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Salvinia molesta: An Assessment of the Effects and Methods of Eradication

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Salvinia molesta:

An Assessment of the Effects and Methods of Eradication

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Abstract

Salvinia molesta is an invasive aquatic fern. It is now the second worse aquatic invader in the world. Since the 1930s, it has invaded most tropical and some temperate countries. *S. molesta* plants grow vegetatively and can increase in size rapidly. *S. molesta* can form thick mats of up to 1-meter-thick. There are a number of ways these thick mats negatively affect the environment: 1) reduce light to benthic organisms, 2) reduce oxygen in the water column for other organisms, 3) accumulate as organic matter at the bottom of the water column, 4) decrease nutrients for other organisms, and 5) change water flow. *S. molesta* not only degrade and alter ecosystems, infestations also increase public health concerns. Dense *S. molesta* mats are ideal breeding grounds for mosquitoes and other insects that carry vector-borne diseases. Countries are negatively affected economically because *S. molesta* hinder use of waterways. Recreational activities, tourism, fishing, and transportation are all impeded due to *S. molesta* infestations. Methods of control are: 1) physical control, 2) chemical control, and 3) biological control. A combination of two or more methods work best for complete eradication. Biological control is the method of choice in tropical areas. Australia was the first to implement biological control via *Cyrtobagous salviniae*. *C. Salviniae* have devastative effects on *S. molesta* plants because both adults and larvae feed on plant parts. Althought *C. savliniae* are very effective, they have some constraints: 1) temperature, 2) nutrients, and 3) *S. molesata* infestation growing stage. *S. molesta* can withstand lower temperatures than *C. salviniae*, so in temperate regions, *C. salviniae* are ineffective. These regions are where other methods of control, such as chemical control, are more effective. *C. salviniae* also require adequate nitrogen concentration for proper development. *S. molesta* infestations also need to be in the primary or secondary growing stage for *C. salviniae* to survive. Tungog Lake in Sabah, Malaysia is heavily infested with S. *molesta* plants that are in the tertiary growing stage. Mats must first be thinned out via chemical control or mechanical removal. *C. salviniae* then should be introduced to the lake. Other recommendations for control overall of *S. molesta* are: 1) more studies in temperate regions should be conducted at specific infestation sites, 2) increase public education to reduce use of *S. molesta* as an ornamental, and 3) ban cultivation sites and sales.

Introduction

Freshwater Aquatic Ecosystems: What are Aquatic Ecosystems and Why are They Important?

Aquatic ecosystems provide many ecosystem services. Ecosystem services are all the processes which provide benefits for all organisms, such as food, habitat, and water. The type of ecosystem determines the ecosystem services. To illustrate, freshwater ecosystems provide a number of services to humans, including direct resources for consumption (i.e. fish, wildlife, plants), uses for transportation, storm buffering, and removal of toxins from a system (Brauman et al., 2007).

Freshwater ecosystems are especially important in developing countries because they provide sustenance for surrounding communities. Communities all over the world rely on fish as their main source of protein (Julien et al., 2002). They also use freshwater ecosystems for transportation. Boats, row or motor, are a much more economical way to travel than planes, trains, and automobiles. Community members are often directly affected when waterways are obstructed. This obstruction can lead to starvation, reduction of economic growth, and displacement of communities (Room, 1983).

Freshwater ecosystems are important to humans and are home to many fish and wildlife species, some of which are threatened or endangered (Cowie and Werner, 1992). Human activity is a significant threat to freshwater ecosystems. Human activity like use of fertilizers and improper waste management transform ecosystems and lead to proliferation of invasive species. Humans add nutrients to freshwater ecosystems by using fertilizers for agriculture and through improper disposal of sewage. Agriculture runoff and waste water then enter freshwater ecosystems causing eutrophication. Eutrophication is excess nutrients in a waterway that causes the overgrowth of plants, algae, and phytoplankton. Invasive species often increase in abundance due to eutrophication.

The Effects of Invasive Species

Invasive species are non-native to a given area and negatively affect the environment, economic growth, and biological organisms of that given area (NISIC, 2016). For a species to become invasive, an alien species must first become established. Established species can grow without intervention and aid. Once established, species often become naturalized and can reproduce on their own. Invasiveness follows naturalization. For a species to become invasive, it must spread outside its initial introduced range. Because invasive species do not have natural predators, they outcompete most native species. Native species are outcompeted for space, food, water, and nutrients.

Invasive species completely alter ecosystems and ecosystem functions. When alien plants become invasive, areas are completely transformed. Vegetation is the foundation of many ecosystems and are habitat for many organisms. When vegetation is altered, composition in all trophic levels is affected, thus decreasing biodiversity and species abundance (Madsen, 2009).

Many factors contribute to the success of invasive species, one being that invasive species do not have natural enemies in their introduced range. Plants are controlled by herbivores and pathogens in their native range. Invasive species have many adaptions to withstand unfavorable conditions, including a wider range of temperature tolerance, higher drought tolerance, and complex dispersal mechanisms.

Aquatic invasive plants are very damaging, devastating countries both ecologically and economically (Table 1). Aquatic invasive plants alter ecosystems by outcompeting native plants and reducing biodiversity (Figure 1). They change a heterogeneous community into a homogenous monoculture. Fish are sensitive to altered ecosystems and are often the first to be affected by invasive aquatic plants. Vegetative growth in waterways hinder fish growth and survival (Dibble, 2009). Excess vegetation reduces water quality and foraging success for many fish species.

Table 1. Ecological and economic impacts of aquatic invasive species on humans. This list does not include ecosystem alterations (Adapted from: Madsen, 2009).

Ecological Impacts	**Economical Impacts**
Impair commercial navigation	Degrade water quality
Disrupt hydropower generation	Reduce species diversity
Increase flood frequency, duration and intensity	Suppress desirable native plants
Impair drinking water (taste and odor)	Increase extinction rate of rare, threatened and endangered species
Habitat for insect-borne disease vectors	Alter animal community interactions
Recreational navigation impairment	Increase detritus buildup
Interfere with safe swimming	Change sediment chemistry
Interfere with fishing	
Reduce property value	
Endanger human health, increase drowning risk	

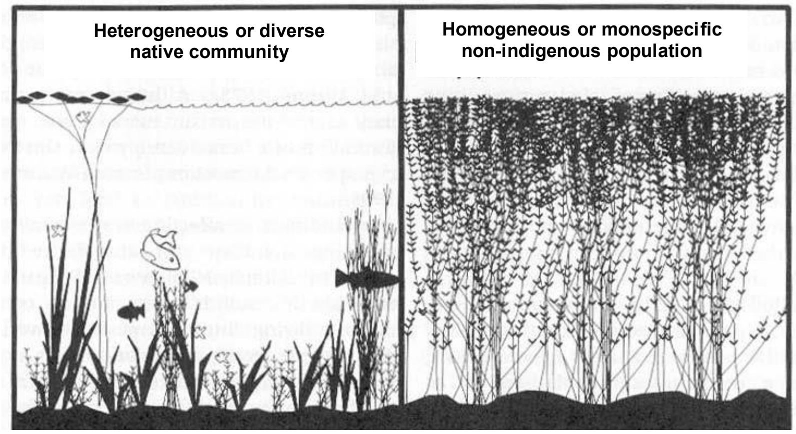


Figure 1. A non-monopolized community is more diverse and heterogeneous. Invasive species lead to monospecific communities where an invasive plant out completes other native plants leading to biodiversity loss through all trophic levels (Source: Madsen, 2009).

Although it costs millions of dollars to control and eradicate invasive plants, costs for uncontrolled invasive species is even more. Countries lose millions of dollars annually because of invasive species. This puts developing nations in unpleasant financial situations. Even developed nations find it difficult to fund invasive species control programs. The United States spends approximately $876 million on invasive species annually (Allen et al., 2014). The second most invasive aquatic plant in the world is *Salvinia molesta* (*Eichhornia crassipes*, water hyacinth, being the first) (Madsen, 2009).

Invasive Aquatic Plant Species: *Salvinia molesta*

Salvinia molesta is a free-floating aquatic fern. Free floating plants are not anchored into sediment and float in the water column, enabling plants to have greater effects on their surroundings (Madsen, 2009). *S. molesta* is native to southeast Brazil, but since the 1930s it has invaded many other countries (Room and Thomas, 1986). In the past 80 years, globalization has allowed it to spread all over the world. Humans transport this plant excessively which has caused an influx of *S. molesta* infestations in many impoverished countries. Accidental and intentional release has been reported all over the world (Konchi and Aronson, 2015). Because *S. molesta* is phenotypically pleasing, the aquarium and horticultural trade has been profitable. People often purchase plants for fish tanks and gardens and unknowingly dump pieces of *S. molesta* into waterways where it grows uncontrollably and becomes invasive. Once it enters waterways, it spreads to new areas by attaching to boats, fishing gear, humans, and animals (NPS, 2015).

The U.S. Department of Agriculture has listed *S. molesta* as a noxious weed and it is a federal offense to sell, transport, and release *S. molesta* across state lines (NPS. 2015; Nelson, 2009). Researchers hypothesize that *S. molesta* infestations will continue to increase due to availability via mail-order catalog and online commercial vendors (Nelson, 2009). This could be a contributing factor to why *S. molesta* is the second worst aquatic invader in the world and is a Weed of National Significance in Australia (**Horst and Mapes, 2000**, Sullivan and Postle, 2012).

S. molesta does very well outside its native range. Factors that contribute to its success are:

1. Morphological and reproductive strategies increase its ability to spread.
2. Climate change is increasing the range of suitable habitat.
3. Nutrient availability, such as agricultural runoff, increases productivity.
4. There are no natural herbivores outside of its native range.

All these reasons to why and how *S. molesta* can do so well will be outlined in detail in this paper along with the negative effects it has on the environment (NPS, 2015):

1. Increase of *S. molesta* infestations leads to more suitable habitat for vectors for infectious diseases.

2. Loss of use of waterways lead to declines in national economic growth.
3. Water quality degradation leads to changes in species composition and alteration of ecosystem services.

Thesis Statement

S. molesta degrades ecosystems, negatively impacts countries economically, and is a risk to human health; therefore, it should be removed. Methods of removal depend on infestation size and extent. Methods of removal and control include prevention, physical control, chemical control, and biological control. When considering methods of control, climate and nutrients play an important role.

Section I: Plant Description and Distribution

Discovery of *Salvinia molesta*

S. molesta is in Order Hydropteridales and in Family Salvinanceae. Salviniaeceae contains one genus *Salvinia,* which has approximately 12 species all native to South America (Room and Thomas, 1986; Nelson, 2009). *S. molesta* is part of the *Salvinia auriculata* complex which contains four species (Room, 1988). These species are grouped together because they have hair-like structures, called trichomes, that are similar morphologically. *S. molesta* was misidentified as *S. auriculata* for years until 1972 (Julien et al., 2012, Forno, 1983). A more careful examination of herbarium specimen led to the discovery of *S. molesta* as a separate species from *S. auriculata*. Researchers found that some specimen were pentaploid with infertile sporocarps (Room and Thomas, 1986; Julien et al., 2012). In 1978, researchers from Commonwealth Scientific and Industrial Research Organization of Australia (CSIRO) discovered that southern Brazil was the native range of *S. molesta* and soon after discovered its natural herbivores, *Crytobagous salviniae*, *Samea multiplicalis*, and *Paulina acuminata* (Doeleman,1989). These natural herbivores were used as biological control agents thereafter.

Since its discovery *S. molesta* has invaded even more countries and has acquired several common names including giant salvinia, Kariba weed, aquarium water moss, water fern, and giant azalea (McFarland et al., 2004). These nicknames were mainly given by villagers and researchers. It often reflects the environment it inhabits or describes its invasive abilities.

Plant Structure

S. molesta is a free-floating aquatic fern found suspended in the water column. An individual plant is a phenet which is composed of 100 ramets (Room, 1990; Oosterhout et al., 2006). Ramets are the most basic unit of the plant and are connected to each other through horizontal rhizomes (Room, 1990, Julien et al., 2012). Each ramet is about 2.5 to 4.0 cm long consisting of a pair of floating leaves, also called fronds, a submerged modified leaf, and an internode (Figure 2,3) (Julien et al., 2012; Nelson, 2009). Fronds are oval and fold on top of each other as they grow (Nelson, 2009). Fronds have small hair-like structures called trichomes that enable leaves to be hydrophobic and buoyant (Room, 1983; Barthlott et al., 2009). *S. molesta* trichomes are shaped like "egg-beaters" and allow fronds to float back up to the surface if submerged (Figure 4) (Nelson, 2009, Room, 1983). The third modified leaf behaves as a root (Room, 1990). It stabilizes the ramets and absorbs nutrients directly from the water column (Nelson, 2009; McFarland, 2004). Racemes of sterile sporocarps are found embedded within the modified roots (Nelson, 2009; McFarland et al., 2004).

Figure 2. *Salvinia molesta* showing fronds and modified leaves (roots) (Source: www.florabase.dpaw.wa.gov.au).

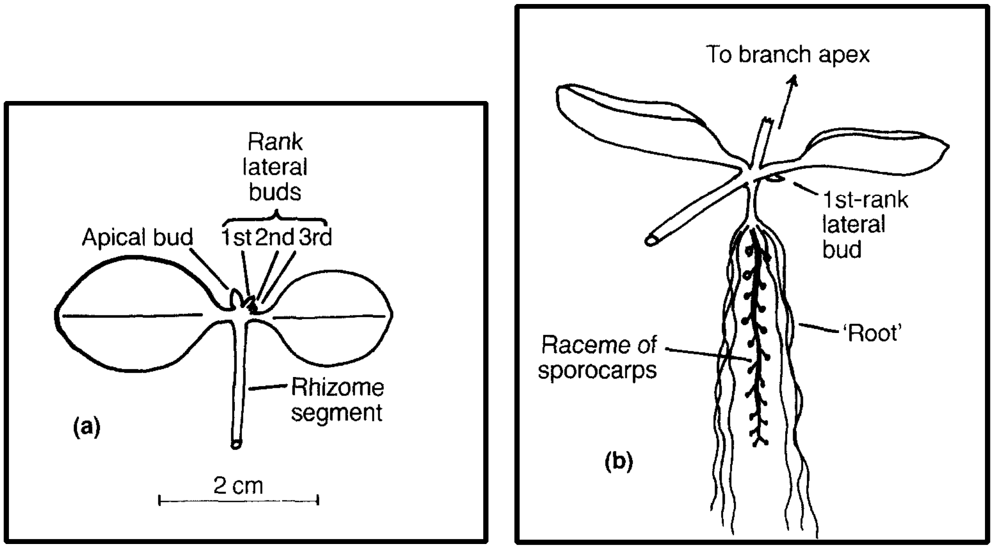


Figure 3. *Salvinia molesta* structure. a) Rhizome and apical bug with 1st, 2nd, and 3rd lateral buds. b) Raceme of sporocarps embedded in modified leaves (roots) (Source: Room, 1990)

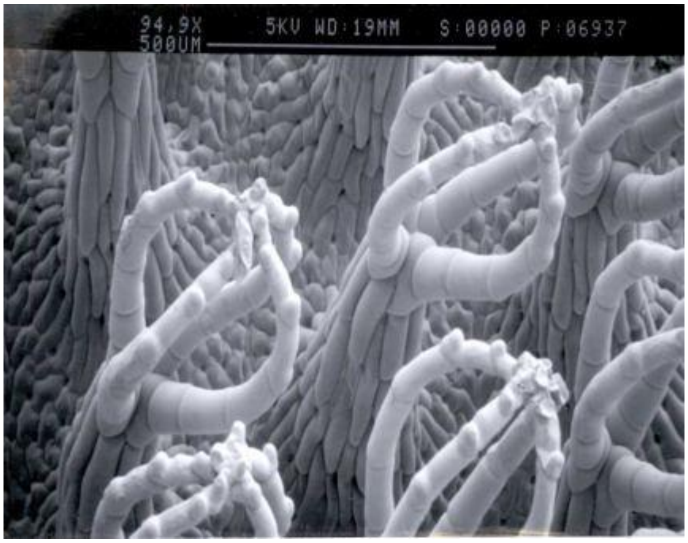


Figure 4. Close up on *S. molesta* trichomes that are shaped like "egg-beaters" (Source: ww2.odu.edu).

Salvinia molesta Reproduction

S. molesta is a fern therefore, it does not produce seeds; it produces spores. The spores however, are sterile and not viable. Since *S. molesta* is pentaplod, its chromosome count is 45, making it genetically unable to reproduce sexually by completing meiosis. *S. molesta* does however reproduce asexually through rhizome fragmentation and bud extension (Julien et al., 2012; Nelson 2009; Room, 1990). Rhizomes easily break apart forming new daughter plants that are genetically identical to parent plants (Room, 1983; Nelson, 2009). Although *S. molesta* plants are genetically identical, studies across Texas and Louisiana have shown morphological and molecular diversity suggesting that *S. molesta* mutate often which allows it to quickly adapt to new environments (Galam et al., 2015). *S. molesta* infestations grow prolifically due to asexual reproduction; plants can double in biomass in 2 to 3 days under favorable conditions (Room, 1990).

Salvinia molesta **Vegetative Growth and Growing Stages**

S. molesta plants form dense colonies that can be categorized into primary (Figure 5a), secondary (Figure 5b), and tertiary (Figure 5c) growing stages. In the primary growing stage, plants have small, oval, and delicate leaves that grow to about 10-15 mm wide. Leaves lay flat on the water surface and plants are spread out with no indication of crowding (Julien et al.,2012; McFarland et al., 2004). In the secondary growing stage, mats of *S. molesta* become more stable. Leaves begin cupping and become 20-50 mm wide. At this stage, the internodes are longer than the leaves. Plants experience some crowding, but no overlapping occurs (Julien et al., 2012). In the tertiary growing stage, leaves get even more cupped and become 60 mm wide. The internodes are now shorter than leaves. Plants start overlapping causing overcrowding. Eventually mats extend down 1-meter into the water (Julien et al.,2012; McFarland et al., 2004). At this stage, plants and grasses begin growing on top of the thick mats. (Oosterhout et al., 2006).

a. Primary growth b. Secondard growth c. Tertiary growth

Figure 5. *S. molesta* growing stages (Source: Julien et al., 2012).

Salvinia molesta Parameters for Growth

S. molesta plants need certain conditions to grow successfully (Julien et al., 2012). Temperature, light, pH, conductivity, salinity, and nutrients are some factors that control the growth of plants. Temperature, light, salinity, nutrients, and pH all influence the growth of *S. molesta* while only temperature and nutrients control rate of growth (Madsen and Wersal, 2008). *S. molesta* growth depends heavily on temperature and nutrients. If temperatures are not ideal (approximately 30°C), then *S. molesta* plants will not grow. If nutrients are not ideal, then plants will not increase biomass as efficiently and rapidly.

Temperature

Temperature is a major controlling factor for growth. *S. molesta* plants are sensitive to temperature changes. The optimum water temperature for *S. molesta* is 30°C (Julien et al., 2012). *S. molesta* has a lower tolerance limit of 5°C and an upper tolerance limit of 40°C (Madsen and Wersal, 2008). When plants are exposed to temperatures lower than -3°C or higher than 43°C, they die within 2 hours (Room and Whitman, 1991). These findings are consistent with their range because plants have not yet invaded northern United States due to extremely low winter temperatures. *S. molesta* has invaded some temperate regions, mainly due to bud dormancy. Once winter passes, ideal temperatures allow dormant buds to grow, leading to the invasion of the waterway once again. With climate change, it is a possibility that it can eventually invade most northern latitudes (Julien et al., 2012).

Light

S. molesta leaves lie flat on the water surface, enabling it to access sunlight readily. Because *S. molesta* can obtain light readily, it has a photosynthetic advantage over other aquatic plants that inhabit an area (Nelson, 2009). *S. molesta* is from the tropics and mostly invades high radiant regions, where it is sunny a majority of the time, therefore, in the tropics light is never a limiting factor (Rani and Bhambie, 1983). However, growth can be inhibited if exposed to too much or too little light. More than 4,500 kcal/m^2 day of exposure can inhibit growth (Madsen and Wersal, 2008). More light often means higher temperatures. When temperature increases, it replaces light as a controlling or inhibiting factor (Rani and Bhambie, 1983). When *S. molesta* plants invade countries with seasons, such as the United States, both light and temperature are controlling factors (McFarland et al., 2004). Light in temperate countries becomes limiting and growth is often inhibited in winter.

Conductivity and Salinity

The range of conductivity that is best suited for *S. molesta* is between 239.3 and 503.5 µS/cm (Room and Gill, 1985). When conductivity is low (100 µS/cm), *S. molesta* becomes chlorotic, this is due to the reduction in chlorophyll production. This is often seen in low nutrient waters where nitrogen is limiting (McFarland et al. 2004). High conductivity damages plant tissues directly and leads to low survival rates (McFarland et al. 2004).

Salinity severely inhibits *S. molesta* growth. Plants cannot withstand more than 3 ppt of exposure. Anything more than 7 ppt is lethal. (Madsen and Wersal, 2008). 34 ppt of exposure (seawater salinity), causes plant death within 30 minutes (McFarland et al., 2004).

pH

S. molesta plants are most productive in water bodies with pH of 6 (Cary and Weerts, 1984). When pH values between 5 and 8 were compared, biomass increased the most at pH of 6 and 6.5 (Maden and Wersal, 2008). When no nutrients were added to the system, biomass increased only at pH of 6.5. Although pH can affect growth of plants, it does not limit its invasiveness. In Lewisville, Texas, *S. molesta* infestations have been seen in ponds with pH levels of 7.5 and 8.5 (McFarland et al. 2004). It survives and retains its invasive tendencies in basic and acidic waters. Malaysia lakes with a pH of 5.2 have *S. molesta* infestations (McFarland et al. 2004).

Nutrients

S. molesta is very sensitive to nutrient changes because it absorbs nutrients directly from the water column. Agricultural runoff into streams, rivers, and lakes increase productivity. Because nitrogen and phosphorus are limiting nutrients in most aquatic ecosystems, any increase in nutrient supply drastically influence plant growth. Nitrogen and phosphorus determine morphological characteristics and growth rates (Room and Thomas, 1986).

Growth is controlled by nitrogen availability. When plants have less than 0.8% of nitrogen, one ramet gives rise to only five new ramets and there is no lateral extension (Figure 6a) (Room 1990). When nitrogen increases to 2.65%, first rank lateral buds grow and one ramet gives rise to 10 new ramets (Figure 6b). When nitrogen increases to 5% or greater, second and third rank lateral buds grow and one ramet gives rise to 30 new ramets (Figure 6c). There are higher chances of *S. molesta* infestations in nutrient rich waters than nutrient poor waters.

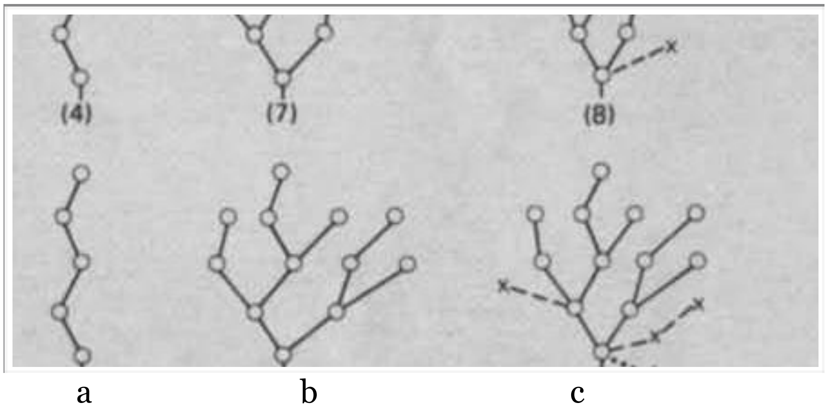


Figure 6. Branching of S. molesta plants based on nutrient availability. a) Nitrogen levels 0.8% or less, linear extension, 5 new ramets. b) Niteogrn levels 2.65%, lateral extension, 10 new ramets. c) Nitrogen levels 5% or greater, lateral bud extension, 30 new ramets (Source: Room, 1990).

Morphology is also affected by nutrients. When nutrients are low, rhizomes are tough and do not fragment. This toughness allows plants to retain high nitrogen concentration inside tissues as opposed to losing them when cells divide; this process of not fragmenting allows plants to survive in low nutrient environments (Room and Thomas, 1986). Also, there are longer modified roots, larger leaves, and more sporocarps (Julien et al., 2012). When nutrients are high, rhizomes are brittle and fragment easily increasing the rate and chance of dispersal (Cary and Weerts, 1983).

Relationship Between pH and Nutrients

When comparing pH levels and nutrient loading, researchers found that nutrients have a greater effect on biomass than pH; however, there is a threshold for effects of nutrient loading (Figures 7, 8) (Madsen and Wersal, 2008). Biomass increased at pH 6.5 when no nitrogen was added. With 9 mg/L of nitrogen added to the system, biomass increased 4 to 7-fold. Plants increased in biomass when 9 mg/L of nitrogen was added but there was no significant change at 13 mg/L (Figure 8). Although pH is an important factor for growth in the early stages, nutrients are much more influential.

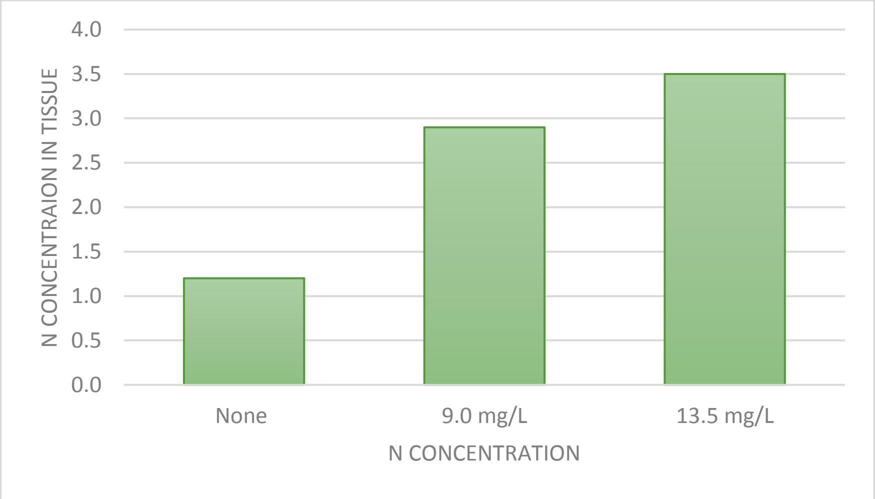


Figure 7. Relationship between nitrogen content in *S. molesta* plant tissue and concentration of nitrogen in the water column. (Adapted from: Madsen and Wersal, 2008).

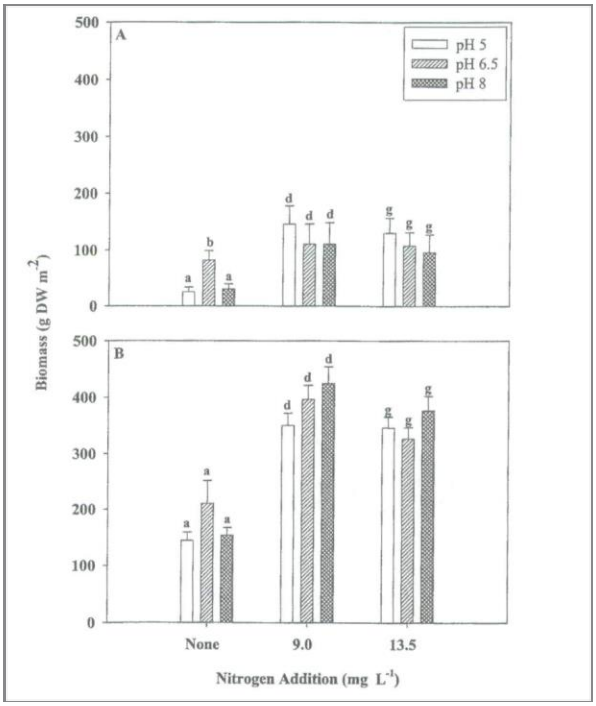


Figure 8. Relationship between nitrogen concentration in the water column and *S. molesta* plant biomass (dry weight/m^2). The same lower case letters represent that there is no significant difference between growth according to the pH levels. A) 14 days after the start of the experiment. B) 84 days after the start of the experiment.

Current Distribution and Spread

S. molesta is native to Southeast Brazil, Uruguay, Paraguay, and Northeast Argentina (Room, 1990). *S. molesta* is now found in other tropical and subtropical regions of the world and has been invading these regions since the 1930s. Recently, within the past 20 years, *S. molesta* has even invaded temperate regions. In most subtropical and temperate countries, *S. molesta* is confined to coastal regions whereas in tropical countries, it is found further inland (Cilliers, 1991).

S. molesta was first discovered outside its native range in 1939 when it invaded rice paddy fields in Sri Lanka. Since then, it has invaded over 20 other countries (Oliver, 1993). In 50 years, it spread to Australia, New Zealand, Fiji, Philippines, India, Indonesia, Malaysia, Singapore, Papa New Guinea, and the United States. It has invaded countries in Africa (the Ivory Republic, Ghana, Zambia, Kenya, Namibia, Botswana, South Africa, Madagascar), South America (Columbia and Guyana), and the Caribbean (Cuba and Trinidad) (Oliver, 1993; McFarland et al, 2004). It has become a serious weed throughout Africa, India, Sri Lanka, South-East Asia, the Philippines, Papua New

Guinea, New Zealand, Fiji, Hawaii and continental United States (CRC Weed Management, 2003). It has been causing serious ecological and economic problems in these countries (Room, 1983).

Australia

S. molesta first invaded Australia in 1950. It was introduced to the region for ornamental purposes to be used in gardens and aquarium tanks. By 1980, it reached New South Wales and Queensland. It also reached all of Western, Eastern, and Northern Australia, both inland and coastal systems. Much of coastal Australia is heavily invaded and some temperate inland regions are also invaded (Figure 9). Australia has been successfully using biological control for years in tropical and subtropical regions; however, it has not been successful in temperate regions such as in Kakadu National Park. Because *S. molesta* does well in the tropics, much of Australia was invaded. Lake Moondarra is another classic example where *S. molesta* invasion occurred and biological control was successful.

Figure 9. Map of *S. molesta* distribution in Australia with blue dots representing *S. molesta* infestations. (Source: Australia's Virtual Herbarium).

United States

In the United States, *S. molesta* is confined to the south ranging from Southern California to North Carolina (McFarland et al., 2004). It can even be found in small ponds in Washington D.C. *S. molesta* is considered naturalized in North Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Texas, Arizona, California, and Hawaii (McFarland et al., 2004).

Although regulations are stricter in the U.S. compared to other parts of the world, *S. molesta* is still cultivated here (Figure 10). *S. molesta* was first discovered outside of its cultivated range in 1995 in South Carolina where it was quickly eradicated with herbicides (Nelson et al., 2001; Tipping et al., 2012). In 1998, it was discovered along state lines between Texas and Louisiana, where it affected interstate commerce and regulations. State officials became concerned that boat transport between states would lead to *S. molesta* infestations in new territories. This concern led to further regulations restricting transport across state lines. *S. molesta* is currently invasive in approximately 12 states (Table 2). *S. molesta* is projected to invade the Atlantic coast plain (Virginia to Florida), the Gulf Coast, Central to Northern California, and Southern Arizona (USDA, 2016).

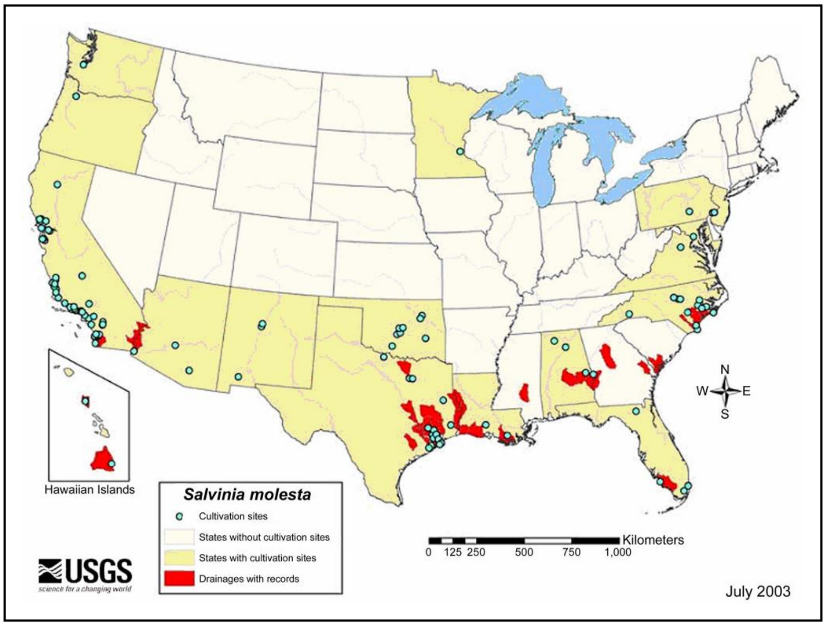
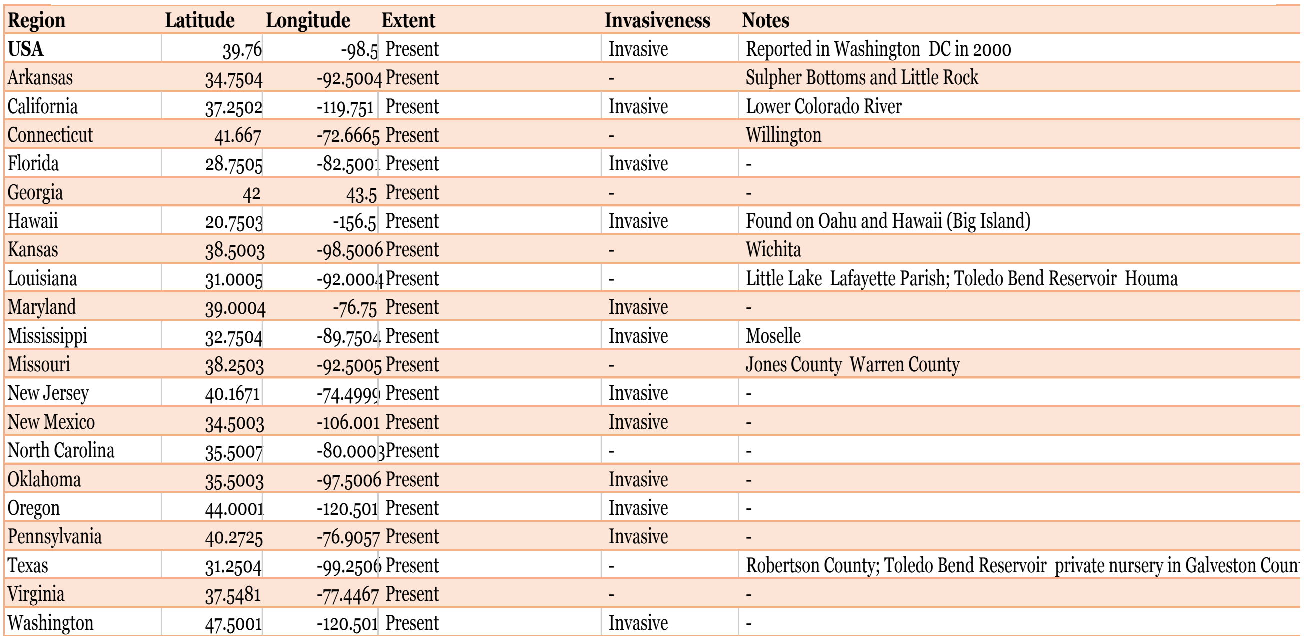


Figure 10. Current distribution of *S. molesta*. Blue dots represent cultivation sites; yellow states are states where cultivation and light yellow are states without. Red represents waterways with infestation (Source: McFarland et al., 2004; USDA, 2016).

In 1999, *S. molesta* invaded Hawaii where it has been negatively affecting endangered birds, such as *Fulica alai* (Hawaiian coot), *Gallinula chloropus* (Hawaiian gallinule), and *Himantopus mexicanus knudseni* (Hawaiian stilt) (McFarland et al., 2004). *S. molesta* took away suitable habitat for these birds and changed the food web dynamics by decreasing their abundance (McFarland et al., 2004).

Table 2. Distribution and status of *S. molesta* in the United States (Adapted from: CABI, 2016).



Region	Latitude	Longitude	Extent	Invasiveness	Notes
USA	39.76	-98.5	Present	Invasive	Reported in Washington DC in 2000
Arkansas	34.7504	-92.5004	Present	-	Sulpher Bottoms and Little Rock
California	37.2502	-119.751	Present	Invasive	Lower Colorado River
Connecticut	41.667	-72.6665	Present	-	Willington
Florida	28.7505	-82.5001	Present	Invasive	-
Georgia	42	43.5	Present	-	-
Hawaii	20.7503	-156.5	Present	Invasive	Found on Oahu and Hawaii (Big Island)
Kansas	38.5003	-98.5006	Present	-	Wichita
Louisiana	31.0005	-92.0004	Present	-	Little Lake Lafayette Parish; Toledo Bend Reservoir Houma
Maryland	39.0004	-76.75	Present	Invasive	-
Mississippi	32.7504	-89.7504	Present	Invasive	Moselle
Missouri	38.2503	-92.5005	Present	-	Jones County Warren County
New Jersey	40.1671	-74.4999	Present	Invasive	-
New Mexico	34.5003	-106.001	Present	Invasive	-
North Carolina	35.5007	-80.0003	Present	-	-
Oklahoma	35.5003	-97.5006	Present	Invasive	-
Oregon	44.0001	-120.501	Present	Invasive	-
Pennsylvania	40.2725	-76.9057	Present	Invasive	-
Texas	31.2504	-99.2506	Present	-	Robertson County; Toledo Bend Reservoir private nursery in Galveston Coun
Virginia	37.5481	-77.4467	Present	-	-
Washington	47.5001	-120.501	Present	Invasive	-

Other Countries

S. molesta is widespread in many regions of the world. It is able to naturally reproduce in many countries, including Sri Lanka. It can even naturally reproduce in the temperate countries such as the United States in Alabama, Arizona, California, Georgia, Hawaii, Louisiana, and Texas It is not reproducing naturally in Mississippi, South Carolina, and Virginia (Table 3). *S. molesta* is present in Australia and in many of the Pacific Islands, such as Fiji and French Polynesia. It is invasive in Australia, New Zealand, and Papua New Guinea (Table 4). It is even invasive in Europe, such as in Austria, Belgium, France, Italy, and Portugal (Table 5). It has become widespread in Cuba and Trinidad (Table 6). *S. molesta* is invasive in India, Indonesia, Malaysia, Pakistan, Singapore, Taiwan, and Thailand (Table 8). In Africa, *S molesta* is invasive in

Benin, Botswana, Burkina Faso, Ghana, Kenya, Mali, Reunion, and Zambia (Table 9). In North America, it is invasive in both Mexico and the United States (Table 10). There are many countries that do not have accurate data which may be the only reason why it is not listed as an invasive in that country. *S. molesta* has been able to adapt to cooler temperatures in the past 80 years. Because it is seen in so many temperate countries where freezing occurs, one can argue that it will soon invade all countries with suitable habitat.

Table 4. Place, year of introduction, and natural reproduction of *S. molesta*. (Adapted from: CABI, 2016).

Introductions	**Column1**	**Column2**
Introduced to	**Year**	**Natural reproduction**
Alabama	1999	Yes
Arizona	1999	Yes
California	1999	Yes
Georgia	1999	Yes
Hawaii	1999	Yes
Kenya	1984	NA
Louisiana	1998	Yes
Mississippi	1999	No
North Carolina	2000	No
Senegal	1999	NA
South Carolina	1995	No
Sri Lanka	1939	Yes
Texas	1998	Yes
Virginia	2004	No

Table 3. Distribution of *S. molesta* in Oceania countries. (Adapted from: CABI, 2016).

Country	**Distribution**	**Invasive**
OCEANIA		
Australia	Widespread	Invasive
Fiji	Widespread	
French Polynesia	Present	
New Caledonia	Present	
New Zealand	Widespread	Invasive
Papua New Guinea	Present	Invasive
Vanuatu	Present	

Table 5. Distribution and status of *S. molesta* in Europe. (Adapted from: CABI, 2016).

Country	Distribution	Invasive
EUROPE		
Austria	Present	Invasive
Belgium	Present	Invasive
Denmark	Present	
France	Present, few occurrences	
-Corsica	Present	Invasive
Germany	Present	
Italy	Present	Invasive
Netherlands	Present	
Portugal	Present	Invasive

Table 6. Distribution of *S. molesta* in Central America and Caribbean (Adapted from: CABI, 2016).

CENTRAL AMERICA AND CARIBBEAN	
Cuba	Widespread
Guatemala	Present
Trinidad and Tobago	Widespread

Table 7. Distribution and status of *S. molesta* in South America (Adapted from: CABI, 2016).

Country	Distribution	Origin	Invasive
SOUTH AMERICA			
Argentina	Widespread	Native	
Brazil	Widespread	Native	Not invasive
Colombia	Widespread		
Guyana	Widespread	Native	

Table 9. *S. molesta* infestations in Asia. Bold are countries and non-bolded are cities. (Adapted from: Cabi, 2016).

Country	Distribution	Origin	First Reported	Invasive
ASIA				
India	Widespread			Invasive
-Uttarakhand	Present	Introduced		
Indonesia	Widespread			Invasive
-Java	Present			Invasive
-Kalimantan	Widespread			Invasive
Israel	Present, few occurrences			Invasive
Japan	Present			
Malaysia	Widespread			Invasive
Pakistan	Present	Introduced		Invasive
Philippines				Invasive
Singapore	Present			Invasive
Sri Lanka	Widespread		1939	Invasive
Taiwan	Present			Invasive
Thailand	Present			Invasive

Table 8. Distribution and status of *S. molesta* in Africa. (Adapted from: CABI, 2016).

Country	Distribution	Origin	First Reported	Invasive
AFRICA				
Benin	Present			Invasive
Botswana	Widespread	Introduced		Invasive
Burkina Faso	Present	Introduced		Invasive
Cameroon	Present			
Congo	Widespread			
Congo Democratic Republic	Present			
Côte d'Ivoire	Present			
Ghana	Introduced, established	Introduced		Invasive
Kenya	Widespread	Introduced	1984	Invasive
Lesotho	Present			
Madagascar	Present			
Malawi	Present			
Mali	Present			Invasive
Mauritania	Present			
Mauritius	Present			
Mozambique	Present			
Namibia	Present			
Nigeria	Widespread			
Réunion	Present			Invasive
Senegal	Present	Introduced	1999	
South Africa	Widespread			
Swaziland	Present			
Tanzania	Present			
Uganda	Present			
Zambia	Widespread	Introduced		Invasive
Zimbabwe	Widespread			

Table 10. *S. molesta* distribution and status in North America. (Adapted from: CABI, 2016).

Country	Distribution	Invasive
NORTH AMERICA		
Mexico	Present	Invasive
USA	Present	Invasive

Mechanism of Spread and Dispersal

S. molesta has spread to many tropical and subtropical countries (Figure 11). It has even spread to many temperate regions such as Europe and North America (Tables 5 and 10). Wherever *S. molesta* has spread, it has gotten invasive.

S. molesta grows vegetatively, enabling it to reproduce rapidly and spread easily. *S. molesta* plants have adaptions to deal with increased water supply. Plants contain aerenchyma tissue which, along with trichomes, increase buoyancy. Buoyancy helps plants stay afloat and spread to larger areas when flooding occurs (Oliver, 1993). With flooding, water level rises and is distributed over a larger area, increasing *S. molesta* range. Flooding also causes plants to fragment more frequently, which escalates range and reproduction (McFarland et al., 2004). Other abiotic factors that increase range are storm events that cause natural disasters, such as landslides, which allow transport of *S. molesta* plants to new areas.

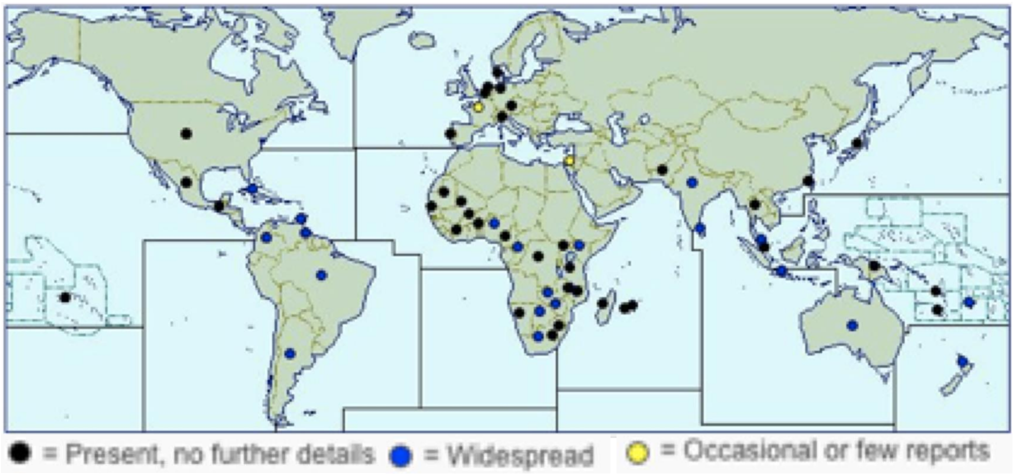


Figure 11. Map of current distribution of *S. molesta*. Black dot represents current distribution. Blue dots represent that it is widespread in that region. Yellow dots represent the few reports in that region. (Source: CABI, 2016).

The biggest contributor to the spreading of *S. molesta* plants are humans. Humans, both intentionally and unintentionally, introduce *S. molesta* to new regions of the world. Cultivation sites have been a huge contributor to accidental release. Accidental release occurs when people are unware of the consequences of *S. molesta* invasions and dump aquarium or garden water containing *S. molesta* fragments into waterways. This dumping leads to overgrowth of *S. molesta* plants in major waterways.

Globalization has also contributed greatly to accidental spread. Globalization is the act of humans traveling and migrating around the world. Plants often get caught in fishing nets, boats, cars, and other moving vehicles which carry plant fragments to new areas (Room, 1990; McFarland et al., 2004). Although animals contribute minimally, Hippopotami and water buffaloes are very successful in spreading *S. molesta* to new parts of Africa (McFarland et al., 2004). Humans are often the cause of new infestations, mostly by using *S. molesta* plants for ornamental purposes. Cases have also been documented where people have intentionally released fragments of *S. molesta* into a waterway (Oosterhout et al., 2006). Intentional cases occur when people who know what *S. molesta* is, dump fragments into waterways because proper disposal might be require more effort. Accidental and intentional release is largely due to the horticultural industry making *S. molesta* available to the general public.

Because *S. molesta* is considered aesthetically pleasing, it has a high demand in the horticultural industry (Oliver, 1993). *S. molesta* has made it to almost every continent. In the United States, it is illegal to transport *S. molesta* across state lines, but you can easily find plants on the internet and order it. There are no strict regulations in developing countries (Oliver, 1993).

Section II: Ecological, economic, and health

Ecological Impacts of *Salvinia molesta*

S. molesta is very detrimental to its environment because plants grow uncontrollably forming dense mats develop in the water column. These thick mats are not only connected vertically through fronds and modified leaves, but are connected horizontally through rhizomes as well. This makes mats impenetrable (Sullivan and Postle, 2012). *S. molesta* is a serious threat to many lakes, ponds, streams, rivers, and other freshwater wetlands such as rice fields. There are a number of ways these thick mats negatively affect the environment including:

1. Reduce light to benthic organisms
2. Reduce oxygen in the water column for organisms
3. Accumulate organic matter at the bottom of the water column
4. Decrease nutrients for other organisms
5. Change water flow.

Once the mats reach tertiary growth, outcomes are devastating because eradication becomes difficult (Sullivan and Postle, 2012).

Light availability becomes a limiting resource for submerged species when *S. molesta* invades systems. Mats decrease light penetration to submerged plants, algae, and phytoplankton; they either receive very little to no sunlight. These organisms do not survive because they cannot photosynthesize without sunlight. When phytoplankton populations are reduced, this affects organisms in all trophic levels, cascading up the food chain. Depleted light in the water column also reduces animal vision which affects foraging (Madsen and Wersal, 2008, Sullivan and Postle, 2012)

S. molesta invasions severely reduce dissolved oxygen content (DO) in aquatic ecosystems. Even though *S. molesta* increases oxygen in the environment through photosynthesis, it is not enough to compensate for all the DO that is consumed during decomposition. When plants die, biomass sink to the bottom, leading to a large buildup of organic matter (Sullivan and Postle, 2012; Madsen and Wersal; 2008, Julien et al., 12012). Bacteria then decompose all organic matter, using up most of the DO in the water column. This decrease in DO levels leave very little oxygen for other organisms (Sullivan and Postle, 2012). The loss of DO has detrimental effects on native plant and animal populations. Fish are especially affected by this phenomenon because they require higher oxygen levels than invertebrates (Dibble, 2009). Fish stocks are reduced which negatively affects human communities as well. (Madsen and Wersal, 2008). Along with reductions of DO, *S. molesta* also decreases overall gas exchange. When gas is not exchanged, carbon dioxide builds up in the water column leading to acidification of the waterway and reduction of pH (Julien et al., 2012)

Along with reducing DO levels, accumulation of organic matter also alters ecosystems physically. Organic matter buildup raises the sediment level which reduces depth, shallowing waterways (Sullivan and Postle, 2012). When waterways become shallower, organisms are negatively affected because their environment has now been altered. Fish nurseries and other breeding pools are also affected; shallow nurseries have higher temperatures and decreased nutrients (Sullivan and Postle, 2012, Julien et al., 2012).

S. molesta has a very high capacity to uptake nutrients: up to 8 mg N/g of dry plant tissue a day (Room, 1986). This process takes nutrients away from other organisms (Sullivan and Postle, 2012, Julien et al., 2012). *S. molesta* invasions create mono-specific communities where richness and abundance in species are severely decreased. When species composition is changed at the bottom of the food web, this affects species in all trophic levels. For an example, *S. molesta* could affect populations phytoplankton which would lead to the depletion of zooplankton, then fish species. *S. molesta* also alters entire ecosystems by offering new habitat for plants to grow on. Cattles often mistake *S. molesta* mats for actual land masses and when they step on to the mats, they fall into the water and drown (McFarland et al., 2004).

When *S. molesta* grows in moving waterways, such as rivers or creeks, it stagnates movement, sometimes stopping in altogether. *S. molesta* mats become dense enough to stop water movement. This endangers small bodies of water to evaporate, leading to the disappearance of that waterway. (Sullivan and Postle, 2012). This would be detrimental to all organisms that use the waterway, especially wildlife and village communities that utilize it for drinking water.

Economic Impacts of *Salvinia molesta*

Invasive species in general cause a lot of economic turmoil in countries all around the world. Developing countries are the most affected because many have neither the means to withstand the damage caused by invasive species nor the means to eradicate them. Developed countries often aid developing countries in research and financial support to begin eradication programs. Australia has helped many countries develop proper *S. molesta* eradication programs, as seen in Sri Lanka, Papa New Guinea, and Zimbabwe.

S. molesta infestations change the ecology and use of the waterbodies. As previously discussed, fish populations are severely affected by *S. molesta* mats (Julien et al., 2012). When fish populations plummet, human communities in developing countries are directly affected. Most communities rely on fish as their main source of sustenance and often lose a healthy source of food and water due to infestations. In the most extreme cases, entire communities are displaced due to lack of resources; this occurred in Papua New Guinea (Room, 1990; Chikwenhere and Keswani, 1997).

Transportation is also hindered by *S. molesta*. Dense mats block boat access and impede recreational activities. Countries that rely on tourism are most affected by this hindrance. Tungog Rainforest Eco Camp in Malaysia has been negatively affected by *S. molesta* infestations. They rely heavily on ecotourism to continue conservation and restoration of the surrounding rainforests. The eco-camp has experienced a decrease in tourism since the adjacent lake, Tungog Lake, was invaded by *S. molesta*.

S. molesta infestations also clog irrigation and drainage canals thus negatively affecting the agricultural industry (Room and Thomas, 1986). It reduces nutrients, space, and water for crops (Julien et al., 2012) and is a noxious weed in rice paddies all over the world (Room and Thomas, 1986). Mats also block access to drinking water for humans, livestock, and wildlife. This hindrance can seriously affect threatened and endangered species, and human communities in developing countries.

In 1939, Sri Lanka experienced economic decline in agriculture due to *S. molesta* infestations. Because the country relies heavily on the production of rice, the losses due to *S. molesta* infestations were devastating. *S. molesta* infestations in rice paddies cost the country between USD$61,000 to USD$152,000 a year. There were other costs associated with *S. molesta* infestations, such as: fishing losses, health costs, environmental costs, and abatement costs (Table 11). The highest cost was from rice paddy losses, followed by losses due to health concerns and abatement. Altogether, Sri Lanka lost between $USD163,000 to $USD375,000 a year.

Zimbabwe has also experienced some economic turmoil because of *S. molesta* infestations. Lakes lost entire species of fish which impacted commercial fisheries, severely impacting the community's livelihood. Although eradication was completed, there were costs associated with reintroduction of fish and wildlife species into the areas that were affected (Chikwenhere and Keswani, 1997).

Table 11. Cost analysis of *S. molesta* infestations in Sri Lanka. Environmental losses cannot be quantified effectively. (Adapted from Doeleman, 1987)

Losses due to *S. molesta* (Annual costs)	USD (thousands)		
Infestation Level	Low	Medium	High
Paddy Losses	61	107	152
Fishing Losses	27	40	54
Other Losses	1.3	2.3	3.3
Losses due to Health	38	58	77
Losses in Environment	NA	NA	NA
Losses due to Abatement	36	63	89
Total Losses	163.3	270.3	375.3

Impacts on Human Health by *Salvinia molesta*

S. molesta not only degrades and alters ecosystems, it also impacts public health concerns. *S. molesta* plants grow in stagnant and slow moving water which are ideal breeding grounds for mosquitoes and other insects that are vectors to some of the most infectious diseases in the world, such as malaria, encephalitis, dengue fever, and rural filariasis (Sullivan and Postle, 2012, Tipping et al., 2008, Oliver, 1993). There are about 500 million new cases of malaria every year worldwide and about 1 million malaria related deaths a year (Cuda, 2009). This raises concerns in countries where vector-borne diseases reduce human populations. At Lake Kariba, Zimbabwe, populations of *Biomaphalaria boissyi* increased due to *S. molesta* infestations. This snail is a vector for schistosomiasis so inhabitants of the surrounding area have a new health concern (McFarland et al. 2004).

Section III: Tungog Lake Case Study

Background

Tungog Lake in Kinabatangan, Sabah Malaysia (5.4110° N, 117.9632° E) is an oxbow lake that was once part of the Kinabatangan River (Figure 12). It is one of 27 oxbow lakes in the region. This lake offers many ecological services to organisms and people living in the surrounding villages. It is a breeding ground for many fish and wildlife species. It is even home to some endangered species such as *Ardea purpurea* (Oriental darter), *Ciconia stormi* (Storm's stork), and three species of otter. *S. molesta* first invaded Kinabatangan in the 1990s and since then it has invaded every oxbow lake in the region. Although natural flooding flushes these lakes out every 6 to 10 years, flooding is also the reason how *S. molesta* is spread to new areas. This occurred at Tungog Lake where *S. molesta* was able to invade the lake due to a flooding episode.

Tungog Lake **Kaboi Lake**

Figure 12. Figure 12. Tungog Lake on the left and Kaboi Lake on the right.

Tungog Lake is the center of Tungog Rainforest Eco Camp (TREC, www.mescot.org), in Kinabatangan, Sabah Malaysia. This eco-camp is run by an organization called Kopel-Mescot which focuses on community based ecotourism that connects the four surrounding villages, including Batu Petah (Mescot-Kopel Organization, 2006). Kopel-Mescot offers ecotourism and homestay programs to tourists. This community based ecotourism approach encourages villagers to be part of the conservation and restoration of the surrounding rainforests and waterways (Mescot-Kopel Organization, 2006).). Tungog Lake is maintained by Kopel-Mescot and is the center of tourist attraction at TREC.

S. molesta entered the lake through a flooding episode in 2001 which resulted in very harmful effects on the ecosystem. Kopel-Mescot received aid from the non-profit organization, Land Empowerment Animals People (LEAP), and the Alexander Abraham

Foundation for restoration. Until recently, funds from the Alexander Abraham Foundation aided in *S. molesta* removal through physical containment and manual/mechanical removal (Figure 13). The funds from the Alexander Abraham Foundation lasted through another flooding episode through 2006. During this time, because funding was available, manual and mechanical control was largely successful and limited *S. molesta* infestations in the lake (Mescot-Kopel Organization, 2006). Unfortunately, the funds have now run out and a more permanent solution is needed. Mechanical and manual removal only controlled the population of *S. molesta*, it did not eradicate it. Now Martin Vogel, director of Kopel-Mescot, is initiating a biological control program using the weevil *Cyrtobagous salviniae*. This control program will be initiated in 2016.

Figure 13. Bamboo and nets to separate *S. molesta* (Source: Mescot-Kopel Organization, 2006).

This study explores some of the ecological effects *S. molesta* has on its environment in Tungog Lake and surrounding waterways, including dissolved oxygen content (DO) and chemical oxygen demand (COD) (June 22nd-24th, 2016). These parameters were measured at Tungog Lake and a reference site, Kaboi Lake (Figure 12). DO is the amount of dissolved oxygen that is present in the water column and is the oxygen available for organisms to use. COD is the measure of organic chemicals in waterways that consume dissolved oxygen (UW Madison)**.** It is often used as a measurement to determine a measurement for the capacity of water to decompose organic matter (Camlab's Blog and Information Datacase, 2009). Kaboi Lake is not the ideal reference site because it is not a true oxbow lake and is connected to the Kinabatangan River by a small tributary. However, it offers parameters of what DO levels should be in Tungog Lake. Unfortunately, there are no ideal reference sites in this region because all oxbow lakes are infested with *S. molesta*.

Objective:

1. To determine DO and COD levels in Tungog Lake.
2. To compare DO and COD levels in *S. molesta*-infested areas to *S. molesta*-free areas of Tungog Lake.
3. To determine DO levels in Kaboi Lake.
4. To compare DO levels of Tungog Lake to Kaboi Lake.

Methods

Data Collection at each sampling location

1. Wind speed (m/s) was measured using a handheld weather meter (Kestrel 4500).
2. Humidity was measured using a handheld weather meter (Kestrel 4500).
3. Air temperature (°C) was measured using a handheld weather meter (Kestrel 4500).
4. pH was measured using a HACH meter (HQ40d).
5. DO (mg/L) was measured using HACH meter (HQ40d).
6. Electrical conductivity (μs/cm) was measured using HACH meter (HQ40d).
7. Water clarity was measured using a transparency tube.
8. Water depth was measured using a Hondex depth meter (PS-7).
9. Longitude and latitude was determined using a handheld GPS unit (eTrex).
10. Water samples were collected at random locations (determined sporadically), which was later tested using Kyoritsu Chemical-Check Lab Pack Test® for nitrites, nitrates, ammonium, and COD (mg/L).

Tungog Lake

A total of 42 samples were taken from Tungog Lake using a small motor/paddle boat.

S. molesta-infested vs. *S. molesta*-free areas (Figure 14):

A small portion of Tungog Lake remains *S. molesta* free due to physical containment via booms and nets. The *S. molesta*-free zone is near the dock of TREC. Eight locations were sampled in the *S. molesta*-free areas and 34 locations were sampled in the *S. molesta*-infested areas. Samples were collected via transects. From a total of 10 transects, 2 transects were in the *S. molesta* free areas and 8 transects in the *S. molesta*-infested areas. For 9 out of 10 transects, 4 locations were sampled in each transect; one on each side of the bank and 2 in the middle. In 1 transect, only 2 locations were sampled in the middle of the lake (Figure 14). Samples were collected by: Arti Lal, Tara Morin, Jenna Rinde, and Edris Arpah.

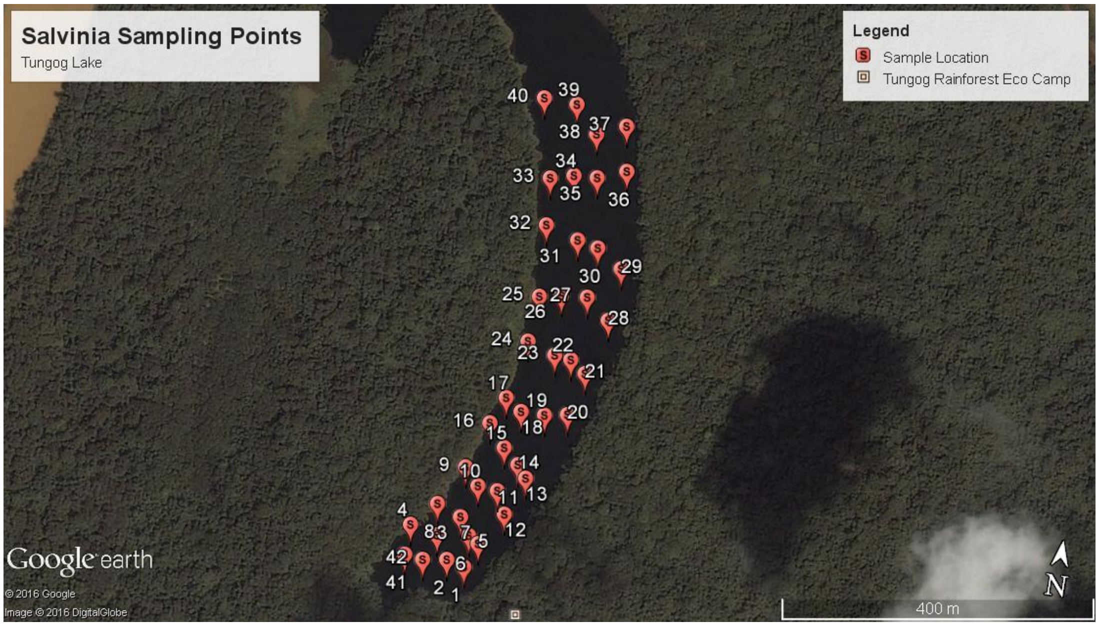


Figure 14. Sampling points in Tungog Lake. A total of 42 samples, 8 in *S. molesta*-free areas and 34 samples in *S. molesta*-infested areas. Map made by Brandon Lum and John Dilger.

Kaboi Lake

Due to time constraints and limited resources, only a small area of Kaboi Lake was surveyed. A total of 8 locations were sampled at Kaboi Lake. GPS location, pH, COD, DO, and water temperature were measured at Kaboi Lake. There were 2 transects with 4 locations sampled in each transect – 2 samples on each side of bank and 2 in the middle (Figure 15). Samples were taken and collected by Rosli Jukrana, water quality research at Kopel-Mescot.

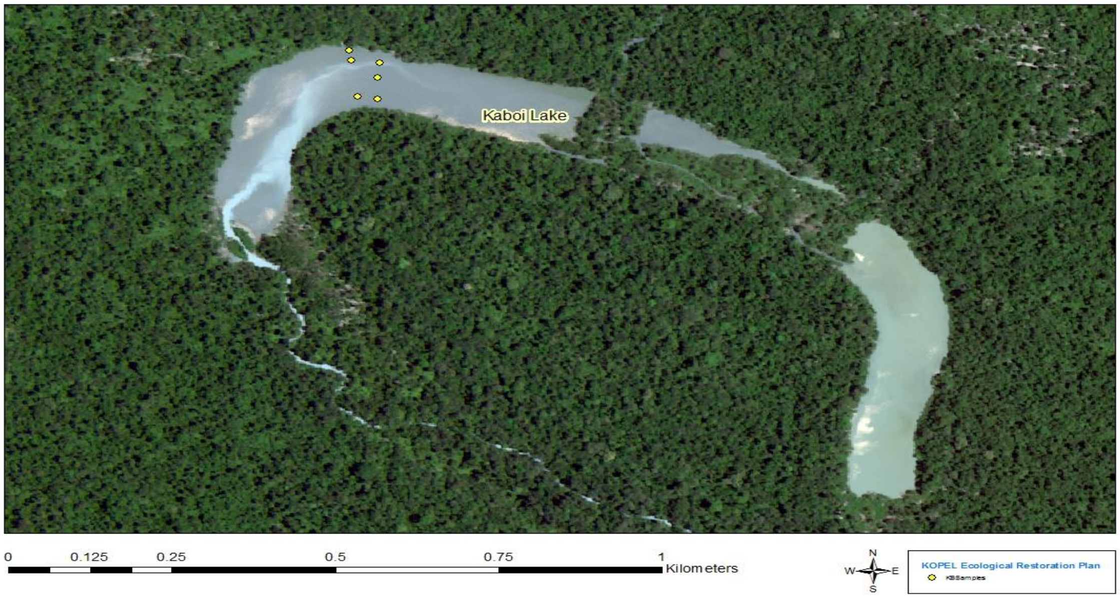


Figure 15. Kaboi Lake sampling locations in yellow. Map made by Brandon Lum and John Dilger.

Statistical Analysis

Null hypothesis 1:

> There is no difference in DO levels between the *S. molesta*-free areas and *S. molesta*-infested areas.

Alternative hypothesis 1:

> There is a significant difference in DO levels between the *S. molesta*-free areas and *S. molesta*-infested areas.

Null hypothesis 2:

> There is no difference in DO levels between Tungog Lake and Kaboi Lake.

Alternative hypothesis 2:

> There is a significant difference in DO levels between Tungog Lake and Kaboi Lake.

Statistical Methods:

8 samples were randomly selected using Random.org (www.random.com) from *S. molesta*-infested areas of Tungog Lake to run a statistical analysis. The two analyses

were conducted via two sample t-test. The two analyses were:

1. DO levels in the *S. molesta*-infested vs. *S. molesta*-free areas of Tungog Lake
2. Average DO levels of Tungog Lake vs. average DO levels of Kaboi Lake.

Results

In Tungog Lake, nutrients were within normal ranges: ammonium was approximately 0.3 mg/L overall, nitrites were less than 0.2mg/L, and nitrates were less than 1.0 mg/L (Table 12). COD was higher in the *S. molesta*-infested areas of the lake (29.33 mg/L) than the *S. molesta*-free parts of the lake (12.50 mg/L) (Figure 16). DO levels were significantly lower in *S. molesta*-infested areas of the lake than they were in the *S. molesta*-free areas of the lake (t-value = 0.03 < p-value of 0.05). DO was about 1.64 mg/L in the *S. molesta*-infested areas and was about 2.42 mg/L in the *S. molesta*-free areas (Figure 17, Table 13). This data suggests that *S. molesta* could be the probable cause of DO depletion in Tungog Lake.

Table 12. Average ammonium, nitrite, and nitrate concentration in Tungog Lake (June 22nd-June 24th, 2016)

Type of Nutrient	Average Concentration	Range
Ammonium	0.3 mg/L	Remained 0.3 mg/L
Nitrite	<0.2 mg/L	0.1-0.3 mg/L
Nitrate	<1 mg/L	0.0-0.1 mg/L

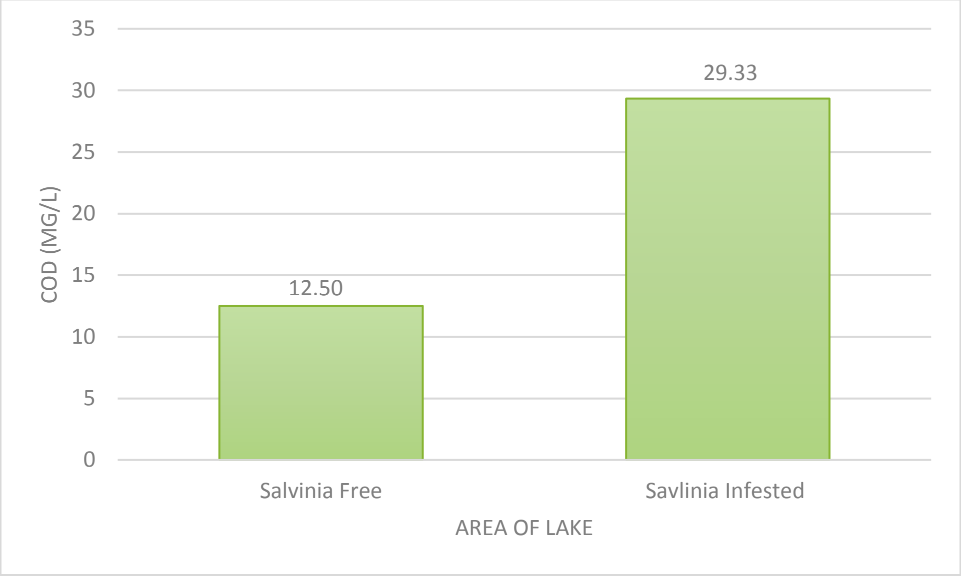


Figure 16. Comparison of chemical oxygen demand (COD) in the *S. molesta*-infested area of the lake to the *S. molesta*-free area.

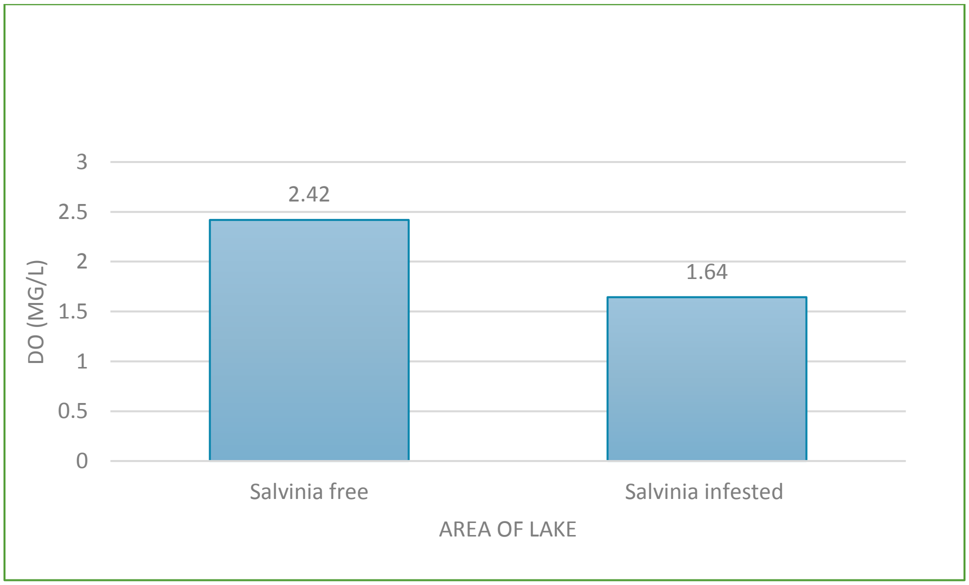


Figure 17. Comparison of DO levels in *S. molesta*-free areas and *S. molesta*-infested areas of Tungog Lake.

Table 13. T-test for two samples. Data is significant with t-value 0.03 (<p-value of 0.05) thus the null hypothesis is rejected and alternative is accepted.

t-Test: Two-Sample Assuming Unequal Variances			
	Salvinia Free		*Salvinia Infested*
Mean	2.42		1.64
Variance	0.28		0.56
Observations	8.00		8.00
Hypothesized Mean Difference		0.00	
df		13.00	
t Stat		2.39	
P(T<=t) one-tail		0.02	
t Critical one-tail		1.77	
P(T<=t) two-tail		0.03	
t Critical two-tail		2.16	

DO levels in Tungog Lake was significantly lower than DO levels in Kaboi Lake (Figure 18, Table 14). Tungog Lake had an average of 2.08 mg/L of DO while Kaboi Lake had an average of 7.76 mg/L. Statistical analysis showed that the t-value was 1.89 x 10^{-6} (< p-value of 0.05). Null hypothesis is rejected and alternative is accepted. Although DO levels are significantly different, water temperature and pH were similar between the two lakes (Table 15).

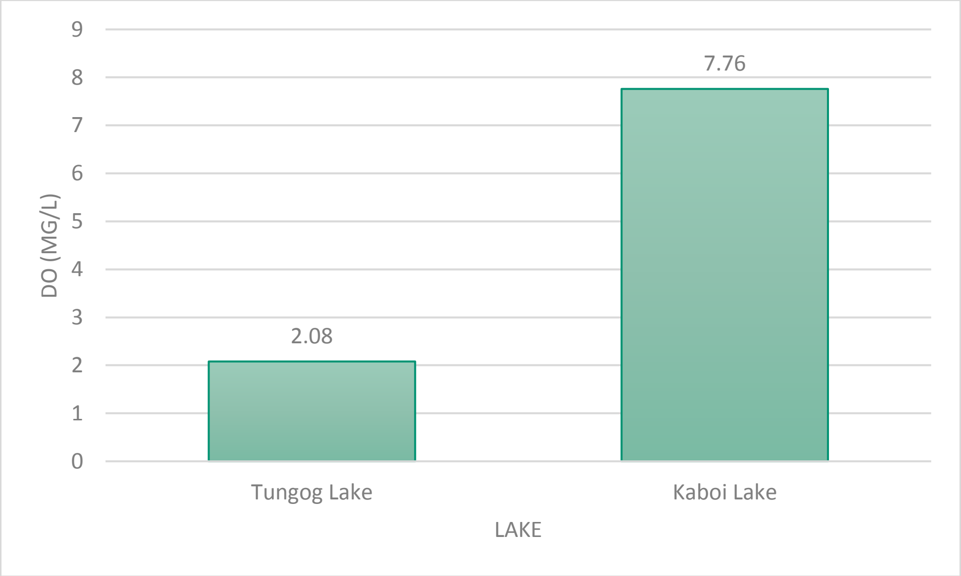


Figure 18. Comparison of DO of Tungog Lake and Kaboi Lake.

Table 14. DO comparison in Tungog Lake and Kaboi Lake.

t-Test: Two-Sample Assuming Unequal Variances			
	Tungog Lake		*Kaboi Lake*
Mean	1.64		7.76
Variance	0.56		2.55
Observations	8.00		8.00
Hypothesized Mean Difference		0.00	
df		10.00	
t Stat		-9.81	
P(T<=t) one-tail		0.00	
t Critical one-tail		1.81	
P(T<=t) two-tail		1.89×10^{-6}	
t Critical two-tail	2.23		

Table 15. Average conditions of Tungog Lake and Kaboi Lake. Water temperature and pH were very similar. DO is significantly different (t-value < p-value).

Parameter	**Tungog Lake**	**Kaboi Lake**
Water Temperature	29.7 ° C	29.7 ° C
Electrical Conductivity	47.9 µS/cm	NA
Dissolved Oxygen	2.08 mg/L	7.76 mg/L
Depth	1.75 m	NA
Clarity	27.6	NA
pH	6.6	6.5

Discussion

Tungog Lake, like many other aquatic ecosystems, is negatively affected by *S. molesta*. This study shows low DO levels in Tungog Lake where *S. molesta* infestations occur. Tungog Lake also shows severely depleted DO levels when compared to another nearby lake that is *S. molesta*-free. Because Kaboi Lake does not have *S. molesta* infestations, it has significantly higher DO levels. This suggests that *S. molesta* is the probable cause for this depletion. Other studies have also shown that *S. molesta* infestations lead to oxygen depletion (Room and Thomas, 1986; Chikwenhere and Keswani, 1997; Flores and Carlson, 2006). Thick mats in Tungog Lake alter the ecosystem services it offers including changes in species composition. New plant species have begun growing on top of *S. molesta* mats (Figure 19). Tungog Lake is home to many fish and wildlife species. There are populations of otters that currently reside in Tungog Lake and *S. molesta* infestations can potentially negatively affect their foraging and swimming activities. Also, there is a probable chance that fish populations have decreased, causing a cascade effect up the food chain.

Figure 19. Plants growing on *S. molesta* mats in Tungog Lake (Photo by: Arti Lal).

Most fish species need about 4-15 mg/L of DO to survive (Fondriest Environmental Inc., 2013). DO levels at Tungog Lake were about 2 mg/L on June 22nd-24th, 2016, indicating that most fish species cannot survive there. Invertebrates need about 1-6 mg/L of DO to survive, so the invertebrates of Tungog Lake probably have not been affected. Light penetration is also a serious concern. With decreased sunlight, phytoplankton populations might have decreased, affecting organisms up the food chain (Fondriest Environmental Inc., 2013).

Currently most of Tungog Lake is covered with *S. molesta*. Only a small portion is *S. molesta*-free and this is due to a physical barrier that was put in place by Kopel-Mescot. This barrier, although successful in keeping *S. molesta* contained, is not a longterm solution. During storm events, the barrier breaks and *S. molesta* invades all of the lake. If left unmanaged, infestations can take over the entire lake. DO levels are significantly different between the *S. molest*- free and *S. molesta*-invaded areas of the lake. According to Rosli Jukrana, a water quality researcher in Kopel-Mescot, the *S. molesta*-free areas of the lake provide habitat for fish and wildlife species that cannot survive in the thick S. *molesta* mats and a more permanent solution of eradication is needed. COD is higher in the *S. molesta*-infested areas when compared to *S. molesta*-free areas, indicating that there is a higher oxygen demand to breakdown organic matter in the infested regions.

Kaboi Lake was used as an imperfect reference site for Tungog Lake. When comparing DO levels of Kaboi Lake to Tungog Lake, there is a significant difference. Freshwater lakes should have an average of about 6 mg/L of DO (Fondriest Environmental Inc., 2013). This average is not seen at Tungog Lake. Villagers of Batu Petah have confirmed that the lake no longer harbors many of the fish species that it once did.

In 2006, when most of Tungog Lake was cleared of *S. molesta* through manual and mechanical removal, wildlife that had left the area returned to the lake, some of which were: *Anhinga melanogaster* (Oriental darter), *Ardea purpurea* (Purple heron), *Aonyx cinerea* (Small clawed otter), and *Crocodylus porosus* (Esturaine crocodile). Fish populations such as, *Oxyeleotris marmorata* (Marbled goby), *Osphronemus goramy* (Giant gouramy), and *Toi dourensis* (Ikan pelian), also increased as *S. molesta* decreased (Mescot-Kopel Organization, 2006). This is evidence that once *S. molesta* is controlled, native species will return to the system.

Section IV: Methods of Control

S. molesta has adverse effects on countries, both economically and ecologically. There are several methods of control and removal practiced all over the world. Because *S. molesta* has such devastating effects on ecosystems, complete eradication is ideal but not always feasible. Although there are several methods of control, only a few strategies eradicate populations completely. Control and eradication methods are dependent on the financial stability of the country, climate, and size of infestation. The following are control methods implemented throughout the world: 1) prevention, 2) habitat alteration, 3) physical control, 4) chemical control, and 5) biological control.

Prevention

Prevention is the first and main method of control that should be implemented everywhere. *S. molesta* is still cultivated in many botanical gardens around the world. There have been many cases of accidental and intentional release of *S. molesta*. Prevention can be implemented and secured through public education. Public education is by far the most effective and least costly method of control (Pimentel et al., 2004). Education increases awareness and cautiousness which will lead people into making more conscious decisions when considering to purchase *S. molesta* plants.

Filling the niche is a method where a species takes place of a former species. If waterways are replaced with native species after *S. molesta* is eradicated, then *S. molesta* is less likely to invade the area again because now another species if filling in all the habitat requirements (McFarland et al., 2004). A native, free floating aquatic plant should only be put in place once the *S. molesta* is eradicated. This "filling in the niche" process will prevent further infestations by *S. molesta*.

Reduction of nutrient loading is another prevention method. Because agriculture is such a huge industry worldwide, loading of nutrients should be closely managed and examined. Reducing nutrients into waterways can reduce *S. molesta* growth, giving managers more time to determine a more effective method of control (McFarland et al., 2004). Some developing countries do not have wastewater treatment plants, adding more nutrients to their waterways. Countries such as Malaysia and other parts of Southeast Asia should assess their water treatment systems properly to ensure better prevention.

Habitat Alteration

Habitat alteration is inexpensive but also ineffective. This is done through water level drawdown where the goal is to destroy *S. molesta* plants by drying or freezing (McFarland et al., 2004). Freezing is not the most effective control for *S. molesta* because even if one fragment survives, vegetative growth can lead to reintroduction of *S.*

molesta in the system when conditions become favorable (Sushilkumar, 2011; Cooke et al., 1986). This method has only showed success in small waterways, such as in Lewisville, Texas, but it may not work well in larger waterways or in the tropics (McFarland et al., 2004; Cooke et al., 1986).

Physical Control

There are three methods that can be categorized under physical control:

1. Manual removal
2. Mechanical removal
3. Physical barriers

Physical control is the act of physically removing or relocating *S. molesta* plants in waterways. Manual removal is removal of *S. molesta* plants by use of hands or simple tools, mechanical removal is removal using machinery and large equipment, and physical barriers are put in place to contain *S. molesta* infestations.

Manual control

Manual control is one of the most economical methods to manage *S. molesta* infestations. Manual control is removal of *S. molesta* by hand or use of simple tools (Figure 20). Plants can also be chopped or cut before they are removed from waterways (Room and Thomas, 1986). Manual removal is only effective if infestations are small and in the primary growing stage. It is ineffective in large infestations because *S. molesta* grows faster than the rate of removal (Room and Thomas, 1986; Room, 1990; CRC Weed Management, 2003). *S. molesta* can double in size within 2 to 3 days, so it becomes impossible for manual removal to keep pace with growing plants. Even during low growing seasons, manual removal is inefficient (Room, 1988).

Figure 20. Manual removal of *S. molesta* using tools in Tungog Lake (Photo by: Sarah Carter).

Mechanical Control

Mechanical removal is removal with heavy equipment and machinery. Mechanical removal works best in small to medium infestations (CRC Weed Management, 2003). Large scale mechanical removal can get expensive for developing countries so some countries do not use this method leading to larger infestations (Mescot-Kopel Organization, 2006). When using mechanical machinery for removal of infestations, piles of plant material are accumulated. If this accumulation is not burned immediately, then regrowth occurs. Paraffin, also known as kerosene, is often needed alongside mechanical removal to burn accumulated plant material (CRC Weed Management, 2003).

Barriers

Containment is a practice that uses barriers to separate infested areas from non-infested areas. A physical barrier can provide suitable habitat for fish and wildlife in *S. molest*- free areas of a lake. Barriers are made of nets, lines, and other materials that can physically keep plants from migrating (McFarland et al., 2004).

Although containment is very economical, it is not a method of eradication. Weather is a concern when using containment because storms often break barriers, leading to the spread of *S. molesta* into uninvaded areas of the water. This issue due to weather occurs at Tungog Lake, Malaysia periodically. Weather events that affect barriers often go unnoticed in countries that do not have funding to set up monitoring (Oliver, 1993). In Lake Moondarra, Australia, wire hawsers that were attached to 200-liter drums and anchored to concrete blocks were used to keep *S. molesta* contained from non-invaded areas (McFarland et al., 2004; Oosterhout et al., 2006). This method of containment was very effective because it was monitored periodically. Lake Kariba, Zimbabwe however, used booms attached to 5-cm steel cables which was ineffective only because monitoring was not put in place. *S. molesta* spread to all parts of the lake because containment was broken and officials did were too late to discover it (Room and Thomas, 1986). Using booms attached to 5-cm steel cables would have been effective if it was monitored periodically. Monitoring is important because if containment is breached, then the issue could be resolved before containment is no longer plausible.

There are several types of physical barriers used for containment. Depending on the size of the infestation, large booms to small ropes can be used (Oliver, 1993; Oosterhout et al., 2006; Mescot-Kopel Organization, 2006). Booms must be 10-cm below and 10-cm above the water for effective containment (Oosterhout et al., 2006). Industrial booms that are used for oil spills are also effective. If a waterway is moving, containment fences might be most effective because it will prevent *S. molesta* plants from spreading downstream (Oosterhout et al., 2006).

Chemical Control

Chemical control with herbicides have been largely successful. Some herbicides are selective and designed for only *S. molesta* plants, others are non-selective and will damage any plant material it is applied on. Most herbicides reduce plant buoyancy so plants sink and die (McFarland et al., 2004). The first successful control with herbicides was in 1940 in Sri Lanka. Pentachlorophenol, a wood preservative, was sprayed and *S. molesta* infestations were successfully controlled (Room and Thomas, 1986). In 1990, a small pond in South Carolina, United States was invaded and through successful use of the herbicide diquat, the infestation was eradicated. Later infestations in Texas, Louisiana, and Hawaii were also controlled with herbicides. Currently, diquat and glyphosate, are used in the United States. Diquat, calcium dodecyl benzene sulfonate, glyphosate, and orange oil are all used in Australia (Room and Thomas, 1986; Oliver, 1993; McFarland et al., 2004)

Although chemical control has been largely successful, there are downsides. Water quality is severely affected through herbicide use, both by contamination and oxygen depletion. When herbicides are not properly used and directions are not followed, it often leads to contamination of a waterway (Oosterhout et al., 2006). Often times the calibration of equipment or dosage applied is incorrect, leading to chemical contamination of the waterway that harm many fish and wildlife species. Deoxygenation is another issue that arises through herbicide use. When herbicides are used to kill *S. molesta* infestations, large populations die all at once, leading to an accumulation of organic matter in the water column. Decomposing bacteria then use most of the DO available to decompose *S. molesta,* leaving very little DO for other organisms (Oosterhout et al., 2006).

Due to fast growth rates of *S. molesta*, a series of treatments is often required for complete eradication (Oosterhout et al., 2006). Australia is the leading country for controlling *S. molesta* infestations and have set the guidelines required for herbicide use. Other countries often follow the rules and regulations that Australia has set in place. Countries also use research conducted in Australia to implement their own methods of control. Below is a detailed description of the most commonly used herbicides around the world.

Diquat (Aquacide ®, Dextrone ®, Aquakill ®)

Diquat, also known as diquat dibromide, is used as a “knockdown agent” and is sprayed on thick *S. molesta* mats that are in the tertiary growing stage. Diquat is used to reduce overcrowding so that another herbicide or method of control can be used to eradicate *S. molesta* populations; once diquat is applied then mats reduce to either the secondary or primary growing stage (Table 15) (Oosterhout et al., 2006). Diquat is a non-selective desiccant that deteriorates all plants that is it applied on and does not only target *S. molesta* plants. Diquat, although frequently used for *S. molesta* infestation control, can be toxic to fish, mammals, and birds (Extension Toxicology Network, 1993).

Calcium dodecylbenzene sulfonate (Immerse ®)

Calcium dodecylbenzene sulfonate is an oil-based surfactant that has a low surface tension which allows it to go through the trichomes of *S. molesta* plants. Calcium dodecylbenzene sulfonate must be applied to the water surface to work effectively so secondary or primary growing stages are ideal for the use. When plants are in the tertiary growing stage, there is overcrowding and no open water for calcium dodecylbenzene sulfonate to be applied on. Once it touches plant material, it reduces plant buoyancy. Diquat and calcium dodecylbenzene sulfonate are often used together on thick mats. Diquat is sprayed first to thin out mats and increase open areas, then calcium dodecylbenzene sulfonate is sprayed on the water sureface (Table 15) (Oosterhout et al., 2006).

Glyphosate (Roundup ®, Rodeo ®, and Pondmaster ®)

Glyphosate is another commonly used herbicide for *S. molesta* eradication. It is absorbed directly by plants and takes a few months to work. Mats must be in the primary or secondary growing stages (Table 15) (Oosterhout et al., 2006). Glyphosate is non-selective and can damage other plants it comes into contact with. It is an organophosphate but unlike other organophosphates, it is not toxic to fish (Extension Toxicology Network, 1993).

Orange oil

Orange oil is the least effective herbicide. It is rarely used but can be used in urban areas where toxicity levels should be minimum (Oosterhout et al., 2006).

Table 16. Herbicides that work well with certain *S. molesta* growing stages, risk level of herbicide, and application rate. (Adapted from: Oosterhout et al., 2006 and CRC Weed Management, 2003).

Type of Herbicide	Growth Stage	Toxicity	Effective Alone	Effectiveness	Application rate (gal/acre)
Diquat	Tertiary	Moderate	No	Excellent	0.5-0.75
Immerse	Secondary or Primary	High	No	Excellent	1.0-2.0
Glyphosate	Secondary or Primary	Moderate	No	Excellent	1.0-2.0
Orange Oil	Secondary or Primary	Low	No	Good	1L/100 L water

Biological Control

Although one of the best methods of control for *S. molesta* is biological control because it is the most effective and least costly, it is also the most controversial method (Allen et al., 2014). Biological control uses living organisms that are the natural predators, herbivores, and/or pathogens of the target species and are used to control its population (Sullivan and Postle, 2012). When species invade an area outside of its native range, biological control agents can be imported to that area to control invasion. However, biological control agents may not eradicate invasive species completely but only reduce populations enough to where eradication is possible through other means. The reason is because biological control agents require biomass of the target species to survive and sustain their own population size. In order for biological control agents to be released into regions outside of their native range, they should go through host-specificity testing.

Host-specificity testing is a series of tests conducted to ensure that the biological control agent will not become invasive itself (Sullivan and Postle, 2012). It must first be established that the control agent will not switch preference to any native species. There have been many cases around the world where the control agent became invasive, such as *Rhinocyllus conicus* (flowerhead weevil), which became invasive in North America. *R. conicus* was released to control *Carduus nutus* (musk thistle), but continued to increase its range and affected native thistle plants. It was known beforehand that the range of the weevil would intersect that of three native thistle plants. Because regulations were not as strict at the time, biological control was still approved, which led to decline in native thistle populations (USDA, 2016). This is not the case for *S. molesta* because the biological control agent used for *S. molesta*, *Cyrtobagous salviniae*, are less likely to only consume on *Salvinia* species.

Section V: *Cyrtobagous Salviniae*

Discovery of *Crytobagous salviniae*

Biological control has been the most successful method of control for *S. molesta* in many tropical and subtropical regions. The biological control agent of preference for *S. molesta* is the Salvinia weevil, *Cyrtobagous salviniae*. Because *S. molesta* was thought to be *S. auriculata* before 1978, the biological control agent used at first was *Cyrtobagous singularis,* an herbivore of *S. auriculata*. Biological control trials on *S. molesta* with *C. singularis* were unsuccessful. After the discovery of *S. molesta*, *C. salviniae* was discovered in Brazil (Room, 1981; Driesche et al., 2002). Because *S. molesta* has several natural herbivores in its native range, host specificity tests were conducted for *C. salviniae, C. singularis, Samea multiplicalis,* and *Paulina acuminata* (Room, 1981; Room et al. 1984). Because *S. molesta* was thought to be a different species at first, the wrong biological control agent, *C. singularis*, was used and implemented for a number of years. Australia was one of the first to implement biological control of *S. molesta* using *C. salviniae* in Lake Moondarra which was heavily infested with *S. molesta* (Room, 1991). This action by Australia led other countries to implement *C. salviniae* as their primary method of control.

Description of *Cyrtobagous salviniae*

C. salivinae are small weevils about 1.5 to 3.0 mm in length with reddish-brown legs and yellow depressions on their backs (Sullivan et al. 2011; Mukherjee et al., 2014). Adults are black and juveniles are brown; all weevils have protruded snouts (Oosterhout et al., 2006, Sullivan et al., 2011). Larvae are white, approximately 3 mm long and are larger than adults. Weevils have several life cycle stages, one being the pupae stage. At this stage, larvae use the fronds of *S. molesta* plants to make cocoons (McFarland et al., 2004; Knutson et al., 2011).

Life Cycle and Habitat

The entire life cycle of *C. salviniae* occur inside *S. molesta* plants; which includes the egg, larvae, pupae, and adult stages. Adult females dig cavities into leaves, petioles, rhizomes and roots where they lay eggs (Cillers, 1991; Knutson et al., 2011). Each adult lays about 2 to 5 eggs a day over a span of 60 days (McFarland et al., 2004, Oosterhout et al., 2006). The optimum temperature range for egg development is between 23 and 31 °C so the most suitable environment for successful reproduction is in tropical and subtropical regions (Cillers, 1991; Sullivan and Postle, 2012). In temperate environments, *C. salviniae* lay eggs only in the spring. If populations can grow and can sustain themselves then overwintering will be successful (Sullivan and Postle, 2012).

As larvae develop, they consume the buds and new leaves of *S. molesta* plants. After about 3 to 14 days, larvae tunnel through the stem, consuming plant parts as they move (Cillers, 1991). The whole larval stage lasts about 25 days. Towards the end of the 25 days, they begin their journey to the base of the leaves. Here larvae will use leaves to make cocoons, initiating the pupae stage. Pupae will remain in the cocoon for 9 to 15 days (Oosterhout et al., 2006; Knutson et al., 2011). Fifteen days later, adults emerge from the cocoons and live out the rest of their lives eating leaves and buds.

C. salviniae are very host-specific and live only on *Salvinia* species. *C. salviniae* are found on *S. minima* plants in Florida, but often only chose *S. molesta* plants (Oosterhout et al., 2006). The best habitat for *C. salviniae* to grow in are thin *S. molesta* mats. They prefer thinner mats because thick, multilayered mats that are in the tertiary growing stage have reduced oxygen and *C. salviniae* are not able to reproduce effectively in oxygen poor environments. Larvae and pupae need oxygen-rich environments and when oxygen levels reach below 40%, *C. salviniae* do not reproduce (Oosterhout et al., 2006, Sullivan and Postle, 2012). *C. salviniae* also prefer little to no shading because reproduction is temperature dependent. This is why shallow waters are unfavorable for *C. salviniae* population growth because it leads to increased temperatures (Sullivan and Postle, 2012). Overall, *C. Salviniae* need large, permanent bodies of water with little to no shading that is 70% covered with *S. molesta* (Sullivan and Postle, 2012).

Plant-weevil Interaction

C. salviniae have devastative effects on *S. molesta* plants because both adults and larvae feed on plant parts. Studies have shown that within a couple of years, entire water bodies can be controlled by *C. salviniae* from *S. molesta* infestations. Once *C. salviniae* are introduced to an infestation site, they begin reducing populations of *S. molesta* within a few months. When plants are damaged from overfeeding, they turn brown, lose buoyancy, and sink to the bottom (McFarland et al., 2004). Adult *C. salviniae* feed on new buds; plants cannot regrow new buds at the rate that they are eaten. Because of *C. salviniae* consumption rates, plants have to use stored energy to grow new buds depleting stored resources. *C. salviniae* populations grow rapidly and because plants cannot keep growing new buds at that rate, plants deteriorate (Oosterhout et al., 2006). Larvae consume stems, rhizomes, and petioles which disrupts the root to shoot connection (Sullivan et al., 2011). When this occurs, nutrient uptake is disrupted leading to malnutrition (Sullivan et al., 2011).

S. molesta and *C. salviniae* portray what is called "alternative stable states". When *C. salviniae* populations increase, consumption by *C. salviniae* also increase. Increased consumption decreases *S. molesta* populations. This in-turn decreases *C. salviniae* populations. When *S. molesta* populations increase again due to decreased consumption, *C. salviniae* populations follow. This feedback loop creates peaks and troughs on population graphs (Stone, 2011). There will eventually be a "biocontrol balance" where neither of the two populations increase or decrease (Stone, 2011, Oosterhout et al. 2012).

Another aspect to alternative stable states is that high densities of *S. molesta* lead to suppressed *C. salviniae* populations. When *S. molesta* is overcrowded, there are few new buds and most growth is vegetative. Because *C. salviniae* need new buds to feed on, they cannot sustain a healthy population size when plants are in the tertiary growing stage (Schooler et al., 2014). Ideal growing stages for *S. molesta* that *C. salviniae* prefer are primary and secondary.

Biological control is not a method of eradication but only a method of control; it must be used concurrently with either physical, mechanical, or chemical controls to effectively eradicate *S. molesta* infestations (Oosterhout et al., 2006). Biological control takes more time than other methods because C. *salviniae* need time to reproduce and establish a healthy population size. It takes about 1 to 3 years for biological control via *C. salviniae* to show results (Oosterhout et al., 2006).

Temperature and Nutrient Constraints on *Crytobagous salviniae*

Temperature and nutrients are both controlling growth factors for *C. salviniae* (Room et al., 1981). When nutrients are limiting or temperature is not optimum, *C. salviniae* cannot grow and spread effectively. When both parameters are ideal, *C. salviniae* populations can grow uncontrollably and reduce populations of *S. molesta* by consumption.

Nutrient Constraints on *Crytobagous salviniae*

Nutrient availability affects both *S. molesta* and *C. salviniae*. When nutrients are high in the water column, *S. molesta* take up and concentrate large quantities in their tissues. When *C. salviniae* feed on these high concentrated tissues, they receive high levels as well. Ideal nutrients concentrations increase *C. salviniae* survival by aiding in development and reproduction. When nutrient levels are low, less is taken up by plants, which affects *C. salviniae* population growth (Oosterhout et al., 2006). Nutrients are important when there are no temperature constraints. Low nutrients with ideal temperatures have occurred in tropical countries such as Sri Lanka and Papa New Guinea (Thomas and Room, 1986). As in the case of Papua New Guinea, temperatures were ideal but nutrient levels were low. This limitation of nutrients impeded *C. salviniae* growth and survival. Once nutrients were added to the system, *C. salviniae* were effective biological control agents, reducing *S. molesta* infestations (Room, 1989).

Temperature Constraints on *Cyrtobagous salviniae*

S. molesta has invaded tropical and temperate environments, whereas *C. salviniae* has only been largely successful in the tropics where it can grow uncontrollably (Room and Fernando, 1992). *C. salviniae* as a biological control agent have successfully controlled populations of *S. molesta* in over 12 tropical countries. Unsuccessful biological control in temperate regions is due to low winter temperatures that *C. salviniae* cannot survive in. *S. molesta* can tolerate lower temperatures than *C.*

salviniae so they often outlive *C. salviniae* in temperate regions during winter (Forno et al., 1983; Room et al., 1989). *S. molesta* plants are also able to adapt to new conditions quickly and grow rapidly. The optimum temperature range for *S. molesta* is between 20 to 30°C, however, *S. molesta* plants have established successfully in regions with temperatures as low as 12 °C, although growth does become limited when temperatures reach below 16 °C (Room, 1986). *C. salviniae* do not have such a wide range of temperature tolerance. *S. molesta* has adaptions that allow it to survive in various environments, including regions that freeze. Buds remain dormant until ideal conditions return. Because *S. molesta* can withstand lower temperatures than *C. salviniae*, biological control has been ineffective in temperate regions (Doelmann, 1989). Chemical control has been the most effective method of control in temperate regions, however, a combination of herbicides and control via *C. salviniae* has shown to be the most effective method of control.

In the 1980s, biological control was also implemented in New South Wales, Australia. Trials here were largely unsuccessful and managers decided to use herbicides instead (Sullivan et al., 2011). In Hawkesbury-Nepean, Australia however, biological control was successful, but only in river systems. Creeks, dams, and lakes were not controlled by *C. salviniae* (Sullivan and Postle, 2010). Earlier studies from the 1980s show that *C. salviniae* can establish in temperate regions but cannot sustain healthy populations. Later studies in the 2000s show that *C. salviniae* can establish and sustain themselves for long periods of time if oviposition occurs at low temperatures (Sullivan et al., 2011; Tipping et al, 2007, Forno et al., 1983).

In Sydney, Australia biological control was successful but timing of release was important. When *C. salviniae* were released in Sydney, populations failed to establish at first. Researchers theorized that the timing of release was an important factor in temperate regions so they released *C. salviniae* in early summer and noticed that they were able to survive through the winter. Being released earlier in the summertime gave *C. savliniae* enough time to develop, reproduce, and ensure a healthy population size to successfully survive through the winter. With this strategy, control of *S. molesta* in Sydney via *C. salviniae* was successful and in 3 years, infestations became manageable (Sullivan et al., 2011).

Biological control trials were successful in Texas and Louisiana in which *C.*

salviniae were able to survive in temperatures below -9 °C (Tipping et al, 2007; Flores and Carlson, 2006). Even with successful trials such as these, biological control has not been largely successful in the United States. Other than temperature, another factor preventing *C. salviniae* survival in the United States could be that there are two distinct populations of *C. salviniae*. One population was brought in from Australia and the other population has been in Florida for many years. Although both populations were originally from Brazil, the Florida population has had many years to adapt and mutate. A chill coma study determined that *C. salviniae* from Australia could tolerate lower temperatures than the Florida population. The chill coma study was conducted by freezing *C. salviniae* then measuring their reproduction afterwards (Mukerjee et al. 2014). This study could provide evidence of why some biological control trials have shown no success while others have.

Low Reproduction of *Cyrtobagous salviniae* in Temperate Climate

Optimum temperature range for *C. salviniae* is between 25 and 30°C (Oosterhout et al., 2006). Adult *C. salviniae* need temperatures above 13°C, with adult females needing 21 °C or higher (Forno et al., 1983; Sullivan and Postle, 2010). When temperatures go below 13°C, all weevils stop eating (Oosterhout et al., 2006, Allen et al., 2014). Egg and larvae require temperatures of about 25°C. Both adults and larvae play important roles in controlling *S. molesta* populations, however, larvae play a slightly bigger role because they tunnel through *S. molesta*, destroying stems in the process (Sullivan and Postle, 2010, Allen et al., 2014). Even with these ranges, *C. salviniae* need a constant temperature range to reproduce and oviposit successfully (Henecke and Postle 2006). When temperatures fluctuate, *C. salviniae* usually relocate to other parts of the plant, looking for areas where temperature is ideal. They cannot always relocate and find optimum temperature ranges because in most temperate regions, there are no areas of the plant with optimum temperature ranges during winter (Oosterhout et al., 2006, Allen et al., 2014). Because *C. salviniae* cannot relocate to other parts of *S. molesta* plants, this leads to slower development and eventually death (Oosterhout et al., 2006).

Climate Implications on *Salvinia molesta* and *Cyrtobagous salviniae*

Climate change is an important issue with invasive plants, especially invasive aquatic plants. *S. molesta* will likely increase its range in South Africa due to climate change (Hoveka et al. 2006). The Intergovernmental Panel on Climate Change predicts that temperatures in South Africa will increase 3-4 °C by 2100 (Allen et al., 2014). Not only will temperatures increase, but extreme weather events will also increase. Suitable habitat for *S. molesta* will increase to Limpopo, Mpunlanga, KwaZulu, Natal, and Eastern Cape and Western Cape Provinces (Figure 21). This pattern will be seen all over the world. Climate change will increase regions of potential invasions by providing new areas with optimum temperature range for *S. molesta* (Figure 22). In the United States, *S. molesta* is currently only present in the southern states. With climate change, however, temperature increase in the northern states will allow *S. molesta* to invade the region (Julien et al., 2012). *S. molesta* range could also decrease in areas that become too warm with climate change.

Figure 21. *S. molesta* current range in South Africa. Blue indicates unsuitable habitat and red indicates very suitable habitat. (Source: Hoveka et al., 2006)

Figure 22. *S. molesta's* projected range for 2080; blue indicating unsuitable habitat and red for suitable habitat in South Africa. (Source: Hoveka et al., 2006).

Climate change will not only affect the distribution and spread of *S. molesta*, but will also affect *C. salviniae*. In general, climate change will have conflicting effects; it will increase the effectiveness of some biological control agents while limiting others. There will be a change in the interactions between biological control agents and their target species (Allen et al., 2014). By 2040, projected temperature increase in South Africa is 1.4°C and 2.5°C in 2080 (Figure 21). *C. salviniae* however, should not be affected negatively because it can tolerate a temperature increase well above 6°C.

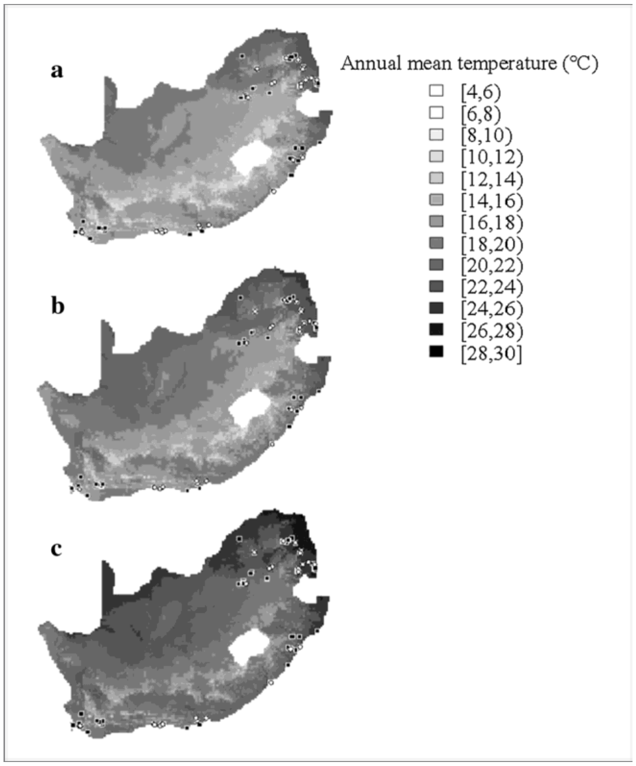


Figure 23. Climate in South Africa.
a) Present climate
b) 2040 climate projection
c) 2080 climate projection

In conclusion, *C. salviniae* will continue to be an effective biological control agent for *S. molesta* in South Africa but only with careful monitoring and re-introduction efforts (Allen et al., 2014). Because *C. salviniae* are ectotherms, external temperatures control populations and climate change could have more detrimental effects on *C. salviniae* than *S. molesta* (Oosterhout et al., 2006).

Description of Other Biological Control Agents for *Salvinia molesta*

Samea multiplicalis is a moth that feeds on both *S. molesta* and *E. crassipes* (water hyacinth) and is native to Brazil (Driesche et al., 2002). These are the two most noxious aquatic invasive plants in the world. *S. multiplicalis* was introduced in Australia as a biological control agent to reduce populations of *S. molesta* but was unsuccessful. In Florida, however, *S. multiplicalis* manages populations of *S. minima*. Although *S. multiplicalis* have a higher dispersal range than *C. salviniae* and can tolerate lower temperatures, *C. salviniae* are ae effective at controlling *S. molesta* populations (Procter, 1983). *S. multiplicalis* were released in Lake Julius, Queensland Australia, where it was not as effective as *C. salviniae* (Mitchell, 2014). In earlier years, *C. singularis* and *S. multiplicalis* were both released in Botswana, Zambia, and Fiji. No results from *S. multiplicalis* were observed but *C. singularis* were able to establish populations.

Paulina acuminata is a semi-aquatic grasshopper native to Brazil, that feed on aquatic plants, including all *Salvinia* species (Driesche et al., 2002). In 1972, *P. acuminata* was released in Lake Kariba, Zimbabwe to control populations of *S. molesta* and was successful, however, it was unsuccessful in other parts of the country (Driesche et al., 2002). *P. acuminata* was also released in several other countries including Kenya, Zambia, Botswana, Sri Lanka, India, and Fiji. Unfortunately, it did not provide sufficient control for *S. molesta*.

As previously discussed, *C. singularis* mostly feeds on *S. auriculata*. *C. singularis* was released in many regions when *S. molesta* was thought to be *S. auriculata*. It was released in Botswana in 1972 and again in 1975 along with *P. acuminata*. *C. singularis* was able to establish in Botswana but nowhere else. This could be an indication that these weevils adapt quickly and that Botswana has nutrient rich soils (Procter, 1983).

Researchers argue that if *C. salviniae* were to be introduced in Botswana now, this could have detrimental effects. One species could outcompete the other. Competition could also reduce both populations which can lead to increased *S. molesta* growth. Also, interbreeding would lead to hybrids with unknown effects. The two species do in fact consume different parts of plants, *C. salviniae* tunnel through the rhizomes and stems while *C. singularis* consume external leaves and stems (Driesche et al., 2002). One reason to why *C. salviniae* are better biological control agents could be that they cause more damage to plants by consuming the internal structures that inhibit plant growth.

When to Use Biological Control

Since *C. salviniae* do not do well on plants that are in the tertiary growing stage, it is most effective to use biological and chemical control concurrently. Herbicides, such as diquat, are first used to thin out *S. molesta* mats from the tertiary growing stage to either secondary or primary. *C. salviniae* are then introduced to the area (Sullivan and Postle, 2012). Over time, as *C. salviniae* grow and establish a healthy population size, *S. molesta* mats will thin out even more. Then the application of calcium dodecylbenzene sulfonate will completely eradicate *S. molesta* infestations. (Sullivan and Postle, 2012, Oosterhout et al., 2006).

Mechanical control is also very effective with biological control. Once *C. salviniae* reduce *S. molesta* populations, machines can then be used to eradicate remaining populations. This method is often preferred over the use of calcium dodecylbenzene sulfonate. Calcium dodecylbenzene sulfonate causes depletion in DO levels because large quantities of plants die all at once; this does not occur with mechanical removal because all plant material that is collected is burned (Sullivan and Postle, 2012). Biological control does not work well when *S. molesta* plants are in the tertiary growth forms (Table 17). Manual removal is only best with primary growth forms because secondary and tertiary forms are too overcrowded for laborers to manually remove. Although mechanical and chemical removal work well with all growth types, the best methods of removal are combinations of two or more methods (Sullivan and Postle, 2012).

Table 17. Type of removal that works best with *S. molesta* growing stage.

Growth Type	Manual Removal	Mechanical Removal	Chemical Removal	Biological Control
Primary	x	x	x	x
Secondary	x	x	x	x
Tertiary			x	

Section VI: Successful Biological Control Program

Biological control has been successful in many countries including, Papua New Guinea, India, Namibia, Sri Lanka, Zimbabwe, and Fiji (Figure 24). *C. salviniae* have been the biological control agent of choice in these regions because it is most effective over *S. multiplicalis, P. acuiminata*, and *C. singularis* (Doelmann et al., 1998). CSIRO has assisted many countries in acquiring *C. salviniae* and releasing it into infested areas. In some regions, however, biological control has been unsuccessful, such as at Kakadu National Park in Australia and the Lower Colorado River in Mexico (Mora-Olivo and Yatskievych, 2009).

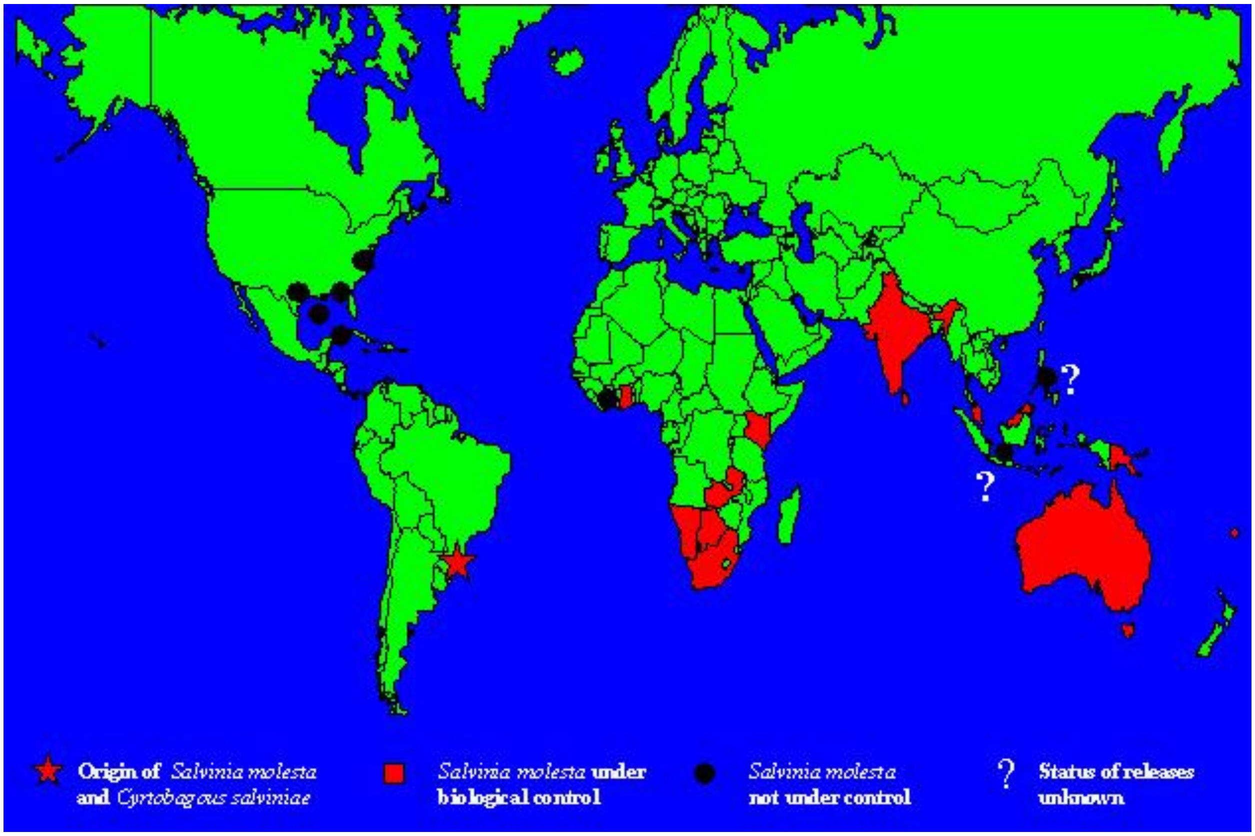


Figure 24. Distribution and location of where biological control via. *C. savliniae* is taking place. Red start represents origin of *S. molesta* in Brazil. Red countries represent where *S. molesta* is under biological control and black dots represent where it is not under control. Question mark represents an unknown status (Source: Julien et al., 2012).

Australia

S. molesta was first seen in Australia in 1952 and it has since invaded many parts of that country. The first successful biological control program for *S. molesta* was conducted in 1979 in Australia via *C. salviniae*. It was first released through CSIRO in 1980 in Lake Moondarra, Queensland Australia, where it effectively reduced *S. molesta* populations within 11 months (Oosterhout et al., 2006; Sullivan and Postle, 2012). *C. salviniae* were able to successfully control over 200-ha of *S. molesta* infestations in 14 months (Room et al., 1981; Thomas and Room, 1986). Biological control in the tropical and subtropical regions of Australia continue to be largely successful.

Sri Lanka

S. molesta was introduced in Sri Lanka in 1939; by 1954 9000-ha of rice paddies were infested with *S. molesta* (Room and Fernando, 1992). After several attempts of physical and chemical control, infestations did not decrease but got worse, leading to 20,000-ha of infested rice paddies (Room and Fernando, 1992). *S. molesta* blocked irrigation for rice paddies and competed for light, nutrients, and space with rice crops. *S. molesta* also interfered with the hydroelectric Mahaweli Scheme that Sri Lanka and Australia worked on together. This was an irrigation system that generated 500-megawatts of electricity. After host-specificity tests confirmed *C. salviniae* success in Australia in 1986, they were taken to Sri Lanka.

In 1986, CSIRO aided Sri Lanka's National Resources Energy and Science Authority (NARESA) in the release of *C. salviniae* in 96 sites. At the time, only 16 sites showed results of successful control (Doelemann, 1989). *C. salviniae* were put in cages which were set around *S. molesta* infestations. Researchers noticed that *C. salviniae* breeding was more efficient in plants that had higher nitrogen concentration (Room and Fernando, 1992). After adding fertilizer inside the cages of *C. salviniae*, they saw an improvement in *C. salviniae* reproduction and survival in all sites. *C. savliniae* eliminated about 10-ha of *S. molesta* in 3 months. Overall, it took about 12 to 24 months for *C. salviniae* to control *S. molesta* infestations. Sites where nitrogen was below 2-3 % took longer to control.

The financial benefits of *C. salviniae* outweighed losses due to *S. molesta* in Sri Lanka. *C. salviniae* was the most cost effective method of control. Losses due to *S. molesta* infestations were between USD$163,000 to USD$375,000 a year (Table 11). The cost to implement control via *C. salviniae* was only USD$ 55,000. Sri Lanka saved about USD$11,000 to USD$32,000 by using *C. Salviniae* for control; the benefit to cost ratio was 53:1.

Papua New Guinea

In 1970, *S. molesta* spread to the Sepik River Delta through accidental release by aquarium tanks. Several homes and businesses had aquarium tanks that contained *S. molesta* plants that were used for ornamental purposes. By 1980, more than 500 km^2 of lakes were infested with *S. molesta* and more than 80,000 people were affected

(Doeleman, 1989). The surrounding communities depended heavily on fishing as their main source of protein, and when *S. molesta* infestations depleted fish stocks, villagers were displaced and had to abandoned their homes (Room, 1990).

The success of Lake Moondarra, Australia brought high hopes to Papua New Guinea. Unfortunately, biological control in Papa New Guinea was unsuccessful at first, *C. salviniae* died in seven months. This was due to low nitrogen levels in the water. Researchers found that nitrogen was less here than in Lake Moondarra where biological control via *C. salviniae* was successful. To increase nitrogen levels, they added urea fertilizer (Room, 1990). Plant nitrogen levels increased 0.2-0.3% leading to *C. salviniae* survival which led to effective control of *S. molesta* infestations.

Zimbabwe

In 1956, Lake Kariba in Zimbabwe was invaded by *S. molesta* and in 1959, biological control was attempted. It was unsuccessful at first because *S. molesta* was misidentified as *S. auriculata* at the time and the biological control agent used was *C. singularis*. After *S. molesta* was identified and *C. salviniae* was discovered, implementation of *C. salviniae* led to successful control of *S. molesta* in all of Lake Kariba (Room and Thomas, 1986).

By the 1980s, *S. molesta* spread to other parts of the country invading lakes, ponds, and rivers. For years, managers were using physical, mechanical, and chemical control for eradication which were not effective. Manual and mechanical control could not keep up with *S. molesta* growth, and chemical control was too expensive to continue. Due to Australia's success (and several other countries including Papua New Guinea, Kenya, Namibia, South Africa, and Zambia) with biological control, researchers initiated this process in Zimbabwe (Chikwenhere and Keswani, 1997).

Chemical, physical, and manual control of *S. molesta* was evaluated and compared to biological control; biological control was the most economic and effective method of control. Estimated cost for manual removal was USD$8.30. Containment costs a were $USD 12.60, with $USD10.55 for the actual barrier and $USD 2.07 for paraffin that was used to burn dry plants. Chemical control cost $USD 38.79, $USD 110.53 of it was on spraying the two dams that were infested and $USD 27.74 for acquiring the amount of glyphosate needed. Biological control cost a total of $USD 6.38 (Chikwenhere and Keswani, 1997).

Not only was biological control the most cost effective, it was also the best at reducing *S. molesta* biomass. In 1992, before releasing *C. salviniae*, 90 percent of the two dams in Tengew River were covered with *S. molesta* which severely impacted the surrounding native communities. Fish populations declined due to *S. molesta* infestations. Manual removal was implemented, but it only solved the issue temporarily because plant growth was faster than the rate of removal. Chemical control was successful at first, but because *S. molesta* regenerates, the cost of herbicidal use was too high.

When biological control was initiated in Zimbabwe, it was very effective. *C. Salviniae* were released in March 1993; 95 percent of plants were damaged in 7 months. A year later in March 1994, large patches of *S. molesta* disappeared and open water became visible. By February 1995, 99 percent of the reservoirs were *S. molesta* free; the cost to benefits ratio was 10.6:1. The rivers were then restocked with fish species that had disappeared (Chikwenhere and Keswani, 1997).

Senegal River

In 2000, the Senegal River between Senegal and Mauritania was heavily invaded by *S. molesta* (Figure 25). It first became invasive in 1994 when it was found outside plant nurseries where it was being grown for ornamental purposes. Authorities implemented biological control via *C. salviniae* after much host-specificity testing. In May 2000, 300 *C. salviniae* were released from Mauritania and 300 from Senegal (Pieterse et al., 2003). Unfortunately, the Senegal population did not survive, but even though there were complications with the Senegal population, the Mauritania population were able to reproduce and establish populations. By April 2002, *S. molesta* was no longer a problem in the Senegal River. (Pieterse et al., 2003). The overall cost for the transport and rearing *of C. salviniae* was USD$1000 (Pieterse et al., 2003).

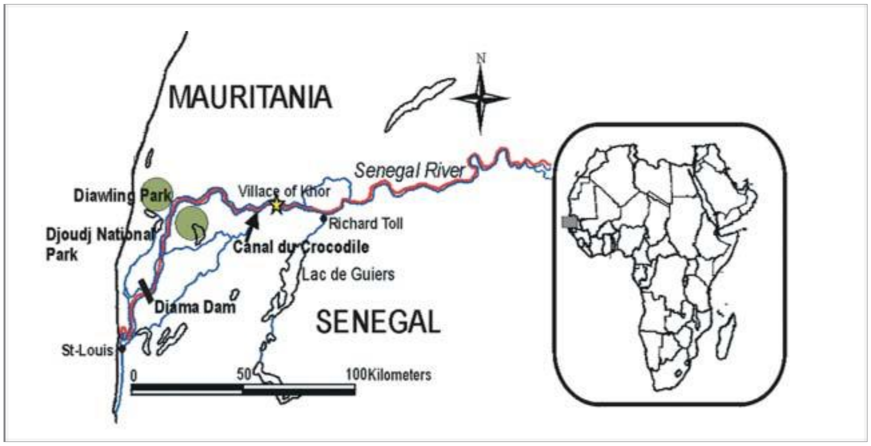


Figure 25. Map of Senegal River between Mauritania and Senegal (Source: Pieterse et al., 2003)

Republic of Congo

The Congo Basin was invaded by *S. molesta* in the 1950s. The infestations across the basin interfered with boat transportation and fishing in small villages. It drastically affected the livelihood of the people living in that area. It also increased vector borne diseases (Mbati and Neuenschwander, 2004). *C. salviniae* was released in Likouala, Cuvette, Kouilou, and Brazzaville in 2000. Residents stated that by 2003 most *S. molesta* infestations were either gone or thinned out (Mbati and Neuenschwander, 2004). Transportation via boats and fishing continued in those regions after *S. molesta* infestations were successfully controlled by *C. salviniae*. Mbondo-Mako had a remarkable recovery from 80% of *S. molesta* coverage to less than 5% between 2000 and 2003 (Table 18).

Table 18. *C. salviniae* release date corresponding to various villages. *S. molesta* was in 2003 in all villages via *C. salviniae* (Source: Mbati and Neuenschwander, 2004).

Village	Release Date	Cleared Date
Cuvette	2001	2003
Bongoye	2001	NA
Motaba	2000	2003
Mbondo-Mako	2000	2003

South Africa

In 1985 the South African Department of Water Affairs funded a biological control program in South Africa. Because *C. salviniae* was so successful in Australia, Papua New Guinea, and Namibia, the program was easily implemented and no host-specificity tests were conducted. Three dams were chosen for the initial three releases. The smallest dam was cleared within 12 months, the medium sized dam was cleared in 13 months, and the largest dam was cleared in 14 months (Cilliers, 1991).

United States

C. salviniae have not been largely successful in temperate ecosystems. A study conducted in Eastern Texas assessed the effects of biological control through *C. Salvinae*. 651,000 larvae, pupae, and adult *C. salviniae* were released into five *S. molesta* invaded sites (Flores and Carlson, 2006). Implementation of biological control was important here because using an herbicide would further reduce oxygen levels in the waterway, leading to dead zones. This is because when herbicides are used, large quantities of plants die all at once, causing a drastic depletion in DO levels when bacteria decompose the organic matter. Although *C. salviniae* also kill plants, they do not kill them at the rate that herbicides do.

The standard average and minimum requirement for aquatic organisms in Texas waterways is about 3.0 to 3.5 mg/L of DO. Unfortunately, due to *S. molesta* infestations, DO in those five sites had dropped to 1.7 mg/L and use of herbicides would further deplete DO levels. After assessing host-specificity requirements*, C. salviniae* was chosen over *S. multiplicalis* and *P. acuminata*. Significant improvements were observed over a course of just nine months. Four out of five sites exhibited dramatic results; *S. molesta* populations dropped to 10 percent. The thick, dense mats turned yellow-brown and sank down to the bottom of the water column. This build-up of organic matter was not as rapid as what would have happened if herbicides were used so DO levels did not decrease significantly (Flores and Carlson, 2006).

One site exhibited delayed results, nonetheless, DO levels increased from 1.7 to 4.3 mg/L. Overall, this study provides evidence that *C. salviniae* can be effective in controlling *S. molesta*, in temperate regions. The study was also able to establish that rearing and releasing *C. salviniae* is not expensive and can be completed at small scale levels (Tipping et al., 2007).

In conclusion, many tropical and subtropical countries have been successful in using *C. salviniae* as a biological control agent; temperate regions, however, have had some difficulties. Australia and the United States have not yet shown biological control success at a large scale. All tropical and subtropical countries that have implemented biological control have shown success (Table 19).

Table 19. Control via *C. salviniae*, country, release date, and status of *S. molesta* infestation.

Country	Release Date	Status
Australia	1980	Control in tropical and subtropical regions, some control in temperate
Botswana	Natural spread from Nambia	Successful control
Congo	2000	Successful control in Cuvette, Motaba, Mbondo-Mako
Cote D'Ivoire	1998	Established, no full control
Fiji	1991	Successful control
Ghana	1996	Successful control
India	1983	Successful control in Banglore and Kerala
Indonesia	1997	Unknown
Kenya	1990	Successful control except where herbicide use
Malaysia	1989	Successful control where released, needs redistribution
Mauritania	2000	Successful control
Nambia	1984	Successful control
Papa New Guniea	1982	Successful control
Philippines	1989	Established in Panay, status unknown
Republic of South Africa	1985	Successful control
Senegal	2000	Successful control
Sri Lanka	1986	Successful control
South Africa	1985	Unknown
USA	2004	Some control in Texas and Louisiana
Zambia	1990	Successful control
Zimbabwe	1992	Successful control

Will Biological Control be Successful in Tungog Lake, Sabah Malaysia?

To determine if *C. salviniae* will be a successful biological control agent in Tungog Lake, Sabah Malaysia, it must first be determined if there will any constraints on *C. salviniae*. *C. salviniae* have three constraints: temperature, nutrients, and *S. molesta* growing stage. *C. salviniae* are not successful when temperatures and nutrients are low, and when *S. molesta* infestations are in the tertiary growing stage. Tungog Lake has ideal water temperatures for both *S. molesta* and *C salviniae* (Table 20). Water temperature of Tungog Lake was between 28 and 30°C on June 22nd-24th, 2016. Nutrients in Tungog Lake were also not limiting. According to Edris Arpah (personal communication), a water quality employee for Kopel-Mescot, the parameters for ammonium, nitrate, and nitrite were normal (Table 12). Nutrients in plant tissue however, were not measured, so to effectively determine if nutrients are limiting in Tungog Lake, plant nitrogen concentration should be measured. *S. molesta* infestation size in Tungog Lake is not ideal for *C. salviniae* survival. Plants in Tungog Lake are in the tertiary growing stage which might inhibit *C. salviniae* development and reproduction. Initial chemical or mechanical removal will be necessary to successfully ensure the survivorship of *C. salviniae*. Because Kopel-Mescot have used mechanical removal in the past, it would be best to use a mechanical-biological combination. Once populations are balanced, then manual removal can maintain *S. molesta* populations.

Table 20. *C. salviniae* constraints in Tungog Lake. Temperature is ideal, nutrients cannot be determined, and infestation size or growing stage is not ideal.

Constraint	**Ideal**
Temperature	Yes
Nutrients	Yes/no
Infestation size	No

Section VII: Conclusion

S. molesta is the second worst aquatic invasive species in the world. Because of its ability to grow rapidly, it can quickly invade new areas, especially tropical and subtropical regions. *S. molesta* is devastating to ecosystems. It impedes ecological processes while altering the daily lives of many who inhabit small villages (Thomas and Room, 1986).

Developing countries such as Zimbabwe, Papua New Guinea, and Sri Lanka have experienced huge economic downfalls due to *S. molesta* invasions. Villages were completely abandoned because their sustenance was disrupted (Thomas and Room, 1986). *S. molesta* takes away habitat from native plants which has a cascading affect through the trophic levels. Humans are the main cause of distribution and spread of *S. molesta*. Plants are still being traded and sold for horticultural purposes with incidents of accidental and intentional release being reported worldwide (McFarland et al., 2004).

Control of *S. molesta* has been very costly for many nations. Decades after initial spread, researchers still have not found the best method of control. Most tropical and subtropical countries have implemented biological control programs successfully. Unfortunately, biological control has not been as successful in temperate regions, including the United States and parts of Australia. Trial studies in both countries have conflicting results (Oosterhout et al., 2006). Some studies have stated that *C. salviniae* will be able to withstand freezing temperatures if monitored carefully (Tipping et al, 2007, Forno et al., 1983). Others have stated that *C. salviniae* must be released at certain times of the year, preferably late spring or early summer, to ensure survivorship (Sullivan et al., 2011). While others have stated that biological control will not be successful in temperate regions because *C. salviniae* cannot tolerate freezing temperatures like *S. molesta* can.

Synthesis reports in Australia and the United States have formulated promising plans. They state that eradication of *S. molesta* in all regions must be completed through a combination of two or more methods of control (Sullivan and Postle, 2012). Some combinations that have worked successfully in the past are: herbicide use and biological control; herbicide use and mechanical removal; and biological control and mechanical removal. Herbicide use and biological control should be implemented in overcrowded, tertiary growth forms. Diquat is used to thin out the infestations, then *C. salviniae* is introduced to reduce populations, and lastly calcium dodecylbenzene sulfonate is used to eradicate remaining populations. Herbicide use and mechanical removal involves diquat to thin out infestations, followed with mechanical removal to eradicate remaining populations. This method has not been as successful because eradication via mechanical removal alone is not as effective as biological and chemical control used concurrently. Mechanical removal and biological control are chosen in two cases. If there is tertiary growth, then mechanical removal can thin out infestations. *C. salviniae* is then introduced to reduce populations. Lastly, mechanical removal eradicates remaining species. If the infestation is in secondary or primary growth, then *C. salviniae* can be introduced first and mechanical removal can be used to eradicate remaining populations (McFarland et al., 2004; Knutson et al., 2011; Sullivan and Postle, 2012).

Biological control is often the method of choice in many developing countries. This is due to mechanical removal and herbicide use being very expensive. Developing countries cannot afford the labor and machinery costs of mechanical removal. They also cannot afford the several applications of herbicides that is required to effectively remove infestations. Most developing countries use only biological control and leave the systems at the "biocontrol balance" stage (Stone, 2011). This is when neither population increases and are at a balance. *S. molesta* infestations are often small and manageable at the balance stage.

Section VII: Recommendations

Spread

S. molesta has spread to almost every continent within that last few decades. Control of spread and distribution has become very critical, especially in developing countries. Even though *S. molesta* is from the tropics, it spreads to both tropical and temperate countries. Plants are adapting new strategies to withstand cold to freezing temperatures and with climate change, it is likely that plants will spread to northern latitudes.

The best method of control is prevention. For prevention to be successful, countries must implement stricter laws and regulations on the cultivation and distribution of *S. molesta* plants. People are able to purchase *S. molesta* from nurseries, the internet, and catalogs. In many countries, the losses due to *S. molesta* infestations outweigh the economic benefits gained from horticultural sales. Cultivation sites must be banned in order to prevent accidental spread. The ecological degradation and human health concerns caused by *S. molesta* are also too detrimental for continued use. Government agencies should focus on eradicating existing infestations and banning use altogether. This will prevent spread by boats, people, and even animals. People should also be able to contact government officials easily to share any concerns linked to *S. molesta* and of any new cases. *S. molesta* should not be allowed to be cultivated in countries where it has an invasive status. New adaptions and mutations might make it even harder to eradicate in the future if prevention methods are not practiced now.

Public education is the best way to implement effective control programs. The public should know of all the harmful effects *S. molesta* poses. Learning the consequences involved with possessing and releasing *S. molesta* will decrease incidents of accidental release. Technology makes it simpler to share knowledge and should be used more frequently. More people around the world have cellphones than access to clean water. Technology, such as applications, can be used to increase knowledge of *S. molesta* effects.

Methods of Control, *Cyrtobagous salviniae*, and Climate Change

To develop a proper control plan, one must first establish the growing stage of the *S. molesta* infestation. If the growing stage is in the primary or secondary stage, then biological control via *C. salviniae* should be implemented in tropical and subtropical regions. There are over 12 countries in the tropics that have shown success in control using *C. salviniae*. If in the tertiary growing stage, biomass should be reduced through either use of herbicides, such as diquat, or mechanical removal. Once infestations are thinned out to secondary or primary growing stage, then *C. salviniae* can be introduced.

In temperate regions, due to the uncertainty and conflicting studies, researchers should do trial studies at all infestation sites to determine if biological control *via C. salviniae* will be successful. Because biological control is the most cost effective, it would be beneficial to run studies to establish when, where, and how to release *C. salviniae*. If possible, biological control should be implemented over other methods of control.

Although studies have stated that *C. salviniae* will survive with climate change in places like South Africa, it is never certain (Allen et al., 2004). Temperatures will eventually increase past the 6°C increase that *C. salviniae* will be able to withstand leading to decreased *C. salviniae* survivorship. If climate change eradicates *C. salviniae* populations worldwide, when countries will experience exponential economic and ecological turmoil. Communities in developing countries will lose drinking water supply, food, and uses of waterways. Developed countries will also be affected. *S. molesta* will invade northern latitudes with no natural herbivore to control it. This increase in range will lead to significant economic loss and ecological degradation via herbicides.

Another way to prevent S. molesta infestations is to by planting native aquatic plants in place of *S. molesta* infestations will reduce chances of re-introduction. In Florida, there are several free-floating aquatic plants that can fill the niche of *S. molesta* infestations including, *Wolffia Columbiana* (Columbian watermeal)*, Lemna valdiviana (*valdivia duckweed), and *Spriodela polyrhiza (*common duckweed). Filling the niche with native plant species will be the best method of prevention and control with climate change. Plans such as these should be put in place now even though climate change is not projected to affect *S. molesta* and *C. salviniae* for another few decades. Uncontrolled *S. molesta* infestations will be devastating worldwide, causing further ecological degradation, economic turmoil, and human health concerns.

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