

Eco-friendly banks (NVOs) and the effects on macroinvertebrates and macrophytes

Thesis study about the effects of NVOs on macroinvertebrates and macrophytes in KRW waterbodies of Waterboard Aa en Maas



Thesis report

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Preface

During my internship from February to June, I worked as an intern at Waterschap Aa en Maas and a fourth year (graduate) student of Applied Biology at Aeres Hogeschool Almere. This report aims to explain the effects of Eco-friendly banks (NVOs, in Dutch: Natuurvriendelijke Oevers) on macroinvertebrates and macrophytes in water bodies of Waterboard Aa en Maas. Results are based on EQR's of the waterbodies that were calculated with Aquokit and statistically analyzed. During my previous internships I had already been introduced to stream and river restoration. During my graduation phase, my interest and experience in the practice of freshwater restoration were sharpened again. Although field visits and contacts were limited during these times of the corona crisis, the ecologists, administrators and other employees at Aa en Maas were very helpful and I was happy that the contact in the department was stimulated with weekly video conversations. I would like to thank Annet Pouw and Joost Rink for their guidance and personal support during my internship and graduation phase. I also want to thank the ecologists Frank van Herpen, Bram Spierings, Marcel Cox, Frank van Herpen and Bart Brugmans for their advice and guidance while writing the report.

Dian Oosterhuis

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Summary

This report focuses on the eco-friendly banks (NVOs) that were expected to restore the natural connectivity of water and land in the management area of Waterschap Aa en Maas. The aim of this study was to determine the effects of NVOs on the biological elements in the management area of Aa en Maas and give people working in the water sector an idea about the effectiveness of NVOs. According to the standard protocol of WFD monitoring, the quality scores of macroinvertebrate and macrophyte data were calculated using the online program Aquo-kit. Statistical data analysis was used in order to define the importance of effects caused by realized NVOs compared to scheduled (not-realized) NVOs, see table 1.

Table 1: Summary describing the effects of realized NVOs compared to scheduled NVOs.

Legend:

- Blue cells: positive significant effects;
- Green cells: not a significant effect but higher in average, thus an increasing trend;
- Yellow cells: not a significant effect but lower in average, thus an decreasing trend.
- NA = means that the sub-metric is not applicable for the water type.

Metrics	sub-metrics	M-types	R-types
Macroinvertebrates	Macroinvertebrate EQR (present and through time)	Green	Green
	Number of individuals (n)	Green	Blue
	Species richness (n)	Blue	Green
	Abundance characteristic (%)	NA	Green
	Abundance characteristic + positive dominant (%)	NA	Yellow
	Abundance positive dominant (n)	Green	NA
	Abundance negative dominant t(%)	Green	Blue
Macrophytes	Total flora EQR	Green	Yellow
	Abundance growth forms EQR	Yellow	Green
	Species composition EQR	Green	Yellow

Realized NVOs do not significantly increase most of the macroinvertebrate scores. In M-types, more positive effects are shown for the EQR's and species of both macroinvertebrates and macrophytes. NVOs also have negative effects on scores, in particular for R-types, but these effects are not significant.

There was assumed that maintenance of NVO's, angle of the banks, stream restoration measures, nutrient emission and isolation of habitats are important factors in seeing not many significant effects. This study has different limitations and small-scale studies are still needed still to define effects of ecological key factors (EKFs) at specific locations.

The conclusion was that realized NVOs in the management area of Aa en Maas have positive but limited effects on the macroinvertebrates and macrophytes in compared with scheduled NVOs. Recommendations for Aa en Maas includes measures that are appropriate for the water type, such as replacing flat banks at R-types with steeper banks and prioritization for the most promising NVO locations. For looking at the effects of maintenance measures on macrophytes and macroinvertebrates at different water types, is therefore recommended using prediction models for NVOs such as from Royal HaskoningDHV (2020).

Samenvatting

Dit rapport focust op de natuurvriendelijke oevers (NVO's) die naar verwachting de natuurlijke connectiviteit van water en land in het beheergebied Waterschap Aa en Maas herstellen. Het doel van deze studie was om de effecten van NVO's op de biologische elementen in het beheergebied van Aa en Maas te bepalen en geïnteresseerden van het water en natuur sector een idee te geven over de effectiviteit van NVO's. Aan de hand van het standaardprotocol van KRW-monitoring werden de kwaliteitsscores van macroinvertebraten en macrofyten berekend met behulp van het online programma Aquo-kit. Statistische data-analyse werd gebruikt om het belang van de effecten bij gerealiseerde NVO's vergeleken met geplande (niet-gerealiseerde) NVO's te bepalen bij M-typen (sloten en kanalen) en R-typen (beken), zie tabel 1.

Tabel 1: Samenvatting van de effecten van gerealiseerde NVO's ten opzichte van geplande NVO's.

Legend:

- *Blauwe cellen: positieve significante effecten;*
- *Groene cellen: geen significant effect maar hoger, dus een stijgende trend;*
- *Gele cellen: geen significant effect maar lager, dus een dalende trend;*
- *NVT = niet van toepassing voor water type.*

Maatlatten	Deelmaatlatten	M-typen	R-typen
Macroinvertebraten	Macroinvertebraten EQR (huidig en door de tijd)		
	Aantal individuen (n)		
	Soortenrijkdom (n)		
	Abundantie karakteristieke taxa (%)	NVT	
	Abundantie karakteristieke + positief dominante taxa (%)	NVT	
	Abundantie positief dominante taxa positive dominant taxa (n)		NVT
	Abundantie negatief dominante taxa (%)		
Overige waterflora	Overige waterflora EQR		
	Groeivormen EQR		
	Soortensamenstelling EQR		

Bij gerealiseerde NVO's zijn de meeste kwaliteitsscores van macroinvertebraten niet significant hoger.

Bij sloten en kanalen zijn meer stijgende trends getoond bij zowel macroinvertebraten als macrofyten. NVO's hebben ook negatieve effecten op scores, met name voor R-typen, maar deze effecten zijn afnemende trends en geen significante effecten.

In deze studie wordt aangenomen dat beheer van vegetatie, talud, de combinatie met andere beekmaatregelen, emissie van nutriënten en isolatie van habitats belangrijke factoren zijn geweest bij de niet-significante effecten. Deze studie had verschillende beperkingen en kleinschalige studies zijn in de toekomst nodig om effecten bij specifieke locaties te definiëren.

De conclusie van dit onderzoek was dat gerealiseerde NVO's in het beheergebied van Aa en Maas over het algemeen positieve maar beperkte effecten hebben op de macroinvertebraten en macrofyten in vergelijking met geplande NVO's. Aanbevelingen voor Aa en Maas omvatten maatregelen die passen bij het watertype, zoals het vervangen van te flauwe oevers bij R-typen door steilere oevers, passend maaibeheer en prioritisering van de meest kansrijke NVO locaties. Om een beter beeld te krijgen van de effecten van gerealiseerde NVO's in de toekomst, wordt kleinschalig onderzoek en analyse met ecologische sleutelfactoren (ESF's) aanbevolen. Zie ook de complete lijst van aanbevelingen in hoofdstuk 6.

1 Introduction

1.1 The importance of restoration

Freshwater resources provide ecosystem services that are essential to human health, economic activity and ecological sustainability. Important ecosystem services include drinking water quality, soil retention, nutrient retention and hydropower (Maes, Liqueste, Teller, et al., 2016). Biodiversity and its resilience that provides the ecosystem services are threatened worldwide by different stressors. The main stressors for wetlands, rivers, lakes and other aquatic systems are caused by modified water regimes, climate change effects and invasive alien species and land-use change in catchments (Moss, 2008; Rieu-Clarke, A., & Moynihan, R. 2015; Timmerman, Matthews, Koepfel et al., 2017; Hofstra et al., 2020). The activities concerning water management are recognized as a key factor for the control of ecosystem services and declines of freshwater biodiversity worldwide (Grantham, Matthews, & Bledsoe, 2019).

Prior to the WFD, the Netherlands had an approach to water management that mainly focused on reducing flood risks and maximizing economic and social functions. This was done by for example regulating water drainage, forms of embankment, dams, draining bogs, canalization, ditches and even new constructions of lakes and watercourses in dry areas. Those management strategies enforced the strong boundary between land and water that made the natural embankment disappear. This decreased the water quality of freshwater ecosystems and caused the loss of species that are characteristic to the aquatic and littoral habit that are present in lotic and lake systems (Carvalho, Mackay, Cardoso, et al., 2019; Duró, Crosato, Kleinhans, et al., 2018). In reaction to this, the Netherlands developed a solution to these problems. The idea of eco-friendly banks (in Dutch: NVO's, Natuurvriendelijke Oevers, see figure 1.1) stands out as it tackles the limited space problems in Dutch water management where the farming industry has a strong say. It aims to naturalize the boundaries between water and land of rivers, streams, canals and ditches. Policies and studies about the effectiveness of NVOs followed, in which can be concluded that different effects can be seen between different management areas, types of freshwater ecosystems and that research has only been a few decades long. This is why further research is needed at regional scale and small-scale as well as time scale to define if the NVO works according the objectives. Hence, each management area has its own ecosystems and could implement NVOs differently regarding reconstruction and maintenance (Tanis & Kamp, 2019; Vossen & Verhagen, 2009). Thus, the following text first elaborates the legislation objectives from European scale into the objectives of freshwater ecosystems of Dutch geographical scale.

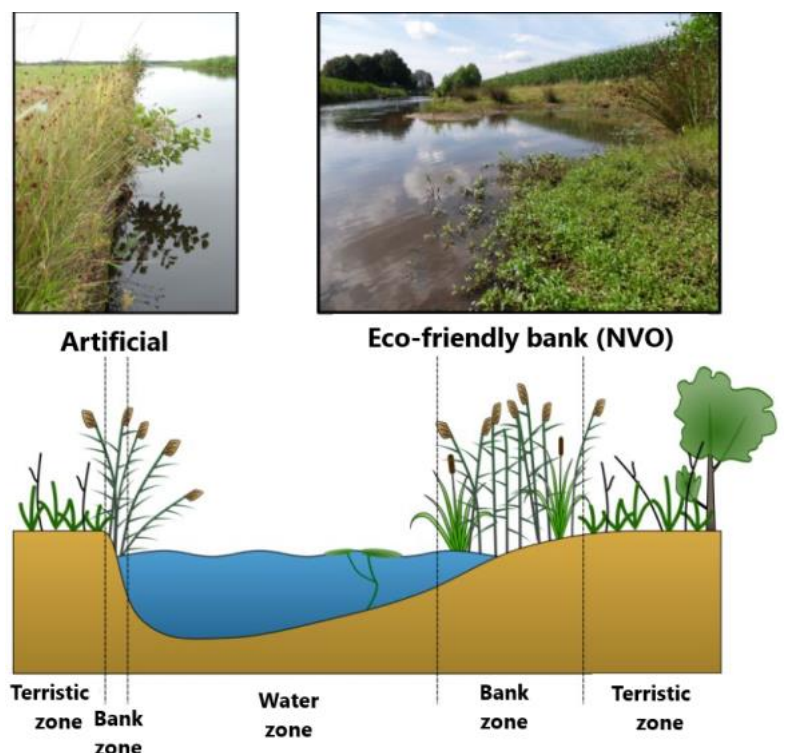


Figure 1.1: The idea of Eco-friendly banks (NVOs) in the Netherlands and the comparison with artificial banks of which the bank zone or bank width is much smaller.

1.2 Objectives for freshwater ecosystems

As awareness of threats to freshwater quality was growing, Europe began to adopt long-term adaptation with new legislation frameworks. The most ambitious European legislation framework is probably the EU Water Framework Directive 2000/60/EC (WFD) (Voulvoulis, Arpon & Giakoumis, 2017; European Commission, n.d.). This directive aims a shift from canalization, contamination and end-of-of-the-pipe solutions towards a sustainable catchment management within river basin management plans. The Directive supports the biodiversity and human health by focusing on groundwater quality, surface water quality, drinking water quality, ecological quality and chemical-physical quality at transboundary scale of lakes, rivers and coast waters (Carvalho, Mackay, Cardoso, et al., 2019). The objectives for inland waters within the KRW are established within national plans of several European member states, that are the so-called River Basin Management Plans (RBMPs). In the Netherlands the WFD is translated into “Kaderrichtlijn Water” (KRW), that is anchored in the Decree on quality requirements and water monitoring 2009 (Besluit kwaliteitseisen en monitoring water 2009; BKMW) of the Law of Water (Dutch: Waterwet). In this agreement the tasks of the waterboards were illustrated. Waterboards are regional government organizations for managing the water. In the Netherlands waterboards make Water Management Plans (WBPs) (Ministerie van Algemene Zaken, 2020, Mostert 2016).

According to the WFD, waterbodies are defined as either artificial waterbodies (AWB) or heavily modified water bodies (HMWB). All over Europe, WFD aims to restore surface water bodies to achieve “a good ecological status” (GES) or good ecological potential (GEP) before 2027 by using the Ecological Quality Ratio (EQR) from Bund & Solimini (2007) as an assessment method (Kail, McKie, Verdonschot, & Hering, 2016). The WFD integrates different reference water types, that provide a basis for designing the outlines of the Programme of Measures (PoM) (European Commission, n.d.). This study focuses on the quality of biological elements that is part of the surface water status. The surface water status consists of different elements that are each given a score that are summed up as the “Surface Water Status”, see also figure 1.2. The elements can be given a score with one of the following five quality classes: high (H), good (G), moderate (M), poor (P), and bad (B) (Squintani, Plambeck & van Rijswick, 2017).

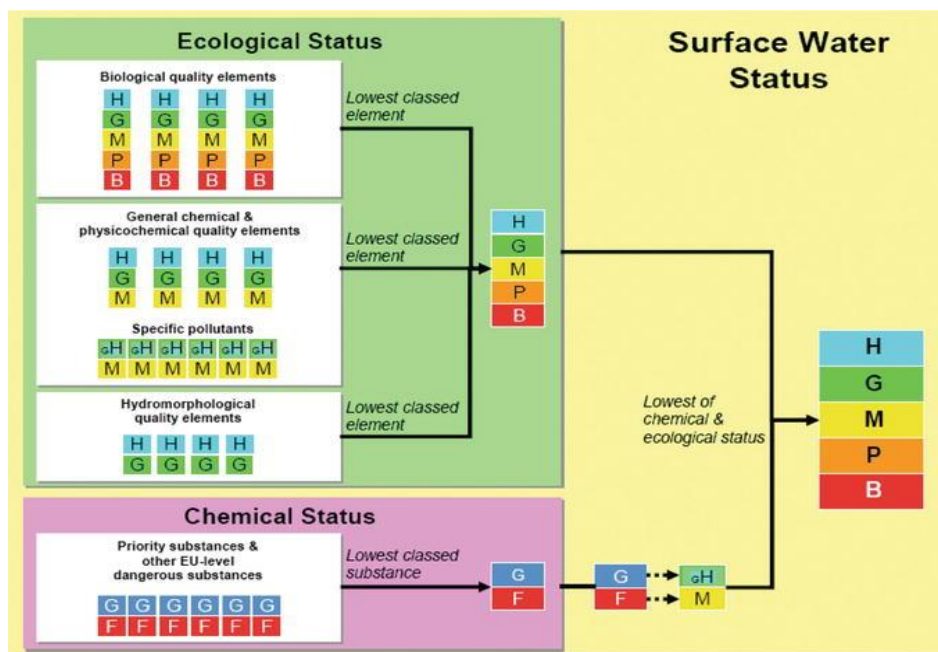


Figure 1.2: The surface water status consists of different elements: the ecological status an chemical status with sub-elements that each can be given a score, which is H = High, G = Good, M = Moderate, P = Poor, B = Bad and F = fail (Squintani et al., 2017).

In the Netherlands, the quality elements of the surface water status are monitored by waterboards or hired experts from laboratories and consultancies. Waterboards are governmental management organisations that are most responsible for the surface water status of regional waters, that exist of different water types such as streams, lakes, small rivers, canals and polder waterways. Waterboards are met with challenges when designing measures that focus on the dynamics of resilient freshwater ecosystems (Mostert, 2016; Ministerie van Algemene Zaken, 2020). Resilient, dynamic ecosystems without many human activities generally have a higher biodiversity. In the case of streams and rivers, there is a high variability of structure and flow in seasons, that provides connectivity of different habitats and species in the length and width of river (Grantham, Matthews, & Bledsoe, 2019; Tanis & Kamp, 2019).

Even though these “natural” ecosystems of river and streams in the Netherlands are almost always modified, all of them have a desired reference situation according to the GEP. The so-called “metrics” of the Dutch research institute STOWA describes the standard characteristics of the most natural freshwater ecosystems. Natural freshwater ecosystems are categorized with codes, which are the R-types with a given number behind the “R” (Ministerie van Infrastructuur en Waterstaat, 2018). STOWA also describes “M-types” that consist of the ditches, canals, lakes and polder ways etcetera (Evers Broek, Buskens, et al., 2018). Each water type has its abiotic and biotic elements, including species, that must be protected in order to have a high ecological quality score (Ministerie van Infrastructuur en Waterstaat, 2018).

1.3 NVO restoration

An eco-friendly bank (NVO) is an artificially constructed bank that is constructed outside or inside the existing profile by widening the watercourse. A gradual transition from deep water to a dry bank is being created where there was previously a hard boundary between water land. (Tanis & Kamp, 2019). “NVO” is a Dutch term, but this freshwater restoration measure can also be compared with other known concepts in other countries and globally, such as “streambed naturalization”, “elimination of river bank protection” (figure 1.3A), “riparian buffers” (figure 1.3B) defined by the organisation Natural Water Retention Measures among others. All of these measures come down to improving the ecological quality of freshwater ecosystems by restoring banks (Natural Water Retention Measures, n.d.).

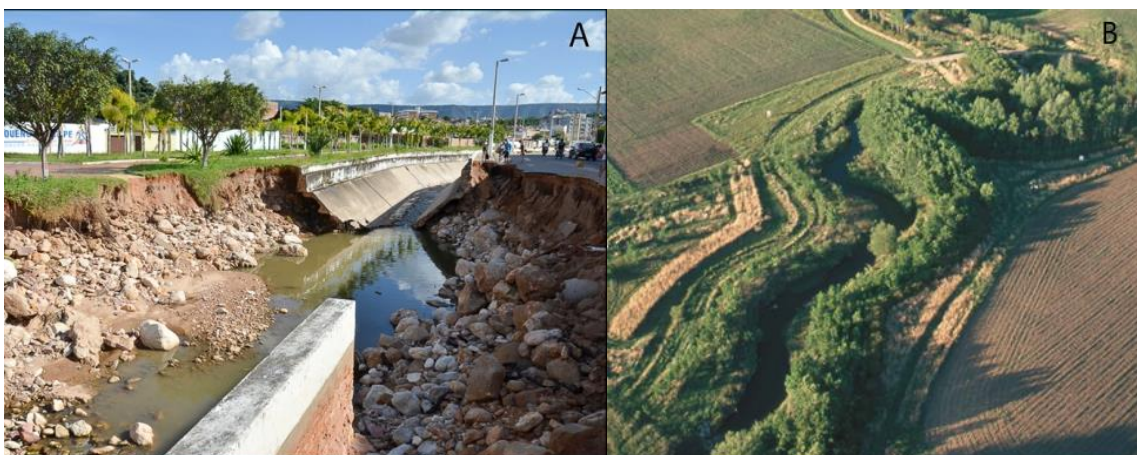


Figure 1.3: Pictures showing similar international concepts as NVOs, elimination of river bank protection in Brazil (A) and riparian buffers in the United States (B) (Natural Water Retention Measures, n.d.).

The effectiveness of the NVOs relies on important ecological key factors as seen in figure 1.4. These factors are also important additional information for the habitat suitability for species and ecological quality (Tanis & Kamp, 2019; Mellor, Verbeek, & Wijngaart, 2017). Banks can have different appearance and vegetation depending on the maintenance and objectives for the water type. When designing NVOs in the Netherlands, bank protections are removed and replaced mostly by a shore with a bigger width and a weak slope, which is most depending on the width of available space for maintenance, distance from neighboring landowners and the water type (Reeze, Winden & Kirstjens, 2015). In addition, decreasing nutrient emissions and toxicants also play a major role for NVOs to be effective, especially in areas with a high intensity of agriculture (Tanis & Kamp, 2019).



Figure 1.4: The Ecological Key Factors that are important for NVOs (Tanis & Kamp, 2019), translated into English.

The construction of an NVO creates more habitats for all kinds of organisms. The NVOs of M-types in the Netherlands is often characterized with three zones as specified in figure 1.5. Firstly, there is an aquatic zone that is suitable for macroinvertebrates and macrophytes. Secondly, the amphibic zone is suitable for water plants and animals favouring swampy conditions. And last, the terrestrial zone that consists of terrestrial plant and animals species (Tanis & Kamp, 2019; Vossen & Verhagen, 2009). NVOs can provide biodiversity for different water types, in particular for straightened waterbeds with artificial or heavily modified banks such as canals (Verhofstad, Zuidam, Bruin et al., 2017; Verhofstad, Herder, Peeters, et al., 2019). In this way, NVOs can act as a connecting corridor along which fish, macroinvertebrates, insects and amphibians can move to move from one habitat to another. This removes barriers. Thus, NVOs can increase the diversity and EQR of plants, macroinvertebrates and fish (Tanis & Kamp, 2019).

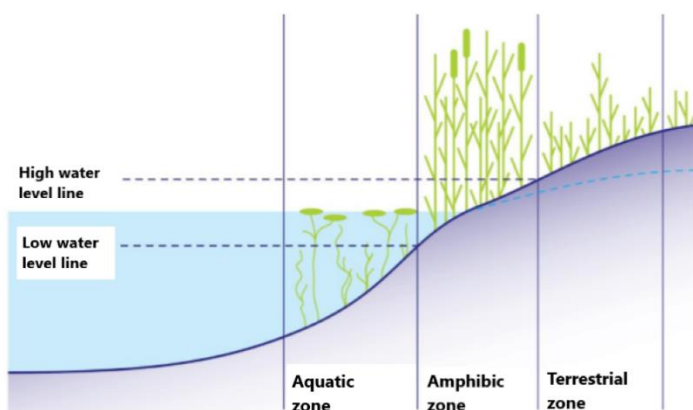


Figure 1.5: The aquatic zone, amphibic zone and terrestrial zone of an NVO (Tanis & Kamp, 2019)

When changing the waterbed of waterways, hydrologists are involved to calculate effects on discharge in terms of flood and drought in other places of the water system. This also accounts for the “ecological connecting zones” (EVZ, in Dutch: Ecologische Verbindingszones) that refer to the Dutch nature networks (NNN, Dutch: Natuur Netwerk Nederland). This connects ecosystems and its species and is a common objective within the Dutch nature legislations. NVOs and EVZs are implemented in different ways for water types and are conditioned on cooperation with adjacent landowners (Hokken & Torenbeek, 2017; Tanis & Kamp, 2019). Studies of NVOs were done by waterboards and research organizations to evaluate the effectiveness on the ecological quality (EQRs) of fish, macroinvertebrate and plant species. There was found that the EQR of M-types (channels and lakes) and R-types (rivers and streams) have increased because of the improved conditions for plants (Verhofstad, et al., 2017; Verhofstad et al., 2019).

However, an NVO as the only measure for restoring stream and rivers may not deliver the desired ecological profits. It is therefore important to apply the combination of, NVOs, ecological connecting zones (EVZs) and so-called small-scale measures (in Dutch: Kleinschalige Maatregelen). The name “small-scale measures” refers to the effective measures that are suitable for stream types at a small scale, also known as small-scale stream development or stream recovery. Examples of the small-scale measures include adding wood into the streams (see also figure 1.6), shading by adding riparian vegetation, profile adjustment, adapted mowing management and a more natural water level management (Verdonschot, Verdonschot, Bauwens, et al., 2017; Reeze, Winden & Kirstjens, 2015).



Figure 1.6: Adding wood and shading to streams (R-types) provides habitats for macroinvertebrates among other ecological benefits (picture made by Dian Oosterhuis at Lactariabeek).

1.4 Waterboard Aa en Maas

Aa en Maas is one of the waterboards that is part of the network of The Netherlands Water Partnership and works together with organizations of the water sector. The management area of the waterboard is located in the North-East of the province of North-Brabant and is divided into four districts: Hertogswetering, Raam, Boven Aa and Beneden Aa, see also figure 1.7.

Aa en Maas is one of the water boards where the monitoring of NVO effects is still a young concept and does not have extensive scientific research yet compared to other North parts of the Netherlands as seen in studies of Verhofstad et al. (2019) and Hokken & Toreneek (2017). Moreover, the waterboard has its own history of the implementation of NVOs. For M-types, the waterboard aims for shallowing the angle of banks in M-types that is known to improve the macrophyte quality (Verhofstad et al., 2019). Previously, the banks at R-types were known to not always be effective for R-types when observing it in the field. This is because instead of shallow and less steep banks, most R-types need more steep banks in order to have a sufficient water flow and good ecological quality (Noord, 2019; Reeze, van Winden, & Kurstjens, 2016). This is why R-types are often included with the small-scale measures that are combined with NVOs (Verdonschot, et al., 2017; Royal Haskoning DHV, 2019). The NVOs in R-types are important in Aa en Maas, because of the relatively high amount of natural water types compared to lower and north parts of the Netherlands. There is a difference in relief, that accompanies streams and small rivers most intensively in the districts of Boven Aa, Beneden Aa and Raam (Royal Haskoning DHV, 2019; Reeze, et al., 2016).

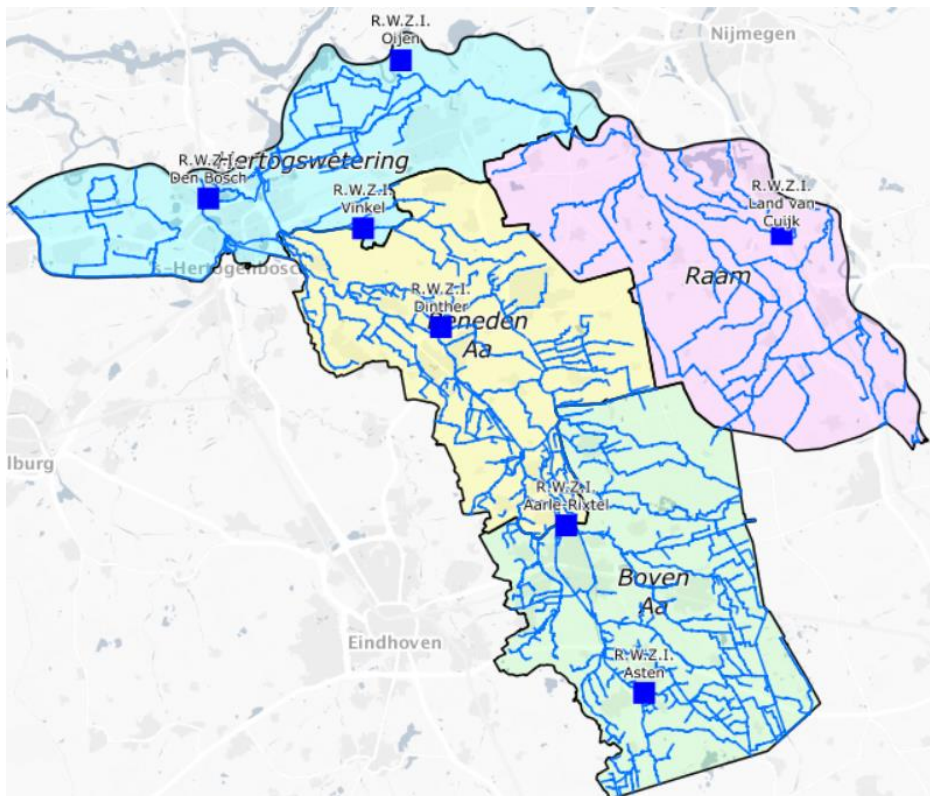


Figure 1.7: Districts of the management area of Waterboard Aa en Maas (Waterschap Aa en Maas, 2016)

In addition, the focus on developing eco-friendly banks (NVOs) has been growing at Waterboard Aa en Maas. The objective of Aa en Maas is to restore about 292 km NVO sections while the currently finished distance is 161 km since May 2020. In order to finish the other planned sections before 2027, the planning will be fastened by categorizing the most promising locations. According to the geographical maps with the programmed measures, the NVOs are marked as realized and scheduled. See also figure 1.8, in which monitoring locations (points) are shown of macrophytes and macroinvertebrates. “Scheduled” in this figure means either that there is an NVO planned, or that there is a NVO that is in practice and not yet appointed as “realized” by ecologists and water system advisors. In reality the NVOs are not yet scientifically studied and reported on the effects of NVOs at the given monitoring locations. This means that more research is needed to determine the effectiveness of the current NVO implementations by the waterboard. Namely, the effects could be different at each water type or effects will change through time. The effects in the water types could be different due to the combination of NVO measures and other measures. In comparison with the measures of NVOs as a whole, small-scale measures of R-types have been extensively studied and applied under the project Small-scale measures of Brabantse Wateren by Waterschap Aa en Maas, Waterschap De Dommel, Waterschap Brabantse Delta and the province of Noord-Brabant. Further effect monitoring and analysis has to determine the ecological profit of NVOs in the management area of Aa en Maas (Brugmans, Verdonshot, Kempen, et al., 2017; Rink, 2020).

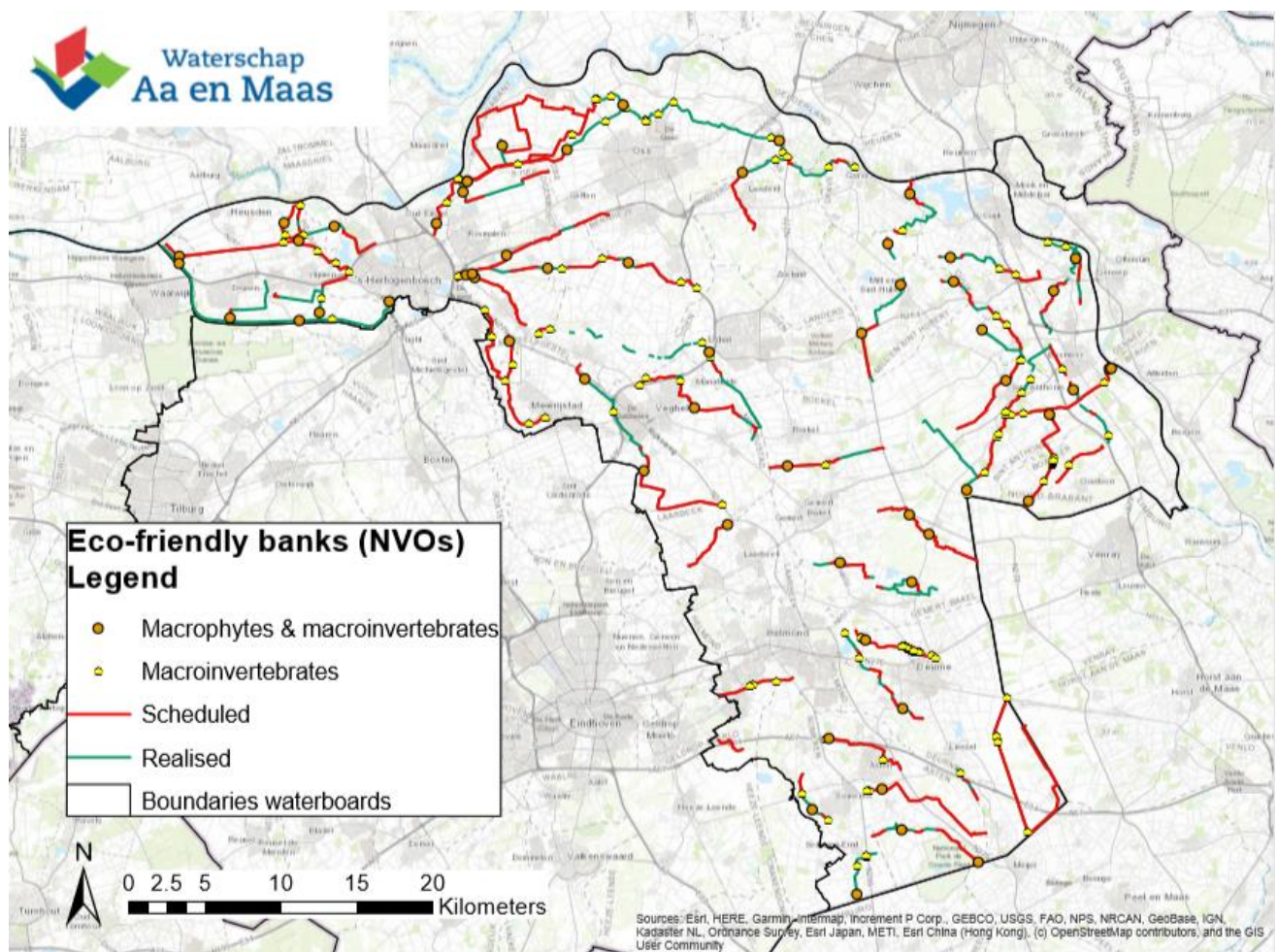


Figure 1.8: Map of the scheduled and realized eco-friendly banks (NVO's) in the management area of Waterboard Aa en Maas according to geographic applications. Source: Programma in beeld (PIB), made by Dian Oosterhuis with ArcMap 10.5.1.

1.5 Aim and research questions

This report focuses on the eco-friendly banks (NVOs) that are a common principle to naturalize banks in water management areas of waterboards since 2000 (Waterschap Aa en Maas, 2016; Tanis & Kamp, 2019). Based on the gaps of knowledge, the aim of this study was to determine the overall situation of the most important biological elements at realized and scheduled NVO monitoring locations (see figure 1.5). This study also gives people who work in the water sector an general idea about the effectiveness of NVOs. The EQRs of macroinvertebrates and macrophytes were determined in M- and R water types. With this information, the general situation of NVOs was pictured and recommendations were made for focusing the development of NVOs and further research. The influence of time gave information about how the macroinvertebrate EQR has developed through time, before realization and after realization. The main research question of this report is:

- What are the effects of the NVO implementation on macroinvertebrates and macrophytes in M- and R water types in the management area of Aa en Maas?

The main research questions is divided into sub-questions. In order to define differences between scheduled and realized NVOs, hypotheses are made for each sub-question as is shown in table 1.1. The hypotheses will be tested with statistical analysis as is explained in the following chapter, that describes the methods (chapter 2). The results (chapter 3) consists of the observations of the EQR for scheduled NVOs vs realized NVOs in the first paragraph. In the next two paragraphs the sub-metrics of macroinvertebrates and macrophytes are explained. In the fourth paragraph the influence of time and before-after NVO realization on macroinvertebrate EQR are described. The last paragraph summarizes the mean differences and significances found with statistical analysis. In chapter 4, the discussion is described. Finally, the conclusion and recommendations are described in chapter 5 and 6.

Table 1.1: The sub-questions and hypothesis of this study.

Sub-question	Hypothesis
What difference in macroinvertebrate and Total Flora EQR is seen between realized and scheduled NVOs?	Null hypothesis (H0): There is no statistical significant difference in the EQR and sub-metrics between scheduled and realized NVO monitoring locations
Which differences in the sub-metrics of macroinvertebrate and macrophytes are seen between realized and scheduled NVOs?	Alternative hypothesis (H1): There is a statistical significant difference in EQR and sub-metrics between scheduled and realized NVOs
Does time have an influence on the macroinvertebrate EQR?	Null hypothesis (H0): there is no significant relationship between 1) time and EQR of NVOs and 2) before-after NVO realization Alternative hypothesis (H1): There is a significant relationship between 1) time and macroinvertebrate EQR of NVOs and 2) before-after NVO realization

2. Methods

2.1 Overview data

In table 2.1 the data variables from available monitoring data is shown that was used for this study in order to define the situation of NVOs. The data includes different types concerning main variables sub-metrics, continuous variables and binomial variables. The continuous variables are the measurements of standard WFD monitoring and results of the assessment of the macroinvertebrates in the software program Aquo-kit (version 12-10-2019). In the following chapters these variables will be further explained.

Table 2.1: Overview of variables used in this study with data type, definition, and relevance in this study indicated with X = in this study and O = not in this study for change in time and difference between scheduled (S) and realized (R).

Category	Variable	Type	Definitions	Difference S and R	Time
Main variables	Macroinvertebrate EQR	continuous	The total ecological quality ratio of macroinvertebrates. This is a number between 0.0 and 1.0.	X	X
	Total flora EQR		The total ecological quality of macrophytes and phytobenthos. This is a number between 0.0 and 1.0.	X	O
	Time (years)		The time in years will be used to describe the EQR's before and after realization and planning of the NVO measures.	O	X
	Current NVO status	binomial	The NVO status includes two different categories: scheduled (0) and realized (1) NVOs.	X	O
	NVO status through time		The NVO status through time includes two different categories: the measure point before realizing the NVO and after realizing the NVO.		X
	Water types	categorical	M-types: M1a (ditches), M3 & M6 (canals). R-types: R4a, R20 and R5 (streams)	X	X
Sub-metrics macroinvertebrates	Abundance of positive dominant + characteristic macroinvertebrate species (%)	continuous	The species that are the most present in the macrofauna abundance according the reference situation of the water type.	X	
	Abundance of negative dominant macroinvertebrate species (%)		The species that are not desired to be present and should be close to a zero according to reference situation of the water type.		
	Abundance of characteristic macroinvertebrate species (%)		The species that are characteristic for a certain WFD water type, in terms of water quality-, substrate- and velocity preferences		
	Species richness (n)		Number of macroinvertebrate species		
	Number of individuals (n)		Number of total macroinvertebrate individuals		
Sub-metrics macrophytes	Species composition EQR		The species composition of macrophytes monitored with the Tansley method. This is a number between 0,0 and 1,0.		
	Abundance growth forms EQR		The diverse growth forms that exist for the macrophytes. This is a number between 0,0 and 1,0.		

The data was presented to Waterschap Aa en Maas using excel documents, Powerpoint presentations, and the tables and figures in the results and annexes (see Annexes I, II, III). These information forms were used in order to explain the most successful and less successful locations in several water types. The less successful locations that are realized or scheduled were appointed for further implementation of NVO measures.

The data of the macroinvertebrates and macrophytes that was used for this study is from the WFD monitoring network and monitoring strategy. Macroinvertebrates are chosen because it is the most relevant data of the biological elements, especially for the R-types. Macrophytes are more important in M-types. Aa en Maas begun developing NVOs from 2000, so from this date the EQR scores from the macroinvertebrates and macrophytes were calculated with the software program Aquo-kit, according the current WFD types and the formulas of the new metrics (Ministerie van Infrastructuur en Waterstaat, 2018; Evers et al., 2018).

2.2 Macroinvertebrates

The EQR of macroinvertebrates is calculated using the standard protocol and sub-metrics (see table 2.1) that is described in the Netherlands by Ministerie van Infrastructuur en Waterstaat, 2018 in the case of the streams and by Evers et al. (2018) in the case of ditches and canals.

For the streams in this study (R4, R20 and R5) Aquo-kit uses the abundance classes of characteristic species, dominant positive and dominant negative taxa. Negative dominant species are species that indicate a poor ecological status while positive dominant species indicate a good ecological status. Characteristic species are species that are species belong to the reference situation of the different water types. The use of abundance classes is necessary, because it prevents extremely high abundances of one or a few species from highly influencing the EQR. The EQR calculation consists of the following parameters that is combined in the formula as shown in figure 2.1. (Evers et al., 2018):

- 1) DN% (abundance); the percentage of individuals belonging to the negative dominant indicators of the sample based on abundance classes;
- 2) KM% (number of taxa); the percentage of characteristic taxa of the sample;
- 3) KM% + DP% (abundance); the sum of the percentage of individuals belonging to the characteristic and positively dominant indicators based on abundance classes.
- 4) KMmax; the percentage of characteristic taxa that are expected in reference conditions of the particular water type. This value is shown in Evers et al. (2018) for each water type.

$$EQR = \left(\frac{\left(200 + \frac{KM\%}{KMmax} \right) + (2 * (100 - DN\%)) + (KM\% + DP\%)}{500} \right)$$

Figure 2.1: The standard formula used for calculating the EQR of macroinvertebrates of the R4 and R5 water types in this study (Evers et al., 2018).

For the ditches and canals in this study (M1, M3, M6), Aquo-kit uses only the abundance of negative dominant and positive dominant taxa. This is because ditches and canals are artificial water bodies, where no species can be defined as naturally characteristic. The EQR calculation consists of the following parameters that is combined in a formula as shown in figure 2.2:

- 1) DN% (abundance); the percentage of individuals belonging to the negative dominant indicators of the sample based on abundance classes
- 2) PT (number of taxa); number of positive taxa (so not the number of individuals).
- 3) PTmax; the number of positive taxa that is expected under the circumstances of the maximal ecological potential (MEP).
- 4) DN%max; the minimum percentage of negative dominant taxa that occurs in the quality class 'Bad'.

The use of 'DN%max' and PTmax is necessary, because it prevents that a very high DN% influences the EKR positively. Both PTmax and DN%max are different for each water type (Ministerie van Infrastructuur en Waterstaat, 2018).

$$EKR = \frac{2x \left(\frac{PT}{PTmax} \right) + \left(1 - \frac{DN\%}{DNmax\%} \right)}{3}$$

Figure 2.2: The standard formula used for the EQR calculation of macroinvertebrates (Ministerie van Infrastructuur en Waterstaat, 2018)

2.3 Total Flora

The calculation of the Total Flora EQR is a procedure that is more complex than the calculation of the macroinvertebrate EQR. The full standard procedure is described in the Netherlands by Ministerie van Infrastructuur en Waterstaat (2018) in the case of the streams and by Evers et al. (2018) in the case of ditches and canals. In these reports the objectives of water types are described. The EQR calculation is dependent of several factors and has more variables in the calculation. The factors that were used by Aquo-kit in this study are summarized below.

In order to characterize the Total Flora EQR and macrophytes, three important components were chosen: the species composition EQR, the abundance growth forms EQR and the phytobenthos EQR. The species composition EQR is based on the cover in percentages of species by using the Tansley method. Both the species and growth forms are monitored in different zones, which are the water zone and the bank zone. The abundance growth forms EQR is based on the growth forms that are expressed in percentages. Below the following growth forms are described (Ministerie van Infrastructuur en Waterstaat, 2018; Evers et al., 2018):

- Submerged: plants with submerged leaves (including submerged thread algae);
- Floating: plants with floating leaves that do not belong to the growth form duckweed or large floating leaf plants;
- Emergent: plants with leaves protruding above the water surface (helophytes)
- Duckweed: small floating plants that can form a sealing layer on the water surface;
- Floating thread algae: it can form an extensive mass on the water surface;
- Bank vegetation: vegetation on the bank between the high and low water lines. For streams (R4, R5, R20), trees that provide shade for the bank zone and water zone

Thus, the calculation of the Total Flora EQR of each monitoring location was based on the averages of species composition EQR, abundance growth forms EQR and the phytobenthos EQR (Ministerie van Infrastructuur en Waterstaat, 2018; Evers et al., 2018).

2.4 Differences between water types

In this study data analysis of EQRs different water types were used that are described by Ministerie van Infrastructuur en Waterstaat (2018). The EQR's of the current situation are studied in M-types and R-types separately. When studying the effects of time, ditches (M1a), canals (M3 and M6) and streams (R4a, R20, R5) are analysed separately. Small-scale measures, water quality, dredging and other environmental explanatory variables that are applied at different locations could have a relation with the ecological quality and in particular NVOs. However, it was too time consuming to include the analysis of possible interaction effects of these factors at all locations and water types. Even though no analysis was done, these factors are discussed in the discussion to explain the findings in the results and the overall background of different NVOs. Thus, the discussion of this study aims to explain what overall background would have played a role in the effectiveness on macroinvertebrate and macrophyte quality in scheduled and realized NVOs.

2.5 Data analysis

Before starting the statistical testing, the so-called pre-liminary analyses were done. This was required, because visual inspections of the data was needed to use the statistical testing that is appropriate for the data. This is further explained in the results and in Annex II. In Annex II is further explained about the choice of statistical tests and interpretation of test results.

2.5.1 Macroinvertebrate and Total Flora EQR in realized and scheduled NVOs

The differences of the most recent EQRs and sub-metric scores of macroinvertebrates and flora between scheduled and realized NVOs were studied. A visual inspection was done using boxplots. This was done in order to characterize the assumptions of meeting the statistical testing, that includes inspecting outliers, normality and shape of the data. This was also done to show the amount of the NVOs that score Very good, Good, Moderate, Poor and Bad and to show the abundance of species according the sub-metrics (see also table 2.1). The Independent-samples T-test was used to test if the difference in mean EQR and sub-metric scores between scheduled and realized monitoring locations was significant. The Independent-samples T-test was used because data was normal, did not have many significant outliers and is the appropriate test for comparing the differences between averages. Transformation with square root and log10 was used to get of non-normality and outliers that affect the reliability of the results (see also Annex II). This analysis gave a general idea about the current situation of the WFD goals between NVOs and scheduled NVOs (Evers, Barten & Scheepens, 2017; Atsma et al., 2016).

2.5.2 Effect of time

The macroinvertebrate data were available through time and year measurements per measure locations were used. The macroinvertebrate EQR of realized NVOs was studied through time (in years), before realization and after realization. Pearson Correlation tests were used to analyse the effect of time on management area level. Individual observations were done to appoint the data of specific KRW water types (R4a, M1a, M3 etc.) that was appropriate for using statistical testing. This approach was done, because data was not always in big amounts available and available for the same locations before and after realising the NVO (Evers, Schipper, Barten et al., 2017; Atsma et al., 2016).

3. Results

3.1 EQRs in scheduled and realized NVOs

In this paragraph, the difference in macroinvertebrate EQR and Total Flora EQR in scheduled and realized NVOs is studied. This is seen in figure 3.1 with the macroinvertebrate EQR and figure 3.2 with the Total Flora EQR. A few observations are made about the differences in percentages of quality classes between scheduled and realized NVOs.

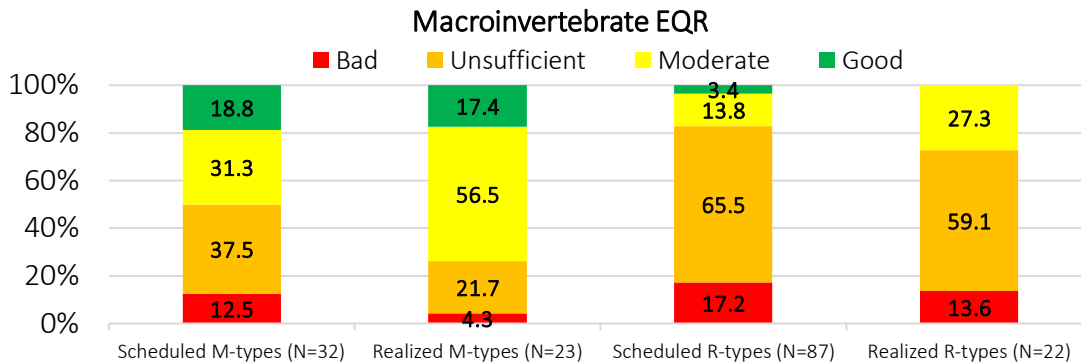


Figure 3.1: The percentages of quality classes found of Macroinvertebrate EQR in M-types and R-types for monitoring locations in scheduled and realized NVOs with N = number of monitoring locations.

Figure 3.1 shows in the second bar that the macroinvertebrate quality at realized NVOs of M-types is generally higher than scheduled NVOs. 56.5% of the 23 monitoring locations of realized M-types have a macroinvertebrate EQR that is moderate. This is about 25% more locations than scheduled NVOs. Moreover, about 16% less insufficient and about 8% less bad quality is seen in realized NVOs. However, the amount of good quality is not very different in realized NVOs, with scheduled NVOs of M-types actually having 6 locations that already have a good quality.

The third bar shows that the macroinvertebrate EQR at realized NVOs of R-types is generally lower than scheduled NVOs. It has no good quality and about 13% more moderate quality is found at the locations of realized NVOs. There are also less is found in realized R-types in compared to scheduled R-types.

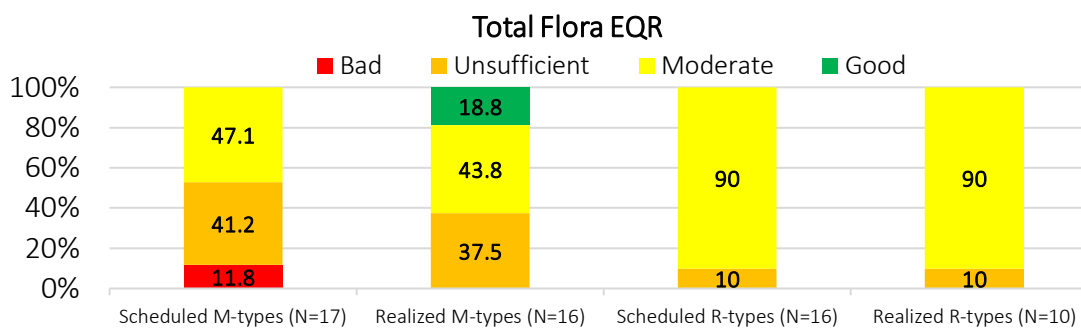


Figure 3.2: The percentages of quality classes found of Total Flora EQR in M-types and R-types for monitoring locations in scheduled and realized NVOs with N = number of monitoring locations.

Figure 3.2 shows in the second bar that the Total Flora EQR in realized M-types is higher. 18.8% of the 16 monitoring locations has a good quality. Realized M-types also have no bad quality, and less insufficient quality and less moderate quality than scheduled M-types. As shown in the third and fourth bar, no difference of the Total Flora EQR between scheduled and realized R-types is seen.

In order to visualize the distribution of the EQR data in realized NVOs is, boxplots were made as seen in figure 3.3 and statistical testing with the T-Independent samples test were done to show the differences between scheduled and realized NVOs.

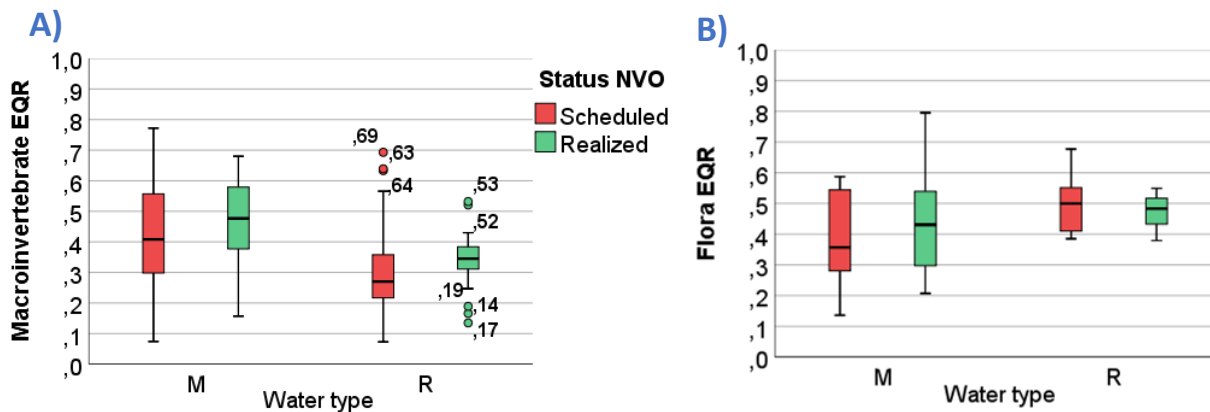


Figure 3.3: Box plots with the difference in A) Macroinvertebrate EQR and B) Total Flora EQR. Boxes represent the 25-75 percentile with the median, the error bars, and the minimum and maximum values. Points outside the boxplot indicate non-significant outlier

A few observations can be made using the figures and statistical testing with the T-independent samples test. In general, the T-independent samples test (Annex II) shows that realized NVOs do not have a significant higher EQR in both R-types ($p = 0.121$) and M-types ($p = 0.159$).

Figure 3.3A shows significant outliers of macroinvertebrate EQR in a few monitoring locations of scheduled NVOs of R-types. These outliers are higher EQRs outside of the boxplot and have a good quality. These outliers are represented by the KRW water type R4a at the water body “Lactariabeek” (monitoring locations codes 140818 and 140820) and the KRW water type R5 which is St. Jansbeek (monitoring location code: 340412).

Figure 3.3A also shows an outlier that has an insufficient macroinvertebrate quality in realized NVOs of the R-types, which is of the KRW water type R4a of water body “Vlier”.

In addition, figure 3.3B shows that the Total Flora EQR does not differ much. Hence, the difference of Total Flora EQR between scheduled and realized at both M-types ($p = 0.399$) and R-types ($p = 0.436$) is not significant.

3.2 Macroinvertebrate sub-metrics scores in scheduled and realized NVOs

The sub-metrics that are part of the total EQR score of macroinvertebrates were also studied in order to know more about the differences between scheduled and realized NVOs. This was done in the same way as mentioned before, with boxplots and statistical testing with T-independent samples test. The boxplots are shown in figure 3.4 with each of the sub-metrics of M-types and R-types. No significant differences were found for EQR, but as shown in this paragraph the sub-metrics did have a few significant differences.

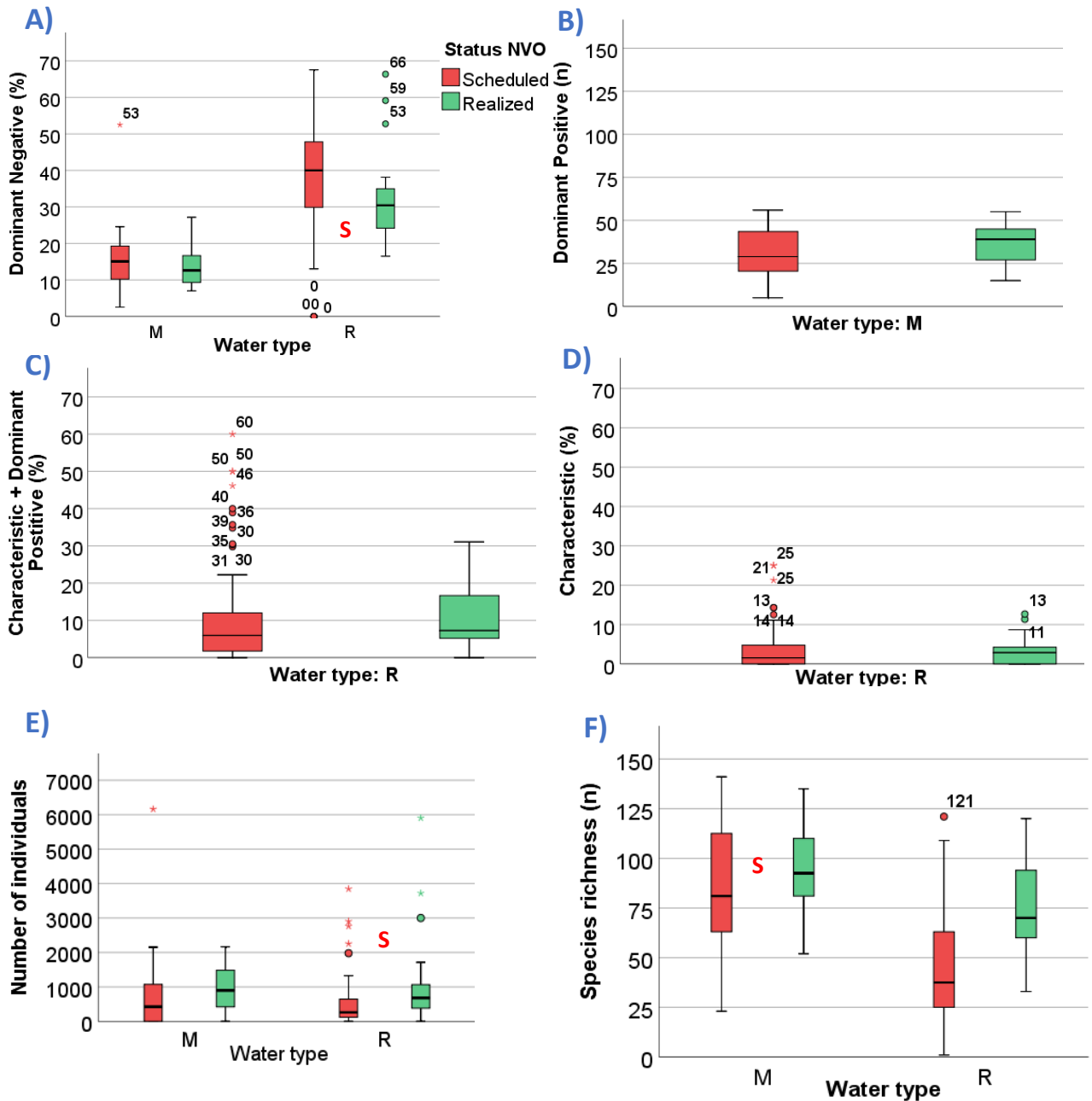


Figure 3.4: Box plots with the difference in A) characteristic + positive dominant taxa, B) characteristic taxa and C) negative dominant taxa expressed in percentages (%), D) positive dominant taxa expressed in numbers (N), E) Number of individuals and F) Species richness (n). Boxes represent the 25-75 percentile with the median, the error bars, and the minimum and maximum values.

“[S]” indicates the significant difference ($P < 0.05$) between scheduled and realized.

* indicates significant outliers and points outside of the boxplot

Points outside the boxplot indicate non-significant outlier

As shown in figure 3.A the abundance of negative dominant taxa at realized M-types do not differ significantly from scheduled NVOs ($p = 0.292$). In R-types, the difference of the abundance of negative dominant taxa are significantly lower in realized ($p = 0.002$).

Figure 3.4B presents the number of positive dominant taxa in M-types dominant species, which shows no significant difference ($p = 0.215$), even though the median is a bit higher in realized NVOs.

Figure 3.4C shows the characteristic + dominant positive taxa of R-types. There was seen that there are significant outliers and non-significant outliers outside the boxplot. No differences are found between scheduled and realized NVOs ($p = 0.846$).

Figure 3.4D shows the characteristic taxa of R-types. Significant and non-significant outliers are found outside of the boxplot. However there is almost no difference between scheduled and realized ($p = 0.962$).

As is seen in figure 3.5E, realized M-types have generally more individuals of macroinvertebrates, even though this is not significant ($p = 0.456$). The realized NVOs of R-types generally have a higher number of species, which is significant ($p = 0.043$).

In figure 3.5F can be seen that the species richness is significantly higher in realized M-types ($p = 0.030$) in realized NVOs. Even though is seen that realized R-types have more macroinvertebrate species, this is not significantly different ($p = 0.067$).

3.3 Macrophyte sub-metric scores between scheduled and realized NVOs

The species composition EQR and abundance growth forms of macrophytes are studied here with boxplots and the T-independent-samples test in order to see differences between scheduled and realized NVOs. Below the boxplots are shown in figure 3.5 of scheduled and realized NVOs of M-types and R-types.

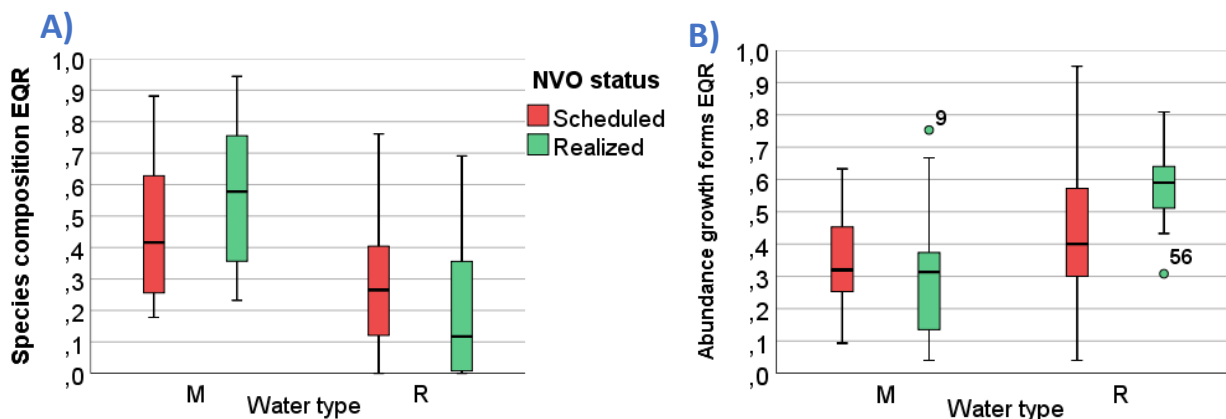


Figure 3.5: Box plots with the difference in A) species composition EQR and B) abundance growth forms EQR between scheduled and realized NVOs in M-types and R-types.

Boxes represent the 25-75 percentile with the median, the error bars, and the minimum and maximum values.

Points outside the boxplot indicate non-significant outlier.

Figure 3.6A shows the species composition EQR of macrophytes. Here is shown that realized M-types have an higher species composition EQR, but this is not significant ($p = 0.150$). Realized NVOs at R-types have a lower species composition EQR, but this also not a significant difference ($p = 0.321$).

Figure 3.6B shows the abundance growth forms EQR of macrophytes. The differences of abundance growth forms EQR in realized M-types does not differ much from scheduled M-types ($p = 0.138$). The abundance growth forms EQR at realized R-types is generally higher than scheduled R-types, but this is not a significant difference ($p = 0.138$).

3.4 Effect of NVO's on macroinvertebrate EQR over time

In this paragraph the effect of time and before and after NVO realization is described. In figure 3.6 the boxplots of M-types and R-types are presented in order to see the differences between macroinvertebrate before and after realization. Figure 3.7 shows the monitoring locations with data before and after the NVO realization.

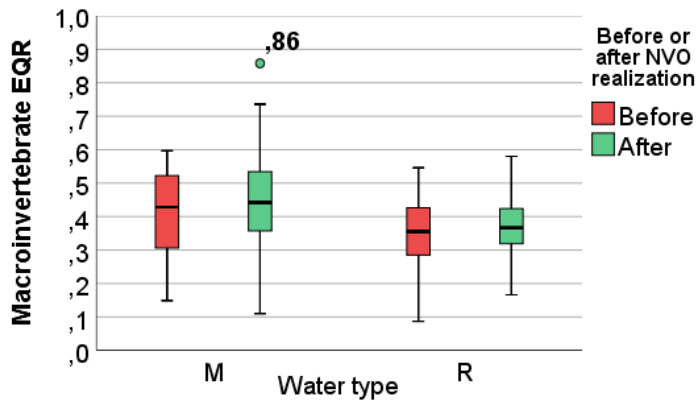


Figure 3.6: Boxplot showing the mean differences of macroinvertebrate EQR between before and after the date of NVO realization (not significant, $p > 0.05$)

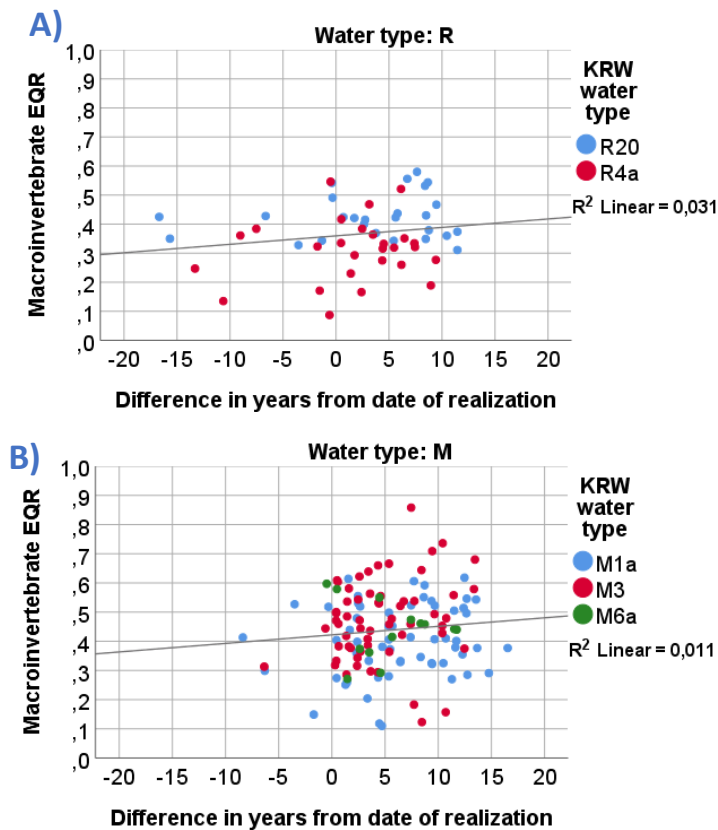


Figure 3.7: Correlation plot showing a small positive significant correlation between macroinvertebrate EQR and time that is not significant in M-types (A) and R-types (B). The negative values are before NVO realization and the positive values are after NVO realization.

As seen in figure 3.6 differences of the EQR before and after realization are not significantly different for M-types and R-types. In figure 3.8 can be seen that there is more data after the NVO realization. In figure 3.8A can be seen that the EQR of M-types have some outliers, even though it has a small overall increase with a linear relationship ($R^2 = 0,011$). This is actually not significant ($p > 0.05$). In figure 3.8B can be seen that R-types have outliers. Even though it shows an linear relationship, there are too many outliers and there is no significant change in EQR over time ($p > 0.05$). The results show that only M-types and the R4a water type have a small correlation with time, whereas no correlation is found between before-after NVO realization in all of the water types. 2.9% ($R^2 = 0.029$) of the M-types is statistically explained by time. R4a type is only 7.7% ($R^2 = 0.077$) statistically explained by time. However, when looking at the individual observations, there are some monitoring locations in water bodies that seemed to have increased. The detailed information about individual observations can be found in the graphs with monitoring points in Annex III.

3.5 Summary

This paragraph summarizes the statistical results of the previous paragraphs by showing the mean differences and significance differences together in tables. The mean differences between scheduled and realized NVOs macroinvertebrates and macrophytes are shown in table 3.1. No significant negative effects are seen in realized NVOs, even though some sub-metric scores are lower in average at realized NVOs. The number of individuals of R-types and species richness of M-types of macroinvertebrates are significantly different in realized NVOs, which are positive effects. Another shown significant positive effect is the decreased abundance of negative dominant taxa in R-types. In table 3.2 and 3.3 the differences over time and before-after NVO realization are found. With “NA” (not applicable) is shown that data is not available, and with “NP” (not possible) is shown or if sample size is too small for statistical testing (Bonett, Douglas & Wright, 2000). In these tables no significant effects are shown before and NVO realization on macroinvertebrate EQR, even though the EQR has increased through time at realized NVOs of M-types and the R4a type.

Table 3.1: Summary table of statistical testing of the EQR and sub-metrics of macroinvertebrates and macrophytes.

Legend:

- N = number of monitoring measure locations
- r = mean value for the NVOs in realized locations
- s = mean value for the NVOs in scheduled locations
- Δ = difference between realized and scheduled
- NA = means that the sub-metric is not applicable for the water type;
- blue cells = significant positive effect;
- green cells = no significant effect but higher in average;
- yellow cells = no significant effect but lower in average.

	M-types			R-types		
	s	r	Δ	s	r	Δ
N (macroinvertebrates)	32	23	9	87	22	65
Macroinvertebrate EQR	0.41	0.47	+0.06	0.30	0.35	+0.05
Number of individuals (n)	728.17	934.10	+205.93	505.58	1099.67	+594.09
Species richness (n)	49.53	78.78	+29.25	41.09	58.50	+17.41
Abundance characteristic taxa (%)	NA	NA	NA	3.55	3.61	+0.06
Abundance characteristic + positive dominant taxa (%)	NA	NA	NA	10.68	10.11	-0.57
Abundance positive dominant taxa (n)	32.15	36.61	+4.46	NA	NA	NA
Abundance negative dominant taxa (%)	16.11	13.26	-2.85	41.45	32.60	-8.85
N (macrophytes)	17	16	1	17	10	7
Total flora EQR	0.40	0.44	+0.04	0.50	0.47	-0.03
Abundance growth forms EQR	0.34	0.30	-0.04	0.45	0.58	+0.13
Species composition EQR	0.45	0.56	+0.11	0.30	0.20	-0.10

Table 3.2: Statistical testing results of the influence of time and before and after NVO realization on macroinvertebrate EQR. Significant values are indicated with bold text.

Type	M				R			
	Before	After	Before-after	Time (years)	Before	After	Before-After	Time (years)
N	8	123			16	38		
Mean EQR	0.41	0.44			0.35	0.38		
Correlation (R)			0.169	0.045			0.080	0.181

Table 3.3: Statistical testing results of the influence of time and before-after NVO realization on macroinvertebrate EQR (all non-significant). NA = not applicable for statistical testing, because sample size is too small (Bonett, Douglas & Wright, 2000).

Water types	N	N (before)	N (after)	Mean (before)	Mean (after)	Correlation (R) Before-After	Correlation (R) time (years)
M1a	65	12	53			0.175	0.235
M3	61	3	58			NP	0.215
M6a	16	4	12			NP	-0,169
R4a	51	32	19			0.157	0.277
R20	40	7	70			NP	-0.006

4. Discussion

4.1 Effects NVOs according to this study

At first sight, NVOs seem to have positive but limited effects and no remarkable negative effects when seeing the coloured cells in table 3.1. Species richness, negative dominant taxa and number of individuals of macroinvertebrates clearly improved significantly. However it was still difficult to determine the importance of the differences of EQR and other sub-metrics that are not statistically different. Despite showing no statistical significance, the “trends” that showed a small positive or negative effect that is not significant were still worth to mention.

4.2 Reasons of effectiveness

The analysis in this study does not involve the reasons why characteristics of the NVOs are effective. Important factors for the effectiveness of NVOs were mentioned in the introduction (see figure 4.1) NVOs are effective or not effective depending on its relations with the habitat preferences of macrophytes and macroinvertebrates. Species could have an interaction with different environmental factors that are not analysed in the present study. These are the ecological key factors water productivity (ESF1), light climate (ESF 2), soil productivity (ESF 3), removal (ESF 6) organic load (ESF 7) and toxicity (ESF 8). These factors are the most important for the planning of NVO restoration measures (Tanis & Kamp 2019). In addition, also other measures could interact with NVOs and its macroinvertebrate and macrophyte quality, such as EVZs, decreasing nutrient emissions and toxicants, ecological networks, and reconstruction of water vegetation zones (Tanis & Kamp, 2019).



Figure 4.1: For NVOs, the nine ecological key factors are important to take in account when deciding its effectiveness and further improvement.

Thus, the following discussion paragraphs will also explain the environmental conditions and background stories of Waterboard Aa en Maas that could have influenced the results. The discussion about this is meant to give people in the water and ecology sectors explain the current effects of NVOs that are found in the management area of Aa en Maas.

4.3 Effects on macroinvertebrates

The positive effects on the macroinvertebrates in M-types of Aa en Maas can be explained by the shallow banks that are present in realized NVOs. NVOs of Aa en Maas are often appointed as realized when the profile of the bank shows a gradual angle and mowing maintenance appears to

complement the vegetation and macrophytes. Even though water quality is very important for macroinvertebrates, the positive effects on species richness may be caused by both the presence of macrophytes and the gradual angle of the bank of NVOs. This also shown in the studies of Hokken & Torenbeek (2017) and Verhofstad et al. (2019).

There are several studies that described if macroinvertebrate quality and abundance increased at NVOs. The most recent study, Verhofstad, Herder, Peeters, et al., (2019), focused on a selection of eco-friendly banks of ditches, canals and streams in the centre and the north of the Netherlands with a total number of 37 monitoring locations. This study also shows results with statistical significance. In addition, some results are similar, both research areas are quite big compared to other studies and both studies show results at M-types and R-types.

Both the present study and Verhofstad et al. (2019) showed that there were no significant higher abundance of indicative species found in for R-types (Verhofstad et al., 2019). The study showed that there were was a significant increase in macroinvertebrate EQR (+0.1) whereas in the present study no significant increase in macroinvertebrate quality was found in R-types (+0.05) and M-types (+0.06). There were 34.2 more taxa and 444.1 more individuals that is both significant, whereas in the present study there is a significant amount of 29.25 more taxa found in M-types and in R-types 17.41 more taxa that was not significant. There was an significant increase of individuals in ditches, whereas in the present study 205.93 more individuals found which was not significant. In R-type there was no significant effect found, whereas in the present study there were 594.09 individuals found which was significant.

4.4 Effects on macrophytes

In this study no significant differences were found, but the findings were similar to the results of Verhofstad et al., 2019. In M-types there were positive effects for macrophytes in M-types, whereas in R-types it actually decreased. The first thing that comes to mind when seeing this is that water types might need appropriate measures. For M-types for example, the maintenance is very important for quality of macrophytes as seen in Hokken & Torenbeek (2017). For R-types, this might also be the case, but R-types often require more than only a nature friendly bank angle as seen in the practice of Aa en Maas.

In the Netherlands, there are studies done nationally by different organisations on the effect of NVOs on macroinvertebrate quality and species groups. The quality of macrophytes in canals and ditches (M-types) is known in other studies to increase when NVOs are reconstructed that have weak slope and shallow bank, even though the most important ecological key factors such as toxicity and organic loads did not have a green light. This might also have been a reason for Aa en Maas why the species richness of macroinvertebrates significantly increased in M-types, and why the species composition EQR of macrophytes have increased even though it is not significant.

As is seen in other studies, NVOs have effects on water plants and fish. In particular, the EQR of water plants in Verhofstad et al. (2019) was significantly higher with +0.05 EQR in NVOs of canals and all NVOs had an average of 8.4 plant species in the bank zone. No significant effects on water plants in water zones, streams and canals were found (Verhofstad et al., 2019; Hokken & Torenbeek, 2017).

4.5 Scheduled NVOs with good quality

In this study was shown that there is quite little data of realized locations compared to scheduled locations. Thus, there are still a lot of NVOs that are appointed as scheduled. Even though no

significant differences were found in M-types and R-types types altogether, there are certainly monitoring locations that have increased EQRs (see also Annex III). In addition, 9 scheduled NVO locations already have a good macroinvertebrate quality as is seen in Annex III. These locations are of the water bodies Kleine Wetering, Nieuwe Vliet, Roode Wetering, Kanaal van Deurne and Buitendijkse loop in M-types, and Lactariabeek and St. Jansbeek in R-types.

An example was described in the results about the macroinvertebrate EQR of waterbodies Lactariabeek, St. Jansbeek and Vlier. In addition, sub-metrics also had outliers. These observations and outliers is logically explained by the effects stream restoration with measures that are less related with NVOs. For example, in the Lactariabeek wood was added to the streambed and at St. Jansbeek re-meandering and construction of EVZs were done in the past. Both waterbodies also have an adapted water level management and mowing maintenance measures that could have had positive effects on the habitat suitability (Reeze, et al., 2016). Thus, further research is needed for these kind of examples at small geographical scale.

4.6 Ineffective measures and bottlenecks

In the present study is seen that many quality scores show no significant differences in realized NVO compared to scheduled NVOs. Hence, for some water bodies there are measures that are not effective due to the fact that it is inappropriate for the water type or there are still bottlenecks for applying the measure. Previously, the NVO applications for R-types were sometimes misunderstood, especially for R4 and R5 types. Shallow and less steep banks were used that are not appropriate for R4 and R5 types, because flat banks lower the flow velocity that is not desired for a good macroinvertebrate quality (Evers, et al., 2018). Even though Verhofstad et al. (2019) and the present study both state a higher species richness in general, no higher abundance of species are found that are indicative for R-types.

This was also seen in the Redundancy Analysis (RDA) of Noord (2019), where positive or characteristic taxa also show no relation with flat banks whereas high abundance and richness did. Unlike flat profiles, meandering caused higher EQR scores in R-types. It could potentially even decrease the macroinvertebrate EQR if the abundance of negative dominant species increases (Noord, 2019). Thus, there can be concluded that construction of flat banks is not a sufficient measure to increase biological quality in R-types.

Moreover, Hokken & Torenbeek (2017) states that the present methods of its mowing maintenance favour the quality of water plants, but is still a bottleneck for macroinvertebrates and fish. This is because of the insufficient removal of water plants that causes low oxygen concentrations. Even though this study did not research about mowing intensities, there were already studies done on the mowing intensities in the management area of Waterschap Aa en Maas. It proves with models and theories that more ecological profits remains to be made in mowing maintenance at Aa en Maas as well. This includes different findings per water type that still require field validation.

Another bottleneck is the connectivity of waterbodies. Some waterbodies do not have large NVO restoration that might not be enough to be effective. In addition, many streams are isolated from the source area and upstreams at Boven-Dommel by land management and urbanisation. This explains the appearance of the data of the R-types. Many characteristic species have never been found in the province of North-Brabant. This had led to problems for many stonefly (*Plecoptera*) species to recolonise and distribute. Because of this, some species disappeared which was problematic due to its feeding behaviour as 'shredders' of coarse particulate matter. Isolation of habitats is also assumed

to be one of the causes of a species composition of macrophytes that is not really different in realized NVOs compared to scheduled NVOs. Connectivity enables the continuation of different species compositions in rivers and streams. Thus, in order to assure new characteristic species and varied species composition (see figure 4.2), accessibility of the waterbodies in the management area of Aa en Maas needs to be improved (Verdonschot & Verdonschot, 2017).



Figure 4.2: Characteristic / positive dominant macroinvertebrate taxa in streams, A) *Hydropsyche* sp. and B) *Gomphus vulgatissimus*. (Source: Maria Judith Sanabria, Aquon).

4.7 Effective measures

When looking at the data, it shows a high distribution of EQR. This means that there are big differences between NVO monitoring locations as is seen in Annex III, which is especially the case for the R-types. This is because R-types have a more dynamic ecosystem that includes more small-scale restoring measures (Verdonschot, Verdonschot, Bauwens, et al., 2017) and environmental factors (Mellor, Verbeek & Wijngaart, 2017), which it makes it different at several monitoring locations.

As is mentioned before, good macroinvertebrate quality requires a good water quality. In addition, other factors such as flow velocity, profile shape, shade, nutrients, vegetation management and other conditions for species can be improved with NVO measures. The most common NVO measures according to Verhofstad et al. (2019) include the removal of artificial construction and shallowing the banks. It also includes reconstructing swamp conditions and bypass or side channels.

In addition, Verhofstad et al. (2019) recommended that banks should be more variable, especially for the R-types. This means that M-types should have more different depths (Evers et al, 2018; Verhofstad et al., 2019). NVOs at R5 and R4 types should include measures like the small-scale measures. These are measures that are meant to complement the natural dynamic of banks due to erosion and sedimentation. This should result in the characteristic banks of these water types, that are steep with inner and outer bends (Ministerie van van Infrastructuur en Waterstaat, 2018; Verdonschot, Verdonschot, Bauwens, et al., 2017).

The present study states that NVOs negative dominant species are significantly lower at R-types. Therefore, there is much known about the good effects of small-scale measures (Verdonschot, Verdonschot, Bauwens, et al., 2017). Future research and effect monitoring needs to prove how the combination of environmental variables and specific measures affect the macroinvertebrate quality at the NVO locations. This is important for the increase and protection of the characteristic + positive dominant taxa at R-types and positive dominant taxa at M-types. Other effective measures for M-

types besides just replacing artificial banks with less steep eco-friendly banks is the implementation of extensive mowing maintenance. Studies by Royal Haskoning DHV (2020) have used prediction models in order to predict effects of maintenance measures at different water types, where it is found that decreasing the mowing intensity across the length of banks often increases EQR's.

4.8 Limitations of this study

There are also other specific reasons for the appearance of data that is regarding with the background and history of Aa en Maas.

Firstly, not many effect monitoring of the NVOs has been done, especially not to establish the reference situation (before realization of the NVO). Secondly, in the past NVOs banks with a gradual angle were constructed at streams where no other stream restoration measures were planned. Shallow banks are not appropriate for most stream types (R-types), but Aa en Maas does aim since last WFD cycle that shallow banks are appropriate for the KRW type R20. Thirdly, the big difference between the present study and other studies, is that the present study uses the reference locations that are called "scheduled". The scheduled locations in the present study are planned to have an NVO in the future or do not have any NVO. The NVO status that is referred by PIB also does not always inform the good and bad qualities of realized NVO as well as scheduled NVO sections. The scheduled NVOs might not be NVOs at all, or are NVOs that are still in development and still not effective according to ecologists and water system advisors.

In addition, some data of the monitoring locations is old and might need more recent monitoring data in order to be reliable.

Finally, this research is done at a big scale and did not specifically analyse NVO effects at the scale of a waterbody and monitoring location.

This means that future research still has to determine the appropriately made NVOs at the given location and water body.

Thus, effect monitoring of NVOs is still a young research method and needs field validation as well as further analysis.

4.9 Effects of time and before/after NVO realization

In the present study, no evidence could be found about the significant effects of time and before-after NVO realization on the macroinvertebrate EQR. This could also be explained by the reasons mentioned before and monitoring of NVOs is still new. Individual observation of monitoring locations was therefore needed to see if the EQR has increased. This was a similar outcome as de La Haye, Verduin, Blom et al. (2011), where no optimum was found a few years after the NVO reconstruction with statistical analysis. Other studies show that the EQR score changes after a few years after the reconstruction of NVOs (Soesbergen & Rozier, 2004; Tanix & Kamp, 2019). A study by Kits, Bruggmans, Verstappen et al. (2011) of 10 years NVOs in the management area of Aa en Maas did not show the development of macroinvertebrates, but did show the development of other important species groups. A species groups close to macroinvertebrates are the dragonflies, that come from the water during the larvae and earlier life cycle stages. It stated that the dragonflies seem to benefit at the NVOs, as the desired conditions at almost all locations were achieved. This was especially the case for locations at Leijgraaf, Peelse Loop and Hertogswetering with a high number of species and rare species (Kits, et al., 2011).

5. Conclusion

The aim of this study was to determine the overall situation of the macroinvertebrates and macrophytes at realized and scheduled NVO monitoring locations in order to give recommendations (see chapter 6) for focusing the research and development of NVOs at certain locations. The main research question was:

- What are the effects of the NVO implementation on macroinvertebrates and macrophytes in M- and R water types of the management area of Aa en Maas?

In the following paragraphs the main research questions is answered by answering the subquestions. The last paragraph describes the ending conclusion and the answer to the main research question.

5.1 What difference in macroinvertebrate and Total EQR is seen between realized and scheduled NVOs?

The present EQR between scheduled and realized NVOs is the same according to statistical significance. However, both the macroinvertebrate and Total Flora EQR has a higher average in realized NVOs.

5.2 Which differences in the sub-metrics of macroinvertebrate and macrophytes are seen between realized and scheduled NVOs?

Overall:

- NVOs have positive effects on macroinvertebrates and macrophytes
- No significant negative effects are seen

Macroinvertebrates:

- In M-types, the species richness is positively affected by NVOs.
- In R-types, there are also positive effects on the number of macroinvertebrate individuals
- In R-types, there is a lower abundance of negative dominant taxa
- Characteristic species and positive dominant species in R-types need to be increased as the species did not significantly increase yet.

Macrophytes:

- The abundance growth forms have not increased in average, which could be explained by maintenance measures that may not have worked correctly.
- In M-types, the shallow bank angles with nature friendly vegetation zones have had positive effects on the species composition of macrophytes.
- Negative but not significant effects are seen on the species composition and EQR of macrophytes are seen in R-types.

5.3 Does time have an influence on the macroinvertebrate EQR?

This study shows that macroinvertebrate quality increased over time only with regard to M-types and the R4a-type, but none of the water types are known to significantly change after NVO realization.

5.4 Ending conclusion: what are the effects of the NVO implementation?

The conclusion of this study is that realized NVOs in the management area of Aa en Maas have positive effects on the macroinvertebrates and macrophytes in compared with scheduled NVOs.

The statistical significance played a role in defining the importance of effects. Even though not many positive effects are significant, there are also no significant negative effects. See also table 5.1 where the overall situation is given of the realized NVOs compared to the scheduled NVOs. In this table the following categories of effects are shown:

- Alternative hypothesis is accepted: positive significant effects indicated with blue cells;
- Null-hypothesis is accepted
 - Not a significant effect but higher in average, thus an increasing trend indicated with green cells (positive effects);
 - Not a significant effect but lower in average, thus an decreasing trend indicated with yellow cells (negative effects).

Table 5.1: Summary table of effectiveness of realized NVOs, describing differences of realized NVOs compared to scheduled NVOs. NA = means that the sub-metric is not applicable for the water type, blue cells = significant positive effect, green cells = no significant effect but higher in average and yellow cells = no significant effect but lower in average.

Species group	Metrics (Dutch: maatlaten)	M-types	R-types
Macroinvertebrates	Macroinvertebrate EQR (present and through time)		
	Number of individuals (n)		
	Species richness (n)		
	Abundance characteristic taxa (%)	NA	
	Abundance characteristic + positive dominant taxa (%)	NA	
	Abundance positive dominant taxa (n)		NA
Macrophytes	Abundance negative dominant taxa (%)		
	Total flora EQR		
	Abundance growth forms EQR		
	Species composition EQR		

In addition, this study gives an overview of the locations where the EQR's still need to be improved and locations where the EQR's are already meet the GEP. In the next chapter with the recommendations these locations are presented with colours, in which the quality classes are shown (Bad, Insufficient, Moderate, Good). The locations that do not meet the GEP (Bad, Insufficient or Moderate) are suggested to need improvement by using the recommendations of NVO measures mentioned in the next chapter. As mentioned in the discussion, there were different limitations and underlying factors that affected the reliability of this research. This study is only a pre-research of the small-scale studies that are needed to define effects at specific locations and water bodies. Thus, recommendations for further research are also mentioned in the next chapter.

6. Recommendations

In the present study, the general situation of NVOs was made clear and recommendations can be made for focusing the development, research and practical measures of NVOs. In the Netherlands, the importance of the monitoring cycle is hereby worth to mention, see figure 6.1. When following this cycle, this study regarding NVOs was initiated because of the information needs about the effectiveness of NVOs (Information needs). At this very moment, Aa en Maas is designing a monitoring strategy that will take place at NVOs. During this research, information was collected and analysis was done to define the importance of the effects (Data Analysis).

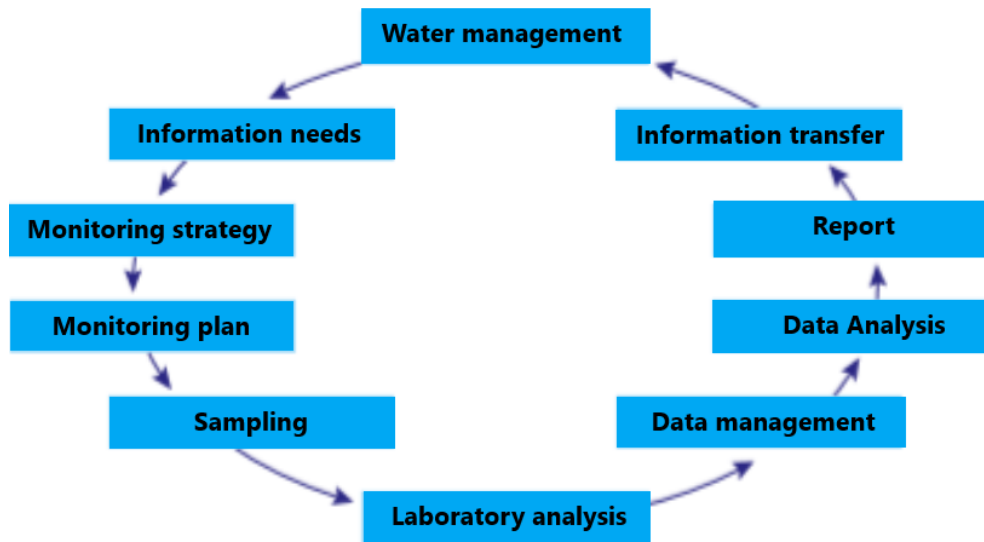


Figure 6.1: The monitoring cycle according to Reeze & Lenssen (2015) in the Netherlands (translated into English).

Even though the areas of this study and the present study cannot be really compared due to other freshwater ecosystems, part of the Netherlands, land and management and other factors, it is still interesting to show the differences on a more national scale. Future research could eventually evaluate the effectiveness of NVOs on a national scale or even bigger geographical scale as is similarly done by the European organisation “Natural Water Retention Measures” with “stream bed naturalization” and other restoration measures.

Below the recommendations for further research and effect monitoring is presented:

- Continue with effect monitoring and future research, because data is either old or not in big amounts.
- More detailed research at a selection of locations (small-scale research) using in order to find trends more efficiently.
- Research that includes ecological explanatory variables, such as substrate, shading, depth, soil productivity, maintenance, type of NVO, profile, ammonium, total phosphor, total nitrogen, shape etcetera. This is highly the case for many R-types, because the monitoring locations show many differences as is described in the discussion.
- Determine effects of the specific KM-measures in order to differentiate different types of NVOs.

This study aimed to give an overview of the locations where NVO measures could be improved. The literature study in the discussion together with the knowledge of the present EQRs showed which possible practical effective measures could be taken into account. The locations where NVOs are implemented or are planned to be implemented are shown in table 6.1 on the following pages. When looking at these locations and taking the discussion into account, the following recommendations are given:

- **Prioritization:** NVO measures at both realized and planned NVOs should be prioritized according different factors, so that in the short term the goals of the next WFD cycle in 2021 will be met. Prioritization should be done according the ecological key factors whether NVOs will be promising to develop (Rink, 2020). If important ESFs are good at one location, this should improve the chance of NVOs being effective. Ecological key factors and EQRs can be prioritized quickly for individual observations by using the method “Afwegingskader TAUW” from Wilhelm & Boon, 2019), which is a model made by ecologists that prioritizes EKFs. This can be used for an efficient selection of measures at NVO locations. The EQR’s for example, should be prioritized so that locations with bad EQR’s come first and after that insufficient. This also means that locations with an aim for implementing an NVO (planned NVO) that have a bad quality should be prioritized. Furthermore, connection of NVOs with other NVOs, natural areas and ecological connecting zones should also be taken into account when prioritizing the development NVOs in order to decrease the isolation of habitats.
- **Definition M-types and R-types:** NVOs at M-types and R-types are recommended to be defined digitally within the short term, which might mean new applications in the practical work field in the long term. This means for example that locations where the bank is too flat for water types R4a and R5, needs to be replaced by more steep banks and if needed combined with other stream restoration measures, such as a more nature-friendly water level management, profile deepening, remove weirs if possible, re-meandering, construct fish migration passages, adding wood and create shading by planting trees. Therefore, R20 is a special case of the streams with a flat zone containing wet bog areas, which do need less steep banks in order to improve the EQR’s. In addition, NVOs at M-types are recommended to have variable depths and angles (Verhofstad et al., 2019; Rink, 2020; Reeze, et al., 2016).
- **Maintenance:** In overall, the mowing intensity should be decreased in the long term to increase EQR’s. However, maintenance measures should validated in the field if it is adapted to the water type. For instance, in areas with many shading, maintenance is often not needed to improve the EQR’s and not maintaining the vegetation could actually let vegetation naturally develop leading to increasing EQR’s. However, caution for negative dominant species, invasive exotic species, and keeping the vegetation diverse is recommended in order to adapt maintenance. Field validation with local managers and ecologists of Aa en Maas is needed to check if the aims of maintenance seen in Reeze et al. (2016) are according the goals of maintenance of each water type. The use of prediction models is recommended in order to predict future effects of NVOs. An example of prediction model used by Waterschap Aa en Maas is Royal HaskoningDHV (2020).

Table 6.1: The EQR's and status of macroinvertebrates and total Flora of every NVO monitoring location in Aa en Maas.

Legend:

- MEPID: identification code of monitoring location;
- Status: planned NVOs are indicated with 0 and realized NVOs are indicated with 1;
- Waterbody: name of the water body in Aa en Maas;
- Date: year-month-day
- Type: KRW water types, consisting of R4a, R5, M1a, M3 and R20;
- MV_EQR: EQR of macroinvertebrates
- MV_status: quality classes macroinvertebrates
- TF_EQR: Total Flora EQR
- TF_status: quality classes of total flora

MEPID	Status	Waterbody	Date	Type	MV_EQR	MV_Status	TF_EQR	TF_status
149728	0	Vlier	2017-05-10	R4a	0.073	Bad		
149726	0	Vlier	2017-05-10	R4a	0.105	Bad		
149707	0	Vlier	2017-05-10	R4a	0.131	Bad		
149704	0	Vlier	2017-07-25	R4a	0.135	Bad		
149717	0	Vlier	2017-07-25	R4a	0.156	Bad		
149695	0	Vlier	2017-07-25	R4a	0.16	Bad		
149695	0	Vlier	2017-07-25	R4a	0.16	Bad		
149702	0	Vlier	2017-07-25	R4a	0.162	Bad		
149720	0	Vlier	2017-07-25	R4a	0.164	Bad		
149694	0	Vlier	2017-07-25	R4a	0.165	Bad		
149727	0	Vlier	2017-07-25	R4a	0.184	Bad		
149703	0	Vlier	2017-07-25	R4a	0.185	Bad		
149725	0	Vlier	2017-07-25	R4a	0.19	Bad		
149721	0	Vlier	2017-07-25	R4a	0.191	Bad		
149700	0	Vlier	2017-07-25	R4a	0.193	Bad		
149712	0	Vlier	2017-07-25	R4a	0.196	Bad		
140274	0	Beekgraaf	2019-04-24	M1a	0.14	Bad		
140284	0	Schijndelse Loop	2005-06-01	M1a	0.14	Bad		
341415	0	Strijpse Beek	2018-08-29	M2	0.187	Bad		
900073	0	Zuid-Willemsvaart	2010-05-20	M6b	0.074	Bad		
900035	0	Kleine Aa	2011-09-28	R4a	0.192	Bad		
140294	0	Kleine Wetering	2019-06-17	M1a	0.615	Good		
900187	0	Buitendijkse Loop	2018-06-20	M1a	0.618	Good	0.357	Unsufficient
900083	0	Kanaal van Deurne	2013-05-21	M3	0.653	Good		
340446	0	Nieuwe Vliet	2011-05-03	M3	0.722	Good		
340454	0	Roode Wetering	2017-05-08	M3	0.607	Good		
340412	0	St. Jansbeek	2017-06-19	R5	0.639	Good		
140818	0	Lactariabeek	2019-12-11	R4a	0.633	Good		
140820	0	Lactariabeek	2019-12-11	R4a	0.693	Good		
342136	0	Nieuwe Loonse Vaart	2018-06-19	M1a	0.468	Moderate		
342410	0	Koningsvliet	2019-05-09	M3	0.564	Moderate		
140373	0	Groote Wetering	2019-06-13	M3	0.567	Moderate	0.544	Moderate

140289	0	Biezenloop	2019-08-20	M1a	0.462	Moderate		
140293	0	Groote Wetering	2005-09-01	M3	0.419	Moderate		
140391	0	Groote Wetering	2019-06-17	M3	0.437	Moderate	0.584	Moderate
140234	0	Kanaal van Deurne	2000-08-31	M3	0.408	Moderate		
340410	0	Sambeekse Uitwatering	2019-08-12	M1a	0.424	Moderate		
990237	0	Sambeekse Uitwatering	2017-09-18	M1a	0.483	Moderate		
159047	0	Landmeerse loop	2000-06-28	R4a	0.453	Moderate		
900243	0	Lactariabeek	2014-08-27	R4a	0.421	Moderate		
900042	0	Oeffeltse Raam	2016-05-09	R20	0.448	Moderate		
149724	0	Vlier	2017-07-25	R4a	0.413	Moderate		
140264	0	Donkersvoortse Loop	2018-07-31	R20	0.467	Moderate		
900049	0	Peelse Loop	2019-04-01	R4a	0.445	Moderate		
900189	0	Peelse Loop	2019-04-01	R4a	0.512	Moderate		
140814	0	Lactariabeek	2019-12-11	R4a	0.477	Moderate		
140815	0	Lactariabeek	2019-12-11	R4a	0.566	Moderate		
140816	0	Lactariabeek	2019-12-11	R4a	0.537	Moderate		
140817	0	Lactariabeek	2019-12-11	R4a	0.566	Moderate		
140824	0	Lactariabeek	2019-12-11	R4a	0.409	Moderate		
142221	0	Kleine Aa	2005-07-12	R4a	0.215	Unsufficient		
340423	0	St. Anthonisloop	2003-08-18	R4a	0.375	Unsufficient		
900004	0	Aa	2013-08-05	R5	0.249	Unsufficient		
140228	0	Eeuwse Loop	2015-05-19	R4a	0.264	Unsufficient		
149709	0	Vlier	2017-07-25	R4a	0.2	Unsufficient		
149699	0	Vlier	2017-07-25	R4a	0.206	Unsufficient		
149729	0	Vlier	2017-05-10	R4a	0.213	Unsufficient		
149697	0	Vlier	2017-07-25	R4a	0.215	Unsufficient		
149690	0	Vlier	2017-07-25	R4a	0.217	Unsufficient		
149691	0	Vlier	2017-07-25	R4a	0.223	Unsufficient		
149696	0	Vlier	2017-07-25	R4a	0.224	Unsufficient		
149708	0	Vlier	2017-07-25	R4a	0.224	Unsufficient		
149706	0	Vlier	2017-07-25	R4a	0.225	Unsufficient		
149711	0	Vlier	2017-07-25	R4a	0.228	Unsufficient		
149723	0	Vlier	2017-07-25	R4a	0.23	Unsufficient		
149698	0	Vlier	2017-07-25	R4a	0.232	Unsufficient		
149710	0	Vlier	2017-07-25	R4a	0.24	Unsufficient		
149715	0	Vlier	2017-07-25	R4a	0.241	Unsufficient		
140810	0	Vlier	2015-06-03	R4a	0.259	Unsufficient		
149701	0	Vlier	2017-07-25	R4a	0.265	Unsufficient		
140808	0	Vlier	2015-06-03	R4a	0.267	Unsufficient		

149722	0	Vlier	2017-07-25	R4a	0.268	Unsufficient		
149692	0	Vlier	2017-07-25	R4a	0.27	Unsufficient		
990235	0	Oploosche Molenbeek	2017-09-18	R5	0.347	Unsufficient		
990236	0	Oploosche Molenbeek	2017-09-18	R5	0.358	Unsufficient		
140222	0	Voordeldonkse Broekloop	2018-05-14	R4a	0.231	Unsufficient		
140223	0	Beekerloop	2018-05-14	R4a	0.263	Unsufficient		
900190	0	Lactariabeek	2018-05-28	R4a	0.297	Unsufficient		
140221	0	Eeuwse Loop	2018-06-12	R4a	0.237	Unsufficient		
340426	0	Ledeackerse Beek	2018-06-13	R4a	0.373	Unsufficient		
341413	0	Lactariabeek	2018-06-25	R4a	0.347	Unsufficient		
140809	0	Vlier	2015-06-03	R4a	0.285	Unsufficient		
140819	0	Lactariabeek	2019-12-11	R4a	0.338	Unsufficient		
140376	0	Snelle Loop	2019-06-20	R4a	0.292	Unsufficient		
900079	0	Biezenloop	2019-08-19	M1a	0.352	Unsufficient		
900054	0	Schijndelse Loop	2011-05-10	M1a	0.341	Unsufficient		
140256	0	Landmeerse loop	2018-06-11	R4a	0.248	Unsufficient		
900188	0	Zijsloot Polderdijk	2019-08-29	M1a	0.303	Unsufficient		
140241	0	Oude Aa	2005-05-09	R4a	0.229	Unsufficient		
140295	0	Wambergse Beek	2012-08-06	R5	0.269	Unsufficient		
140291	0	Wambergse Beek	2018-09-21	R20	0.31	Unsufficient		
140296	0	Groote Wetering	2005-08-30	M3	0.34	Unsufficient		
140364	0	Schijndelse Loop	2019-08-20	M1a	0.293	Unsufficient		
340436	0	Hertogswetering	2019-09-03	M3	0.253	Unsufficient	0.348	Unsufficient
340438	0	Hertogswetering	2019-08-29	M3	0.312	Unsufficient	0.281	Unsufficient
149716	0	Vlier	2017-07-25	R4a	0.287	Unsufficient		
149719	0	Vlier	2017-07-25	R4a	0.287	Unsufficient		
159037	0	Vlier	2000-06-28	R4a	0.293	Unsufficient		
149693	0	Vlier	2017-07-25	R4a	0.303	Unsufficient		
149714	0	Vlier	2017-07-25	R4a	0.305	Unsufficient		
140803	0	Vlier	2015-06-03	R4a	0.309	Unsufficient		
149705	0	Vlier	2017-05-10	R4a	0.313	Unsufficient		
149718	0	Vlier	2017-07-25	R4a	0.317	Unsufficient		
140806	0	Vlier	2015-06-03	R4a	0.318	Unsufficient		
140801	0	Vlier	2015-06-03	R4a	0.33	Unsufficient		
140805	0	Vlier	2015-06-03	R4a	0.332	Unsufficient		
149713	0	Vlier	2017-07-25	R4a	0.345	Unsufficient		
140802	0	Vlier	2015-06-03	R4a	0.346	Unsufficient		
140807	0	Vlier	2015-06-03	R4a	0.364	Unsufficient		

140242	0	Vlier	2019-05-02	R4a	0.365	Unsufficient		
140804	0	Vlier	2015-06-03	R4a	0.368	Unsufficient		
144305	0	Eindhovens Kanaal	2015-09-07	M3	0.394	Unsufficient		
900009	0	Beekgraaf	2019-06-18	M1a	0.266	Unsufficient		
341428	0	Peelkanaal	2019-04-24	R20	0.346	Unsufficient		
341422	0	Peelkanaal	2018-05-28	M3	0.394	Unsufficient		
349100	0	Laarakkerse Waterleiding	2013-09-17	R20	0.331	Unsufficient		
340422	0	Tochtsloot	2018-09-05	R20	0.377	Unsufficient		
900189	0	Peelse Loop	2016	R4a			0.478	Moderate
140391	0	Groote Wetering	2019	M3			0.584	Moderate
900187	0	Buitendijkse Loop	2018	M1a			0.357	Unsufficient
140373	0	Groote Wetering	2019	M3			0.544	Moderate
340438	0	Hertogswetering	2017	M3			0.281	Unsufficient
340436	0	Hertogswetering	2017	M3			0.348	Unsufficient
340452	1	Teeffelense Wetering	2017-09-12	M3	0.157	Bad		
900185	1	Kleine Aa	2019-06-11	R4a	0.189	Bad		
140224	1	Diepenhoekse Loop	2005-05-12	R4a	0.135	Bad		
140220	1	Kievitsloop	2018-05-24	R4a	0.166	Bad		
340445	1	Nieuwe Vliet	2017-08-07	M3	0.772	Good		
343521	1	Nieuwe Bossche Sloot	2018-06-19	M1a	0.618	Good		
140742	1	Hertogswetering	2011-05-02	M3	0.66	Good		
140369	1	Hertogswetering	2017-06-12	M3	0.68	Good	0.437	Moderate
340440	1	Hertogswetering	2003-07-07	M3	0.609	Good		
343502	1	Hedikhuizen Maas	2018-05-07	M1a	0.406	Moderate		
342401	1	Luisbroekse Wetering	2018-09-03	M1a	0.452	Moderate		
343513	1	Nieuwe Bossche Sloot	2003-08-13	M1a	0.543	Moderate		
343504	1	Virdsche Graaf	2016-06-29	R4a	0.417	Moderate		
147273	1	Leijgraaf	2004-08-25	R20	0.425	Moderate		
900181	1	Snelle Loop	2017-05-16	R4a	0.521	Moderate		
900023	1	Hedikhuizen Maas meander	2010-05-27	M1a	0.55	Moderate		
342406	1	Drongelens Kanaal	2017-07-05	M6a	0.442	Moderate		
342408	1	Drongelens Kanaal	2013-06-06	M6a	0.474	Moderate		
900184	1	Drongelens Kanaal	2017-09-26	M6a	0.44	Moderate		

340442	1	Hoefgraaf	2017-06-12	M3	0.558	Moderate	0.222	Unsufficient
140367	1	Drongelens Kanaal	2018-06-20	M6a	0.579	Moderate	0.283	Unsufficient
140292	1	Groote Wetering	2019-05-09	M3	0.579	Moderate		
340439	1	Hertogswetering	2005-06-07	M3	0.536	Moderate		
140375	1	Leijgraaf	2017-06-28	R20	0.43	Moderate		
900085	1	Peelkanaal	2018-10-01	M3	0.479	Moderate		
900048	1	Peelkanaal	2016-05-23	R20	0.532	Moderate		
999969	1	Lage Raam	2019-06-12	M1a	0.523	Moderate		
343514	1	Munsche Wetering	2018-06-13	M1a	0.251	Unsufficient		
343509	1	Virdsche Graaf	2002-09-10	R4a	0.247	Unsufficient		
140273	1	Leijgraaf	2005-08-31	R20	0.35	Unsufficient		
140261	1	Goorloop	2005-08-22	R20	0.381	Unsufficient		
140286	1	Dungense Loop	2005-09-01	M1a	0.203	Unsufficient		
343515	1	Lorregraaf	2019-08-29	M1a	0.377	Unsufficient		
140281	1	Biezenloop	2001-08-28	M1a	0.28	Unsufficient		
140272	1	Leijgraaf	2005-08-30	R20	0.343	Unsufficient		
900022	1	Groote Wetering	2019-06-17	M3	0.375	Unsufficient		
340413	1	Oeffeltse Raam	2004-06-14	R20	0.328	Unsufficient		
340415	1	Oeffeltse Raam	2019-06-12	R20	0.374	Unsufficient		
900192	1	Oeffeltse Raam	2019-06-12	R20	0.311	Unsufficient		
340430	1	Oploosche Molenbeek	2004-06-14	R5	0.384	Unsufficient		
900046	1	Oude Aa	2016-05-30	R4a	0.277	Unsufficient		
140236	1	Astense Aa	2013-05-21	R4a	0.334	Unsufficient		
340409	1	St. Anthonisloop	2019-06-12	R4a	0.321	Unsufficient		
140371	1	Oude Aa	2011-09-28	R4a	0.323	Unsufficient		
349758	1	Ossemeer	2009-09-07	M3	0.365	Unsufficient		
343512	1	Tochtsloot	2005-06-21	R4a	0.361	Unsufficient		
900037	1	Laarakkerse Waterleiding	2019-04-01	R20	0.379	Unsufficient		
340442	1	Hoefgraaf	2017	M3			0.222	Unsufficient
140367	1	Drongelens Kanaal	2017	M6a			0.283	Unsufficient
140369	1	Hertogswetering	2017	M3			0.437	Moderate

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ANNEXES

Annex I: Guide additional documents

The documents that were used for this study are shown below in table 1:

Table 1: The documents with document type, name and contents that were used for this study.

Document type	Name	Contents document
CSV document	Data ecologie IM metingen Totaal vanaf 2000 csv	includes total monitoring data set of Aa en Maas
Excel document	All Data_NVOsthesis	<ul style="list-style-type: none">▪ Overview of present EQRs▪ Graphs for present EQRs▪ Input for SPSS: macroinvertebrate EQR, total flora EQR and sub-metrics▪ Input for Aquo-kit: macroinvertebrates and total flora▪ All growth forms▪ Output of Aquo-kit: results of the EQR and sub-metric scores of macroinvertebrates and macrophytes
Power Point Presentation	InternshipNVOS_DianOosterhuis	Includes main stories, findings and background of the thesis

Annex II: Explanation of statistics and output of SPSS

This annex includes the detailed statistical analysis with SPSS (version 25) that was done for the present study. Everything of the following text and the decisions taken was done and refer to the guides from the program and guides from Laerd Statistics (©2018 Lund Research Ltd, <https://statistics.laerd.com/>). Some of the text is cited in order to provide explanation. In this annex there is also referred to the results (chapter 3) of this report and to documents of SPSS. The statistical analysis tests the null and alternative hypotheses as shown in the introduction (chapter 1).

II.1 Macroinvertebrates: Realized NVOs vs scheduled NVOs

In order to compare the EQR and sub-metrics between scheduled and realized NVOs, the study designs of the Independent-samples t-test and the Mann-Whitney U are appropriate statistical testing methods. In this paragraph will be explained which steps are taken to use correct test out of these two tests and how the output of SPSS was interpreted. This is done by using assumptions, which are the requirements of the data that has to meet in order to run the test successfully. When assumptions are “violated”, other decisions can be taken in order to have reliable results.

The Mann-Whitney U and the Independent-samples t-test have a similar design, but there are different because the first one is more appropriate for data with a high non-normal distribution while the second one is for data with a normal distribution. However, the Independent-samples t-test is a parametric test that is more appropriate for most of the data of the present study. This is because: 1) parametric tests can provide trustworthy results with distributions that are skewed and a little bit non-normal (sample size of the data is big enough), 2) Parametric tests can provide trustworthy results when the groups have different amounts of variability, 3) Parametric tests have greater statistical power, and 4) this t-test is appropriate because showing the means and mean differences is the main goal of the present study. Namely, to show if realized NVOs are actually effective in compared with NVOs that are not yet realized or are still scheduled. Even though the t-test is more desirable, because is useful to check if Mann-Whitney shows differences in results when one or a few assumptions of the t-test are violated. The procedure behind making the decisions for choosing the results of one of the two tests is explained in this Annex later on.

The Mann-Whitney U and the Independent-samples t-test have a similar study design, because the tests have three assumptions in common. These assumptions are shown below.

Assumption #1: **one dependent variable** that is measured at the **continuous** level = EQR for both R-types and M-types, and sub-metrics:

- M-types: 1) number of individuals (n), 2) species richness (n), 3) abundance of negative dominant taxa (%), 4) number of positive dominant taxa (n)
- R-types: 1) number of individuals (n), 2) species richness (n), 3) abundance of negative dominant taxa (%), 4) abundance of characteristic taxa (%), 5) abundance of characteristic + dominant positive taxa

Assumption #2: **one independent variable** that consists of **two categorical, independent groups** (i.e., a **dichotomous variable**) = NVO status (scheduled = 0, realized = 1)

Assumption #3: **independence of observations**, this is met, because all monitoring locations all have data from one (the most recent) date, which means that there is no relationship between the observations.

Next, the assumptions are explained that are characteristic for the Mann-Whitney U test.

Assumptions #4: find out if the **distributions of the two groups** (scheduled and unrealized) have the **same shape**. An example of two groups is shown below in figure 1.

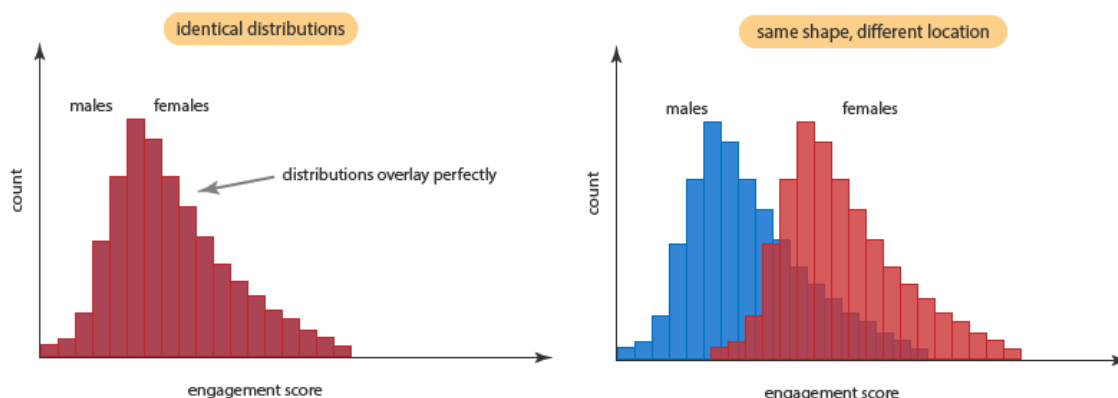


Figure 1: Assumption of the Mann-Whitney U test about similar or different shapes.

In the two diagrams above, the **distribution** of scores for 'males' and 'females' have the **same shape**. In the diagram on the left, you cannot see the distribution of scores for 'males' (illustrated in blue on the diagram on the right) because the two distributions are **identical** (i.e., both distributions are identical, so they are 'on top of each other' in the diagram, with the blue-coloured male distribution underneath the red-coloured female distribution). However, in the diagram on the right, even though both distributions have the **same shape**, they have a **different location** (i.e., the distribution of one of the groups of the independent variable has higher or lower values compared to the second distribution – in our example, females have "higher" values than males, overall).

When you analyse your own data, it is extremely unlikely that your two distributions will be identical, but they may have the same (or a "similar") shape. If they do have the same shape, you can use SPSS Statistics to carry out a Mann-Whitney U test to compare the **medians** of your dependent variable (e.g., engagement score) for the two groups (e.g., males and females) of the independent variable (e.g., gender) you are interested in. However, if your two distribution have a **different shape**, you can only use the Mann-Whitney U test to compare **mean ranks**.

In figure 1 and 2 below is shown how the design of the present study looks like in SPSS.

Visible: 31 of 31 Variables

datum	Einddatum	Compartiment	Aantalmonsters	KRWWatertype	MacrofaunakwaliteitDIMLS	MacrofaunakwaliteitDIMLS_A	AanwezigheidMacrofaunasombundantieklassen	SoortenrijkdomMacrofaunasoortkenmerken	SoortenaandeelMacrofaunasoortdominantnegatief
Jun-19		OW		R20	,374	Ontoereikend	161	18,010	30,430
Apr-19		OW		R20	,346	Ontoereikend	110	15,320	28,830
Apr-19		OW		R20	,379	Ontoereikend	194	19,070	26,290
Jun-19		OW		R20	,311	Ontoereikend	131	6,110	32,820
May-19		OW		R4a	,365	Ontoereikend	220	7,730	32,730
Jun-19		OW		R4a	,292	Ontoereikend	138	4,350	36,230
Dec-19		OW		R4a	,477	Matig	14	35,710	35,710
Dec-19		OW		R4a	,566	Matig	16	50,000	31,250
Dec-19		OW		R4a	,537	Matig	18	38,890	22,220
Dec-19		OW		R4a	,566	Matig	16	50,000	31,250
Dec-19		OW		R4a	,633	Goed	20	40,000	45,000

Figure 1: The data used in SPSS

Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure
4 Watertype	Numeric	8	0	W...	{0, M}...	None	8	Right	Nominal
5 Meetpunt_date	String	12	0		None	None	12	Left	Nominal
6 monitoringlocation_year	String	11	0		None	None	11	Left	Nominal
7 year	Numeric	4	0		None	None	12	Right	Scale
8 aantalindividuenn	Numeric	18	3	Nu...	None	None	15	Right	Scale
9 MonsterObject	String	14	0		None	None	14	Left	Nominal
10 Begindatum	Date	10	0		None	None	11	Right	Scale
11 Einddatum	Numeric	8	2		None	None	12	Right	Nominal
12 Compartiment	String	2	0		None	None	2	Left	Nominal
13 Aantalmonsters	Numeric	8	2	Aa...	None	None	12	Right	Nominal
14 KRWWatertype.code	String	3	0		None	None	3	Left	Nominal
15 MacrofaunakwaliteitDIMLS	Numeric	17	3	Ma...	None	None	12	Right	Scale
16 MacrofaunakwaliteitDIMLS_A	String	12	0	Ma...	None	None	12	Left	Nominal
17 AanwezigheidMacrofaunasombundantieklassenDIMLS	Numeric	3	0	Aa...	None	None	12	Right	Scale
18 SoortenrijkdomMacrofaunasoortkenmerkenofdominantpositief	Numeric	18	3	Ch...	None	None	12	Right	Scale
19 SoortenaandeelMacrofaunasoortdominantnegatief	Numeric	18	3	Do...	None	None	12	Right	Scale
20 SoortenrijkdomMacrofaunasoortdominantpositiefn	Numeric	2	0	Do...	None	None	12	Right	Scale
21 SoortenrijkdomMacrofaunasoortkenmerkend	Numeric	18	3	Ch...	None	None	12	Right	Scale
22 Soortenrijkdomn	Numeric	3	0	So...	None	None	12	Right	Scale
23 soortenrijkdomn	Numeric	3	0	Sp...	None	None	12	Right	Scale
24 soortenrijkdomtotaaln	Numeric	3	0	Sp...	None	None	12	Right	Scale
25 individuals_lg10	Numeric	8	2		None	None	18	Right	Scale
26 macevEQR_sqrt	Numeric	8	2		None	None	15	Right	Scale
27 macevEQR_lg10	Numeric	8	2		None	None	15	Right	Scale
28 CharDP_sqrt	Numeric	8	2		None	None	13	Right	Scale
29 CharDP_lg10	Numeric	8	2		None	None	13	Right	Scale
30 Char_sqrt	Numeric	8	2		None	None	11	Right	Scale
31 Char_lg10	Numeric	8	2		None	None	11	Right	Scale
32 DN_sqrt	Numeric	8	2		None	None	10	Right	Scale
33 DN_lg10	Numeric	8	2		None	None	10	Right	Scale

Figure 2: The SPSS document with the variables used, showing variable types (nominal, scale / continuous).

In the following text the assumptions are explained that are characteristic for the Independent-samples t-test.

Assumption #5: There should be **no significant outliers** in the two groups the independent variable in terms of the dependent variable. Outliers can have a large negative effect on the results because they can exert a large influence (i.e., change) on the mean and standard deviation for that group, which can affect the statistical test results. When using the boxplots as seen in the results (chapter 3) of the present study, there was seen that there are significant outliers. So, **this assumption is violated in this study**, so transformation square root (sqrt) and log10 are used In SPSS (see also figure 2 with for example log10 and square root transformations, such as Char_lg10 that is log 10 of the abundance of characteristic taxa). Even though the data was made normal again, the transformation still had outliers. So, in addition, there is checked if the results are different when removing outliers, and using the non-parametric alternative of the independent-samples t-test, which is the Mann-Whitney U test. Thus, both tests are used to see if there are significant differences.

Assumption #6: Your dependent variable should be approximately **normally distributed** for each group of the independent variable. **Shapiro-Wilk test was used to test the normality**. This is a numerical method for testing normality and is run using the **Explore...** procedure in SPSS Statistics. In table 1 the results of the normality test with Shapiro-Wilk (with p / Sig. > 0.05) test are shown of both groups (scheduled and realized) of M-types. In the table can be seen that the macroinvertebrate EQR, species richness (n) and number of dominant positive taxa (n) already has an normal distribution (marked with green). In the results was seen that all boxplots had a maximum of only one outlier. However, number of individuals (n) was transformed with lg10 (individuals_lg10) and Dominant Negative (%) was transformed into square root (DN_sqrt), in order to make a normal distribution. Though both the variables were used for the testing results in order to be more sure of the reliability.

Table 1: Normality test with SPSS for the M-types, with data in green that has **normal** distributions (p / Sign. > 0.05) for both groups.

	NVO status	Shapiro-Wilk			
		Statistic	Statistic	df	Sig.
Macroinvertebrate EQR	Scheduled	,092	,978	18	,928
	Realized	,136	,953	19	,436
Number of individuals	Scheduled	,270	,616	18	,000
	Realized	,151	,947	19	,345
individuals_lg10	Scheduled	,116	,972	18	,830
	Realized	,124	,941	19	,270
Dominant Negative (%)	Scheduled	,199	,770	18	,001
	Realized	,161	,906	19	,062
DN_sqrt	Scheduled	,173	,912	18	,095
	Realized	,137	,941	19	,278
	Realized	,107	,963	19	,624
Dominant Positive (n)	Scheduled	,130	,952	18	,464
	Realized	,112	,962	19	,603
Species richness (n)	Scheduled	,225	,905	18	,071
	Realized	,094	,975	19	,875

In table 2 the normality test of the R-types is seen. The data that has normal distributions in both groups (scheduled and realized) are shown. Dominant negative (%) and species richness (n) already has an normal distribution. The other continuous variables all needed to be transformed with log10 in order to have an normal distribution that is appropriate for the Independent-samples t-test.

Table 2: Normality test with SPSS of the R-types, with data in green that has **normal** distributions (p / Sign. > 0.05) for both groups.

		Tests of Normality					
		Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	NVO status	Statistic	df	Sig.	Statistic	df	Sig.
Macroinvertebrate EQR	Scheduled	,134	37	,090	,888	37	,001
	Realized	,162	12	,200*	,905	12	,183
macevEQR_lg10	Scheduled	,092	37	,200*	,962	37	,228
	Realized	,155	12	,200*	,921	12	,299
Number of individuals	Scheduled	,174	37	,006	,778	37	,000
	Realized	,322	12	,001	,620	12	,000
individuals_lg10	Scheduled	,109	37	,200*	,967	37	,326
	Realized	,208	12	,161	,884	12	,099
Characteristic + Dominant Positive (%)	Scheduled	,196	37	,001	,836	37	,000
	Realized	,184	12	,200*	,886	12	,106
CharDP_lg10	Scheduled	,059	37	,200*	,975	37	,549
	Realized	,160	12	,200*	,958	12	,757
Characteristic (%)	Scheduled	,292	37	,000	,666	37	,000
	Realized	,161	12	,200*	,924	12	,323
Char_lg10	Scheduled	,162	37	,016	,960	37	,204
	Realized	,118	12	,200*	,964	12	,836
Dominant negative (%)	Scheduled	,100	37	,200*	,969	37	,380
	Realized	,140	12	,200*	,964	12	,839
Species richness (n)	Scheduled	,118	37	,200*	,966	37	,311
	Realized	,176	12	,200*	,938	12	,470

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Transformations will generally only work when the distribution of scores in both groups are the same shape. There is seen in the data that all shapes are the same, which is also another reason why was chosen to also run the **Mann-Whitney U test** (see paragraph 1.1) to see the difference in results.

Assumption #7

You have **homogeneity of variances** (i.e., the variance is equal in each group of your independent variable). The assumption of homogeneity of variances states that the population variance for each group of your independent variable is the same. **In the present study this assumption is not violated**. This assumption was automatically tested when running main independent-samples t-test procedure. Below in table 3 is the descriptive statistics (mean, standard error, N = number of

monitoring locations, etcetera) of the M-types is seen, whereas the same is seen in table 4 for R-types. The mean of scheduled (s) and realized (r) was used with the difference between this means as seen in the results of this report (chapter 3).

Table 3: The descriptive statistics of M-types (output SPSS independent-samples t-test).

Group Statistics					
	NVO status	N	Mean	Std. Deviation	Std. Error Mean
Macroinvertebrate EQR	Scheduled	32	,41372	,171118	,030250
	Realized	23	,47478	,132514	,027631
Number of individuals	Scheduled	32	728,16625	1181,955163	208,942128
	Realized	23	934,10157	678,191906	141,412788
individuals_lg10	Scheduled	19	2,9304	,37574	,08620
	Realized	19	2,9896	,25793	,05917
Dominant negative (%)	Scheduled	32	16,10750	9,276567	1,639881
	Realized	23	13,26696	5,175972	1,079265
DN_sqrt	Scheduled	32	3,8767	1,05512	,18652
	Realized	23	3,5806	,68293	,14240
	Realized	23	1,0933	,16187	,03375
Dominant Positive (n)	Scheduled	31	32,16	14,116	2,535
	Realized	23	36,61	10,974	2,288
Species richness (n)	Scheduled	32	49,53	51,074	9,029
	Realized	23	78,78	42,853	8,935

Table 4: The descriptive statistics of R-types (output SPSS independent-samples t-test).

Group Statistics					
	NVO status	N	Mean	Std. Deviation	Std. Error Mean
Macroinvertebrate EQR	Scheduled	87	,30380	,119114	,012770
	Realized	22	,34718	,103992	,022171
macevEQR_lg10	Scheduled	87	-,5469	,15824	,01697
	Realized	22	-,4819	,15149	,03230
Number of individuals	Scheduled	87	505,57526	680,566711	72,964410
	Realized	22	1099,66523	1422,501810	303,278405
individuals_lg10	Scheduled	74	2,5577	,42712	,04965
	Realized	17	3,0069	,33697	,08173
Characteristic + Dominant Positive (%)	Scheduled	87	10,67782	12,957011	1,389137
	Realized	22	10,11000	8,294934	1,768486
CharDP_lg10	Scheduled	76	,8855	,41703	,04784
	Realized	19	,9675	,32835	,07533
Dominant negative (%)	Scheduled	87	41,44897	11,683544	1,252607
	Realized	22	32,59773	12,437457	2,651675
Characteristic (%)	Scheduled	87	3,55425	5,232261	,560957

	Realized	22	3,61045	3,715268	,792098
Char_lg10	Scheduled	49	,6741	,32442	,04635
	Realized	16	,5999	,29949	,07487
Species richness (n)	Scheduled	87	41,09	27,559	2,955
	Realized	22	58,50	40,433	8,620

Results of the M-types

In table 5 the results of the homogeneity of variances and Independent-samples t-test is seen. So, an independent-samples t-test was run to determine if there were differences in EQR, number of individuals, abundance of dominant negative taxa (%), number of dominant positive taxa (n) and species richness between scheduled and realized NVOs. In the table the important values are marked with bold text. The rows are marked with yellow that are the most important in the present study. When the p-value (Sig. 2-tailed) is below 0.05, it means that it is significantly different. The mean difference was used in the results (chapter 3) of the present study. Hence, the test shows that only the species richness has a significant difference. In order to be completely sure of the results of the statistical testing, the Mann-Whitney U test was also run, which is shown in table 6. In this table the important value is marked with yellow, that shows that according to a non-parametric test, the species richness is not significantly different. However, the species richness meets all the assumptions for the Independent-samples t-test and desirable to use as is mentioned before for several reason.

Table 5: The results of the Levene's Test for equality of variances and the independent samples t-test in SPSS for M-types.

	Independent Samples Test								
	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper	
Macroinvertebrate EQR	1,387	,244	-1,430	53	,159	-,061064	,042715	-,146739	,024611
Number of individuals	,837	,364	-,750	53	,456	-205,935315	274,461974	-756,436320	344,565689
individuals_lg10	1,874	,179	-,566	36	,575	-,05920	,10456	-,27125	,15285
Dominant negative (%)	2,246	,140	1,326	53	,191	2,840543	2,142987	-1,457743	7,138830
DN_sqrt	1,747	,192	1,179	53	,244	,29611	,25125	-,20784	,80005
Dominant Positive (n)	3,214	,079	1,255	52	,215	-4,447	3,545	-11,561	2,666
Species richness (n)	3,705	,060	2,237	53	,030	-29,251	13,076	-55,478	-3,025

Table 6: The results of the Mann-Whitney U test in SPSS for M-types.

	Macroinvertebrate EQR	Number of individuals	individuals_lg10	Dominant negative (%)	DN_sqrt	Dominant Positive (n)	Species richness (n)
Mann-Whitney U	280,500	261,000	159,000	299,000	299,000	289,000	256,000
Wilcoxon W	808,500	789,000	349,000	575,000	575,000	785,000	784,000
Z	-1,493	-1,853	-,628	-1,177	-1,177	-1,182	-1,940
Asymp. Sig. (2-tailed)	,135	,064	,530	,239	,239	,237	,052
Exact Sig. [2*(1-tailed Sig.)]			,544 ^b				

Results of the R-types

In table 7 the results of the homogeneity of variances and independent-samples t-test is seen. The rows are marked with yellow that are the most important in the present study. In the table the two yellow values ($p < 0.05$) show that species richness (n) and number of individuals (n) do not have equality of variances, which means that these variables violate the assumption for the t-test. However, the 'Sign' (p-value) can be used in the row with 'equal variances not assumed'. This is why is marked with green which 'Sign' values are used. The significant values ($p < 0.05$) are marked with bold text. In table 8 the results of the Mann-Whitney U test are shown.

Table 7: The results of the Levene's Test for equality of variances and the independent samples t-test in SPSS for R-types.

		Independent Samples Test								
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2- tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Macroinvertebrate EQR	Equal variances assumed	,810	,370	-	107	,121	-,043377	,027754	-,098396	,011642

	Equal variances not assumed			- 1,695	36,271	,099	-,043377	,025586	-,095254	,008500
macevEQR_lg10	Equal variances assumed	,981	,324	- 1,737	107	,085	-,06504	,03745	-,13928	,00921
	Equal variances not assumed			- 1,783	33,562	,084	-,06504	,03648	-,13921	,00914
Number of individuals	Equal variances assumed	9,556	,003	- 2,838	107	,005	- 594,089963	209,324512	-1009,051393	-179,128532
	Equal variances not assumed			- 1,905	23,482	,043	- 594,089963	311,932038	-1238,638178	50,458252
individuals_lg10	Equal variances assumed	2,287	,134	- 4,050	89	,000	-,44921	,11091	-,66959	-,22884
	Equal variances not assumed			- 4,698	29,121	,000	-,44921	,09563	-,64476	-,25367
Characteristic + Dominant Positive (%)	Equal variances assumed	1,505	,223	,195	107	,846	,567816	2,907476	-5,195916	6,331548
	Equal variances not assumed			,252	50,239	,802	,567816	2,248832	-3,948564	5,084197
CharDP_lg10	Equal variances assumed	1,846	,177	-,796	93	,428	-,08198	,10296	-,28643	,12247
	Equal variances not assumed			-,919	34,114	,365	-,08198	,08923	-,26330	,09934
Dominant negative (%)	Equal variances assumed	,360	,550	3,134	107	,002	8,851238	2,824369	3,252256	14,450220

	Equal variances not assumed			3,018	31,040	,005	8,851238	2,932645	2,870386	14,832091
Characteristic (%)	Equal variances assumed	,748	,389	-,047	107	,962	-,056202	1,186320	-2,407942	2,295539
	Equal variances not assumed			-,058	44,607	,954	-,056202	,970614	-2,011595	1,899191
Char_lg10	Equal variances assumed	,000	,983	,809	63	,421	,07427	,09175	-,10909	,25763
	Equal variances not assumed			,843	27,438	,406	,07427	,08806	-,10627	,25481
Species richness (n)	Equal variances assumed	5,632	,019	-2,390	107	,019	-17,408	7,283	-31,845	-2,971
	Equal variances not assumed			-1,910	26,136	,067	-17,408	9,113	-36,135	1,319

Table 8: Mann-Whitney U test for R-types

	Test Statistics ^a									
	Macroinvertebrate EQR	macevEQR_lg10	Number of individuals	individuals_lg10	Characteristic + Dominant Positive (%)	CharDP_lg10	Dominant negative (%)	Characteristic (%)	Char_lg10	Species richness (n)
Mann-Whitney U	678,500	678,500	665,500	263,000	847,500	603,000	528,000	853,000	339,000	675,500
Wilcoxon W	4506,500	4506,500	4493,500	3038,000	4675,500	3529,000	781,000	4681,000	475,000	4503,500
Z	-2,103	-2,103	-2,206	-3,727	-,828	-1,107	-3,239	-,812	-,807	-2,130

Asym p. Sig. (2- tailed)	,036	,036	,027	,000	,408	,268	,001	,417	,419	,033
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a. Grouping Variable: NVO status

When looking at both the Independent-samples T test (table 7) and the Mann-Whitney (table 8) test a few decisions were made, see table 9. Both an old and new procedure of SPSS is seen in this table. There was chosen to follow the results of the independent-samples t-test because this test is based on mean differences. Mann-Whitney U only shows the significance of distributions and mean ranks, which is less appropriate to use for the present study.

Table 9: The differences of the statistical results between the Independent-samples t-test and the Mann-Whitney U test (old and new procedure of SPSS).

Variable	M-types			R-types		
	Significance according to Independent-samples t-test	Significance according to Mann-Whitney U test	Significance according to Mann-Whitney U test (NEW PROCURE)	Significance according to Independent-samples t-test	Significance according to Mann-Whitney U test	Significance according to Mann-Whitney U test (NEW PROCURE)
Macroinvertebrate EQR	,159	NS	NS	-,043377	S	S
Number of individuals (n)	,456	NS	NS	-594,089963	S	S
Species richness (n)	-29,251	S	NS	-17,408	S	NS
Negative Dominant (%)	,191	NS	NS	8,851238	S	S
Positive Dominant (n)	-4,447	NS	NS	NA	NA	NA
Characteristic (%)	NA	NS	NS	-,056202	NS	NS
Characteristic + positive dominant (%)	NA	NA	NA	,567816	NS	NS

II.2 Total flora and macrophytes: Realized NVOs vs scheduled NVOS

Assumption #1: **one dependent variable** that is measured at the **continuous** level = total flora EQR, and **sub-metrics**: species composition (EQR) and Abundance growth forms (EQR).

Assumption #2: **one independent variable** that consists of **two categorical, independent groups** (i.e., a **dichotomous variable**) = NVO status (scheduled = 0, realized = 1)

Assumption #3: **independence of observations**, this is met, because all monitoring locations all have data from one (the most recent) date, which means that there is no relationship between the observations.

Assumption #4: There should be **no significant outliers** in the two groups the independent variable in terms of the dependent variable. Outliers can have a large negative effect on the results because they can exert a large influence (i.e., change) on the mean and standard deviation for that group, which can affect the statistical test results. When using the boxplots as seen in the results (chapter 3)

of the present study, there was seen that there are significant outliers. So, **this assumption is violated in this study**, so transformation square root (sqrt) was used for the Species composition EQR (SpeciesC_sqrt). In addition, there is checked if the results are different when removing the outliers, and using the non-parametric alternative of the independent-samples t-test, which is the Mann-Whitney U test. Thus, both tests are used to see if there are significant differences.

Assumption #6: Your dependent variable should be approximately **normally distributed** for each group of the independent variable. **Shapiro-Wilk test was used to test the normality**. This is a numerical method for testing normality and is run using the **Explore...** procedure in SPSS Statistics. In table 10 the results of the normality test with Shapiro-Wilk (with p / Sig. > 0.05) test are shown of both groups (scheduled and realized) of M-types. In the table can be seen that the Total Flora EQR, Abundance growth forms EQR and Species composition EQR already has a normal distribution (marked with green).

Table 10: Normality test of the M-types

	NVO status	Shapiro-Wilk		
		Statistic	df	Sig.
Flora EQR	Scheduled	,929	17	,209
	Realized	,954	16	,558
Abundance growth forms EQR	Scheduled	,983	17	,977
	Realized	,919	16	,163
Species composition EQR	Scheduled	,925	17	,179
	Realized	,942	16	,370

In table 11 the normality test of the R-types is seen. The Species composition needed to be transformed with square root (SpeciesC_sqrt) in order to have a normal distribution that is appropriate for the Independent-samples t-test.

Table 11: Normality test of the R-types

	NVO status	Shapiro-Wilk		
		Statistic	df	Sig.
Flora EQR	Scheduled	,915	17	,120
	Realized	,944	10	,598
Abundance growth forms EQR	Scheduled	,963	17	,691
	Realized	,981	10	,970
Species composition EQR	Scheduled	,926	17	,187
	Realized	,829	10	,032
SpeciesC_sqrt	Scheduled	,915	17	,122
	Realized	,877	10	,122

Assumption #7

You have **homogeneity of variances** (i.e., the variance is equal in each group of your independent variable). The assumption of homogeneity of variances states that the population variance for each

group of your independent variable is the same. **In the present study this assumption is not violated.** This assumption was automatically tested when running main independent-samples t-test procedure. Below in table 12 is the descriptive statistics (mean, standard error, N = number of monitoring locations, etcetera) of the M-types is seen, whereas the same is seen in table 13 for R-types. The mean of scheduled (s) and realized (r) was used with the difference between this means as seen in the results of this report (chapter 3).

Table 12: Descriptive statistics of the M-types

Group Statistics					
	NVO status	N	Mean	Std. Deviation	Std. Error Mean
Flora EQR	Scheduled	17	,39518	,150163	,036420
	Realized	16	,44394	,176718	,044179
Abundance growth forms EQR	Scheduled	17	,34294	,151312	,036699
	Realized	16	,30538	,203488	,050872
Species composition EQR	Scheduled	17	,44718	,218310	,052948
	Realized	16	,56100	,224144	,056036

Table 13: Descriptive statistics of the R-types

Group Statistics ^a					
	NVO status	N	Mean	Std. Deviation	Std. Error Mean
Flora EQR	Scheduled	17	,49935	,096166	,023324
	Realized	10	,47270	,058504	,018500
Abundance growth forms EQR	Scheduled	17	,45135	,231889	,056241
	Realized	10	,57580	,139684	,044172
Species composition EQR	Scheduled	17	,28965	,231499	,056147
	Realized	10	,19570	,235284	,074403

Results of the M-types

In table 12 the results of the homogeneity of variances and Independent-samples t-test is seen. So, an independent-samples t-test was run to determine if there were differences in EQR, Abundance of growth forms EQR and Species composition EQR between scheduled and realized NVOs. In the table the important values are marked with bold text. The rows are marked with yellow that are the most important in the present study. When the p-value (Sig. 2-tailed) is below 0.05, it means that it is significantly different. The mean difference was used in the results (chapter 3) of the present study. Hence, the test shows that only the species richness has a significant difference. In order to be completely sure of the results of the statistical testing, the Mann-Whitney U test was also run, which is shown in table 13. In this table the important value is marked with yellow, that shows that according to a non-parametric test, the species richness is not significantly different. However, the species richness meets all the assumptions for the Independent-samples t-test and desirable to use as is mentioned before for several reason.

Table 12: Independent-samples T-test results of the M-types

		Independent Samples Test ^a								
		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Flora EQR	Equal variances assumed	,113	,739	-,856	31	,399	-,048761	,056967	-,164947	,067425
	Equal variances not assumed			-,852	29,530	,401	-,048761	,057256	-,165771	,068249
Abundance growth forms EQR	Equal variances assumed	,505	,482	,604	31	,550	,037566	,062165	-,089220	,164353
	Equal variances not assumed			,599	27,653	,554	,037566	,062727	-,090998	,166130
Species composition EQR	Equal variances assumed	,060	,808	-1,478	31	,150	-,113824	,077031	-,270929	,043282
	Equal variances not assumed			-1,476	30,757	,150	-,113824	,077094	-,271109	,043462

a. Water type = M

Table 13: Mann-Whitney U test for M-types

	Test Statistics ^{a,b}			
	Flora EQR	Abundance growth forms EQR	Species composition EQR	SpeciesC_sqrt
Mann-Whitney U	120,000	111,500	91,500	91,500
Wilcoxon W	273,000	247,500	244,500	244,500
Z	-,576	-,883	-1,603	-1,603
Asymp. Sig. (2-tailed)	,564	,377	,109	,109
Exact Sig. [2*(1-tailed Sig.)]	,581 ^c	,382 ^c	,110 ^c	,110 ^c

a. Water type = M

b. Grouping Variable: NVO status

c. Not corrected for ties.

Results of the R-types

In table 14 the results of the homogeneity of variances and independent-samples t-test is seen. The rows are marked with yellow that are the most important in the present study. There is seen that none of the sub-metrics show a significant difference between scheduled and realized NVOs. In table 15 the results of the Mann-Whitney U test are shown, where is shown that only the Abundance Growth forms shown significant differences. However, the Independent T-test is chosen as the most reliable result because of the same reasons as mentioned for the macroinvertebrates earlier.

Table 14: Independent-samples T-test results of the R-types

		Independent Samples Test ^a								
		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Flora EQR	Equal variances assumed	3,482	,074	,791	25	,436	,026653	,033700	-,042754	,096060
	Equal variances not assumed			,895	24,926	,379	,026653	,029770	-,034669	,087975
Abundance growth forms EQR	Equal variances assumed	2,560	,122	-1,534	25	,138	-,124447	,081126	-,291529	,042635
	Equal variances not assumed			-1,740	24,950	,094	-,124447	,071514	-,271748	,022853
Species composition EQR	Equal variances assumed	,059	,810	1,012	25	,321	,093947	,092804	-,097187	,285081

Equal variances not assumed			1,008	18,749	,326	,093947	,093211	-,101323	,289217
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a. Water type = R

Table 15: Mann-Whitney U test for R-types

	Test Statistics ^{a,b}			
	Flora EQR	Abundance growth forms EQR	Species composition EQR	SpeciesC_sqrt
Mann-Whitney U	73,000	45,500	66,500	66,500
Wilcoxon W	128,000	198,500	121,500	121,500
Z	-,603	-1,988	-,932	-,932
Asymp. Sig. (2-tailed)	,547	,047	,351	,351
Exact Sig. [2*(1-tailed Sig.)]	,570 ^c	,046 ^c	,359 ^c	,359 ^c

a. Water type = R

b. Grouping Variable: NVO status

c. Not corrected for ties.

II.3 Effect of NVO's on macroinvertebrates over time in the management area

In this part of the statistical analysis the influence of time and before-after NVO realization on the macroinvertebrate EQR is described. For analysing this was chosen to use Pearson Correlation Coefficients for the effect of time in years. Some of the KRW water types have only a small amount of data or a sample size that is too small, which why it was not possible to run statistical texts. In the study of Bonett, Douglas & Wright (2000) is described how big the samples sizes must be in order to give reliable results. For the effect of before and after NVO realization was chosen for the Point-biserial correlation, which is a specific type of Pearson Correlation test.

Below the assumptions and results of meeting those assumptions is shown. Later on the results of the Pearson Correlation test is shown and discussed.

Assumption #1: two variables measured on a **continuous** scale, this are the time in years and the macroinvertebrate EQR. For the bi-serial Pearson Correlation this is = continuous variable is macroinvertebrate EQR and a dichotomous (nominal) variable = before / after NVO realization.

Assumption #2: Two continuous variables should be **paired**, which means that each case has two values: one for each variable. However, in this study the monitoring locations were not paired over the years, so it was chosen to put the data together as the M-types and R-types. In addition, the data of separate R-types (R4a, R20, R5) and M-types (M1, M2, M6) was checked. Thus, the water types were considered as the "paired observations".

Below the assumptions of the Pearson Correlation is explained together with the findings and characteristics of the macroinvertebrate data. There is explained if those assumptions are violated regarding the macroinvertebrate EQR.

Assumption #3: There needs to be a **linear relationship** between the two variables. In the results (chapter 3) was shown that both the M-types ($R^2 = 0.011$) and R-types ($R^2 = 0.031$) in overall have a linear relationship. In Annex II the graphs of the separate water types (R4a, M1a etc.) are shown that have a linear relationship or not. When the line through the data is straight, there is no linear relationship.

Assumption #4: There should be **no significant outliers**. The present study the data does have significant outliers, however this is ignored for the statistical results, because when the data is normal it will not have big impact on the results. Transformation with sqrt and log10 was tried, but this did not help much to mitigate the outliers.

Assumption #5: bivariate normality. This is tested by doing normality test as is shown below in table 16. In the table is shown with bold text when the data is not normal according to the p-value (Sig.). In addition, the data of water types that have a too small sample size for the statistical testing is shown in red (Bonett, Douglas & Wright, 2000). This data is not usable for the results and is marked with "NA" (Not Applicable) in chapter 3. In the table is also seen that most of the data is normal, except for EQR of KRW water type R20. Transformation done to make this data have a normal distributions. Even though the linear relationship it this EQR was small, it was still tested with Pearson and Spearman in order to see if there were differences.

Table 16: The statistical results of the normality test of all water types, also showing bivariate normality (shown as before and after).

		Tests of Normality ^a			
Water type		Before or after NVO realization	Shapiro-Wilk		
			Statistic	df	Sig.
M	Macroinvertebrate EQR	Before	,959	8	,797
M		After	,993	123	,815
M	Macroinvertebrate EQR		,992	142	,644
R	Macroinvertebrate EQR		,984	93	,302
R	Macroinvertebrate EQR	Before	,949	16	,478
R		After	,980	38	,732
R	Macroinvertebrate EQR		,984	93	,302
M1a	Macroinvertebrate EQR	Before	,937	12	,463
M1a		After	,973	53	,262
M1a	Macroinvertebrate EQR		,973	65	,161
M3	Macroinvertebrate EQR	Before	,778	3	,062
M3		After	,992	58	,959
M3	Macroinvertebrate EQR		,992	61	,969
M6a	Macroinvertebrate EQR	Before	,807	4	,116
M6a		After	,962	12	,806
M6a	Macroinvertebrate EQR		,954	16	,559
R20	Macroinvertebrate EQR	Before	,929	21	,131
R20		After	,922	19	,123
R20	Macroinvertebrate EQR		,930	40	,017
R20	Macroinvertebrate EQR (log10)		,946	40	,056

R4a	Macroinvertebrate EQR	Before	,984	32	,902
R4a		After	,971	19	,801
R4a	Macroinvertebrate EQR		,988	51	,876

*. This is a lower bound of the true significance.

a. Main type = M

b. Lilliefors Significance Correction

In the following tables the Pearson Correlation tests are seen for M-types, R-types and the separate KRW water types. The significant values are marked with bold text. The transformation of log10 is also seen that is compared with the normal EQR. The results show that there was no relationship between before-after NVO realization, but the M-types and specifically the R4a KRW water type did have an relationship with time (in years). By using the coefficient of determination (R^2) there could be determined how much of the monitoring locations time has a positive correlation with EQR. For M-types, this means that 2.9% of the M-types is statistically explained by time. For the R4a types, this means that only 7.7% is statistically explained by time.

Correlations: M-types

		Before/After	Time (in years)
Macroinvertebrate EQR	Pearson Correlation	,145	,169
	R^2		,029
	Sig. (2-tailed)	,086	,045
	Sum of Squares and Cross-products	,912	16,397
	Covariance	,006	,116
	N	142	142
macevEQR_lg10	Pearson Correlation	,121	,136
	Sig. (2-tailed)	,151	,107
	Sum of Squares and Cross-products	,889	15,353
	Covariance	,006	,109
	N	142	142

Correlations M1a

		Before/After	Time (in years)
Macroinvertebrate EQR	Pearson Correlation	,175	,235
	Sig. (2-tailed)	,164	,060
	Sum of Squares and Cross-products	,523	11,318
	Covariance	,008	,177
	N	65	65
macevEQR_lg10	Pearson Correlation	,145	,215
	Sig. (2-tailed)	,249	,086
	Sum of Squares and Cross-products	,562	13,403
	Covariance	,009	,209
	N	65	65

Correlations M3

		Time (in years)
Macroinvertebrate EQR	Pearson Correlation	,215
	Sig. (2-tailed)	,096
	Sum of Squares and Cross-products	7,501
	Covariance	,125
	N	61
macevEQR_Ig10	Pearson Correlation	,114
	Sig. (2-tailed)	,382
	Sum of Squares and Cross-products	4,306
	Covariance	,072
	N	61

Correlations M6a

		Time (in years)
Macroinvertebrate EQR	Pearson Correlation	-,169
	Sig. (2-tailed)	,531
	Sum of Squares and Cross-products	-1,105
	Covariance	-,074
	N	16
macevEQR_Ig10	Pearson Correlation	-,120
	Sig. (2-tailed)	,658
	Sum of Squares and Cross-products	-,820
	Covariance	-,055
	N	16

Correlations: R-types

		Before/After	Time (in years)
Macroinvertebrate EQR	Pearson Correlation	,080	,181
	Sig. (2-tailed)	,446	,082
	Sum of Squares and Cross-products	,404	14,924
	Covariance	,004	,162
	N	93	93
macevEQR_Ig10	Pearson Correlation	,115	,176
	Sig. (2-tailed)	,274	,092
	Sum of Squares and Cross-products	,801	20,049
	Covariance	,009	,218
	N	93	93

Correlations R20

		Time (in years)
Macroinvertebrate EQR	Pearson Correlation	-,006
	Sig. (2-tailed)	,969
	Sum of Squares and Cross-products	-,178
	Covariance	-,005
	N	40
macevEQR_Ig10	Pearson Correlation	-,028
	Sig. (2-tailed)	,866
	Sum of Squares and Cross-products	-,770
	Covariance	-,020
	N	40

Correlations R4a

		Before/After	Time (in years)
Macroinvertebrate EQR	Pearson Correlation	,157	,277
	R ²		0.077
	Sig. (2-tailed)	,273	,049
	Sum of Squares and Cross-products	,371	9,463
	Covariance	,007	,189
	N	51	51
macevEQR_Ig10	Pearson Correlation	,181	,249
	Sig. (2-tailed)	,204	,078
	Sum of Squares and Cross-products	,688	13,660
	Covariance	,014	,273
	N	51	51

Annex III: Macroinvertebrate EQR before and after realization

