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Addressing Water Hyacinth (Pontederia crassipes) Impacts on Aquatic Biota in Lake Okeechobee

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

Addressing Water Hyacinth (*Pontederia crassipes*) Impacts on Aquatic Biota in Lake Okeechobee

By

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Abstract

The incursion of water hyacinth, *Pontederia crassipes* in Lake Okeechobee has resulted in management systems to be implemented to reduce the coverage of the invasive macrophyte. Its residence in the Lake Okeechobee ecosystem and the effects it has on organisms in the lake, whether it be positive or harmful is unknown. This study attempted to assess the potential effects that water hyacinth has on aquatic biota in Lake Okeechobee. Biotic data were collected on open water, water hyacinth covered, and native vegetation covered habitats via hook-and-line fishing, electrofishing, baited minnow traps, and the sampling of plant roots over a thirteen-month span. A total of 10,795 freshwater fish, representing 24 species, and 13,419 invertebrates, representing 38 distinct groups were recorded.

9,258 individuals were caught using the baited-minnow traps, with a total of 17 species identified. 2,903 individuals were sampled using a Smith Root LR-20B electrofisher, with 26 species recorded. Hook-and-line angling only recorded 6 individuals total, each being a different species. Invertebrate sampling caught 36 different taxa for a total of 12,047 individuals. All sampling methods resulted in no significant differences in fish/invertebrate communities when comparing water hyacinth infested areas with native/open water treatment groups. Even though water hyacinth has negative impacts on shipping channels, recreational angling, human vector contact, and aesthetics, we did not detect any specific negative impact in aquatic biota communities in Lake Okeechobee.

Keywords:

Lake Okeechobee, *Pontederia crassipes*, populations, invasive, communities

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1.0 Introduction

Non-native species are organisms that do not naturally occur in an area but are introduced as a result of anthropogenic activities. Non-native species have the potential to inhibit the survival of other organisms in the ecosystem. When this happens, these non-native species can be classified as invasive. Invasive species are considered successful when they have colonized a wide foreign geographical range, becoming a dominant figure of the ecosystem it's invaded (Thompson 1991).

Characteristics of invasive plant species that allow for domination of native flora in foreign ecosystems include higher specific leaf area (SLA), flowering duration, and maximum height (Gallagher et al. 2015). Additionally, the range of mean annual temperatures and precipitation for invasive species have been shown to be higher than their native counterparts (Gallagher, Randall, and Leishman 2015). Invasive alien aquatic plants (IAAPs) tend to have higher reproductive potential, often regrowing from small (1 cm or greater) plant fragments (Hussner et al. 2017). Furthermore, possession of traits that are similar in nature to native vegetation such as resource use efficiency, allelopathy, and phenotypic plasticity, has been shown to result in negative effects on the native community (Kuehne et al. 2016). Free-floating IAPPs have been shown to cause drastic declines in aquatic macroinvertebrate taxa abundance and diversity (Motitsoe et al. 2022). Dense mats of floating macrophytes reduce biodiversity, decrease ecosystem functioning, increase siltation, and have the potential to impede irrigation canals and pumps (Strange, Hill, and Coetzee 2018). In addition, when water depth decreases, fish abundance has been shown to decline in vegetated areas that contain successfully invaded macrophytes (Schultz and Dibble 2018). Through the formation of dense floating mats, IAAPs can decrease atmospheric exchange with water, limiting the concentrations of dissolved oxygen (Schultz and Dibble 2018). High plant densities also give rise to increased organic material and decomposition in benthic zones, consuming oxygen that may be available (Fleming and Dibble 2014). With most aquatic animals being sensitive to low dissolved oxygen levels, species assemblage underneath invasive floating macrophytes are expected to be different in composition when compared to those found under native vegetation. IAAP species compete for light and nutrients with phytoplankton, epiphyton, and other native aquatic organisms, giving rise to energy flow disruptions to higher trophic level organisms (Motitsoe et al. 2022).

1.1 Water Hyacinth Ecology

Water hyacinth (*Pontederia crassipes*) is an invasive floating macrophyte originating from the Amazon. Facilitated by anthropogenic activities, water hyacinth has now spread to Africa, Asia, Australia, Europe, and North America (Bhattacharya, Halder, and Chatterjee 2015). The invasive plant has a destructive impact on both environmental and economic factors (Harun et al. 2021). Being seen as a desirable botanical specimen, water hyacinth was frequently distributed as gifts to decorate ornamental ponds (Williams 2015). The plants however, escaped cultivation from local ponds due to winds transferring plants to new bodies of water, as well as human release into native ecosystems. This resulted in water hyacinth spreading rapidly across the globe. As early as 1957, water hyacinth has been kept in cities in East Africa, such as Nairobi and Mombasa, as ornamental plants (Mailu 2001)**.** In Southeast Asia, the Inle Lake has experienced a fluctuating spatial extent of hyacinth, ranging from 250-1300 hectares from 2000-2011 (Mund, Murach, and Parplies 2014). Currently, water hyacinth is the most distributed aquatic weed across the world (Elenwo and Akankali 2016). The aquatic plant has been categorized as one of the top 100 most aggressive invasive species, as well as top 10 on the most invasive aquatic weeds (Gezie et al. 2018). Water hyacinth has been seen to inhabit different forms of aquatic ecosystems; being present in rivers, canals, lakes, dams, ponds, and other freshwater bodies. Typically found floating, mature specimens are characterized by broad, glossy leaves, comprised of a thick petiole, with pale lilac or violet flowers at the center of each rosette of leaves. The large, pale flowers are often decorated with purple and yellow spots, scattered sporadically on the petals (Navarro, Luis, and Kanyama-Phiri 2000). The leaves can grow to 10-20 centimeters across and may rise as tall as one meter above the water's surface (Sharma et al. 2015). The plant can be divided into three distinct sections; a fleshy leaf (leaves) that are responsible for photosynthesis, a semi-succulent stem that is greenish in nature and often inflated with air bladders towards the base, and a brown, fibrous root network that is submerged (Ndimele, Kumolu-Joh, and Anetekhai 2011). Water hyacinth reproduces both sexually via seed dispersal, and vegetatively by budding and stolen production. By having the ability to reproduce both sexually and asexually, water hyacinth plants can produce large quantities of offspring. Flowering occurs 10-15 weeks after germination. An inflorescence containing 20 flowers can produce 3000 seeds and up to four inflorescences can be produced from a single rosette over a 21-day span (Barrett 1980). A typical flowering season lasts for five to nine months, allowing for

large numbers of inflorescences per year per plant. Reproduction occurs so frequently that a single water hyacinth plant can produce 140 million new hyacinths within one year (Su et al. 2018). In ideal conditions, an individual water hyacinth can double its mass every five days (Degaga 2019). The optimal mean temperature growth for water hyacinth is between 25°C and 27°C, with growth halting if the water temperature falls below 10 °C or rises above 40 °C (Tellez et al. 2008). The pH range for water hyacinth is from 4.0 to 8.0, with optimum growth occurring between a pH of 5.8-6.0 (El-Gendy et al. 2004). Growth of water hyacinth is influenced by a variety of factors, including space available, water nutrient concentrations, starting biomass, temperature, light, and other limiting factors (Yan and Guo 2017).

1.2 Water Hyacinth Anthropogenic Effects

Water hyacinth often becomes so abundant that it takes over natural streams, impeding run-off and increasing backwater and flood conditions in many areas of living (Penfound and Earle 1948). Furthermore, water hyacinth increases evapotranspiration, roughly 3 times greater than open water, resulting in significant water loss (IUCN, n.d.). Lake Tana, the largest lake in Ethiopia, was shown to have a net annual water loss of 526,221 km^3in 2019 because of water hyacinth (Damtie et al. 2021). In countries such as Egypt, where water hyacinth has become dominant in irrigation and drainage canals, the water loss by evapotranspiration from water hyacinth is 3.5 billion m^3per year (Eid and Shaltout 2017). Additionally, a large mat of water hyacinth can halt navigation along water channels, impact fishing in infested bodies of water, and block dams (Lahon et al. 2023). The presence of water hyacinth has been shown to impede the fishing of tilapia in Lake Victoria (Segbefia, Honlah, and Appiah 2019). Hyacinth mats in Lake Victoria have reduced fishermen's catch by covering grounds, delayed access to fishing markets, and increased fishing costs due to effort needed to clear waterways (Kateregga and Sterner 2009). Thick mats of hyacinth can damage fishing boats by slowing boats down and causing engines to operate under greater stress, impacting the fuel efficiency of the boat (Simpson et al. 2020). The presence of water hyacinth in bodies of water where fisheries occur has the potential to disrupt these industries by inducing changes in either fish community composition, or the relative catchability of harvested species (Villamagna and Murphy 2010). In 1997, media agencies reported that there was a 70% decline in economic activities at the Kenyan port city, Kisumu, because of water hyacinth (Mailu 2001). On the River Tano and Tano Lagoon

of Ghana, smallholder farmers were asked how they had been impacted by water hyacinth, with a significant majority (92.7%) claiming negative effects on their activities (Honlah et al. 2019).

Water hyacinth presence can also result in large economic costs to remove the plant from non-native waters. The cost to remove one hectare (2.5 acres) of hyacinth is estimated between USD $2,400-30,000 (Williams 2006). In 1997, it was estimated that to clean the intake screens at the Owen Falls hydroelectric power plant in Jinja, Uganda, would cost USD $1 million annually (Mailu 2001). In some cases, the economic impacts are so significant that they require the use of control techniques, as seen in the State of Florida, in the United States, which spent more than USD $43 million between 1980 and 1991 on the suppression of water hyacinth (Schmitz et al. 1993).

The plant can also act as a refuge for invertebrates, impacting humans who live in the nearby area. Floating hyacinth mats have led to worsening health conditions for people living near Lake Tana, in Northwestern Ethiopia, as the plants provide breeding grounds for mosquitoes, worms, and snails. These animals often act as vectors capable of transferring harmful pathogens such as malaria and human schistosome infections (Enyew, Workiyie, and Ayenew 2020).

1.3 Water Hyacinth Environmental Effects

Water hyacinth can have impacts on biodiversity, as plants reduce phytoplankton productivity, which in turn decreases zooplankton abundance, affecting higher trophic levels (Degaga 2019). As mentioned previously, water hyacinth has been shown to disrupt zooplankton abundance, with multiple species of rotifers and microcrustaceans occurring at a significantly lower number under hyacinth mats (Meerhoff et al. 2003). Water hyacinth has also been documented to trap phytoplankton and detritus, with phytoplankton abundance being higher than at sites that lack hyacinth (Brendonck et al. 2003). Macroinvertebrates found in the digestive tracts of fish in the California Delta, showed that the community assemblage of invertebrates differed depending on if the fish fed on macroinvertebrates near water hyacinth patches, indicating that water hyacinth alters community assemblage to a degree (Mats being defined in the study as having a surface area of 30.96 m^2) (Toft et al. 2003). Large dense mats can displace hydrophytes as well, resulting in algal blooms (Ndimele, Kumolu-Joh, and Anetekhai 2011). It has been proposed that due to water hyacinths dense mat-forming nature, cyanobacteria cells could build up and become extremely concentrated resulting in cyanobacteria blooms and

lowering overall water quality (Corman et al.2023). Water hyacinth can disrupt lower trophic level fish diets as well due to changes in prey availability from the presence of water hyacinth (Villamagna and Murphy 2010).

Water hyacinth growth has been shown to be directly correlated with nutrient concentrations, with increases in nitrogen and phosphorus directly leading to increases in water hyacinth biomass (Coetzee, Byrne, and Hill 2007). Bodies of water that are high in nitrogen and phosphorus levels are at risk of water hyacinth invasions (Coetzee, Byrne, and Hill 2007). Water hyacinth has been shown to remove arsenic, a common chemical element that has been shown to carcinogenics in humans (Misbahuddin and Fariduddin 2002). Misbahuddin and Fariduddin placed water hyacinth plants in 10L containers of water with 400 ppb (parts per billion) of arsenic. Results showed that an individual water hyacinth plant was capable of removing arsenic completely, with the fibrous roots removing 81% of arsenic (Misbahuddin and Fariduddin 2002). In Lake Tana, Ethiopia, water hyacinth infested areas showed to have significantly higher levels of water conductivity and total dissolved solids, whereas pH and dissolved oxygen levels were significantly lower when compared to open water habitats (Gezie et al. 2018). Water hyacinth mats can induce hypoxia and reduce overall dissolved oxygen levels in the bodies of water where they reside. In the Atchafalaya River Basin of Louisiana, water hyacinth beds induced hypoxia because of the shading of the water column and inhibition of surface turbulence (Troutman, Rutherford, and Kelso 2011). The turbidity of water in areas containing hyacinth has also been shown to be greater than areas lacking hyacinth. Rommens et al. (2003) demonstrated that the water turbidity was higher in a highly eutrophic man-made reservoir near Harare, Zimbabwe, than areas in the reservoir that were absent of water hyacinth, which can prevent light from penetrating and reaching benthic vegetation.

1.4 Water Hyacinth Management

Several control programs are used across the globe to manage and control water hyacinth, with three being physical, chemical, and biological control methods. Physical control is the removal of water hyacinth using ones hands or specialized equipment. Chemical control utilizes herbicides, and biological control uses organisms that will target and feed on the invasive hyacinth. Management methods can be combined and used together, which is often referred to as integrated control (Xu et al. 2022). Common herbicides that are used to treat water hyacinth include glyphosate (formulation: 53.8% isopropylamine salt of glyphosate and 46.2% water),

adjuvant (99% heavy range parafinnic oil, polyethoxylated derivatives, and polyol fatty acid esters), 2,4-D, Penoxsulam, and Diquat (Prtilla and Sharon 2020; Smith et al. 2021). Common biological control agents of water hyacinth include *Eccritotarsus catarinensis* (Hemiptera: Miridae), *Megamelus scutellaris* (Hemiptera: Delphacidae), *Neochetina eichhorniae*, and *Neochetina bruchi* (Coleoptera: Curculionidae) (Wilson et al. 2017; Hill, Coetzee, and Ueckermann 2012; Smithet al. 2021). The biological control agents often feed on the furled and partially unfurled emerging leaves, as they are often very rich in nitrogen content (Moran 2004). *M. scutellaris* has been shown to be an ideal biological control for water hyacinth, as controlled studies have shown *M. scutellari*s to have a 100% oviposition rate on water hyacinth (Tipping et al. 2010). Heavy usage of chemical pesticides and herbicides, as well as highly eutrophic conditions can lead to increased target plant growth rates, limiting the effectiveness of biological control agents (Hopper et al. 2021).

1.5 Water Hyacinth Utilization

Water hyacinth has a strong potential for being utilized as a feedstock for furfural production, a chemical compound that has a structure that can be used to produce biofuels (Poomsawat et al. 2019). Dried water hyacinth can be used as feed, as dry water hyacinth matter is comprised of 10-20% of crude protein, making it a potential source of animal forage (Su et al. 2018). Water hyacinth is also capable of absorbing calcium, magnesium, phosphorus, sulfur, ferric, manganese, boron, copper, aluminum, zinc, nitrogen, molybdenum, and potassium. By being able to absorb a variety of elements, water hyacinth has the potential to be an ideal source of economic feed (Shu, QuanFa, WeiBo 2014). Additionally, water hyacinth has also been shown to transform free copper ions into less toxic forms via organic complexation (Sierra-Carmona et al. 2022). Turning water hyacinth into compost seems promising, as the plant is abundant in countries that it inhabits and can be prepared as compost in the dry season where available labor is more abundant (Tibebe et al. 2022). Water hyacinth can be utilized to generate liquid fertilizer, as seen in the Lake Tondano area, which produces 591,300 tons of liquid organic fertilizer annually (Sumual et al. 2018). Utilization of porous carbon from water hyacinth has been used as a composite cathode in lithium sulfur batteries (Nurhilal et al. 2023). By converting water hyacinth to a magnetic biochar, it can act as an effective absorbent to remove lead ions Pb(II) from industrial wastewater (Tran et al. 2022). The blending of water hyacinth and sheep waste can be used to generate energy by creating biogas which could possibly reduce

conventional fossil fuel use (Patil et al. 2014). Water hyacinth can be transformed into bio-based building materials by turning the plant into thermal insulation particle boards, which could be viable in the construction sector (Ilo et al. 2020).

1.6 Lake Okeechobee

Measuring in at 700 square miles (1,800 sq. km), Lake Okeechobee is the largest freshwater lake in the southeastern United States. The lake is located in the center of southern Florida and is characterized by its shallowness. The average depth of the lake is less than 9 feet (3 m) and is about 35 miles (55 km) long (Bass and Machmuller 2022). The lake serves multiple purposes, including flood control, agricultural water use, urban and industrial water supply, fish and wildlife habitat, groundwater recharge, navigation, recreation, as well as water supply for environmental restoration (Steinman et al. 2002). The lake's primary water source is from the Kissimmee River, which is located directly north of Lake Okeechobee. Over the last century, anthropogenic development has altered the lake, affecting its role with the surrounding ecosystem. In the mid 1900's, the construction of a dike around the lake reduced the size of the pelagic zone by roughly 30%, resulting in a reduction of average water levels. Havens and Gawlik (2005) emphasized the impacts of the dike on water levels in the lake. Under natural conditions, water in the lake was able to expand and recede across a large-low gradient marsh that spread from the west to the south. However, when the lake's stage exceeds 4.6 meters, water rises over the smaller littoral zone, flooding it to an even greater depth. Inversely, when the lake stage falls below 3.4 meters, the entirety of the littoral zone dries up, resulting in an absence of lateral expansion of water. Consequently, extremely high, or low lake levels of any duration can cause significant harm to the surrounding ecosystem.

During the 1980s and 1990s and even today, the lake has suffered from high levels of phosphorus, resulting in extensive and frequent algal blooms. Primary sources of phosphorus have been from non-point source agricultural runoff, specifically from beef cattle ranching and dairy farming (Flaig and Reddy 1995). Ecological changes such as shifts in benthic invertebrate community structures towards pollution-tolerant oligochaetes, as well as increased shoreline algal blooms have been linked to excessive phosphorus concentrations (Havens and James 2005).

1.7 Fish Community Assemblage

Fish communities can be used as ecological indicators to describe the impacts of habitat deterioration, invasive species, fisheries stability, and climate change (Corpuz, Paller, and Ocampo 2016). To monitor and manage freshwater fishes, understanding the species assemblages and the characteristics of the habitats their habitats is critical. Additionally, how assemblages are defined can vary, with factors influencing composition including structural features, hydraulic conditions, water quality, and at large scale interactions, even climate and altitude (Hamilton, Pollino, and Walker 2016). Structural conditions include debris, banks, pools, and aquatic vegetation, all of which can affect the composition of surrounding fish populations (Bond and Lake 2003). Hydraulic factors such as water flow and depth, as well as water quality factors like conductivity and turbidity, can additionally affect species assemblage (Hamilton, Pollino, and Walker 2016). To accurately portray fish assemblages, a multitude of gear/sampling methods must be used, as fishes can occur at a multitude of depths/habitat types. Sampling methods for fishes in shallow freshwater habitats often include back-pack electrofishing, seines, baited minnow traps, trap nets, and experimental gill nets (Fischer and Quist 2014). Lentic habitats such as lakes require multiple sampling methods, as lakes can contain distinct physiochemical zones (i.e., littoral, limnetic, pelagic). Seasonal movement can also occur, as species such as Red Shiners (*Cyprinella lutrensis*), and Blacktail Shiners (*C. venusta*) exhibited spring and fall peaks for relative catch per unit while electrofishing in a littoral zone of Lake Texoma (Pope and Willis 1996). By utilizing multiple sampling methods, one can reduce the amount of bias present to accurately portray biological assemblages, as differences in construction materials/sampling gear have demonstrated to result in different species of fish that vary in size (Fischer and Quist 2014). Multiple methods additionally reduce bias by detecting species that are hard to catch and may often be underrepresented (Neebling and Quist 2011).

1.8 Factors Affecting Fish Community Assemblage

Factors that affect fish community assemblage are often classified in a hierarchical organization, with primary or secondary variables (Dembkowski and Miranda 2012). Primary variables can consist of physical lake characteristics, which can influence secondary variables such as water quality and primary productivity, affecting fish community composition. Fish diversity and abundance have been shown to be related to local water shed characteristics, water quality, and physical characteristics of the lake (Carlson et al. 2022). The size and productivity of an ecosystem has shown to significantly account for the variability in the trophic level of fish,

which was seen in 30 lakes found throughout China's Eastern Plain Lake Zone (Jia et al. 2021). A significant negative relationship was found between elevation and total species richness in small lakes in Gatineau Park, Québec (Chapleau, Findlay, and Szenasy 1997). In tropical aquatic ecosystems, predation has been shown to be a major contributor in affecting community structure, influencing species composition, abundance, and biomass (Sá-Oliveira et al. 2016). In lakes that had fisheries management, total fish species richness (α-diversity) as well as the number of predatory species was significantly higher than those without fisheries management (Matern et al. 2022). Fish composition varies significantly between different seasons, with certain fish species labeled as diagnostic species of each season (Jaureguizar et al. 2004).

1.9 Lake Okeechobee Fish Community Assemblage

Fish richness has been shown to be highest in areas containing floating/emergent plants, and intermediate in Maidencane (*Panicum hemitomon*) and Hydrilla (*Hydrilla verticillata*) habitats (Johnson, Allen, and Havens 2007). Species in Lake Okeechobee are diverse, with common native species including Eastern Mosquitofish (*Gambusia holbrooki)*, Least Killifish (*Heterandria formosa*), Sailfin Molly (*Poecilia latipinna*), Bluefin Killifish (*Lucania goodei*), Golden Topminnow (*Fundulus chrysotus*), and Tadpole Madtoms (*Noturus gyrinus*), comprising the lower levels of the trophic system. Blue Tilapia (*Oreochromis aureus*), Oscar Cichlid (*Astronotus ocellatus*), Spotted Tilapia (*Pelmatolapia mariae),* and Red Jewel Cichlids *(Hemichromis bimaculatus*) are larger invasive species that are found throughout Lake Okeechobee. Eastern Mosquitofish have been shown to dominate fish communities, which was seen in a population survey of fish in the Everglades agricultural area conducted by Pearlstine et al. (2007). Results showed that Eastern Mosquitofish were the most numerous fish species caught, as 14,624 out of 18,993 fish caught (roughly 77%) were Eastern Mosquitofish. Invasive species like the Mayan Cichlid have been able to adapt to Lake Okeechobee, as they possess an opportunistic feeding behavior, as well as a tolerance for a wide range of salinities (Matamoros, Chin, and Sharfstein 2005). In limnetic areas of Lake Okeechobee, species such as Threadfin Shad (*Dorosoma petenense*), Bluegill (*Lepomis macrochirus*), and Black Crappie (*Pomoxis* nigromaculatus), accounted for 92% of all fish documented (Bull et al. 1995).

1.10 Water Hyacinth Affecting Invertebrate Community Assemblage

Invasive aquatic plants have been shown to influence invertebrate composition in freshwater bodies. In a study on the impacts of three invasive macrophytes on invertebrate

composition, uninvaded ponds contained 17 different families, compared to invaded ponds that contained ten, nine, and fourteen families respectively (Stiers et al. 2012). Epiphytic invertebrates that reside in native pennywort (*Hydrocotyle umbellate*) have been shown to have differing densities depending on the site and month when compared to hyacinth. In the month of June 1998, water hyacinth exhibited greater concentrations of *Crangonyx floridanus* when compared to native pennywort (Toft et al. 2003). Taxa richness of epiphytic invertebrates in pennywort was slightly greater than that of water hyacinth and had a larger amount of diversity than hyacinth in the month of June (Toft et al. 2023). Studies on macroinvertebrate communities in Florida, demonstrated that water hyacinth, in combination with the native submersed vegetation *Sagittaria kurziana,* exhibited significantly greater macroinvertebrate abundance than sites without hyacinth during the autumn and winter (Villamagna and Murphy 2010). The roots of water hyacinth can also act as refuge for invertebrates as invertebrates found on water hyacinth roots occurred less frequently in fish diets than those associated with native pennywort (Barker, Hutchens, and Luken 2014).

Overall, this research aims to provide insight on the effects of a widely distributed invasive aquatic macrophyte on the communities of organisms in Lake Okeechobee. With water hyacinth having such negative effects on foreign environments, and with little research done on how hyacinth affects aquatic biota, we predict that hyacinth would disrupt animal communities in Lake Okeechobee.

2.0 Materials and Methods

Minnow trap samples were collected in Lake Okeechobee at thirteen sites, designated by the USDA-SAR Invasive Plant Laboratory in Davie, Florida to develop integrative weed management methods for water hyacinth while reducing herbicide input into sensitive aquatic environments (Figure 1).

At each site, the date, time of arrival, water depth (in centimeters), conductivity (HM Digital COM-100 Electrical Conductivity Reader), and dissolved oxygen reading (Fisherbrand™ Traceable™ Portable Dissolved Oxygen Meter) were recorded.

2.1 Site Composition

We sampled thirteen USDA-designated sites that were homogenous in water depth, bottom substrate composition, and temperature. Sites ranged in size from 200-500 m^2 and consisted of three treatment groups: open water, water hyacinth, or native vegetation. Native vegetation varied from pennywort (*Hydrocotyle umbellata*), water primrose (*Ludwigia* spp.), alligator flag (*Thalia geniculata*), and water lettuce (*Pistia stratiotesi*).

As for electrofishing sites, sites were established by myself, as well as other peers on my team in areas along Torrey Island that had ideal patches of hyacinth, pennywort, and open-water areas. Ideal sites were those that had the three treatment groups in the same body of water. A total of seven sites were sampled at/near the Torrey Island area. The area of each site sampled was roughly 40 m^2.

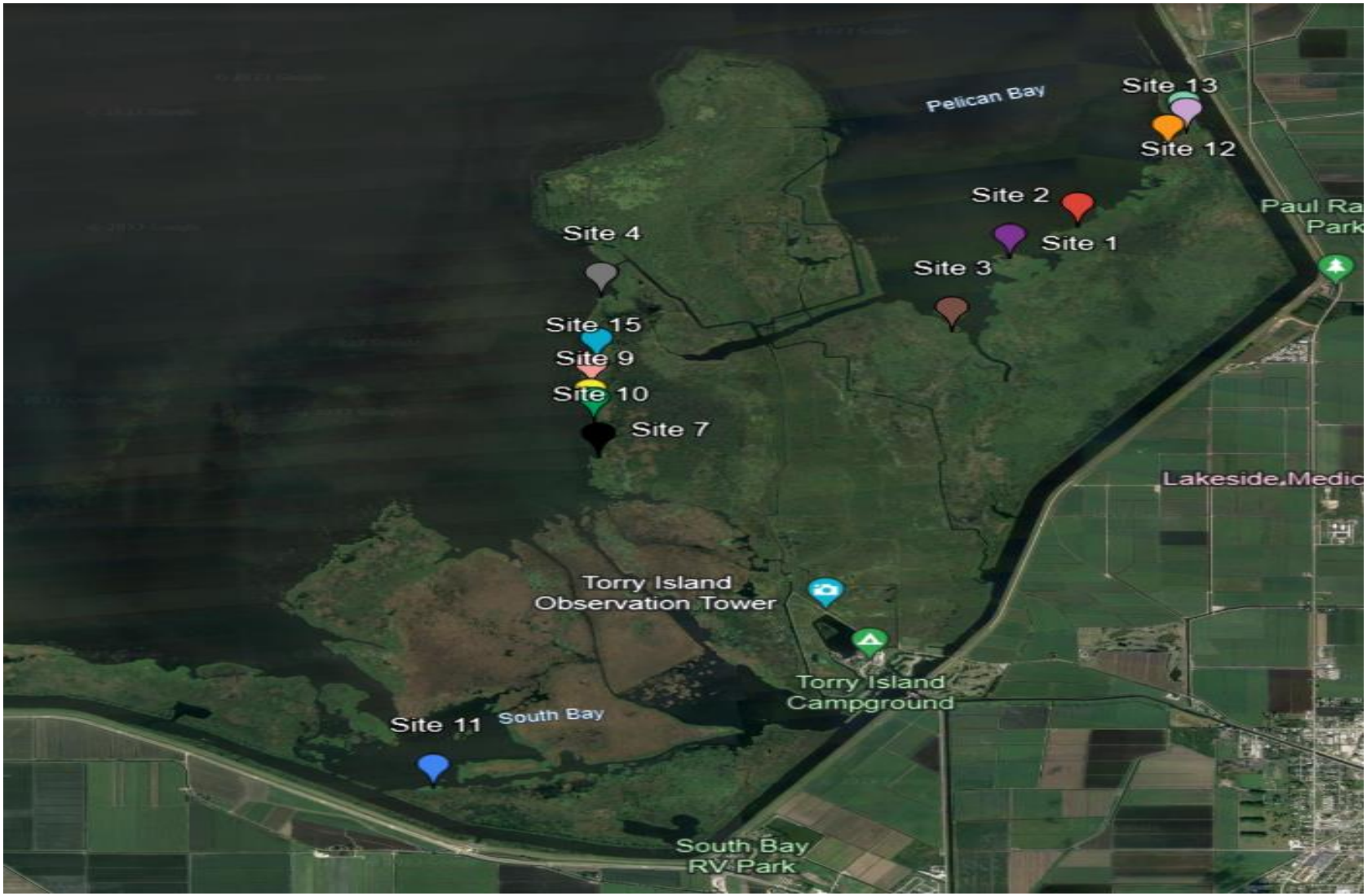

Figure 1. Minnow sites that were sampled at in the surrounding Torrey Island area, located in Lake Okeechobee, Florida (Salerno 2023)

2.2 Baited Minnow Traps

To assess the portion of the fish community including smaller fishes, baited minnow traps (Gee G-40 Minnow Traps) were deployed for 24 hours during each round of sampling at each site. Two minnow traps were deployed at each treatment group, for a total of six traps at each site. Traps were tied with a polypropylene rope that was clipped to a zip tie and a labeled float on a PVC-pole that was pushed into the ground. The two traps were clipped to the PVC-pole via zip ties with both a short and long line of rope to sample a 40 cm apart distance in depth. Minnow traps were baited with roughly five pieces of Pedigree dog food. After a 24-hour period, traps were retrieved. Traps were emptied into a 50 cm x 75 cm mesh PVC-framed basket where species were identified, counted, and recorded. Fish species that were not easily identified upon initial catch were photographed for later identification. Traps that experienced a large quantity of fish were photographed to be recorded and counted on a computer. Live pictures of specimens caught were also taken by placing the caught fish in a Wild Fish Conservancy photarium to capture the specimens' full hues of color. This was done as the use of ethanol for preservation purposes often drowns out and fades away the colors of the specimen. Pictures (12 megapixels) were taken on a WG-6 Digital Camera, Model: R02050, as well as on an iPhone 12. Images were examined on the computer with ImageJ (Java 1.8.0_345 (64-bit)). Using ImageJ's cell counter feature, a colored number corresponding to each species being counted was displayed every time I clicked an image on the screen. The cell counter feature allowed for a rapid means of counting large quantities of fish. However, the cell counter required confirmation by eye as some fish in the images were in proximity of each other, and in some cases, on top of each other.

One to four representatives of each species caught were collected for permanent archiving as voucher specimens. Representative specimens were euthanized humanely by placing them in a collection jar containing a mixture of lake water and tricaine mesylate (MS-222), buffered with baking soda. Up to four representative samples of each species were taken during sampling. Once euthanized, specimens were stored in ethanol for preservation. Specimens were then brought back to the Ecology, Evolution, and Environment Laboratory, in the Parker building at the Nova Southeastern University (NSU) main campus in Davie, Florida. Specimens were placed in glass jars, which were then labeled with the species.

All sampling of live fishes occurred under the prior approval of NSU Institutional Animal Care and Use Committee protocol [2021.08.JH3] to PI J M Hoch.

2.3 Hook and Line Angling

To assess the community of tertiary and secondary consumer fish species found in the lake, hook-and-line angling was used. Hook-and-line angling occurred at sites that were sampled with baited minnow traps. Two traditional fishing rods were deployed at each of the three treatment groups per site for a total of six lines cast per site. Lines sat in the water at each area for five minutes each and were reeled in once the five-minute period had ended. Standard two-inch hooks were used with a bobber placed roughly 15.2 cm from the hook. Hooks were baited with locally purchased nightcrawlers. Nightcrawlers were used until either a bite occurred, and the nightcrawler was taken, or we moved to a new site. To record fish caught, photographs of the specimens caught were taken next to a meter stick for size reference. After recording, specimens were released back into the lake. No specimens were recaught as we did not catch more than one fish at each site when sampling.

2.4 Electrofishing

A Smith Root LR-20B electrofisher was used to assess fish and invertebrate populations at different sampled sites. Each site consisted of a water hyacinth, native pennywort, and an open water treatment group. To ensure randomness, each treatment group within a site was assigned a number of one, two, or three. Using the Google™ online random number generator, a number would be selected, representing one of the three treatment groups. This process was done again to determine which treatment group would be sampled second. The electrofisher was then used at each area in eight, 30 second rounds totaling about 5-meter transects, where the electrofisher would shock for 30 seconds continuously as the operator of the unit moved the anode pole to sweep through each treatment group. Next to the operator of the unit, two catchers with long nets would sweep the water surrounding the electrofisher, with one person sweeping near the anode ring, and the other sweeping at the back near the cathode tail. Organisms caught were dumped onto a 50 cm x 75 cm PVC net-screen after each 30-second repetition. Organisms were identified and recorded in a waterproof field notebook. The electrofisher settings were kept the same at all sites: frequency was set to 45 pulses per second, duty cycle was at 10%, and voltage was 250 V.

2.5 Invertebrate Collection

Invertebrate collection occurred at eight of the thirteen sites used for baited minnow traps. At each site, a handful of water hyacinth was taken out of the water and placed into a 10-liter bucket containing one liter of ethanol and nine liters of water. Once placed in the bucket, the

plant was then submerged and shaken thoroughly in the solution for ten seconds. After shaking for ten seconds, the plant was then discarded. The leftover contents in the bucket were poured and seined through a mesh net into the other empty 10-liter bucket to collect invertebrates that were residing on/in the plant. The contents of the net were then placed into a small plastic container. A solution of Rose Bengal and 70% ethanol (to dye the organisms) was then poured into the plastic container until it was full (Toft et al. 2003). The lid of the container was then marked with the site, date, and what plant it was collected from. This procedure would then be repeated using a handful of pennywort found at the same site. The contents of the bucket containing the water-ethanol solution were reused for every site and sample. Once sampling had concluded, the contents of the 10-liter bucket were brought back to the lab and disposed of appropriately. The samples were also brought back to the lab to be stored, identified, and sorted later.

Invertebrate sorting started by first grabbing a sample and emptying it onto a sorting tray to sort the invertebrates from plant matter. Ethanol was frequently added to the sorting trays to prevent drying. Rose Bengal dyed organisms or figures that strongly resembled an organism were removed from the remaining plant material using a pair of tweezers. Sorting occurred by looking at the contents with either the naked eye and light or a dissecting microscope. Samples were thoroughly picked to prevent leaving out specimens. Organisms were then identified and photographed using a Panasonic digital camera, or digital microscope for record keeping. Organisms were identified by comparing the photos of specimens, or those under the microscope to corresponding ID guides, or by utilizing the "Invertebrate Identification Guide", by the Florida International University Aquatic Ecology Lab (Robertson et al. 2006). They were then counted and placed into microcentrifuge tubes filled with ethanol. The tubes were labeled with the type of organism using a shortened ID tag name "CODE" and the number of the organism. As organisms were counted, a clicker was utilized to keep track of the quantity. Terrestrial organisms and fish found were excluded from the count and placed in separate microcentrifuge tubes away from the invertebrates. Organisms that could not be identified at the time were left unmarked and stored with other microcentrifuge tubes to be reexamined later. Those that could not be identified even after thorough analysis were given a code name such as "unidentified snail 1" so each unidentified organism could be expressed as a different organism for subsequent data analysis. Once a sample had been completely sorted and identified, the quantified information was

recorded on paper and put into an Excel spreadsheet. The information recorded included the number of organisms, site, host plant, the name of the person sorting, and the date the sample was collected and sorted. The microcentrifuge tubes were then placed in a container labeled with the sample's information and stored. The remaining contents on the sorting tray that were left over were placed back into the original plastic container and stored.

2.6 Statistical Analysis

2.6.1 Minnow Trap Analysis

Statistical analyses on the Minnow Trap data ere performed with using the statistical analysis program PRIMER v7. The data was first given a pretreatment Log(X+1) transformation to reduce the skew from species that were present in large numbers, as a large amount of the sites sampled contained few to no fish. A range of univariate indices was then calculated through the DIVERSE function. Indices such as Shannon-Weiner and Simpson were calculated. Shannon measures the amount of entropy in a system, serving as a measure of predicting the identity of the next sampled individual (Hill et al. 2003), and Simpson, measures the probability that two individuals from a sample, when chosen at random, will be the same species (Fath 2018). The averages were calculated for both Shannon and Simpson indices. Rarefaction data was calculated to compare species richness under the three different treatment groups (Invasive, Native, and Open). The RESEMBLANCE function to generate a Bray-Curtis dissimilarity matrix. Similarities between samples were calculated and run through the Analysis of Similarity (ANOSIM) to test whether communities between treatment groups are similar. A two-way nested ANOSIM (B within A), testing for similarities between Treatment groups (Invasive, Native, and Open) within Sites was run. The SIMPER (Similarity Percentage) function was used to find the average contributions of each species to the average overall dissimilarity between different treatment methods. The SUMMARY function was used on the raw data to calculate the sum of each species, the minimum, the maximum, and the number of each species caught. A non-metric MDS plot was generated to display dissimilarities between treatment groups.

SAS (version 9.2) was used to conduct a two-way ANOVA that tested run for differences in mean Shannon and Simpson index values using type and site as predictor variables.

2.6.2 Electrofishing Analysis

Statistical analysis was performed using PRIMER v7 on the data sampled using the Smith Root LR-20B electrofisher. The data was first given a pre-treatment Log(X+1) transformation to reduce the skew once again from large samples. Data was then run through the DIVERSE function to yield the above-mentioned indices. The averages were calculated for both Shannon and Simpson indices. Using rarefaction values, a rarefaction curve for the three treatment methods (Invasive, Native, and Open) was generated to visualize species richness. The transformed data was further analyzed using the SIMPER function. The ANOSIM function tested a two-way nested ANOSIM, with Treatment (Invasive, Native, and Open) nested within Site. A non-metric MDS plot was then generated to visualize dissimilarities between sites. The SUMMARY function was used on the raw data to calculate the sum of each species, and the minimum and maximum of each species.

SAS version was used again to run a two-way ANOVA that tested for differences in mean Shannon and Simpson index values using type and site as predictor variables. As a result of lack of replication for the electrofishing sampled data, only the effects of treatment and site, excluding the interaction between the two, were tested for both average Shannon and Simpson index values.

2.6.3 Angling Analysis

Angling data did not receive statistical analysis, as the sample population consisted of only 6 individuals, which would not be enough to accurately represent communities within the lake. Instead, a table highlighting the species with quantity caught, as well as the number of bites recorded was generated.

2.6.4 Invertebrate Analysis

Invertebrate data received statistical analysis treatment using PRIMER v7 as well. A Log(X+1) pre-treatment transformation was first applied to the raw data to reduce skew from large samples. The DIVERSE function was used to calculate the Shannon, Simpson, and rarefaction values for the invertebrate data. The averages were calculated for the Shannon and Simpson indices. A rarefaction curve was created for each treatment method (Invasive, Native, and Open). Transformed data was run through the RESEMBLANCE function to cover similarity. Similarities between samples were calculated and then ran using One-Way ANOSIM testing for differences in invertebrate communities between invasive water hyacinth and native pennywort

treatments. A SIMPER analysis using a Bray-Curtis resemblance was also performed utilizing the DIVERSE data to find the average similarity/dissimilarity of species under Invasive/Native treatments.

3.0 Results

3.1 Minnow Trap Analysis

A total of 9,258 individuals were sampled over a thirteen-month span, comprising of seventeen documented fish species (Table 1). Of the seventeen species, five species accounted for 98.7% (roughly 9,216 individuals) of the total number of fish caught in the minnow traps.

The two-way nested ANOSIM testing for differences between treatment groups across all sites showed that there were no statistically significant differences between treatment groups (R=-0.009, P-value=0.602). Both these tests yielded values that support the idea that water hyacinth does not significantly affect freshwater fish communities in Lake Okeechobee that were caught using minnow traps.

The two-way ANOVA testing for differences in mean Shannon index values using treatment and site as predictor variables showed that mean Shannon diversity index values when testing for Site were statistically significant (p=0.0001) (Table 2). Mean Shannon diversity index values tested with treatment (Invasive, Native, Open) were not statistically significant (p=0.7174). Site crossed with treatment showed to not have a significant statistical effect on mean Shannon diversity index values.

The two-way ANOVA testing for differences in mean Simpson index values using Treatment and site as predictor variables found there to be no significant variation in mean Simpson values (Table 3).

Table 1. Total species caught in minnow traps and number of each individual species caught.

Species Name	Percentage of Total Fish Caught	Common Name	Count
Astronotus ocellatus	0.06%	Oscar Cichlid	6
Clarias batrachus	0.01%	Walking Catfish	1
Etheostoma fusiforme	0.01%	Swamp Darter	1
Fundulus chrysotus	0.14%	Golden Topminnow	12
Gambusia holbrooki	56.5%	Eastern Mosquitofish	5,230
Hemichromis bimaculatus	0.01%	Red Jewel Cichlid	1
Heterandria formosa	4.2%	Least Killifish	389
Lepomis gulosus	0.18%	Warmouth	17
Lepomis spp.	0.14%	unidentified sunfish species	12
Lucania goodei	22.3%	Bluefin Killifish	2,064
Mayaheros urophthalmus	14.2%	Mayan Cichlid	1,318
Noturus gyrinus	0.03%	Tadpole Madtom	3
Oreochromis aureus	0.37%	Blue Tilapia	34
Pelmatolapia mariae	0.09%	Spotted Tilapia	7
Poecilia latipinna	1.52%	Sailfin Molly	141
Pterygoplichthys multiradiatus	0.24%	Ornico Sailfin Catfish	22
Total Sum			9,258

Table 2. Two-way ANOVA testing for differences in mean Shannon index values using treatment and site as predictor variables.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	12	13.57163	1.130969	3.77	<0.0001
Treatment	2	0.200004	0.100002	0.33	0.7174
Site*Treatment	21	4.081634	0.194364	0.65	0.8754

Table 3. Two-way ANOVA testing for differences in mean Simpson index values using treatment and site as predictor variables.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	12	3.540016	0.295001	1.72	0.0702
Treatment	2	0.043355	0.021677	0.13	0.8814
Site*Treatment	21	2.909383	0.138542	0.81	0.7062

A SIMPER test using a Bray-Curtis similarity resemblance and a cut-off percentage for low contributions of 70% was run for the three treatment groups (Invasive, Native, and Open) crossed with Site (Table 4). *G. holbrooki* accounted for the greatest dissimilarity between Native and Open treatments (27.38%), followed close behind by *L. goodei* (26.87%).

Native and Invasive groups had the greatest dissimilarity arise from *L. goodei* (23.66%), followed by *G.holbrooki* (22.86%). The Open and Invasive group had the greatest dissimilarity from *L.goodei* (29.06%), followed by *G.holbrooki* (28.17%).

A non-metric MDS plot was generated to highlight relationships between samples. Samples 47, 109, 115, 121, & 153 were omitted as they were outliers and made visualizing the dissimilarities between samples difficult.

A rarefaction curve of the data was plotted to assess species richness under the three different treatment groups (Figure 2). Species richness was highest under the native treatment, but the invasive water hyacinth treatment was very close in comparison.

Table 4. SIMPER analysis test results highlighting average Similarity and Dissimilarity between different treatment groups for Minnow Trap data.

Group N						
Average similarity: 31.56						
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
GAMHOL	1.74	11.54	0.82	36.58	36.58	
MAYURO	1.43	10.12	0.80	32.06	68.64	
LUCGOO	1.23	5.83	0.55	18.48	87.12	
Group O						
Average similarity: 44.57						
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
LUCGOO	1.97	18.62	1.18	41.78	41.78	
GAMHOL	2.28	15.78	0.85	35.41	77.19	
Group I						
Average similarity: 32.01						
Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%	
MAYURO	1.41	12.91	0.78	40.33	40.33	
GAMHOL	1.63	10.68	0.71	33.36	73.69	
Groups N & O						
Average dissimilarity = 63.62						
	Group N	Group O				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
GAMHOL	1.74	2.28	17.42	1.27	27.38	27.38
LUCGOO	1.23	1.97	17.09	1.04	26.87	54.25
MAYURO	1.43	1.10	12.55	0.88	19.72	73.97
Groups N & I						
Average dissimilarity = 65.60						
	Group N	Group I				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
LUCGOO	1.23	1.05	15.52	0.84	23.66	23.66
GAMHOL	1.74	1.63	15.00	1.12	22.86	46.52
MAYURO	1.43	1.41	14.29	0.86	21.78	68.30
HETFOR	0.65	0.56	7.59	0.81	11.56	79.86
Groups O & I						
Average dissimilarity = 66.25						
	Group O	Group I				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
LUCGOO	1.97	1.05	19.25	1.09	29.06	29.06
GAMHOL	2.28	1.63	18.66	1.29	28.17	57.23
MAYURO	1.10	1.41	12.94	0.93	19.54	76.77

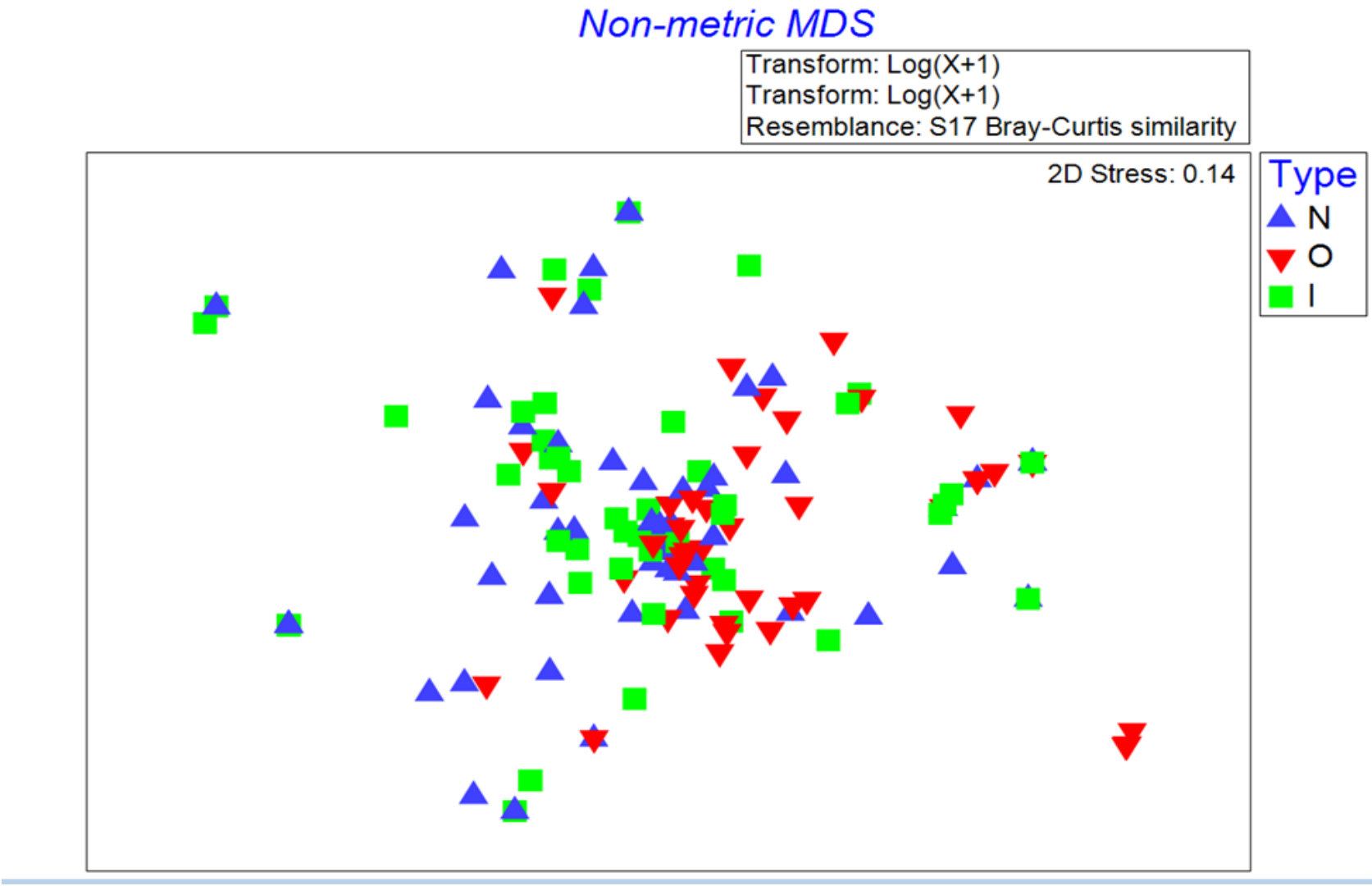

Figure 2. A non-metric MDS plot with a (Log X+1) transformation highlighting similarities/differences between treatment groups (Invasive, Native, and Open) for Minnow-Trap sampled data, excluding outliers from samples 47, 109, 115, 121, & 153.

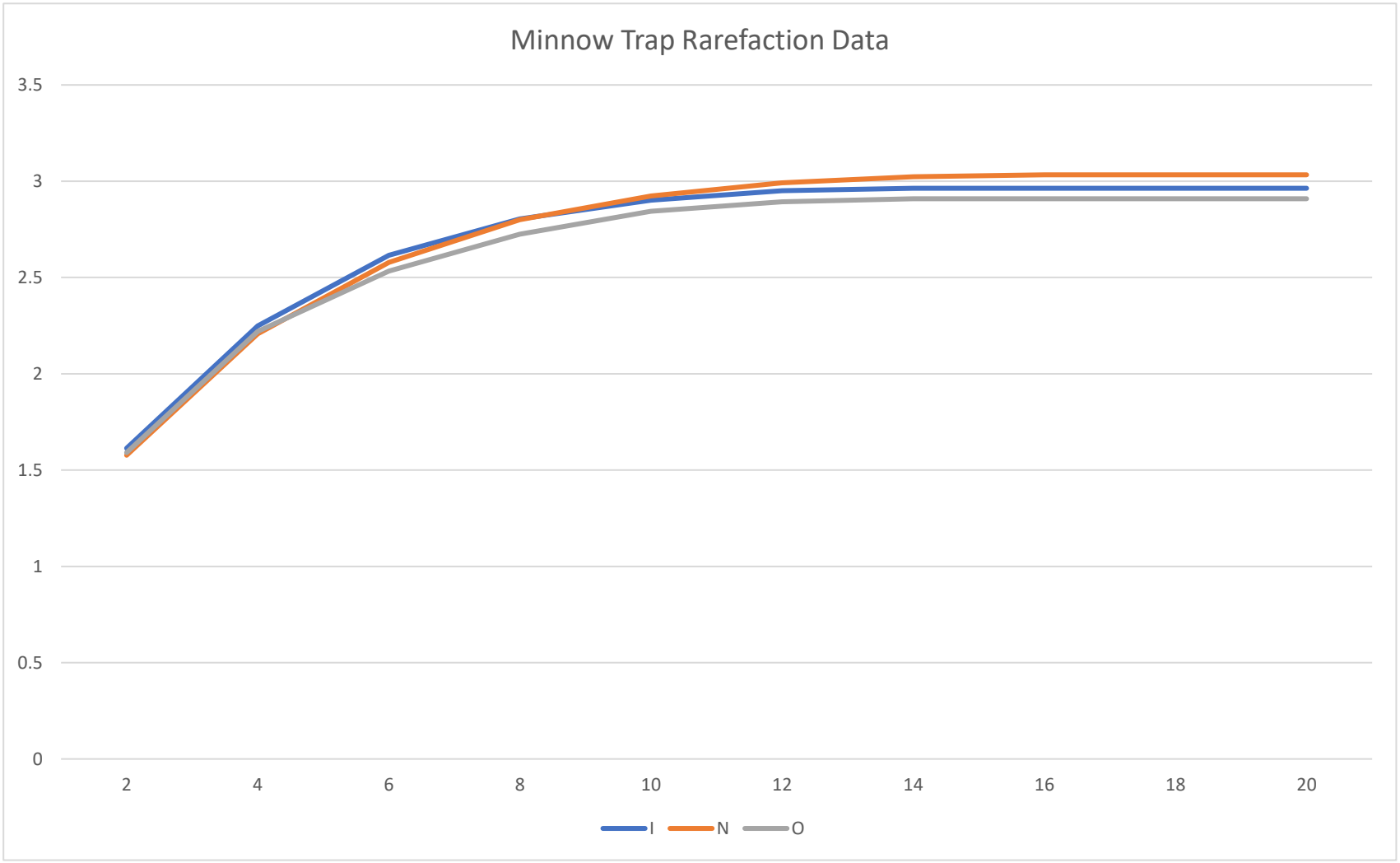

Figure 3. Rarefaction data for the three treatment groups (Invasive, Native, and Open) using the sampled minnow trap data.

3.2 Electrofishing Data Analysis

2,903 individuals comprising of 26 species were sampled using a Smith Root LR-20B electrofisher (Table 5). The most abundant species caught over the three-month span was *Palaemonetes paludosus* at 1,252 individuals (43.1%). *G. holbrooki* followed behind with 986 individuals (34%). *M. urophthalmus* had 180 individuals sampled (6.2%). *Heterandria formosa* had 166 individuals recorded (5.7%). *L. goodei* had 96 individuals sampled (3.3%). These five species made up 92.2% of the 2,906 individuals sampled.

The two-way nested ANOSIM testing for differences between treatment groups nested within site showed that there were no statistically significant differences between treatment groups (R=0.321, P-value=0.20), indicating that there were no differences in fish and invertebrate communities under water hyacinth when compared to native pennywort.

A two-way ANOVA due to a lack of replication testing for treatment and site on mean Shannon/Simpson index values was run (Tables 6 and 7), without testing the interaction of site and treatment. For the mean Shannon index values, there was a significant effect of site on mean Shannon diversity index values (p=0.0195). Treatment was shown to be non-significant on the mean Shannon index values. Mean Simpson index values showed to be non-significant when testing for both treatment and site.

A SIMPER test using a Bray-Curtis similarity resemblance and a cut-off percentage for low contributions of 70% was run for the three treatment groups (Invasive, Native, and Open) crossed with Site (Table 8). *P. paludosus* accounted for the greatest dissimilarity between Native and Open treatments at 19.42%, followed by *H. Formosa* at 12.47%. *P. paludosus* once again accounted for the greatest dissimilarity between Native and Invasive treatment groups, with it being responsible for 15.20% of the dissimilarity between Native and Invasive treatments. *L. goodei* had the second greatest dissimilarity between the two groups at 9.93%. Open and Invasive groups had the greatest dissimilarity from *L. goodei* (14.34%), followed by *H. Formosa* (13.48%).

A non-metric MDS plot with a Log(X+1) transformation was generated for the electrofishing data to highlight dissimilarities between treatment groups (Figure 4).

A rarefaction curve of the data was plotted to assess species richness under the three different treatment groups (Figure 4). The species richness was highest under the invasive

treatment group, indicating that more species are expected to be found initially under the invasive treatment than the native treatment.

Table 5. Total species caught using the Smith Root LR-20B electrofisher and number of each individual species caught.

Species	Common Name	Count
Ameiurus nebulosus	Brown Bullhead Catfish	1
Belostoma spp.	Belostoma Species	10
Unidentified beetle larvae *(COLEOA)*	unidentified beetle larvae	13
Ephemeroptera spp.	Mayfly species	29
Etheostoma fusiforme	Swamp Darter	4
Fundulus chrysotus	Golden Topminnow	3
Fundulus seminolis	Seminole Killifish	1
Gambusia holbrooki	Eastern Mosquitofish	986
Heterandria formosa	Least Killifish	166
Lepomis gulosus	Warmouth	4
Lepomis macrochirus	Bluegill	10
Lepomis punctatus	Spotted Sunfish	3
Lepomis spp.	unidentified sunfish species	12
Lucania goodei	Bluefin Killifish	96
Mayaheros urophthalmus	Mayan Cichlid	180
Microphis brachyurus	Opossum Pipefish	3
Micropterus salmoides	Largemouth Bass	1
Micropterus spp.	unidentified Bass Species	1
Odonata spp.	Dragonfly Species	33
Oreochromis aureus	Blue Tilapia	3
Palaemonetes paludosus	Glass Shrimp	1,252
Pelocoris femoratus	Gator Flea	7
Pelmatolapia mariae	Spotted Tilapia	10
Poecilia latipinna	Sailfin Molly	38
Procambarus spp.	unidentified crayfish species	28
Pterygoplichthys multiradiatus	Orinoco Sailfin Catfish	9
Total Sum		2,903

Table 6. A two-way ANOVA testing for differences in mean Shannon index values using treatment and site as predictor variables.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	6	3.22989484	0.53831581	4.01	0.0195
Treatment	2	0.23806599	0.11903299	0.89	0.4375

Table 7. A two-way ANOVA testing for differences in mean Simpson index values using treatment and site as predictor variables.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	6	0.23752292	0.03958715	0.90	0.5263
Treatment	2	0.07449782	0.03724891	0.85	0.4533

Table 8. SIMPER analysis test results highlighting average dissimilarity between different treatment groups for electrofishing sampled data.

Groups N & O						
Average dissimilarity = 50.67						
	Group N	Group O				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
PALPAL	3.90	2.20	9.84	1.11	19.42	19.42
HETFOR	1.65	1.30	6.32	2.03	12.47	31.89
GAMHOL	3.11	2.61	5.54	0.90	10.93	42.82
LUCGOO	1.42	1.12	5.47	1.31	10.80	53.62
MAYURO	1.58	1.04	3.97	1.58	7.84	61.45
BELSPP	0.16	0.43	2.61	0.70	5.14	66.60
PROSPP	0.65	0.33	2.46	1.04	4.86	71.46
Groups N & I						
Average dissimilarity = 33.06						
	Group N	Group I				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
PALPAL	3.90	2.81	5.03	0.65	15.20	15.20
LUCGOO	1.42	0.89	3.28	1.41	9.93	25.12
PROSPP	0.65	0.81	3.21	0.69	9.70	34.83
POELAT	0.98	0.45	2.98	0.77	9.01	43.84
MAYURO	1.58	1.65	2.62	0.97	7.94	51.77
HETFOR	1.65	1.18	2.13	0.94	6.43	58.20
ODOSPP	0.61	0.55	2.02	0.53	6.11	64.32
LEPMAC	0.10	0.50	1.97	0.67	5.97	70.29
Groups O & I						
Average dissimilarity = 50.05						
	Group O	Group I				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
LUCGOO	1.12	0.89	7.18	2.27	14.34	14.34
HETFOR	1.30	1.18	6.75	1.49	13.48	27.83
PALPAL	2.20	2.81	6.28	0.81	12.55	40.38
GAMHOL	2.61	3.02	5.59	0.67	11.17	51.54
PROSPP	0.33	0.81	3.91	1.34	7.81	59.35
MAYURO	1.04	1.65	3.24	0.80	6.48	65.83
BELSPP	0.43	0.10	2.49	0.67	4.97	70.80

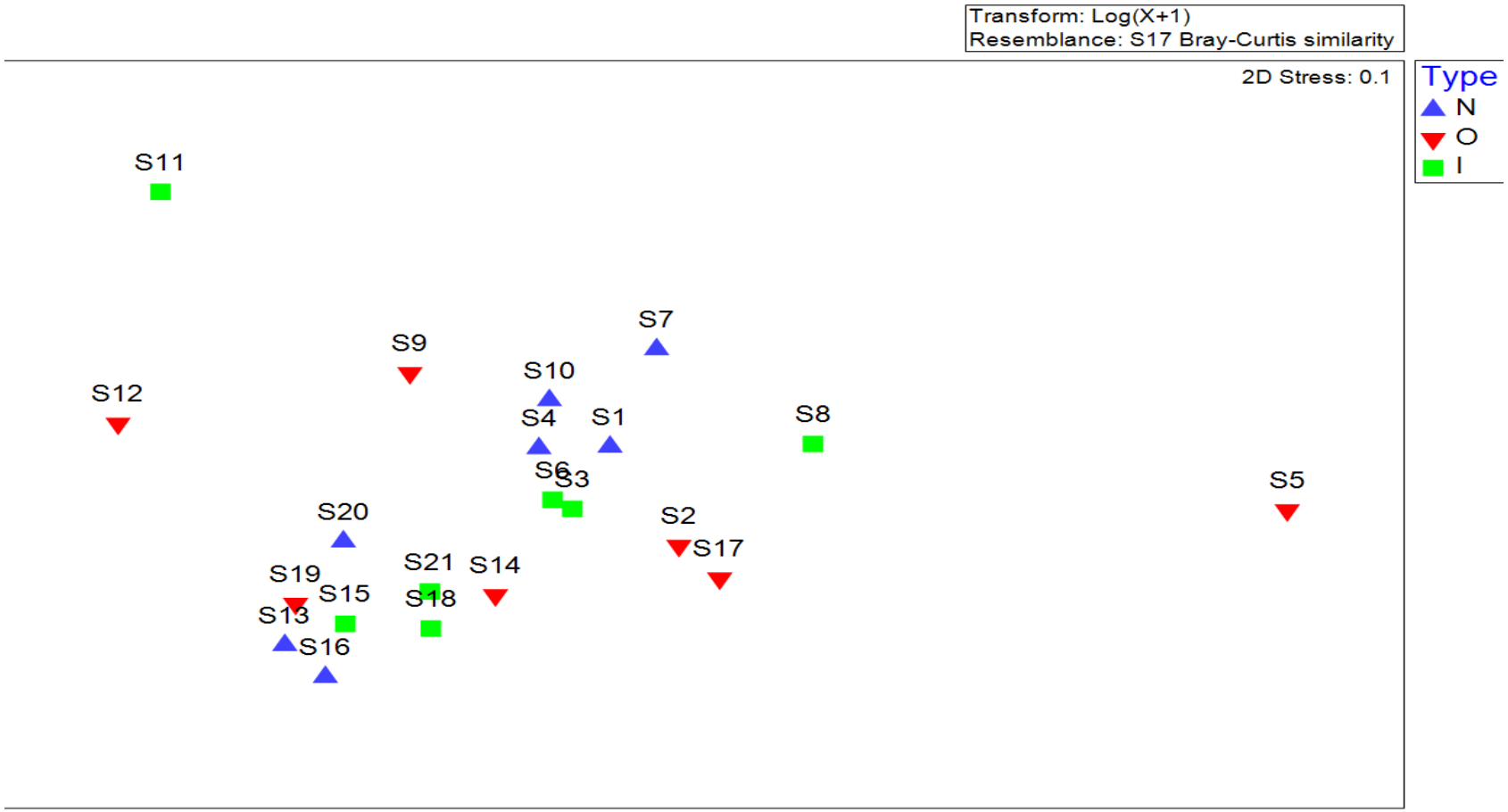

Figure 4. A non-metric MDS plot with a (Log X+1) transformation highlighting similarities/differences between treatment groups (Invasive, Native, and Open) for Smith Root LR-20B electrofisher sampled data.

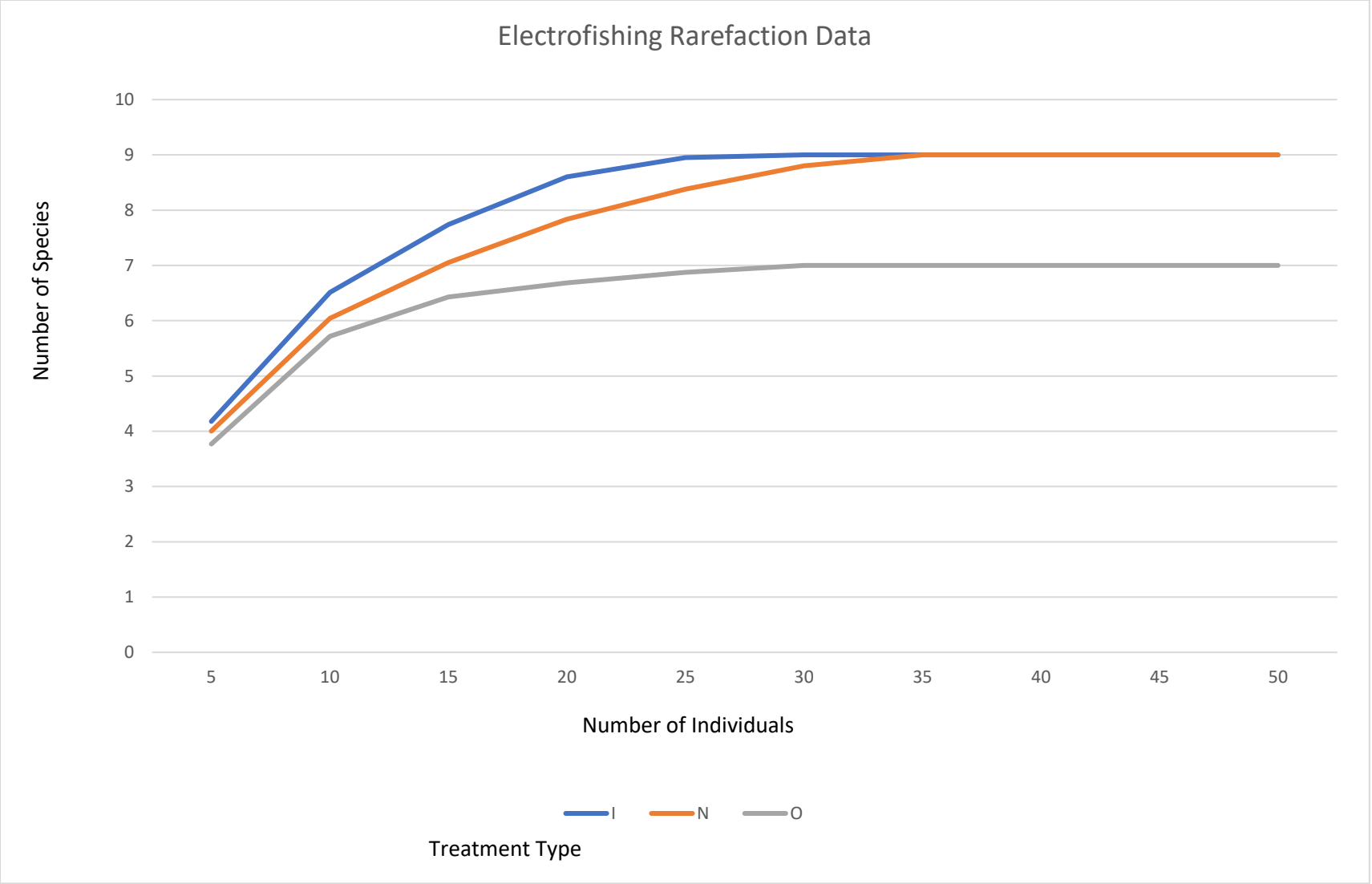

Figure 5. Rarefaction data highlighting the species richness under the three different treatment methods (Invasive, Native, and Open) for Smith Root LR-20B electrofisher sampled data

3.3 Angling Data Analysis

Only a small quantity of individuals was caught using the angling method, making statistical analysis not ideal. A total of six individuals were caught, with each individual being a distinct species. Table 9 highlights the six species caught, as well as the number of bites recorded.

Table 9. Total species caught angling and number of each individual species caught angling.

Species Name	Common Name	Treatment	Count
Ictalurus punctatus	Channel Catfish	Invasive	1
Clarias batrachus	Walking Catfish	Open	1
Mayaheros urophthalmus	Mayan Cichlid	Open	1
Hoplosternum littorale	Brown Hoplo	Native	1
Astronotus ocellatus	Oscar Cichlid	Native	1
Ameiurus nebulosus	Brown Bullhead Catfish	Native	1
Bites (Unknown Species)	NA	NA	10
Total Sum			16

3.4 Invertebrate Data Analysis

36 taxa, representing a total of 12,047 individuals were sampled under pennywort and water hyacinth plants (Table 6). *Hyalella azteca* comprised of the greatest percentage of total individuals caught, comprising of 95% of total individuals. Cyclopoid copopods were the second most abundant group caught, comprising of 1.3% of total individuals caught. Ostracods were the third most abundant group caught, representing 1.1% of the sample population.

A two-way ANOVA due to a lack of replication testing for treatment and site on mean Shannon/Simpson index values was run (Tables 11 and 12), without testing the interaction of both site and treatment on the invertebrate data. For the mean Shannon index values, there was no significant effect of site, as well as treatment on mean Shannon diversity index values. Mean Simpson index values showed to be non-significant when testing for both treatment and site as well.

A two-way nested ANOSIM testing for differences between Treatment groups nested within site found that there was no statistical difference in invertebrate populations underneath Invasive water hyacinth when compared to native pennywort ($R=0.03$, $P=0.48$).

A SIMPER test using a S17 Bray-Curtis similarity resemblance and a cut-off percentage for low contributions of 70% was run for the two treatment groups (Invasive and Native) crossed with Site (Table 7). Freshwater amphipods contributed to the greatest dissimilarity between Native and Invasive treatment groups, at 10.35%. Ostracods contributed to the second highest dissimilarity at 9.05%. The *Hirudinea* group followed behind the Ostracods, having a dissimilarity of 7.34%.

The non-metric MDS plot (Figure 6) generated to visualize differences between Invasive and Native treatment groups showed that there was a great deal of distance between each treatment group at each site. For example, S1 and S2 represent Site 3 Invasive and Native treatment. The distance between the two samples is a great enough distance away, indicating that there is a dissimilarity between the species found under water hyacinth compared to pennywort.

A rarefaction curve (Figure 6) displayed the species richness under native and invasive treatments. The species richness was much higher under the invasive water hyacinth than the native pennywort, indicating that more species were expected to be found under water hyacinth when sampling than compared to pennywort.

Table 10. Total species caught for the Invertebrate sampling under Pennywort and invasive Water Hyacinth with species code names.

Identification	Common Name	Count
Acaridae (ACARIN)	Aquatic Mites	5
Hyalella azteca (AMPHIP)	Freshwater Amphipods	11,443
Aphaostracon pachynotus (APHPAC)	Dense Hydrobe	5
Copepoda (CALANO)	Calanoid	2
Ceratopogonidae (CERATO)	Unidentified Biting Midge Larvae	3
Chironomidae (CHIRON)	Unidentified Midge Larvae	24
Coenagrionidae (COENAG)	Narrow-winged Damselflies	16
Insecta (COLEOA)	Aquatic Adult Beetle	7
Culicidae (CULICI)	Mosquito Larvae	1
Cybister spp. (CYBFIM)	Predaceous Diving Beetle	2
Copepoda (CYCLOP)	Cyclopoid	159
Diptera (DIPTER)	Unknown Fly Larvae	2
Enochrus spp. (ENOSPP)	Water Scavenger Beetle Larvae	1
Ephemeroptera (EPHEME)	Mayfly Nymph	11
Erythemis simplicicollis (ERYSIM)	Pondhawks	1
Haitia spp. (HAISPP)	Physid Snail	1
Copepoda (HARPAC)	Harpacticoid	1
Hirudinea (HIRUDI)	Leeches	95
Hydrocanthus spp. (HYDSPP)	Aquatic Beetle Larvae	3
Unknown Isopod (ISOPOD)	Unknown Isopod (ISOPOD)	36
Laevepax pennisulae (LAESPP)	Peninsula Ancylid	5
Lepidoptera (LEPIDO)	Butterfly and Moth Larvae	1
Littoridinops monoroensis (LITMON)	Cockscomb	3
Macrothricidae (MACROT)	Cladoceran	28
Noteridae (NOTERL)	Unidentified noterid beetle larvae	5
Oligochaete (OLIGOC)	Oligochaetes	25
Ostracoda (OSTRAC)	Ostracod	129
Pachydiplax longipennis (PACLON)	Blue Dasher	1
Pelocoris femoratus (PELFEM)	Alligator Flea	12
Planorbella spp. (PLASPP)	Planorbid Snail	1
Procambarus spp. (PROSPP)	Crayfish Species	1
Pseudosuccinae columella (PSECOL)	Mimic Pond-snail	3
Sphaeriidae (SPHAER)	Fingernail Clam	2
Tanytarsini (TANSPP)	Midge Larvae	2
Tanypodinae (TANYPO)	Midge Larvae	9
Trichoptera (TRICHO)	Caddis Fly Larvae	2
Total Sum		12,047

Table 11. A two-way ANOVA testing for differences in mean Shannon index values using treatment and site as predictor variables.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	7	3.05939205	0.43705601	1.62	0.2862
Treatment	1	0.35555766	0.35555766	1.32	0.2944

Table 12. A two-way ANOVA testing for differences in mean Simpson index values using treatment and site as predictor variables.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Site	7	0.22345506	0.03192215	1.18	0.4299
Treatment	1	0.00445358	0..00445358	0.16	0.6996

Table 13. SIMPER test results for Invertebrate data testing treatment type within Site.

Groups Hyacynth & Pennywart						
Average dissimilarity = 48.56						
	Group Hyacynth	Group Pennywart				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum.%
AMPHIP	6.69	5.73	5.02	1.15	10.35	10.35
OSTRAC	1.52	0.09	4.39	1.18	9.05	19.39
HIRUDI	1.04	1.11	3.57	1.02	7.34	26.73
ISOPOD	0.79	0.57	3.19	0.78	6.58	33.31
COENAG	0.63	0.48	2.99	1.50	6.16	39.47
OLIGOC	0.28	0.58	2.76	0.94	5.68	45.14
CYCLOP	1.26	0.09	2.39	0.65	4.93	50.07
CHIRON	0.48	0.30	2.25	0.62	4.64	54.71
PELFEM	0.50	0.43	1.78	1.33	3.66	58.37
COLEOA	0.50	0.09	1.74	1.38	3.58	61.95
EPHEME	0.63	0.09	1.50	0.78	3.10	65.05
LAESPP	0.10	0.26	1.16	0.59	2.39	67.44
DIPTER	0.20	0.00	1.03	0.56	2.12	69.56
APHPAC	0.10	0.26	0.97	0.77	2.00	71.55

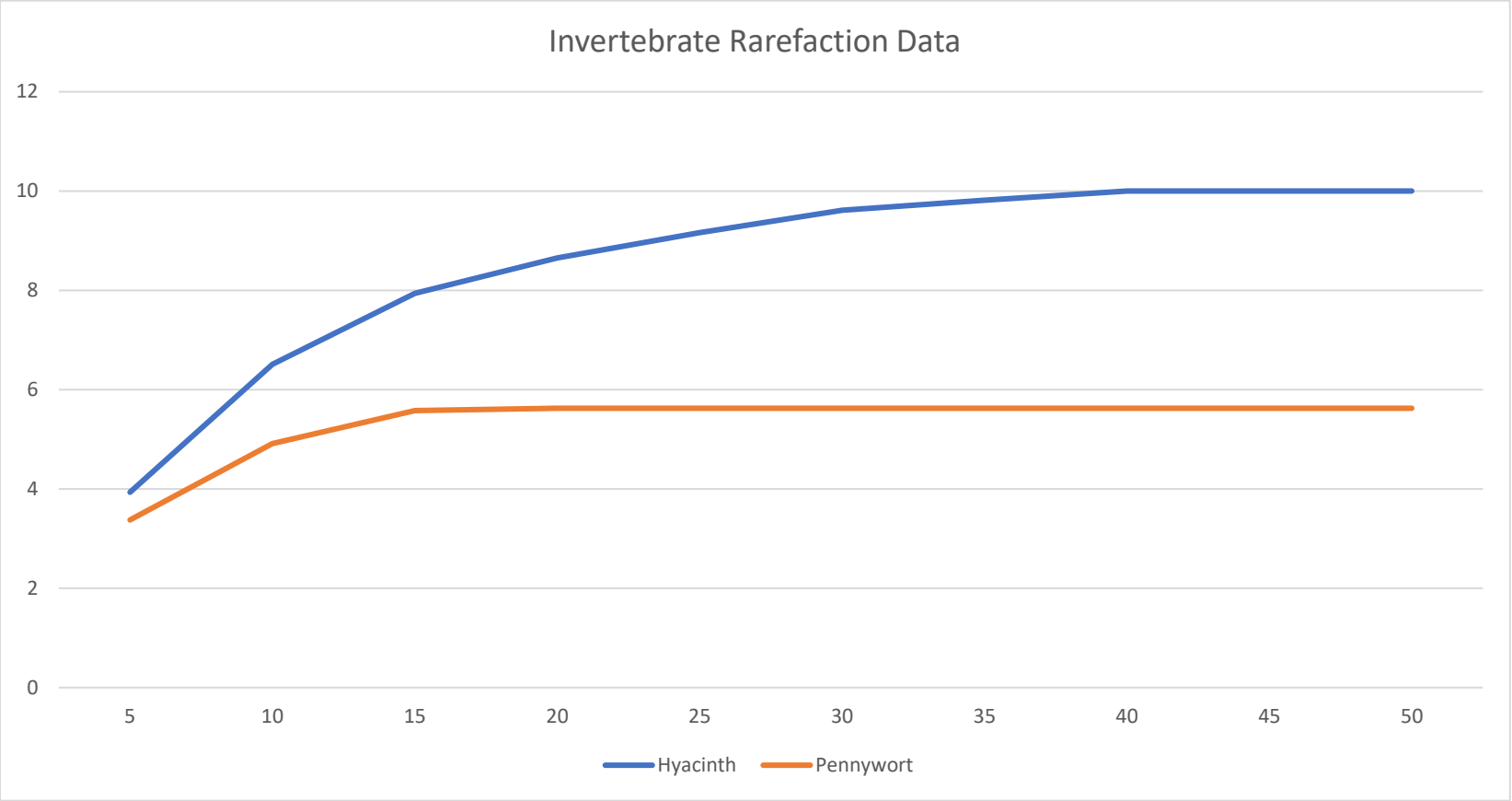

Figure 6. Rarefaction data highlighting the species richness under Native and Invasive treatment groups for Invertebrate sampled data.

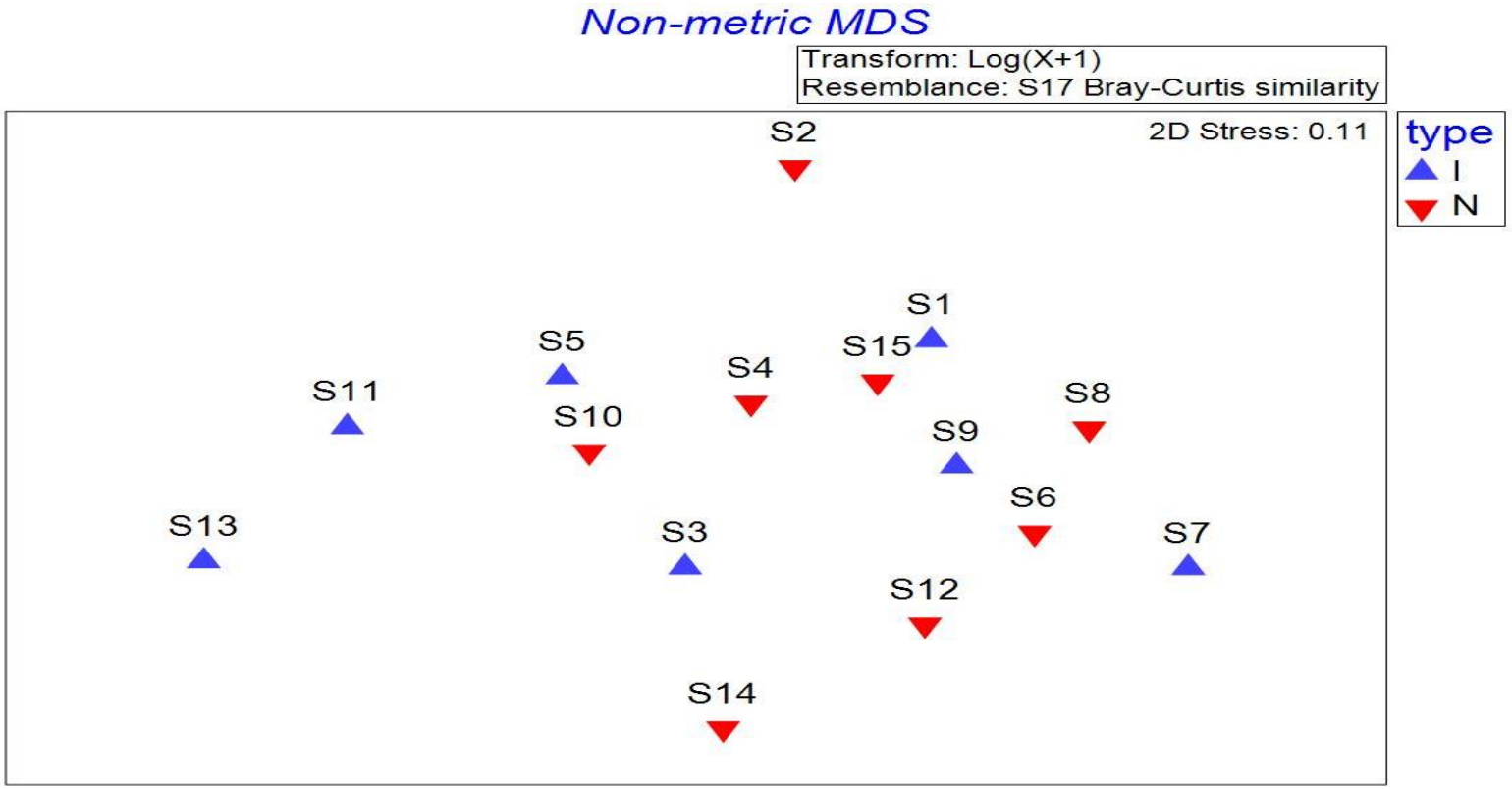

Figure 7. Non-metric MDS with a Log(X+1) transformation highlighting the similarities between Native and Invasive treatment groups.

4.0 Discussion

Water hyacinth has proven to be a prominent invader of freshwater ecosystems across the world. Although, some species of fish and invertebrates accounted for greater dissimilarity between water hyacinth and native vegetation than others no statistical analyses displayed a significant difference in sample populations under hyacinth versus native vegetation. My research aims to serve as a baseline for understanding the effects that water hyacinth has on fish and invertebrate populations in bodies of water with similar eutrophic conditions to Lake Okeechobee. By working in cooperation with USDA-ARS Invasive Plant Research Laboratory, my research will allow for characterization of how different management strategies can affect fish communities found under water hyacinth.

With water hyacinth having some complicated implications on surrounding environments such as blocking shipping channels, affecting recreational fishing, and other negative consequences, it's an ongoing effort to reduce the biomass of the plant seen in Lake Okeechobee. Due to the issues that have arisen from water hyacinth, federal agencies, such as the United States Department of Agriculture (USDA) are attempting to find ways to reduce the biomass of the plant in waterways across the United States. The USDA-ARS Invasive Plant Research Laboratory in Ft. Lauderdale has evaluated four biological control agents -- *Neochetina eichorniae, Neochetina bruchi*, *Niphograpta albiguttalis,* and *Megamelus scutellaris* -- to reduce water hyacinth biomass. The impact that these insects have had has led to a 50-70% decline in biomass and a reduction greater than 90% in seed production. With continued success, it could be viable to introduce these species to other areas of the United States plagued by water hyacinth if they were to prove to solely feed on the invasive macrophyte. Furthermore, in April 2022, the Florida Fish and Wildlife Conservation Commission (FWC), in partnership with the Florida Department of Environmental Protection, U.S. Army Corps of Engineers, and the South Florida Water Management District, agreed on a project to utilize mechanical harvesters to remove water hyacinth from a 35-acre area of Lake Okeechobee.

By sampling over a thirteen-month span, variations in water depth, temperature, and site composition occurred. The summer of 2022 sampled a great number of freshwater fish and invertebrate species utilizing the minnow traps. However, a very large increase in water depth due to rainfall occurred around the beginning of November 2022. Site depths had gone from an

average depth of 45.77 centimeters, to an average depth of 144.11 centimeters. Following November 2022, the months after (December 2022-February 2023) recorded water depth averages of roughly 71.85 centimeters. During this period, few to no species were often caught in the minnow traps, resulting in zero fish caught at sites. This observed disappearance in species because of water depths increasing in Lake Okeechobee could be the result of the boldness of species such as *G. holbrooki* and *F. chrysotus*. Hoch et al. (2019) showed that species such as *G. holbrooki* and *F. chrysotus* were bolder when water levels fluctuated and were superior explorers of unknown environments. Areas that became inundated might have caught the attention of these bolder species causing them to migrate to newly flooded areas.

Additionally, site appearance varied throughout the year, depending on weather conditions and nutrient availability. In early November 2022, South Florida experienced Hurricane Nicole which passed through Lake Okeechobee. As a result, the patches of vegetation were disrupted at some sites. Sites that were previously sampled lost either one species of plant, or both, affecting the number of sites that could be sampled at the time. The sites sampled were all relatively close proximity to each other. It may be worthwhile to conduct an experiment of a similar procedure to test the effects of water hyacinth on fish/invertebrate communities but over a larger spatial scale. A study that would test over the scale of multiple miles for example, as our traps were very close to each other in relatively small patches of floating vegetation, allowing for fish to move freely between treatment sites.

Nitrogen and phosphorous have both been linked to eutrophication of Lake Okeechobee in recent years. As a result, there has been a major emphasis on curtailing the amount of nitrogen and phosphorus that leak into the lake. Excess phosphorus has been shown to be the greatest factor to control to prevent algal species, such as *Anabaena circinalis* from causing mass outbreaks in highly eutrophic lakes (Rechcigl 1997). The low levels of dissolved oxygen and high ammonia concentrations created from the algal bloom has been shown to kill virtually all invertebrate life in the surrounding area (Rechcigl 1997). Large blooms of *Anabaena* were seen during June and July of 2023. The large blooms seen of the noxious blue-green algae could have affected invertebrate populations at sampling sites, lowering overall catch.

Angling data yielded a total of only six individuals. Although this data may not be robust enough to accurately represent communities under different treatment methods, fishing under

water hyacinth or pennywort treatment groups is significantly harder than open water treatments. To fish in water hyacinth or pennywort sites, the airboat was maneuvered into the patch of vegetation. This maneuver could startle any potential fish that could be caught using a hook-and-line method. Additionally, a lot of the sites angled earlier during the sampling period had shallower depths, which could have limited larger species of fish that would typically be caught on a hook-and-line. Nightcrawlers were the only form of bait used to try and catch fish, which could have influenced what species went for the bait. In future studies, it may be beneficial to use other forms of bait such as smaller fish or shrimp.

Upon review of the invertebrate data, there was a noticeable absence of gastropods in the data, having only five total individuals recorded. Desautels et al. (2022) demonstrated that snail reproduction was extremely rare under water hyacinth when compared to control groups. Non-vector snails were also shown to be significantly less attached to water hyacinth when compared to open water sites (Ofulla et al. 2010). It could be possible that when the plants were removed from the water for the invertebrate sampling, snails trapped within the roots fell into the water before being captured. Using a net to scoop the plants and prevent snails from falling, as well as resampling over a larger sample period could be performed to accurately determine how water hyacinth in Lake Okeechobee affects gastropod populations.

The root structure of water hyacinth can play a role in which species can reside among the invasive macrophyte. Water hyacinth roots in the water column have been shown to have a significantly higher surface area (S.A.) than pennywort roots, having roughly three times greater S.A. (Toft et al. 2003). The roots are also more complex in structure compared to pennywort. The complex nature of the hyacinth roots, as well as the larger S.A. could explain for differences in the invertebrate species found in hyacinth versus pennywort roots, by providing more habitat opportunities for invertebrates to feed and seek refuge in.

Dissolved oxygen levels can influence the structure and abundance of fish communities. In Singing Springs, Florida, the distribution, and relative abundance of fish communities were examined in relation to dissolved oxygen levels (McKinsey and Chapman 1998). The most abundant species observed was *G. holbrooki*, showing to be the most tolerant of lower dissolved oxygen levels. My own study found *G. holbrooki* to be the most abundant species caught, which could explain how tolerant *G. holbrooki* can be of low dissolved oxygen levels. We would only

expect to find species that are very tolerant of these low dissolved oxygen levels, such as *G. holbrooki* under areas of water hyacinth.

In a study on the effects of water hyacinth in an estuarine habitat, in the upper Terrebonne Bay, in Louisiana, water hyacinth was found to not alter the overall species richness or diversity, when compared to open water sites (Hill et al. 2021). The study utilized funnel traps to sample fish species in the estuary. The study by Hill et al. (2021) found results similar to what was found in this study, namely that water hyacinth does not significantly affect species richness or composition when compared to native pennywort or open-water sites.

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