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Initial Assessment of Salinity Tolerance in the Invasive Aquatic Weed Frogbit (*Hydrocharis laevigata*)

Adrian Renshaw | Michael J. M. Franklin

School of Science, Western Sydney University, Penrith, New South Wales, Australia

Correspondence: Adrian Renshaw (a.renshaw@westernsydney.edu.au)

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ABSTRACT

Frogbit (*Hydrocharis laevigata*) is an aquatic invasive species with a high capacity to disperse, which can lead to rapid colonisation and potential domination of the littoral zone of affected freshwater systems. Little is known about the tolerance of this species to saline waters, or whether it has the potential to impact estuaries or tidal sections of rivers. On receipt of a communication that Frogbit had been observed in an estuary in south-eastern Australia, we conducted a glasshouse experiment with the aim of making an initial assessment of the salinity tolerance of Frogbit. Mature and juvenile Frogbit ramets were placed in open 1-L opaque, cylindrical containers with water and six sodium chloride concentrations (control (0 g/L), 1, 2, 5, 10 and 20 g/L) replicated 12 times each. Ramets were assessed as alive or dead at seven, 14 and 21 days. Bayesian logistic regression was used to model Frogbit responses to salinity. At 21 days, all except two ramets remained alive in the control, 1 g/L and 2 g/L NaCl treatments. At 7 days, the probability of mature and juvenile survival began to decline sharply at 5 g/L and was approximately zero at 15 g/L on average. The probability of survival reduced further with time at these intermediate concentrations. All ramets were dead in the 20 g/L solution at day 21. Our results show that while Frogbit may tolerate slightly elevated salinity for short periods, it is unlikely to persist in parts of estuaries where seawater prevails. However, further research is urgently required to understand the extent to which Frogbit can occupy tidal waterways with regular influxes of fresh water on receding tides.

1 | Introduction

The recent Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) report on invasive alien species (IAS) and their control identified the global estimated cost of biological invasions (for 2019) to be $US423 billion, a cost that has quadrupled each decade since 1970 (IPBES 2023). They also reported that IAS contributed to 60% of global species extinctions and that 80% of invasions resulted in negative impacts on human quality of life. One key message from the IPBES report was that the most cost-effective options against IAS were prevention and preparedness. Prevention is the initial stage (prevention, eradication, containment, and long-term management) in the classic invasion curve (Lodge et al. 2016). Preparedness includes surveillance, early detection, and rapid response, all necessary to reduce rates of IAS establishment (Leung et al. 2002; IPBES 2023; Diagne et al. 2021). Furthermore, Cuthbert et al. (2022) and Ahmed et al. (2022) outline the additional high cost brought about by early inaction or lack of preparedness. Critical to preparedness is biological and ecological knowledge of the invasive species.

Aquatic IAS (e.g., water hyacinth, *Pontederia crassipes* Mart.) can have significant negative impacts including impeding vehicle and water passage, increasing overland flow and flooding, changing habitat structure and complexity, reducing biodiversity and reducing light to benthic organisms, and impacting fisheries and aquaculture enterprises (Lovell et al. 2006;

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Kovalenko et al. 2010). Once established, costly control measures include the use of herbicides, mechanical or manual harvesting and biocontrol, or various combinations depending on the site characteristics.

Hydrocharis laevigata (Humb. and Bonpl. ex Willd.) Byng and Christenh. (Hydrocharitaceae) is native to Central and South America (Cook and Urmi-König 1983; Yang et al. 2023), but naturalised populations now occur on every continent (Garcia-Murillo 2023). The ornamental plant trade is responsible for the introduction of *H. laevigata* (hereafter, Frogbit) to the environment in Australia, Zimbabwe, Japan and Spain (Garcia-Murillo 2023). The species is invasive in Australia, Asia and North America (Howard et al. 2016). In America, where its invasiveness has been likened to water hyacinth, further spread is likely (Anderson and Akers 2011). Across Europe, naturalised populations occur in Spain and Hungary, with detections recorded further north in Belgium, Poland and the Netherlands (Garcia-Murillo 2023). Cold temperatures may limit the establishment and spread of Frogbit in locations such as Poland (Pliszko and Górecki 2021). Frogbit is an aquatic floating perennial that may become rooted in mud. It reproduces asexually or sexually, developing long stolons that give rise to leaves in rosettes. Leaves are ovate to circular, to 9 cm long and 8 cm wide, and may develop both floating and aerial leaves. The abaxial side of floating leaves is characterised by a patch of aerenchymous tissue up to 1 cm thick (Cook and Urmi-König 1983). The elongated stolons and root mats knit strongly together to form dense floating mats (Garcia-Murillo 2023).

Frogbit is currently recognised as prohibited matter under the Biosecurity Act NSW 2015 (Department of Primary Industries 2024), and is a relatively new aquatic invader in Australia. An anecdotal report (Chris Dewhurst, Director of Hawkesbury River County Council, pers. comm.) was received by the first author that Frogbit had been detected within estuarine mangroves on the Georges River, Sydney, Australia. Mangroves are unique plants occupying both the aquatic and terrestrial domains and are regularly exposed to high levels of salinity that most plants would not survive. They are also regarded as important ecosystems providing services such as shelter, erosion control, and protection from storm and cyclone surges (Vo et al. 2012). The range of salinities that mangroves typically experience is 5–20 ppt; however, most mangrove species survive salinities up to 35 ppt (Kodikara et al. 2017). The potential for Frogbit to occupy the intertidal sections of waterways is poorly understood and requires investigation to support strategic management of this invasive species.

Research on salinity tolerance in aquatic plants compared to terrestrial plants is less common (Kaijser et al. 2019). Plant adaptations to salinity include a tolerance to accumulate or exclude Na^+ or Cl^- and a tolerance to osmotic stress, whereas plants responses to salinity include a rapid, osmotic phase inhibiting the growth of young leaves, and an ionic phase that increases the rate of senescence of mature leaves (Munns and Tester 2008). Altered salinity levels can induce salt stress or salt shock in plants. Salt stress occurs when plants are exposed to low levels of salt and/or increasing levels of salt, whereas salt shock is a form of stress that occurs when plants are exposed to sudden high salinity levels (Shavrukov 2013).

This study aimed to provide a preliminary estimate of the likelihood of Frogbit persisting in estuaries and tidal sections of rivers by capturing baseline quantitative information on the salinity tolerance of Frogbit. In March 2022, the NSW Department of Primary Industries (DPI) supplied Western Sydney University (WSU) with a large environmental sample of Frogbit that was subsequently held in a glasshouse in a 2000-L tank (DPI Biosecurity Permit granted). The Frogbit sample then actively grew with heavily intertwined stems, a very dense root mat, and produced several male flowers. This material was used to conduct an experiment using water treated with sodium chloride at a range of concentrations as a proxy for estuarine waters.

2 | Materials and Methods

In August 2022, experimental ramets were removed from the larger sample by cutting through the stolons adjacent to nodes. These ramets were further divided into mature ramets with leaf diameter >2 cm and juvenile ramets with leaves <1 cm in diameter. Juvenile ramets were obtained from terminal or near-terminal nodes, whereas mature ramets were obtained from nodes away from the terminal node. All ramets had roots and 4–7 fully expanded leaves. Six salinity treatments were prepared using sodium chloride (NaCl) and main supply drinking (tap) water (0 g/L (control), 1, 2, 5, 10 and 20 g/L of added NaCl). These six treatments were replicated 12 times each for both mature and juvenile ramets. Tap water had a baseline electrical conductivity of 0.22 mS/cm, which is approximately equivalent to 0.1 g/L NaCl in solution. The source of the tap water was the North Richmond Delivery System. Tap water was used instead of Type I or II laboratory grade water because it typically contains small quantities of phosphorous (0.003–0.004 mg/L), nitrate (0.21–0.42 mg/L) and potassium (2.00–2.86 mg/L) (Sydney Water 2025), to provide a low level of support for plant growth.

Seventy-two 1 L plastic containers were labelled and filled to the 800 mL mark, and a single mature and juvenile ramet was placed into each container (day 0). The 72 containers were randomly assigned to a bench in a shade-covered glasshouse without heating (Figure 1a). The containers were randomly reshuffled weekly after assessing the ramets. The mean minimum and maximum temperature for Richmond in August is 4.7°C–19.9°C and September is 8.0°C–23.0°C. The ramets were scored as alive (still green but may otherwise have localised necrotic regions or some whole leaves dead) or dead (all brown, without green tissue) at day 0, 7, 14 and 21. All ramets were completely green at the start of the experiment (day 0).

To assess the tolerance of Frogbit to salinity, we fitted a Bayesian logistic regression model with Stan (Stan Development Team 2024) using the brms package (Bürkner 2017) and R (R Core Team 2024). The binary response variable labelled Alive represented whether an individual was dead (0) or alive (1). Predictor variables were (1) Stage, which was a factor with two levels (mature, juvenile), (2) Day, which was a factor with three levels (7, 14, 21), and (3) Treatment, which was the NaCl concentration of the solution. A random intercept for container was also included in the model. Normal (0.5) priors were used for the intercept and predictor variables, and priors for standard deviation parameters were exponential (1). The model was run



FIGURE 1 | Photographs of the experiment showing (a) the array of containers in the glasshouse on day one, (b) necrotic changes in tissues commencing at the leaf margin in juvenile ramets on day seven, and (c) a green meristem held within older, necrotic tissues on day seven.

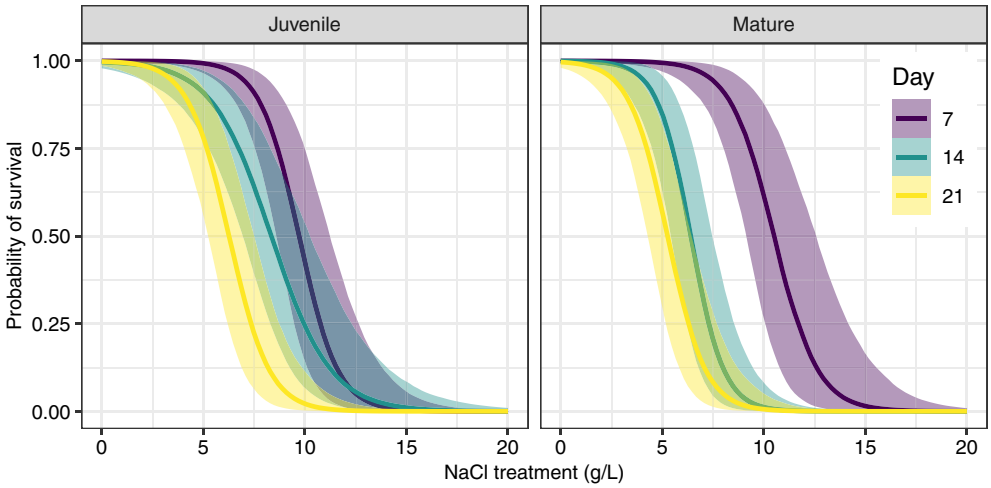


FIGURE 2 | The probability that juvenile and mature Frogbit ramets survived at seven, 14 and 21 days after placement in water with different sodium chloride (NaCl) concentrations. Lines and shaded bands indicate means and 90% of distributions of posterior predictions, respectively.

using four MCMC (Markov chain Monte Carlo) chains to obtain 2000 posterior samples for each parameter, having discarded the initial 2000 warmup samples and thinning the remaining 10000 samples at the rate of 20 (Gelman et al. 2013). Model convergence was assessed through inspection of the Gelman–Rubin statistic and the number of effective samples. Posterior predictive checks were also conducted (Gelman et al. 2013). The median lethal concentration (LC50) of NaCl was estimated for mature and juvenile ramets on different days using model coefficients (Schabenberger and Pierce 2001).

3 | Results

At the completion of the experiment (21 days) all juvenile and all but a total of two mature ramets remained alive in the control, 1 and 2 g/L NaCl treatments. Two juvenile ramets remained alive in the 10 g/L solution at day 21, but all corresponding mature ramets had died. All juvenile and mature ramets had died in the 20 g/L solution at day seven, but at weaker concentrations, ramet death was preceded by a decline in plant health that was observed to occur over days. Leaf necrosis typically started at the leaf margins and progressed across the lamina towards the leaf petiole (Figure 1b). The meristem in all ramets did not grow but remained green within surrounding tissues (Figure 1c). The meristems were the last tissues to turn brown.

TABLE 1 | Sodium chloride (NaCl) concentrations at which 50% of Frogbit ramets were predicted to have died (LC50).

Growth stage	Day	LC50 (NaCl g/L)
Juvenile	7	9.7
Juvenile	14	8.25
Juvenile	21	6.25
Mature	7	10.47
Mature	14	6.37
Mature	21	5.3

The probability of survival of mature and juvenile Frogbit ramets was reduced with increasing NaCl concentration and through time (Figure 2, Table 1). After 7 days in solution, the probability of mature and juvenile survival began to decline

sharply at 5 g/L NaCl and was approximately zero at 15 g/L, on average. At 14 days, the decline in the probability of mature ramet survival occurred at substantially lower NaCl concentrations than at day seven (Figure 2). The mature ramet LC50 at 21 days was approximately half the NaCl concentration at 7 days (Table 1). Although the reduction in the probability of juvenile survival was similar to mature ramets at seven and 21 days, only juveniles had some clear probability of surviving at 14 days at concentrations above 10 g/L NaCl (Figure 2).

4 | Discussion

The design of the experiment in terms of duration and NaCl concentrations effectively captured the salinity tolerance range of Frogbit ramets in treatments. The results suggest that Frogbit would not survive in sea water. However, Frogbit ramets exhibited some tolerance to low levels of salinity, at least for several days, so the threat posed by this species in the lower reaches of rivers, where the tidal influence is weak, remains uncertain. Furthermore, the flushing effect of tides coupled with high rain events and/or microclimatic factors such as proximity to freshwater inflows might enable Frogbit to persist and reproduce in an intertidal zone.

The changes in plant tissues observed during the experiment were likely to have been driven by mechanisms of plant salinity tolerance. An initial osmotic phase may have inhibited growth (Munns and Tester 2008), but did not immediately kill the meristems, which remained green for longer within older, senesced tissues, where they may have been buffered from immediate salt effects. We also observed a pattern of leaf necrosis that corresponded to the thickness of leaf aerenchyma. Necrosis commenced at the leaf margin, where aerenchyma is absent (Cook and Urmi-König 1983), and progressed inwards across the lamina towards the petiole, where aerenchyma is thickest. In an ionic phase, Na^+ or Cl^- may have accumulated to toxic levels in the leaf's thinnest extremities first (i.e., leaf margins) (Munns and Tester 2008). Progressive accumulation of ions across the thicker part of the leaf would cause subsequent cell death in those locations. Osmotic shock might also account for the same pattern, and the two processes would act simultaneously, being more rapid in the higher concentration treatments, which was potentially reflected in the results.

With an increasing frequency of flood events in eastern Australia and other parts of the world (Power and Callaghan 2016), the spread of aquatic weeds is more likely. Floods can occur at any time of the year and Frogbit may be transported downstream to lodge in mangrove or estuarine environments in different water temperatures. Future experiments could vary water temperatures and might simulate tides with salt and freshwater flushes and/or could also use river/estuarine water from different reaches within a river system. Harvesting ramets from the source material required manual handling and this would have stressed/shocked the ramets. Future experiments could acclimatise ramets by simulating passage from a freshwater system through increasing salt concentrations as would be encountered in a river system that drains to the sea.

Our study has found that Frogbit is somewhat tolerant of slightly brackish water. As such, there is a risk that Frogbit can persist in locations in rivers at the upper limits of tidal influence. In addition, Frogbit invasion may occur in inland waterways where soils are naturally saline, or where they are subject to elevated salt levels from adjacent human land use. Frogbit is unlikely to flourish at salinity levels representing those where seawater dominates estuaries at the peak of the incoming tide. However, salinity levels fluctuate in these locations, so further research is required to determine whether Frogbit can persist through regular cycles of low and high salinity.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The study data will be uploaded to a reputable data repository and made publicly available on acceptance of the article for publication.

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Supporting Information

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