LIFE RIPARIAS surveillance plan

Life team surveillance (C1.2-C1.3), version 1.3

The RIPARIAS project has received funding from the LIFE Programme of the European Union

RIPARIAS

Reaching Integrated and Prompt Action in Response to Invasive Alien Species

Beneficiaries responsible for the implementation of action C1:

Main action responsible: SPW-ARNE.

Other beneficiaries directly involved in IAS field surveillance: BE (Brussels), ANB & VMM (Flanders), CRD, CRDG & CRS (Wallonia).

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EXECUTIVE SUMMARY

This document provides general guidelines for field data acquisition by regional Life teams involved in LIFE RIPARIAS enhanced surveillance actions C1.2 and C1.3. It aims at standardising the way surveys are conducted in the field so that data can be easily aggregated later, integrated in the decision support tool and used for the monitoring activities of the project.

The riparian zone is the main focus for enhanced surveillance of the LIFE RIPARIAS project; it consists of rivers, riverbanks, floodplains and related waterbodies. Different surveillance intensity levels are defined, with increasing searching effort for emerging species compared to widespread ones, in spatial units located close to the river system and in river sub-units benefiting from an official protection status. Special attention is paid to riparian Natura 2000 habitats, including water bodies (3130-3150), rivers (3260), tall herb communities (6430) and alluvial forests (91E0*).

Field work to be performed by the life teams consists of characterising historical and new suspected sites colonised by target IAS, surveying their immediate vicinity and conducting additional inventory samplings in under-prospected areas. For widespread invasive plants, source populations in upstream river sections will be identified and their distribution will be updated considering management actions carried out in the past.

Separate guidelines are provided for the surveillance of riparian plants, aquatic plants (macrophytes) and crayfish. In each case, the surveillance plan details spatial units to be considered, sampling device and timing, abundance assessment and management-related information to be collected in the field.

1. Introduction

Rationale: The enhanced surveillance action (C1) aims at improving IAS detection rate and distribution within the LIFE RIPARIAS territory especially during the 2 first years of the project to feed the decision support tool (A4). Collected data will help in identifying areas (sites and river sub-units or RSUs) wherein management will be implemented through C2 and C3 actions from year 3 to 5.

Enhanced surveillance is expected to feed both the rapid eradication and the management workflows. Prompt detection and validation of new populations will be of uttermost importance for emerging IAS while enhanced surveillance will be especially useful to update current distribution of widespread IAS at RSU scale, including the measurement of the effect of management actions initiated before the beginning of the LIFE RIPARIAS project. C1 outcome will also provide the baseline 2022 distribution (incl. abundance) of widespread plants, that will be compared with the situation reached after management during the last year of the project (2026), and 5 years later at the end of the afterlife period (2031) (action D2).

Increasing IAS detection rate

During the 2000-2015 period, the ongoing overall detection rate of IAS in the whole pilot area was about 506 observations/year. The average species detection rate amounted to 62.5 data per year and per species for widespread taxa and only 0.6 data per year and per species for emerging taxa within the LIFE RIPARIAS area. As compared to the detection baseline described above, **ongoing detection rate is expected to be multiplied by 3 for emerging plant and crayfish species and by 2 for widespread ones** during the LIFE RIPARIAS project.

C1 action will also allow to collect management-related information useful for selecting the most appropriate technique to control IAS populations in the different sites (e.g. IAS abundance or density, water bodies peculiarities, site access, risk of spread towards neighbouring sites, presence of protected species, etc.).

The purpose of this document is to standardize as much as possible and provide general guidelines for field data acquisition by the Life teams across the 3 administrative regions involved in the project, so that data can be easily aggregated afterwards, integrated in the decision support tool and used for monitoring activities.

As this plan focuses on field surveys to be carried out by the Life teams, it is related to sub-actions C1.2 and C1.3. They were slightly reorganised since project proposal for better organisation of surveillance activities and were renamed as follows:

C1.2 - Surveillance of riparian plants by the Life teams

C1.3 - Surveillance of water weeds and crayfish in ponds by the Life teams

Action C1.1 will be initiated only during the second year of the project, after determination of the composition of the alert list and preparation of identification sheets for citizen. It will be described in a separate document.

The following spatial units are considered in the surveillance plan:

- Rivers, riverbanks and river-connected waterbodies (up to 5 m on both sides of the river)
- Floodplains (from 5 m to a distance between 15 m and 250 m from the river)
- Remote habitats like ponds or gardens located outside the floodplain

The riparian zone covers all habitats included in floodplains, rivers and riverbanks.

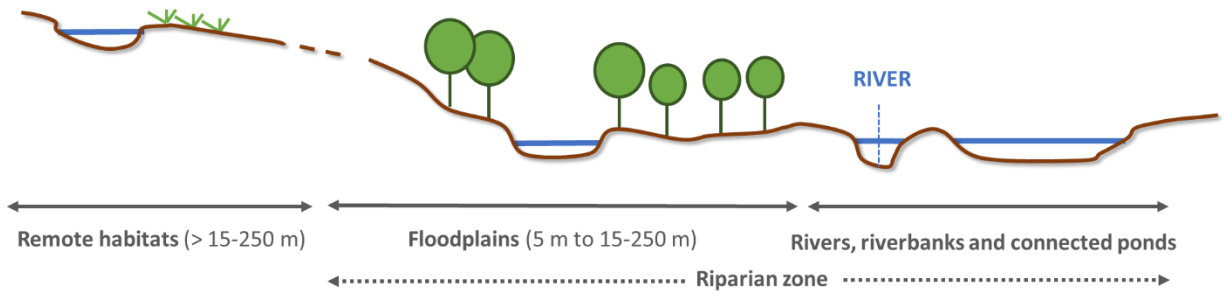

Figure 1 - Spatial units to consider in the LIFE RIPARIAS surveillance plan

2. Definitions and specificities of the LIFE RIPARIAS project

2.1 Definition of key terms

Alert list IAS: alien aquatic plants, riparian plants and crayfish not yet present or with very limited distribution in the LIFE RIPARIAS territory that are likely to pose a threat to biodiversity, and for which dedicated surveillance and monitoring are recommended to foster prompt response in the case of arrival and spread. The alert list will be defined during the 1st year of the project and alien species like *Aponogeton dystachyos, Pontederia cordata* and *Sagittaria latifolia* (aquatic plants) or *Faxonius immunis, F. juvenilis, F. rusticus* and *Procambarus acutus* (crayfish) are typical candidates for it.

Baseline distribution: IAS spatial occupation defined by cumulative observation data collected during the 2000-2015 period (baseline 2015 from the project proposal). It will be updated to include (i) new observations recorded between 2016 and 2020 (baseline 2020) and (ii) additional data collected during the enhanced surveillance period of the project (2021-2022) (baseline 2022).

Emerging IAS: invasive species poorly established in Belgium (≤ 3% 5x5 km UTM squares) including both species of EU concern and species from the alert list. Ten emerging IAS of EU concern are targeted by the LIFE RIPARIAS project, including 3 riparian plants (*Heracleum persicum*, *H. sosnowskyi* and *Lysichiton americanus*), 4 aquatic plants (*Cabomba caroliniana, Lagarosiphon major, Ludwigia peploides, Myriophyllum heterophyllum*) and 3 crayfish (*Faxonius virilis*, *Procambarus clarkii* and *P. virginalis*).

Floodplain: area of land adjacent to a stream or river which stretches from the banks of its channel to the base of the enclosing valley walls, and which experiences flooding during periods of high discharge. Its width is defined based on low flood hazard (return period of once every 10 years) and varies between 30 m and 500 m depending on river categories. Floodplain width is acknowledged to be much larger in Flanders than in Wallonia because of different positions occupied in river basins, as shown by differences observed in lateral distribution of Himalayan balsam, a typical riparian species.

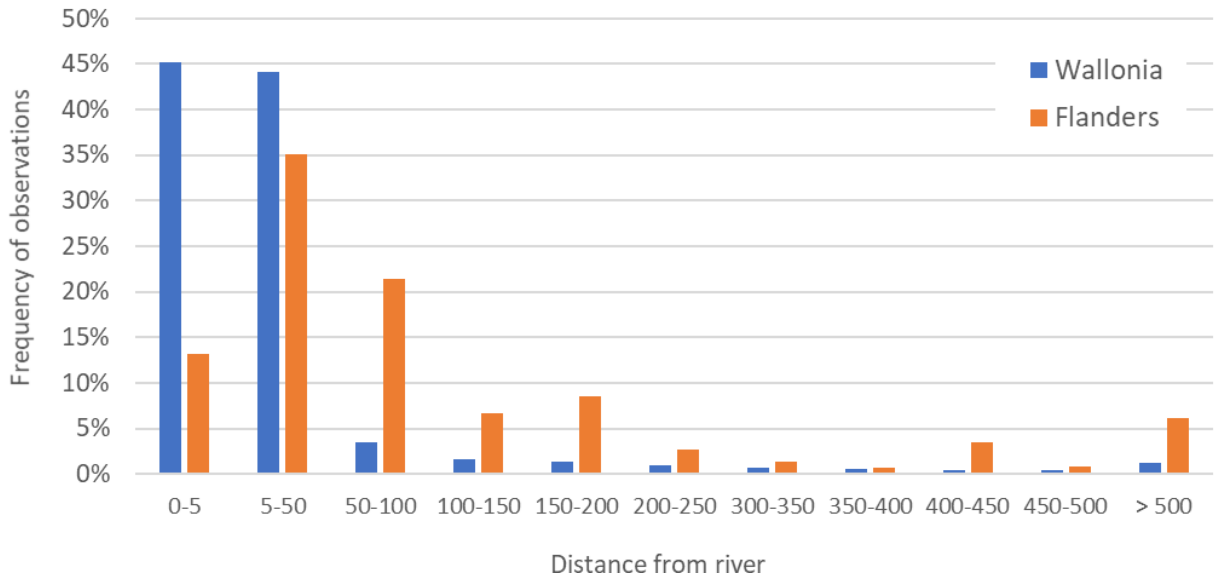

Figure 2 – Frequency of Himalayan balsam observations during the 2000-2015 reference period in the Flemish and Walloon regions of the LIFE RIPARIAS territory according to their distance to water courses.

Historical sites: sites wherein IAS presence was recorded during the baseline 2015 (+ baseline 2020) period.

Priority area for surveillance purposes: areas made of RSUs wherein management of widespread IAS (especially I. *glandulifera* and *H. mantegazzianum*) is likely to be conducted during the RIPARIAS project, due either (i) to high conservation value (extent of riparian zones benefiting from an official protection status greater than 10% of river linear within individual RSU) or (ii) to upstream location within river basins.

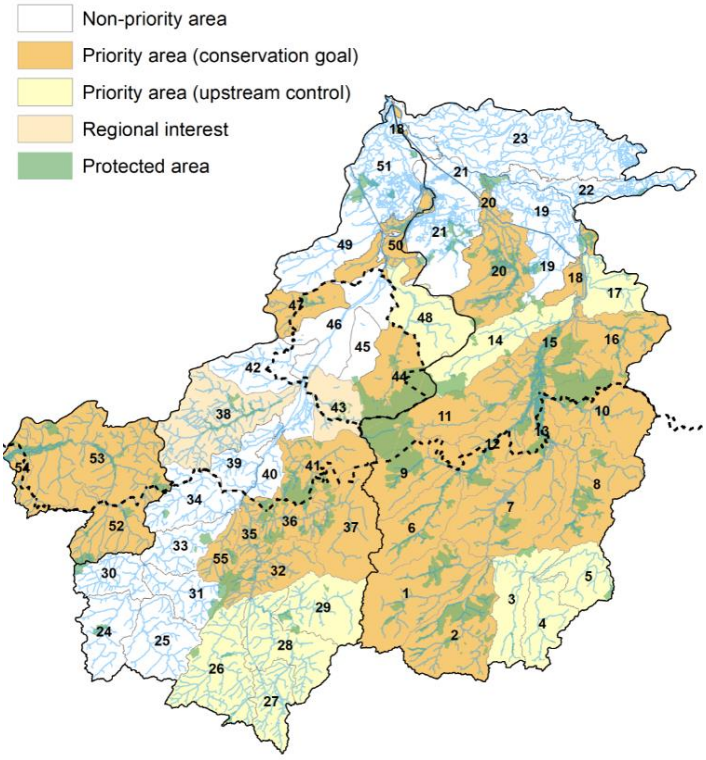

Figure 3 - Priority RSUs for surveillance and management of widespread IAS within the LIFE RIPARIAS territory. Two RSUs (38 and 43) with insufficient coverage of protected area were added based on requests by water managers (regional interest).

Riverbank: land at either edge of a river, expanding up to 5 meters from it.

River sub-unit (RSU): hydrological unit resulting from the finest sub-division of river catchment areas by water managers, used as a functional elementary unit to design containment measures of widespread IAS in the LIFE RIPARIAS project.

Source population: furthest upstream populations identified along each river or tributary within individual RSUs based on historical observation data and supposed to act as the source of propagules colonising downstream areas; several source populations may be identified along the same river providing that no historical observation is recorded along a stretch of at least 20 km[1].

Widespread IAS: invasive species widely established in Belgium (> 3% 5x5 km UTM squares). Five widespread plant species are targeted by the LIFE RIPARIAS project, including 2 riparian plants (*Impatiens glandulifera* and *Heracleum mantegazzianum*) and 3 aquatic plants (*Hydrocotyle ranunculoides, Ludwigia grandiflora* and *Myriophyllum aquaticum*). Three other widespread IAS, the waterweed *Elodea nuttallii*, and the crayfish *Faxonius limosus* and *Pacifastacus leniusculus*, are not directly addressed by the project because of low detectability and very low effectiveness of containment measures.

2.2 Specificities of the LIFE RIPARIAS territory, target IAS and habitats

The LIFE RIPARIAS territory covers 2,631 km^2 and is subdivided into 3 river basins and 55 river sub-units each of them covering an average of 48 km^2. The total river length included in this territory is 4,111 km.

The total number of ponds is estimated at 6,428. About 26% of them are connected to watercourses, 38% are in floodplains and 36% in remote areas. The occupancy rate of ponds by LIFE RIPARIAS target IAS is lower than 3% based on data currently available.

Target Natura 2000 habitats as defined in the project proposal include water bodies (3130-3150), rivers (3260), tall herb communities (6430) and alluvial forests (91E0*). All together, they cover a surface of 1,812 ha within Natura 2000 sites, 78% of which is alluvial forest. In Wallonia, these habitats extend up to 50 m from the river; this distance exceeds 100 m in Flanders because they occupy the lowest part of river basins.

Table 1 – River length, pond number and area of target Natura 2000 habitats within the 3 regions of the LIFE RIPARIAS territory.

	Brussels	Flanders	Wallonia	**Total**
River length	102 km	2,341 km	1,668 km	**4, 111 km**
Pond number	333	3,565	2,530	**6,428**
Target Natura 2000 habitats	12 ha	1,437 ha	363 ha	**1,812 ha**

[1] Furthest river dispersal recorded for *Impatiens glandulifera* according to Wadsworth et al. (2000)

Table 2 – Spatial distribution of ponds within the LIFE RIPARIAS territory

	Brussels	Flanders	Wallonia	**Total**
River-connected	136	443	1,114	**1,693**
Floodplain	58	1,878	503	**2,439**
Remote	139	1,257	907	**2,303**

2.3 Habitat preferences and introduction pathways of target IAS

Target IAS occupy different semi-natural habitats including ponds, rivers, riverbanks and riparian habitats. Habitats occupied by the largest number of species are ponds (see table 3).

Most of target IAS were imported for ornamental purposes and were introduced in or nearby garden and fishing ponds, from which they are likely to spread towards neighbouring areas using watercourses as migration corridors. These habitats should therefore be especially targeted by surveillance actions.

Table 3 – Habitat preferences of target IAS. Caption: x: preferred habitat, (x): tolerated habitat.

	Ponds	Rivers	Banks	Tall herbs	Alluvial forests
WIDESPREAD PLANT SPECIES					
Impatiens glandulifera			x	x	x
Heracleum mantegazzianum			x	x	(x)
Hydrocotyle ranunculoides	x	x			
Ludwigia grandiflora	x				
Myriophyllum aquaticum	x				
EMERGING PLANT SPECIES					
Heracleum persicum			x	x	(x)
Heracleum sosnowskyi			x	x	(x)
Lysichiton americanus	(x)	(x)	x		x
(Gunnera tinctoria)	(x)	(x)	x		
Cabomba caroliniana	x	(x)			
Lagarosiphon major	x	(x)			
Myriophyllum heterophyllum	x	(x)			
EMERGING CRAYFISH SPECIES					
Faxonius virilis	x	x			
*(Pacifastacus leniusculus)**	x	x			
Procambarus clarkii	x	(x)			
Procambarus virginalis	x	(x)			

* An emerging species only in RSUs located in Brussels and Flanders.

2.4 Baseline distribution data of target IAS

Cumulative observation data collected in 2000-2015 provided the baseline distribution of target IAS for the project proposal and were used to define quantitative targets to reach as described in the project proposal (= baseline 2015). This information will have to be updated twice during the project in order to include (i) new observations recorded between 2016 and 2020[2] and (ii) additional data collected during the enhanced surveillance period of the project (2021-2022).

3. Intensity levels of surveillance

Three levels of surveillance intensity were derived from partner expectations collected through an online survey (summarised in Appendix 2) and were proposed to design the LIFE RIPARIAS surveillance plan for both emerging and widespread IAS, as referred to in the table below:

1) The **first level** of intensity corresponds to low priority for surveillance and management and is equivalent to data gathering by citizen science only, without field visit by the LIFE RIPARIAS team.
2) The **second level** is equivalent to level 1 supplemented by field visits by the life teams of historical and new suspected sites, including their immediate vicinity within at least a 25 m radius. In case of populations established in watercourses and on riverbanks, searching effort will be extended up to 1 to 3 km downstream[3] from source populations and 0,5 km upstream. The objective of field visit is to validate species presence, assess organism abundance and potentially also collect information on site specificities useful for management. In case of widespread riparian plants, this approach will be adapted as described below.
3) The **third level** is equivalent to level 2 supplemented by inventory samplings in additional sites to increase searching effort. River-connected and floodplain ponds will be especially at target as they may shelter the most dispersive populations. Additional sampling of widespread riparian plants will be also conducted along riverbanks in priority areas poorly covered by pre-existing surveillance actions.

[2] Historical distribution of target IAS within the LIFE RIPARIAS territory should cover the period 2000-2020.
[3] Half distance of giant hogweed and Himalayan balsam dispersal along rivers (Wadsworth *et al* 2000).

Table 4 – Intensity levels of surveillance to be conducted based on RIPARIAS partner consultation. Caption: * only for populations detected in connected water bodies without invasion of the water course; ** to be performed by private owners and managers as far as possible. Widely spread crayfish species are theoretically not targeted by the project but could be exceptionally controlled in poorly invaded RSUs.

	Spatial units			
	River, riverbanks and connected waterbodies	Floodplain	Remote ponds	Remote terrestrial habitats
EMERGING SPECIES (PRIORITY AND NON-PRIORITY AREAS)				
Water plants	level 3	level 3	level 2	-
Riparian plants	level 3	level 3	-	level 2**
Crayfish	level 3*	level 2-3	level 2	-
WIDESPREAD SPECIES (PRIORITY AREAS ONLY)				
Water plants	level 3	level 3	level 2	-
Riparian plants	level 2-3	level 2	-	level 1**
(Crayfish)	(level 2*)	(level 1)	(level 1)	-

4. Surveillance of riparian plants

4.1 Widespread riparian plants

The surveillance effort will focus on riverbanks and floodplains within priority areas only as defined previously. A systematic survey of any historical and new suspected sites occupied by *Impatiens glandulifera* and *Heracleum mantegazzianum*, supplemented by their immediate vicinity, will however be much too labour intensive. As illustrated in figure 4, it is rather proposed to focus field visits on:

- the identification of source populations in upstream river sections[4], from which subsequent management should start from,
- the validation and characterization of invaded plots in floodplains,
- the surveillance of priority RSUs where control actions were conducted before the onset of the LIFE RIPARIAS project to check for plant eradication,
- the survey of riverbanks by additional inventory samplings in under-prospected areas within priority RSUs, with a focus on Natura 2000 habitats (both within and outside special areas of conservation) and soil types most suitable for riparian plant establishment.

Surveillance (and management) of widespread IAS in remote habitats is expected to be mainly conducted through citizen science (CS) and enhanced by communication actions targeting the general public.

[4] Extensive search for new specimens should be conducted up to a distance of 500 m of the furthest upstream historical record. Note that several source populations may be identified along the same river providing that no historical observation is recorded along a stretch of at least 20 km.

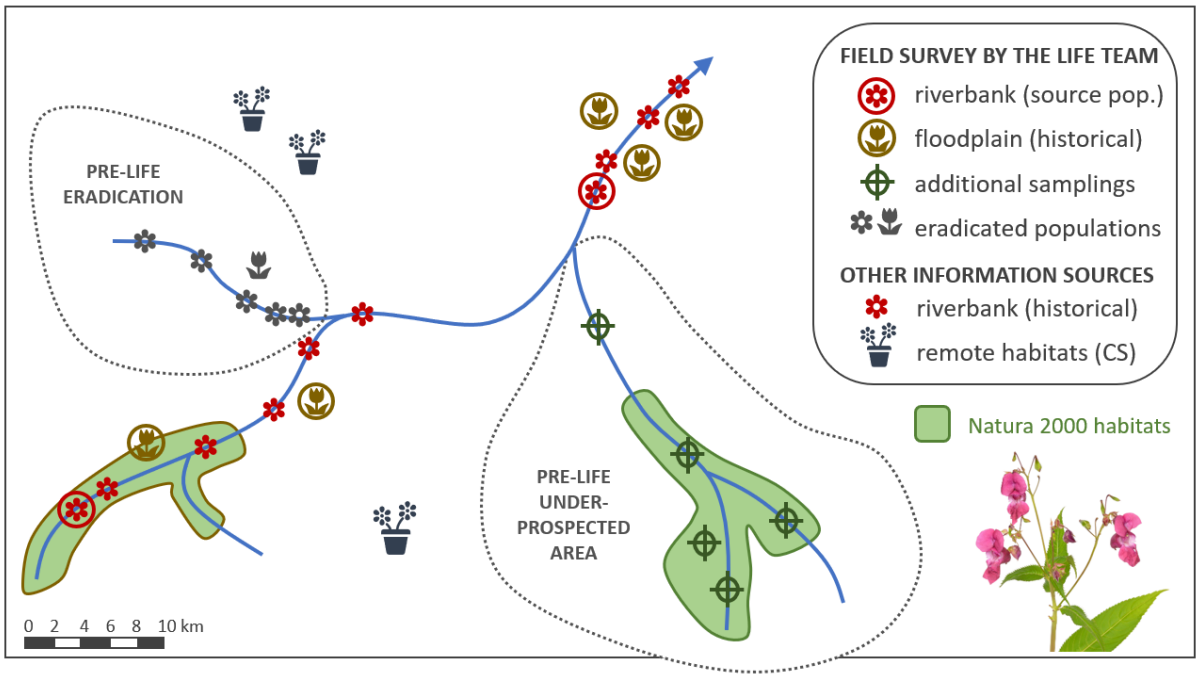

Figure 4 – Field survey to be conducted for the surveillance of widespread riparian plants.

Abundance values to be used for selecting RSUs to be managed through action C3 will be assessed from the density of historical observations per river kilometre and the analysis of metadata from existing databases. Accurate abundance data required for management efficiency monitoring (baseline 2022 in action D2) based on a linear abundance scale applied to river stretches as described in project proposal will be recorded only in year 3[5], by field manager teams as they make their way along the river system. A calibration work will be done during years 1-2 along some river samples to allow transforming observation densities into abundance data.

4.2 Emerging riparian plants

The surveillance effort will focus on historical and new suspected sites occupied by these species in riverbanks, floodplains and remote habitats, within priority and non-priority areas. Also their immediate vicinity will be surveyed as indicated in section 3 (level 2).

Additional inventory sampling will focus on sites within the riparian zone where historical introductions of alien plants could have been made. In practice, it is proposed to search for the presence of emerging riparian plants in the vicinity of ponds that will be considered for additional inventory sampling of water plants and crayfish (see below).

4.3 Seasonality

Survey of riparian plants will be preferably conducted between 1st July and 15th October to increase species detectability. However, specific surveys can be conducted from 1st April onwards, e.g. for the validation of the status of historical populations of giant hogweed or the surveillance of Himalayan balsam seedlings.

[5] In this way, time consuming river stretch inventories will be only conducted in RSUs under management.

Field surveys will be mainly conducted during years 1 and 2 of the project. For Himalayan balsam, they could be also conducted at the beginning of year 3, at the onset of the management actions (see above).

Specific attention should be drawn to IAS targeted by ongoing management activities: in this case, inventories should be conducted before management is implemented in the field. It means actors involved in ongoing management should be identified and contacted by the Life team.

4.4 Sampling device and abundance assessment

No specific sampling device is needed to collect riparian plants and abundance will be assessed through direct visual inspection. Three different scales will be used to assess plant abundance depending on species and habitat considered.

a) Abundance assessment of Himalayan balsam using polygonal abundance scores

In case of distinct extensive invaded patches found within the floodplain or in islands, plant abundance will be assessed in collecting information of the area invaded (i.e. the area including most of the plant specimens except for outliers) combined with a simple abundance score.

Area invaded - The area invaded will be roughly assessed in square meters either directly in the field or from digital maps as illustrated below.

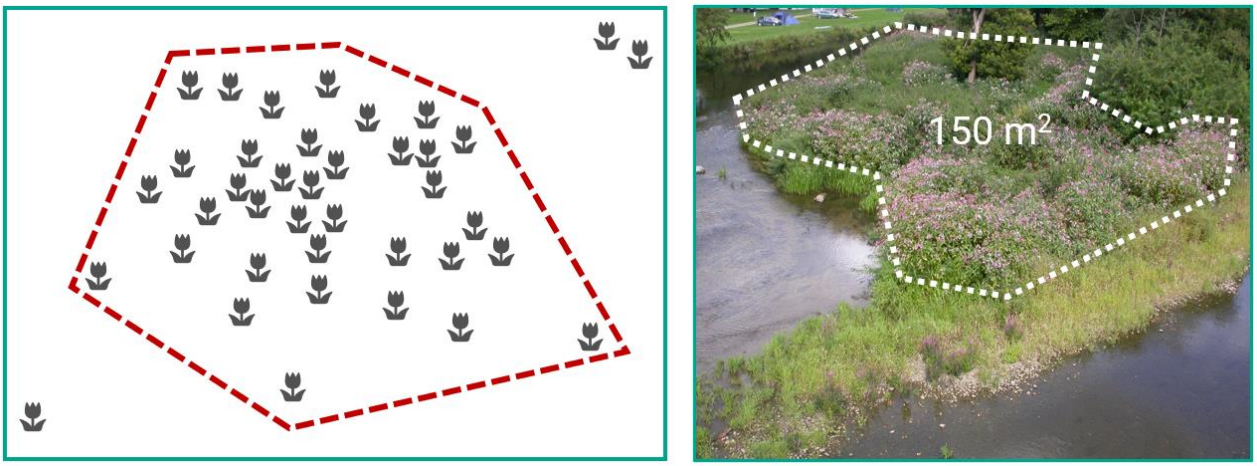

Figure 5 – Delineation of the invaded area. Picture: Etienne Branquart.

Abundance score - The simple 4-level scale illustrated in table 5 below will be used to assess plant abundance within patches. The visual representation of the different plant cover scores of this abundance scale provided below can be used to facilitate field assessment.

Table 5 – The polygonal abundance scale proposed for invasive plant assessment

Score	Abundance	% plant cover	Description
3	Dominant	> 50%	Continuous cover of the invasive plant all over the area
2	Abundant	25% - 50%	Intermediate cover of the invasive plant
1	Occasional	< 25%	Sparse cover of the invasive plant
0	Absent	0%	No invasive plant detected

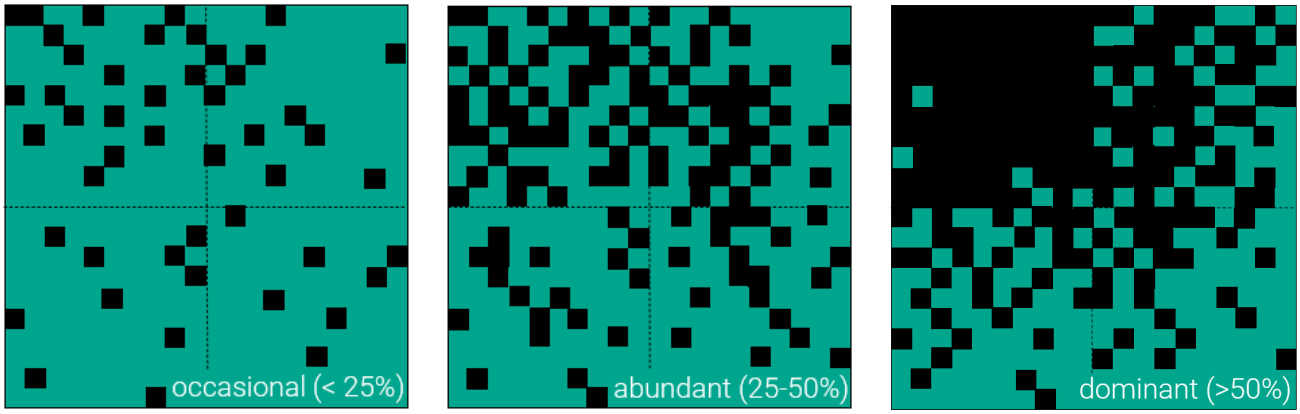

Figure 6 – Visual representation of the different abundance classes

b) Abundance assessment of Himalayan balsam using linear abundance scores (shorelines)

Abundance assessments of Himalayan balsam along riverbanks will be carried out along 50-m long river stretches using the following simple linear abundance scale already in use in Wallonia to guide management operations:

Table 6 – The linear abundance scale proposed for Himalayan balsam (HB) assessment along riverbanks

Score	Abundance	Description
3	Dominant	Riverbank stretch invaded by extensive dense patches of HB whose management requires intensive mechanical control (brushcutter).
2	Abundant	Frequent occurrence of isolated HB plants and presence of a few dense patches along the riverbank stretch, to be managed through intensive hand-pulling operations.
1	Occasional	Few isolated HB plants spread along the riverbank stretch that can be easily managed by light hand-pulling operations.
0	Absent	No HB plant detected.

c) Abundance assessment of large conspicuous riparian plants using plant counting scores

For large and conspicuous plants like giant hogweeds and skunk cabbage, counting scores may be easier to use to assess local abundance than the polygonal abundance scores. Indeed, this method doesn't require to assess the invaded area. The scale in table 7 may be used in replacement or in complement to abundance assessment proposed in a). It is already in use in Wallonia for several years and may be directly linked to manpower required for management.

Table 7 – The plant counting scale for abundance assessment of giant hogweed

Score	Plant number (> 1 year old)
4	> 1000
3	101-1000
2	11-100
1	1-10
0	Absent

Only plants older than one-year need be considered as highlighted in the figure below. Seedlings (< 1-year-old) may be easily recognised thanks to rounded and poorly divided leaves.

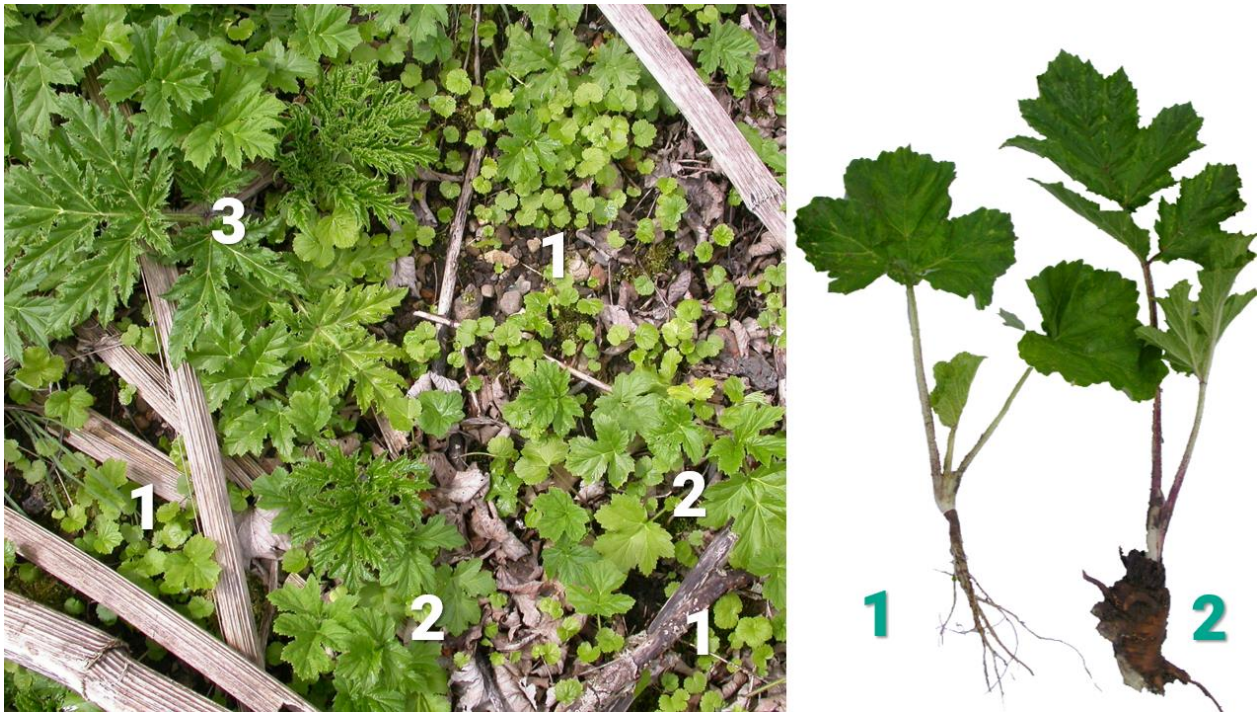

Figure 7– Distinction between different age classes of giant hogweed in spring and summertime (1 = seedlings less than one-year-old, 2 = plants between 1 and 2-year-old, 3 = plants over 2-year-old). Pictures: Etienne Branquart.

4.5 Management-related information

Site-specific information useful for management should be gathered in addition to plant abundance, including ownership, accessibility, etc.

5. Surveillance of aquatic plants (macrophytes)

5.1 Widespread aquatic plants

The surveillance effort will focus on watercourses and ponds within priority areas only as defined previously. A systematic survey of any historical and new suspected sites occupied by *Hydrocotyle ranunculoides, Ludwigia grandiflora* and *Myriophyllum aquaticum* will be conducted in these areas (including remoted ponds) in order to validate species presence, assess their abundance and collect management-related information.

Also their immediate vicinity will be subjected to field surveillance, including other ponds found within a radius of at least 25 m as well as river sections and river-connected ponds up to 1 to 3 km downstream from source populations.

5.2 Emerging aquatic plants

The surveillance effort for emerging aquatic plants will focus on historical and new suspected sites supplemented by their immediate vicinity as described above, but within priority and non-priority areas.

Additional inventory sampling will focus on sites within the riparian zone where historical introductions of alien plants could have been made. As multiple introductions of aquatic alien plants frequently occur in fishing and ornamental ponds[6], emerging aquatic plants will be searched for in sites wherein presence of other taxa was reported in the past. Historical observation data of candidate aquatic plant species for alert list will be considered to this purpose, including the following taxa: *Aponogeton distachyos, Crassula helmsii, Egeria densa, Gunnera manicata, Hydrilla verticillata, Mimulus guttatus, Myriophyllum 'brasiliensis'* (red stem), *Petasites japonicus, Pistia stratiotes, Pontederia cordata, Sagittaria latifolia, Saururus cernuus* and *Zantedeschia aethiopica*. Also occurrence of casual species of the EU list will be considered, i.e. *Eichhornia crassipes* and *Gunnera tinctoria*.

5.3 Seasonality

Survey of aquatic plants will be preferably conducted between 1st June and 15th October to increase species detectability. Field surveys will be mainly conducted during years 1 and 2 of the project, with a focus on year 2 as alert list and new identification tools will not be available before the beginning of this year.

Specific attention should be drawn to IAS targeted by ongoing management activities: in this case, inventories should be conducted before management is implemented in the field. It means actors involved in ongoing management should be identified and contacted by the Life team.

5.4 Sampling design and abundance assessment

Macrophyte sampling is a rather time-consuming task requiring specific expertise. Material to be used includes boots and telescopic rake (4 m). As identification using plant fragment is not always easy, it will sometimes require laboratory examination ("bag & tag" approach as advised by Kevin Scheers)[7].

Particular attention should be paid to biosecurity measures to avoid any new accidental introduction of IAS and associated pathogens (crayfish plague), using adequate biosecurity such as cleaning and disinfection of sampling material before using it in new sites.

The polygonal abundance scale proposed for riparian plants (table 5) will be used for the abundance assessment of aquatic plants. It will be applied at the scale of water bodies, 50-m long river stretches or invaded patch in case of a limited invasion spot. In any case, plant abundance will be supplemented with information related to the approximate size of invaded patch and colonised pond, together with GPS coordinates.

[6] See e.g. Delbart E. (2012) Etat des lieux actualisé des plans d'eau envahis par *Crassula helmsii, Hydrocotyle ranunculoides, Ludwigia grandiflora, L. peploides* et *Myriophyllum aquaticum* à l'échelle de la Wallonie. Rapport Gembloux Agro-Bio Tech, 36 pp. ; de Boer *et al.* (2016) Assessment of the risks to Norwegian biodiversity from the import and keeping of aquarium and garden pond plants. Opinion of the Panel on Alien Organisms and Trade in Endangered Species (CITES) of the Norwegian Scientific Committee for Food Safety. VKM Report.

[7] Based on INBO's expertise, between 2 and 20 ponds can be surveyed within a day depending on site accessibility and pond size.

Figure 8– Delineation of the invaded area will be done either at the scale of the invaded patch (limited invasion) or at the pond scale (extended invasion). Pictures: Etienne Branquart.

5.5 Management-related information

The following information will be collected in the field in addition to species abundance in order to assess site manageability and choose for the best management technique(s):

- Site use (fish pond, ornamental purpose, etc.)
- Site accessibility
- Pond size
- Pond water supply and drawdown possibilities
- Pond siltation degree
- Presence of protected species (e.g. plants and amphibians)
- Risk of IAS spread towards neighbouring sites (window of opportunity)

6. Surveillance of emerging crayfish

6.1 Emerging crayfish

Crayfish surveillance will mainly focus on emerging species listed under the IAS Regulation, i.e. *Faxonius virilis*, *Procambarus clarkii* and *P. virginalis,* to which alert list species will be added like *Procambarus acutus*. Also *Pacifastacus leniusculus* populations located in Brussels or in Flanders deserve special attention as this species is currently poorly represented in these regions. No active surveillance of *Faxonius limosus* and *Pontastacus leptodactylus* (not EU listed) will be conducted but species presence will be recorded when detected through the surveillance of emerging species.

As for emerging aquatic plants, the surveillance effort for emerging crayfish will target historical and new suspected sites supplemented by their immediate vicinity within priority and non-priority areas (see above), with a focus on fishing and ornamental ponds.

Additional inventory sampling will focus on ponds that should be surveyed for macrophyte sampling sites in the riparian zone (see section 5 here above), especially when they are connected to rivers and may act as a propagule source for downstream areas.

6.2 Seasonality

Crayfish surveys with traps will be mainly conducted during years 1 and 2 of the project. Trapping will be conducted during the crayfish activity period, i.e. preferably when water temperature exceeds 10°C (approximately from 1st April and 15th October).

Additional e-DNA surveys will be envisaged for year 2 depending on availability of additional budgets (see below).

6.3 Sampling design and abundance assessment

Trapping: Crayfish surveillance will be mainly conducted by baited trapping. Cylindrical metal traps will be favoured as they consist of solid devices with small mesh. They are robust, discrete (black colour) and easy to open. Their cylindrical shape is well suited to throw into the water. As they can be split in tow, they are also easy to transport and crayfish extraction from traps is facilitated. Addition of a perforated plastic container into the traps is recommended for bait addition (see picture). This trap device has been already successfully used for crayfish surveys by DEMNA and INBO.

Figure 9– Example of baited trap recommended for crayfish surveillance. Picture : Kevin Scheers.

Field sampling will be organised as follows, based on guidelines provided by Larsen & Olden (2016)[8]. Single short trapping sessions (1 night) will be performed per site during crayfish activity period, making use of at least 5 traps per pond or per 50-m long river stretch. Additional traps will be added to survey large bodies to reach up to 25 traps per site separated by a minimum distance of 3 to 10 meters. They should be installed to sample all micro-environmental conditions and substrates found in the ecosystem. Traps will be baited systematically to increase capture rate, using preferably frolic dry dog food as bait. The personnel in charge of trapping shall have a proper authorisation issued by the regional fishery authority.

The number of captures per trap and per night will be recorded for the different crayfish species in order to determine catch per unit effort (CPUE).

[8] Larson, E. R., & Olden, J. D. (2016). Field sampling techniques for crayfish. Biology and ecology of crayfish, 287, 324.

Trapped crayfish will be killed on-site with a knife or through freezing. After each trapping session, traps will be systematically disinfected using e.g. Virkon to avoid spreading crayfish plague and chytridiomycosis between sites.

e-DNA: As crayfish detection from DNA analysis of water samples is increasingly found to be quite effective to conduct year-round monitoring and detect rare species in lentic and lotic habitats[9], its use as a complementary crayfish sampling technique will be considered during the second year of the project depending on first results of survey and budget available at that time. The possibility to detect submerged macrophytes from the same water sample using metabarcoding techniques will be investigated as well[10].

6.4 Management-related information

The same information as this collected for macrophytes is required to assess site manageability and choose for the best management technique(s) (see above).

7. Contacts with private owners

Numerous ponds and riparian habitats are located in private domains and difficult to access. Field personnel will have to comply with regional rules to conduct surveillance in these domains. IAS surveillance and management in private properties are for example described through a specific provision in the regional IAS Decree in Wallonia[11]; it requires identifying the site owner and contacting him/her before accessing private terrain. Note that conducting management in private properties will also require establishing a convention with site owners for a duration of at least 10 years based on LIFE rules.

[9] Komai, T., Gotoh, R. O., Sado, T., & Miya, M. (2019). Development of a new set of PCR primers for eDNA metabarcoding decapod crustaceans. Metabarcoding and Metagenomics, 3, e33835; Chucholl, F., Fiolka, F., Segelbacher, G., & Epp, L. S. (2021). eDNA detection of native and invasive crayfish species allows for year-round monitoring and large-scale screening of lotic systems. Frontiers in Environmental Science, 9, 23.

[10] Kuzmina, M. L., Braukmann, T. W., & Zakharov, E. V. (2018). Finding the pond through the weeds: eDNA reveals underestimated diversity of pondweeds. Applications in plant sciences, 6(5), e01155; Coghlan, S. A., Shafer, A. B., & Freeland, J. R. (2021). Development of an environmental DNA metabarcoding assay for aquatic vascular plant communities. Environmental DNA, 3(2), 372-387

[11] Article 24 du Décret wallon du 02 mai 2019 (MB 16.10.2019) : « Les fonctionnaires et agents désignés par le Gouvernement, les agents et personnes chargés de la de la mise en œuvre des mesures d'éradication et de gestion ainsi que les agents et personnes chargés de la surveillance sont autorisés, en vue d'exercer leurs missions, à pénétrer en tout lieu, non constitutif d'un domicile au sens de l'article 15 de la Constitution, pour y faire toutes recherches ou constatations utiles et pour procéder aux opérations nécessaires à la mise en œuvre des missions précitées. Ils peuvent se faire communiquer tous les renseignements qu'ils jugent utiles. L'accès est permis en tout temps, moyennant un avertissement préalable des propriétaires ou des occupants au moins quarante-huit heures avant l'intervention (…). Lorsqu'il s'agit d'un domicile au sens de l'article 15 de la Constitution, l'accès est subordonné au consentement écrit des propriétaires ou des occupants ou, à défaut, à une autorisation du juge d'instruction.

RIPARIAN PLANTS

EMERGING RIPARIAN PLANTS

	RIVER, RIVERBANKS AND CONNECTED WATERBODIES	FLOODPLAINS	REMOTE HABITATS
I	Historical and opportunistic data (basic citizen science inventories)		
II	Field visit of historical and new suspected sites (+ immediate vicinity)		
III	Inventory samplings in additional sites (incl. enhanced citizen science inventories)		No additional sampling

WIDESPREAD RIPARIAN PLANTS
In priority areas only!

	RIVERBANKS	FLOODPLAINS	REMOTE HABITATS
I	Historical and opportunistic data (basic citizen science inventories)		
II	Identification of source pops and confirmation of pre-LIFE eradications	Field visit of historical and new suspected sites (+ immediate vicinity)	No field visit
III	Inventory samplings in under-prospected areas (focus on Natura 2000)	No additional sampling	

AQUATIC PLANTS AND CRAYFISH

EMERGING AQUATIC PLANTS

	RIVER, RIVERBANKS AND CONNECTED WATERBODIES	FLOODPLAINS	REMOTE HABITATS
I	Historical and opportunistic data (basic citizen science inventories)		
II	Field visit of historical and new suspected sites (+ immediate vicinity)		
III	Inventory samplings in additional sites (incl. enhanced citizen science inventories)		No additional sampling

WIDESPREAD AQUATIC PLANTS
In priority areas only!

	RIVER, RIVERBANKS AND CONNECTED WATERBODIES	FLOODPLAINS	REMOTE HABITATS
I	Historical and opportunistic data (basic citizen science inventories)		
II	Field visit of historical and new suspected sites (+ immediate vicinity)		
III	Inventory samplings in additional sites (incl. enhanced citizen science inventories)		No additional sampling

EMERGING CRAYFISH

	River, riverbanks and connected waterbodies	Floodplains	Remote ponds
I	Historical and opportunistic data (basic citizen science inventories)		
II	Field visit of historical and new suspected sites (+ immediate vicinity)		
III	Inventory samplings in additional sites (incl. CS)	No additional sampling	

Appendix 2 – Results of the online survey: expectations from project partners

An online survey was sent on 29th January 2021 to LIFE RIPARIAS partners in order to refine spatial units and define priorities for surveillance and management. The main results of this survey may be summarised as follows:

1) Priorities for surveillance and management were identified based on type of organisms and spatial units within the LIFE RIPARIAS territory. Highest priority organisms were water plants followed by riparian plants and by crayfish (water plans > riparian plants > crayfish). LIFE RIPARIAS partners considered that emerging IAS have a higher priority for surveillance and management than widespread IAS. Spatial units located closer to watercourses were considered a higher priority than remote ones (riverbanks > floodplain > remote ponds).

2) Although IAS populations located in remote terrestrial habitats as gardens or fallow lands (spatial unit 4) are not explicitly included into target areas of the LIFE RIPARIAS project, partners agreed that they should be somehow managed. Site owners will be prompted to take action to get rid of invasive populations through communication actions and/or specific obligations depending on regional context.

3) RSUs were endorsed as elementary units for subsequent management of the 5 widespread invasive plants (i.e. *Impatiens glandulifera, Heracleum mantegazzianum, Hydrocotyle ranunculoides, Ludwigia grandiflora* and *Myriophyllum aquaticum*). Small adaptations of a few RSUs were proposed by project partners in order to better cope with the hydrological structure of the territory. A final RSU subdivision was adopted on this basis.

4) LIFE RIPARIAS partners agreed that systematic surveillance and management of the 5 widespread IAS should be primarily conducted in so-called priority areas based on the extend of the riparian zone benefiting from an official protection status defined by regional nature conservation instruments (mainly Natura 2000 sites). A threshold of 10% of river linear under Natura 2000 status was considered to this purpose, to which 2 additional RSUs were added by partners due to regional interest.

5) LIFE RIPARIAS partners chose for a moderate intensity riverbank surveillance of widespread IAS during the two first years of the project in order to safeguard resources to survey other spatial units and target organisms. The distribution of the 5 widespread plants along riverbanks will be assessed within priority areas through the analysis of historical data supplemented by additional field survey of under-prospected areas and field validation of source populations for the different species. Abundance values to be used for selecting RSUs to be managed (action A5) will be assessed from the density of historical observations per river kilometre; accurate abundance data required for management efficiency monitoring (baseline in action D2) will be recorded only in a second step.