EUPHRESCO DeCLAIM Final report

A State-of-the-art June 2011

Hydrocotyle ranunculoides L.f.

Plant Protection Service
Plant Research International, Wageningen UR
Aquatic Ecology and Water Quality Management Group, Wageningen UR
Centre for Ecology and Hydrology- Wallingford
This report contains the result of research on *Hydrocotyle ranunculoides* and was part of a larger project EUPHRESCO DeCLAIM (Decision Support Systems for Control of Alien Invasive Macrophytes). A project initiated to generate a prototype decision support system for optimal control measures for the four most troublesome invasive alien aquatic weeds at present in the UK and NL.

*Cabomba caroliniana*, a representative for the Myriophyllids growth form, representing 35% of the import volume of aquarium plants in The Netherlands. In 2009 it was found at three sites in The Netherlands, posing serious problems at one.

*Hydrocotyle ranunculoides*, a representative for the Stratiotids *s.l.* growth form, is at present the most troublesome invasive alien aquatic weed in the United Kingdom and The Netherlands, and is showing increased distribution in neighbouring countries as well as in the Australia, Uganda and Zimbabwe.

A second representative for the Stratiotids *s.l.* growth form, *Ludwigia grandiflora*, has demonstrated significant detrimental ecological impact in France and is gaining importance in The Netherlands. In the UK early intervention management strategies using herbicides have been developed and deployed (DEFRA website).

A third representative of the Stratiotids *s.l.* growth form is *Myriophyllum aquaticum*. This species has been sold extensively by the aquatic nursery trade as an ornamental species for domestic ponds. It is now present in many natural lowland static water sites in the UK. The species is still very popular in The Netherlands, and the number of infestations is increasing.

The overall project was a joint effort between four partners.

**Plant Protection Service**

**Plant Research International, Wageningen UR**

**Aquatic Ecology and Water Quality Management Group, Wageningen UR**

**Aquatic Plant Management Group, Centre for Ecology and Hydrology- Wallingford**

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This research was funded by the Ministry of Agriculture, Nature and Food safety of the Netherlands and the Department of Environment Food and Rural Affairs of the United Kingdom
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1. **Executive Summary**

- **Range:** In its native range, *Hydrocotyle ranunculoides* L.f. (Floating pennywort) occurs in, and at the margins of, slowly flowing, warm nutrient rich water in Argentina, Brazil and Paraguay, also in southern states of the USA. *H. ranunculoides* is an invasive aquatic weed in North Western Europe and several other countries worldwide, including Chile, Australia and Uganda. In Europe, it is found in and around canals, lakes, rivers, streams, ditches, and garden ponds.

- **Growth characteristics:** In an aquatic environment the species forms floating mats, in riparian vegetation it behaves as a helophyte. Growth in North Western Europe starts in early spring from small plants or fragments when air and water temperatures rise. They grow slowly in spring and form small, up to 10 cm² large leaves, which mostly float on the surface water (Hussner & Lösch, 2007). With increasing temperatures, photoperiod and light intensity, leaves grow larger and petioles reach a height of up to 40 cm above water level (EPPO, 2006). The growth rate of *H. ranunculoides* is greatest in June and July. The stems root freely from nodes at about 4-15 cm intervals. With decreasing temperatures and light availability in autumn, smaller leaves develop and some of the leaves die due to night frost. At this time plants will form floating and submerged leaves. The latter are able to survive the low water temperatures during the winter (Hussner & Lösch, 2007). From these small submerged plants and leafless overwintering stolons plants will grow out again in spring.

- **Areas at Risk:** In Britain, we were able to produce risk maps based on altitude which may predict areas at risk where suitable habitats exist, temperature is implied in this distribution but not specifically dealt with as day degree values were not calculated. In the Netherlands, due to the emphasis on the detail on the biotic factors limiting or enhancing *H. ranunculoides* growth, and the lack of relevant information, we were not able to identify the localities at risk or make maps of areas at risk. However, we can consider all shallow slow flowing and still waters in the Netherlands to be potentially at risk.

- **Reproductive strategy:** The species reproduces primarily by vegetative reproduction in Europe though spread by seed has been observed through sewage treatment works (e.g. Pevensey Levels). It can regenerate from small stolon fragments which must contain at least one node. It flowers in July–October in its native and introduced range. Flowers are white in colour, small and held above the water in the axes of stolons and petioles on 5 – 15 mm stems, with a group of 5 – 15 individual flowers in an umbel. Although establishment by seed is suspected at Pevensey Levels (UK), the production of viable seeds in this site has not yet been observed. Seeds do form, but seem not to mature, remaining white in colour rather than the brown of mature seeds in the Americas. This is probably due to cold temperatures at the time when seeds should mature in autumn. Vegetative reproduction is thought to be favoured in both flowing conditions at the edge of the mat, and during late November through to January when the plants start to
decompose and small fragments are produced in very large numbers. The regenerative capacity of nodal material under favourable conditions is close to 100%, and colonisation is inevitable in suitable habitats (Newman, unpubl.). With decreasing temperatures and light availability in autumn, smaller leaves develop and some of the leaves die due to night frost. At that time plants form floating and submerged leaves. The latter are able to survive the low water temperatures during the winter. From these small submerged plants and leafless overwintering stoloniferous plants will grow out again in spring.

- **CHARISMA:** Only a hand full of scientific articles describe the characterization of the growth conditions for *H. ranunculoides* in more detail and make an effort to quantify the growth under these conditions. In those studies, the leaf area index (LAI), total dry weight, dry weight of leaves, petioles, shoots and roots, the total shoot length, the number of nodes, the total number of leaves and the average leaf size between naturally occurring stands in habitats with low and high nutrient levels. It was concluded that with increasing nutrient content of the soil, all these parameters were higher than for stands in a habitat with a lower nutrient contents of the soil. In an aquatic environment the species forms floating mats, in riparian vegetation it behaves as a helophyte. CHARISMA accurately predicts growth patterns and biomass accumulation when in an unmanaged state, with annual biomass maxima c. 1,200 g dry weight m⁻². However, it was not possible to model the effects of management using this system in this project; as a result biomass maxima may occur on more than one occasion per year, resulting in peak release of vegetative fragments on a more regular basis, especially if plant material is cut, releasing fragments at optimal times of the year for regrowth and colonisation, rather than natural fragmentation which tends to occur in fast flowing environments and in the winter, both less suitable for rapid regrowth.

- **Management:** Mechanical control is the main method of management of this species, with cutting and removal of large floating mats the most common operation. This has been shown to produce benefits over time with regular maintenance, resulting in a much reduced final biomass in Dutch canal systems. In UK situations, mechanical control has probably perpetuated the presence of the plant in several locations, primarily as a result of the timing of cutting and release of vegetative fragments at optimum times for regeneration of fragments. In studies on the comparison between mechanical and chemical costs, herbicides are approximately 50% cheaper than mechanical control, and result in a better overall reduction in plant biomass in the following year. Novel techniques using hydrogen peroxide, flame throwers, adjuvants and combined mechanical and chemical techniques all show promise. Methods using heat and hydrogen peroxide have been tested in greenhouses during the project in the Netherlands. The prospects for flame weeding are positive, as a result of which one of the Belgian waterboards will test this control option under practical conditions in the growth season and investigate the optimal timing of application in relation to growth stage. Trials using herbicides and adjuvants in combination with mechanical control are ongoing in the UK. It appears that site requirements and conditions govern the choice of technique and optimisation of those techniques in particular situations will still require up to date advice based on experience.
• **DSS:** The main objective of this project was to produce a support system that could be used by field operatives and office based managers to identify the species accurately, and to enable a rapid risk assessment to be made in the field that could be reported in a consistent manner, enabling a rapid response to be made against the species with the aim of preventing further spread and eventually eradication the species from the affected watercourse. In order to make the response to aquatic non-native species consistent and proportionate a pictorial field and office guide has been produced that provides descriptive photographs of characteristic features, areas at risk, typical habitat types, and available management techniques. We have deliberately left out costs of management as these vary within each country and certainly between countries. In addition, each species chapter will be made available at [www.declaim.eu](http://www.declaim.eu)

1. The DSS was submitted to the Non Native Species Secretariat in the UK for comment before being used by managers. The comments received were positive and helpful and led to developments in the current version. In the Netherlands the DSS was submitted to representatives of various waterboards involved in control of invasive macrophytes.

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1 This domain is not hosted within any of the project organisations and should be migrated to a partner organisation web site as soon as possible.
2. Habitat Requirements and Areas At Risk

2.1. Objectives

- Analyse from literature what are the (a)biotic factors that characterize the habitat of *Hydrocotyle ranunculoides*.
- These (a)biotic factors will be used to identify in existing databases (STOWA-databases on ditches, streams, canals, lakes and ponds for The Netherlands; MTR, SNIFFER, LEAFPACS and existing CEH databases for the UK) localities at risk.
- Using these data maps of “at risk areas” of The Netherlands and the UK will be produced.

2.2. Literature Analysis of Growth conditions

Floating Pennywort (*H. ranunculoides*), occurs in and at the margins of slowly flowing, warm and nutrient rich water in Argentina, Brazil and Paraguay, also in the southern states of the USA (Baas & Holverda, 1996; Hussner & Lösch, 2007). In an aquatic environment the species forms floating mats, in riparian vegetation it behaves as a helophyte. Growth in North Western Europe starts in early spring from small plants or fragments after ice melt. They grow slowly in spring and form small, up to 10 cm² large leaves, which mostly float on the surface water (Hussner & Lösch, 2007). With increasing temperatures, photoperiod and light intensity, leaves grow larger and petioles reach a height of up to 40 cm above water level (EPPO, 2006). The growth rate of *H. ranunculoides* is highest in June and July. The stems root freely from nodes at about 4-15 cm intervals. Flowering and fruit formation takes place between May and October (Hussner & Lösch, 2007). Flowers are hermaphrodite, white and grouped (5-10) together in a small umbel. Fruits are almost round and flat, brownish, with faint ribs and are divided into two halves (EPPO, 2006, Hussner & Lösch, 2007). Floating Pennywort primarily reproduces by fragmentation. With decreasing temperatures and light availability in autumn, smaller leaves develop and some of the leaves die due to night frost. At this time plants will form floating and submerged leaves. The latter are able to survive the low water temperatures during the winter (Hussner & Lösch, 2007). From these small submerged plants and leafless overwintering stolons plants grow out again in spring.

Floating Pennywort has a high regenerative capacity and is able to form new shoots from small stem fragments of about 1 cm in length with at least one node with or without leaves. Formation of new shoots is not possible from single leaves or internode fragments of the shoots. The presence of leaves increases the speed with which new shoots are formed. Shoot development from fragments with one node and one leaf takes 1 week, while shoot development from fragments with one node without leaves attached will take significantly longer (Hussner & Lösch, 2007).

A large amount of sources report on the growth conditions for *H. ranunculoides*. Most of them report that the species occurs in slowly flowing, warm and nutrient rich water (Baas & Holverda, 1996). In canal systems in which water is flowing very slowly, the plant can be expected to be transported downstream and to form patches at the side of these waterways, which are convenient places for the species to settle. In the Netherlands, it occurs predominantly in flowing water that receives waste water from municipalities or
agriculture (Baas & Duistermaat, 1998). On most of these sites the species is spreading by vegetative growth only. Some sources report about different requirements for vegetative and generative growth. Under poor nutrient rich conditions vegetative growth will be relatively slow, and generative growth will be promoted (Baas & Holverda, 1996). Detailed data on the growth rate is however lacking. Because vegetative growth is the main route of spread for pennywort, the species mainly occurs in water bodies that are characterized by large amounts of waste water (Pot, 2008). It is important that these waterways remain open and water can pass them freely because of this function. Unfortunately H. ranunculoides favours this kind of relatively open and nutrient rich waterways. Under these favourable conditions a vegetative growth of 20 cm per day has been reported (EPPO, 2006). Only a hand full scientific articles describe the characterization of the specific growth conditions for H. ranunculoides in more detail and make an effort to quantify the growth under these conditions (Hussner & Lösch, 2007) (Hussner & Meyer, 2009). In those studies, the leaf area index (LAI), total dry weight, dry weight of leaves, petioles, shoots and roots, the total shoot length, the number of nodes, the total number of leaves and the average leaf size between naturally occurring stands in habitats with low and high nutrient levels. It was concluded that with increasing nutrient content of the soil, all these parameters were higher than for stands in a habitat with a lower nutrient contents of the soil. The Relative growth rate (RGR) varied from 0.005 g g⁻¹ d⁻¹ under low nutrient supply to 0.132 g g⁻¹ d⁻¹ under high nutrient supply systems. Light saturation of the species is reached at 800 mol photons m⁻² s⁻¹, or approximately 40% of full sunlight From the list of required parameters to model the growth of H. ranunculoides with CHARISMA, parameter values were only available for: leaf conductance, LAI (Leaf Area Index), SLA (Specific Leaf Area), RGR (Relative Growth Rate), internode length, gas exchange, total dry weight, relative dry weight allocated to leaves, relative dry weight allocated to petioles, relative dry weight allocated to shoots, relative dry weight allocated to roots, and maximum total shoot length (Appendix 1).

In Germany two natural H. ranunculoides stands were investigated. The two locations differed in nutrient content of the soil: one with a relatively low and one with a relatively high nutrient content of the soil. Several parameters were determined (Table 1). The total dry weight, the leaves, shoots, roots dry weight, and the total length of the shoots, the number of nodes, and the total number of leaves were higher of the stand on a location with high nutrient conditions of the soil (Hussner & Löhse, 2007).
Table 1. Biomass sampling at two locations with different nutrient levels. From: Hussner and Lösch (2007).

<table>
<thead>
<tr>
<th>Nutrient conditions of the soil</th>
<th>Total Dry weight (g/m²)</th>
<th>Leaves</th>
<th>Shoots</th>
<th>Roots</th>
<th>Distance of two nodes (cm)</th>
<th>Total length of shoots (m)</th>
<th>Total number of nodes</th>
<th>Root:shoot ratio</th>
<th>Total number of leaves</th>
<th>LAI (m²/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>312.2</td>
<td>71.1</td>
<td>78.9</td>
<td>38.0</td>
<td>7.33</td>
<td>141.0</td>
<td>1924</td>
<td>0.15</td>
<td>1549</td>
<td>4.36</td>
</tr>
<tr>
<td>High</td>
<td>532.4</td>
<td>104.1</td>
<td>129.9</td>
<td>72.6</td>
<td>7.77</td>
<td>207.0</td>
<td>2664</td>
<td>0.16</td>
<td>2488</td>
<td>5.47</td>
</tr>
</tbody>
</table>

An experiment was performed in which the relative growth rate of *H. ranunculoides* growing at different substrates was determined. The substrates differed in NH₄-N, NO₃-N, P₂O₅, and P₂O₅-P₂tot. The relative growth rate was significantly lower at a substrate with lower nutrient contents (Table 2). These growth rates were measured in a pot experiment over a period of 5 weeks (Hussner & Lösch, 2007). In Great Britain a maximum stolon extension rate of 20 cm per day has been observed (EPPO, 2006).

Table 2. Relative growth rate (RGR) of *H. ranunculoides* on different substrates. After: Hussner and Lösch (2007).

<table>
<thead>
<tr>
<th>Nutrient content of the substrate (mg/kg soil)</th>
<th>low</th>
<th>high</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄-N</td>
<td>1.9</td>
<td>3.9</td>
</tr>
<tr>
<td>NO₃-N</td>
<td>6.0</td>
<td>103.9</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.2</td>
<td>31.1</td>
</tr>
<tr>
<td>P₂O₅-P₂tot</td>
<td>1.5</td>
<td>61.2</td>
</tr>
</tbody>
</table>

| RGR (g g⁻¹d⁻¹) | 0.005 | 0.132 |

The water level will influence the growth rate as well; the relative growth rate is significantly higher when the soil is saturated with water (water level 5 cm above the soil surface) than under conditions where the soil is semi-drained (water level 17 cm under the soil surface) or completely drained (moist, but airy soil) (Hussner & Meyer, 2009). The relative biomass of roots compared to the other plant parts is not influenced by the water level (Hussner & Meyer, 2009).

Work undertaken by Newman & Duenas (2011) on the biometric response of aquatic *Hydrocotyle ranunculoides* to nutrient conditions in the Pevensey Levels, UK in the autumn
of 2008 at 5 sites with different nutrient concentrations showed that various metrics of *H. ranunculoides* were also correlated with nutrient concentrations.

The environmental parameters measured were soluble reactive Phosphorus, Total Phosphorous, NH$_4$, Cl, NO$_3$, Total Dissolved Nitrogen, water temp, Specific Conductivity, and pH. Plant parameters measured included internode length, internode diameter, petiole length, petiole weight (fresh and dry) leaf weight (fresh and dry), leaf area, root length, root weight (fresh and dry), flower numbers and total biomass were expressed on a per square metre basis.

Data from Duenas and Newman (2011) are given in Table 3. Plant samples from five sites on the Pevensey levels were collected and analysed for biometric differences correlated with environmental differences, mainly nutrient parameters, but channel width, depth and flow characteristics were also collected. The data in the table are the summarised ranges obtained from these sites in November 2008.

**Table 3: Summary nutrient data and plant biometrics of *H. ranunculoides* growing on the Pevensey levels, November 2008. From Duenas and Newman (2011, in prep)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRP µg/l</td>
<td>7 - 296</td>
<td></td>
</tr>
<tr>
<td>TP µg/l</td>
<td>126 - 440</td>
<td></td>
</tr>
<tr>
<td>NH$_4$ mg/l</td>
<td>0.014 – 0.184</td>
<td></td>
</tr>
<tr>
<td>Cl mg/l</td>
<td>51.0 – 105.0</td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$ mg/l</td>
<td>0.1 – 36.5</td>
<td></td>
</tr>
<tr>
<td>Temp</td>
<td>8.92 – 10.83</td>
<td></td>
</tr>
<tr>
<td>Specific Conductivity µS</td>
<td>545 - 630</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.61 – 6.90</td>
<td></td>
</tr>
<tr>
<td>Internode length (cm)</td>
<td>3.0 – 16.0</td>
<td></td>
</tr>
<tr>
<td>Internode diameter (cm)</td>
<td>0.3 – 0.82</td>
<td></td>
</tr>
<tr>
<td>Root Length (cm)</td>
<td>5.0 – 117.0</td>
<td></td>
</tr>
<tr>
<td>Petiole length (cm)</td>
<td>6.0 – 47.4</td>
<td></td>
</tr>
<tr>
<td>Leaf Area(cm$^2$)</td>
<td>0.946 – 29.801</td>
<td>Correlated with NO$_3^-$</td>
</tr>
<tr>
<td>leaves FW gm$^{-2}$</td>
<td>73.60 – 693.60</td>
<td></td>
</tr>
<tr>
<td>Leaves DW gm$^{-2}$</td>
<td>8.86 – 62.65</td>
<td></td>
</tr>
<tr>
<td>Petioles FW gm$^{-2}$</td>
<td>203.20 – 4,925.60</td>
<td></td>
</tr>
<tr>
<td>Petioles DW gm$^{-2}$</td>
<td>14.27 – 169.18</td>
<td></td>
</tr>
<tr>
<td>Stolons FW gm$^{-2}$</td>
<td>1,388.18 – 8,156.69</td>
<td></td>
</tr>
<tr>
<td>Stolons DW gm$^{-2}$</td>
<td>32.97 – 449.22</td>
<td></td>
</tr>
<tr>
<td>Roots FW gm$^{-2}$</td>
<td>549.93 – 9,102.88</td>
<td>Inversely correlated with SRP</td>
</tr>
<tr>
<td>Roots DW gm$^{-2}$</td>
<td>38.18 – 533.26</td>
<td></td>
</tr>
<tr>
<td>Whole Plant FW gm$^{-2}$</td>
<td>2,940.8 – 13,852.8</td>
<td></td>
</tr>
<tr>
<td>Whole Plant DW gm$^{-2}$</td>
<td>95.06 – 766.11</td>
<td></td>
</tr>
<tr>
<td>Specific Leaf Area cm$^2$ g$^{-1}$</td>
<td>1.189 – 5.414</td>
<td></td>
</tr>
<tr>
<td>Specific Root Length cm g$^{-1}$</td>
<td>0.372 – 8.210</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. A graph of TP(μg/l) (Y axis) against root length (cm) (X Axis) is shown below. Root length increases with decreasing TP, presumably because a greater surface area is required to assimilate sufficient P in low nutrient conditions.

Figure 2. A graph of TDN (mg/l) (Y axis) against root length (cm) (X Axis) is shown below. Root length increases with decreasing TDN, although there is a distinct shoulder above 4 mg/L TDN where root length is less sensitive to increasing nitrogen concentrations.
Figure 3. The relationship of leaf area to TP and TDN (Figure 4) is similar. The following graphs show an increase in leaf area with increasing TP (μg/L), and TDN respectively (mg/L).
This results in the general appearance of the plant in low nutrient concentrations of small leaves with long roots, and in eutrophic conditions of large leaves with short roots. It also means that larger plants with more reproductive tissues grow best in eutrophic conditions, probably resulting in more rapid spread in eutrophic conditions due to the plant architecture.

Total plant biomass does not vary widely between sites with different nutrient concentrations, as can be seen from Figure 5 of biomass (g) (x axis) plotted against TP (μg/L). The biomass of various components changes to compensate for lower values in other components. This strategy allows for domination of a wide variety of habitats, occupying space to exclude other macrophyte species.

**Figure 5. Total Plant biomass (DWt gm⁻²) (x axis) plotted against TP (μg/L).**

Soil Nutrient effects. Only a hand full scientific articles describe the characterization of the terrestrial growth conditions for *H. ranunculoides* in more detail and make an effort to quantify the growth under these conditions (Hussner and Losch, 2007; Hussner & Meyer, 2009). In these studies, the leaf area index (LAI), total dry weight, dry weight of leaves, petioles, shoots and roots, the total shoot length, the number of nodes, the total number of leaves and the average mat size between naturally occurring stands in habitats with low and high nutrient levels. It was concluded that with increasing nutrient content of the soil, all these parameters were higher than for stands in a habitat with a lower nutrient contents of the soil. The relative growth rate (RGR) varied from 0.005 g g⁻¹d⁻¹ under low nutrient supply to 0.132 g g⁻¹d⁻¹ under high nutrient supply systems. Light saturation of the species was reached at 800 mol photons m⁻²s⁻¹ under these conditions. From the list of required parameters to model the growth of *H. ranunculoides* with CHARISMA, parameter values were only available for: leaf conductance, LAI (Leaf Area Index), SLA (Specific Leaf Area), RGR (Relative Growth
Rate), internode length, gas exchange, total dry weight, relative dry weight allocated to leaves, relative dry weight allocated to petioles, relative dry weight allocated to shoots, relative dry weight allocated to roots, and maximum total shoot length.
**Table 4. Growth parameters of terrestrial Hydrocotyle ranunculoides under North-West European conditions**

<table>
<thead>
<tr>
<th>parameter</th>
<th>value</th>
<th>unit</th>
<th>area</th>
<th>remark</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaf conductance</td>
<td>0.4*</td>
<td>1.5**</td>
<td>mol m⁻¹s⁻¹</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td>LAI (Leaf Area Index)</td>
<td>4.36</td>
<td>5.47</td>
<td>-</td>
<td></td>
<td>(Hussner &amp; Lösch, 2007)</td>
</tr>
<tr>
<td>SLA (Specific Leaf Area)</td>
<td>330*</td>
<td>353**</td>
<td>cm² g⁻¹ dw</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td>RGR (relative growth rate)</td>
<td>0.078*</td>
<td>0.097**</td>
<td>g g⁻¹ dw d⁻¹</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td>internode length</td>
<td>3.04*</td>
<td>7.07**</td>
<td>cm</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td>gas exchange</td>
<td>18</td>
<td>μmol CO₂ m² s⁻¹</td>
<td></td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td>Total dry weight</td>
<td>79**</td>
<td>18*</td>
<td>g</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td></td>
<td>312.2 ± 31.5₅</td>
<td>532.4 ± 14.2₅</td>
<td>g m⁻²</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td>relative dry weight allocated to leaves</td>
<td>21*</td>
<td>22**</td>
<td>% of total dw</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td></td>
<td>22.78₅</td>
<td>19.55₅</td>
<td>% of total dw</td>
<td></td>
<td>(Hussner &amp; Lösch, 2007)</td>
</tr>
<tr>
<td>relative dry weight allocated to petioles</td>
<td>12*</td>
<td>21**</td>
<td>% of total dw</td>
<td></td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td></td>
<td>39.78₅</td>
<td>42.39₅</td>
<td>% of total dw</td>
<td></td>
<td>(Hussner &amp; Lösch, 2007)</td>
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<tr>
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<td>area</td>
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<td>reference</td>
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<td>----------------------------------------</td>
<td>-------------</td>
<td>-------------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------</td>
<td></td>
</tr>
<tr>
<td>relative dry weight allocated to shoots</td>
<td>low: 48*</td>
<td>high: 38**</td>
<td>% of total dw</td>
<td>Westphalia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Germany, North Rhein Westphalia</td>
<td>levels, highest value for high nutrient levels</td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td></td>
<td>low: 25.27$</td>
<td>high: 24.40$</td>
<td>% of total dw</td>
<td>Westphalia</td>
<td>lowest value for low nutrient levels, highest value for high nutrient levels</td>
</tr>
<tr>
<td>relative dry weight allocated to roots</td>
<td>low: 19*</td>
<td>high: 19**</td>
<td>% of total dw</td>
<td>Westphalia</td>
<td>(Hussner &amp; Meyer, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Germany, North Rhein Westphalia</td>
<td>levels, highest value for high nutrient levels</td>
<td>(Hussner &amp; Lösch, 2007)</td>
</tr>
<tr>
<td>maximum total shoot length</td>
<td>low: 141</td>
<td>high: 207</td>
<td>m shoot m$^{-2}$</td>
<td>Westphalia</td>
<td>lowest value for low nutrient levels, highest value for high nutrient levels</td>
</tr>
</tbody>
</table>

*) drained conditions  

**) waterlogged conditions  

This work was carried out on plants growing in contact with wet or dry mud, and used data for soil nutrients to compare growth rates for *Hydrocotyle* between these habitat types.
2.3. Areas at Risk

The current distribution of *H. ranunculoides* in the UK is shown in Figure 6.

![Figure 6. Current distribution of H. ranunculoides in the UK](image)

The increase in the number of locations during the previous 30 years is set out in the following figures (Figure 7). The rate is similar for all time periods, indicating that the rate of spread or introduction is similar. If we assume this is so, then the rate of new introductions will be approximately $150 / 24 = 6.25$ new sites per year.
Figure 7. New occurrences of *H. ranunculoides* prior to 1986, between 1987 and 1999 and between 2000 and 2010.

In order to predict areas at risk we used altitude data for known occurrences and extrapolated areas of the UK which matched available data. The only available data at the time of writing was for extrapolated altitude data, which while giving useful information about the altitude and slope of watercourses infested with *H. ranunculoides*, did not sufficiently accurately match climatic conditions. We are currently obtaining climate data with the aim of optimising the predictions based on day degrees, or other surrogate temperature matched systems.

Figure 8 on the following page shows areas shaded in pink that are at risk of colonisation by *H. ranunculoides*, based simply on altitude data. A more accurate figure is in preparation based on day degree data from the UK and the Netherlands.
Figure 8. Areas at risk of colonisation by H. ranunculoides
The recent distribution of *H. ranunculoides* in the Netherlands is shown in Figure 9.

*Figure 9. The distribution of *H. ranunculoides* in the Netherlands*
3. Ecology and Growth Modelling using CHARISMA

3.1. Objectives

- Review ecological literature on the life cycle of Hydrocotyle ranunculoides.
- Based on the available information come to a preliminary parameterization of the individual based macrophyte model CHARISMA (Van Nes et al. 2003).
- Gaps in published literature will be identified and attempts to gain information required for CHARISMA will be made.

Most literature on this species relates to geographical distribution or occurrence in the flora of particular states, territories or areas. This information, while useful for climate matching is not helpful in determining growth conditions as often physical and chemical data are not recorded, or not correlated with exact locations. Data on nutrient response are provided in section 2.1 of this document.

3.2. Life Cycle

A literature search was performed to understand the lifecycle of Hydrocotyle ranunculoides in North-West Europe. Several sources described the life cycle. H. ranunculoides, is probably native to South America, is an invasive aquatic weed in North Western Europe and several other countries worldwide, including Chile, Australia and Uganda. In an aquatic environment the species grows as an amphibious plant, rooting freely at the margins of watercourses, where the perennial nature of the species is perpetuated, and from which floating mats are formed, with very dense intertwined stoloniferous floating canopies, with petioles up to 40 cm long and very dense leaf canopies. Growth in Europe starts in early spring from small plants or fragments after the melt of ice either at the margins or from submerged material at the bottom of ditches, lakes and canals. These plants or fragments grow slowly in spring and form small leaves, which float flat on the water surface. With increasing temperatures, photoperiod and light intensity, leaves grow larger and petioles can reach a height of up to 40 cm above water level. The growth rate of H. ranunculoides is highest in June and July. The stems root freely from nodes at about 4-15 cm intervals. Flowering and fruit formation takes place between May and October. Flowers are hermaphrodite, white and grouped (5-10) together in a small umbel. Fruits are almost round and flat, brownish, with faint ribs and are divided into two halves. Floating Pennywort primarily reproduces vegetatively. With decreasing temperatures and light availability in autumn, smaller leaves are developed and some of the leaves will die due to night frost. At that time plants will form floating and submerged leaves. The latter are able to survive the low water temperatures during the winter. From these small submerged plants and leafless overwintering stolons plants will grow out again in spring.
Reproductive strategy

The species reproduces primarily by vegetative reproduction in Europe though spread by seed has been observed through sewage treatment works. It can regenerate even from small stolon fragments containing at least one node. It flowers in July–October in its native range. Chromosome number: 2n = 24. There is a wide range of polyploids within the genus *Hydrocotyle*, with up to 15-ploidy (Moore, 1971, Federov, 1974). Newman (unpubl.) found four distinct groups of *H. ranunculoides* in the UK population which can be separated by AFLP analysis. There is uncertainty about the extent to which different levels of ploidy within and between populations influences invasiveness.

Flowers are white in colour, small and held above the water in the axes of stolons and petioles on 5 – 15 mm stems, with a group of 5 – 15 individual flowers in an umbel. Although establishment by seed is suspected at Pevensey Levels (UK), the production of viable seeds in this site has not yet been observed. Seeds do form in European conditions, but seem not to mature, remaining white in colour rather than the brown of mature seeds in the Americas. This is probably due to cold temperatures at the time when seeds should mature in autumn. However, seed production is monitored regularly in nuisance populations. The relationship between the number of flowers and seeds m$^{-2}$ and nutrient concentration is under investigation.

In canal systems in which water is flowing very slowly, plant fragments can be expected to be transported downstream and to form patches at the side of these waterways, which are convenient places for the species to colonise. In the Netherlands, it occurs predominantly in flowing water that receives waste water from municipalities or agriculture. On most of these sites the species is spreading by vegetative growth only. Some sources report about different requirements for vegetative and generative growth. Under poor nutrient rich conditions vegetative growth will be relatively slow and generative growth will be promoted. Detailed data on the rate of growth is however lacking. Because vegetative growth is the main route of spread for pennywort, the species favours relatively open and nutrient rich waterways. Under these favourable conditions a vegetative growth of 20 cm per day has been reported (Newman et al. 2002).

Vegetative reproduction is thought to be favoured in both flowing conditions at the edge of the mat, and during late November through to January when the plants start to decompose and small fragments are produced in very large numbers. In a study undertaken in 1995, (Newman, unpubl) 100 nodes were taken from material collected in December 2004 and potted into damp compost in a glasshouse at 20°C with 16:8 day to night. The nodes were visibly brown with no green material. Out of 100 nodes, 99 developed into small plants after 21 days in these conditions. The regenerative capacity under favourable conditions is assumed to be close to 100%, and colonisation is inevitable in suitable habitats.
3.2.1. Life Cycle Diagrams:

The following diagrams illustrate the growth form through the seasons

*Figure 10. Hydrocotyle ranunculoides in Spring*

In spring, single stems grow from overwintering shoots. Leaves are usually below the water surface or lying flat on the water surface. Usually single stems grow from the bank.
In late Spring, the plant is usually well established and emergent stems start to grow from prostrate creeping stems. The stems usually have many branches by now, with creeping prostrate leaves at the edge of the mat, and emergent stems growing from further back for the stem tips.
Figure 12. *Hydrocotyle ranunculoides* in early Summer

Large clumps have developed by this stage, with predominantly emergent leaves and petioles. The clumps are usually distinct and only a few have joined up. Navigation between patches for control purposes is usually still possible.
Figure 13. *Hydrocotyle ranunculoides* in late Summer and Autumn (July – November)

All the separate mats have usually coalesced to produce complete coverage in channels of less than 15 – 20 m in width. In wider channels, often with faster velocities, growth may be restricted to the margins, as the limiting velocity for growth in the centre of the channel is usually exceeded. The limiting velocity is usually reached due to the presence of the dense marginal mats of *Hydrocotyle*, which tend to narrow the effective channel width and increase the discharge in the unimpeded channel area, restricting further growth of *Hydrocotyle*. However, fragmentation due to shear forces at the edge of the mat is usually increased, resulting in rapid spread within this type of large waterbody.
Winter growth form is determined by wash out of the floating mats after storm water surges. The mats become brittle and easy to break up physically when overnight temperatures are close to 0°C. The remaining vegetation is characterised as overwintering vegetation. Usually this takes the form of small rooted plants with leaves that become submerged under rising water levels, or of small leaved semi-terrestrial plants that survive in decaying marginal vegetation. The plants remain green and physiologically active throughout winter in this condition. The plants do not lose leaves or form specialised overwintering structures, they adopt a survival growth strategy that allows rapid regrowth when spring conditions allow.
3.3. CHARISMA Model development

The objective of this work package is to review ecological literature on the life cycle of *Hydrocotyle* and based on the available information come to a preliminary parameterization of the individual based macrophyte model CHARISMA (Van Nes et al. 2003). Gaps in published literature will be identified and attempts to gain information required for CHARISMA will be made.

The basis of this spatially explicit model is the seasonal cycle. Plants can survive the winter as shoots and as different types of overwintering structures. At a pre-set day in spring, growth is initiated and the plants get a certain amount of energy from the overwintering structures and an increasing amount from primary production. At a pre-set age, the macrophytes start allocating a part of their biomass to overwintering structures. At the end of the growing season, this part of the plants is transformed into biomass and the plants die off.

We used the literature review to parameterize the CHARISMA model. The model has been parameterized for *Potamogeton pectinatus*, *P. perfoliatus* and *Chara aspera* (Van Nes et al. 2002, 2003; Coops et al. 2002) and can readily be applied to *Cabomba*. These parameter sets will be used as basis. In discussion with partners we will come to a preliminary parameterization of the model. For *Hydrocotyle*, that has a different growth form, the aim will be to come to an appropriate design of the model.

For several growth parameters insufficient parameter values were available in the literature to run the CHARISMA model accurately. Most papers only give a general description of favourable or unfavourable conditions for *H. ranunculoides* growth. To be able to further adapt the model CHARISMA to accommodate for *H. ranunculoides* growth, this information should be obtained from experimental work.

3.3.1. The CHARISMA model

The CHARISMA model is an individual-based and spatially explicit model in which individual plants and overwintering structures are positioned on grid cells (figure 15). This allows modelling spatial ecological processes such as seed or tuber dispersal. CHARISMA allows modelling of 3D competition for light and nutrients between two or more aquatic plant species.

The model is based on a seasonal cycle. Plants survive the winter as shoots or overwintering structures. In the spring, growth is initiated from the energy from the overwintering structures. At the beginning of the summer, primary production and respiration determine plant growth. In the fall, individual plants start allocating biomass to overwintering structures. At the end of the growing season, shoot biomass is transferred to overwintering structures and the plants die off.
Figure 15. The CHARISMA model.

3.3.1.1. Growth form

The weight of plants is determined by a fixed root/shoot ratio. Part of the biomass during the growing season will be allocated to reproductive organs. The length of young shoots increases underwater proportionally with biomass. After reaching the water surface, there is a proportional increase of the biomass over the whole length of the plant. Optionally, a fraction of the biomass can be allocated to spread just under the water surface.

3.3.1.2. Growth rate and mortality

The daily growth rate depends mainly on the gross photosynthesis rate and the respiration rate. Mortality can be caused by high plant densities, wave damage, grazing, or seasonal die-off.

3.3.1.3. Environmental variables

The CHARISMA model allows you to modify a wide array of environmental variables such as light levels, temperature, turbidity, bicarbonate concentrations in the water and water levels (figure 16). Model parameters, including comments on significant gaps.
An exhaustive list of all the parameters that can be adjusted in CHARISMA is presented in the annex 1. Table 5 presents the different parameters that have been adjusted specifically for modelling the life cycle of *Hydrocotyle ranunculoides*.

![Seasonal cycle and environmental variables in the CHARISMA model](image)

**Figure 16. Seasonal cycle and environmental variables in the CHARISMA model**

### 3.3.2. Summary of available data for CHARISMA variables

Table 5 contains the different parameters that have been adjusted specifically for modeling the life cycle of *H. ranunculoides*.

The $P_{\text{Max}}$, the maximal gross photosynthetic rate at the plant canopy at 20°C in the absence of light limitation, has been adjusted for *H. ranunculoides* to 0.088/h, based on measurements of 3500 μmol of CO$_2$·h$^{-1}$·dry weight·g$^{-1}$ (Hussner 2009). The light compensation point ($h_{\text{PhotoLight}}$) was fixed at the default value (52 microE/m$^2$/s), as well as the plant $K$ (0.02 m$^2$/g). The respiration rate was fixed at 0.022/day (calibrated). The average root/shoot ratio (14%) has been calculated by compiling data from Hussner and Lösch 2007 and data collected in the field in September 2009 by Newman and Duenas (2011 in prep).

The seed biomass – 0.0039807g – was estimated from size measurements (1.3mm X 2.3mm) available from images of seeds from the USDA website (fig.1, USDA 2010).
The fraction of biomass allocated to seeds has been calculated from the following information. The number of inflorescences has been estimated on field to 218.5 inflorescences per m² (field data, Newman and Duenas September 2009). At the time of sampling, all inflorescences had immature fruits. According to Klemm et al (1993), each inflorescence has nine flowers. Each flower develops two fruits and each fruit produces two seeds, but just one is viable (Marchant 1987), which is consistent with Webster (1994) who found that around 50% of seeds are viable (which will be the SeedGermination parameter in CHARISMA). This allowed us to estimate 3933 viable seeds/m² or 7866 seeds/m² in total. This last number represents 2.5% of 1250.75 g/m² of total biomass (field data, Newman and Duenas September 2009).

The flowering and fruiting period has been fixed to May 1st – October 1st (Hussner and Lösch 2007) which is entered in the model as Age 30 for SeedStartAge, the beginning of seed production. Age 30 represents May 1st since the plants of *H. ranunculoides* start to grow on April 1st. The seed production ends on October 1st, five months later, when the plants are at age 180 (SeedEndAge).

From data in Hussner and Lösch (2007), we calculated 0.18g as the average dry weight of segments of one node (including leaf-petiole-shoot-root weight). In CHARISMA, regeneration fragments (any vegetative wintering structure) will be considered as a tuber. Thus 0.18g will be considered the average TuberBiomass in the model. 0.18g will also be considered as the “frond weight” (WeightFond) as *H.ranunculoides* is modeled as a floating plant in CHARISMA, which was based on *Lemma* species (default floating species).

The fraction of biomass allocated to tuber has been fixed to 38% (TuberFraction).This percentage has been estimated from the % of biomass allocated only to root and shoot (NOT to leaves and petioles) from Hussner and Lösch 2007. This is considering that in the winter, most of the leaves died at the first night frosts in Germany (Hussner and Lösch 2007), although leaves take longer to die in UK conditions, with at least three night air frosts required for leaf death in Reading, UK (Newman, pers. obs.).

The germination day of the overwintering fragments (TuberGerminationDay) has been fixed to April 1st, while the seeds germinate two weeks later on April 15th (SeedGerminationDay). This is to represent the advantage that overwintering structures have on seeds: an earlier germination because overwintering structures are already photosynthetic structures. 100% of the biomass of the overwintering fragments (cTuber=1) turns into photosynthetic shoots on April 1st, the germination day – the fragments are not storing any resources as oppose to tubers which release only a proportion of their biomass everyday for the production of shoots. Because *H. ranunculoides* starts producing only at the end of the growing season biomass that will overwinter, we decided to set the age of the plant when it starts to make overwintering structures on September 1st (at age 153 days=5 months, May-September). The end of the production of fragments, October 1st (at age 182 days=6
months, May-October), is also the end of the growing season (reproDay) which represents the day when seeds and tubers are dispersed.

We also decided for the duration of the simulations not to have any import of new seeds (seed import=0) nor fragments (tuber import=0).

**Table 5. Values of the parameters adjusted for modelling the growth of Hydrocotyle ranunculoides**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>value</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMax</td>
<td>0.088 /h</td>
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</tr>
<tr>
<td>hPhotoLight</td>
<td>52 microE/m2/s</td>
<td>default</td>
</tr>
<tr>
<td>PlantK</td>
<td>0.02 m2/g</td>
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<tr>
<td>Resp20</td>
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</tr>
<tr>
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<td>Day#274</td>
<td>#day of dispersal (seeds and tubers): October 1st</td>
</tr>
<tr>
<td>SeedGerminationDay</td>
<td>Day#105</td>
<td>April 14th</td>
</tr>
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<td>weight of one fragment</td>
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<tr>
<td>Weight Fond</td>
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</table>
3.3.3. Results

After adjusting in the model all the required parameters, we ran simulations of the growth of \textit{H. ranunculoides} over time with and without other competing species (\textit{Chara aspera, Potamogeton pectinatus}).

The aim of the first set of simulations without competition was to understand better the growth patterns of \textit{H. ranunculoides} within the seasonal cycles. Because of its high photosynthetic rate, the species can grow to high densities up to more than 2 kg/m$^2$ (figure 17a). This corresponds to 2 times the value of 1250.73 g/m2 from the data collected on the field in September 2009 (Newman and Duenas). Because this seems to be unrealistic, we ran again the same simulations but this time with a nutrient limitation for the growth of \textit{H. ranunculoides} (half-saturation of nutrient concentration of growth: 0.4 mg/L). The species then grows up to 1200 g/m2 (figure 17b), which is very close to what was observed on the field.

\textit{Figure 17. Simulations of the growth of \textit{H. ranunculoides} over ten years without competition. a) with no nutrient limitation. b) with a nutrient limitation. The red line is the shoot biomass, the blue line the wintering biomass (‘tuber biomass’ in CHARISMA’) and the green line, the seed biomass.}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig17a.png}
\caption{Fig 17a}
\end{figure}
Fig. 17b
We then performed simulations of the growth of *H. ranunculoides* with two competing species, *Chara aspera* and *Potamogeton pectinatus* on a 10 years period. We can see that *H. ranunculoides* completely out-competes the other species and dominates the macrophyte community from the very first growing season (figure 18).

![Figure 18. Simulations of the growth of Hydrocotyle ranunculoides with competition by Chara aspera and Potamogeton pectinatus over ten years.](image)

### 3.4. Discussion

**Model Accuracy**

The model accurately predicts relative maximum biomass measurements made in the field for both high and low nutrient status, although the biomass achieved in low nutrient conditions was $515 \pm 196$ g dry weight m$^{-2}$, about half of the predicted biomass. This may be at the range of biomass values as data are from only one site. The data on biomass were modelled using data from summer 2008, and peak biomass in the high nutrient reflect these data, however, when data from the end of season is used, a maximum biomass of $643 \pm 120$ g dry weight m$^{-2}$ was only achieved. This may be due to inaccuracies of photosynthetic rates for this type of habitat, or difference in loss rates due to weather and water velocity conditions. Further work is required at different times of the year to assess the effects of nutrients on maximum plant biomass and biometrics.
The ability of the model to predict overwintering biomass is also limited, due to lack of reported data on this aspect. This is partly confused by different overwintering strategies between sites, with some occupying riparian vegetation overwinter, and some plant remaining small under the ice.

Effects of Management

The model was not asked to predict the effects of management on the species. There are a number of effects that could have influenced the outcome of the model.

- Cutting and fragmentation. Fragmentation is known to occur after mechanical cutting operations. This usually reduces biomass to less than 5% of that before the operation. It is not known what percentage of the cut and collected 95% is lost as fragments. However, if we assume that all fragments with a node are viable, and that about one third of stolon fragments will have nodes, and assume that about 1% of the cut material is lost as fragments, then the amount of plant material available for recolonisation would be about 5 – 20 g per square metre, equivalent to about 20 – 80 nodes per square metre. Each one of these will grow to form a new plant as demonstrated by Newman (unpubl.), resulting in a very high propagule pressure at times independent of natural reproductive processes. Further work is required on viable fragment numbers released during management activities in order to model population response.

- The slightly different maximum biomass values produced by the model and the actual reported values are not of great concern. The model accurately predicts rapid colonisation and dominance of habitats, which is observed, and exclusion of other species in a relatively short time period, which is also observed. What is not really known, and what has not been modelled, is the potential to produce reproductive fragments under different nutrient conditions. We have assumed that propagule pressure will be the same and independent of nutrient status and in any further developments this aspect needs to be addressed.
4. Management

4.1. Objectives

The objective of this work package is to derive from the results of Work packages 2 and 3 new practical control options that can be tested by water boards and other bodies involved in management of surface water.

Since chemical weed control in an aquatic environment is extremely restricted in the Netherlands and because the results should be of practical use for both NL and UK, and other EU countries, the practical control options will focus on prevention and non-chemical methods. Data on suitable habitat characteristics and life cycle will be used to:

- Indicate how and when in the season the colonization of Hydrocotyle ranunculoides can be prevented or markedly restricted by influencing the growth requirements (substrate, light conditions and other environmental characteristics that result from Work package 2).

- Conceptualize physical control options that combine a very high control efficacy with a minimum dispersal of vegetative parts of the aquatic weed. The current mechanical control options generally strongly induce further vegetative reproduction.

- Test at least one concept management option output under practical field conditions.

4.2. Control Methods in the Netherlands

4.2.1. Daily practice

Since the introduction of H. ranunculoides in the Netherlands and Belgium, several organisations have made an effort to control the species in their management area. All of them apply both mechanical and manual control. Mechanical control is performed when large floating mats over large areas are formed. During the mechanical control fragmentation and further spread of the vegetative parts is prevented. This prevention takes place by shielding off the treatment area during the control activities and by manual removal of small floating fragments shortly after mechanical control. The mechanical control that is used does not make use of cutting devices, only machines that grab the floating masses and pull them onto land are used. Cutting is avoided to prevent fragmentation. Manual control is used in areas where a small amount of H. ranunculoides is present or in areas that are vulnerable for disturbance such as nature areas. The workers are instructed to remove all plants, also the root systems should be removed. However, at the same
time managers instruct sub-contractors not to disturb the soil and avoid clouding of the water. These two instructions seem to be contradictory. Often, the payment of the subcontractor is dependent on the water quality after control and the complete removal of the plants. However, the first is easier to control than the second, and the expectation is that not all plant material will be removed. In all cases, large amounts of material are removed, several tons per year in each district. However, this does not result in a decrease of the species in the next season. The plants are able to regrow from the root pieces left behind and plants remaining in the bank vegetation. One of the water boards performed an experiment in which the soil of the waterway and river banks was removed to a depth of 11 cm. However, eleven weeks after treatment, the plant showed regrowth again. The species has been described rooting to a depth of 15 cm (Ruiz-Avila & Klemm, 1996). This can explain why the species was able to regrow relatively quickly after removal of the top 11 cm of the substrate, although in general rooting depth is probably related to soil structure with deeper rooting is sandy and silty soils than on clay soils. Regrowth took twice as long in that experiment than after regular mechanical removal in the area, which usually occurs within 6 weeks during late spring and summer.

The frequency at which mechanical and manual removal is performed differs between waterway and year. The frequency is usually dependent on the magnitude of the problems in the waterway and the available time to remove the plants. Table 6 gives an example of the frequencies, removed material and costs. The costs per waterboard (the Netherlands) or province (Belgium) to control *H. ranunculoides* varies from € 96.000 to € 168.000 per year.
Table 6. Examples of control frequency in several districts in the Netherlands in 2009. Source: personal communication Dutch Waterboards.

<table>
<thead>
<tr>
<th>District</th>
<th>Frequency</th>
<th>Tons material</th>
<th>Annual Costs €</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 times, of which 3 times mechanical and 4 manual</td>
<td>78 wet material</td>
<td>22,776</td>
</tr>
<tr>
<td>2</td>
<td>4 mechanical treatment, supplemented with manual control once a month</td>
<td>50 wet material</td>
<td>10,000</td>
</tr>
<tr>
<td>3</td>
<td>18 mechanical treatments from week 35 until 49.</td>
<td>172 wet material</td>
<td>134,800</td>
</tr>
<tr>
<td>4</td>
<td>1 mechanical treatment, several manual removal treatments of small fragments</td>
<td>6 wet material</td>
<td>unknown</td>
</tr>
</tbody>
</table>

Some waterboards have tried to control the species with liquid nitrogen. The experiments received some attention in the local press, but a description of the treatments or results were never reported in official documents. The idea of the liquid nitrogen is that it controls all cells that are exposed to the liquid nitrogen. It will not have a systemic mechanism and will not control plant parts below the water surface. The waterboards that tried these treatments were not satisfied with the results and will not repeat the treatments.

4.2.2. An outlook to the future

At the moment, a combination of mechanical and manual removal in combination with environmental management options has most promise for a new control program for *H. ranunculoides* in the Netherlands (UK and other EU-countries). Environmental management options such as the alteration of the nutrient availability of the habitats and the adjustment of the water level may be options to combine with the current mechanical and manual control programmes. However, to be able to combine these management options, we need knowledge of their individual effects on the growth and spread of *H. ranunculoides*. As we show in chapter 2, little is known about quantitative effects of nutrient levels on the growth rate of *H. ranunculoides*. We now simply know that the species favours nutrient rich conditions. We do not now the minimum amount of NH₄-N, NO₃-N, P₂O₅ and P₂O₅-
$P_{\text{tot}}$ required for the growth of *H. ranunculoides*. As a result, we do not know what the maximum nutrient levels are that can be tolerated in infected waterways to prevent the species from its rapid growth and spread. The same is true for the water level. We know that the water level influences the growth of the species, but we do not know what the effect of the water level is for an extended period. Growth will be reduced by a low or very low water level, but we do not know the magnitude of the effect, nor the influence of the growth season (plant stage) in which the water is lowered. Effects in winter or summer can be quite different.

Based on the few data we have, we can conclude that several management programmes can be tested to answer some or all of these questions. These management programmes should at least include various frequency levels of mechanical and manual control, several water levels during the growth season and winter, various flow rates of the current, and several nutrient levels, both in the substrate as well as the water. As parameters for the control efficacy the percentage cover and the biomass of the *H. ranunculoides* should be used.

Another option to include in the management programmes could be control with Hydrogen peroxide (H$_2$O$_2$). Hydrogen peroxide is registered as a crop protection product (www.ctgn.nl) to control bacteria, yeast and fungi. Hydrogen peroxide breaks down into water and oxygen (2H$_2$O$_2$ $\rightarrow$ 2H$_2$O + O$_2$) and has a half life of several hours up to days, depending on the presence of micro-organisms and temperature. It is known that the photosynthesis and assimilation of terrestrial plants is reduced after exposure to hydrogen peroxide. However, there are no data available on the duration of the exposure or the dosage, or on the effects on aquatic plants. Algae are known to be sensitive to solutions of hydrogen peroxide. A small trial was performed by one of the water bodies in the Netherlands (figure 19) in which *H. ranunculoides* was exposed to solutions of hydrogen peroxide. *H. ranunculoides* plants were collected during the winter from a natural stand and some weeks prior to the experiment placed in three plastic containers at 10-15°C in water from the natural stand. During these couple of weeks the plants started to produce new leaves. In an undefined growth-stage the natural water was removed from the containers, leaving the plants without water. Directly after this removal, 200 ml of a 3% hydrogen peroxide solution was added to one of the containers. To the other two containers 200 ml water and 1 L water were added. The 200 ml water treatment served as a control, the 1 L water treatment was used to investigate the possible effects of low (200 ml) water levels. Directly after treatment, the plants treated with the water peroxide started to wilt. A few days after treatment, the plants did not recover from this treatment. No difference between the containers with 200 ml and 1 L water was observed.

Next to this container experiment, *H. ranunculoides* was exposed to droplets of a 30% hydrogen peroxide solution. In total 100 ml of the solution was sprayed over a 10-L bucket filled with water from the natural stand and *H. ranunculoides* plants that were removed and stored according to the same conditions as in the container trial. At the moment of treatment, approximately 50% of the plant was present above the
water surface. Within a couple of days 40% of the leaves died. No recovery was observed during the week after treatment.

We cannot make proper conclusions from this small trial, without replications and a poor description of the treatments, origin and growth stage of the plants and short period in which recovery was observed. However, it might be a good option to investigate the possible use of hydrogen peroxide in a proper experiment to determine the proper dosage, way of exposure, and recovery.

![Figure 19. H. ranunculoides plants treated with 200 ml water (left), 200 ml 3% hydrogen peroxide solution (middle) and 1 L water (right).](image)

4.3. Control Methods in the UK

4.3.1. Environmental control

There are several methods that may be used, none of which give a complete solution. Shade may be an effective method of control as the plant does not establish well in shaded conditions, and is best achieved by planting trees on the
south side of the water body. This is unlikely to be practical to implement on larger water bodies. Increasing flow will restrict the growth of *H. ranunculoides* in situ but may increase the spread of the plant downstream. Increasing rooting depth to below 1 metre may reduce the ability of *H. ranunculoides* to root at the margins. This, however, is unlikely to be a feasible option. Reducing the amount of suitable rooting substrate by piling or preventing access to suitable areas by netting off sections may prove effective. All these environmental options are likely to be expensive to implement and are untested.

4.3.2. Non Chemical control

*H. ranunculoides* can be cut with weed cutting buckets or boats. These techniques will only offer a short-term reduction in the local extent of the plant, as it is capable of growing back rapidly from single nodes. Re-cutting will be necessary throughout the growth season. However, without thorough removal of all cut material the inevitable spread of the plant within the watercourse will be exacerbated. Considerable release of viable fragments occurs after mechanical removal and manual hand picking of these is necessary to prevent rapid recolonisation.

The success of mechanical control is dependent on timing and habitat type. When control is practiced before the end of August, regrowth is rapid from any fragments that are inevitably left behind. Mechanical removal from September onwards gives much better control because regrowth is much less vigorous. However, unless all fragments are removed by follow up hand removal, regrowth will occur in the following spring. Regrowth usually takes the form of growth from isolated fragments which grow rapidly and coalesce into dense mats, often covering 100% of smaller channels. There is no evidence that continued mechanical control will lead to eradication of this species, and mechanical control can only be effective as an annual treatment.

In rivers, as opposed to flowing drainage channels (c.f. River Soar vs. Pevensey Levels), mechanical control is much more effective. This is probably due to the flow causing fragments to be washed downstream without remaining in the cut area. Considerable success has been achieved in the River Soar with intensive mechanical removal programmes during 2008 and 2009 (Harding, 2010), followed up by herbicide treatment. In terms of cost, herbicide treatment has been shown to be about 40% of the cost of mechanical control (Harding 2011).

Where cutting is deemed appropriate, the risk of downstream infestation should be carefully considered. Mechanical removal can be practised to reduce the biomass for subsequent chemical treatment and to ease access for herbicide application, especially in where dense mats are present.

A better option is to remove as much of the plant biomass as possible and then to go over the area handpicking the remaining fragments. This technique has eradicated the plant for the upper reaches of the River Chelmer in Essex and the River Cam in Cambridge.
4.3.3. Chemical control

Herbicides containing glyphosate work well on this plant, but they must be used in conjunction with the aquatic approved adjuvants TopFilm or Codacide Oil. The spraying programme is presented in Table 1. The herbicide most often used for control is Glyphosate (usually the product with the lowest ecotoxicological profile, e.g. Roundup pro Biactive 360 g/L formulation), combined with either TopFilm before August, or with Codacide oil afterwards.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyphosate herbicide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>2,4-D amine herbicide</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>TopFilm (Adjuvant)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Codacide Oil (Adjuvant)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

James *et al.* (2011) showed no significant differences between the impacts on invertebrates of either glyphosate or 2,4-D amine compared with control effects, although data were collected before mechanical control and then after chemical application (without differentiating the effects of mechanical control alone), which, as no differences were detected between herbicide effects, is expected to have the highest impact on invertebrate communities in mechanically managed watercourses.

Decomposition of the remaining plant material is often slow, as *H. ranunculoides* typically forms extensive beds, and may take as long as six weeks in slow flowing water bodies. As *H. ranunculoides* forms such thick beds of vegetation conventional spray applications may not reach all the leaves at the first attempt. Small leaves under the main canopy may be shaded from the herbicide by those above leading to incomplete control and a source from which the plant will regrow.

It is therefore essential to plan a follow-up treatment in any chemical control programme which allows spot treatment or removal by hand, of any remaining stands of *H. ranunculoides* about 2 to 4 weeks after the first herbicide application. Although the growth of *H. ranunculoides* is noticeable throughout the season (it may completely cover small slow flowing channels or ditches in the late summer) it does not usually reach nuisance proportions on larger water bodies until later in the summer or early autumn, with the peak growth starting in early July. However, treatment earlier in the season will reduce man-hours, equipment and chemicals needed to control the weed at a later date. Agreement must be obtained from the local Environment Agency office before application of herbicides in, on or near controlled waters.
4.3.4. Best practice

For effective long-term control of *H. ranunculoides* a thorough control programme mechanical removal followed up by intensive hand-picking or chemical treatment of remaining patches or fragments

should be used. Where physical or mechanical cutting techniques are deemed necessary the affected areas should be fenced off to prevent the downstream spread of the plant. All cut foliage should be removed from the water body. Physical control methods are likely to have little effect other than a short-term reduction in the local extent of the plant. It is very difficult to completely control this plant and it may prove impossible to eradicate it in areas where it has formed extensive stands. Remove this plant as soon as it is observed.

Work undertaken by the Environment Agency on the River Soar (Harding 2011) showed that targeted herbicide application was just as successful as mechanical control at about half the cost, on both complete mats and when using application after mechanical control.

4.4. Experiences from around the world

Various methods are used throughout the world, appropriate to prevailing conditions and regulations in place at the time.

In Australia a two-weekly control program was started shortly after the explosive growth and spread of floating pennywort after its introduction to the Swan River in 1991 (Ruiz-Avila & Klemm, 1996). Every two weeks the floating pennywort mats were manually cut into smaller pieces with large knives. These pieces were collected and pulled onto the banks. At the start of this control program, an estimated 175 tons of *H. ranunculoides* was present in the area. After one year the amount of pennywort was estimated at 420 tons. Although the control seemed effective in the growth season, the cutting resulted in a further spread of the pennywort in the area. In 1993 two new control programmes were started: a short term control program and a long term control program. The applied techniques in the short term program were almost identical to the first program of 1991. In addition to the removal of the large mats, glyphosate (Round-up) was applied at the remaining plant sites in the banks. The aim of this treatment was prevention of regrowth from marginal plants. Glyphosate was chosen because of its systemic mechanism. Roots of *H. ranunculoides* can reach a depth of 15 cm and a systemic compound can affect these plant parts as well. The applied dosage was 360 g active ingredient per ha. In 1994 the dosage was increased to 450 g active ingredient per ha. The same treatments were performed in the long term program, but now supplemented with ecological techniques. These techniques were aimed at the reduction of the nutrient supply to the waterways and the removal of the nutrient rich sediment. The short term program was not effective enough. The treatments with glyphosate could not
prevent regrowth from the marginal plants, at least not at the applied dosages. The results of the long term program are not published.

### 4.5. Prospects for biological control

Natural enemies are not present in the areas where *H. ranunculoides* is introduced (EPPO, 2006), although the presence of a phytoplasm associated with *H. ranunculoides* on the River Soar ion Leicestershire, UK is of great interest (Harding, 2011). The absence of plant predators is probably one of the reasons for the rapid growth and success of the species after introduction. Biological control agents are currently not used in areas where floating pennywort has been introduced. Worldwide 75 *Hydrocotyle* species are described, of which one is known to be native to Europe: *H. vulgaris* (Sheppard et al., 2006). However, in 1982 a beetle, native to South-America, was described as a possible biological control agent: *Listronotus elongatus* (Cordo et al., 1982). Adults cause damage to the upper leaf surface and lay their eggs in the petioles. The larvae move down into the stolons over a distance of approximately 15 cm. The result is that the leaves start wilting and eventually die. After some time, the infected stolons sink. CABI and the CEH collected adults of *L. elongatus* in the winter of 2005 and performed laboratory experiments in Great Britain in 2006 to determine the host status of several related native species, including *H. vulgaris* (described in Newman, 2009). In those experiments, adult beetles were offered either *H. ranunculoides* or *H. vulgaris*. *H. vulgaris* was found to be a poor host to the beetle. Experiments in which *L. elongatus* adults were allowed to choose between several plant species showed that it strongly prefers *H. ranunculoides* as a food source. Oviposition tests showed that adults did not lay eggs on *H. vulgaris*. However, the larvae were able to feed on this species, after transfer to its leaves. Before a biological control program can be started, a broader host status screening is required (Newman, 2009; Sheppard et al., 2006).

Other natural enemies of *H. ranunculoides* that were found in CABI in Argentina include two *Cercospora* like fungi (these require further determination) and a stolon mining fly (*Eugarix* sp.).
5. Decision Support System

5.1. Objectives

The objective of this work package is to derive from the current practices in aquatic weed management and from the results of Work packages 2, 3 and 4 a prototype DSS. This DSS will be disseminated amongst bodies involved in water management for review.

The DSS will permit the application of best practice derived from fundamental understanding of the ecology and growth strategy of the species in question, and the application of existing management techniques to achieve optimum control.

Can you identify the species?

No

Yes

Assess the risk of spread
Use risk assessment protocols

Low Risk
Low Priority

Medium Risk

High Risk
Low Priority

High Risk
High Priority

If the species is not part of the Declaim project, please contact a local expert to confirm identity

Use the Field ID sheet or Office Guide to confirm identity

Isolation must take place as soon as possible after the species has been noted and should remain in place until after the plant has been eradicated.

This situation represents a greater risk to watercourse function and to the ecosystem of the ponds. The infestation has probably been present for at least one year and has completed a life cycle. The ability to spread is demonstrated by the occurrence of more than one patch in different parts of the watercourse and action should be taken to remove as much as possible.

There are several large and small patches spread within a drainage system, spread over a large area, in different parts of the channel and in nearby ponds. The sections can be isolated and there are no critical watercourse functions at risk.

There are several large and small patches spread within a drainage system, spread over a large area, in different parts of the channel and in nearby ponds. The sections cannot be isolated and there are critical watercourse functions at risk.
5.2. Information for use in the Field

5.2.1. Identification

*Hydrocotyle ranunculoides* (Floating Pennywort)

**Field Recognition Guide**

*Preferred habitat:* most often in slow flowing or static shallow watercourses, ponds, ditches, but also at the margins of larger rivers. The plant visible from April onwards

*Key features:* leaves kidney shaped with single slit to petiole, usually flaccid, between 5 and 15 cm in diameter

*Reporting:* Line Manager. Non Native Species Recording Program, at: https://secure.fera.defra.gov.uk/nonnativespecies/index.cfm?sectionid=75

*Further Action:* Assess the risk of the population you have observed using the risk assessment sheet provided in this pack.
5.2.2. Risk assessment

**LOW RISK**
The occurrence is limited to a few square metres at one location.

**ACTION:**
1. Inform appropriate manager, authority, national organisation
3. Ensure no fragments get out of the side channel into the main river, or spread to nearby ponds.

**MEDIUM RISK**
There are several small patches of less than ten square meters spread within a short distance, but in different parts of the channel and in nearby ponds.

**ACTION:**
1. Inform appropriate manager, authority, national organisation
3. Ensure no fragments spread form the small patches by isolating side channels or retaining patches in large channels by using floating barriers etc.
4. Monitor for regrowth at 4 week intervals
HIGH RISK – LOW PRIORITY
There are several large and small patches spread within a drainage system, spread over a large area, in different parts of the channel and in nearby ponds. The sections can be isolated and there are no critical watercourse functions at risk.

**ACTION:**
1. Inform appropriate manager, authority, national organisation
2. Arrange to isolate the section from water movement for as long as possible.
3. Arrange for treatment by manual / hand picking of small patches, mechanical removal of large patches followed by hand picking or by herbicide treatment
4. Ensure no fragments spread form the small patches by isolating side channels or retaining patches in large channels by using floating barriers etc.
5. Monitor for regrowth at 4 week intervals

HIGH RISK AND HIGH PRIORITY
There are several large and small patches spread within a drainage system, spread over a large area, in different parts of the channel and in nearby ponds. The sections cannot be isolated and there are critical watercourse functions at risk.

**ACTION:**
1. Inform appropriate manager, authority, national organisation
2. Arrange for removal by manual / hand picking and by any means possible. Try to contain each patch to prevent fragmentation and spread.
3. Protect pumps, sluices and other structures from becoming blocked by vegetation.
4. Ensure no fragments spread form the small patches by isolating side channels or retaining patches in large channels by using floating barriers etc.
5. Monitor for regrowth at 4 week intervals
5.3. Information for use in the Office

This information is produced as a separate document, both in printed form and in PDF form for access via the website http://www.q-bank.eu/Plants/. Scroll for header: Control

*Hydrocotyle ranunculoides* L. *f.*

A guide to Identification, Risk Assessment and Management
Background and Ecology

What is it?

Hydrocotyle ranunculoides L.f, the Floating Pennywort, is a native of North America but has become naturalised in Central and South America and also occurs in the Netherlands and in southern mainland Europe. It was first brought to Europe in the 1980’s by the aquatic nursery trade to sell as a plant for tropical aquaria and garden ponds. The first note of concern over its potential to become a weed was published in 1936 (Mathias, 1936).

Reproduction is thought principally to be asexual and vegetative in northern Europe, and the plant is capable of forming extensive mats from the smallest root fragment, although introduction by seed may have occurred in at least two sites through sewage treatment works. In Australia, H. ranunculoides doubles its biomass in 3 days, and in the UK doubling times vary between 4 and 7 days in summer, depending on the availability of nitrate and phosphate. The plant exhibits a seasonally variable growth rate in the UK, with maximum growth in the late summer when it typically forms extensive floating mats of vegetation. It overwinters in the margins below the water surface and as an emergent on banks as a much flatter and smaller plant.

Where does it grow?

H. ranunculoides roots in the shallow margins of slow-flowing water bodies, particularly ditches, slow flowing dykes, canals and lakes and forms dense interwoven mats of vegetation which can quickly cover the water surface interfering with the ecology and amenity uses of the water body. Under European conditions, mats of vegetation have been observed to grow up to 15 metres from the bank in a single season, growing at approximately 20 cm per day.

Morphological description

Amphibious plant, glabrous, up to 40 cm tall. Stem creeping or floating, rooting at nodes. Leaves alternate, held on long fleshy petioles, not peltate, almost circular to kidney shaped, shallowly to deeply 3-7 lobed, lobes rounded, crenate or lobulate and subequal, (20-)40-100(-180) mm diameter. Flowers 5-10(-15), up to 3 mm diameter, grouped on a short stalk in the axil of a leaf, sepals absent, petals 5, white. Fruits suborbicular and flat, divided into 2 halves with a persistent style.
Leaf Details

Usually 8 – 10 veins with small pale spot in centre of leaf. Deeply cut to point of insertion at petiole, often 5 – 6 lobed overlapping at margin, pale to dark green, thin leaves, never succulent.
Flower Details

Small umbels of 5 - 15 florets held above water on short stalks, 5 – 15 mm.
Not to be confused with:

<table>
<thead>
<tr>
<th>Hydrocotyle vulgaris</th>
<th>Hydrocotyle umbellata</th>
<th>Ranunculus spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General Appearance</strong></td>
<td>On damp mud, rarely in water</td>
<td>General Appearance</td>
</tr>
<tr>
<td><strong>Leaf detail</strong></td>
<td>Never larger than 50mm, leaf not split to petiole, normally 9 – 12 lobed</td>
<td>Normally 12 – 14 lobed</td>
</tr>
<tr>
<td><strong>Flower Detail</strong></td>
<td>Flower stalks longer than petioles</td>
<td>White flowers with yellow centre, held above water</td>
</tr>
</tbody>
</table>
Life cycle

*Hydrocotyle in Spring*

In spring, single stems grow from overwintering shoots or nodal fragments. Leaves are usually below the water surface or lying flat on the water surface. Usually single stems grow from the bank.

**Management Restrictions:** There are restrictions on mowing, dredging re-profiling and cutting between the middle of March and the end of May, and these activities are not recommended between June and the middle of July.

**Action:** Manual removal of small colonies is possible at this stage, but mechanical control using excavators to remove plant material and topsoil is also possible. Chemical treatment using glyphosate mixed with TopFilm is possible at this stage, but only when leaves are floating at the surface. Retreatment will be necessary. See chemical control section later in this document.
In late Spring, the plant is usually well established and emergent stems start to grow from prostrate creeping stems. The stems usually have many branches by now, with creeping prostrate leaves at the edge of the mat, and emergent stems growing from further back for the stem tips.

**Management Restrictions:**
There are restrictions on mowing, dredging re-profiling and cutting between the middle of March and the end of May, and these activities are not recommended between June and the middle of July.

**Action:** Manual removal of small colonies is still possible at this stage, but mechanical control using excavators to remove plant material and topsoil is recommended. Follow this up by manual checking and removal of any remaining fragments or stems. Chemical treatment using glyphosate mixed with TopFilm is possible at this stage, but retreatment will be necessary. Chemical treatment now is usually more effective than earlier treatment, although is earlier spray have been applied, a retreatment will usually be necessary again. An early application of herbicide tends to delay the climax state of biomass, and may delay this for up to 6 weeks, allowing additional effective management activity later in the season. The main aim of early control is to allow further control. See chemical control section later in this document.
Hydrocotyle in early Summer

Large clumps have developed by this stage, with predominantly emergent leaves and petioles. The clumps are usually distinct and only a few have joined up. Navigation between patches for control purposes is usually still possible.

**Management Restrictions:** There are no restrictions on mowing, dredging re-profiling and cutting should only be undertaken after the middle of July. Please be aware that cutting has no long term effect on this species and may assist spread. If this is the only method available then be aware that repeat cutting operations will be required at least twice a year every year.

**Action:** Manual removal of small colonies is usually no longer possible at this stage, with biomass reaching between 20 and 30 kg m⁻² but mechanical control using excavators to remove plant material is recommended. Follow this up by manual checking and removal of any remaining fragments or stems. Chemical treatment using glyphosate (at least 1.8 kg ha⁻¹) mixed with TopFilm (1.2 l ha⁻¹) is very effective at this stage, but retreatment may be necessary if new growth occurs. Chemical treatment now is usually more effective than earlier treatment, although if an earlier spray has been applied, a retreatment will usually be necessary again. See chemical control section later in this document.
All the separate mats have usually coalesced to produce complete coverage in channels of less than 15 – 20 m in width. In wider channels, often with faster velocities, growth may be restricted to the margins, as the limiting velocity for growth in the centre of the channel is usually exceeded. The limiting velocity is usually reached due to the presence of the dense marginal mats of *Hydrocotyle*, which tend to narrow the effective channel width and increase the discharge in the unimpeded channel area, restricting further growth of *Hydrocotyle*. However, fragmentation due to shear forces at the edge of the mat is usually increased, resulting in rapid spread within this type of large waterbody.

**Management Restrictions:** There are no restrictions on mowing, dredging re-profiling and cutting should only be undertaken after the middle of July. Please be aware that cutting has no long term effect on this species and may assist spread.

**Action:** Control at this stage can either be mechanical or by herbicide, although the risk of deoxygenation is very high at this stage and treated patches should be separated by the same length of the treated section, usually 500 m maximum, to avoid deoxygenation of the watercourse. This prevents rapid control and therefore mechanical removal followed by chemical control or manual removal is more effective in reducing biomass fast enough to achieve satisfactory management levels.
Management Techniques

Manual Hand Picking

This technique should be used when patches are small in early spring and as a tidying up technique after mechanical or chemical control later in the season. It is an essential part of an eradication campaign as control cannot be achieved by either gross mechanical removal or by herbicides alone.

Mechanical Control

Mechanical control can be either by using an excavator equipped with a cutting bucket, or by using weed boats with rakes. Excavators tend to cut the fringe on the opposite bank, unless dredging and removing topsoil with the bucket, leaving viable fragments that regrow very rapidly. Manual hand picking should follow any mechanical control technique. Weed boats are more often used in Holland, raking the mats out of intermingled marginal vegetation. However, even this technique leaves viable fragments which must be removed to prevent or severely restrict any regrowth.

<table>
<thead>
<tr>
<th>Weed Boat – raking margins</th>
<th>Excavator with cutting bucket</th>
</tr>
</thead>
<tbody>
<tr>
<td>Note inaccessible areas under power lines and behind trees where regrowth can occur</td>
<td></td>
</tr>
</tbody>
</table>
Weed boats often leave an intact marginal fringe and create floating fragments. Excavators tend to create large piles of weed, although these rot away within 2 – 3 weeks, the risk of reintroduction to the channel is high.

Mechanical excavation by barge can be used in faster flowing rivers, Cut material is highly palatable to livestock.

Chemical Control

Although it is possible to use two active ingredients on Hydrocotyle, the use of 2,4-D amine is not recommended due to difficulties caused by rapid translocation and excretion of the herbicide by the plant. The use of glyphosate at normal spray volumes of 200 litres of water per hectare without an approved adjuvant is also not recommended as the herbicide is also rapidly excreted from the plant.

There are two adjuvants suitable for improving control of Hydrocotyle when used with appropriate glyphosate formulations\(^2\), TopFilm (www.topfilm-uk.com) and Codacide Oil (www.microcide.co.uk). TopFilm is made from microcrystalline sponges of soya protein, with almost all the oil removed. TopFilm absorbs the herbicide and sticks it to the leaf surface for up to three weeks, resulting in excellent rain-fastness and a long slow release pattern. This prevents the herbicide being excreted rapidly and results in better control

\(^2\) Please see www.pesticides.gov.uk for formulations registered for aquatic use
early in the season (before mid August). After August, better control is achieved by using Codacide Oil, a vegetable oil that rapidly dissolves the waxy leaf cuticle and results in very rapid absorption of the herbicide, overwhelming the plant’s ability to excrete the herbicide, and a disruption of the ability to regulate transpiration by the leaves, resulting in fairly rapid cell necrosis and plant death.

<table>
<thead>
<tr>
<th>Using herbicides with a long lance and non-hazardous glyphosate formulation reduces the need for PPE to a minimum</th>
<th>Symptoms (leaf yellowing) are usually visible within days of application using glyphosate and adjuvants</th>
</tr>
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<tbody>
<tr>
<td>Large areas can be treated in short time</td>
<td>Collapse of the mat usually occurs in 4 – 7 days</td>
</tr>
<tr>
<td>Easy access to marginal fringes is possible using small boats or canoes</td>
<td>Plant death occurs with 3 – 4 weeks after treatment</td>
</tr>
</tbody>
</table>
Although initial assessments may give the impression that herbicide treatment has been successful, the ability of dormant nodes to produce new shoots should not be underestimated. Often regrowth from apparently dead mats of plant material occurs within 6 – 8 weeks after treatment, requiring retreatment or mechanical removal of dead mats. Continuous monitoring should occur in the first year of treatment, followed by monitoring of any regrowth in the following spring and summer. Overwintering of untreated material should be avoided at all costs, as this results in very rapid spread within a catchment.
The occurrence of an invasive species in a new area should always be a case of low risk, because the isolated presence of a small amount of biomass does not present a risk to watercourse function or ecology. However, it should be a high priority to remove or isolate the infested area and to eradicate the species from the area as soon as possible.

In the situation described in the diagram to the left, eradication from the pond would be relatively easy. The patch in the channel should be isolated from the rest of the ditch network and removed as soon as possible. The isolation can take the form of a temporary dam, weighted net or other structure that does not represent a flood risk.

Isolation must take place as soon as possible after the species has been noted and should remain in place until after the plant has been eradicated, and probably for at least 1 year after no more plants have been observed in the area. This is to ensure that a re-occurrence does not occur, caused either by fragmentation of upstream patches, or by deliberate planting.
Medium Risk

There are several small patches of less than ten square meters spread within a short distance, but in different parts of the channel and in nearby ponds.

This situation represents a greater risk to watercourse function and to the ecosystem of the ponds. The infestation has probably been present for at least one year and has completed a life cycle. The ability to spread is demonstrated by the occurrence of more than one patch in different parts of the watercourse and action should be taken to remove as much as possible.

Sections of the watercourse that can be isolated must be isolated immediately. Removal of as much as possible of all the patches should be undertaken within 6 months of the first observation. A management plan for removal and eradication of the species could be used to prioritise resources for future observation and monitoring and immediate removal.
High Risk – Low Priority

There are several large and small patches spread within a drainage system, spread over a large area, in different parts of the channel and in nearby ponds. The sections can be isolated and there are no critical watercourse functions at risk.

This situation represents perhaps an agricultural drainage network with no pumps, sluices, weirs or risk of flooding to populated areas. The infested section is either contained within an isolated section of watercourse, or can be easily contained.

The spread within this section can be easily monitored and a strategy for eradication or reduction can be implemented as and when resources are available.

Consideration should be given to the impact of the non-native species on the ecology of the drainage network, in terms of angling, bird and invertebrate populations.

Careful disposal of the biomass removed from the watercourse is required to prevent reinfestation of the cleared channel, or any channels along the transport route to the disposal site.
High Risk and High Priority

There are several large and small patches spread within a drainage system, spread over a large area, in different parts of the channel and in nearby ponds. The sections cannot be isolated and there are critical watercourse functions at risk.

This is a situation that should be rare, and results often from inappropriate management of small infestations, the presence of a very aggressive species, or as a result of favourable environmental conditions resulting in rapid spread within a system in less than one year.

Navigation functions are at risk, both from an inability to navigate and because movement of boats and ships will transport fragments of the species elsewhere in the network.

Fishing may be prevented by excessive growth of the target species.

Sluices, locks, weirs, pumps and other critical watercourse management structures may be at risk.

There is a serious risk of flooding of houses and commercial property as a result of the presence of this species.

Rapid and immediate management should take place to reduce the biomass of the target species. Sections, once cleared should be isolated to prevent further spread, and in the main channel a follow up maintenance operation should be undertaken, usually involving manual removal of fragments. Consideration should be given to educational notices and public awareness campaigns in the local are to encourage reporting of additional sites not normally monitored by the responsible authorities.
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