Phenology and Water Quality Impacts of an Invasive Water Chestnut (Trapa bispinsoa Roxb. Var iinumai Nakano) in Northern Virginia, USA and Evaluation of Early Detection/Rapid Response (EDRR) Practices in Its Control

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at George Mason University

by

Sujata Poudel
Master of Science
University of Massachusetts, Boston, 2012
Master of Environmental Studies
University of Tokyo, 2005

Director: R. Christian Jones, Professor
Department of Environmental Science and Public Policy

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George Mason University
Fairfax, VA

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Artificial Intelligence .... AI
Alkalinity ....Alk
Alien Invasive Species .... AIS
Analysis of Variance ....Anova
Average Rosette Diameter .... ARD
Animal and Plant Health Inspection Service ....APHIS
Aquatic Nuisance Species ....ANS
April .... Apr
August ....Aug
Aquatic Nuisance Species Task Force ....ANSTF
Biological Oxygen Demand.. BOD
Chemical Oxygen Demand .... COD
Convention on Biological Diversity .... CBD
Custom and Border Protection .... CBP
Corona Virus Disease ....COVID
Early Detection and Rapid Response ....EDRR
Environmental Protection Agency .... EPA
District of Columbia .... DC
Department of Homeland Security ....DHS
Dissolved Oxygen ....DO
Dissolved Oxygen (% Saturation) .... DO%
Dissolved Organic Carbon .... DOC
Early Detection and Distribution .... EDD
Electronic Logging Device ....ELD
Executive Order .... EO
Early Warning System .... EWS
Fairfax County Park Authority ....FCPA
Fairfax County Storm Water.. FFXSW
Federal Interagency Committee for the Management of Noxious and Exotic Weeds ....FICMNEW
Flowers per Rosette.. FLPR
Flowers per Quadrat..FLPQ
Fruits per Rosette ....FRPR
Geographic Information System .... GIS
Geographic Positioning System.. GPS
June .... Jun
July..Jul
Invasive Plant Atlas of New England ....IPANE
Invasive Species Advisory Committee .... ISAC
Mid Atlantic Early Detection Network .... MAEDEN

Nonindigenous Aquatic Species ....NAS
Nonindigenous Aquatic Nuisance Prevention and Control Act .... NANPCA
National Agricultural Pest Information System..NAPIS
National Wildlife Health Center .... NWHC
National Invasive Species Act of 1996 .... NISA
National Invasive Species Management .... NISC
Northern Virginia Regional Park Authority ....NVRPA
November...Nov
Not Significant .... NS
Potomac Science Center .... PSC
Precipitation .... PPN
October..Oct
Remotely Operated Vehicle ....ROV
Rosette Reproductive Phenology ....RRP
Submerged Aquatic Vegetation ....SAV
Secretariat of the Convention on Biological Diversity ....SCBD
September .... Sept
Specific Conductivity...SC
Species ....sp.
Trapa bispinosa ....*Trapa*
Temperature .... Temp
Total Dissolved Solid..TDS
Trapa Pond Coverage .... TPC
Trapa Quadrat Coverage.. TQC
Turbidity .... NTU
The Wildlife Society ....TWS
Unarmed Aerial Vehicle .... UAV
United Nations Environment Program..UNEP
United States Department of Agriculture ....USDA
Water Quality..WQ
2, 4-Dichlorophenoxyacetic acid ....2,4 D

ABSTRACT

PHENOLOGY AND WATER QUALITY IMPACTS OF AN INVASIVE WATER CHESTNUT (TRAPA BISPINSOA ROXB. VAR IINUMAI NAKANO) IN NORTHERN VIRGINIA, USA AND EVALUATION OF EARLY DETECTION/RAPID RESPONSE (EDRR) PRACTICES IN ITS CONTROL

Sujata Poudel, Ph.D.

George Mason University, 2021

Dissertation Director: Dr. R. Christian Jones

Species of the genus *Trapa*, specifically *Trapa natans*, have plagued the northeastern US, including the tidal Potomac for over 100 years. Much has been learned about the ecology and management of *T. natans*, during this period. In 2014, a new species of *Trapa* identified as (*T. bispinosa Roxb. var iinumai Nakano*) was discovered in the tidal Potomac River and in subsequent years it has spread to nearby waterbodies. This species is different from *T. natans* as it has pink flowers instead of white flowers of *T. natans*; two horned fruits instead of four horned fruits; and reddish underside of leaves instead of green leaves of *T. natans*. *T. bispinosa* (hereafter referred as *Trapa*) has been identified at over 65 different locations in VA, since 1995.

The purpose of this study is to describe the vegetative and reproductive phenology of *T. bispinosa* in a group of Fairfax County ponds and to assess its impact on WQ variables

such as temperature, dissolved oxygen (DO), pH, specific conductance (SPC) and turbidity (NTU). The final goal is to evaluate laws, regulations, incentives, knowledge gaps, and to assess the usefulness of Early Detection and Rapid Response (EDRR) as a policy option in its control. A total of 8 *Trapa* ponds were sampled over the two-year study (2019 and 2020). Some had a history of herbicide treatment while others did not. A total of 4 non-*Trapa* ponds were also sampled in 2020 and WQ was compared with *Trapa* ponds. In untreated ponds with dense and healthy *Trapa*, the beds rapidly developed in spring and remained robust until fall. Rosette diameters were bigger in July and August. *Trapa* flowers, fruit and reproductive phenology were also higher in late summer and early fall. *Trapa* growth was highly impacted by herbicide applications in treated ponds resulting in more erratic plant development, with fewer flowers and fruits. Even though *Trapa* appeared later in treated ponds compared to untreated, maximum flower and fruit phenology was during the similar months. Herbicide did not completely block the flowering and fruiting capacity of *Trapa*.

All phenological parameters were negatively correlated with DO (mg/L). WQ variables also varied according to the density of *Trapa* and were more consistent in untreated ponds compared to treated ponds except pH. DO ((mg/L) and %) was significantly depleted in untreated *Trapa* ponds compared to treated *Trapa* ponds and non-*Trapa* ponds. This indicated that the dense carpet of *Trapa* was obstructing reaeration and photosynthesis in the underlying layers. pH was low in fall and alkalinity was low in spring in *Trapa* ponds compared to non-*Trapa* ponds. DO (mg/L) was mostly below 5 but above 2 in summer in the most intensely studied untreated pond, VGA-VA where *Trapa* was

healthy, dense and abundant and DO % was above 25 in summer but higher than 40 in spring and rest of the fall months. The short-term hydroponic experiment assessing the effect of low densities of *Trapa* found that *Trapa* could sustain higher DO levels in uncrowded conditions due to photosynthesis by underwater leaves. *Trapa* management is a multijurisdictional issue in VA. Counties, researchers, and volunteers have initiated some management options for its control. EDRR could help manage the distribution and spread of *Trapa* and efforts are being made to apply EDRR to this situation. This study by providing some basic ecological and phenological information on *Trapa* growth in both treated and untreated ponds will inform the EDRR process.

Keywords: *Trapa natans*, *Trapa bispinosa*, Phenology, Fairfax County, EDRR

LITERATURE REVIEW ON TRAPA BISPINOSA

<u>Introduction</u>

The invasive Water Chestnut (*Trapa bispinsoa* Roxb. var *iinumai* Nakano) was first reported from the Pohick Bay area of the Potomac River, VA in 2014 by Virginia Game and Inland Fisheries biologists (Dodd et al., 2019 & Chorak et al., 2019). This variety is different from *Trapa natans* (another common form historically in VA) as it has pink flowers instead of white flowers of *Trapa natans*; two horned fruit instead of four horned fruit; and reddish underside of leaves instead of green leaves of *Trapa natans*. *T. bispinosa* was identified at over 65 different locations in VA since 1995 (Pfingsten & Rybicki, 2020). As *T. bispinosa* is a recently identified species in VA, certain parameters such as its prevalence, phenology and its effects on water quality have not been documented so far; and assumptions are being made with reference to its closest relative *T. natans*. Distinguishing characteristics between these two *Trapa* species in US is important not only for the documentation, spread and identification of new population but also for threat assessment: timing, efficacy of physical, chemical, and biological control method. The following sections provide an overview of background information on

Trapa and its invasion history in US & its consequences; and propose a research problem that identifies knowledge gaps, goals, objectives, research questions and set of methods to address the research problem. Since there were very few studies that documented *T. bispinosa* of VA, the background information is focused on its closest relatives; mostly *T. natans* and similar other varieties.

Invasive Species

The term "Non-native species" refers to the plants, animals or microorganisms which become established outside of their natural range due to the activities of humans. They may or may not disrupt the natural functions and processes of the native ecosystems.

In the history of U.S., almost 50,000 nonnative species are estimated to have been introduced from elsewhere. Introduced species and cultivars including corn, wheat, rice and other food crops as well as cattle, poultry and livestock provide more than 98% of the U.S. food system at a value of approximately $800 billion per year (USBC, 2001 & Pimentel et al., 2004). Other non-native species have been used for landscape restoration, biological pest control, sports, pets and food processing. However, many of those species have spread rapidly causing major economic losses in agriculture, forestry, and ecosystem function (Pimentel et al., 2004). Plants such as Chinese tallow tree, insects like Emerald ash-borer, clams and mussels such as Asiatic clam, Zebra and Quagga mussels and fish such as Northern Snakeheads are some of nonnative species which became invasive in US.

An invasive species is a species that is nonnative to ecosystem or country; but which when introduced proliferates dramatically. This proliferation may cause economic, environmental or human health impacts. Invasive species of plants and animals can wreak havoc on native environment: displacing native species, permanently changing habitats, and causing loss of billions of dollars in terms of economics (Stuart, 2012). Not all nonnative species will become invasive and undesirable. But even the species that may at first appear to be non-invasive may indeed become invasive given the right local habitat conditions. This might include the introduction of another non-native species, environmental changes, or other factors that give it a biological advantage to allow for invasive proliferation. Such changes can occur over a long-time lag or quite suddenly. Thus, any new introductions into the local environment warrant scrutiny over time (AIS Management Plan, 2003). The cost of invasive species to the American economy is almost about $138 billion/year (Pimentel, 2002).

Control of Invasive Species

Although multimillion-dollar control programs have been launched in the US to manage these species (Cooke et al., 1993), early detection is often difficult and control measures are used only after a species has spread to nuisance proportions. Many of the lakes where such invasive species are predominant are privately owned. Therefore, stakeholder education and observations, as well as predictive dispersal capabilities are both important in early detection and management. New generation mapping software such as Geographic Information Systems (GIS), coupled with a growing availability of computerized taxonomic and online herbarium databases, may provide aquatic plant ecologists and managers with a new set of tools to observe via computer simulation, dispersal across time and space (Boylen et al., 2006).

Water Chestnut *(Trapa natans)*

Taxonomy

The Water Chestnut, genus *Trapa*, is currently classified in the *Lythraceae* family (Integrated Taxonomic Information System, 2016). Previously, Water Chestnut was classified as a separate family, *Trapaceae* (Crow and Hellquist, 2000; Muenscher, 1944) or *Hydrocaryaceae* (Gleason and Cronquist, 1963), with one genus, *Trapa*. There are between 2 and 11 species listed by various sources under the *Trapa* genus, the most common being *T. natans* and *T. bicornis*. *Trapa* species should not be confused with the "Chinese Water Chestnut" (*Eleocharis dulcis*) used in Chinese cuisine (www.oars3rivers.org).

Biology of Water Chestnut & Seed Bank

T. natans prefers nutrient and humus rich habitats such as muddy lakes, ponds or oxbow lakes (1-2 meters in depth), as the calm shallow water warms up rapidly in spring. Water temperature of 12-15 °C is absolutely necessary for the fruit gemination and, at least 20 °C is required for the flower development. The critical water temperature for the spring seed germination was estimated as 10 ºC. The plant flowers in July and August. In autumn, the leaves change color from green to purple- brown, rosettes dismantle, and fruits sink to the bottom of the lake and anchor with their thorns in the silty sediments (Karge, 2006).

The study done by Kuni et al. (1998), with *T. japonica Flerov*, has shown that seeds left at room temperature remained viable for more than five years. Among the ungerminated buried viable seeds, some showed germination in an outdoor water tank two years after they were collected from the pond bottom. In a glass bottle settled under room temperature, seed germination occurred not synchronously but intermittently and none of the seeds remained viable for more than 5 yrs. The *Trapa sp*. identified in this study was *T. japonica Flerov.* with two-spined fruit form (Kuni et al., 1998). The *T. natans* seeds showed a higher germination rate compared to *T. japonica Flerov.*: up-to 87% in the field (Kurihara & Ikusima, 1991). *T natans* seeds remained viable in sediments for upto 10 years, if not allowed to dry (Hummel and Kiviat, 2004). According to Phartyal et al. (2015), seeds tolerated freezing down to -14 ºC and the seeds with emerged hypocotyl were highly sensitive and exhibited freezing down to -4 ºC. The non-dormant seeds germinated 75-100% under all env conditions of constant 22 ºC and from low 10 ºC to high 30 ºC under light, darkness, oxic or hypoxic condition (Phartyal et al., 2018).

Distribution

Trapa natans is an aggressive annual aquatic plant which is native to much of the Old World (Europe, Asia and Africa) and invasive to much of New World (North America, New Zealand & Australia). It can rapidly expand by natural intra-watershed dispersal of seeds. Nutrient-rich shallow lakes and rivers are the favorable environment for its growth. *Trapa* is found in shallow (less than 16 feet deep) areas of freshwater lakes and ponds, and slow-moving streams and rivers, where it forms dense mats of floating vegetation. It prefers the nutrient-rich waters with a pH range of 6.7 to 8.2 and an alkalinity of 12 to 128 mg/l of calcium carbonate (Adkar et al., 2014).

***Trapa* bispinosa**

Trapa bispinosa is an annual floating leaf macrophyte found in tropical, subtropical and temperate zones of the world. Its natural range of growth includes Southern Europe, Africa and Asia. It has been grown in Europe since Neolithic times. It was commonly used as food by ancient Europeans as an easy growing plant. In the Chinese Zhou Dynasty, water caltrop was considered as an important food for worship as prayer offerings. The rites of Zhou (2nd century BC) mentioned that a worshipper is supposed to use a bamboo basket containing dried water caltrops in certain occasions. In India, it is known as Singhara or Paniphal (eastern India) and is widely cultivated in freshwater lakes. The fruits are eaten as raw or boiled. The dried fruit is also ground into powder (aka Singahara ka atta), which is eaten during religious fasting of Hindu festival of "Navaratri" (Adkar et al., 2014).

Trapa bispinosa fruit has a great quantity of antioxidants, such as flavonoids, flavones, and total phenol contents (Adkar et al., 2014). Water Chestnut powder is also sold in eBay and Amazon in US as a detoxing agent. *T. bispinosa* flowers found in India are few, auxiliary, solitary, pure and white. Fruits are ovoid, angular, 2.2 -5 cm long and broad with very sharp spinous horns in each side (Adkar et al., 2014). The plant is also abundant in Indonesia, southeast Asia, the southern part of China and in the eutrophic waters of Japan, Italy, and tropical America. It is found in slow moving rivers, ponds, lakes, transitional zone between shore and open water and is widely cultivated in Asia. (Wu et al., 2007 & Deng et al., 2014; Adkar et al., 2014).

Morphology

One of the varieties of *Trapa natans*; *T. natans* Linn has two types of leaves; finely divided feather-like submerged leaves borne along the length of the stem, and undivided floating leaves borne in a rosette at the water's surface (Mendhekar & Rachh, 2019). The floating leaves are about 2-6.5 cm diameter, rhomboid, fan-shaped with toothed edges. *T. natans* is capable of producing three primary rosettes. The first one arises from the center, the second one from the side opposite of hypocotyl and the third between the first shoot and hypocotyl (Groth et al., 1996). The submerged floating stem of *T. natans* attaches to the buoyant rosette of leaves. Rosettes are borne on long stems bearing early deciduous leaves that are replaced after about 2 weeks by highly dissected green structures growing from the leaf nodes (Fig. 1). These adventitious structures are referred both as roots (Sculthorpe, 1971) and leaves (Muenscher, 1944; Vasilev, 1978 & Groth et al.,1996). Their functional role is primarily nutrient absorption although their developmental origin remains unclear. *T. natans* has no primary root system unlike many aquatic plants (Arber,1920 & Groth et al., 1996); but adventitious roots extend from the hypocotyl and later form the lower stem and serves for the anchorage of plants.

The fruit of *T. natans* has four horns and a prominent crown, whereas the fruit of *T. bispinosa* has two horns and lacks a crown (Fig 5, Fig 6). *T. natans* has white flower petals whereas *T. bispinosa* has pink flowers (Fig 2, 3 & 4) (Chorak et al., 2019).

Fig. 1. *Trapa natans* with floating leaves (top); submerged leaves (bottom left) and nut (right) (Source: nyis.info/invasive species/Water Chestnut).

Fig. 2. ***Trapa natans*** **with white flowers (Source: bentonswcd.org).**

Fig. 3. ***Trapa bispinosa*** **with pink flowers discovered in VA (Source: Rybicki, 2018).**

Fig. 4. ***Trapa bispinosa*** **with pink flowers (2019).**

Fig. 5. ***Trapa bispinosa*** **with two spined fruits (Source: Rybicki, 2018).**

Fig. 6. *Trapa bispinosa* with different stages of fruit in VA (2019).

In midsummer, the stems become long, and the lower sections lie on the substratum and the decumbent stems produce additional adventitious roots anchoring the stem at one or more spots. The stem is cordlike, spongy and buoyant and can reach up to 16 ft (Jain et al., 2012).

Reproductive Structures

The flowering time of the genus *Trapa* varies from one place to another (Yanglem et al., 2016). The flowering of the variety *T. natans* begins in early July and continues until October when the plant senesces. The white flowers of *T. natans* are four petaled and insect pollinated (Fig. 2). The fruit is deciduous, fleshy green pericarp with two to four sharp spines derived from the sepals (Lawrence, 1951 & Groth, 1996) (Fig. 1). In most of the sources, *Trapa* fruit is referred as a nut (Methe et al., 1993 & Groth, 1996). In spring, the plant emerges as rosette from seeds that remain viable up-to 12 years. The flowering of *T. bispinonsa* was observed from early June until early October during our experimental study in 2019 and 2020 in Fairfax County, VA. Water temperature of 12-15 °C is absolutely necessary for the fruit germination and promote seedling to grow, whereas 20 °C is required for development of flower in *T. natans L.*, *sensu lato* (Yanglem et al., 2016). More than 40% of the total plant biomass maybe concentrated in the top 30 cm during the growing season from late May until September and other submerged macrophytes can grow in or under the *T. bispinosa* canopy (Deng et al., 2014; Galanti et al., 1990).

Importance and Uses in the Native Range

In its native range in Asia, *Trapa* is used in many Ayurvedic (holistic) preparations to serve as nutrient, appetizer, astringent, diuretic, aphrodisiac, antidiarrheal and tonic. It is also considered useful in rabies, poisonous animal bites, diarrhea and dysentery, sore throats, bronchitis, leucorrhoea, bad teeth, fatigues, inflammation, tuberculosis, malaria, pregnancy problems and other medicinal ailment conditions (Hummel and Kiviat, 2004; Yanglem et al., 2016). Fruits are also used to treat rheumatism, sunburn, recurrent herpes genitalis and labialis and also claimed to have the cancer preventing properties (Jain et al., 2012 & Adkar et al., 2014; Hijikata et al., 2007 & Yanglem et al., 2016).

Archaeological excavation study in Southern Germany has showed that *T. natans L*. was used as a food resource during the 4^{th} to 1^{st} millennia BC (Karg, 2006). Until 1880 AD, *Trapa natans* was available at many markets all over Europe (Jaggi,1883 & Karg, 2006). In northern Italy, the nuts were roasted as sweet chestnuts (*Castanea sativa* Mill.) & offered, which are still sold today. At many places in Europe, sweet chestnut fruits were known and used for human food until the beginning of 20^{th} century (Brockmann-Jerosch, 1914; Karg, 2006). Nowadays, it is considered as a very rare plant because of its extinction due to climatic fluctuations, changes in the nutrient contents of the water bodies and drainage of many wetlands, ponds and oxbow lakes (Karg, 2006).

The study done by Kousar & Puttaiah (2009) indicates that *T. bispinosa* is highly efficient in treating the pulp and paper industry effluent and also possess an outstanding ability to assimilate nutrients and heavy metals. The potential to accumulate metals like iron, nickel, manganese and copper by *T. bispinosa* was studied by subjecting them to different effluent concentrations of pulp and paper industry under laboratory conditions. In this study, *T. bispinosa* caused significant reduction in various parameters like dissolved oxygen (DO), biological oxygen demand (BOD), chemical oxygen demand (COD), total alkalinity, total hardness, chloride, and sulphate (Koursar & Puttaiah, 2009). *T. natans* has no evidence of having Nitrogen fixing capacity, and it is also not in a plant family known to contain nitrogen-fixing species (Martin & Dowd, 1990). However, some of the literatures mention that *T. natans* can fix nitrogen and phosphorus: and can be used as a unique biological tool to reduce eutrophication and environmental reclamation (Yanglem et al., 2016).

One of the varieties: *T. bispinosa* Roxb. is reported to have various physiological functions including antioxidant, antimicrobial, and antiulcer activity. In addition, recent studies have demonstrated that the *Trapa* genus is known for inhibition of the formation of cross-linking and carbonylation of α-crystallin by glycation *in vitro*. In the study done in the diabetic rats, oral intake of the combination of the *T. bispinosa* extract and lutein significantly inhibited the progression of cataractogenesis in the eye lens of diabetic rats, even at low doses, and the combination was more effective than individual treatment (Kinositha et al., 2019).

Impact Potential

Water Chestnut creates a canopy that interrupts the passage of light through water (Tall et al., 2011; Groth et al., 1996) which inhibits photosynthesis and prevents oxygenation in the deeper layer (Strayer, 2010). The colonies displace native species (Strayer et al., 2003; Hummel & Kiviat, 2004) and pose problems in recreational waters. The aggressive growth of *Trapa* influences aquatic ecosystem biodiversity and function; and impedes hydroelectric generation, irrigation and recreation (Naylor, 2003). Emergent plants of *T. natans* release oxygen directly into the atmosphere while depleting oxygen from the surrounding water (Tall et al., 2011) resulting in hypoxia and anoxia (USDA, 2016). The floating-leaved growth habit of *Trapa* may outcompete native submerged aquatic vegetation through shading and degrading waterfowl habitat (Chorak et al., 2019). The dispersal of *Trapa* is prone to both natural (Swearingen et al., 2002; Hummel & Kiviat, 2004; Pemberton, 2002) and human-mediated (Dementeva & Petushkova, 2010; Hummel & Kiviat, 2004) vectors. Its barbed seeds can attach to fish nets (Dementeva & Petushkova, 2010), boats (Hummel & Kiviat, 2004), water currents (Van der Pijl, 1982; Pemberton, 2002), birds (Swearingen et al., 2002; Hummel & Kiviat, 2004), Canada geese (Hummel & Kiviat, 2004), animals (Swearingen et al., 2002; Hummel & Kiviat, 2004), larger birds, beavers and otters (Karg, 2006). The 2 inch long, hard, and spiny pods also known as caltrops, pierce the feet of beachcombers (Chorak et al., 2019 & Dodd et al., 2019). *Trapa* poses danger to public, including injury from stepping into barbed fruits (Kaufman & Kaufman, 2007; Hummel & Kiviat, 2004) and drowning in its thick growth (Hummel & Kiviat, 2004).

History of *Trapa* Invasion in the US

Trapa natans was first observed in North America, growing "luxuriantly" in Sanders Lake, Schenectady, New York in 1884 (Wibbe, 1886). The plant subsequently spread to many other areas in the Northeastern United States including Connecticut, Delaware, Maryland, Massachusetts, New Hampshire, Pennsylvania, Vermont, Virginia, and Washington D.C. The plant is also present in the Great Lake Basin and recently has been found in Quebec, Canada (Wibbe, 1886 & Adkar et al., 2014). The specific geographic origins of the *T. natans* genotype(s) that has become a problem in the United States are unknown. It is thought to be from Eurasia, but recent work considers it of Asian origin (Crow & Hellquist, 2000, www. invasive.org).

In the mid-Atlantic region, *T. natans* was first reported from the Bird River, Maryland in 1955 and believed to be eradicated by the use of herbicide (2, 4-D), but reappeared after 10 years. Currently, the most problematic areas include the Bird and Sassafras Rivers in Maryland, Hudson River, Connecticut River valley and Lake Champlain (Naylor, 2003). *T. natans* is a serious pest within the US (Gupta, 2011; Groth et al., Ding and Blossey, 2005; Ding et al., 2006; Countryman 1977; Pemberton, 2002) but it is declining in the other areas of the world. For example, in Europe and the former Soviet Union, *T. natans* is in an apparent natural decline (Dementeva & Petushkova, 2010; Gupta, 2011) possibly due to the loss of the natural habitat (Gupta, 2011).

In US, the complete decline of *T. natans* has been observed in the Potomac River, due to extensive management using underwater mowing (Orth and Morre, 1984; Carter and Rybicki, 1994). In 1923, it was reported from Oxon Run, across the Potomac from Alexandria (Gwathmey, 1945). In response to the Water Chestnut expansion in the Potomac River in 1930's, a massive removal effort was conducted by the U.S. Army Corps of Engineers from 1939 to 1945 at an estimated cost of $3.7 million. This coupled with follow-up removal by hand until from 1950 to1965 resulted in the eradication of *T. natans* from the tidal Potomac. Mechanical removal and herbicide application (2,4-D) were also used to control the population by the Maryland Department of Game and Inland Fish and Tidewater Fisheries in Bird River and Sassafras River. *T. natans* continues to be found in Maryland portion of Chesapeake Bay and throughout northeast US. *T. bispinosa* was recently discovered at Pohick Bay area of Potomac River in 2014 (Chorak et al., 2019).

Management Options

For the successful management of aquatic plants, the following two points should be noted: As excessive aquatic plant growth is promoted by nutrient-rich water, efforts should be made on the reduction of nutrients in water. This may be done by reducing and treating stormwater runoff, reducing nutrient from wastewater treatment facilities, eliminating runoff from lawn fertilizer, and other sources of nutrients into waterbody. Second, aquatic ecosystems should be managed as complex ecosystems with the focus on maintaining a healthy and continuous diverse system.

Unlike many invasive plants, Water Chestnut is strictly an annual. It has been successfully controlled or eliminated in some water bodies, but only after persistent efforts. The most important aspects of successful Water Chestnut management are commitment to adaptive management, ongoing monitoring, and long-term maintenance (www.oars3rivers.org).

Hand pulling

Pulling Water Chestnuts out by hand or with a rake is an easy technique for *Trapa* control. It is frequently used alongside a mechanical harvester to pick up the plants difficult to access. Hand-pulling is a possible option for volunteer as it requires minimal training and equipment (Mass. DCR, 2007; www.oars3rivers.org). Hand pulling is normally the most effective treatment for smaller population (Groth et al., 1996; Countryman, 1977).

Mechanical Harvesting and Hydroraking

Mechanical aquatic weed harvesters are like lawnmowers for aquatic weeds. Aquatic plant harvesters are hydraulically driven with reciprocating knives mounted on the harvesting head to cut the aquatic vegetation. The vegetation is then transferred onto the conveyor located on closed deck barge. The harvested plants are then transferred to the shoreline or into a dump truck via a shore conveyor. Compared to hand pulling, mechanical harvesting is faster (0.2–0.6 acres per hour) and requires fewer people (Wagner, 2004). However, it can be challenging to find a suitable launch and offloading site. Harvesters generally need a minimum of 2–3 feet of water depth. Once harvested, rosette might resprout, thus it requires a second cutting later in the season. Mechanical removal is useful on larger population and to open up the clogged waterways (Naylor, 2003).

A Hydrorake is similar to floating backhoe with several different size and functioning rake attachment. The hydrorake can operate in water as shallow as 1.0–1.5 ft and as deep as 1.5 ft to 10 ft." Hydrorakes don't have on-board storage, so they deposit weeds either on-shore or require a barge. Hydrorake pulls the entire plant with roots out of the sediment, which might tend to accumulate silt along with the plants.

Weed Disposal for Hand-pulling and Mechanical Harvesting

The harvested weed must be disposed by composting or incineration to control its further spread. The plant biomass may be piled near the harvest site and allowed to dry for a few days to weeks before final disposal as this method will decrease the volume and weight, making the disposal easier. However, the pile should be kept away from the water's edge to avoid washing back into the water. Composting is usually the least expensive option if a suitable site is accessible nearby (www.oars3rivers.org).

Drawdowns

Winter drawdowns are not recommended for control of Water Chestnut because the seeds are likely to survive in the sediments (Wagner, 2004) for upto 10 years (Hummel and Kiviat, 2004). Summer drawdowns have been used occasionally to control Water Chestnut. Summer drawdown is recommended after late May/early June when Water Chestnut has sprouted, and water levels are drawn down far enough to dry the sediment and kill the vegetation (www.oars3rivers.org). Lake drawdowns are widely used for the management purposes of the lakes in some part of Europe and Russia. This method has resulted in the disappearance of native *Trapa natans* in these areas (Phartyal et al., 2018).

Drawdowns can be cost effective and might have broad impacts on other plant and animal species. There are possibilities of recolonization from nearby areas (Wagner, 2004). Summer drawdowns might affect nearby wells and fire-fighting ponds, which could be critical during the lower-flow summer months (www.oars3rivers.org).

Dredging

Sediment dredging has been used successfully for weed control, in the areas that will not rapidly re-accumulate sediment. Dredging can control Water Chestnut by physically removing its seed bank, nutrients, soft sediments and increasing the depth of water body. Dry dredging, wet dredging, conventional wet dredging and hydraulic dredging are different methods of dredging. In dry dredging, conventional excavation equipment is used to drawdown waterbody to expose the sediment by controlling inflows during the process. Wet dredging may involve a partial drawdown with the use of specialized excavation equipment. Conventional wet dredging might result in turbidity and requires steps to limit downstream movement of the sediments.

Hydraulic dredging involves a suction type of dredge to remove a slurry of sediments. Permitting requirements and costs for dredging are generally higher than for other management options (www.oars3rivers.org).

Benthic Barriers

Benthic barriers might be used to prevent growth of rooted aquatic plants by limiting light and other growth factors in the application area (Mass. DCR, 2007). Clay, silt, sand, gravel, or sheets of artificial material (e.g., polyethylene, polypropylene, fiberglass, or nylon) can be used as barriers. The use of benthic barriers is limited because they are expensive and hard to maintain (www.oars3rivers.org).

Chemical Method

The most widely used chemical for *Trapa* control is herbicide 2, 4- D which is secondary plant growth regulator. However, the concentration of 2, 4- D necessary to control growth is harmful to native flora and fauna (Hummel & Kiviat, 2004). 2, 4- D has been in use in the United States since 1940s and was evaluated for re-registration in 2005 by the United States Environmental Protection Agency (U.S. EPA). The carcinogenic effects of 2,4- D were evaluated by U. S. EPA in 1988, 1992 and in 2004. Each evaluation has concluded that "the data were not sufficient to conclude that there is a cause-and-effect relationship between the exposure to 2,4-D and non- Hodgkin's Lymphoma.” 2,4-D was categorized as “Group D-not classifiable as to human carcinogenicity” in 2004 (Jervais et al., 2008).

Another auxin-like systemic herbicide, triclopyr (3,5,6- trichloro-2-pyridinyloxyacetic acid), might be a candidate for selective control of water *T. natans*. Poovey & Getsinger (2007) demonstrated that some control at growing rosette phase of *Trapa natans* can be achieved using subsurface applications of both triclopyr and 2,4-D amine at rates of 0.5 to 2.0 mg ai L^{-1} with exposure times of 24 to 48 hrs., without harming other aquatic monocot vegetations (Carpentier et al.,1988; Getsinger et al., 1997; Parsons et al., 2001; Poovey et al., 2004; Poovey & Getsinger et al., 2007).

Currently, Flumioxazin is widely used herbicide for *Trapa* control. Flumioxazin has been used in agricultural control since 2001 and was conditionally registered for aquatic use in 2010. Clipper, Schooner and Red Eagle herbicide are some of the trade names for water dispersible granule containing 51% of active ingredient of Flumioxazin. Flumioxazin can be used as a direct foliar application to control emergent and floating-leaf plants. It is a broad-spectrum contact herbicide. It interferes with the plants’ production of chlorophyll. Treated plants will respond quickly to treatment and rapidly decompose. For larger treatments or in dense vegetation, split treatments (2 weeks apart) are recommended to prevent fish suffocation from low oxygen due to decaying plants. Flumioxazin needs to be applied in young plants in early spring as they begin to grow. It should not be used in very hard-water lakes with pH over 8.5. Moving water bodies such as rivers or streams or waterbodies containing outlets should not be treated with flumioxazin. Tests on bluegill and rainbow trout indicate that flumioxazin is slightly to moderately toxic to fish and moderately to highly toxic to aquatic invertebrates. It is non-toxic to birds, small mammals, and bees. The risk of acute exposure would be primarily to chemical applicators. Concentrated flumioxazin has no inhalation risk but can cause some skin and eye irritation. Recreational users of a water body should not be exposed to concentrated flumioxazin. Flumioxazin is not carcinogenic according to chronic health effect studies. In some studies, adverse effects were seen on reproduction and development, including reduced offspring viability, malformation in cardiac and skeletal development, and anemia. Flumioxazin does not bioaccumulate in mammals, majority of it excretes within a week (dnr.wi.gov).

Biological Method

Biological removal with the leaf beetle *Galerucella birmanica* (*Coleoptera; Chrysomeliadae*) has shown promising results in the experimental tests (Ding et al., 2006). *G. brimanica* is host specific and its preference for the leaves of *T. natans* continues even after the plant is defoliated (Cornell Chronicle, 2016). Study done by Blossey et al. (2018) has showed that this biological method is not harmful to non-target species. The study was done by modeling the study on *G. birmanica* and *G. nymphaeae* (Blossey et al., 2018). The use of the beetle *G. nymphaeae* for biological control of *Trapa natans* has also been investigated (Ding et al., 2006). Ding et al. (2006) found that the impact of leaf beetle grazing can be significant and can act as a promising biological control agent for *Trapa*. However, plant compensatory growth can reduce the impact of grazing, as plants may grow faster or produce more seeds after being grazed (Ikeda and Nakasuji, 2003). In total, the grazing impact of *G. nymphaeae* on plant growth was assumed to be small (Tsuchiya et al., 1987). Grazing by *G. nipponensis* did not seem to affect the abundance of *T. japonica* & *T. natans* in the shallow eutrophic lakes in Japan (Saito et al., 2019). It is important to take extreme precaution before applying any chemical or biological control method to *Trapa* species as it might affect the non-target species. A North American beetle, *Pyrrhalta nymphaeae*, has also been reported to graze on *T. natans* in the Hudson River, but not extensively enough to inhibit nut production (Schmidt, 1986). More than 50 larvae per rosette were needed to negatively impact *T. natans* (Ding et al., 2006b; www.oars3rivers.org).

***Trapa* and Water Quality (WQ)**

When the water characteristics of *Trapa japonica* were compared with the mixed stands of submerged aquatics there was a wide range of nutrient level, steeper extinction of light, higher concentration of dissolved organic carbon (DOC), lower concentration of dissolved oxygen (DO) on the bottom (Takamura et al., 2003; Hummel & Findlay, 2006), lower concentrations of nitrate, nitrite and soluble reactive phosphorus (SRP) than other vegetation types (Takamura et al., 2003). *T natans* both produces and consumes dissolved oxygen (DO) with the balance depending on the photosynthetic capacity of plants and the release of oxygen into water or atmosphere. Under favorable conditions, Water Chestnut is capable of overgrowing 100% of Submerged Aquatic Vegetation (SAV): such as Water Celery (*Vallisneria americana*), clasping pondweed (*Potamogeton perfoliatus*) and

Eurasian watermilfoil (*Myriophyllum spicatum*) (Kiviat,1987, 1993; Groth et al., 1996; Hummel & Findlay, 2006). Duckweeds (*Lemna minor L*., *Spirodela polyrhiza L., Wolffia spp*.) and filamentous algae grow among the *Trapa* rosettes. Narrowleaf cattail (*Typha angustifolia L*.), pickerelweed (*Pontederia cordata L*.), and spatterdock (*Nuphar advena Aiton f*.) are unaffected by the presence of Water Chestnut (Kiviat 1987, 1993).

The dense canopy of Water Chestnut beds may reduce gas exchange between the water column and atmosphere. In addition, Water Chestnut bed may reduce turbidity by enhancing settling of suspended solids and contributing to local accumulation of fine sediment. A mesocosm study on *Trapa* and other SAV showed that *Trapa natans* has the potential to remove potassium and calcium from water (Shrivastava et al., 2009).

According to Goldhammer and Findlay (1988), the water flowing into and out of the tidal cove covered with Water Chestnut (Tivoli South Bay, NY) exhibited a reduction in suspended matter leaving the cove when compared to incoming water (Hummel & Findlay, 2006). A lab study done in Japan showed that DO was consistently low under dense Water Chestnut beds & 85% of dissolved inorganic nitrogen could be removed from the water column by dense Water Chestnut beds (Tsuchiya & Iwakuma, 1993). There is an evidence of direct correlation between rates of photosynthesis and increases in the dissolved organic carbon (DOC) in aquatic plant beds (Wetzel, 1969; Hummel & Findlay, 2006). The large beds of *T. natans* would decrease turbidity, NO_3, NH_4 and PO_4 and increase in DOC whereas the smaller beds would have lesser or no effect on such parameters (Hummel & Findlay, 2006).

Similarly, in a temperate shallow lake in central Japan, the formation of dense *Trapa* beds during summer resulted in hypoxia, leading to a decrease in the abundance of Chironomidae and Oligochaeta in the benthic community, as well as that of Calanoida among zooplankton (Kato et al., 2016). When dense *T. japonica* beds formed, other microinvertebrates resistant to hypoxia, such as Cladocera, Cyclopoida, Ostracoda, and Nematoda were favored. In addition, when the dense *Trapa* beds formed during summer, the concentration of chlorophyll *a* declined across the lake. The decline in *Trapa* beds from autumn to spring resulted in increased dissolved oxygen concentration, chlorophyll *a* concentration, and invertebrate abundance (Chironomidae, Oligochaeta and Calanoida), as well as a decrease in taxa utilizing the dense *Trapa* beds. This study suggests how the phenology of dense *Trapa* beds can drastically change the seasonal dynamics of physicochemical conditions and lower the components of food web in a shallow lake ecosystem (Kato et al., 2016).

N_2 production from denitrification was extremely high (37-71 mmol N m^{-2} $d\text{-}^{1}$) in the beds with invasive floating-leaved plants (*T. natans*) but was insignificant in submersed native vegetation (*Vallisneria Americana*) in the tidal Hudson River. An estimate of summertime N_2 production in *Trapa* beds, based on continuous measurement of oxygen and temperature by moored sondes, suggested that these beds were a major seasonal hot spot for nitrogen removal. This study showed that even though the large *Trapa* beds represented only 2.7% of the total area of the tidal Hudson, they removed between 70% and 100% of the total N contained in this river reach during summer month (Tall et al., 2011).

Policies and Laws Associated with Invasive *Trapa*

The current state of the law regarding invasive species is an amalgam of disparate state regulations; some of which are created by Congress and federal regulatory agencies (Stuart, 2012). US Constitution does not explicitly mention invasive species. However, it grants Congress authority to legislate, deliver appropriate funds, and authorize federal agencies to take action and issue rules and regulations related to invasive species. As a result, the executive branch can create policy, guidelines, and programs related to invasive species (Rodriguez & Burgiel, 2020). The United States Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is the primary agency responsible for protecting U.S. plant and animal resources from invasive pests and diseases. Since its establishment in 1972, a large part of APHIS's mission has been to protect commercial crops and native ecosystems in the United States from invasive species (Stewart & Schenewerk, 2004).

The threat of Alien Invasive Species (AIS) has also been recognized globally. 150 government leaders from different countries have adopted the Convention on Biological Diversity (CBD) to address this growing concern (SCBD, 2000). The CBD was approved in December 1992 during the Rio Earth Summit and obliges signatory countries to prevent biological invasions and develop countermeasures in their territories (UNEP, 1993) (Beric & Macissac, 2015). Authorities for aquatic invasive species include the Nonindigenous Aquatic Nuisance Prevention and Control Act of 1990 (NANPCA), as amended by the National Invasive Species Act of 1996 (NISA), and the River and Harbor Act of 1958, as amended by the Water Resources Reform and Development Act of 2014.

NANPCA created the Aquatic Nuisance Species (ANS) Task Force (known as ANSTF). It is charged with developing and implementing a program for waters of the United States ‘‘to prevent the introduction and dispersal of aquatic nuisance species; to monitor, control, and study such species; and to disseminate related information’’ (16 USC §4721) (Rodriguz and Burgiel, 2020).

The National Invasive Species Management Plan (NISC, 2008) defines four phases for the management of invasive species: prevention, early detection & rapid assessment and rapid response (EDRR), control and management, and restoration. Prevention involves identifying potential threats before invasives are introduced (Kolar and Lodge, 2002), and making investments to keep them out.

The Wildlife Society (TWS) is an international non-profit organization founded in 1937. The headquarter is located in Bethesda, MD which is involved in wildlife conservation through science and education. TWS supports the state and federal legislation that address the importation, transportation and mitigation of invasive plants and animals (www.wildlife.org).

Currently, Water Chestnut (*T. natans*) is considered as a noxious weed in 35 states of the US. In these states, it is illegal to propagate, sell or transport this weed. Water Chestnut is not regulated in the Commonwealth of Virginia but is listed as an occasionally invasive species. In Maryland, Water Chestnut is listed as a noxious weed, and sale is not permitted. However, Water Chestnut is not regulated in the nearby District of Colombia (DC) (Naylor, 2003).

Statement of Problem

If *Trapa bispinosa* is allowed to establish in ponds, it could soon spread back to tidal waters and thereby create an epic control challenge that would undo past decades of successful eradication and undermine more recent estuarine water quality improvements. Thus, continuous monitoring and early detection and rapid response (EDRR) is needed to control its invasion. In addition, the phenology of *Trapa bispinosa* in the US has not been studied in details and the assumptions are made with the reference to its closest relative *Trapa natans*. Thus, it is important to study the vegetative and reproductive phenology such as flowering and fruiting season in order to successfully control its spread as *Trapa natans* seeds are viable in the sediment up to 10 years. In addition, it is important to document how *Trapa bispinosa* affects the water quality and *vice versa* for the successful implementation of management options.

VEGETATIVE AND REPRODUCTIVE PHENOLOGY OF TRAPA BISPINOSA IN SELECTED PONDS OF FAIRFAX COUNTY, VIRGINIA

Introduction

Water Chestnut (*Trapa bispinosa*) was first noticed in tidal Potomac River in Pohick Bay in 2014 (Chorak et al., 2019 and Dodd et al., 2019). Another species of Water Chestnut (*Trapa natans*) was historically found in Potomac River and currently it occurs broadly in the northeastern U. S. *T. bispinosa* has pink flowers and two horned fruit whereas *T natans* has white flowers and four-horned fruit. Currently, *T bispinosa* has been verified at 68 locations in Virginia since 1995 to 2019 (Pfingsten and Rybicki, 2020) and is spreading rapidly. Dense population of *Trapa* create a canopy that interrupts the passage of light through water (Tall et al., 2011; Groth et al., 1996) which inhibits photosynthesis and prevents oxygenation in the deeper layer and may kill fish (Strayer, 2010). In addition, it shades out and impedes the growth of other macrophytes (Markovic et al., 2015); displaces native species (Strayer et al., 2003; Hummel and Kiviat, 2004) and poses problems in recreational waters (Markovic et al., 2015). Intensive decay of its fruit increases eutrophication and deteriorates water quality (Borojevic, 2009). The spiny fruit of *Trapa* can pierce the feet and poses danger to public (Chorak et al., 2019; Dodd et al., 2019; Kaufman and Kaufman, 2007; Hummel and Kiviat, 2004).

The purpose of this chapter is to describe the vegetative development, rosette size, flowering, and fruit phenology of *Trapa bispinosa* found in some of the ponds of Fairfax County, Virginia, USA and to examine the interrelations among water quality, sampling months, herbicide use and *Trapa* phenology. Thus, five ponds were selected for the study in 2019 and six ponds in 2020. Among these ponds, some were in the jurisdiction of the Fairfax County Storm water (FFXSW) division and were managed with herbicide by FFXSW. The others were private or regional park authority ponds, so were not treated at all. This chapter provides a description of study area, herbicide information, frequency of sampling, results, discussion, and conclusions based on the study of *T. bispinosa* pond coverage and density. It also describes the extent of coverage in the quadrat used in sampling, rosette size, and the counts of flowers and fruits measured weekly or bi-weekly. Finally, it describes the interrelation between the phenology with water quality variables (WQ). This study was conducted from June to October 2019 and April to November 2020.

The hypotheses of this chapter are:

1. In ponds free from herbicide treatment, *Trapa bispinosa* will follow a predictable and rapid pattern of growth and reproduction dominating the shallow water habitat, in some cases the whole pond, by early to midsummer and remain in place until fall.
2. In ponds that are subjected to herbicide, *Trapa bispinosa* will follow a more erratic pattern of growth and reproduction that can be related generally or in some cases specifically to the application of herbicides.

Materials and Methods

1. Study Area

The study area was located in the western part of Fairfax County, Virginia (Fig. 7 and Fig. 8). A total of 8 ponds were sampled over the two-year study (2019 and 2020). Some had a history of herbicide treatment and others had no known treatment history.

Table 1. Description of Study Area.

Pond Name	Treated (Yes/No)	Latitude (°)	Longitude (°)	Area (ha)	Jurisdiction	Period of Study	Frequency of Observation
VGA-VA	No	38.82867	-77.40161	0.50	Private	2019/2020	weekly
HP-VA	No	38.83673	-77.42897	0.05	Private	2019/2020	bi-weekly
WP-VA	No	38.87362	-77.33995	0.64	FCPA	2019	bi-weekly
HO-VA	No	38.76963	-77.40743	0.18	NVRPA	2019	bi-weekly
ML-VA	Yes	38.84241	-77.39618	0.76	FFXSW	2019/2020	bi-weekly
GCP-VA(A)	Yes	38.8545	-77.35328	0.39	FFXSW	2020	bi-weekly
GCP-VA(A)	Yes	38.85495	-77.35157	0.37	FFXSW	2020	bi-weekly
WL-VA	Yes	38.84416	-77.41006	0.84	FFXSW	2020	bi-weekly

(Note: FCPA- Fairfax County Park Authority, NVRPA-Northern Virginia Regional Park Authority, FFXSW- Fairfax County Stormwater)

A. Untreated Ponds (Ponds 1-4)

1. Virginia Golf Academy (VGA-VA)

VGA-VA is located in 5801 Clifton Road, Clifton, VA, 20124 adjacent to a driving range (Figs.7 and 8). The pond water looked clear, and the vegetation was dominated by *Trapa*, with some water primrose (*Ludwigia repens*), water buttercup (*Ranunculus aquatilis*) and grasses. The shore had some grasses, sage grass (*Andropogon virginicus*) and blackberry shrubs. There were frogs (*Anura*) and tadpoles, dragonflies (*Anisoptera*) inside the pond and some birds such as great blue heron (*Ardea Herodias*), green heron (*Butorides virescens*) and northern cardinal (*Cardinalis cardinalis*) spotted sometimes in the surroundings (Table 1). No geese were ever observed at this pond.

2. H-Mart Pond (HP-VA)

HP-VA is located in 13818 Braddock Rd, Centerville, VA 20121 adjacent to the shopping complex of H-mart and commercial buildings (Fig. 7 and 8). This pond is a favorite place for Canada geese (*Branta canadensis*), which were frequently spotted around the site during the sampling period. According to a local resident, 3 mallards and a pair of geese stay all year, and many resident Canadian geese were spotted all summer in 2019. The pond and its shore were littered with goose feces. There was moderate amount of duckweed (*Lemnoideae*) and water silk (*Spirogyra*) inside the pond. Sage grass (*Andropogon virginicus*), knotweed (*Polygonum*) and American water willow grass (*Justicia americana*) were also noticed along the shore. Some frogs (*Anura)* and tadpoles, dragonflies (*Anisoptera*), nematodes, oligochaetes, leeches (*Hirudinea*) were also observed during sampling (Table 1).

3. Waples Mill Pond (WP-VA)

WP-VA is located in Waples Mill Meadow Park, Oakton, VA 22124 (Figs. 7 and 8). This pond is surrounded by mostly shrubs and grasses. During our sampling, deers (*Cervidae*) were sometimes grazing nearby, and Canada geese (*Branta canadensis*) were also noticed inside the pond. Some of the other plants inside the pond were carnivorous bladderwort (*Utricularia*), little duckweed (*Lemnoideae*) and water primrose (*Ludwigia repens*) (Table 1).

4. Hemlock Overlook Regional Park (HO-VA)

HO-VA is located in 13220 Yates Ford Rd, Clifton, VA 20124 on the grounds of Hemlock Overlook Regional Park and the pond is fringed with overhanging trees (Figs. 7 and 8). There were lilies (*Lilium*), cattails (*Typha*), stinging nettle (*Urtica dioica*) and grasses around the shore (Table 1).

B. Treated Ponds (5-7)

The following ponds had been subjected to herbicide treatment conducted by Solitude Lake Management and under contract from Fairfax County.

5. Myrtle Leaf Pond (ML-VA)

ML-VA is located in 5130 Myrtle Leaf Drive, Fairfax, VA 22030 near a residential complex in Fairfax (Figs. 7 and 8). The water looked clear with some *Trapa* growth during the peak season accompanied by creeping primrose-willow (*Ludwigia repens*), Carolina fanwort (*Cabomba caroliniana*), waterthyme (*Hydrilla verticillata*) and water lilly (*Nymphaea*). The shore of ML-VA had yellow water lilly (*Nuphar lutea*), arrow arum (*Peltandra virginica*), iris violet (*Iris versicolor*) and sage grass (*Andropogon virginicus)* around.

ML-VA was treated with herbicide (Flumioxazin) in 2017, 2018 and 2020. In 2017, ML-VA was treated partially on August 1st and again treated for the remainder of the lake on September 13th. In 2018, it was treated on June 29th which resulted in 100% control for few weeks on September 21st again the regrowth was treated. In 2019, it was not treated. In 2020, it was treated only once on July 29th (Tables 1 and 2). Thus, this pond had only small, isolated patches of *Trapa* near the shore. *Trapa* died abruptly in 2020 and the next batch of *Trapa* grew in a different location, which was also used during the sampling process.

6. Government Center Pond (GCP-VA(A) and GCP-VA(B))

GCP-VA(A) and GCP-VA(B) are located adjacent to 11851 Monument Drive Fairfax, VA 22033. These two sites are located close to each other between the residential complex and the Fairfax Government center and connected by a small creek: with GCP-VA(A) being upstream and GCP-VA(B) being downstream (Figs. 7 and 8,

Table 1). The water of GCP-VA(A) was clear and there was no other noticeable vegetation. There were schools of fish observed during the sampling procedures. The water of GCP-VA(B) was murky and had unknown yellowish color. There were tadpoles, dragonflies and duckweeds noticed in this pond.

GCP-VA (A) was treated with herbicide (Flumioxazin) once every year in 2015, 2016, 2017, 2018 and 2020 resulting in 100% control right after the treatment. GCP-VA (B) was treated twice in 2014 and once in 2016. In 2017, it was treated on August 1^{st} to achieve partial control and again on August 31^{st}. In 2020, it was treated on September 15^{th} resulting in partial control and was retreated on October 5^{th} (Tables 1 and 2).

7. Wood Lilly Pond (WL-VA)

WL-VA is located near 13431 Wood Lily Lane, Centerville, VA 20120 in the vicinity of a residential area (Figs. 7 and 8). The pond looks like an extended wetland, which has *Trapa* in only one portion. 45% of that portion of the pond was covered by *Trapa* and the remaining 55% was covered by Water Lilly (*Nymphaeaceae*) during the peak growing season. During the sampling period, water was very murky and had an unknown yellowish color. There were tall sage grasses (*Andropogon virginicus),* sedges (*Cyperaceae*) and other types of grasses on the shore making it difficult to access. WL-VA was treated with herbicide (Flumioxazin) once in mid-September 2020 for *Trapa* removal (Tables 1 and 2).

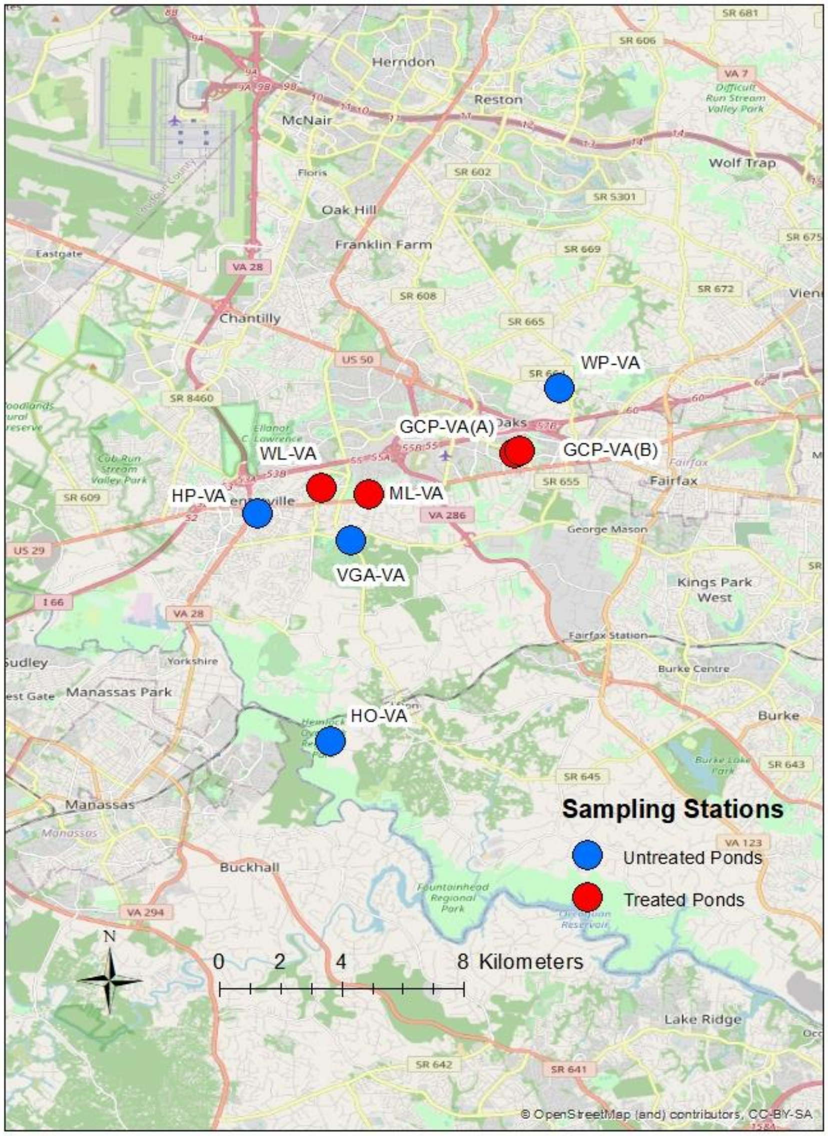


Fig. 7. Study Area showing Untreated and Treated Ponds in *T. bispinosa* Research (2019 and 2020).

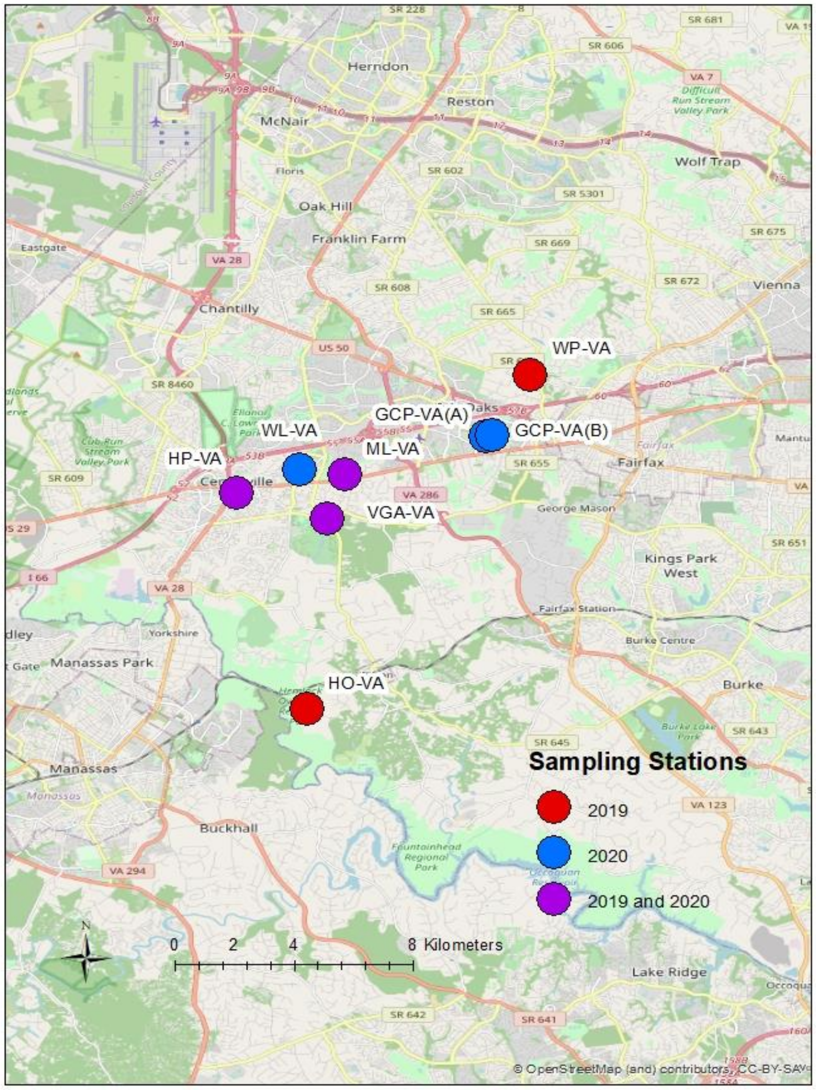


Fig. 8. Study Area showing the date of the study for each pond in *T. bispinosa* research (2019 and 2020).

Management Procedures to control *Trapa bispinosa*

Fairfax County Stormwater Division is responsible for the maintenance of these ponds. Solitude.org has been under contract for the maintenance of these ponds since 2020. The information about herbicides, amount, location and the time of the treatment is listed in Table 2 below. Solitude plans to start the herbicide treatment as early as June and no later than mid- September when *Trapa* fruits start to mature but sometimes it is not that precise (Source: Solitude.org).

Clipper, Schooner and Red Eagle herbicide are the trade names for water dispersible granule containing 51% active ingredient of Flumioxazin. Clipper herbicide is very rapidly absorbed by target plants and breaks down rapidly in water with a pH greater than 8. pH of water surrounding mats of submersed vegetation can exceed 8.5 by early to mid-day due to the photosynthetic process. Application of Clipper herbicide under these conditions may only provide partial weed control and rapid regrowth is likely. Thus, it should be applied in the early morning to actively growing aquatic weeds and early in the season before surface matting occurs (www.epa.gov).

Schooner is a fast-acting contact herbicide that controls selected submersed, emergent, and floating aquatic weeds. It is most effective when applied to young, actively growing weeds with a pH less than 8.5. Red Eagle is used to control weeds in crop fields such as alfalfa, artichoke, asparagus, bushberries, cabbage, cactus, cucurbit vegetables and many others as well as aquatic environment (www.epa.gov).

L1700 is a soy-oil derived, non-ionic penetrating surfactant that reduces off-target spray drift and reduces spray water pH. Unique formulation technology and quality ingredients separate L1700 from the imitators (www.lovelandproducts.com).

Cide-Kick II is a wetting agent, sticker, activator and penetrant all in one. It is a low viscosity oil which helps break down the waxy cuticle on the leaf surface and helps penetrate the bud and bark area (of the woody brush), allowing a more effective uptake of herbicide (Cide-Kick II, Specimen Label- Brewer International).

Methylated Seed Oil (MSL) is a spray adjuvant for post-emergence applications with herbicides. In post emergence herbicide spray mixture of this product may be used as a replacement for non-ionic surfactant and crop oil (petroleum oil) (Specimen Label- Southern Agricultural Insecticides, Inc.,).

Tribune is a nonvolatile herbicidal chemical which contains active ingredient of diquat dibromide and pyrazinedium dibromide as 37.3% and other ingredients as 62.7%. Diquat herbicide consists of active ingredient of diquat dibromide and pyrazinedium dibromide as 37.3% and another ingredient as 62.7%. It is used to control weeds in aquatic areas, noncrop or nonplanted areas on farms and landscapes (Specimen Label - Syngenta Crop Protection, LLC.).

Table 2. Herbicide Treatment for *Trapa* Removal (Source: Solitude Lake Management, 2021).

Pond	Date	Product 1	Rate	Product 2	Rate	Product 3	Rate	Notes
ML-VA	8/1/2017	Schooner	8 oz	L1700	2 oz			Partial Treatment
ML-VA	9/13/2017	Schooner	1.5 lb	L1700	4 oz			Treated remainder of lake
ML-VA	6/29/2018	Schooner	12 oz	L1700	4 oz	Tribune	0.5gal	Achieved 100% control
ML-VA	9/21/2018	Schooner	2 lb	MSO	6 oz			Treatment of regrowth
ML-VA	7/29/2020	Red Eagle	6 oz					Spot treatment for *Trapa* removal
GCP-VA(A)	6/30/2015	Clipper	2 lb	L1700	12 oz			Achieved 100% control
GCP-VA(A)	7/27/2016	Schooner	2 lb	L1700	10 oz			
GCP-VA(A)	8/31/2017	Schooner	1 lb	L1700	4 oz			Achieved 100% control
GCP-VA(A)	9/21/2018	Schooner	10 oz	MSO	3 oz			Follow up to Procella COR treatment, achieved 100% control
GCP-VA(A)	10/5/2020	Red Eagle	16 oz	L1700	4 oz			Achieved 100% control
GCP-VA(B)	9/30/2014	Clipper	2 lb	Diquat	0.25gal	L1700	3 oz	Achieved partial control
GCP-VA(B)	10/22/2014	Clipper	1 lb	Cidekick	4 oz			Retreatment
GCP-VA(B)	8/23/2016	Schooner	38 oz	L1700	2 oz			Achieved 100% control
GCP-VA(B)	8/1/2017	Schooner	8 oz	L1700	2 oz			Achieved partial control

GCP-VA(B)	8/31/2017	Schooner	16 oz	L1700	2 oz			
GCP-VA(B)	9/15/2020	Red Eagle	32 oz					Achieved partial control
GCP-VA(B)	10/5/2020	Red Eagle	16 oz					Retreatment
WL-VA	9/15/2020	Red Eagle	24 oz	L1700	8 oz			

(Note: Products 1, 2 and 3 are the trade names of herbicide (Flumioxazin), Product 2- L1700, MSO, Diquat, Cidekick and Product 3-Tribune and L1700 used for *Trapa* removal)

2. Sampling Methods

1. Frequency

Sites VGA-VA, HP-VA, ML-VA, WP-VA and HO-VA were sampled in 2019. In 2020, sites VGA-VA, HP-VA and ML-VA were sampled again. Some of the newer sites GCP-VA(A), GCP-VA(B) and WL-VA were added in 2020 and WP-VA and HO-VA were dropped off due to sampling inconvenience and other limitations. VGA-VA was sampled weekly whereas the rest of the sites were sampled bi-weekly in both years. In 2019, the sampling period was from early June until late October. In 2020, the sampling period was from late April until early November. VGA-VA was visited frequently from February to April to note the onset of *Trapa* in 2020.

2. Percentage Pond Coverage of *Trapa* and Density Class

Upon arrival at the study site, a digital picture of the whole pond was taken, and the study area was sketched noting the % of water body with vegetation. *Trapa* pond coverage and density class were averaged by month at each site and represented as percentage Pond coverage of *Trapa* and Density class (Table 3-6). The density of *Trapa* rosettes within the area occupied by *Trapa* over the whole pond was assessed and classified as follows:

Class 1-very sparse with canopy cover <10%

Class 2-10-40% of canopy cover

Class 3- 40-70% of canopy cover

Class 4 >70% of canopy cover

3. Quadrat Study

Three locations (A, B, C) were chosen either along a particular stretch of the shoreline or scattered around the perimeter and three replicate quadrats were sampled at each location. These locations were noted on the pond sketch. Efforts were made to sample the same general area during each pond visit. Coverage of *Trapa* was assessed by using 1 m^2 quadrat divided into 100 (10 by 10) cells (Fig. 9). Cells with any *Trapa* leaves present were noted and tallied to get a number from 0 to 100 (the total number of cells) for each quadrat. This was recorded as *Trapa* quadrat coverage. Total number of *Trapa* flowers and other SAV present were also determined for each quadrat. The data were processed using excel and average percentage of *Trapa* in each quadrat were calculated. *Trapa* quadrat coverage at all sampling stations in 2019 and 2020 are represented in terms of percentage. The quadrat study quantified the density of rosettes within the areas of colonization and was a more rigorous measure of density than the 1-4 class ratings above.

Fig. 9. Quadrat sampling of *Trapa* (2019 and 2020).

4. Phenological Study

To study the phenology of *T. bispinosa*, 3 representative rosettes were chosen randomly from each quadrat, making the total number of 9 (n=9). In all the treated ponds, the rosettes sampled were occasionally fewer than 9 (n*<9) due to scanty coverage of *Trapa* and difficulties to access. Any debris on the rosette was rinsed and the plants were placed in Ziploc bags. The Ziploc bags were brought back to the lab in 2019 for analysis. In 2020, due to the COVID circumstances, strict social distancing was practiced during sampling procedures and the plants were brought back home and further analysis was done in the backyard (Fig. 10). The color of the leaves on both sides were noted. The diameter of individual rosettes was measured using a ruler and tabulated. The % of 9 rosettes that were fruiting, and flowering were assessed and recorded. The flowers were counted including flower buds and recently submerged flowers without petals. Flower petal colors and the number of spines in the fruit were also noted and the presence of any of the reproductive structures such as flower bud, immature (<30mm in size) or mature fruit (>30 mm in size) was tabulated in excel. If there were any broken stubs of the fruits, it was also counted as a mature fruit during tabulation. Reproductive phenology was also noted. This was based on the condition that if a plant had either bud, flower or fruit, it was considered "reproductive" and the percentage of the 9 rosettes that were reproductive was calculated by date. Similarly, the number of flowers, petal colors and fruits per rosette were counted and average of the 9 rosettes was tabulated by date. The data was processed using Microsoft Excel by averaging and graphical analysis was done by using Sigma plot 14.5.

Fig. 10. Photos show two representative rosettes of *Trapa*.

<u>5. Correlation Analysis Between Phenological and Water Quality (WQ) Variables</u>

The correlation among the different phenological attributes; *Trapa* Pond Coverage (TPC), *Trapa* Quadrat Coverage (TQC), Flowers per Quadrat (FLQ), Average Rosette Diameter (ARD), Rosette Reproductive Phenology (RRP), Flowers per Rosette (FLPR), and Fruits per Rosette (FRPR) were analyzed using SYSTAT. The correlations of the phenology attributes of *Trapa* and Water Quality (WQ) of the ponds were also analyzed using SYSTAT.

Results

1. *Trapa* Pond Coverage (TPC) and Density Class

A. Untreated Ponds

Trapa Pond Coverage (TPC) in terms of percentage was estimated by visual observation (Tables 3 and 4). In 2019, sampling started in June and by that time TPC was already 80% with the (density class-4) in VGA-VA. In 2020, the onset of *Trapa* was observed on April 23 in VGA-VA.

TPC was not greater than 1% in April, reached its maximum in August in both years for three of the four untreated sites, except HP-VA and decreased afterwards. Maximum and minimum density seemed more variable among the sites and was 2 or less in April and May at two untreated sites sampled, increased to the maximum of 4 (100%) in summer and decreased from maximum of 3 or 4 in mid-summer to 2 or 3 in October in untreated sites.

Table 3. *Trapa* Pond coverage (TPC%) of pond Surface Area and Density class estimated by visual observation (VGA-VA and HP-VA, 2019 and 2020).

Site	Month	*Trapa* Pond coverage (TPC% 2019)	Density class	*Trapa* Pond coverage (TPC% 2020)	Density class
VGA-VA	April	-	-	0.5	1
VGA-VA	May	-	-	35	2
VGA-VA	June	80	4	65	4
VGA-VA	July	80	4	75	4
VGA-VA	August	85	4	85	4
VGA-VA	September	80	3	80	3
VGA-VA	October	70	2	80	3
HP-VA	April	-	-	1	1
HP-VA	May	-	-	40	2
HP-VA	June	100	4	60	3
HP-VA	July	100	4	95	4
HP-VA	August	95	3	95	4
HP-VA	September	95	2	85	4
HP-VA	October	90	2	85	3

Table 4. *Trapa* Pond coverage (TPC %) of pond Surface Area and Density class estimated by visual observation (WP-VA and HO-VA, 2019 and 2020).

Site	Month	*Trapa* Pond coverage (TPC% 2019)	Density class	Site	*Trapa* Pond coverage (TPC% 2019)	Density class
WP-VA	June	75	4	HO-VA	25	4
WP-VA	July	80	4	HO-VA	20	4
WP-VA	August	85	3	HO-VA	30	3
WP-VA	September	75	2	HO-VA	25	2
WP-VA	October	65	2	HO-VA	20	2

B. Treated Ponds

In treated ponds, *Trapa* did not appear until May or June. In some cases, coverage remained low (less than 5%) while in two ponds (WL-VA and GCP-VA (B)), it increased to higher levels but was not as stable at those levels as in the untreated ponds (Tables 5 and 6).

In 2020, in ML-VA, *Trapa* grew sporadically for a short period. ML- VA was not treated with herbicide in 2019. In 2019, TPC was less than 1% and consistent during the entire sampling period, whereas the density was maximum 4 (100%) during summer which declined to 3 and 2 in September and October. In 2020, the maximum coverage was less than 0.5% and the density was inconsistent.

In GCP-VA (B), *Trapa* appeared in June with the coverage of 20%, peaked in August with 95% of TPC and gradually declined through November. It had the maximum density of 4 (100%) through June and July and decreased in late summer into 3 and 2 in the fall. In WL-VA, *Trapa* appeared in May with the coverage of 30%, peaked in July with 45% and gradually decreased afterwards. The density remained at maximum 4 (100%) from May through August and declined to 1 in September. In WL-VA only a portion of the pond was covered with *Trapa*. *Trapa* covered 45 % of the pond while the remaining 55% of the pond was covered with water lily (*Nymphaeaceae*).

In GCP-VA(A), TPC was 5% and density was 4. *Trapa* declined completely in September, which was prior to the herbicide application on October 5th (Table 2 and 6). In the ponds that received herbicide, rosettes turned brownish in color and remained decayed for about two weeks after the herbicide application and finally disappeared. In WL-VA and GCP-VA (B), *Trapa* plants were very robust and green until the herbicide was applied.

Table 5. *Trapa* coverage (%) of Pond Surface Area and Density class estimated by visual observation in Treated Pond (ML-VA, 2019 and 2020).

Site	Month	*Trapa* Pond coverage (TPC % 2019)	Density class	*Trapa* Pond coverage (TPC % 2020)	Density Class
ML-VA	April	-	-	-	-
ML-VA	May	-	-	0.1	2
ML-VA	June	-	4	0.5	3
ML-VA	July	0.5	4	0.2	2
ML-VA	August	0.5	4	0.1	1
ML-VA	September	0.5	3	0	0
ML-VA	October	0.5	2	0	0

Table 6. *Trapa* Pond coverage (TPC %) of pond Surface Area and Density class estimated by visual observation in Treated Ponds (GCP-VA (A and B) and WL-VA, 2020).

Site	Month	*Trapa* Pond coverage (TPC% 2020)	Density class
GCP-VA(A)	April	0	0
GCP-VA(A)	May	0	0
GCP-VA(A)	June	1	1
GCP-VA(A)	July	3	3
GCP-VA(A)	August	5	4
GCP-VA(A)	September	0	0
GCP-VA(A)	October	0	0
GCP-VA(B)	April	0	0
GCP-VA(B)	May	0	0
GCP-VA(B)	June	20	4
GCP-VA(B)	July	70	4
GCP-VA(B)	August	95	3
GCP-VA(B)	September	60	2
GCP-VA(B)	October	45	2
GCP-VA(B)	November	10	2
WL-VA	April	0	0
WL-VA	May	30	4
WL-VA	June	30	4
WL-VA	July	45	4
WL-VA	August	40	4
WL-VA	September	20	1
WL-VA	October	0	0

2. *Trapa* Quadrat Coverage (TQC)

A. Untreated Ponds

Among the untreated ponds, the pattern of TQC was similar from June through the end of September in both years with some exception. In 2020, TQC increased steadily from 0% in April to 100% by early June. In VGA-VA, from early June through early October, the coverage was 100% in both years (Fig. 11). In HP-VA, from mid-June until early September, the coverage was 100% in both years. In 2020 in VGA-VA, *Trapa* continuously increased from late April until early June whereas in HP-VA the increase was rather erratic (Fig. 11, A and B). The decline in *Trapa* started in September in 2019 in VGA-VA and both years in HP-VA whereas it started in October in 2020 in VGA-VA. WP-VA and HO-VA were sampled only in 2019. In WP-VA and HO-VA, TQC started to increase from mid-June until early August and started to decline until September (Fig. 11C).

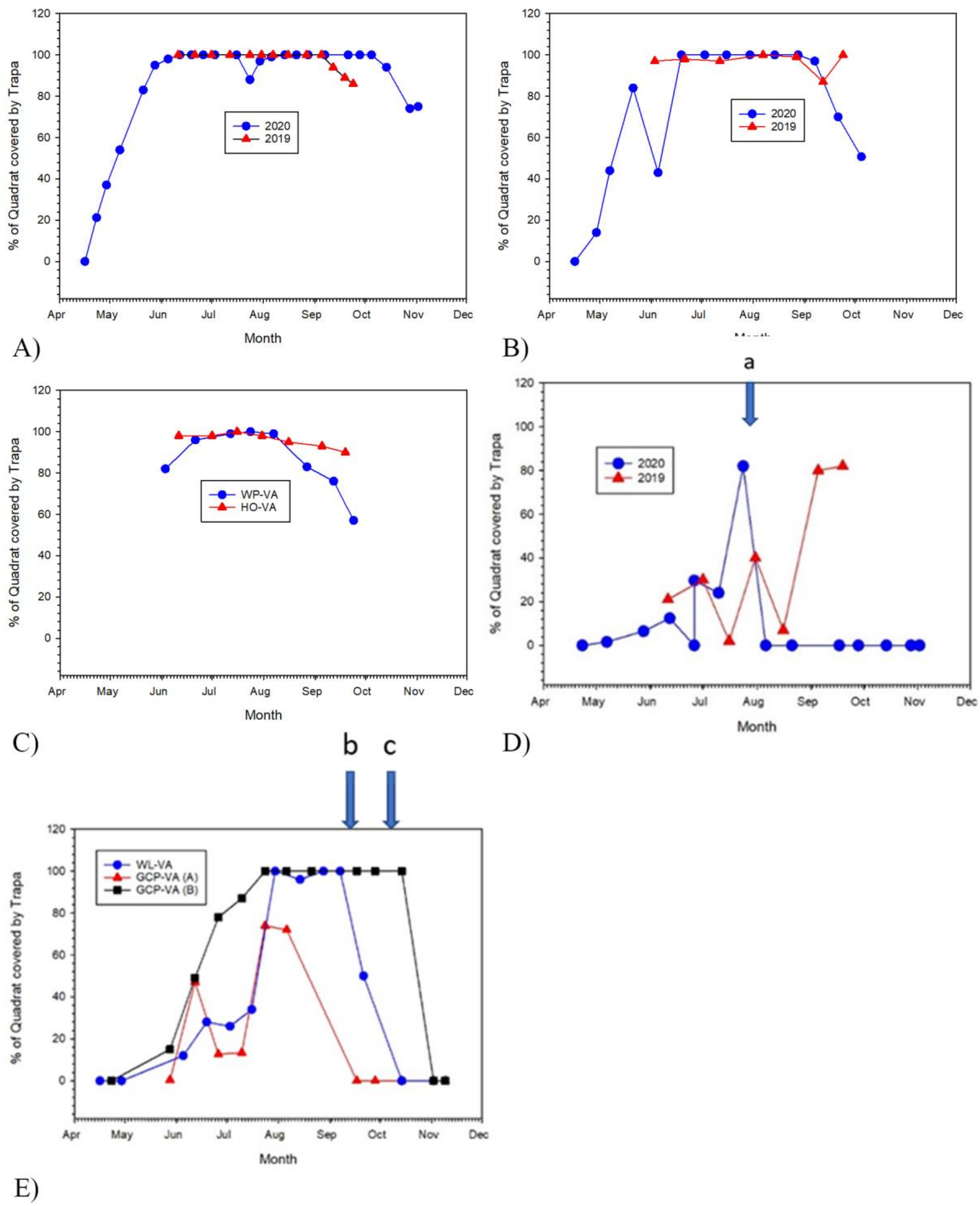


Fig. 11. A-E show *Trapa* Quadrat Coverage (TQC) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively.

(Note: a, b and c= Herbicide Treatment Codes, a= July 29 at ML-VA, b= Sept 15 at WL-VA and GCP-VA(B), c=Oct 5 at GCP-VA (A and B))

B. Treated Ponds

Among the treated ponds, in ML-VA, *Trapa* was only sporadically distributed within the pond (Table 6). In 2019 in ML-VA, *Trapa* started to increase from mid-June followed by erratic pattern of increase and decrease and finally increasing up to 80% in September. In 2020, few *Trapa* were growing from early May followed by abrupt death during the end of June. The death of some *Trapa* was before the application of herbicide on July 29th (Table 2). A new set of *Trapa* was growing in a different location of pond from early June which were dead by the first week of August due to herbicide application on July 29th (Table 2, Fig. 11D).

In GCP-VA (A), *Trapa* increased in mid- June, declined in July, increased in August and again declined completely in late August. The *Trapa* death was prior to the herbicide application on October 5th (Table 2). This could be due to the effect of yearly herbicide treatment from 2015 to 2018 (Table 2, Fig. 11E).

In WL-VA, GCP-VA(A) and GCP-VA(B), *Trapa* started to appear in the first week of June. In WL-VA, *Trapa* generally increased upto 100 % by the end of August but *Trapa* turned brown in color, decreased in September and completely disappered during early October. This was probably because of the herbicide application in mid-September (Table 2, Fig. 11E).

In GCP-VA(B), *Trapa* generally increased to 100 % during the end of July which remained 100% for an extended period until late October. After late October, *Trapa* was dead due the herbicide application on September 15th and October 5th (Table 2). Even though there was not a complete death of *Trapa*, leaves had changed into brown from green from the mid- September, due to the effect of herbicide (Fig. 11E).

3. Flowers per Quadrat (FPQ)

A. Untreated Ponds

In untreated ponds, flowers started to appear in quadrat samples in June or July. The abundance of flowers generally increased during June, July and September. Among the untreated ponds, the first set of flowers appeared during the end of June in VGA-VA and the average number of flowers per quadrat varied from 1 to 14 through October. There appeared to be some definite peaks, but no consistent pattern in both years. In HP-VA, flowers arrived later and were generally less numerous with the average number of 2 to 6. In WP-VA, the number of flowers varied from 1 to 4 per quadrat and were present from June to September. In HO-VA very few flowers were spotted and only occasionally, perhaps due to shady conditions from overhanging trees (Fig. 12, A-C).

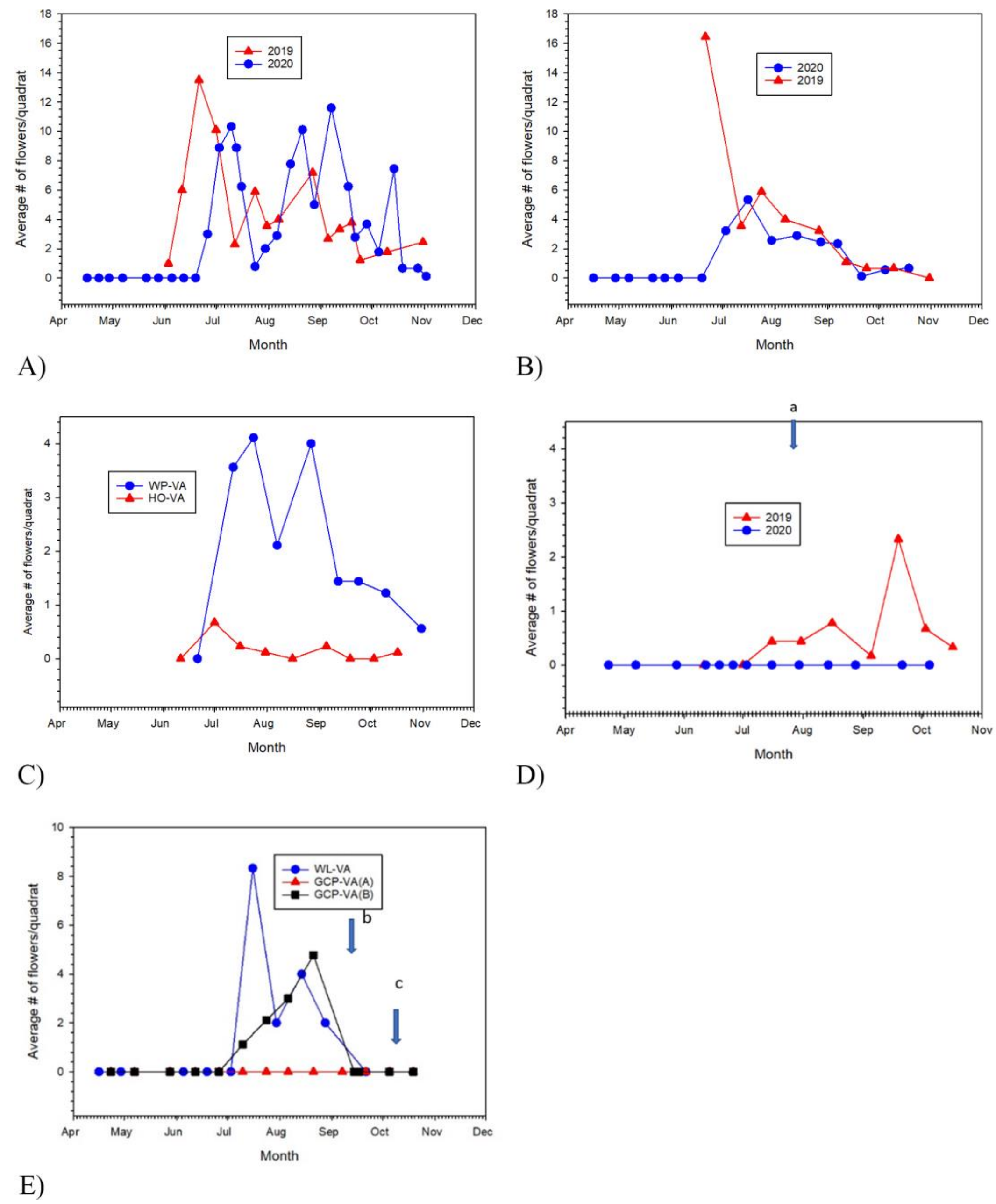


Fig. 12. A-E show Flowers per Quadrat (FPQ) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively.

(Note: a, b and c=Herbicide Treatment Codes, a= July 29 at ML-VA, b= Sept 15 at WL-VA and GCP-VA(B), c=Oct 5 at GCP-VA (A and B))

B. Treated Ponds

Among the treated ponds, ML-VA was treated in 2020 but not in 2019 and WL-VA, GCP-VA(A) and GCP-VA (B) were treated in 2020 (Table 2). In 2020, ML-VA and GCP-VA(A) had very low pond coverage in a portion of pond this year (Tables 5 and 6). The plants were dead by early July 2020 in ML-VA and in late August in GCP-VA(A) without blooming. In treated ponds, there were fewer flowers and the pattern was inconsistent with maximum flowers in July and late August. Since many rosettes died off without reaching maturity, the complete comparision of the flower pattern of untreated and treated ponds was rather difficult. WL-VA and GCP-VA(B) showed an inconsistent pattern of flower no. with maximum no./quadrat being 8 and 4 respectively. No flowers were observed at GCP-VA(A), as *Trapa* died off without reaching its maturity (Fig. 12, D and E).

4. Average Rosette Diameter (ARD)

A. Untreated Ponds

In untreated ponds, *Trapa* leaves were green in color with a pink underside. Average rosette diameter increased steadily from April through September in 2020 and followed a similar pattern in 2019. Rosettes started to decrease in diameter from early October. The average rosette diameter varied between 2- 23 cm. In late April the rosettes were very small (2cm) when *Trapa* just started to grow. In September, the size of the rosette was maximum upto (23cm) when the plant was completely mature (Fig. 13, A-C).

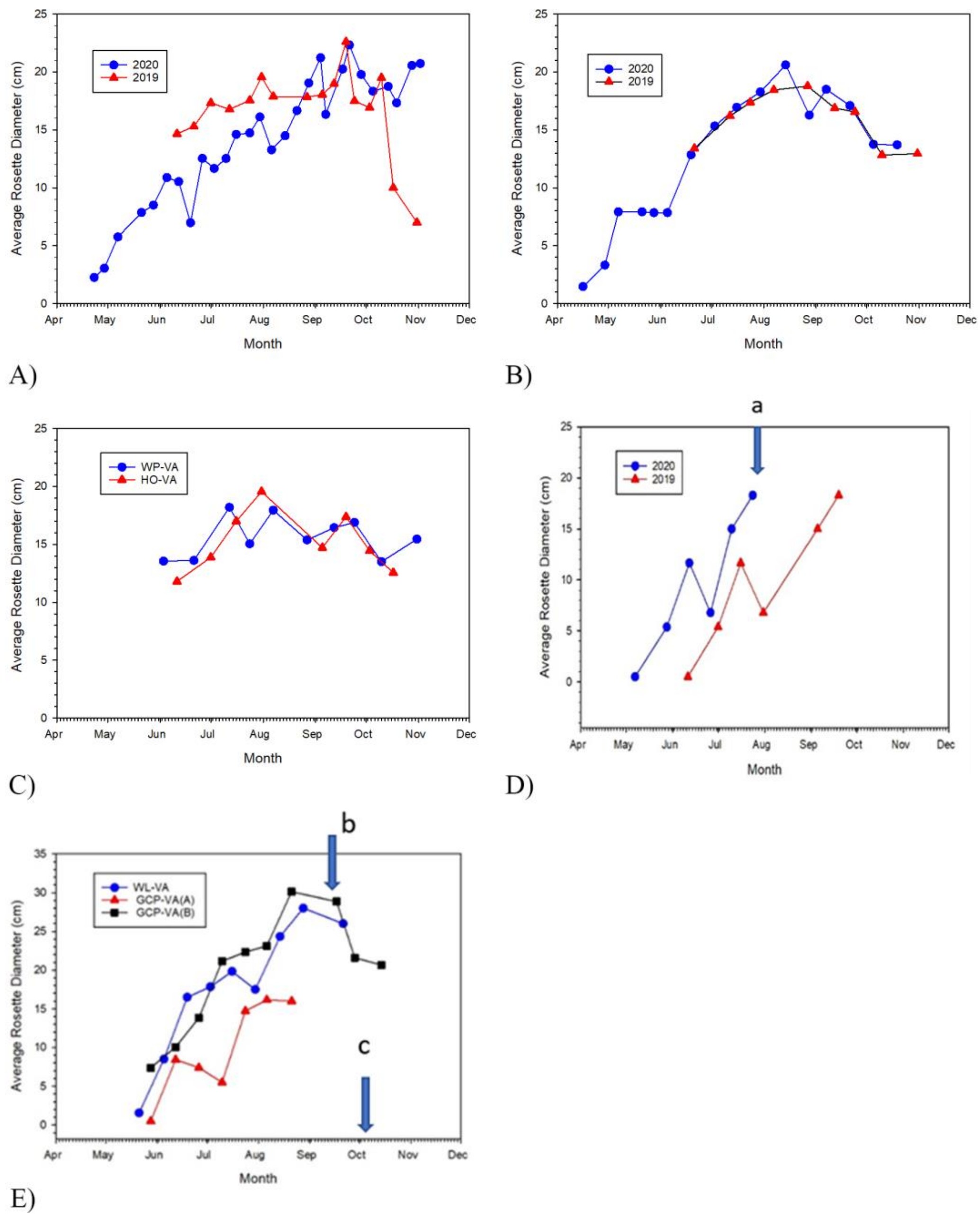


Fig. 13. A-E show Average Rosette Diameter (FPQ) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively.

(Note: a, b and c= Herbicide Treatment Codes, a= July 29 at ML-VA, b= Sept 15 at WL-VA and GCP-VA(B), c=Oct 5 at GCP-VA (A and B))

B. Treated Ponds

Among the treated ponds, *Trapa* leaves were green in color with the pink underside before the herbicide application. The leaves turned brown in color after the herbicide application and stayed for about two weeks and started to disintegrate. In some of the treated ponds, the rosette size followed the same general pattern as in the untreated ponds, consistently increasing from May through August. In ML-VA, the rosettes were very similar in size both in 2019 and 2020 and the size was ~19cm during their peak growth. But *Trapa* was dead after July due to the herbicide application on July 29, 2020 (Table 2, Fig. 13D). Thus, samples could not be collected from August 2020 in ML-VA.

In WL-VA and GCP-VA(B), rosettes were robust with the size ~ 30 cm. The rosette size of *Trapa* of GCP-VA(A) was about half of GCP-VA(B), even though these two sites are located next to each other. The smaller size of the rosette in GCP-VA(A) could be due to the effect of herbicide. In GCP-VA(A) *Trapa* were dead by late August and could not be sampled further which is visible in graph (Fig. 13E).

5. Rosette Reproductive Phenology (RRP)

A. Untreated Ponds

In most of the untreated ponds, RRP (percent of rosette with either flower, fruit or other reproductive parts) started to increase from early June and became 100% after July and remained 100 % until October in both years (Figs. 14, A-C). In VGA-VA, in late October, RRP decreased to 80% (Fig. 14 A).

WP-VA and HO-VA were sampled only in 2019. In WP-VA and HO-VA, RRP was almost similar after August when it reached the peak (100%) followed by a decrease in early September and again increased to 100% until late October. In HO-VA and WP-VA, RRP declined to less than 80% and 90% respectively, in early September (Fig. 14C).

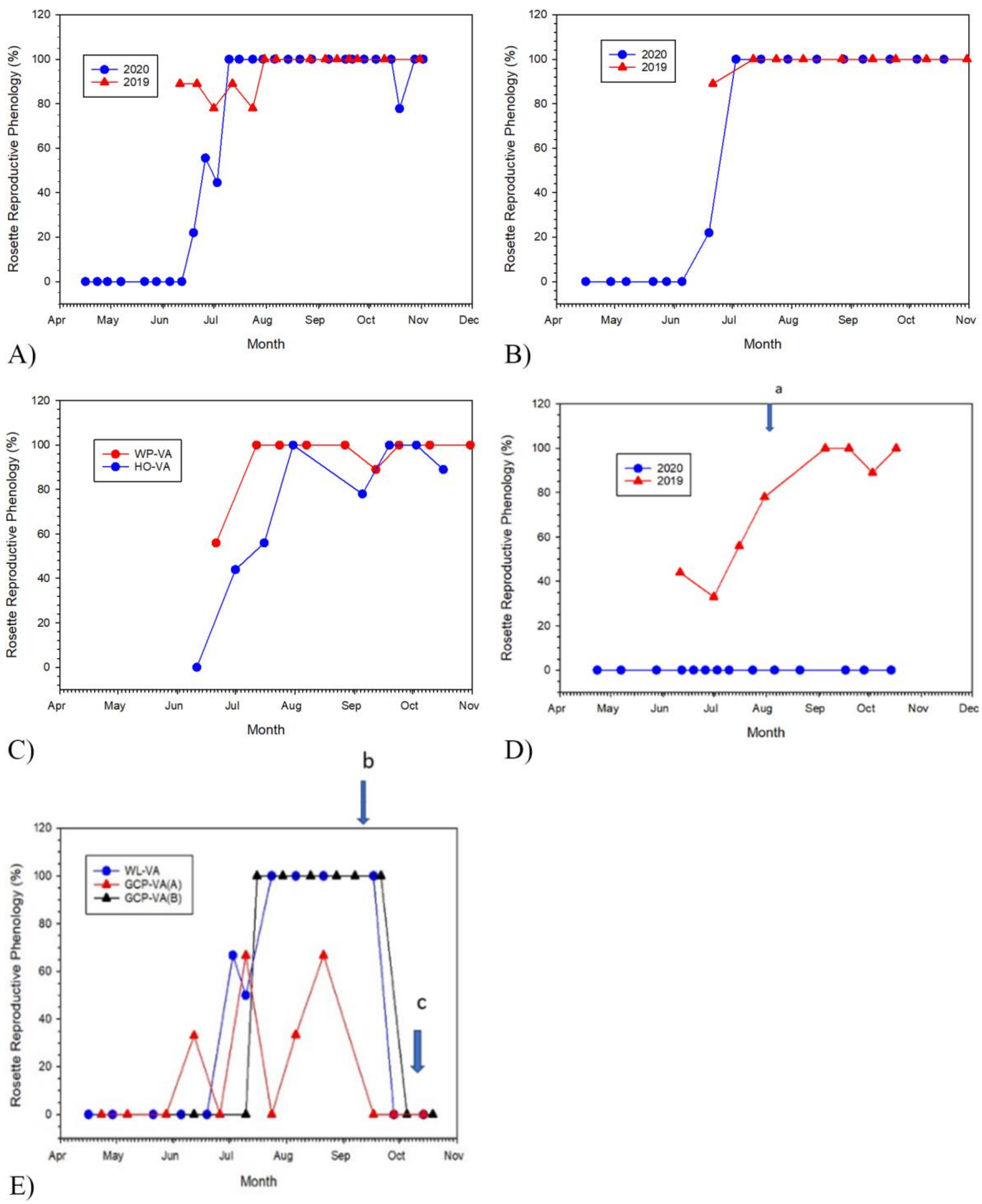


Fig. 14. A-E show Rosette Reproductive Phenology (RRP) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively.

(Note: a, b and c= Herbicide Treatment Codes, a= July 29 at ML-VA, b= Sept 15 at WL-VA and GCP-VA(B), c=Oct 5 at GCP-VA (A and B))

B. Treated Ponds

In ML-VA, in 2019, average RRP was 45% in early June which continued to increase until late September (100%) but decreased afterwards. In 2020, *Trapa* died off very early without reaching maturity due to herbicide application on July 29, 2020, and couldn't be sampled afterwards (Table 2, Fig. 14D).

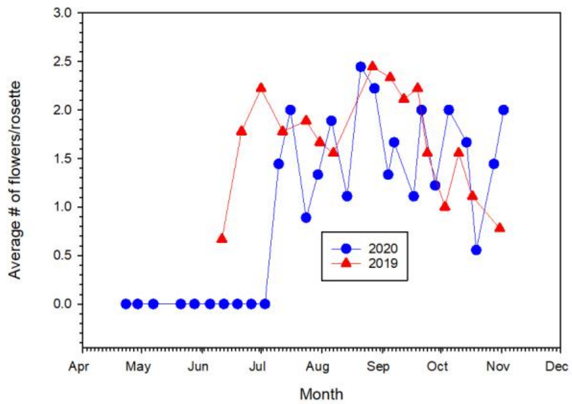
In WL-VA and GCP-VA(B), RRP started to increase from late June and reached 100% from mid-July until late September and started to decline afterwards. *Trapa* was all dead by late September in WL-VA after the herbicide treatment on 15th September 2020 (Table 2, Fig. 14E).

In GCP-VA(A), RRP was 0% until early July, reached its peak (60%) in mid-July and again declined to 0% in early September. The plants were dead by September in GCP-VA(A) and early October in GCP-VA(B) due to herbicide treatment on 15th, September 2020 and 5th October 2020 (Table 2, Fig.14E).

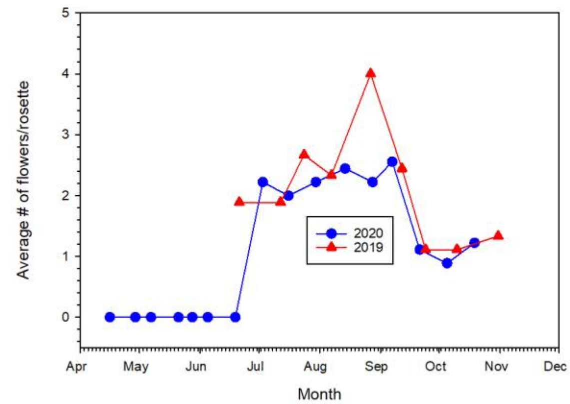
6. Flowers per Rosette (FLPR)

A. Untreated Ponds

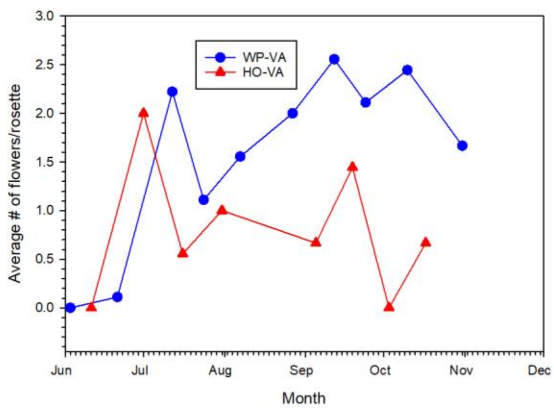
The increase and decrease in rosette flower depended on its life cycle with some of the flowers reaching their maturity and the next batch getting ready. In VGA-VA, flowers appeared in June in 2019 and July in 2020. From flower onset through October, *Trapa* continued to flower at a rate of about 1 to 2 flowers per rosette. A similar pattern was observed at HP-VA, WP-VA and HO-VA (Figs. 15, A-C).



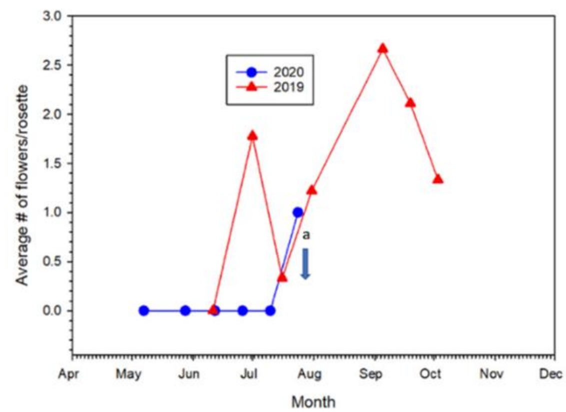
A)



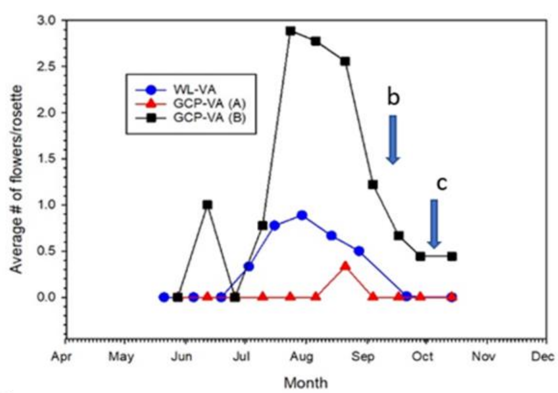
B)



C)



D)



E)

Fig. 15. A- E show the Average Flowers per Rosette (FLPR) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively.

(Note: a, b and c= Herbicide Treatment Codes, a= July 29 at ML-VA, b= Sept 15 at WL-VA and GCP-VA(B), c=Oct 5 at GCP-VA (A and B))

B. Treated Ponds

In treated ponds, FLPR were fewer in number and the pattern of increase and decrease was inconsistent. Flower counts were higher in mid-July and early September than other months. The maximum FLPR was 3 in treated ponds. In 2019, in ML-VA, the onset of flowers was during the end of June which generally increased until September followed by the decrease. The average no. of flowers varied between 1 to 2 through September. In 2020 in ML-VA, *Trapa* flowers did not appear because of their premature death due to the herbicide treatment (Fig. 15D).

In GCP-VA(B), the onset of flowers was in early June, increasing rapidly and steadily until late July followed by a steady decline through October. In GCP-VA (A) and WL-VA, the average flowering pattern was rather inconsistent and *Trapa* died abruptly by mid-September and late September respectively (Fig. 15E).

7. Fruits per Rosette (FRPR)

A. Untreated Ponds

In untreated ponds, FRPR was fairly consistent with fruits first appearing in late June or early July and generally increased within a month to the levels of 2 to 4 FRPR. In VGA-VA, FRPR increased rapdily from June through November in 2019. However, in 2020, the onset of fruit was little later in July which increased rapidly during September and October reaching 5 (Fig. 16A). In HP-VA, the fruits started to appear during early July and followed a consistent pattern through November (Fig. 16B). In WP-VA, the FRPR continued to increase in July which started to decline until early September. In HO-VA, FRPR started to increase slowly and steadily until September followed by the decrease through October. The maximum no. of fruits were 5 and 4 in VGA-VA and HP-VA respecitvley and it was 3 in WP-VA and HO-VA (Fig. 16C).

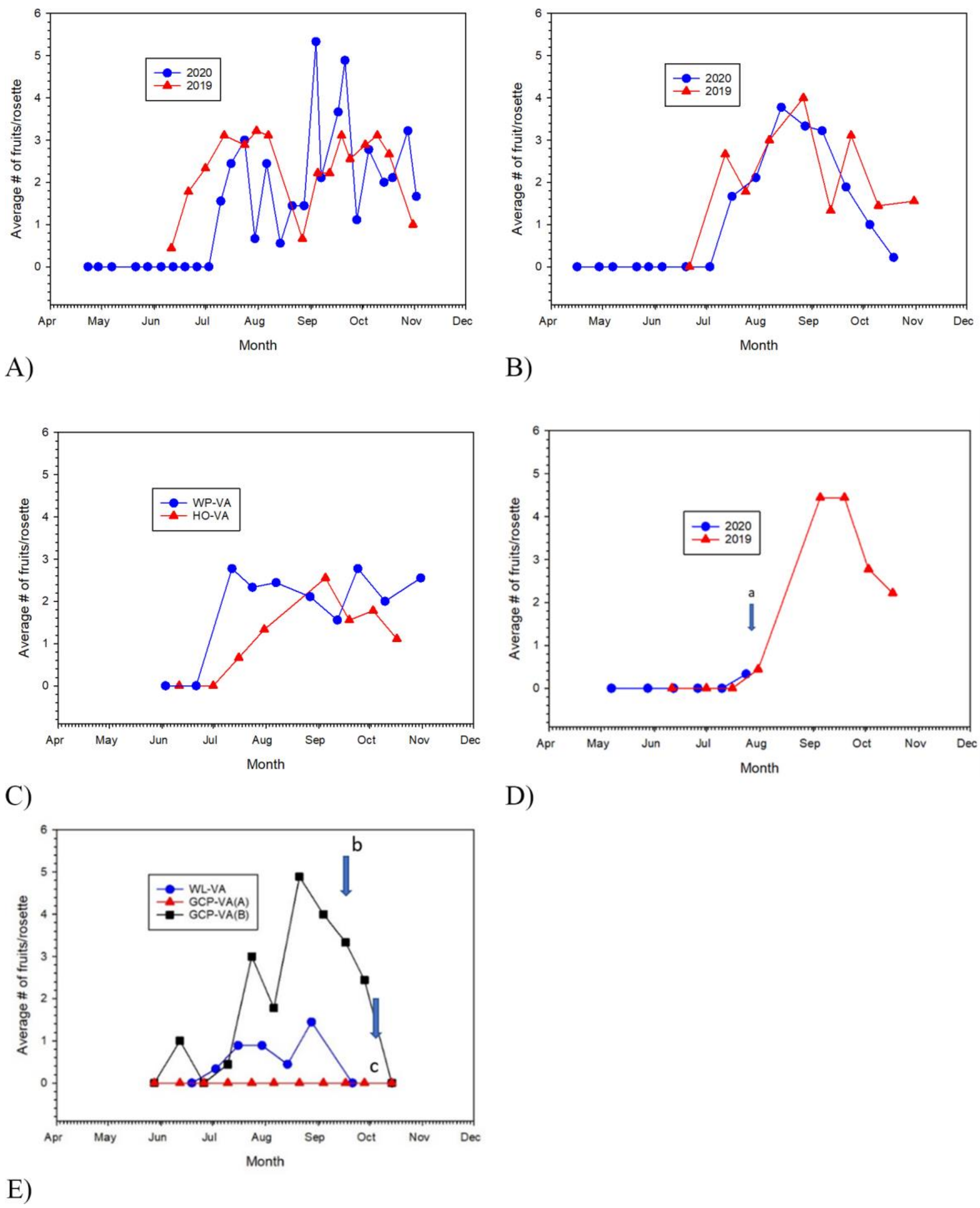


Fig. 16. A-E show Average number of Fruits per Rosette (FRPR) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively.

(Note: a, b and c= Herbicide Treatment Codes, a= July 29 at ML-VA, b= Sept 15 at WL-VA and GCP-VA(B), c=Oct 5 at GCP-VA (A and B))

B. Treated Ponds

In treated ponds, FRPR was inconsistent. Maximum fruits were noticed during late August and early September. The maximum number of fruits was about 5. In ML-VA in 2019, the FRPR started to increase rapidly from August through September to about 4 followed by the steady decline through October (Fig. 16D). In GCP-VA(B), FRPR generally increased until September followed by decrease through October. WL-VA and GCP-VA(A) had very few fruits, as they suffered premature death due to the application of herbicide (Fig. 16E).

8. Correlation Analysis Among Phenological Attributes; Phenological Attributes and Water Quality (WQ) Variables

Correlation analysis revealed significant positive relationship among the phenological attributes and between the phenological attributes and WQ variables. The following tables (Tables 7 to 12) show the Spearmen Corrleation Coefficients of intercorrelation of phenological attributes and between the WQ variables using data from all (untreated and treated) ponds.

Table 7. Spearman Correlation Coefficients (Untreated and Treated Ponds).

Phenological Attributes	TPC	TQC	FPQ	ARD	RRP	FLPR	FRPR
Trapa Pond Coverage (TPC)	1						
Trapa Quadrat Coverage (TQC)	0.69	1					
Flowers/Quadrat (FPQ)	0.738	0.602	1				
Avg Rosette Diameter (ARD)	0.529	0.489	0.589	1			
Rosette Repro Phenology (RRP)	0.736	0.582	0.709	0.748	1		
Flowers Per Rosette (FLPR)	0.735	0.556	0.785	0.641	0.8	1	
Fruits Per Rosette (FRPR)	0.616	0.48	0.675	0.657	0.782	0.776	1

($r \geq 0.3$ is Statistically Significant ; n=123 to 150)

The field observation showed that phenological attributes of *Trapa* were interrelated with each other in all ponds. As TPC started to increase TQC, ARD, RRP, FLPR and FRPR also increased and vice versa. The Spearman correlation test also revealed a significant correlation among all of phenological attributes ($r \geq 0.3$) in both untreated and treated ponds (Table 7).

TPC and TQC of all ponds were negativley correlated with SPC. All the phenological attributes were negatively correlated with DO (mg/L). TPC was negatively correalted with pH and NTU (Table 8).

Table 8. Spearman Correlation Coefficient (Phenological attributes and WQ Variables) of (Treated and Untreated Ponds).

Phenological Attributes	Temp	SPC	DO%	DO mg/L	pH	NTU	Alk
TPC	NS	-0.347	NS	-0.713	-0.34	-0.356	NS
TQC	NS	-0.30	NS	-0.565	NS	-0.317	NS
FPQ	NS	NS	NS	-0.517	NS	NS	NS
ARD	NS	NS	NS	-0.398	NS	NS	NS
RRP	NS	NS	NS	-0.625	NS	NS	NS
FLPR	NS	NS	NS	-0.523	NS	NS	NS
FRPR	NS	NS	NS	-0.514	NS	NS	NS

(Note: $r \geq 0.3$ is Statistically Significant, NS- Not significant; n=84 to150; Temp-Temperature, SPC-Specific Conductance, DO-Dissolved Oxygen, NTU-Turbidity, Alk-Alkalinity)

A. Untreated Ponds

In untreated ponds, as TPC started to increase TQC, ARD, RRP, FLPR and FRPR also increased and vice versa. The Spearman correlation test also showed a significant positive correlation among all of the phenological attributes ($r \geq 0.3$). In untreated ponds, TPC, ARD, RRP, FLPR and FRPR were positively correlated with month ($r \geq 0.3$) (Table 9).

Table 9. Spearman Correlation Coefficients of Untreated Ponds (Phenological Attributes).

Phenological Attributes (Untreated Ponds)	Month	TPC	TQC	FPQ	ARD	RRP	FLPR	FRPR
TPC	0.362	1						
TQC	NS	0.461	1					
FPQ	NS	0.609	0.627	1				
ARD	0.504	0.545	0.42	0.584	1			
RRP	0.679	0.669	0.35	0.516	0.717	1		
FLPR	0.414	0.683	0.431	0.661	0.69	0.708	1	
FRPR	0.545	0.501	0.349	0.575	0.746	0.667	0.59	1

(r≥0.3 is Statistically Significant ; n=74 to 89)

Table 10. Spearman Correlation Coefficients of Unreated Pond (Phenology vs. WQ Variables).

Phenological Attributes (Untreated Ponds)	Temp	SPC	DO%	DO mg/L	pH	NTU	Alk
TPC	NS	0.406	NS	-0.485	NS	NS	NS
TQC	NS	NS	NS	-0.394	NS	NS	NS
FPQ	NS	NS	NS	-0.325	NS	NS	NS
ARD	NS	NS	NS	-0.45	NS	NS	NS
RRP	NS	NS	NS	-0.574	NS	NS	NS
FLPR	NS	NS	NS	-0.559	-0.338	NS	NS
FRPR	NS	NS	NS	-0.416	NS	NS	NS

(Note: r≥0.3 is Statistically Significant, NS- Not significant; n=84 to 90; Temp-Temperature, SPC-Specific Conductance, DO-Dissolved Oxygen, NTU-Turbidity, Alk-Alkalinity).

Among the WQ variables, TPC was positively correlated with SPC. All of the phenological attributes were negatively corrleated with DO (mg/L) and FLPR was negatively correlated with pH in untreated ponds (Table 10).

B. Treated Ponds

In treated ponds, RRP and FLPR were significantly correlated (r≥0.3) with month (Table 11). Most phenological attributes were not correlated with month for treated ponds due to herbicide disruption of normal phenological progression. But intrecorrelations among phenological attributes remained intact.

Table 11. Spearman Correlation Coefficients of Treated Ponds (Phenological Attributes).

Phenological Attributes (Treated Pond)	Month	TPC	TQC	FPQ	ARD	RRP	FLPR	FR PR
TPC	NS	1						
TQC	NS	0.519	1					
FPQ	NS	0.466	0.325	1				
ARD	NS	0.753	0.557	0.639	1			
RRP	0.44	0.709	0.46	0.7	0.762	1		
FLPR	0.309	0.595	0.464	0.736	0.644	0.766	1	
FRPR	NS	0.583	0.395	0.701	0.643	0.782	0.878	1

(n= 48 to 60, r≥0.3)

TPC and ARD were negatively correlated with temperature. TPC, ARD and FLPR were negatively correalted with SPC. TPC was positively correlated with DO (%

saturation). TPC and ARD were negatively correlated with DO (mg/L) and FLPR was positively correlated with pH (Table 12). In treated ponds, fewer negative correlation was noted with DO (mg/L) than in untreated due to disruption of continous *Trapa* cover.

Table 12. Spearman Correlation Coefficients of Treated Pond (Phenology and WQ Variables).

Phenological Attributes (Treated Pond)	Month	Temp	SPC	DO%	DO mg/L	pH	NTU	Alk
TPC	NS	-0.513	-0.413	-0.476	-0.509	NS	NS	NS
TQC	NS	NS	NS	NS	NS	NS	NS	NS
FPQ	NS	NS	NS	NS	NS	NS	NS	NS
ARD	NS	-0.352	-0.344	NS	-0.295	NS	NS	NS
RRP	0.44	NS	NS	NS	NS	NS	NS	NS
FLPR	0.309	NS	-0.38	NS	NS	0.313	NS	NS
FRPR	NS	NS	NS	NS	NS	NS	NS	NS

(Note: $r \geq 0.3$ is Statistically Significant, NS- Not significant; n=49 to 60 ; Temp-Temperature, SPC-Specific Conductance, DO-Dissolved Oxygen, NTU-Turbidity, Alk-Alkalinity)

Discussion

1. Phenology

In untreated ponds, the increase in TPC and TQC followed a consistent pattern compared to treated ponds. In untreated ponds FPQ and FLPR followed a similar pattern in both sampling years. However, in treated ponds, there were fewer flowers and the pattern was inconsistent. Even though *Trapa* appeared later in treated ponds compared to untreated, maximum flower phenology occurred at about the same time. In untreated ponds, the rosettes were bigger and consistent in both sampling years. In some of the treated ponds, rosette sizes were inconsistent. Some of the rosette sizes were smaller and some were even bigger compared to untreated ponds.

In untreated ponds, RRP was consistent after July and August, and was 100%. However, in treated ponds, RRP was not consistent and reached 100% for only a short interval in few sites. In untreated ponds, fruit phenology was more consistent compared to treated ponds. The correation analysis showed that all the phenological attributes of treated and untreated ponds were significantly intercorrelating even though some of the attributes- the number of flowers, fruits and rosette diameter of treated ponds were not as high as untreated ponds and the flowering and fruiting pattern was less consistent.

The pattern of TQC, ARD, FLPR and FRPR is plotted in the conceptual plot below (Fig. 17). It is based on the general pattern of *Trapa* phenology of two of the untreated sites VGA-VA and HP-VA. TPC increased rapidly from May through June, peaked in early June and remained constant until late August and started to decline in fall. TQC started to increase rapidly from May through early September, remained constant through early October and finally declined afterwards. FLPR started to appear from late June, increased rapidly through summer, peaked in early and late August and started to decline in the fall. FRPR started to appear in early July, increased gradually through August, peaked in early September, and declined in the fall. This phenological synthesis will aid in the management and control of *Trapa bispinosa* as there is not much relevant information available.

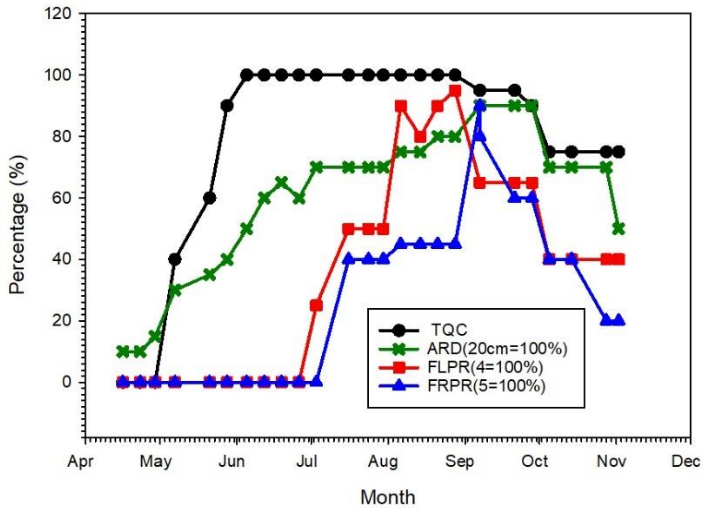


Fig. 17. Conceptual Plot showing the general patterns of Total Quadrat Coverage (TQC), Rosette Diameter (ARD), Flowers per Rosette (FLPR) and Fruits per Rosette (FRPR) of untreated ponds (derived from VGA-VA and HP-VA).

2. Phenology and WQ Correlation

A. Untreated Ponds

In untreated ponds, TPC, ARD, RRP, FLPR and FRPR were positively correlated with month. This is because, these attributes increase as the growing season progresses. TPC, ARD, RRP, FLPR and FRPR were higher in the summer month of June, July, August and early September compared to April and May (Table 9). In untreated ponds, TPC was positively correlated to sampling month and SPC (Table 10). All vegetative and reproductive characteristics were negatively correlated with DO (mg/L) (Table 10). These *Trapa* attributes were also interrelated to each other. As the amount of *Trapa* increases in the quadrat, the floating leaves of *Trapa* block the passage of sunlight in the lower level which interferes with photosynthesis and depletes the oxygen. pH is negatively correlated with flowers per rosette (Table 10).

The positive correlation between the *Trapa* pond coverage and SPC and negative correlation of pH with flowers per rosette are more difficult to explain.

B. Treated Ponds

In treated ponds, the correlation of *Trapa* phenology with WQ variables was unclear. RRP and FLPR were positively correlated with temperature (Table 12). This is because TPC, TQC, ARD, RRP, FLPR and FRPR of *Trapa* started to develop and increase as the *Trapa* growing season progressed (June, July and August). TPC and ARD were negatively correlated to temperature. In most of the treated ponds, TPC was less and *Trapa* started to die off along with the increase in temperature in summer. Most of these treated ponds were treated with herbicide in late July, mid- September, and early October (Table 2). RRP and FLPR were positively correlated with month. TPC, ARD and FLPR were negatively correlated with SPC. This could be due to the uptake of ions by *Trapa* as it metabolizes. This result is similar to our result of one day hydroponic experiment of *Trapa* (Chapter 4). There was a positive correlation between the FLPR and pH which is difficult to explain.

TPC was negatively correlated with DO% and DO mg/L. Contrary to the untreated ponds, the rest of phenological attributes did not show the significant correlation with DO (mg/L). This might be due to the less amount and short life span of *Trapa* in treated ponds which did not deplete oxygen level in the water underlying the

Trapa as in the untreated ponds. There was no significant correlation between the phenological attributes with rest of the WQ variables in treated ponds (Table 12).

This study supports the initial hypothesis of more rapid, predictable and consistent pattern of growth and reproduction in untreated ponds compared to treated ponds.

Conclusions

In untreated ponds, the pond coverage and quadrat coverage of *Trapa* followed a more consistent pattern compared to treated ponds. TPC was higher and denser in summer, and it started to decrease and thin out after early September. TQC was increasing rapidly until June, reached as high as 100% until August and early September. In all untreated ponds, rosette diameter, the number of flowers and fruits, rosette reproductive phenology were comparatively greater and more consistent than the treated ponds. In untreated ponds rosette diameter reached its maximum in July and remained high in August and September whereas in treated ponds, the maximum rosette diameter did not reach until August and September.

Even though *Trapa* appeared later in treated ponds compared to untreated, the maximum flower and fruit phenology occurred during the similar months. In addition, the number of flowers and fruits of some of these treated ponds were similar to untreated ponds and in some cases, it was even higher in treated ponds. This leads to the fact that herbicide treatment might delay the onset of *Trapa,* but it does not necessarily block the flowering and fruiting capacity of *Trapa*.

In untreated ponds, most of the phenological attributes were positively correlated with month and all phenology were negatively correlated with DO (mg/L) whereas in treated ponds, only TPC was negatively correlated with DO (mg/L). In some of the treated ponds, *Trapa* phenology had negative correlation with temperature whereas in untreated ponds, there was no correlation. *Trapa* pond coverage had a positive correlation with SPC in untreated ponds, whereas in treated ponds three of the *Trapa* phenology had a negative correlation with SPC. In untreated ponds, FLPR had a positive correlation with pH whereas, it had a negative correlation in treated ponds (Tables 11 and 12). The reason behind this is more difficult to explain.

WATER QUALITY (WQ) SAMPLING OF *TRAPA* AND *NON-TRAPA* PONDS

Introduction

Water Chestnut (*T. bispinosa*) creates a canopy which blocks the passage of light through water which inhibits photosynthesis and prevents oxygenation in the deeper layers which may kill fish and other organisms (Strayer, 2010). A dense canopy of Water Chestnut may reduce gas exchange between the water column and atmosphere allowing depletion of oxygen in the lower levels. In addition, Water Chestnut bed may reduce turbidity by enhancing settling of suspended solids and contributing to local accumulation of fine sediment (Shrivastava et al., 2009). Thus, *Trapa* affects water quality and *vice versa*.

The purpose of this chapter is to describe the water quality (WQ) parameters - temperature, dissolved oxygen (DO (mg/L and % saturation)), pH, specific conductance (SPC) and turbidity (NTU) of *Trapa* ponds (untreated and treated with herbicides) and a group of non-*Trapa* ponds.

The hypothesis of this study are:

1. WQ of *Trapa* ponds will vary according to the density of *Trapa* coverage.
2. WQ of untreated *Trapa* ponds will be more consistent compared to WQ of treated *Trapa* ponds.
3. WQ of non-*Trapa* ponds will have more DO and differ in other WQ parameters with *Trapa* ponds.

Materials and Methods

1. Sampling Dates

Water quality was assessed on the same dates as stations were visited for vegetation analysis. The sampling stations for 2019 for *Trapa* ponds were grouped into sets that were sampled on the same day. VGA-VA was considered its own sample set since it was sampled weekly (Table 13). The other sites HP-VA, WP-VA and HO-VA, ML-VA were sampled in an alternating pattern biweekly and grouped into two additional sets.

Table 13. Sampling dates and sampling stations for *Trapa* ponds in 2019.

Station Sets	Set 1- VGA-VA	Set 2- HP-VA & WP-VA	Set 3-HO-VA & ML-VA
Date			
6/3/19	X	X	
6/11/19	X		X
6/21/19	X	X	
7/12/19	X	X	
7/16/19	X		X
7/24/19	X	X	
7/31/19	X		X
8/7/19	X	X	
8/16/19	X		X
8/27/19	X	X	
9/5/19	X		X
9/12/19	X	X	
9/19/19	X		X
9/24/19	X	X	
10/10/19	X	X	
10/17/19	X		X

(Note: X is sampling date)

In 2020, a similar sampling pattern was followed but there was a change in some of the biweekly stations. VGA-VA was considered its own sample set since it was sampled on all field dates (Table 14). The other sites HP-VA, WL-VA and ML-VA, GCP-VA(A), GCP-VA(B) were considered as a different set and generally sampled in alternating weeks.

Table 14. Sampling dates and sampling stations for *Trapa* ponds in 2020.

Station Sets	Set 1-VGA-VA	Set 2- HP-VA, WL-VA	Set 3-ML-VA, GCP-VA(A), GCP-VA(B)
Date			
4/16/20	X	X	
4/23/20	X		
4/29/20	X	X	X
5/7/20	X	X	X
5/21/20	X	X	
5/28/20	X	X	X
6/5/20	X	X	
6/12/20	X		X
6/19/20	X	X	
6/26/20	X		X
7/3/20	X	X	
7/10/20	X		X
7/16/20	X	X	
7/24/20	X		X
7/30/20	X	X	
8/6/20	X		X
8/14/20	X	X	
8/21/20	X		X
8/28/20	X	X	
9/4/20	X		
9/7/20	X	X	
9/17/20	X		X
9/21/20	X	X	
9/28/20	X		X
10/5/20	X	X	
10/14/20	X		X
10/19/20	X	X	
10/28/20	X		
11/2/20	X		

(Note: X is sampling date)

2. Water Quality Measurement Methods

YSI sonde Pro DDs was used to measure the water temperature, dissolved oxygen (DO mg/L and DO%), pH, specific conductance (SPC) and turbidity (NTU) in this research. A single location was assayed at each pond about 2 m offshore at a depth of 0.3m.

Alkalinity

Alkalinity is water's ability to neutralize acids. According to USGS, alkalinity is the buffering capacity of a water body; a measure of the ability of the water body to neutralize acids and bases to maintain a fairly stable pH level.

Alkalinity of water was tested in each of the *Trapa* and non-*Trapa* ponds sampled in 2020. A water sample was collected from each pond and alkalinity was tested immediately on return to the vehicle. To measure the alkalinity, 45 ml of water sample was collected in a clean conical flask. One packet of Bromocresol Methyl green indicator was used which gave the water sample green color. Sulphuric Acid (0.035N) was added to the water sample mixed with indicator to note the change in color. The number of drops needed to change the color to pink was observed and noted. Total alkalinity (mg/L as $CaCO_3$) was estimated using the experimental result using the following formula. The alkalinity of water is represented in the graphs in the result section.

Total alkalinity=Number of drops of H_2SO_4 consumed *2.

3. Statistical Analysis

For the statistical analysis of the field data, the software packages Systat 13.2 and Sigma plot 14.5 were used.

Results

Water Quality Data of *Trapa bispinosa* Ponds

1. Water Temperature

Water temperature of all sampling stations in 2019 and 2020 are represented in the graph below (Fig.18, A-E).

A. Untreated Ponds

The pattern of water temperature was very similar in 2019 and 2020 and followed the expected air temperature patterns (Fig. 18, A-C). The water temperature increased in the spring peaking in July and August and gradually declined afterwards in VGA-VA and HP-VA in both sampling years. In WP-VA and HO-VA, the water temperature increased until early July followed by the gradual decline until early October. Among the untreated ponds, the highest temperature was 29.1°C in WP-VA (mid-July 2019) and the lowest temperature was 9.7 °C (early November 2020) in VGA-VA (Appendix, Table 35).

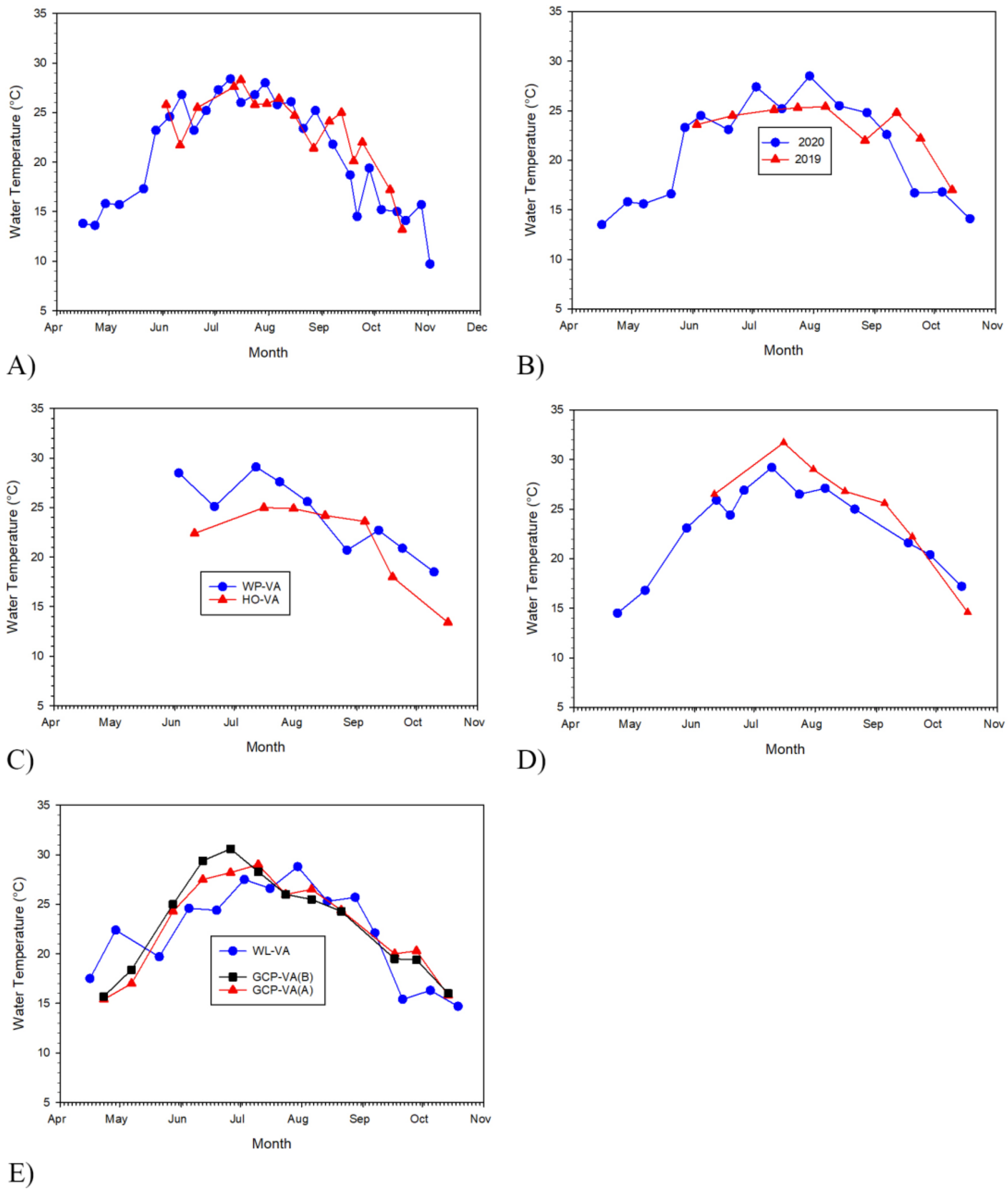


Fig. 18. A-E show the water temperature of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively in 2019 and 2020.

B. Treated Ponds

Among the treated ponds, water temperature followed a similar seasonal pattern both in 2019 and 2020 (Fig. 18, D and E). The water temperature increased until early July and decreased afterwards until late October in these ponds. In ML-VA, most of 2019 was slightly warmer compared to 2020 but October was slightly warmer in 2020 compared to 2019. WL-VA, GCP-VA(A) and GCP-VA(B) were sampled only in 2020. In 2019, the maximum temperature was 31.7 °C in mid- July in ML-VA and the minimum temperature was 14.6°C in mid-October in ML-VA (Appendix, Table 42). In 2020, the lowest temperature was of WL-VA during the end of October (14.7 °C) and the highest was of GCP-VA(B) during the end of June which was 30.6° C.

2. Dissolved Oxygen (mg/L)

A. Untreated Ponds

The analysis of DO (mg/L) will focus on the periods when DO (mg/L) was less than the water quality standard of 5 mg/L (WQ-5) or below the hypoxia threshold (<2 mg/L). In 2019, DO (mg/L) of VGA-VA was generally below the hypoxia level in late July, August and early September and above the hypoxia level in the rest of the months (Fig.19A). In 2020, DO (mg/L) of VGA-VA was higher than 5 (mg/L) in April and May. DO (mg/L) fluctuated from June until August when the level was below 5 (mg/L) but well above the hypoxia threshold. DO (mg/L) declined below the hypoxia threshold in early and mid-September in 2020 (Appendix, Table 36).

At HP-VA in 2019, DO (mg/L) was at or slightly above the hypoxia threshold from June until August (Fig. 19B). WQ declined below the hypoxia threshold in September and October. DO was below the WQ-5 for the majority of the sampling periods at HP-VA. In 2020, DO (mg/L) of HP-VA was higher than WQ-5 in April and late May but below WQ-5 for the rest of the year until October (Appendix, Table 36).

The DO (mg/L) of HO-VA and WP-VA was sampled only in 2019. DO (mg/L) of WP-VA and HO-VA was generally slightly above the hypoxia level from June until August (Fig. 19C). After August, DO (mg/L) in both sites declined below the hypoxia threshold level (Appendix, Table 36).

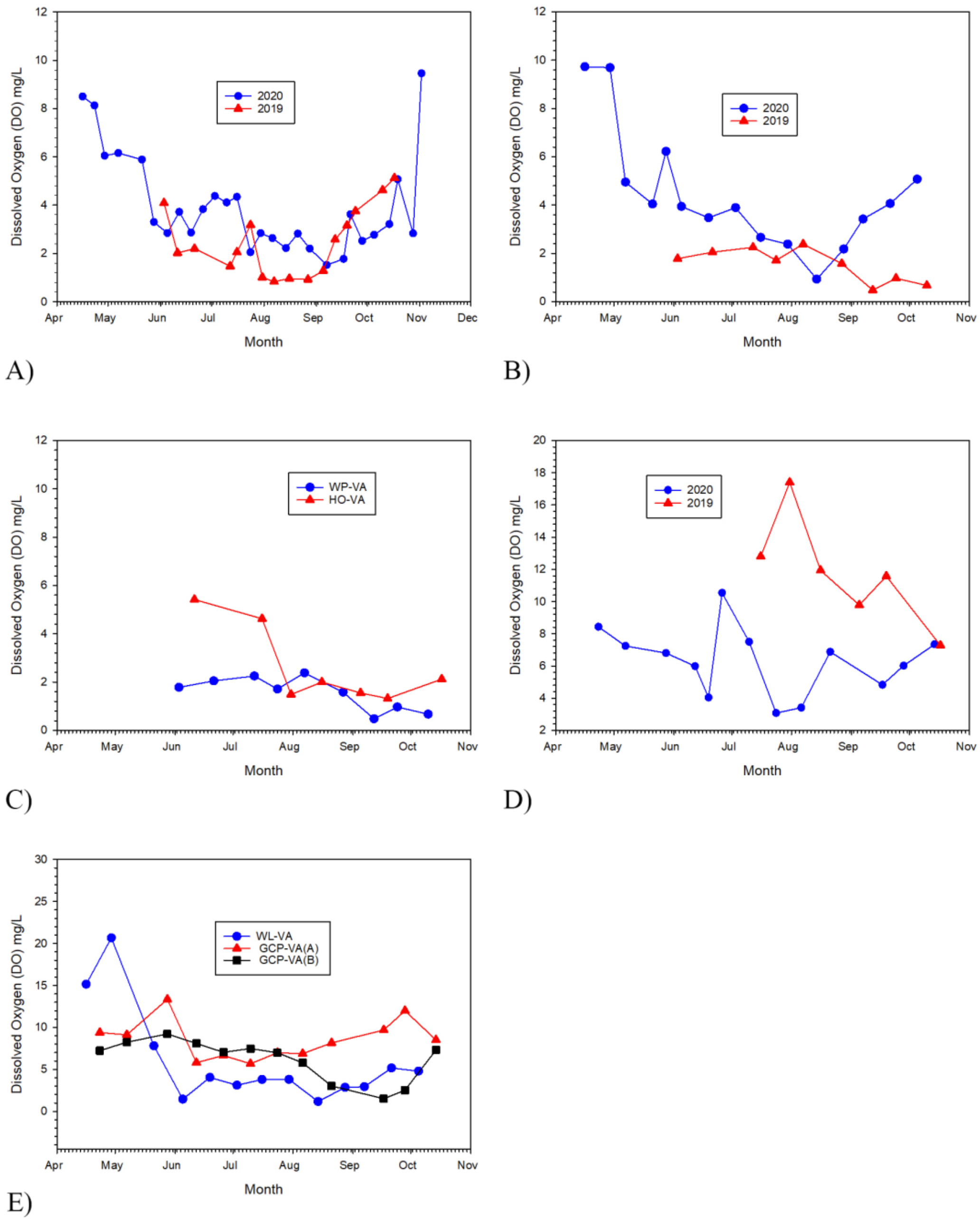


Fig. 19. A-E show DO (mg/L) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA and GCP-VA (A and B) respectively in 2019 and 2020.

B. Treated Ponds

Among the treated ponds, the pattern of DO (mg/L) of ML-VA in 2019 was higher compared to 2020. In 2019, in ML-VA, DO was well above the hypoxia threshold and above WQ-5 and ranged as high as 17 mg/L (Fig.19D). In 2020, DO was well above the hypoxia threshold in all the months and was above WQ-5 in most of the months. DO depleted below WQ-5 in June, end of July and the beginning of August in 2020 in ML-VA (Appendix, Table 43).

WL-VA, GCP-VA(A) and GCP-VA(B) were sampled only in 2020 (Fig. 19E). DO (mg/L) was high after the mid-April which generally declined until October in all three sites. At WL-VA, DO was above the WQ-5 in April and May. After June, DO (mg/L) declined but remained mostly above the hypoxia threshold and below WQ-5 and well above the hypoxia threshold in all the months.

At GCP-VA(A), DO (mg/L) was higher than the WQ-5 and well above the hypoxia threshold in all the months (Fig. 19E).

At GCP-VA(B), DO (mg/L) was higher than the WQ-5 from April until the beginning of August (Fig. 19E). After August, DO (mg/L) declined to below WQ-5 in mid-September, DO (mg/L) was below the hypoxia threshold then increased slightly afterwards (Appendix, Table 43).

3. Dissolved Oxygen (DO% saturation)

A. Untreated Ponds

DO (% saturation) of VGA- VA was generally lower in 2019 compared to 2020 but both years followed a similar seasonal pattern (Fig. 20A). In 2020, DO (%) started high (82.4%) during April and generally declined until September. After September, there was a slight increase in DO (%) until November. DO (%) of 2020 was typically higher than 2019 in summer until the beginning of September but it was lower than 2019 in September, October, and November. In 2020, DO (%) was the highest in mid- April (82.4 %) and the lowest in the beginning of November (9.5) whereas in 2019, lowest values were observed in August (Appendix, Table 37).

DO (%) of HP- VA in 2019 was lower compared to 2020 (Fig. 20B). In 2019, DO (%) was already low (~25 % range) when sampling began in June, never rose above 40% and declined to below 10% after early August. In 2020, the DO (%) of HP-VA was high (94 %) in the beginning of April and then showed a general decrease until September to below 20%. DO (%) increased steadily until the end of October (Appendix, Table 37).

WP-VA and HO-VA were sampled only in 2019. At both sites, DO (%) was high in the beginning (June), but showed a general decrease into the fall (Fig. 20C). DO (%) was in a wider range in WP-VA whereas in HO-VA the range was narrower. In WP-VA, the range of DO (%) was between 8-105. In HO-VA, the range was 15-65% (Appendix, Table 37).

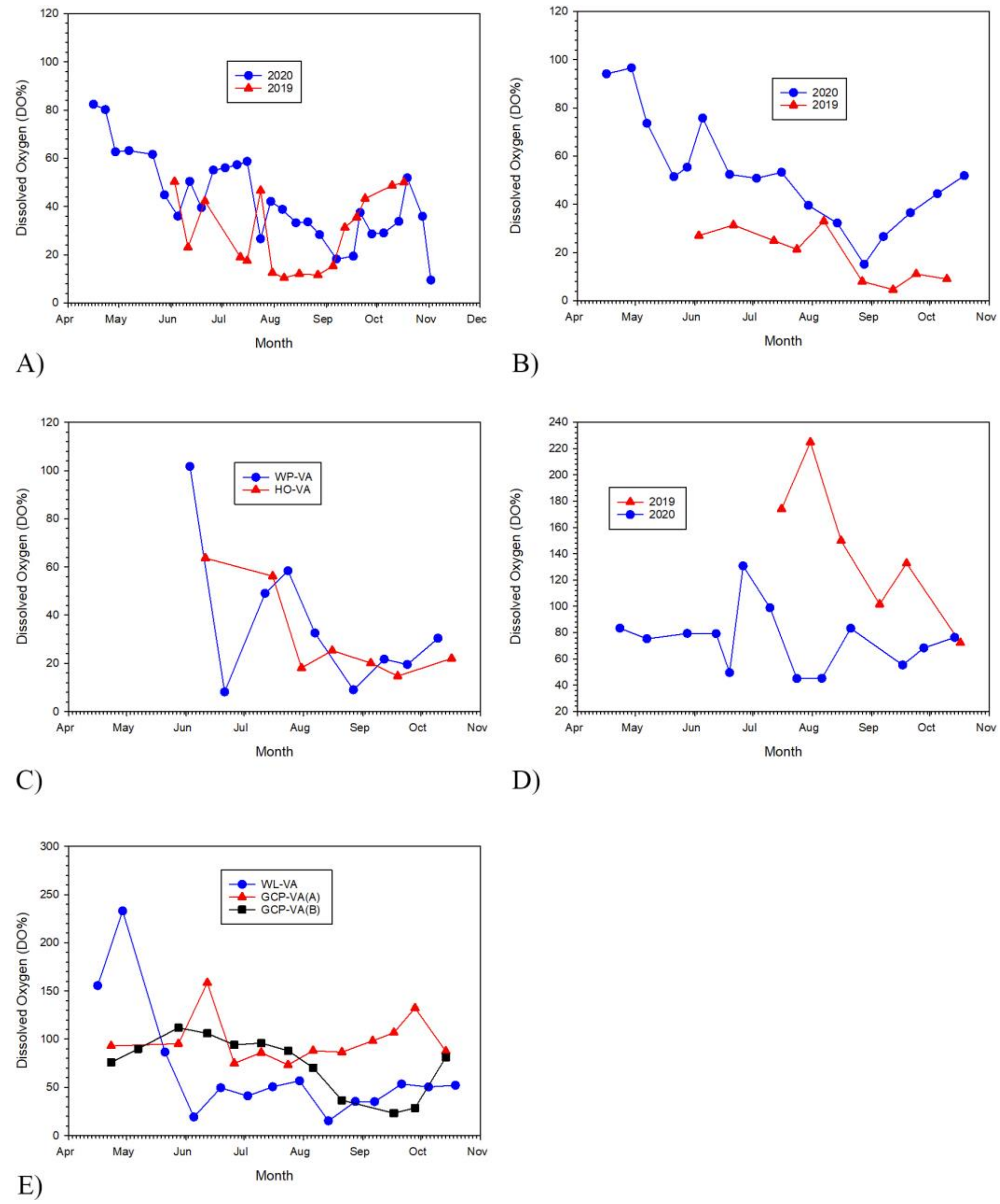


Fig. 20. A-E show the DO% of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively in 2019 and 2020.

B. Treated Ponds

In ML-VA, DO% was completely different in two years. In 2019, DO% started very high and continued to decline gradually until the end of October (Fig. 20D). In 2020, DO% started low and remained between 50-100 in most of the year. DO% was higher in late June and July in ML-VA which decreased afterwards (Appendix, Table 44).

In WL-VA, GCP-VA(A) and GCP-VA(B), data were collected only in 2020 (Fig. 20E). DO% was high in April and May which continued to decline slowly until October (Appendix, Table 44). DO% was higher in May in WL-VA compared to rest of the months.

4. pH

A. Untreated Ponds

Comparing 2019 and 2020, the values of pH for VGA-VA was consistently higher in 2020 (Fig. 21A). In 2020, pH of VGA-VA was about 7 in the beginning and generally increased to about 8.5 in mid-July. There was a decline through August and then another increase in October. In 2019, there was a more stable pattern ranging from 6.5 to 7.5 (Appendix, Table 38).

At HP-VA, in 2019, pH generally increased seasonally from about 5.8 to 6.6 (Fig. 21B). The values of pH were consistently higher in 2020 compared to 2019 ranging from about 7 to 8.5 both years (Appendix, Table 38).

WP-VA and HO-VA were sampled only in 2019 (Fig. 21C). pH values of WP-VA and HO-VA followed a similar pattern and pH of both sites were within the similar range of 5.9-7.2. Lowest values were observed in July and highest values in October (Appendix, Table 38).

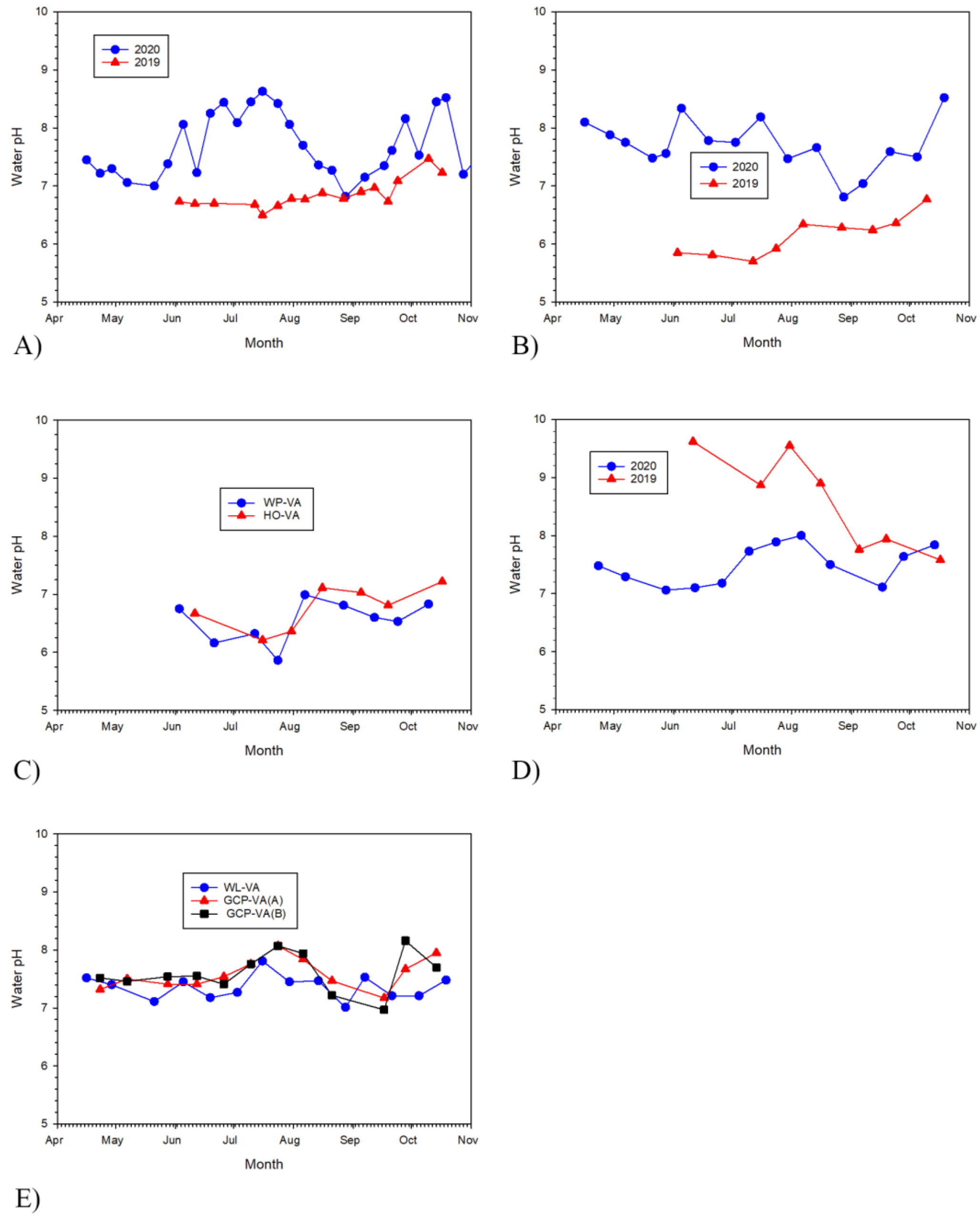


Fig. 21. A-E show the pH of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively in 2019 and 2020.

B. Treated Ponds

At ML-VA in 2019, pH generally decreased from June until October from about 9.5 to about 7.5. In 2020, pH value of ML-VA occupied a narrow range from 7 to 8 (Fig. 21D). During the end of October, the pH values of both years converged at the same point (Appendix, Table 45).

WL-VA, GCP-VA (A) and GCP-VA (B) were sampled only in 2020. pH of WL-VA, GCP-VA(A) and GCP-VA(B) followed a similar pattern and was within the range of 7-8 with slight variation (Fig. 21E, Appendix, Table 45).

5. Specific Conductance (SPC)

A. Untreated Ponds

At VGA-VA, the pattern and range of SPC was similar between 2019 and 2020 ranging between 131-212 (Fig. 22A). In 2019 SPC was about 160 from June to mid-July which declined during the end of July followed by a steep rise in August. In 2020, the values generally hovered at about 150 through August, rising somewhat in October and November.

SPC of HP-VA was quite variable during both years (Fig. 22B). In 2019, in HP-VA, there was a steep decline in SPC during early July and at the end of August.

WP-VA and HO-VA were sampled only in 2019. SPC started to increase from June until October both in WP-VA and HO-VA (Fig. 22C). The pattern was almost similar, but the range of SPC of WP-VA was higher compared to HO-VA in 2019 (Appendix, Table 38).

In all of these ponds, there was a steep decline in SPC from time to time which could be possibly due to the precipitation during the period of sampling which is shown in the table below. All of the steep declines corresponded with the amount of precipitation (Table 15).

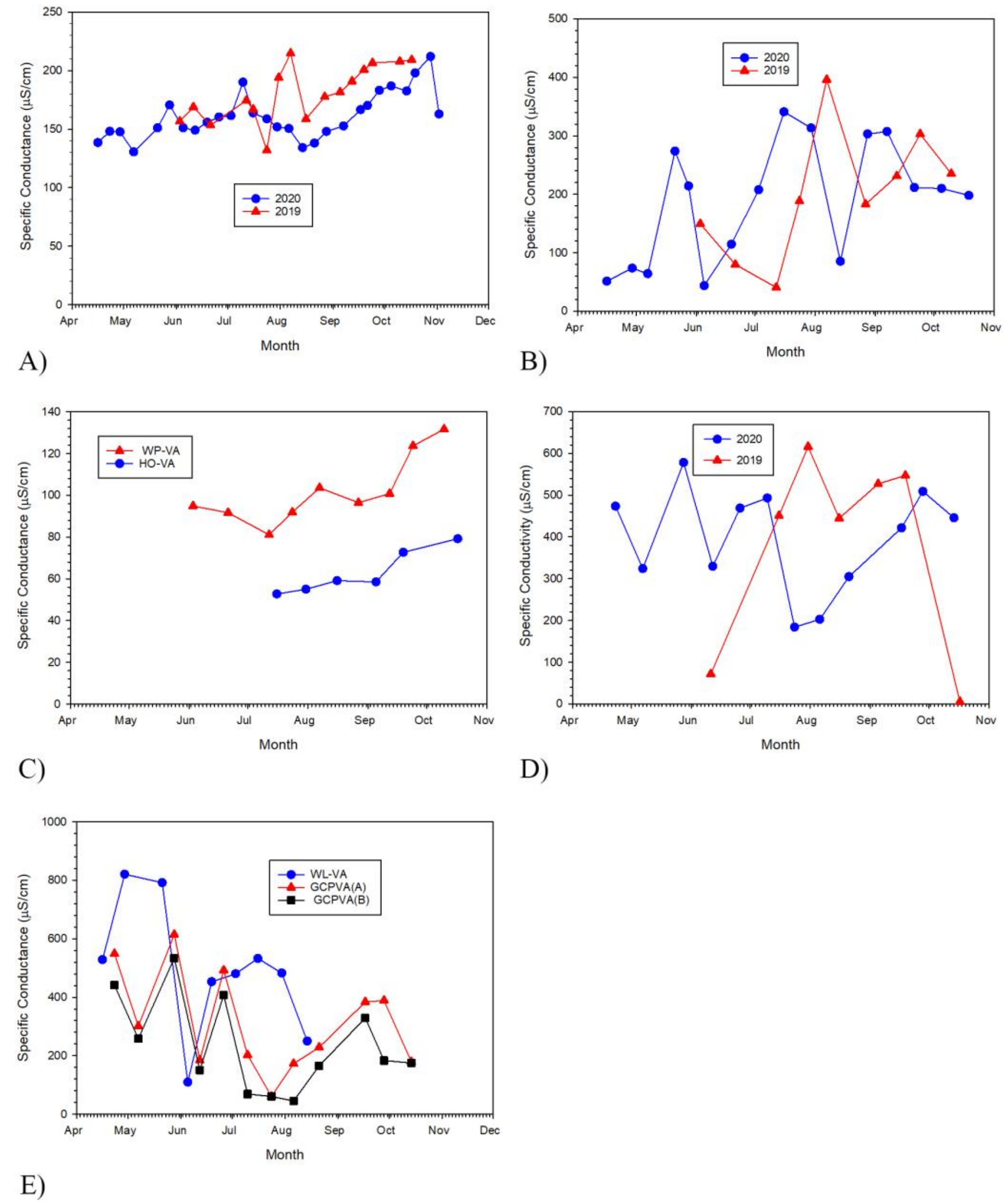


Fig. 22. A- E show the Specific Conductance (SPC) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively in 2019 and 2020.

B. Treated Ponds

There was no consistent pattern of SPC in 2019 and 2020 in ML-VA (Fig. 22D). In 2019, SPC of ML-VA started off very low, increased strongly during summer and levelled off in the fall. In 2020, SPC in ML-VA varied from 200-600 µS/cm.

At WL-VA, GCP-VA (A and B), from April to July, there was an alternating pattern of peaks and low points, probably related to precipitation (Fig. 22E, Table 15). In July and early august, SPC was very low and then increased somewhat in September and October. At WL-VA, there was somewhat higher SPC levels.

SPC of treated ponds followed an inconsistent trend which varied with the amount of rainfall.

Table 15. Low points and Peaks of SPC, NTU and Alkalinity with the corresponding dates of Precipitation.

					Precipitation (cm)		
Pond	Sampling Date	SPC	NTU	Alk	Avg.	3-day	7-days
VGA-VA	7/10/2020	190.1	105	-	.01	.01	6.3
VGA-VA	7/24/2019	132	41.29	-	0.94	1.22	1.23
VGA-VA	9/21/2020	170.2	139.8	-	0	0	1.17
VGA-VA	10/14/2020	182.6	45.34	-	0	0.87	1.55
VGA-VA	10/19/2020	198	139.6	-	0	0	0
HP-VA	7/12/2019	40.7	-	-	0	2.24	12.80
HP-VA	8/27/2019	183.0	-	-	.01	.03	1.02
HP-VA	5/28/2020	214.1	1.56	-	0.2	0.22	2.44
HP-VA	6/5/2020	43.7	3.93	-	3.2	4.52	4.55
HP-VA	8/28/2020	303.1	0.78	-	3.71	3.71	3.87
ML-VA	8/16/2019	444.7	4.36	-	0	0.52	0.83
ML-VA	5/7/2020	324	10.13	-	0.01	0.67	3.49
ML-VA	6/12/2020	329.7	18.78	-	0	0.97	0.99
ML-VA	7/24/2020	183.9	39.16	-	1.09	7.32	9.02
ML-VA	8/21/2020	-	34.87	-	.01	0.24	3.75
WL-VA	6/5/2020	-	418.52	-	3.2	4.52	4.55
WL-VA	8/14/2020	-	235.5	-	0.15	2.31	2.35
GCP-VA(B)	6/26/2020	-	150.1	-	0	0.01	1.22
WP-VA	7/24/2019	-	74.66	-	0.94	1.22	1.23
VGA-VA	6/19/2020	-	-	72	.03	2.20	2.21
VGA-VA	10/5/2020	-	-	60.5	.05	.025	1.10
HP-VA	7/30/2020	-	-	37.5	0.38	0.39	1.49
ML-VA	5/28/2020	-	-	27	0.2	0.22	2.44
GCP-VA(B)	9/28/2020	-	-	60	.03	0.89	1.91
WL-VA	6/19/2020	-	-	65	.03	2.20	2.21

(Note: SPC -Specific conductance, NTU-Turbidity, Alk-Alkalinity, Avg. -Average)

6. Turbidity (NTU)

A. Untreated Ponds

In 2019 and 2020 at VGA-VA, turbidity was generally less than 20 NTU. In 2020, turbidity varied between 10-140 with a sharp increase in between. There was a sharp increase in early July, late September and late October to above 100 NTU in 2020, which could be due to precipitation (Fig. 23A, Table 15).

Turbidity of HP-VA remained below 10 NTU from April until the end of September both in 2019 and 2020 (Fig. 23B). WP-VA and HO-VA were sampled only in 2019. At WP-VA and HO-VA, SPC was below 10 NTU for most of the months (Fig. 23C). At WP-VA, NTU increased sharply during the end of July. The spikes in turbidity corresponded with the precipitation, except in VGA-VA in late October (Table 15).

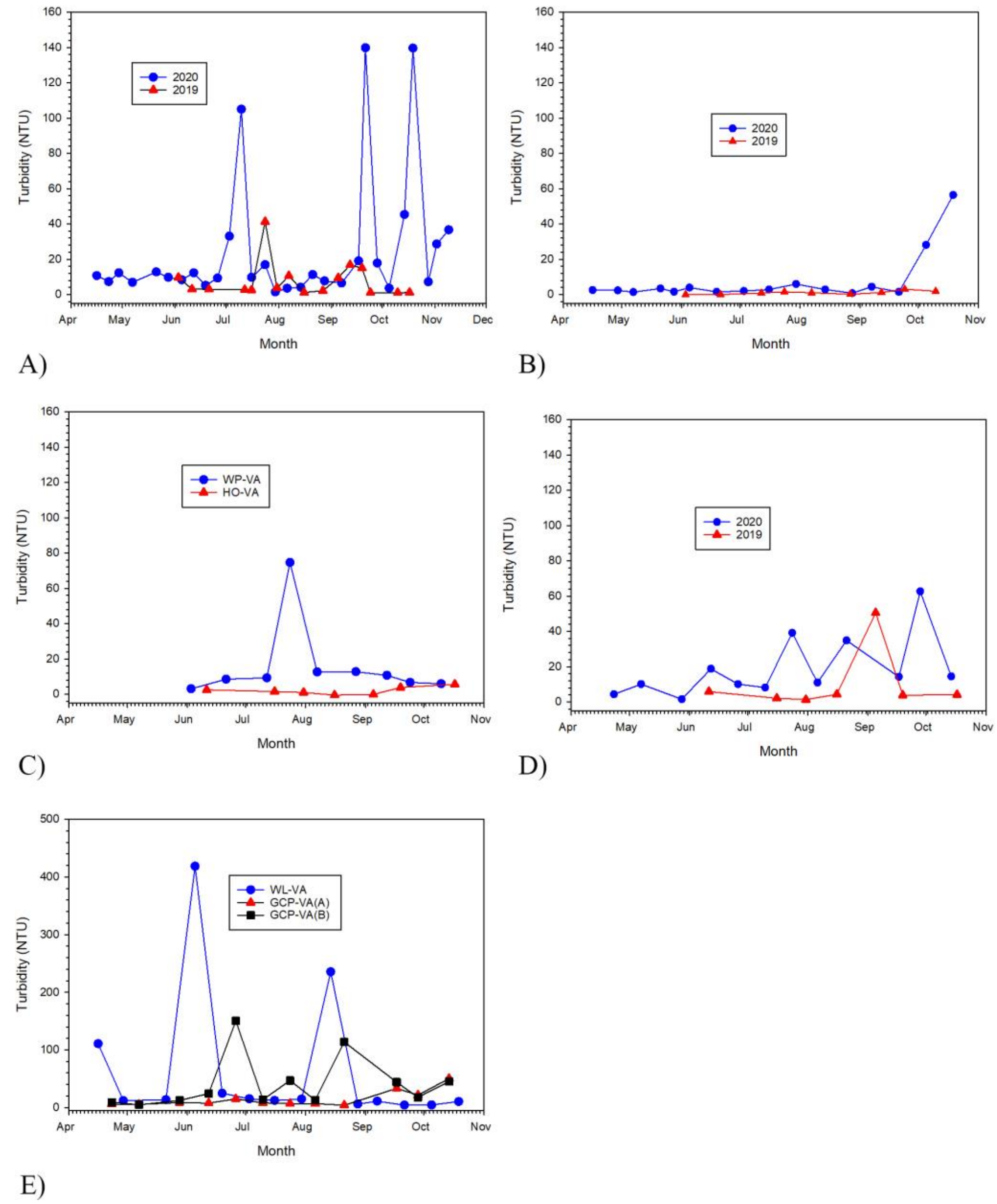


Fig. 23. A-E show the Turbidity (NTU) of VGA-VA, HP-VA, WP-VA and HO-VA, ML-VA and WL-VA, GCP-VA (A and B) respectively in 2019 and 2020.

B. Treated Ponds

Turbidity of ML-VA showed a similar range from the beginning of June until the beginning of September both in 2019 and 2020 (Fig. 23D). In 2019, most turbidity samples at ML-VA were less than 10 NTU. In 2020, the range of turbidity was between 4-63 with continuous increase and decrease.

WL-VA, GCP-VA(A) and GCP-VA(B) were sampled only in 2020. The turbidity of WL-VA varied significantly between the range of 10-420 NTU from April until October. There were big spikes in the first week of June (418.5) and mid-August (235.5) followed by the decrease until the third week of October (10.5) (Fig. 23E).

In GCP-VA(A), turbidity generally remained constant most of the time within the range of 6-50 NTU. Even though, A and B are located next to each other the turbidity range of B was higher compared to A at the same sampling dates. The spikes in turbidity corresponded with the amount of precipitation (Table 15).

7. Alkalinity (mg/L as $CaCO_3$)

A. Untreated Ponds

At VGA-VA, alkalinity of water was tested weekly from April until November in 2020 (Fig. 24A). Alkalinity varied between 43-72 during the sampling period. There was an occasional increase and decrease of alkalinity in between.

The alkalinity of HP-VA generally increased from April until mid-October. On three dates, early June, early August and late September, this general increase was interrupted by a distinct decline (Fig. 24B). The increase in alkalinity corresponded with the precipitation in VGA-VA and HP-VA (Table 15).

B. Treated Ponds

The alkalinity of ML-VA, GCP-VA(A) and GCP-VA(B) was in the range of 15-30 for most of the year (Fig. 24C). The alkalinity was higher in early April and May in ML-VA and the end of September in GCP-VA(B). There was a steep decline of alkalinity in late May ML-VA which corresponded with precipitation (Table 15).

The range of alkalinity in WL-VA was a lot higher (range 65-225) than other sampling stations (Fig. 24D). In WL-VA, there was a big variation in alkalinity, which was highest during the end of May and followed by a steep decline during the third week of June which corresponded with precipitation (Table 15). At GCP-VA(B) the increase in turbidity during the end of September corresponded with the precipitation (Table 15).

In untreated ponds, alkalinity increased along with the precipitation, in treated ponds, it decreased with precipitation in ML-VA and WL-VA but increased in GCP-VA(B).

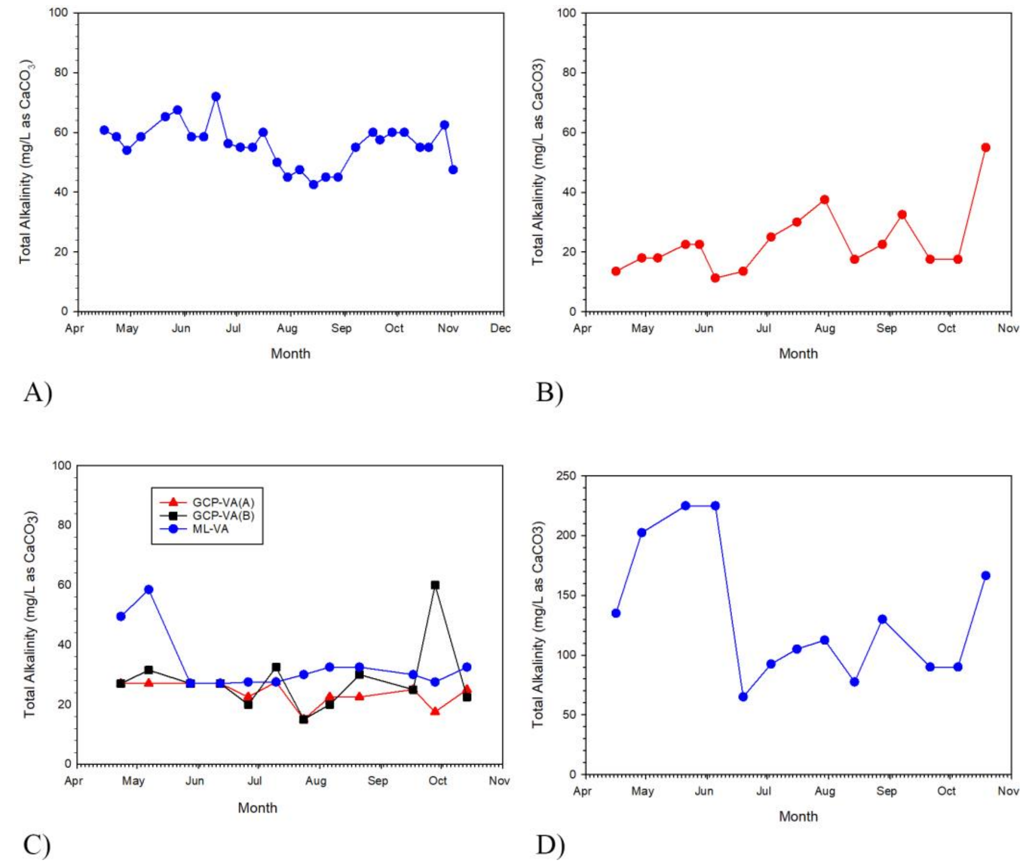


Fig. 24. A-D show the alkalinity of VGA-VA, HP-VA, ML-VA and GCP-VA (A and B), and WL-VA respectively in 2019 and 2020.

NON-*TRAPA* PONDS

Sampling procedures of non-*Trapa* ponds

Study Area

Ponds without *Trapa bispinosa* were sampled in the general vicinity of *Trapa* ponds to determine if there was a consistent difference between *Trapa* and non-*Trapa* ponds. Overall, 18 ponds were inspected in western Fairfax County and 4 non-*Trapa* ponds were selected from them for sampling. The following is a description of each of 4 non-*Trapa* ponds chosen for this study (Fig. 25). Those ponds were sampled twice in 2020. The first sampling period was during early June, when plants were limited even in *Trapa* dominated ponds and the second sampling was done during early September, which was the peak season of *Trapa* growth.

1. Zasada Lake (ZL-VA)

ZL-VA is located in 4203 Summit Manor Ct, Fairfax, VA 22033. It is a pond with an area of 1.87 ha and latitude and longitude of 38.86987 and -77.381535 respectively (Fig. 25). This pond is nearby the residential complex with walking trails and trees. The pond water looked clean with a fountain. There was no significant vegetation noticed inside the pond.

2. Fair Lake Circle Pond (FLC-VA)

FLC-VA is at 12450 Fair Lakes Circle, Fairfax, VA 22033. It is located nearby a residential complex. This pond has an area of 1.64 ha with latitude and longitude of 38.861064 and -77.38025 respectively (Fig. 25). The water looked clean. There was no significant vegetation inside the pond.

3. Eleanor Lawrence Park Pond (ELP-VA)

ELP-VA is located at 5040 Walney Rd, Chantilly, VA 22033. It is a pond with an area of 0.18 ha and latitude and longitude of 38.860903 and -77.430656 respectively. It is located within the EC Lawrence Park with walking trails and other recreational activities (Fig. 25). This pond had water lily (*Nymphaeaceae*) inside the pond and arrow arum (*Peltandra virginica*), iris violet (*Iris versicolor*), sage grass (*Andropogon virginicus)* and cattails (*Typha*) along the shore.

4. Brookfield Pond (BR-VA)

Brookfield Pond is located nearby 4598 Brookfield Corporate Drive, VA 22033. It has an area of 0.34 ha and latitude and longitude of 38.881127 and -77.445908 respectively (Fig. 25). This pond had a lot of creeping primrose-willow (*Ludwigia repens),* Joe Pye weed (*Eutrochium purpureum*) and filamentous algae along the shore. There were Canada geese (*Branta canadensis*), schools of sunfish (*Centrarchidae*) and few goldfish (*Carassius auratus*) spotted inside the pond.

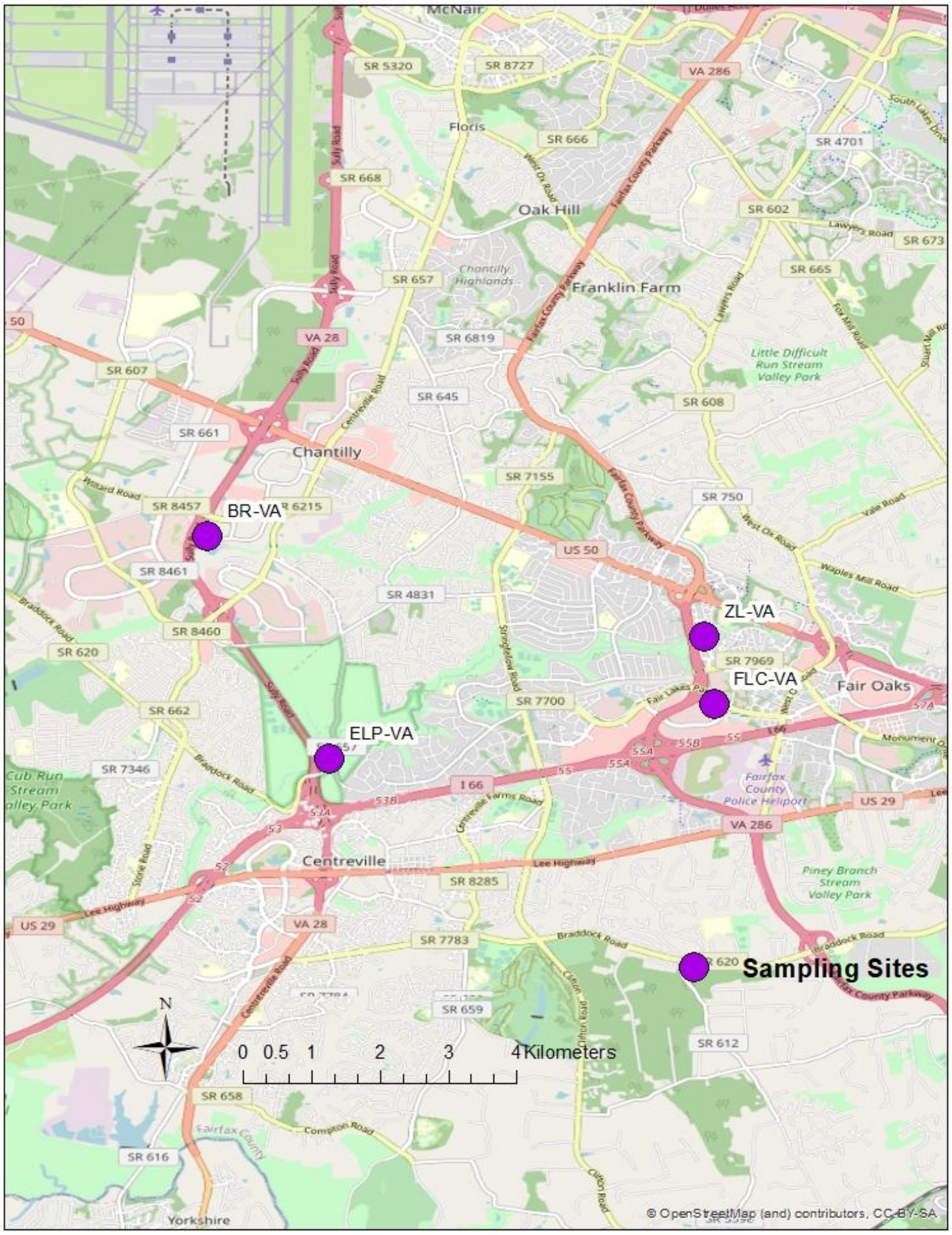


Fig. 25. Water Quality (WQ) sampling stations of ponds without *Trapa bispinosa* in 2020.

WATER QUALITY OF NON-*TRAPA* PONDS

Materials and Methods

The average Water Quality (WQ) of non-*Trapa* ponds was compared with the average WQ of untreated *Trapa* ponds sampled around the similar dates. The non-*Trapa* ponds were sampled on June 3rd and September 6th, 2020. The WQ of VGA-VA sampled on May 28, June 5, and the WQ of HP-VA sampled on May 28 and June 5, 2020, was averaged for comparison with the non-*Trapa* pond WQ of June 3, 2020. The WQ of VGA-VA sampled on August 28, September 4, and September 7 and WQ of HP-VA sampled on August 28 and September 7, 2020, was averaged, and compared with the non-*Trapa* pond WQ of September 6, 2020 (Table 16 and Table 17).

Results

The following are the tables of WQ of *Trapa* ponds sampled around the similar dates with non-*Trapa* ponds.

Table 16. WQ of *Trapa* ponds sampled during the similar time of non-*Trapa* Ponds (Spring Sampling).

Site	Date	Temp	DO mg/L	DO%	pH	SPC	NTU	Alkalinity
VGA-VA	5/28/2020	23.2	3.3	44.8	7.38	170.6	9.8	30
VGA-VA	6/5/2020	24.6	2.84	36	8.06	151.1	8.34	26
VGA-VA	6/12/2020	26.8	3.72	50.4	7.23	149.1	12.3	26
HP-VA	5/28/2020	23.3	4.04	55.4	7.56	214.1	1.56	10
HP-VA	6/5/2020	24.5	6.22	75.8	8.34	43.7	3.93	5
Average		24.48	4.024	52.48	7.71	145.72	7.186	19.4

Table 17. WQ of *Trapa* ponds sampled during the similar time of non-*Trapa* Ponds (Fall Sampling).

Site	Date	Temp	DO mg/L	DO%	pH	SPC	NTU	Alkalinity
VGA-VA	8/28/2020	25.2	2.2	28.3	6.82	148	7.71	36
VGA-VA	9/4/2020	23	1.8	20.1	7	150.3	7.5	40
VGA-VA	9/7/2020	21.8	1.51	18.2	7.15	152.7	6.5	44
HP-VA	8/28/2020	24.8	0.93	15.1	6.81	303.1	0.78	18
HP-VA	9/7/2020	22.6	2.18	26.6	7.043	307.3	4.4	26
Average		23.48	1.724	21.66	6.96	212.28	5.38	32.8

Table 18. Anova (Kruskal-Wallis test) results of *Trapa* vs. non-*Trapa* Ponds.

WQ Variable	P value (Spring)	P value (Fall)
Temp	NS	NS
DO mg/L	0.028	0.014
Do %	0.028	0.014
pH	NS	0.014
SPC	NS	NS
NTU	NS	NS
Alkalinity	0.014	NS

(Note: $p \leq 0.05$ values for null hypothesis of no significant difference among treatments at a given time)

1. Water Temperature

In early June 2020, the average water temperature of non-*Trapa* was 23.82°C (Table 16). The average temperature of *Trapa* ponds was slightly higher and 24.48 °C (Table 16 and 19). In early September 2020, the average water temperature of non-*Trapa* ponds temperature was 25.05°C (Table 17 and 19) and the average water temperature of *Trapa* ponds was 23.48 °C. Thus, the water temperature of non-*Trapa* compared to *Trapa* ponds was less than 1 °C in June and greater than 1 °C in September.

Table 19. Temperature of non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. Temp
6/3/20	23.2	24.5	23.5	24.1	23.82
9/6/20	24.3	25.2	23.7	27	25.05

2. Dissolved Oxygen (DO mg/L)

In early June, the average DO (mg/L) of non-*Trapa* ponds (8.07 mg/L) was higher than the average DO (mg/L) of *Trapa* ponds 4.02 (Tables 16 and 20).

Table 20. DO (mg/L) of non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. DO (mg/L)
6/3/20	6.86	9.87	6.17	9.4	8.07
9/6/20	8.56	11.16	2.62	10.6	8.24

In early September, the average DO (mg/L) of non-*Trapa* ponds (8.24) was substantially higher than the average DO mg/L of *Trapa* ponds (1.72 mg/L) (Tables 17 and 20).

3. Dissolved Oxygen (% Saturation)

In early June, the average DO % of non-*Trapa* ponds higher (96.4) than the average DO% of *Trapa* pond (52.48) (Tables 16 and 21). In early September, the average DO% of non-*Trapa* ponds (101.6) was a lot higher than the average DO% of *Trapa* ponds (21.66) (Table 17 and 21). Among the non-*Trapa* ponds, the DO% was slightly higher in September compared to June.

Table 21. DO% of non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. DO %
6/3/20	82.9	117.8	73	111.8	96.4
9/6/20	104	134.2	34.2	133.9	101.6

4. pH

In early June, the average pH of non-*Trapa* ponds (7.58) was slightly lower than the average pH of *Trapa* ponds (7.72) (Tables 16 and 22). In early September, the average pH of non-*Trapa* ponds was higher (7.45) than the average pH of *Trapa* ponds (6.96) (Tables 17 and 22).

Table 22. Average pH of the non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. pH
6/3/20	7.36	7.46	7.9	7.63	7.58
9/6/20	7.48	7.37	7.2	7.77	7.45

5. Specific Conductivity (SPC)

In early June, the average SPC of non-*Trapa* ponds (288) was higher than the average SPC of *Trapa* ponds (145.72) (Tables 16 and 23). In early September, the average SPC of non-*Trapa* ponds was lower (170.1) than the average SPC of *Trapa* ponds (212.28) (Tables 17 and 23). Among the non-*Trapa* ponds, SPC was a lot higher in June compared to September (Table 23).

Table 23. Average SPC of non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. SPC
6/3/20	331.1	513	95.1	212.5	288
9/6/20	189.9	303.3	61.8	125.5	170.1

6. Turbidity (NTU)

In early June, the average NTU of non-*Trapa* ponds (6.03) was lower than the average SPC of *Trapa* ponds (7.18) (Tables 16 and 24). In early September, the average NTU of non-*Trapa* ponds was lower (4.03) than the average NTU of *Trapa* ponds (5.38) (Tables 17 and 24).

Table 24. Average NTU of Non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. NTU
6/3/20	8.2	6.69	4.36	5.09	6.08
9/6/20	3.93	5.84	4.25	2.11	4.03

7. Alkalinity (mg/L as $CaCO_3$)

In early June, the average alkalinity of non-*Trapa* ponds (43.2) was higher than the average alkalinity of *Trapa* ponds (19.4) (Tables 16 and 25). In early September, the average alkalinity of non-*Trapa* ponds (32) was almost similar to the alkalinity of *Trapa* ponds (32.8) (Tables 17 and 25). Among the non-*Trapa* ponds, alkalinity was higher in June compared to September (Table 25).

Table 25 Average Alkalinity of non-*Trapa* Ponds.

Date	ZL-VA	FLC-VA	ELP-VA	BR-VA	Avg. Alkalinity
6/3/20	36	45	31.5	63	43.9
9/6/20	27.5	37.5	17.5	45	32

Discussion

1. Water Temperature

In general, water temperature followed a similar consistent and seasonal pattern in both treated and untreated ponds. The location of the pond with full access to sunlight and the time of sampling during day might have been responsible for the slight variation in temperature. For example, VGA-VA, WP-VA, and HP-VA are located in the sunny area with full access to sunlight and HO-VA is located in the shady area with lot of overhanging vegetation. In addition, the sampling was generally conducted in the morning in VGA-VA, HO-VA, HP-VA and ML-VA and in the afternoon in WP-VA, WL-VA and GCP-VA (A and B).

Water temperature of non-*Trapa* ponds also followed a slight seasonal variation in temperature in early June and early September. There was not a significant difference in temperature in *Trapa* vs. non-*Trapa* ponds (Table 18).

2. Dissolved Oxygen (mg/L)

In untreated ponds, DO (mg/L) was higher than the WQ-5 in April, May, and November and decreased below it during June, July, August. In September, it decreased below 2 mg/L. This is due to the dense *Trapa* growth during those months. DO (mg/L) recovered and increased during the month of October and November when the dense *Trapa* beds started to thin out followed by the death. The depletion of DO (mg/L) was somewhat less in ponds with thin *Trapa* coverage. As for example, HO-VA had about 25% of the total *Trapa* coverage and DO (mg/L) level was still above the WQ-5 in June and generally above hypoxia threshold in other months of *Trapa* peak growth in July, August and October (Ch-2, Table 4). Among the treated ponds, DO was higher than the untreated ponds and the difference was higher during the peak growth. This could be because GCP-VA(A) and ML-VA had very little coverage of *Trapa* due to the herbicide treatment. ML-VA had the average *Trapa* coverage of 0.5% and GCP-VA (A) had 5% (Tables 5 and 6, Ch-2).

The average DO (mg/L) of non-*Trapa* ponds was higher than the average DO of *Trapa* ponds both in June and September. DO was higher than standard WQ-5 with the exception of ELP-VA whose DO mg/L was 2.62 mg/L in September. This might be due to the floating leaved water lily (*Nymphaeaceae*) covering up-to 30% of this pond in September (Tables 16, 17 and 20).

There was a significant difference between the DO (mg/L) in *Trapa* vs non-*Trapa* of ponds (Table 18). *Trapa* creates a canopy that interrupts the passage of light through water which inhibits photosynthesis and prevents oxygenation in the deeper layer and floating leaves of *Trapa* release oxygen directly to the atmosphere (Strayer 2010 and Tall et al., 2011). This finding is similar to the previous studies done on dense *T. natans* beds by Caraco et al., 2002, Takamura et al., 2003, Hummel & Findlay, 2006 and Kato et al., 2016.

3. DO (% Saturation)

In untreated ponds, the amount of DO (%) was higher in the months when there were fewer *Trapa* compared to the months when there were dense *Trapa*. The dense *Trapa* growth during those months was responsible for the DO depletion as explained in the previous section.

The seasonal variation of DO (%) of untreated ponds was more consistent in both 2019 and 2020 and followed a linear pattern with some exceptions. The range was lower in 2019 compared to 2020. In VGA-VA, the DO (%) depleted highly during November when the density and the coverage of *Trapa* was already depleting (Appendix, Table 37). This phenomenon is difficult to explain. In general, the treated ponds had higher DO (%) than the untreated ponds.

The average DO (%) of non-*Trapa* ponds was generally higher than the average DO (%) of *Trapa* ponds both in June and September. Thus, the higher level of DO in non-*Trapa* ponds and lower level of DO (%) in *Trapa* ponds could be due to the dense *Trapa* growth in *Trapa* ponds. However, in ELP-VA whose DO (%) was lot less than other non-*Trapa* ponds. The low DO (%) of ELP-VA during September could be attributed to the growth of the floating leaved water lily (*Nymphaeaceae*) inside the pond (Tables 16, 17 and 21). DO % was significantly lower in *Trapa* ponds compared to non-*Trapa* ponds (Table 18). This result is similar to the study done by Akobari et al., 2016 in lake Inba Japan where DO % was lower in the *T. natans* beds compared to open water without *Trapa*.

4. pH

In untreated ponds, the range of pH was less consistent in both sampling years except in WP-VA and HO-VA. pH was generally higher in 2020 compared to 2019. Among the treated ponds, the pH of ML-VA followed a wider trend whereas the pH of WL-VA, GCP-VA(A), GCP-VA(B) followed a consistent trend. The average pH of non-*Trapa* ponds was slightly lower than the average pH of *Trapa* ponds in June whereas in early September, the average pH of non-*Trapa* ponds was higher than the average pH of *Trapa* ponds (Tables 16, 17 and 22). Overall, pH varied very little pH in spring but the pH difference in fall was statistically significant in *Trapa* vs non-*Trapa* (Table 18). The reason behind this is difficult to explain.

5. Specific Conductivity (SPC)

SPC followed the consistent pattern in both sampling years in untreated ponds, whereas it was inconsistent in treated ponds. There was an occasional decline in SPC along with the precipitation in both type of ponds.

The average SPC of non-*Trapa* ponds in June was higher than the average SPC of *Trapa* ponds in June and in September it was lower than the *Trapa* ponds (Tables 16, 17 and 23).

There was no significant difference in SPC between *Trapa* vs. non-*Trapa* ponds (Table 18).

6. Turbidity (NTU)

In general, there was an occasional increase and decrease in turbidity which correlated with the rainfall pattern in both untreated and treated ponds. The pattern was more consistent in untreated ponds compared to treated ponds. Among the untreated ponds, turbidity pattern was smoother in 2019 compared to 2020 (Tables 16, 17 and 24).

The turbidity of most of the non-*Trapa* ponds was slightly less than the turbidity of *Trapa* ponds. There was no significant difference between the NTU of *Trapa* vs non-*Trapa* ponds in both season (Table 18).

7. Alkalinity (mg/L as $CaCO_3$)

In general, alkalinity of untreated and treated ponds decreased towards the end. However, in the untreated pond (HP-VA), it increased towards the end. Alkalinity occasionally increased and decreased along with the precipitation in both treated and untreated ponds.

The average alkalinity of non-*Trapa* ponds was higher than the average alkalinity of *Trapa* ponds both in June and September. However, the range of difference was higher in June compared to September (Tables 16, 17 and 25). There was a significant difference in alkalinity in Spring in *Trapa* vs. non-*Trapa* ponds. The reason behind this is difficult to explain. It might be possible that underlying geology such as bedrock containing

$CaCO_3$, precipitation and the runoff from the lawns containing $CaCO_3$ could be responsible for the increase in alkalinity.

Overall, the result of this study showed the significant depletion of DO (mg/L) and DO (%) in *Trapa* ponds compared to non-*Trapa* ponds which is similar to previous studies. There was no significant difference in SPC and NTU between these two types of ponds. pH of *Trapa* pond was less than the pH of non-*Trapa* ponds in fall. Alkalinity of *Trapa* ponds was less than the alkalinity of non-*Trapa* ponds in spring. The result of NTU was different from the previous study done by Akobari et al. (2016), in dense *T. natans* bed in Lake Inba Japan which showed a lower NTU in the *Trapa* beds compared to open water.

Conclusions

Thus, the WQ parameters varied according to the density of *Trapa* growth. Most of the WQ parameters were more consistent in untreated ponds compared to treated ponds except pH which was more consistent in treated ponds compared to untreated ponds. Dissolved Oxygen (mg/L and %) was significantly depleted in *Trapa* ponds compared to non-*Trapa* ponds. pH was less in fall and alkalinity was less in spring in *Trapa* ponds compared to non-*Trapa* ponds.

SHORT TERM DIURNAL EXPERIMENT ON *T. BISPINOSA*

Introduction

When growing robustly, *Trapa* create a canopy that interrupts the passage of light through water (Tall et al., 2011; Groth et al., 1996) inhibiting photosynthesis and preventing oxygenation in the deeper layer (Strayer, 2010). Floating leaved plants of *T. natans* release oxygen directly into the atmosphere while depleting oxygen from the surrounding water (Tall et al., 2011) resulting in hypoxia and anoxia (USDA, 2016). In a large (900,000 m^2) *T. natans* bed in the Hudson River, DO oscillated with the tide between 0 and 8.2 mg/L at the edge of the bed and between 0 and 6.0 mg/L at the inner site with extremely low DO values (below 2.5 mg/l) 42% of the time (Caraco & Cole, 2002; Hummel & Findlay, 2006). DO less than 5mg/L negatively affects sensitive fish and invertebrates. Most fish are negatively affected in DO below 2.5 mg/L (Frodge et al., 1990).

T. natans both produces and consumes dissolved oxygen (DO) with the balance depending on the photosynthetic capacity of plants and the release of oxygen into water or atmosphere. Since the lower portion of *Trapa* shoots have chlorophyll containing tissues, it is possible that at lower densities, *Trapa* can actually contribute substantial amount of oxygen in water by conducting photosynthesis and thus raise DO concentrations. There is evidence of direct correlation between rates of photosynthesis and increase in the dissolved organic carbon (DOC) in aquatic plant beds (Wetzel, 1969; Hummel & Findlay, 2006). The large beds of *T. natans* would decrease turbidity, NO_3,

NH_4 and PO_4 and increase DOC whereas the smaller beds would have lesser or no effect on such parameters (Hummel & Findlay, 2006).

According to Goldhammer and Findlay (1988), the water flowing into and out of a tidal cove covered with *T. natans* (Tivoli South Bay, NY) exhibited a reduction in suspended matter leaving the cove when compared to incoming water (Goldhammer & Findlay, 1988; Hummel & Findlay, 2006). Because sediment deposition increases as flow is decreased, *Trapa* beds may enhance settling of suspended solids thus reducing turbidity and contributing to the sediment accumulation (Hummel & Findlay, 2006). The effect of aquatic plants on water velocity has direct implications for transport of water column constituents such as particulate matters, plankton, and detritus (Abdelrhman, 2003, Hummel & Findlay, 2006). A mesocosm study on *Trapa* and other SAV showed that *T. natans* also has the potential to remove potassium and calcium from water (Shrivastava et al., 2009).

The effect of *T. japonica* study in water quality of Japanese temperate lake Mikata showed that formation of dense *Trapa* beds during summer resulted in hypoxia and the growth of benthic communities resistant to hypoxia such as Cladocera, Cyclopoida, Ostracoda and Nematoda. But the decline in *Trapa* beds from autumn to spring resulted in increased dissolved oxygen concentration and invertebrate abundance (Kato et. al, 2016).

When the water characteristics of *T. japonica* was compared with the mixed beds of submerged aquatic vegetation, there were a wide range of nutrient level, steeper extinction of light, higher concentration of dissolved organic carbon (DOC) and lower concentration of dissolved oxygen (DO) on the bottom of the *Trapa* beds than in the SAV (Takamura et al., 2003; Hummel & Findlay, 2006). The percentage of DO and turbidity were lower in *T. natans* beds than the open water without it in a study done in Lake Inba Japan (Akabori et al., 2016).

An experiment to assess phytoremediation capacity of *T. natans* on municipal wastewater revealed that *T. natans* significantly reduced total dissolved solids (TDS), SPC, biological oxygen demand (BOD), chemical oxygen demand (COD), total Kjeldahl nitrogen (N), phosphate (P), sodium (Na), potassium (K) and calcium (Ca) (Kumar and Chopra, 2018). There are many studies on the of uptake of inorganic nutrients by *Trapa* in lakes and ponds in Europe and Japan (Juget & Rostan, 1973; Kaspati & Pomogyi, 1979). The majority of the studies examined the effects of environmental factors on the growth and productivity of *Trapa* beds (Nakano & Seki, 1981; Kadono, 1982; Sastroutomo, 1982; Hamashima, 1983; Tsuchiya & Iwakuma, 1993), rather than the effects of *Trapa* on its environment (Hummel & Findlay, 2006).

Hypothesis

There will be a diurnal effect of *Trapa* on water quality and this effect will vary with *Trapa* density.

Materials and Methods

On August 20, 2020, a one-day hydroponic experiment was conducted to examine the effect of *Trapa* on WQ parameters. These WQ parameters were temperature, DO (% saturation and mg/L), pH, SPC and NTU. The experiment was set up in white plastic dish pans (10.8 L and dimension of 36.7cm* 31.9cm*14.4 cm) that were placed in a 3 by 5 array outdoors on a flat surface with full sunlight at Potomac Science Center (PSC) parking garage upper level (on the roof) in Fairfax County, VA. A random number generator was used to assign the pans randomly to one of three treatments. Four to six pans were established at each of three levels of *Trapa* biomass: no *Trapa*; Low *Trapa* (1 rosette) and Medium *Trapa* (3 rosettes). These levels simulated low to moderate densities of *Trapa*.

The following were the sets of pans:

No *Trapa* (Treatment 0)- Pan 1, 2,3, 4, 5, 6 (6 became 0 instead of 1 due to experimental error).

Low *Trapa* (1 *Trapa* rosette per pan-Treatment 1)- Pan 7, 8, 9,10.

Medium *Trapa* (3 *Trapa* rosettes per pan- Treatment 3)- Pan 11, 12, 13, 14, 15.

The following was the placement of pans randomly on the flat surface.

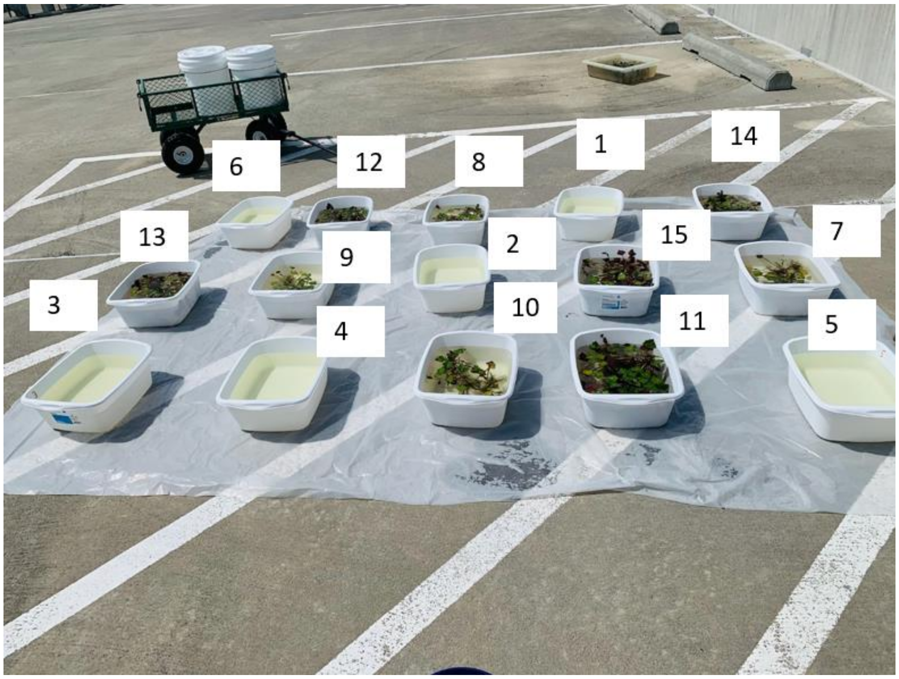


Fig. 26. ***Trapa*** **Diurnal Experiment at Potomac Science Center (PSC) Parking Garage -Upper level.**

(Note-No. 1-15 are the number assigned to experimental pans, 8/20/2020).

Fig. 27. ***Trapa*** **Diurnal Experiment at Potomac Science Center (PSC), Medium** ***Trapa*** **(3 rosettes).**

Fig. 28. *Trapa bispinosa* with floating leaves (top); submerged leaves (bottom) (Source: nas.er.usgs.gov).

These pans were filled with 8 liters of water from a small pond on the Potomac Science Center (PSC) site. Each pan was stocked with the appropriate level of *Trapa* as given in the experimental design noted above. These experimental *Trapa* were collected from VGA-VA in the late afternoon of August 19, 2020 and stored in water from the PSC pond at ambient temperature in the dark overnight. While collecting the plants, they were selected to include 0.3 m shoot sections which contained underwater photosynthetic tissue such as submerged leaves (Fig. 28).

The experiment was launched in the morning and the measurements were taken at four times. Time 1 was before *Trapa* addition and started at 9:45 am and took about 15 minutes to complete measurements. Time 2 was recorded immediately after *Trapa* addition at 10:20 am. Time 3 was at mid-day (1:50pm), and Time 4 was at 5:20 pm (1 hour before sunset) (Table 26). Temperature, DO, pH, SPC and NTU were recorded in each pan at Times 1-4 using YSI sonde Pro DDS. At the end of the experiment, *Trapa* were harvested from each pan, dried at 70°C, and weighed so that the exact biomass of *Trapa* is known for each dish pans. Results of the experiment were analyzed using the non-parametric Kruskal Wallis analysis of variance (ANOVA) routine in SYSTAT with significant multiple comparisons assessed using Conover-Inman test.

Table 26. Time Codes with Experimental Design.

Time Codes	Time*	Treatment
Time 1	9:45 am	Water only (Blank or Control)
Time 2	10:20 am	Water + *Trapa* treatment (mid-morning)
Time 3	1:50 pm	Water + *Trapa* treatment (mid-day)
Time 4	5:20 pm	Water + *Trapa* treatment (before sunset)

(Note: Time*- Measurement Start Time)

Table 27. Biomass of *Trapa* from each Replicate.

Replicates	Dry Biomass (g)
Pan 1-6	0
Pan 7	2.0
Pan 8	3.0
Pan 9	1.5
Pan 10	2.0
Pan 11	11.5
Pan 12	8.0
Pan 13	5.5
Pan 14	5.0
Pan 15	8.5

(Note: Biomass=Net dry weights of *Trapa* plants; Pans 1-6- No *Trapa*; Pans 7,8, 9, 10- Low *Trapa*; Pans 11, 12, 13, 14, 15- Medium *Trapa*)

Results

Several parameters were different among the three times or three plant density treatments. Temperature and DO (% saturation), peaked at times 3 and 4 with a slight decrease at time 4 compared to time 3 (Figs. 29A and 29D). DO (mg/ L) decreased steadily at treatment 0, whereas at treatment 3, it increased slightly at time 3 and decreased slightly at time 4 (Fig. 29C). SPC, pH and NTU did not vary much which ranged from 48.14 to 53.55, 7.23 to 8.21 and 0.66 to 2.26 respectively (Table 28, Figs. 29B, 30A and 30B). Turbidity was low and there was a little pattern related to treatment or time. pH dipped at all density at time 2 (Fig. 30B). DO (mg/L) was always above a level detrimental for fish. DO (mg/L) was greater than 8 mg/L and DO (% saturation) was greater than 100 % (Figs. 29C and 29D). According to Kruskal- Wallis Anova result, there was not a significant effect of temperature, DO (mg/L), DO (% saturation), SPC, pH and NTU at Time 1 (Table 29). Temperature, DO% and SPC had a significant effect at Time 2, 3 and 4. DO mg/L was significant at Time 3 and 4. pH and NTU had no significant effect at all times.

The relationship between the DO (% saturation) and dry biomass of *Trapa* at mid-day and late afternoon sampling is shown by the graph of Lowess Smoother curve (Fig. 31). During the mid-day, there was a rapid increase in DO (% saturation) along with the increase of *Trapa* dry weight. During late afternoon, there was also an increase in DO (% saturation) along with *Trapa* dry weight, but not as strongly as during the mid-day (Fig. 31). DO (% saturation) and *Trapa* biomass were highly correlated during mid-day and late afternoon with Pearson correlation value of 0.86 and 0.77 respectively ($p<0.01$, $n=15$) (Source; Statistical Methods, 1946).

Table 28. Mean value of Temp, DO mg/L, DO%, pH, SPC and NTU versus *Trapa bispinosa* density Treatment and Time.

Treatment	Time Code	Mean Temp	Mean DO mg/L	Mean DO%	Mean pH	Mean SPC	Mean NTU
0	1	25.8	8.54	105.1	7.74	49.9	1.44
0	2	26.7	8.57	107.2	7.23	50.1	1.55
0	3	33.3	8.26	115.4	7.78	52.2	1.41
0	4	32.2	8.07	111.0	8.08	53.6	1.32
1	1	25.8	8.53	104.9	7.81	49.7	1.01
1	2	26.9	8.62	108.1	7.25	49.9	1.17
1	3	34.4	8.65	123.0	7.79	50.9	1.25
1	4	33.4	8.56	119.6	8.12	51.1	0.66
3	1	25.8	8.65	106.1	7.79	49.8	1.39
3	2	26.9	8.75	109.8	7.34	50.4	2.26
3	3	34.5	8.94	127.3	7.83	49.7	1.53
3	4	33.8	8.75	123.2	8.21	48.1	1.12

(Note: Time code 1-9:25 am, Time Code 2- 10:20 am, Time Code 3- 1:50 pm, Time Code 4- 5:20pm; Treatment 0-No *Trapa* or Control, Treatment 1- Low (1) *Trapa* and Treatment 3-Medium (3) *Trapa*)

Table 29. P values of Anova (Kruskal-Wallis) Results.

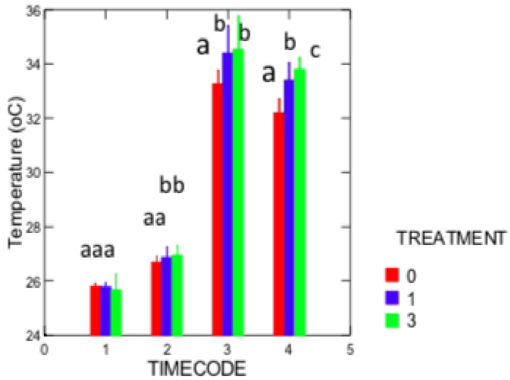
Variables	Time 1	Time 2	Time 3	Time 4
Temperature	NS	0.05	0.009	0.003
DO mg/L	NS	NS	0.002	0.003
DO %	NS	0.027	0.002	0.002
SPC	NS	0.027	0.003	0.002
pH	NS	NS	NS	NS
NTU	NS	NS	NS	NS

(Note: $p \leq 0.05$ values for null hypothesis of no significant difference among treatments at a given time)

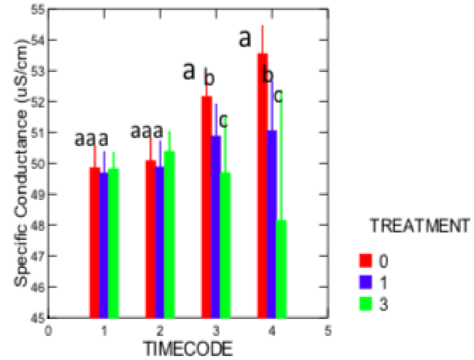
Table 30. Significance of Pairwise Comparisons among Individual Plant Density Treatments (0, low and medium) at each of four time Periods for each Water Quality Parameter.

		p for Time 1	p for Time 2	p for Time 3	p for Time 4
Treatments Compared		Temperature	Temperature	Temperature	Temperature
0	1	NS	NS	0.002	0.001
0	3	NS	0.016	0.001	0.000
1	3	NS	NS	NS	0.012
		Do mg/L	Do mg/L	Do mg/L	Do mg/L
0	1	NS	NS	0.000	0.001
0	3	NS	NS	0.000	0.000
1	3	NS	NS	0.001	0.020
		Do%	Do% (.027)	Do% (.002)	Do% (.002)
0	1	NS	NS	0.000	0.001
0	3	NS	0.004	0.000	0.000
1	3	NS	0.048	0.001	0.006
		SPC	SPC	SPC	SPC
0	1	NS	NS	0.001	0.001
0	3	NS	NS	0.000	0.000
1	3	NS	NS	0.007	0.002
		pH	pH	pH	pH
0	1	NS	NS	NS	NS
0	3	NS	NS	NS	NS
1	3	NS	NS	NS	NS
		NTU	NTU	NTU	NTU
0	1	NS	NS	NS	NS
0	3	NS	0.046	NS	NS
1	3	NS	0.054	NS	NS

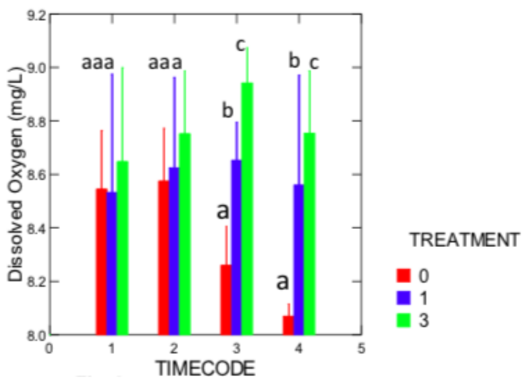
(Note: Time 1-9:25 am, Time 2- 10:20 am, Time 3- 1:50 pm, Time 4- 5:20pm; NS- Not Significant; p=Conover-Inman p value; $p \leq 0.05$. Significance of pairwise comparison among individual treatment is shown on the three lines below the headings for each parameter and time)



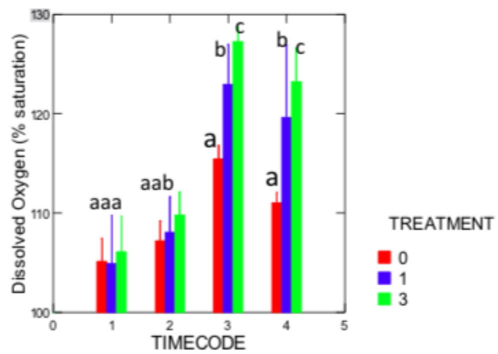
A) Temperature



B) Specific Conductance (SPC)

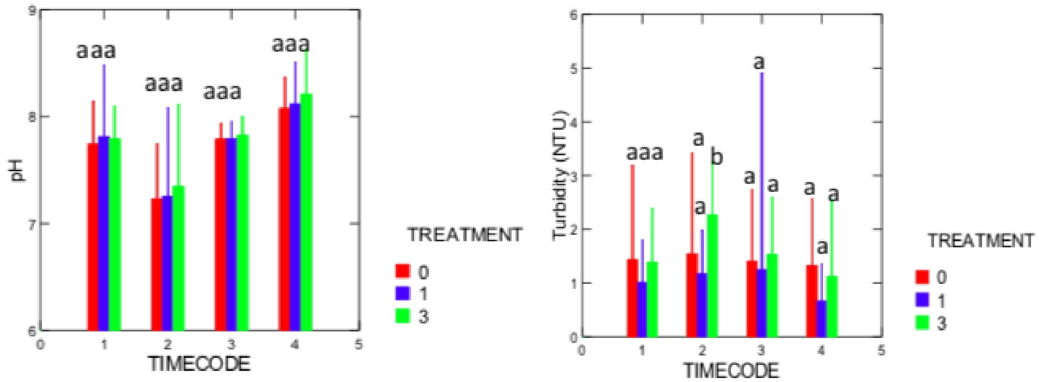


C) Dissolved Oxygen (mg/L)



D) Dissolved Oxygen (% saturation)

Fig. 29. A- D show Temperature, Specific Conductance (SPC), Dissolved Oxygen (mg/L) and Dissolved Oxygen (% saturation).



A) pH B) Turbidity (NTU)

Fig. 30. A and B show pH and Turbidity (NTU).

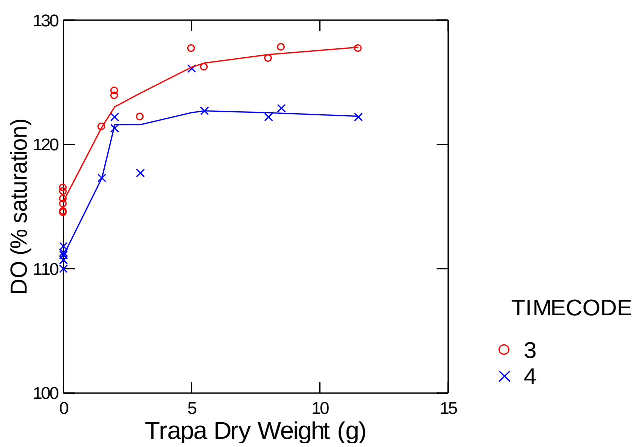


Fig. 31. Relationship between DO (%) and dry biomass of *Trapa* at mid-day and afternoon Sampling.

<u>Discussion</u>

Water temperature varied significantly with *Trapa* density in later part of the day. Temperature of water inside the pans increased because darker leaves of *Trapa* could absorb more sunlight raising the temperature of its habitat along with the increase in solar intensity gradually from mid-morning to mid-day and finally one hour before the sunset (Tables 29, 30 and Fig. 29A).

DO (mg/L) and DO (% saturation) of the medium density of *Trapa* increased strongly from morning until mid-day but decreased in the late afternoon. DO (mg/L) and DO (% saturation) at Time 3 and Time 4 showed significant differences among all the treatments. This result suggests that mid-day solar radiation was effective in raising oxygen concentration in *Trapa* treatment by photosynthesis of submerged leaves which excrete their oxygen into water (Table 30 and Figs. 29C and 29D). Further evidence for this was provided by the positive relationship between DO (% saturation) and *Trapa* biomass. Even though, *Trapa* roots were excluded in this experiment, the submerged leaves might have been able to conduct photosynthesis and release oxygen as its byproduct in water. DO (% saturation) was slightly lower with the low density of *Trapa* at Time 1. This low DO (% saturation) being less than the control could be due to depletion of oxygen by *Trapa* respiration and not being able to replenish it by conducting photosynthesis during early morning (Fig. 29D).

NTU and pH varied very little during this experiment and were not statistically significant almost all times or treatments. NTU was significant only at Time 2 with medium *Trapa* (Table 30). SPC was significant among treatments at Time 3 and 4. The lowest level of SPC at Time 4 with the medium *Trapa* and the highest at Time 4 without *Trapa* suggests that *Trapa* might be responsible for the decrease in SPC. In addition, sunlight might play some role in its decrease, but it does not require the maximum sunlight of mid-day (solar noon) (Table 30, Fig. 29B). The most obvious mechanism for the decrease in SPC in the *Trapa* treatments relative to control would be uptake of ions by *Trapa* as it metabolizes. The increase in SPC in the control is more difficult to explain.

Overall, these findings are in contrast with field observations done in the dense *Trapa* beds from the literature outlined in the introduction of this section. This difference may be due to the fact that our *Trapa* densities were lower than normally used in the field studies which allowed substantial light to penetrate to the submerged leaves. The field studies focused on very dense *Trapa* beds. Even our field studies showed consistently lower DO (mg/L and % saturation) in ponds with high proportion of *Trapa* coverage during summer (June, July and August) compared to spring and fall. In our field experiment, DO (mg/L) was mostly below WQ-5 but above 2mg/L in June, July, August and September in VGA-VA where *T. bispinosa* was healthy, dense and abundant. DO (% saturation) was above 25 in June, July, August and September but higher than 40 in spring and rest of the fall months in VGA-VA (Ch-3, WQ). In this experiment turbidity was very low (1 NTU) and not affected by *Trapa*. The result of this experiment is consistent to the previous studies done in *T. natans* in Hudson River by Hummel and Findlay (2006) and Akobari et al. (2016) in Lake Inba, Japan in terms of DO and NTU. Both of these studies showed that smaller amount of *Trapa* had less effect on DO and NTU.

Conclusions

This study demonstrated the diurnal effect of none, one, and three rosettes of *T. bispinosa* on dissolved oxygen and temperature in a 10. 8-liter container experiment.

Temperature and DO (% saturation) increased during the afternoon period and one hour before the sunset. Overall, DO (mg/L) increased in the treatments whereas it decreased steadily in the control. DO (mg/L) was always more than 8mg/L which was well above the standard water quality (5mg/L) and hypoxia (2mg/L) level. DO (% saturation) was above 100%. This leads to the fact that less amount of *Trapa bispinosa* in the container does not deplete the DO level, but it leads to an increase. SPC, NTU and pH varied very little. The impact of *Trapa* in raising the water temperature has not been documented in previous studies. This study will add significant relevance to the literatures since there is not much information in the effect of *T. bispinosa* in the WQ of the US.

EARLY DETECTION AND RAPID RESPONSE (EDRR) AS A POLICY TOOL FOR *TRAPA BISPINOSA* CONTROL

Literature Review

Early Detection and Rapid Response (EDRR) is a guiding principle for minimizing the impact of invasive species in a rapid, effective and cost-efficient manner. ‘‘Detection’’ is the process of observing and documenting an invasive species. “Response’’ is the process of reacting to the detection once the organism has been authoritatively identified and response options assessed. The ideal outcome of invasive species detection is the eradication even though there is a narrow window of opportunity for success due to the self-perpetuating nature of invasive species (Reaser et al., 2019 and Martinez, 2020). The success rate of the response options towards the invasive species depends on the area it covers. The larger the population and area it covers, the greater the likelihood that response options will no longer be feasible (Simberloff, 2003 and Martinez, 2020). It is not possible to stop all invasive species, even with the best prevention methods. However, EDRR can slow the range of expansion and avoid the need of costly long-term efforts. EDRR has been cited as the best management practice against the establishment of invasive species (Westbrooks 2004; Wittenberg & Cock 2001; Crall et al., 2012).

In the US, three presidential executive orders (EO) have explicitly recognized and focused on the threats posed to national security by harmful non-native species, tasking federal agencies to coordinate a high-level effort and cost-efficient approach to invasive species prevention, eradication, and control (Executive Office of the President

1977, 1999, 2016). Invasive species are detected and responded along invasion pathways into the country or at US national borders, prior to entering the country. The federal government bears primary leadership responsibility for these actions. When these pathway management and border control efforts fail to intercept harmful non-native species, the costs of action increase and the burden of defense falls upon land management and transportation agencies across all levels of government, private and public sector (Reaser, 2020).

Thus, the federal government must coordinate and use applicable federal frameworks, investments, assets, and expertise to detect and respond to invasive species incursions in an effective and cost-efficient manner. This is only possible by coordination, integration, and communication among the agencies with EDRR-related activities. Legal and institutional capacity-building agencies should harmonize and expand the legal and institutional frameworks necessary to enable the rapid detection and response towards invasive species while at the port of entry or after entering it. Planning and decision support agencies should support EDRR-related planning approaches to increase the speed and effectiveness of invasive species detection and response measures. Data collection agencies should collect and share EDRR-relevant information including data on non-native species occurrence, identification, biology, risks and impacts, response options and effectiveness making it available to researchers and the general public. In addition, scientific and technological agencies should enhance EDRR efforts by carrying out and supporting relevant research and technology innovation and transfer (Reaser, 2020).

Effective EDRR depends upon the timely ability to answer some critical questions such as: what is the species of concern; is the species authoritatively identified; where is it located and likely to spread; what is the possible harm caused by the species; what are the possible actions; who has the needed authority and resources and how will the efforts be funded (www. Invasive.org). The following flow chart describes about the useful procedures for a successful EDRR (Fig. 32).

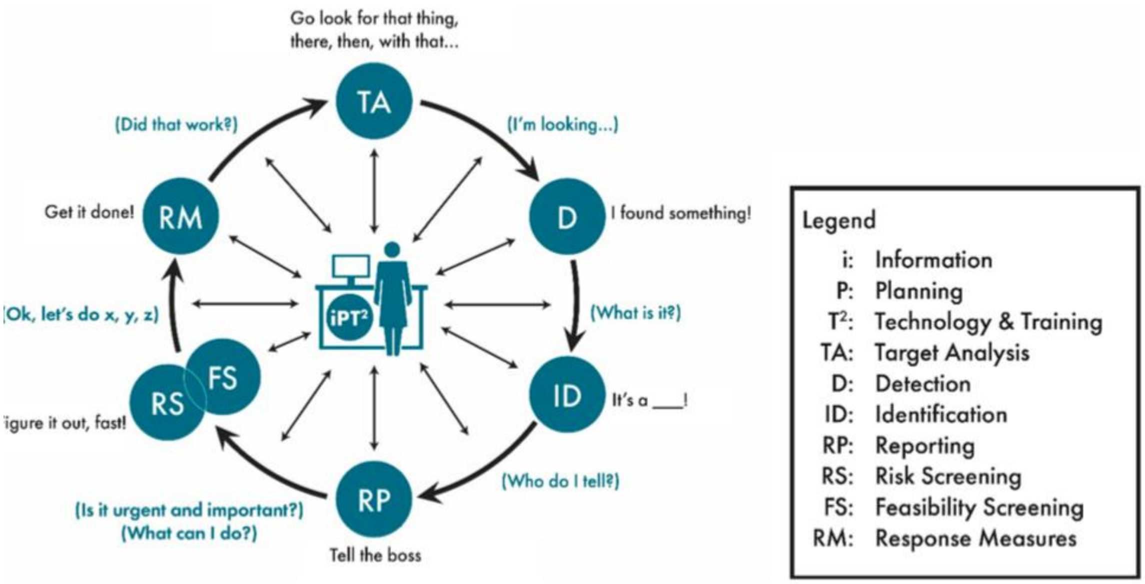


Fig. 32. Early Detection and Rapid Response (EDRR) (Source: Reaser et al., 2020).

In this model, blue circles represent the primary actions that need to be enacted in a step-wise manner for successful EDRR. At the core of the process, it is represented by the person or workstation. The legend shows the meaning of the letters in the circles.

Arrows point in both direction because the information and output generated by one component are strategically utilized by other components for a sustainable EDRR.

In response to the Healthy Forest Restoration Act of 2003, USDA Forest Service has monitored a framework known as Early Warning System (EWS). EWS is developed for early detection and response to environmental threats to forest lands in the United States. The EWS focuses on the key steps such as identification and detection of actual threats, impact assessment and response to minimize the environmental threats (www.fs.fed.us).

According to Federal Interagency Committee for the Management of Noxious and Exotic Weeds (FICMNEW), EDRR is the most cost-effective and environmentally sound approach. The overall goal of FICMNEW National EDRR system for invasive plants is to minimize the establishment and spread of new species through a coordinated framework of public and private partners and processes through early detection and reporting of suspected new plant species to appropriate officials. After full implementation across the US, an EDRR system will provide an important second line of defense in the US port of entry. The first line of defense against invasive species is US Custom and Border Protection (CBP) (www.doi.gov). CBP regulates and facilitates international trade, import, enforces U.S. regulations, including trade, customs, and immigration (www.invasive.org).

USDA's Animal and Plant Health Inspection Service (APHIS) was charged with implementing Section 10007; (initially part of the 2014 Farm Bill) to prevent the introduction or spread of plant pests and diseases that threaten U.S. agriculture and the environment. Under the Farm Bill, APHIS provides funding to strengthen the nation's infrastructure for pest detection and surveillance, identification, and threat mitigation, while working to safeguard the nursery production system. In addition, USDA has launched certain tools for the surveillance of the aquatic plants in the great lakes and other regions (www.invasivespeciesinfo.gov). The Invasive Plant Atlas of New England's (IPANE) mission is to create a comprehensive web-accessible database of invasive and potentially invasive plants in New England that can be continually updated by a network of professionals and trained volunteers (www.eddmaps.org/ipane/).

National Agricultural Pest information system (NAPIS) is the database for Cooperative Agricultural Pest Survey and related pest detection surveys. More than 5 million records summarize survey results for 6,385 insects, pathogens, weeds, mollusks and biological control organisms. Pest detection survey observations recorded in NAPIS emphasize exotic pests that may impact exports of U.S. agricultural products; or damage in agricultural production and natural resources (www.napis.ceris.purdue). Pest Tracker (exotic pest reporting) is a program which suggests the residents to keep their eyes open for damage to trees and plants in the yards and gardens. If someone is not familiar with the insect pests, or disease symptoms, the report can be sent to a pest tracker program which helps to investigate if there are any exotic pests. There are various home pest tracking programs such as CAPS and Zpest Tracker programs in different States, which help in EDRR by sorting out the unwanted species as incidental, indicator, nuisance, pest or predator required to control its spread (www. pest.ceris.purdue.edu).

USGS Nonindigenous Aquatic Species (NAS) is a central repository for spatially referenced biogeographic accounts of introduced aquatic species of United States. The program provides scientific reports, online/real-time queries, spatial data sets, distribution maps, and general information. The data can be used by biologists, interagency groups, and the general public (www.nas.er.usgs.gov).

EDRR programs require an understanding of vector and species spatial dynamics to prioritize monitoring sites and efficiently allocate resources. EDDR can be effective in early detection and removal of aquatic nonindigenous species (ANS) (Wimbush et al. 2009), but they face many challenges, such as the delay in publishing useful tools and disseminating knowledge once a study has been finished (Darbyson et al., 2009 & Rothlisberger et al., 2010). The quality and availability of knowledge used to inform and prioritize EDRR efforts occur along a spectrum, from very low (educated guesses on potential ANS and their associated vectors) to very high (complete understanding of potential ANS and their associated vectors) but generally present toward the low end of the spectrum (Davidson et al., 2015). Even though a national model for effective EDRR has been described for invasive plants (FICMNEW, 2003 & Crall et al., 2012); its implementation is hampered by insufficient financial resources and the difficulty in management (US General Accounting Office, 2001 & Crall et al., 2012). There are very few examples of successful eradication of plant species using EDRR and also the efforts that seek to protect the entire ecosystem (Hulme, 2006 & Crall et al., 2012).

Technology innovation plays an important role in the advancement of early detection and rapid response to invasive species. While applying the technology in the context of EDRR, decision makers and invasive species practitioners need to consider that technological application is governed by policies and regulations which foster or hinder EDRR efforts (Burgos- Rodriguez and Burgiel, 2019: Martinez et al., 2020) and social acceptance is necessary for enacting EDRR (Kendal and Ford 2017, 2018; van Putten et al., 2018 and Martinez et al., 2020). Accurate, accessible, up-to-date information is necessary to support every aspect of EDRR (Reaser et al., 2019; Wallace et al., 2019 and Martinez et al., 2020). Artificial intelligence (AI**)** is the ability of machines to acquire and apply information and social media. It can be used to understand the invasive species biology and occurrence data necessary to inform detection and response strategies (Martinez et al., 2020).

Internet commerce (e-commerce) is a vital part of the US economy that has been used widely recently. Some of the federal entities have jurisdiction over aspects of e-commerce and recently, the National Invasive Species Council's Invasive Species Advisory Committee (ISAC) recommended USDA, USFWS, and the Department of Homeland Security (DHS) expand the use of web crawlers to detect invasive species moving through e-commerce (ISAC, 2014). A ''web crawler'' is a program which ''scrapes'' the World Wide Web for specific information based on keywords or codes in an automated manner, which is helpful in tracking the internet-based sale of invasive species (Martinez et al., 2020)

A Montana based company (AIS Solutions) is testing a ''geo-fencing'' technology for accurate monitoring and tracking of watercraft to prevent the spread of zebra and quagga mussels. The technology includes relatively inexpensive electronics outfitted on recreational watercraft, with a small, waterproof battery, and solar-powered geographic positioning system (GPS) or electronic logging device (ELD) (Martinez et al., 2020).

Recently developed applications and accessories for Smart- phones contain multiple sensors (Lane et al., 2010), such as microphones, cameras, altimeters, accelerometers, barometers, gyroscopes, proximity sensors, compasses, bluetooth network devices, and GPS sensors which can enable real-time linkages between field-based visual observations and internet-based identification, reporting, and mapping (Martinez et al., 2020).

In addition, the programs, and apps like i-naturalist, Ecoquest challenge, Early Detection & Distribution Mapping System (EDD) maps, Plant net, Mid- Atlantic Early Detection Network (MAEDN), and other smartphone apps can be used to document the distribution of invasive species across the United States and help identify leading edges of new infestations. US Geological Survey's Nonindigenous Aquatic Species program phone app can be used on iPhone and Android for invasive species detection (nas.er.usgs.gov).

There are various other methods and technologies such as acoustic, light-based and chemical detection e-nose devices used for detection of invasive species. For example, USGS FORT and the National Wildlife Health Center (NWHC) has used an ultraviolet light to detect an invasive microscopic fungus (*Pseudogymnoascus destructans*) which causes the devastating white-nose syndrome in hibernating bats (Martinez, 2020).

Unmanned aerial vehicles (UAVs), underwater remotely operated vehicles (ROVs) and drones are also used for the EDDR process. Some of the invasive species such as yellow flag iris (*Iris pseudacorus*, Baron et al., 2018), invasive grasses (*Cenchrus ciliaris* and *Triodia spp*., Sandino et al., 2018) and Burmese pythons (*Python bivittatus,* Gomes 2017) were detected through the use of drones (Martinez et al., 2020).

Case Studies on EDRR in Action

Spotted Lanternfly (*Lycorma delicatula*)

Spotted Lanternfly (*Lycorma delicatula*), a member of the insect order Hemiptera, was first discovered in Berks County, Pennsylvania in the fall of 2014. It has been found in other neighboring counties as well. It is an invasive species in the US which feeds on more than 70 species of plant hosts, including grapes, apples, other stone fruits, and pines. It can threaten both agricultural and natural areas. The spotted lanternfly is a planthopper that feeds on plants by sucking that sap from the plant's phloem. Adults are ~1" in length with one set of spotted greyish wings and another set of red, black and white hind wings that are visible in flight. The female lays its egg masses on flat outdoor surfaces (~30-50 eggs per mass). In the US, nymphs hatch in late April to early May. The spotted lanternfly is native to some parts of southern China and subtropical regions of southeast Asia, India and Vietnam. In its native range, the adult lanternfly prefers Tree of Heaven (*Ailanthus altissima*) also an exotic invasive as its host. Even though this tree is a widespread invasive tree throughout the US, the lanternfly does not limit its impacts to the Tree of Heaven (www. doi.gov).

Impacts

The feeding habits of the spotted lanternfly can leave trees with ‘wounds’ that weep sap from their trunks. These wounds make the tree susceptible to fungal mats, stunted growth, and impacts by other organisms that feed on the sap. The insect poses a potential threat to a range of agricultural and forestry enterprises. Economic projections have indicated that the spotted lanternfly could adversely impact the value of the following industries in Pennsylvania alone: grapes - $20.5 million, apples - $134 million, stone fruits - $24 million, and hardwoods - $12 billion.

Research is still ongoing regarding the pathway for the spotted lanternfly’s introduction. The movement of a very wide range of natural and man-made objects (e.g., building materials, outdoor household and recreational items, garden and landscaping equipment, firewood, plants) from infested areas could serve as a pathway for its initial and further spread as the lanternflies lay eggs outdoor.

Spotted lanternfly could become a ‘poster child’ for early detection and rapid response to invasive species as its distribution in the US is recent and limited. There is still an opportunity to eradicate it from the US (www. doi.gov).

EDRR Actions and Investment

Currently, spotted lanternfly is under quarantine in parts of four Pennsylvania counties (Berks, Bucks, Chester, and Montgomery) which forbid the movement of outdoor household articles, construction waste, firewood, yard waste, and other organic debris outside of the quarantine zone. Research is ongoing regarding the insect's potential range; major constraints are likely to include limits on availability of host species and cold temperature extremes. Citizens are asked to be on the lookout for the insect and to scrape, bag, and destroy any egg masses. In 2015, USDA provided $1.4 million to the Pennsylvania Department of Agriculture for research, control and surveillance, in addition to allocation of general state and federal resources (www. doi.gov).

Quagga and Zebra Mussels (*Dreissenids*)

Non-native aquatic mussels, such as quagga and zebra mussels (*Dreissena rostriformis bugensis* and *Dreissena polymorpha*) were first introduced into the Great Lakes region in the 1980s, and by the 1990s had spread through all of the lakes. The native range of these mussels are Black and Caspian Seas. Zebra and quagga mussels are freshwater filter feeders about the size of 1-5 cm. The mussels can attach themselves to the submerged substrates, including populations of native clams. This tendency has led to the near extirpation of native unionid clams in Lake St. Clair and western Lake Erie. Quagga and zebra mussels were initially introduced to the Great Lakes through the exchange of ballast water of ships crossing the Atlantic. In addition, these mussels have been spread through the movement of infested recreational boats to new water bodies. Currently they are concentrated in the great lake region and have spread to 29 states mostly in the eastern US and few Western states (www.doi.gov).

Impacts

If invasive *Dreissenids* were to establish in the Columbia River Basin, projected infrastructural costs are estimated to include $2 million per hydroelectric facility to install treatment systems, with an additional annual maintenance costs of $100,000 per unit. Direct and indirect costs for Idaho alone are projected at over $94 million.

Parts of Western US are working hard to keep their waterbodies and waterways free of invasive mussels (www.doi.gov).

EDRR Action & Investment

There are a series of efforts and initiatives, such as the 100th Meridian Initiative and Building Consensus in the West, to control the spread of invasive *Dreissenid* mussels to western water bodies. Many of those activities are being implemented in line with the Quagga-Zebra Mussel Action Plan (QZAP), for Western U.S Waters initially adopted by the Aquatic Nuisance Species Task Force (ANSTF) in 2010 and reapproved in 2015. Watercraft inspection and decontamination is a critical step for ensuring effective early detection/rapid response within the region. To fully implement the recommended actions of the QZAP total ~$47 million in up-front costs and additional annual expenditures of $60 million is estimated. These estimates include prevention and early detection/rapid response activities, along with support to states for implementing their aquatic nuisance species management plans. To date, federal agencies have provided some technical and financial resources to support efforts to address the spread of invasive *Dreissenids* into western waters (www. doi.gov).

On Jan 4, 2021, USDA allocated $70 million in Fiscal Year (FY) 2021 to protect Agriculture and Natural resources from Plant Pest and disease. The FY 2021 includes 29 projects funded through National Clean Plant Network (NCPN). Some of the projects funded by FY 2021 include Asian giant hornet research and eradication efforts with $944,116 in Washington and other states; exotic fruit fly and detection with $5,575,000 in Florida and California, Honeybee, and pollinator health with $1,337,819 to protect honeybees, bumble bees and other important pollinators from harmful pests etc. In addition, $1,339,183 fund is allocated for Biosecurity to Texas to monitor for pests in agricultural shipments at ports of entry. Fund is also allocated for the detection and control of forest pest in various states such as sudden oak death pathogen (*Phytophthora ramorum*) and related species, protection of Solanaceous plants (including the tomato), for the support of agriculture detector dog teams (www. aphis.usda.gov). In addition, Trump administration had announced a new interagency conservation agreement to protect western water from invasive zebra and quagga mussels in November 2020 (www.invasivespeciesinfo.gov).

Barking up the Right Tree

Barking up the Right Tree is a unique program run by the Agricultural Research Service on Fort Pierce, FL. It uses trained dogs to detect invasive pathogens of trees on orchards. The canine-detection method has an accuracy rate of 99 percent. The dogs have been used to locate the endangered species such as mammals, cats, dogs and marten families in Europe and North America (www.invasiveinfo.gov).

Caulerpa taxifolia

Caulerpa taxifolia is an attractive species of macro alga (seaweed), commonly used as an ornamental decoration in marine aquariums. In 2000, *C. taxifolia* was discovered near San Diego and Seagate Lagoons, prompting one of the first marine rapid response efforts in the US. The native range of *C. taxifolia* is Indian Ocean. *C. taxifolia* produces dense mats with the potential to displace native aquatic plants, other algal species, and marine invertebrates. It can grow as fast as a centimeter a day, and severed parts are capable of establishment and further growth. *C. taxifolia* is one of two alga listed in IUCN's 100 of the World's Worst Invasive Alien Species and also as a Federal Noxious Weed by USDA. Ship anchors, fishing nets, and other marine equipment can spread *C. taxifolia* by transporting their fragments to new areas. *C. taxifolia* might have arrived in the US from aquarium trade or dumping of small aquariums. Economic impacts can include a reduction in catch for commercial fishermen, as well as the costs of repairing fishing equipment, anchors, and boat propellers. The introduced population of *C. taxifolia* of San Diego were officially eradicated in 2006. Currently, the only verified population of *C. taxifolia* in the US is in the State of Louisiana (www.doi.gov).

EDRR Action & Investment

The EDRR activities were undertaken by governmental and non-governmental organizations after the onset of *C. taxifolia* in San Diego in 2000. The underwater infestation was covered with Tarps filled with chlorine. National Oceanic and

Atmospheric Administration (NOAA), the US Department of Agriculture (USDA), the California Department of Fish and Game, the San Diego Regional Water Quality Control Board, and the Santa Ana Regional Water Quality Control Board participated in the removal efforts. Total costs for the suite of early detection/rapid response activities associated with the removal of *C. taxifolia* are estimated at $7.7 million. The eradication of *C. taxifolia* demonstrates the success story of early investment in EDRR before causing much substantial harm (www.doi.gov).

Zika Virus (genus *Flavivirus*)

The Zika virus is transmitted by mosquitoes and is a human health concern, particularly for infants. It was first isolated in Uganda in 1947 but recently has spread throughout the Americas. Even though, initially limited to the equatorial belt, Zika virus has currently spread throughout Central and South America, Sub-Saharan Africa, Southeast Asia, and the Pacific. Its range is limited to that of its vectors (Aedes mosquitoes) and hosts (i.e., monkeys and humans).

Since January 2016, some infected individuals have been identified in the US, although they were likely infected by mosquito bites during travels to regions with the virus. Mosquitoes of the genus *Aedes* can serve as vectors for transmitting the virus to new hosts.

The World Health Organization (WHO) has designated the virus as a Public Health Emergency of International Concern (www.doi.gov).

Impacts

The human health impacts include increased risk of microcephaly in infants, Guillain-Barré syndrome (a neurological disorder) and symptoms including fever, rash, joint pain, and conjunctivitis (www.doi.gov).

EDRR Action and Investment

The Centers for Disease Control (CDC) has initiated work on Zika virus. Initial efforts were focused on surveillance and identification of infections in humans. The CDC has developed guidance to assist local authorities and other government agencies to monitor the individuals who may already have contracted the virus and for the mosquitoes carrying the virus. Infected individuals should take precautions against mosquito bites as they could serve as reservoirs for introducing the Zika virus into new populations. Longer term work on diagnostics, vector control, vaccines and therapeutics is also underway. In 2016m President Obama had submitted an emergency request to Congress for $1.8 billion to support further control, research and educational activities on the Zika virus (www.doi.gov).

EDRR for Water Chestnut control

Early Detection and Rapid Response (EDRR) and Early Warning and Rapid Response (EWRR) programs can significantly reduce the negative impacts of plant invasions and are crucial for effective management and successful eradication (Genovesi et al., 2010). Monitoring sensitive sites, mapping, and reporting new infestations, involving the public are all key actions within many national strategies. However, species identification is often difficult, which limits the applicability of early detection methods. This is due to the high phenotypic plasticity of many aquatic plants in response to environmental factors and site conditions (Arber, 1920; Dorken and Barrett, 2004; Riis et al., 2010; Eusebio Malheiro et al., 2013) and hybrids between native and alien plants, (Hussner et al., 2015) and fewer taxonomists. The Wildlife Society (TWS) supports the state and federal legislation that address the importation, transportation and mitigation of invasive plants and animals (www.wildlife.org).

Currently, Water Chestnut (*T. natans*) is considered as a noxious weed in 35 states of US. In these states, it is illegal to propagate, sell or transport this weed. Water Chestnut is not regulated in the Commonwealth of Virginia but is listed as an occasionally invasive species. In Maryland, Water Chestnut is listed as a noxious weed, and sale is not permitted. However, Water Chestnut is not regulated in the nearby District of Colombia (DC) (Naylor, 2003).

Thus, improvement in research, data mobilization, stakeholder and public awareness and participation, investment in technological innovation can result in better and successful EDDR. Invasive species EDRR need to work across multiple jurisdictions. There should be careful delineation of legal authorities, regulations, and policies for federal agencies to enable EDRR.

Water Chestnut Removal Efforts in the Past in the US

According to Gwathmey (1945), Water Chestnut (*T. natans*) was first reported in the US near Scotia, NYC in 1884. In 1923, it was reported from Oxon Run, across the Potomac from Alexandria. The US fish and wildlife service (known as the Bureau of Biological Survey then) worked for its eradication during the succeeding decade trying various chemicals, manual methods and even searing the plants with fire. The Potomac River Water Chestnut Eradication Committee established in 1938 procured funding to promote an extensive governmental effort to get rid of the Water Chestnut. Comprehensive surveys in 1939 determined that the best method for the removal was the use of the cutting machines to harvest the dense beds before they could reproduce themselves. In 1940, the Virginia Commission of Game and Inland Fisheries authorized the use of the commission's boat equipment and warden force along the Potomac. It was discovered that Water Chestnut reseeded 7 times as rapidly in downstream areas compared to upstream areas. Hence the method of working downstream was adopted. Manual removal efforts were conducted by using underwater weed cutter for few years afterwards (Gwathmey, 1945).

For the complete removal of Water Chestnut, the treatment options should be applied before the maturity of fruit, as the fruit is viable up to 10 years (Hummel and Kiviat, 2004). In a mesocosm study, done by Rector et al. (2015), the fruit collected from herbicide (2,4-D, and/or glyphosate) treated area had a significantly fewer and a different germination rate than those collected from untreated areas. The result of this study indicates that herbicide application may have a significant impact on surviving plants' ability to produce viable fruit. The seeds produced from the fruits in treated area were either nonviable or of smaller mass and lack vigor. This study indicates that herbicide applications may provide additional control through impacts on seed germination (Rector et al., 2015).

Phartyal et al. (2018) studied the seed germination ecology of *T. natans*. Non-dormant seeds were very sensitive to desiccation and could not tolerate even a brief period of drying (Muenscher, 1936 and Pharytal et. al, 2018). These results supported the theory that *T. natans* seeds were not able to persist drying water bodies but might survive if fully covered by wet mud. The inability of *T. natans* seeds to tolerate a short period of drying could be the reason behind the disappearance of this species in some part of Europe and Russia where lakes are drawn down for the management purposes (Phartyal et al., 2018). Hence, the method of lake drawdown could be considered as an option for the control of *Trapa* species in the US.

The application of weed biocontrol method could be possible application for control of *Trapa* species. However, the use of such methods, can result in harmful environmental effects and damage in non-target species. Blossey et al. (2018) studied the impact of possible biocontrol agent (Beetle- *Garelucella birmanica*) on non-target species. They assessed the effect of G. *nymphaeae* on non-target host *T. natans* and the effect of *G. birmanica* on target host *T. natans*. The demographic assessments correctly predicted that G. *birmanica* attack on *T. natans* populations can lead to rapid and severe population growth rate declines whereas *G. nymphaeae* did not affect *T. natans*. Use of herbivore impact studies and demographic models would be an important tool to evaluate efficacy and safety of potential biocontrol agents, which are completely unutilized despite their promise (Blossey 2016b and Blossey 2018). Application of demographic approaches in forecasting species-agent efficacy and potential impacts on non-target species will increase the accountability of such methods rather than making the weed biocontrol risky. This information should be delivered to society, decision makers and regulators to make the invasive species management and stewardship more successful (Blossey 2016a, b; Hare and Blossey 2014 and Blossey 2018).

Plant Protection Quarantine for *T. natans*

Plant Protection and Quarantine (PPQ) regulates noxious weeds under the authority of the Plant Protection Act (7 U.S.C. § 7701-7786, 2000) and the Federal Seed Act (7 U.S.C. § 1581-1610, 1939). A noxious weed is defined as "any plant or plant product that can directly or indirectly injure or cause damage to crops, livestock, poultry, or other interests of agriculture, irrigation, navigation, natural resources of the US, public health, or the environment" (7 U.S.C. § 7701- 7786, 2000). USDA and Aphis used the PPQ weed risk assessment (WRA) process (PPQ, 2015) to evaluate the risk potential of those plants, which are newly detected in the United States and the those emerging as weeds elsewhere and ready to import into the US. The PPQ WRA process includes three analytical components that together describe the risk profile of a plant species (risk potential, uncertainty, and geographic potential; PPQ, 2015) (www. aphis.usda.gov). The predictive model is geographically and climatically neutral and can be used to evaluate the risk of any plant species for the entire US or for any area within it. Geographic Information System (GIS) is used to evaluate the areas that may be suitable for the establishment of such plant species (www.aphis.usda.gov).

T. natans **Analysis using PPQ**

1. Establishment/Spread Potential

The seeds have a high germination rate of up to 87 percent in the field (Kurihara and Ikusima, 1991). There is a low amount of uncertainty for this risk element. Risk score = 18 Uncertainty index = 0.07 (Source: aphis.usda.gov).

2. Impact Potential

There is no evidence of impacts in agricultural systems. There is a very low amount of uncertainty for this risk element.

Risk score = 3.4 Uncertainty index = 0.06 (Source: aphis.usda.gov).

3. Geographic Potential

About 82 percent of the United States is suitable for the establishment of *T. natans*. *T. natans* prefers temperate to tropical water bodies in sluggish areas with slower water flow (Hummel and Kiviat, 2004) (Source: aphis.usda.gov).

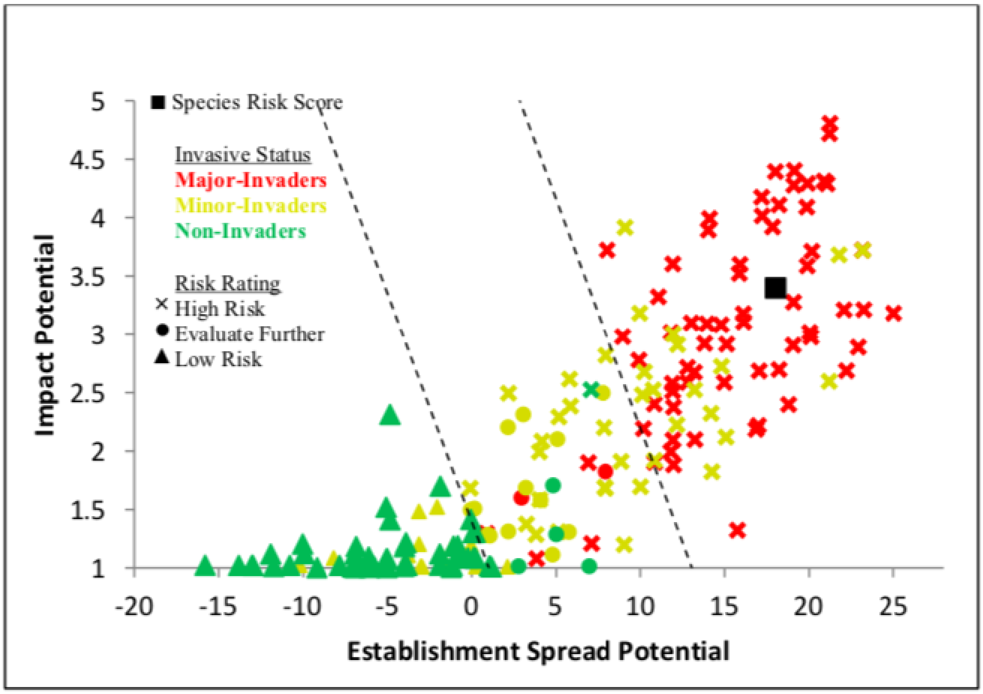


Fig. 33. *T. natans* risk score (black box) relative to the risk scores of species used to develop and validate the PPQ WRA model (other symbols) (Source: aphis.usda.gov).

The result of the weed risk assessment for *T. natans* is High Risk (Fig. 33). Comparing with the known weeds used to validate the WRA model, this species is ranked among with other High-Risk weeds (www.aphis.usda.gov).

T. natans has been the focus of several management and eradication programs, mostly within Lake Champlain in the northeastern US and within Maryland, on or near the Chesapeake Bay (Naylor, 2003). In Lake Champlain, between 1982 and 2003, more than $5 million was spent for its control (Kaufman and Kaufman, 2007). The states of New York and Vermont, the U.S. Fish and Wildlife Service, the Army Corps of Engineers, and the Lake Champlain Basin Program have collaborated on this management program for decades. The Maryland Department of Natural Resources (2003 Management Plan) outlined a $27,000 plan for control and management of *T. natans*. Additional funds were also allocated for prevention and educational activities (Naylor, 2003). $2.8 million has been spent in the past 20 years for *Trapa* control and monitoring programs in the Chesapeake Bay (Eyres, 2009; www.aphis.usda.gov).

EDRR on *Trapa bispinosa* in VA

Recently *T. bispinosa* has been reported from 68 sites in Virginia. In the past, the management of Water Chestnut was in the jurisdiction of the Corps of Engineers (COE), now it is a multijurisdictional issue. Table 1 shows some of the management efforts to control *T. bispinosa* in Northern Virginia area. In this table, address of the waterbody, jurisdiction, methods of treatment procedure and the initial year is listed. The initial year is the year when *Trapa* was first noticed.

After the discovery of *T. bispinosa*, Virginia Game and Inland Fisheries (VGIF) coordinated a harvest in Pohick Bay by hand pulling in September 2014. The amount of harvested *T. bispinosa* was 7200 lbs. in 2014 which continued to diminish over the yearly harvest and became 60 lbs. in 2019. In 2020, there were just two plants reported from Pohick Bay. Fairfax County of Virginia uses herbicides and manages stormwater ponds in its jurisdiction.

In 2017, *Trapa bispinosa* was first noticed on the Industrial Road, in Springfield, VA (Vulcan Cement) with the largest colony (26,000 square meters) found to date in Northern Virginia. In 2016, *Trapa bispinosa* bed was reported from a federal facility on

Wellington Road, Fairfax, VA covering 8600 square meters and reported to USGS on Fall 2017. The herbicidal treatment plan was initiated in 2018 on the Industrial Roadbed and in 2019 on Wellington Road, Fairfax. Some of the privately owned ponds with *Trapa bispinosa* are also harvested by volunteers in Virginia. (Based on Personal Communication with Dr. Rybicki on May 04, 2021).

In Nutley Pond (VCB-VA), there was no herbicide treatment, but *Trapa* died out naturally, possibly due to poor water clarity in 2018 which came back in Spring 2020. For Twin Lake Golf course pond management, golf course had hired a company (DCS Aquatic Solutions) to treat the 2 large lakes, but the company was not as efficient and experienced and did a poor job for a decade. The cost was $5,000 per year. They were able to eliminate *Trapa* in the lower lake with the pumping stations for about 2 years. In 2019, they tried the herbicide Triclopyr monthly from May to September and hand pulling was also conducted by 10 volunteers including Dr. Rybicki and Dr. Jones. The treatment effort in the lower lake was able to control *Trapa* in 2019 but not in the upper lake. In 2020, there were no possible management procedures due to covid circumstances. Twin Lake Golf course has run out of money in recent years and there are no other treatment procedures currently. In 2021, Dr. Rybicki and her team were working with them to get a new sustainable plan for the control of *T. bispinosa* (Rybicki and Pfingsten, 2020).

Table 31. Ongoing EDRR process for the control of *Trapa bispinosa* in Northern VA.

Water body	Jurisdiction	Treatment	Initial Year
Brookfield Pond (BP-VA)	FFXSW	Herbicide	2017
Fairfax County Bus Depot (FBD-VA)	FFXSW	Herbicide	2017
Lee Highway Costco (LHC-VA)	FFXSW	Herbicide	2017
Myrtle Leaf Pond (ML-VA)	FFXSW	Herbicide	2016
Pohick Bay (PB-VA)	NVRP	Mechanical	2013
Pfitzner Stadium (PS-VA)	Prince William	Herbicide	2014
Clifton Pond (CP-VA)	Private	Mechanical	2010
Fairfax Station Pond on Daysailor Drive (DD-VA)	Private	Herbicide	2014
Fairfax Station Pond on 11309 Hunting Horse Drive (HH-VA)	Private	Mechanical	2018
Industrial Pond (IR-VA)	Private	Herbicide	2000
NV Community College, Annandale (NVCC)	Private	Mechanical	2016
Nutley Pond (VCB-VA)	Private	Died Out by itself-	2010
Miller Drive Pond (MD-VA)	Private	Herbicide and Mechanical	2014
Brook Hill Dr. Pond (BH-VA)	Private	Herbicide and Mechanical	2014
Occoquan Reservoir (OR-VA)	Tributary	Mechanical	2010
Forest Hills Community Pond (FH-VA)	Private	Herbicide and Mechanical	2010
Woollily Pond, 13431 Woollily Ln, Centerville (WL-VA)	Private	Herbicide	2018
Twin Lake Golf Course (TL-VA)	FCPA	Partial Treatment	2000
Government Center Pond (A- Upstream and Downstream)	FFXSW	Herbicide	2012

An organized effort for early detection and rapid response could help manage the distribution and spread of *T. bispinosa*, but there is no specific management plan for the implementation.

APPENDICES

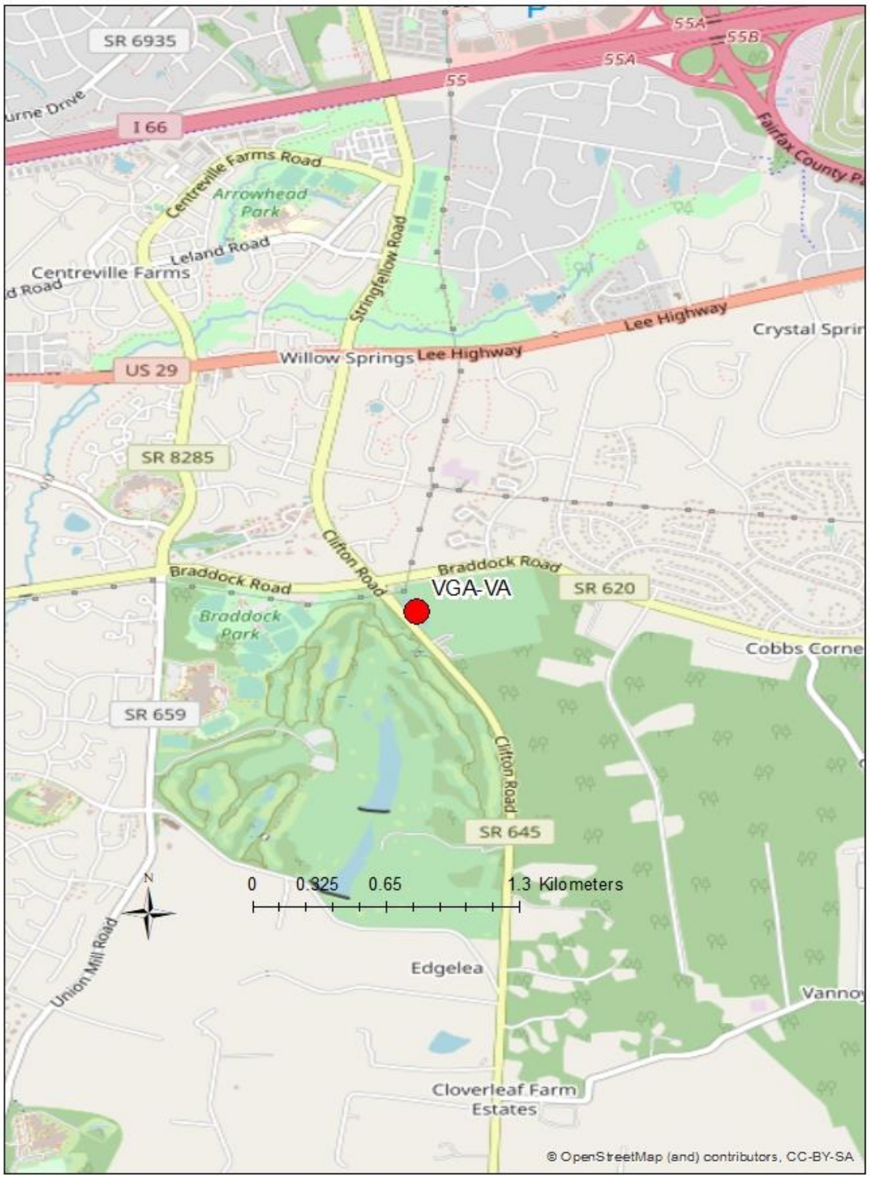


Fig. 34. Virginia Golf Academy (VGA-VA).

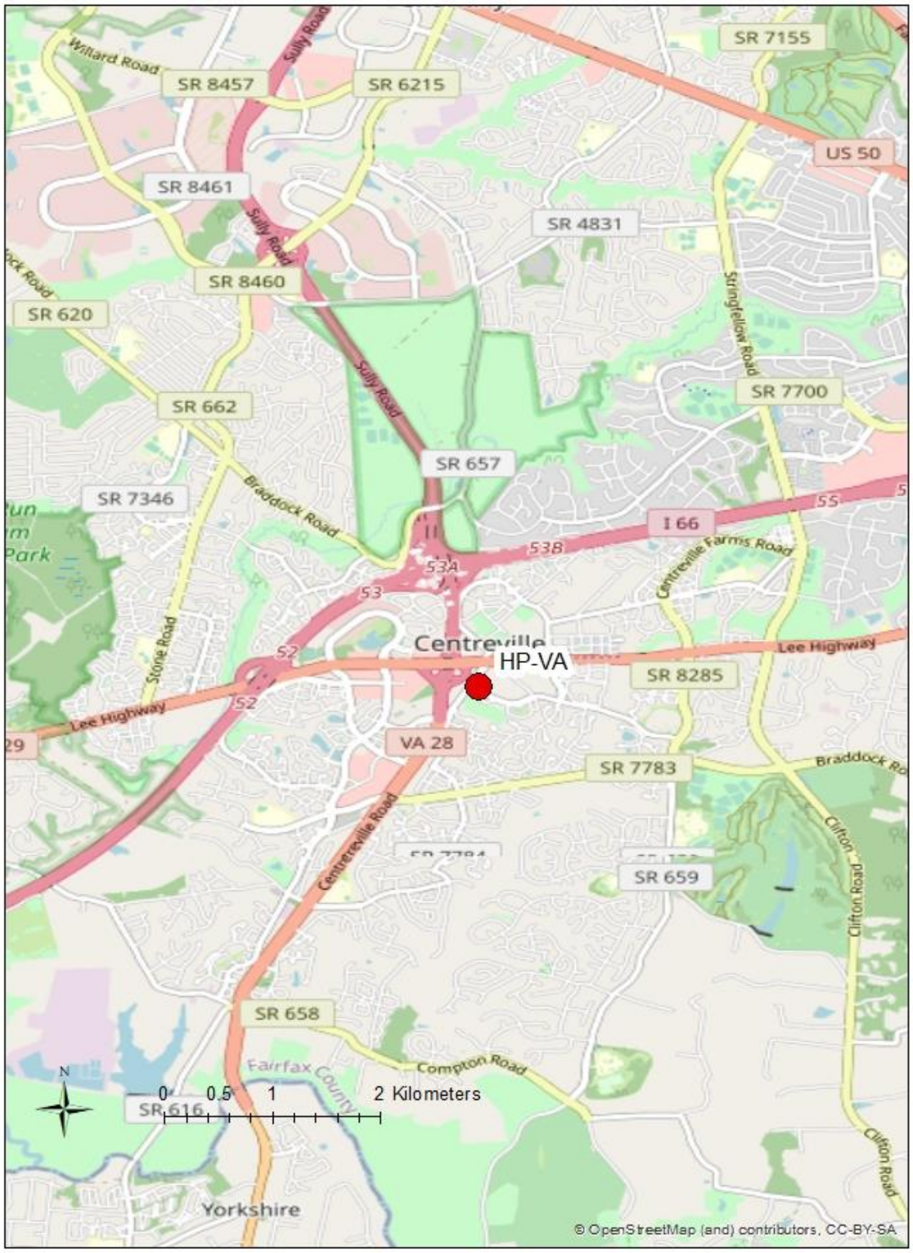


Fig. 35. H-Mart Pond (HP-VA).

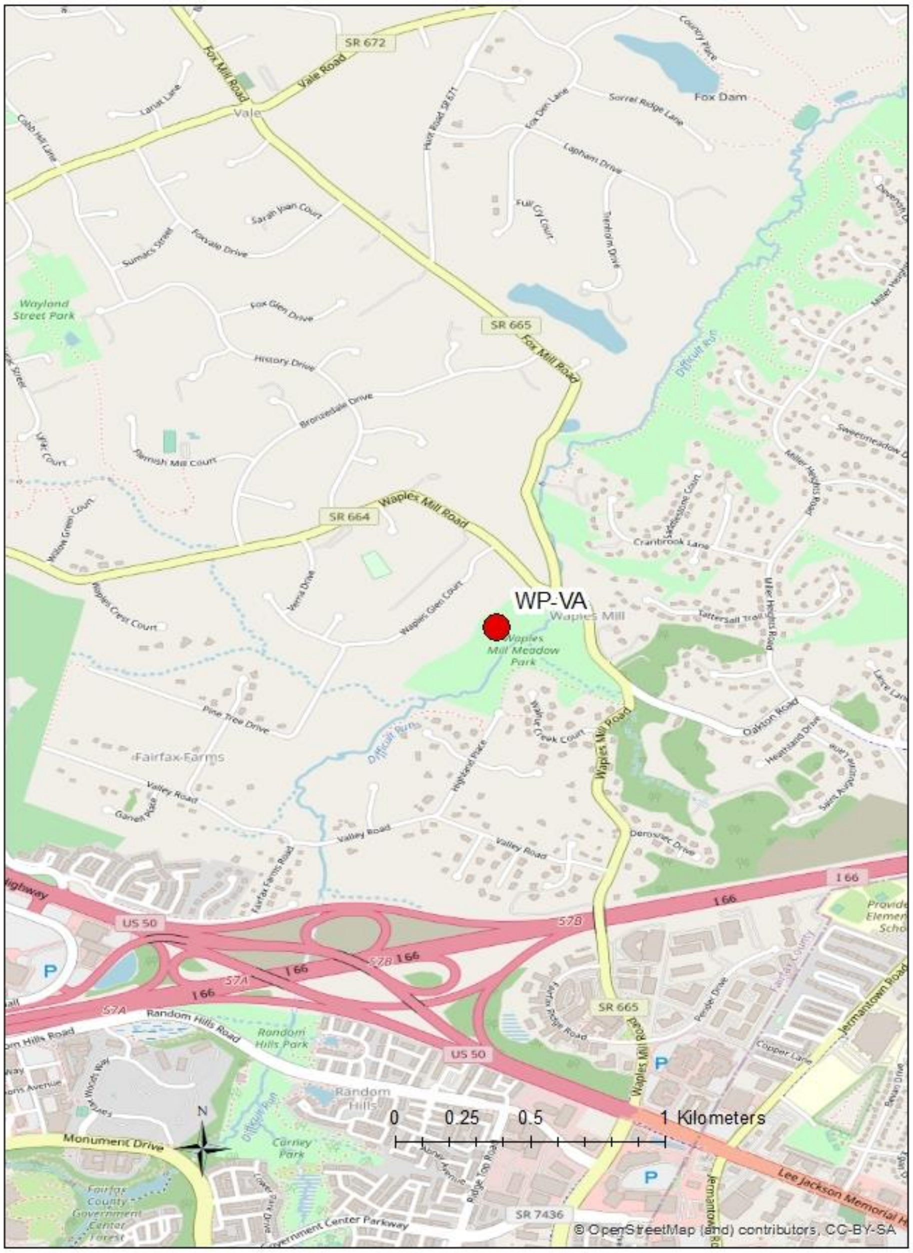


Fig. 36. Waples Mill Pond (WP-VA).

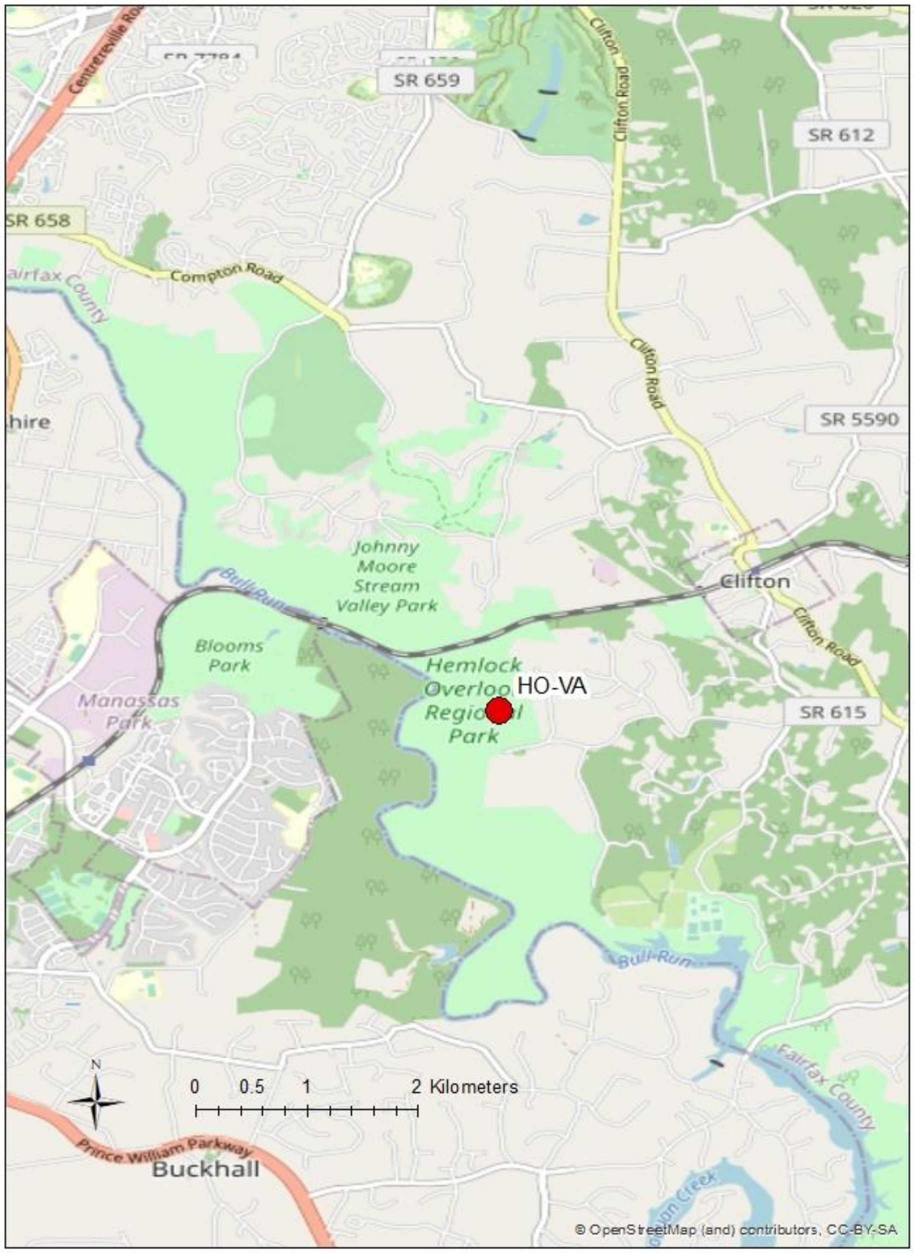


Fig. 37. Hemlock Overlook Regional Park (HO-VA).

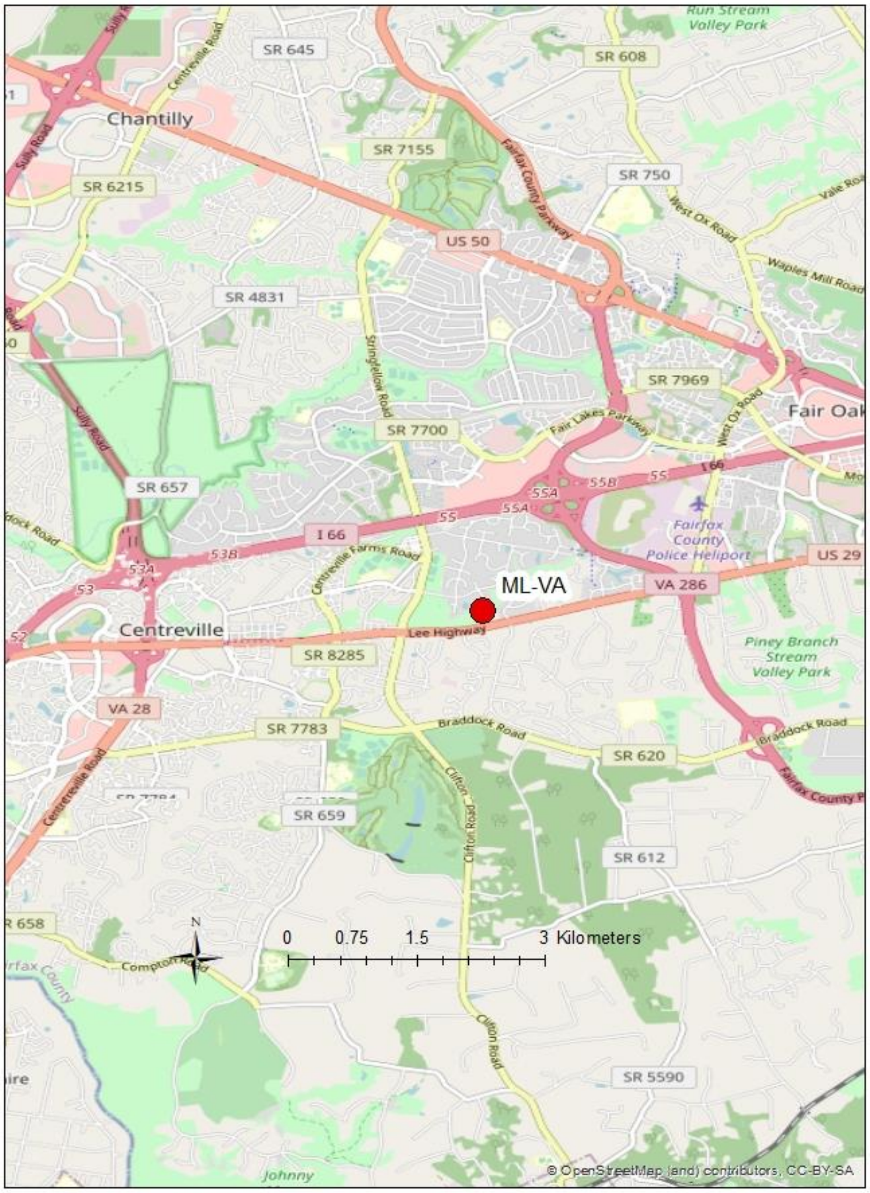


Fig. 38. Myrtle Leaf Pond (ML-VA).

Fig. 39. Government Center Pond (GCP-VA (A and B)).

Table 32. Average March to December air temperature and precipitation data for 2019, 2020 and 1971-2000.

Month	2019 Avg. Air Temp	2020 Avg. Air Temp	Long-term Avg. Monthly Air Temp 2019	Long-term Avg. Monthly Air Temp 2020	2019 Avg. Total PPN (cm)	2020 Avg. Total PPN (cm)	Long-term Avg. total Monthly PPN (cm) 2019 and 2020
Mar.	8.2	11.8	8.1	8.1	10.16	5.87	9.1
Apr.	16.9	12.9	13.4	13.4	5.69	16	7
May	21.7	17.7	18.7	18.7	12.62	6.32	9.7
Jun.	24.7	25	23.6	23.6	10.85	8.92	8
Jul.	27.8	28.8	26.2	26.2	16.48	16.54	9.3
Aug.	26.7	26.4	25.2	25.2	5.05	22.17	8.7
Sept.	24.7	21.2	21.4	21.4	0.64	14.05	9.6
Oct.	17.8	17.1	14.9	14.9	16.92	11.56	8.2
Nov.	7.8	16.6	9.3	9.3	3.48	3.48	7.7
Dec.	5.7	12.4	4.2	4.2	8.33	8.33	7.8

Source: Local Climatological Data. National Climatic Data Center, National Oceanic and Atmospheric Administration.

https://forecast.weather.gov/product.php?site=JAX&issuedby=DCA&product=CF6&format=CI&version=5&glossary=1;(Note: Avg. is average)

Table 33. Weather data of 2019 from National Weather Station (Temperature and Precipitation for 3 and 7 days).

Dates	Avg. Air Temp (°C)	Avg. 3day Air Temp (°C)	Avg. 7day Air Temp (°C)	Avg. PPN (cm)	3day PPN (cm)	7day PPN (cm)
6/3/19	19.4	23.1	25.3	0.00	0.03	1.24
6/11/19	22.8	21.9	23.6	0.36	1.63	1.66
6/21/19	25.6	26.9	26.3	0.01	0.23	5.70
7/12/19	27.2	27.0	26.7	0.00	2.24	12.80
7/16/19	28.9	28.5	27.8	0.01	0.01	2.25
7/24/19	25.6	26.3	29.1	0.94	1.22	1.23
7/31/19	27.8	29.1	27.7	0.03	0.03	0.05
8/7/19	28.3	27.8	27.8	2.29	2.29	2.32
8/16/19	27.8	28.0	26.8	0.00	0.52	0.83
8/27/19	22.8	22.2	24.4	0.01	0.03	1.02
9/5/19	25.0	26.9	26.4	0.00	0.05	0.18
9/12/19	30.0	28.0	25.6	0.08	0.10	0.11
9/19/19	20.0	22.0	23.7	0.00	0.00	0.01
9/24/19	23.3	26.5	23.6	0.00	0.00	0.00
10/10/19	20.0	18.9	19.1	0.00	0.01	0.06
10/17/19	13.3	14.8	16.7	0.00	3.43	3.73

Note: 3-day average temperature is the average of the sampling day and 2 previous days. 7-day average temperature is the average of the sampling day and 6 previous days. 3-day precipitation is the cumulative of sampling day plus 2 previous days and 7-day precipitation is the cumulative of sampling day plus 6 previous days. Avg.- Average, PPN-Precipitation

Source: Local Climatological Data. National Climatic Data Center, National Oceanic and Atmospheric Administration. https://forecast.weather.gov/product.php?site=JAX&issuedby=DCA&product=CF6&format=CI&version=5&glossary=1

Table 34. Weather data of 2020 from National Weather Station (Temperature and Precipitation for 3 and 7 days).

Date	Av Air Temp (°C)	Av 3-day Air Temp	Av 7day Air Temp	PPN (cm)	3-day PPN (cm)	7-day PPN (cm)
4/16/20	9.4	9.1	11.7	0.00	0.76	7.23
4/23/20	12.2	11.9	11.4	1.88	1.93	2.11
4/29/20	16.1	13.0	13.0	0.00	0.28	4.42
5/7/20	13.9	12.6	15.7	0.01	0.67	3.49
5/21/20	16.7	16.1	18.7	0.00	0.01	0.04
5/28/20	24.4	23.0	21.8	0.20	0.22	2.44
6/5/20	26.1	27.0	23.6	3.20	4.52	4.55
6/12/20	25.6	27.0	25.9	0.00	0.97	0.99
6/19/20	25.0	23.1	22.5	0.03	2.20	2.21
6/26/20	25.6	26.1	25.9	0.00	0.01	1.22
7/3/20	27.8	26.1	27.6	0.00	0.30	0.37
7/10/20	28.3	28.1	29.0	0.01	0.01	6.30
7/16/20	27.8	27.6	28.2	0.00	0.00	0.39
7/24/20	27.8	29.1	30.3	1.09	7.32	9.02
7/30/20	28.9	30.2	29.2	0.38	0.39	1.49
8/6/20	26.1	26.5	27.3	1.57	7.87	9.40
8/14/20	27.8	27.6	27.8	0.15	2.31	2.35
8/21/20	25.0	24.8	24.3	0.01	0.24	3.75
8/28/20	28.3	28.0	27.9	3.71	3.71	3.87
9/4/20	27.8	27.8	25.9	0.01	1.00	3.57
9/7/20	23.3	23.0	25.2	0.00	0.00	1.28
9/17/20	21.1	18.7	21.0	0.99	0.99	1.02
9/21/20	14.4	14.4	17.1	0.00	0.00	1.17
9/28/20	23.3	21.9	20.1	0.03	0.89	1.91
10/5/20	16.7	15.6	17.3	0.05	.025	1.10
10/14/20	16.7	17.06	17.8	0	0.87	1.55
10/19/20	18.3	14.8	16.2	0.00	0.00	0.00
10/28/20	19.4	16.3	21.1	0.00	0.005	0.46
11/2/20	7.23	8.5	10.6	0.005	0.175	0.44

Note: Avg.- Average; PPN- Precipitation
Source: Local Climatological Data. National Climatic Data Center, National Oceanic and Atmospheric Administration.
https://forecast.weather.gov/product.php?site=JAX&issuedby=DCA&product=CF6&format=CI&version=5&glossary=1

Table 35. Average water temperature of untreated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2019			24.34	27	24.1	22.8	15.2	
VGA-VA	2020	14.4	18.7	25	27.3	25.13	18.6	15	9.7
HP-VA	2019			24.05	25.2	23.7	23.5	17	
HP-VA	2020	14.65	18.5	23.8	27	25.15	19.65	15.45	
WP-VA	2019			26.8	28.35	23.15	21.8	18.5	
HO-VA	2019			22.4	24.95	24.2	20.8	13.4	

Table 36. Average DO mg/L of untreated *Trapa ponds* by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2019			2.77	1.92	0.9	2.7	4.87	
VGA-VA	2020	7.56	5.12	3.31	3.54	2.47	2.35	3.47	9.46
HP-VA	2019			1.91	1.98	1.98	0.72	0.67	
HP-VA	2020	9.71	5.07	3.7	2.98	1.5	3.74	5.07	
WP-VA	2019			1.92	1.98	1.98	0.73	0.67	
HO-VA	2019			5.42	3.05	2	1.44	2.12	

Table 37. Average dissolved oxygen (DO%) of untreated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2019			38.53	23.9	11.37	31.35	49.35	
VGA-VA	2020	75.1	56.55	45.25	48.14	33.48	25.93	37.65	9.46
HP-VA	2019			29.2	23.1	20.5	7.85	9	
HP-VA	2020	95.35	60.13	64.1	47.87	23.65	31.5	48.15	
WP-VA	2019			54.9	53.7	20.8	20.6	30.4	
HO-VA	2019			63.7	37.15	25.3	17.4	22	

Table 38. Average pH of untreated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2019			6.7	6.65	6.81	6.92	7.35	
VGA-VA	2020	7.32	7.15	7.8	8.34	7.29	7.57	7.92	7.4
HP-VA	2019			5.83	5.81	6.31	6.3	6.77	
HP-VA	2020	8	7.6	8.06	7.8	7.23	7.32	8.01	
WP-VA	2019			6.46	6.09	6.9	6.56	6.83	
HO-VA	2019			6.67	6.28	7.11	6.92	7.22	

Table 39. Average SPC of untreated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2019			159.6	136.6	183.7	168.2	195	
VGA-VA	2020	144.8	150.7	154.1	165.2	142.67	168.2	194.9	162.9
HP-VA	2019			114.6	114.5	289.5	267.1	235.4	
HP-VA	2020	62.5	184	79.1	287.4	194.15	259.3	204	
WP-VA	2019			93.3	86.5	100.05	112.25	131.7	
HO-VA	2019			40.3	53.85	59.11	65.6	79.2	

Table 40. Average Turbidity of untreated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2019			5.28	12.57	4.65	10.58	1.14	
VGA-VA	2020	10.12	9.84	8.8	33.22	6.67	45.79	48.95	32.6
HP-VA	2019			0.02	1.2	0.6	2.08	1.84	
HP-VA	2020	2.45	2.12	2.73	3.39	1.805	2.95	42.26	
WP-VA	2019			5.78	41.96	12.65	8.67	5.9	
HO-VA	2019			2.45	1.28	0.5	1.97	5.63	

Table 41. Average Alkalinity of untreated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
VGA-VA	2020	57.75	63.75	61.31	53	45	58.125	58.125	47.5
HP-VA	2020	15.75	21	12.34	30.83	20	25	36.25	

Table 42. Average Temperature of treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct
ML-VA	2019	-	-	26.5	30.35	26.8	23.9	14.6
ML-VA	2020	14.5	19.95	25.73	27.85	26.05	21	17.5
GCP-VA(A)	2020	15.4	20.65	27.85	27.5	25.45	20.15	15.8
GCP-VA(B)	2020	15.7	21.7	30	27.15	24.9	19.45	16
WL-VA	2020	19.95	19.7	24.5	27.6	25.5	18.75	15.5

Table 43. Average DO (mg/L) of treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	June	July	Aug	Sept	Oct	Nov
ML-VA	2019	-	-	24.11	15.1	11.94	10.68	7.28	-
ML-VA	2020	8.43	7.02	6.85	5.3	3.4	6.88	5.43	7.34
GCP-VA(A)	2020	9.38	11.24	6.23	6.32	7.51	7.85	8.51	-
GCP-VA(B)	2020	7.23	8.73	7.57	7.23	4.4	2.01	7.3	-
WL-VA	2020	17.91	17.81	2.75	3.56	2.01	4.05	4.79	-

Table 44. Average DO% of treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct
ML-VA	2019	-	-	294.4	199.4	149.9	117.1	72.2
ML-VA	2020	83.3	77.25	86.43	71.9	64.2	61.8	76.3
GCP-VA(A)	2020	93	95.2	116.65	79.6	87.25	119.55	87.4
GCP-VA(B)	2020	76	100.8	100	91.95	53.5	25.9	81.2
WL-VA	2020	194.2	86.5	34.35	49.4	25.2	44.2	102.5

Table 45. Average pH of the treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct
ML-VA	2019	-	-	9.62	9.21	8.9	7.85	7.58
ML-VA	2020	7.48	7.17	7.14	7.81	7.75	7.37	7.84
GCP-VA(A)	2020	7.32	7.45	7.47	7.91	7.65	7.42	7.95
GCP-VA(B)	2020	7.52	7.5	7.48	7.92	7.58	7.56	7.7
WL-VA	2020	7.46	7.11	7.32	7.51	7.24	7.37	7.35

Table 46. Average SPC of the treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct
ML-VA	2019	-	-	71.7	533.45	444.7	537	5.52
ML-VA	2020	473.4	451	399.4	338.5	253.65	465.25	445.7
GCP-VA(A)	2020	549	457.8	338.1	131.25	200.45	386.25	179
GCP-VA(B)	2020	441.8	396	278.8	64.5	104.95	246.1	175.1
WL-VA	2020	675	792	281.25	498.73	475.5	532.5	537

Table 47. Average NTU of the treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct
ML-VA	2019	-	-	5.87	1.75	4.36	27.18	4.19
ML-VA	2020	4.36	5.85	14.4	23.67	22.93	38.4	14.51
GCP-VA(A)	2020	6.06	7.085	11.45	7.63	5.53	27.4	50.28
GCP-VA(B)	2020	9.3	8.67	87.22	30.33	63.23	30.93	45.21
WL-VA	2020	61.65	13.62	221.67	14.08	120.85	7.71	7.5

Table 48. Average Alkalinity of the treated *Trapa* ponds by month in 2019 and 2020.

Pond	Year	Apr	May	Jun	Jul	Aug	Sept	Oct
ML-VA	2020	49.5	42.75	27.25	28.75	32.5	28.75	32.5
GCP-VA (A)	2020	27	27	24.75	21.25	22.5	21.25	25
GCP-VA (B)	2020	27	29.25	23.5	23.75	25	42.5	22.5
WL-VAV	2020	168.75	225	145	103.34	103.5	90	128.25

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BIOGRAPHY

Sujata Poudel was born and raised in Kathmandu, Nepal. She received her Bachelor of Environmental Science from Tribhuvan University, Nepal. She received her Master of Environmental Studies from University of Tokyo in 2005 and Master of Science in Biology from University of Massachusetts, Boston in 2012. After her Masters, she worked at Virginia Department of Environmental Quality (VADEQ) for three years. She received her PhD from George Mason University, Fairfax in 2021. After her graduation, she is going to teach Environmental Science as an adjunct faculty at George Mason University, Fairfax and Northern Virginia Community College, Annandale.