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# Environmental Factors Influencing Blooms of a Neurotoxic Stigonematalan Cyanobacterium Responsible for Avian Vacuolar Myelinopathy

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**PURPOSE:** Managers of southeastern reservoirs have been challenged by the introduction of non-native aquatic plants and subsequent ecological consequences. The authors of this technical note have linked avian vacuolar myelinopathy (AVM), a disease killing waterbirds and raptors, to an epiphytic cyanobacterium which grows primarily on nonindigenous submerged aquatic vegetation (SAV). Waterbirds, especially American coots and herbivorous waterfowl populations, increase on reservoirs with abundant invasive aquatic plants. Once they consume the epiphytic cyanobacteria growing on SAV and become neurologically impaired, birds of prey (especially eagles) readily consume the sick and dead birds. Research studies have suggested that the causative agent is a neurotoxin produced by a previously undescribed Stigonematalean cyanobacterium (UCB) that grows epiphytically on submerged, primarily non-native, aquatic plants. Plant collections from Texas to North Carolina indicate that the range of the suspect cyanobacterium is expanding as invasive aquatic plants colonize new systems, especially those in close proximity to AVM sites. The authors' research seeks to guide management of AVM reservoirs by investigating relationships between environmental factors and the presence of the cyanobacterium. Field surveys conducted from 2001-2010 documented the geographic distribution of the Stigonematalan species and confirmed that invasive aquatic plants supported dense colonies of this species. The authors then created a generalized linear model using temperature, oxygen, turbidity, and previous colonization data to explain site-specific variation of Stigonematales densities in an AVM positive site. These field surveys and preliminary models provide insights into predicting the seasonal prevalence of Stigonematales in reservoirs. The findings in this technical note suggest that the density of the invasive plants plays a key role in creating environmental conditions that are favorable to the dominance of the novel cyanobacterium linked to this emerging avian disease.

**BACKGROUND:** The authors' research to date supports the hypothesis that a cyanobacterial epiphyte in the order Stigonematales produces an uncharacterized toxin resulting in Avian Vaculolar Myelinopathy (AVM), a neurological disease in waterbirds and their avian predators in the Southeast. This cyanobacterium is especially abundant on invasive nonindigenous submerged aquatic vegetation (SAV), including hydrilla (*Hydrilla verticillata*), Brazilian elodea (*Egeria densa*) and Eurasian watermilfoil (*Myriophyllum spicatum*); it is also present on native species (Wilde et al. 2005). The cyanobacterium can cover up to 95% of the surface area of invasive SAV leaves.

The undescribed species is a filamentous, heterocystous, true branching cyanobacterium. Morphological characteristics place the cyanobacterium in section V, order Stigonematales. All known members of this order can fix nitrogen. Many of the species within this order occur in wet soil and rice fields but can withstand extensive periods of desiccation. In general, with increasing temperature, the algal group with the highest growth rate changes from diatoms to green algae to cyanobacteria. Cyanobacteria can also

survive in high UV exposure or in low light environments (Whitton and Potts 2000). They can survive at very low oxygen conditions, use water as a hydrogen source, and produce oxygen.

The 16S rRNA sequence identity of this unknown Stigonematalan species was determined using denaturing gradient gel electrophoresis (DGGE). The 16S rRNA sequence data were aligned with additional cyanobacteria sequences to determine designations for probe development, to advance understanding of the species' phylogeny, and to lay groundwork for its formal description. Phylogeny data confirm that the species is in section V, order Stigonematales. Phylogeny also infers that the species is most genetically similar to a *Stigonema* sp. Scanning and transmission electron microscopic documentation of field-collected and cultured isolates have been compiled in preparation for the pending formal species description. Real-time PCR assays were developed for rapid, specific detection of the Stigonematales species from environmental samples. The genetic probe and taxonomic information produced by this study will help test the hypothetical link between these cyanobacteria and AVM, and therefore help guide decisions on managing hydrilla and other nonindigenous SAV in AVM-affected waterbodies (Williams et al. 2007).

AVM is a neurological condition resulting in the diffuse, spongy degeneration of the white matter of the central nervous system (CNS). Researchers can make a definitive AVM diagnosis only after confirmation of the distinctive microscopic lesion, an intramyelinic edema, in the organisms' CNS tissue, most commonly the brain (Thomas et al. 1998). AVM-afflicted birds display erratic behavior; clinical symptoms include head tremors, fatigue, weight loss, and ataxia, manifested as inverted swimming and compromised flight (Thomas et al. 1998, Larsen et al. 2002). Affected species include American coots (*Fulica americana*), Canada geese (*Branta canadensis*), mallard ducks (*Anas platyrhynchos*), buffleheads (*Bucephala albeola*), killdeer (*Charadrius vociferous*), bald eagles (*Haliaeetus leucocephalus*), and great horned owls (*Bubo virginianus*) (Augspurger et al. 2003, Birrenkott et al. 2004, Rocke et al. 2005, Wilde et al. 2005, Fischer et al. 2006, Wiley et al. 2007). Scientists believe that during the winters of 1994-1995 and 1996-1997, 30-65% of the over-wintering eagles on DeGray Lake, AR (34°14'17.33"N, 93°10'5.34"W) died from AVM (Thomas et al. 1998). Affected eagles captured for rehabilitation all died within 24 hours. AVM was confirmed at the U.S. Army Corps of Engineers J. Strom Thurmond Lake (JSTL; 33°40'3.20"N, 82°12'38.79"W) in 1998 and has resulted in a loss of 15 out of 16 active bald eagle nests (Tom Murphy and Jim Ozier, unpublished data). Because of its geographic location and repeat AVM epizootics, this reservoir has served as the authors' primary study site. During a survival study during an AVM epizootic on JSTL, none of the banded symptomatic coots (n=56) were resighted after one week. Over half of the banded asymptomatic coots (n=13) were re-sighted over a two-month period (Haynie 2008).

Several anthropogenic compounds such as hexachlorophene, triethyltin, and bromethalin can cause intramyelinic lesions in domestic animals and humans. However, thorough testing of AVM-positive (AVM+) site water, sediment, and resident bird tissues demonstrated no consistency in the presence of these compounds (Thomas et al. 1998, Rocke et al. 2002, Dodder et al. 2003, Wilde et al. 2005). Two exotic plant species, *Stypandra imbricate* and *Heliochrysum argyrosphaerum,* can cause intramyelinic lesions but are not present in North America (Fischer et al. 2003). The only documented commonalities among all AVM positive lakes are their general geographic location, the presence of invasive SAV and an epiphytic cyanobacterium within the Order Stigonematales (hereafter referred to as undescribed cyanobacterium, UCB) (Wilde et al. 2005, Williams et al. 2007).

This UCB is a true-branching filamentous cyanobacterium that grows as epiphytic colonies on SAV leaf surfaces. Monocultures of the targeted species developed using standard cyanobacteria media without nitrogen ($BG\text{-}11_0$) indicated that it is capable of nitrogen fixation. When cultured in this liquid media, it grows rapidly at 27º C (Williams et al. 2007). Aseptic UCB cultures were established and maintained for over a year, but have proven difficult to mass culture and sustain long-term. Williams et al. (2007) was able to isolate the organism and develop a 16S rRNA polymerase chain reaction (PCR) probe. As a result, it is possible to molecularly screen aquatic plant samples suspected of containing UCB colonies for definitive identification, an essential tool in understanding the distribution of this species within aquatic systems.

Wilde et al. (2005) hypothesized that aquatic birds were ingesting SAV and associated UCB colonies, thereby ingesting a compound that promotes AVM development. Birrenkott et al. (2004) demonstrated that farm-raised mallards developed AVM after consuming field-collected hydrilla with dense UCB colonies (50-95 % leaf coverage). The mallards consumed ~ 1 kg (wet weight) of aquatic plants per day during the feeding trials. Gavage trials with a methanol extract of the hydrilla-UCB demonstrated that extract would cause clinical symptoms and AVM lesions in the treatment mallards (Wiley et al. 2007). While Larsen et al. (2003) was unable to induce AVM in mallards through direct contact with AVM+ coots, Fischer et al. (2003) successfully induced AVM in healthy red-tailed hawks (*Buteo jamaicensis*) by feeding them AVM+ coot tissue. These studies demonstrate that the ingestion of the SAV/UCB complex was the initial point of exposure, and the consumption of AVM+ tissues confers the condition through higher trophic levels.

Members of the Order Stigonematales produce cyanotoxins, which are often secondary metabolites (Mazur-Marzec 2006). For example, *Hapalosiphon hibernicus* produces microcystin-LA, a phosphatase-inhibitor that affects the liver and gastrointestinal tract (Prinsep et al. 1992). While little data exist on neurotoxic genera within this order, one species, *Hapalosiphon fontinalis*, produces a cytotoxic alkaloid (Moore et al. 1984). Cyanobacterial neurotoxins (saxitoxins, anatoxins, cylindrospermopsin, etc.) have been attributed to *Anabaena spp.*, *Aphanizomenon spp.*, *Planktothrix spp*. and *Cylindrospermopsis spp.*, respectively (Zurawell et al. 2005, Mazur-Marzec 2006) and there is increasing evidence that cyanobacteria are capable of producing compounds that cause microscopic lesions similar to those observed in AVM+ tissues. BMAA (β-N-methyloamino-L-alanine), a non-protein amino acid that causes the degeneration of central nervous tissue, has been detected in a wide variety of cyanobacterial cultures including *Trichodesmium*, *Nostoc*, *Lyngbya*, *Myxosarcina*, *Chroococcidiopsis*, and *Cylindrospermopsis*, and has been detected in the brain tissue of Canadian Alzheimer's patients and Amyotrophic lateral sclerosis (ALS) sufferers in Guam (Cox et al. 2005). Samples of hydrilla with the associated UCB and pure UCB cultures contained elevated levels of BMAA (Bidigare et al. 2009). There is still no evidence that mammals can contract AVM. Beavers (*Castor canadensis*), raccoons (*Procyon lotor*), gray fox (*Urocyon cinereoargenteus*) captured on AVM positive (AVM+) reservoirs and domestic swine that underwent feeding trials did not exhibit clinical or histological AVM symptoms (Lewis-Weiss et al. 2004, Fischer et al. 2006; Haynie 2008).

AVM researchers have primarily focused on epidemiology and not on the environmental factors that may contribute to the suspected causative UCB-produced neurotoxin. The present paper reports on the potential significance of environmental parameters on the occurrence of this UCB in waterbodies. The authors investigated the potential relationship(s) between environmental factors, including water quality, seasonality, and SAV species, and the abundance of UCB through an extensive database analysis (2001-

2010). The authors then conducted an intensive survey at JSTL and created a generalized linear model using temperature, oxygen, and previous colonization data to better explain site-specific variation of UCB densities in an AVM+ site.

**MATERIALS AND METHODS:** Initial SAV and algal surveys conducted from 2001-2010 included samples from all 18 waterbodies where AVM has been confirmed. The authors also received or collected 62 SAV samples from additional waterbodies in the Southeast with similar characteristics, including the presence of SAV and waterbirds. SAV samples included all submerged plants present at the waterbody. These were primarily three nonindigenous invasive species (hydrilla, Brazilian elodea, Eurasian watermilfoil) but also included ten native species (*Najas marina, Brasenia schreberi, Ceratophyllum demersum, Chara sp, Utricularia spp., Nymphaea odorata, Potamogeton spp, Bacopa caroliniana, Vallisneria americana, Nelumbo lutea*). Using a rake, personnel collected samples from 3-20 random sites (the number of samples was dependent on waterbody size) within each waterbody. Up to 3 slides were prepared for each SAV species from each site. For species with cauline leaves, such as hydrilla and elodea, five randomly selected leaves were removed from stems from different nodes, mounted on glass slides, and viewed using light and epifluorescent microscopy (Figure 1; Nikon Eclipse Ti, Nikon Corp., Melville, NY USA, 11747-3064). For species with large entire leaves (*Nymphaea*) or blades (*Vallisneria*), three small sections were dissected, using a razor blade, and aligned on a slide for viewing. When examining each slide, the screener recorded the sample's geographic information, plant species and enumerated all algae present, at least to genus and, if possible, to species. The screener, using a Rhodamine red filter set, then recorded the approximate percent leaflet surface area covered by UCB colonies. Surface area coverage estimates were conducted on intact leaves, as the epiphytic UCB species was not readily removed by agitation or sonification (Wilde et al. 2005). Personnel verified the presence of the UCB species using a real-time PCR assay developed for the species (Williams et al. 2007).

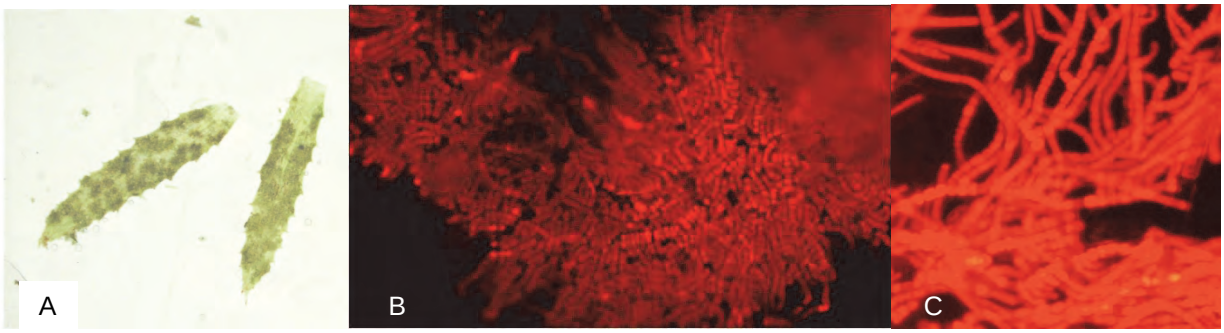


Figure 1. (A) UCB species colonies (10x) growing epiphytically on hydrilla leaflets; (B) UCB species colonies (200x) fluoresce under a Rhodamine red filter set; (C) View (400X) of the cyanobacterium's true-branching cells.

Additional sampling was initiated in Florida during fall 2009 to determine whether the suspect AVM toxin could be ultimately transferred from aquatic vegetation to apple snails, (*Pomacea sp.*) to bald eagles (*Halieetus leucocephalus*) or federally endangered snail kites (*Rostrhamus sociabilis*). The authors conducted field monitoring to document the occurrence of the UCB throughout sites where both apple snails (*Pomacea sp.*) and snail kites occur since native and invasive apple snails are a primary food source for the endangered snail kite. Florida hydrilla sites included five lakes; Lake Orange, Lake Tohopekeliga, Lake Cypress, Lake Hatchineha, and Lake Kissimmee. These lakes support significant

growth of hydrilla, attract large numbers of coots, and currently have large populations of both snail kites and eagles. The authors visited 6 sites on an initial trip, taken on 23 September 2009, and collected 16 samples for analysis. Based on screening results, the authors returned to Lake Toho on 16 November 2009 and collected an additional 14 samples for analysis. In 2009, hydrilla was present in Tohopekaliga (3035 ha), Cypress (817 ha), Hatchineha (263 ha), and Kissimmee (1093 ha) (Jeff Schardt, FFWCC). A cooperative survey by U.S. Army Engineer Research and Development Center, University of Florida Center for Aquatic and Invasive Plants, Osceola Co., Florida Fish and Wildlife Conservation Commission, and ReMetrix was conducted on the Kissimmee chain of lakes in Fall 2009.

During fall 2009, additional surveys were also conducted on JSTL. Hydrilla, first discovered in JSTL in 1994, is the most prevalent SAV species. Prior to the introduction of hydrilla, the lake did not support a substantial community of any native aquatic plants. Since 1998, the Southeastern Cooperative Wildlife Disease Study (SCWDS) has documented AVM bird mortalities annually on this reservoir. The authors selected 20 sites based on SAV and algae surveys conducted from 2003 to 2008 (Figure 2). Data were collected from a boat at twenty sites, monthly, from August to December 2009. The abundance of the UCB at each site was determined, in the laboratory, from an approximately 1-kg (wet weight) hydrilla sample. Hydrilla was collected using a rake; the position in the water column from which the sample was collected varied throughout the season depending on water level fluctuations and plant senescence. The samples were transported to the Nuisance Aquatic Species and Algae Laboratory at the University of Georgia where they were processed for microscopy as described previously. UCB abundance was expressed as percent surface area coverage for each of the leaves, and the mean value for each of the five samples was calculated. The mean percent surface area coverage was then grouped into five classes: 1) 0 - 10%, 2) 10% - 25%, 3) 25% - 50%, 4) 50%-75 and 5) 75-100% surface coverage.

Due to its role as a host for the UCB, the growth pattern and abundance of hydrilla was initially evaluated at each site utilizing a four point scale: (1) hydrilla sprouting to the lower half of the water column, growing at a low density; (2) hydrilla covering at least two-thirds of the water column, growing at a medium density; (3) hydrilla growing to the surface of the water column vertically, at a moderate to high density; and (4) hydrilla growing to the surface of the water column and extending horizontally, at a high density. Classes 2 and 3 were later aggregated, resulting in three hydrilla abundance classes (low, medium, high). The authors used yearly hydrilla distribution data for JSTL to approximate the length of time the survey point had been infested with hydrilla (Figure 2).

The authors collected water quality data and water samples, for nutrient and turbidity analyses, from each of the 20 sites concurrent with monthly hydrilla sampling. Water quality measurements, turbidity and nutrient samples were taken in depth-stratified increments: surface, 1 meter and near the bottom. The authors measured dissolved oxygen and water temperature using a YSI 55 dissolved oxygen meter (Yellow Springs Instruments, Yellow Springs, OH, USA). The water samples were collected in 500 mL opaque bottles and transported on ice to the South Carolina Department of Natural Resources Chemistry Laboratory for analyses. Turbidity samples were analyzed within 24 hrs using a Hach DR/2000 spectrophotometer (Hach Company, Loveland, CO, USA). Each sample was evaluated for nutrients, zinc, fluoride, chloride, nitrate, sulfate, and phosphate, using a Dionex ICS-2000 Ion Chromatograph system (Dionex, Bannockburn, IL, USA).

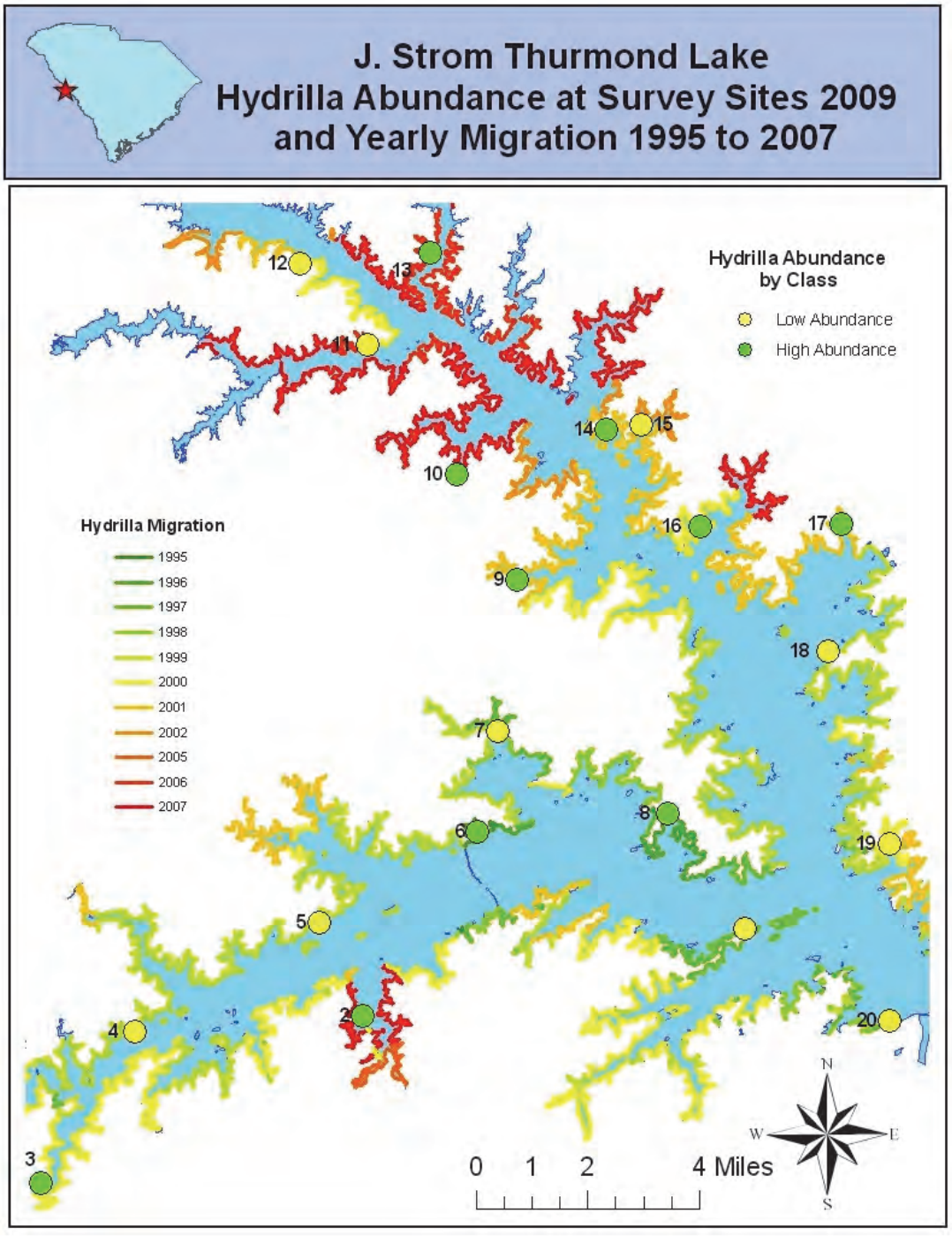


Figure 2. JSTL monthly sampling points for the intensive survey of water quality parameters and UCB species abundance during August 2009 to December 2009. Summary of hydrilla abundance by site number during this time period indicated as high abundance (green filled circle) or low abundance (yellow filled circles) on JSTL from August 2009 to December 2009 and hydrilla migration by yearly survey from 1995 to 2007.

Relationships between UCB abundance and the environmental parameters were analyzed using generalized linear modeling (GLM) with a cumulative logit link function because the response data represented five ordinal classes. Analyses were conducted in two steps. First, the significance of main variable effects associated with the abundance of UCB was tested using both forward and backward stepwise variable entry. Overall model significance was assessed by determining the difference in likelihood values between a fitted model and an intercept-only model. The statistical significance of individual ecological factors was tested with a Wald chi-square test and Bonferroni corrected follow-up test. Non-significant variables were then removed, and the data were re-analyzed using the significant variables and two-way interaction effects among the variables.

As UCB abundance on a given sample date may be a function of its abundance on a previous date, its abundance in the previous month was added as an additional factor in the GLM analyses. This meant that the response data included samples from September to December, with the August data serving only to provide previous month abundance information. Because of an anomalously high UCB abundance in December, Site 10 was flagged as an outlier and eliminated from the data set. December was the final sampling date for this study since the hydrilla had completely senesced by the end of the month. Finally, preliminary analyses indicated highly significant correlations among data from the surface, mid-level, and bottom for most variables, with Pearson r values regularly exceeding 0.80. Therefore, only the surface data were utilized in the model building process under the assumption that the sampling protocol resulted in the majority of UCB being harvested from the water column's surface.

Potential feedbacks between hydrilla density and other environmental factors were assessed by: (1) calculating mean values for each significant factor identified in the GLM for August-September (Late Summer) and October-November (Fall), and (2) testing for differences in these values among the three hydrilla classes for both time periods using a one-way analysis of variance (ANOVA). The nature of significant results was clarified using a Bonferroni corrected follow-up test (when classes had homogeneous variances) or Dunnett's T3 test (when class variances were unequal). All analyses were conducted using SPSS version 18.0.

**RESULTS AND DISCUSSION:** The authors' field surveys conducted from 2001-2010 documented AVM in five states: North Carolina, South Carolina, Georgia, Arkansas and Texas. New hydrilla infestations (Lake Horton, Lake Varner, Smith Reservoir, Upper Towaliga Reservoir) in Georgia represent the most recent sites where SCWDS has confirmed AVM (Table 1, Figure 3). The authors have discovered the UCB in SAV samples from nine states: North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Arkansas, and Texas.

Dense (>75% coverage) UCB coverage on SAV has been documented in all sites where AVM has been confirmed (Table 1).

The UCB has been identified from SAV samples collected from waterbodies where AVM has not yet been observed. These additional locations within Table 2 are highlighted in blue and include several water supply and recreation reservoirs in Georgia and a location in central Florida (Lake Tohopekeliga) (Table 2, Figure 4, 5).

**Table 1. AVM+ locations where plant samples have been screened for the UCB species with state, waterbody, area, SAV type, management, abundance (4=dominant, 3=abundant, 2=common,1=rare, 0=not present), and year AVM was confirmed at the site.**

| State | Waterbody | Hectares | SAV | Management | UCB abundance | AVM confirmed |
|---|---|---|---|---|---|---|
| **AR** | DeGray | 5587 | *Hydrilla* | Herbicides, *Hydrellia* flies | 4 | 1994 |
| **AR** | Ouachita | 17004 | *Hydrilla* | Herbicides, *Hydrellia* flies | 4 | 1996 |
| **AR** | Hamilton | 2915 | *Myriophyllum* | | 2 | 1996 |
| **AR** | Greeson | 555 | *no plants noted | **Hydrilla* in nearby site | | 1996 |
| **TX** | Sam Rayburn | 46356 | *Hydrilla* | Herbicides | 2 | 2000 |
| **NC** | Coachman's Trail | 7 | (*Hydrilla*) | Grass carp | 4 | 2005 |
| **NC** | Woodlake | 457 | (*Hydrilla*) | Grass carp | 4 | 1998 |
| **GA** | Juliette | 1457 | *Hydrilla*, *Egeria*, *Myriophyllum* | Herbicides | 4 | 1998 |
| **GA** | Emerald | 4 | *Hydrilla* | | 4 | 2004 |
| **GA** | Horton | 316 | (*Hydrilla*) | Grass carp | 4 | 2005 |
| **GA** | Smith | 101 | *Hydrilla* | | 1 | 2005 |
| **GA** | West Point | 10486 | *no plants noted | **Hydrilla* in nearby ponds | | 2007 |
| **GA** | Varner | 334 | *Hydrilla* | Herbicides | 4 | 2007 |
| **GA** | Upper Towaliga | 445 | *Hydrilla* | Grass carp | 4 | 2010 |
| **GA/SC** | Thurmond | 28745 | *Hydrilla* | Herbicides | 4 | 1998 |
| **SC** | Davis Pond | 2 | (*Hydrilla*) | Grass carp | 4 | 2003 |
| **SC** | L Lake | 405 | *Myriophyllum, Hydrilla* | | 2 | 1998 |
| **SC** | Murray | 20243 | *Hydrilla* | Grass carp | 3 | 1998 |
| **SC** | Par Pond | 1069 | *Myriophyllum* | | 3 | 1998 |

*(Hydrilla) indicates effective control*

Hydrilla collection in Florida included four lakes where invasive apple snails (*Pomaceae insularum*) and snail kites (*Rostrhamus sociabilis plumbeus*) co-occur; Lake Tohopekaliga, Lake Cypress, Lake Hatchineha, and Lake Kissimmee. ReMetrix sampling effort documented 1015 points in Lake Tohopekaliga with hydrilla and the densest infestation concentrated in the northeast grassy island area (ReMetrix, unpublished data, Figure 4).

UCB species was abundant on site 237 within Lake Tohopekeliga (Figure 5) on the sampling competed 23 September 2009. On 16 November 2009, UCB species was abundant (>50% leaf coverage) at two sites (GA5, GA10), and present/common at 5 more (Figure 5, 6). The authors noted additional potentially toxic cyanobacterial species growing epiphytically on hydrilla leaves, including *Pseudanabaena*, *Anabaena*, *Nostoc*, *Oscillatoria*, *Lyngbya*, *Microcystis*, and *Cylindrospermopsis*. The authors screened hydrilla, and *Utricularia* leaves for all algal species, but cyanobacteria were the dominant group, with 87 species identified.

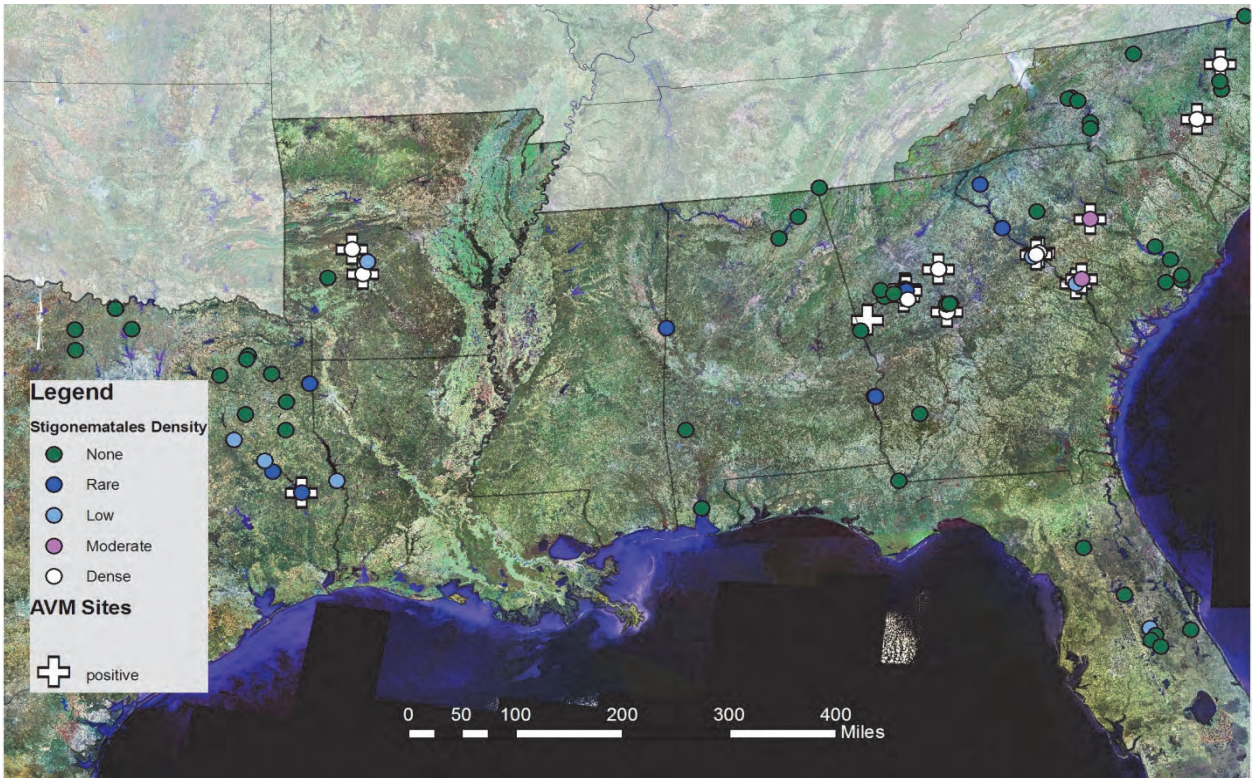


Figure 3. AVM confirmed locations (white plus sign) and UCB density (filled circles), on SAV leaves collected from reservoirs across the southeastern United States.

**Table 2. Locations where UCB species were found on plant samples with state, waterbody, acreage, SAV, management, abundance (4=dominant, 3=abundant, 2=common, 1=rare, 0=not present). Blue highlighted locations are AVM suspect based on density of UCB.**

| State | Waterbody | Hectares | SAV | Management | UCB |
|---|---|---|---|---|---|
| TX | Jacksonville | 489 | *(Hydrilla)* | Grass carp, Herbicides, *Hydrellia* flies, revegetation | 2 |
| TX/LA | Caddo | 10283 | *Hydrilla* | Herbicides | 1 |
| TX | Kurth | 294 | *Hydrilla* | | 1 |
| TX | Nacogdoches | 891 | *(Hydrilla)* | Herbicides | 2 |
| TX/LA | Toledo Bend | 74899 | *Hydrilla* | Herbicides | 2 |
| AL/MS | Aliceville | 3360 | *Hydrilla* | | 2 |
| FL | Tohopekaliga | 9190 | *Hydrilla* | Herbicides | 2 |
| AL/GA | Walter F. George (lower basin) | 10195 | *Hydrilla* | Herbicides, Grass carp | 1 |
| GA | Brittany | 2 | *Hydrilla* | | 1 |
| GA | Petersburg Pond | 2 | *Hydrilla* | | 2 |
| GA | Piedmont NWR (Pond 11A) | 2 | *Hydrilla, Najas* | | 4 |
| GA | Summer Grove Golf Course | 40 | *(Hydrilla)* | Grass carp | 1 |

| | | | | | |
|---|---|---|---|---|---|
| GA<br>GA | Longbranch<br>Tribble Mill | 112<br>44 | *Hydrilla*<br>*Hydrilla* | | 3<br>3 |
| GA | Heads Creek | 121 | *Hydrilla* | | 4 |
| GA | Kedron | 202 | *Hydrilla* | | 2 |
| GA | Redwine | 121 | *Hydrilla* | | 1 |
| GA | Peachtree | 93 | *Hydrilla* | | 1 |
| GA/SC | Russell | 10729 | *Hydrilla* | Water level fluctuations | 1 |
| SC | Keowee | 7490 | *Hydrilla* | Herbicides | 1 |

Blue highlighted rows indicate AVM suspect locations
*(Hydrilla) indicates effective control*

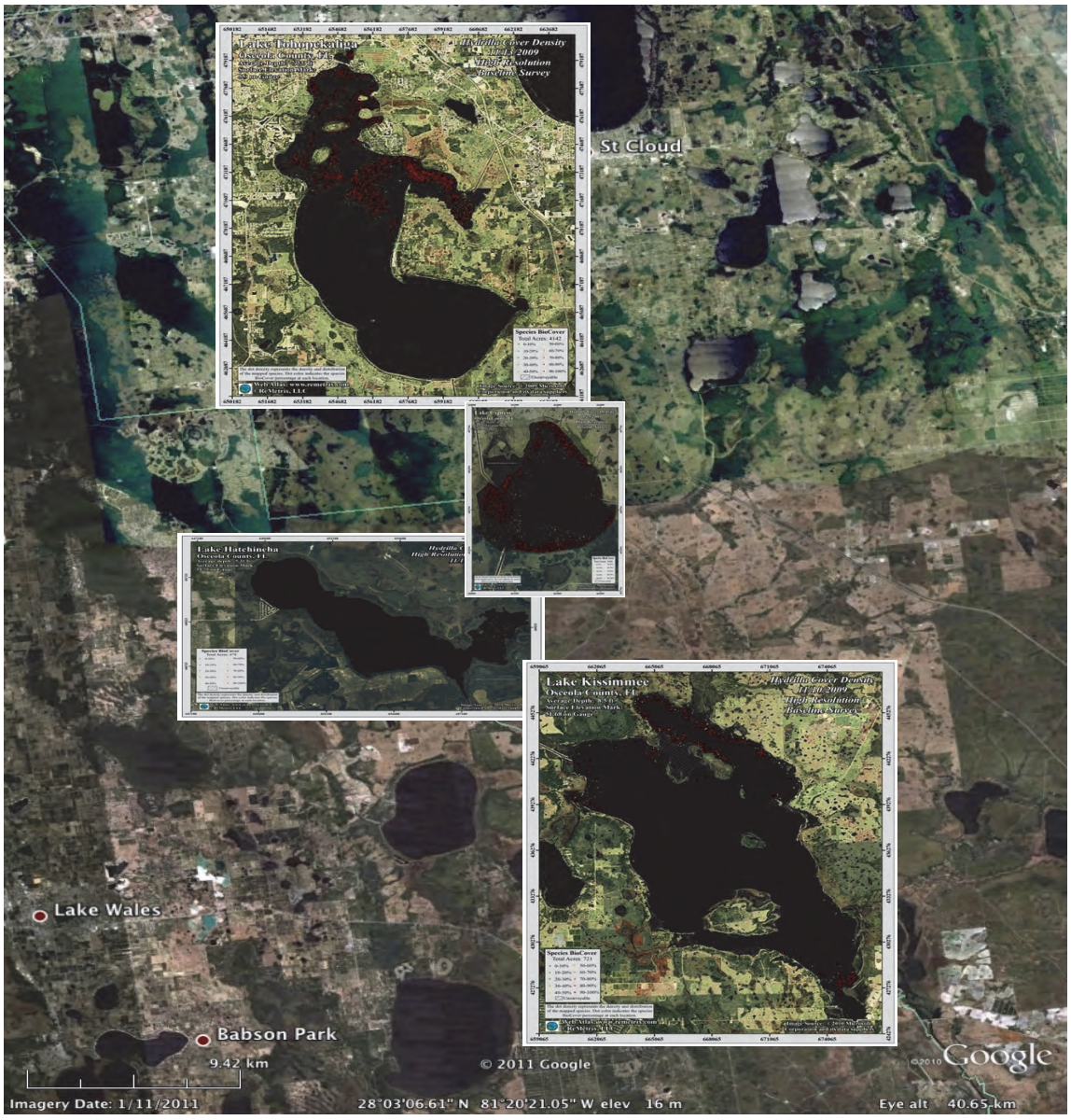


Figure 4. Surveys conducted by ReMetrix during September 2009 confirmed higher hydrilla density in Tohopekaliga and Cypress relative to Hatchineha and Kissimmee within Kissimmee chain of lakes.

23 September 2009 16 November 2009


Figure 5. Florida Lake Tohopekeliga hydrilla collection sites on 23 September 2009 (a) and 16 November 2009 (b) (red stars indicate the presence of UCB species).

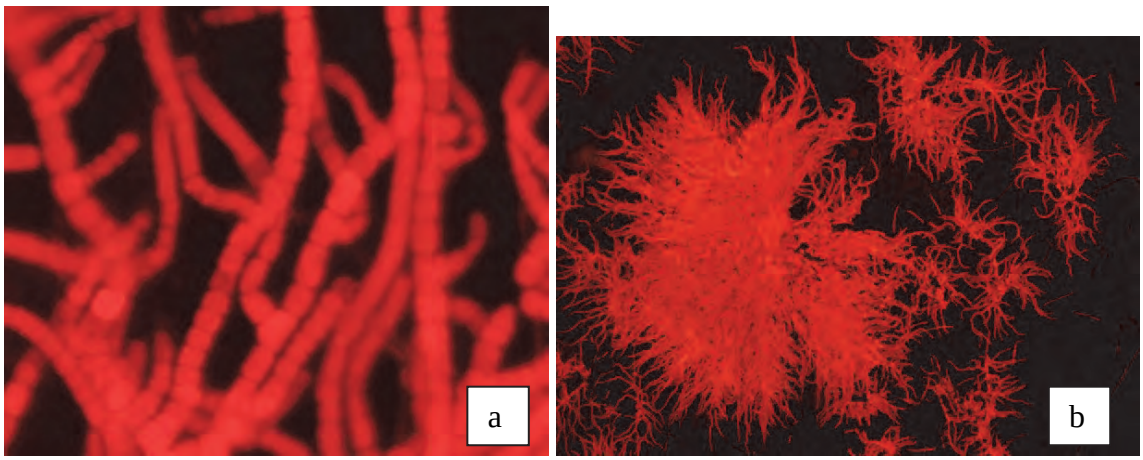


Figure 6. Colonies of the suspect toxin-producing UCB species cyanobacterium, at 400x (a) and 200x (b), growing epiphytically on hydrilla leaflets collected from Lake Tohopekeliga, Florida, 23 September 2009.

There were native aquatic plants present at 30 out of 101 total sites sampled (AVM+ and control) in the survey of Southeast waterbody plant/algal database from 2002-2010. The authors found the UCB growing only in sites where nonindigenous SAV was also present. At these sites, the authors found the UCB growing on native aquatic plants co-occurring with the three nonindigenous species, Hydrilla, Eurasian watermilfoil, and Brazilian elodea. However, coverage on the native species was not as dense as on the nonindigenous SAV (Table 3). UCB density was, on average, two times greater on hydrilla than on *N. lutea*, *V. americana*, and *Potamogeton* sp., and up to 20X greater relative to *N. marina*. (Table 3). These findings suggest that the UCB can be easily spread as nonindigenous SAV invades new waterbodies.

**Table 3. Average maximum Stigonematalan density, measured as percent leaf coverage, and associated 95% confidence limits. The nonindegineous plants have the highest prevalance of UCB species.**

| | Plant | Average Maximum | 95% LCL | 95% UCL |
|---|---|---|---|---|
| Native | *Najas marina* | 2.00 | NA | NA |
| | *Brasenia schreberi* * | 5.00 | NA | NA |
| | *Ceratophyllum demersum* | 9.57 | 0.42 | 18.73 |
| | *Chara sp* | 12.50 | 0.00 | 100.00 |
| | *Utricularia sp* | 17.80 | 9.46 | 26.14 |
| | *Nymphaea odorata* * | 18.33 | 0.00 | 40.01 |
| | *Potamogeton illinoensis* | 18.64 | 9.98 | 27.30 |
| | *Potamogeton pusillus* | 18.67 | 0.00 | 55.51 |
| | *Bacopa caroliniana* | 19.00 | 11.14 | 26.86 |
| | *Vallisneria americana* | 20.87 | 4.08 | 37.65 |
| | *Nelumbo lutea* * | 22.50 | 0.00 | 100.00 |
| Exotic | *Myriophyllum spicatum* | 26.97 | 17.93 | 36.00 |
| | *Egeria densa* | 44.23 | 35.47 | 53.00 |
| | *Hydrilla verticillata* | 45.94 | 43.84 | 48.04 |

(*) indicates floating leaved plants

Initial laboratory results indicated that the UCB species would grow best at high temperatures and with high nutrients (Williams 2007). A monoculture of the targeted UCB species was established using BG-$11_0$ media. This medium does not contain nitrogen and the ability of the UCB species to out-compete co-occurring algae in BG-$11_0$ may relate to its nitrogen-fixing capability. The UCB species grew at a much faster rate at 27°C than at 25°C, suggesting that warmer temperature is optimal. At 25°C, the cyanobacterium took approximately one month to become established and double the population size on agar medium. When transferred to 27°C, the culture doubled in approximately 2 weeks.

Consistent monthly surveys (July-January) from 2002-2009 at JSTL revealed a seasonal variation in average UCB coverage on hydrilla leaves. While initial late summer (August, September) temperatures were within the range of laboratory maximal growth rates (27 ºC), the highest average percent coverage of UCB in JSTL was during October-December as temperatures declined below 20ºC (Figure 7). Hydrilla maintains a surface canopy through October, providing maximal substrate for the UCB near the surface. The hydrilla leaves have also begun to senesce and may release nutrients available to the epiphytic cyanobacteria.

Sampling conducted at JSTL during fall 2009 included depth-specific water quality data and hydrilla density and UCB species coverage. Trends in surface water temperatures and dissolved oxygen concentrations followed general expectations. Water temperatures were highest in August and cooled gradually until December, while dissolved oxygen readings were lowest in August and generally increased throughout the survey period. Turbidity measures increased slightly from August through November and then more noticeably in December. High rainfall coinciding with hydrilla senescence during December likely increased the turbidity in the reservoir. Concentrations of most nutrients varied minimally, but sulfate and nitrate concentrations both increased steadily throughout the study period (Figure 8).

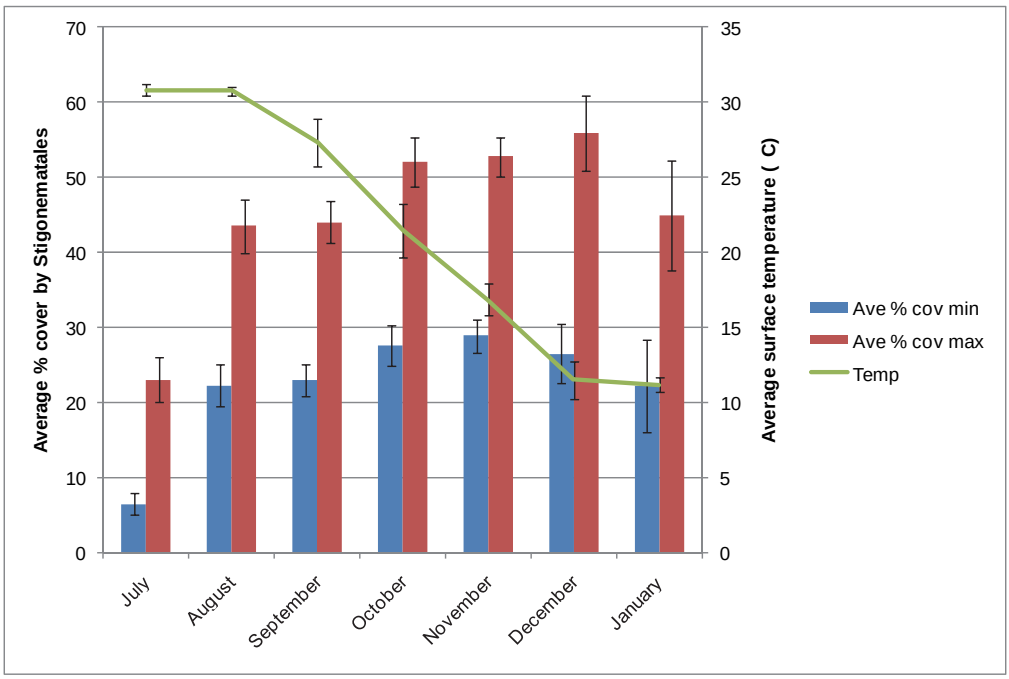


Figure 7. Average monthly temperature and percent minimum/maximum coverage by UCB species (+/-SE) at JSTL (2002-2009).

The average leaf surface coverage of UCB species on a given sample date ranged from 2-86% per site surveyed. Many sites had peak values in August and September, although some were highest in December (Figure 9).

The full model of UCB species abundance, which was highly significant (Likelihood Ratio Chi-square = 43.60; d.f. = 11; $p < 0.00001$), contained five main effects and four interaction effects (Table 4). The main effects related to UCB species abundance were hydrilla density, surface water temperature, dissolved oxygen, prior UCB species cover, and turbidity (Table 5). The coefficients for temperature (0.75) and dissolved oxygen (1.578) were both positive, indicating that UCB species abundance was positively related to these measures (Table 5). Hydrilla Class 1 (low density) and Class 2 (moderate density) had negative coefficients (-3.657 and -2.918, respectively), indicating that UCB species abundance declined with decreasing hydrilla density. Conversely, the negative coefficient for surface turbidity (-0.159) indicated that UCB species abundance declined as turbidity increased (Table 5).

The interaction effects clarified the complex interactions among the variables, particularly the role of prior UCB species values (Table 5). Sites with low hydrilla density generally tended to have lower levels of UCB species abundance (based on the main effects), but on sites where it was better established, greater abundances tended to persist until hydrilla dieback occurred in November. On sites with high hydrilla density, which tended to favor better overall growth of UCB species, there was little relationship between the abundance in the current and previous months, and patterns tended more to reflect the seasonal peak and decline of UCB species and hydrilla (Table 5).

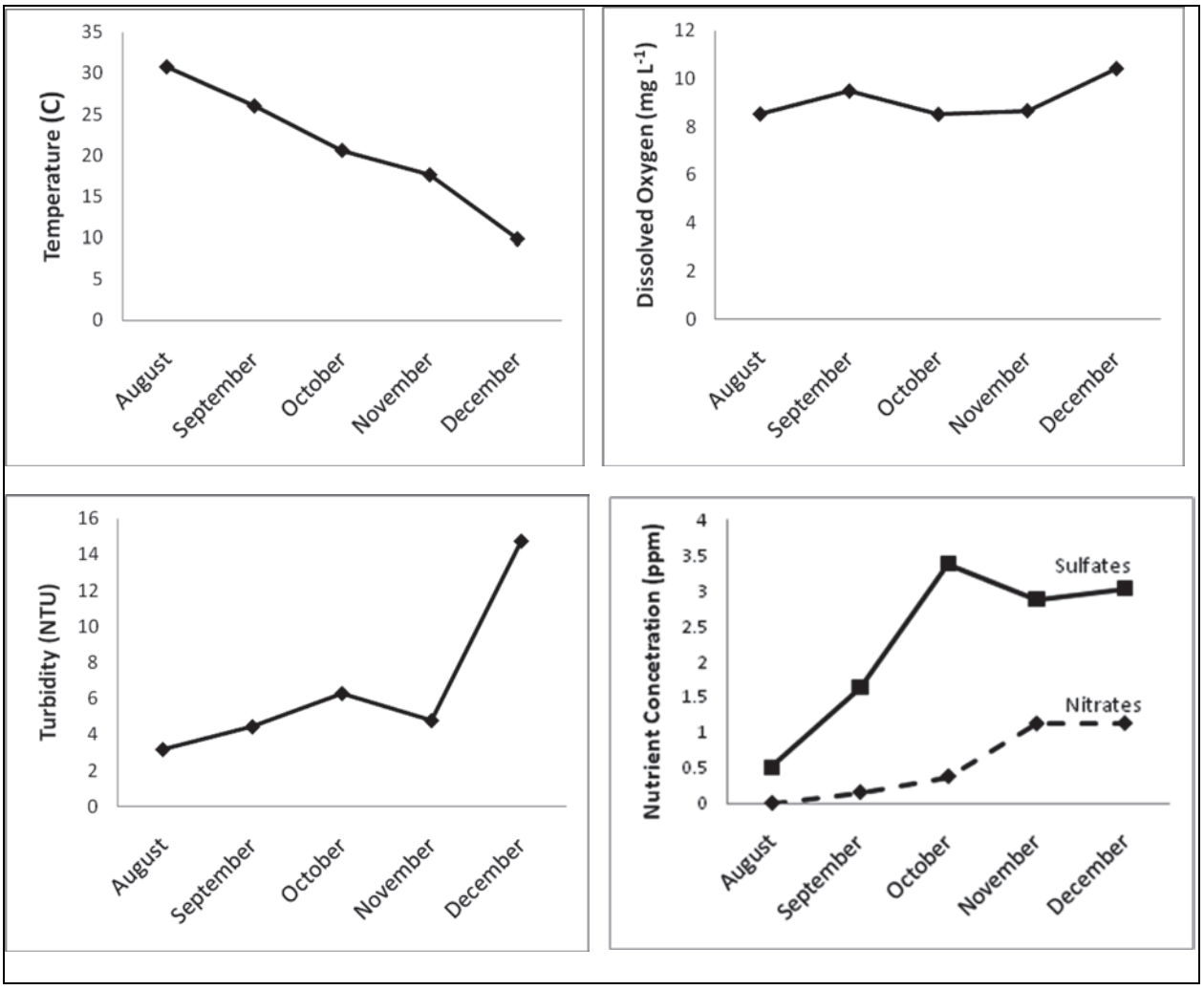


Figure 8. Temperature, oxygen, turbidity levels and nutrient concentrations during Fall 2009 hydrilla monitoring study at J. Strom Thurmond Reservoir.

The interaction effect between prior UCB species abundance and surface temperatures showed a significant seasonal component. At the end of the growing season (in September and especially October), there was a significant positive relationship between UCB species abundance in the current and previous months (Figure 10). This indicated that establishment and growth earlier in the season translated into greater abundance in subsequent months. However, at the end of the growing season (November), senescence led to declines of UCB species at all sites, regardless of prior abundance, and thus little effect of prior abundance (Figure 10).

Finally, the significant interaction effect between temperature and dissolved oxygen concentrations on UCB species showed a similar seasonal component. When surface water temperatures were higher (Late summer), greater UCB species abundance was positively associated with higher dissolved oxygen levels (sites with $\geq 9$ mg $L^{-1}$). It is possible that this finding was more a reflection of greater photosynthesis by hydrilla on sites where both had greater abundance than an actual control over UCB species growth. When water temperatures declined and both species senesced, there was no difference in UCB species abundance between sites with high or low ($< 9$ mg $L^{-1}$) dissolved oxygen concentrations (Table 6).

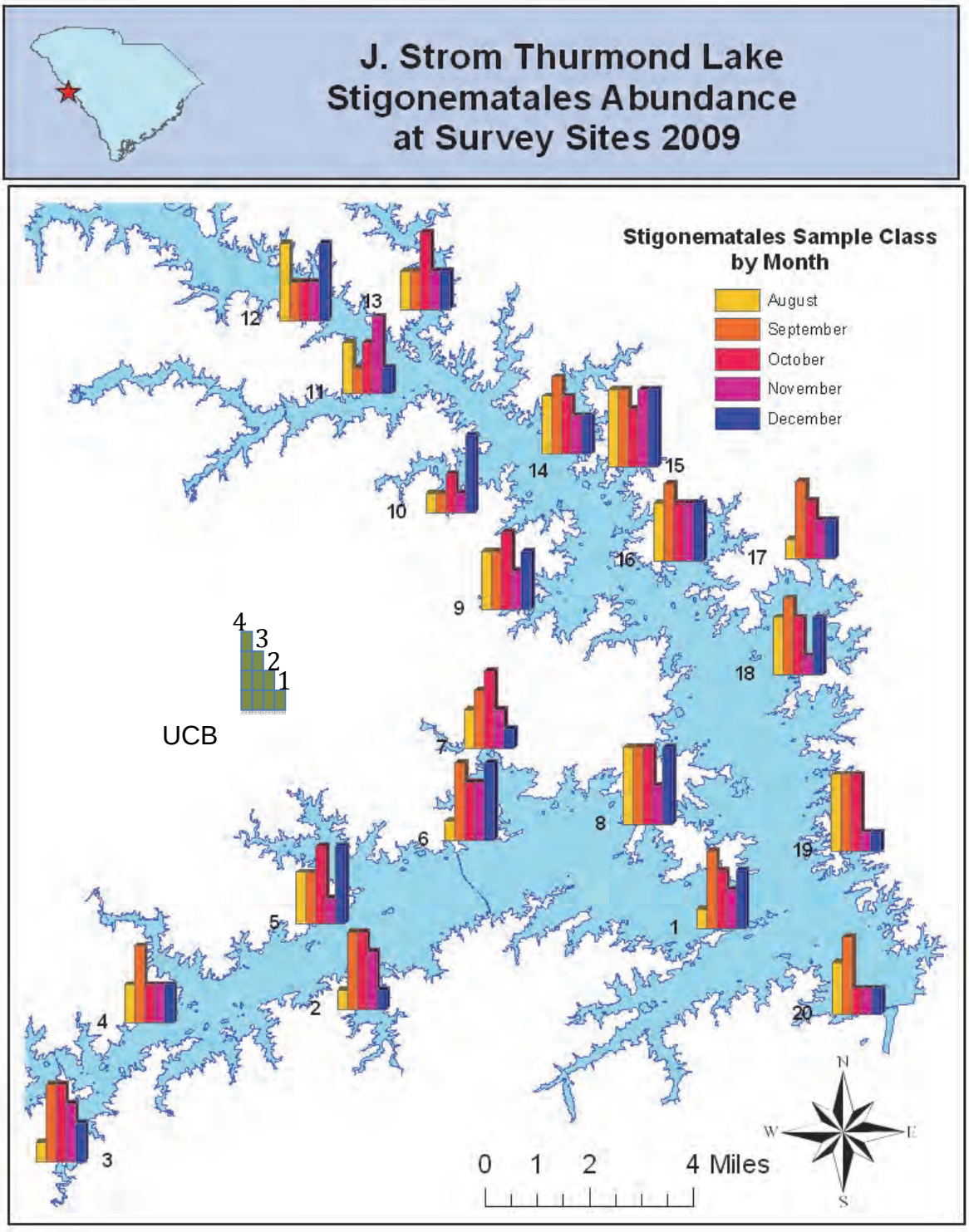


Figure 9. Relative abundance of UCB species for survey sites on J. Strom Thurmond Lake from August 2009 to December 2009 (1: 0 – 10%, 2: 0% - 25%, 3: 25% - 50%, and 4: 50%-100%).

**Table 4. Statistical significance of individual ecological factors was tested with a Wald chi-square test and Bonferroni corrected follow-up test.**

| Source | Wald Chi-Square | d.f. | Sig. |
|---|---|---|---|
| ***Main Effects:*** | | | |
| Hydrilla Density | 14.67 | 1 | < 0.001 |
| Prior UCB species Abundance (% cover) | 6.28 | 1 | 0.012 |
| Surface Temperature (C) | 5.03 | 1 | 0.025 |
| Surface Dissolved Oxygen (mg/L) | 4.31 | 1 | 0.038 |
| Surface Turbidity (NTU) | 3.90 | 1 | 0.048 |
| ***Interaction Effects:*** | | | |
| Turbidity x Prior UCB species Abund. | 11.76 | 1 | 0.001 |
| Hydrilla Abund. x Prior UCB species Abund. | 9.98 | 1 | 0.007 |
| Surface Temp. x Prior UCB species Abund. | 5.79 | 1 | 0.016 |
| Surface Temp. x Surface Dissolved Oxygen | 5.71 | 1 | 0.017 |

**Table 5. Significant ecological factors were reanalyzed using the significant variable and two-way interaction terms effects among the variables.**

| Source | B | Std. Error | 95% Wald Confidence Interval | |
|---|---|---|---|---|
| | | | Lower | Upper |
| ***Main Effects:*** | | | | |
| Hydrilla Abundance: | | | | |
| Class 1 | -3.657 | 1.017 | -5.650 | -1.664 |
| Class 2 | -2.918 | 0.911 | -4.703 | -1.133 |
| Class 3 | 0 | | | |
| Prior UCB species Abundance | -0.182 | 0.063 | 0.305 | 0.058 |
| Surface Temperature | 0.750 | 0.334 | 0.095 | 1.405 |
| Surface Dissolved Oxygen | 1.578 | 0.760 | 0.089 | 3.066 |
| Surface Turbidity | -0.159 | 0.081 | 0.317 | 0.001 |
| ***Interaction Effects:*** | | | | |
| Turbidity x Prior UCB Abund. | 0.007 | 0.002 | 0.003 | 0.011 |
| Hydr. Abund. x Prior UCB Abund. | | | | |
| Class 1 | 0.056 | 0.198 | 0.017 | 0.095 |
| Class 2 | 0.047 | 0.018 | 0.011 | 0.082 |
| Class 3 | 0 | | | |
| Surface Temp. x Prior UCB Abund. | 0.006 | 0.003 | 0.001 | 0.011 |
| Surface Temp. x Surface DO | -0.074 | 0.031 | 0.134 | 0.013 |

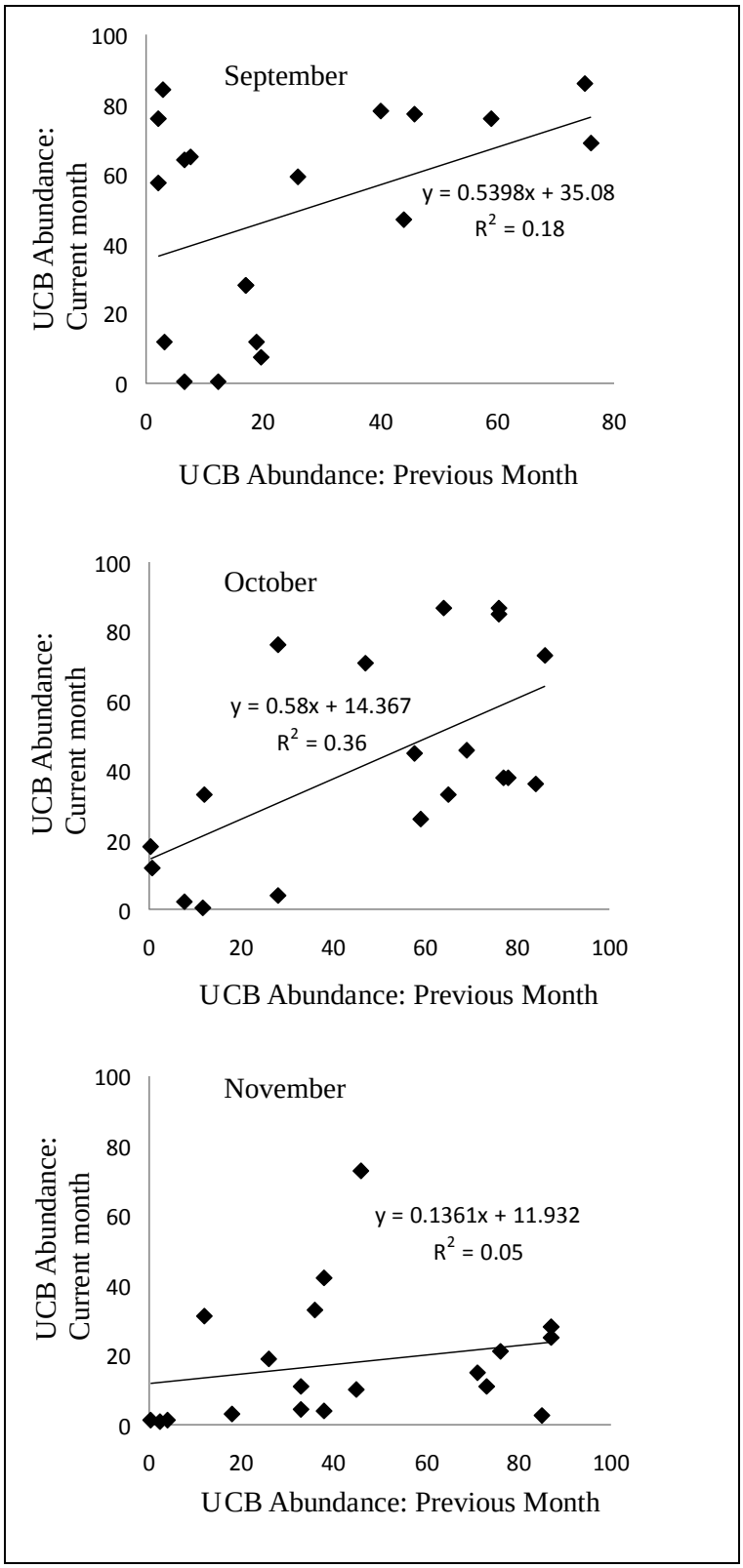


Figure 10. Relationship of UCB abundance in the current month relative to UCB abundance in the previous month at JSTL.

**Table 6. Mean values of water temperature, dissolved oxygen concentrations, and turbidity for sites with low (Class 1), moderate (Class 2) and high (Class 3) hydrilla abundance based on late summer (August-September) and fall (October-November) samples at JSTL. Values indicated by bold and superscripted numbers are statistically significant (p< 0.05).**

| | Late Summer | | | Fall | | |
|---|---|---|---|---|---|---|
| | Hydrilla | | | Hydrilla | | |
| Source | Class 1 | Class 2 | Class 3 | Class 1 | Class 2 | Class 3 |
| ***Temperature*** (C) | | | | | | |
| Surface | **27.53**[a] | **28.15**[ab] | **29.36**[b] | 19.08 | 19.00 | 19.28 |
| Mid-depth | 27.21 | 27.44 | 27.11 | 18.97 | 18.90 | 18.98 |
| Bottom | 26.98 | 27.45 | 26.53 | 18.81 | 18.69 | 18.48 |
| ***Dissolved Oxygen*** (mg/l) | | | | | | |
| Surface | **7.92** [a] | **8.75** [ab] | **10.58** [b] | 8.07 | 8.56 | 9.17 |
| Mid-level | 7.75 | 8.13 | 8.50 | 8.50 | 8.69 | 9.33 |
| Bottom | **6.93** [a] | **7.05** [ab] | **4.88** [b] | 8.25 | 8.62 | 8.77 |
| ***Turbidity*** (NTU) | | | | | | |
| Surface | 4.16 | 3.19 | 4.33 | 5.83 | 4.81 | 6.25 |
| Mid-level | 4.67 | 4.75 | 5.42 | 6.08 | 4.44 | 6.83 |
| Bottom | 6.42 | 5.63 | 5.50 | 5.92 | 5.63 | 9.75 |

As hydrilla beds mature and grow in size, they become denser and possibly provide a more suitable habitat for UCB species. While changes in temperature and turbidity driven by seasonal variations clearly constrained the abundance of UCB species across JSTL, hydrilla density appears to have influenced site-level conditions (and thus UCB species abundance) at finer temporal and spatial scales. In August and September, surface temperatures averaged nearly 2° C warmer in dense hydrilla patches than in patches with low hydrilla density. Dissolved oxygen concentrations were similarly higher in the surface within patches of greater hydrilla abundance during late summer, but much lower at the bottom layer. Collectively, patterns of temperature and dissolved oxygen suggest that stratification occurred in high density hydrilla patches and created three different layers in temperature and dissolved oxygen levels. In contrast, low density hydrilla patches had less variability in temperature and dissolved oxygen levels among the three different sampling layers (Table 6).

**CONCLUSIONS:** The highest UCB species densities documented during field surveys conducted from 2001-2010 were in reservoirs with dense hydrilla in the late fall.

Water temperatures in the field during the highest average percent coverage on hydrilla leaves were lower than the optimal laboratory temperatures for rapid growth. Even if they are not growing at maximal rates, UCB species colonies may be promoted on hydrilla leaves relative to other epiphytic species under the conditions present in late fall in these AVM reservoirs. When UCB species is dominant on the leaves, the diversity and abundance of diatoms and other cyanobacteria are reduced. During the same time frame (late fall), diatoms and other species of cyanobacteria generally are abundant and diverse on plant leaves in non-AVM (control) reservoirs (Wilde et al 2005). Dense hydrilla mats can affect localized water quality and may actually out-compete native species due to this

alteration (Pesacreta 1988). UCB species may benefit from this alteration as well and grow at an accelerated rate within the warm, stagnant hydrilla mat.

Temperature, dissolved oxygen, previous hydrilla density, and turbidity significantly correlated with the abundance of UCB species in this study, but the authors expect that there are more factors affecting UCB species' growth patterns. Turbidity levels had a significantly negative relationship with UCB species abundance and turbidity is frequently invoked as a factor inhibiting hydrilla growth (Bowes et al. 1979, Pesacreta 1988). Shannon (2008) found significantly lower turbidity, specific conductivity and pH in AVM+ lakes than in AVM- lakes.

A long-term objective for additional research would be to develop a predictive model for examining UCB species abundance in reservoirs where it has been documented or to predict the probability of its occurrence in undocumented lakes. The majority of the nutrient and water quality parameters measured in the 2009 Thurmond study were not significantly related to UCB species distributions, but might be significant in a multi-reservoir comparison. Building the model using data from additional AVM+ and AVM- sites would provide insight into factors related to the presence of the UCB species. This model could be applied by gathering temperature data by remote sensing techniques and continued addition of water quality data converted into useable layers in ArcGIS. As more factors that affect the abundance of UCB species are discovered, they could be added into the model for greater accuracy in predicting the abundance of UCB species.

A key finding with the authors' research is the strong field correlation between abundant invasive vegetation with dense UCB species colonies and high avian mortality. The predictive model based on empirical data from one season at J. Strom Thurmond supports this association and indicates that the hydrilla density alone can optimize growth of the toxic cyanobacterial species associated with AVM.

## FUTURE RESEARCH:

The collection method used for this study was raking hydrilla from the top meter of the water column; this allowed the authors to assess accurately only UCB species coverage in the surface layer. In current studies, an amended collection protocol is being used to assess vertical variation in UCB species growth by collecting the entire hydrilla stem from surface to bottom. This will allow the authors to incorporate the water column profiles in water quality parameters to explain additional variation in UCB species abundance.

The development of a predictive model would help identify areas prone to high abundance of UCB species and would enable managers to focus efforts on the SAV which host the cyanobacterium. This would reduce the labor and cost associated with large-scale treatments of SAV by allowing the managers to target specific sites/reservoirs. Reducing invasive SAV where UCB species can become dense and herbivorous waterbirds are present should result in less avian mortality from AVM. Continued surveillance and monitoring of AVM sites with ongoing hydrilla management will allow the authors to document changes as invasive vegetation is controlled. The authors plan to use ongoing management efforts test the effectiveness of varying levels of plant control in decreasing incidence of avian disease in known AVM locations.

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