# Summary

# Restoration of lowland streams through the reintroduction of macroinvertebrate species

## Reason and object

The changes in the landscape have created huge barriers for the dispersion of aquatic insects, often rendering it no longer possible for species to move from one drainage basin to another (Malmqvist 2000; Nijboer 2004). It so happens that movements mainly take place upstream and downstream, whereas lateral movements to other streams seldom occur across larger distances. There have been indications that stream macroinvertebrate are generally not capable of moving more than 5 kilometers (Sundermann et al. 2011). The lower reaches of the stream, where exchanges of individuals between drainage basins could take place, are precisely the most affected, making exchanges virtually impossible. In addition, the small populations that remain do not generate enough colonists with which to successfully colonize new areas, because the animals that disperse themselves laterally face a high risk of dying before finding a new stream.

The only solution is either to restore stream ecosystems in their entirety, removing the dispersion barriers in that way and increasing the dispersion opportunities for species, and then wait for colonization to take place. In addition to the fact that this is often impossible due to the scale on which measures must be taken, and that this is therefore usually in conflict with the adjacent use of the land, it is also a process that occurs in the long to very long term (at least decades, but more likely centuries). An alternative concerns the (re)introduction of species that do not disperse easily at the spots within the natural area of distribution that are once again environmentally suitable after taking the restoration measures.

The human activity of moving species from one water body to the other is called translocation. Moving animals to locations where they do not occur can take place within different contexts (Olden et al. 2011; Schwartz et al. 2012; IUCN 2013). Reintroduction concerns releasing species within the historical area of distribution at locations where the species were not (or are no longer) found. Species additions, in which small populations are strengthened by acquiring fauna elsewhere also falls within this category. Relocation or assisted colonization (or migration) concerns moving populations of species or genotypes to locations outside the historical area of distribution with an aim to protect a species against the influences of environmental changes, such as climate change.

There are various locations at which the circumstances have improved compared to a few decades ago, creating new, potentially suitable habitats for the species that have disappeared. Yet these species usually do not return. In a biological sense, the results of many stream restoration projects in the Netherlands are disappointing as well. In some cases, the scale of the project was too small, or the project did not deal with all of the pressure factors, or the restoration time was too short. But there are also many locations at which these aspects are not at issue. Here, the restoration appears not to take place due to biological factors, such as the dispersion- and colonization-capacity of the desired species. The goal of reintroduction as a stream restoration measure is therefore not so much aimed at restoring a historical situation or a desired reference, but rather to improve the functioning of the lowland stream ecosystem by increasing the diversity of functional groups.

## Preparations to be able to carry out a reintroduction

Before a start can be made with a reintroduction, it is essential that a sufficient number of individuals of the selected species are available. Based on previous reintroductions, the guideline is to consider 500 individuals as the minimum number required to start a new population. For the groups studied, larvae or adults appeared to be the most suitable life stages to collect in the field. Field research has shown that the random sampling of communities was found to yield insufficient individuals per species. Not because of the mortality rates during sampling, transport or storage, but rather due to the much too low probability of actually collecting the desired species. Two options appeared to be quite promising: collecting the target species by hand in the field and rearing the species in the laboratory. In the case study, the former was successfully applied.

When a species is relocated to a new environment, interactions occur with the community already present. Animals must position themselves in relation to, for example, food sources and shelter, whereas these are already (potentially) occupied by other species. To gain more insight into the consequences for the introduced species in a situation of this kind, a long-term experiment was carried out in artificial streams (so-called mesocosms, being any outdoor experimental system that examines the natural environment under controlled conditions) using the caddisfly community of the case study. Larvae of *L. basale* were placed in a community consisting of six other species of caddisflies under different environmental conditions (habitat heterogeneity and flow rate were varied). It was then examined how many individuals successfully emerged and whether the adults differed in terms of their general fitness. In the end, 57% of the *L. basale* larvae that were introduced into the mesocosms reached the adult stage. It was also found that a high habitat heterogeneity had a positive effect on the survival rate, indicating that stream sections with substrate mosaics were the most suitable locations for the introduction of this species.

Another important aspect of the reintroduction is the manner in which the animals are introduced into the stream. How this is done may influence the number of animals that survives. For example, if larvae are carried downstream with the flow, this could mean that they end up in an unsuitable habitat, resulting in mortality. This principle has been tested for *L. basale* in artificial streams by applying three different release techniques subject to an increasing level of flow and substrate roughness: release directly into the water column, placing the species on the bottom substrate by hand and indirectly releasing the species using pre-colonized artificial substrates. Under low flow conditions, it does not really matter which release technique is used, whereas at higher flow velocities, the substrate type in particular influenced which technique yielded the best results. Overall, the pre-colonized artificial substrate proved to be the most efficient choice.

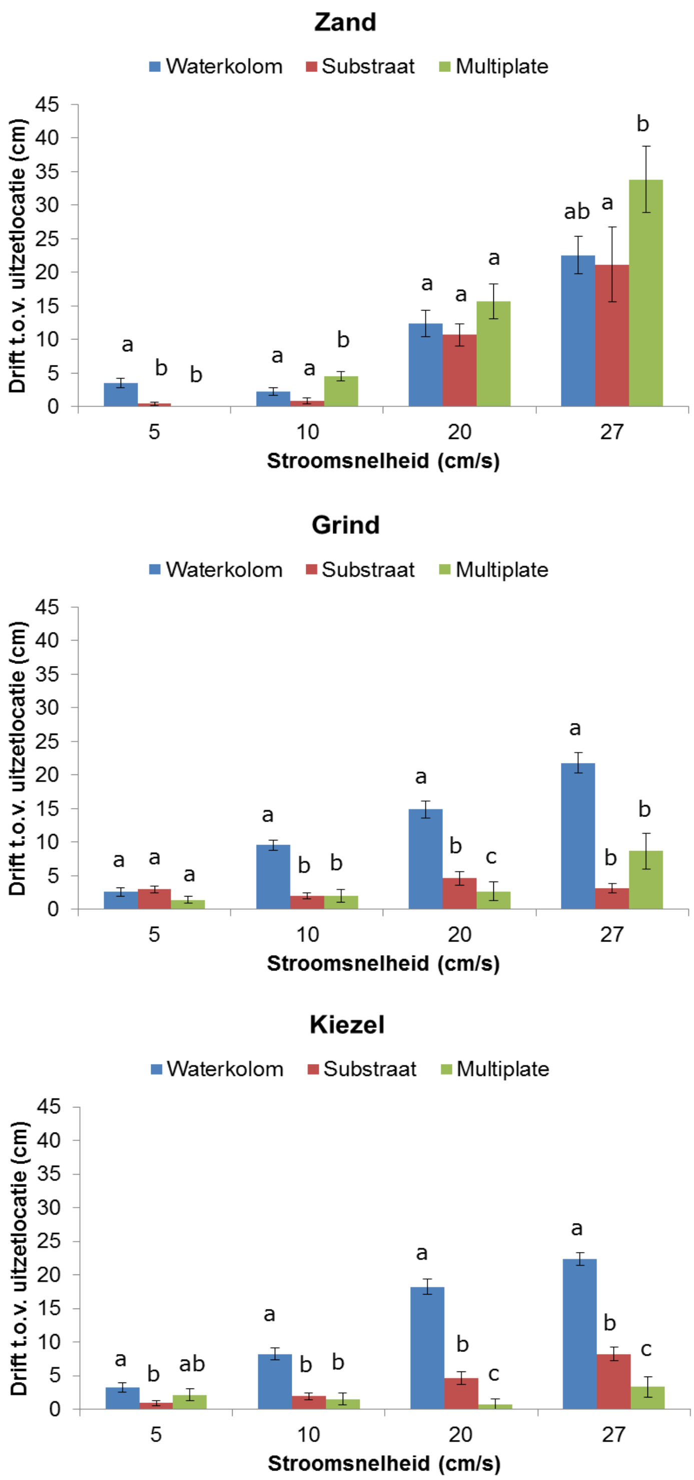


***Figure 1**: Distances drifted by larvae of Lepidostoma basale for three introduction methods (released directly into the water column, placed on the bottom substrate by hand, and released using the Multiplate-sampler) at 4 current velocities and 3 substrate types. Letters indicate significant differences between methods per current velocity.*

**Explanation**: Zand = Sand / Grind = Gravel / Kiezel = Silicon / Drift tov uitzetlocatie (cm) = Drift in relation to release location (cm) / Stroomsnelheid (cm/s) = rate of flow (cm/s) / Waterkolom = Water column / Substraat = Substrate / Multiplate = Multiplate

## Approach

Literature studies show the number of coordinated and documented reintroductions of invertebrates in European streams and rivers to be low and frequently unsuccessful. Therefore, a generic framework for reintroductions as a stream restoration measure was developed in this study based on descriptions in scientific literature and supported by laboratory and field studies. The application of the framework is illustrated by a case study in which the potential for the reintroduction of mayflies, stoneflies and caddisflies in the stream *Heelsumse beek* on the southern Veluwe was examined. The framework consists of two main components, a step-by-step plan describing the procedure for assessing the feasibility of a reintroduction and for assessing the species that are eligible for a reintroduction and a protocol for the actual reintroduction in which the methodology and practical aspects are described.

***Table 1****: Overview of the components that play an essential role in determining the feasibility of a reintroduction project*.

| **Component** | **Answers the question** |
|---|---|
| Selection of species that are eligible for reintroduction in a stream process (step 1) | Which species are currently present in the target stream?<br>Which species are not present in the target stream, but are present in the region?<br>Which species that are characteristic of the target stream are lacking? |
| Selection of functionally important species (step 2) | Which absent species are most suitable for a possible reintroduction? |
| Auto-ecology selected species (step 3) | What do these species require to successfully go through their life cycle? |
| Assessment target system (step 4) | Is the target stream suitable for the species that has been selected? |
| Determination of the current dispersion of the species and selection of source population (step 5) | Is a source population available that can serve as donor without the source population being negatively influenced? |
| Estimate of risk of undesired species hitching a ride (step 6) | Is there a risk of the dispersion of exotic species when animals are relocated from the donor stream to the target stream? |

## Step-by-step plan for species selection

The first step concerns selecting the species that are eligible for reintroduction into the restored stream. This involves determining which species are still present in the target system and which are absent. The starting point in this respect is the species pool that is characteristic of the target stream type. This characteristic pool is compared to the species that can be observed in the area in which the target-stream is located, being on a higher scale level (a larger drainage area) than the stream itself. An interspersion distance of five kilometres between streams was used as the maximum distance which could be bridged by dispersing individuals.

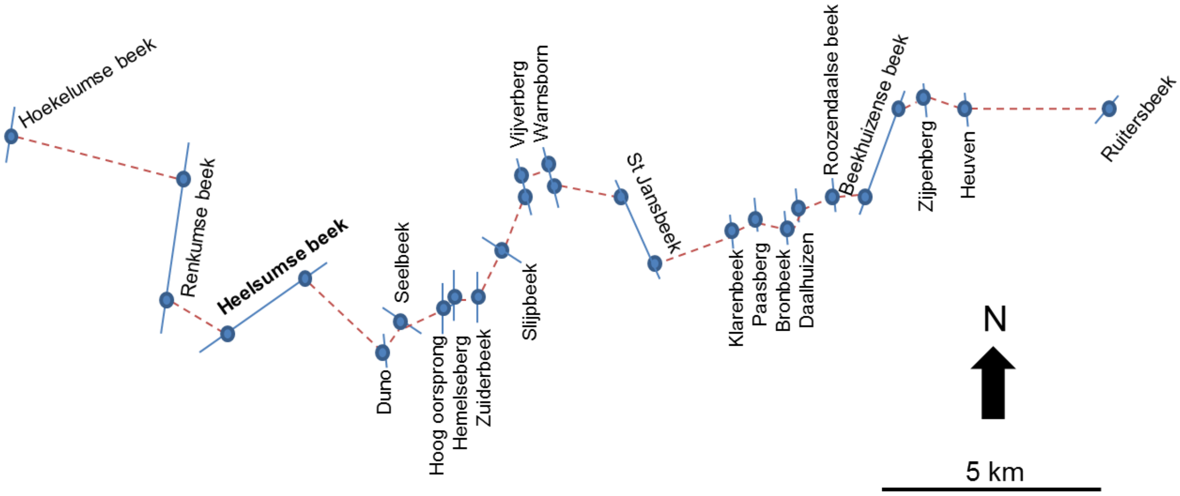


***Figure 2***: *Streams containing the species pool of the south Veluwe. A maximum interspersion distance of five kilometres between streams (orange dotted lines) was used to determine the size of the study area.*

The second step involves examining the functional role of the species that are lacking based on the functional feeding groups. Concerning the functioning of the eco-system, the best candidates for a reintroduction are the species that have a functional role that is lacking in the stream and that generally occur in high densities and/or have a high biomass.

The third step concerns a risk analysis for both the target and the source stream. The (a)biotic habitat requirements are summarized for the selected species in a habitat suitability model, which can then be used to determine the suitability of the target system for the selected species. In the Heelsumse beek case study involving three insect orders, only one species appeared to be a suitable candidate for reintroduction, namely the caddisfly *Lepidostoma basale*.

The fourth step concerns applying the system- and habitat suitability model that was developed for the species that is to be introduced to the stream system, so that any potential bottlenecks can be identified before the animals are actually introduced. Should it be found that there are no solutions for the potential bottlenecks, then the system is simply not suitable for the introduction of the species concerned. Knowledge of the system based on data acquired through multiple years is naturally crucial in this respect.

***Table 2****: Conditions in the target system, the stream Heelsumse beek at location x=206.383, y=350.463. Factors used in the table are based on the habitat-suitability-model for the species. Colours indicate suitability of the key factors: red = not suited, yellow = moderately suited, green = suited. Minimum current velocity and maximum water temperature in April-June (pupal-phase) are based on monthly measurements. The frequency of peak discharges is based on measurements with 30-minute intervals. g.g. no data.*

| **Period** | **Parameters habitat- and system suitability model** | | | | | |
|---|---|---|---|---|---|---|
| | Dimensions | Stagnation $V_{min}$ | Peak discharge frequency | Water temperature | Substrates | Ligneous vegetation |
| 2000-2004 | upper-middle course | 0 | g.g. | 13.1 | roots, branches, stones present | Strip with *Alnus, Salix* en *Quercus* |
| 2004-2008 | upper-middle course | 2 | g.g. | 13.7 | roots, branches, stones present | Strip with *Alnus, Salix* en *Quercus* |
| 2009-2013 | upper-middle course | 8 | 2 in 5 years; (not both in the same year) | 13.2 | roots, branches, stones present | Strip with *Alnus, Salix* en *Quercus* |

Step 5 concerns gaining a good impression of the streams or catchment areas where the species are still present before proceeding to reintroduce them. It should be noted in this respect that the locations where a species can currently be found do not necessarily entail the optimal habitat (IUCN 2013). After all, a species may have been forced back into habitats with sub-optimal conditions after the original habitat became degraded.
When the reintroduction takes place using material (eggs, larvae, pupa) that has been collected directly from a stream, then it is important to know what exactly is being translocated from one stream to the other (step 6). It so happens that undesired species may hitch a ride with the collected material, including certain exotics, for example. The risk increases when complete standard net samples, Surber samples or colonized artificial substates are transported from one stream to the other, as there is always uncertainty regarding which species and how many are precisely present in the sample used.

## Heelsumse beek

The *Heelsumse beek*, one of the largest streams bordering on the southern region of the Veluwe has been used as the target system (Figure 3 below). Much is known about this stream system, as it is a much used object of study for students at the former Landbouwhogeschool (now the Wageningen University) in Wageningen and has been studied in detail in the past. The source of the Heelsumse beek and upper course, consisting of multiple springs, are relatively acidic and nutrient-poor. The stream becomes less acidic and richer in nutrients farther downstream. In the seventies of the past century, the stream system had become highly degraded due to the discharge of unpurified water used for industrial processes and a decrease in the water drainage as a result of water draw-off. The unpurified discharges ceased in 1979/1980. At that time, the system was highly organic and chemically affected, with an extremely high pH. In the decades to come, various measures were taken to improve the water quality, including hydrological and morphological measures. The quality of the stream has since significantly improved compared to the situation in the seventies.

***Figure 3**: The stream Heelsumse beek near the Kerkweg in the village of Heelsum.*

### The actual reintroduction

Following the actual reintroduction, there are three points in time from which the success of the reintroduction can be derived:

1. Successful settlement at the location of the reintroduction. The presence of offspring of the species that has been reintroduced at the release location is a sign that the species has established itself, in the short term in any event.
2. The coming about of a population that can maintain itself. The higher the number of generations to stem from the reintroduced population, the stronger the indication that a viable population has come about that is also capable of survival in the long term.
3. Expansion of the species. The species spreads to other locations within the drainage basin, establishing new sub-populations. This is a sign that the reintroduction has been successful.

On 12 March 2014, 2400 larvae of *L. basale* were released in the stream Heelsumse beek, all in the same stream section. The most optimal release site was determined on the basis of the requirements described in the habitat suitability model and the presence of a heterogeneous mosaic of substrate types. The animals were released on artificial substrates. Immediately following the release, the larvae spread downstream across the stream bottom (they were up to 25 meters downstream after 1 day). No drift in the water column was recorded. One month later, the species was found to still be in the larval stage. The following summer, empty pupal cages were found, indicating that the animals had reached the adult stage. In the years to come, monitoring in early spring and late spring-summer is necessary to detect larvae and adults respectively, providing an indication of whether or not the species has successfully established itself. Also, the adjacent streams should be included in the monitoring scheme, so as to record any expansion along the edge of the southern Veluwe. Furthermore, it could also be examined whether the reintroduction of the species, which is regarded as an ecosystem engineer, has a significant impact on the functioning of the stream ecosystem, for example, by following leaf decomposition and nutrient flows.

## Findings

It is obvious from this study that the reintroduction of macrofauna is complicated. It requires a sound foundation based on potentially present and actually present groups of species, the functional role and the auto-ecology of species and the (a)biotic limiting conditions of the target system. The most important findings of this study have been generalized in a protocol for releasing macrofauna, consisting of two flow charts. The first examines preparing for the reintroduction of macrofauna, the second concerns the actual reintroduction, including the monitoring following the reintroduction. The latter is based on the subsequent phases that make up the development of a population when a species establishes itself at a new location. The protocol must be preceded by a step-by-step plan for selecting suitable species and for assessing the feasibility of a reintroduction. This step-by-step plan and the two flow charts referred to above are included in this summary at the end of this document (Figures 4, 5 and 6).

Two important problems that were encountered in the course of the study and that will play a role in any introduction concerns the fact that many species are so rare in the Netherlands (or have disappeared) that it is not possible to collect these in large numbers and/or it is difficult to collect large numbers of a species in a stream. Regular sampling techniques were found to yield insufficient numbers and collecting species by hand is only possible for species that are easily recognized. The latter is quite problematic for the majority of the macrofauna. The solution to both these problems is to cultivate these species. How to precisely go about this falls outside the scope of this study. However, there are studies that show that such cultivations are difficult and time-consuming, and that the cultivated macroinvertebrates are often weak and much smaller than normal; they most likely like a certain nutrient that is available in their natural habitat. It is therefore recommended that subsequent research relating to this subject matter be aimed at optimizing the cultivation process of macrofauna species so that it is possible to generate large numbers of a desired species. At the same time, this raises the question of whether or not there will be genetic consequences for the cultivated populations (how to prevent inbreeding, where do the cultivation species initially come from). These genetic aspects should be taken into account when setting up cultivation lines.

**Images of nature in relation to the release of stream macrofauna**
Finally, there is one more aspect that frequently came up in the course of the study, namely differences of opinion concerning the necessity of releasing macrofauna. The image that people have of nature differs from one individual to another. Some people feel it is justified to interfere in an ecosystem in this way; after all, we have caused degradation and the disappearance of species for more than a century now. If a species is not capable of returning to a restored system on its own, then it certainly deserves a bit of help, particularly if the species concerned can make a positive contribution to te functioning of the system. Others reject this idea; they feel that nature should be left to go its own way and they consider the release of species as a type of ‘gardening’. Species should return to restored systems on their own, anything else is unnatural. There were also people who objected against the use of a reference ecological community from the past and to claim that species were missing based on that, as ecosystems develop in a changing world (climate change, for example) and are therefore never the same as they were in the past. It is important to keep these differences of opinion in mind in subsequent studies and to ensure adequate and open communications between all of the parties involved.

## In conclusion

The initial positive results achieved in the case study — in which an 'ambassador' for natural lowland streams in Northwest Europe was used with respect to the environment and the habitat requirements and that is also recognized as an ecosystem engineer with a significant influence on the functioning of the ecosystem — indicate that the set-up developed in this study appears to be a major step in the right direction.

Of course, a long-term success cannot be concluded until after a number of years, but that is intrinsic to this type of innovating and unique studies. After all, this is the first time that an introduction of macrofauna under controlled conditions and in such detail has taken place in the Netherlands. It is therefore concluded that the protocols developed here can serve as a template for future introductions of macrofauna in other stream systems.

The two main components of the framework developed, namely a step-by-step plan describing the procedure for assessing the feasibility of a reintroduction and for assessing the species that are eligible for a reintroduction are shown below, as well as a protocol for the actual reintroduction in which the methodology and practical aspects are described. A translation of the text in the figures is provided below each figure.

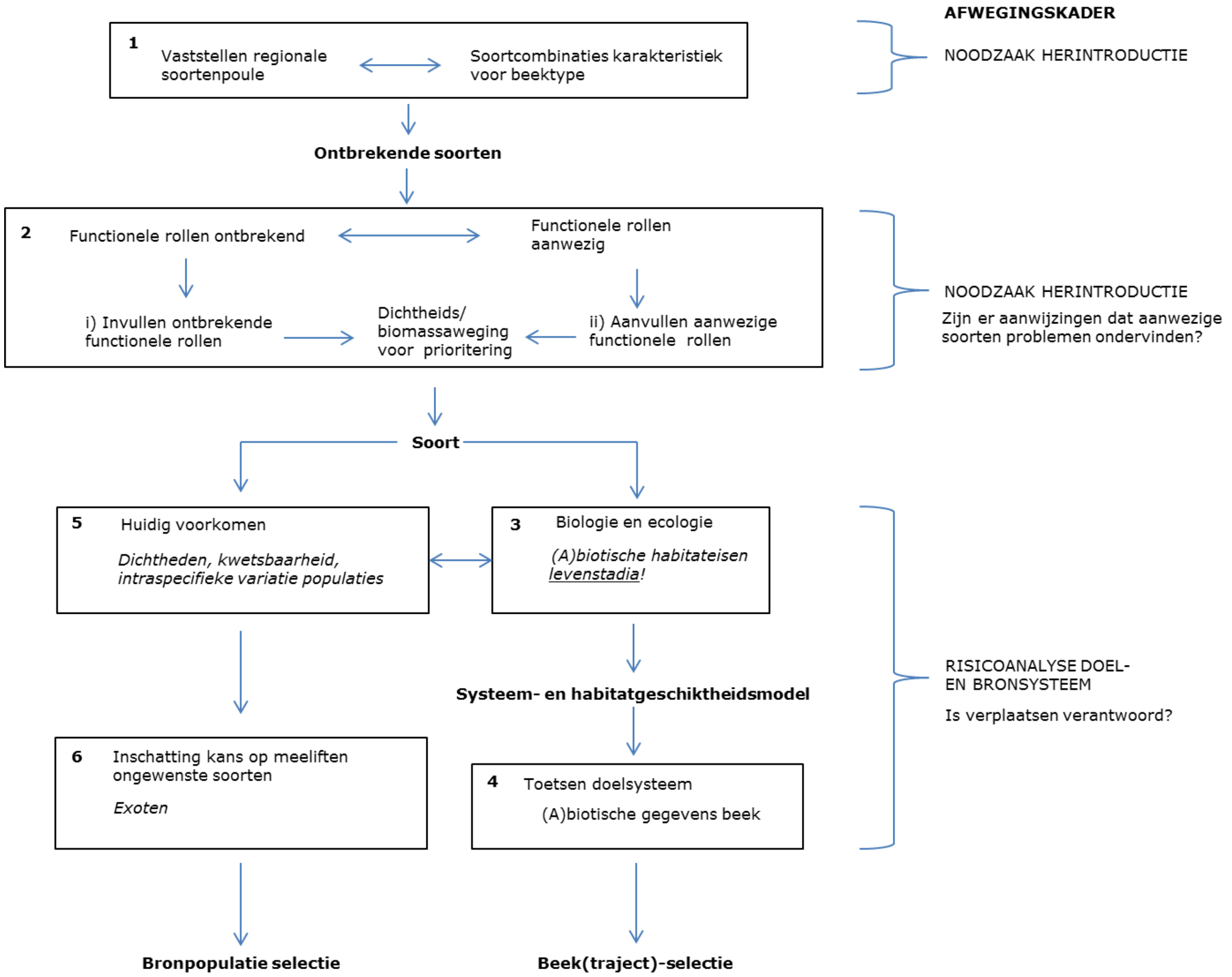


**Figure 4**: *Step-by-step plan in preparation of selecting suitable species and assessing the feasibility of the reintroduction of the selected species when reintroducing macroinvertebrates in a restored stream*.

**Translation text figure 4 (above)**

1. Determination of regional species group ← → Species combinations characteristic of stream type

Assessment Frame work
Necessity of reintroduction

Absent species

2. Functional roles missing / present
i) Description missing functional roles
Density/weighing of biomass for prioritization purposes
ii) Supplement functional roles present

Necessity of reintroduction
Are there any indications that the species present are facing problems?

Species

3 Biology and ecology ((A)biotic habitat requirements life stages)
System- and habitat suitability model

4 Assessment target system
(A)biotic data stream

5 Present occurrence
Densities, vulnerability, intraspecific variation populations

6 Estimated risk of undesired species hitching a ride
Exotics

3/4/5/6 Risk analysis target- and source system Is relocation a sound option?

Source population selection

Stream (section) selection

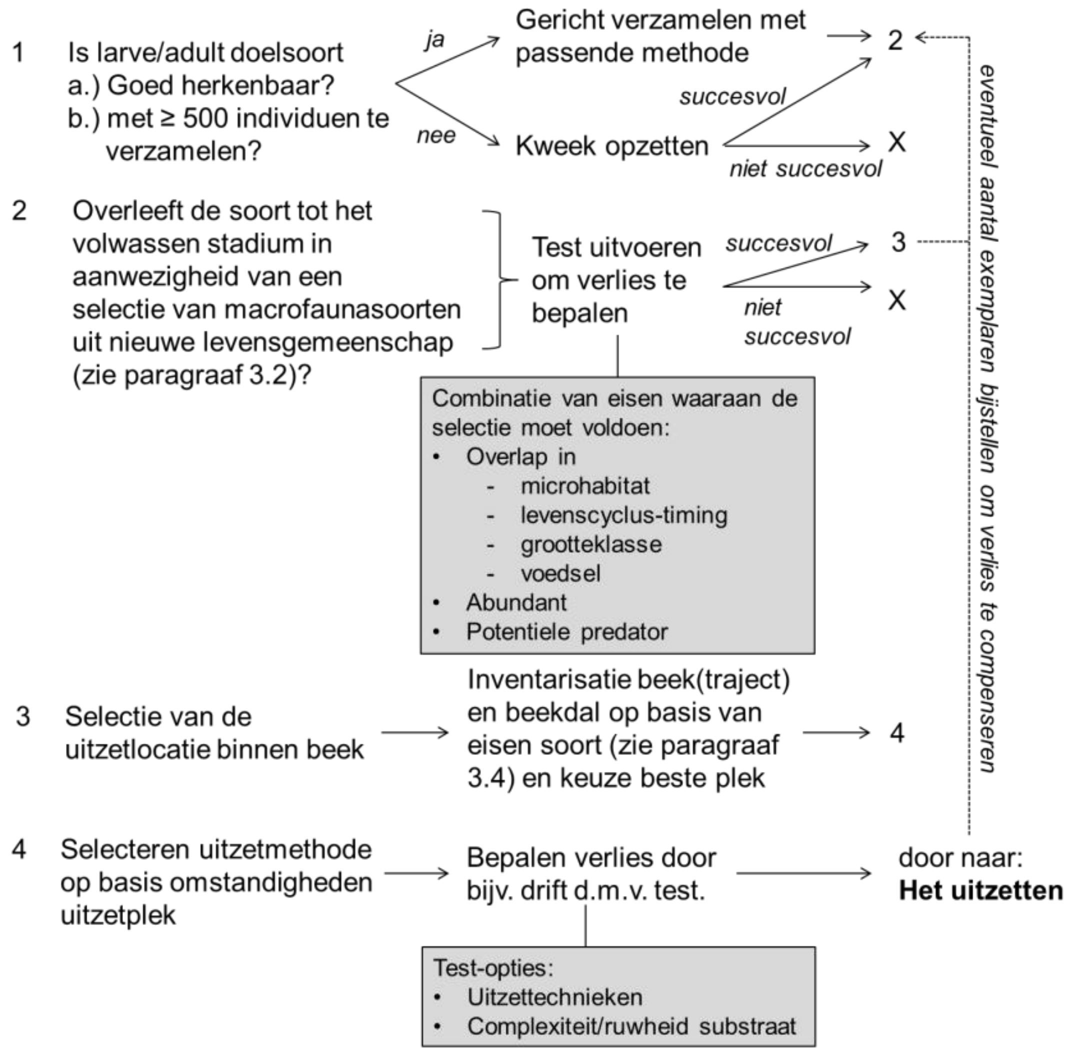


***Figure 5***: *Flowchart describing the preparations needed to increase the success rate when releasing macroinvertebrates in a stream.*

<u>Translation text figure 5 (above)</u>
1 Regarding the larva/adult of the target species a) is it easily recognizable? b) is it possible to collect > 500 individuals?
Yes no
Collect individuals using suitable method
Set up a cultivation
Successful Unsuccessful
2 Does the species survive up to the adult stage in the presence of a selection of macrofauna species from the new biotic community?
Conduct test to determine loss
Successful Unsuccessful
Combination of requirements that the selection must meet:
Overlap in – micro-habitat – life cycle timing – size category – food
Abundant
Potential predator
3 Selection of the release location in the stream
Inventory of stream (section) and stream valley based on species requirements and choice of best location
4 Selection of release method based on circumstances at release location
Test to determine loss due to, for example, drift
Continue to the release
Test options – release techniques – complexity/roughness of substrate
If necessary, adjust number of species to compensate for the loss

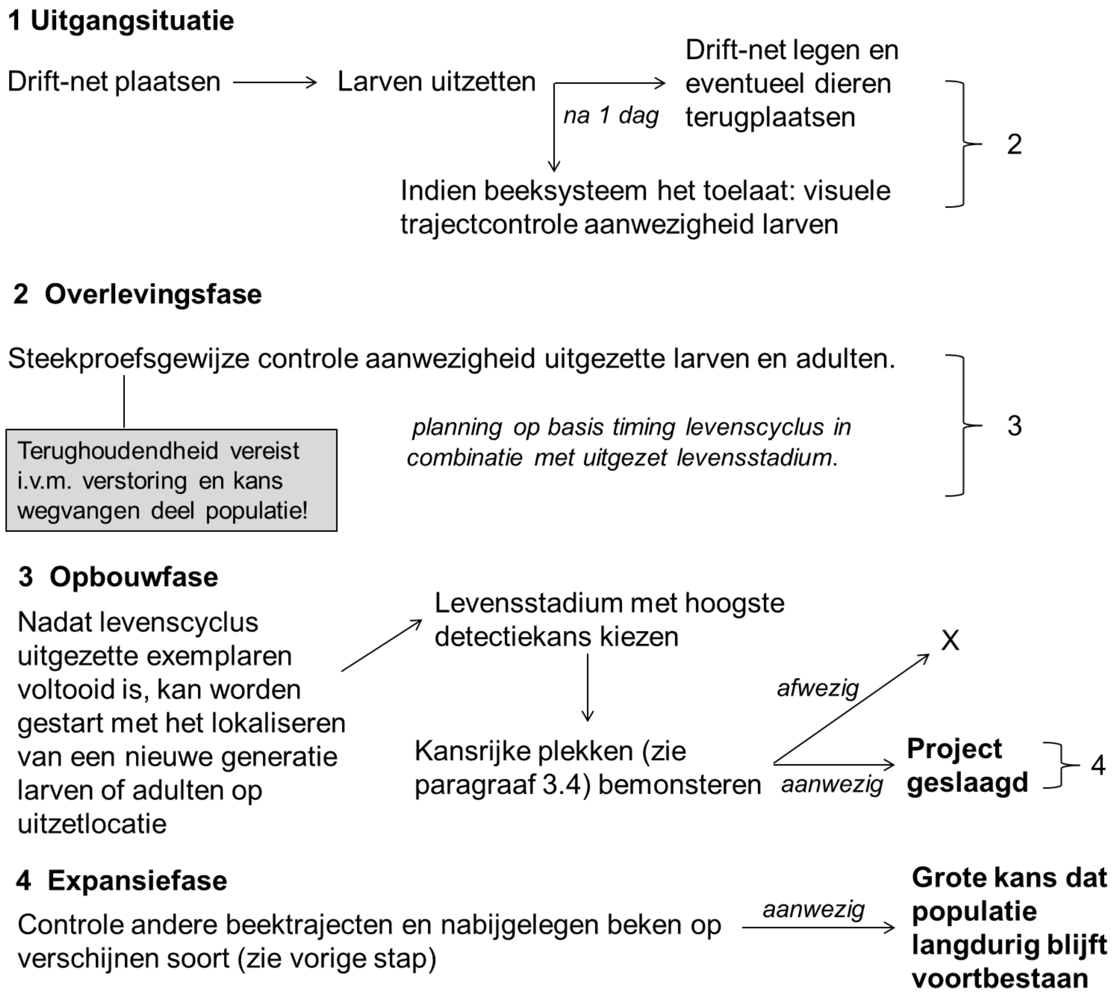


***Figure 6***: *Flowchart describing the release procedure for macroinvertebrates and the post-release monitoring scheme, based on the phases of population development characteristic for species that establish themselves in new areas.*

<u>Translation text figure 6 (above)</u>
1 Situation at the start
Position drift nets
Put out larvae
Empty drift net and put individuals back, if necessary
*After 1 day*
If the stream system allows: visual inspection of presence larvae in stream (section)
2 Survival phase
Random inspection of presence of larvae and adults
Restraint is to be exercised to avoid disruption and catching part of the population!
*Planning based on timing life cycle in combination with stage in life of larvae put out*
3 Build-up phase
Once the life cycle of the released individuals has been completed, a start can be made with the localisation of a new generation of larvae or adults at the release location
Choose the stage of life most likely to be detected
Sample favourable locations
*Present absent*
Project successful
4 Expansion phase
Inspection of other stream sections and adjacent streams for the occurrence of the species (see previous step)
*Present*
Highly likely that the population will continue to maintain itself for a prolonged period

## Literature

IUCN 2013
Malmqvist 2000
Nijboer 2004
Olden et al. 2011
Schwartz et al. 2012
Sundermann et al. 2011

**Rapport nr. 2015/OBN199-BE:**
[Herstel van laaglandbeken door het herintroduceren van macrofauna]