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Seasonal Resource Allocation and Accumulated Degree Day Estimation for Cuban Bulrush (Oxycarum cubense) in the Southeastern U.S.

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Seasonal Resource Allocation and Accumulated Degree Day Estimation for Cuban Bulrush (*Oxycarum cubense*) in the Southeastern U.S.

By

Allison Squires

A Thesis
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Seasonal Resource Allocation and Accumulated Degree Day Estimation for Cuban
Bulrush (*Oxycarum cubense*) in the Southeastern U.S.

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Cuban bulrush (*Oxycaryum cubense* (Poepp. & Kunth) Lye) is a non-native aquatic plant from South America and the West Indies, first introduced into the United States in 1878. This study focused on the resource allocation and accumulated degree day modeling of the monocephalous and polycephalous Cuban bulrush populations in Mississippi, Louisiana, and Florida. Environmental factors such as photoperiod and temperature were analyzed on its effect on plant growth. From October 2021- September 2022 Cuban bulrush biomass was harvested monthly from two 0.04 Ha (0.1 ac) plots in each state. Height was measured during each monthly event. Environmental monitoring of photoperiod and air temperature were also measured monthly. Starch analyses were completed using the amylase/amyloglucosidase method via a commercially available STA-20 starch assay kit. Linear and polynomial regressions were conducted between Cuban bulrush biomass, starch content, and environmental variables. A Kruskal-Wallis One-Way ANOVA was conducted on total, inflorescence, emergent, and submersed biomass and starch to determine if each state had an effect. Accumulated degree day modeling was done using the Single Sine method by Snyder et al. (1999). Almost all Mississippi biomass and starch had negative relationships with photoperiod and air temperature. All biomass had a significant state effect ($P<0.01$), while total and emergent starch had a significant effect between Mississippi and Florida ($P<0.01$). Starch content was not prominent in any one tissue (<1.4% DW). Phenological differences were identified between the monocephalous and polycephalous biotype where the monocephalous had more allocation to total biomass, while the polycephalous focused more on inflorescence. Monocephalous inflorescence peaked in the summer, while polycephalous peaked in the winter. Model results show base threshold temperatures below 0°C, and the potential of Cuban bulrush to expand north. Future recommendations include future growth studies based on modeling data from this study.

Chapter I

Cuban bulrush [*Oxycaryum Cubense* (Poepp. & Kunth) Lye] literature review

TAXONOMY AND MORPHOLOGY

Cuban bulrush, or *Oxycaryum cubense* (Poepp. & Kunth) Lye is native to the tropical Americas (Bryson et al. 2008). Taxonomically, it is the only species within the genus *Oxycaryum* of the Cyperaceae family (Fernandez 2013). Though more recent data through embryography, morphology, and ontogeny has placed the species in the *Cyperus* genus (Larridon et al. 2011, Ribeiro et al. 2021). For example, the embryo of Cuban bulrush is similar to *Cyperus* sect. *Anosporum,* and *Cyperus gardneri;* the spikelets are similar to *Cyperus gardneri*, while also having many other key characteristics originating in *Cyperus* (Larridon et al. 2011). Cuban bulrush is now considered to be a heterotypic or taxonomic synonym with this suggested taxonomic change (Schoch et al. 2020). However, several sites including the Integrated Taxonomic Information System (ITIS) has yet to accept the new name change (ITIS 2023), therefore *Oxycaryum cubense* will be used.

Cuban bulrush grows approximately 0.3 to 0.9 m tall and has triangular stems (Watson and Madsen 2014). It has spirally arranged scales or glumes within the inflorescence, which has been mistaken as a burhead sedge (*Scirpus cubensis*) (Bryson et al. 2008). Multiple inflorescences, either umbellate (multiple) or monocephalous (one), are produced at the apical portion of each stem (Bryson and Carter 2008). Inflorescences are surrounded by 5.08 to 15.24 cm long leaf-life bracts (Godfrey and Wooten 1979). Seeds are spiked, buoyant achenes that form from spring to fall and are adapted to dispersal via water (Bryson et al. 2008). It forms transient floating mats and rafts that can have red-like stolons that form at the base and are the means of vegetative spread (Bryson et al. 2008).

DISTRIBUTION

The first recording of Cuban bulrush in the U.S. was in 1878 on the eastern side of Mobile Bay in Alabama (SERNEC 2022); however, it is also thought to have arrived in Florida around the same time (Chapman 1889). Cuban bulrush was likely introduced by migratory birds or ship ballasts from the tropical Americas. Cuban bulrush has spread to parts of Africa, Mexico, and throughout the southeastern United States where both types of terminal inflorescence are found (Bryson et al. 2008; Bryson and Carter 2008; Carter 2005; Grippo et al. 2014; Sartain et al. 2014). Historic records of the 1878 specimen found in Alabama showed presence of both inflorescence forms, both from the tropical Americas (SERNEC 2022).

The plant is commonly found along the water's edge, or floating freely within freshwater ditches, ponds, lakes, rivers, swamps, and marshes (Bryson et al. 2008, Grippo et al. 2014). The monocephalous inflorescence is found in northern Florida, southern Georgia, Alabama, and Mississippi (GISD 2023). The polyephalous form is predominately found in peninsular Florida, Louisiana, and coastal Texas (GISD 2023). Cuban bulrush forms large floating islands, or tussocks, on top of native or invasive species for buoyancy; this includes water hyacinth [*Eichhornia crassipes* (Mart.) Solms], water fern (*Salvinia minima* Baker), and hydrilla [*Hydrilla verticillata* (L. f.) Royle] (Watson and Madsen 2014). However, once the tussock root/rhizome network of Cuban bulrush assimilates enough sediment the tussock becomes self-supportive reproducing via buoyant, vegetative fragments that break off from the tussock (Robles et al. 2007, Turnage 2018, Watson and Madsen 2014).

IMPACTS

With the ability to rapidly colonize waterways, Cuban bulrush infestations impact multi-use lakes, reservoirs, flowing waters that provide drinking water, hydro-electric power, fish, outdoor recreational activities, and navigation for commercial and military vessels (Watson and Madsen 2014, Turnage and Shoemaker 2017). The floating islands cause blockages at lake-accesses and make any sort of river navigation and recreation almost impossible (Madsen 2004, Mallison et al. 2001, Turnage 2018, Watson and Madsen 2014). Infestations alter drainage in ditches or canals, thus increasing flood risk, reducing hydropower generation, and increasing evaporative loss along surface waters from lack of flow (Madsen 2004, Mallison et al. 2001, Turnage 2018, Watson and Madsen 2014). The mats negatively impact aquatic ecosystem function by crowding, shading, and displacing native or desirable aquatic plants (Turnage 2018). Low light penetration from increased Cuban bulrush organic matter decreases dissolved oxygen from a lack of photosynthesis from plants shaded below; an increase in decomposition also occurs when the plant masses die (Boylen et al. 1999, Kuehne et al. 2016, Watson and Madsen 2014).

PHENOLOGY, LIFE HISTORY, AND RESOURCE ALLOCATION

Phenology is the study of seasonal timing or life cycle in plants. Several environmental variables can be used to describe events in a plant's life cycle (Wersal and Madsen 2018). For instance, temperature influences physiological processes in plants, and can determine phenology and resource allocation (Madsen 1991). Aquatic plants tend to increase productivity in higher temperatures due to increased photosynthesis (Riis et al. 2012). For example, water hyacinth (*Eichornia crassipes*) reproduction and growth are strongly influenced by air and water temperature, where an increase in temperature correlates to more growth (Wilson et al. 2005). Additionally, light is a limiting factor for aquatic macrophytes and is correlated to increasing or decreasing temperature (Barko et al. 1986); this includes photosynthetically active radiation (PAR), or the duration of light during the day (photoperiod). Light can also determine plant zonation within a water body during times of the year (Seabloom et al. 1998). For example, a Mississippi population of parrotfeather (*Myriophyllum aquaticum*) during winter months increased in submersed biomass as irradiance and temperature decreased (Wersal et al. 2011). Another aquatic macrophyte that also shows some inverse relationships to light and temperature is Cuban bulrush.

Cuban bulrush is an epiphytic perennial invasive plant that exploits other aquatic plants or structures for habitat and does not directly rely on sediment roots or belowground biomass for most of its life cycle (Turnage 2018). Vegetative reproduction is done through stolons, while sexual reproduction is through corky buoyant achenes via water dispersal (Bryson and Carter 2008, Grippo et al. 2014, Watson and Madsen 2014).

Cuban bulrush relies on plants like water hyacinth (*Eichornia crassipes*) for seed germination in its leaf axils (Bryson et al. 2008). However, the achenes may have low fertility due to sporadic distribution (Bryson and Carter 2008, Watson and Madsen 2014). In addition, the inflorescences and seeds of Cuban bulrush are not ideal starch storage structures due to being small, low in biomass, and short lived (Clarke et al. 2023). To date, specialized storage structures, such as tubers, turions, or bulbils, have not been identified in Cuban bulrush. Carbohydrate storage is commonly described in two ways: reserve formation and accumulated reserves.

Reserve formation is when carbohydrates are stored for growth and defense (Chapin et al. 1990, Pennington and Systma 2009). Accumulated reserves are carbohydrates stored in excess when the supply exceeds the demand for growth and maintenance (Chapin et al. 1990, Pennington and Systma 2009). Carbohydrates are stored in three forms: free sugars, starch, and structural carbohydrates (Madsen et al. 1993). Nonstructural carbohydrates are soluble or free sugars, or long-term storage (Madsen et al. 1993). Free sugars store and provide energy for cells as well as transport energy throughout tissues of the plant (Madsen et al. 1993). In most cases, free sugars are turned into starch for use later (Madsen et al. 1993). Starch is one of the easiest carbohydrates to study as it is a long-term storage molecule (Madsen 1993, Madsen et al. 1993). In some aquatic plants, starch can be stored at 40 percent or more in various tissues (Madsen 1993, Madsen et al. 1993, Haram and Wersal 2023). The storage, management, and designation of resources such as carbohydrates is called resource allocation (Bazzaz et al. 1987). For instance, carbohydrates are allocated to different tissues such as emergent, submergent, or inflorescence biomass; biomass is one of the most widely used measurements in resource allocation studies (Bazzaz et al. 1987). Biomass tends to have an inverse relationship with starch when energy is being utilized for seasonal growth (Stitt and Zeeman 2012). Biomass and carbohydrate storage vary seasonally, mostly driven by its surrounding environment (Stitt and Zeeman 2012).

In most cases, starch storage peaks in the summer or fall, but depletes after plant growth in the spring; this includes most tissues in parrotfeather (*Myriophyllum aquaticum*)*,* emergent tissues of flowering rush *(Butomus umbellatus)* and most tissues of water hyacinth (*Eichornia crassipes*) (Madsen et al. 1993, Marko et al. 2015, Wersal et al. 2011, Wersal and Madsen 2018). Some plants follow a different cycle; for example, Brazilian Egeria (*Egeria densa* Planch.), an invasive to the west coast and southern United States, shows nonstructural carbohydrate levels peaking during the months of November-March, and was lowest during the months of April-June (Pennington and Systma 2009); hydrilla populations in Texas also follows a slightly different seasonal cycle, where storage is lowest during late June to mid-July when most reserve carbohydrates were utilized for spring regrowth (Owens and Madsen 1998). Cuban bulrush has also been found to have unique allocation patterns.

In Lake Columbus, Mississippi monocephalous Cuban bulrush did not store starch in large concentrations (total starch: <4%) (Clarke et al. 2023). Overall, starch was not disproportionately stored in any one tissue (<1.5%) (Clarke et al. 2023). However, peak biomass allocation corresponded with colder temperatures at a max total biomass of 309.0 g DW m^{-2} (November-January) (Clarke et al. 2023). Further analyses then revealed negative correlations with water temperature between all Cuban bulrush biomass tissues (Clarke et al. 2023). However, inflorescence tissues had a positive correlation with water temperature and incident light (Clarke et al. 2023). For instance, the least biomass allocation was in inflorescence tissues, peaking in late summer or early fall (Clarke et al. 2023). According to the same study, it was suggested that the affinity to colder temperature may also be a biological response to have a competitive advantage over other plants that senesce around the same time (Clarke et al. 2023). To our knowledge, there is no record of any native species within the area eliciting a similar response. In addition, there has not been a study looking at the phenology of Cuban bulrush outside of Mississippi, or the polycephalous biotype.

DEGREE DAYS AND PREDICTED SEASONAL BIOMASS

The global average surface temperature has warmed by 0.85°C between 1880 and 2012 (IPCC 2022). Temperatures in the U.S. increased 1.8°C from 1895 to 2016 with 38% of that increase (0.7°C) occurring in the last 35 years of that period, suggesting many invasive species could spread further north (Kriticos and Brunel 2016, Vose et al. 2017). For example, an invasion of threadleaf water-crowfoot (*Ranunculus trichophyllus*) into several high-elevation lakes in the Himalayas has been associated with a decrease in the length of ice cover due to climate change (Rahel and Olden 2008). Several invasive species of the Pooideae subfamily on the sub-Antarctic Marion Island also showed marked increases in photosynthesis with temperature (Ripley et al. 2019). Several studies including Spencer et. al (2000) have modeled plant growth and expansion utilizing temperature data using an accumulated degree day (ADD) approach.

Accumulated degree days (ADD) are heat units that are a measure of the time duration at various temperatures used to predict the length of time (i.e., calander days) it takes to achieve the occurrence of certain life stages (sprouting, peak biomass, flowering, senescence, etc.) in a plant's life cycle (Snyder 1985, Snyder et al. 1999, Spencer et al. 2000). Degree day-based modeling and equations have been helpful in forecasting sprouting and emergence from aquatic vegetative propagules such as *Hydrilla verticillata* (L.f.) Royle, *Potamogeton pectinatus* L., *Potomageton nodosus* Poiret, and *Vallisneria americana* L. (Spencer et al. 2000, Spencer and Ksander 2001). One of the first attempts for *Arundo donax* not only accurately predicted emergence but was able to predict emergence patterns from one year to the next (Spencer et al. 2006). Accumulated degree days have also been used to model growth and development of various terrestrial weeds such as yellow nutsedge (*Cyperus esculentus*) (Wilen et al. 1996).

Cuban bulrush has already been observed spreading farther northward into the mid-southern regions of the United States (Fernandez 2013, Grippo et al. 2014, Rahel and Olden 2008, Vose et al. 2017). Currently, it has been observed as far north as Tennessee (McLaurin et al. 2023). To date, there has only been one study documenting the phenological effects of temperature and light on Cuban bulrush biomass (Clarke et al. 2023), and one study modeling ADD using a limited geographic range (Squires et al. *In Review*).

Originally, it was assumed that Cuban bulrush could not tolerate freezing temperatures (Grippo et al. 2014); however, recent Cuban bulrush ADD data indicated growth could initiate at a base threshold temperature of -4°C (24.8°C) in Mississippi

(Squires et al. *In Review*). In addition, Cuban bulrush emergent biomass was found to occur in the colder months in the two years of the most recent study (December-January) (Clarke et al. 2023). Cuban bulrush biomass peaked when temperatures were near 5°C in Overall, this suggests that Cuban bulrush can expand further north than was previously expected; states such as Missouri have an average January temperature near -2°C (NOAA 2022).

PROPOSED RESEARCH

ADD modeling, starch allocation, and phenology studies have mostly occurred in the state of Mississippi, where the focus was on herbicide management, suitable habitat, and distribution (Clarke et al. 2023, Squires et al. *In Review*, Turnage 2018, Watson and Madsen 2014). However, geographic location can also impact life history characteristics for a plant. For example, northern populations of curlyleaf pondweed (*Potamogeton crispus*) have a winter annual life history strategy; however, southern populations follow more of an evergreen perennial life history strategy (Turnage et al. 2018, Woolf and Madsen 2004). The overall goals of this research are to expand on previous studies on Cuban bulrush by conducting a phenology study and accumulated degree day models on other biotypes within the southeastern U.S.

Phenology

The aim of this research is to provide a better understanding of the biomass and starch allocation of Cuban bulrush with differing temperatures and photoperiod at various field sites in Mississippi, Louisiana, and Florida. In addition, another objective of this study is to determine the phenological patterns between the monocephalous (MS) and polycephalous (LA and FL) biotypes found within the three states. It is hypothesized that Cuban bulrush will have a negative relationship with temperature and light, as seen in a prior phenological study (Clarke et al. 2023). It is also hypothesized that there will not be a significant difference in starch allocation due to previous observation in already low starch amounts (<4%).

Accumulated Degree Days

As invasive species continue to colonize new areas, significant resources are often exploited to manage infestations of aquatic weeds, therefore stressing the importance of developing new knowledge for management of species such as Cuban bulrush (Madsen and Wersal 2017). For instance, ADD can be used to predict peaks in biomass, and model geographic expansion of a species (Squires et al. *In Review*). The goal of this study is to expand the geographic extent of ADD research to two southeastern states that also have a differing biotype compared to a previous study (Squires et al. *In Review*). It is hypothesized that Louisiana and Florida, despite a different biotype, will show a similar preference to colder temperatures as the populations in Mississippi. In other words, the polycephalous form will also have a negative base threshold as seen in a prior study in Mississippi (Squires et al. *In Review*).

LITERATURE CITED

Barko JW, Adams MS, Clesceri NL. 1986. Environmental factors and their consideration in the management of submersed aquatic vegetation: a review. J. Aquat. Plant. Manage 24: 1-10.

Bazzaz FA, Chiariello NR, Coley PD, Pitelka LF. 1987. Allocating resources to reproduction and defense. BioSci. 37:58–67.

Boylen CW, Eichler LW, Madsen JD. 1999. Loss of native aquatic plant species in a community dominated by Eurasian watermilfoil. Hydrobiologia. 415:207-211.

Bryson CT, Carter R. 2008. The significance of Cyperaceae as weeds. Monogr Syst Bot Missouri Bot. Gard. 108:15-10.

Bryson CT, Maddox VL, Carter R. 2008. Spread of Cuban club-rush (*Oxycaryum Cubense*) in the southeastern United States. Invasive Plant. Sci. and Manag. 1:326–329.

Burnett DA, Champion PD, Clayton JS, Ogden J. 2007. A system for investigation of the temperature responses of emergent aquatic plants. Aquat. Bot. 86:187–190.

Chapin FS, Schulze E, Mooney HA. 1990. The ecology and economics of storage in plants. Ann. Rev. Ecol. Syst. 21:423-447.

Chapman AW. 1889. Flora of the southern states. 2nd ed. New York: Ivison, Blakeman and Company.

Clarke M, Wersal RM, Turnage G. 2023. Seasonal phenology and starch allocation patterns of Cuban bulrush (*Oxycaryum cubense*) growing in Mississippi, USA. Aquat. Bot. 186:103627.

Fernandez, AL. 2013. Dispersal and management of invasive aquatic plants in Mississippi waterways. Thesis, Mississippi State University, Starkville, Mississippi, USA.

Global Invasive Species Database (GISD).2023. Species profile: *Oxycaryum cubense*. http://www.iucngisd.org/gisd/species.php?sc=1231. Accessed 20 June 2023.

Grippo M, Fox L, Hayse J, Hlohowskyj I, Allison T. 2014. Risk of adverse impacts from the movement through the CAWS and establishment of aquatic nuisance species in the Great Lakes and Mississippi River basins. U. S. Army Corps of Engineers. 2: Appendix E.

Godfrey RK, Wooten JW. 1979. Aquatic and wetland plants of southeastern United States: monocotyledons. University of Georgia Press, Athens.

Haram AM, Wersal RM. 2023. Seasonal starch allocation of starry stonewort (*Nitellopsis obtusa*) growing in Lake Koronis, MN. Journal of Freshwater Ecology 38:2211086.

IPCC. 2022. Framing and context. In global warming of 1.5°C: ipcc special report on impacts of global warming of 1.5°c above pre-industrial levels in context of strengthening response to climate change, sustainable development, and efforts to eradicate poverty. pp. 49-92 Cambridge: Cambridge University Press. doi:10.1017/9781009157940.003.

ITIS. 2023. Integrated taxonomic information system. https://www.itis.gov/servlet/SingleRpt/SingleRpt. Accessed 15 June 2023.

Kriticos DJ, Brunel S. 2016. Assessing and managing the current and future pest risk from water hyacinth, (*Eichhornia crassipes*), an invasive aquatic plant threatening the environment and water security. PLoS One 11:1-18.

Kuehne LM, Olden JD, Rubenson ES. 2016. Multi-trophic impacts of an invasive aquatic plant. Freshw. Biol. 61:1846–1861.

Larridon I, Reynders M, Huygh W, Bauters K, Vrijdaghs A, Leroux O, Muasya M, Simpson D, Goetghebeur P. 2011. Taxonomic changes in C3 *Cyperus* (Cyperaceae) supported by molecular data, morphology, embryography, ontogeny and anatomy. Plant Ecol. Evol. 144:327–356.

Madsen JD. 1991. Resource allocation at the individual plant level. Aquat. Bot. 41:67–86.

Madsen JD. 1993. Control points in the phenological cycle of Eurasian watermilfoil. Aquatic Plant Control Research Program, Vol A-93-1. US Army Engineer Waterways Experiment Station, Vicksburg, MS.

Madsen JD. 1997. Seasonal biomass and carbohydrate allocation in a southern population of Eurasian watermilfoil. J. Aquat. Plant Manage. 35:15-21.

Madsen JD, Luu KT, Getsinger KD. 1993. Allocation of biomass and carbohydrates in waterhyacinth (*Eichhornia crassipes*): pond-scale verification. Technical Report A-9393, Vicksburg, MS: U. S. Army Engineer Waterways Experiment Station.

Madsen JD, Owens CS. 1998. Seasonal biomass and carbohydrate allocation in dioecious hydrilla. J. Aquat. Plant Manage. 36:138-145.

Madsen, JD. 2004. Invasive aquatic plants: a threat to Mississippi water resources. In 2004 Proceedings, Mississippi Water Resources Conference. pp. 122-134.

Mallison CT, Stocker RK, Cichra CE. 2001. Physical and vegetative characteristics of floating islands. J. Aquat. Plant Manage. 39:107-111.

Marko M, Madsen JD, Smith R, Sartain B, Olson C. 2015. Ecology and phenology of flowering rush in the Detroit Lakes chain of lakes, Minnesota. J. Aquat. Plant Manage. 53:54-54.

Mclaurin CS, Wersal RM, Daniel WM. 2023. *Cyperus blepharoleptos.* In: Nonindigenous Aquatic Species.

https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=2819&msclkid=23f9c944c5dc11ecb699ef587ca71b41. Accessed 15 June 2022.

NOAA National Centers for Environmental information. 2022. Climate at a glance: statewide mapping. In: National Climatic Data Center. https://www.ncdc.noaa.gov/cag/statewide/mapping/110/tavg/202201/1/value. Accessed 26 Apr 2022.

Oke TR. 1987. Boundary layer climates. Routledge, London England.

Owens CS, Madsen JD. 1998. Phenological studies of carbohydrate allocation in hydrilla. J. Aquat. Plant Manage. 36:40-44.

Pennington TG, Sytsma MD. 2009. Seasonal changes in carbohydrate and nitrogen concentrations in Oregon and California populations of Brazilian Egeria (*Egeria densa*). Invas. Plant Sci. Manage. 2:120–129.

Rahel FJ, Olden JD. 2008. Assessing the effects of climate change on aquatic invasive species. Conserv. Biol. 22:521–533.

Ribeiro AR, Pereira-Silva L, Vieira JP, Isabel L, Ribeiro VS, et al. 2021. *Cyperus prophyllatus*: An endangered aquatic new species of *Cyperus L*. (Cyperaceae) with a exceptional spikelet disarticulation pattern among about 950 species, including molecular phylogenetic, anatomical and (micro) morphological data.

Riis T, Olesen B, Clayton JS, et al. 2012. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. Aquat. Bot. 102:56–64.

Ripley BS, Edwardes A, Rossouw MW, et al. 2019. Invasive grasses of sub-Antarctic Marion Island respond to increasing temperatures at the expense of chilling tolerance. Annals. of Bot.125:765–773.

Robles W, Madsen JD, Maddox VL, Wersal RM. 2007. The invasive status of giant salvinia and hydrilla in Mississippi. pp. 109–113 In: Proceedings of the 37th Annual Mississippi Water Resources Conference, Jackson, MS.

Sartain BT, Turnage G, Madsen JD. 2014. Aquatic plant community and invasive plant management assessment of the Ross Barnett reservoir, MS in 2013. Geosystems Research Institute Report 5062.

Schoch CL, et al. 2020. Taxonomy browser (*Cyperus blepharoleptos*). In: National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=76484. Accessed 16 Jun 2022.

Seabloom EW, van der Valk AG, Moloney KA. 1998. The role of water depth and soil temperature in determining initial composition of prairie wetland coenoclines. Plant Ecology 138:203–216.

SERNEC. 2022. Southeastern regional network of expertise and collections. http://sernecportal.org/portal/collections/index.php. Accessed 24 May 2023.

Snyder R. 1985. Hand calculating degree days. Agric. For Meteorol. 35:353–358.

Snyder RL, Spano D, Cesaraccio C, Duce P. 1999. Determining degree-day thresholds from Field Observations. Int. J. of Biometeorol. 42:177–182.

Spencer DF, Ksander GG, Madsen JD, Owens CS. 2000. Emergence of vegetative propagules of *Potamogeton nodosus*, *Potamogeton pectinatus*, *Vallisneria Americana*, and *Hydrilla verticillata* based on accumulated degree-days. Aquat. Bot. 67:237–249.

Stitt M, Zeeman SC. 2012. Starch turnover: pathways, regulation and role in growth. Curr. Opin. in Plant Biol. 15 (30); 282-92.

Turnage G. 2018. Control of Cuban bulrush (*Oxycaryum cubense*) through submersed herbicide applications- Final Report. Geosystems Research Institute Report 5080.

Turnage G, Madsen JD, Wersal RM. 2018. Phenology of curlyleaf pondweed (*Potamogeton crispus* L.) in the southeastern United States: A two-year mesocosm study. J. Aquat. Plant Manage. 56:35-38.

Turnage G, Shoemaker C. 2017. 2017 Survey of aquatic plant species in Mississippi waterbodies. Geosystems Research Institute Report 5077.

Vose RS, Easterling DR, Kunkel KE, LeGrande AN, Wehner MF. 2017. Climate science special report: fourth national climate assessment, volume I. U.S. Global Change Research Program, Washington, DC.

Watson, AL, Madsen JD. 2014. The effect of the herbicide and growth stage on Cuban club-rush (*Oxycaryum cubense*) control. J. Aquat. Plant Manage. 52:71-74.

Wersal RM, Cheshier JC, Madsen JD, Gerard PD. 2011. Phenology, starch allocation, and environmental effects on *Myriophyllum aquaticum*. Aquat. Bot. 95:194–199.

Wersal RM, Madsen JD. 2018. Designing and using phenological studies to define management strategies for aquatic plants. J. Aquat. Plant Manage. 56s:83-89.

Wilson JR, Holst N, Rees M. 2005. Determinants and patterns of population growth in *E. crassipes*. Aquat. Bot. 81:51–67.

Woolf TE, Madsen JD. 2003. Seasonal Biomass and Carbohydrate Allocation Patterns in Southern Minnesota Curlyleaf Pondweed Populations. J. Aquat. Plant Manage. 41:113– 118.

CHAPTER II

Seasonal phenology and starch allocation patterns of *Oxycaryum Cubense* in the Southeastern U.S.

ABSTRACT

Most knowledge about Cuban bulrush (*Oxycaryum cubense* (Poepp. & Kunth) Lye comes from studies done in Mississippi with the monocephalous form. However, there has not been a study comparing phenological differences between Cuban bulrush populations in other states, specifically ones that have the polycephalous form (Louisiana and Florida). Therefore, seasonal patterns of biomass and starch allocation for the aquatic invasive species (AIS) Cuban bulrush were quantified in populations from Lake Columbus on the Tennessee-Tombigbee Waterway in Mississippi, Lake Martin in Louisiana, and Orange Lake in Florida. Monthly samples of Cuban bulrush were harvested from two plots per state from October 2021-September 2022. During each sampling period, air temperature and photoperiod were recorded. Starch allocation patterns were similar among all states, with starch not being disproportionately stored in any one tissue (<1.5% DW for all plant tissues). Biomass allocation was different between all states with Mississippi (monocephalous form) having greater total biomass compared to the other states at 600.71 g DW m^{-2} ± 37.76 SE; Louisiana (polycephalous form) and Florida (polycephalous form) at 392.25 g DW m^{-2} ± 62.14 SE and 233.85 g DW m^{-2} ± 7.33 SE respectively. Peak inflorescence biomass occurred in the winter for the LA and FL biotypes, and the summer for the MS biotype. Mississippi inflorescence

biomass had a positive relationship with air temperature (r^2=0.53). However, emergent and submersed biomass within each state was generally negatively related with photoperiod and temperature (r^2=0.022-0.7789). Therefore, it is suggested that this species is able to expand northward regardless of biotypes.

INTRODUCTION

Cuban bulrush is an epiphytic perennial invasive plant that exploits other aquatic plants or structures for habitat and does not directly rely on sediment roots or belowground biomass for most of its life cycle (Turnage 2018). It is native to the tropical Americas and is found throughout the southeastern United States where both types of terminal inflorescences are found: polycephalous (many) and moncephalous (one) (Bryson et al. 2008; Bryson and Carter 2008; Carter 2005; Grippo et al. 2014; Sartain et al. 2014). Vegetative reproduction is done via stolons, while sexual reproduction is through corky buoyant achenes via water dispersal (Bryson and Carter 2008, Grippo et al. 2014, Watson and Madsen 2014). Cuban bulrush relies on plants like water hyacinth (*Eichornia crassipes*) as seed germination occurs in water hyacinth leaf axils (Bryson et al. 2008). However, the achenes may have low fertility and observed sporadic distribution within the country (Bryson and Carter 2008, Watson and Madsen 2014). Moreover, the phenology, or the seasonal timing of critical life stages like biomass allocation in Cuban bulrush, is relatively unique compared to most aquatic plants (Clarke et al. 2023, Wersal and Madsen 2018).

During a plant's life cycle, resources such as carbohydrates are stored or used for critical functions like growth, maintenance, and photosynthesis (Chapin et al. 1990, Pennington and Sytsma 2009). How a plant stores, manages, and assigns these resources is called resource allocation. Biomass and carbohydrate storage vary seasonally, where biomass tends to have an inverse relationship with starch when energy is being utilized for seasonal growth; this can be determined by several environmental variables such as temperature and light (Stitt and Zeeman 2012, Wersal and Madsen 2018).

Temperature influences physiological processes in plants and can determine the phenology of biomass and starch allocation (Madsen 1991). However, light is often the most important factor in aquatic macrophytes and is often positively correlated with increasing or decreasing temperatures (Barko et al. 1986). This includes photosynthetically active radiation (PAR), or the duration of light during the day (photoperiod). How these factors influence aquatic plants can cause biomass accumulation to differ. For example, water hyacinth (*Eichornia crassipe*s) was observed to peak during late summer/early fall due to a strong positive influence with air temperature (Wilson et al. 2005). In Minnesota, *P. crispus* biomass peaked in May or June which coincided with peak turion production and was followed by little to no growth during icy conditions (Woolf and Madsen 2003). However, *Potamageton crispus* in Mississippi biomass peaked in February due to a strong positive correlation with water temperatures between 10-20°C and photoperiod of 10-12 hours (Turnage et al. 2018). A similar timing of peak biomass was found with Mississippi populations of Cuban bulrush (Clarke et al. 2023).

There has been one study in Lake Columbus on the Tennessee -Tombigbee Waterway in Mississippi researching starch allocation patterns in tissues of monocephalous Cuban bulrush (Clarke et al. 2023). Peak biomass corresponded with colder temperatures with a max total biomass of 309.0 g DW m^{-2} (November-January) (Clarke et al. 2023). Further analyses revealed negative correlations between all Cuban bulrush tissues but inflorescence with water temperature and PAR (Clarke et al. 2023). Cuban bulrush only stored starch at 3.7% DW, with peak storage occurring from August to September. Currently, there has not been a study looking at the phenology of Cuban bulrush outside of Mississippi or the monocephalous biotype.

Geographic distribution can change life cycles for a plant, as seen in curlyleaf pondweed (*Potamogeton crispus*) (Turnage et al. 2018, Woolf and Madsen 2003). Therefore, the objectives of this research were to expand the spatial extent of the previous phenology study (Clarke et al. 2023) and investigate the phenological differences in Cuban bulrush populations over a one-year period in MS, LA, and FL. Additionally, resource allocation patterns were related to environmental metrics such as photoperiod and temperature. Considering that there is a polycephalous inflorescence form in Louisiana and Florida, another goal was to also compare starch allocation and phenology between the monocephalous and polycephalous Cuban bulrush populations. It is hypothesized that most Cuban bulrush tissues will have a negative relationship with temperature and light, further supporting the prior phenological study (Clarke et al. 2023). Similarly, it is hypothesized that there will not be a significant difference in total starch allocation between states or inflorescence form.

METHODS

Biomass data collection

The study was conducted on existing populations of Cuban bulrush infesting Lake Columbus on the Tennessee-Tombigbee Waterway in NE Mississippi (33°32' 2.76" N, 88°29'27.60" W), Lake Martin near Baton Rouge, Louisiana (30°13' 23.2572" N, 91°54'22.284" W), and Orange Lake near Orlando, Florida (29°27' 32.39" N, 82°10'23.40" W). Plots in Mississippi were created by placing PVC posts into the lakebed at the corners of each plot; each corner post was then geotagged in case of damage from flooding or other natural events. Louisiana and Florida plots were marked with plot centers that were also geotagged. Sites were located in backwater areas that were less likely to be affected by disturbance. From October 2021-September 2022 Cuban bulrush was harvested monthly from two 0.04 Ha (0.1 ac) plots in each state. Twelve Cuban bulrush biomass and height samples were collected randomly using a 0.1 m^2 (33 cm x 33 cm) PVC frame placed on the water surface over the plants at each site. Within the frame, height was collected using a meter stick from the water surface to the top of the plant. Once the PVC frame was randomly placed, all Cuban bulrush plants were cut within the perimeter of the PVC frame, placed in labeled 3.79-L Ziploc® bags, stored in a cooler, and transported from each site in the three states to Mississippi State University where the plants were separated into emergent, submergent, and inflorescence tissues. Plant tissues were placed into labeled paper bags and dried at 70°C for five days in a forced air oven. Once dried, the biomass samples were weighed to determine g m^{-2} for each plant for each month.

Environmental monitoring

One onset HOBO pendant was placed on a pole 1-2 meters above the water surface at each site to collect air temperature (°C) and photoperiod every hour for the duration of the study. Any data that was unattainable due to weather events or equipment failure was supplemented with temperature data from nearby weather stations such as the MSU RR Foil Plant Science Research Station for Mississippi (33°28' 12.15" N, 88°46' 48.10"W), the Lafayette Regional Airport for Louisiana (30°12' 11.40" N, 91°59' 8.99" W), and the Gainesville Regional Airport for Florida (29°41' 30.12" N, 82°16' 33.70" W).

Starch analysis

Cuban bulrush biomass was used to assess the seasonal allocation patterns of starch in emergent, submergent, and inflorescence tissues. Dried biomass samples from each site were composited into three groups of four (biomass samples 1-4 into tissue sample 1; 5-8 into sample 2, 9-12 into sample 3) to obtain three tissue samples for each plant at each sample location of every month (Clarke et al. 2023, Wersal et al. 2011, Wersal et. al 2013). Compositing samples ensured that there was enough tissue mass available for starch analysis.

Once the samples were composited, they were ground at MSU (Mississippi State University) using a Cyclone Sample Mill (UDY Corporation, Fort Collins, Colorado) to pass through #40 mesh screen (1 mm). Ground tissues were shipped to Minnesota State University- Mankato (MNSU) for starch analysis. At MNSU, approximately 60 mg of the ground samples were used for starch analysis. Starch extraction and determination was conducted using the Amylase/Amyloglucosidase method through a commercially purchased STA20 starch assay kit from Sigma Aldrich (Sigma Aldrich, St. Louis, Missouri) utilized by several studies including Clarke et al. (2023), Wersal et al. (2011), and Haram and Wersal (2023). Wheat and corn starch standards that were included with the kits as 89% and 93% pure starch, and two sets of duplicated Cuban bulrush samples were assayed per field site and per month to determine the reliability of starch data. Standard curves were developed to ensure that starch data were within the range of what the kits could detect, and to assess the relative accuracy of starch data. The precision of our assays as determined by the percent difference of our duplicate samples was 11.13% ± 1.23 (SE). Accuracy as determined by our standard curves was an R^2=0.98. Average corn and wheat starch recovery was 94.75% ± 1.68 SE and 101.90% ±2.24 SE.

Statistical analysis

Monthly averages for biomass, plant heights, and environmental variables (photoperiod and temperature) were computed for each plot in each state (MS, LA, and FL). To better observe seasonal trends in biomass, a three-point moving average was calculated. A Kruskal-Wallis One-Way ANOVA was conducted on total, inflorescence, emergent, and submersed biomass and starch, followed by a Dunn's All- Pairwise Comparisons post-hoc test to determine if a state effect existed. Regression analyses were used to determine relationships between environmental variables (photoperiod and air temperature) and plant metrics. Regression models were sequentially fit, beginning with a linear model. Polynomial terms were then added one at a time to improve model fit if needed. All analyses were conducted at a $\alpha \leq 0.05$ significance level using Statistix 10 or SigmaPlot 12.5.

RESULTS

Seasonal biomass and environmental factors

There was a significant state effect on total, inflorescence, and emergent biomass ($p<0.01$) in which all biomass metrics were different in each state. A significant state effect was found on submersed biomass ($p<0.01$) where biomass in Louisiana was lower than the other states. Total biomass was greatest in Mississippi, where peak biomass was 600.71 g DW m^{-2} ± 37.76 SE in October (Figure 2.1). Total biomass in Louisiana peaked at 392.25 g DW m^{-2} ± 62.14 SE in September (Figure 2.1). Total biomass in Florida peaked at 233.85 g DW m^{-2} ± 7.33 SE in May (Figure 2.1). Peak inflorescence biomass in Mississippi, Louisiana, and Florida was 19.53 g DW m^{-2} ± 0.68 SE in August, 138.53 g DW m^{-2} ± 5.76 SE in December, and 22.00 g DW m^{-2} ± 6.13 SE, in January respectively (Figure 2.1). All average emergent peak biomass went from 336.78 g DW m^{-2} ± 31.21 SE in Mississippi, 250.33 g DW m^{-2} ± 9.00 SE in Louisiana, and 168.76 g DW m^{-2} ± 24.81 SE in Florida within August to October (Figure 2.1). Submersed biomass showed differences in peak timings where Mississippi, Louisiana, and Florida peaked at 258.79 g DW m^{-2} ± 71. 62 SE, 76.63 g DW m-2 ± 1.78 SE, and 183.77 g DW m^{-2} ± 29.20 SE in October, January, and May, respectively (Figure 2.1).

Inflorescence biomass from Mississippi had a positive relationship with air temperature ($r^2= 0.53$) and photoperiod ($r^2=0.31$) (Figure 2.2). There were no significant relationships between total biomass, emergent biomass, submersed biomass, and the environmental variables from Mississippi (Table 2.1). Inflorescence and submersed biomass from Louisiana had a negative relationship with photoperiod (r^2= 0.62 and 0.78, respectively) and submersed biomass had a negative relationship (r^2=0.64) with air temperature (Figure 2.3). There was no relationship between emergent biomass and the environmental variables from Louisianna (Table 2.11). Inflorescence biomass in Florida had a negative relationship with photoperiod (r^2=0.45) (Table 2.1, Figure 2.4). However, there was a positive relationship between submersed biomass and air temperature (Table 2.1, Figure 2.4). There were negative relationships among the rest of the tissues (Table 2.1, Figure 2.4). There was no significant relationship between Florida total biomass and the environmental variables (r^2<0.1) (Table 2.1, Figure 2.4). Mississippi height had a positive relationship with air temperature (r^2=0.1846), and Florida height had a negative relationship with photoperiod (r^2=0.4835) (Figure 2.5). There was no significant relationship between Louisiana height and the environmental variables (Table 2.1).

Seasonal starch and environmental factors

A significant state effect was found between Mississippi and Florida for total and emergent starch (p<0.01). A significant state effect was not found for inflorescence and submersed starch (p=0.57, 0.27). Peak total starch (%DW) was low among states between 2.00-2.8% DW within the months of October and February (Figure 2.6). Low points in starch generally occurred from May-September for all three states (0.38- 1.00% DW) (Figure 2.6). Inflorescence starch ranged from 0.09%-1.2% among all three states (Figure 2.6) Starch in plant samples from Louisiana and Florida had more starch in the winter months, while Mississippi had more starch later in the summer (Figure 2.6).

Overall, starch was not predominately stored in any one tissue during the study (<1.4% DW per tissue).

Emergent starch from Mississippi had a negative relationship with air temperature (r^2= 0.43); there were no significant relationships with total and inflorescence starch (Figure 2.7) (Table 2.1). Inflorescence starch from Louisiana had a negative relationship with photoperiod (r^2=0.41); all other relationships were negative. Emergent starch in Louisianna had no significant relationships (p=0.87-0.95) (Figure 2.8) (Table 2.1). Starch in Florida samples had negative relationships between air and photoperiod with all tissues (Figures 2.9 and 2.10) (Table 2.1).

DISCUSSION

Seasonal biomass and environmental factors

The polycephalous form was found in Louisiana and Florida in this study, while the monocephalous form was found in Mississippi. Both populations in Louisiana and Florida inflorescence showed a change in phenology compared to Mississippi by peaking between November and January and having higher inflorescence biomass. Moreover, the monocephalous form was shown to allocate more resources to generating total biomass than the polycephalous form, showing a difference in resource allocation between the two inflorescence types. The differing phenology and higher allocation of inflorescence biomass in Louisiana and Florida may be due to higher stresses from other aquatic species. For instance, both Florida and Louisiana are ranked first and second for introduced species, including aquatic species (USGS 2023). Many introduced plants become invasive, causing high competition for other plants in the same area (Gérard and Triest 2018, USGS 2023). Interspecific competition can cause niche differentiation, such as differing phenology (Uchida et al. 2018). Sexual reproduction is also hypothesized to be more beneficial than clonal propagation for plants growing at high density, because it promotes dispersal; it is known that Cuban bulrush utilizes sexual reproduction for a similar purpose (Turnage 2020, Zhang and Zhang 2007). This may explain why there was a difference in biotypes. Since this is the first study to observe phenological differences of Cuban bulrush within the southeastern United States, there are several environmental variables that could also influence phenology (Barko et al. 1986, Burnett et al. 2007).

Light (photoperiod or PAR) can be one of the most limiting factors for submersed and emergent macrophytes (Barko et al. 1986). In all three states, it is seen that Cuban bulrush tends to have a negative relationship with photoperiod. In other words, as photoperiod increases, Cuban bulrush biomass tends to decrease. Increasing photoperiod can result in stress on aquatic plants due to transpiration exceeding water uptake in emergent plants (Salvucci and Bowes 1982). It is possible that Cuban bulrush increased in transpiration in more southerly populations, however, this was not directly measured. From this study, the hypothesis that most Cuban bulrush biomass will have a negative relationship with temperature and light is supported. For instance, in Mississippi, light availability is reduced in the fall and winter months, where photoperiod ranges from 11-12 hours; other than the inflorescence tissue, most Mississippi biomass corresponded with this lower light period. Louisiana and Florida populations had several negative relationships with photoperiod. For instance, both populations had several tissues with higher biomass during a photoperiod between 10-11 hours of light. The exposure to more intense and consistent photoperiod is seen to occur as populations move closer to the equator. Therefore, it is suggested that the further south a population grows, the less biomass allocation there was due to increased rates of photorespiration and a decrease in carbon assimilation (Salvucci and Bowes 1982). The influence of light on aquatic macrophytes is not the only factor and tends to be coupled with temperature. For example, light is often positively correlated with temperature (Barko et al. 1986, Bornette and Puijalon 2010).

Depending on the growth form of an aquatic plant (i.e., free-floating, emergent, etc.), the effect of air temperature can vary (Dhir 2015). Temperatures between 20 and 35 °C are considered optimal for most aquatic plant species (Santamaria and van Vierssen 1997). Other emergent plants like Cuban bulrush such as cattail (Typha spp.) biomass increased positively with temperatures (Dhir 2015). However, it is seen that the higher total biomass was observed to occur in colder months where average temperatures in December was 9.41-13.55°C and January was 6-7.22°C, 12.20-18.42°C in December and 9.00-10.53°C in January, and 16.10-17.28°C in December and 14.20-14.70°C in January for Mississippi, Louisiana, and Florida, respectively.

Seasonal starch allocation and environmental factors

Total starch storage was different between Mississippi and Florida, though as reported previously Cuban bulrush does not store starch in large quantities (Clarke et al. 2023). In several other aquatic plants such as coontail (*Ceratophyllum demersum* L.), wild celery (*Vallisneria americana* Michx.), curlyleaf pondweed (*Potamogeton crispus* L.), and Eurasian watermilfoil (*Myriophyllum spicatum* L.) store total nonstructural carbohydrates between 11% and 66% depending upon the species and plant structure (Best and Visser 1987, Kunii 1989, Titus and Adams 1979, Woolf and Madsen 2003). However, this study saw concentrations below 1.5% DW in each biomass tissue in each state, further confirming that starch is not a major component of carbohydrate reserves for this species (Clarke et al. 2023). Similar results have been seen with plants such as water hyacinth, where starch is less than 5% (Madsen et al. 1993).

CONCLUSION

Overall, total starch allocation is similar if there are not much latitudinal differences (i.e., Louisiana and Florida). Cuban bulrush may prefer lower light durations due to a preference for periods of light between 10-12 hours of light compared to 14-16 hours, which is seen in all states. Among all three states, Cuban bulrush also survived and grew during colder months where most plants senesce. Given the differences (or lack thereof) of the resource allocation of Cuban bulrush populations in the three studied states and two biotypes, it is also suggested that it has a broad temperature tolerance, and high adaptability. Therefore, the northern expansion of this species is of concern. Cuban bulrush has already been shown to spread further north than previously expected, which elicits the question of how far north Cuban bulrush can expand (Bryson et al. 2008).

LITERATURE CITED

Barko JW, Adams MS, Clesceri NL. 1986. Environmental factors and their consideration in the management of submersed aquatic vegetation: a review. J. Aquat. Plant. Manage 24: 1-10.

Best EP, Visser HW. 1987. Seasonal growth of the submerged macrophyte *Ceratophyllum demersum* L. in Mesotrophic Lake Vechten in relation to insolation, temperature and reserve carbohydrates. Hydrobiologia 148:231–243.

Bornette G, Puijalon S. 2010. Response of aquatic plants to abiotic factors: A Review. Aquat. Sci. 73:1–14.

Bryson CT, Carter R. 2008. The significance of Cyperaceae as weeds. Monogr Syst Bot Missouri Bot. Gard. 108:15-10.

Bryson CT, Maddox VL, Carter R. 2008. Spread of Cuban club-rush (*Oxycaryum Cubense*) in the southeastern United States. Invasive Plant. Sci. and Manag. 1:326–329.

Burnett DA, Champion PD, Clayton JS, Ogden J. 2007. A system for investigation of the temperature responses of emergent aquatic plants. Aquat. Bot. 86:187–190.

Carter, R. 2005. An introduction to the sedges of Georgia. Tipularia. 20:15-44.

Chapin FS, Schulze E, Mooney HA. 1990. The ecology and economics of storage in plants. Ann. Rev. Ecol. Syst. 21:423-447.

Clarke M, Wersal RM, Turnage G. 2023. Seasonal phenology and starch allocation patterns of Cuban bulrush (*Oxycaryum cubense*) growing in Mississippi, USA. Aquat. Bot. 186:103627.

Dhir B. 2015. Status of aquatic macrophytes in changing climate: A perspective. Journal of Environmental Science and Technology 8:139–148.

Gérard J, Triest L. 2018. Competition between invasive *Lemna minuta* and native *L. minor* in indoor and field experiments. Hydrobiologia. 812(1):57-65.

Grippo M, Fox L, Hayse J, Hlohowskyj I, Allison T. 2014. Risk of adverse impacts from the movement through the CAWS and establishment of aquatic nuisance species in the Great Lakes and Mississippi River basins. U. S. Army Corps of Engineers. 2: Appendix E.

Haram AM, Wersal RM. 2023. Seasonal starch allocation of starry stonewort (*Nitellopsis obtusa*) growing in Lake Koronis, MN. Journal of Freshwater Ecology 38: 1-11.

Hussner A. 2009. Growth and photosynthesis of four invasive aquatic plant species in Europe. Weed Research 49:506–515.

Kunii H .1989. Continuous growth and clump maintenance of *Potamogeton Crispus* L. in Narutoh River, Japan. Aquatic Botany 33:13–26.

Madsen JD, Luu KT, Getsinger KD. 1993. Allocation of biomass and carbohydrates in waterhyacinth (*Eichhornia crassipes*): pond-scale verification. Technical Report A-9393, Vicksburg, MS: U. S. Army Engineer Waterways Experiment Station.

Madsen JD. 1991. Resource allocation at the individual plant level. Aquat. Bot. 41:67–86.

Pennington TG, Sytsma MD. 2009. Seasonal changes in carbohydrate and nitrogen concentrations in Oregon and California populations of Brazilian Egeria (*Egeria densa*). Invas. Plant Sci. Manage. 2:120–129.

Salvucci ME, Bowes G. 1982. Photosynthetic and photorespiratory responses of the aerial and Submerged Leaves of *Myriophyllum brasiliense*. Aquat. Bot. 13:147–164.

Santamaría L, van Vierssen W. 1997. Photosynthetic temperature responses of fresh- and brackish-water macrophytes: A Review. Aquatic Botany 58:135–150.

Sartain BT, Turnage G, Madsen JD. 2014. Aquatic plant community and invasive plant management assessment of the Ross Barnett reservoir, MS in 2013. Geosystems Research Institute Report 5062.

Stitt M, Zeeman SC. 2012. Starch turnover: pathways, regulation and role in growth. Curr. Opin. in Plant Biol. 15 (30); 282-92.

Titus JE, Adams MS. 1979. Comparative carbohydrate storage and utilization patterns in the submersed macrophytes, *Myriophyllum spicatum* and *Vallisneria Americana*. American Midland Naturalist 102:263.

Turnage G, Madsen JD, Wersal RM. 2018. Phenology of curlyleaf pondweed (*Potamogeton crispus* L.) in the southeastern United States: A two-year mesocosm study. J. Aquat. Plant Manage. 56:35-38.

Turnage G. 2018. Control of Cuban bulrush (*Oxycaryum cubense*) through submersed herbicide applications- Final Report. Geosystems Research Institute Report 5080.

Turnage G. 2020. INVASIVE PLANT FACTSHEET Cuban bulrush (Oxycaryum cubense (Poepp. & Kunth) Lye).

Uchida, K, Fujimoto H, Ushimaru A. 2018. Urbanization promotes the loss of seasonal dynamics in the semi-natural grasslands of an East Asian megacity. Basic and Applied Ecology 29:1-11.

USGS. 2023. NAS graphs and charts: aquatic species by state. In: Nonindigenous Aquatic Species. https://nas.er.usgs.gov/graphs/State.aspx. Accessed 14 November 2023.

Watson, AL, Madsen JD. 2014. The effect of the herbicide and growth stage on Cuban club-rush (*Oxycaryum cubense*) control. J. Aquat. Plant Manage. 52:71-74.

Wersal RM, Cheshier JC, Madsen JD, Gerard PD. 2011. Phenology, starch allocation, and environmental effects on *Myriophyllum aquaticum*. Aquat. Bot. 95:194–199.

Wersal RM, Madsen JD, Cheshier JC. 2013. Seasonal biomass and starch allocation of common reed (*Phragmites australis*) (haplotype I) in Southern Alabama, USA. Invasive Plant Science and Management 6:140–146.

Wersal RM, Madsen JD. 2018. Designing and using phenological studies to define management strategies for aquatic plants. J. Aquat. Plant Manage. 56s:83-89.

Woolf TE, Madsen JD. 2003. Seasonal Biomass and Carbohydrate Allocation Patterns in Southern Minnesota Curlyleaf Pondweed Populations. J. Aquat. Plant Manage. 41:113– 118.

Zhang Y, Zhang D. 2007. Asexual and sexual reproductive strategies in Clonal plants. Frontiers of Biology in China 2:256–262.

Table 1. Regression (r^2) analyses for Cuban bulrush plant metrics and environmental factors from two sampling locations in Mississippi, Louisiana, and Florida from October 2021 to September 2022.

		Environmental Factor			
		Air Temperature		Photoperiod	
State	Plant Metric	P Value	R^2 Value	P value	R^2 Value
	Total Biomass	0.4	0.0321	0.33	0.0439
	Inflorescence Biomass	<0.01	0.5312	<0.05	0.3095
	Emergent Biomass	0.49	0.022	0.23	0.0649
	Submersed Biomass	0.65	0.0106	0.48	0.0218
Mississippi	Total Starch	0.43	0.0299	0.09	0.1327
	Inflorescence Starch	0.66	0.0145	0.7	0.0113
	Emergent Starch	<0.01	0.4338	<0.05	0.2673
	Submersed Starch	<0.05	0.2625	<0.05	0.2094
	Height	<0.05	0.1846	0.84	0.0018
	Total Biomass	0.39	0.0337	<0.05	0.3669
	Inflorescence Biomass	<0.01	0.3184	<0.01	0.6245
	Emergent Biomass	0.49	0.0216	0.31	0.047
	Submersed Biomass	<0.01	0.6413	<0.01	0.7789
Louisiana	Total Starch	<0.05	0.2356	<0.01	0.3747
	Inflorescence Starch	0.25	0.1013	<0.05	0.4095
	Emergent Starch	0.73	0.006	0.77	0.0043
	Submersed Starch	0.1	0.1285	<0.05	0.216
	Height	0.76	0.0045	0.15	0.0907
	Total Biomass	0.44	0.075	0.095	0.2098
	Inflorescence Biomass	<0.01	0.4522	<0.01	0.4503
	Emergent Biomass	0.64	0.0099	0.18	0.0815
	Submersed Biomass	<0.05	0.3354	<0.01	0.404
Florida	Total Starch	<0.01	0.6617	<0.01	0.5838
	Inflorescence Starch	<0.01	0.631	<0.05	0.453
	Emergent Starch	<0.05	0.2511	<0.05	0.1722
	Submersed Starch	<0.01	0.4194	<0.01	0.4617
	Height	<0.05	0.1671	<0.01	0.3312

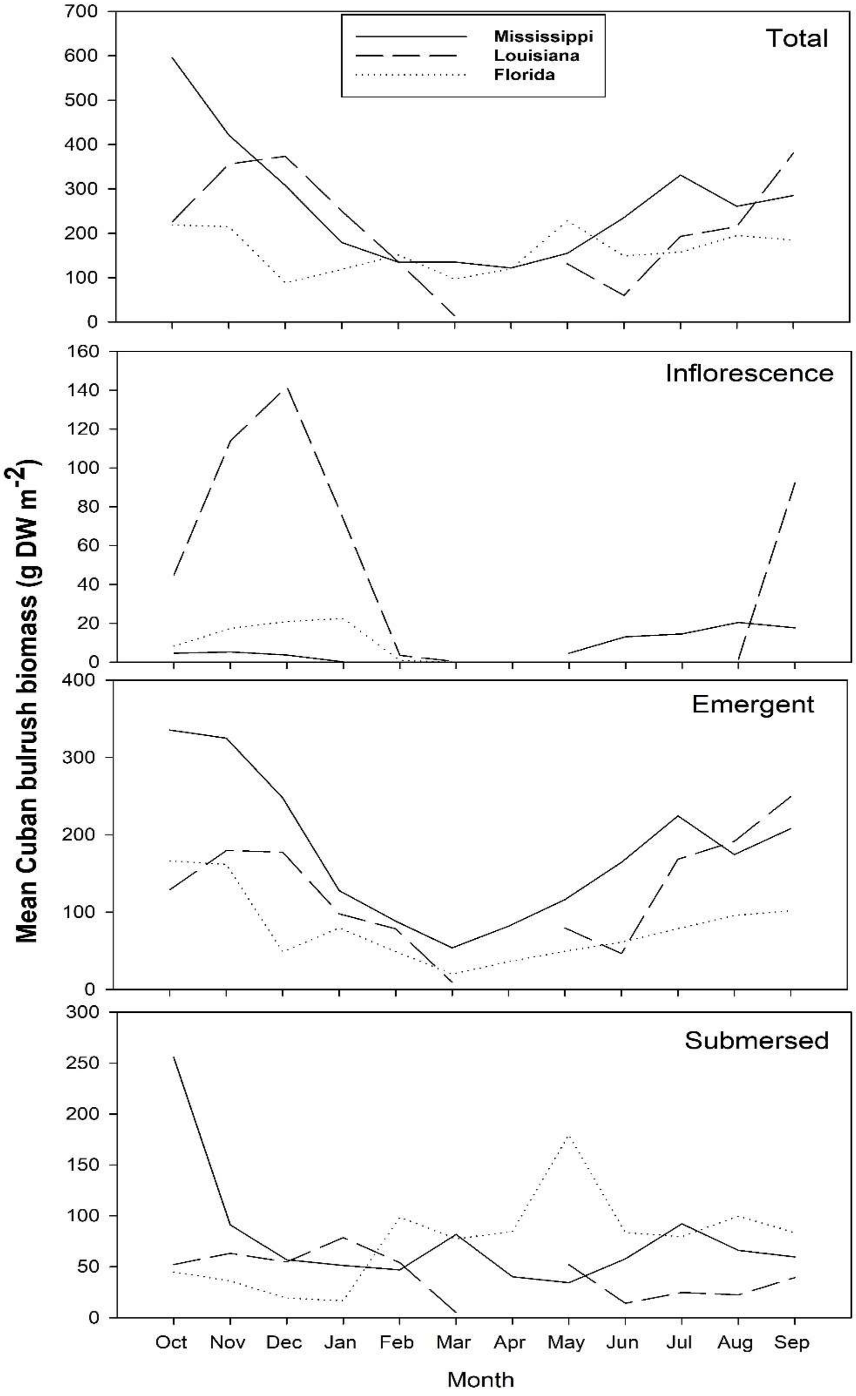

Figure 2.1. Mean Cuban bulrush seasonal total biomass (g DW m^{-2}) from two sampling locations in Mississippi, Louisiana, and Florida from October 2021 to September 2022.

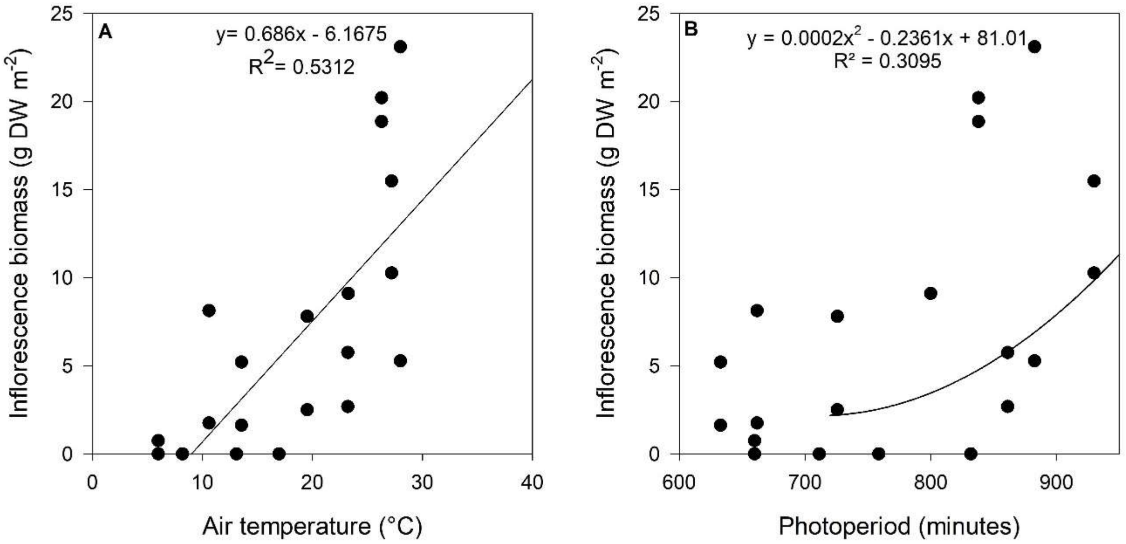

Figure 2.2 Cuban bulrush inflorescence biomass (g DW m^{-2}) samples collected in Lake Columbus, MS from October 2021-September 2022; (A) Cuban bulrush inflorescence biomass (g DW m^{-2}) by air temperature (°C); (B) Cuban bulrush inflorescence biomass (g DW m^{-2}) by photoperiod (minutes).

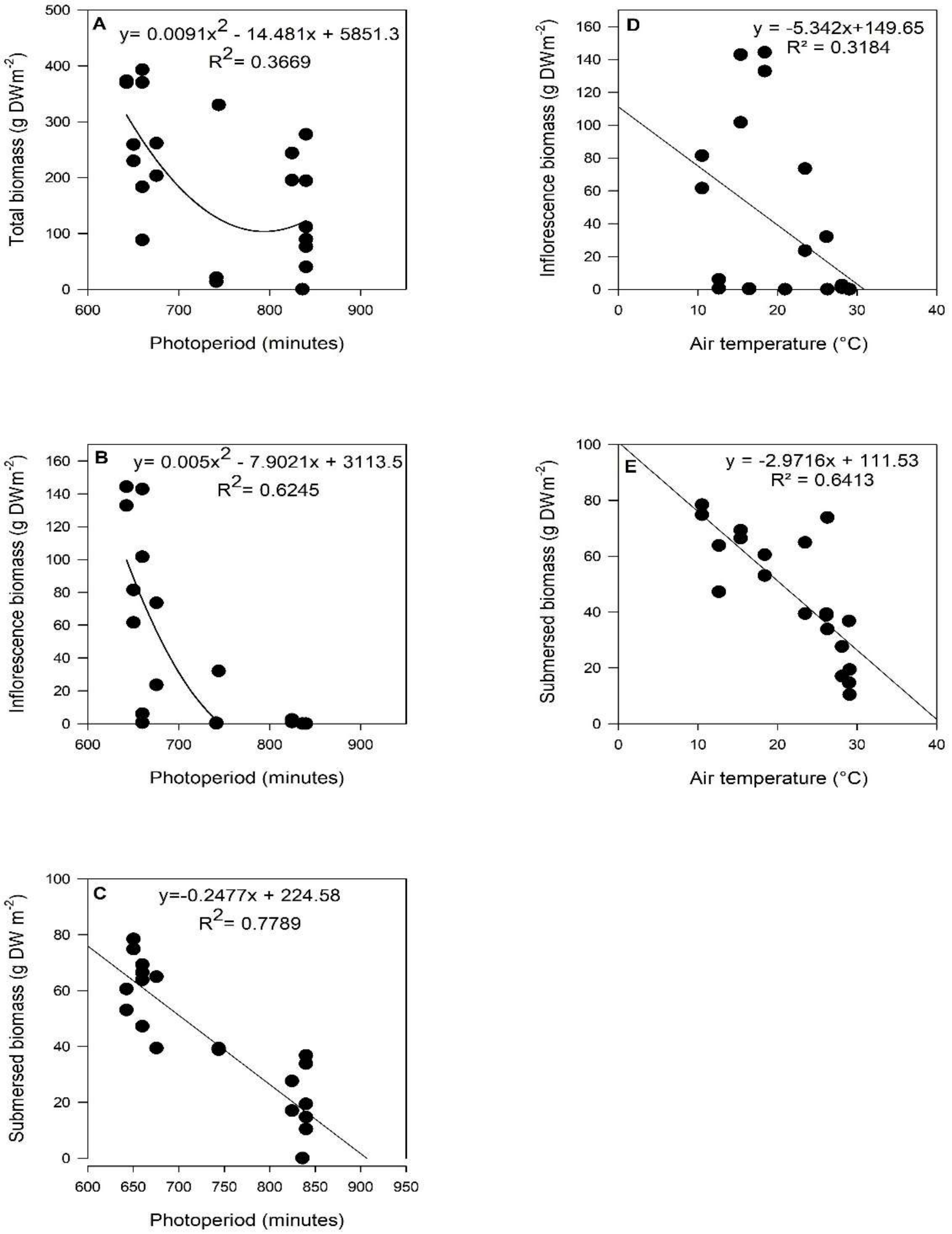

Figure 2.3 Cuban bulrush total, inflorescence, and submersed biomass (g DW m^{-2}) samples collected in Lake Martin, LA from October 2021-September 2022; (A) Cuban bulrush total biomass (g DW m^{-2}) by photoperiod (minutes); (B) Cuban bulrush inflorescence biomass (g DW m^{-2}) by photoperiod (minutes); (C) Cuban bulrush submersed biomass (g DW m^{-2}) by photoperiod (minutes); (D) Cuban bulrush inflorescence biomass (g DW m^{-2}) by air temperature (°C); (E) Cuban bulrush submersed biomass (g DW m^{-2}) over air temperature (°C).

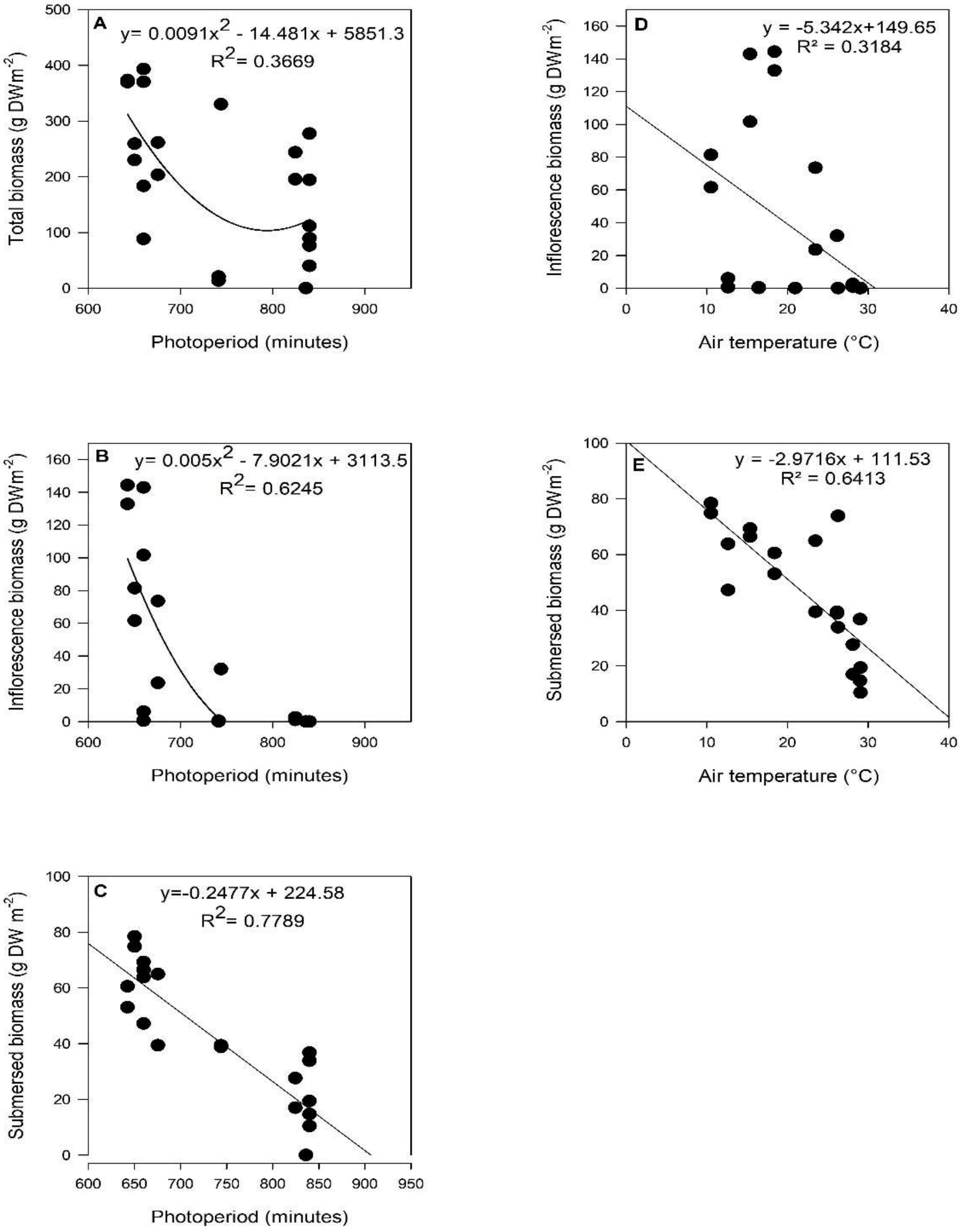

Figure 2.4 Cuban bulrush inflorescence, emergent and submersed biomass (g DW m^{-2}) samples collected in Orange Lake, FL from October 2021-September 2022; (A) Cuban bulrush inflorescence biomass (g DW m^{-2}) by photoperiod (minutes); (B) Cuban bulrush emergent biomass (g DW m^{-2}) by photoperiod (minutes); (C) Cuban bulrush submersed biomass (g DW m^{-2}) by photoperiod (minutes); (D) Cuban bulrush inflorescence biomass (g DW m^{-2}) by air temperature (°C); (E) Cuban bulrush submersed biomass (g DW m^{-2}) over air temperature (°C).

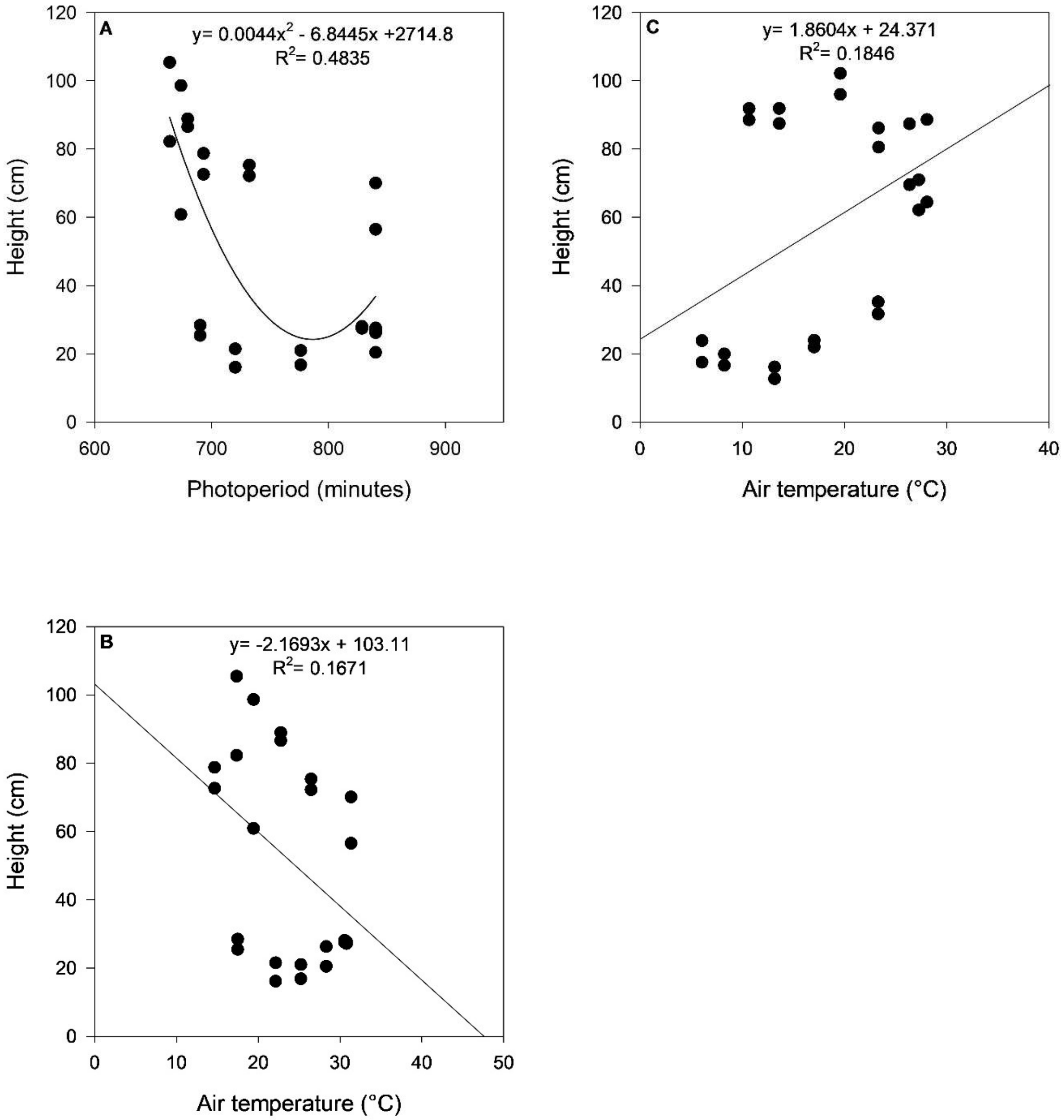

Figure 2.5 Cuban bulrush height (cm) recorded in Orange Lake, FL from October 2021-September 2022; (A) Cuban bulrush height (cm) over photoperiod (minutes); (B) Cuban bulrush height over air temperature (°C); (C)Cuban bulrush height (cm) recorded in Lake Columbus, MS from October 2021-September 2022 over air temperature (°C).

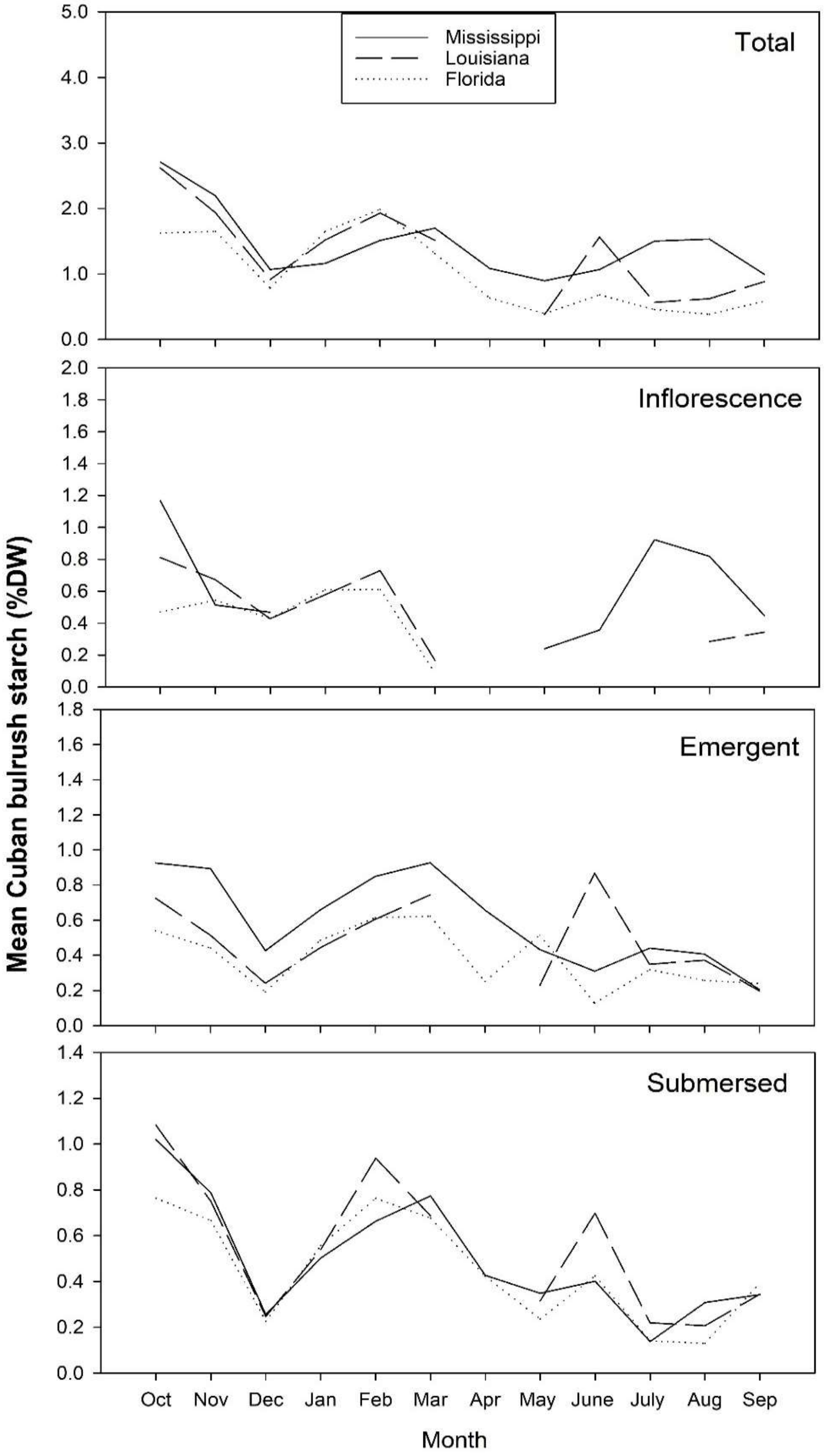

Figure 2.6 Mean Cuban bulrush starch (%DW) from two sampling locations in Mississippi, Louisiana, and Florida from October 2021 to September 2022.

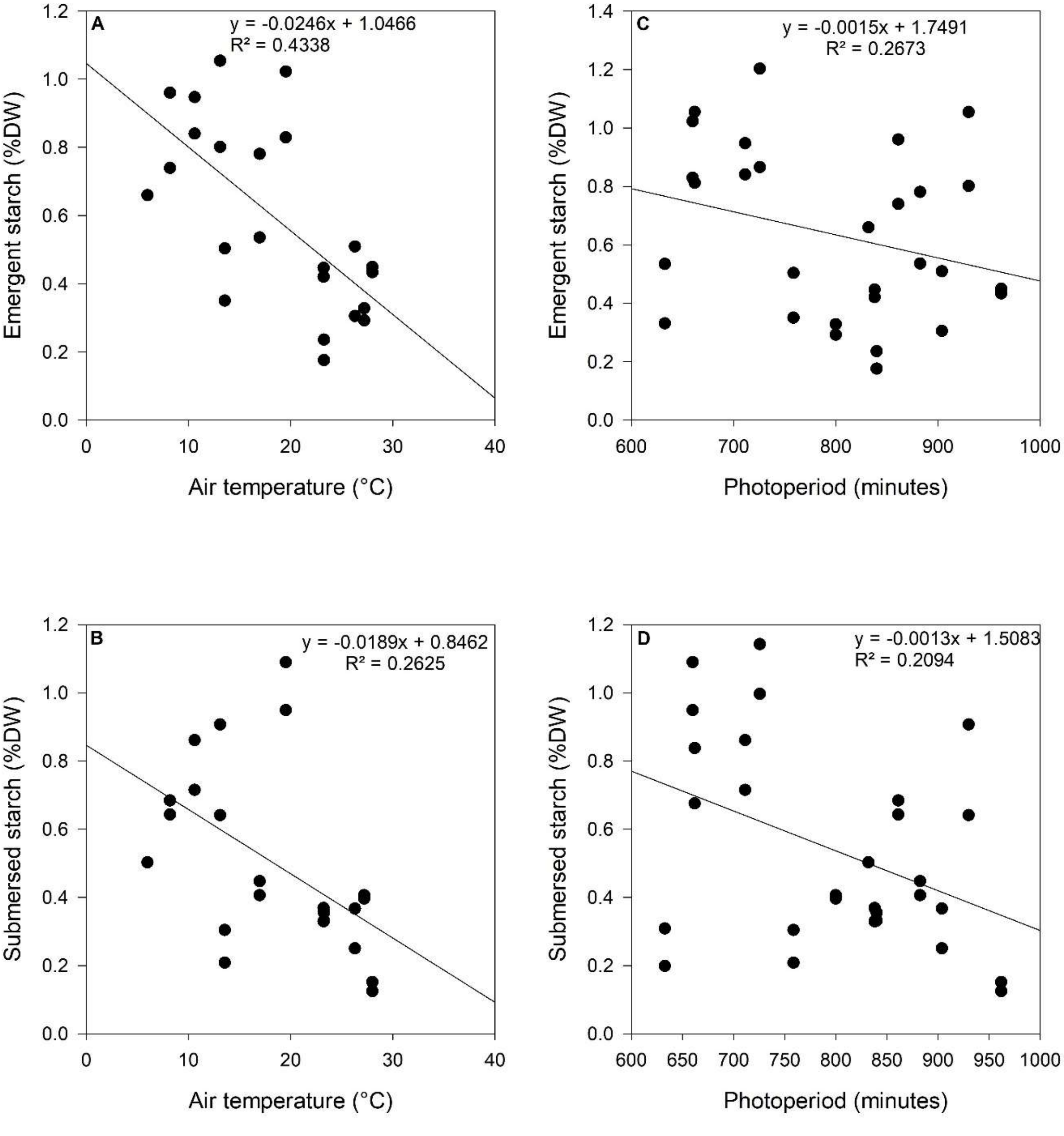

Figure 2.7 Cuban bulrush emergent and submersed starch (% DW) samples collected in Lake Columbus, MS from October 2021-September 2022; (A) Cuban bulrush emergent starch (% DW) by air temperature (°C); (B) Cuban bulrush submersed starch (% DW) by air temperature (°C); (C) Cuban bulrush emergent starch (% DW) by photoperiod (minutes); (E) Cuban bulrush submersed starch(% DW) by photoperiod (minutes).

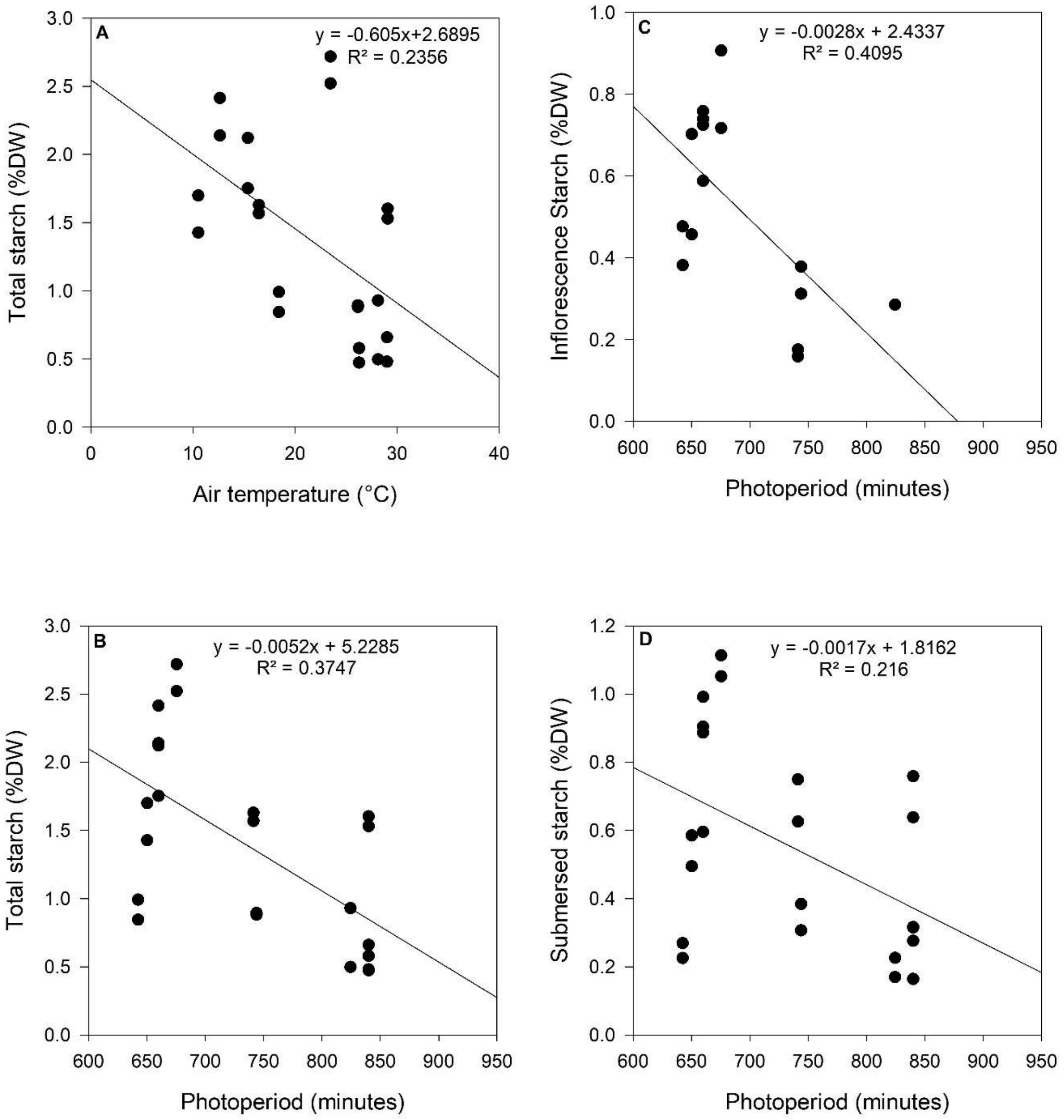

Figure 2.8 Cuban bulrush total, inflorescence, and submersed starch (% DW) samples collected in Lake Martin, LA from October 2021-September 2022; (A) Cuban bulrush total starch (%DW) by air temperature (°C); (B) Cuban bulrush total starch (% DW) by photoperiod (minutes); (C) Cuban bulrush inflorescence starch (% DW) by photoperiod (minutes); (D) Cuban bulrush submersed starch (% DW) by photoperiod (minutes).

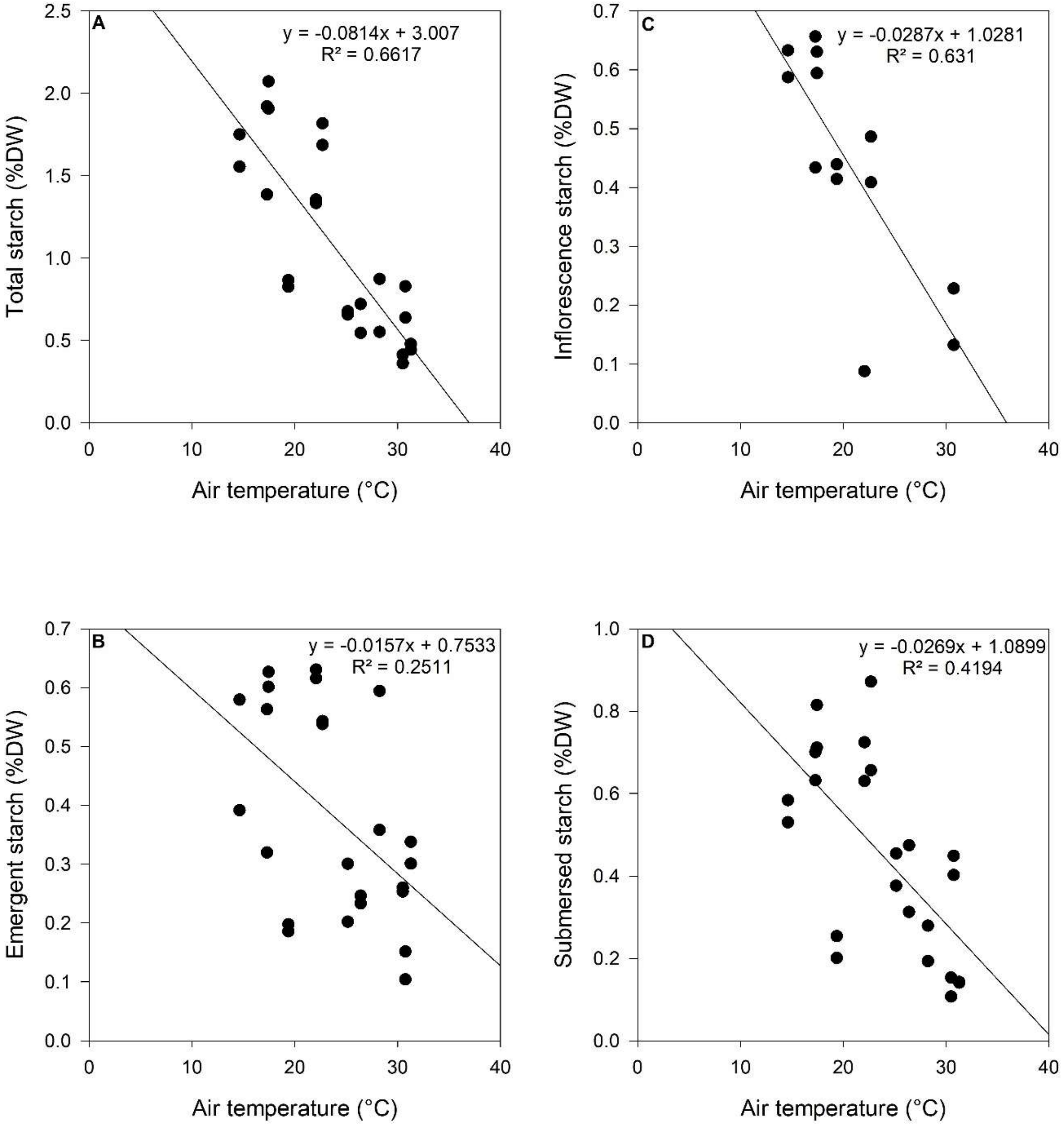

Figure 2.9 Cuban bulrush total, inflorescence, emergent, and submersed starch (% DW) samples collected in Orange Lake, FL from October 2021-September 2022; (A) Cuban bulrush total starch (%DW) by air temperature (°C); (B) Cuban bulrush emergent starch (% DW) by air temperature (°C); (C) Cuban bulrush inflorescence starch (% DW) by air temperature (°C); (D) Cuban bulrush submersed starch (% DW) by air temperature (°C).

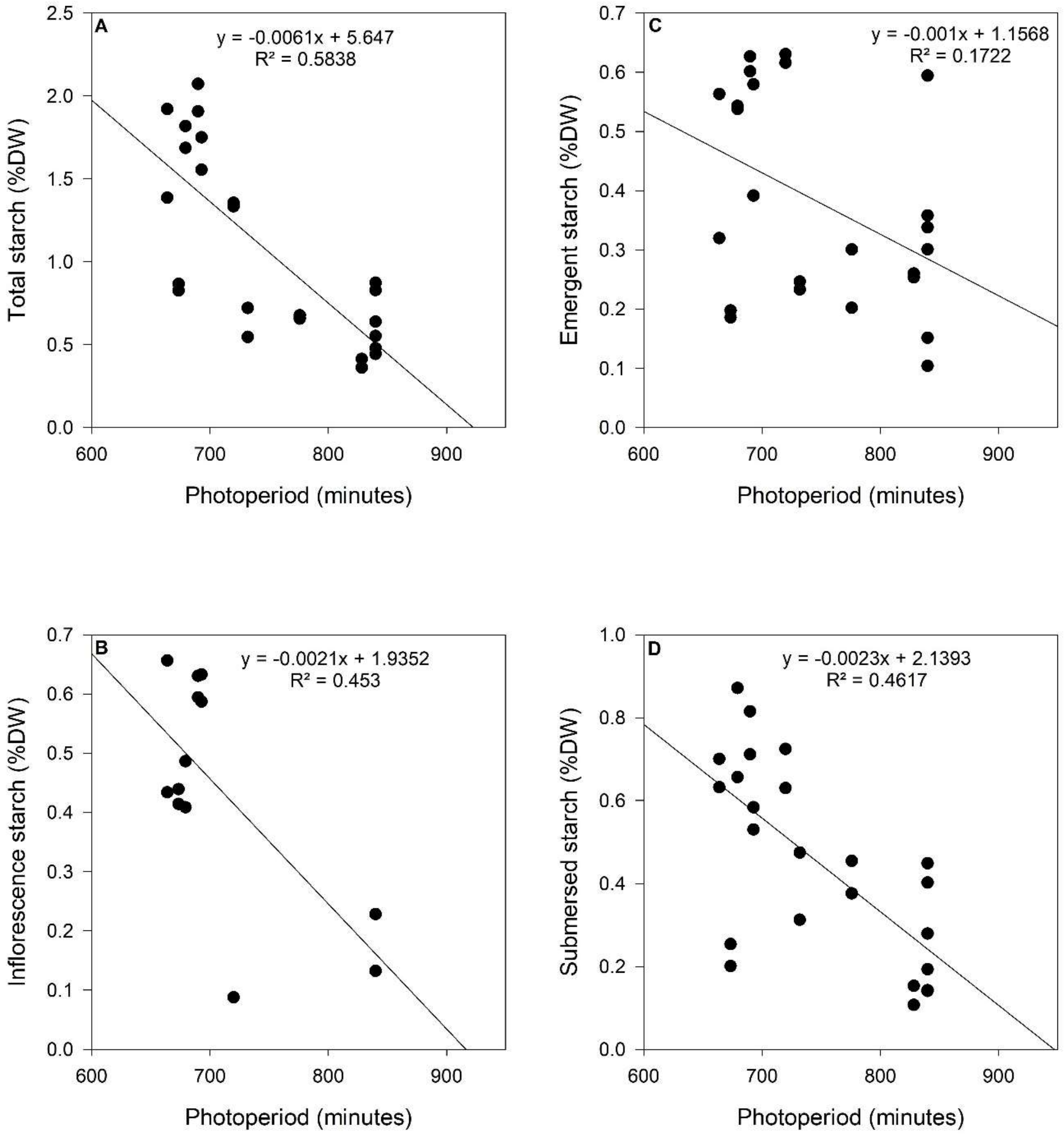

Figure 2.10 Cuban bulrush total, inflorescence, emergent, and submersed starch (% DW) samples collected in Orange Lake, FL from October 2021-September 2022; (A) Cuban bulrush total starch (%DW) by photoperiod (minutes); (B) Cuban bulrush inflorescence starch (% DW) by photoperiod (minutes); (C) Cuban bulrush emergent starch (% DW) by photoperiod (minutes); (D) Cuban bulrush submersed starch (% DW) by photoperiod (minutes).

CHAPTER III

Modeling accumulated degree-days for the invasive aquatic plant *Oxycaryum Cubense* in the Southeastern U.S.

ABSTRACT

Cuban bulrush (*Oxycaryum cubense* [Poepp. & Kunth] Lye) is a floating aquatic plant that has negative ecological and economic impacts in the southeastern U.S. Temperatures in the U.S. have increased over recent decades. Increases in temperatures can result in increased productivity and expansion of invasive plant colonies giving rise to concerns that several invasive plants in North America (including Cuban bulrush) may expand their ranges further north. Accumulated degree-days (ADD) were utilized to develop a predictive model for Cuban bulrush growth from harvested biomass collected over one year in Mississippi, Louisiana, and Florida. Peak biomass occurred in the fall and was maintained into winter. Models suggest Cuban bulrush has a lower air temperature limit that restricts growth between -2 and -6 C in winter months. Predictive ability of ADD models weakened for populations further south (Florida), likely due to warmer winter temperatures and year-round growth of Cuban bulrush in these sites. Results from the models imply that Cuban bulrush has an affinity for warmer temperatures, but also suggest that it is capable of expanding its invaded range in the U.S. as far north as northern Missouri or southern Iowa.

INTRODUCTION

Temperatures in the U.S. increased 1.8°C from 1895 to 2016 with 38% of that increase (0.7°C) occurring in the last 35 years of that timeframe (Kriticos and Brunel 2016, Vose et al. 2017). Several climate models predict an increase of another 3-9°C in the United States over the next century (Finch et al. 2021). Increases in temperature typically result in increased plant productivity due to increases in physiological activities like photosynthesis and respiration (Riis et al. 2012). This suggests the ranges of several invasive species may expand if mean temperatures continue to rise. Other invasive species to expand closer to the planetary poles include buffelgrass (*Pennisetum ciliare* (L)) and members of the Pooideae family (Archer and Predick 2008, Ripley et al. 2019). Similarly, threadleaf water-crowfoot (*Ranunculus trichophyllus* Chaix) may expand its range as its invaded many high-elevation lakes in the Himalayas due to a decrease in ice cover following increased temperatures from climate change (Rahel and Olden 2008). Increases in temperatures across the U.S. suggest a possible northern expansion of aquatic invasive species (IPCC 2022, Kriticos and Brunel 2016, Vose et al. 2017). Phenological models assessing temperature metrics have been used to inform management of several invasive aquatic plants (Wersal and Madsen 2018) and may be useful for Cuban bulrush monitoring. One such modelling technique includes the use of accumulated degree-days to predict stages of plant growth throughout the year for invasive aquatic plants (Spencer et al. 2000).

Accumulated degree-days (ADD) are a measure of heat units needed for a life stage to occur and can be used to predict the occurrence (as calendar days) of certain life stages (sprouting, peak biomass, flowering, senescence, etc.) in a plant's life cycle at different geographical locations (Snyder 1985, Snyder et al. 1999, Spencer et al. 2000). Since ADD is based on temperature accumulation (rather than time), the predictive capability is somewhat immune to changes in geographic location within a region when upper and lower threshold temperatures are incorporated. For example, the single sine method of calculating ADD utilizes the minimum and maximum temperature each day to produce a sine curve estimating degree-days by calculating the area under the curve that is also above and below maximum and minimum temperature thresholds (respectively) at which growth no longer occurs (Spencer et al. 2000, Snyder et al. 1985, Snyder et al. 1999). Therefore, ADD needed to complete a life stage at one location should be comparable to ADD estimated at another location even though calendar days needed to achieve the life stage may be different at each site (Spencer et al. 2000, Snyder et al. 1985, Snyder et al. 1999).

To date, only one study has documented the phenological effects of air temperature and photoperiod on Cuban bulrush biomass in the U.S. (Clarke et al. 2023) with a second study utilizing the same dataset to determine ADD for predictive modeling of Cuban bulrush biomass (Squires et al. *In Review*). Grippo et al. (2014) assumed that Cuban bulrush could not tolerate freezing temperatures; however, Squires et al. (*In Review*) suggests Cuban bulrush may be metabolically active at air temperatures as low as -4°C (24.8°F). In addition, Cuban bulrush emergent biomass peaked in colder months (December-January) in populations growing on Lake Columbus, MS (Clarke et al. 2023). However, Cuban bulrush growth was only assessed at one site in the invaded North American range (Clarke et al. 2023) suggesting that further work is needed to determine the utility of ADD modelling at greater spatial scales. The objective of this study was to compare ADD models from Cuban bulrush samples collected at different geographical locations in North America (Mississippi, Florida, and Louisiana).

METHODS

Biomass data collection

This study was conducted on existing populations of Cuban bulrush infesting Lake Columbus on the Tennessee-Tombigbee Waterway in NE Mississippi (33°32' 2.76" N, 88°29' 27.60" W), Lake Martin near Breaux Bridge, Louisiana (30°13' 23.2572" N, 91°54' 22.284" W), and Orange Lake near Gainesville, Florida (29°27' 32.39" N, 82°10' 23.40" W). From October 2021 to September 2022, Cuban bulrush was harvested monthly from two 0.04 Ha (0.1 ac) plots on each lake. Plots in Mississippi were created by placing PVC posts into the lakebed into the corners of each plot; each corner post was then geotagged in case of damage from flooding or other natural events. Louisiana and Florida plots were marked with plot centers that were also geotagged. Cuban bulrush biomass was harvested from 12 random points per plot. Harvesting consisted of randomly placing a 0.1 m^2 (33 cm x 33 cm) PVC frame placed on the water surface then collecting all Cuban bulrush biomass within the frame. Harvested biomass from all states was placed in labeled 3.79-L Ziploc® bags, stored on ice in a cooler, and transported to Mississippi State University where the plants were separated into emergent, submersed, and inflorescence tissues. Plant tissues were placed into labeled paper bags, dried at 70°C for five days in a forced air oven, then biomass was weighed, and weights recorded.

Environmental monitoring

Onset HOBO pendants were placed 1-2 meters off above the water surface at each site to collect air temperature and photoperiod every hour for the duration of the study. Any data that was unattainable due to weather events or equipment failure was supplemented with temperature data from nearby weather stations in Mississippi (33°28' 12.15" N, 88°46' 48.10"W), Louisiana (30°12' 11.40" N, 91°59' 8.99" W), and Florida (29°41' 30.12" N, 82°16' 33.70" W) allowing for calculation of ADD for later modelling (Spencer et al. 2000).

Accumulated degree days

An analysis of variance (ANOVA) was used to detect differences in monthly Cuban bulrush biomass. If differences were detected, a Fisher Exact test was used to separate monthly means in order to identify the month at which peak and minimum biomass occurred. Binary values were assigned to each month to depict months that did or did not have peak biomass. ADD calculations started the day that lowest minimum temperatures were recorded and ended the last month peak biomass was recorded (Snyder et al. 1985, Snyder et al. 1999). Calculations for ADD followed the methodology of Snyder et al. (1999), which utilizes an iterative Single Sine Method to simultaneously estimate a combination of 1) ADD needed to reach a life stage and 2) the base temperature threshold that best estimates the lower temperature for a species growth. The combination of ADD and base threshold that provides the lowest root mean squared error (RMSE) is assumed to be the best model for predicting the amount of heat needed for a life stage to occur. The life stage of interest in this study was peak biomass and was defined as the 50% of samples attaining maximum growth within a month for locations within each state (Snyder et al. 1999).

Statistical analyses

Logistic regression analyses were conducted to assess the relationships between ADD and the proportion of Cuban bulrush plots that reached peak biomass each month. Hosmer and Lemeshow tests (2013) were used to evaluate the goodness of fit for regression models. Spearman and Kendall correlations were also utilized to determine the relationship between peak biomass occurrence and ADD (Spencer et al. 2000). Correlation strength was defined as 0 to 0.1 = no correlation, 0.1 to 0.4 = weak correlation, 0.4 to 0.6 = moderate correlation, 0.6 to 0.9 = strong correlation, and 0.9 to 1.0 = perfect correlation (Dancy and Reidy 2004). All analyses were conducted in R statistical software (R Core Team 2021).

RESULTS

Model fit

Accumulated degree days had a strong positive relationship with Cuban bulrush growth in Mississippi ($r^2=0.65$) and a moderate positive relationship in Louisiana ($r^2=0.50$). The goodness of fit, or how well predicted data matches observed, for Cuban bulrush populations in Louisiana and Mississippi ($p>0.05$) suggests model predictions were similar to observed growth in those states (Table 3.1). Florida biomass could not be modeled accurately due to a lack of discrete life stages from year-round growth within the population (Table 3.1). Accumulated degree days had strong or moderate positive correlations with plant growth in MS (Rho=0.65; Tau=0.55) and FL (Rho=0.65; Tau=0.55), respectively, and moderate positive correlations with Cuban bulrush plant growth in LA (Rho=0.57, Tau=0.48, $p<0.01$ for all, Table 3.1).

Accumulated degree days

Biomass peaked in the fall for each state as air temperatures started to cool and was maintained into winter months ($p<0.01$, Tables 3.2-3.4). Cuban bulrush growth was associated with colder temperatures in each state (Tables 3.2-3.4). Accorded to the model, accumulated degree days needed for Cuban bulrush to reach peak biomass ranged from 6,469 (MS) to 7,903 (LA) (Tables 3.2-3.4). Calendar days needed for Cuban bulrush to reach peak biomass ranged from 292 (MS) to 334 (FL) (Tables 3.2-3.4).

DISCUSSION

Temperature significantly influences aquatic plant growth, and in most cases, warmer temperatures enhance plant growth and biomass accumulation (Burnett et al. 2007, Wilson et al. 2005). For instance, previous studies assessing the relationship of water hyacinth phenology and air temperature found a strong positive linear relationship between biomass and increasing air temperature (Wilson et al. 2005, Villamagna and Murphy 2010). Cuban bulrush biomass was able to increase through the summer months, reached peak growth in the fall, and maintained peak biomass levels into winter months, which is similar to that recorded by Squires et al. (*In Review*). Cuban bulrush growth was negatively correlated to water temperature, air temperature, and photoperiod (Clarke et al. 2023) suggesting that it can survive and grow in environments that other aquatic plants, like water hyacinth, cannot survive.

It is important to note, however, that the plant may be sensitive to changes in annual temperatures. For instance, Squires et al. (*In Review*) observed peak Cuban bulrush biomass in the winter months, whereas peak biomass was recorded in the fall months (Lake Columbus).

However, it is seen that the higher total biomass was observed to occur in colder months where average temperatures in December was 9.41-13.55°C and January was 6-7.22°C, 12.20-16.73°C in December and 8.67-9.16°C in January, and 16.10-17.28°C in December and 14.20-14.70°C in January for Mississippi, Louisiana, and Florida, respectively. This suggests the data of observed peak growth for Cuban bulrush could shift from year to year based on annual temperature cycles (e.g., warmer years vs. colder years). Our data suggest Cuban bulrush can tolerate air temperatures below freezing, it has potential to expand its invaded range further north in the U.S.

Another factor that Cuban bulrush that may be sensitive to is photoperiod. Photoperiod, which is used as an environmental cue to several seasonal developmental processes in plants, is considered to be a direct influence on temperature (Jackson 2008). Light (photoperiod or PAR) can be one of the most limiting factors for submersed and emergent macrophytes (Barko et al. 1986). For instance, in Mississippi, light availability is reduced in the fall and winter months, where photoperiod ranges from 11-12 hours. In Louisiana and Florida, 10-11 hours of light were observed. All three state populations had higher biomass around lower light levels for each area compared to max levels (Mississippi: 16 hours, Louisiana: 14 hours, Florida: 14 hours). Therefore, it is suggested that Cuban bulrush may have a low light saturation point (Clarke et al. 2023).

Models estimated 6,469 ADD at -6°C to reach peak Cuban bulrush biomass for plants growing in MS, 7,903 ADD at -3°C for plants in LA, and 7,643 ADD at -2°C for plants in FL. Squires et al. (*In Review*) estimated a base threshold temperature of -4°C for plants in MS which is similar to the model predictions here. In this study, two types of inflorescence forms were used: multiple head or polycephalous (*Oxycaryum cubense* forma *cubense*) and single head or monocephalous (*Oxycaryum cubense* forma *paraguayense*) (Bryson et al. 2008, Bryson and Carter 2008). The mono form was in Mississippi, while the poly form was in Louisiana and Florida. The differences in abiotic factors such as photoperiod and temperature may have influenced the differing model results. However, a difference of almost 1200 ADD and a 3°C difference between the mono and poly forms suggest a biotype influence, including the impact in morphology of the inflorescence.

Predictions of plant growth based on ADD estimates can be useful in development in long-range management strategies attempting to prevent further spread of invasive species. Such models have been used to predict sprouting and emergence of aquatic vegetative propagules for species like *Hydrilla verticillata* (L.f.) Royle, *Potamogeton pectinatus* L., *Potomageton nodosus* Poiret, and *Vallisneria americana* L. (Spencer et al. 2000, Spencer and Ksander 2006). As seen in Florida however, states that have longer, less variable or incremental growing seasons may not be good areas for estimating ADD requirements. For instance, *Potamogeton crispus* L. phenology is different in Mississippi due to a longer growing season than populations growing in Minnesota (Turnage et al. 2018, Woolf and Madsen 2003). This trend is also seen in a previous modeling study where a population of long-leaf pondweed (*Potamogeton nodosus*) peaked earlier and more gradually in California than in Texas (Spencer et al. 2000).

Future models for Cuban bulrush growth should assess more plots across the invaded range; particularly sites near the northern invasion front as this model suggests the potential for Cuban bulrush to spread as far north as Missouri for Florida populations (mean January temperature approximately -2°C; NOAA 2023). Mississippi and Louisiana populations may also have the potential to spread up to Iowa (mean January temperature approximately -4°C) (NOAA

2023). Future management should prioritize monitoring of states that may be at most risk of invasion (i.e., freshwater environments with temperatures above -6°C) to prevent invasion of Cuban bulrush before it establishes large colonies. Future efforts should also conduct a growth study based on the model's temperature estimates.

LITERATURE CITED

Archer SR, Predick KI. 2008. Climate change and ecosystems of the southwestern United States. Rangelands 30:23–28.

Burnett DA, Champion PD, Clayton JS, Ogden J. 2007. A system for investigation of the temperature responses of emergent aquatic plants. Aquat. Bot. 86:187–190.

Clarke M, Wersal RM, Turnage G. 2023. Seasonal phenology and starch allocation patterns of Cuban bulrush (*Oxycaryum cubense*) growing in Mississippi, USA. Aquat. Bot. 186:103627.

Finch DM, Butler JL, Runyon JB, et al. 2021. Effects of climate change on invasive species. Invasive Species in Forests and Rangelands of the United States 57–83.

Grippo M, Fox L, Hayse J, Hlohowskyj I, Allison T. 2014. Risk of adverse impacts from the movement through the CAWS and establishment of aquatic nuisance species in the Great Lakes and Mississippi River basins. U. S. Army Corps of Engineers. 2: Appendix E.

Hosmer DW, Lemeshow S, Sturdivant RX. 2013. Applied Logistic Regression. Wiley, Hoboken, NJ.

IPCC. 2022. Framing and context. In global warming of 1.5°C: IPCC special report on impacts of global warming of 1.5°c above pre-industrial levels in context of strengthening response to climate change, sustainable development, and efforts to eradicate poverty. pp. 49-92 Cambridge: Cambridge University Press. doi:10.1017/9781009157940.003.

Jackson SD. 2008. Plant responses to photoperiod. New Phytologist 181:517–531.

Kriticos DJ, Brunel S. 2016. Assessing and managing the current and future pest risk from water hyacinth, (*Eichhornia crassipes*), an invasive aquatic plant threatening the environment and water security. PLoS One 11:1-18.

NOAA National Centers for Environmental information. 2023. Climate at a glance: statewide mapping. In: National Climatic Data Center. https://www.ncdc.noaa.gov/cag/statewide/mapping/110/tavg/202201/1/value. Accessed 01 Aug 2023.

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Www.R-project.org/.

Rahel FJ, Olden JD. 2008. Assessing the effects of climate change on aquatic invasive species. Conserv. Biol. 22:521–533.

Riis T, Olesen B, Clayton JS, et al. 2012. Growth and morphology in relation to temperature and light availability during the establishment of three invasive aquatic plant species. Aquat. Bot. 102:56–64.

Ripley BS, Edwardes A, Rossouw MW, et al. 2019. Invasive grasses of sub-Antarctic Marion Island respond to increasing temperatures at the expense of chilling tolerance. Annal. Bot. 125:765–773.

Snyder R. 1985. Hand calculating degree-days. Agric. For Meteorol. 35:353–358.

Snyder RL, Spano D, Cesaraccio C, Duce P. 1999. Determining degree-day thresholds from field observations. Int. J. Biometerol. 42:177–182.

Spencer DF, Ksander GG, Madsen JD, Owens CS. 2000. Emergence of vegetative propagules of *Potamogeton nodosus*, *Potamogeton pectinatus*, *Vallisneria Americana*, and *Hydrilla verticillata* based on accumulated degree-days. Aquat. Bot. 67:237–249.

Spencer DF, Ksander GG. 2006. Estimating *Arundo donax* ramet recruitment using degree-day-based equations. Aquat. Bot. 85:282–288.

Turnage G, Madsen JD, Wersal RM. 2018. Phenology of curlyleaf pondweed (*Potamogeton crispus* L.) in the southeastern United States: A two-year mesocosm study. J. Aquat. Plant Manage. 56:35-38.

Villamagna AM, Murphy BR. 2010. Ecological and socio-economic impacts of invasive *E. crassipes* (*Eichhornia crassipes*): a review. Freshw. Biol. 55:282–298.

Vose RS, Easterling DR, Kunkel KE, LeGrande AN, Wehner MF. 2017. Climate science special report: fourth national climate assessment, volume I. U.S. Global Change Research Program, Washington, DC.

Wersal RM, Madsen JD. 2018. Designing and using phenological studies to define management strategies for aquatic plants. J. Aquat. Plant Manage. 56s:83-89.

Wilson JR, Holst N, Rees M. 2005. Determinants and patterns of population growth in *E. crassipes*. Aquat. Bot 81:51–67.

Table 3.1 Relationship of Cuban bulrush peak biomass occurrence to ADD for each state.

Model	Statistic	Test Statistic	p-value
Mississippi	Logistic Regression (R^2)	0.65	<0.01
	Hosmer-Lemeshow (RMSE)	0.6	0.28
	Spearman (Rho)	0.65	<0.01
	Kendall (Tau)	0.55	<0.01
Louisiana	Logistic Regression (R^2)	0.50	<0.01
	Hosmer-Lemeshow (RMSE)	0.56	0.43
	Spearman (Rho)	0.57	<0.01
	Kendall (Tau)	0.48	<0.01
Florida	Logistic Regression (R^2)	1.00	<0.01
	Hosmer-Lemeshow (RMSE)	0	<0.01
	Spearman (Rho)	0.65	<0.01
	Kendall (Tau)	0.55	<0.01

Table 3.2 Mississippi ADD calculated for base temperatures of 0 and -6°C (RMSE=0.00) for each month from October 2021 to September 2022 ADD models suggest 6,469 ADD (292 calendar days) were needed for Cuban bulrush to reach peak biomass.

MONTH	**ADD (base = 0°C)**	**ADD (base = -6°C)**
October	404.7	584.25
November	653..45	1003.25
December	882.05	1410.7
January	1077.65	1743.6
February	1536.5	2387.4
March	2024.05	3054.15
April	2669.45	3885.55
May	3430.25	4826.35
June	4263.7	5845.8
July	5073.35	6835.45
August	5807.3	7755.4
September	6424.5	8558.6

Table 3.3 Louisiana ADD calculated for base temperatures of 0 and -3°C (RMSE=0.27) for each month from October 2021 to September 2022; ADD models suggest 7,903 ADD (331 calendar days) were needed for Cuban bulrush to reach peak biomass.

MONTH	**ADD (base = 0^{O}C)**	**ADD (base = -3^{O}C)**
October	727.5	820.5
November	1162.3	1371.95
December	1710.1	2035.85
January	2062.85	2456.5
February	2448.15	2894.5
March	2942.05	3497.8
April	3587.45	4216.8
May	4402.95	5125.3
June	5275.05	6087.4
July	6174.15	7079.5
August	7017.1	8044.3
September	7830.75	8919.1

Table 3.4 Florida ADD calculated for base temperatures of 0 and -2°C (RMSE=0.45) for each month from October 2021 to September 2022; ADD models suggest 7,643 ADD (334 calendar days) were needed for Cuban bulrush to reach peak biomass.

MONTH	ADD (base = 0^{O}C)	ADD (base = -2^{O}C)
October	591.4	651.4
November	967.5	1082.7
December	1367.35	1543.5
January	1813.25	2043.9
February	2378.95	2671.6
March	2957.5	3310.15
April	3669.95	4084.6
May	4466.1	4940.75
June	5309.8	5846.45
July	6140.05	6736.7
August	6943.3	7601.95
September	7643.55	8364.2

Chapter 4

CONCLUSIONS AND RECOMMENDATIONS

Phenology and resource allocation

Most knowledge about Cuban bulrush (*Oxycaryum cubense* (Poepp. & Kunth) Lye comes from studies done in Mississippi with the monocephalous form. However, there has not been a study comparing phenological differences between Cuban bulrush populations in other states, specifically ones that have the polycephalous form (LA and FL). Therefore, seasonal patterns of biomass and starch allocation for the aquatic invasive species (AIS) Cuban bulrush were quantified in populations from Lake Columbus on the Tennessee-Tombigbee Waterway in Mississippi, Lake Martin in Louisiana, and Orange Lake in Florida. Monthly samples of Cuban bulrush were harvested from two plots per state from October 2021-September 2022. During each sampling period, air temperature and photoperiod were recorded. Starch allocation patterns were similar among the different states, with starch not being disproportionately stored in any one tissue (<1.5% DW for all plant tissues); however, biomass allocation was different between all three states with Mississippi (monocephalous form) having greater biomass compared to the other states at 600.71 g DW m^{-2} ± 37.76 SE; Louisiana (polycephalous form) and Florida (polycephalous form) at 392.25 g DW m^{-2} ± 62.14 SE and 233.85 g DW m^{-2} ± 7.33 SE respectively. Peak inflorescence biomass occurred in the winter for the LA and FL biotypes, and the summer for the MS biotype. Mississippi inflorescence biomass had a positive relationship with air temperature (r^2=0.53). However, emergent and submersed biomass within each state was generally negatively related with photoperiod and temperature (max r^2=0.78, r^2=0.66). These data indicate that Cuban bulrush may have the capability to expand further northward than previously thought.

Accumulated degree day modeling

Accumulated degree day predictive modeling was applied to Cuban bulrush (*Oxycaryum cubense* (Poepp. & Kunth) Lye) emergent biomass that was harvested from October 2021-September 2022 in Mississippi, Louisiana, and Florida. All states observed emergent biomass peak in early to mid-fall (September-October). The models estimated a base threshold temperature of -6°C in Mississippi, -3°C in Louisiana, and -2°C in Florida. ADD needed for Cuban bulrush in Mississippi to reach peak biomass was 6,469 ADD compared to 7,903 ADD in Louisiana and 7,643 ADD in Florida. Prediction strength of each model decreased as populations moved south from Mississippi, with Florida showing lower reliability of results. Results from the models imply that Cuban bulrush may grow well in colder temperatures if physiological demands are met within the growing season.

Future recommendations

There are some clear differences between both inflorescence biotypes. For instance, LA and FL biotypes peaked in inflorescence biomass earlier in the year than the monocephalous form. However, the MS biotype accrued higher overall total biomass. The inflorescence biotype differentiation in Louisiana and Florida suggests reallocation of resources more towards sexual reproduction, which is seen in many competitive environments (Zhang and Zhang 2007). In addition, the lack of starch allocation found in Cuban bulrush suggests more of a focus on constant growth using free sugars, not long-term storage for future use (Clarke et al. 2023). This may explain why Cuban bulrush tends to be a very competitive plant.

Based on ADD modeling, the low temperature thresholds suggest a high likelihood for northern expansion, especially with increases in temperatures in the U.S. (Kriticos and Brunel 2016, Vose et al. 2017). Cuban bulrush has already been spreading farther northward into the mid-southern regions of the United States in the last 20 years (Bryson et al. 2008, Fernandez 2013, Rahel and Olden 2008, Grippo et al. 2014, Vose et al. 2017). Possible range expansion could be to the northern edge of Missouri for the Florida biotype, while the Louisiana and Mississippi populations could spread to Iowa (mean January temperature approximately -2°C and -4°C, NOAA 2022 and 2023), due to the base threshold temperatures estimated to being -6°C, -3°C, and -2°C (Figure 4.1). As temperatures increase, northern expansion could be even further north than Missouri. Therefore, it would be useful to implement the estimated temperature ranges from this study and conduct controlled growth studies to narrow down the optimum temperature for Cuban bulrush growth (Figure 4.1).

LITERATURE CITED

Bryson CT, Maddox VL, Carter R. 2008. Spread of Cuban club-rush (*Oxycaryum Cubense*) in the southeastern United States. Invasive Plant. Sci. and Manag. 1:326–329.

Clarke M, Wersal RM, Turnage G. 2023. Seasonal phenology and starch allocation patterns of Cuban bulrush (*Oxycaryum cubense*) growing in Mississippi, USA. Aquat. Bot. 186:103627.

Fernandez, AL. 2013. Dispersal and management of invasive aquatic plants in Mississippi waterways. Thesis, Mississippi State University, Starkville, Mississippi, USA.

Grippo M, Fox L, Hayse J, Hlohowskyj I, Allison T. 2014. Risk of adverse impacts from the movement through the CAWS and establishment of aquatic nuisance species in the Great Lakes and Mississippi River basins. U. S. Army Corps of Engineers. 2: Appendix E.

Kriticos DJ, Brunel S. 2016. Assessing and managing the current and future pest risk from water hyacinth, (*Eichhornia crassipes*), an invasive aquatic plant threatening the environment and water security. PLoS One 11:1-18.

NOAA National Centers for Environmental information. 2022. Climate at a glance: statewide mapping. In: National Climatic Data Center. https://www.ncdc.noaa.gov/cag/statewide/mapping/110/tavg/202201/1/value. Accessed 26 Apr 2022.

NOAA National Centers for Environmental information. 2023. Climate at a glance: statewide mapping. In: National Climatic Data Center. https://www.ncdc.noaa.gov/cag/statewide/mapping/110/tavg/202201/1/value. Accessed 26 Apr 2023.

Rahel FJ, Olden JD. 2008. Assessing the effects of climate change on aquatic invasive species. Conserv. Biol. 22:521–533.

Vose RS, Easterling DR, Kunkel KE, LeGrande AN, Wehner MF. 2017. Climate science special report: fourth national climate assessment, volume I. U.S. Global Change Research Program, Washington, DC.

Watson, AL, Madsen JD. 2014. The effect of the herbicide and growth stage on Cuban club-rush (*Oxycaryum cubense*) control. J. Aquat. Plant Manage. 52:71-74.

Zhang Y, Zhang D. 2007. Asexual and sexual reproductive strategies in Clonal plants. Frontiers of Biology in China 2:256–262.

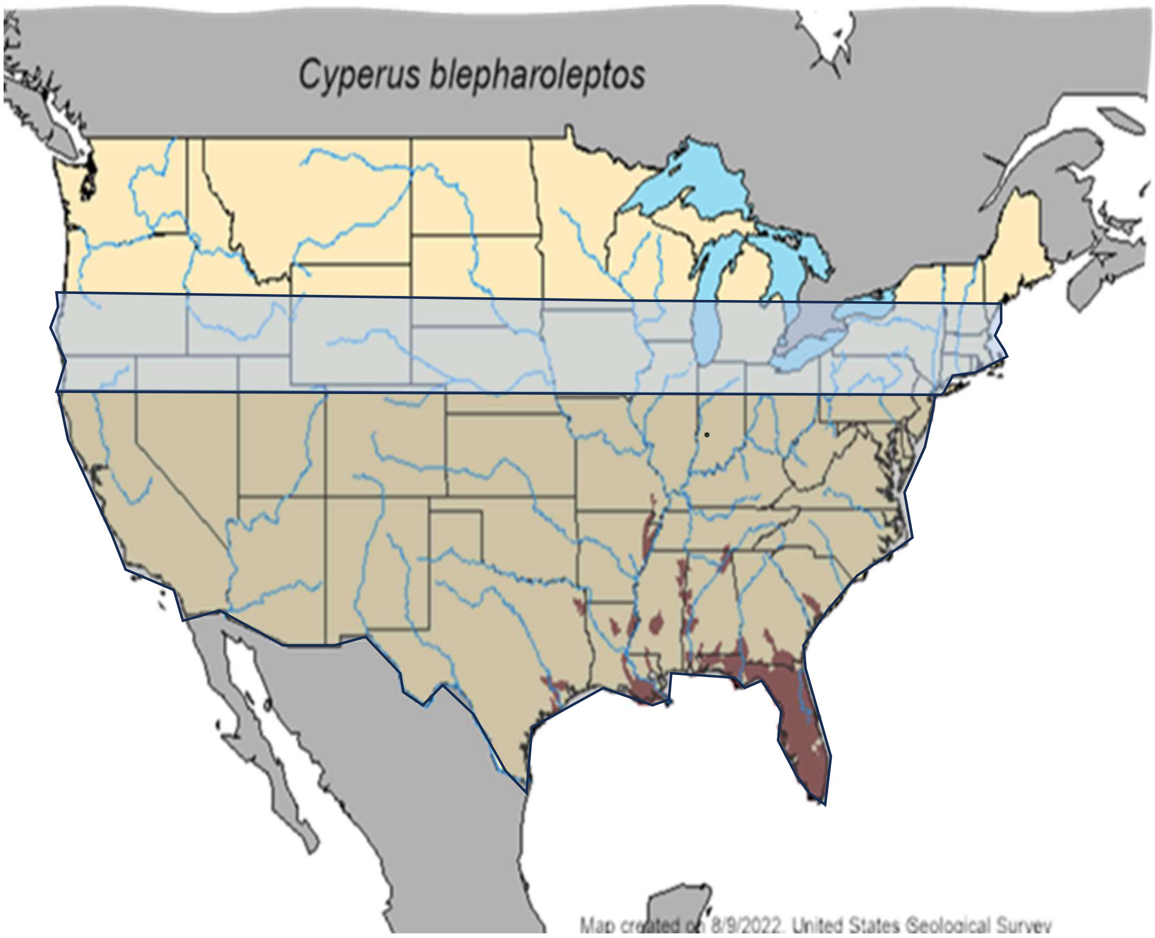

Figure 4.1 Current (red) and projected latitudinal range of Florida populations (overlaid in gray), and Mississippi and Louisiana populations (overlaid in blue) of Cuban bulrush. Map of current range provided by USGS Invasive Species Database.

APPENDIX A

MAP OF CUBAN BULRUSH BIOMASS SAMPLING LOCATIONS WITHIN THE SOUTHEASTERN U.S.

Figure A. Location of Cuban bulrush sampling sites on Lake Columbus in Mississippi, Lake Martin in Louisiana, and Orange Lake in Florida from October 2021-September 2022.

APPENDIX B

STARCH ASSAY METHOD

SIGMA-ALDRICH®
sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Starch Assay Kit
(Amylase/Amyloglucosidase Method)

Catalog Number **STA20**
Storage Temperature 2–8 °C
(Do Not Freeze)

TECHNICAL BULLETIN

Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industries. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore, ideal for analytical purposes. Because of the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.[1-9]

This kit is for the quantitative, enzymatic determination of starch in food and other materials. Several publications have noted use of this kit on such samples as plant leaves,[10] tomato,[11] and algae.[12]

Principle

$$\text{Starch} + (n\text{-}1)\ H_2O \xrightarrow{\alpha\text{-Amylase, Amyloglucosidase}} n\ (\text{D-glucose})$$

$$\text{D-Glucose} + H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{D-Gluconic Acid} + H_2O_2$$

$$H_2O_2 + \text{Reduced } o\text{-Dianisidine (colorless)} \xrightarrow{\text{Peroxidase}} \text{Oxidized } o\text{-Dianisidine (brown)}$$

$$\text{Oxidized } o\text{-Dianisidine (brown)} \xrightarrow{H_2SO_4} \text{Oxidized } o\text{-Dianisidine (pink)}$$

- The hydrolysis of starch to glucose is catalyzed by α-amylase and amyloglucosidase.
- Glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase.
- Hydrogen peroxide reacts with *o*-dianisidine in the presence of peroxidase to form a colored product.
- Oxidized *o*-dianisidine reacts with sulfuric acid to form a more stable colored product.
- The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration.

Reagents

1. α-Amylase for Starch Assay Kit (Catalog Number A4582): This heat stable α-amylase is supplied as a solution in 25% propylene glycol and is ready-to-use.

2. Starch Assay Reagent (Catalog Number S9144): Reconstitute the contents of the vial with 20 mL of water. After addition of water, stopper the vial and mix several times by inversion. **Do Not Shake**.

   Each vial, when reconstituted with 20 mL of water, contains 50 units/mL of amyloglucosidase from *Aspergillus niger* and buffer salts.

   The reconstituted reagent is stable in the absence of visible microbial growth for 7 days at 18–26 °C and for 4 weeks at 2–8 °C. The dry reagent should be discarded under these circumstances:
   - if the vial exhibits caking because of possible moisture penetration,
   - if the vial contents do not dissolve completely upon reconstitution,
   - or if the solution appears turbid.

3. Glucose Oxidase/Peroxidase Reagent (Catalog Number G3660): Each capsule contains 500 units of glucose oxidase from *Aspergillus niger*, 100 purpurgalin units of horseradish peroxidase, and buffer salts. Store the unopened reagent at 2–8 °C.

   Empty the contents of the capsule into an amber bottle. Dissolve the contents in 39.2 mL of water. The solution is stable up to one month at 2–8 °C and for at least 6 months frozen at –20 °C. Discard if turbidity develops.

4. *o*-Dianisidine Reagent (Catalog Number D2679): The preweighed vial contains 5 mg of *o*-dianisidine dihydrochloride. Store the reagent at 2–8 °C and minimize exposure to light.

   Reconstitute the contents of the *o*-dianisidine vial with 1.0 mL of water. Invert the vial several times to dissolve the contents. The solution is stable for 3 months at 2–8 °C. Store the solution to minimize exposure to light.

5. Glucose Assay Reagent: Add 0.8 mL of the reconstituted *o*-Dianisidine Reagent to the amber bottle which contains the 39.2 mL of the reconstituted Glucose Oxidase/Peroxidase Reagent. Invert the bottle several times to mix. Store the reagent at 2–8 °C, and minimize exposure to light. The solution is stable up to 1 month at 2–8 °C. Discard if turbidity develops or color forms.

6. Glucose Standard Solution (Catalog Number G3285): D-Glucose, 1.0 mg/ml in 0.1% benzoic acid. This standard is **traceable to an NIST standard** and is supplied ready-to-use. It is stable at 2–8 °C for at least six months. Discard if turbidity develops.

7. Wheat Starch, Standard for Starch Assay Kit (Catalog Number S1520): This standard is used as a control to ensure assay reliability. The dry reagent is stable for at least 2 years when stored desiccated at room temperature. Moisture content will vary depending on storage conditions.

8. Corn Starch, Standard for Starch Assay Kit (Catalog Number S5296): This standard is used as a control to ensure assay reliability. The dry reagent is stable for at least 2 years when stored desiccated at room temperature. Moisture content will vary depending on storage conditions.

Reagents and Equipment Required but Not Provided

- 6 M Sulfuric Acid Solution – Prepared by a 3-fold dilution in water of concentrated (18 M) sulfuric acid, ACS Reagent (e.g. Catalog Number 258105)
- 80% Ethanol Solution – Prepared by dilution of 95% ethanol with water.
- Dimethyl Sulfoxide (DMSO), ACS Reagent (e.g. Catalog Number 154935)

Apparatus

- Spectrophotometer suitable for measuring absorbance at 540 nm
- Cuvettes
- Test Tubes, 16 mm × 120 mm
- Pipettes capable of accurately dispensing 10 μL to 10 mL
- Boiling water bath
- Water bath capable of maintaining temperatures at 60 ± 1 °C and 37 ± 1 °C
- Analytical centrifuge
- Analytical balance
- Vortex mixer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

Grind the sample to < 0.5 mm (No. 40 mesh). Weigh 50–100 mg samples to 0.1 mg accuracy. Transfer the samples to appropriately marked test tubes.

For wheat and corn starch controls, and samples with high starch content, reduce the sample size to 1–10 mg.

Samples that contain glucose or maltodextrins must be extracted with ethanol to remove these substances.

1. Add 5.0 mL of the 80% Ethanol Solution to the sample.
2. Incubate at 80–85 °C for 5 minutes.
3. Mix the contents of the tube and add another 5.0 mL of the 80% Ethanol Solution.
4. Centrifuge tube for 10 minutes at 1,000 × *g*. Discard the supernatant.
5. Resuspend the pellet in 10 mL of the 80% Ethanol Solution and mix. Centrifuge for 10 minutes at 1,000 × *g*. Carefully pour off the supernatant and discard.
6. Proceed with starch digestion in the next section.

For samples that contain resistant starch:

1. Add 2 mL of DMSO to each sample.
2. Mix and incubate for 5 minutes in a boiling water bath.
3. Proceed with starch digestion in the next section.

Procedure

Starch Digestion

1. Add 0.2 mL of the 80% Ethanol Solution to each sample and to an empty tube labeled "Starch Digestion Blank" and mix.
2. Pipette 3.0 mL of water and 0.02 mL of the α-Amylase (Reagent 1) into each sample and blank tube.
3. Mix and incubate for 5 minutes in a boiling water bath.
4. Remove the tubes from the water bath and cool to room temperature.
5. Bring the volume in each tube up to 10 mL with water and mix.
6. To 1.0 mL of each test and blank solution from step 5, add 1.0 mL of the Starch Assay Reagent (Reagent 2).
7. Mix and incubate for 15 minutes in a 60 °C shaking water bath.
8. Remove the tubes from the water bath and cool to room temperature.
9. Dilute 1.0 mL of each sample and blank to 10 mL with water.
10. Proceed with glucose determination in the next section.

Glucose Assay

Avoid prolonged exposure of the Glucose Assay Reagent (Reagent 5) to bright light.

1. Pipette the following solutions into the appropriately marked test tubes:

Reagent	Standard Blank	Standard	Reagent Blank	Test
Water (mL)	1.0	0.950	---	---
Glucose Standard - Reagent 6 (mL)	---	0.05	---	---
Blank from Starch Digestion (mL)	---	---	1.0	---
Sample from Starch Digestion (mL)	---	---	---	1.0

2. At time zero, start the reaction by adding 2.0 mL of the Glucose Assay Reagent (Reagent 5) to the first tube and mix. Allow 30–60 second intervals between addition of Glucose Assay Reagent (Reagent 5) to each subsequent tube.
3. Incubate each tube exactly 30 minutes at 37 °C. Stop each reaction at 30–60 second intervals by adding 2.0 mL of the 6 M Sulfuric Acid Solution into each tube. Carefully mix each tube thoroughly.
4. Measure the absorbance of each tube at 540 nm.

Calculations

$\Delta A_{\text{STANDARD}} = A_{\text{STANDARD}} - A_{\text{STANDARD BLANK}}$

$\Delta A_{\text{TEST}} = A_{\text{TEST}} - A_{\text{REAGENT BLANK}}$

%Starch

$$= \frac{(\Delta A_{\text{TEST}})(F)(V)(SF)(SDF)(VGA)(MWF)(100)}{(\text{Conversion Factor for } \mu\text{g to mg})\ (\text{sample Weight in mg})}$$

$$= \frac{(\Delta A_{\text{TEST}})(50/\Delta A_{\text{STD}})(10)(2)(10)(1.0)(0.9)(100)}{(1000)(\text{Sample Weight in mg})}$$

$$= \frac{(\Delta A_{\text{TEST}})(900)}{(\Delta A_{\text{STD}})(\text{Sample Weight in mg})}$$

F = μg glucose in standard ÷ $\Delta A_{\text{STANDARD}}$ at 540 nm = $50/\Delta A_{540}$
V = Initial Sample Volume (from sample preparation)
SF = Total Assay Volume from Starch Assay/Sample Volume from Starch Assay
SDF = Dilution Factor from end of Starch Assay
VGA = Initial Sample Volume from Glucose Assay
MWF = Molecular Weight of Starch monomer/Molecular Weight of Glucose = 162/180 = 0.9

References

1. *Approved Methods of the American Association of Cereal Chemists*, 9th Edition. American Association of Cereal Chemists – Approved Methods Committee (St. Paul, MN), Method 76-13 (1995).
2. Karkalas, J., *J. Sci. Food Agric.*, **36(10)**, 1019-1027 (1985).
3. Beutler, H.O., "Starch", in *Methods of Enzymatic Analysis*m 3rd ed. (H.U. Bergmeyer, ed.). Academic Press (New York), Volume 6, pp. 2-10 (1984).
4. MacRae, J.C. *et al.*, *J. Sci. Food Agric.*, **25(12)**, 1465-1469 (1974).
5. Thivend, P. *et al.*, "Determination of Starch with Glycoamylase", in *Methods in Carbohydrate Chemistry*, Volume 6: General Carbohydrate Method (R.L. Whistler and J.N. BeMiller, eds.). Academic Press (New York and London), pp. 100-105 (1972).
6. Bergmeyer, H.U. and Bernt , E., *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.). Academic Press (New York, NY), 2nd ed., pp. 1205-1212 (1974).
7. *Official Methods of Analysis*, 16th Edition. AOAC International, Sections 32.2.05 and 44.7.12 (1995).
8. Raabo, E. and Terkildsen, T.C., *Scand. J. Clin. and Lab. Invest.*, **12(4)**, 402-407 (1960).
9. Washko, M.E. and Rice, E.W., *Clin. Chem.*, **7(5)**, 542-545 (1961).
10. Wang, Y. *et al.*, *Plant Cell*, **25(4)**, 1383-1399 (2013).
11. Nguyen, C.V. *et al.*, *Plant Cell*, **26(2)**, 585-601 (2014).
12. Ramanan, R. *et al.*, *Plant Physiol.*, **177(3)**, 1050-1065 (2018).

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APPENDIX C

STANDARD CURVE FOR STARCH ASSAY METHOD

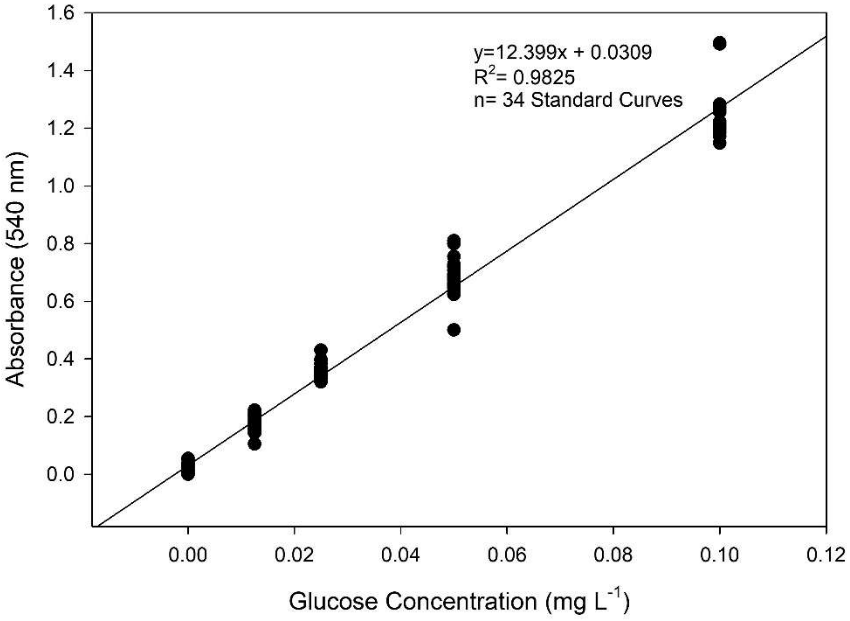

Figure C. Standard curve for starch assays using the STA-20 kit.

APPENDIX D

STARCH STANDARD ASSAY FOR THE STA-20 KIT

Table D.1. Percent starch recovery of corn standards provided in the STA-20 kit.

Sample	Type	Mass (mg)	% Starch	% Purity	% Recovery
15	Corn	10	91.08	93	97.94
30	Corn	10	75.11	93	80.76
43	Corn	10	77.65	93	83.50
58	Corn	10	88.01	93	94.63
73	Corn	10	77.76	93	83.62
88	Corn	10	107.34	93	115.42
103	Corn	10	77.78	93	83.63
117	Corn	10	81.54	93	87.68
132	Corn	10	82.23	93	88.41
146	Corn	10	80.11	93	86.14
159	Corn	10	79.05	93	85.00
170	Corn	10	75.46	93	81.14
185	Corn	12	79.35	93	85.32
200	Corn	10	96.26	93	103.50
212	Corn	10	79.51	93	85.50
227	Corn	10	90.55	93	97.37
241	Corn	10	94.03	93	101.11
248	Corn	10	85.79	93	92.24
259	Corn	10	97.19	93	104.51
270	Corn	10	88.78	93	95.46
282	Corn	11	97.32	93	104.65
297	Corn	10	101.79	93	109.45
304	Corn	10	86.00	93	92.47
319	Corn	10	87.66	93	94.26
334	Corn	10	90.41	93	97.21
360	Corn	10	106.16	93	114.15
375	Corn	10	89.71	93	96.46
382	Corn	10	81.61	93	87.75
397	Corn	10	81.93	93	88.09
413	Corn	10	93.65	93	100.70
421	Corn	10	93.40	93	100.43
436	Corn	10	104.48	93	112.34
451	Corn	10	96.40	93	103.66
462	Corn	10	80.90	93	86.99
Mean (± 1 SE)			88.12 ± 1.56		94.75 ± 1.68

Table D.2. Percent starch recovery of wheat standards provided in the STA-20 kit.

Sample	Tissue	Mass (mg)	Purity	% Starch	% Purity	% Recovery
14	Wheat	10	89	94.14	89	105.78
29	Wheat	10	89	78.86	89	88.61
42	Wheat	10	89	74.60	89	83.82
57	Wheat	10	89	93.13	89	104.64
72	Wheat	10	89	90.25	89	101.41
87	Wheat	10	89	105.68	89	118.74
102	Wheat	10	89	93.19	89	104.71
117	Wheat	10	89	89.34	89	100.38
133	Wheat	10	89	74.85	89	84.10
145	Wheat	10	89	98.65	89	110.85
158	Wheat	10	89	72.95	89	81.96
169	Wheat	12	89	71.94	89	80.84
184	Wheat	10	89	76.38	89	85.82
199	Wheat	10	89	98.01	89	110.12
211	Wheat	10	89	77.79	89	87.40
226	Wheat	10	89	98.52	89	110.70
242	Wheat	10	89	89.87	89	100.97
247	Wheat	10	89	87.82	89	98.68
258	Wheat	10	89	81.58	89	91.67
269	Wheat	10	89	76.70	89	86.18
281	Wheat	10	89	101.65	89	114.21
296	Wheat	10	89	92.78	89	104.24
303	Wheat	11	89	90.30	89	101.46
318	Wheat	10	89	97.02	89	109.01
333	Wheat	10	89	85.38	89	95.94
344	Wheat	10	89	90.82	89	102.04
359	Wheat	10	89	118.00	89	132.59
374	Wheat	10	89	92.45	89	103.88
381	Wheat	10	89	78.23	89	87.89
396	Wheat	10	89	107.47	89	120.75
412	Wheat	10	89	102.05	89	114.66
420	Wheat	10	89	88.02	89	98.89
435	Wheat	10	89	118.95	89	133.66
450	Wheat	10	89	96.27	89	108.16
461	Wheat	10	89	89.17	89	100.19
Mean (± 1 SE)				90.65± 2.00		101.90±2.24

APPENDIX E

PERCENT DIFFERENCE OF DUPLICATE CUBAN BULRISH STARCH SAMPLES

Table E. Percent difference between duplicate *Oxycaryum cubense* starch samples.

Month	Year	Site/Plot	Tissue	Mass	% Starch	% Difference
October	2021	FL/1	Emer Dup	50	0.65	
October	2021	FL/1	Emer	52	0.55	17.08
October	2021	MS/1	Sub Dup	60	0.94	
October	2021	MS/1	Sub	52	1.17	19.34
October	2021	LA/1	Inf Dup	50	1.33	
October	2022	LA/1	Inf	50	1.30	2.04
November	2021	MS/1	Emer Dup	60	0.79	
November	2021	MS/1	Emer	52	0.88	10.23
November	2021	LA/1	Sub Dup	50	1.05	
November	2021	LA/1	Sub	50	1.12	6.82
November	2021	FL/2	Inf Dup	60	0.48	
November	2021	FL/2	Inf	60	0.40	18.75
December	2021	MS/1	Emer Dup	60	0.26	
December	2021	MS/1	Emer	60	0.28	7.69
December	2021	LA/2	Sub Dup	60	0.39	
December	2021	LA/2	Dup	60	0.48	18.18
December	2021	FL/2	Inf Dup	60	0.45	
December	2021	FL/2	Inf	50	0.45	1.46
January	2022	MS/2	Emer Dup	52	1.47	
January	2022	MS/2	Emer	50	1.53	3.85
January	2022	LA/2	Sub Dup	61	0.36	
January	2022	LA/2	Dup	50	0.45	18.86
January	2022	LA/1	Inf Dup	61	0.42	
January	2022	LA/1	Inf	50	0.39	8.07
February	2022	MS/2	Emer Dup	50	0.53	
February	2022	MS/2	Emer	50	0.46	16.67
February	2022	LA/2	Sub Dup	50	0.85	
February	2022	LA/2	Sub	52	0.72	17.87
February	2022	FL/2	Inf Dup	62	0.29	
February	2022	FL/2	Inf	52	0.26	12.01
March	2022	MS/2	Emer Dup	60	1.12	
March	2022	MS/2	Emer	60	1.35	16.95
March	2022	MS/2	Sub Dup	60	0.63	
March	2022	MS/2	Sub	60	0.58	7.69
March	2022	LA/2	Inf Dup	61	0.22	
March	2022	LA/2	Inf	62	0.18	27.43
April	2022	MS/2	Emer Dup	61	0.78	
April	2022	MS/2	Emer	60	0.99	20.58
April	2022	FL/2	Sub Dup	61	0.38	

Table E (continued)

Month	Year	Site/Plot	Tissue	Mass	% Starch	% Difference
April	2022	FL/2	Sub	61	0.47	19.05
May	2022	MS/1	Emer Dup	60	0.37	
May	2022	MS/1	Emer	60	0.32	14.29
May	2022	LA/2	Sub Dup	60	0.28	
May	2022	LA/2	Sub	60	0.23	20.00
May	2022	MS/1	Inf Dup	60	0.22	
May	2022	MS/1	Inf	60	0.22	0.00
June	2022	MS/2	Emer Dup	60	0.20	
June	2022	MS/2	Emer	60	0.20	0.00
June	2022	LA/2	Sub Dup	61	0.87	
June	2022	LA/2	Sub	61	0.85	2.63
June	2022	FL/2	Inf Dup	60	0.25	
June	2022	FL/2`	Inf	61	0.22	11.83
July	2022	MS/2	Emer Dup	60	0.67	
July	2022	MS/2	Emer	60	0.63	7.41
July	2022	LA/2	Sub Dup	62	0.11	
July	2022	LA/2	Sub	60	0.10	14.37
July	2022	MS/1	Inf Dup	60	1.78	
July	2022	MS/1	Inf	60	1.51	17.91
August	2022	MS/2	Emer Dup	60	0.29	
August	2022	MS/2	Emer	60	0.31	7.14
August	2022	LA/2	Sub Dup	60	0.18	
August	2022	LA/2	Sub	60	0.20	11.11
August	2022	LA /1	Inf Dup	60	0.30	
August	2022	LA /1	Inf	60	0.28	4.23
September	2022	FL/2`	Emer Dup	62	0.13	
September	2022	FL/2`	Emer	62	0.13	0.00
September	2022	LA/1	Sub Dup	60	0.41	
September	2022	LA/1	Sub	60	0.42	3.11
September	2022	MS/2	Inf Dup	62	0.49	
September	2022	MS/2	Inf	62	0.47	4.76
Mean (±SE)						11.13 ± 1.23
95% CI						10 <> 12.46