Amazon Frogbit - *Limnobium laevigatum*

Ex-situ Experiments and Field Investigations

SERCUL

South East Regional Centre for Urban Landcare

Acknowledgments

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For further information contact:

Dr Rose Weerasinghe
Ecologist
SERCUL
1 Horley Road
Beckenham, WA, 6107
Telephone: 9458 5664
Email: roseweerasinghe@sercul.org.au
Website: www.sercul.org.au

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Background

Limnobium laevigatum is a perennial herbaceous aquatic plant belonging to the family Hydrocharitaceae. This family contains the genus *Hydrilla* (water thyme) and *Eichhornia* (water hyacinth) which are well-known as some of the world's worst submersed-type aquatic weeds. *L. laevigatum* originates from the fresh water habitats of tropical and subtropical regions in Mexico, Central and South America and the Caribbean (Akers, P., 2015; CABI, 2020).

This species is described as generally free-floating, though it will also grow rooted in mud in shallow water or on wet shorelines (Howard et al., 2016; WA-US, 2020). Young plants resemble duckweed (*Lemna* spp) before developing into a rosette stage and finally a mature phase with stalked emergent leaves. Mature plants grow up to 50 cm tall and have emergent leaves borne on petioles that are not swollen or inflated like the spongy leaf stalks of water hyacinth. However, adult plants can often be confused with water hyacinth (Aponte and Pacherres, 2013; NSW-DPI). A key identification attribute of *L. laevigatum* plant is the aerenchyma (plant tissue containing air spaces) located on the underside of the leaf.

The species can reproduce sexually through flower pollination and seed production. Flowers are small and pale green to white in colour and approximately 1.3 cm in diameter. Fruits are fleshy capsules containing small bare seeds that are about 1 mm long and hairy (NT Gov, 2018; CABI, 2020). The small, floating seeds easily disperse via water and wind once produced and can germinate underwater (Akers, P., 2015). There is not enough information regarding the survival time of seeds, however, a few reports explained that the seed bank can be retained for three to four years (Akers, P. 2015, WA-US, 2020). *L. laevigatum* can also reproduce vegetatively through fragmentation of stolon segments which connect rosettes. The floating rosettes send stolons out into the water, the ends of which bear ramets (juvenile new plants) (CABI, 2020). Vegetative propagation through large numbers of stolons and new juveniles may be the main source of new plants where flowers are unknown.

L. laevigatum is commonly known as Amazon Frogbit, South American Spongeplant, Smooth Frogbit or West Indian Sponge Plant (WA-US, 2020). In the aquarium industry it is largely referred to as 'Amazon Frogbit' and has been kept and traded for use in aquariums, garden ponds, fish tanks and other water features. In this report, hereafter we will use the name 'Amazon Frogbit'. The plant has become a favourite freshwater aquarium plant, which may explain the recent spread of this South American species to many parts of the world. This species is a highly invasive aquatic plant that impacts on water quality and aquatic biodiversity by forming a large mat across the water surface in freshwater habitats. It can spread rapidly due to its high reproductive ability and high propensity for dispersal (Anderson and Akers, 2011) and has the high potential to result in significant environmental and economic costs (Akers, 2013; CABI, 2020; NSW-DPI). Amazon Frogbit is now regulated as a noxious weed and subject to eradication efforts by many countries. There are invasiveness records of this species outside of its native range in America, Australia, Indonesia, Japan Zambia and Zimbabwe.

Amazon Frogbit was imported to Australia as an ornamental aquarium plant and now poses a serious threat to Australian waterways due to its explosive rate of spread (NSW –DPI). The species is recorded in New South Wales, Queensland, Northern Territory and Western Australia, and in New South Wales Amazon Frogbit is a declared weed and regarded as prohibited matter (NSW –DPI; NT Gov., 2018). A '*Limnobium*' species was found in NSW in 2007 (NIWA, 2008). However, the first known and reported occurrence of Amazon Frogbit in NSW was at Green Point near Forster in 2017. Illegal dumping of aquarium or pond plants in waterways has been the main cause of Amazon Frogbit infestations in NSW. In Queensland, this plant is already naturalised in some areas and the regulations are different according to councils. In the Brisbane City Council, this weed is declared as Class E, indicating for early detection and eradication. Amazon Frogbit has been declared a Class C (not to be introduced) weed under the Weeds Management Act in the Northern Territory since January 2018. It is illegal to grow, trade, import, sell or transport this plant species in the Northern Territory, and any known existing plants must be destroyed.

In Western Australia, Amazon Frogbit was initially discovered in 2013 at the Liege St. Wetlands and the following year in Bannister Creek, both in the City of Canning. In subsequent years there have been outbreaks in Ballanup Drain (City of Armadale), Yangebup Lake (City of Cockburn), Baileys Drain (City of Armadale), Bayswater Brook (City of Bayswater), South Belmont Main Drain (City of Belmont), Little Rush Lake (City of Cockburn), Noble Falls (City of Swan), Rockingham Central Main Drain (City of Rockingham) and Lamberita Creek (City of Canning). Each infestation has been an isolated incident thought to have resulted from residents inappropriately disposing of their aquarium into the stormwater network or directly into the waterway. Amazon Frogbit was listed as a declared pest in Western Australia in October 2018 and was placed on the Western Australian Organism List. Unfortunately, it was placed under the S22 (2) no control or exempt keeping category. Due to its current declaration status there is no legal obligation for land managers to manage it within their assets and nothing to prevent the continued sale of Amazon Frogbit.

This fast growing aquatic weed can quickly invade any waterway, obstructing drainage networks or stream flow, and threatening the stormwater infrastructure. The weed species can form dense mats that choke native aquatic plants and impact the wetland ecosystem and food webs. It can also provide a good habitat for mosquitoes, increase vector mosquito populations and the mosquito borne disease risk. If Amazon Frogbit spreads out of control in Western Australia, not only can it impact both environmental and human health it also can cause economic impacts. More data is needed on effective control methods for this spreading invader, as current management practices are not preventing proliferation. SERCUL has commenced a research project on Amazon Frogbit to minimise the knowledge gap and to improve management of the species. This preliminary report outlines the ex-situ experiments and brief field investigation activities undertaken in the project.

Objective

To investigate Amazon Frogbit's potential spread to, and establishment in, rivers with different salinity levels and to understand other ecological conditions to maximise control methods.

Method

1. Field investigations

   Field visits were conducted on 24th and 27th March 2020 to collect in-situ ecological information from locations where Amazon Frogbit infestations were reported (Map 1). Measurements of pH, temperature and conductivity were taken in the surface layer of the water column, generally between the surface and a depth of 30 cm, using a pre-calibrated YSI ProPlus water quality meter. Other relevant habitat characteristics were recorded during the visits.

2. Ex-situ experimental procedures

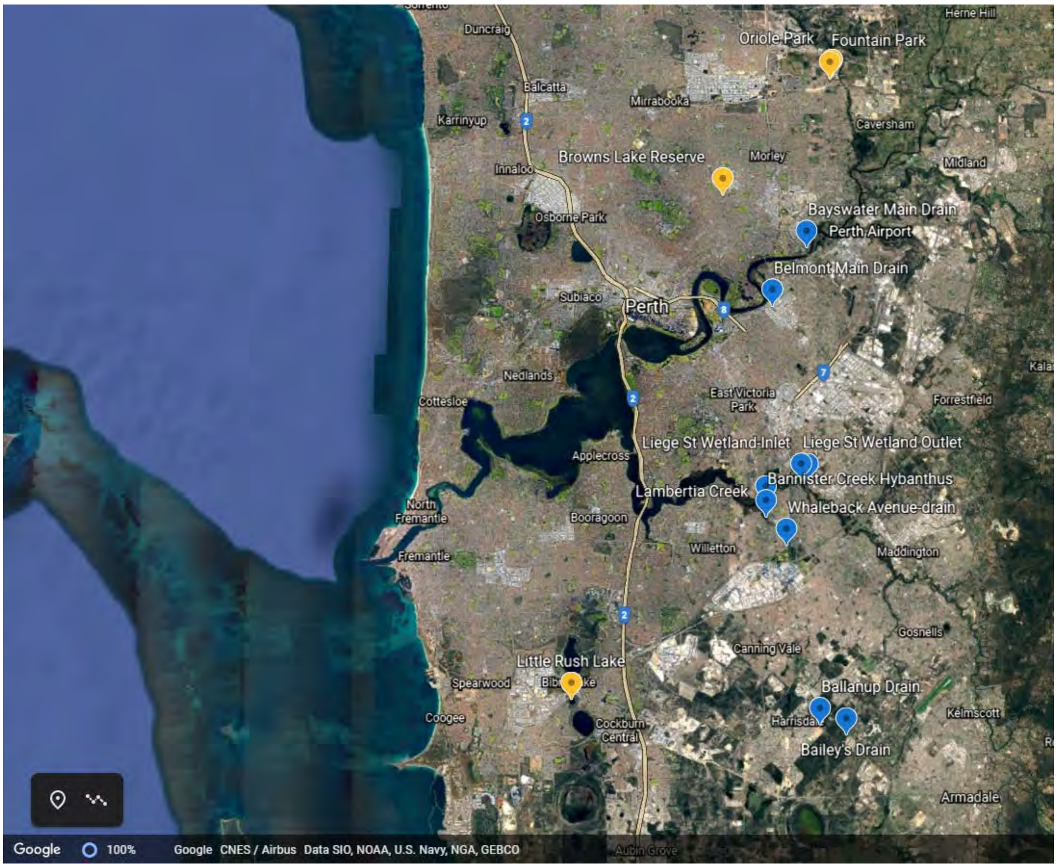
   Different mesocosms were created at the SERCUL premises in Beckenham using readily available materials to perform four different Amazon Frogbit experiments (refer to Pictures). A mesocosm is a created small ecosystem, bridging the gap between ex-situ experimental work and field studies. For the experiment, 500 small seedlings (about 0.4 -0.5 cm width) of Amazon Frogbit were collected from Little Rush Lake (City of Cockburn) foreshore prior to weed control. All plants were allowed four weeks establishment time in a 100L capacity tub filled with the lake water (refer to Pictures).

View of Little Rush Lake foreshore. Photo taken in February 2020.

Amazon Frogbit plants were allowed four weeks establishment time in a 100L capacity tub before being used in the experiment.

3. Data collection
    - Due to the limitations of time, budget and resources, the field investigations were conducted by way of one visit to each location and the measurement of physiochemical parameters: pH, temperature and conductivity. Monitoring locations at each site were determined after investigating the behaviour of the water body.

- For the purposes of this project, the conductivity unit mS/cm (millisiemens/centimetre) was converted to salinity ppt (parts per thousand) to discuss the results.
- Salinity conditions followed the Rivers and Estuaries Science, Biodiversity and Conservation Science, Department of Biodiversity, Conservation and Attractions (DBCA) guidelines: fresh <5ppt, brackish 5-25ppt, saline 25-35ppt, hypersaline >35ppt.
- The experimental data was collected two times per week from 20th March to 6th October 2020.
- Experiments were influenced by predictive rainfall and temperature measurements obtained from the Bureau of Meteorology (BOM).
- Visual observation was carried out to record the plant health. This health code was used: 4 - whole plant green; 3 - <50% yellow and remain green; 2 - >50% yellow and remain green; 1 - brown and yellow, some still remain green; 0 – whole plant brown/dead.



Map 1. Field investigation locations where Amazon Frogbit has been recorded.

Amazon Frogbit growing in Belmont Main Drain. Photo taken before control activities, February 2019.

Amazon Frogbit growing in Lambertia Creek, City of Canning. Photo taken before control activities, August 2019.

2.1. Mesocosm 1

An experiment to assess Amazon Frogbit survival in river water with different salinity levels. Experiment period: 20th March 2020 to 16th June 2020.

Water was obtained from the Canning River at two locations, under the Riverton Bridge and downstream of Kent Street Weir, and from one location along the Swan River, close to the Eric Singleton Bird Sanctuary, and was used as treatments to test the ability of Amazon Frogbit to grow in river water with differing salinity levels. Water from Little Rush Lake was used as a control for the experiment. The mesocosm set up consisted of three replicates of 60L samples of river water for each of the four locations outlined above. This consisted of a total of twelve rectangular plastic tubs, each with a capacity of 100 L, placed outdoors behind the SERCUL shed (refer to Pictures).

Selecting Amazon Frogbit plants for experimental mesocosms.

Collecting water samples for experimental mesocosms

Experimental mesocosms at SERCUL premises, behind SERCUL shed.

At each location, conductivity, temperature and pH were measured onsite prior to water being collected. This water was emptied in to the designated tubs and conductivity, temperature and pH were recorded again. The weather parameters corresponded to the Beckenham area. Twenty Amazon Frogbit plants of a similar size range and possessing two to three leaves were placed in each tub. The width of each plant (rosette) was recorded from two different angles along with the root length before they were placed in to the replicate containers. All replicates were covered using transparent plastic to protect from rain, sunlight and wind and also to protect them from escaping to the environment through removal by birds. Water level was maintained by adding additional water collected from the same location and on the same date to the replicates throughout the experiment period.

The replicates were checked two times per week to record the following physical and ecological parameters until all of the plants placed in the treatment tubs were dead.

1. Physical parameters of water: Conductivity ms/cm , pH and Temperature °C
2. Plant health – determined according to the health code outlined previously under Data Collection.
3. Size of the plant : Rosette size (length x width) cm
4. Root growth: Length cm
5. New growth: shoot/leaf/ramets (juvenile new plants)/roots

Amazon Frogbit plant with two ramets (juvenile plants) attached to stolons.

2.2 Mesocosm 2

An experiment to assess the survival of Amazon Frogbit under complete submersion in natural light and low light conditions.

Experiment period: 3rd April 2020 to 16th September 2020 (at which we were still collecting data).

Water from Yule Brook was emptied in to two 20 L buckets and placed outdoors behind SERCUL's shed. Two large coffee jars containing plants of approximately the same size were filled with water from Yule Brook and then inverted and one placed in each bucket to hold the plants under suspension. One bucket was covered with a lid and the other kept open throughout the experiment period. The percentage of plant deterioration in each bucket was recorded two times per week.

2.3. Mesocosm 3

a. An experiment conducted on a warm day to assess Amazon Frogbit survival when plants remain out of water under direct sunlight for a period of time before being placed back in water.

Experiment period: 3rd April 2020 to 24th April 2020.

b. An experiment conducted on a cloudy day to assess Amazon Frogbit survival when plants remain out of water under shade conditions for a period of time before being placed back in water.

Experiment period: 28th April 2020 to 2nd June 2020.

The same method was used for both experiments. A 32 cell hard-plastic seedling tray was placed in a rectangular plastic 20 L container filled with water from Yule Brook. 25 plants of approximately the same size were selected for each experiment and for experiment a) 24 plants were left out of water under direct sunlight and for experiment b) 24 plants were left out of water under shady conditions. For each experiment an individual plant was returned to the water every 15 minute interval from 9 am to 3 pm and the health code was recorded. The first plant was directly transferred to the water as a control without any drying. The health code of each plant was recorded two times per week up to a month.

2.4 Mesocosm 4

Experiments to assess shading (solarisation) as a control method for Amazon Frogbit

a. Grown in water (to replicate the open water of a wetland)

b. Grown in mud (to replicate the foreshore).

Experiment period: 7th April 2020 to 5th June 2020.

Two plastic trays measuring 45 x 31.5 x 0.58 cm, and mud and water from Little Rush Lake were used to create an open water wetland and foreshore conditions for the experiment. One tray was filled with a mud layer and then water and the other tray with wet mud only. 115 plants of approximately the same size (with 2 to 3 leaves) were planted in each tray and covered with black plastic sheets. Both trays were monitored two times per week. The percentage of plants remaining and general observations were recorded until all plants were dead.

Results and Discussion

1. **Weather during project period**

   Perth Airport Station data from the Bureau of Meteorology (BoM) was utilised for rainfall and temperature referencing for the project. Figures 1 and 2 illustrate monthly average rainfall and temperature data during the experiment period from March to September 2020.

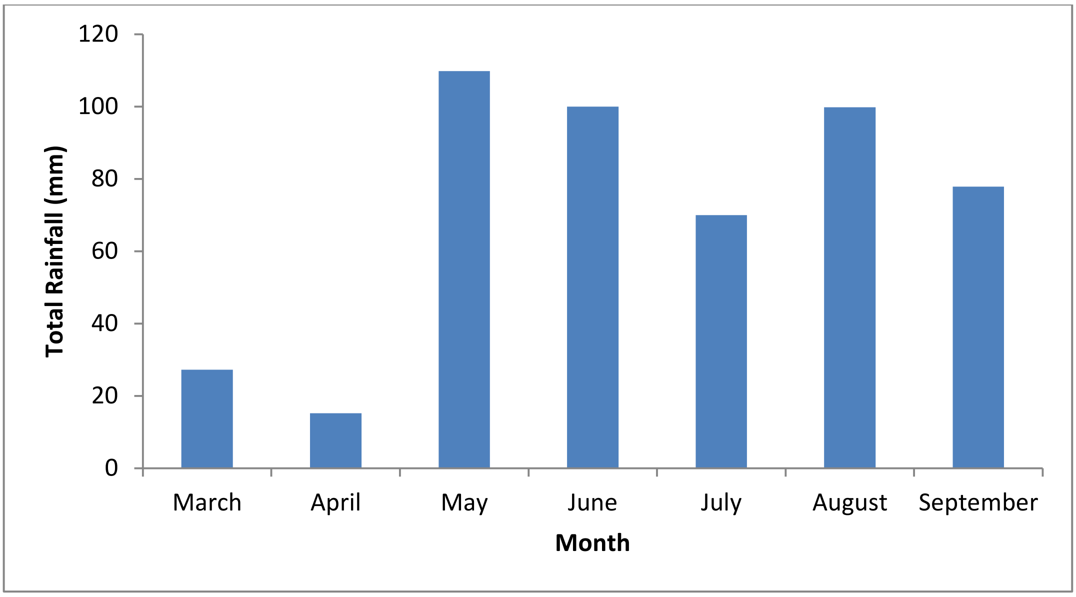


Figure 1. Monthly rainfall data during experiment period. Source: Bureau of Meteorology rainfall data (BoM 2020)

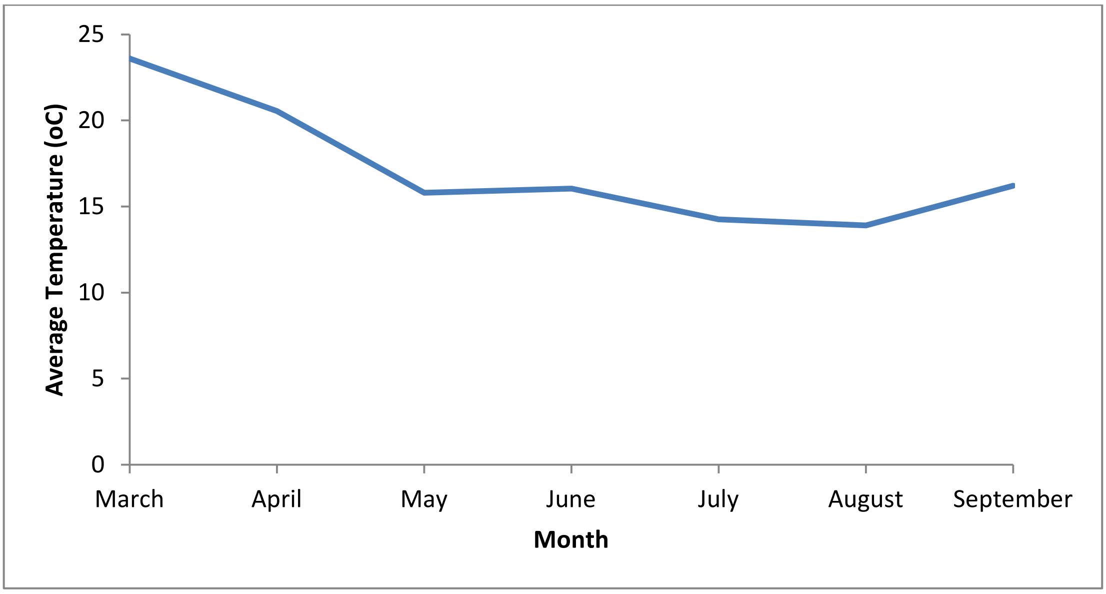


Figure 2. Monthly average temperature data during experiment period. Source: Bureau of Meteorology temperature data (BoM 2020)

The highest temperature of 23.75°C was recorded when the experiment was started in March. The average temperature then decreased during the experiment, dropping to 13.55 °C in August. Total rainfall data ranged from a minimum value of 15.2 mm in April to its highest value of 109.8 mm in May. Due to limited facilities and resources, we could not control environmental conditions such as sunlight, temperature and evaporation levels within the experimental mesocosms. These can fluctuate with daily weather changes. It should be noted that these changes could influence the mesocosm data that was collected. However, Amazon Frogbit can be exposed to similar environmental stresses in real field in-situ situations and thus these environmental changes were treated as natural.

1. **Field investigations**

Location	Date-Visited	Parameters		
		pH	Salinity ppt	Temperature °C
Liege St Wetland-Inlet, CoC	27/03/2020	7.28	0.515	24.2
Liege St Wetland-Outlet, CoC	27/03/2020	7.04	0.529	23.1
Lambertia Creek, CoC	27/03/2020	7.34	0.925	25.3
Bannister Creek/Hybanthus, CoC	27/03/2020	7.38	0.731	24.5
Whaleback Avenue –drain, CoC	27/03/2020	7.16	0.753	24.9
Little Rush Lake, CoCB	27/03/2020	8.76	1.227	27.0
Ballanup Drain,Co Armadale	27/03/2020	6.83	0.409	23.1
Bailey's Drain, Co Gosnells	27/03/2020	7.40	0.884	26.6
Belmont Main Drain, Co Belmont	24/03/2020	7.22	0.355	23.9
Fountain/Oriole Park, Co Swan	24/03/2020	8.15	0.252	25.5
Browns Lake, Co Bayswater	24/03/2020	7.24	0.402	24.5
Bayswater MainDrain, Co Bayswater	24/03/2020	7.33	0.389	24.5

Table 1. Summary of water physiochemical information of locations where Amazon Frogbit has been recorded (CoC = City of Canning; CoCB = City of Cockburn).

In total, 12 locations where Amazon Frogbit infestations have been reported were visited. Water physiochemical parameters of the site were sampled on one occasion (Table 1). The pH was close to neutral in all habitats. The lowest pH of 6.83 was recorded at Ballanup Drain, Armadale, and the highest pH value of 8.76 was reported at Little Rush Lake. At all locations, the salinity readings were less than 5ppt and were identified as fresh water habitats. Little Rush Lake provided open habitats exposed to full sunlight and therefore had the highest temperature value. These values can be changed by weather, stormwater influence, or from any other physical disturbances. A few studies reported that Amazon Frogbit can grow in full sun or shade and prefers water temperatures between 15 - 28˚ C, pH of 6 to 8, and prefers salinity up to 10 ppt (Perryman 2013; CABI 2020). The recorded parameters are close or similar to the available information.

A primary aim of this field investigation was to understand the general characteristics of the habitats in which Amazon Frogbit has been recorded in the Perth region. We identified that it has grown in a wide range of habitats including shallow drains, ditches, wetlands and creeks. Open to shady, weed infested storm water infrastructures were the main type of recorded habitats. Most sites except Little Rush Lake and Browns Lake in Bayswater had shallow, static to intermediate flow characteristics. However, flow patterns could change according to the rainfall and stormwater influence. We identified deep to shallow water and boggy foreshore to slightly wet habitats within Little Rush Lake.

It should be noted that these field assessments should be considered a snapshot only, as to obtain a full understanding of the ecology of Amazon Frogbit locations, sampling should occur during different seasons.

2. Mesocosm Experiments

2.1. An experiment to assess Amazon Frogbit survival in river water

Locations of water collected	Date	Parameters			
		pH	Temprature °C	Salinity ppt	Water condition
Little Rush Lake (Control)	31/03/2020	8.84	27.0	1.227	Fresh
Canning River-under Riverton Bridge (Treatment)	31/03/2020	7.89	22.8	28.438	Saline
Canning River -Kent St Weir down Stream (Treatment)	31/03/2020	7.26	25.6	22.784	Brackish
Swan River-close to Bayswater main drain (Treatment)	31/03/2020	7.56	23.1	29.4	Saline

Table 2. In-situ water physiochemical information of selected-locations for mesocosms.

Table 2 illustrates the in-situ pH, salinity and temperature parameters of selected locations for mesocosms: control and three different treatments. The highest salinity was recorded at the Swan River, Bayswater location, and the second highest was at the Canning River, under the Riverton Bridge. The Canning River's Kent Street Weir location had brackish conditions at the time water was collected for the mesocosms. These parameters were used as the baseline for the experiment. The Little Rush Lake water, which was a control for the experiment, was recorded as having a salinity reading of less than 5ppt, which made it a suitable fresh water control for our purposes. It should be noted that we didn't add any nutrients or control any other parameters during the experiment.
As shown in Figure 3, the change in average temperature of treatment and control mesocosms over time showed a decreasing trend. This pattern reflected weather changes during the experiment period (Figures 1 & 2). All mesocosms had favourable temperatures for the growth of Amazon Frogbit.

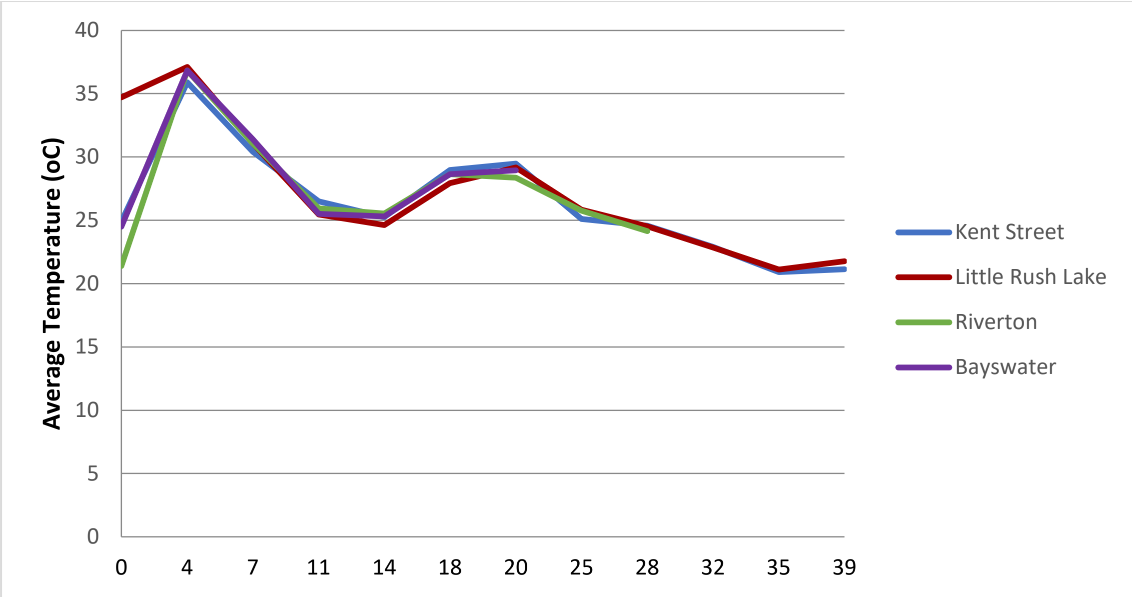


Figure 3. Change in average temperature of treatment and control mesocosms over time (days).

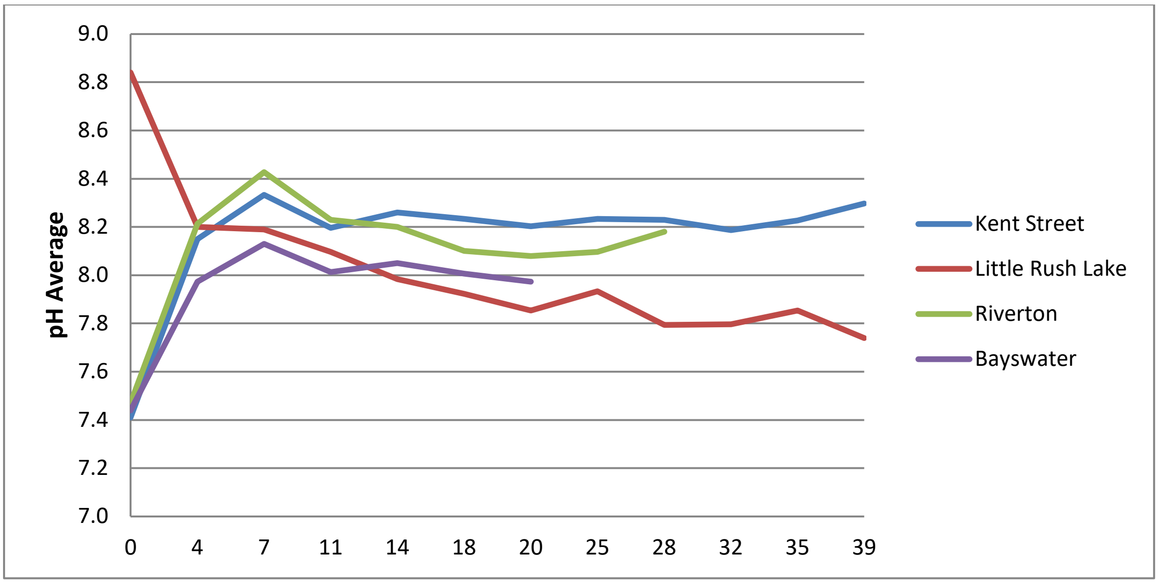


Figure 4. Average pH for each treatment and control mesocosm over time.

Kent Street had the highest average pH of 8.16, and Bayswater had the lowest at 7.94. The average pH for Bayswater on the final day of the experiment increased by 0.53 in comparison to day 0, Kent Street's average pH increased by 0.89 compared to the initial pH, and Riverton's pH increased by 0.71. The final pH for Little Rush Lake was lower than what it was initially, with a decrease of 1.10. The pH average for each location never dropped below 7, and each location apart from Little Rush Lake experienced an increase in average pH over the first seven days (Figure 4).

It is important to note that pH only becomes a dominant factor at very high or low pH, at which point the productivity of most plants and animals is limited. Wetland scientists have shown that small changes in acidity/alkalinity rarely have large effects on aquatic systems (DBCA). Therefore, we consider that the above slight changes did not affect the plant health code of the experiment.

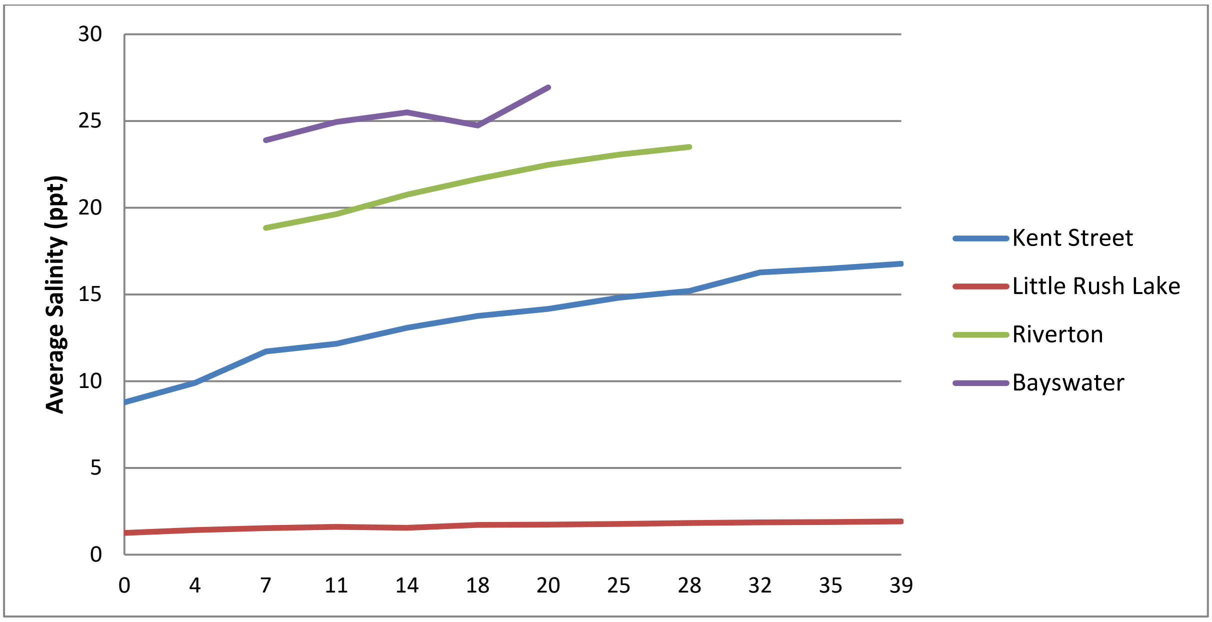


Figure 5. Average Salinity (ppt) for each treatment and control mesocosms over time.

The average salinity for each mesocosm differed, where Bayswater was the most saline with an average salinity range of 22.72 -26.93 ppt, and Little Rush Lake the least saline with an average salinity of 1.67 ppt. Each mesocosm had an increase in average salinity on the final day in comparison to initially (Figure 5). Bayswater had the highest increase in average salinity between the first and final day, with an increase of 16.57 ppt, and Little Rush Lake had the smallest increase of just 0.67 ppt (Figure 5). Riverton's average salinity (range 18.87 – 24.9 ppt) showed brackish to saline conditions and Bayswater was saline which did not increase to hypersaline. Kent Street was brackish and the control mesocosm of Little Rush Lake was fresh throughout the experiment (Figure 5).

In natural ecosystems (in-situ), salinity data is often presented together with water depths as it is directly affected by evapo-concentration. Even though the water level was maintained by adding the location water to each of the replicates, increased salinity can be due to evaporative loss. It is clear that consequently, salt accumulated in mesocosms throughout the experimental period. However, all the mesocosms showed similar in-situ salinity changes to what river systems could experience with daily tidal movements and weather variables.

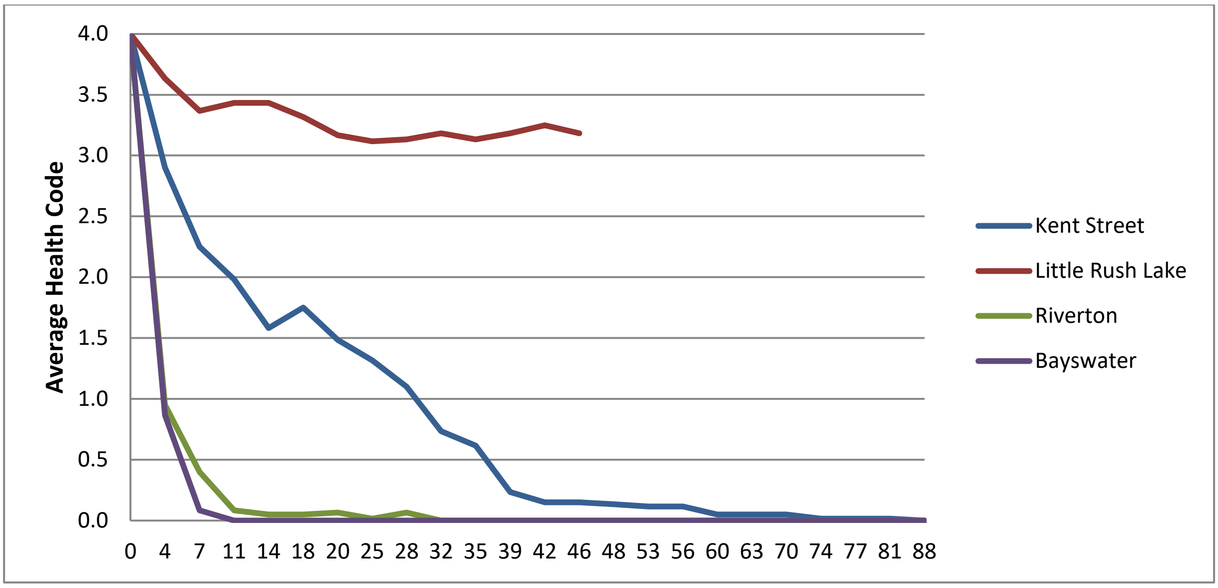


Figure 6. Change in average health code of Amazon Frogbit over time. A value of 0 indicates health code 'dead', and a value of 4 indicates health code 'healthiest'.

Salinity is the main focus of the experiment to understand Amazon Frogbit's potential spread to, and survival, in river systems. A health code was used as a measure to determine the relationship of this weed with different salinity conditions in river systems. Bayswater was the first location to demonstrate an average health code of 0 (dead) at 11 days, followed by Riverton at 32 days and Kent Street at 84 days. The average health code of Little Rush Lake was not recorded after day 46 as it experienced little change. This saved time that was necessary to spend monitoring each plant. Each mesocosm had a lower average health code by the end of the experiment, and all locations apart from Bayswater experienced an increase in average health code at some point (Figure 6).

The original plants had on average 2.0 x 2.5 cm size rosettes and 3 to 13 cm length roots at the beginning of the experiments. We observed that sizes of plant rosettes and roots increased from the first week in control mesocosms: the recorded average rosettes size was 4.0 x 4.8 cm and 15 to 38 cm length roots by day 46. In contrast to the control, all treatment mesocosms' rosettes and root sizes decreased and showed drastic differences in health code, believed to be a response to salinity levels. After four days, Kent Street's original roots had fallen off and new roots started. At this stage all plants survived but health decreased. Figure 7 shows the percentage of original plant survival in all treatment mesocosms and the control.

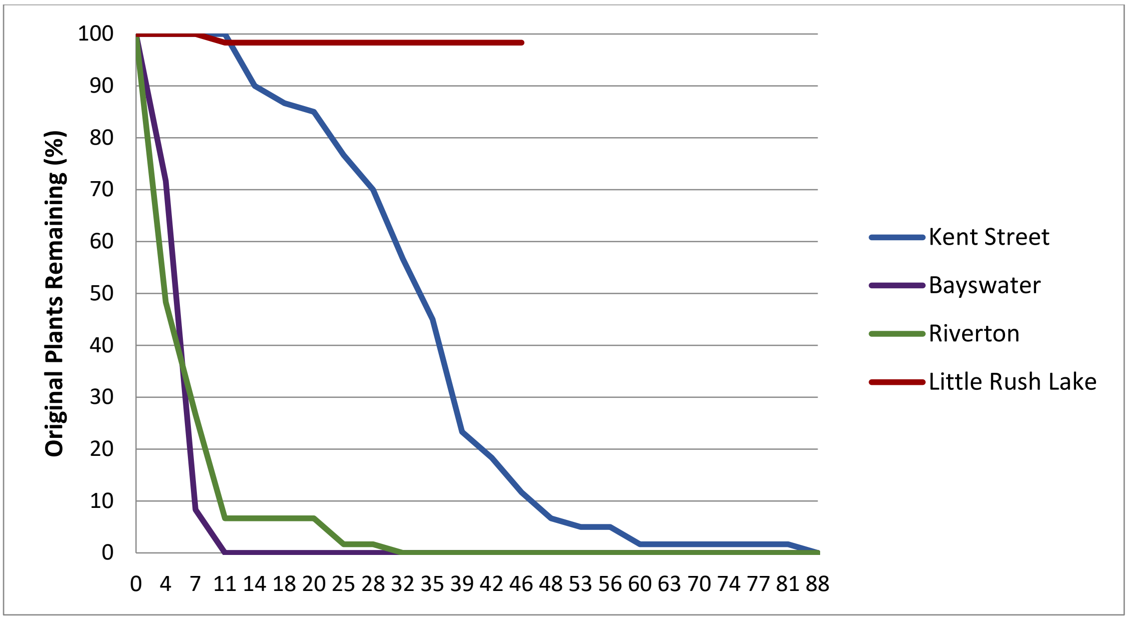


Figure 7. Percentage original plant remaining in each treatment and control mesocosm.

Plant mortality was recorded in Bayswater (30%) and Riverton (38%) by day four (Figure 7). A few plants were lost due to being eaten by aquatic snails in the control mesocosms, and was the reason for the observed decrease of original numbers on the 7th day (Figure 7). In the Kent Street and Riverton mesocosms, the leaves and roots started to fall off from day four but new leaves and roots were grown on Kent Street plants till 60 days had passed, and on Riverton plants until 28 days (Appendices 1-4). This repeated pattern maintained longer survival of plants in Kent Street and Riverton but with a poor health code (Figures 6 and 7).

Both the Little Rush Lake and Kent Street mesocosms had new plants (ramets/juvenile new plants) grow at some point. Kent Street had three new plants on day four, and Little Rush Lake first had new plants on day seven, and had a total of 29 new plants across each replicate by day 46 (Appendices 1-4). Some researchers have identified salinity stress in aquatic plants is caused by short-term exposure to high sodium concentrations, which affects plants through imbalanced osmotic pressure and causes wilting (Hussain et al., 2019; Cheng et al., 2020). The observed trend of decreased plant health due to salinity stress clearly reflected physiological responses according to the different salinity levels in the treatment river water. Whilst in our study, we did not intend to study plant response to short-term salinity stress through the experimental period, similar in-situ salinity was long enough to assess Amazon Frogbit’s survival, re-growth and vegetative propagation. In stressful situations where survival probability is low, normally plants tend to assign more resources to reproductive output. However, if the stress persists, affected plants will eventually die (Zhu et al. 2014; Cheng et al., 2020). It is clear that under saline stress, Kent Street, Riverton and Bayswater plant numbers, sizes and health code changed and decreased clearly compared to the control, Little Rush Lake (Figure 6 & 7).

The relationship between the Amazon Frogbit plant and salinity has not been previously documented. A report from the US mentioned the plants can handle salinity up to 10 ppt (Perryman 2013). This experiment identified that Amazon Frogbit can survive in river water over 35 days in the 12.16 to 19.60 ppt salinity range with brackish conditions, and a short period about 14 days at salinity range 22.72 -26.93 ppt (Figure 6 & 7). These findings suggest that the maximum salinity tolerated by Amazon Frogbit is close to 19.0 ppt. Flowers were not found in any mesocosms during the experiment period. However, we found that even with saline stress, Amazon Frogbit could reproduce asexually in salinity higher than 10 ppt which differed to the US report mentioned.

View of treatments and control mesocosms on 14th day of the experiment.

Healthy Frogbit plant (Health code = 4) from Little Rush Lake, control mesocosm.

2.2. Mesocosm 2

An experiment to assess the survival of Amazon Frogbit under complete submersion in natural light and low light conditions.

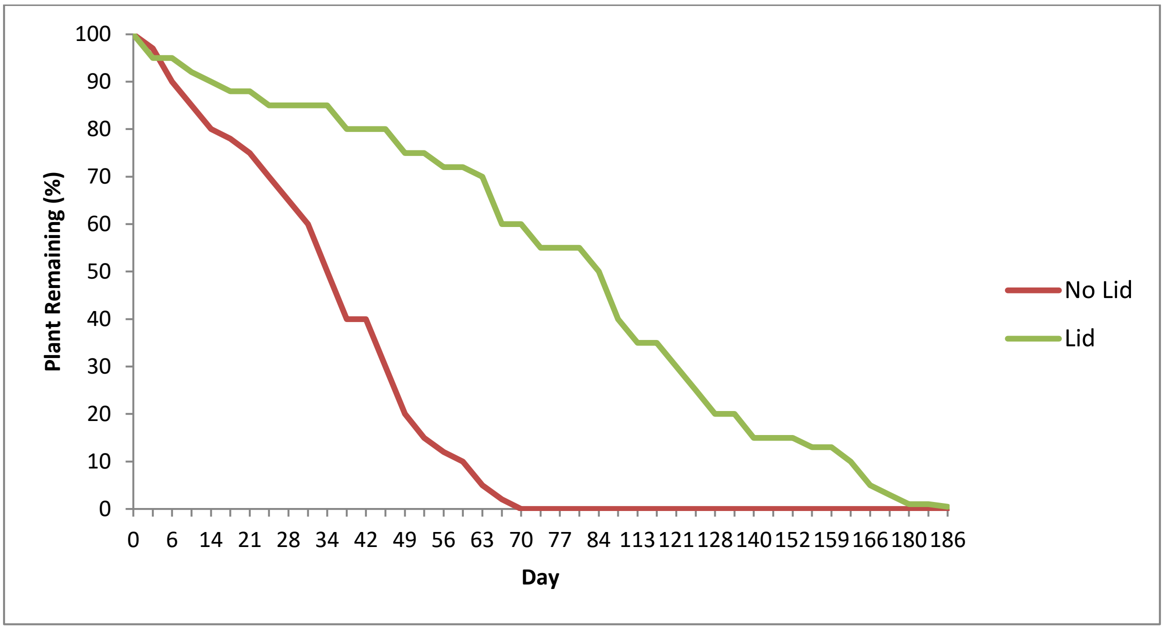


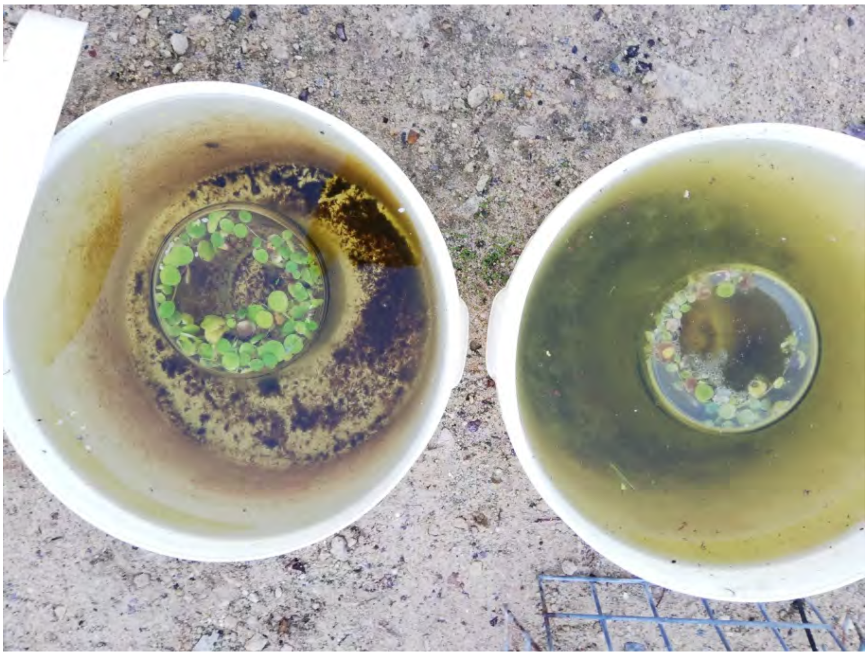
Figure 8. Decline in submersed Amazon Frogbit over time in natural light (no lid) and low light (lid) conditions.

An open mesocosm was created to mimic Amazon Frogbit's submersed growing possibility close to the surface of a water column that still receives natural sunlight (Picture). Another mesocosm with a white plastic lid mimicking low sunlight aquatic habitats where Amazon Frogbit plants are submersed as they are growing under other plant communities in shady conditions was also constructed. Physiochemical information was not collected from these mesocosms except pre and post conductivity to make sure both mesocosms maintained the fresh water condition. Visual decline of percentage plant cover was recorded two times per week throughout the experiment period (Figure 8).

Submerge test mesocosms: No lid (open) and lid (covered).

The decline in Amazon Frogbit after four days was similar for both the lidded and non-lidded mesocosms, with a reduction of 5% and 3% respectively (Figure 8). The non-lidded mesocosm experienced more rapid plant deterioration than the lidded one, and was completely eliminated by day 70, whereas the lidded mesocosm had 60% of the plant remaining. The lidded test had at least 90% of plants remaining until 14 days had passed, had 20% plant remaining after 133 days and had 5% plants remaining after 166 days. 1% of the plants still survived in the lidded mesocosm up to six months when we stopped the experiment (186 days; Figure 8). Neither mesocosm experienced an increase in plants remaining, but both experienced no change in the percentage of plants remaining at some point, with this happening once in the non-lidded test and multiple times in the lidded test (Figure 8).

After 18 days, the non-lidded test contained algae which increased in size until the Amazon Frogbit was completely eliminated (Picture). The lidded test first experienced algae on day 42 and had low coverage of algae growth by day 186. Each test demonstrated removal of old leaves and the growth of new leaves on the plants, starting on day 11 and multiple days afterwards, but new leaves and roots grew more frequently and to a greater extent in the lidded test. On all occasions, leaves grew smaller than the original fallen leaves. Both mesocosms had a number of new plant (ramets/juvenile new plants) growth at some point but neither test demonstrated an increase in the percentage of plant remaining.



Comparison of algae levels in lid and no lid mesocosms on May 19.

Many environmental factors could affect Amazon Frogbit's submersed growth in our small scale mesocosms; such as oxygen, carbon dioxide, light, temperature and nutrition. Due to limited facilities and capacity, we could not maintain, control or measure any of these factors. However, we assume that both mesocosms experienced the same factors, except the natural or low sunlight condition that was the main limiting factor for submersed plant growth.

Direct sunlight, providing suitable temperature, quantity, and quality of light, could be the reason for early algal growth in the non-lidded mesocosm. The algal growth could have been a reason for the rapid decline and early mortality of plants in the non-lidded mesocosm, by reducing carbon dioxide, sunlight, and other resources for the Amazon Frogbit. In contrast, the other mesocosm (low light) received minimal sunlight through the white plastic lid, which was enough for photosynthesis but not enough for rapid algal growth in a similar way to the non-lidded mesocosm. These conditions helped the plants survive a very long period in the low light mesocosm. This is similar in real in-situ aquatic ecosystems; water clarity and the maximum depth of light penetration typically drive submersed plant distribution and abundance. The results showed that not only does Amazon Frogbit have the ability to survive and grow in a completely submersed condition with minimum light and other resources, it also can proliferate by vegetative propagation.

2.3. Mesocosms 3a and 3b

a. An experiment on a warm day to assess Amazon Frogbit survival when plants remain out of water under direct sunlight for a period of time before being placed back in water.

b. An experiment on a cloudy day to assess Amazon Frogbit survival when plants remain out of water under shaded conditions for a period of time before being placed back in water.

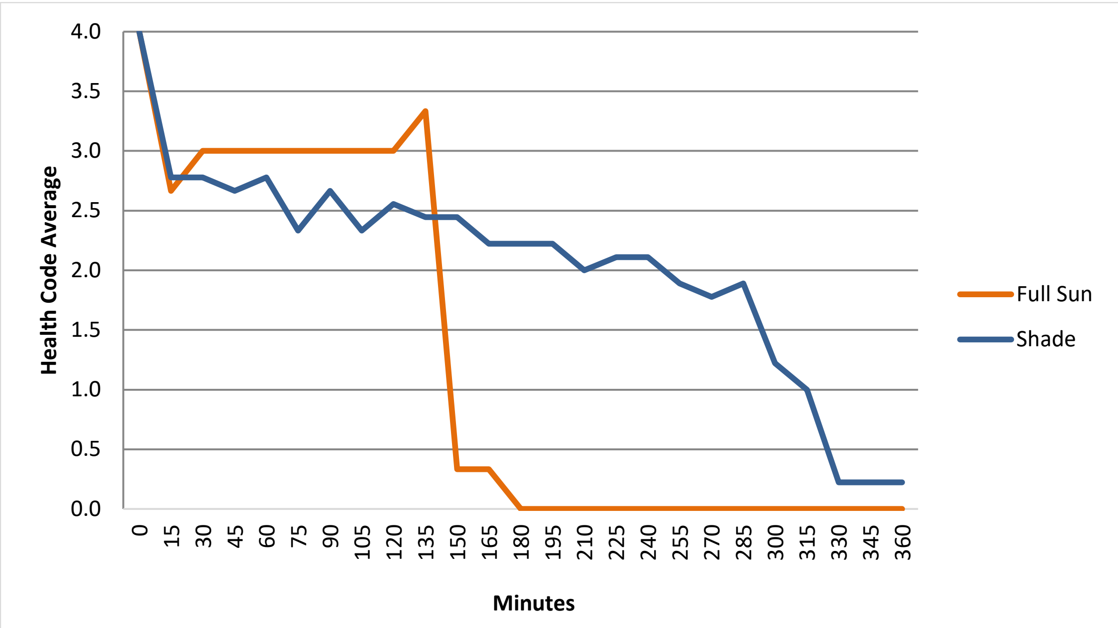


Figure 9. Change in average health code of Amazon Frogbit over time out of water (minutes). A value of 0 health code indicates dead, and a value of 4 indicates plants in the best condition.

These full sun and shaded mesocosms were created to test Amazon Frogbit re-growth possibility if plants escaped back to the waterways after physical or mechanical control on sunny and cooler days.

Full sun mesocosm 3a and shade mesocosm 3b showing differences of Amazon Frogbit wilting after being out of water.

The full sun test was conducted in the temperature range from 28 °C to 38 °C and shade experiment continued at 16.5 °C to 21.6 °C temperature range. Both tests experienced an initial drop in health code after the Amazon Frogbit had been out of the water for 15 minutes, and the full sun test then had an increase in average health code. Each test experienced multiple increases in average health code at different lengths of time out of water, and neither test had an average health code that was as high as the initial average (Figure 9). The full sun test displayed a dramatic drop in average health code after 150 minutes out of water, and was completely eliminated with an average health code of 0 after 180 minutes out of water, whereas the shade test's average health code was 2.2 after 180 minutes. The shade test displayed a more gradual decline in average health code until 285 minutes out of water, after which the average health code fell by 1.7 in 45 minutes, and had an average health code of 0.2 after 360 minutes out of water.

Due to the limited facilities, there were no replicates for this experiment. Even though similar sized plants were selected for both the experiments, the observed multiple increase of health code can be due to individual plants adaptive capacity under the stress condition. No other research data could be found on the effect of drying or leaving the plant out of water, and then reintroducing it to water. This test demonstrated Amazon Frogbit has ability to survive on cooler days (< 20 °C), including plants left out of water to dry for up to six hours. The plants need to be dried for a minimum of three hours on warmer days (>28°C) to kill them completely. Further investigation with more replicates and a more controlled setup is necessary to obtain a clear conclusion.

However, the four week investigation of this experiment noticed that the 48% of plants that survived in the full sun test mesocosm rapidly improved their health code by producing new leaves, roots and ramets. These plants were left out of water for less than three hours before they were reintroduced to water. In the shade test mesocosm, 96% of plants survived and improved their health code rapidly when they were in water over a few days.

This experiment identified the re-establishment potential of Amazon Frogbit removed using mechanical and hand removal methods if not disposed of properly. Therefore, it is important to follow strict disposal guidelines and thoroughly wash down equipment to prevent spread to same or other sites after control activities.

2.3. Mesocosm 4

Experiments to assess shading (solarisation) control method on Amazon Frogbit .

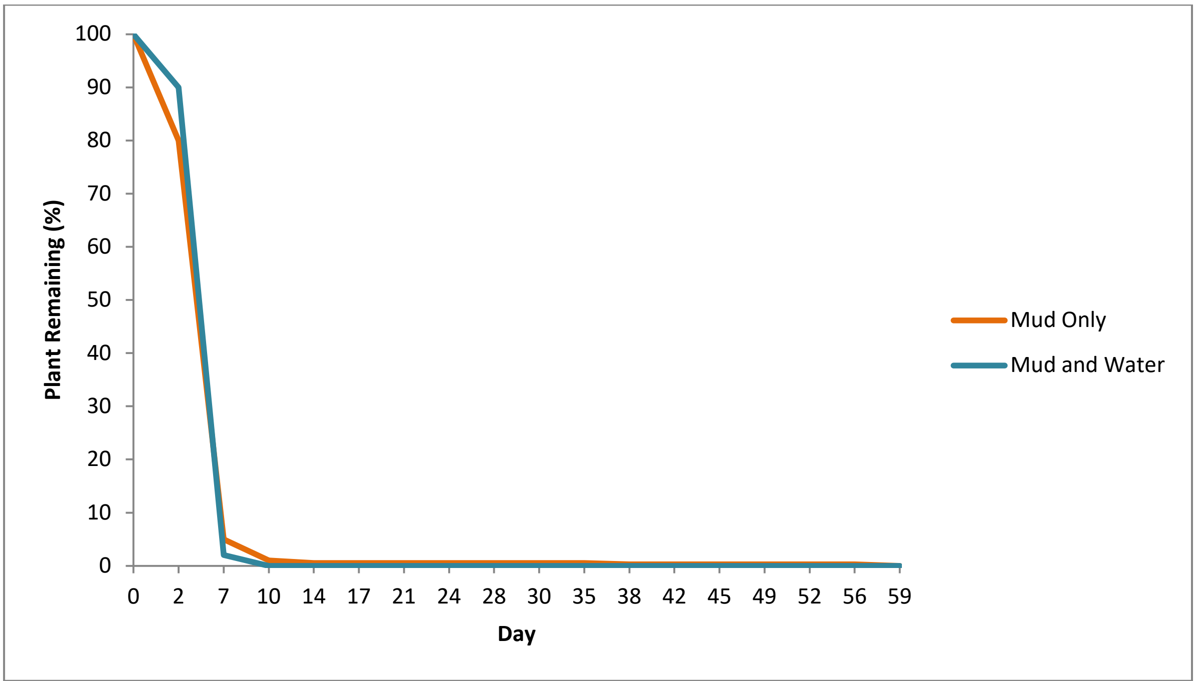
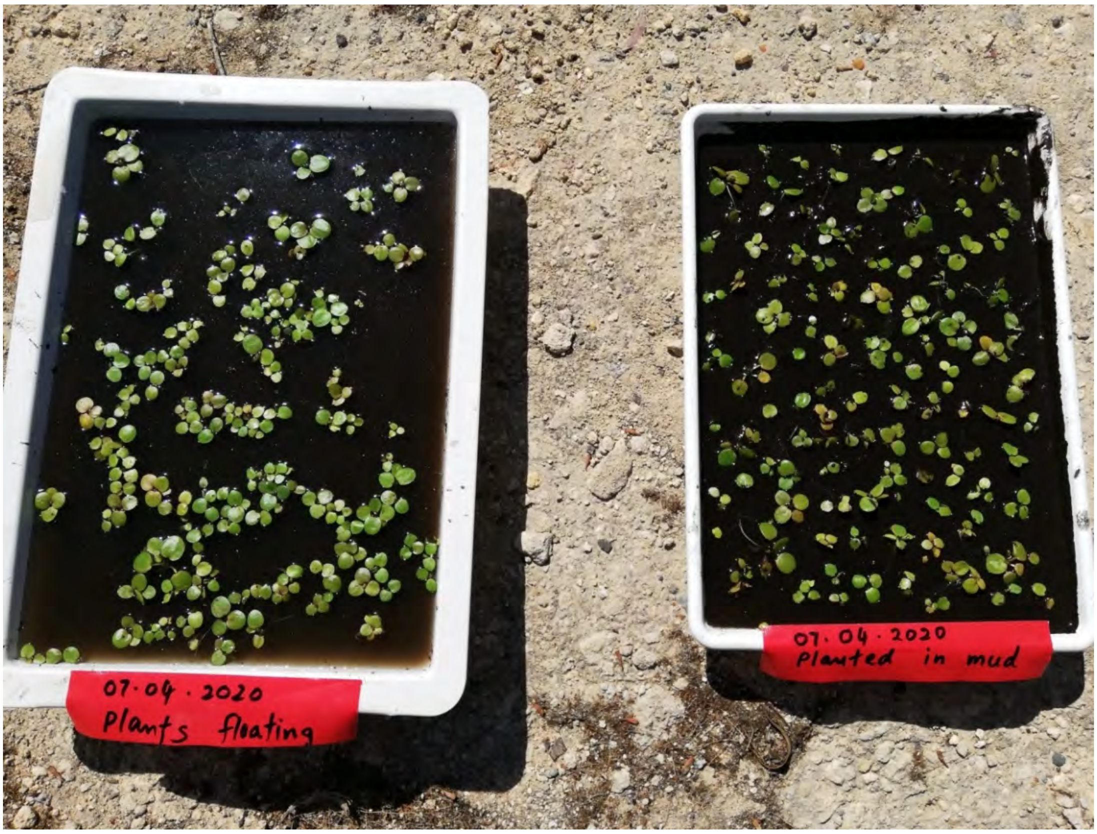


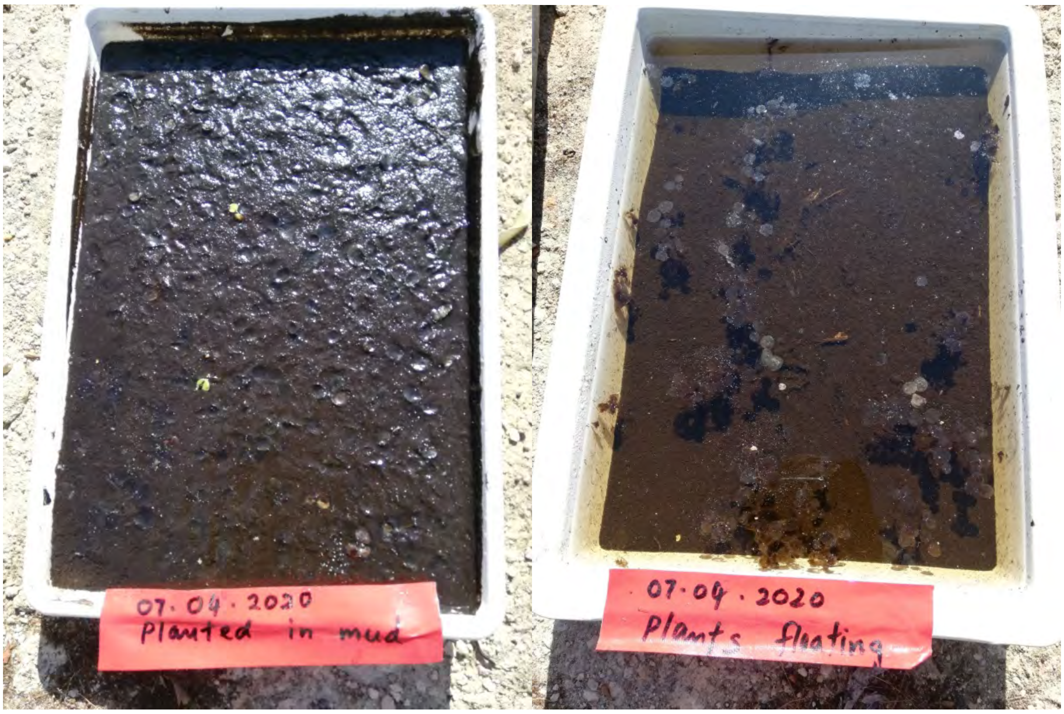
Figure 10. Decline in Amazon Frogbit over time in shaded mesocosms.

Shading as a control method for Amazon Frogbit was tested in both foreshore (mud only) and wetland (mud and water) mesocosm experiments (Picture). After two days, the mud and water test experienced a decline in Amazon Frogbit remaining of 10%, and the mud test experienced a decline of 20%. Both tests experienced a rapid decline in the amount of Amazon Frogbit remaining after seven days, with the mud test possessing a total of 5% of plants remaining, and the mud and water test containing just 2% of the original plants (Figure 10). The mud and water test had no remaining plants after 10 days, and the mud test had no remaining plants after day 59, with the amount of remaining plants being less than 1% from day 14 onwards. Neither test demonstrated an increase in the percentage of plant remaining (Figure 10).



Experiment set up before cover mesocosms from black plastic, to assess shading (solarisation) control method on Amazon Frogbit .

Shading has not been regularly used for controlling aquatic plants to date, with few invasive aquatic plant management cases utilising this method (Schooler S S , 2008; Zhu et al., 2014). Zhu et al (2014) investigated shading as a control method for European Frogbit (*Hydrocharis morsus-ranae*) and concluded that a moderately high density shade can effectively remove European Frogbit, likely with minor impact on the environment. The data from both mesocosm experiments showed shading reduced 95% and 98% of Amazon Frogbit within a week. Therefore, shading can be used as an effective tool for removing invasive Amazon Frogbit with less man power. However, entirely covering a wetland or lake may also have significant negative environmental effects. This management technique can be used to eradicate Amazon Frogbit from storm water ponds, drains and wetlands foreshore areas. However, detailed investigation is necessary to understand possible applicability of shading as a suitable control method for Amazon Frogbit .



Mud only and mud and water mesocosms observation after 7th day of the experiment set up.

Summary

L. laevigatum was described as a floating or emergent macrophyte in most of the research. This study found that this plant can float on the water surface and also grows submerged or partly submerged or emergent.

Although Amazon Frogbit originates from fresh water habitats, this study showed it can survive and vegetatively propagate in saline waters with less than 19ppt. This shows it has the potential to rapidly spread and seriously degrade our ecosystems if it is established in the Canning River or other rivers where water has fresh to brackish conditions.

It should be noted that in this experiment, due to limited facilities and capacity, measurement or control of environmental conditions such as sunlight, temperature, dissolved carbon dioxide, oxygen, or nutrients was not possible. In order to evaluate the salt tolerance of Amazon Frogbit comprehensively and intuitively, other variables should be controlled. Therefore, data from this study was not statistically tested. In this study, visual ratings allowed for the evaluation of the survival of Amazon Frogbit in experimental mesocosms, which was the main focus. Despite these limitations and challenges, evidence for Amazon Frogbit's possible survival in river systems within the Perth region was found. The three treatment locations, Swan River, Bayswater and the Canning River (both under Riverton Bridge and downstream of the Kent Street Weir) had different salinity ranges and showed a predictable pattern of invasion and survival length of the species. The short survival period of Amazon Frogbit in river systems is enough for them to easily re-disperse to fresh water habitats by wind, currents, tidal action, birds or recreational activities. As the stormwater network systems connect with rivers, this can be rapidly spread to many areas through drainage infrastructure and could grow with other weeds without being noticed. However, if it is spread, controlling the infestations in the river systems will be a challenge due to tidal flows, net river flows, and the likelihood of widely dispersed populations, some of which will be hidden under other plants.

This experiment identified that Amazon Frogbit has the ability to grow and propagate (asexually) in a completely submersed condition with lower levels of light and other resources. These findings are alarming as Amazon Frogbit can grow submerged whilst hidden by other aquatic native or weed vegetation in waterways, especially in stormwater drains or wetlands. The combination of shallow enough water that sunlight can penetrate, and nutrients, makes the ideal habitat for submerged growth possibility of this weed in the Perth region waterways. Therefore, control methods need to consider possible growth of Amazon Frogbit in submerged conditions.

Further, this experiment identified the re-establishment potential of Amazon Frogbit from mechanical and hand removal materials if not disposed of properly. Therefore, it is important to follow strict management guidelines to prevent spread to same or other sites after control activities.

One factor important to mechanical and hand removal control methods that were looked at but not reported on here, is that vegetative division to establish a new plant was only possible if the node was present. New plants were not seen to establish from parts of leaves, roots alone or parts of stolons. This means that leaving small parts of the plant behind when removing infestations by hand or mechanical means will only lead to regrowth if the plant part is attached to the node. This knowledge will allow those removing Amazon Frogbit to save time as they will only have to ensure no nodes remain behind, rather than removing every single piece of leaf, root or stolon.

Control methods integrating shading are likely to inhibit the growth and spread of this invasive species in stormwater systems. However, this can be difficult to use as a large-scale, longer period control option for natural waterways since its application can have negative impacts on native plant growth and would be detrimental to many other aquatic organisms. More experiments under different percentage of shading with larger scales and longer time periods or in-situ trials are recommended for further investigation. Some studies in lake mesocosms and greenhouses have demonstrated that a moderately high density of shading, such as that achieved with the 70% shade cloth, can serve as an effective control for European Frogbit (*Hydrocharis morsus-ranae*) with minor impacts on the environment (Zhu et al.,2014).

Therefore, if Amazon Frogbit is established, shading may be considered by aquatic plant managers as one of the possible control methods. In addition, shade may be an effective eradication method with low environmental impact when compared with chemical control. This strategy of using shade to prevent the accumulation of Amazon Frogbit seed bank is a key toward the eventual eradication of the weed. This will also reduce the necessary chemical load or labour for physical removal. Physical removal or chemical control can be then applied for any remaining plants after the shading control method has been applied for a short period.

It is important to note that the practice of integrated Amazon Frogbit, or any other aquatic weed, management is site-specific in nature, with individual methods determined according to ecology of habitats. Where appropriate, each site should have in place a management strategy for prevention, monitoring and suppression of Amazon Frogbit populations.

It is noteworthy to mention that prevention is the single best solution for Amazon Frogbit management, just as it is for many other invasive species. The best solution is, declare Amazon Frogbit as a declared pest S22 (2) with a minimum C3 Management Control Category and Prohibited Keeping (currently a Declared pest S22(2) No Control Category, Exempt Keeping Category). Amazon Frogbit is a declared weed and regarded as prohibited matter in New South Wales and the Northern Territory.

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Appendices

Appendix 1. Canning River, downstream of Kent Street Weir, treatment mesocosms observational records throughout the experiment (NL = new leaf, NR = new roots, Ramets = juvenile new plants, HC = Health Code, 4 =best condition, 0 = dead).

Date	Day	NL	NR	Ramets	Plants Remaining (%)	Plants With New Growth (%)	New Plants Total	Average HC Status
20/Mar	0	0	0	0	100	0	0	4
24/Mar	4	0	6	0	100.00	10.00	0	2.90
27/Mar	7	39	40	0	100.00	75.00	3	2.25
31/Mar	11	58	51	4	100.00	93.33	3	1.98
03/Apr	14	55	31	2	90.00	92.59	0	1.58
07/Apr	18	41	32	1	86.67	92.31	0	1.75
09/Apr	20	59	35	3	85.00	98.04	0	1.48
14/Apr	25	25	20	1	76.67	82.61	0	1.32
17/Apr	28	11	16	0	70.00	54.76	0	1.10
21/Apr	32	12	5	1	56.67	44.12	0	0.73
24/Apr	35	3	2	3	45.00	18.52	0	0.62
28/Apr	39	2	1	3	23.33	14.29	0	0.23
01/May	42	3	0	1	18.33	27.27	0	0.15
05/May	46	0	0	2	11.67	0.00	0	0.15
07/May	48	0	0	1	6.67	0.00	0	0.13
12/May	53	0	0	1	5.00	0.00	0	0.12
15/May	56	1	0	1	5.00	33.33	0	0.12
19/May	60	1	0	1	1.67	100.00	0	0.05
22/May	63	0	0	1	1.67	0.00	0	0.05
29/May	70	0	0	1	1.67	0.00	0	0.05
02/Jun	74	0	0	0	1.67	0.00	0	0.02
05/Jun	77	0	0	0	1.67	0.00	0	0.02
09/Jun	81	0	0	0	1.67	0.00	0	0.02
16/Jun	88	0	0	0	0.00	0.00	0	0.00

Appendix 2. Canning River, under the Riverton Bridge, treatment mesocosms observational records throughout the experiment (NL = new leaf, NR = new roots, Ramets = juvenile new plants, HC = Health Code, 4 = best condition, 0 = dead).

Date	Day	NL	NR	Ramets	Plants Remaining (%)	Plants With New Growth (%)	New Plants Total	HC Status
20/Mar	0	0	0	0	100	0	0	4.00
24/Mar	4	0	0	0	48.33	0.00	0	0.95
27/Mar	7	7	0	0	26.67	43.75	0	0.40
31/Mar	11	3	0	0	6.67	75.00	0	0.08
03/Apr	14	0	0	0	6.67	0.00	0	0.05
07/Apr	18	0	0	0	6.67	0.00	0	0.05
09/Apr	20	0	0	0	6.67	0.00	0	0.07
14/Apr	25	0	0	0	1.67	0.00	0	0.02
17/Apr	28	0	0	0	1.67	0.00	0	0.07
21/Apr	32	0	0	0	0.00	0.00	0	0.00
24/Apr	35	0	0	0	0.00	0.00	0	0.00
28/Apr	39	0	0	0	0.00	0.00	0	0.00
01/May	42	0	0	0	0.00	0.00	0	0.00
05/May	46	0	0	0	0.00	0.00	0	0.00
07/May	48	0	0	0	0.00	0.00	0	0.00
12/May	53	0	0	0	0.00	0.00	0	0.00
15/May	56	0	0	0	0.00	0.00	0	0.00
19/May	60	0	0	0	0.00	0.00	0	0.00
22/May	63	0	0	0	0.00	0.00	0	0.00
29/May	70	0	0	0	0.00	0.00	0	0.00
02/Jun	74	0	0	0	0.00	0.00	0	0.00
05/Jun	77	0	0	0	0.00	0.00	0	0.00
09/Jun	81	0	0	0	0.00	0.00	0	0.00
16/Jun	88	0	0	0	0.00	0.00	0	0.00

Appendix 3. Bayswater, treatment mesocosms observational records throughout the experiment (NL = new leaf, NR = new roots, Ramets = juvenile new plants, HC = Health Code, 4 = best condition, 0 = dead).

Date	Day	NL	NR	Ramets	Plants Remaining (%)	Plants With New Growth (%)	New Plants Total	HC Status
20/Mar	0	0	0	0	100	0	0	4
24/Mar	4	0	0	0	71.67	0.00	0	0.87
27/Mar	7	0	0	0	8.33	0.00	0	0.08
31/Mar	11	0	0	0	0.00	0.00	0	0.00
03/Apr	14	0	0	0	0.00	0.00	0	0.00
07/Apr	18	0	0	0	0.00	0.00	0	0.00
09/Apr	20	0	0	0	0.00	0.00	0	0.00
14/Apr	25	0	0	0	0.00	0.00	0	0.00
17/Apr	28	0	0	0	0.00	0.00	0	0.00
21/Apr	32	0	0	0	0.00	0.00	0	0.00
24/Apr	35	0	0	0	0.00	0.00	0	0.00
28/Apr	39	0	0	0	0.00	0.00	0	0.00
01/May	42	0	0	0	0.00	0.00	0	0.00
05/May	46	0	0	0	0.00	0.00	0	0.00
07/May	48	0	0	0	0.00	0.00	0	0.00
12/May	53	0	0	0	0.00	0.00	0	0.00
15/May	56	0	0	0	0.00	0.00	0	0.00
19/May	60	0	0	0	0.00	0.00	0	0.00
22/May	63	0	0	0	0.00	0.00	0	0.00
29/May	70	0	0	0	0.00	0.00	0	0.00
02/Jun	74	0	0	0	0.00	0.00	0	0.00
05/Jun	77	0	0	0	0.00	0.00	0	0.00
09/Jun	81	0	0	0	0.00	0.00	0	0.00
16/Jun	88	0	0	0	0.00	0.00	0	0.00

Appendix 4. Little Rush Lake control mesocosms observational records throughout the experiment (NL = new leaf, NR = new roots, Ramets = juvenile new plants, HC = Health Code, 4 = best condition, 0 = dead).

Date	Day	NL	NR	Ramets	Plants Remaining (%)	Plants With New Growth (%)	New Plants Total	HC Status
20/Mar	0	0	0	0	100	0	0	4.00
24/Mar	4	0	0	7	100.00	0.00	0	3.63
27/Mar	7	29	0	8	100.00	46.67	2	3.37
31/Mar	11	65	1	7	98.33	79.66	2	3.43
03/Apr	14	23	0	7	98.33	30.51	3	3.43
07/Apr	18	66	0	7	98.33	62.71	3	3.32
09/Apr	20	23	1	9	98.33	36.67	7	3.17
14/Apr	25	64	0	25	98.33	74.58	5	3.12
17/Apr	28	48	1	21	98.33	59.32	11	3.13
21/Apr	32	38	0	23	98.33	64.41	14	3.18
24/Apr	35	29	0	26	98.33	49.15	19	3.13
28/Apr	39	35	0	32	98.33	58.33	29	3.18
01/May	42	26	0	23	98.33	44.07	36	3.25
05/May	46	25	0	31	98.33	42.37	39	3.18
07/May	48	-	-	-	-	-	-	-
12/May	53	-	-	-	-	-	-	-
15/May	56	-	-	-	-	-	-	-
19/May	60	-	-	-	-	-	-	-
22/May	63	-	-	-	-	-	-	-
29/May	70	-	-	-	-	-	-	-
02/Jun	74	-	-	-	-	-	-	-
05/Jun	77	-	-	-	-	-	-	-
09/Jun	81	-	-	-	-	-	-	-
16/Jun	88	-	-	-	-	-	-	-