Spey Fishery Board

CONTROL OF *RANUNCULUS* ON THE RIVERS SPEY, DEE AND DON

Final Report

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1 INTRODUCTION

1.1 Background

In recent decades, various species of water crowfoot (*Ranunculus* spp., subgenus *Batrachium*) have become widely established on the Rivers Spey, Dee and Don, where they are non-native, covering large areas of the river bed during the summer months (SFB, 2013). The extensive growth of *Ranunculus* has been reported as having a potentially negative impact on freshwater pearl mussels and juvenile salmonids (Laughton *et al.*, 2004; SFB, 2013). It is also believed to negatively impact the spawning success of adult salmonids. Furthermore, there is a perceived economic loss as a result of the direct impacts on the level of salmon angling activity.

Ranunculus control has traditionally been carried out on the Spey, Dee and Don by manual cutting; however this is no longer considered feasible due to the considerable labour intensive resources required to conduct a catchment-wide control programme. From the late 1970s, *Ranunculus* was controlled on the Spey, Dee and Don using diquat alginate herbicide, a gel formulation containing the active ingredient that adhered to the leaf surface in flowing water. The active ingredient has subsequently been withdrawn by the European Commission for use in aquatic habitats as a result of the Plant Protection Products Directive (EU 91/414).

The objective of the current project is to determine the efficacy of using a potential alternative herbicide, the glyphosate-based preparation Roundup® Pro Biactive®, with the adjuvant Topfilm™ to control *Ranunculus,* and to provide the information that would be required for demonstrating its acceptability for use in the three rivers in question. In particular, this will involve determining if the herbicide would be effective when used on submerged species and the ecotoxicological effects of the preparation on the qualifying interests of the Rivers Spey and Dee Special Areas of Conservation (SAC): Atlantic salmon *Salmo salar*, otter *Lutra lutra*, freshwater pearl mussel *Magaritifera margaritifera* and sea lamprey *Petromyzon marinus*. Due to the SAC status of the rivers in question, any activities need to be compatible with the Conservation (Natural Habitats, &c.) Regulations 1994, and therefore demonstrate that no adverse impact on the integrity of sites will occur, unless the activity in question can be justified as being 'directly necessary for or connected with the management of the SAC'.

1.2 Project tasks

The project objectives are:

- To undertake a literature review on the efficacy of Roundup® Pro Biactive® (and other similar herbicides) in controlling aquatic plants, including *Ranunculus* spp., in riverine environments. In addition, to undertake a review of the ecotoxicological effects of the preparation on non-target organisms (Atlantic salmon, otter, freshwater pearl mussel and sea lamprey);
- Provide a report summarising the results of the literature review; and
- Provide recommendations as to the type/extent of future scoping study/experimental investigations that may be required based upon the outcomes of the literature review.

2 LITERATURE REVIEW

The search terms and resources used during the literature review are shown in **Appendix I**. Information was also provided directly by the product's manufacturer Monsanto (via Technical.helpline.uk@monsanto.com) and from Dr Jonathan Newman (Head of the Aquatic Plant Management Group at the Centre for Ecology and Hydrology; CEH).

2.1 Roundup® Pro Biactive®

Roundup® Pro Biactive® is a translocated[1] herbicide, containing the active ingredient glyphosate (Monsanto, 2011a), with two adjuvants, which (i) extend the period of glyphosate uptake from the treated leaf; and (ii) strongly binds to the leaf surface and ensures minimal glyphosate is locked up in the leaf, maximising efficacy (Monsanto, 2009). Glyphosate, the active ingredient in Roundup® Pro Biactive®, controls plants by blocking the plant's protein production system via the shikimic acid pathway (Monsanto, 2012a). Glyphosate degrades readily in the environment and does not accumulate (Monsanto, 2012a; Soloman & Thompson, 2003). When used according to approved uses, it has no negative effects on wildlife (Monsanto, 2012a). There are currently 93 products approved for use in or near water containing glyphosate, and two products containing 2,4-D Amine[2].

In Scotland, the regulatory body for herbicide applications in water is SEPA. Some general guidelines for the use of aquatic herbicides in Scotland may be found in the Farming and Watercourse Management Handbook (Wood-Gee, 2000). No mention is made of the requirement for the operative to be certified, but it is recommended to gain SEPA's advice on requirements for consent due to the age of the handbook. Guidance on the application process for aquatic herbicide use in Scotland is detailed on the website http://www.sears.scotland.gov.uk/Herbicide.aspx, and more information on controlling weeds and using herbicides is available on the Chemicals Regulations Directorate (CRD) website (www.pesticides.gov.uk; also applies to aerial herbicide use in Scotland). Guidelines regarding the safe use of herbicides are also given by the EA in their guidance notes for the use of herbicides in or near water (EA, 2010). The EA document also contains details of dosage rates.

2.1.1 Efficacy of Roundup Pro Biactive®

Glyphosate, the active ingredient in Roundup® and Roundup® Pro Biactive®, is intended for aquatic use only on emergent or floating leaved aquatic plants, as its efficacy requires contact between the herbicide and the target plant to allow penetration (ICID, 2002). Glyphosate is effective in controlling grassy, broad-leaved weeds & sedges. It is equally effective on annual & perennial weeds (ICID, 2002). It is very effective in controlling emergent vegetation where glyphosate can be applied directly on the foliage. When applied to foliage, glyphosate will translocate through the plant and destroy the roots and rhizomes systems even though they are under water. However, if all the vegetation is under water and glyphosate is applied to the surface of the water, it will not provide effective control (ICID, 2002).

[1] i.e. one which is transported around the plant
[2] https://secure.pesticides.gov.uk/pestreg/, accessed October 2013

Glyphosate is recommended for use in the control of the following aquatic plants: duckweed (*Lemna* sp.), water fern (*Azolla filiculoides*), reeds, rushes and sedges (i.e. emergents) (Newman, 1997). However, the manufacturer's guidelines state that Roundup®[3] is not effective against submerged aquatic plants, including common water crowfoot (*Ranunculus aquatilis*) (Monsanto, 2013). Generally speaking, water crowfoots (Ranunculus sp.) are submerged, with approximately 10% of leaves floating on the surface. Thus, although they are generally considered to be 'submerged aquatic plants', they are partially floating, which means herbicide application may be possible, albeit less effective than on emergent plants. Based on multiple searches of scientific and 'grey' literature, no instances of effective glyphosate use on *Ranunculus* have been reported (see **Appendix I**). This is confirmed by Jonathan Newman (*pers. comm.*), as well as The Herbicide Handbook (English Nature, 2003), which lists dichlobenil, diquat, and terbutryn as the only herbicides shown by researchers to have proved effective for the control of *Ranunculus* spp. (but these are no longer approved for aquatic use; see **Section 2.2**). The one trial known to CEH (the home of the Aquatic Plant Management Group) involving the use of glyphosate on submerged aquatic plants (Barrett, 1981) used glyphosate (as Roundup®) and glyphosate with 3% alginate (a preparation which sticks to the plant and releases herbicide slowly) as a method of control for water milfoil (*Myriophyllum spicatum*). The trial concluded that glyphosate/Roundup® was not an effective herbicide for submerged aquatic macrophytes, unless used at a dilution rate of 1:1 water to glyphosate. While the improved formulation of Roundup® Pro Biactive® may prove to be more effective than Roundup® alone, the manufacturer's own guidance suggests the product is not suitable for submerged plants (Monsanto, 2013), therefore it can be concluded that Roundup® Pro Biactive® is unlikely to be effective in the control of water crowfoots (*Ranunculus* spp.). There is a minimum contact time required between the diluted herbicide and the target plant leaf to allow enough herbicide to be taken up into the plant to provide control. When applied to plants underwater, there is effectively no contact time because the herbicide is diluted immediately and not enough herbicide can be absorbed by the plant. The target species in this case is *Ranunculus* spp., the growth pattern of which is predominantly submerged, but a proportion of submerged leaves reach the surface and flowers are produced. The very small surface area of exposed leaves would probably not provide enough surface area to take up sufficient herbicide to provide control. There are no data to support the use of glyphosate on this type of aquatic plant.

However, there may be improved effects when Roundup® Pro Biactive® is used alongside Topfilm™. Topfilm™ is an adjuvant preparation, which is used to improve rain-fastness[4] and herbicide delivery (see http://www.topfilm-uk.com/). It was designed for use on submerged macrophytes and is non-toxic to the environment (Newman, 2009). According to a supplier website[5] Roundup® Pro Biactive® (with the adjuvant Topfilm™) can be used on "plants with floating leaves", although no

[3] Roundup Pro Biactive/Roundup ProBio, Roundup Biactive, Roundup Flex and Roundup ProBiactive 450 have label approval for use in aquatic areas and will be referred to throughout this document as Roundup®

[4] i.e. Topfilm® reduces the amount of herbicide lost due to rain washing it off

[5] http://www.water-land.co.uk/submerged%20rooted%20weeds.htm

evidence is presented to support this, or to specify whether this applies to submerged plants with some floating leaves, such as *Ranunculus* spp. According to the Roundup® Pro Biactive® product guide (Monsanto, 2011b), for floating weeds the addition of Topfilm™ may improve control of species where herbicide wash-off is a problem, but the list of example species does not include submerged plants such as *Ranunculus* spp. No known trials on the use of Topfilm™ alongside glyphosate on the control of Ranunculus have been undertaken, thus the efficacy of *Ranunculus* control using Roundup® Pro Biactive® with Topfilm™ cannot be confirmed, and will need to be investigated further by laboratory experimentation and/or field trials.

2.1.2 Review of ecotoxicological effects on non-target organisms

Searches were performed for peer-reviewed (and 'grey') literature on the use of Roundup® and Roundup® Pro Biactive®, and its toxicity to the following non-target organisms (due to their presence as qualifying features of the Rivers Spey and Dee SAC):

- Otter;
- Atlantic salmon;
- Freshwater pearl mussel/mussel; and
- Sea lamprey/lamprey.

No records were found for the use of Roundup® and its impact on otter, freshwater pearl mussel and lamprey. Soloman & Thompson (2003) conclude that glyphosate is of small acute toxicity to mammals and fish. In all the organisms tested by Monsanto during development, including earthworms, birds, mammals and arthropods, glyphosate exhibited only low toxicity at typical application rates[6]. The toxicity of Roundup® to (other species of) mussels is discussed below.

Of the records found for salmon, the closest match was a journal article which tested three different commercial glyphosate preparations (none of which were Roundup® Pro Biactve®) on *Oncorhynchus mykiss* rainbow trout and *Carassius auratus* goldfish (Antón *et al.*, 1994). The experimental concentrations of the commercial herbicides were higher than the recommended doses to control weeds, and showed a very low toxicity to both species of fish; therefore the current use of this herbicide "would probably not be too hazardous to these fish species" (Antón *et al.*, 1994). Giesy (2000) reported an increased toxicity of Roundup® compared to technical glyphosate on fish; however, the impact was still below the hazard quotient[7], and Giesy concluded that Roundup® can be used safely in the restoration of aquatic habitats (Giesy, 2000). Glyphosate has been used extensively to control aquatic weeds and restore ecosystems affected by introductions of exotic weeds. During this period of use, there have been no documented cases of adverse effects on fish (Giesy, 2000). In addition to this, channel catfish, rainbow trout, bluegill, marsh clams, and crayfish did not bioconcentrate glyphosate when exposed under laboratory conditions (WHO 1994). Folmar *et al.* (1979) found that the application of Roundup®, at recommended

[6] http://www.glyphosate.eu/glyphosate-safety-profile-non-target-wildlife-and-plants

[7] calculated by dividing the maximum environmental exposure concentration derived from modelling or environmental monitoring data by the greatest level of Roundup or glyphosate found to have no effect on survival, growth, or reproduction of the most sensitive non target organisms.

rates, along ditchbank areas of irrigation canals should not adversely affect resident populations of fish or invertebrates. Technical glyphosate was considerably less toxic than Roundup® or the surfactant (Folmar *et al.*, 1979), highlighting the importance of using commercial formulations when evaluating toxicity to non-target organisms.

Very few relevant records were found when searching the peer-reviewed literature for impacts of Roundup® on 'mussel(s)', and it is debateable whether these results can be extrapolated to assess the impacts on freshwater pearl mussels. One experimental study found that freshwater mussels native to the United States are among the most sensitive invertebrates tested to date with glyphosate-containing compounds, and that the toxicity of Roundup® could not be attributed to surfactant alone (*Bringolf et al.*, 2007). Glyphosate has been found to affect the metabolism of golden mussel *Limnoperna fortunei*, an invasive freshwater bivalve of China and South-east Asia origin, when present in environmentally relevant concentrations (Iummato *et al.*, 2013). This may have implications for the freshwater pearl mussel population of the Rivers Spey and Dee, should treatment with Roundup® Pro Biactive® go ahead. The Safety Data Sheet for Roundup® Pro Biactive® (Monsanto, 2012b) does not include toxicity information for mussels or any other bivalves.

In their risk assessment paper (including assessment of Roundup® and Rodeo®, a Monsanto manufactured glyphosate product used in the United States) Soloman & Thompson (2003) concluded that glyphosate is of small acute toxicity to animals and wildlife, including mammals, birds, fish, and aquatic invertebrates. This is based on numerous extensive and thorough reviews by a number of international and national regulatory agencies (Soloman & Thompson, 2003). Risks from the use of glyphosate + MON 0818 (Roundup®) were slightly greater than those from glyphosate (Rodeo®) and surfactants such as LI 700; however, in deliberate or accidental over-water uses they were considered small (Soloman & Thompson, 2003).

Given the lack of bioaccumulation and non-persistence of Roundup® Pro Biactive® in the environment, and the fast-flowing, riverine nature of the target areas, should Roundup® Pro Biactive® prove to be effective against water crowfoot (*Ranunculus* spp.), it is unlikely to have a negative impact upon the specified non-target organisms. Tsui & Chu (manuscript in preparation, quoted in Tsui & Chu, 2003) found that Roundup® Biactive® (a formulation with an undisclosed surfactant manufactured by Monsanto Co., Australia) is about 14 times less toxic than Roundup® to *Ceridaphnia dubia* (a water flea). Similar, independent, tests do not appear to have been carried out specifically on Roundup® Pro Biactive®; however, Monsanto commissioned tests resulted in toxicity levels for Roundup® Pro Biactive® of 35.5 times less than Roundup® on an alga (*Selenastrum capricornutum*), and 120 times less than Roundup® on rainbow trout. The Environmental Impact Assessment for Roundup® Pro Biactive® (Monsanto, 2011a) states that it shows low toxicity to mammals, and is not considered 'harmful to aquatic life', being of low toxicity to fish, aquatic invertebrates (e.g. water fleas) and green algae. Further information on the tests undertaken to determine this are available in the Safety Data Sheet (Monsanto, 2012b).

Giesy (2000) concluded that Roundup® can be used safely for aquatic habitat restoration, but requires consideration of items such as application rate, depth of water, and vegetation coverage. Given the fact that the additional ingredients within herbicides (such as surfactants) can be more toxic than the herbicide itself (Perkins *et al.*, 2000; Tsui & Chu, 2002), as mentioned earlier, it is important to base ecotoxicological conclusions on specific tests of the herbicide in question (i.e. Roundup® Pro Biactive® and its constituents), rather than on the main ingredient only (i.e. glyphosate). Therefore some of the evidence presented in studies may be of limited use to this study.

In summary, the literature available shows that Roundup® generally has low toxicity, highlighted by the fact that it is licensed for use in aquatic environments. However, there is no information on the toxicity of Roundup® specifically on freshwater pearl mussels, and one study (Bringolf *et al.*, 2007) showed that bivalves are amongst the most sensitive invertebrates tested with glyphosate compounds. Therefore it cannot be concluded that Roundup® is proven to be safe for use in rivers containing freshwater pearl mussels. SEPA, as the competent authority under the Conservation (Natural Habitats, &c.) Regulations, need to consent any herbicide applications in accordance with the Conservation Regulations. Specifically SEPA needs to be able to determine 'no adverse effect on site integrity' as a result of the plan or project. Given the uncertainty over impacts, APEM consider it questionable whether SEPA could conclude no adverse effect on site integrity, with such a conclusion by SEPA resulting in their declining consent. However, this is solely APEM's view and engagement with SEPA would be required to seek a more definitive view. Furthermore, SEPA's view would be required regarding the level of proof needed in order for the herbicide application to be consented, and hence the nature and scale of any studies undertaken to provide evidence.

Experience shows that regulatory bodies are often reluctant to give definitive advice on what would constitute no adverse effect on site integrity; nonetheless, engagement with SEPA (and SNH as statutory consultee under the Regulations) on this topic is an essential component of scoping the next phase of study (experimental phase). Such a phase was outside of the scope of this study, but it is intended that this report will inform the next stage.

2.2 Other methods for controlling *Ranunculus*

As previously mentioned, glyphosate and 2,4-D amine are currently the only approved herbicides for use in water in the UK[8], thus it may be necessary to investigate other methods of control. These include, but are not limited to:

- Use of dyes;
- Biocontrol (e.g. herbivorous fish, insects or pathogens);
- Increased shading; and
- Mechanical removal:
    - Cutting/harvesting;
    - Chaining; and
    - Netting.

8 Depitox (2,4-D) and 2,4-D Amine 500 are currently approved by the CRD for use in aquatic situations, however the revocation date for this aquatic use allows the storage and use of these products labelled for aquatic use until 31st August 2014 (EA, 2013)

Dyes have been mooted by stillwater fishery owners and others (e.g. see ICID, 2002) as a method of aquatic plant control, sometimes alongside herbicides to increase their effect. They work by reducing light penetration, which limits photosynthesis and therefore plant growth. However, the use of dyes in high flow rate environments is not possible due to the flushing action of the water preventing the dye from remaining *in situ* long enough for noticeable effects to occur; or the volumes of dye required to be effective would be unfeasible.

Biocontrol using herbivorous fish (such as grass carp) is only permissible under relevant legislation (Import of Live Fish Act 1980, and associated orders) in fully enclosed systems due to the potential impact on the ecosystem (e.g. grass carp are non-native to the UK), thus their use is not relevant to the Rivers Spey, Dee and Don. For this reason, the use of grass carp is not considered further. Furthermore, the use of any biocontrol methods in an SAC should be treated with extreme caution due to the risk of undesired effects, but possibilities other than fish (e.g. plant pathogens) could be looked into further. New technologies for controlling aquatic plants are currently in development, including the use of terrestrial fungi in a binder matrix, which has proved successful for control of *Hydrilla* (a submerged aquatic plant) in the USA (Newman, 2009).

Increased shading could help to prevent overgrowth of certain aquatic plants in narrow channels but is unlikely to be highly effective at the scale required to remove *Ranunculus* from wide rivers such as the River Spey, where increased shading would have a limited impact on plant growth in the centre of the channel.

According to Newman (2009), mechanical control of aquatic plants “continues apace, primarily because it is the only sensible option available”. This is in part due to the lack of effective herbicides currently available for use in aquatic environments. Mechanical control has already been ruled out by SFB on the Spey, Dee and Don due to the scale of the operation needed for it to be effective. However, this option may have to be re-considered as an ongoing control measure while other methods of control are being developed, or if chemical control does not prove to be effective and environmentally acceptable. There are several advantages to using mechanical control, including the low environmental impact compared to other methods; and the lack of risk of impacts on adjacent/connected waterbodies (ICID, 2002). However, there are also disadvantages, including impacts on biota attached to the removed weed, such as macroinvertebrates (e.g. see Dawson *et al.*, 1991), and the relatively high time and financial costs. Limited effectiveness has also been reported for this method (Barrett, 1978; ICID, 2002), but this is dependent on the desired outcome and which mechanical method is applied. It is likely that multiple control techniques will be needed to control *Ranunculus* spp. in the Rivers Spey, Dee and Don to the desired level.

3 RECOMMENDATIONS FOR FUTURE STUDY

From this literature review it is apparent that no chemical solution currently exists that is specifically recommended for the control of submerged aquatic plants such as *Ranunculus* spp., and that the use of Roundup® Pro Biactive®, appears likely to result in limited success.

There are two main issues with the use of Roundup® Pro Biactive®:

1. Efficacy – this is likely to be low, although more could be done to establish this; and
2. Acceptability in terms of use in an SAC – no adverse effect on the integrity of the SAC will need to be confirmed; in particular, potential effects on freshwater pearl mussels are a concern.

A stepwise approach to addressing the use of Roundup® Pro Biactive® in the rivers concerned is recommended, as follows:

1. **Discuss with SEPA (and SNH) the feasibility of field trials in the Don, and the burden of proof that would be required in terms of showing both efficacy and no adverse impact on site integrity**. Field trials would seek to a) determine the efficacy of the treatment and b) determine concentrations of glyphosate and associated compounds in the river (water and sediments) resulting from the application. Providing SEPA and SNH were supportive, the next stage would be to design a field trial.
2. **Design an experimental field trial**. This would need to be undertaken with a clear understanding of the objectives, based on discussion with SEPA and SNH, but it is envisaged that it would address a) the efficacy of Roundup® Pro Biactive® in inhibiting *Ranunculus* growth and b) determine the environmental concentrations of Roundup® Pro Biactive® present immediately above the river bed, and to which freshwater pearl mussels would be exposed, and the concentration profile over time (i.e. exposure duration). Information on actual concentrations in the field would both inform risk assessment, and feed into a laboratory study if undertaken, i.e. the concentrations recorded in the field would inform the treatment exposures in the laboratory.

   The intention would be to undertake field trials on the River Don, as approval is more likely given the absence of freshwater pearl mussels, and the fact that the Don is not an SAC, is not therefore subject to the provisions of the Habitats Regulations and is thus more likely to result in approval for trials being granted. A 'randomised block' type experiment is likely to be appropriate for the field study. The design would require careful allocation of treatment and control reaches, in particular given the downstream effect whereby control and treatment reaches downstream of others will be subject to their direct treatment, plus the residual (and cumulative) effects from upstream treatments. Such effects can be accounted for by direct measurement of Roundup® Pro Biactive® concentration in each experimental and control reach. Furthermore, the complexity of multiple herbicide application locations and times will reflect what might transpire in reality, and thus provides a basis of evaluating such cumulative or 'in combination' effects. Indeed, consideration of 'in combination' effects is a specific requirement of a Habitats Regulations Assessment, and this aspect will thus need to be considered in order to satisfy SEPA and SNH's requirements.

3. **Undertake field trials.** Field Trials would be undertaken in the late spring/summer, at which time *Ranunculus* growth would be sufficient to enable a trial to progress. Higher temperatures prevalent at this time of year and higher growth rate of the plant will also ensure that trials are conducted during conditions likely to optimise Roundup® Pro Biactive® efficacy. Die back and subsequent regrowth of *Ranunculus* would have to be monitored in the days, weeks and months following application, in control and treatment reaches, and an assessment made of the efficacy of treatment. A strategy for sampling water during application and in the minutes and hours following application would be devised, with samples being processed by an appropriate analytical laboratory to determine concentrations of the main Roundup® Pro Biactive® constituents (i.e. glyphosate).

4. **Design laboratory experiments on the ecotoxicology of Roundup® Pro Biactive® with Topfilm® on freshwater pearl mussels**.

   The merits of laboratory trials would need to be carefully considered, given the biology of freshwater pearl mussels. Freshwater pearl mussels are very long lived, which has two significant implications: firstly, any individual in the wild would be subject to repeated annual exposure to glyphosate – a situation which would not be able to be replicated in a relatively short term laboratory experiment; and secondly, any biological response to chemical exposure may not occur in the laboratory but may potentially lead to a subtle chronic reduction in health in the long term, e.g. by affecting breeding success. It is recommended to ascertain SEPA's requirements before designing the experiment in order to ensure what they require is technically and practically feasible.

   The need to undertake targeted ecotoxicological studies of the effects of Roundup® Pro Biactive® on freshwater pearl mussel will depend partly on whether field trials prove it to be effective in controlling *Ranunculus*. Given that design of an appropriate experiment will be a significant task in itself, if this is to take place in 2014, it may be desirable to plan such an experiment concurrently while the field trials are being planned and undertaken. Waiting for the results of the field trial before commencing planning laboratory trials may result in significant delay, although this would have to be balanced against the possibility that planning the laboratory trials in advance of the outcome of the field trials may result in abortive efforts (i.e. if field trials proved no efficacy). The relative importance of project timescales and available budget will need to be considered in making such an evaluation. Some of the potential complexities of a laboratory trial are:

   - Securing suitable aquaria, laboratory facilities and project personnel;
   - Identifying a suitable freshwater pearl mussel population, and obtaining licences to remove freshwater pearl mussels for experimental purposes;

- Agreeing project objectives and evidence requirements from SEPA and SNH;
- Designing the experiment itself (husbandry conditions, experimental treatments (e.g. concentrations, frequency of application), number of replicates, monitoring responses of freshwater pearl mussel, duration of study); and
- Agreeing how results will be interpreted in terms of acceptable levels of impact and how this will be incorporated within an appropriate assessment.

5. **Undertake laboratory experiments on the ecotoxicology of Roundup® Pro Biactive® with Topfilm® on freshwater pearl mussels** - according to the experimental design outlined above.

These suggested next steps are summarised in Figure 3.1, with a proposed timeline (indicative only) shown in Figure 3.2.

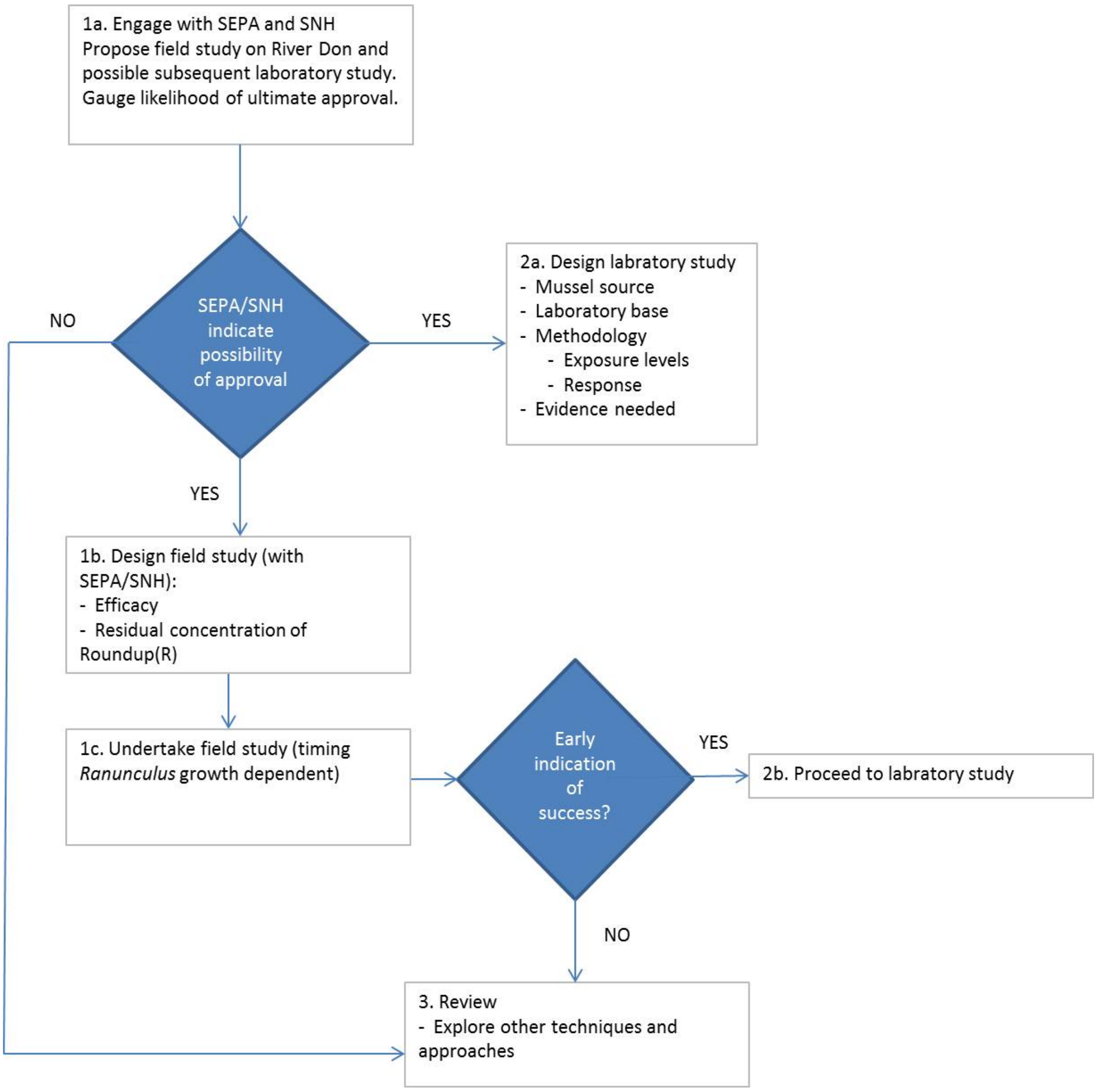

Figure 3.1 - Suggested next steps

Task	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Engage with SEPA/SNH	■										
Field study design		■	■								
Field study				■	■						
Lab study design		■	■	■	■						
Field study review					■						
Lab study						■	■	■	■	■	
Lab study review											■

Figure 3.2 - Suggested timeline for field and laboratory studies (indicative only)

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APPENDIX I – LIST OF SEARCH TERMS USED

Resource	Search terms used[9]
Web of Knowledge	Roundup Pro Biactive AND Ranunculus
	Roundup Pro Biactive AND aquatic weed*
	Roundup Pro Biactive AND aquatic
	Ranunculus AND control
	Aquatic weed AND control AND Roundup
	Roundup AND otter*
	Roundup AND salmon
	Roundup AND freshwater mussel
	Roundup AND mussel
	Roundup AND sea lamprey
	Roundup AND lamprey
	Glyphosate AND lamprey
	Herbicide AND lamprey
Google scholar	“Chemical control of Ranunculus in water”
	Ranunculus control
	Roundup aquatic weed

* wildcard search e.g. weed* will find ‘weed’ and ‘weeds’

[9] Including those with zero returns