

THE RELATIONSHIP BETWEEN INVASIVE AQUATIC PLANTS, CYANOTOXINS, AND  
FRESHWATER TURTLES IN THE SOUTHEASTERN UNITED STATES

by

Albert Donald Mercurio

(Under the Direction of John C. Maerz and Sonia M. Hernandez)

ABSTRACT

We explore how the invasive aquatic plant *Hydrilla verticillata* may impact freshwater turtles. We used a laboratory feeding trial to show that painted turtles (*Chrysemys picta*) could develop vacuolar myelinopathy (VM), a neurologic disease caused by an undescribed cyanotoxin produced by a cyanobacterium commonly found on *Hydrilla*. We also estimated patch occupancy patterns of turtles in five reservoirs to determine if *Hydrilla* invasion affects turtle habitat use or abundance. *Hydrilla* was positively associated with the detection probability of painted turtles and yellow-bellied sliders (*Trachemys scripta*) and positively associated with patch occupancy of common musk turtles (*Sternotherus odoratus*), indicating *Hydrilla* density is likely positively influencing patch abundance of these three turtle species. *Hydrilla* was not correlated with detection or occupancy of common snapping turtles (*Chelydra serpentina*). These results suggest *Hydrilla* is positively affecting habitat use or abundance of turtles inhabiting reservoirs; however, if occupancy of *Hydrilla* invaded habitats increases turtle exposure to cyanotoxins, then *Hydrilla* invasions may be creating an ecological trap for turtle populations.

INDEX WORDS: Vacuolar myelinopathy, Painted turtle, Common musk turtle, Common snapping turtle, Yellow-bellied slider, Habitat use, *Chrysemys picta*, Stigonematales, *Hydrilla verticillata*, Occupancy modeling, Habitat management, Invasion ecology

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by

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BS, James Madison University, 2009

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment  
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2014

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## ACKNOWLEDGEMENTS

My entire committee has been instrumental in facilitating this research by providing guidance, logistical, and moral support. The Graduate School at the University of Georgia, the Southeastern Cooperative Wildlife Disease Study, and the D.B. Warnell School of Forestry and Natural Resources have provided logistical and financial support via my assistantship, travel grants, and research grants. The National Institutes of Health, the U.S. Army Corps of Engineers, and the U.S. Fish and Wildlife Service provided funding for these projects. The Wildlife Disease Association and the South Carolina Aquatic Plant Management Society have supported me via scholarships.

I would like to thank the entire staff at the Henry County Water Authority, Newton County Water Authority, and Lynn and Mark Garrett for logistical support, providing access to sampling locations, and assistance with data collection. Without you all, this thesis would have never been possible and I am truly appreciative of your support.

Numerous students and staff at the University of Georgia have assisted with data collection and in particular I would like to thank Matthew Walter, Leslie Shelnett, Amanda Coleman, Bridget Altman, Krisitn Hinkson, the University of Georgia Herpetological Society, and members of the Maerz, Hernandez, Wilde, Nibbelink, and Bettinger Laboratories. I would also like to thank the University of Georgia Herpetological Society and various University of Georgia College of Veterinary Medicine students that have contributed to this thesis.

The Southeastern Cooperative Wildlife Disease Study (SCWDS) provided logistical support and facilities for necropsies. The Poultry Diagnostic Research Center in Athens, GA and

the University of Georgia Golf Course provided technical and logistical assistance. I also would like to thank the U.S. Army Corps of Engineers for collecting *Hydrilla* samples from J. Strom Thurmond Reservoir.

I would like to thank my family and friends for their support. Finally, I acknowledge the animals that contributed to this research project, some of which did not survive the experience. I am eternally grateful for their sacrifice.

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## CHAPTER 1

### INTRODUCTION AND LITERATURE REVIEW

The invasion of non-indigenous plant species is one of the primary threats to biodiversity and the integrity, resilience, and function of ecosystems (Usher 1988, Drake et al. 1989, Macdonald et al. 1989, Congress 1993, Randall and Marinelli 1996, Vitousek et al. 1997, Westbrooks 1998, Blossey 1999, Westbrooks 2004). Invasive plants can change fire regimes, biogeochemical cycling, geomorphological processes, hydrological cycles, prevent recruitment or reproduction of native species, hybridize with native species, affect human health, and facilitate wildlife diseases (Randall and Marinelli 1996, Gordon 1998, Westbrooks 1998, Blossey 1999, Mack et al. 2000, Crooks 2002, Rocke et al. 2005, Schultz and Dibble 2012). The rate of plant invasions in natural systems has increased by several orders of magnitude in the last century, bringing this issue to the forefront of ecological and conservation research (Mooney and Drake 1986, Drake et al. 1989, Soulé 1990, Pysek 1995, Gordon 1998).

Approximately 5,000 plant species are naturalized in the United States, 10% of which are seriously invasive (Congress 1993, Blossey 1999), thus, predictions and quantifications of changes in ecosystem processes and effects to wildlife are lacking for most of these species. However, generally speaking, it is expected that invasive species alter the habitat they invade, likely affecting all trophic levels because plants play a major role in the construction and maintenance of ecosystems, support food webs, regulate microclimates, and influence energy flow through systems by altering habitat structure and composition (D'Antonio and Vitousek 1992, Mack et al. 2000, Carvalheiro et al. 2010, DeVore and Maerz 2014). Three primary

mechanisms by which invasive plants in terrestrial and aquatic systems can affect consumers are the alteration of the flow, availability, or quality of nutrient resources within biogeochemical cycles, alteration of trophic resources within food webs, and alteration of physical resources such as living space, sediment, light, or water (Vitousek 1996, Crooks 2002, DeVore 2011).

Invasion by nonnative aquatic macrophytes is also a common driver of freshwater plant community change (Bunn and Arthington 2002, Abell et al. 2009). Many tropical species of plants from Africa, Asia, and South America are notably adventitious in freshwater systems within the U.S. including alligatorweed (*Alternanthera philoxeroides*), Eurasian watermilfoil (*Myriophyllum spicatum*), water hyacinth (*Eichhornia crassipes*), and *Hydrilla verticillata* (USDA, 2010). These plants change aquatic habitat structure, outcompete many native plant species, alter flow regimes, can affect native fish and wildlife that depend on native flora for forage or cover, and have caused hundreds of millions of dollars in damages to affected ecosystems (Pimentel et al. 2005, Rahel et al. 2008, Abell et al. 2009).

*Hydrilla verticillata* was introduced to the United States in the 1950's and 1960's from Southeast Asia to southern Florida. By the late 1970's, *Hydrilla* had spread through most of Florida's freshwater systems, continued to expand, and was designated a federal noxious weed by the United States Department of Agriculture (Langeland 1996, Puri et al. 2007, Regulations and Kaplan 2011). Aquarium releases, contaminations by boats, trailers, nets, fishing gear as well as mechanical transfer by migratory aquatic birds have all contributed to this widespread expansion (MacDonald et al. 2008). *Hydrilla* rapidly out-competes native macrophytes for resources by rapidly growing to the surface of the water and spreading laterally which blocks available light for other species. It also can propagate via seeds, cuttings, or tubers, can colonize areas with low light levels and high turbidity, and can persist in dry sediment when water levels

vary whereas many native plants cannot (Langeland 1996). *Hydrilla* can be managed over time with herbicides and/or triploid grass carp (*Ctenopharyngodon idella*), yet resistant varieties are increasingly observed (Puri et al. 2007).

*Hydrilla* invasions can lower water quality, reduce phytoplankton diversity, and alter aquatic invertebrate community structure (McCann et al. 1996, Colon-Gaud et al. 2004, Wilde et al. 2005). In addition, *Hydrilla* forms thick mats throughout the water column that displace or eliminate many fish species (Langeland 1996). Despite these negative effects to native flora and fauna, there is controversy surrounding management actions because in some cases, *Hydrilla* can provide vital ecosystem services to water bodies that lack natural vegetation, such as anthropogenic impoundments. *Hydrilla* is considered a good food source for waterfowl and provides cover for juvenile sport fish including *Macropterus* and *Lepomis* spp. (Colle and Shireman 1980) though see (Langeland 1996, Perret 2007). Migratory waterfowl use lakes with abundant aquatic plants (including *Hydrilla*) as stopovers during migration and overwintering habitats and densities of waterfowl tend to increase as *Hydrilla* density increases (Johnson and Montalbano 1987, Esler 1990). Until the effects of *Hydrilla* on all freshwater taxa are fully understood, the effects of *Hydrilla* invasions and management are hard to predict.

The southeastern U.S., the nidus of *Hydrilla* invasions in the United States, hosts 10% of the world's freshwater turtle diversity (Langeland 1996, Buhlmann et al. 2009). Some specialist turtle species are influenced by overharvesting, road mortality, and habitat loss and degradation, such as the impoundment of rivers (Gibbons et al. 2000, Buhlmann et al. 2009). Historically, the southeastern U.S. had few natural lakes or permanent water bodies (Lydeard and Mayden 1995). Instead, the landscape was dominated by isolated and riparian wetlands, streams, and rivers. Most isolated wetlands have been lost to agriculture, and the creation of more permanent water

bodies including “farm” ponds and river impoundments for recreational use, hydropower production, or drinking water have dramatically altered the freshwater landscape of the southeastern U.S (Bunn and Arthington 2002). This loss of wetlands and the degradation of streams and rivers has impacted habitat specialist turtle species (Bodie and Semlitsch 2000, Bodie et al. 2000, Bodie 2001, Anderson et al. 2002, Moll 2004). However, habitat generalists often occupy impounded waters at high densities (DonnerWright et al. 1999, Rizkalla and Swihart 2006, Tucker et al. 2012).

Freshwater impoundments are much more susceptible to plant invasions than natural rivers (Bunn and Arthington 2002). In general, it is known that habitat changes resulting from *Hydrilla* invasions and the impoundment of rivers work independently and synergistically to simplify the geomorphology of the channel, the biotic community, and the interactions among biota, but how these invasions affect turtles in freshwater impoundments has been relatively understudied (Bjorndal et al. 1997, Bunn and Arthington 2002, Fields et al. 2003, Moll 2004, Tucker et al. 2012). Freshwater turtles directly consume aquatic plants, macroinvertebrates and fish that feed on or reside in patches of aquatic plants, and use areas of plants for cover (Ernst and Lovich 2009). Many turtle species will consume *Hydrilla* directly, however it has a lower nutritional content than many of the native plant species that it replaces (Bjorndal et al. 1997, Fields et al. 2003). Studies have documented that turtles may be attracted to areas with dense plant cover, and abundance of some turtle species indeed increases with primary productivity (Galbraith et al. 1988, Buhlmann and Vaughan 1991, DonnerWright et al. 1999, Hartwig and Kiviat 2007a, Ernst and Lovich 2009). However, thick aquatic vegetation can also impede turtle movement, reducing the habitat suitability of extremely dense patches and decreasing the overall abundance of turtles within these water bodies (Black 2000, Marchand and Litvaitis 2004).

Furthermore, poor water quality resulting from *Hydrilla* invasions can significantly reduce phytoplankton diversity and alter aquatic invertebrate communities that turtles may depend on for food (McCann et al. 1996, Colon-Gaud et al. 2004). *Hydrilla* may also reduce the abundance of fish species on which some turtle species prey (Langeland 1996, Perret 2007).

In this study, we aimed to identify the importance of *Hydrilla* cover on turtle communities in southeastern U.S. reservoirs. Because freshwater turtle species partition themselves within microhabitats based on habitat preferences or requirements, such as plant density, an analysis of habitat use within patches of varying *Hydrilla* density can provide insight into the effects of *Hydrilla* invasions on freshwater turtles (Brewster and Brewster 1991, Harrel et al. 1996, Hartwig and Kiviat 2007b, Steen et al. 2007, Vecchio et al. 2011, Anthonysamy et al. 2014). Occupancy modeling estimates the probability that a species is occupying a certain pre-defined patch of habitat (MacKenzie et al. 2003, MacKenzie 2006). Is a viable method for species with low recapture rates, such as freshwater turtles, because occupancy patterns can provide insight into habitat use and is also an efficient proxy to more time intensive measurements of abundance or demographic rates such as survival or reproduction (MacKenzie et al. 2003, MacKenzie 2006, Mazerolle et al. 2007). We used patch occupancy after accounting for incomplete detection to measure the effects of *Hydrilla* density and other factors on turtle use of habitats within southeastern U.S. reservoirs for two omnivorous-highly herbivorous species (eastern painted turtle, *Chrysemys picta*; yellow-bellied slider, *Trachemys scripta*), and two carnivorous species (common snapping turtle, *Chelydra serpentina*; common musk turtle, *Sternotherus odoratus*). We hypothesized that invasive plant density would be negatively correlated with the probability of occupancy for all of the species studied.

In addition to habitat changes, the recent emergence of a disease associated with *Hydrilla* presence, vacuolar myelinopathy (VM) has increased concern about how *Hydrilla* invasions affect aquatic wildlife. Consumption of *Hydrilla* material colonized by a yet undescribed toxin-producing species of cyanobacteria (hereafter referred to as *Hydrilla*+UC) causes vacuolations within the white matter of the central nervous system resulting in lethargy and ataxia in birds, which may lead to death (Thomas et al. 1998). Since its discovery in 1994, the mortality of more than 100 bald eagles (*Haliaeetus leucocephalus*) and thousands of American coots (*Fulica americana*) are attributed to VM from consuming *Hydrilla*+UC and the disease has been documented in eight other species of birds (Rocke et al. 2002, Augspurger et al. 2003, Lewis-Weis et al. 2004, Fischer et al. 2006). Until recently it was believed only birds were susceptible to VM. Clinical signs (and death) consistent with VM caused by the consumption of *Hydrilla*+UC has also been demonstrated both experimentally and naturally in grass carp, which are used as a biological control for *Hydrilla* (Haynie et al. 2013). VM has not been documented in mammals (Lewis-Weiss et al. 2014). The two remaining taxa potentially susceptible to VM are amphibians and reptiles.

Freshwater turtles have a number of characteristics that, if susceptible to the putative UCB toxin(s), make them likely candidates to develop VM. Turtles and crocodylians are members of the Archosauria and therefore are the closest extant relatives to birds (Chiari et al. 2012). The vast majority of turtles in the southeastern U.S. occur in freshwater, many species are omnivorous or herbivorous, and several feed extensively on submerged aquatic vegetation including *Hydrilla*, or on invertebrates that graze on epiphytic algae (Ernst and Lovich 2009), and turtles are known to be susceptible to other food chain-linked cyanotoxins (Kozlowsky-Suzuki et al. 2012). Therefore, in this study we also tested the hypothesis that turtles fed

*Hydrilla* colonized by the UCB and verified to be neurotoxic to birds would develop clinical signs of neurologic disease and histologic lesions similar to those of described in birds with vacuolar myelinopathy.

Recently there has been an increase in the efforts to manage the impacts, develop early detection programs, and prevent the further invasion of exotic species (Westbrooks 2004, Puri et al. 2007). Objections to controlling non-indigenous species can be grouped into 5 categories, (1) economic, (2) ecological, (3) aesthetic, (4) ethical, and (5) risks associated with the development of biological weed control (Blossey 1999). Even though costs associated with *Hydrilla* invasions via the loss of freshwater recreation revenue, ecosystem services, etc total hundreds of millions of dollars annually (Pimentel et al. 2005), direct economic costs of managing *Hydrilla* are also extremely costly and are wrought with complications from indirect ecosystem effects (Richardson 2008). Therefore, management of *Hydrilla* requires weighing social, ecological, and economic concerns and comprehensive, empirical evidence provided by this study will help inform management decisions.

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CHAPTER 2

EXPERIMENTAL FEEDING OF *HYDRILLA VERTICILLATA* COLONIZED BY  
STIGONEMATALES CYANOBACTERIA INDUCES VACUOLAR MYELINOPATHY IN  
PAINTED TURTLES (*CHRYSEMYS PICTA*)<sup>1</sup>

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<sup>1</sup> Mercurio, Albert D., Sonia M. Hernandez, John C. Maerz, Michael J. Yabsley, Angela E. Ellis, Amanda L. Coleman, Leslie M. Shelnutt, John R. Fischer, and Susan B. Wilde. *Plos One* 9.4 (2014): e93295.  
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## ABSTRACT

Vacuolar myelinopathy (VM) is a neurologic disease primarily found in birds that occurs when wildlife ingest submerged aquatic vegetation colonized by an uncharacterized toxin-producing cyanobacterium (hereafter “UCB” for “uncharacterized cyanobacterium”). Turtles are among the closest extant relatives of birds and many species directly and/or indirectly consume aquatic vegetation. However, it is unknown whether turtles can develop VM. We conducted a feeding trial to determine whether painted turtles (*Chrysemys picta*) would develop VM after feeding on Hydrilla (*Hydrilla verticillata*), colonized by the UCB (Hydrilla is the most common “host” of UCB). We hypothesized turtles fed Hydrilla colonized by the UCB would exhibit neurologic impairment and vacuolation of nervous tissues, whereas turtles fed Hydrilla free of the UCB would not. The ability of Hydrilla colonized by the UCB to cause VM (hereafter, “toxicity”) was verified by feeding it to domestic chickens (*Gallus gallus domesticus*) or necropsy of field collected American coots (*Fulica americana*) captured at the site of Hydrilla collections. We randomly assigned ten wild-caught turtles into toxic or non-toxic Hydrilla feeding groups and delivered the diets for up to 97 days. Between days 82 and 89, all turtles fed toxic Hydrilla displayed physical and/or neurologic impairment. Histologic examination of the brain and spinal cord revealed vacuolations in all treatment turtles. None of the control turtles exhibited neurologic impairment or had detectable brain or spinal cord vacuolations. This is the first evidence that freshwater turtles can become neurologically impaired and develop vacuolations after consuming toxic Hydrilla colonized with the UCB. The southeastern United States, where outbreaks of VM occur regularly and where vegetation colonized by the UCB is common, is also a global hotspot of freshwater turtle diversity. Our results suggest that further investigations into the effect of the putative UCB toxin on wild turtles in situ are warranted.

## INTRODUCTION

Vacuolar myelinopathy (VM) is a neurologic syndrome that primarily affects birds associated with freshwater habitats. The effects of VM on wild birds are documented for American coots (*Fulica americana*), bald eagles (*Haliaeetus leucocephalus*), mallards (*Anas platyrhynchos*), ring-necked ducks (*Aythya collaris*), buffleheads (*Bucephala albeola*), Canada geese (*Branta canadensis*), great horned owls (*Bubo virginianus*), and killdeer (*Charadrius vociferus*) in approximately 20 southeastern U.S. reservoirs ranging from Texas to North Carolina (Thomas et al. 1998, Augspurger et al. 2003, Lewis-Weis et al. 2004, Fischer et al. 2006). It is thought that birds develop VM by directly or indirectly consuming aquatic vegetation colonized by a novel species of epiphytic cyanobacteria in the order Stigonematales (hereafter “UCB” for “uncharacterized cyanobacterium”) that produces a yet to be described toxin(s) (Wilde et al. 2005, Williams et al. 2007). The UCB grows in high abundance on *Hydrilla* (*Hydrilla verticillata*), a widespread invasive exotic plant, although it can also grow on several native aquatic plant species (Wilde et al. 2005). Birds may acquire the toxin(s) directly by ingesting plants that are colonized with the UCB or indirectly by feeding on herbivorous prey such as invertebrates (Robertson 2012) or other bird species that have fed on plants that are colonized with the UCB (Fischer et al. 2003). Affected birds develop microscopic vacuoles in the white matter of the central nervous system. Lesions tend to be most prominent in the optic tectum but can occur in the cerebrum, cerebellum, brain stem, or spinal cord. Degenerative lesions in peripheral nerves have rarely been reported. Ultrastructurally, vacuolation is due to splitting of myelin lamellae at the intraperiod line, consistent with intramyelinic edema. These lesions result in variable neurologic dysfunction that in severe cases can result in death within a few days (Thomas et al. 1998, Larsen et al. 2002, Rocke et al. 2002, Augspurger et al. 2003).

A number of studies stress the need to evaluate the risk that consumption of vegetation colonized by the UCB poses to other taxa (Birrenkott et al. 2004, Wiley et al. 2009). Previous work showed that grass carp (*Ctenopharyngodon idella*) experimentally fed *Hydrilla* colonized with the UCB developed vacuolations consistent with avian models, yet domestic pigs (*Sus domesticus*) and laboratory mice did not (Lewis-Weis et al. 2004, Rocke et al. 2005, Haynie et al. 2013). To date, there are no reports for any species representing the remaining major vertebrate lineages (amphibians or reptiles).

Freshwater turtles have a number of characteristics that, if susceptible to the putative UCB toxin(s), make them likely candidates to develop vacuolar myelinopathy. Turtles and crocodylians are members of the Archosauria and therefore are the closest extant relatives to birds (Chiari et al. 2012). The southeastern United States, the current location of VM outbreaks, is a global hotspot of freshwater turtle diversity with ~10% of the world's turtle species occurring in the region (Buhlmann et al. 2009). The vast majority of these turtles occur in freshwater, many species are omnivorous or herbivorous, and several feed extensively on submerged aquatic vegetation including *Hydrilla*, or on invertebrates that graze on epiphytic algae (Ernst and Lovich 2009); and turtles are known to be susceptible to other food chain-linked cyanotoxins (Kozlowsky-Suzuki et al. 2012). Therefore, the objective of this study was to test the hypothesis that turtles fed *Hydrilla* colonized by the UCB and verified to be neurotoxic to birds would develop clinical signs of neurologic disease and histologic lesions similar to those of described in birds with vacuolar myelinopathy.

#### *Focal Turtle Species*

We selected painted turtles (*Chrysemys picta*) as our focal species for this study. Painted turtles are one of the most thoroughly studied turtle species in the world, and their husbandry

protocols are well established (Johnson 2004, Ernst and Lovich 2009). They are abundant and readily available in regions where they occur and adapt well to captivity (Davis and Burghardt 2007). As a member of Emydidae, they are related to most of the turtle species in the southeastern U.S., and they are omnivorous but highly herbivorous as adults. We have documented *Hydrilla* in the gut contents of painted turtles in reservoirs experiencing VM epornitics (Mercurio et al. unpublished data) and they are known to feed on invertebrates that graze on epiphytic algae (Ernst and Lovich 2009).

## MATERIALS AND METHODS

### *Ethics Statement*

All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (A2012 02-001-Y2-A4). Field studies did not involve endangered or protected species and wildlife collections were permitted by the U.S. Fish and Wildlife Service (MB779238-0) and the Georgia Department of Natural Resources (29-WBH-12-95), which allow for the collection of wildlife from Georgia public state lands. In addition, the Henry County Water and Sewerage Authority provided permission to access Upper Towaliga Reservoir (33.3472°, -84.2145°). The University of Georgia Golf Course permitted access to a pond in Athens, GA (33.9041°, -83.3674°).

### *First Hydrilla Collection*

Approximately enough *Hydrilla* to fill three, 50 gallon coolers was collected from J. Strom Thurmond Reservoir (33.6972°, -82.2540°) on the border of Georgia and South Carolina during a VM epornitic and Lake Seminole on the Georgia-Florida border in December 2011. *Hydrilla* was transported within clean zip top plastic bags (3.79 l) on ice to the University of Georgia (Wiley et al. 2009). VM positive birds have been recovered in late fall from J. Strom

Thurmond Reservoir annually since 1998 and *Hydrilla* in this reservoir is consistently colonized by the UCB (Wilde et al. 2005, Fischer et al. 2006). Lake Seminole has never experienced a VM case, and the UCB has never been detected at this site (Wilde et al. 2005). Light and epifluorescent microscopy were used to confirm the presence/absence of the UCB following established methods (Wilde et al. 2005, Williams et al. 2007). Briefly, 5 representative leaves were wet mounted on a glass slide. Light microscopy and a rhodamine filter set were used to visualize cyanopigments on *Hydrilla* leaves and the presence/absence of the UCB colonies were documented via visual assessment. The rest of the *Hydrilla* was frozen at -20°C for 48 hours. To lyophilize the material, one gallon sized paper bags of frozen *Hydrilla* were then placed in the lyophilizing chamber of a Labconco Freeze Dryer 5 (Labconco, Kansas City, MO) at ~5 mm Hg for 48 hours or until completely dry. Once dry, *Hydrilla* was stored in sealed plastic bags in a temperature controlled facility at 26.6°C.

#### *Validation of the Toxicity of the First Hydrilla Collection*

Because some plant samples with the UCB do not induce VM when consumed, a feeding trial was conducted to determine the toxicity of the *Hydrilla* (Williams et al. 2009). Domestic chickens (*Gallus gallus domesticus*) are susceptible to VM through dietary exposure of aquatic vegetation collected from sites where VM has been documented in wild birds (Lewis-Weis et al. 2004). A chicken feeding trial was conducted as previously described with the first collection of *Hydrilla* to assess its potential to induce VM (Lewis-Weis et al. 2004). Briefly, 4-week-old specific pathogen free leghorn chickens (0.8-1.5 kg, n=10) were housed at the University of Georgia Poultry Diagnostic and Research Center in Horsfal (isolation) units. Once a week, chickens were weighed and received a full physical and neurologic exam consistent with previous trials (Lewis-Weis et al. 2004, Lightfoot and Harrison 2006, Clippinger et al. 2007).

Mentation, posture, attitude, movement, gait, postural reactions, spinal reflexes, and cranial nerve function were assessed. Limbs were palpated to evaluate asymmetry, masses, tenderness, contour, and tone. Birds were also weighed and observed for any superficial injuries. Chickens were allowed to acclimate to laboratory conditions for four days and were fed a non-medicated starter feed produced by the University of Georgia feed mill *ad libitum* (~30g/kg bw/day) out of ceramic bowls. All chickens were in good body condition and no physical or neurologic abnormalities were noted at the beginning of the trial. Chickens were then randomly assigned to two treatment groups. Five treatment group birds were fed 30g/kg bw/day of poultry starter feed and 2g/kg bw/day of lyophilized *Hydrilla* colonized with the UCB from J. Strom Thurmond Reservoir for 28 days. The other five control group birds were fed the same volume of poultry starter feed and lyophilized *Hydrilla* free of the UCB from Lake Seminole. Each bird was monitored twice daily for clinical signs of VM (difficulty standing or ambulating, ataxia, loss of balance, limb paresis and/or head droop) and were weighed twice a week (Larsen et al. 2002).

All chickens were humanely euthanized on day 28 with CO<sub>2</sub> followed by cervical dislocation. Calvaria were opened and partially removed with rongeurs to expose the dorsal surface of the brain. Brains were removed intact from the calvaria using a scalpel and/or scissors and immediately placed into 10% neutral buffered formaldehyde. Following ten days of fixation, brains were halved longitudinally. A single longitudinal section was then made 1-2 mm lateral to midline and an additional 1-2 transverse sections were made through the optic lobe. Resulting sections were placed whole into a cassette. These sections were routinely processed, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin prior to light microscopic examination by a veterinary pathologist (Thomas et al. 1998). All treatment chickens fed *Hydrilla* from J. Strom Thurmond Reservoir were bright, alert, responsive and eating well

throughout the entire trial but developed very mild neurologic clinical signs (mild ataxia beginning on day seven until the end of the trial) and developed vacuolations consistent with VM, whereas none of the control birds fed *Hydrilla* from Lake Seminole developed clinical signs or vacuolations.

In December 2012, we collected more fresh *Hydrilla* (same volume) from Upper Towaliga Reservoir in Henry County, GA and from Lake Seminole (30.7428°, -84.8776°). Like J. Strom Thurmond Reservoir, Upper Towaliga Reservoir undergoes annual VM outbreaks and *Hydrilla* in this reservoir is routinely colonized by the UCB. This *Hydrilla* was collected during a VM epornitic at Upper Towaliga Reservoir and was transported on ice to the University of Georgia as previously described. The presence/absence of the UCB was verified via light and epifluorescent microscopy as previously described. VM lesions were verified in coots recovered from Upper Towaliga Reservoir during the fall of 2012 (Fischer et al. unpublished data). *Hydrilla* was frozen at -20°C in zip top plastic bags (3.79 l) and was thawed as needed.

#### *Turtle Feeding Trial*

Adult painted turtles (*Chrysemys picta*, straight carapace length >7 cm: 7 females, 3 males) were collected using canned sardine (Crown Prince, City of Industry, CA) baited hoop traps (model TN210; Memphis Net and Twine Co, Inc., Memphis, TN) from a pond in Athens, GA where an VM outbreak has never been documented and the UCB has never been documented. Turtles were individually transported in clean plastic bins to the Whitehall Herpetology Laboratory, a climate controlled facility, where we completed physical and neurologic exams as described below. The ten turtles were selected from a larger sample and were determined to be neurologically and physically normal. The turtles were housed individually in 37.8 l (50.8 cm x 25.4 cm x 30.48 cm) glass tanks following standard husbandry

protocols (Johnson 2004). Briefly, incandescent lights provided a 12 hour light cycle, the ambient temperature was maintained in the room at 26.6° C, water temperature at 24.4° C, and basking surfaces (clay bricks) at ~32.2° C. Fresh city water was supplied as needed to maintain a depth of 20 cm. Water quality was maintained using aquarium filters (Fluval Nano; Rolf C. Hagen Corp, Mansfield, MA). Each week the water was removed, the gravel was rinsed, the tank was scrubbed with a mild dish detergent and rinsed thoroughly, one half of the old water was replaced, and the tank was filled up to 20 cm with fresh water. Turtles were monitored daily for gross appearance, behavior, food consumption, mentation, and were allowed to acclimate to laboratory conditions and to our feeding delivery method for a minimum of 15 days. During this time they were fed ReptoMin floating turtle sticks (Spectrum Brands Inc., Melle, Germany) homogenized into a uniform powder and packed into transparent gelatin capsules (Capsuline Corporation, Pompano Beach, FL and Torpac Inc., Fairfield, NJ) at 0.02 kcal/g body weight/day, calculated using an allometric food calculator developed by the University of Georgia College of Veterinary Medicine Teaching Hospital (Mader 2006).

Five turtles were randomly assigned into either a treatment *Hydrilla* or a control *Hydrilla* group. *Hydrilla* was fed to turtles in two ways to maximize consumption. The *Hydrilla* from the first collection with confirmed UCB toxicity status was packed into transparent gelatin capsules. To increase palatability and provide additional nutrition, capsules were coated in a mixture of sardine oil and ReptoMin prior to feeding. Each turtle was offered ~6 g/kg bw/day of their assigned *Hydrilla* diet in floating gelatin capsules. ReptoMin was also provided as needed to maintain each turtle's body weight relative to the start of the study. Starting on day 30 of the feeding trial, 50 g of intact floating *Hydrilla* from the second collection with confirmed UCB toxicity status was added to each tank each day to maximize *Hydrilla* consumption by turtles.

The amount of the floating *Hydrilla* consumed each day was measured to the nearest 10 g. More accurate monitoring of material consumed was not possible because the turtles shredded *Hydrilla* during normal feeding activities.

#### *Physical and Neurologic Exams*

Turtles underwent a complete physical and neurologic exam once per week. Turtles were weighed and were observed for any obvious injuries, lesions, dysecdysis, or abrasions. A neurologic exam as described for reptiles in (Mariani 2007) and (Mader 2006) was performed to assess the mental status, attitude, general activity, head and body posture, limb movement and coordination, gait, position in the water while swimming, and sensory and motor responses. Briefly, the turtles were first observed from a distance within their tanks for coordination while swimming, posture, and mentation prior to handling. The turtles were then removed from their tanks and held by the observer. The limbs were palpated to determine musculoskeletal symmetry, tone, strength, and tenderness. Reflexes were described as absent, reduced, normal, or clonus (where applicable) unless otherwise stated. Leg and head withdrawal reflexes and the ability to maintain their head position in a horizontal plane while rotated and listed in midair were assessed. The function of cranial nerves II, IV, and VII was assessed by inciting a menace response in a standard manner by obscuring the vision in one eye and making a slow threatening hand gesture to the other eye (Mader 2006). Cranial nerves III, IV, and VI functions were assessed by observing for strabismus (present/absent). Cranial nerve V function was evaluated by assessing mandibular movement during feeding (normal/abnormal). Cranial nerve VIII function was assessed by observing for nystagmus by moving the turtle's head side to side in a horizontal plane and observing the resulting movement of eyes. The presence/ absence of nystagmus when the turtle was held stationary was also assessed. Cranial nerve VIII function

was also assessed by observing for head tilting, rolling, and the righting reflex. The function of cranial nerves IX, XI, and XII was assessed by looking for signs of dysphagia. The turtles were then allowed to ambulate to evaluate symmetry of movement, gait, and posture.

#### *Detection of Vacuolations*

At the conclusion of the trial (97 days) or if an individual developed neurologic signs, humane euthanasia was performed using an injection of sodium pentobarbital (100 mg/kg) (Mader 2006) with a 22 gauge needle into the subcarapacial vein followed immediately by complete necropsy. Briefly, the plastron was removed using a striker saw to expose the coelomic cavity. Representative samples of liver, lungs, kidney, heart, spleen, gonads, stomach, and intestine were excised and placed into 10% neutral buffered formalin. An approximately 5 mm segment of skeletal muscle and peripheral nerve was excised from a rear leg. Brains were removed in a fashion similar to that previously described for the chickens with the exception that the proximal 2-5 mm of the spinal cord was also removed with the brain. The brain was halved longitudinally and halves were immediately placed into fixative (either 10% buffered formaldehyde for histopathology or chilled 2% glutaraldehyde, 2% paraformaldehyde, and 0.2% picric acid in a 0.1 M cacodylate buffer (pH 7.2) for transmission electron microscopy (EM)). Following a fixation time of approximately 30 days, the formalin fixed half of the brain was sectioned transversely at approximately 2 mm intervals, resulting in 5 total sections that were placed into a divided cassette. Spinal cord was also sectioned transversely, resulting in 3-5 sections that were placed into a second cassette. Formalin fixed tissues were routinely processed, embedded, and stained as previously described for the chickens and were subsequently examined by a pathologist with experience in chelonian histopathology following previously described methods (Fischer et al. 2003). Subsequently one treatment and one control turtle were randomly

selected for EM examination at the Electron Microscopy Laboratory at the University of Georgia (Lewis-Weis et al. 2004). Transmission electron microscopy specimens were post-fixed in 1% osmium tetroxide, serially dehydrated, infiltrated in an acetone/epoxy plastic, and then embedded in a plastic mold. Plastic blocks were cut with an ultramicrotome, and thick sections were stained with toluidine blue to identify optimal areas for thin sectioning. Thin sections were cut at 55-60 nm, placed on copper grids, and stained with uranyl acetate and lead citrate.

### *Statistical Analysis*

A Student's t-test for paired samples was used to determine if all turtles increased in weight from the beginning of the trial to the end. A Student's t-test was then used to determine if the change in weight over time significantly varied between treatment groups. An Analysis of Covariance was also used to determine if the average amount of *Hydrilla* consumed per day varied between treatment groups while accounting for body mass (Quinn and Keough 2002). Statistical analyses were completed in IBM SPSS Version 21.

## RESULTS

### *Turtle Feeding Trial*

The weight of the turtles increased significantly throughout the trial ( $t_{\alpha 0.05, 9} = -2.788$ ,  $p = 0.021$ ) with no difference between the treatment and control groups ( $t_{\alpha 0.05, 7} = -0.030$ ,  $p = 0.977$ ). Control turtles consumed an average of 2.76 g of lyophilized *Hydrilla*/kg bw /day (SE  $\pm 0.73$  g) and turtles fed *Hydrilla* with UCB consumed an average of 1.58 g of lyophilized *Hydrilla*/kg bw/day (SE  $\pm 0.13$ g). The main effect of treatment group was not significant,  $F(1,6) = 0.65$ ,  $p = 0.45$ ,  $\eta^2 = 0.10$ , nor was body mass,  $F(1,6) = 0.52$ ,  $p = 0.49$ ,  $\eta^2 = 0.08$ , nor was the interaction between mass and treatment group,  $F(1,6) = 0.39$ ,  $p = 0.55$ ,  $\eta^2 = 0.06$ . For floating *Hydrilla*, control turtles consumed an average of 14.5 g/kg bw/ day (SE  $\pm 3.22$ g) whereas turtles

fed *Hydrilla* with UCB consumed an average of 8.55 g/kg bw/day (SE ± 1.03g). The main effect of treatment group was not significant,  $F(1,6)=1.11$ ,  $p=0.33$ ,  $\eta^2=0.156$ , nor was body mass,  $F(1,6)=0.27$ ,  $p=0.63$ ,  $\eta^2=0.04$ , nor was the interaction between mass and treatment group,  $F(1,6)=0.71$ ,  $p=0.43$ ,  $\eta^2=0.11$ .

All turtles appeared healthy until day 82 of the trial. Between days 82-89, the five treatment turtles began displaying neurologic dysfunction, including, but not limited to, various degrees of ataxia (mild gait asymmetry to severe limb dragging- Video S1) and inability to right themselves, in addition to performing poorly on one or more aspects of the neurologic exam (Table 1). Three of the turtles were euthanized on day 82. Two turtles (#85 and 119) only displayed mild neurologic deficits on day 89, the first observation of deficits. We maintained these turtles, which were still alert and eating well, until day 97 to observe the progression of clinical signs. During this time, the feeding volume decreased to anorexia in #85, however, its gait improved to normal by day 94. Turtle 119 continued to eat and intermittently displayed mild neurologic deficits. Both turtles were subsequently euthanized on day 97. Control turtles appeared healthy throughout the entire trial.

#### *Diagnostic Findings*

No gross abnormalities were observed for any turtles at necropsy. All turtles were in good body condition and contained food in the gastrointestinal tract, with the exception of the three turtles in the treatment group that displayed anorexia or reduced feed intake towards the end of the trial. Significant histologic abnormalities were not observed in any of the controls. However, all turtles in the treated group had severe, diffuse vacuolation of white matter throughout the entire brain, including cerebrum, cerebellum, and brain stem, and spinal cord with no single area appearing to be consistently more or less affected than other areas. Mild multifocal

inflammatory lesions consisting of lymphocytic perivascular cuffing were noted in peripheral nerves. However, these were present and similar in both the control and treatment groups and may have been related to schistosomes which were an incidental finding in several turtles. Significant lesions were not present in any of the other examined organs (kidney, liver, heart, lung, spleen, gonad, and gastrointestinal tract) in both treatment groups. Light microscopic changes were present throughout the white matter of the brain and spinal cord of treatment group turtles and consisted of coalescing, round to ovoid, clear vacuoles that were approximately 5-40  $\mu\text{m}$  in diameter (Figure 1.1). Similar but less widespread vacuolation was also noted in the Purkinje and inner granular cell layers of the cerebellum. However, perikarya were unaffected. In the cerebral gray matter, scattered vacuoles, either individually or in small clusters, were also observed but this tended to occur at white matter interfaces. Electron microscopic findings in the brain of the treatment group turtle consisted of axonal swelling and degeneration with splitting of myelin at the intraperiod line (Figure 1.2). No significant histologic (Figure 1.3) or electron microscopic abnormalities were noted in the brain of the control turtles.

## DISCUSSION

Our study demonstrates that a common freshwater turtle species, the painted turtle, can develop neurologic signs and vacuolations consistent with VM from consuming *Hydrilla* with UCB. Clinical signs in turtles were consistent with avian models, presenting as varying degrees of neurologic and physical impairment (Larsen et al. 2002, Lewis-Weis et al. 2004). A subjective attempt was made to correlate lesions with neurologic severity and/or type of neurologic signs. However, with the exception of the cerebellar lesions, all affected turtles appeared to have similar, severe, widespread lesions and no such correlations could be identified. While some variation in distribution and severity was present among the cerebellar lesions, this did not

appear to correlate with any differences in the clinical signs. These findings are similar to those described in birds with VM (Thomas et al. 1998, Larsen et al. 2002).

Though the specific agent or agents that cause VM have not been identified, we believe that our results provide strong evidence that the same active agent(s) that induce VM in birds and are associated with ingestion of the UCB induce the lesions and associated neurologic disease in painted turtles. There might be a generalized effect among these two closely related taxa. A previous study demonstrated that grass carp fed toxic *Hydrilla* also developed vacuolations, suggesting the toxin(s) produced by the UCB may have broad neurologic effects among vertebrates [10]. Although two studies of domestic pigs and one study of laboratory mice fed toxic *Hydrilla* failed to find evidence of neurologic signs or detectable vacuolations, the authors of those studies emphasize the dose and/or duration of toxin(s) exposure may vary among taxa and experimental design, and may not have been sufficient to induce disease (Lewis-Weis et al. 2004, Rocke et al. 2005, Haynie et al. 2013). Our results support the hypothesis that taxa may vary in the required dosage or exposure duration to induce neurologic lesions. Standard avian trials are less than 30 days in length, and birds are often symptomatic within a few days. Grass carp euthanized 37 days post exposure to colonized *Hydrilla* had vacuolations, although no clinical signs were noted [10]. In our study, chickens fed *Hydrilla* colonized by the UCB exhibited mild neurologic signs within 7 days; however, turtles fed the same *Hydrilla* did not exhibit detectable clinical signs until 82 days. Possible explanations for this difference are the slower metabolism of ectotherms when compared to endotherms, differences in digestive efficiency, different metabolic pathways, an innate resistance to the toxin, or some other unknown factor.

We caution that while turtles may be sensitive to the UCB toxin, it remains to be determined whether turtle populations are vulnerable to the UCB's spread and invasions of freshwaters. Vulnerability incorporates both sensitivity and exposure. Many ponds and reservoirs in the southeastern U.S. have dense *Hydrilla* or native submerged aquatic vegetation that supports abundant concentrations of the UCB (Wilde et al. 2005, Fischer et al. 2006). In those systems, a diet consisting of large amounts of *Hydrilla* may be biologically realistic, particularly for highly herbivorous turtles (e.g., *Trachemys* and *Pseudemys* spp.) (Bjorndal et al. 1997, Fields et al. 2003). However, VM epornitics occur during late fall-winter, leading some to suggest that toxin production is related to season (Rocke et al. 2002). Most turtle species in the southeastern U.S. exhibit limited activity in the late fall to winter and may limit feeding during the cooler months of peak VM epornitics. To date, no large-scale die offs of aquatic turtles have been reported in reservoirs where VM die offs were reported for birds. Dead turtles may sink, decompose, or become scavenged in the water, which may contribute to low detection rates of impaired turtles. Moreover, our observations were that impaired turtles could show some motor recovery despite significant lesions in the brain. Turtles that have lesions but are not clearly distressed may not be reported (Fischer et al. 2003), and the dominant effects of ingesting the UCB may be subacute and not associated with high mortality. It is also not known whether turtles can recover longer term from the neurologic damage associated with ingesting the UCB. Clearly, more studies will be needed to elucidate important details on the epidemiology and vulnerability of the UCB to turtles and other wildlife. We propose a near term need for sensitivity studies of wider suites of taxa including those feeding directly or indirectly on UCB host plants, and studies of the seasonality of toxin production relative to seasonal variation in foraging rates of exposed taxa to determine potential population level vulnerabilities.

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Table 1.1. Clinical signs observed in the treatment group turtles after the first observed deficits on day 82 of the experiment.

ID #	Anorexic?	Gait and movement Normal?	Able to Swim?	Mentation	Spinal and other Reflexes Normal?	Could keep head in horizontal plane when rotated and listed?
107	Yes	Would not ambulate	Floating upside down	Stupor	No attempt to right itself	Yes
104	Yes	Ataxia	No	Stupor	Unable to right itself	Reduced ability
118	No	Ataxia	Yes	Depressed	No head withdrawal, no attempt to right itself	Reduced ability
85	Yes	Ataxia	Yes	Alert	Unable to right itself and head withdrawal reflex was reduced	Yes
119	No	Ataxia	Yes	Alert	Unable to right itself	Yes

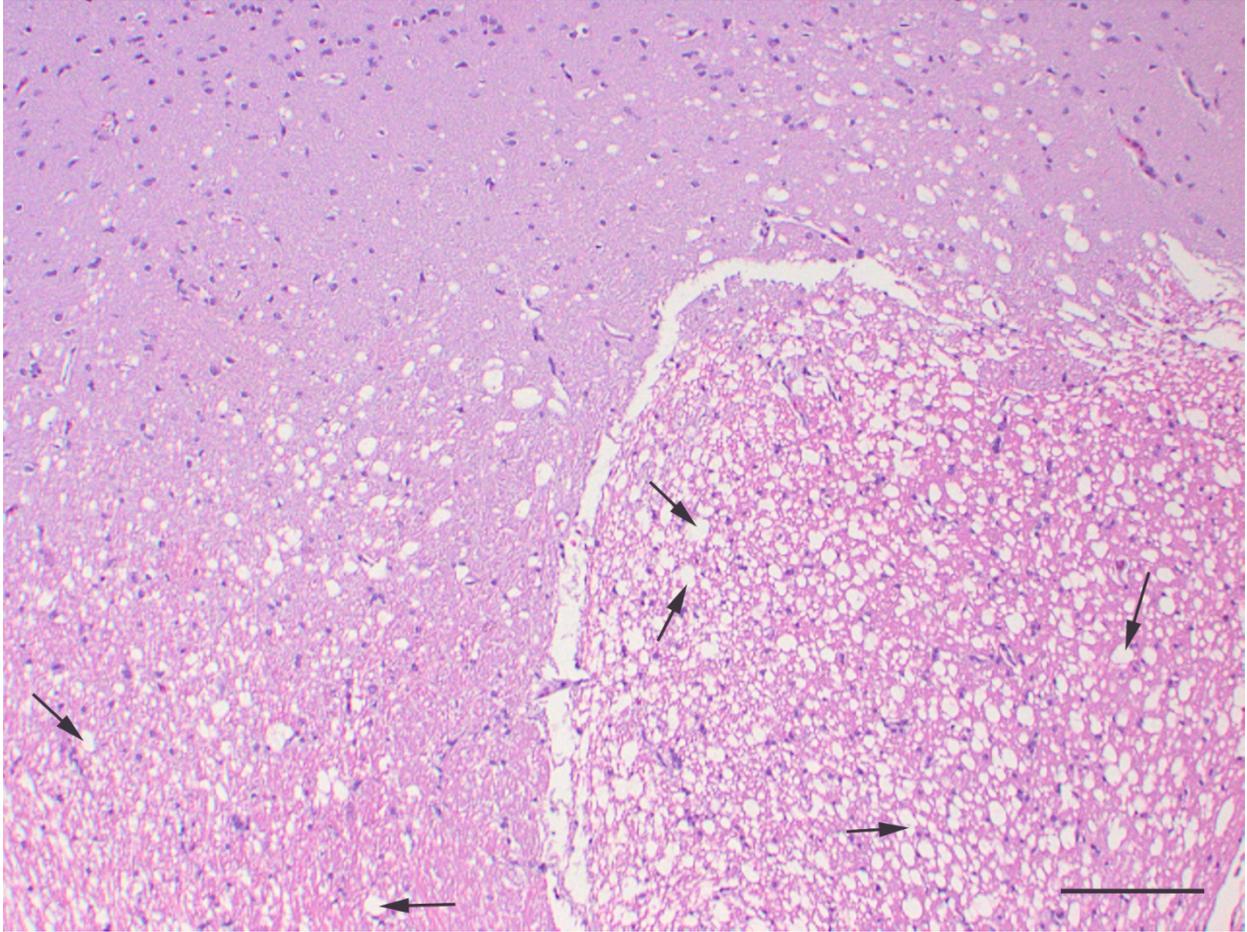


Figure 1.1: Painted turtle (*Chrysemys picta*), brain: Numerous clear vacuoles (black arrows) representing myelin degeneration and dilation of axonal sheaths are present in the white matter of a turtle treated with toxic *Hydrilla*. H&E, 100X. Scale bar is 100  $\mu\text{m}$ .

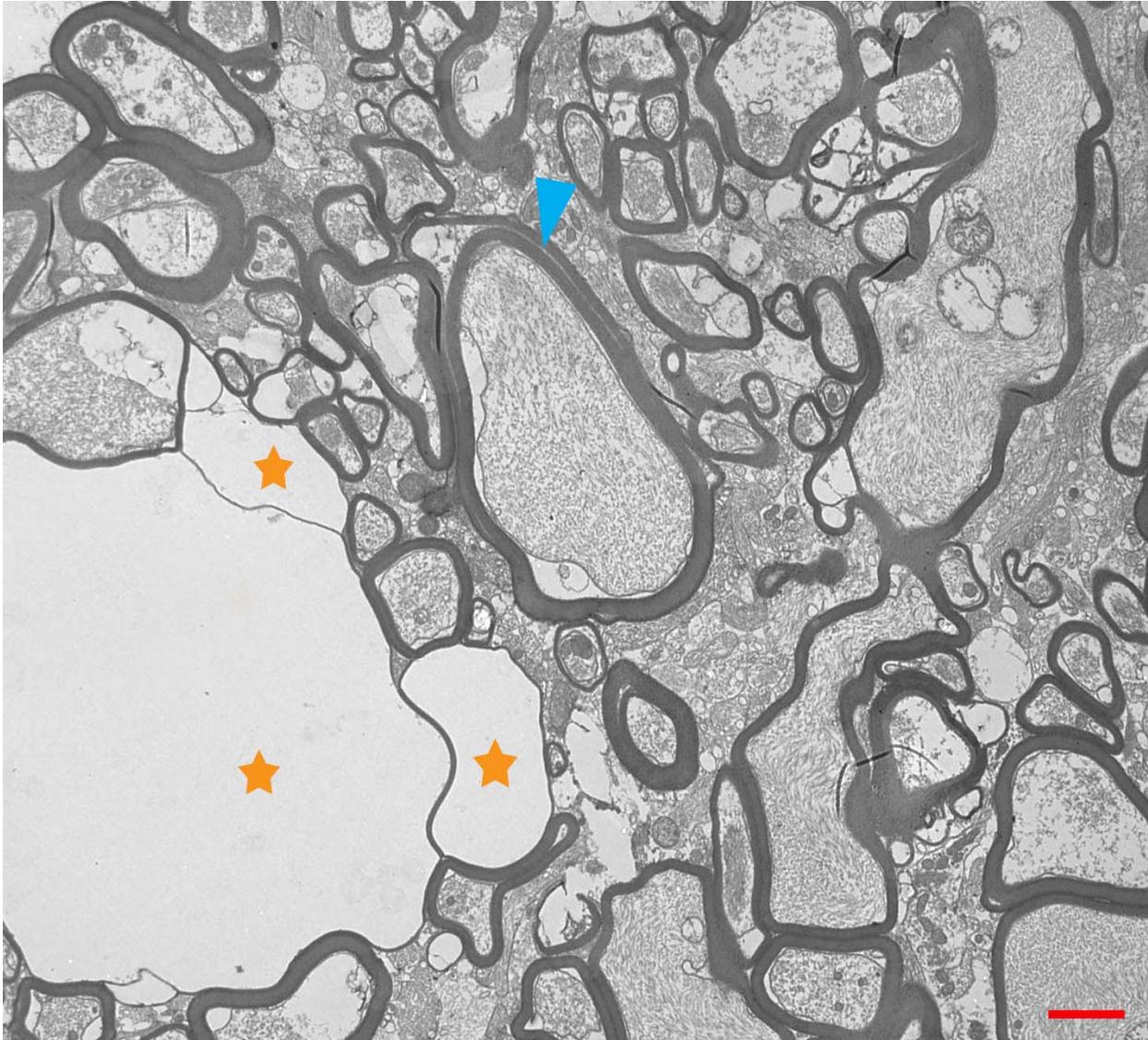


Figure 1.2: Electron Microscopy, painted turtle (*Chrysemys picta*), brain: Axons are swollen and degenerate and myelin sheaths are frequently disrupted by large, clear, intramyelinic vacuoles (orange stars). In less severely affected axons, splitting can be seen to occur at the intraperiod lines (blue arrow). Scale bar is 2  $\mu\text{m}$ .

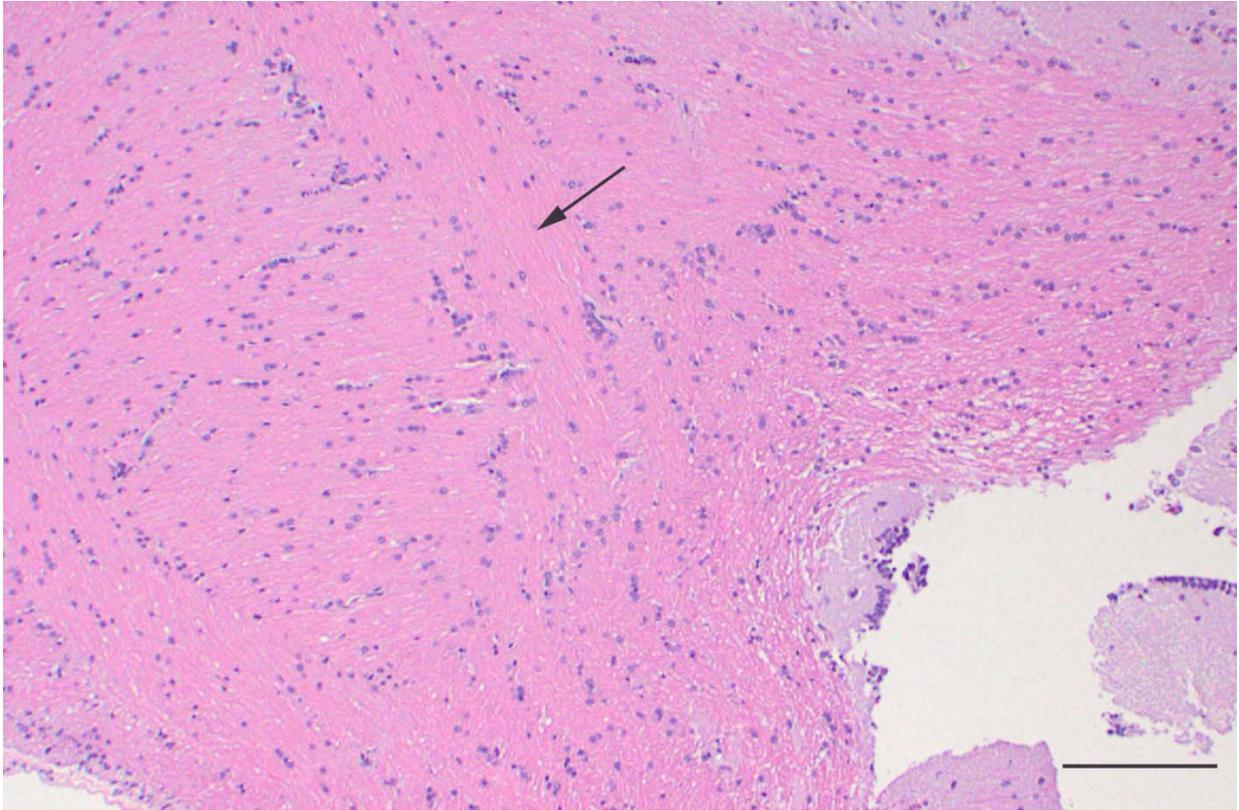


Figure 1.3: Painted turtle (*Chrysemys picta*), brain: white matter, indicated by black arrows, appears normal with no evidence of vacuolation or myelin degeneration. H&E, 100X. Scale bar is 100  $\mu\text{m}$ .

## CHAPTER 3

# INVASIVE PLANT EFFECTS ON PATCH OCCUPANCY PATTERNS OF FRESHWATER TURTLES IN SOUTHEASTERN U.S. RESERVOIRS

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<sup>1</sup> Mercurio, Albert D., Sonia M. Hernandez, John C. Maerz, Michael J. Yabsley, Jeffrey Hepinstall-Cymerman, and Susan B. Wilde. To be submitted to the *Journal of Wildlife Management*.

## ABSTRACT

*Hydrilla verticillata* is a submerged aquatic plant that has invaded much of the continental United States. *Hydrilla* changes aquatic habitat structure, composition, chemistry, and can facilitate vacuolar myelinopathy, a neurologic disease of wildlife. We estimated patch occupancy patterns in five reservoirs to determine if *Hydrilla* invasion affects turtle habitat use or abundance. *Hydrilla* was positively associated with patch occupancy or detection for painted turtles, yellow-bellied sliders (*Trachemys scripta*), and common musk turtles (*Sternotherus odoratus*), but not common snapping turtles (*Chelydra serpentina*). *Hydrilla* beds appear attractive to three of the four species studied. These results suggest *Hydrilla* is positively affecting habitat use or abundance of turtles inhabiting reservoirs; however, if occupancy of *Hydrilla* invaded habitats increases turtle exposure to cyanotoxins, then *Hydrilla* invasions may be creating an ecological trap for turtle populations.

## INTRODUCTION

The invasion of non-indigenous plant species is one of the primary threats to biodiversity and the integrity, resilience, and function of ecosystems (Usher 1988, Drake et al. 1989, Macdonald et al. 1989, Congress 1993, Randall and Marinelli 1996, Vitousek et al. 1997, Westbrooks 1998, Blossey 1999, Westbrooks 2004). Invasive plants can change fire regimes, biogeochemical cycling, geomorphological processes, hydrological cycles, prevent recruitment or reproduction of native species, hybridize with native species, affect human health, and facilitate wildlife diseases (Randall and Marinelli 1996, Gordon 1998, Westbrooks 1998, Blossey 1999, Mack et al. 2000, Crooks 2002, Rocke et al. 2005, Schultz and Dibble 2012).

The rate of plant invasions in natural systems has increased by several orders of magnitude in the last century, bringing this issue to the forefront of ecological and conservation

research (Mooney and Drake 1986, Drake et al. 1989, Soulé 1990, Pysek 1995, Gordon 1998). Approximately 5,000 plant species are naturalized in the United States, 10% of which are seriously invasive (Congress 1993, Blossey 1999), thus, predictions and quantifications of changes in ecosystem processes and effects to wildlife are lacking for most of these species. However, generally speaking, it is expected that invasive species alter the habitat they invade, likely affecting all trophic levels because plants play a major role in the construction and maintenance of ecosystems, support food webs, regulate microclimates, and influence energy flow through systems by altering habitat structure and composition (D'Antonio and Vitousek 1992, Mack et al. 2000, Carvalheiro et al. 2010, DeVore and Maerz 2014). Three primary mechanisms by which invasive plants in terrestrial and aquatic systems can affect consumers are the alteration of the flow, availability, or quality of nutrient resources within biogeochemical cycles, alteration of trophic resources within food webs, and alteration of physical resources such as living space, sediment, light, or water (Vitousek 1996, Crooks 2002, DeVore 2011).

Invasion by nonnative aquatic macrophytes is also a common driver of freshwater plant community change (Bunn and Arthington 2002, Abell et al. 2009). Many tropical species of plants from Africa, Asia, and South America are notably adventitious in freshwater systems within the U.S. including alligatorweed (*Alternanthera philoxeroides*), Eurasian watermilfoil (*Myriophyllum spicatum*), water hyacinth (*Eichhornia crassipes*), and *Hydrilla verticillata* (USDA, 2010). These plants change aquatic habitat structure, outcompete many native plant species, alter flow regimes, can affect native fish and wildlife that depend on native flora for forage or cover, and have caused hundreds of millions of dollars in damages to affected ecosystems (Pimentel et al. 2005, Rahel et al. 2008, Abell et al. 2009).

*Hydrilla verticillata* was introduced to the United States in the 1950's and 1960's from Southeast Asia to southern Florida. By the late 1970's, *Hydrilla* had spread through most of Florida's freshwater systems, continued to expand, and was designated a federal noxious weed by the United States Department of Agriculture (Langeland 1996, Puri et al. 2007, Regulations and Kaplan 2011). Aquarium releases, contaminations by boats, trailers, nets, fishing gear as well as mechanical transfer by migratory aquatic birds have all contributed to this widespread expansion (MacDonald et al. 2008). *Hydrilla* rapidly out-competes native macrophytes for resources by rapidly growing to the surface of the water and spreading laterally which blocks available light for other species. It also can propagate via seeds, cuttings, or tubers, can colonize areas with low light levels and high turbidity, and can persist in dry sediment when water levels vary whereas many native plants cannot (Langeland 1996). *Hydrilla* can be managed over time with herbicides and/or triploid grass carp (*Ctenopharyngodon idella*), yet resistant varieties are increasingly observed (Puri et al. 2007).

*Hydrilla* invasions can lower water quality, reduce phytoplankton diversity, and alter aquatic invertebrate community structure (McCann et al. 1996, Colon-Gaud et al. 2004, Wilde et al. 2005). In addition, *Hydrilla* forms thick mats throughout the water column that displace or eliminate many fish species (Langeland 1996). Despite these negative effects to native flora and fauna, there is controversy surrounding management actions because in some cases, *Hydrilla* can provide vital ecosystem services to water bodies that lack natural vegetation, such as anthropogenic impoundments. *Hydrilla* is considered a good food source for waterfowl and provides cover for juvenile sport fish including *Macropterus* spp. and *Lepomis* spp. (Colle and Shireman 1980) however see (Langeland 1996, Perret 2007). Migratory waterfowl use lakes with abundant aquatic plants (including *Hydrilla*) extensively as stopovers during migration and

overwintering habitats and densities of waterfowl tend to increase as *Hydrilla* density increases (Johnson and Montalbano 1987, Esler 1990). Until the effects of *Hydrilla* on all freshwater taxa are fully understood, the effects of *Hydrilla* invasions and management are hard to predict.

The southeastern U.S., the nidus of *Hydrilla* invasions in the United States, hosts 10% of the world's freshwater turtle diversity (Langeland 1996, Buhlmann et al. 2009). Some specialist turtle species are influenced by overharvesting, road mortality, and habitat loss and degradation, such as the impoundment of rivers (Gibbons et al. 2000, Buhlmann et al. 2009). Historically, the southeastern U.S. had few natural lakes or permanent water bodies (Lydeard and Mayden 1995). Instead, the landscape was dominated by isolated and riparian wetlands, streams, and rivers. Most isolated wetlands have been lost to agriculture, and the creation of more permanent water bodies including "farm" ponds and river impoundments for recreational use, hydropower production, or drinking water have dramatically altered the freshwater landscape of the southeastern U.S (Bunn and Arthington 2002). This loss of wetlands and the degradation of streams and rivers has impacted habitat specialist turtle species (Bodie and Semlitsch 2000, Bodie et al. 2000, Bodie 2001, Anderson et al. 2002, Moll 2004). However, habitat generalists often occupy impounded waters at high densities (DonnerWright et al. 1999, Rizkalla and Swihart 2006, Tucker et al. 2012).

Freshwater impoundments are much more susceptible to plant invasions (Bunn and Arthington 2002). In general, it is known that habitat changes resulting from *Hydrilla* invasions and the impoundment of rivers work independently and synergistically to simplify the geomorphology of the channel, the biotic community, and the interactions among biota, but how these invasions affect turtles in freshwater impoundments has been relatively understudied (Bjorndal et al. 1997, Bunn and Arthington 2002, Fields et al. 2003, Moll 2004, Tucker et al.

2012). Freshwater turtles directly consume aquatic plants, macroinvertebrates and fish that feed on or reside in patches of aquatic plants, and use areas of plants for cover (Ernst and Lovich 2009). Many turtle species will consume *Hydrilla* directly, however it has a lower nutritional content than many of the native plant species that it replaces (Bjorndal et al. 1997, Fields et al. 2003). Studies have documented that turtles may be attracted to areas with dense native plant cover, and abundance of some species indeed increases with primary productivity (Galbraith et al. 1988, Buhlmann and Vaughan 1991, DonnerWright et al. 1999, Hartwig and Kiviat 2007a, Ernst and Lovich 2009). However, thick aquatic vegetation can also impede turtle movement, reducing the habitat suitability of extremely dense patches and decreasing the overall abundance of turtles within these water bodies (Black 2000, Marchand and Litvaitis 2004). Furthermore, poor water quality resulting from *Hydrilla* invasions can significantly reduce phytoplankton diversity and alter aquatic invertebrate communities that turtles may depend on for food (McCann et al. 1996, Colon-Gaud et al. 2004). *Hydrilla* may also reduce the abundance of fish species on which some turtle species prey (Langeland 1996, Perret 2007).

In addition to habitat changes, the recent emergence of a disease associated with *Hydrilla* presence, vacuolar myelinopathy (VM), has increased concern about how *Hydrilla* invasions affect aquatic wildlife. Consumption of *Hydrilla* material colonized by a yet undescribed toxin-producing species of cyanobacteria [hereafter referred to as *Hydrilla*+UC] causes lethargy and ataxia in birds, which eventually leads to their death (Thomas et al. 1998). Since its discovery in 1994, deaths of greater than 100 bald eagles (*Haliaeetus leucocephalus*) and thousands of American coots (*Fulica americana*) are attributed to VM from consuming *Hydrilla*+UC and the disease has been documented in eight other species of birds (Rocke et al. 2002, Augspurger et al. 2003, Lewis-Weis et al. 2004, Fischer et al. 2006). Clinical signs (and death) consistent with

VM caused by the consumption of *Hydrilla*+UC has also been demonstrated both experimentally and naturally in grass carp and painted turtles (*Chrysemys picta*) (Haynie et al. 2013, Mercurio et al. 2014).

In this study, we aimed to identify the importance of *Hydrilla* cover on turtle communities in southeastern U.S. reservoirs. Because freshwater turtle species partition themselves within microhabitats based on habitat preferences or requirements, such as plant density, an analysis of habitat use within patches of varying *Hydrilla* density can provide insight into the effects of *Hydrilla* invasions on freshwater turtles (Brewster and Brewster 1991, Harrel et al. 1996, Hartwig and Kiviat 2007b, Steen et al. 2007, Vecchio et al. 2011, Anthonysamy et al. 2014). Occupancy modeling estimates the probability that a species is occupying a certain pre-defined patch of habitat (MacKenzie et al. 2003, MacKenzie 2006). It is a viable method for species with low recapture rates, such as freshwater turtles, because occupancy patterns can provide insight into habitat use and is also an efficient proxy to more time intensive measurements of abundance or demographic rates such as survival or reproduction (MacKenzie et al. 2003, MacKenzie 2006, Mazerolle et al. 2007). We used patch occupancy after accounting for incomplete detection to measure the effects of *Hydrilla* density and other factors on turtle use of habitats within southeastern U.S. reservoirs for two omnivorous-highly herbivorous species (eastern painted turtle, *Chrysemys picta*; yellow-bellied slider, *Trachemys scripta*), and two carnivorous species (common snapping turtle, *Chelydra serpentina*; common musk turtle, *Sternotherus odoratus*). We hypothesized that invasive plant density would be negatively correlated with the probability of occupancy for all of the species studied.

## MATERIALS AND METHODS

### *Study area*

During July and August of 2012 we sampled turtles at 53 littoral zone locations dispersed within five reservoirs in the Ocmulgee River Basin in central Georgia, USA (Fig.3.1). The reservoirs had little native aquatic vegetation cover, which was typical of reservoirs in the region.

Long Branch Reservoir (LBR; 33.3155°N, -84.1321°W) is a 277-acre reservoir for drinking water constructed in 1997 and is surrounded by a forested buffer and service roads. Submerged aquatic vegetation was first noted in 2007 but not identified as *Hydrilla* until October 2010 (S. Wilde pers. comm). VM was confirmed at this site in 2011 in wild American coots and sentinel mallards (*Anas platyrhynchos*). No treatments are currently being conducted to control the *Hydrilla* infestation in Long Branch and it is closed to public use.

Upper Towaliga Reservoir (UTR; 33.3293° N, -84.2066°W) is a 1100-acre reservoir and is surrounded primarily by a forested buffer with some agricultural fields and one golf course. *Hydrilla* was first noted in 2008 and has since become established throughout the coves and along the entire shoreline of the lake. VM was first documented in a bald eagle during 2010, and since then in wild American coots and sentinel mallards (S. Wilde, pers comm). Current management includes triploid grass carp and copper sulfate treatments to control *Hydrilla*. Upper Towaliga was closed to public use during this study.

Tussahaw Reservoir (THW; 33.3445°N, -83.8646°W) is the largest of the five reservoirs (1466 acres) and the only location at which *Hydrilla* has not yet been reported. Tussahaw is primarily surrounded by a forested buffer zone with a few scattered private residences. This reservoir was created and filled in 2005, is used for drinking water and is currently open to recreational angling and limited to the use of electric trolling motors.

*Lake Varner* (LV; 33.6639° N, -83.8076°W) is an 850-acre drinking water reservoir that was built in 1991. *Hydrilla* was documented in this lake in 2008 and VM has been documented in bald eagles and american coots since 2007(S. Wilde, pers comm.). Lake Varner is primarily surrounded by a forested buffer zone and agricultural land with a few scattered private residences. The lake is also used for recreational angling.

Lake Walton is a 200-acre privately owned reservoir and is surrounded by private residences and two large subdivisions, forests, and agricultural land. The lake has been used by residents for livestock watering and recreational fishing since its creation in 1994. The earthen dam on the south side of the lake was destroyed by Hurricane Opal in 1995, but was quickly repaired the following year. *Hydrilla* was first noticed in Lake Walton during the summer of 2008 and has since spread throughout the reservoir. VM has not been documented on this lake.

#### *Site Selection*

Within the five study reservoirs, we used visual surveys to identify patches of distinct vegetation or non-vegetated habitat within the reservoir. Patches could vary in size, minimum patch size was approximately 0.5 acres of surface water area, and were defined as portions of the reservoir margin that were visibly uniform in the presence or absence of native plants or *Hydrilla*. We classified patches to encompass as much variation as possible in *Hydrilla* density, substrate type, number of basking surfaces, and whether the location was on exposed shoreline or in a cove. To reduce spatial autocorrelation amongst patches, we geographically stratified the patches we sampled in each reservoir to maximize the distances between plots. Patches were at least 200m apart, and we sampled 8-15 patches per reservoir (Figure 3.2).

### *Turtle Trapping*

We used hoop net traps (model TN210; Memphis Net and Twine Co, Inc., Memphis, TN) baited with sardines to capture turtles. Within each patch, we placed two traps 100 m apart. To estimate detection rates, we trapped each patch daily for three consecutive days, and we assumed that patches were closed to immigration or emigration during that three-day period. We pooled capture data for the two traps in each patch, and generated species-specific detection strings for each patch in our analyses. We also individually marked nearly all of the turtles captured during this study although mark-recapture analyses were not conducted.

### *Estimating Habitat Variables*

We measured plant cover and a suite of other variables known to affect turtle abundance or habitat use (Table 3.1). We measured percent dissolved oxygen (DO, mg/L), water temperature (H<sub>2</sub>O Temp, °F), turbidity (Turb, Nephelometric Turbidity Units), and pH at every patch (average of three measurements during one survey at three points within the patch, one at each trap and one equidistant between traps) using a YSI multiparameter system (YSI 556 MPS, YSI Inc/Xylem Inc, Yellow Springs, OH) and a turbidimeter (Model 2100p; Hach Inc, Loveland, CO). We counted the number of basking surfaces (Bask) visible within a 100 m radius from the center of each patch. We measured substrate type (Substrate) at three locations within each patch and categorized substrate types as primarily organic soil, inorganic soil (hard packed clay), sand, sand/inorganic soil, or rocky. We estimated the density of *Hydrilla* cover in each patch by submerging a five gallon bucket with the bottom removed into three points at each patch (one at each trap location and one equidistant between the two traps), and collecting all plant material within the bucket. *Hydrilla* was separated from all other material, dried and weighed to obtain a

dry mass per 15 gallons of lake volume, which we used as an index of *Hydrilla density* at each patch.

### *Modeling Detection and Occupancy*

To estimate detection (p) and occupancy (psi) rates, we analyzed the detection/non-detection data using Program PRESENCE 5.5 (<http://www.mbr-pwrc.usgs.gov/software/presence.html>). PRESENCE uses information on detection probability and likelihood-based log-linear models to estimate occupancy of a site by a species and also allows you to model detection and occupancy as a function of survey level and site level covariates (MacKenzie et al. 2003). Survey data were run using single-season models.

Because turtles are less likely to be trapped on extremely hot days or during severe weather events, daily maximum air temperature (MaxTemp, °F) for each sampling date was collected from the Josnesboro, GA weather station from the National Weather Service and daily precipitation (Precip, inches per 24 hour period) was obtained from the National Weather Service for the respective counties of each reservoir (<http://www.nws.noaa.gov/climate/xmacis.php?wfo=ffc>). Because abundance can influence detection, we also included habitat variables in our detection models. We developed competing models that included combinations of covariates to account for factors that may influence detection, which represented hypothesized influences on turtle detection rates (Table 3.2). We included a null model [psi(.)p(.)] to determine if these covariates explained detection rates better than a model that assumed a constant detection rate across all surveys. The best-supported detection model was then applied to all models representing turtle occupancy rates. We assessed collinearity among the survey and site covariates using Spearman rank correlations in R (R v. 2.11.1 Development Core Team 2014). None of our variables were highly correlated ( $r \geq 0.60$ ).

We used variables in Table 3.1 to create a series of single-species occupancy models that reflected hypothesized influences on turtle occupancy (Table 3.3) to ensure that our results are biologically meaningful (Burnham and Anderson 2002). The number of basking sites and *Hydrilla* density were normalized to a mean of 0 and a standard deviation of 1 to facilitate model convergence (MacKenzie 2006). We determined if blocking by lake had a stronger support vs. a null occupancy model [ $\psi(\cdot)$ ] with detection being modeled with the optimum covariates determined previously.

We ranked our models using Akaike's Information Criteria adjusted for small sample sizes (AIC<sub>c</sub>; (Hurvich and Tsai 1989, Burnham and Anderson 2002). We calculated AIC<sub>c</sub> weights for each model and assessed the fit of each model by ranking these models from highest to lowest weight (Burnham and Anderson 2002). Models less than two AIC<sub>c</sub> units from the top ranked model were considered to have equal support. Logistic regressions were plotted for all parameter estimates that were significant (95% Confidence interval did not cross 0). We assessed the fit of the global model for each species by comparing the observed Pearson's chi-square statistic to the expected chi-square statistic generated with 10,000 bootstraps (MacKenzie and Bailey 2004).

## RESULTS

### *Detection Models*

We detected painted turtles in 17 of 53 sites, common snapping turtles in 10 of 53 sites, yellow-bellied sliders in 46 of 53 sites, and common musk turtles in 14 of 53 sites for naïve occupancy estimates of 32%, 19%, 87%, and 26%, respectively. The model that included *Hydrilla* density was the best supported and had moderately high support for painted turtles ( $w=0.3725$ , Table 3.4) and yellow-bellied sliders ( $w=0.4779$ , Table 3.5). The best supported

model for common snapping turtles included the number of basking surfaces as a covariate ( $w=0.269$ , Table 3.6) The best supported model for estimating common musk turtle detection rates included MaxTemp and Precip as covariates and had moderately high support ( $w=0.3607$ , Table 3.7). The values of the  $\beta$  coefficients which represent the direction of the effects of covariates on detection probability indicated that detection rates of painted turtles and yellow-bellied sliders increased with *Hydrilla* density ( $\beta=1.38$ , 95% CI: 0.65- 2.12, Figure 3.3, Table 3.8;  $\beta=0.43$ , 95% CI: 0.02-0.83, Figure 3.4, Table 3.8, respectively). The confidence intervals for the  $\beta$  estimate representing the effect of basking surfaces on common snapping turtle detection rates crossed 0 (Table 3.8). Common musk turtle detection increased with MaxTemp ( $\beta=0.132$ , 95% CI: 0.110- 0.155, Figure 3.5 Table 3.8) but was not correlated with Precip ( $\beta=4.3445$ , 95% CI: -2.403- 11.092, Table 3.8). Other models had equal support to the top model for all species (they were less than two AICc units from the top model), however the  $\beta$  coefficients included 0 in the 95% confidence intervals, indicating parameter estimate uncertainty was high (Table 3.8). Therefore, for painted turtles and yellow-bellied sliders, the top model including *Hydrilla* as a covariate was used in our occupancy analysis. The model that kept detection constant across sites was used in our occupancy analysis of common snapping turtles. For common musk turtles, we chose the top model which included MaxTemp and Precip covariates in our occupancy analysis. These models estimated patch occupancy probabilities of 59% (95% CI = 0.34 - 0.80) for painted turtles, 76% (95% CI 0.003-1.000) for common snapping turtles, 94% (95% CI 0.71-0.99) for yellow-bellied sliders, and 34 % for common musk turtles (95% CI 0.204 - 0.517).

### *Occupancy Models*

In our occupancy analysis of painted turtles and common snapping turtles, the top model included the number of basking surfaces ( $\psi(\text{Bask})$   $w= 0.5792$  and  $w = 0.4785$ , respectively,

Table 3.9). For yellow-bellied sliders two models had equal support ( $\psi(\cdot)$   $w=0.5005$ ;  $\psi(\text{Bask})$   $\Delta\text{AICc}=1.56$   $w=0.229$ , Table 3.11). The  $\beta$  coefficients which represent the direction of effects of these covariates on occupancy probability included 0 in the 95% confidence intervals, indicating parameter estimate uncertainty was high (Table 3.12). In our occupancy analysis of common musk turtles, the top two models had equal support ( $\psi(\text{Hydrilla})$   $\Delta\text{AICc}=0$ ,  $w=0.588$ ;  $\psi(\text{Bask},\text{Hydrilla})$   $\Delta\text{AICc}=1.86$ ,  $w=0.232$  Table 3.13). Within both models the  $\beta$  coefficients representing of the effect of *Hydrilla* on patch occupancy probability were positive and the confidence intervals did not cross 0 (Table 3.12, Fig 3.6). However, the  $\beta$  coefficients of the effect of the number of basking surfaces on occupancy probability included 0 in the 95% confidence intervals (Table 3.12).

#### *Assessing Model Fit*

All global models fit when we compared the observed Pearson's chi-square statistic to the expected chi-square statistic generated with 10,000 bootstraps (painted turtle  $p \geq 0.089$ ,  $\hat{c} \leq 1.580$ ; common snapping turtle:  $p \geq 0.851$ ,  $\hat{c} \leq 0.351$ ; yellow-bellied slider:  $p \geq 0.921$ ,  $\hat{c} \leq 0.466$ ; common musk turtle:  $p \geq 0.732$ ,  $\hat{c} \leq 0.579$ ).

## DISCUSSION

Our results indicate *Hydrilla* density was positively correlated with detection probability for painted turtles and yellow-bellied sliders and patch occupancy of common musk turtles. We did not find any correlations between detection rates or patch occupancy and the number of available basking surfaces, substrate type, or water quality parameters for any species studied, which are habitat variables frequently considered relevant to shape the distribution and abundance of these species either directly or indirectly. Therefore, we reject our hypothesis that *Hydrilla* density is negatively correlated with patch occupancy of these four species. Detection is

determined by two factors, catchability and abundance (Seber 1986). Increases in *Hydrilla* density might increase catchability if more cover increases the activity of turtles. However, it is more likely that increasing *Hydrilla* density would negatively affect catchability. Dense plants slow water flow and increase baffling of chemicals dispersing throughout the water, which should reduce the effective range of baited traps in dense plant stands (Webster and Weissburg 2009). Dense plants can also impede the movement of some turtles (Black 2000, Marchand and Litvaitis 2004). We marked most turtles we captured, but recaptured less than 1% of individuals. This is strong evidence that *Hydrilla* was not increasing individual capture rates, but rather contains a higher density of individuals. Though *Hydrilla* density was not related to detection of musk turtles, we found a positive relationship between *Hydrilla* density and musk turtle patch occupancy that also suggests a positive relationship between *Hydrilla* invasion and turtle abundance. Thus, for three of our four focal species, *Hydrilla* invasion appears positively related to turtle abundance.

Differences in whether detection or occupancy was related to *Hydrilla* invasion were likely related to differences in the natural history of our focal species. First, using patch occupancy as an index of abundance among small patches is sensitive to differences in home range size among focal taxa. Species with larger home ranges are more likely to spend a portion of their time outside study patches. In our case, musk turtles were the smallest species and have small home ranges compared to painted turtles and yellow-bellied sliders, which are known to move large distances in short periods of time (Schubauer et al. 1990, Rowe 2003, Ernst and Lovich 2009). Therefore, we could have detected individuals in low *Hydrilla* density habitats that were merely transients or in portions of their home range that were not indicative of the animal's core habitat. Second, patch occupancy will be an insensitive measure for relating

abundance to patch characteristics if a focal species is so abundant as to be supersaturated within an environment (He and Gaston 2000). Painted turtles and yellow-bellied sliders are the most abundant species found within these reservoirs, so they are likely to occur in most patches though abundance may vary locally. Common snapping turtles have the largest documented home ranges of all species studied (Obbard and Brooks 1981, Galbraith et al. 1987), and overall, had a detection rate of 9% resulting in poor estimates of patch occupancy. Therefore, the absence of any measurable relationship between *Hydrilla* invasion and snapping turtle detection or abundance may reflect an absence of any effect of the plant on this turtle species or be related to the low abundance and large home range of this species.

The question that remains is whether the positive association between *Hydrilla* invasion and turtle detection or occupancy reflects a positive, benign, or negative effect of the plant on freshwater turtle populations. *Hydrilla* might serve as a resource for turtles in reservoirs, particularly in the absence of dense native plants. The abundance of freshwater turtle species is positively correlated with native plant cover (Galbraith et al. 1988, DonnerWright et al. 1999, Belleau 2008, Picard 2008), and *Hydrilla* is shown to be positively associated with some other freshwater taxa (Colle and Shireman 1980, Johnson and Montalbano 1987, Esler 1989, Esler 1990). However, if foraging within *Hydrilla* invaded patches increases turtle exposure to the cyanotoxins that cause vacuolar myelinopathy (VM) (Mercurio et al. 2014), then *Hydrilla* invasions could be creating an ecological trap (Schlaepfer et al. 2002) for turtles and other wildlife within reservoirs. While turtles are vulnerable to developing VM from consuming *Hydrilla* (Mercurio et al. 2014), exposure to the cyanotoxin and evidence of disease have not been documented in the wild. We suggest a near term need for sensitivity studies of wider suites

of taxa including those feeding directly or indirectly on *Hydrilla* with the suspect cyanobacterium.

Currently we have equivocal information on the potential impacts of *Hydrilla* invasion on freshwater turtles and other wildlife, so additional research is needed to inform management. The plant exposes turtles and other wildlife to neurological disease, but is nonetheless positively correlated with the abundance of turtles and some fish in reservoirs. We would advocate that local demographic studies and *in situ* experiments could be used to determine whether *Hydrilla* patches are beneficial to turtles or function as ecological traps. In addition, surveys of broader sets of reservoirs with a wider range of times since *Hydrilla* invasion might provide a clearer indication of any negative effects of the plant on turtle abundance. All sites in our study were invaded less within the past five years, and it is likely that any potential negative effects of *Hydrilla* invasions may not yet be detectable. Other studies of the effects of invaders have noted that it can take years for effects of invasive species to be detectable (Crooks 2005, Ewers and Didham 2006, Strayer et al. 2006). Therefore, future studies should explore turtle communities within water bodies with the longest time since invasion.

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Table 3.1. Covariates to occupancy probability and detection probability included in models both at the patch scale and reservoir scale for occupancy surveys of freshwater turtles in freshwater impoundments throughout central Georgia, USA in July and August of 2012. Hypothesized relationship and justification from published literature for each covariate are included where applicable.

Covariate	Abbreviation	Hypothesis	Support
<b>Patch covariates to occupancy and detection probability</b>			
Dissolved oxygen, water temperature, turbidity, pH	DO, H <sub>2</sub> O Temp, Turb, pH	Lower water quality may indirectly affect turtle populations via loss of invertebrate forage.	(Findlay and Houlihan 1997, DonnerWright et al. 1999, Russell et al. 2002)
Number of basking surfaces	Bask	Basking surfaces are necessary for thermoregulation and cover.	(Marchand and Litvaitis 2004)
Substrate type	Substrate	Certain substrate types provide better cover from predators or substrate for invertebrate prey.	(Galbraith et al. 1988, Marchand and Litvaitis 2004)
<i>Hydrilla</i> density	<i>Hydrilla</i>	<i>Hydrilla</i> density is negatively correlated with turtle abundance.	**The primary question for this research**
<b>Reservoir scale covariates to occupancy and detection probability</b>			
Block by lake	BlockbyLake	Blocking is always desirable in field studies to account for any extraneous variation.	(Feinsinger 2001)
<b>Covariates to detection probability</b>			
Daily maximum air temperature	MaxTemp	Turtles less likely to be trapped on extremely hot days.	(Mazerolle et al. 2007, Ernst and Lovich 2009)
Daily precipitation	Precip	Turtles less likely to be trapped during severe storms.	(Mazerolle et al. 2007, Ernst and Lovich 2009)

Table 3.2. Description of models used to model painted turtle (*Chrysemys picta*), yellow-bellied slider (*Trachemys scripta*), common musk turtle (*Sternotherus odoratus*), and common snapping turtle (*Chelydra serpentina*) detection rates. Occupancy ( $\psi$ ) was held constant for all models.

Model	Hypothesis
p (.)	Detection probability is constant across all sites
pBlockbyLake)	Detection probability is correlated with the lake sampled
p(MaxTemp)	Detection probability decreases with increasing temperature
p(Precip)	Detection probability decreases with increasing precipitation
p(MaxTemp+Precip)	Detection probability decreases with increasing temperature and precipitation
p ( <i>Hydrilla</i> )	Detection probability decreases with density of <i>Hydrilla</i>
p (Bask)	Detection probability increases with number of basking surfaces
p (Bask, <i>Hydrilla</i> )	Detection probability increases as a function of increased basking surfaces and decreased <i>Hydrilla</i> density
p (Substrate)	Detection probability increases as substrate quality increases.
pBask,Substrate)	Detection probability increases with number of basking surfaces and increasing substrate quality.
p ( <i>Hydrilla</i> ,Substrate)	Detection probability increases as a function of decreased <i>Hydrilla</i> density and increasing basking surfaces.
p (Bask,Substrate, <i>Hydrilla</i> )	Detection probability increases as a function of decreased <i>Hydrilla</i> density, increasing substrate quality, and increasing basking surfaces.
p(DO,H <sub>2</sub> O Temp,pH,Turb)	Detection probability increases as water quality increases.
p(Bask,Substrate, <i>Hydrilla</i> ,DO,pH,Turb,H <sub>2</sub> O Temp)	Detection probability increases as a function of decreased <i>Hydrilla</i> density, increasing substrate quality, increasing water quality, and increasing basking surfaces.
p(Bask,Substrate, <i>Hydrilla</i> ,DO,pH,Turb,H <sub>2</sub> O Temp,MaxTemp,Precip)	Detection probability increases as a function of decreased <i>Hydrilla</i> density, increasing substrate quality, increasing water quality, increasing basking surfaces, air temperature, and precipitation.

Table 3.3 Description of models used to model painted turtle (*Chrysemys picta*), yellow-bellied slider (*Trachemys scripta*), common musk turtle (*Sternotherus odoratus*), and common snapping turtle (*Chelydra serpentina*) occupancy rates and their hypothesized relationship. Probability of detection ( $p$ ) was modeled with different covariates depending on the species studied.

Model	Hypothesis
psi(.)	Occupancy probability is constant across all sites
psi(BlockbyLake)	Occupancy probability is correlated with the lake sampled
psi( <i>Hydrilla</i> )	Occupancy probability decreases with density of <i>Hydrilla</i>
psi(Bask)	Occupancy probability increases with number of basking surfaces
psi(Bask, <i>Hydrilla</i> )	Occupancy probability increases as a function of increased basking surfaces and decreased <i>Hydrilla</i> density
psi(Substrate)	Occupancy probability increases as substrate quality increases.
psi(Bask,Substrate)	Occupancy probability increases with number of basking surfaces and increasing substrate quality.
psi( <i>Hydrilla</i> ,Substrate)	Occupancy probability increases as a function of decreased <i>Hydrilla</i> density and increasing basking surfaces.
psi(Bask,Substrate, <i>Hydrilla</i> )	Occupancy probability increases as a function of decreased <i>Hydrilla</i> density, increasing substrate quality, and increasing basking surfaces.
psi(DO,H <sub>2</sub> O Temp,pH,Turb)	Occupancy probability increases as water quality increases.
psi(Bask,Substrate, <i>Hydrilla</i> ,DO,pH,Turb,H <sub>2</sub> O Temp)	Occupancy probability increases as a function of decreased <i>Hydrilla</i> density, increasing substrate quality, increasing water quality, and increasing basking surfaces.

Table 3.4 Model selection results for painted turtle (*Chrysemys picta*) detection rates.  $\Delta AICc$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Occupancy was held constant [psi(.)] for all models. \* indicates top ranking models ( $\Delta AICc \leq 2.0$ )

Model	AICc	$\Delta AICc$	$w$	Likelihood	K	$-2\text{Log}(L)$
p( <i>Hydrilla</i> )*	129.88	0	0.3725	1	3	123.39
p(BlockbyLake)*	130.81	0.93	0.234	0.6281	7	114.32
p(Bask, <i>Hydrilla</i> )*	130.92	1.04	0.2215	0.5945	4	122.09
p(Bask,Substrate, <i>Hydrilla</i> )	132.63	2.75	0.0942	0.2528	9	110.44
p( <i>Hydrilla</i> ,Substrate)	135.74	5.86	0.0199	0.0534	8	116.47
p(.)	136.58	6.7	0.0131	0.0351	2	132.34
p(Bask)	136.68	6.8	0.0124	0.0334	3	130.19
p(Bask,Substrate)	137.61	7.73	0.0078	0.021	8	118.34
p(DO, H <sub>2</sub> OTemp,pH,Turb)	137.65	7.77	0.0077	0.0205	6	123.82
p(Precip)	137.92	8.04	0.0067	0.018	3	131.43
p(MaxTemp)	138.04	8.16	0.0063	0.0169	3	131.55
p(MaxTemp,Precip)	139.59	9.71	0.0029	0.0078	4	130.76
p(Bask,Substrate, <i>Hydrilla</i> ,DO, pH,Turb, H <sub>2</sub> OTemp)	142.04	12.16	0.0009	0.0023	13	106.71
p(Substrate)	144.23	14.35	0.0003	0.0008	7	127.74
p(Bask,Substrate, <i>Hydrilla</i> ,DO, pH,Turb,H <sub>2</sub> OTemp,MaxTemp, Precip)	148.53	18.65	0	0.0001	15	105.56

Table 3.5 Model selection results for common snapping turtle (*Chelydra serpentina*) detection rates.  $\Delta\text{AICc}$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Occupancy was held constant [ $\psi(\cdot)$ ] for all models. \* indicates top ranking models ( $\Delta\text{AICc} \leq 2.0$ ).

Model	AICc	$\Delta\text{AICc}$	$w$	Model Likelihood	K	$-2\text{Log}(L)$
p(Bask)*	82.65	0	0.269	1	3	76.65
p(.)*	83.61	0.96	0.1665	0.6188	2	79.61
p(Precip)*	83.83	1.18	0.1491	0.5543	3	77.83
p(Bask, <i>Hydrilla</i> )*	84.65	2	0.099	0.3679	4	76.65
p(MaxTemp)	84.92	2.27	0.0865	0.3214	3	78.92
p(MaxTemp,Precip)	85.22	2.57	0.0744	0.2767	4	77.22
p(DO, H <sub>2</sub> O Temp,pH,Turb)	85.25	2.6	0.0733	0.2725	6	73.25
p( <i>Hydrilla</i> )	85.6	2.95	0.0616	0.2288	3	79.6
p(Bask,Substrate)	89.57	6.92	0.0085	0.0314	8	73.57
p(Bask,Substrate, <i>Hydrilla</i> )	91.55	8.9	0.0031	0.0117	9	73.55
p(Bask,Substrate, <i>Hydrilla</i> ,DO, pH,Turb,H <sub>2</sub> O Temp,MaxTemp, Precip)	91.88	9.23	0.0027	0.0099	7	77.88
p(Substrate)	91.88	9.23	0.0027	0.0099	7	77.88
p(BlockbyLake)	92.45	9.8	0.002	0.0074	7	78.45
p( <i>Hydrilla</i> ,Substrate)	93.88	11.23	0.001	0.0036	8	77.88
p(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb, H <sub>2</sub> O Temp)	94.69	12.04	0.0007	0.0024	13	68.69

Table 3.6 Model selection results for yellow-bellied slider (*Trachemys scripta*) detection rates.  $\Delta\text{AICc}$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Occupancy was held constant [psi(.)] for all models. \* indicates top ranking models ( $\Delta\text{AICc} \leq 2.0$ )

Model	AICc	$\Delta\text{AICc}$	$w$	Model Likelihood	K	$-2\text{Log}(L)$
p( <i>Hydrilla</i> )*	212.95	0	0.4779	1	3	206.46
p(Bask, <i>Hydrilla</i> )	215.03	2.08	0.1689	0.3535	4	206.2
p(.)	215.43	2.48	0.1383	0.2894	2	211.19
p(Precip)	217.37	4.42	0.0524	0.1097	3	210.88
p(MaxTemp)	217.4	4.45	0.0516	0.1081	3	210.91
p(Bask)	217.59	4.64	0.047	0.0983	3	211.1
p(MaxTemp,Precip)	219.47	6.52	0.0183	0.0384	4	210.64
p( <i>Hydrilla</i> ,Substrate)	219.58	6.63	0.0174	0.0363	8	200.31
p(Substrate)	221.33	8.38	0.0072	0.0151	7	204.84
p(DO, H <sub>2</sub> O Temp,pH,Turb)	221.62	8.67	0.0063	0.0131	6	207.79
p(BlockbyLake)	221.65	8.7	0.0062	0.0129	7	205.16
p(Bask,Substrate, <i>Hydrilla</i> )	221.72	8.77	0.006	0.0125	9	199.53
p(Bask,Substrate)	223.57	10.62	0.0024	0.0049	8	204.3
p(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb, H <sub>2</sub> O Temp)	230.69	17.74	0.0001	0.0001	13	195.36
p(Bask,Substrate, <i>Hydrilla</i> ,DO, pH,Turb,H <sub>2</sub> O Temp,Max Temp, Precip)	238.3	25.35	0	0	15	195.33

Table 3.7 Model selection results for common musk turtle (*Sternotherus odoratus*) detection rates.  $\Delta AICc$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Occupancy was held constant [psi(.)] for all models. \* indicates top ranking models ( $\Delta AICc \leq 2.0$ ).

Model	AICc	$\Delta AICc$	$w$	Model Likelihood	K	$-2\text{Log}(L)$
p(MaxTemp,Precip)*	112.58	0	0.3607	1	4	103.75
p(Precip)*	113.41	0.83	0.2382	0.6603	3	106.92
p(DO, H <sub>2</sub> O Temp,pH,Turb)	114.63	2.05	0.1294	0.3588	6	100.8
p( <i>Hydrilla</i> )	115.07	2.49	0.1039	0.2879	3	108.58
p(MaxTemp)	116.52	3.94	0.0503	0.1395	3	110.03
p(Bask, <i>Hydrilla</i> )	116.82	4.24	0.0433	0.12	4	107.99
p(.)	117.09	4.51	0.0378	0.1049	2	112.85
p(Bask)	117.38	4.8	0.0327	0.0907	3	110.89
p(Bask,Substrate)	123.85	11.27	0.0013	0.0036	8	104.58
p(BlockbyLake)	123.93	11.35	0.0012	0.0034	7	107.44
pp( <i>Hydrilla</i> ,Substrate)	125.9	13.32	0.0005	0.0013	8	106.63
p(Bask,Substrate, <i>Hydrilla</i> )	126.5	13.92	0.0003	0.0009	9	104.31
p(Substrate)	127.46	14.88	0.0002	0.0006	7	110.97
p(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb, H <sub>2</sub> O Temp)	134.06	21.48	0	0	13	98.73
p(Bask,Substrate, <i>Hydrilla</i> ,DO, pH,Turb,H <sub>2</sub> O Temp,MaxTemp, Precip)	134.56	21.98	0	0	15	91.59

Table 3.8. Parameter estimates for occupancy models results to determine detection as a factor of covariates for the painted turtle (*Chrysemys picta*), common snapping turtle (*Chrysemys picta*), yellow-bellied slider (*Trachemys scripta*), and common musk turtle (*Sternotherus odoratus*) in freshwater reservoirs in central Georgia in 2012. Models with  $\Delta AICc < 2$  are shown.

Model	Parameter	$\beta$	SE	95% CI	
<i>C.picta</i>					
<i>psi(.)p(Hydrilla)</i>					
	<i>Hydrilla</i>	1.38	0.38	0.65	2.12
<i>psi(.)p(BlockbyLake)</i>					
	LV	5.70	11.98	-17.79	29.18
	LW	4.34	11.97	-19.12	27.80
	TH	-23.52	307504.89	-602733.10	602686.06
	LB	3.48	11.97	-19.98	26.94
	UT	5.42	11.97	-18.03	28.87
<i>psi(.)p(Bask,Hydrilla)</i>					
	Bask	0.30	0.26	-0.21	0.82
	<i>Hydrilla</i>	1.32	0.38	0.58	2.06
<i>C.serpentina</i>					
<i>psi(.)p(bask)</i>					
	Bask	0.50	0.28	-0.05	1.05
<i>psi(.)p(Precip)</i>					
	Precip	-0.99	1.03	-3.02	1.04
<i>psi(.)P(Bask,Hydrilla)</i>					
	<i>Hydrilla</i>	0.02	0.32	-0.61	0.65
	Bask	0.50	0.28	-0.05	1.05
<i>T. scripta</i>					
<i>psi(.)p(Hydrilla)</i>					
	<i>Hydrilla</i>	0.43	0.21	0.02	0.83
<i>S.odoratus</i>					
<i>psi(.)p(MaxTemp,Precip)</i>					
	MaxTemp	0.13	0.01	0.11	0.16
	Precip	4.34	3.44	-2.40	11.09
<i>psi(.)p(Precip)</i>					
	Precip	3.52	2.83	-2.02	9.05

Table 3.9. Model selection results for painted turtle (*Chrysemys picta*) occupancy rates..  $\Delta AICc$  represents the change in  $AICc$ ,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Probability of detection (p) included *Hydrilla* density as a covariate.

Model	AICc	$\Delta AICc$	$w$	Model Likelihood	K	$-2\text{Log}(L)$
psi(Bask)*	126.55	0	0.5792	1	4	117.72
psi(Bask, <i>Hydrilla</i> )	128.75	2.2	0.1928	0.3329	5	117.47
psi(.)	129.88	3.33	0.1096	0.1892	3	123.39
psi(Bask,Substrate, <i>Hydrilla</i> )	131.31	4.76	0.0536	0.0926	10	106.07
psi( <i>Hydrilla</i> )	131.65	5.1	0.0452	0.0781	4	122.82
psi(Bask,Substrate)	134.38	7.83	0.0115	0.0199	9	112.19
psi(BlockbyLake)	136.97	10.42	0.0032	0.0055	8	117.7
psi(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb, H <sub>2</sub> O Temp)	137.38	10.83	0.0026	0.0044	14	98.33
psi(DO,H <sub>2</sub> O Temp,pH,Turb)	138.19	11.64	0.0017	0.003	7	121.7
psi(Substrate)	140.87	14.32	0.0005	0.0008	8	121.6
psi( <i>Hydrilla</i> ,Substrate)	143.38	16.83	0.0001	0.0002	9	121.19

Table 3.10. Model selection results for common snapping turtle (*Chelydra serpentina*) occupancy rates..  $\Delta AICc$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Probability of detection ( $p$ ) was kept constant for all models.

Model	AICc	$\Delta AICc$	$w$	Model Likelihood	K	$-2\text{Log}(L)$
psi(Bask)*	80.89	0	0.4785	1	3	74.4
psi(Bask, <i>Hydrilla</i> )	83.19	2.3	0.1515	0.3166	4	74.36
psi(Bask,Substrate)	83.33	2.44	0.1413	0.2952	8	64.06
psi(.)	83.85	2.96	0.1089	0.2276	2	79.61
psi( <i>Hydrilla</i> )	84.82	3.93	0.0671	0.1402	3	78.33
psi(DO,H2OTemp,pH,Turb)	86.61	5.72	0.0274	0.0573	6	72.78
psi( <i>Hydrilla</i> ,Substrate)	88.21	7.32	0.0123	0.0257	8	68.94
psi(Bask,Substrate, <i>Hydrilla</i> )	88.23	7.34	0.0122	0.0255	9	66.04
psi(Substrate)	95.08	14.19	0.0004	0.0008	7	78.59
psi(BlockbyLake)	95.7	14.81	0.0003	0.0006	7	79.21
psi(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb,H2O)	97.54	16.65	0.0001	0.0002	13	62.21

Table 3.11. Model selection results for yellow-bellied slider (*Trachemys scripta*) occupancy rates.  $\Delta\text{AICc}$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log}(L)$  = the negative 2 log likelihood. Probability of detection ( $p$ ) included *Hydrilla* density as a covariate.

Model	AICc	$\Delta\text{AICc}$	$w$	Model Likelihood	K	$-2\text{Log}(L)$
psi(.)*	212.95	0	0.5005	1	3	206.46
psi(Bask)*	214.51	1.56	0.2294	0.4584	4	205.68
psi( <i>Hydrilla</i> )	215.06	2.11	0.1743	0.3482	4	206.23
psi(Bask, <i>Hydrilla</i> )	216.44	3.49	0.0874	0.1746	5	205.16
psi(Substrate)	223.53	10.58	0.0025	0.005	8	204.26
psi(BlockbyLake)	224.18	11.23	0.0018	0.0036	8	204.91
psi(DO,H2OTemp,pH,Turb)	224.45	11.5	0.0016	0.0032	7	207.96
psi(Bask,Substrate)	225.28	12.33	0.0011	0.0021	9	203.09
psi( <i>Hydrilla</i> ,Substrate)	225.91	12.96	0.0008	0.0015	9	203.72
psi(Bask,Substrate, <i>Hydrilla</i> )	227.46	14.51	0.0004	0.0007	10	202.22
psi(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb, H <sub>2</sub> O Temp)	228.3	15.35	0.0002	0.0005	14	189.25

Table 3.12. Parameter estimates for occupancy models results to determine occupancy as a factor of covariates for the painted turtle (*Chrysemys picta*), common snapping turtle (*Chrysemys picta*), yellow-bellied slider (*Trachemys scripta*), and common musk turtle (*Sternotherus odoratus*) in freshwater reservoirs in central Georgia in 2012. Models with  $\Delta AICc < 2$  are shown.

Model	Parameter	$\beta$	SE	95% CI	
<i>C. picta</i>					
<i>psi(Bask)p(Hydrilla)</i>					
	Bask	1.56	0.99	-0.39	3.51
<i>psi(Bask,Hydrilla)p(Hydrilla)</i>					
	Bask	1.65	1.02	-0.34	3.65
	<i>Hydrilla</i>	-0.30	0.65	-1.58	0.97
<i>C. serpentina</i>					
<i>psi(Bask)</i>					
	Bask	7.57	6.94	-6.03	21.17
<i>T. scripta</i>					
<i>psi(Bask)p(Hydrilla)</i>					
	Bask	0.77	1.08	-1.35	2.89
<i>S. odoratus</i>					
<i>psi(Hydrilla),p(MaxTemp,Precip)</i>					
	<i>Hydrilla</i>	1.28	0.53	0.24	2.32
<i>psi(Hydrilla,Bask),p(MaxTemp,Precip)</i>					
	<i>Hydrilla</i>	1.22	0.55	0.14	2.30
	Bask	0.38	0.46	-0.53	1.28

Table 3.13. Model selection results for common musk turtle (*Sternotherus odoratus*) occupancy rates.  $\Delta\text{AICc}$  represents the change in AICc,  $w$  = model weight,  $K$  = number of parameters in the model,  $-2\text{Log(L)}$  = the negative 2 log likelihood. MaxTemp+Precip covariates were included in detection ( $p$ ) for all models.

Model	AICc	$\Delta\text{AICc}$	$w$	Model Likelihood	K	$-2\text{Log(L)}$
psi( <i>Hydrilla</i> )*	107.57	0	0.588	1	5	96.29
psi(Bask, <i>Hydrilla</i> )*	109.43	1.86	0.232	0.3946	6	95.6
psi(Bask)	110.7	3.13	0.1229	0.2091	5	99.42
psi(.)	112.58	5.01	0.048	0.0817	4	103.75
psi(Bask,Substrate)	117.82	10.25	0.0035	0.0059	10	92.58
psi(BlockbyLake)	118.97	11.4	0.002	0.0033	9	96.78
psi(DO,H2OTemp,pH,Turb)	119.49	11.92	0.0015	0.0026	8	100.22
psi( <i>Hydrilla</i> ,Substrate)	119.79	12.22	0.0013	0.0022	10	94.55
psi(Bask,Substrate, <i>Hydrilla</i> )	121.52	13.95	0.0005	0.0009	11	93.08
psi(Substrate)	123.55	15.98	0.0002	0.0003	9	101.36
psi(Bask,Substrate, <i>Hydrilla</i> , DO,pH,Turb,H2Otemp)	133.81	26.24	0	0	15	90.84

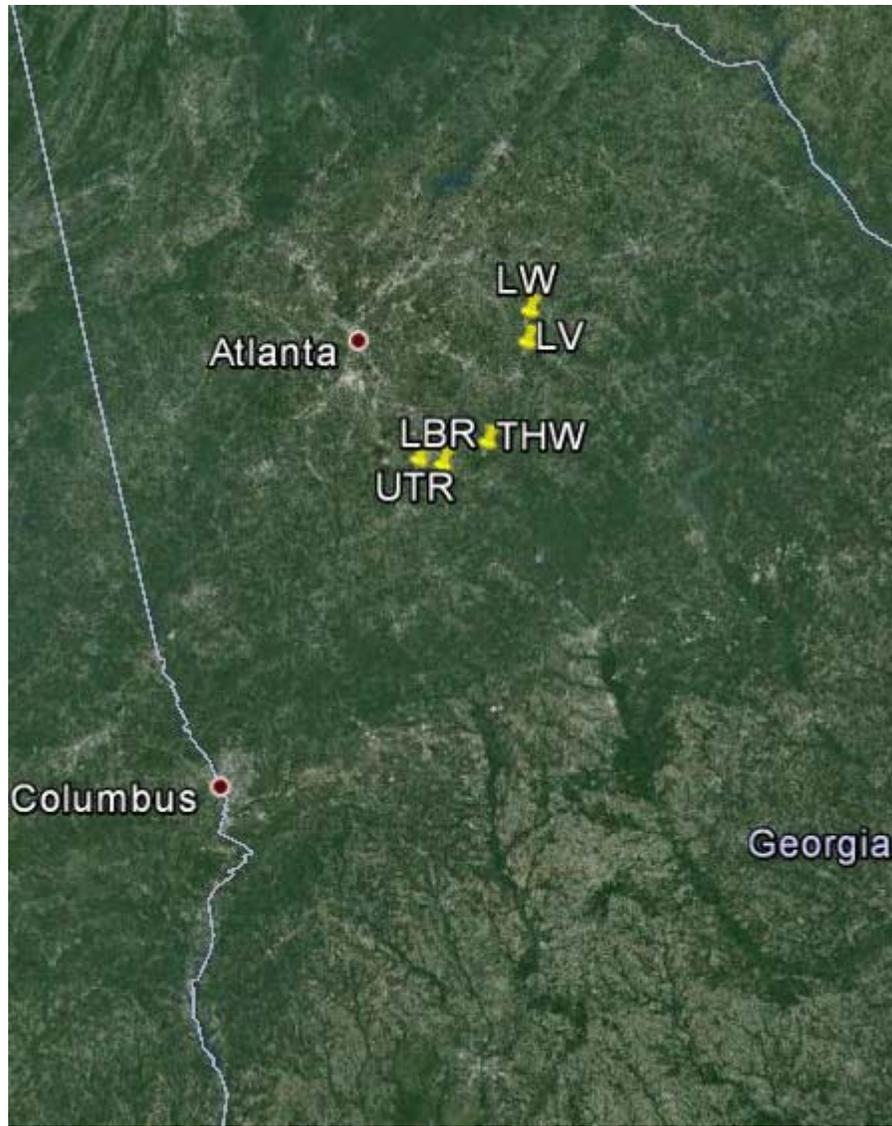


Figure 3.1 Map of the five study reservoirs in Georgia, USA where occupancy surveys for two omnivorous-highly herbivorous species (eastern painted turtle, *Chrysemys picta*; yellow-bellied slider, *Trachemys scripta*), and two carnivorous species (common snapping turtle, *Chelydra serpentina*; common musk turtle, *Sternotherus odoratus*), were conducted. LW= Lake Walton, LV=Lake Varner, THW=Tussahaw Reservoir, UTR=Upper Towaliga Reservoir, LBR=Long Branch Reservoir.



Figure 3.2. Survey locations within Long Branch Reservoir in Georgia, USA. Plots were surveyed on three consecutive days to determine presence or apparent absence of two omnivorous-highly herbivorous turtle species (eastern painted turtle, *Chrysemys picta*; yellow-bellied slider, *Trachemys scripta*), and two carnivorous species (common snapping turtle, *Chelydra serpentina*; common musk turtle, *Sternotherus odoratus*), which was used to analyze detection and occupancy rates within the reservoir patches

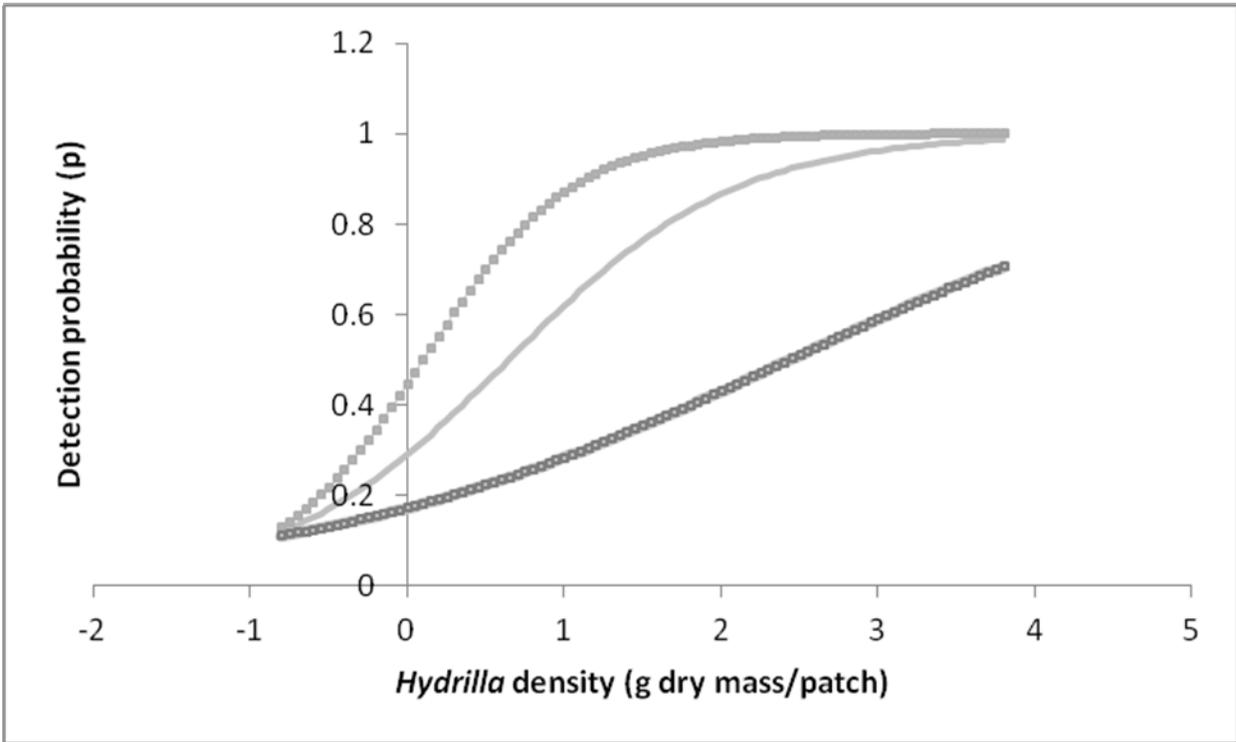


Figure 3.3 The relationship between painted turtle (*Chrysemys picta*) detection rates and an index of *Hydrilla verticillata* (z-score transformation of total grams of dry *Hydrilla* within 15 gallons of lake water per patch) within freshwater reservoirs in central Georgia in 2012. The dotted lines indicate the upper and lower 95% confidence intervals.

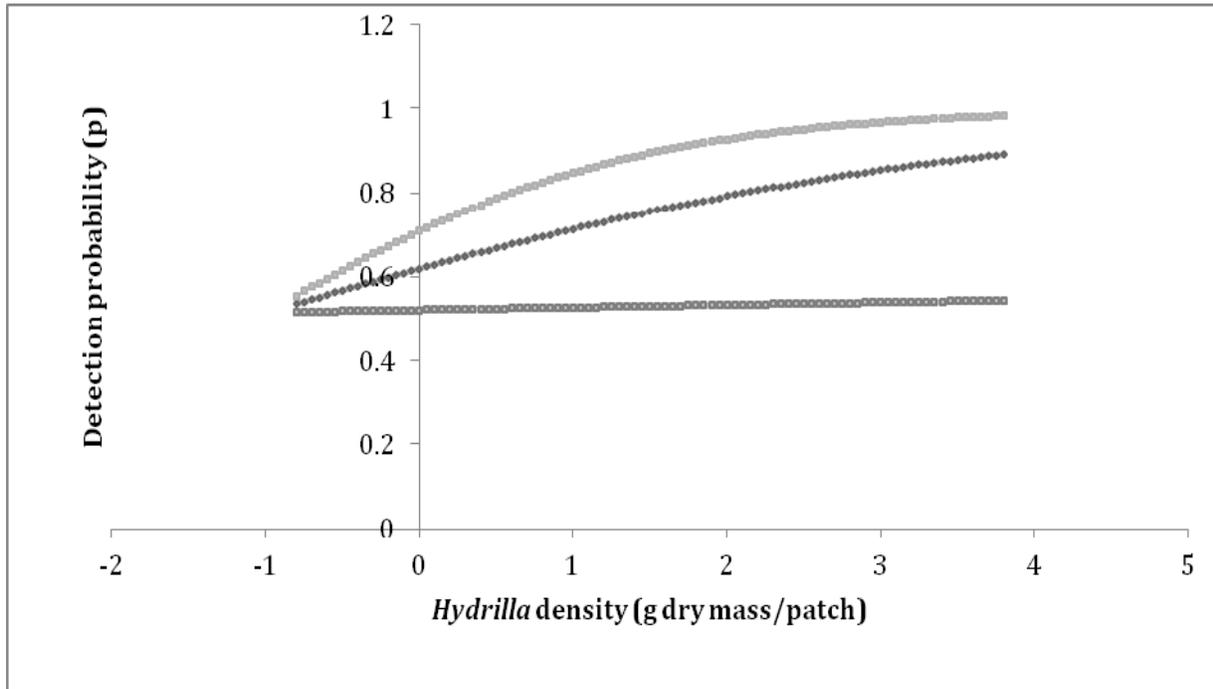


Figure 3.4 The relationship between yellow-bellied slider (*Trachemys scripta*) detection rates and an index of *Hydrilla verticillata* (z-score transformation of total grams of dry *Hydrilla* within 15 gallons of lake water per patch) within freshwater reservoirs in central Georgia in 2012. The dotted lines indicate the upper and lower 95% confidence intervals.

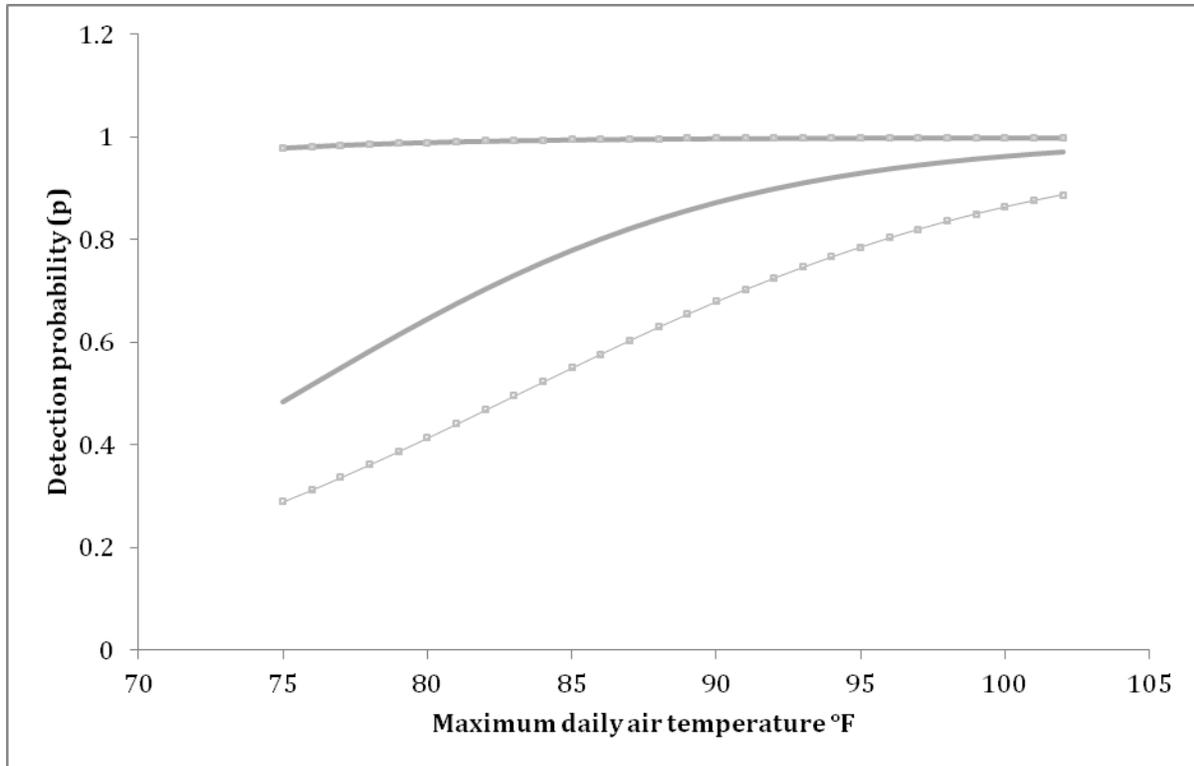


Figure 3.5. The relationship between common musk turtle (*Sternotherus odoratus*) detection rates and maximum daily air temperature given a mean condition of precipitation (0.684 inches/24hours) during an occupancy survey within freshwater reservoirs in central Georgia in 2012. The dotted lines indicate the upper and lower 95% confidence intervals.

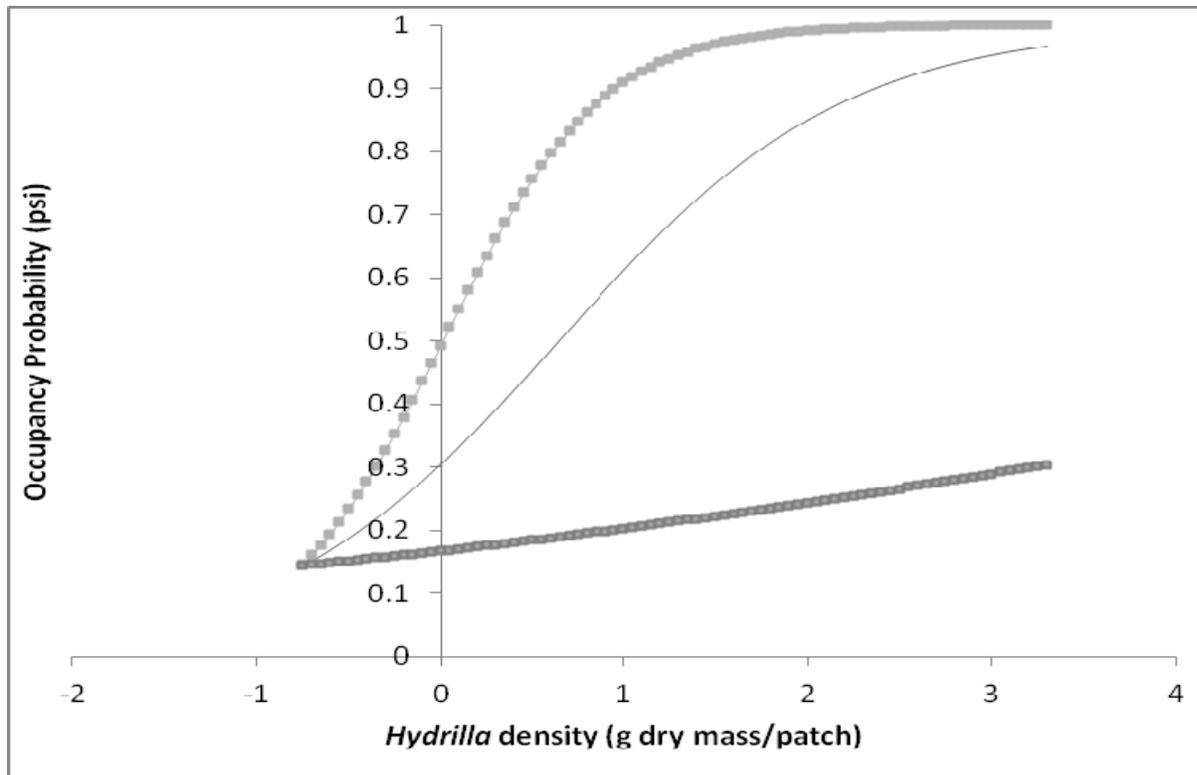


Figure 3.6 The relationship between common musk turtle (*Sternotherus odoratus*) occupancy rates and an index of *Hydrilla verticillata* density (z-score transformation of total grams of dry *Hydrilla* within 15 gallons of lake water per patch) within freshwater reservoirs in central Georgia in 2012. The dotted lines indicate the upper and lower 95% confidence intervals.

## CHAPTER 4

### CONCLUSIONS

As part of evaluating the potential for *Hydrilla* invasions to impact freshwater turtles, a key goal of our research was to determine whether turtles are susceptible to the cyanotoxin(s) that induce Vacuolar Myelinopathy (VM) in other wildlife. Turtles were known to consume *Hydrilla*, associated epiphytes, and invertebrates grazing on those epiphytes, so were likely exposed to the cyanotoxin(s). The results of our feeding trial were conclusive in showing that painted turtles feeding on cyanotoxin positive *Hydrilla* develop VM, with a similar clinical presentation and diagnostic lesions as described in birds. We believe that our results provide strong evidence that the same active agent(s) that induce VM in birds and fish and are associated with ingestion of the uncharacterized cyanobacterium (UCB) induce the lesions and associated neurologic disease in painted turtles and there might be a generalized effect among these taxa. Our results also support the hypothesis that taxa may vary in the required dosage or exposure duration to induce neurologic lesions as the time to develop clinical signs has varied from a few days in domestic chickens (*Gallus domesticus*), to 82 days in our painted turtles. Possible explanations for this difference are the slower metabolism of ectotherms when compared to endotherms, differences in digestive efficiency, different metabolic pathways, an innate resistance to the toxin, or some other unknown factor.

While turtles may be sensitive to the UCB toxin, it remains to be determined whether turtle populations are vulnerable to the UCB's spread and invasions of freshwaters. Vulnerability incorporates both sensitivity and exposure. Many ponds and reservoirs in the southeastern U.S.

have dense *Hydrilla* or native submerged aquatic vegetation that supports abundant concentrations of the UCB (Wilde et al. 2005, Fischer et al. 2006). In those systems, a diet consisting of large amounts of *Hydrilla* may be biologically realistic, particularly for highly herbivorous turtles (e.g., *Trachemys* and *Pseudemys* spp.) (Bjorndal et al. 1997, Fields et al. 2003). However, VM epornitics occur during late fall-winter, leading some to suggest that toxin production is related to season (Rocke et al. 2002). Most turtle species in the southeastern U.S. exhibit limited activity during this period and may limit feeding during the cooler months of peak VM epornitics. To date, no large-scale die offs of aquatic turtles have been reported in reservoirs where VM die offs were reported for birds which may be a result of low detection rates, mortality rates, or a combination of both. Moreover, our observations were that impaired turtles could show some motor recovery despite significant lesions in the brain. Turtles that have lesions but are not clearly distressed may not be reported (Fischer et al. 2003), and the dominant effects of ingesting the UCB may be subacute and not associated with high mortality. It is also not known whether turtles can recover longer term from the neurologic damage associated with ingesting the UCB. Clearly, more studies will be needed to elucidate important details on the epidemiology and vulnerability of the UCB to turtles and other wildlife. We propose a near term need for sensitivity studies of wider suites of taxa including those feeding directly or indirectly on UCB host plants, and studies of the seasonality of toxin production relative to seasonal variation in foraging rates of exposed taxa to determine potential population level vulnerabilities.

Next, we addressed whether *Hydrilla* invasion was associated with patch occupancy of freshwater turtles within southeastern U.S. reservoirs. We found that all turtles studied used areas invaded by *Hydrilla*. *Hydrilla* density was positively correlated with detection rates, but not occupancy rates of painted turtles and yellow-bellied sliders and was positively correlated with

patch occupancy of common musk turtles. No trend was found with common snapping turtles, however some features about their natural history may make trends difficult to detect via occupancy analysis. These results imply that *Hydrilla* invasions have the potential to increase the occurrence of some species within invaded habitats. This may increase exposure of some species that directly or indirectly consume *Hydrilla* to VM. Although we did not document any negative effects of *Hydrilla* to an important freshwater taxa, we caution that further research is needed to inform management decisions and future research directions because single-species occupancy modeling has some limitations that limit inferences. The transitory nature of species with large home ranges can affect patch occupancy patterns. In addition, species with high population sizes can also affect occupancy patterns and occupancy analysis. A small number of individuals may be captured in poor habitat yet occupancy analysis may not be able to distinguish the low population size within those patches from other abundant patches. Future studies should explore abundance patterns in reservoirs related to patch occupancy and the effect of the transitory nature of species, perhaps via radiotelemetry or mark recapture. Furthermore, future studies should focus on reservoirs that have the longest time since invasion, as effects may take more time to be detectable than the five year time since invasion of our study sites.

The impact of vacuolar myelinopathy (VM) on susceptible wildlife populations is largely unknown (Birrenkott 2003, Wiley 2007) examined the impact of VM on South Carolina and Georgia's bald eagle population, concluding that VM has significantly affected local populations. Since eagles in these states are non-migratory and their breeding seasons coincide with VM epizootics, VM could pose a significant risk to statewide populations if it should spread to other reservoirs. It appears that VM is not regulating american coot populations (Haynie 2008). However, there are many other species affected by VM (Augsburger et al. 2003, Lewis-

Weis et al. 2004, Fischer et al. 2006). From our research, we can conclude that turtles are susceptible to negative effects of *Hydrilla* when it co-occurs with toxic cyanobacteria, and that wild turtles are exposed to this risk because they occupy habitats with dense *Hydrilla*. What we do not yet know is whether wild turtles are sufficiently exposed to *Hydrilla*-associated cyanotoxins to cause harm or impact wild populations. Turtles may not be sufficiently active or feeding during periods of peak toxicity, which would limit their natural exposure. Further, we do not know whether turtles can tolerate or recover from the effects VM, and we do not know whether, in the absence of dense areas of native vegetation, *Hydrilla* offers some compensatory benefits to turtles. Future research should explore the effects of *Hydrilla* invasions or VM on other demographic rates such as survival or reproduction and a risk assessment should be conducted for susceptible taxa.

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