Estimating the minimum salinity level for the control of New Zealand Pygmyweed *Crassula helmsii* in brackish water habitats

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SUMMARY

Crassula helmsii is a semi-aquatic, invasive macrophyte, which has become abundant in wetland habitats across Europe. This species is of conservation concern because heavy invasions form dense carpets within which few other plants species occur. *C. helmsii* is known to be killed by inundation with seawater, but published information on its response to inundation by less saline water is limited. Growth trials were conducted to investigate the levels of salinity required to kill this species. We found a linear negative relationship between growth rate and salinity across the range from 2 – 8 ppt, but that 8 ppt was required to kill *C. helmsii*. These findings suggest that *C. helmsii* growth could be controlled by inundation with saline water of 8 ppt. This may present a method for reducing the negative effects of salt water on co-occurring species, and thus the next stage will be to determine the efficacy of this method in field trials.

BACKGROUND

The semi-aquatic plant New Zealand Pygmyweed *Crassula helmsii* has invaded and become abundant within many nature reserves across Europe where land is managed as wetland habitat, such as ponds, shallow pools, reedbeds, and grazing marsh (Langdon *et al.* 2004; Bridge 2005; Gomes 2005; Wilton-Jones 2005). *C. helmsii* can spread rapidly across bare mud (personal observation), and heavy invasions resemble thick green carpets with few other plant species occurring in amongst the dense vegetation (Dawson & Warman 1987; EPPO 2007; Minchin 2008). Degrading the invasive vegetation using herbicides, heating, or covering with light eliminating material, can decrease the abundance of *C. helmsii*. However, complete eradication is rarely achieved due to subsequent re-growth of remaining vegetative fragments (Bridge 2005; Gomes 2005; Wilton-Jones 2005).

The use of seawater inundation has been found to be a practical option for the control of *C. helmsii* in coastal habitats. For example, Charlton *et al.* (2010) found that *C. helmsii* was eradicated from grazing marsh at RSPB Old Hall Marshes by flooding the area with seawater. At this site, the benefit of *C. helmsii* eradication was carefully assessed in relation to the potential negative impacts on co-occurring native species (Charlton *et al.* 2010; Gardiner & Charlton 2012). Such a consideration is important as saltwater inundation is a non-targeted method of control, so any other organisms occurring within an inundation zone would also experience a sudden rise in salinity; those which cannot tolerate the rise in salinity would also be locally eradicated. Reviews of invasive plant control efficacy have also highlighted that the costs to native species is an important consideration when planning best practice control attempts (Kettenring & Adams 2011).

There is limited published information on salinity tolerance in *C. helmsii*. Whilst it can be deduced that this invasive plant has some tolerance to saline water due to its invasion of coastal habitats (EPPO 2007), this species is not a brackish habitat specialist and its UK distribution is more associated with freshwater (Preston & Croft 1997). Knowledge of the environmental tolerances of an invasive species can be used to create conditions that are less favourable for the invader (Davis 2009). In the present context, knowing the threshold between freshwater and saline water at which *C. helmsii* can no longer grow would allow manipulation of site salinity to be used as a management tool. Furthermore, such information has utility when predicting how the abundance and distribution of the invasive plant may change if site management is altered in a way which affects the salinity (Thouvenot *et al.* 2012).

The aim of this investigation was to determine the levels of salinity required to kill *C. helmsii*. In doing so, we aimed to provide information on the increase in salinity needed at a site in order to eradicate *C. helmsii*, whilst minimising saline toxicity in co-occurring native species. We conducted growth trials with the objective of finding the lowest level of salinity that kills *C. helmsii*. The concentration of plant available nutrients was also controlled, as nutrient availability in tank experiments has previously been found to affect the growth rate of *C. helmsii* (Hussner 2009).

ACTION

The growth trials were conducted outdoors at Bournemouth University, Dorset, UK. *C. helmsii* was grown in conditions specifically modified to produce three different levels of salinity; 2, 4, and 8 parts/thousand ('ppt'). By contrast, seawater has a salinity of greater than 30 ppt. The three test levels of 2, 4 and 8 ppt were chosen based on field observations of the distribution of *C. helmsii* in brackish water pools at Pett Level in East Sussex (OS grid ref: TQ 903 147),

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where this plant was restricted to water of 4 ppt and below.

To set up the growth trials, we used 5 L plastic storage containers, lined with horticultural sand to 3 cm depth and filled with 1 L of distilled water, to mimic shallow water habitat conditions suitable for *C. helmsii*. Fresh cut samples of *C. helmsii* weighing 10 g were added to each 5 L container. The correct salinity was achieved at the start of the experiment by mixing 2, 4, or 8 g of salt (sodium chloride) to the 1 L of distilled water. A solution containing all required mineral elements for growth (Taiz & Zeiger 2006), was applied to the water + salt solutions at the start of the experiment. The nutrient solution was applied at three different dilutions; 0.125, 0.25 and 0.5 times the full strength solution. Each salinity level was combined with each nutrient dilution, making nine different salinity/nutrient treatments, and each treatment was replicated four times in a separate container. To act as a control, additional containers were set up in which 10 g fresh weight samples of *C. helmsii* were grown within the same three nutrient dilutions but with no added salt, replicated three times each. In total, therefore, *C. helmsii* was grown in 45 separate containers.

The experiment lasted for 31 days, during September and October 2011. Meteorological Office records of climate data from Hurn weather station (approximately 4 miles north-east of the trial location) show that in September 2011 daily temperature averages ranged from 19.8 °C to 11.3 °C, with 0 days air frost, and in October 2011 daily temperature averages ranged from 17.3 °C to 8.6 °C, with 1 day air frost (© Crown copyright 2011). At the end of the experiment, the *C. helmsii* was removed from each container and thoroughly rinsed with tap water. In order to obtain a dry weight value for the amount of *C. helmsii* growing in each container at the end of the experiment, the vegetation was dried at 90 ˚C for 48 hrs before being weighed. A dry weight value for the start of the experiment was estimated by weighing out twenty additional *C. helmsii* samples of 10 g fresh weight, drying them at 90 ˚C for 48 hours, and taking the average dry weight of these samples. Having the average 'start dry weight', and the 'end dry weight' for each *C. helmsii* sample allowed for the relative growth rate to be calculated for each replicate. This was done using the formula:

Relative growth rate = (lnW2 – lnW1) / (t2 – t1)

Where W1 is the start and W2 the end dry weight, ln is the natural logarithm, and t1 is the start and t2 the end time measured in days. The formula describes the proportional increase of a plant in grams, expressed per gram per day (g g^{-1}/day^{-1}; Hunt, 2003).

CONSEQUENCES

A significant difference was found in average growth rate between the salinity treatments (two-way ANOVA: $F_{(3, 33)}$ = 197.705, $p < 0.001$; Figure 1). The highest average growth rates were recorded within the control (0 ppt; Table 1). Growth was recorded at 4 ppt but the average growth rates were lower than at 2 ppt or in the control. In the 8 ppt treatments, all but one replicate was found to have a negative growth rate which is indicative of a loss of biomass. By the end of the experiment, it was observed that the *C. helmsii* within the 8 ppt salinity treatments had died and was in the early stages of decomposition. The nutrient dilution had no significant effect on the growth rate of *C. helmsii* in this experiment (two-way ANOVA: $F_{(2, 33)} = 0.115$, $p = 0.892$).

DISCUSSION

The results of the growth trials show a negative linear relationship between *C. helmsii* growth and salinity, indicating that *C. helmsii* growth is inhibited by increasing salinity across the range from 2 to 8 ppt. Importantly, *C. helmsii* died in tanks of 8 ppt, suggesting that this is beyond the salinity tolerance of this species. Thus, based on the findings of these growth trials, we suggest that if the salinity of a *C. helmsii* invaded site was raised to 8 ppt or above, this could result in similar eradication efficacy to that produced by seawater of 30 ppt. Furthermore, if a site was permanently maintained at 8 ppt or higher, this might provide a long-term solution for the prevention of re-invasion in sites where *C. helmsii* had been eradicated, or

Table 1. The average start dry weight, end dry weight, and growth rate values for replicate trials of *Crassula helmsii* when exposed to three different levels of salinity (2, 4, and 8 parts/thousand) plus a control, combined with three different nutrient dilutions (0.5, 0.25, and 0.125 x full strength nutrient solution).

Salinity (ppt)	Nutrient dilution (x full strength)	Average values Start dry weight (g)	End dry weight (g)	Growth Rate (g g^{-1}/day^{-1})
0	0.125	0.840	2.790	0.048
0	0.25	0.840	3.067	0.052
0	0.5	0.840	3.093	0.052
2	0.125	0.840	1.288	0.014
2	0.25	0.840	1.268	0.013
2	0.5	0.840	1.445	0.017
4	0.125	0.840	1.033	0.007
4	0.25	0.840	1.013	0.006
4	0.5	0.840	0.923	0.003
8	0.125	0.840	0.678	-0.008
8	0.25	0.840	0.593	-0.012
8	0.5	0.840	0.653	-0.009

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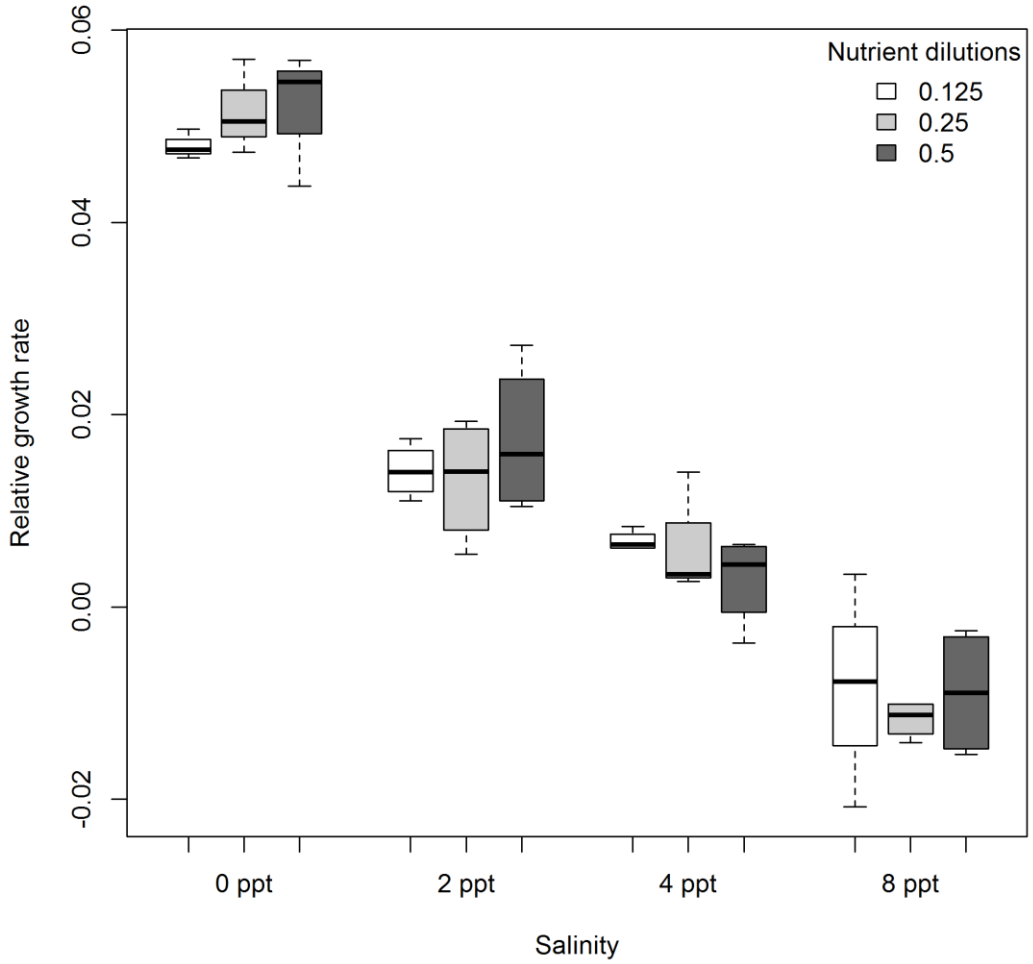

Figure 1. The relative growth rate of *Crassula helmsii* when grown in tanks, with a factorial design combining three treatment levels of salinity (2, 4, and 8 ppt) plus a control, and three treatment levels of nutrient dilution (0.5, 0.25, and 0.125 x full strength nutrient solution). Boxplots falling below the 0.00 line represent biomass loss. Units for salinity were ppt, and for relative growth weight were grams per gram/day (g g^{-1}/d^{-1}).

prevent its spread within habitats where the species has a patchy distribution.

Using the lowest effective salinity for *C. helmsii* eradication may represent a way to minimise the impacts of saltwater inundation. In particular this method may minimise impacts in naturally brackish habitats, where an increase to 8 ppt may be enough to inhibit *C. helmsii* whilst favouring native brackish habitat specialists. For example, plant species such as saltmarsh goosefoot (*Chenopodium chenopodioides*), brackish water-crowfoot (*Runnunculus baudotii*), sea clubrush (*Scirpus maritimus*) and greater sea-spurrey (*Spergularia media*) have all been recorded as co-occurring with *C. helmsii* at Pett Level in East Sussex. In addition, the salt tolerance of common reed (*Phragmites australis*; Preston & Croft 1997; Chambers *et al.* 2003) may make brackish water (8 ppt) inundation an appropriate method of *C. helmsii* eradication in mature reedbed habitats.

This paper represents a demonstration of concept. We acknowledge that the next step will be to test whether *C. helmsii* is successfully eradicated in the field using inundation with water at 8 ppt, and whether control of salinity levels is a feasible management option. Additional tank-based growth trials could also be conducted to estimate the minimum length of time inundated at 8 ppt, which is required to ensure a total kill. We recommend that field trials be conducted at sites where the predominant co-occurring plant community are tolerant of brackish conditions. Furthermore, monitoring the abundance of co-occurring native species in such field trials would provide evidence of whether *C. helmsii* is more greatly inhibited at 8 ppt than plants which are brackish habitat specialists.

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