, Flint River	
Total egg masses counted	1	2	4	194	
Number of plant species	9	3	3	15	
Submerged				<i>Cabomba caroliniana</i> <i>Ceratophyllum demersum</i> <i>Hydrilla verticillata</i> <i>Potomageton illinoensis</i>	
Floating				<i>Limnium spongia</i>	
Shoreline and wetland	<i>Alternanthera philoxeroides</i> <i>Hydrocotyle umbellata</i> <i>Sagittaria latifolia</i> <i>Typha latifolia</i> <i>Ludwigia alterniflora</i> <i>Peltandra virginica</i> <i>Panicum repens</i> <i>Carex sp.</i> <i>Polygonum densiflorum</i>	<i>Alternanthera philoxeroides</i> <i>Sagittaria latifolia</i> <i>Panicum hemitomon</i>	<i>Zizaniopsis aquatica</i> <i>Salix caroliniana</i> <i>Taxodium distichum</i>	<i>Alternanthera philoxeroides</i> <i>Ludwigia alterniflora</i> <i>Nuphar luteum</i> <i>Sagittaria latifolia</i> <i>Carex sp.</i> <i>Leersia sp.</i> <i>Panicum repens</i> <i>Salix caroliniana</i> <i>Taxodium distichum</i>	
Algae				<i>Lyngbya sp.</i>	

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CHAPTER 3

ALTERNATE FOOD-CHAIN TRANSFER OF THE BIOTOXIN LINKED TO AVIAN VACUOLAR MYELINOPATHY (AVM)²

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ABSTRACT

Avian Vacuolar Myelinopathy (AVM) is a neurologic disease that causes yearly mortality events of bald eagles (*Haliaeetus leucocephalus*) and American coots (*Fulica americana*) at reservoirs and small impoundments in the southeastern United States. Since 1994, AVM has caused the deaths of over 150 bald eagles and thousands of American coots, and has been identified in several other species of wild birds. Previous studies link AVM to an uncharacterized biotoxin that can transfer up the food chain. The toxin is transferred trophically when water birds that consume submerged aquatic vegetation and a certain species of epiphytic cyanobacterium develop AVM and pass the toxin to their avian predators. This previously undescribed cyanobacterium (UCB) is in the Order Stigonematales and grows epiphytically on submerged aquatic vegetation in all reservoirs where AVM deaths have occurred. The UCB was recently identified growing abundantly on SAV in Lake Tohopekaliga (Toho) in central Florida. Toho supports a breeding population of a federally endangered raptor, the Florida snail kite (*Rostrhamus sociabilis*), and a dense infestation of an exotic herbivorous aquatic snail, the Island apple snail (*Pomacea insularum*), a primary source of food for the resident snail kites. This study investigated the potential for AVM transmission in a new food chain: from apple snails fed SAV-UCB material to the Florida snail kite. In a 14-day laboratory feeding study, apple snails that ate SAV-UCB material were fed to domestic chickens (*Gallus gallus domesticus*), which were used as a surrogate host for snail kites. A control group of chickens were fed apple snails that ate SAV without the UCB. Only the chickens in the treatment group (3/5) displayed clinical signs and all five developed characteristic brain lesions unique to AVM. This is the first study to demonstrate transfer of the AVM toxin to birds through an invertebrate. The presence of the

UCB in Lake Toho may present significant risk to the already diminished population of endangered Florida snail kites.

INTRODUCTION

Avian Vacuolar Myelinopathy (AVM) is a neurologic disease that affects several species of waterbirds and their avian predators associated with reservoirs in the southeastern United States. Since the initial epizootic event at DeGray Lake, Arkansas in the winter of 1994-1995, AVM has been responsible for the deaths of over 150 bald eagles (*Haliaeetus leucocephalus*) and thousands of American coots (*Fulica americana*) in five southeastern states. Several other species are known to be susceptible to AVM including mallards (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*), buffleheads (*Bucephala albeola*) Canada geese (*Branta canadensis*), great horned owls (*Bubo virginianus*) and killdeer (*Charadrius vociferus*) (Rocke et al. 2002, Augspurger et al. 2003, Fischer et al. 2003).

Birds affected by AVM become uncoordinated, exhibit limb paresis and lose their righting reflex (Thomas et al. 1998). The characteristic loss in motor coordination can cause death in affected birds that become unable to swim, fly or obtain food. These behaviors are consistent with damage to the central nervous system, where the only microscopic abnormality apparent in diseased birds is found. Neurospinal damage manifests as vacuolation of white matter in the brain and spinal cord, and is usually most severe in the optic lobe (Thomas et al. 1998). The microscopic vacuoles, specifically referred to as intramyelinic edema, are formed from the splitting and swelling of the myelin sheath of neural cells (Thomas et al. 1998). Initial field and laboratory studies examined the environmental characteristics of water bodies where AVM affected birds have been collected, but failed to detect consistent or significant amounts of any anthropogenic chemical or toxicant associated with water, sediments, invertebrates or

vegetation (Thomas et al. 1998, Dodder et al. 2003, Rocke et al. 2005). AVM is site-specific and diseased birds are observed and collected during the winter months, suggesting that the toxin is available seasonally (Rocke et al. 2002, Augspurger et al. 2003, Fischer et al. 2006). Red-tailed hawks and chickens that were fed tissues of affected coots in laboratory studies developed AVM lesions, indicating a food-chain transfer of the AVM toxin (Fischer et al. 2003, Lewis-Weis et al. 2004). Additionally, one study found evidence that birds feeding only on the gastrointestinal tract of diseased birds caused AVM, but feeding on muscle or nervous tissue did not (Lewis-Weis et al. 2004). Subsequent feeding studies have implicated a toxin associated with submerged aquatic vegetation growing in water bodies where AVM-positive birds have been diagnosed (Birrenkott et al. 2004, Lewis-Weis et al. 2004, Rocke et al. 2005).

Surveys of environmental parameters revealed one marked commonality among AVM sites. All sites are dominated by dense infestations of one of three types of invasive exotic SAV: hydrilla (*Hydrilla verticillata*), Brazilian elodea (*Egeria densa*) and Eurasian watermilfoil (*Myriophyllum spicatum*) (Wilde et al. 2005). Additionally, SAV in each site serves as a substrate for a previously undescribed cyanobacterium in the Order Stigonematales. This species (hereafter referred to as UCB) dominates the epiphyte community on the leaves of SAV in AVM sites, but is rare or not present in sites where AVM deaths have not occurred (Wilde et al. 2005, Williams et al. 2007). Recent studies have implicated a neurotoxin produced by the UCB as the probable causative agent of AVM (Wiley et al. 2007, Wiley et al. 2009). Indeed, the working hypothesis of all ongoing AVM investigations is that waterbirds develop AVM by ingesting SAV containing the UCB either directly or via predation of other birds that have recently fed on SAV and the UCB.

It is unknown if fish, reptiles or invertebrates that feed on SAV in AVM sites are affected by or capable of transmitting the AVM toxin to their predators. In a field and laboratory study, triploid Chinese grass carp (*Ctenopharyngodon idella*), which are frequently used as a biological control agent for exotic SAV, were fed hydrilla from an AVM site. The grass carp developed suspicious brain lesions, but chickens that consumed those grass carp tissues in the lab did not develop AVM (Haynie 2008). No studies have investigated the possible transfer of the AVM toxin to birds through herbivorous invertebrates. Aquatic snails of the genus *Pomacea* have been shown to bioaccumulate cyanobacterial toxins, but the transfer of these toxins to other wildlife has not been studied (Berry and Lind 2010).

In 2009, researchers identified colonies of the UCB growing on hydrilla in Lake Tohopekaliga (Toho), in Kissimmee, Florida, and confirmed its identity with modifications to a previously validated PCR protocol (Williams et al. 2007). A 2007 survey of cyanobacterial epiphytes in 47 Florida lakes that included Toho, the UCB was found only in Lake Huntley, a small lake approximately 100 miles south of Toho (Williams Jr et al. 2009). The discovery of the UCB on Toho hydrilla in 2009 may indicate that its growth has increased to detectable levels. In fact, during a more recent survey of Toho hydrilla, the UCB was documented from 15 of 30 sites throughout the lake (Wilde unpubl. data). Toho supports a dense infestations of both hydrilla and an exotic herbivorous snail, the Island apple snail (*Pomacea insularum*), which is known to consume large amounts of hydrilla (Baker et al. 2010). Toho also supports a nesting population of the federally endangered Florida snail kite (*Rostrhamus sociabilis*). Less than 400 breeding pairs of snail kites remain in Florida, and a large percentage of the remaining birds nest at Toho (David et al. 2010, Bowling et al. 2012). The Florida snail kite feeds almost exclusively on the native Florida apple snail, *P. paludosa*, but readily consumes the larger exotic *P. insularum*

where native snails are unavailable (Sykes 1987, Darby et al. 2007, Cattau et al. 2010). Although AVM has never been documented at Toho, the presence of the UCB associated with AVM there may pose another threat to snail kites if exotic apple snails are capable of transferring the AVM toxin to their predators.

Two laboratory studies have confirmed that the AVM toxin can be transferred up the food chain from herbivorous waterbirds to predatory raptors (Fischer et al. 2003, Lewis-Weis et al. 2004). Because the AVM toxin is uncharacterized, an avian bioassay is the only available method to illustrate toxicity of SAV-UCB material. Through a series of laboratory feeding trials, this study tested the hypothesis that the AVM toxin can be acquired via an alternate food web: from herbivorous island apple snails to their avian predators. An initial feeding trial using only hydrilla with or without the associated UCB was first necessary to ensure that the AVM toxin was present in the treatment hydrilla collection before proceeding with the subsequent snail tissue feeding trial. We then investigated if island apple snails feeding on hydrilla and the UCB that was confirmed to contain toxin can transfer the AVM toxin to predatory birds. The already imperiled Florida snail kite may be at risk of developing a fatal neurologic disease if this toxin-transfer pathway is valid and the AVM toxin exists in the snail kites' increasingly limited habitat.

METHODS

SAV collection and screening

Although other species of native and invasive SAV can host the UCB, we used hydrilla (*Hydrilla verticillata*) because treatment and control reservoirs containing hydrilla were within a reasonable driving distance. Hydrilla is also the dominant vegetation in all sites where AVM bird deaths have been documented since 2000 (Wilde et al. 2005). "Treatment hydrilla" was

collected on December 8, 2010 from J. Strom Thurmond Reservoir (JSTL), SC (33.706112, -82.343082), a 28,700-ha reservoir on the Savannah River with a history of yearly AVM epornitics since 1998 (Fischer et al. 2006, SCWDS unpubl. data). “Control hydrilla” was collected from Lake Hatchineha, FL (28.021663, -81.405260), a 2,697-ha lake where no AVM deaths have ever been documented. Hydrilla from both sites was collected at depths of 0-1m using a throw rake and immediately frozen at -20°C for use in the snail and avian feeding trials. Random samples of hydrilla leaflets from each collection site were examined under light microscopy for the presence of UCB colonies to confirm that treatment hydrilla contained the UCB and that control hydrilla did not. Subsamples of hydrilla to be used in the feeding studies from both sites were then screened with a PCR probe recently modified from a previously published method for detecting the UCB (Williams et. al 2007, Wilde, unpubl. data).

Snail feeding trial

Approximately 150 adult island apple snails (*Pomacea insularum*) were collected from a small pond in St. Mary’s, Georgia (30.7335, -81.54533) and transported to the UGA Whitehall Fisheries Laboratory, Athens, GA in plastic bins filled with continuously aerated water. Snails were divided into treatment and control groups of equal number (~75) and held separately in large tanks filled with dechlorinated tap water with continuous aeration. Water changes (50 to 75% exchange) were performed daily in order to maximize the amount of vegetation the snails could consume while not compromising water quality. During water changes solid wastes were siphoned from the bottom of the tanks, collected over a screen and autoclaved before disposal. The snails received a diet of exclusively treatment or control hydrilla over a 7-day feeding period, to ensure that the snails retained only hydrilla in their gastrointestinal tracts. After the 7-day feeding period the snail tissues were coarsely chopped, frozen and lyophilized to facilitate

delivery and increase palatability in the chicken feeding trial. The uncharacterized AVM neurotoxin has been previously shown to be stable and active after freezing and lyophilization (Wiley et al. 2008).

Avian feeding trials

We conducted two laboratory feeding trials, with hydrilla or snail tissues as dietary treatments, using 4-6 week old specific pathogen-free (SPF) Leghorn chicks at UGA's Poultry Research Diagnostic Center (PDRC). Chickens have been previously validated as an appropriate laboratory surrogate for AVM feeding studies (Lewis-Weis et al. 2004). Chicks in both trials were randomly divided into control (n=5) and treatment (n=5) groups and housed in individual isolation units (Horsfal units), to prevent exposure to exogenous toxicants or stressors and to monitor diet consumption. At the conclusion of each trial, all remaining birds were euthanized and necropsied and the tissues were prepared for AVM diagnosis as described below.

Hydrilla-UCB dietary treatment trial

Ten chicks were used in this trial, begun in June 2011. Each chick received a diet of hydrilla and commercial poultry feed for 24 days. The birds received approximately 35-40 mg hydrilla per g of body weight (bw) per day in addition to 30 mg/g bw commercial poultry diet per day (Table 3.1). Commercial poultry feed diets were based on recommendations made by animal care professionals at PDRC. Treatment diet measurements were based on previous feeding studies (Lewis-Weis et al. 2004, Wiley et al. 2008). This initial trial was necessary to ensure the AVM toxin is present in the collection before proceeding with the snail feeding trial (Birrenkott et al. 2004; Wilde et al. 2005; Wiley et al. 2007).

Snail tissue dietary treatment trial

The snail tissue trial began in September 2011. Ten chicks received a diet of snail tissue and commercial poultry feed for 14 days. The birds received approximately 20 mg/g bw of either control or treatment snail tissue per day in addition to 30mg/g bw commercial poultry feed per day (Table 3.1).

Table 3.1: Groups and diets of chickens in laboratory feeding trials

Group	Number	Diet	Duration
Control hydrilla	5	35-40 mg/g bw AVM – hydrilla/UCB	24 d
Treatment hydrilla	5	35-40 mg/g bw AVM+ hydrilla/UCB	24 d
Control snail tissue	5	20 mg/g bw AVM – snail tissue	14 d
Treatment snail tissue	5	20 mg/g bw AVM+ snail tissue	14 d

Animal care and necropsy

Birds were monitored twice daily for clinical signs of AVM manifested as ataxia (difficulty walking, standing, loss of balance, limb paresis), wing and/or head droop, and any other signs of neurologic impairment. On day 22 of the hydrilla trial, one treatment chicken displayed clinical signs of AVM and was euthanized and necropsied. All other chickens were euthanized and necropsied on day 24 of the hydrilla trial and on day 14 of the snail trial. At the conclusion of each chicken feeding trial, birds were euthanized by CO₂ asphyxiation followed by cervical dislocation. Upon necropsy, brain tissues were removed and preserved in 10% neutral buffered formalin. Brain tissues were embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Slides of brain sections were examined under light microscopy for the presence of AVM lesions by Dr. Susan Williams (UGA-PDRC) and Dr. John Fischer confirmed AVM diagnosis (UGA-Southeastern Cooperative Wildlife Disease Study).

RESULTS

Hydrilla-UCB dietary treatment trial

Two of five (2/5) treatment chickens in the hydrilla study displayed clinical signs of AVM before the conclusion of the study. One chicken appeared lethargic on day 21 and on day 22 displayed unsteadiness in gait and difficulty standing. This chicken was euthanized and necropsied the same afternoon. A second chicken displayed mild ataxia beginning on day 22 and by day 24 was very unsteady upon standing and was unable to hold its tail erect. The remaining treatment chickens were euthanized and necropsied on day 24. Vacuolar lesions unique to AVM were present in the optic tectum, brain stem and cerebellum in all five (5/5) chickens in the treatment group. Three treatment chickens (3/5) also had mild vacuolar lesions in the cerebrum. No vacuolar lesions were present in the brain tissue of any of the chickens (0/5) in the control group (Table 3.2).

Snail tissue dietary treatment trial

Three of five (3/5) treatment chickens that ate treatment snail material displayed clinical signs of AVM including limb paresis before the conclusion of the 14 day study. From days 4 to 14, three chickens displayed varying signs of ataxia. Two chickens were frequently sedentary and displayed unsteadiness in gait upon standing. On day 14, both exhibited unsteadiness in gait and rested on the metatarsi when attempting to stand. A third chicken displayed unsteady gait and drooping wings from days 4 to 14 of the trial. All chickens were sacrificed and necropsied on day 14. Vacuolar lesions were present in the optic tectum, brainstem, and cerebrum of all five (5/5) chickens in the treatment group (Figure 3.1). Four of five (4/5) chickens also had lesions in the cerebellar tissue, but the cerebellar tissue of the fifth chicken was not examined due to a processing error (Figure 3.2). One chicken from the control group had a few medium sized

vacuoles in the white matter of the cerebellum (1/5). No vacuolar lesions were present in the brain tissue of any of the other chickens from the control group (Table 3.2).

Table 3.2: Results of histological analysis of brain section slides from feeding trials.

Group	Total (n)	Clinical signs	AVM-positive	Optic tectum lesions	Brain stem lesions	Cerebrum lesions	Cerebellum lesions
Control hydrilla	5	0	0	0	0	0	0
Treatment hydrilla	5	2	5	5	5	3	5
Control snail tissue	5	0	0	0	0	0	1
Treatment snail tissue	5	3	5	5	5	5	4

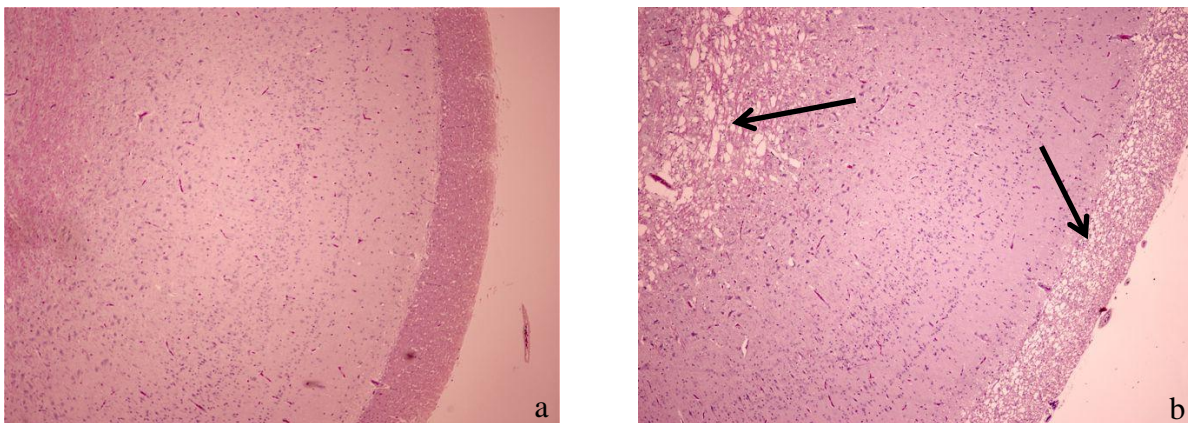


Figure 3.1: (a) Light micrograph of optic tectum and optic lobe (40x) of chicken fed control snail material. (b) Light micrograph of optic tectum and optic lobe (40x) of chicken fed treatment snail material. Arrows indicate vacuolar lesions.

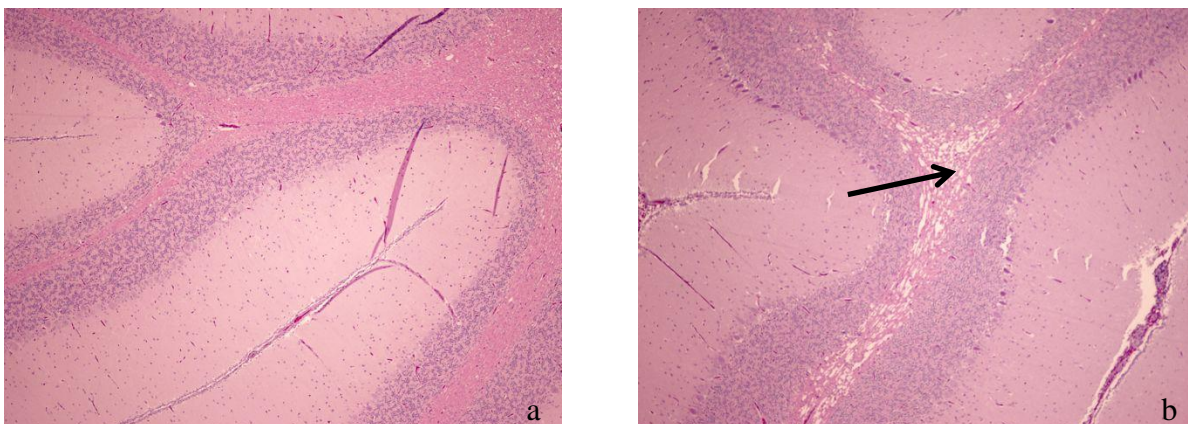


Figure 3.2: (a) Light micrograph of cerebellum (40x) of chicken fed control snail material. (b) Light micrograph of cerebellum (40x) of chicken fed treatment snail material. Arrows indicate vacuolar lesions.

DISCUSSION

All chickens in this study fed tissue from apple snails that consumed hydrilla *with* the UCB developed microscopic lesions in the white matter of the brain consistent with AVM. These results confirm that the uncharacterized cyanobacterial toxin linked to AVM can be transferred to birds through the herbivorous Island apple snail (Figure 3.3). Dietary transfer of the AVM toxin via an avian prey item has been previously demonstrated in laboratory feeding trials; predatory birds ingest the toxin through direct consumption of the gastrointestinal tract of AVM affected birds (Fischer et al. 2003). This is the first study to demonstrate that the toxin can transfer in an invertebrate-to-bird food chain.



Figure 3.3: Potential route of AVM toxin transfer from cyanobacterial colonies to snail kites.

The presence of UCB in Lake Toho may threaten the nesting snail kite population that resides there. UCB colonies were initially documented only on hydrilla collected in a cove in the northeastern section of Lake Toho, but an October 2011 survey identified colonies on hydrilla throughout the lake (Wilde, unpubl. data). Further, preliminary data from a May 2012 trial suggest that the UCB is capable of producing toxin in that environment (Wilde unpubl. data). However, because the AVM toxin is uncharacterized, avian feeding trials are the only method currently available to confirm that the AVM toxin is present in plant material or animal tissue. AVM epornitics have only been documented in the winter months at man made reservoirs and

ponds in the Piedmont region of the southeastern United States, where the most intense research efforts are directed. It would greatly expand the potential geographic range of AVM if the UCB is indeed producing the putative toxin in Toho or other regions of the country, where environmental and hydrologic conditions are vastly different from southeastern Piedmont reservoirs.

The density of UCB colonies observed on Toho hydrilla are comparable to those in AVM reservoirs in South Carolina and Georgia (Wilde, unpubl. data). Exotic apple snails are distributed lake-wide in Toho, including areas where the highest densities of UCB have been observed. Exotic apple snails are also more abundant than the native Florida apple snail in all areas of Toho most frequently used by snail kites for nesting and foraging (Cattau 2008). Due to the combination of these factors, snail kites are probably regularly consuming exotic apple snails that have fed recently and/or exclusively on hydrilla containing dense colonies of the UCB. In our feeding study, each chicken was fed snail tissue to equal approximately one adult snail per day. However, adult snail kites at Toho can consume an average of 1.1 exotic apple snails per hour and 3.9 native apple snails per hour while foraging (Cattau et al. 2010). This indicates that the kites' average rate of apple snail consumption should be more than sufficient to induce AVM if the UCB is producing toxin at Toho and the snails are consuming this material. The native Florida apple snail has as similar feeding preference to the exotic snails, but does not feed as heavily (Morrison and Hay 2010, 2011). We did not test the ability of native apple snails to transfer the AVM toxin in this study. Because of its similar physiology and feeding habits, the Florida apple snail may also be capable of transferring the AVM toxin to snail kites.

Although no snail kites have yet been documented exhibiting neurologic signs consistent with AVM, it is possible that diseased birds have not been detected. In AVM studies in

Arkansas, Georgia and South Carolina, mortality of bald eagles and coots occurred within 1 to 5 days of the animals being observed with clinical signs or as quickly as five days after being introduced to a disease site (Thomas et al. 1998, Rocke et al. 2002, Haynie 2008). There are also many cases of bald eagle mortalities where AVM was the suspected cause, but never confirmed, because the carcasses were not recovered in time to perform necropsy and diagnosis (Fischer et al. 2006). Wild animals are notoriously stoic when sick and will often seek heavy cover making carcass recovery difficult or impossible even when an active monitoring and recovery protocol is in place (Haynie 2008). AVM deaths of other less-studied bird species may have also gone unnoticed. Herbivorous or molluscivorous birds at Lake Toho may also be susceptible to toxin exposure through direct consumption of apple snails (limpkins, *Aramus guarana*) or hydrilla (American coots).

In 2010, the Florida Fish and Wildlife Conservation Commission reduced chemical treatments of hydrilla at Toho by 60% from previous years (David et al. 2010). A reduction in hydrilla treatment continued through the 2011 season. This was done in response to data showing declined nest success of snail kites on Toho in past years when hydrilla coverage was reduced in the early spring (David et al. 2010). Snail kite biologists believe that decreasing chemical treatments of hydrilla provides kites easier access to exotic apple snails near the water's surface in the hydrilla canopy and may lead to higher nest success of kites at Toho. Though this management strategy may provide higher availability of food to snail kites, it could also increase the risk of kites' exposure to the UCB and the associated AVM toxin. It may be necessary for lake managers to resume chemical treatments of hydrilla in areas of the lake where the UCB is found with hydrilla. Only additional research will reveal if hydrilla management strategies need to be altered and if the snail kite is indeed susceptible to the AVM toxin at Toho.

Further avian feeding trials are required to determine if the AVM toxin is present and moving through food webs in Toho. Since the AVM toxin is still uncharacterized, the only method to confirm the presence of toxin is a laboratory avian bioassay. This study established that exotic apple snails are capable of retaining the AVM toxin in their bodies and transferring it to birds, but another avian feeding study using exotic apple snails collected from hydrilla beds at Toho will be more conclusive and environmentally relevant to the snail kite's critical habitat. In the event that neurotoxicity is confirmed in Toho hydrilla material, lake managers may need to immediately alter chemical treatments to mitigate risks to avifauna in this habitat. Such a finding would also inform current research on the environmental requirements of the UCB for toxin production, since its toxicity has only been documented in a limited geographic range.

Apple snails are already known to be involved in disease transmission in other freshwater ecosystems. Invasive apple snails of the genus *Pomacea* can bioaccumulate cyanotoxins responsible for paralytic shellfish poisoning and are known hosts for a parasite that has caused outbreaks of eosinophilic meningitis in humans in the Pacific Islands, Southeast Asia and China (Yen et al. 1990, Lv et al. 2009, Berry and Lind 2010). The interaction of invasive species and native or introduced parasites is well studied and plays a role in the emergence or spread of infectious disease in freshwater ecosystems (Okamura and Feist 2011, Poulin et al. 2011). The role of biological invasions in the occurrence of non-infectious diseases caused by biotoxins or chemical contaminants is complex and less understood. AVM may represent a unique case of multiple aquatic invaders increasing the prevalence of wildlife disease, since it does not involve an infectious disease agent that can be transmitted from one host to another. The invasive macrophyte hydrilla has a known role in the transmission of AVM to wildlife, and exotic apple snails may provide a second mechanism by which wild birds can acquire the disease. Invasive

species management will likely be key in prevention of additional disease pathways that may ultimately affect native freshwater biodiversity.

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CHAPTER 4

CONCLUSIONS

The purpose of this study was to add to the current body of research on an important aquatic invader and to assess its detrimental effects to aquatic ecosystems in Georgia and Florida. Island apple snails (IAS) (*Pomacea insularum*) are established in at least nine locations in southern Georgia and, according to our predictive model, have not yet spread throughout the full extent of their potential range. At Lake Tohopekaliga (Toho) in central Florida, breeding populations of the Florida snail kite (*Rostrhamus sociabilis*) may be in danger of ingesting a neurotoxic cyanobacterium along with IAS, which could further reduce snail kite populations in Florida. Island apple snail infestations have the potential to damage sensitive wetland habitats in Georgia and may threaten the already diminished population of snail kite in central Florida.

The IAS has been established in Georgia since at least 2005, and continues to spread. Even at the conclusion of this study, another new population of IAS was discovered in a neighborhood pond in Kingsland, Georgia (Tim Bonvechio, pers. comm). Although the spread of IAS is limited by cold temperatures, they have not reached their equilibrium distribution within Georgia and may be able to spread further north. In addition, their potential range may extend further north with the global increase in temperatures due to climate change (Hellmann et al. 2008). Acidic waters with low calcium concentrations are inhospitable to IAS, and the acidity of the Okefenokee Swamp in Georgia will likely prevent invasion by apple snails. However, IAS can still persist in relatively low pH (<5.5) and blackwater streams and wetlands in Georgia remain vulnerable to invasion (Ramakrishnan 2007). Models of IAS distribution potential agree

that IAS will not be able to inhabit acidic areas such as the Okefenokee, and have not reached their northern limit for temperature tolerance (Byers et. al, in review). The salinity of one pond where IAS were found in St. Simons was higher than their reported tolerance limit, but their distribution is likely to be restricted to freshwater with a consistent salinity below 7.0 ppt. In their current range in southern Georgia, where cold temperatures do not limit their distribution, IAS may have the potential to become established in any water body with sufficient pH and food supply. Their current distribution in Georgia probably does not reflect habitat requirements, but simply the rate of introduction and spread.

The widespread invasion of IAS across Florida is affecting native wildlife associated with aquatic habitats (Conner et al. 2008, Cattau et al. 2010), and the presence of the UCB associated with Avian Vacuolar Myelinopathy (AVM) on Toho hydrilla (*Hydrilla verticillata*) may threaten the nesting snail kite population that resides there. This study confirmed that the uncharacterized cyanotoxin linked to AVM can be transferred to birds through the herbivorous IAS. It would greatly expand the potential geographic range of AVM if the UCB is indeed producing toxin in Toho or other regions of the country, where environmental and hydrologic conditions are vastly different from southeastern Piedmont reservoirs. Other herbivorous or molluscivorous birds at Toho such as limpkins (*Aramus guarana*) or American coots (*Fulica americana*) may also be susceptible to toxin exposure through direct consumption of apple snails or hydrilla. Further avian feeding trials are required to determine if the AVM toxin is present and available at various trophic levels in Lake Toho. Only additional studies will reveal if hydrilla management strategies need to be altered and if the snail kite is indeed exposed to the AVM toxin at Lake Toho.

Invasive mollusks are one of the most important threats to freshwater ecosystems in the U.S. (Strayer 1999). Many species have documented effects in specific systems, but the ultimate

population level impacts of recent invaders such as exotic apple snails are not yet fully understood. The data collected from these studies provides valuable information on probable detrimental effects of IAS to freshwater ecosystems and native wildlife in the southeastern U.S. Continued monitoring of IAS populations, especially along their northern range in Georgia and South Carolina will provide additional information on their ability to adapt to limiting environmental factors and increase their distribution. Further experiments are necessary to determine if the putative toxin is present in the Toho system and thus available to the imperiled Florida snail kites via their most plentiful prey item, the IAS. The presence of the IAS in all the major river drainages in southern Georgia and its ability to accumulate at least one cyanotoxin illustrate the need for continued surveillance and research of this exotic species.

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