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# **Development of the freshwater fish assemblage in the Mark watershed, the Netherlands**

Final thesis report



Name: Maarten Fritz

Phone: +316 53568702

Student number: a51308

Supervisor at Waterboard Brabantse Delta: Marco Beers

Supervisor at Royal Dutch Angling Organisation: Willie van Emmerik

Supervisor at University of Algarve: José Pedro Andrade

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## Abstract

Effectiveness of recently applied management strategies on developments of the freshwater fish assemblages in the Mark watershed has been unclear. Therefore, the objective of this study was to identify developments within the freshwater fish assemblages of the Mark watershed. Furthermore, the identified developments in fish assemblages were analyzed for correlations with physical-chemical parameters and implementation of fish passages and habitat restoration in streams as explanatory variables. Fish assemblage data of 498 surveys have been collected between 1994 and 2014, and were analyzed to detect developments in diversity indices over time. Furthermore, 30 routine sampling stations for annual mean of physical-chemical variables, 8 fish passages and 5 remaining projects were used to explain the differences in diversity indices. Finally, dominant proportions of guilds and fish communities in small streams of the Mark watershed were categorized and studied for correlations to the physical-chemical variables.

Altogether 25 native and 6 exotic fish species were detected in the Mark watershed. Species number and diversity varied significantly between watercourses and year of sampling. The Bavelse Leij, Bijloop-Turfvaart, Chaamse Beken, Kleine Beek, Molenbeek and Merkske showed no significant differences in diversity indices over the period of 1994 till 2014. Small streams of the Galderse Beek and Strijbeekse Beek were characterized by a significant decreasing linear trend in average diversity. Diversity significantly decreased from 1.34 to 0.93 between 1994 and 2005 for the Galderse Beek and 1.56 to 0.82 between 1994 and 2006 for Strijbeekse Beek. Diversity at the Galderse Beek significantly decreased due to an exponential increase in the numbers of nine spined stickleback and stone loach, accounting for 83.5% of the total number of individuals. Decreased diversity for the Strijbeekse Beek was caused by an increase in the number of gudgeon, accounting for 77.1% of the total number of individuals. After 2006, average diversity decreased gradually further, however no significant differences were obtained anymore. Mark en Vliet was characterized by a significant linear decrease in average species richness and increase in average Pielou's evenness over the studied period. Diversity at Mark en Vliet was characterized by a significant depression of 1.15 to 0.81 between 2005 and 2008. After 2008, diversity recovered to original values of approximately 1.20. Depression at Mark en Vliet could not be explained and the reason remain unclear. The Aa of Weerijns showed a significant gradual increasing linear trend in average diversity indices. The average species richness gradually increases over the period of 1994 to 2013. Together with an significant improvement of Pielou's evenness in 2013, diversity significantly increases from 1.32 to 1.67 for the Aa of Weerijns between 2010 and 2013. The Boven Mark showed a fluctuating character in average diversity indices between 1994 and 2013, and a significant linear increase in average species richness was observed. Average species richness increased from respectively 6.06 species in 1994 to 8.08 species in 2013. However, diversity showed no significant differences due to variation in the distribution among species.

Redundancy analysis and Pearson correlation analysis for the Aa of Weerijis and Boven Mark revealed a positive correlation between the construction of fish passages and diversity. However, it is assumed that the overall environmental conditions improved, allowing a greater diversity to exist, and increased connectivity by fish passages functions as a promoter. Habitat restoration projects showed no significant connection to the diversity indices. Furthermore, the physical-chemical variables conductivity, total phosphorus, total nitrogen, chloride and saturated oxygen concentration showed significant correlations to the diversity indices, where conductivity, total phosphorus, total nitrogen and chloride have a negative correlation and saturated oxygen concentration a positive correlation.

Non-metric dimensional scaling for the Mark watershed revealed differences between the fish communities collected at different watercourses and by used fishing gear. Focus on the small streams of the Mark watershed, showed the distribution of dominant fish communities in the samples. The small streams are relatively well represented by the occurrence of characteristic stream fish species such as, stone loach, gudgeon, three-spined stickleback and nine-spined stickleback. However, undesirable fish communities of perch & roach and pike & rudd exist in the small streams, and indicate the points for future improvements. Pearson correlation analysis of the fish communities showed that the physical- chemical variables temperature, total nitrogen, total phosphorus, saturated oxygen concentration and conductivity have a significant correlation. The number of perch in the small streams negatively correlates to total nitrogen, total phosphorus, and saturated oxygen concentration, while the number of gudgeon positively correlates. Furthermore, the numbers of three-spined stickleback and stone loach respectively correlate positively and negatively to water temperature of the small streams. Finally, conductivity at the small streams showed a positive correlation to the numbers of nine-spined stickleback.

## Resumo

A eficácia de estratégias recentemente recentemente para o desenvolvimento de comunidades de peixes de água doce na bacia hidrográfica de Mark não tem sido clara. Assim, o objetivo deste trabalho foi identificar desenvolvimentos dentro das comunidades de peixes de água doce da bacia hidrográfica de Mark. Ademais, os desenvolvimentos identificados nestas comunidades foram analisados em correlações com parâmetros físico-químicos e implementação de passagens para peixes e restauração de habitat em cursos de água como variáveis explicativas. Dados de comunidades de peixes de 498 investigações foram recolhidos entre 1994 e 2014, e foram analisados para detectar desenvolvimentos em índices de diversidade ao longo do tempo. Além disso, foram usadas 30 estações de amostragens de rotina para médias anuais de variáveis físico-químicas, 8 passagens para peixes e 5 projectos residuais para explicar diferenças nos índices de diversidade. Por fim, as associações e comunidades dominantes de peixes nos pequenos cursos de água da bacia hidrográfica de Mark foram categorizadas pelo número de peixes e estudadas as correlações para as variáveis físico-químicas.

Ao todo, 25 espécies nativas e 6 não-nativas de peixe foram detectadas na bacia hidrográfica de Mark. O número de espécies e a diversidade variaram significativamente entre cursos de água e ano de amostragem. Os cursos de água de Bavelse Leij, BIjloop-Turfvaart, Chaamse Beken, Kleine Beek, Molenbeek e Merkske não mostraram diferenças significativas nos índices de diversidade no período de tempo entre 1994 e 2014. Pequenos ribeiros de Galderse Beek e Strijbeekse Beek caracterizaram-se por uma redução linear significativa na diversidade média. A diversidade baixou significativamente de 1,34 para 0,93 entre 1994 e 2005 em Galdarese Beek e de 1,56 para 0,82 entre 1994 e 2006 em Strijbeekse Beek. A diversidade em Galderse Beek reduziu significativamente devido ao aumento exponencial em número de esgana-gatas e verdemãs-da-pedra, que corresponderam a 83,5% do número total de indivíduos. A queda da diversidade em Strijbeekse Beek foi causada por um aumento em número de góbios, contando com 77,1% do número total de indivíduos. Depois de 2006, a diversidade média continuou a diminuir gradualmente, contudo, não se obtiveram diferenças significativas. Mark en Vliet foi caracterizado por uma redução linear significativa em riqueza específica média e um aumento na equitabilidade média de Pielou durante o período estudado. A diversidade em Mark en Vliet foi definida por uma significativa depressão de 1,15 para 0,81 entre 2005 e 2008. De 2008 em diante, a diversidade foi restaurada para os valores originais de 1,20 aproximadamente. A depressão em Mark en Vliet não pôde ser explicada e as suas causas permanecem indefinidas. Em Aa de Weerij, houve uma tendência linear gradual crescente significativa nos índices médios de diversidade. A riqueza específica média cresceu gradualmente de 1994 a 2013. Em conjunto com uma melhoria na equitabilidade de Pielou em 2013, a diversidade aumentou significativamente de 1,32 para 1,67 em Aa de Weerij entre 2010 e 2013. Boven Mark demonstrou flutuações em índices médios de diversidade entre 1994 e 2013, e um crescimento linear significativo em riqueza específica média foi observado. A riqueza específica média aumentou de 6,06

para 8,08 espécies, de 1994 a 2013 respectivamente. No entanto, a diversidade não demonstrou diferenças significativas devido à variação na distribuição entre espécies.

A análise de redundância e a correlação de Pearson para Aa de Weerij e Boven Mark revelaram uma correlação positiva entre a construção das passagens para peixes e diversidade. Contudo, assumiu-se que as condições ambientais em geral melhoraram, permitindo a existência de uma maior diversidade, e o aumento da conectividade pelas passagens funcionou como um promotor. Projectos de restauração do habitat não mostraram uma ligação significativa com os índices de diversidade. Mais, as variáveis químicas de condutividade, fósforo total, azoto total, concentração de cloro e oxigénio saturado demonstraram correlações significativas para com os índices de diversidade, onde a condutividade, fósforo total, azoto total, e cloro tiveram uma correlação negativa, e a concentração de oxigénio saturado uma correlação positiva.

A escalagem dimensional não-métrica para a bacia hidrográfica de Mark revelou diferenças entre as comunidades de peixes amostrados em diferentes cursos de água e com diferentes materiais de pesca. A focagem nos pequenos cursos de água da bacia de Mark mostrou a distribuição das comunidades de peixes dominantes nas amostras. Estes pequenos ribeiros são relativamente bem representados pela ocorrência de espécies de peixes características, tais como verdemãs-da-pedra, góbios, e esgana-gatas. Contudo, comunidades indesejáveis de percas e pardelhas, e lúcius e escardínios existem nestes pequenos cursos de água, e indicam os pontos para melhoramentos futuros. A correlação de Pearson nas comunidades piscícolas demonstrou que as variáveis químicas, temperatura, azoto total, fósforo total, concentração de oxigénio saturado e condutividade se correlacionam significativamente. O número de percas nos pequenos cursos de água correlaciona-se negativamente com o azoto total, fósforo total e concentração de oxigénio saturado, enquanto que o número de góbios correlaciona-se positivamente. Mais, os números de esgana-gatas e verdemãs-da-pedra correlacionam-se positiva e negativamente, respectivamente, com a temperatura da água nos pequenos rios. Por fim, a condutividade nos pequenos cursos de água demonstrou uma correlação positiva com o número de esgana-gatas.

## Samenvatting

De effectiviteit van het huidige toegepaste beleid op de ontwikkelingen in de visstand van het Mark stroomgebied is nog steeds niet geheel duidelijk. De doelstelling van het onderzoek was om de meest belangrijke ontwikkelingen in de visstand van het Mark stroomgebied te analyseren. De waargenomen verschillen zijn getest middels een correlatie analyse, voor het aantonen van verbanden met fysisch-chemische variabelen, de aanleg van vispassages en herinrichting van de waterlopen in het gebied. In totaal zijn er 498 bemonsteringen van de visstand uitgevoerd in het Mark Stroomgebied tussen 1994 en 2014. Gegevens van de visstand bemonsteringen zijn gebruikt voor het waarnemen van ontwikkelingen in diversiteit indexen. De verklarende variabelen voor de analyses bestonden uit 30 routinematige, fysisch-chemische meetpunten voor het berekenen van jaargemiddelden, acht vispassages en vijf beekherstel projecten. Tot slot is er dieper ingegaan op de visstand van de kleine beken van het Markstroomgebied. Dominante gilden en visgemeenschappen zijn aangeduid en geanalyseerd voor verbanden met jaargemiddelden van de fysisch-chemische variabelen.

In totaal zijn er 25 inheemse en 6 exotische vissoorten aangetroffen in het Markstroomgebied tussen 1994 en 2014. Het aantal aangetroffen vissoorten en de diversiteit verschilde significant tussen waterlichamen onderling en de jaren van bemonstering. De Bavelse Leij, Bijloop-Turfvaart, Chaamse Beken, Kleine Beek, Molenbeek en het Merkske vertoonden geen significante verschillen in diversiteit tussen 1994 en 2014. De Galderse Beek en Strijbeekse Beek vertoonden een significante daling in gemiddelde diversiteit tussen 1994 en 2015. De gemiddelde diversiteit van de Galderse beek daalde significant van 1.34 tot 0.93 tussen 1994 en 2005 door een sterke toename in het aantal tiendoornige stekelbaars en biermje, dat 83.5% bedroeg van het totaal aantal gevangen vissen in 2005. De gemiddelde diversiteit van de Strijbeekse Beek daalde significant van 1.56 tot 0.82 tussen 1994 en 2006 door een toename in het aantal riviergrondels dat 77.1% bedroeg van het totaal aantal gevangen vissen in 2006. Na 2006 daalde de gemiddelde diversiteit geleidelijk verder, maar er werden geen significante verschillen meer waargenomen. Het Mark en Vliet vertoonden een significante lineaire daling in gemiddelde soortenrijkdom en significante lineaire stijging in gemiddelde Pielou's evenness tussen 1994 en 2014. De diversiteit van het Mark en Vliet toonde een significante depressie van 1.15 tot 0.81 tussen 2005 en 2008. De depressie op het Mark en Vliet in 2008 kon niet verklaard worden tijdens deze studie, maar in 2011 was de gemiddelde diversiteit weer hersteld tot het originele niveau. De Aa of Weerijs wordt gekenmerkt door een geleidelijke toenemende trend in de diversiteit indexen tussen 1994 en 2013. De gemiddelde diversiteit steeg significant tussen 2010 en 2013. De significante stijging in diversiteit werd veroorzaakt door een geleidelijke stijging in gemiddelde soortenrijkdom en significante toename in Pielou's evenness in 2013. De diversiteit indexen van de Boven Mark zijn gekenmerkt door een fluctuerend karakter. De gemiddelde soortenrijkdom vertoont een significante lineaire toename van 6.06 soorten in 1994 tot 8.08 soorten in 2013. De gemiddelde diversiteit index vertoont echter geen significante verschillen door tegengestelde veranderingen in Pielou's evenness.

De redundantieanalyse en Pearson correlatie analyse voor de Aa of Weerijns en Boven Mark toonde een positieve correlatie tussen de aangelegde vispassages en diversiteit. Er wordt echter aangenomen dat een toenemende connectiviteit door de aanleg van vispassages een bijdrage heeft geleverd aan een toenemende diversiteit, maar dat de diversiteit ook is toegenomen door een verbetering in de algemene milieuomstandigheden. Er kon geen significant verband aangetoond worden tussen beekherstelprojecten en de diversiteit indexen. Ook de fysisch-chemische variabelen geleidbaarheid, totaal fosfor, totaal stikstof, chloride en verzadigd zuurstofgehalte vertoonden significante correlaties met de diversiteit indexen. Hierbij toonden de fysische-chemische variabelen geleidbaarheid, totaal fosfor, totaal stikstof en chloride een negatieve correlatie met de diversiteit indexen en had verzadigd zuurstofgehalte een positieve correlatie.

Multidimensionale scaling van de bemonsteringen in het Mark stroomgebied toonde een verschil in de visgemeenschappen, afhankelijk van de verschillende wateren en het type vangtuig. De kleine beken in het Mark stroomgebied vertoonden de aanwezigheid van dominante visgemeenschappen in de bemonsteringen. De kleine beken zijn relatief goed vertegenwoordigd door het voorkomen van kenmerkende vissoorten zoals biermpje, riviergrondel, driedoornige stekelbaars en tiendoornige stekelbaars. Desondanks zijn er ook ongewenste visgemeenschappen van baars & blankvoorn en snoek & ruisvoorn aanwezig in de kleine beken, die een indicatie geven voor locaties die in de toekomst verbeterd kunnen worden. De Pearson correlatie analyse toonde significante correlaties tussen vissoorten en de fysisch-chemische variabelen temperatuur, totaal stikstof, totaal fosfor, verzadigd zuurstofgehalte en geleidbaarheid. De aantallen baars en riviergrondel in de kleine beken toonde respectievelijk een negatieve en positieve correlatie met de variabelen totaal stikstof, totaal fosfor en verzadigd zuurstofgehalte. Verder is er een positief verband aangetoond tussen temperatuur en het aantal driedoornige stekelbaarzen en een negatief verband tussen het aantal biermpjes en temperatuur. Tot slot is er voor de kleine beken een positief verband aangetoond tussen het aantal tiendoornige stekelbaarzen en de geleidbaarheid.

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## 1. Introduction

During the last centuries, environmental systems worldwide have been influenced by human developments and activities. Recently, an increased awareness of the environmental degradation has been considered, and increased management effort has been applied to maintain and improve the environmental quality and quantity of systems. Additionally, The Water Framework Directive (WFD) was implemented in 2000, aiming at the protection and improvement of water quality throughout Europe to increase sustainable usage of environmental resources. According to the WFD, fish fauna is accounted as one of the biological quality elements for assessing the ecological status of waters. During the last 20 years, more ecosystem based water management was applied by Regional Water Authority Brabantse Delta. Fish migration routes were established, rivers and streams were remaindered, and the overall water quality improved. Changes in spatial planning (e.g. remaindering, fish passages) and improved physical-chemical quality (e.g. total phosphorus, total nitrogen) are assumed to have a positive effect on the aquatic system, however, adaptation of flora and fauna to new situations could considerably take longer periods. This recently applied water management approach is expected to have improved the overall environmental quality and thus have a positively effect on developments of the freshwater fish assemblages.

For the Mark watershed, the Regional Water Authority Brabantse Delta and Royal Dutch Angling Organization have collected a large amount of fisheries data between the period of 1994 and 2014. The Regional Water Authority also recorded general water management information from the Mark watershed, such as physical-chemical variables and implemented measures. In the current state, understanding of developments in freshwater fish assemblages of the Mark watershed is unclear. Knowledge regarding the developments of the freshwater fish assemblages could be used as a proper tool to explain the effect and efficiency of executed measures and changes in biotic and abiotic conditions. Furthermore, the Regional Water Authority could use this information to improve their water management strategies.

Therefore, this study is focusing on developments of the freshwater fish assemblages in the Mark watershed between 1994 and 2014. Goal of this study was to identify the most important developments in diversity indices of the fish assemblages. Furthermore, the correlation between observed developments in diversity indices and physical-chemical variables, fish passages and habitat restoration activities was explored. Additionally, the fish assemblages of the small streams were studied to assess fish communities, and indicate the most important promoting physical-chemical variables for the small streams of the Mark watershed.

## 1.1 Environmental impact and management

Freshwater systems worldwide have been influenced by human developments and activities. Anthropogenic influences, such as, intensive agriculture and rapid urbanization have significantly affected the environmental quality and biotic composition of aquatic systems in almost all regions of the world (Lenat and Crawford, 1994). Expansion of the human population and accessory urban landscape alteration has shown significant effects on the quality and quantity of water and thus aquatic biota (Brown et al., 2005; Limburg and Schmidt, 1990; Helms et al., 2005). Currently, degradation of aquatic systems due to anthropogenic influences is associated with habitat degradation, environmental pollution, exploitation of species, and degradation of the capacity to support biodiversity (Herlihy et al., 1998; Paul and Meyer, 2001; Helms et al., 2005; Revenga et al., 2000).

The WFD was implemented in 2000, forming a key initiative aimed at protecting and improving water quality throughout Europe (European Union, 2000). The directive states that water should be protected as a heritage. Goal of the WFD is to guarantee sustainable water usage and achieve a good ecological and chemical status of all inland surface waters, transitional waters, coastal waters and groundwater. In order to achieve the formulated goals of a good ecological and chemical status of waterbodies, each member state of the European Union is responsible for management and monitoring of the waters on their territory (European Union, 2010). For the Netherlands, the national and regional water authorities are responsible for implementing the WFD goals, including the classification of water types, endeavor the formulated goals, frequent monitoring of waterbodies, and identification of the chemical and ecological status. According to the WFD, fish fauna is accounted as one of the biological quality elements for assessing the ecological status of waters.

## 1.2 Freshwater fish assemblages as monitoring tool

Watershed conditions are reflected by biological communities since they are sensitive for changes in a wide range of environmental factors. Fish in general, are influenced by various environmental conditions and characteristics of their habitat (Karr, 1981). In addition, seasonal shifts in temperature, rainfall and river flow have showed a direct effect on fish life cycle (Grossman et al., 1998; Allan, 2004). Fish are particularly useful for assessing long-term environmental impacts because they integrate multiple trophic levels in aquatic communities, are relatively long-lived and easily sampled and determined to species level (Karr, 1987; Barbour et al., 1999). Fish assemblages of a system may degrade by various factors, such as, changes in food availability, nutrient delivery, hydrological conditions, habitat, sediment, pesticides, and metal or organic contaminants (Helms et al., 2005; Lenat and Crawford, 1994; Wang et al., 2003; Paul and Meyer, 2001). Therefore, increased environmental stress of river basins is often associated with decreased overall fish richness, abundance and diversity, and may result in assemblages shifts from intolerant to tolerant and /or exotic species (Klein, 1979; Walters et al., 2003; Helms et al., 2005).

Many stream fish ecology studies have been performed to examine the fish assemblage structure in response to their environment (Matthews, 1998). Studies regarding the fish assemblage structure differ from a single stream and its tributaries (Humpl and Pivnicka 2006; Taylor et al., 2006) to several large hydrographical units (Rathert et al., 1999; Oberdorff et al., 2001; Pont et al., 2006; Lamouroux and Cattaneo 2006; Suárez et al., 2011). Most papers conclude that fish species distributions and occurrence in stream communities is related to numerous local conditions, of which many are correlated with each other (Angermeier and Winston, 1998). Environmental factors that could influence the fish assemblage structure are associated with hydraulic (Marchetti and Moyle, 2001; Lamouroux and Cattaneo, 2006), physical (Humpl and Pivnicka, 2006) and chemical conditions (Matthews et al., 1992; Lappalainen and Soinen, 2006; Herpen et al., 2015). It is assumed that freshwater fish assemblages are influenced by a combination of environmental factor acting at different scales (Matthews, 1998; Ferreira et al., 2007). In general, spatially complex habitats typically support more species than simple homogeneous habitats, predation may often restrain the distribution of species (Taylor, 1996; Power et al., 1985; Gilliam et al., 1993).

A freshwater fish assemblage is also influenced by the geographical and hydro morphological structure of the watershed. Most watersheds offer a large variability of environmental conditions, favoring different fish species along a longitudinal upstream-downstream gradient (Matthews, 1998). In theory, fish species can migrate throughout the watershed, however, in reality fish occur at the most suitable environment that is accessible for their biological needs. When focused on the entire watershed, the fluvial ecosystems are primarily altered by water construction, like dams, weirs and channelization (Mueller et al., 2011). These constructions modify the natural dynamic of water, nutrients, and sediment, disconnect river segments and floodplains, and change the overall physical, chemical and biological structure and function of river systems (Wang et al., 2011). Several studies concluded that fish assemblage structure and richness is affected by spatial position of the tributaries within the watershed (Grenouillet et al., 2004). In addition, length of the tributary and a combination of complex habitat types, especially bottom vegetation, have shown to be the most important condition for high diversity of fish species in the tributaries (NCN, 2009).

Knowledge regarding the distribution and developments of a freshwater fish assemblage in response to their environment is especially useful for management purposes. Recently applied management is aiming to improve the chemical and ecological quality of the watershed (European Union, 2000). Improvement of the chemical and ecological quality should be reflected by all the biological communities, and thus the fish assemblages (Karr, 1981). Therefore, studying the fish assemblage of the Mark watershed in response to environmental conditions could provide relevant information to evaluate the relative effect of recently applied management.

### **1.3 Fish assemblages of the Mark watershed**

In 1994, the Mark watershed was monitored and references were established for the fish assemblages (Quak et al., 1996). Since implementation of the WFD, references for the freshwater fish assemblages of streams were slightly adapted (Vriese and Beers, 2004) and criteria for the evaluation method of fish assemblages were formed (STOWA, 2015).

#### **Establishment of a reference situation**

In 1994, the Mark watershed was firstly monitored to gather general information of the fish communities in the area and to indicate bottlenecks and opportunities for future (Quak et al., 1996). As a result, the first reference situation of fish assemblages was described for the Mark watershed.

Mark en Vliet is characterized by large dimensions and monotone structures, being mostly used for shipping transport. In the reference situation, fish assemblage of Mark en Vliet is expected to be dominated by the species roach, bleak and bream. Furthermore, the species ide, white bream, ruffe and smelt are present through the whole watercourse. Subpopulations of roach, bleak and ide travel during the spawning period to upstream parts (Boven Mark and Aa of Weerijis) of the watershed. Pike, zander and perch exists as piscivorous species, and dominance of species is mostly regulated by transparency and availability of vegetation. Rudd, tench, bitterling, spined loach and crucian carp are less abundant in Mark en Vliet, but do occur frequently at habitats with the availability of vegetation.

Fish assemblages of the Aa of Weerijis and Boven Mark show comparable compositions, with largest difference that the Aa of Weerijis is connected to artificial streams and the Boven Mark to natural streams. Both streams are characterized by variable communities due to a gradient of environmental conditions. The species rudd, tench, crucian carp and bitterling are generally common and occur mostly at vegetated parts of the watercourse. Most abundant species in the Aa of Weerijis and Boven Mark are roach and perch, who can complete their entire life cycle in the streams. Also sunbleak, white bream, bleak and Ide are generally abundant and migrate to upstream parts during spawning period. Pike is the most important piscivorous specie in the Aa of Weerijis and Boven Mark due to the small sized perch populations. Furthermore, the species dace, burbot and chub occurred at the streams, but in relatively low numbers and at specific habitats. Gudgeon, stone loach and spined loach are abundant in lower frequencies and prefer shallow habitats with leaves, brunches and obstacles. However, stone loach, spined loach and dace are not as abundant in the Aa of Weerijis as in the Boven Mark.

The small streams of the Mark watershed have different characteristics and are most comparable by dimensions of the watercourses. Fish fry is often observed at downstream parts of the watercourses where they enter the Aa of Weerij of Boven Mark. The small watercourses are characterized by the dominance of stone loach, gudgeon, nine-spined stickleback and three-spined stickleback, where sticklebacks are more abundant in the upstream parts. Habitats with gravel and rocks could be inhabited by small populations of European bullhead and brook lamprey. Small populations of burbot occur at the deeper parts of the small streams, close to the available inundated areas. Spined loach could occur at the most vegetated parts of the watercourses. Furthermore, pike, roach and perch find spawning habitats at the entrance of the small watercourses. Small populations of chub, dace and Ide occasionally use the small streams during summer periods. European eel occurs throughout the whole Mark watershed, however, abundances are relatively low.

### **Current reference situation**

Since 1996, several adaptations were reported for the reference situation of fish assemblages in the Mark watershed (Vriese and Beers, 2004). The currently used reference situation of the fish assemblages in water types of the Mark watershed are shown in table 1.

Fish fauna of the small streams are restricted, with three-spined stickleback as most common species. Furthermore, populations of nine-spined stickleback exist and stone loach and gudgeon are locally present. Brook lamprey could occur in this type of small streams, depend on local conditions (type of substrate and availability of detritus). During spring it is possible that that minnow reaches the upstream parts of the small streams.

Fish assemblages of the large streams are characterized by the occurrence of small rheophilic species such as, stone loach, dace, gudgeon, European bullhead, while also eurytopic species exist due to limited velocities in the streams. Phytophilic fish species, such as, pike, sunbleak, spined loach and nine-spined stickleback occur locally at habitats with less current.

Fish assemblages of rivers are characterized by the occurrence of rheophylic species such as, ide, chub, stone loach, dace, gudgeon and European bullhead. Due to limited stream velocities, also eurytopic species as pike, perch and roach are abundant. Sunbleak, stone loach, nine-spined stickleback and pike exist at habitats with the absence of current, such as, part of the shores and side rivers. River lamprey could occur at habitats with enough substrate in the form of gravel and rock.

Table 1. Overview of the characterizing fish species for watercourses of the Mark watershed in accordance to the adapted reference situation for WFD water types (Vriese and Beers, 2004).

| <b>Fish species</b>      | <b>Small streams</b> | <b>Large streams</b> | <b>Rivers</b> |
|--------------------------|----------------------|----------------------|---------------|
| Bleak                    |                      |                      | R             |
| Perch                    |                      | R                    | R             |
| Brook lamprey            | RB                   | RB                   | RB            |
| Stone loach              | R                    | R                    | R             |
| Roach                    |                      | R                    | R             |
| Bream                    |                      | V                    | V             |
| Three-spined stickleback | R                    | R                    | R             |
| Mirrow                   | RB                   |                      |               |
| Spined loach             |                      | R                    | R             |
| Chub                     |                      | RB                   | R             |
| European eel             |                      | R                    | R             |
| European bullhead        | RB                   | RB                   | RB            |
| Gudgeon                  | R                    | R                    | R             |
| River lamprey            |                      |                      | RB            |
| Dace                     |                      | R                    | R             |
| pike                     |                      | R                    | R             |
| Nine-spined stickleback  | R                    | R                    |               |
| Sunbleak                 |                      | R                    | R             |
| Ide                      |                      |                      | R             |

R Species always occurs in the reference situation.

RB Species could occur in the reference situation, depend of regional differences, presence/absence of suitable substrate or only in a specific stage of the lifecycle.

V Species often occurs in the reference situation.

## WFD evaluation

The criteria for the evaluation method of fish assemblages is reported in STOWA, 2015. The current evaluation criteria for streams and rivers is based on three metrics: fish species composition, abundance of migration sensitive fish species and abundance of habitat sensitive fish species.

Combination of the three evaluation metrics, results in the overall score and is a number between zero and one. Species composition is based on the proportion of rheophylic fish species in terms of total species richness. Abundance of migration and habitat sensitive fish species is based on the proportion of habitat sensitive fish and migratory sensitive fish from the total number of individuals. All watercourses of the Mark watershed are evaluated by the same metrics of fish abundances, however, different targets are stated for the three types of water (appendix 1).

## **Historical assessment**

Surveys from 1994 revealed that the freshwater fish community structure of larger streams and rivers of the Mark watershed were dominated by eurytopic fish species, and less rheophilic and limnophilic species were observed (Quak et al., 1996). Notably, the freshwater fish assemblage was mainly dominated by the year class 0+ of bream, roach and perch. Despite the good condition of fish with younger year classes, older fish (year class 2+) showed to have a deteriorated condition, probably caused by the competition for food with the large amount of young fish. Number of piscivorous fish were minimum during the surveys in 1994 and dominated by perch, only few pike and zander were observed. The small amount of large piscivorous fish was assumed to fail in effective predation to reduce the numbers of younger year classes.

The smaller upstream parts of the Mark watershed are mainly characterized by the occurrence of rheophilic species which represent around fifty percent of the catch during the surveys in 1994. Eurytopic fish species were secondly most dominant in the catch, and limnophilic fish species were caught non-frequently and in small numbers. The rheophilic guild were mainly characterized by the species gudgeon and stone loach, while more intolerant species only were observed occasionally.

## **Recent Assessment**

The Aa of Weerij, Boven Mark and small streams of the Mark watershed are most recently monitored in 2010 and 2013 (Koole, 2013). Fish surveys revealed that the small streams are increasingly dominated by rheophylic fish species, accounting for approximately 60% of the total fish assemblages. However, the small streams differ greatly among each other in terms of fish assemblage. Stone Loach was indicated as most contributing rheophylic fish species, but in some streams gudgeon was more abundant. Fish assemblages at five of the small streams of the Mark watershed show an increase in total numbers to fish between 2010 and 2013. The Aa of Weerij (21%) and Boven Mark (4%) are characterized by remarkably lower proportion of rheophylic fish species. Fish assemblages at the Aa of Weerij and Boven Mark show a strong decrease in total numbers between 2010 and 2013, and could be caused by a cold Spring in 2013 that resulted in unsuccessful reproduction. However, no obvious developments or shifts were observed for the distribution of guilds during recent periods. In this study, a longer period of fish surveys were included to obtain developments of the fish assemblages in the Mark watershed.

## 2. Material and methods

The materials and methods used in this study includes a description of the study area (chapter 3.1), data collection (chapter 3.2), data construction (chapter 3.3) and data analysis (chapter 3.4).

### 2.1 Study area

The Mark watershed is a transboundary system located in the Netherlands and Belgium. More specific, the Mark watershed is situated between Antwerp and Breda, with a total surface of 74.500 ha, of which 56% is located in Belgium (Brevé, 2007). The origin of several small streams is situated in the north of Belgium and south of Breda. The small streams enter the main drainage system of the Boven Mark and Aa of Weerrijs and flow further north to Breda. The Aa of Weerrijs and Boven Mark combine in the channels of Breda, and continue further north as the Mark. This small river becomes the Dintel and eventually flows into the Volkerak. The origin of the Molenbeek is also located in Belgium. Specifically, the origin of the Molenbeek is located between Kalmhout and Achterhoek, from where it flows to the Netherlands and enters into the Vliet. The study area includes all waterbodies directly connected to the Mark watershed which are located within the management area of the Regional Water Authority Brabantse Delta (figure 1; appendix 2). Abbreviations that were used for the individual waterbodies are shown in table 2.

The waterbodies included for this study are typical lowland streams, meaning that precipitation in winter forms most of the water supply. During the 1960s and 1970s, the streams were normalized, straightened and dredged for fast drainage of rainwater and easier shipping transport at the larger waterbodies. Stream normalization decreased the natural sponge effect of wetlands in upstream parts of the basin. Weirs and other constructions were built to have a manageable control for the availability of water during dry periods. These manipulations by water management has changed the natural river characteristics and dynamics, resulting in a disequilibrium of geomorphological processes. In addition, the water quality was influenced by anthropogenic influences (e.g. agricultural activities) for centuries and the availability of complex habitat types degraded within the basin.

During the last decades, increasing management efforts was applied to achieve a good chemical and ecological status of the Mark watershed. Several projects were performed to restore the natural characteristics and dynamics. Weirs and dams were replaced or adapted to achieve sustainable fish migration by the construction of fish passages and parts of the rivers/streams were remaindered or bottom elevated for habitat restoration. Furthermore, wastewater treatment plants were improved and activities in the area were more strictly accounted for discharge of wastewater to improve the chemical status of the Mark watershed. All these activities are assumed to have a positive effect on the aquatic system, however, adaptation of ecosystems to new situations could considerably take long periods.

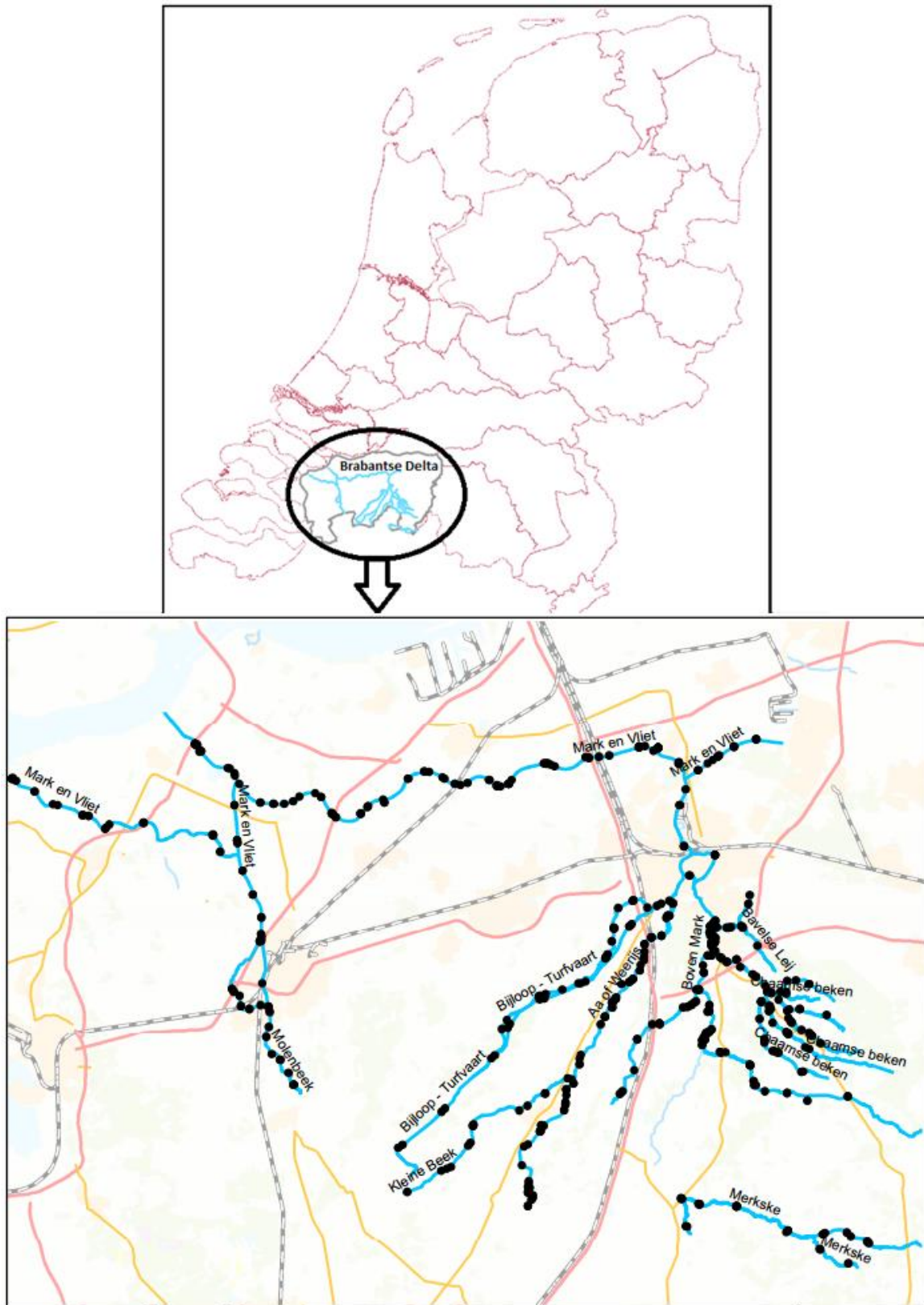


Figure 1. Overview of the study area. Picture on top presents the Netherlands and the management area of the Regional Water Authority Brabantse Delta. Picture below shows all waterbodies directly connected to the Mark Watershed. The black dots represent the samples that were included for this study.

Table 2. Overview of the waterbodies included for this study and the used abbreviations during the performed analyses.

| <b>Name of watercourses</b> | <b>Abbreviation</b> |
|-----------------------------|---------------------|
| Aa of Weerij                | AW                  |
| Bavelse Leij                | BA                  |
| Boven Mark                  | BM                  |
| Bijloop-Turfvaart           | BT                  |
| Chaamse Beken               | CH                  |
| Galderse Beek               | GA                  |
| Kleine Beek                 | KL                  |
| Mark en Vliet               | MV                  |
| Merkske                     | ME                  |
| Molenbeek                   | MO                  |
| Strijbeekse Beek            | ST                  |

## 2.2 Data collection

For this project, data of fish surveys, physical-chemical variables and implemented measures were collected. Fish data were provided by the Regional Water Authority Brabantse Delta and the Royal Dutch Angling Organization. Survey data of the freshwater fish assemblages were collected between 1994 and 2014. Data of the physical-chemical parameters and executive measures were provided by the Regional Water Authority Brabantse Delta. Additionally, it was also attempted to include hydro morphological data for the analysis. However, by lack of time during the project, these data were not included for the analysis.

### Fish assemblage data

The study area represents a relative gradient in environmental characteristics, requiring different methods and techniques for fish sampling. Dependent on size, structure and accessibility of the sampling location, different methods of sampling were applied, such as, electro fishery with waders, electro fishery from a boat, purse seines fisheries, and bottom trawler fisheries. Between 1994 and 2014, a total of 498 samples were collected. During the fish surveys, all individuals were determined to species level and registered as number of fish per length (rounded as cm). In 1994, the first fish survey in the river basin was performed by the Organization for the Improvement of Inland Fisheries (OVB), the predecessor of the Royal Dutch Angling Organization. Aim of this survey was to gather general information of the freshwater fish assemblages in the area and to indicate bottlenecks and opportunities for future developments of the fish communities. The sampling event of 1994 was most intensive, where 8 waterbodies of the river basin were sampled by 108 individual trajectories of approximately 300 meters. Results of the sampling event in 1994 were only available on paper and have been digitalized in the Piscaria software before start of the analysis.

Since implementation of the WFD in the year 2000, the fish assemblages were sampled using standardized fishing procedures. Since 2000, the data was collected by consultancy firms hired by the Regional Water Authority Brabantse Delta. Sampling events occurred on a more or less regular basis between 2000 and 2014, with approximately time intervals of three years. However, the year of sampling differ between the individual waterbodies. Conform handboek hydrobiology (2010), it is assumed that the distribution of fish is more homogeneous in summer. For this reason, fish surveys have to be performed in the period of mid-July and mid-September. Nevertheless, the periods in which the fish data was collected covers a wider range (appendix 3). In the standardized fishing procedures of handboek hydrobiology (2010), trajectories of 250 meters are sampled, with an intensity where at least 7.5% of the total surface of the total waterbody is sampled. Trajectories at the small streams of the Mark watershed were processed in ArcGIS to visualize the performed samplings (appendix 4).

### Chemical/physical data

The chemical/physical data for this study was provided by the Regional Water Authority Brabantse Delta. Chemical/physical data was obtained from routine sampling stations for the period of 1994 to 2014. For each waterbody, at least two routine sampling stations (one upstream and one downstream) were selected for collection of physical-chemical variables. A total of 13 physical-chemical parameters (table 3) were obtained from respectively 30 sampling stations. The chemical/physical variables were selected conform quantitative availability of the data and expected contribution to the ecological quality of the river basin system.

Table 3. Overview of the chemical/physical parameters which were collected.

| Parameter | Description                 |
|-----------|-----------------------------|
| NH4       | Ammonium (mg/l)             |
| Cl        | Chloride (mg/l)             |
| ZICHT     | Transparency (Secchi depth) |
| PO4       | Phosphate (mg/l)            |
| Ptot      | Total phosphorus (mg/l)     |
| GELDHD    | Conductivity (mS/m)         |
| NO2       | Nitrite (mg/l)              |
| NO3       | Nitrate (mg/l)              |
| Ntot      | Total nitrogen (mg/l)       |
| Temp      | Water temperature (°C)      |
| pH        | NA                          |
| O2        | Oxygen concentration (mg/l) |
| O2per     | Oxygen saturation (%)       |

## Implemented measures

Data regarding implemented measures was provided by the Regional Water Authority Brabantse Delta. The analysis focused on the construction of fish passages and remaindering projects within the watershed. The year of construction of fish passages and remaindering project were obtained from ArcGIS and registered by a binary notation.

## 2.3 Dataset construction

One dataset was constructed for the fish data (dependent variables) to perform the analysis. Another dataset was constructed for the chemical/physical parameters and executive measures (independent variables) to perform the explanatory analysis in relation to the fisheries data.

### Fish assemblage dataset

During the first phase of the analysis the data was extracted from the Piscaria software and prepared for analysis. The output from Piscaria is registered as the number of individuals per species per length (rounded as cm). Biomass could be extrapolated from historical data and number of individuals per fish species per length. However, due to lack of time and no clear differences in the results of the first analysis, this report only focuses on the numbers of fish. The Piscaria output was processed as the total number of individuals per species for each sample and with a division into length categories (table 4). The division of length categories for most fish species is based on feeding behavior, assuming that small fish is primarily planktivorous and larger fish become benthivorous or piscivorous. However, the length categories for pike differ from the others and is more based on habitat preference, where small pike prefers the shelter of vegetation and larger pike prefers to live in the open water. A 0+ length category for one summer old fish was not considered in the analysis due to variability in growth rate of species in different streams streams.

Table 4. Division of the length categories for fish species applied in this study (Handboek hydrobiologie, 2010). During the analysis, the abbreviations were used to refer to the different length categories.

| General length category | Length category for pike ( <i>Exos lucius</i> ) | Abbreviation |
|-------------------------|---|--------------|
| 0 - 15 cm               | 0 - 15 cm                                       | LK1          |
| 16 - 25 cm              | 16 - 35 cm                                      | LK2          |
| 26 - 40 cm              | 35 - 45 cm                                      | LK3          |
| >40 cm                  | 45 - 55 cm                                      | LK4          |
| NA                      | > 55 cm   | LK5          |

Furthermore, the dataset was cleaned up by removing the outliers and sample events with non-representative sample size, period or collected number of individuals. A total of 498 samples were registered before the clean-up of the dataset (appendix 3). Samples with a total number of individuals less than 10 were assumed to be non-presentative and were removed from the dataset, resulting in 460 samples remaining. Finally, the sampling events with sample size lower than three samples, and sampling period between January and June were removed. In total, 6 more samples were removed from the dataset, remaining a total of 454 samples for the analysis. An overview of the sampling intensity and frequency of the remaining samples is shown in table 5.

Finally, the data was transformed before the analysis to improve normality. The raw data used for the analysis of fish communities was transformed by a square root transformation. For the diversity indices analysis, the data was mostly transformed to improve uniformity. Because the sampled area was not registered for the data of 1994 and it was not possible to calculate the area, it was impossible to apply the fished surface method (bevist-oppervlak method (BOM)) that is commonly used in the Netherlands (Handboek hydrobiologie, 2010). Therefore, the data was transformed to the proportions of each species in contrast to the total number of individuals (1).

$$\text{proportion of a species} = \frac{\text{Numbers of 1 specie}}{\text{Total numbers of individuals}} * 100 \quad (1)$$

Table 5. Sampling intensity and frequency for the remaining samples after clean-up for the different waterbodies over the years.

|           | 1994 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2013 | 2014 |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|
| <b>AW</b> | 25   | -    | 11   | -    | -    | 12   | -    | -    | 14   | -    | 13   | -    |
| <b>BA</b> | 4    | -    | -    | -    | -    | -    | -    | -    | 4    | -    | 2    | -    |
| <b>BM</b> | 18   | -    | 8    | -    | -    | 6    | -    | -    | 14   | -    | 12   | -    |
| <b>BT</b> | -    | -    | -    | -    | -    | 7    | -    | -    | 11   | -    | 11   | -    |
| <b>CH</b> | 12   | -    | -    | -    | 8    | 6    | -    | -    | 8    | -    | 11   | -    |
| <b>GA</b> | 5    | -    | -    | 5    | -    | -    | -    | -    | 1    | -    | 4    | -    |
| <b>KL</b> | -    | -    | -    | -    | -    | -    | -    | 7    | -    | -    | 7    | -    |
| <b>ME</b> | 3    | -    | -    | -    | 6    | -    | -    | -    | 6    | -    | 8    | -    |
| <b>MO</b> | -    | 5    | -    | -    | -    | -    | 9    | -    | -    | 6    | -    | 7    |
| <b>MV</b> | 26   | -    | -    | 18   | -    | -    | 16   | -    | -    | 44   | -    | 41   |
| <b>ST</b> | 2    | -    | -    | -    | 3    | -    | -    | -    | 4    | -    | 4    | -    |

## Dataset of physical-chemical variables and implemented measures

The physical-chemical variables were extracted from the database of the Regional Water Authority Brabantse Delta. First, the annual average of each physical-chemical parameter was calculated for the 33 sampling stations. According to the location of the 30 stations, fish samples were clustered and linked to a specific station (figure 2). Finally, the annual mean of the physical-chemical parameters per station was combined with the linked cluster of the fisheries data for the analysis.

The dataset construction of implemented measures was performed for the Aa of Weerijns and Boven Mark and includes the construction of fish passages and remaindering projects. These waterbodies are directly connected to the Mark en Vliet and were expected to show most effect of increased connectivity and habitat restoration of the system. Data from implemented measures carried out by the Regional Water Authority Brabantse Delta were extracted from ArcGIS. For the fish passages, the fish data was clustered according to connectivity with Mark en Vliet.

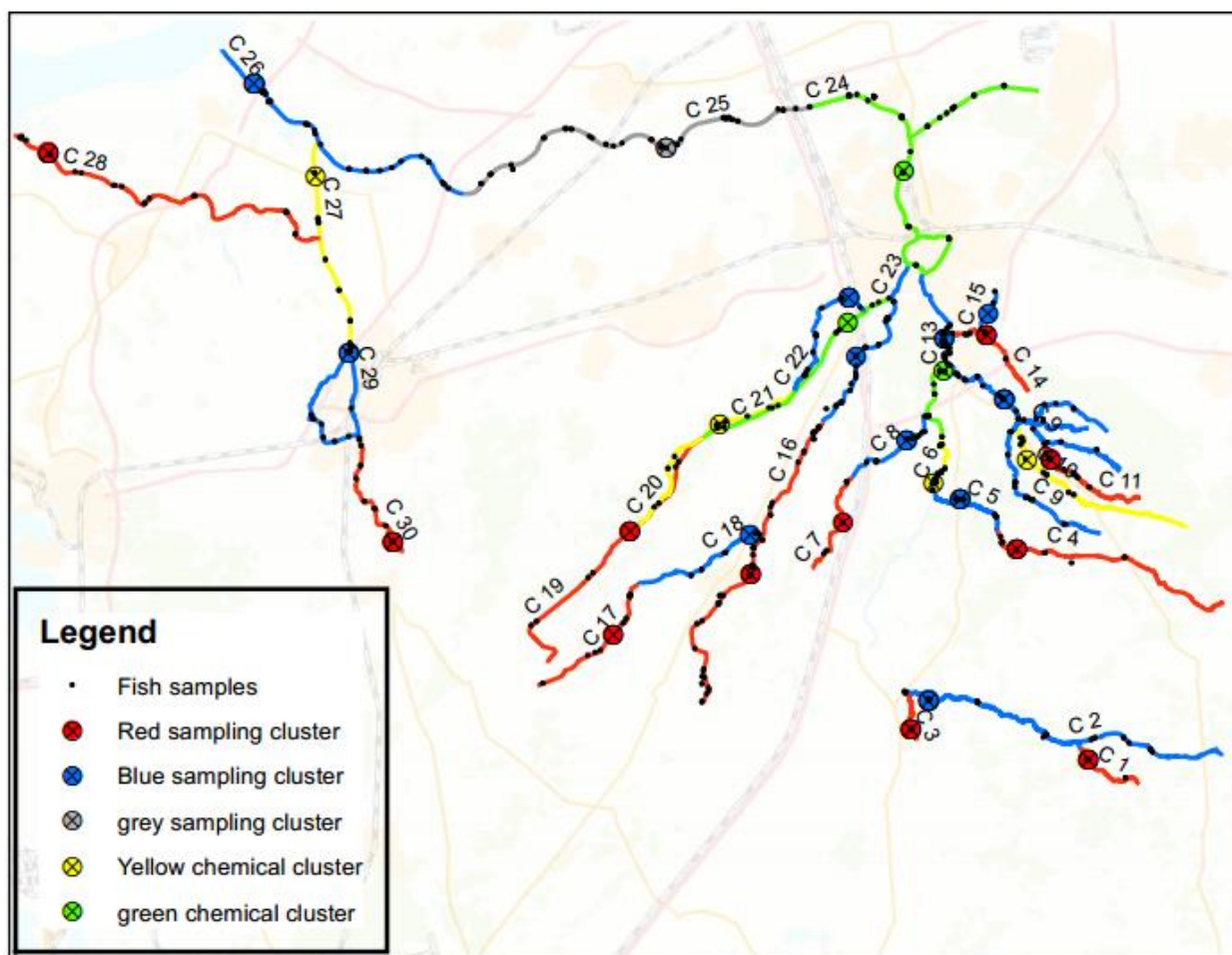


Figure 2. Overview of the 30 physical-chemical stations and clustering of the fish samples in the study area. The physical-chemical stations have different colors and correspond to the part of a waterbody with the corresponding color. All 30 clusters are shown by the character C with the corresponding number of the cluster.

The linkage between implemented measures and fish samples were established by two ways. The remaining projects have a more local effect compared to fish passages. For this reason, only fish samples that were exactly located within the area of the remaining project were linked to this measure. The construction of fish passages were interpreted according to the year of construction and increased connectivity, where 0 means no fish passage / no connectivity with Mark en Vliet and 1 means realization of connectivity (figure 3).

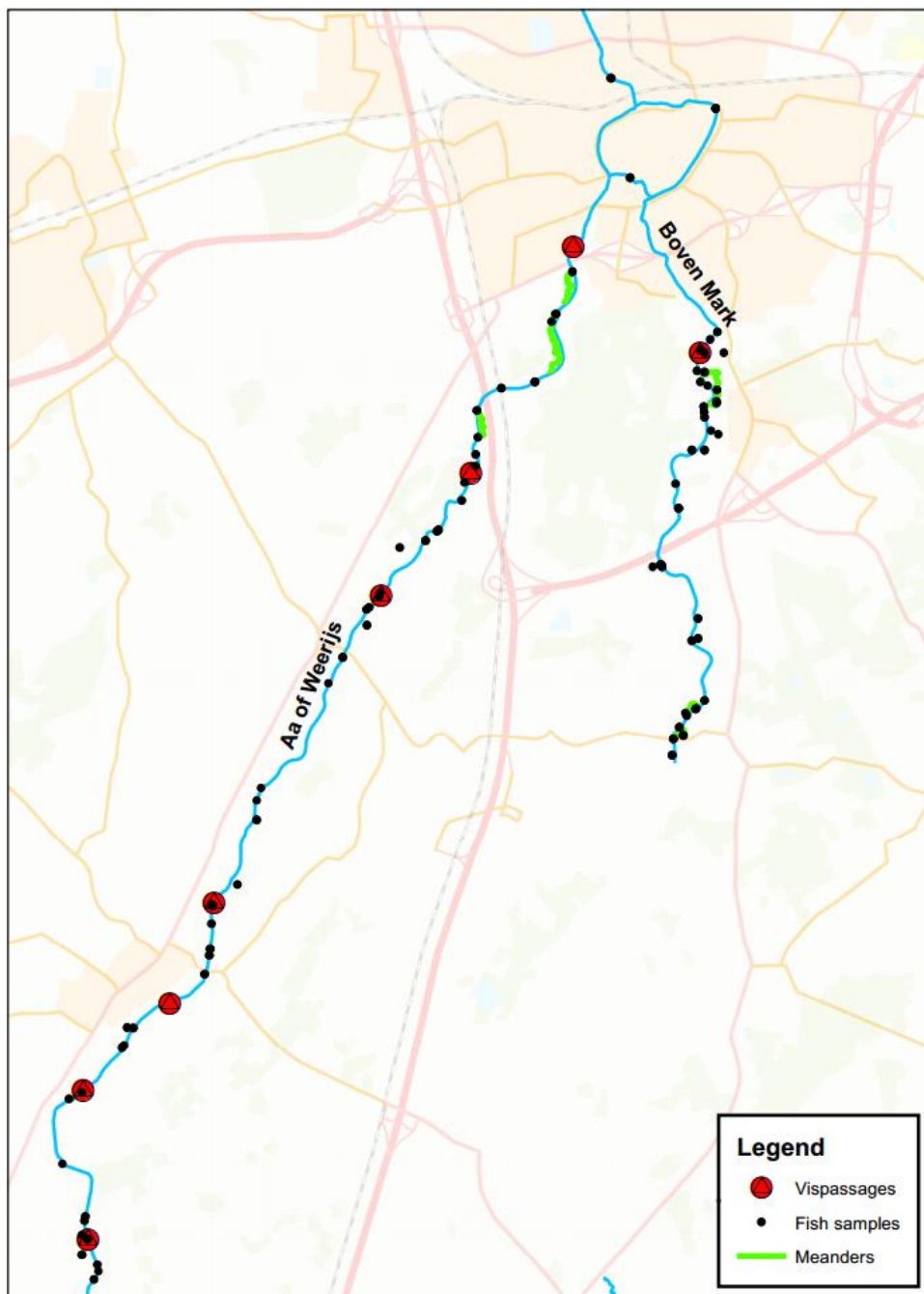


Figure 3. Overview of the implemented measures that were included for the analysis during this study. Respectively for the Aa of Weerijis and Boven Mark, 7 and 1 fish passages showed an increased connectivity, and for both streams 3 remaining projects were included.

## 2.4 Data analysis

The data analysis was performed by two phases, i.e. an exploratory analysis and explanatory analysis. The statistical analysis was performed with R version 3.2.2 (R Core Team, 2015), with the packages Vegan (Baselga et al, 2013) and Betapart (Oksanen et al, 2015).

### Exploratory analysis

Species diversity was assessed for each sample using three different indices: species richness, Pielou's evenness and Shannon's diversity. First, species richness was determined by noting presence/absence of each species (S  arez et al., 2011). Second, the Shannon Wiener diversity index (2) was calculated for each sample, where  $p_i$  is the proportion of the population belonging to specie  $i$  (Beals et al., 2000). The Shannon Wiener diversity index considers both the number of species and the distribution of individuals among species. The Shannon Wiener diversity index is commonly used to characterize species diversity in a community. Shannon Wiener diversity index is a revision, derived from community structures, and is often applied to reflect environmental quality in terms of diversity.

$$H = - \sum_{i=1}^s p_i * \log p_i \quad (2)$$

Third, Pielou's evenness (3) was calculated to assess information regarding the distribution of individuals among species, where  $H$  is the result of the Shannon Wiener diversity index and  $R$  is the total number of species (Beals et al., 2000). Pielou's evenness assumes and reflects that an equal distribution among species within a communities is more diverse compared to communities which are dominated by one or two species.

$$E = \frac{H}{\ln R} \quad (3)$$

In the first phase of the exploratory analysis, Species richness, Pielou's evenness and Shannon Wiener diversity index were analyzed by a linear model for each waterbody to obtain differences over the period of 20 years. Waterbodies with a significant linear trend over time were further analyzed by calculating the average species richness, Pielou's evenness and Shannon's diversity for the years of sampling to increase resolution. Statistical comparison of the means was performed by a two sample t-test. Each waterbody was analyzed individually and the occurred developments in diversity index are described. Finally, the significant differences in species richness, evenness and diversity were explained by scoping onto the observations of the fisheries data.

## **Explanatory analysis**

The explanatory analysis was performed by multi-variate ordination techniques (Baselga et al, 2013). Multi-variate analyses are generally used to filter and narrow complex data sets to easier interpretable data. Ordination techniques are used for the ordering of many samples, each having several variables, along multiple dimensions or axes. The dimensions of data sets with many variables can be reduced to two or three derived axes, which may explain relationships among samples if some of the original variables contribute strongly to these axes. The axes account for the greatest amount of variation in the data and reduce the number of dimensions that should be considered (Holland, 2008). As result, similar samples will be close together and dissimilar samples will be not close together. Multivariate methods are often useful for exploring the structure of data sets and generating relevant ecological hypotheses (Fausch et al., 1990). The explanatory analysis was performed by two multi-variate ordination methods, i.e. Non-Metric Dimensional Scaling (NMDS) and Redundancy analysis (RDA).

## **Diversity indices**

In this study, the RDA was a proper ordination technique to assess the influence of physical-chemical variables on the diversity indices (Grossman, 1998). RDA is a method to extract and summarize the variation in a set of response variables that can be explained by a set of explanatory variables. In contrast to the NMDS technique, the RDA technique assumes linear relationships between the response and explanatory variables. For this reason, the influence of the explanatory variables on the response variables is easier assessable for interpretation and reconstruction. The RDA was performed to explain relationships between the response variables (species richness, Pielou's evenness and Shannon's diversity) and the explanatory variables (physical-chemical variables and executive measures). Fish passages and remaindering projects were only included as explanatory variables for the Aa of Weerijis and Boven Mark.

## **Pearson correlations**

The variables with a significant contribution to the RDA were further analyzed by a Pearson correlation test to confirm significance of the correlations. Only diversity indices and exploratory variables shown in the RDA were analyzed by the Pearson correlation analysis. Furthermore, the significant correlations ( $p < 0.05$ ) and their correlation coefficient ( $r$ ) were included in the results.

## **Fish communities**

The NMDS was a proper ordination technique to compare the samples of the freshwater fish assemblages (Holland, 2008). In contrast to other ordination techniques, NMDS assumes no fixed model, and is a numerical technique that iteratively seeks the best solution to fit the data. For this reason, the NMDS technique is suited for a wide variety of data compared to other ordination techniques (Holland, 2008). Fish species that occur in less than 3% of the samples were excluded for the ordination to overcome overweighting of rare species (table 13). The input for the NMDS consisted of all remaining fish species divided into length categories, transformed by a square-root transformation to improve normality and fitting of the data to the NMDS models. The NMDS was performed by a Bray-Curtis similarity calculation, which is often used in ecologists. Ideal properties of the Bray-Curtis matrix are the robustness against the change in units, addition/removal of species or communities, and recognition of differences in total abundance when relative abundance is equal.

First, all fisheries data was processed to identify differences in used fishing gear and the waterbodies of the Mark watershed. Furthermore, the different waterbodies and fishing gears were analyzed individually to obtain the differences more clearly. Second, The small streams of the Mark watershed were analyzed more accurately because more differentiation in fish assemblages was expected compared to the larger waterbodies. First, the samples were selected for dominance of guilds (appendix 5). The samples were categorized into rheophilic, limnophylic and eurytopic guilds when respectively 50% or more of the sampled fish belonged to a specific guild. Finally, the samples of the small streams were further divided into fish communities of BV & BA, RG & BE, DD & TD and SK & RV, based on the most frequent combination of occurrence in the samples, when respectively 40% or more of the collected fish belonged to the two species of a specific community.

## **Simper analysis**

Results of fish assemblage ordinations were further explored by use of a similarity percentage analysis (simper). A simper analysis was performed to identify the most important contrasts in fish assemblages between different groups showed in the ordinations. Results of the simper analysis, present which species are most important for the distinction between groups. In this study, the simper analysis was performed to distinguish the most contributing fish species for differences between the used fishing gears and waterbodies.

## **Pearson correlations**

The physical-chemical variables with a significant contribution to NMDS of the fish communities in the small streams were also further analyzed by a Pearson correlation. Only physical-chemical variables shown in the NMDS of fish communities in the small streams were analyzed by the Pearson correlation analysis. Significant correlations ( $p < 0.05$ ) and their correlation coefficient ( $r$ ) were included in the results.

### 3. Results and discussion

This chapter includes the results and discussion of the diversity indices (chapter 4.1) and results of the freshwater fish assemblages (chapter 4.2).

#### 3.1 Diversity indices

For the exploration of the diversity indices a linear model was applied on the time series of fisheries data. The linear model of species richness, Pielou's evenness and Shannon's diversity over time for all waterbodies is presented in appendix 6. Patterns of diversity in the river basin differed greatly among waterbodies. Five of the eleven waterbodies showed a significant linear trend over time for at least one of the diversity indices. In this paragraph, the results and discussion of the diversity indices is explained separately for each waterbody with a significant trend. First, the most important general observations are described, followed by the results and discussion for individual diversity indices. Results of diversity indices are further analyzed for correlations to physical-chemical variables and executive measures in paragraph 4.2. The Chaamse Beken, Bavelse Leij, Molenbeek, Bijloop-Turfvaart, Merkske and Kleine Beek are not included because no significant trend was observed in the diversity indices.

##### 3.1.1 Galderse Beek

The linear model for diversity indices at the Galderse Beek shows a significantly decreasing trend in Pielou's evenness and Shannon's diversity between 1994 and 2013 (figure 4). A more detailed overview of the developments in average species richness, Pielou's evenness and Shannon's diversity for each sampling event is shown in table 6. The diversity indices are characterized by a decrease between 1994 and 2013, where species richness decreased strongly between 2005 and 2013 and Pielou's evenness decreased between 1994 and 2005. Despite of a significant decreasing trend over time, only Pielou's evenness showed a significant decrease between 1994 and 2005. Shannon's diversity decreases strongly over time, however, no significant differences were obtained between sampling events.

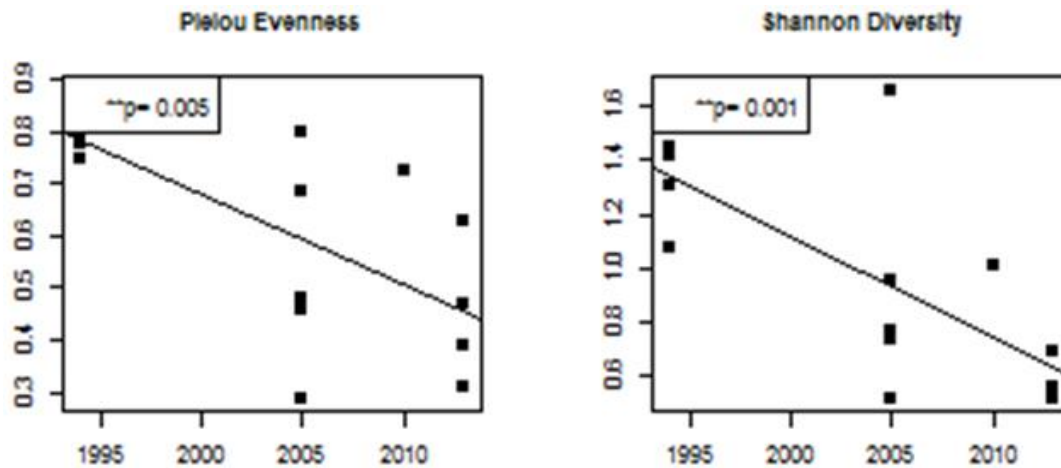


Figure 4. Linear model of Pielou's evenness and Shannon's diversity for the Galderse Beek between 1994 and 2013. The P-values of the linear model is presented in the top left corner of the plots (\*\*,  $P < 0.01$ ).

Table 6. Average species richness, Pielou's evenness and Shannon's diversity of the samples collected at the Galderse Beek between 1994 and 2013. Significant P-values indicate a difference compared to the previous sampling event (\*,  $P < 0.05$ ).

| Waterbody     | Year | Species Richness | Pielou's Evenness | Shannon's Diversity |
|---------------|------|------------------|-------------------|---------------------|
| Galderse Beek | 1994 | 5,40             | 0,80              | 1,34                |
| Galderse Beek | 2005 | 5,60             | 0,54*             | 0,93                |
| Galderse Beek | 2013 | 4,00             | 0,45              | 0,57                |

A total of 2.897 individuals and eleven species were recorded for the Galderse Beek over the period of 1994 to 2013. The total number of individuals for 1994, 2005 and 2013 were respectively 332, 941 and 1.607. Most common species in the samples were nine-spined stickleback and stone loach with an occurrence of 47.5% and 35.8% of the total individuals. Sampling event of 2010 was excluded during analysis of individual sampling events, because only one sample remained after exclusion of samples with less than ten individuals. However, during this period the freshwater fish assemblage clearly deteriorated with a total of 26 individuals observed by four performed trajectories. Dimensions of the Galderse Beek are relatively small and shallow. It is possible that the cold winter of 2010 is responsible for the decreased number of fish in the Galderse Beek in 2010 (Oldenborgh, 2015). However, it remains questionable because none of the other small streams in the Mark watershed indicates the same trend. The species European eel, stone loach, three-spined stickleback, gudgeon, pike and nine-spined stickleback were observed during all sampling years. The species roach, perch and common carp were present in 1994, and tench and rudd were present in 2005. These five species were all absent during the sampling event of 2013.

The average species richness of the samples was comparable for 1994 and 2005 and decreased by 1.6 specie between 2005 and 2013. Total species richness for the Galderse Beek was respectively 9, 8 and 6 species for the years 1994, 2005 and 2013 (appendix 7). Results of the total species richness and average species richness are matching, concluding that species richness in the Galderse Beek decreased over time. The decrease in average species richness between 1994 and 2013 is caused by absence of the species roach, common carp, tench and rudd. Disappearance of these eurytopic and limnophylic species possibly indicates that habitats in the Galderse Beek are developing and getting less appropriate for eurytopic and larger limnophylic fish species and more appropriate for rheophilic species and nine-spined stickleback. This assumption is amplified by the strong increase of stone loach for the same period. Furthermore, the species European eel, stone loach, three-spined stickleback, gudgeon, pike and nine-spined stickleback were present in all sampling years but not in every sample. For this reason, the average species richness was always lower than six species.

The average evenness showed a significant decline between 1994 and 2005. Between these sampling events, the total number of individuals increased from 332 to 941 individuals and nine-spined stickleback became dominant. In 2005, the fish species nine-spined stickleback and stone loach increased from respectively 23 and 52 individuals, to 672 and 114 individuals, together accounting for 83.5% of the total number of individuals. Comparable results were observed at the sampling event of 2013, where stone loach increased to 871 individuals and nine-spined stickleback remained relatively equal in numbers, together accounting for 96.5% of the total number of individuals. However, in 2010 almost no individuals were collected at the Galderse Beek, and could be related to a cold winter. Stone loach and nine-spined stickleback are relatively short living species, making it possible that they benefitted from the disappearance of almost all fish in 2010, and rapidly repopulated the Galderse Beek. However, more data collection in future is required to confirm this assumption.

As a result of the declining species richness and Pielou's evenness, the Shannon diversity shows an strong decrease from 1.34 to 0.57 between 1994 and 2013. Small streams are typically inhabited by lower numbers of species due to the limiting dimensions. Additionally, Small streams are highly variable according to climate, temperature, morphology, water supply and discharge (Fieseler and Wolter, 2006). In the Galderse Beek, a fish passage was constructed in 2011, and ecological maintenance was performed since 2007. However, the Shannon's diversity decreased to a very low value of 0.57, referring to a low number of species with an unequal distribution among species. Low diversity of the fish species in the Galderse Beek could be less robust to environmental fluctuations or anthropogenic influences. The low number of fish species and unequal distribution among species are known to be less adaptive and resistant compared to more diverse systems. On the other hand, The Galderse Beek is categorized as a small river system, prioritizing the occurrence of small rheophilic fish species, such as, stone loach and gudgeon, for the WFD evaluation. However, the robustness/resistance of communities in the Galderse Beek could degrade and should be considered.

### 3.1.2 Strijbeekse Beek

The linear model for diversity indices at the Strijbeekse Beek shows a significantly decreasing trend in Pielou's evenness and Shannon's diversity between 1994 and 2013 (figure 5). A more detailed overview of the developments in average species richness, Pielou's evenness and Shannon's diversity for each sampling event is shown in table 7. Pielou's evenness and Shannon's diversity strongly decreases between 1994 and 2006. Despite of a significant trend over time, the only significant decrease was obtained for Shannon's diversity between 1994 and 2006. Species richness and Pielou's evenness both decreases non significantly in the same period. Furthermore, the diversity indices remains relatively constant between 2006 and 2013.

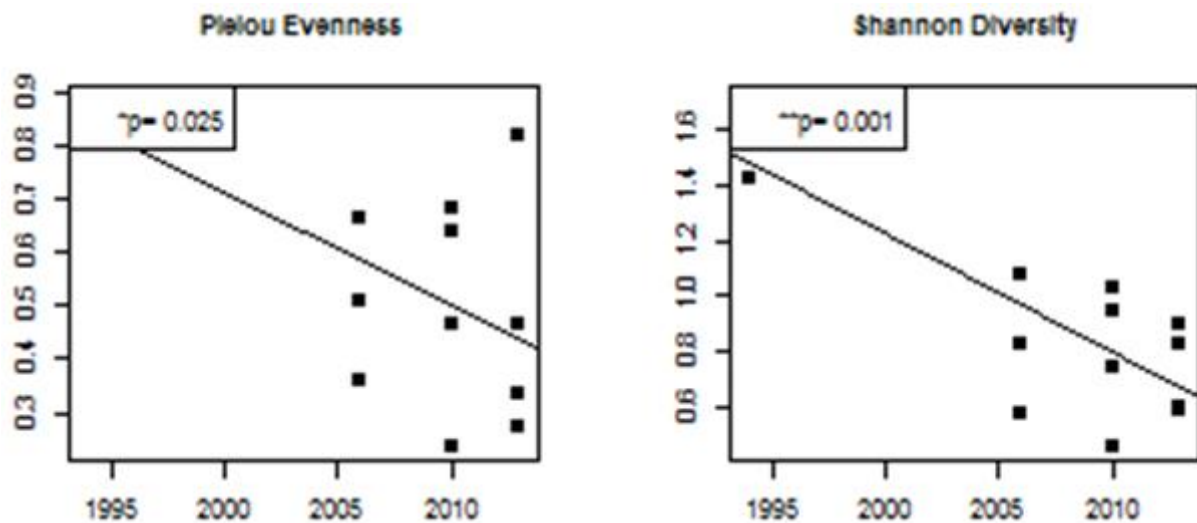


Figure 5. Linear model of Pielou's evenness and Shannon's diversity for the Strijbeekse Beek between 1994 and 2013. The P-values of the linear model is presented in the top left corner of the plots (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ).

Table 7. Average species richness, Pielou's evenness and Shannon's diversity of the samples collected at the Strijbeekse Beek between 1994 and 2013. Significant P-values indicate a difference compared to the previous sampling event (\*,  $P < 0.05$ ).

| Waterbody        | Year | Species Richness | Pielou's Evenness | Shannon's Diversity |
|------------------|------|------------------|-------------------|---------------------|
| Strijbeekse Beek | 1994 | 6,00             | 0,88              | 1,56                |
| Strijbeekse Beek | 2006 | 5,00             | 0,51              | 0,82*               |
| Strijbeekse Beek | 2010 | 5,25             | 0,51              | 0,80                |
| Strijbeekse Beek | 2013 | 6,00             | 0,47              | 0,73                |

A total of 2.672 individuals and twelve species were recorded at the Strijbeekse Beek between 1994 and 2013. The total number of individuals increases over time, for 1994, 2006, 2010, 2013 were respectively 68, 385, 985, 1.234 individuals collected. Most common species were gudgeon and stone loach with an respectively occurrence of 77.2% and 23.5% of the total individuals. The species stone loach, gudgeon and pike were observed during all sampling years. European eel, perch, common carp, tench and pumpkinseed were all absent in the sampling event of 1994, but occurred in later sampling events. Rudd was the only specie that occurred in 1994 and not in the years after.

The average species richness remained relatively comparable between 1994 and 2013. Species richness decreased from 6 species to 5 species between 1994 to 2006 and increased from 5 species in 2010 to 6 species in 2013. The total species richness in 1994, 2006, 2010 and 2013 existed of respectively 7, 6, 7 and 10 species (appendix 7). Sampling intensity was relatively equal compared for the sampling events. Furthermore, many fish species were observed with very low abundance, such as, one individual for common carp, roach and three-spined stickleback and two individuals for perch, pumpkinseed and European eel in 2013. For this reason, it is assumable that coincidence is part of the explanation. On the other hand, it is notable that all species with low abundances (common carp, roach, three-spined stickleback, perch, pumpkinseed and European eel) are eurytopic, therefore, it could be possible that coincidence is not the only reason for the increased total species richness. Nevertheless, the number of fish in samples at the Strijbeekse Beek are still dominated by rheophylic species, making it is difficult to understand the increase of total species richness in 2013. For a better understanding of the developments of total species richness in 2013, the collection of more fish data is required.

The average species evenness decreased strongly between 1994 and 2006 and remained comparable until 2013. In 1994, the species occurred in relatively balanced numbers with a maximum of 22 individuals and a minimum of 4 individuals per species. The number of gudgeon increased exponentially from 20 to 297 individuals between 1994 and 2006 and accounted for 77.1% of the total individuals in 2006. Therefore, Pielou's evenness decreased in average from 0.88 to 0.51. Between 2010 and 2013, Pielou's evenness decreased slightly more due to observations with one or two individuals of the species common carp, roach, three-spined stickleback, perch, pumpkinseed and European eel in 2013. The total number of individuals collected at the Strijbeekse beek increased over time, and individual specimen were more often observed. Therefore, it is logically that Pielou's evenness decreases as effect of the more unbalanced distribution among species.

The average Shannon's diversity significantly decreased from 1.56 to 0.82 between 1994 and 2006. This was caused by an decreasing average species richness and Pielou's evenness. Furthermore, Shannon's diversity gradually decreases further to 0.73 in 2013. The Strijbeekse Beek is a small stream and characterized by inhabitation of less species compared to larger streams. Shannon's diversity were higher at the Strijbeekse Beek compared to the Galderse Beek. Therefore, it could be expected that the fish assemblages of the Strijbeekse Beek are more resistant for variability in environmental conditions. Nevertheless, a decrease from 1.56 to 0.73 in (bio)diversity is considerably much. As for the Galderse Beek, also the Strijbeekse Beek is categorized as a small stream system, requiring the presence of rheophylic fish as characteristic species. The occurrence of stone loach and gudgeon are according the WFD goals of the Strijbeekse Beek. Also the increased total numbers of individuals indicates a positive development and increased robustness of the Strijbeekse Beek system. It seems that environmental requirements of rheophylic species are demoting for the occurrence of many species. Rheophylic species occurring in the Mark watershed are chub, ide, stone loach, gudgeon and European bullhead. The small stream dimensions are probably limiting for the occurrence of larger rheophylic species such as, chub and ide. In contrast, European bullhead occurs mostly at MV and was only once observed at the Chaamse Beken in 2013. Volunteering fisheries from Natuurvereniging Mark & Leij reported European bullhead recently in the downstream part of the Bavelse Leij. However due to a migration barrier at Blauwe Kamer, fish cannot colonize the upstream parts of the Boven Mark and thus reach the Galderse Beek and Strijbeekse Beek.

### 3.1.3 Aa of Weerijjs

The linear model of diversity indices with a significant trend for the Aa of Weerijjs is shown in figure 6. The Aa of Weerijjs shows a significant ( $p < 0.01$ ) increasing trend in species richness, Pielou's evenness and Shannon's diversity between 1994 and 2014. A more defined overview of developments in species richness, Pielou's evenness and Shannon's diversity for each sampling event is shown in table 8. Despite a significant linear trend over time for all diversity indices, only significant differences were obtained for Pielou's evenness and Shannon's diversity for the sampling events in 2013, compared to the sampling event in 2010.

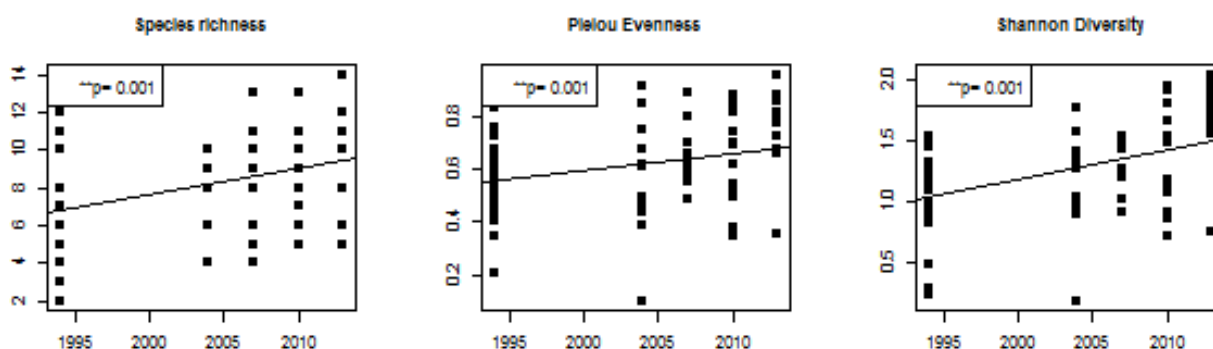


Figure 6. Linear model of the diversity indices species richness, Pielou's evenness and Shannon's diversity for the Aa of Weerijjs between 1994 and 2013. P-values of each linear model are presented in the top left corner of the plots (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ).

Table 8. Average species richness, Pielou's evenness and Shannon's diversity of the samples collected at the Aa of Weerijjs between 1994 and 2013. Significant P-values indicate a difference compared to the previous sampling event (\*,  $P < 0.05$ ).

| Waterbody      | Year | Species Richness | Pielou's Evenness | Shannon's Diversity |
|----------------|------|------------------|-------------------|---------------------|
| Aa of Weerijjs | 1994 | 6,92             | 0,57              | 1,08                |
| Aa of Weerijjs | 2004 | 7,91             | 0,57              | 1,15                |
| Aa of Weerijjs | 2007 | 8,41             | 0,64              | 1,31                |
| Aa of Weerijjs | 2010 | 9,00             | 0,61              | 1,32                |
| Aa of Weerijjs | 2013 | 9,85             | 0,74*             | 1,67*               |

A total of 24.904 individuals and 24 different fish species were recorded for the Aa of Weerijis between 1994 to 2013. The total number of individuals for 1994, 2004, 2007, 2010 and 2013 were respectively 4.074, 12.130, 1.857, 5.219 and 1.624 individuals. Most common species for the Aa of Weerijis are roach, perch, gudgeon, sunbleak and bream with an respectively occurrence of 50.2%, 13.3%, 10.0%, 6.3% and 4.9% of the total individuals. Native bitterling and exotic asp were only present in the sampling events of 2010 and 2013. The species crucian carp only occurred in the samples of 1994 and were absent in all other sampling years. The species bleak only occurred in 2007 and 2010 and nine-spined stickleback occurred since 1994 but was absent in 2013. Species three-spined stickleback, prussian carp and exotic topmouth gudgeon were present until 2007 and disappeared afterwards. Exotic pumpkinseed was observed during sampling years in 1994, 2007 and 2013. All exotic species were observed in relatively low numbers per sampling event.

The average species richness of the samples increased gradually from 6.92 to 9.85 species over the period of 1994 to 2014. No significant differences were obtained by the comparison of individual sampling events. However, the total number of the species found in the Aa of Weerijis for the sampling events in the years 1994, 2004, 2007, 2010 and 2013 were respectively 20, 17, 20, 19 and 18 (appendix 7). The average number of species collected per sample has increased, despite of the decreasing total species richness. An average increase in the number of species per samples indicates that the distribution of fish species within the Aa of Weerijis has increased between 1994 and 2013. One of the requirements for fish species to expand their distribution within a system is connectivity and thus the opportunity to migrate. Connectivity in the Aa of Weerijis has increased since 2003 and most fish passages were realized in 2005, achieving complete connectivity within management area of the Regional Water Authority Brabantse Delta. Therefore, the increased species richness after 2004 could be assumed to be related to the improved connectivity in the Aa of Weerijis. However, this assumption isn't explaining the increased species richness between 1994 and 2004, meaning that other factors also have contributed to the increased species richness. Literature describes that species richness is strongly related to the environmental quality of a system. In addition, the period between 1994 and 2004 was the longest period without sampling events of the freshwater fish assemblages. Between 1994 and 2004, the annual mean of total phosphorus, ammonium and pH decreased and the oxygen concentration increased (appendix 8). Therefore, it is possible that species richness has increased between 1994 and 2004 due to the improved chemical water quality in the Aa of Weerijis. Improvement of chemical variables takes considerably long periods and could also explain why no significant difference was obtained in average species richness in this period.

The average Pielou's evenness shows comparable ratios between species in the samples from 1994 to 2010, and significantly increases ( $p < 0.05$ ) between 2010 and 2013. In 2010, the samples were mostly dominated by the species roach and perch which contained 64.9% of the total individuals. In 2013, the numbers of roach and perch decreased and accounted for 36.9% of the total number of individuals. For the same period, sum of the bream, rudd, gudgeon, ruffe and pike increased respectively from 24.5% to 48.5% of the total number of individuals. Furthermore, the frequency of occurrence of fourteen species (including bream, rudd, gudgeon, ruffe and pike) increased between 2010 and 2013 and decreased for six species (including roach and perch) resulting in a significant increase of Pielou's evenness. Pielou's evenness started to increase since 2007, exactly the first sampling event after construction of most fish passages in the Aa of Weerijs. The increased connectivity and possibility to migrate seems to have an positive effect on the distribution of species. The significant increase of Pielou's evenness between 2010 and 2013 could not be directly explained by the data. Probably the evenness has increased by a combination of factors such as, construction of fish passages and decrease in the concentration of total phosphorus. It is assumed that also other factors have contributed to the increased evenness, which were not included for this study (see paragraph 4.2). For this reason, it is difficult to explain the observations.

Shannon's diversity show an gradual increase from 1.08 to 1.67 during the period of 1994 and 2013. This is mainly caused by the gradual increase of species richness in the Aa of Weerijs. However, the combination of an increasing species richness and significantly increasing Pielou's evenness has resulted in a significant increase ( $p < 0.05$ ) of Shannon's diversity in 2013. The increase in Shannon's diversity indicates overall positive developments of the freshwater fish assemblages in the Aa of Weerijs.

### 3.1.4 Boven Mark

The Boven Mark only shows in species richness a significant ( $p < 0.01$ ) increasing trend between 1994 and 2013 (figure 7). A more defined overview of developments in species richness, Pielou's evenness and Shannon's diversity for each sampling event is shown in table 9. Despite a significantly increasing linear trend over time for species richness, no significant difference was obtained for species richness between the individual sampling events. However, Pielou's evenness shows a significant increase between the sampling event of 2010 and 2013.

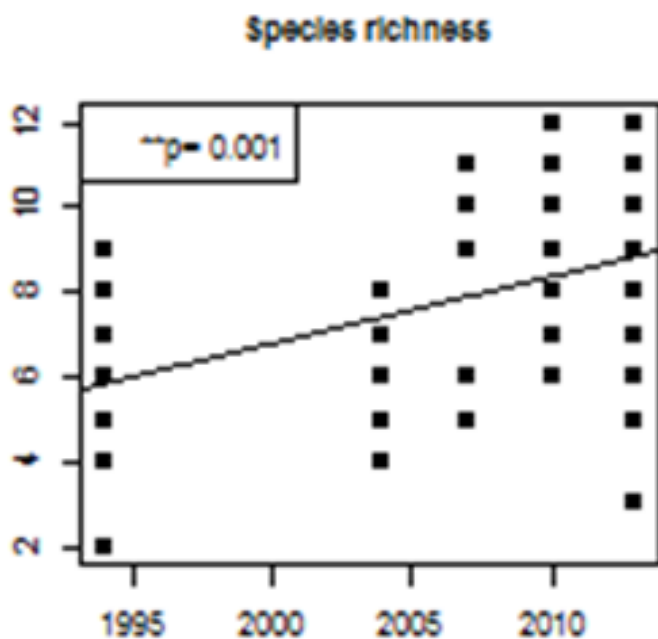


Figure 7. Linear model of species richness for the Boven Mark between 1994 and 2013. The P-values of the linear model is presented in the top left corner of the plots (\*\*,  $P < 0.01$ ).

Table 9. Average species richness, Pielou's evenness and Shannon's diversity of the samples collected at the Boven Mark between 1994 and 2013. Significant P-values indicate a difference compared to the previous sampling event (\*,  $P < 0.05$ ).

| Waterbody  | Year | Species Richness | Pielou's Evenness | Shannon's Diversity |
|------------|------|------------------|-------------------|---------------------|
| Boven Mark | 1994 | 6,06             | 0,66              | 1,19                |
| Boven Mark | 2004 | 5,88             | 0,69              | 1,2                 |
| Boven Mark | 2007 | 7,83             | 0,67              | 1,31                |
| Boven Mark | 2010 | 9,79             | 0,48              | 1,07                |
| Boven Mark | 2013 | 8,08             | 0,63*             | 1,29                |

For the Boven Mark, a total of 27.906 individuals and 25 species were recorded between 1994 and 2013. The total number of individuals collected for 1994, 2004, 2007, 2010, 2013 were respectively 3.296, 600, 692, 18.562 and 4.756. Most common species were roach, perch and bream with an respectively occurrence of 47.7%, 19.8% and 15.7% of the total individuals. Furthermore, the exotic grass carp and native chub were only observed in 1994 and remained absent all other sampling events. Exotic asp and pumpkinseed were observed in the Boven Mark since 2007, and all sampling events after. Also one specimen of exotic pumpkinseed was observed in 1994. The species sunbleak, zander, white bream show a comparable pattern over time, where they appear in all sampling years with exception of 2007. Most probably this is caused by the low sampling intensity (six samples) in 2007 and low frequency of occurrence what makes them difficult to catch.

The average species richness slightly decreases from 6.06 to 5.88 between 1994 and 2004. Over the period of 2004 and 2010 the average species richness of samples increases to 7.83 and 9.79 species in 2007 and 2010. Between 2010 and 2013 species richness decreases with an average of 1.71 species per sample. However, no significant differences were obtained for species richness in the individual sampling events at the Boven Mark. The average species richness shows relatively large fluctuation, meaning that no significant difference were obtained due to much variation between species richness in the samples. Total species richness of the Boven Mark for the years 1994, 2004, 2007, 2010 and 2013 were respectively 20, 14, 16, 19 and 20 species (appendix 7). The lower total species richness was probably (partly) the consequence of the lower sampling intensity in 2004 and 2007. Despite of the lower total species richness, the average species richness of the samples increased in 2007. An average increase in the number of species per samples indicates that the distribution of fish species within the Boven Mark has increased in 2007. Large parts of the Boven Mark still suffers for lack of connectivity due to the weirs at Blauwe Kamer and Galder. Therefore, these developments are assumed to occurred due to an improvement of the environmental quality. Moreover, the average increase in richness of the samples indicate that some species are able to complete their lifecycle and grow in numbers, despite of the barriers which are still present. The annual mean of physical-chemical variables total nitrogen, total phosphorus and pH decreased between 1994 and 2007. Optionally, the decreased physical-chemical concentrations could have contributed to the increased species richness. Contrastingly, the annual mean of total phosphorus, total nitrogen and pH increased between 2010 and 2013 and species richness decreased. This observation supports the argument that species richness in the Boven Mark is influenced by the concentrations total phosphorus, total nitrogen and pH (see paragraph 4.2). However, many other environmental factors that were not included in this study and could also influence species richness.

The average Pielou's evenness remained equal between the years 1994 and 2007. Between 2007 and 2010 the average Pielou's evenness decreases from 0.67 to 0.45 and between 2010 and 2013 the average evenness significantly increased to 0.63. The species roach, perch and bream account for 78.0% of the total individuals in 2007. In 2010, roach, perch and bream account for 86.6% of the total individuals and during the last sampling event in 2013, roach, perch and bream account for 75.3%. The occurrence of perch decreased from 25.2% to 9.1% between 2010 and 2013, while the occurrence of sunbleak increased from 1.6% to 11.1%. The increase of sunbleak and decrease of perch have mostly accounted for the significant increase of evenness between 2010 and 2013. Furthermore, The high numbers of roach, perch and bream in 2010 have accounted for the decrease in evenness compared to 2007. In 2010, the high numbers of roach, perch and bream were dominated by the length category of 0 – 15 cm. Moreover, sampling events of 2007, 2010 and 2013 were all performed in August. The winter of 2010 was a relatively cold compared to other years (Oldenborgh, 2015). It could be possible that the cold winter of 2010 has delayed the spawning season of most fish species, explaining the higher densities of the smaller length categories in the samples. It could also be possible that 2009 was a successful year for reproduction of most species, explaining the high numbers in 2010. Probably the dominant species produced more offspring, becoming even more dominant in numbers, and causing a decrease in the average evenness of the samples in 2010. However, a specific analysis of the younger year classes should be performed to confirm these assumptions.

The diversity index increased between 1994 and 2007, decreases for the sampling event in 2010, and again increased in 2013. Despite the significant increase in evenness between 2010 and 2013, no significant differences in diversity index were obtained due to a decreasing average species richness between 2010 and 2013. The average Shannon's diversity shows an increasing trend with exception of 2010, what is caused by a decreasing Pielou's evenness. Excluding 2010's sampling event, the Shannon's diversity were relatively comparable for all years and increases with approximately 0.1 when comparing 1994 and 2004 to 2007 and 2013, indicating positive developments for the Boven Mark. However, compared to developments in the Aa of Weerij, it seems that something limited the increase of Shannon's diversity at the Boven Mark. Possibly, lack of connectivity in waterbody of the Boven Mark was a contributing limiting condition. However, many other factors such as hydro-morphological and biological parameters were not included and could influence developments of the fish assemblages.

### 3.1.5 Mark en Vliet

The linear model for diversity indices at Mark en Vliet shows a significantly decreasing species richness and increasing Pielou's evenness (figure 8). A more detailed overview of the developments in average species richness, Pielou's evenness and Shannon's diversity for each sampling event is shown in table 10. Species richness significantly decreases according the linear models, however, this observation is overrated due to an extreme decrease in 2008. Pielou's evenness increases according to the linear model, but no significant changes were observed between the years. Decreased average species richness and Pielou's evenness have contributed to a significant decrease in Shannon's diversity for the sampling event of 2008. In 2011, species richness and Pielou's evenness were again equally to its original level in 2005. A clear deterioration in diversity indices is observed, indicating a global change for the fish assemblages in Mark en Vliet for 2008.

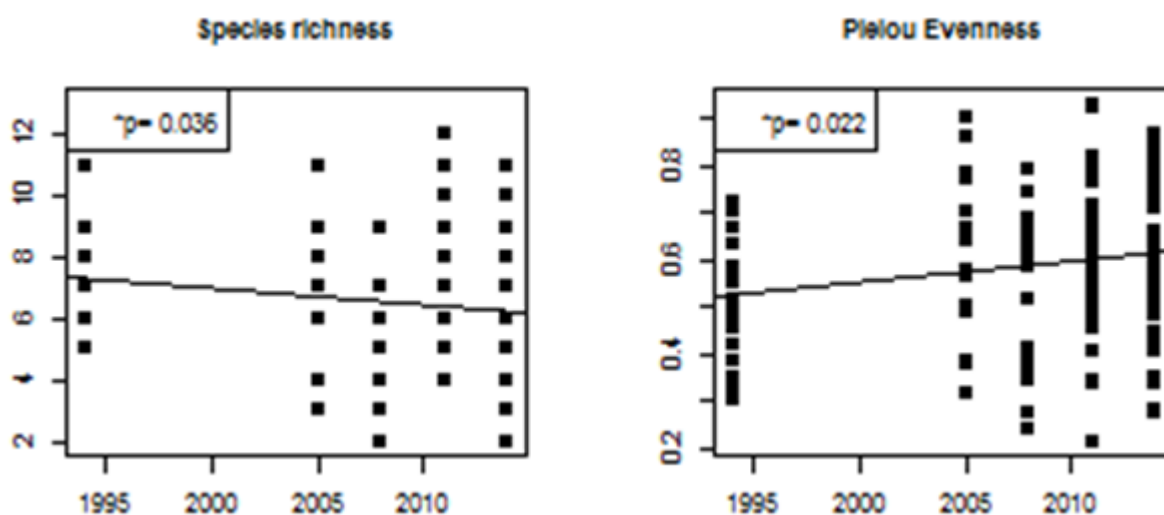


Figure 8. Linear model of species richness and Pielou's evenness for Mark en Vliet between 1994 and 2013. The P-values of the linear model is presented in the top left corner of the plots (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ).

Table 10. Average species richness, Pielou's evenness and Shannon's diversity of the samples collected at the Mark en Vliet between 1994 and 2014. Significant P-values indicate a difference compared to the previous sampling event (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ).

| Waterbody     | Year | Species Richness | Pielou's Evenness | Shannon's Diversity |
|---------------|------|------------------|-------------------|---------------------|
| Mark en Vliet | 1994 | 7,62             | 0,52              | 1,05                |
| Mark en Vliet | 2005 | 6,83             | 0,61              | 1,15                |
| Mark en Vliet | 2008 | 4,94**           | 0,53              | 0,81**              |
| Mark en Vliet | 2011 | 6,41*            | 0,60              | 1,07**              |
| Mark en Vliet | 2014 | 6,66             | 0,62              | 1,12                |

A total of 95.959 individuals and 28 different species were recorded for Mark en Vliet between 1994 and 2014. The total numbers of individuals collected for 1994, 2005, 2008, 2011 and 2014 were respectively 31.180, 1.485, 2.462, 33.769 and 27.062. Most common species in the samples were roach, bream, perch and ruffe with an respectively occurrence of 24.7%, 37.1%, 17.5% and 11.0% of the total individuals. The species gudgeon, prussian carp and crucian carp only occurred in 1994 and disappeared afterwards. The rheophilic species ide occurred in all sampling years, and European bullhead since 2004. Exotic species developed during recent years, where round goby and tubenosed goby were firstly observed in 2014, and pumpkinseed occurred in 2011 and 2014. Exotic asp developed earlier and was firstly observed during the sampling in 2005.

The average species richness of the samples was highest in 1994 with 7.62 species per sample. The average species richness decreased to 6.83 species per sample in 2005. Between 2005 and 2008, the average species richness of the samples decreased significantly to 4.94 species per sample. However, in 2011 the average species richness recovered and increases significantly to 6.41 species per sample and remained relatively comparable in 2014. Total species richness for 1994, 2005, 2008, 2011 and 2014 was respectively 20, 19, 13, 20 and 23 species (appendix 7). The average species richness and total species richness both decreased drastically between 2005 and 2008. The species bleak, three-spined stickleback and tench show a comparable pattern, being present in all sampling years except 2008. Furthermore, the species common carp, bitterling, spined loach and smelt were present in some of the sampling years but remained absent in the samples of 2008. The tench, bitterling and spined loach are species that require the presence of vegetation for shelter. Optionally, disappearance of these species in 2008 could indicate a correlation to a decreasing coverage of vegetation at Mark en Vliet. However, this data was not included for this study and a relation could not be proven.

Moreover, sampling events in 2011 and 2014 had higher sampling intensities compared to the other sampling years. Therefore, it is assumable that the higher total species richness in 2011 and 2014 could be correlated to the higher sampling intensity. Additionally, also three more subareas were sampled which are directly connected to Mark en Vliet and could have influenced the results of 2011 and 2014. For this reason, the diversity indices were also calculated per subarea to have a more transparent overview of the developments (appendix 9). However, no significant influence was observed on the global results when including these subareas.

The average Pielou's evenness of the samples fluctuated between 1994 and 2014, with an increase in 2005 and 2011 and decrease in 2008. Minimum average Pielou's evenness was 0.52 in 1994, and maximum evenness was 0.62 in 2014. Between 2005 and 2008 the biggest difference, and the only decrease in evenness was observed. Pielou's evenness decreases for the samples in 2008, because the numbers of perch and bream increased to respectively 11.9% and 51.9% of the total number of individuals. The distribution among species has been improved between 1994 and 2005, and remained relatively equal when there is not accounted for 2008s sampling event.

Shannon's diversity significantly decreased and increased between 2005 and 2008 and 2008 and 2010. The decreasing average Pielou's evenness and species richness have contributed to the significant decrease in Shannon's diversity between 2005 and 2008. The significant increase in diversity index from 2008 to 2011 was also caused by the average increase of species richness and Pielou's evenness. It is clear that the sampling event of 2008 is extraordinary compared to all other sampling years. Information at the Regional Water Authority Brabantse Delta, indicated that in 2006 and 2007 a blue-green algae bloom occurred in the western part of Mark en Vliet. Additionally, the winter of 2007 was characterized by higher temperature (Oldenborgh, 2015), possibly promoting the algae bloom. For this reason, the flow in the Mark en Vliet was increased by inlet from Amer for dilution and improvement of the water quality. It is possible that the fish assemblages of Mark en Vliet were affected by the increased flow. It is also possible that the blue-green algae bloom restricted the developments of macrophytes, leading to the disappearance of phytophilic fish species in 2008. Finally, it could be possible that fish migrated to the eastern part of Mark en Vliet where conditions were more suitable. Nevertheless, no evidence could be retrieved from the data to confirm this hypothesis regarding migration of the fish assemblages. Furthermore, no global fish mortality was recorded at Mark en Vliet in these periods. Therefore, no clear reason was found during this study to amplify this hypothesis or to explain this explicit decrease of the diversity indices.

### 3.2 Correlation of diversity indices and environmental variables

A Redundancy analysis (RDA) was performed to explain the developments in diversity indices by the available explanatory variables. Datasets of the Galderse Beek and Strijbeekse beek were inadequate for the performance of an RDA due to the small amount of samples. Therefore, the RDA was only performed for Mark en Vliet, and the Boven Mark and Aa of Weerij. Samples collected at the Boven Mark and Aa of Weerij indicated to have a comparable fish assemblage structure (see chapter 4.3) and were therefore clustered together in the analysis. The RDA for the Aa of Weerij and Boven Mark is shown in figure 9 and for the Mark en Vliet the RDA is shown in figure 10. The RDA biplots present only the variables that had a significant contribution for variation within the dataset. The contribution of the variables is represented by length of the arrows in the biplot, where longer arrows have a larger contribution. Furthermore, the explanatory variables are shown by the blue arrows and the response variables (diversity indices) are shown by the red arrows. For the RDA in this chapter, no distinction or correction was applied for the used fishing gear. However, the division into fishing gears showed no obvious differences compared to the total dataset (Aa of Weerij and Boven Mark), or gave less significant results to explain the variation in the data (Mark en Vliet).

#### Aa of Weerij and Boven Mark

The first and second axis explain respectively 72.7% and 19.1% of the total variation in the data of diversity indices for the Aa of Weerij and Boven Mark. Remaindering projects were also included in the RDA for the Aa of Weerij and Boven Mark. However, remaindering projects showed no significant contribution to the model and were therefore excluded in the biplot. The main reason for no significant effect of remaindering projects was probably due to the small amount of samples that were collected in meanders.

For the Aa of Weerij and Boven Mark, all diversity indices showed to have a significant contribution to the model and correspond to both axes. The shorter arrow of Pielou's evenness indicates that variability is less explained by the physical-chemical variables. The diversity indices point in the same direction as the fish passages and saturated oxygen concentration, indicating a positive correlation. Contrastingly, total phosphorus and total nitrogen point in the opposite direction, indicating a negative correlation with the diversity indices. Both relationships are confirmed by their Pearson correlation coefficient and probability value (table 11). Additionally, chloride and conductivity also showed to have a negative correlation with the diversity indices. The physical-chemical variables of pH and transparency showed to have no significant correlation with the diversity indices. In contrast to the diversity indices, the physical-chemical variables of pH corresponds positively and negatively on axis one and axis two. These opposite correlations counteract each other, resulting in no significant correlation of pH to the diversity indices. The arrow of transparency is very short and has therefore no significant correlation to the diversity indices.

These findings indicate that the construction of fish passages and increased oxygen concentrations for the systems of the Boven Mark en Aa of Weerij's contributes for a higher species richness and increased Shannon's diversity. The construction of fish passages has increased the connectivity within the Mark watershed. Connectivity allows the immigration and remigration of dispersal-limited species or inferior competitors (Mouquet and Loreau, 2002 & 2003; Codotte, 2006). Therefore It is assumed that an increased connectivity allows more and different species to inhabit these waterbodies from other parts of the watershed, leading to an increased average species richness and thus Shannon's diversity. Furthermore, oxygen is stated as one of the most important parameter for quality assessments of aquatic environments. The concentration of dissolved oxygen affects many physiological, biochemical and behavioral processes in fish and could change the composition of fish assemblages (Smale and Rabeni 1995; Davis 1975; Kramer 1987). High dissolved oxygen concentrations show a generally consistency with the occurrence of more intolerant fish species. On the other hand, low oxygen concentrations are more characterized by the presence of tolerant species. For the Aa of Weerij's and Boven Mark, an increased concentration of dissolved oxygen was positively correlated to the increase in species richness and Shannon's diversity.

The physical-chemical variables total phosphorus, total nitrogen, conductivity indicated to correlate negatively to species richness and Shannon's diversity and chloride to Pielou's evenness and Shannon diversity. The Shannon diversity is derived from Pielou's evenness and species richness. Therefore, the Shannon's diversity correlates to more physical-chemical variables than Pielou's evenness and species richness. Literature widely explains that biological communities reflect the condition of watersheds (Karr, 1981; Matthews, 1998; Angermeier and Winston, 1998). Degradation of systems is often associated with decreases fish richness, abundances and diversity and could lead to community shifts from intolerant to tolerant species (Klein, 1979; Walters et al., 2003; Helms et al., 2005). The physical-chemical variables total phosphorus and total nitrogen combine the most important nutrients and reflect the productivity of a system. Furthermore, conductivity is widely used as a measure for pollutions by salts and metals in aquatic systems, including chloride. For this reason, increased levels of total phosphorus, total nitrogen, conductivity and chloride are associated with degradation of the environmental quality, and therefore a decrease in species richness and Shannon diversity.

Conclusively, For the Aa of Weerij's and Boven Mark, an increased concentration of dissolved oxygen and the construction of fish passages were positively correlated to the increase in species richness and Shannon's diversity. Furthermore, decreasing concentrations of the physical-chemical variables of total phosphorus, total nitrogen, conductivity and chloride were negatively correlated to the increase in species richness and Shannon's diversity. Results obtained from the RDA of the Aa of Weerij's and Boven Mark are indicative, but clear in accordance to the theoretical backgrounds.

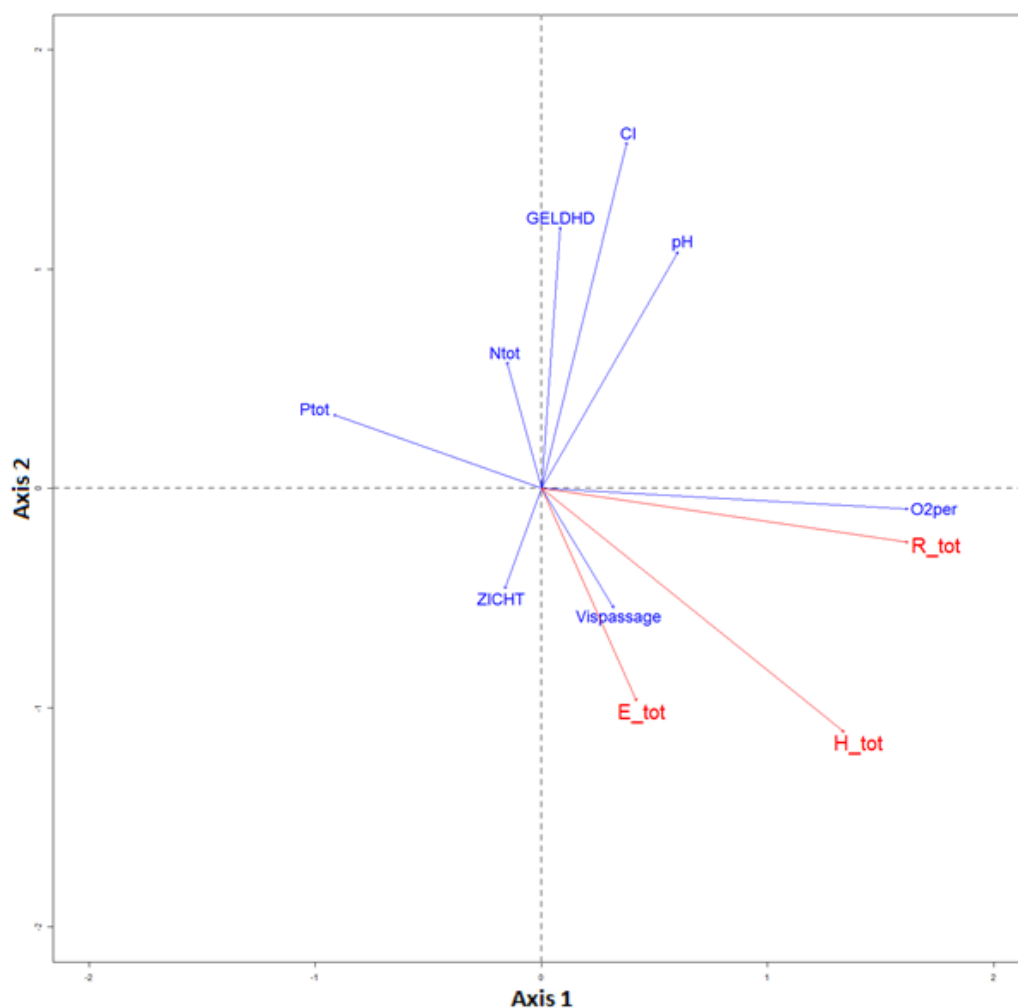


Figure 9. RDA of the response variables (diversity indices) and explanatory variables (physical-chemical data and executive measures) for the Aa of Weerijns en Boven Mark (N = 132). The diversity indices are shown by the red arrows, where E\_tot is Pielou's evenness, R\_tot is species richness and H\_tot is Shannon's diversity. The explanatory variables are shown by the blue arrows.

Table 11. Significant Pearson correlation coefficients (r) and their probability value (p) for the diversity indices and the relevant explanatory variables for the Aa of Weerijns and Boven Mark.

| Variable 1          | Variable 2                     | r     | p-value |
|---------------------|--------------------------------|-------|---------|
| Species richness    | Total phosphorus               | -0.42 | < 0.001 |
| Species richness    | Total nitrogen                 | -0.39 | < 0.001 |
| Species richness    | Fish passage                   | 0.38  | < 0.001 |
| Species richness    | Saturated oxygen concentration | 0.35  | < 0.001 |
| Species richness    | Conductivity                   | -0.24 | 0.006   |
| Pielou's evenness   | Chloride                       | -0.20 | 0.020   |
| Shannon's diversity | Saturated oxygen concentration | 0.31  | < 0.001 |
| Shannon's diversity | Total phosphorus               | -0.27 | 0.002   |
| Shannon's diversity | Fish passage                   | 0.26  | 0.003   |
| Shannon's diversity | Conductivity                   | -0.24 | 0.006   |
| Shannon's diversity | Total nitrogen                 | -0.21 | 0.015   |
| Shannon's diversity | Chloride                       | -0.17 | 0.049   |

## Mark en Vliet

The first and second axis of the RDA explain respectively 85.2% and 10.3% of the total variation in the data of diversity indices for the Mark en Vliet (figure 10). Shannon's diversity was included in the RDA analysis, but showed no significant contribution to the model and was excluded in the biplot. As shown in the biplot, species richness and Pielou's evenness point in the opposite direction, indicating a negative correlation. The Pearson correlation amplifies this relationship ( $r = -0.21$ ,  $p = 0.01$ ).

Therefore, Shannon's diversity which is derived from species richness and Pielou's evenness will have no significant contribution in the model for Mark en Vliet. No constructions of fish passages or remaindering project were performed at Mark en Vliet, so only the available physical-chemical variables were included for the analysis.

Species richness and Pielou's evenness showed a significant contribution to the model of the Mark en Vliet and correspond strongly to axis 1. Species richness and Pielou's evenness point in the opposite direction, meaning that correlations with the physical-chemical variables are also adverse. Species richness points in the same direction as conductivity, pH, chloride and saturated oxygen, indicating a positive correlation. The other way around, Pielou's evenness indicates to correlate negatively to the physical-chemical variables of conductivity, pH, chloride and saturated oxygen. The Pearson correlation coefficient and probability value amplify most of these relationships (table 12). However, the different direction of the saturated oxygen arrow and shorter length of the pH arrow shows to have no significant correlation to Pielou's evenness. Moreover, the physical-chemical variables ammonium, total phosphorus, temperature and transparency show to have no significant correlation with species richness and Pielou's evenness. Arrows of ammonium, total phosphorus, temperature and transparency are relatively short, indicating a moderate contribution to species richness and Pielou's evenness. Therefore, no significant correlations were observed between species richness and Pielou's evenness.

The RDA indicates that species richness increases when the concentrations of GELDHD, pH, Chloride and saturated oxygen increases. The positive correlation between species richness and dissolved oxygen is logically explainable. However, a positive correlation between species richness and the physical-chemical variables conductivity, pH and chloride is less understandable. Increased values of physical-chemical variables conductivity, pH and chloride are generally associated with environmental degradation. Therefore, it would be expected that species richness would decrease as a result of increasing values of conductivity, pH and chloride. However, the annual mean concentration of total phosphorus and total nitrogen decrease over time (appendix 8), while average species richness also decreases (table 10). fish assemblages are affected by much more factors than were included for this study. Therefore, it could be assumed that more variables should be included in order to conduct an explanation for the observed developments in species richness. Moreover, the results of the diversity indices for Mark en Vliet showed a depression of the freshwater fish assemblage during the sampling event of 2008. It is assumed that this depression is caused by external conditions that were not included in the analysis because no extreme concentrations of the physical-chemical variables were noted. This depression in 2008 probably has influenced the results of the RDA. RDA assumes linear relationships between response and explanatory variables. It is possible that the direction of the correlation becomes reversed in case of a depression in the middle of a time series analysis. This assumption could also explain the opposite direction of the arrows in the biplot for species richness and Pielou's evenness. Another explanation could be that more subareas were sampled in 2011 and 2014. The addition of these subareas, with different values for the physical-chemical variables could also reduce accuracy of the linear assumptions.

The physical-chemical variables conductivity, pH and chloride indicate to correlate negatively to Pielou's evenness. This result is comparable to the results obtained from the data of the Aa of Weerijis and Boven Mark. As mentioned before, increase of the physical-chemical variables conductivity, pH and chloride are generally associated with environmental degradation. Environmental degradation affects the fish assemblages and is often described by shifts from intolerant to tolerant species that become dominant. Therefore it is assumable that the fish assemblages of Mark en Vliet becomes more dominated by tolerant species when values of conductivity, pH and chloride increases. For this reason, the abundances among species become less similar, leading to a decrease in Pielou's evenness.

Conclusively for Mark en Vliet, a decreased concentration of the physical-chemical variables chloride, conductivity, pH and saturated oxygen concentration were positively correlated to the decrease in species richness. Furthermore, decreased concentrations of the physical-chemical variables chloride and conductivity were negatively correlated to the increase of Pielou's evenness. Results obtained from the RDA of Mark en Vliet are indicative and difficult to explain in accordance to the theoretical backgrounds.

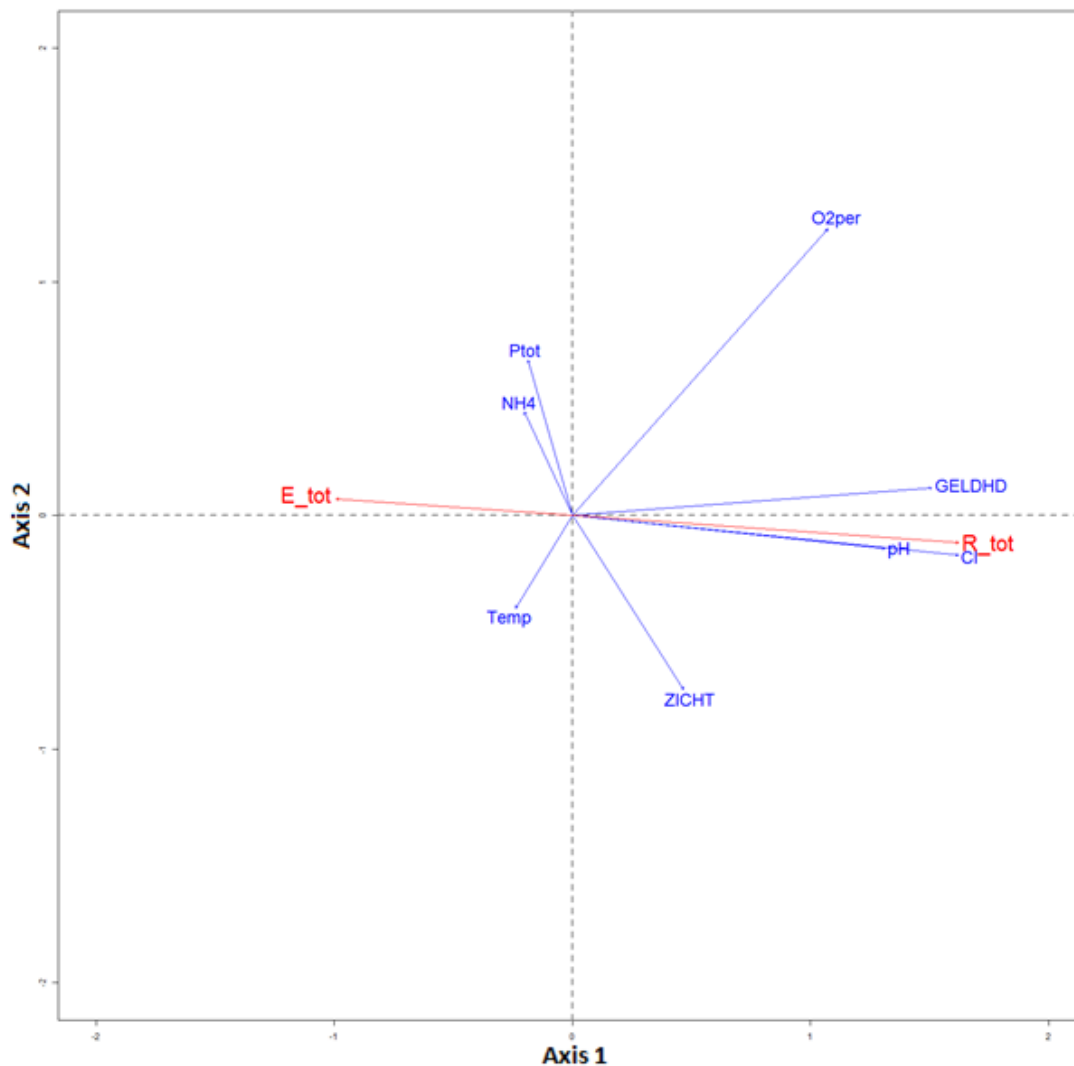


Figure 10. RDA of the response variables (diversity indices) and explanatory variables (physical-chemical data) for Mark en Vliet (n = 145). The diversity indices are shown by the red arrows, where E\_tot is Pielou's evenness and R\_tot is species richness. The contributing explanatory variables of the model are shown by the blue arrows.

Table 12. Significant Pearson correlation coefficients (r) and their probability value (p) for the diversity indices and the relevant explanatory variables for Mark en Vliet.

| Variable 1        | Variable 2                     | R     | p       |
|-------------------|--------------------------------|-------|---------|
| Species richness  | Chloride                       | 0.34  | < 0.001 |
| Species richness  | Conductivity                   | 0.38  | < 0.001 |
| Species richness  | pH                             | 0.28  | < 0.001 |
| Species richness  | Saturated oxygen concentration | 0.21  | 0.013   |
| Pielou's evenness | Chloride                       | -0.21 | 0.010   |
| Pielou's evenness | Conductivity                   | -0.18 | 0.034   |

### 3.3 NMDS of the freshwater fish assemblages

A total of 206.945 individuals and 32 different fish species were recorded in the 454 included samples for this study. Roach, bream and perch were most abundant species and accounted for respectively 30.9%, 23.4% and 14.1% of the total number of individuals (table 13). Furthermore, abundance of the fish species gudgeon, rudd, ruffe, stone loach, nine-spined stickleback, sunbleak, ide, white bream and bleak accounted for 1% or more of the total individuals. Relative occurrence of the fish species in the samples shows that gudgeon, roach, bream, perch, European eel, pike, rudd, stone loach occur in most samples with a frequency higher than 30%. Contrastingly, bitterling, topmouth gudgeon, grass carp, chub, tubenosed goby, crucian carp, smelt and round goby show to have lowest relative occurrence in the samples with a frequency less than 3%, and were not included to overcome overweight of rare species. Additionally, a total of 427 individuals of hybrids fish were observed in the 454 samples, accounting for 0.2% of the total individuals. Hybrids are not accounted as a specific fish species and were excluded during the analysis.

Table 13. Relative occurrence and proportion of the fish species in the samples (N=454). The Dutch, English and scientific names are included.

| Species code | Common Dutch name         | Common English name      | Latin name                              | Frequency in samples (%) | % of the total individuals |
|--------------|---------------------------|--------------------------|---|--------------------------|----------------------------|
| AL           | Alver                     | Bleak                    | <i>Alburnus alburnus</i>                | 6.1                      | 1.4                        |
| BA           | Baars                     | Perch                    | <i>Perca fluviatilis</i>                | 75.2                     | 14.1                       |
| BE           | Bermpje                   | Stone Loach              | <i>Barbatula barbatula</i>              | 32.2                     | 2.6                        |
| BI           | Bittervoorn               | Bitterling               | <i>Rhodeus sericeus amarus</i>          | 2.6                      | 0.0                        |
| BV           | Blankvoorn                | Roach                    | <i>Rutilus rutilus</i>                  | 70.7                     | 30.9                       |
| BD           | Blauwband                 | Topmouth gudgeon         | <i>Pseudorasbora parva</i>              | 2.2                      | 0.0                        |
| BR           | Brasem                    | Bream                    | <i>Abramis brama</i>                    | 40.0                     | 23.4                       |
| DD           | Driedoornige stekelbaars  | Three spined Stickleback | <i>Gasterosteus aculeatus aculeatus</i> | 15.9                     | 0.6                        |
| GI           | Gibel                     | Prussian Carp            | <i>Carassius gibelio</i>                | 3.3                      | 0.0                        |
| GK           | Graskarper                | Grass Carp               | <i>Ctenopharyngodon idella</i>          | 0.2                      | 0.0                        |
| KA           | Karper                    | Common Carp              | <i>Cyprinus carpio carpio</i>           | 11.8                     | 0.0                        |
| KM           | Kleine modderkruiper      | Spined Loach             | <i>Cobitis taenia</i>                   | 5.9                      | 0.0                        |
| KB           | Kolblei                   | White Bream              | <i>Blicca bjoerkna</i>                  | 17.4                     | 1.0                        |
| KV           | Kopvoorn                  | Chub                     | <i>Leuciscus cephalus</i>               | 2.2                      | 0.0                        |
| KK           | Kroeskarper               | Crucian Carp             | <i>Carassius carassius</i>              | 0.7                      | 0.0                        |
| MG           | Marmergroundel            | Tubenosed Goby           | <i>Proterorhinus marmoratus</i>         | 1.3                      | 0.0                        |
| PA           | Paling                    | European Eel             | <i>Anguilla anguilla</i>                | 47.0                     | 0.9                        |
| PO           | Pos                       | Ruffe                    | <i>Gymnocephalus cernua</i>             | 29.8                     | 5.3                        |
| RD           | Rivierdonderpad           | European Bullhead        | <i>Cottus gobio</i>                     | 4.8                      | 0.0                        |
| RG           | Riviergrondel             | Gudgeon                  | <i>Gobio gobio</i>                      | 42.6                     | 5.7                        |
| RB           | Roofblei                  | Asp                      | <i>Aspius aspius</i>                    | 10.0                     | 0.1                        |
| RV           | Ruisvoorn                 | Rudd                     | <i>Scardinius erythrophthalmus</i>      | 43.9                     | 5.7                        |
| SK           | Snoek                     | Pike                     | <i>Esox lucius</i>                      | 60.4                     | 0.7                        |
| SB           | Snoekbaars                | Zander                   | <i>Sander lucioperca</i>                | 13.5                     | 0.5                        |
| SP           | Spiering                  | Smelt                    | <i>Osmerus eperlanus</i>                | 0.7                      | 0.0                        |
| TD           | Tienddoornige stekelbaars | Nine-spined Stickleback  | <i>Pungitius pungitius</i>              | 22.2                     | 3.2                        |
| VE           | Vetje                     | Sunbleak                 | <i>Leucaspius delineatus</i>            | 15.4                     | 1.8                        |
| WI           | Winde                     | Ide                      | <i>Leuciscus idus</i>                   | 29.8                     | 1.2                        |
| ZE           | Zeelt                     | Tench                    | <i>Tinca tinca</i>                      | 29.4                     | 0.6                        |
| ZB           | Zonnebaars                | Pumpkinseed              | <i>Lepomis gibbosus</i>                 | 10.2                     | 0.1                        |
| ZW           | Zwartbekgrondel           | Round goby               | <i>Neogobius melanostomus</i>           | 1.7                      | 0.0                        |

The NMDS in figure 11 was performed as orientation tool to distinguish the differences between water types and fishing gears. A simpler analysis was performed to note the biggest differences between the fishing gears and water types (appendix 10). Waterbodies were categorized into water types according to their dimensions, and shown by the different colors in the NMDS biplot. Differences between fishing gear is shown by the hulled lines. The NMDS reveals that community structure of the freshwater fish assemblage differs between the type of water and type of fishing gear. More defined NMDS are presented in appendix 11 and 12, where water types were separated and presented by the used fishing gears.

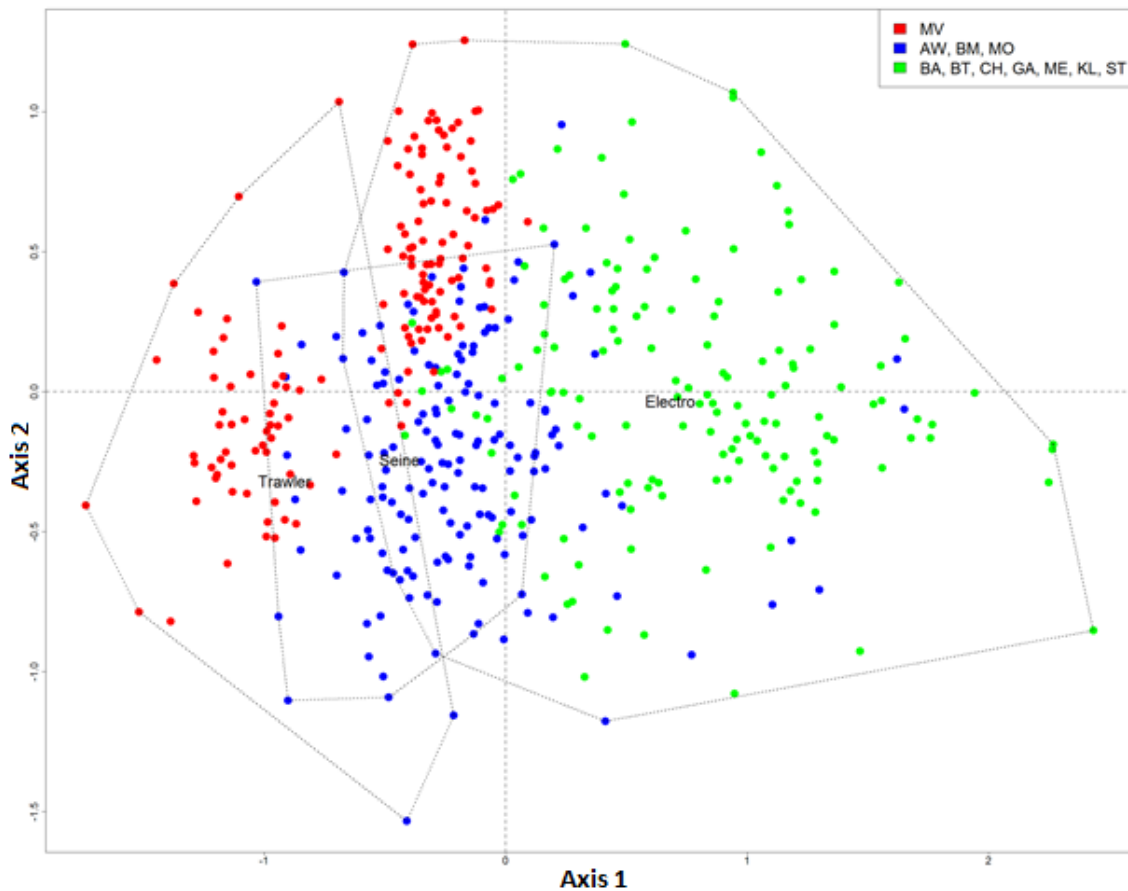


Figure 11. NMDS of all samples ( $n = 454$ ) divided into fishing gear (hulled line per gear) and waterbodies (colors) with relatively comparable community structure of the freshwater fish assemblages. Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted in two dimensions with 0.185 stress.

Samples collected by trawl nets and electro fishing are clearly different from each other and have almost no overlap. Samples collected by the purse seine are located in between the collected samples by the trawl nets and electro gear. The small streams (green) of the watershed exist of seven different waterbodies and were all sampled by electro fishing, mostly wadably. Variation in fish assemblages of the seven small streams is expressed by dispersion of the samples in the NMDS biplot. The small rivers (blue) were sometimes sampled by trawl nets, but mostly by purse seine and electro gear. The large river (red) was sampled by trawl nets and electro fishing gear and shows two distinct clusters for the used fishing gears. For the clusters of waterbodies, the simpler analysis shows that fish samples of Mark en Vliet are characterized by higher abundances of perch, bream and ruffe compared to the other waterbodies. The Aa of Weerijs, Boven Mark and Molenbeek are characterized by higher abundances of roach and rudd. The seven small streams of the Mark watershed are characterized by higher abundances of gudgeon, stone loach and nine-spined stickleback.

The percentage of the total individuals per species shows that the freshwater fish assemblages of the Mark watershed are mostly dominated by the fish species perch, roach and bream (table 13). Simper analysis showed that these species occur mostly in the large and small rivers. These larger waters often contain higher densities of fish compared to the small streams and mainly eurytopic species, leading to a dominant position of perch, roach and bream of the total freshwater fish assemblage. moreover, the applied sampling method and fishing gear is chosen by the dimensions and accessibility of the waters. Therefore, Mark en Vliet is the only waterbody that was sampled frequently by the trawl gear, and perch, bream and ruffe are very abundant in samples collected at Mark en Vliet and the trawl gear. Furthermore, the differences in fishing gear could be explained by the sampling location. Electro trajectories in wider streams are applied close at the shores, while trawler and purse seines are more used in the open water. Species specific habitat preference explains therefore much of the differences obtained by the fishing gears.

The purse seine gear is located in the middle of the NMDS, indicating comparability with both the electro and trawler gear. This could be explained by the fact that with purse seine nets parts of the open water and shores are both covered. Therefore, it is explainable that samples of the purse seine are located between the samples collected by trawl nets or electro gear. Moreover, contrast shows that roach, perch and pike are the most common species in the samples collected by purse seine fishery (appendix 10). These species occur throughout the whole river basin, including the small streams. contrast of bream in the samples is lower because they are mostly not present in the small streams. On the other hand, the species nine-spined stickleback, three-spined stickleback, stone loach and gudgeon are mostly absent or less present in larger waters and show for that reason a lower frequencies in the samples.

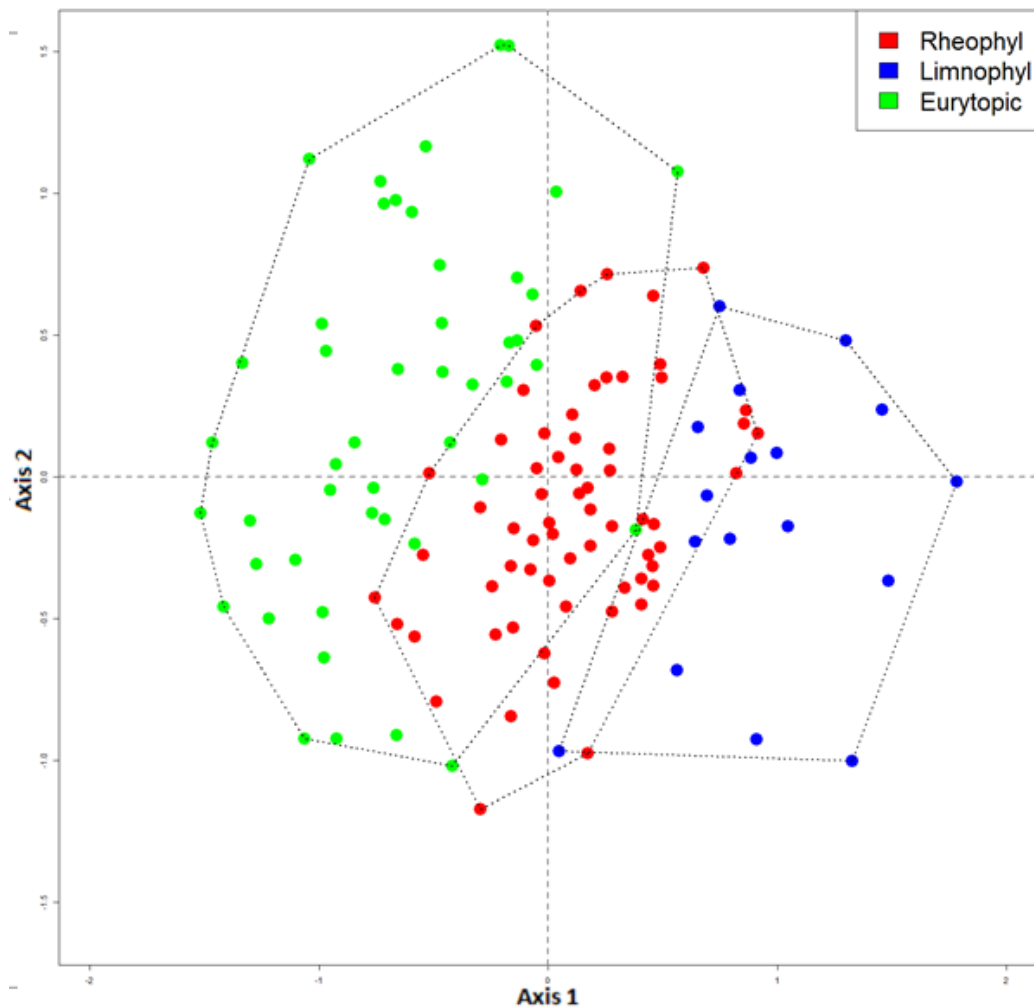


Figure 12. NMDS of all samples from the small streams ordered by the dominance of guilds. Dominant guild of a sample was accounted if more than 50% of the fish species belongs for a specific guild (N = 132). Locations of the samples within the NMDS are based on a square root transformation of the total numbers of fish species, plotted in two dimensions with 0.192 stress.

Dispersion of the small streams was further analyzed by a NMDS divided into dominant guilds (figure 12). In total, 17 samples were excluded because these samples existed of a mixture of at least two guilds that didn't form any cluster, causing noise in the plot. The NMDS shows a clear division with no overlap between the cluster of eurytopic and limnophylic guilds. The rheophylic guild is located in between the eurytopic and limnophylic guild, and shows more overlap with the eurytopic guild than with the limnophylic guild. Eurytopic fish species have no specific habitat requirements compared to limnophylic fish species. Therefore, more overlap between rheophylic and eurytopic dominated samples can be explained. Moreover, samples were all collected in the small streams of the river basin. Fish species of the rheophylic guild are relatively well represented in the samples due to environmental characteristics of the streams. Therefore, the location of the rheophilic guild is in the middle of the NMDS is logical.

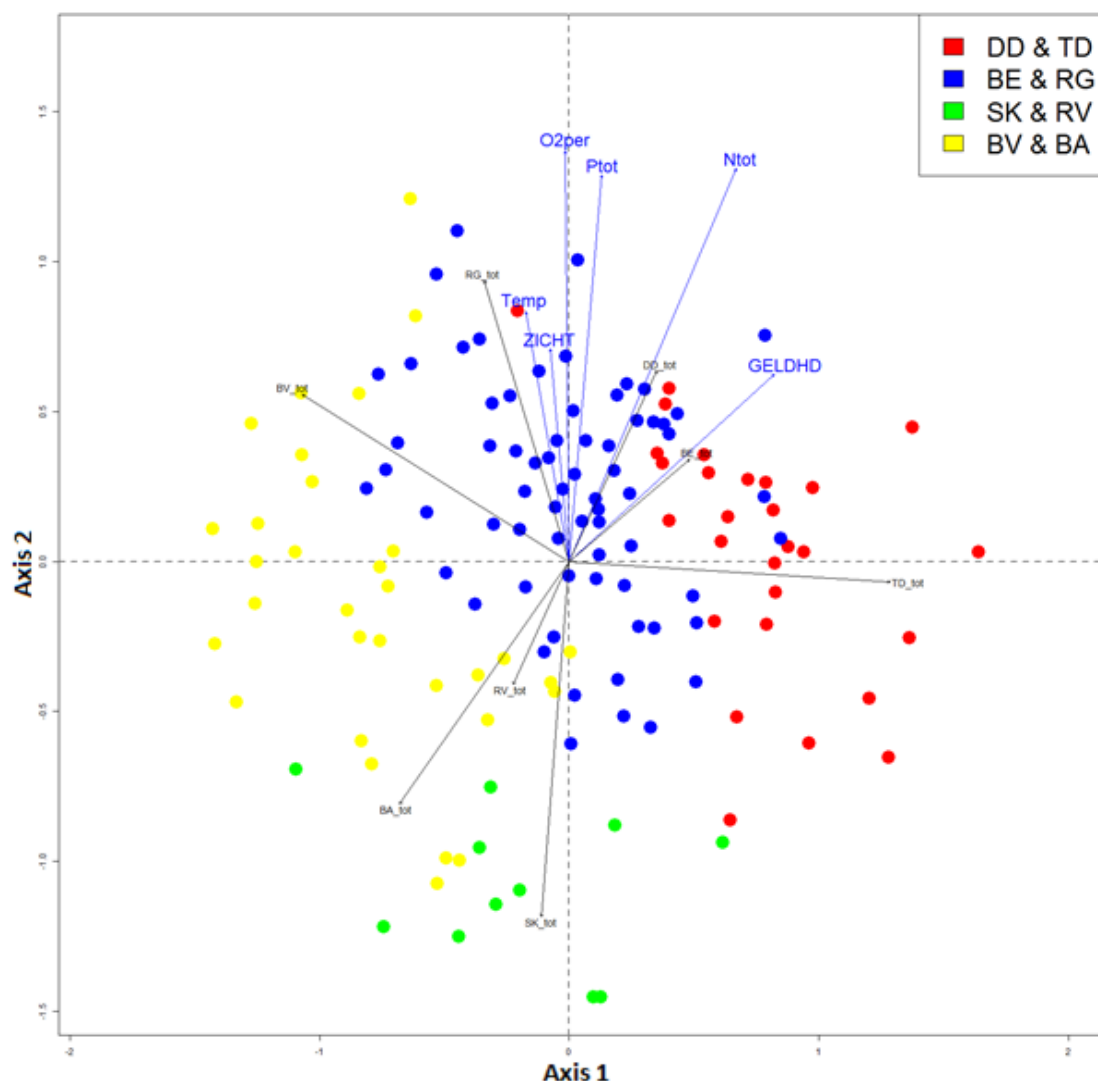


Figure 13. NMDS of all samples from the small streams where more than 40% of the fish accounted for a specific fish community (N = 147). Locations of the samples within the NMDS are based on a square root transformation of the total numbers of fish species, plotted in two dimensions with 0.199 stress.

Table 14. Significant Pearson correlation coefficients (r) and their probability value (p) for fish communities in the small streams of the Mark watershed and physical-chemical variables.

| Physical-chemical parameter    | Fish specie              | r     | p      |
|--------------------------------|--------------------------|-------|--------|
| Total phosphorus               | perch                    | -0.19 | 0.022  |
| Total nitrogen                 | perch                    | -0.28 | <0.001 |
| Saturated oxygen concentration | perch                    | -0.32 | <0.001 |
| Temperature                    | three-spined stickleback | 0.16  | 0.045  |
| Temperature                    | stone loach              | -0.25 | 0.002  |
| Conductivity                   | nine-spined stickleback  | 0.20  | 0.014  |
| Total nitrogen                 | gudgeon                  | 0.24  | 0.003  |
| Saturated oxygen concentration | gudgeon                  | 0.47  | <0.001 |

The small streams were further divided into fish communities to understand the relative influence of physical-chemical parameters on the most common fish species (figure 13). Fish communities were accounted when 40% of the total number of individuals accounted for a specific community. The communities DD & TD and BE & RG were selected according to literature, where is described that DD & TD communities are mostly observed in upstream parts and BE & RG communities mostly in downstream parts of small watercourses. Fish communities BV & BA and SK & RV were selected according to observation of the data because they often occurred together. All fish communities show a clustering pattern in the ordination, where DD & TD communities are located on the right, BV & BA communities on the left, SK & RV below, and BE & RG around the center of the NMDS. All the physical-chemical variables point in the same direction of the NMDS. Two clear separation in clusters can be distinguished, where SK & RV and BE & RG are separated, and DD & TD and BV & BA are separated. This plot indicates that samples of the small streams are partly explained by the physical-chemical variables. The fish species gudgeon, three-spined stickleback and stone loach indicate to correlate positively to the physical-chemical variables. On the other hand, pike, perch, rudd point in the opposite direction, indicating a negative correlation to the physical-chemical variables. The numbers of roach and nine-spined stickleback correlate stronger to axis one compared to the other fish species. This indicates that conductivity, the strongest to axis one correlating physical-chemical variable, could positively correlate to the number of nine-spined stickleback and negatively to the number of roach.

Pearson correlation analysis shows that 8 significant correlations exist between the fish species of the fish communities and the physical-chemical variables for the small streams (table 14). The species perch significantly correlates negatively to physical-chemical variables of total nitrogen, total phosphorus and saturated oxygen. Contrastingly, gudgeon significantly correlates positively to the physical-chemical variables of total nitrogen and saturated oxygen. These correlations indicate that concentrations of total phosphorus, total nitrogen and saturated oxygen significantly contribute to the presence/absence of perch and gudgeon. However, fish species are affected by much more conditions than only physical-chemical variables. Therefore, this information will be only applicable when the effect of more variables on the fish assemblages are studied. Moreover, the species three-spined stickleback and stone loach indicate to significantly correlate with temperature of the aquatic system. Temperature is known to be a key factor for metabolism, survival, recruitment and behavior of freshwater fish species and affects many other factors. Biological needs of these species seems to consequently differ in terms of temperature. Finally, the species nine-spined stickleback significantly correlates positively to conductivity. conductivity is applied as a measure for pollution by salts within an aquatic environment. nine-spined stickleback is known to be tolerant for high salinity and also occurs in brackish environments. Therefore, a positive correlation between conductivity and the numbers of nine-spined stickleback could be expected.

## 4. Conclusion

The analysis of diversity indices has resulted in several conclusions for the Mark watershed between 1994 and 2014. Trends in the linear model of diversity indices greatly differ among waterbodies of the Mark watershed. Watercourses of the Bavelse Leij, Chaamse Beken, Kleine Beek, Molenbeek, Bijloop-Turfvaart and Merkske showed no significant developments between 1994 and 2014 according to the linear model of diversity indices. Diversity indices of the Galderse Beek show a significant decrease between 1994 and 2013, caused by higher abundances of stone loach and nine-spined stickleback and disappearance of common carp, roach, rudd and tench. The Strijbeekse Beek showed a significant decrease in Pielou's evenness and Shannon's diversity between 1994 and 2013, caused by an exponential increase in the numbers of gudgeon and stone loach. Mark en Vliet showed a decreasing trend in diversity indices between 1994 and 2014, caused by a significant depression of the diversity indices in 2008. Species richness and Pielou's evenness for Mark en Vliet correlate respectively positive and negative to the physical-chemical variables Chloride, conductivity, oxygen and pH. Diversity indices for the Aa of Weerijis show a significant gradual increase between 1994 and 2013. Diversity indices for the Boven Mark show a variable pattern between 1994 and 2013, where species richness increases and Pielou's evenness and Shannon's diversity fluctuate. Diversity indices for the Aa of Weerijis and Boven Mark correlate positively to fish passages and oxygen concentration, and negatively to the concentrations of total phosphorus, total nitrogen, conductivity and chloride.

More conclusions were formed during the analysis of fish assemblages in the Mark watershed. The species bream, roach and perch showed highest proportions of the total number of individuals in samples of the Mark watershed. The species pike, roach and perch showed highest occurrence in samples of the Mark watershed. Fish assemblages differ significantly among waterbodies within the Mark watershed. Fish assemblage of Mark en Vliet is characterized by high abundances of bream, perch and ruffe. Fish assemblages of most small streams within the Mark watershed are characterized by high abundances of gudgeon, stone loach and nine-spined stickleback. Fish assemblages of the Aa of Weerijis, Boven Mark and Molenbeek are characterized by higher abundances of roach and rudd compared to the other watercourses. Samples collected at the small streams of the Mark watershed were successfully categorized by dominant fish communities of TD & DD, BE & RG, SK & RV and BA & BV. Concentrations of total phosphorus, total nitrogen and oxygen correlates positively to the number of gudgeon and negatively to the number of perch in the small streams of the Mark watershed. Temperature correlates positively to the numbers of three-spined stickleback and negatively to the numbers of stone loach in the small streams of the Mark watershed. Conductivity correlates positively to the numbers of nine-spined stickleback in the small streams of the Mark watershed.

## 5. Recommendations

After performance of this study, several recommendations were formed for practical performance of fish surveys and perspectives for further research.

### Methodological recommendations

During this study it became clear that most of the data was obtained by the performance of random sampling within the waterbodies. Sampling intensity where 7.5% of the total surface of a waterbody is sampled by trajectories of 250 meters are assumed to give a representative indication of the fish assemblages. Exceptionally, Mark en Vliet where sampling intensity often is lower due to the large dimensions of the watercourse. However, some of the small streams of the Mark watershed are characterized by much environmental differentiation resulting in many different microhabitats. Each specific microhabitat favors the occurrence of different species and abundances. It is questionable if sampling of different locations in the small streams, every sampling year is the best solution to collect comparable and reliable information. To improve comparability and reliability of the samples over time, it would be recommended for the small streams of the Mark watershed to sample the same locations repeatedly over time (if practicably possible). The small and large rivers are characterized by a more monotone character which is less affected by environmental variation. Therefore, sampling at the same locations is potentially less important for the larger waterbodies in the Mark watershed. However, also for these waterbodies the preference is to sample the same locations repeatedly over time.

The sampling period showed much variation between sampling events, where all samples were collected between May and December and most intensive between July and August. Due to this inadequate sampling period it is expectable that seasonal changes in fish assemblages occurred within the data. Seasonal variation in the data is undesirable for studying time series, and makes interpretation of the results difficult. To improve the data collection for time series analysis, it would be recommended to always sample in the same period of the year to avoid seasonal variation. This methodology is performed since the implementation of handboek hydrobiology (2010) and is intended to be proceeded in following years. Furthermore, analysis of the collected fish assemblages showed a clear difference between the type of fishing gears used. Therefore, as handboek hydrobiologie (2010) describes, it would be recommended to consider previous sampling events and to use the same type of gear. Additionally, it would be recommended to measure physical-chemical, ecological and hydro-morphological parameters at the same moment of the fish surveys. However, fish assemblages are sampled once every three year and adapt during longer periods, making it difficult to find the best fit model for comparison to physical-chemical variables. In current study, annual year averages were used as explanatory variables, lacking resolution of the data the specific fish survey events. It's most important that sampling of the fish assemblages is organized according to the aim of the assessment.

## Further research

This study focuses on the number of fish species divided into length categories. However, the provided data shows more resolution and thus potential for further research. Moreover, the fish assemblages are planned to be monitored very intensively in 2016. Addition of this data could be used to get more insights in recent developments of the freshwater fish assemblages.

From this point of study, it would be recommended to use more relevant explanatory variables. Freshwater fish assemblages are known to be affected by many factors, such as, water flow, velocity, vegetation and shading. Inclusion of those parameters in a study would improve the statistical models and explain the developments in fish assemblages more accurate. When more explanatory variables are included, it becomes possible to analyze the interaction between explanatory variables and their combined effect on the freshwater fish assemblages. Furthermore, fish species will react differently to explanatory variables. For this reason, it could be recommended to perform a study on specific target species to understand their interaction to explanatory variables and to adapt the applied management purposes to the desired target species.

Since implementation of the WFD, surveys are performed during the summer period after spawning season of most fish species. In this period, fishes often occur in high numbers due to spawning events and are relatively equal distributed within waterbodies. Environmental fluctuations of controlling factors for fish can lead to significant differentiations in reproductive success. During this study, the number of fish species separated into length categories were used for the analysis. However, the length and numbers of individual species can be used to calculate biomass of the collected fishes, providing another dimension within the data. Advantage of using biomass instead of numbers is that overweight of small individuals in samples are balanced due to their small weight. On the other hand, this methodology is sensitive overweight of large individuals. Nevertheless, it could be possible that fish biomass shows improved relationships to environmental variables compared to the numbers of fish.

The planned fish surveys for 2016 could help to support the assumptions / explanation that were formulated in this study. For the Galderse Beek it is still unclear why the total number of individuals were remarkably low in 2010. The data of 2016 could be used to indicate more accurately if stone loach and nine-spined stickleback, as relatively short living species, benefitted from a cold winter in 2010 and grew to dominant numbers in 2013. Moreover, resistance of the populations of stone loach and nine-spined stickleback at the Galderse Beek could indicate that environmental quality improved for these species. Furthermore, data collection in 2016 could be used to see if the increased total number of individuals at small streams is a temporary occurrence or serious trend over time as effect of environmental changes. Finally, sampling locations in 2016 should be chosen in accordance to previous events. Addition of the 2016 data, provides time series of data for some specific locations. It could also be recommended to perform a time series analysis on specific locations to understand site specific developments in fish assemblages.

In recent years, the exotic fish species Tubenosed Goby and Round Goby have been observed in the Mark en Vliet. In 2014 the total numbers were relatively low, however, these species are known to be very opportunistic. Moreover, the species are known to be very territorial, aggressive and to predate on fish eggs and larvae. Therefore, these species are assumed to form an optional treat for native bottom species such as, Stone Loach, Gudgeon and European Bullhead. The exotic fish species pumpkinseed also colonized the Mark watershed and was firstly observed in the Aa of Weerijns and Boven Mark in 1994. Nowadays, pumpkinseed is also observed in the Chaamse Beken, Strijbeekse Beek, Merkske, Molenbeek and Mark en Vliet. Between 1994 and 2014, pumpkinseed was observed more often and higher numbers, meaning that reproduction is successfully in the Mark watershed. Sampling event of 2016 will result in more information regarding the distribution and development of this exotic species in the Mark watershed. Consequently, it would be recommended to closely observe developments of these species and try to predict future developments of the species in order to formulate antecedent management goals.

Four of the seven small streams in the Mark watershed showed an increased total number of individuals over time (appendix 13). This development could indicate a positive trend over time for the stream fish assemblages, optionally caused by an increased environmental quality. Moreover, these observations indicate that in some of the streams, a dominant position is possessed by some of the desired, characterizing fish communities. Due to lack of time during this study it wasn't possible to scope for the developments in fish communities in individual streams. However, observations of shifts in total number of individuals and fish communities could provide useful information for management. For further research it would be recommended to focus more on developments of individual species and fish communities in relation to environmental variables for better understanding of the interacting mechanisms.

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## Appendix 1 WFD evaluation metrics for abundances

An overview of the evaluation metrics for abundances of fishes in the watercourses of the Mark watershed is shown in table 15.

Table 15. Overview of the evaluation metrics for fish abundances at the studied watercourses.

|                           | Excellent | Good  | Moderate | Insufficient | Bad   |
|---------------------------|-----------|-------|----------|--------------|-------|
| <b>Small streams</b>      |           |       |          |              |       |
| Habitat sensitive species | 20-90     | 15-20 | 10-15    | 5-10         | 0-5   |
| Migratory species         | 95-100    | 95-85 | 50-85    | 30-50        | 10-30 |
| <b>Large streams</b>      |           |       |          |              |       |
| Habitat sensitive species | 50-90     | 40-50 | 30-40    | 20-30        | 5-20  |
| Migratory species         | 95-100    | 90-95 | 60-90    | 20-60        | 0-20  |
| <b>Rivers</b>             |           |       |          |              |       |
| Habitat sensitive species | 70-90     | 50-70 | 30-50    | 20-30        | 5-20  |
| Migratory species         | 95-100    | 90-95 | 60-90    | 20-60        | 0-20  |

## Appendix 2 Map of the Mark watershed

Overview of the Mark watershed including names of the waterbodies and large cities in the area (figure 14).

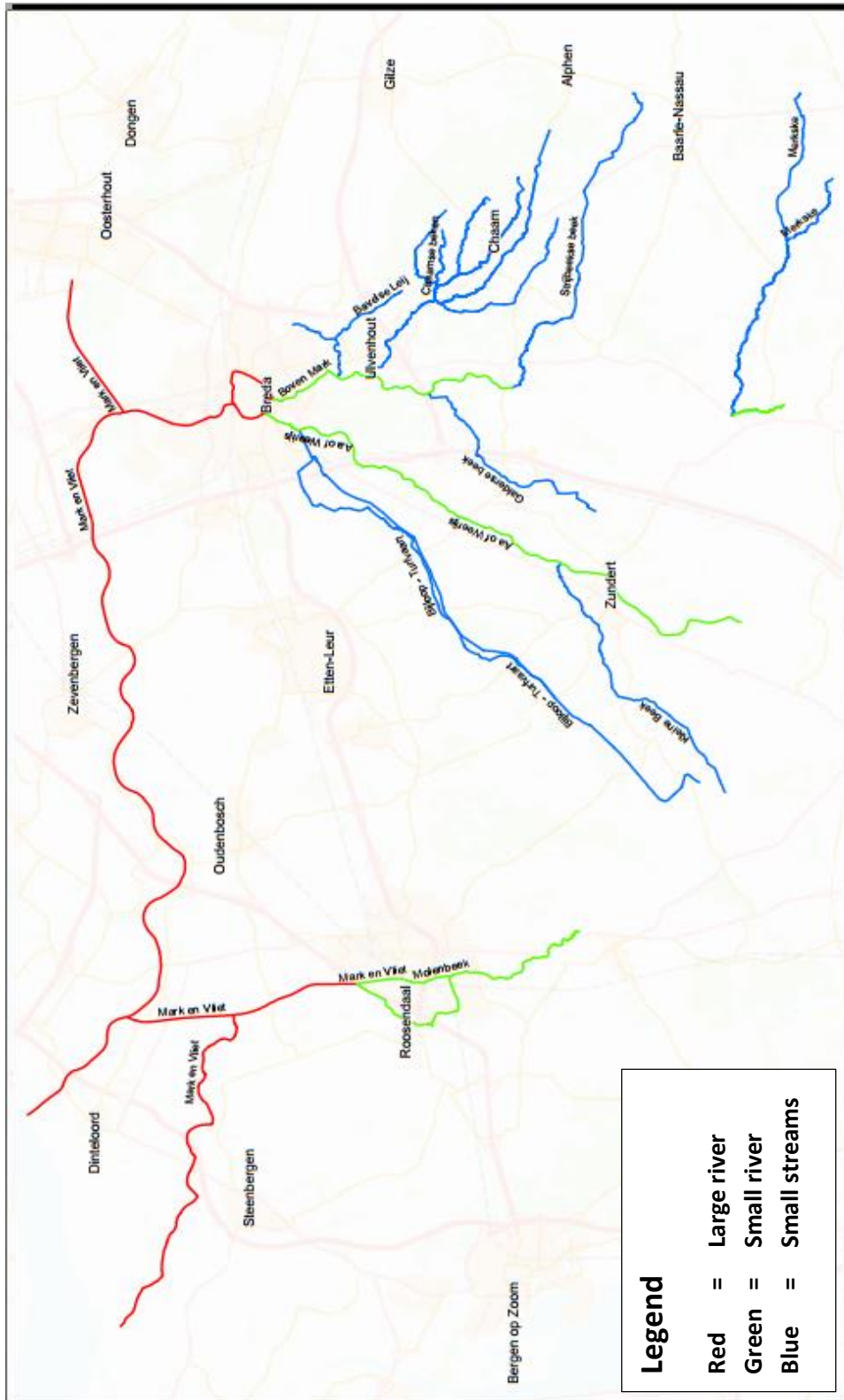


Figure 14. Map of the Mark watershed, including all the sampled waterbodies.

### Appendix 3 General sampling information

An overview of all samples collected within the study area is shown in table 16. Furthermore, frequency of the used fishing gear for each waterbody (table 17) and period of sampling for the different sampling years (table 18). Finally, the total number of fishes and proportion of the total catch is shown in table 19.

Table 16. Sampling intensity and frequency of all samples (n = 498) collected for the different waterbodies between 1994 and 2014.

|    | 1994 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2013 | 2014 |
|----|------|------|------|------|------|------|------|------|------|------|------|------|
| AW | 25   |      | 11   |      |      | 12   |      |      | 14   |      | 14   |      |
| BA | 5    |      |      |      |      |      |      |      | 4    |      | 3    |      |
| BM | 18   |      | 8    |      |      | 6    |      |      | 15   |      | 12   | 2    |
| BT |      |      |      |      |      | 9    |      |      | 12   |      | 12   | 2    |
| CH | 21   |      |      |      | 10   | 7    |      |      | 11   |      | 11   |      |
| GA | 5    |      |      | 5    |      |      |      |      | 3    |      | 4    |      |
| KL |      |      |      |      |      |      |      | 7    |      |      | 7    |      |
| ME | 4    |      |      |      | 6    |      |      |      | 8    |      | 8    |      |
| MO |      | 6    |      |      |      |      | 10   |      |      | 6    |      | 7    |
| MV | 26   |      |      | 18   |      | 2    | 16   |      |      | 45   |      | 44   |
| ST | 4    |      |      |      | 3    |      |      |      | 5    |      | 5    |      |

Table 17. Frequency of the used fishing gears at the different waterbodies.

|             | AW | BA | BM | BT | CH | GA | KL | ME | MO | MV | ST |
|-------------|----|----|----|----|----|----|----|----|----|----|----|
| Electro     | 53 | 12 | 33 | 35 | 60 | 17 | 14 | 26 | 22 | 95 | 17 |
| Purse seine | 21 | -  | 28 | -  | -  | -  | -  | -  | 7  | 4  | -  |
| Trawler     | 2  | -  | -  | -  | -  | -  | -  | -  | -  | 52 | -  |

Table 18. Period of the year in which sampling occurred for the sampling events.

|      | May | July | August | September | October | November | December |
|------|-----|------|--------|-----------|---------|----------|----------|
| 1994 |     |      |        | 108       |         |          |          |
| 2003 |     |      |        | 6         |         |          |          |
| 2004 |     |      |        |           | 19      |          |          |
| 2005 |     |      |        |           |         | 5        | 18       |
| 2006 |     |      |        |           |         | 19       |          |
| 2007 |     |      | 21     | 15        |         |          |          |
| 2008 |     | 1    | 15     |           |         | 10       |          |
| 2009 | 1   | 6    |        |           |         |          |          |
| 2010 |     |      | 72     |           |         |          |          |
| 2011 |     |      | 51     |           |         |          |          |
| 2013 |     | 17   | 59     |           |         |          |          |
| 2014 |     |      | 51     |           | 4       |          |          |

Table 19. Overview of the total number of individuals per fish species collected in all samples (n = 454), and relative frequency per fish species of the total catch.

|                          | <b>Total number of individuals</b> | <b>Frequency of the total number of individuals (%)</b> |
|--------------------------|------------------------------------|---|
| Bleak                    | 2899                               | 1,4   |
| Perch                    | 29156                              | 14,1  |
| Topmouth gudgeon         | 45                                 | 0,0   |
| Stone loach              | 5369                               | 2,6   |
| Bitterling               | 43                                 | 0,0   |
| Bream                    | 48344                              | 23,4  |
| Roach                    | 63886                              | 30,9  |
| Three-spined stickleback | 1245                               | 0,6   |
| Prussian carp            | 40                                 | 0,0   |
| Grass carp               | 1                                  | 0,0   |
| Common carp              | 97                                 | 0,0   |
| White bream              | 2096                               | 1,0   |
| Crucian carp             | 5                                  | 0,0   |
| Spined loach             | 75                                 | 0,0   |
| Chub                     | 43                                 | 0,0   |
| Tubenosed goby           | 28                                 | 0,0   |
| European eel             | 1863                               | 0,9   |
| Ruffe                    | 10935                              | 5,3   |
| Asp                      | 149                                | 0,1   |
| European Bullhead        | 58                                 | 0,0   |
| Gudgeon                  | 11766                              | 5,7   |
| Rudd                     | 11697                              | 5,7   |
| Zander                   | 1022                               | 0,5   |
| Pike                     | 1479                               | 0,7   |
| Smelt                    | 10                                 | 0,0   |
| Nine-spined stickleback  | 6596                               | 3,2   |
| Sunbleak                 | 3765                               | 1,8   |
| Ide                      | 2423                               | 1,2   |
| Pumpkinseed              | 173                                | 0,1   |
| Tench                    | 1168                               | 0,6   |
| Round goby               | 37                                 | 0,0   |
| <b>Total</b>             | <b>206945</b>                      | <b>100</b>  |

## Appendix 4 Maps with sampled trajectories of the small streams in the Mark watershed.

This appendix shows the sampled trajectories of the small streams of the Mark watershed between 1994 and 2014. Maps are included for the Chaamse Beken (figure 15), Merkske (figure 16), Strijbeekse Beek (figure 17), Bijloop-Turfvaart (figure 18), Bavelse Leij (figure 19), Kleine Beek (figure 20) and Galderse Beek (figure 21).

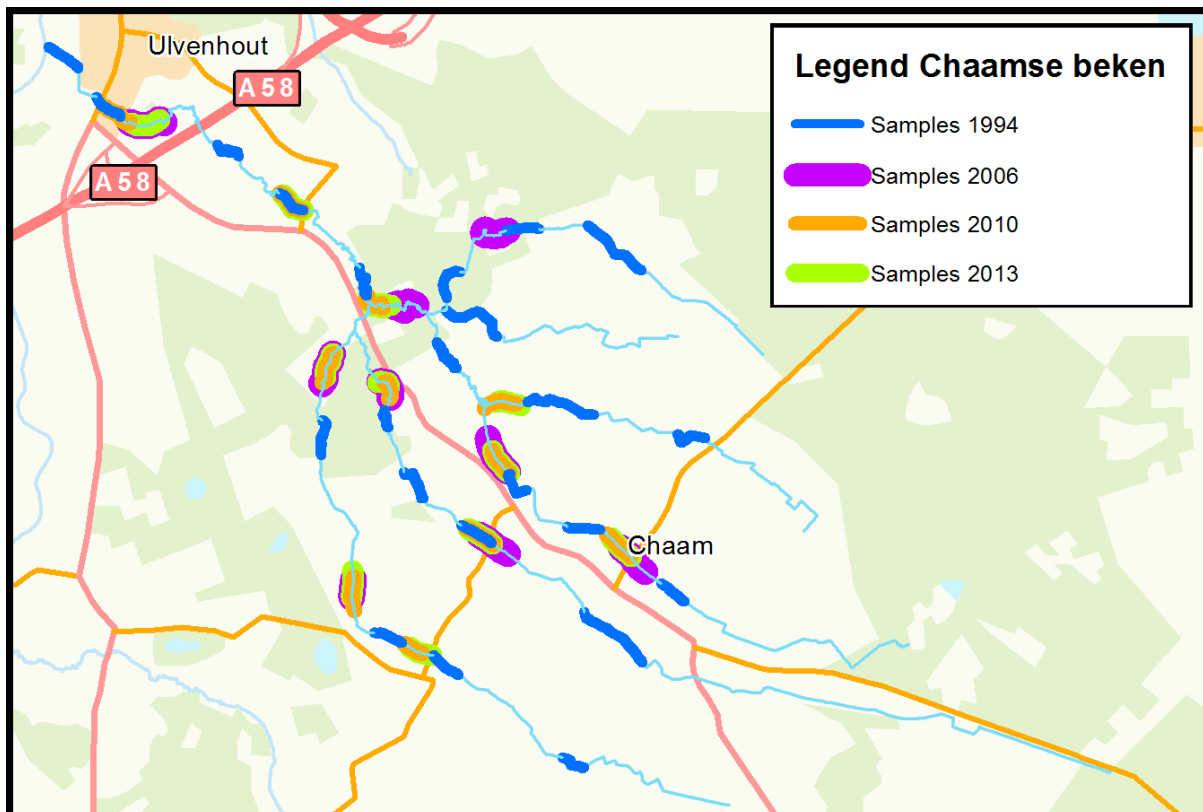


Figure 15. Overview of the sampled trajectories in the Chaamse beken for 1994, 2006, 2010 and 2013.

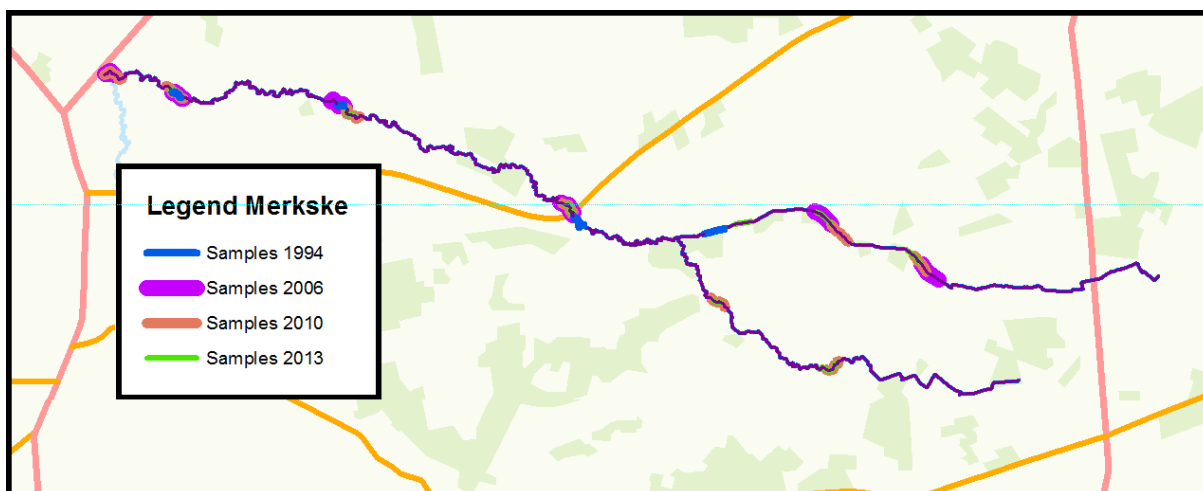


Figure 16. Overview of the sampled trajectories at Merkske for 1994, 2006, 2010 and 2013.



Figure 17. Overview of the sampled trajectories at the Strijbeekse Beek for 1994, 2006, 2010 and 2013.

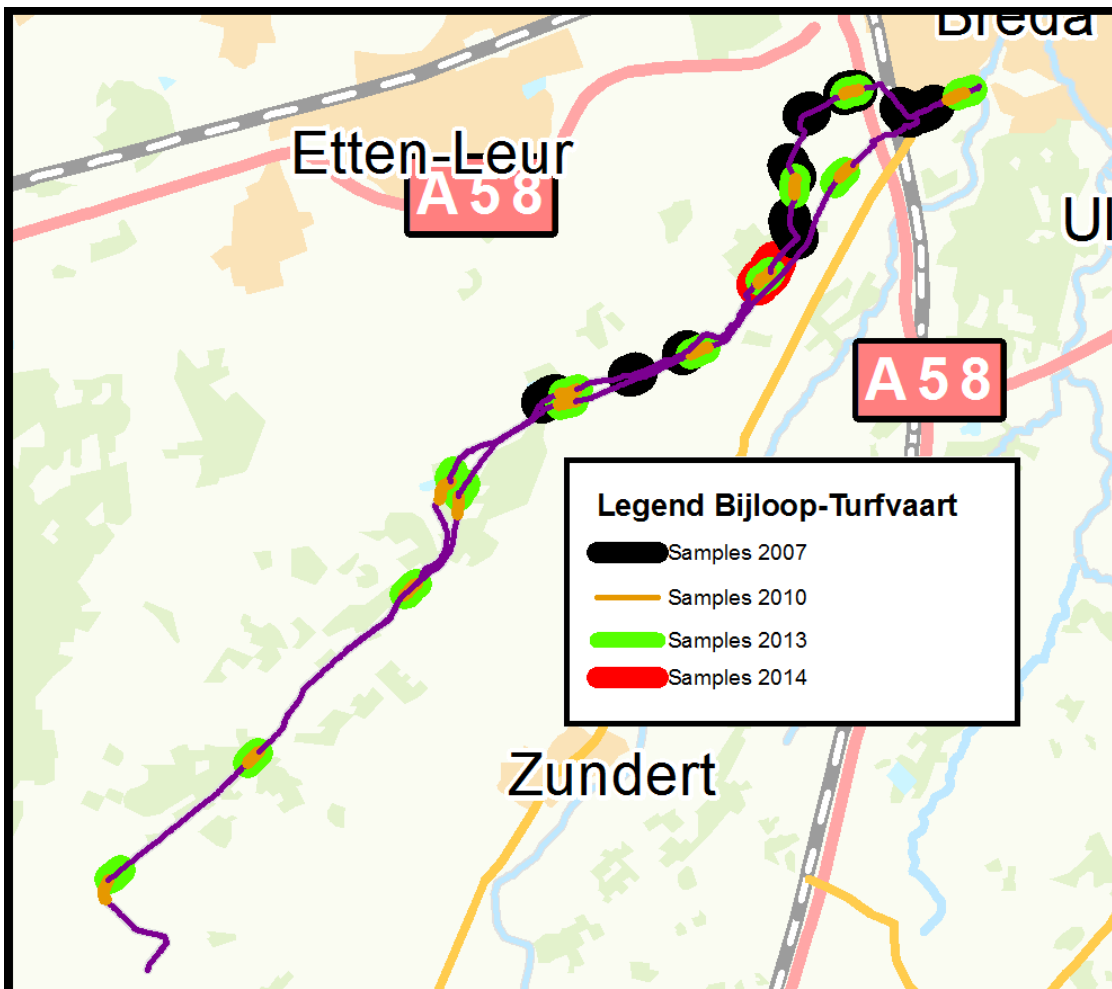


Figure 18. Overview of the sampled trajectories at Bijloop-Turfvaart for 2007, 2010, 2013 and 2014.

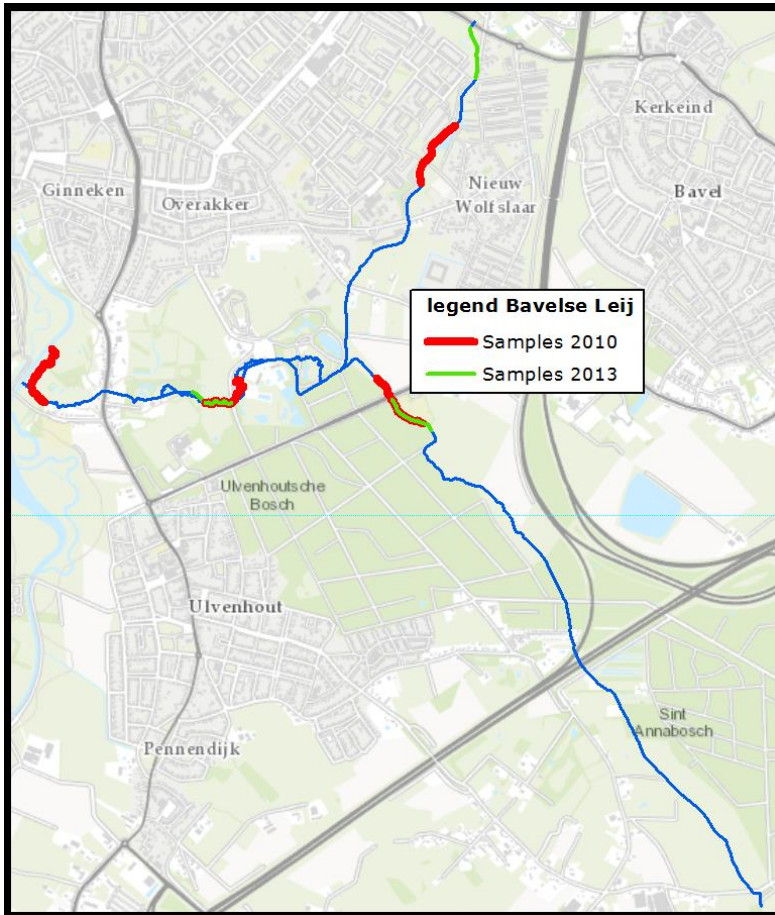


Figure 19. Overview of the sampled trajectories at Bavelse Leij for 2010 and 2013.

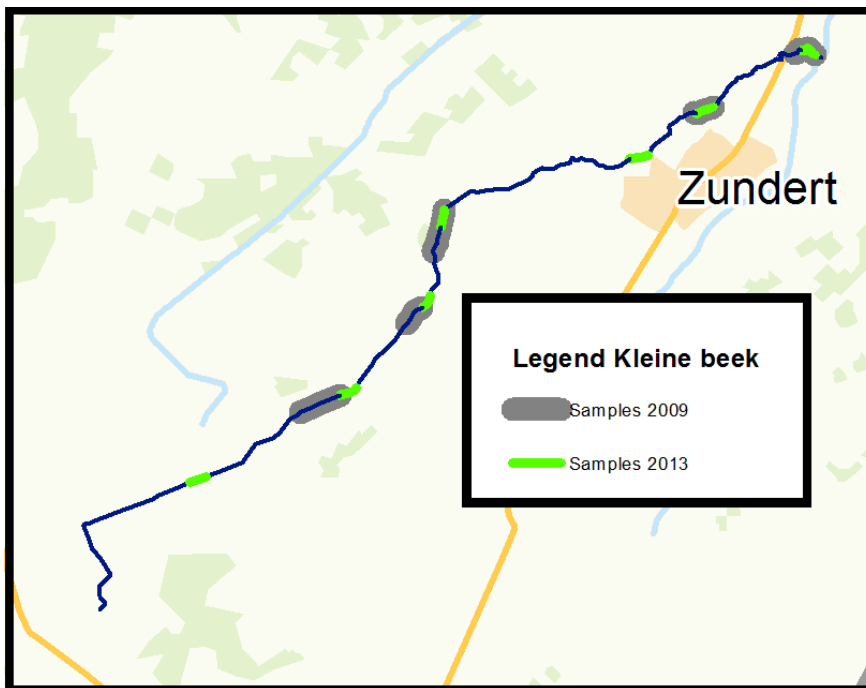


Figure 20. Overview of the sampled trajectories at the Kleine Beek for 2009 and 2013.

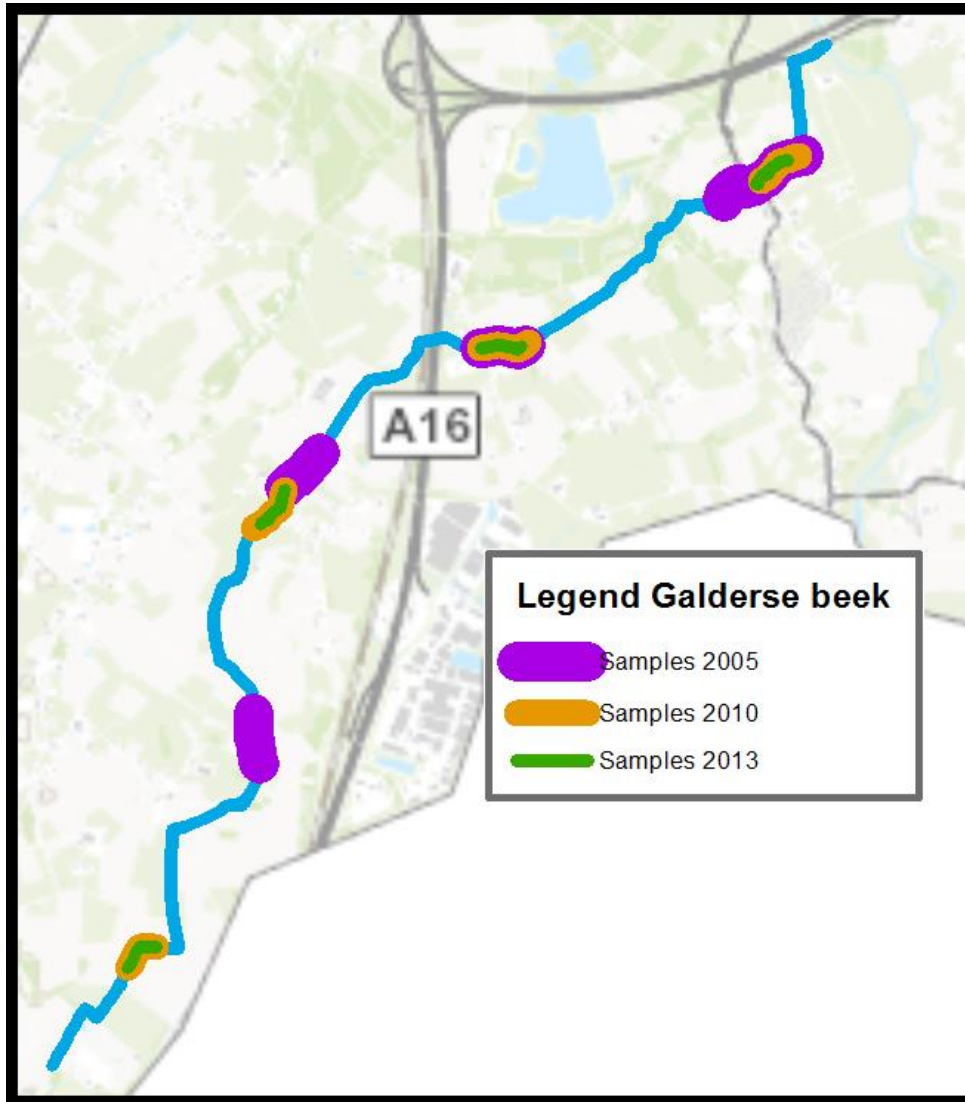


Figure 21. Overview of the sampled trajectories at the Galderse Beek for 2005, 2010 and 2013.

## Appendix 5 General information of fish species

This appendix shows an overview with information regarding the fish species that occur in the Mark watershed (table 20).

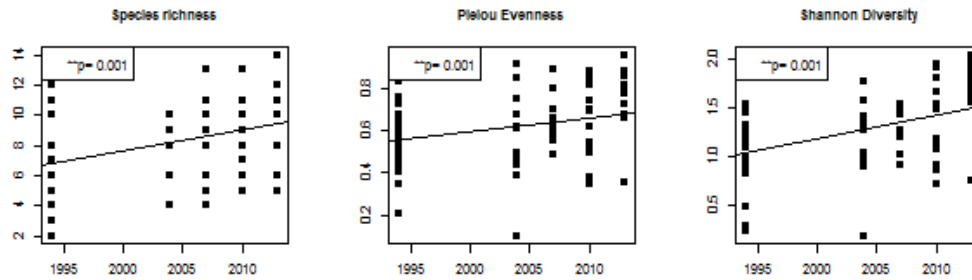
Table 20. Overview of the selected fish species for the Dutch waters in accordance to the total European FAME-List (Noble & Cowx 2002). The table includes trophic level and feeding behavior, (BENT = benthivorous, PISC = piscivorous, HERB = herbivorous, OMNI = omnivorous), guilds (LI = limnophilic, RH = rheophilic and EURY = eurytopic) and tolerance for habitat degradations (TOLE = tolerant, INTE = intermediar and INTOL = intolerant).

| Species code | Common Dutch name         | Common English name     | Latin name                     | Trophic   | Guilds | Habitat degradation |
|--------------|---------------------------|-------------------------|--------------------------------|-----------|--------|---------------------|
| AL           | Alver                     | Bleak                   | <i>Alburnus alburnus</i>       | OMNI      | EURY   | TOLE                |
| BA           | Baars                     | Perch                   | <i>Perca fluviatilis</i>       | BENT/PISC | EURY   | TOLE                |
| BE           | Bermpje                   | Stone Loach             | <i>Barbatula barbatula</i>     | BENT      | RH     | INTE                |
| BI           | Bittervoorn               | Bitterling              | <i>Rhodeus sericeus amarus</i> | HERB      | LI     | INTOL               |
| BV           | Blankvoorn                | Roach                   | <i>Rutilus rutilus</i>         | OMNI      | EURYO  | TOLE                |
| BR           | Brasem                    | Bream                   | <i>Abramis brama</i>           | OMNI      | EURY   | TOLE                |
| DD           | Driedoornige stekelbaars  | Threespined Stickleback | <i>Gasterosteus aculeatus</i>  | OMNI      | EURY   | TOLE                |
| GI           | Giebel                    | Prussian Carp           | <i>Carassius gibelio</i>       | OMNI      | EURY   | TOLE                |
| KA           | Karper                    | Common Carp             | <i>Cyprinus carpio carpio</i>  | OMNI      | EURY   | INTE                |
| KM           | Kleine modderkruiper      | Spined Loach            | <i>Cobitis taenia</i>          | BENT      | EURY   | INTE                |
| KB           | Kolblei                   | White Bream             | <i>Blicca bjoerkna</i>         | OMNI      | EURY   | TOLE                |
| KV           | Kopvoorn                  | Chub                    | <i>Leuciscus cephalus</i>      | OMNI      | RH     | INTOL               |
| KK           | Kroeskarper               | Crucian Carp            | <i>Carassius carassius</i>     | OMNI      | LI     | TOLE                |
| PA           | Paling                    | European Eel            | <i>Anguilla anguilla</i>       | BENT/PISC | EURY   | INTE                |
| PO           | Pos                       | Ruffe                   | <i>Gymnocephalus cernua</i>    | BENT      | EURY   | TOLE                |
| RD           | Rivierdonderpad           | European Bullhead       | <i>Cottus gobio</i>            | INSV      | RH     | INTOL               |
| RG           | Riviergrondel             | Gudgeon                 | <i>Gobio gobio</i>             | BENT      | RH     | INTE                |
| RB           | Roofblei                  | Asp                     | <i>Aspius aspius</i>           | PISC      | EURY   | INTE                |
| RV           | Ruisvoorn                 | Rudd                    | <i>Scardinius</i>              | OMNI      | LI     | INTE                |
| SK           | Snoek                     | Pike                    | <i>Esox lucius</i>             | PISC      | EURY   | INTOL               |
| SB           | Snoekbaars                | Zander                  | <i>Sander lucioperca</i>       | PISC      | EURY   | INTE                |
| SP           | Spiering                  | Smelt                   | <i>Osmerus eperlanus</i>       | PISC      | LI     | INTE                |
| TD           | Tienddoornige stekelbaars | Tenspined Stickleback   | <i>Pungitius pungitius</i>     | OMNI      | LI     | INTE                |
| VE           | Vetje                     | Sunbleak                | <i>Leucaspis delineatus</i>    | OMNI      | LI     | INTE                |
| WI           | Winde                     | Ide                     | <i>Leuciscus idus</i>          | OMNI      | RH     | INTE                |
| ZE           | Zeelt                     | Tench                   | <i>Tinca tinca</i>             | OMNI      | LI     | INTOL               |

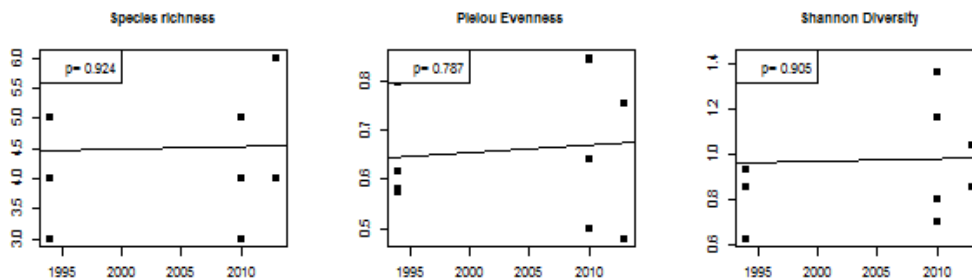
## Appendix 6 Diversity indices of all waterbodies over time

Linear model of species richness, Pielou's evenness and Shannon diversity for the period 1994 - 2014 for the waterbodies included in this project. P-values of the linear model are represented in the top left corner of the plots.

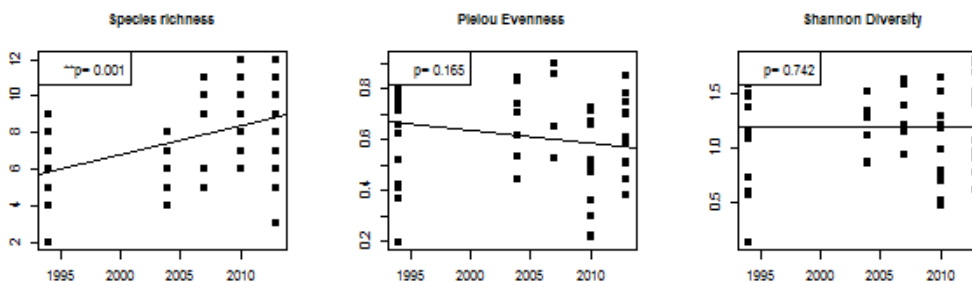
### Aa of Weerij



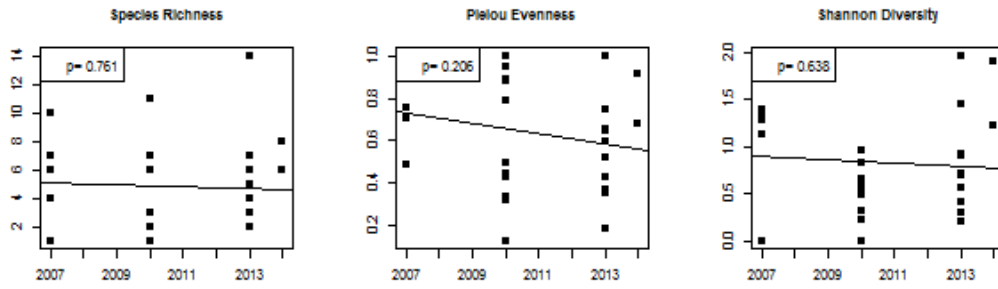
### Bavelse Leij



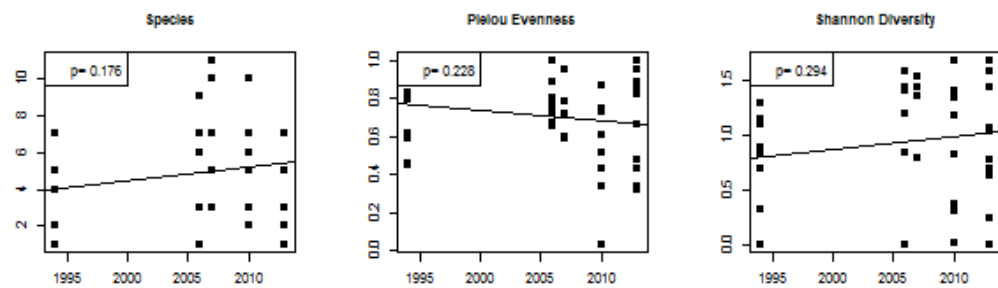
### Boven Mark



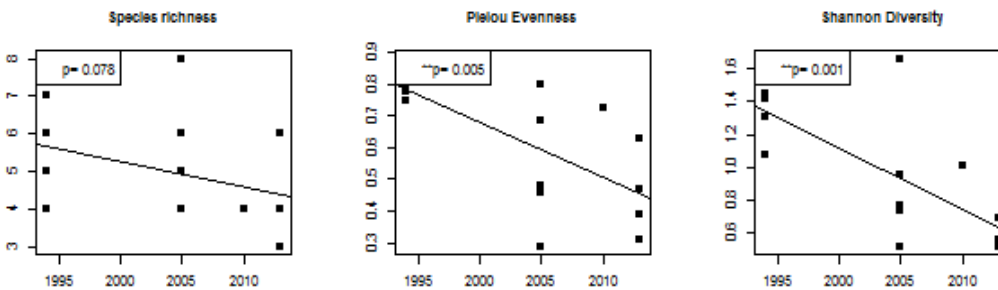
### Bijloop-Turfvaart



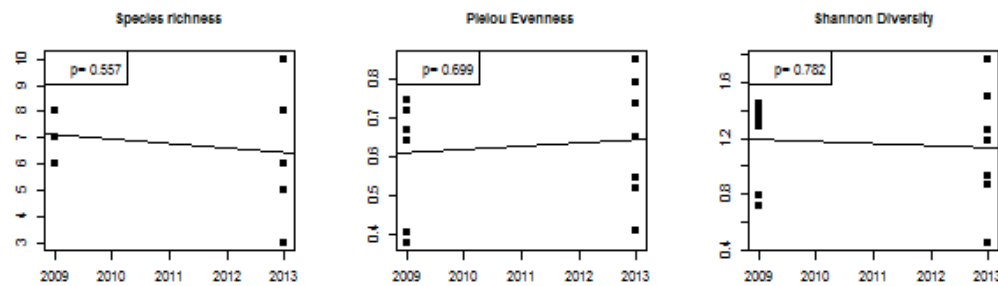
### Chaamse beken



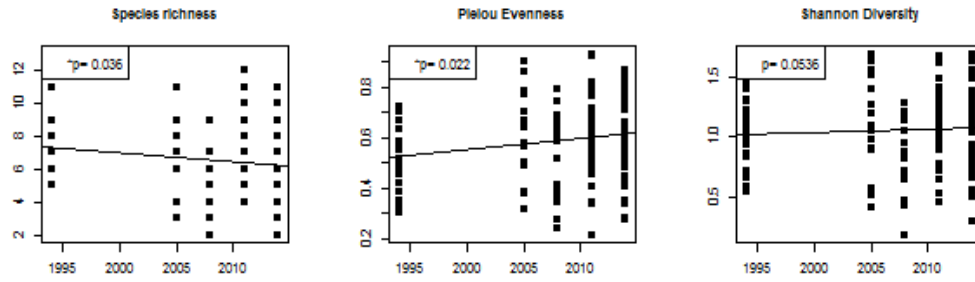
### Galderse beek



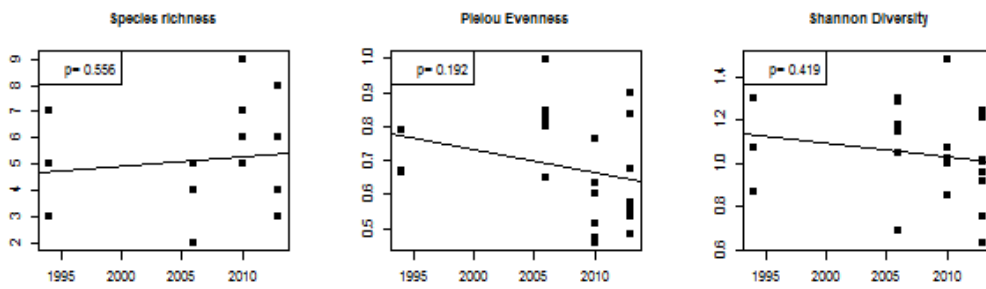
### Kleine beek



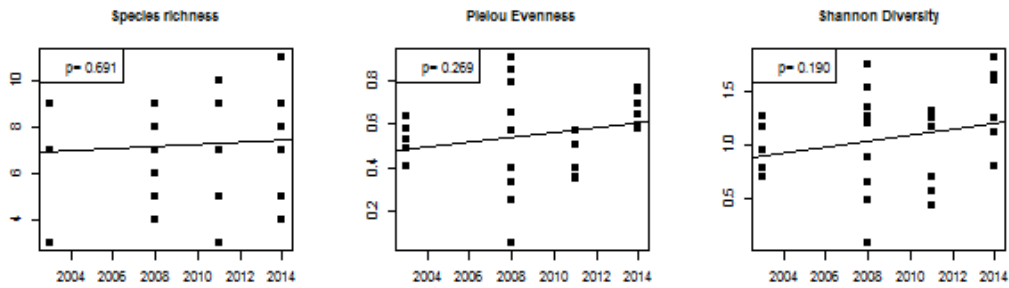
### Mark en Vliet



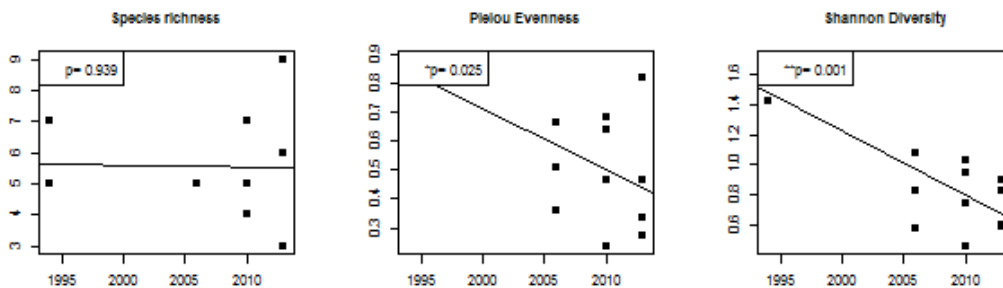
### Merkske



### Molenbeek



### Strijbeekse beek



## Appendix 7 Overview of the total number of fish per sampling event

For all waterbodies of the Mark watershed an overview is shown of the total number of individuals per species for each sampling year. The watercourses included are: the Aa of Weerijs (table 21), Bavelse Leij (table 22), Bijloop-Turfvaart (table 23), Boven Mark (table 24), Galderse Beek (table 25), Chaamse Beken (table 26), Kleine Beek (table 27), Strijbeekse Beek (table 28), Mark en Vliet (table 29) and Merkske (table 30).

Table 21. Total number of collected fishes per sampling event for the Aa of Weerijs

| <b>Fish species</b>      | <b>1994</b> | <b>2004</b>  | <b>2007</b> | <b>2010</b> | <b>2013</b> |
|--------------------------|-------------|--------------|-------------|-------------|-------------|
| European Eel             | 7           | 8            | 16          | 16          | 15          |
| Bleak                    |             |              | 11          | 1           |             |
| Perch                    | 204         | 479          | 405         | 1943        | 284         |
| Stone loach              | 41          | 14           | 12          | 38          | 5           |
| Bitterling               |             |              |             | 8           | 5           |
| Roach                    | 2668        | 7639         | 612         | 1445        | 309         |
| Topmouth Gudgeon         |             | 5            | 2           |             |             |
| Bream                    | 39          | 403          | 38          | 529         | 213         |
| Three-spined stickleback | 5           |              | 11          |             |             |
| Prussian Carp            | 1           | 1            | 2           |             |             |
| Common carp              | 4           | 3            | 1           | 3           | 5           |
| Spined loach             | 2           |              | 33          | 10          | 19          |
| White Bream              | 3           | 343          | 1           | 3           | 10          |
| Crucian carp             | 3           |              |             |             |             |
| Ruffe                    | 66          | 14           | 22          | 37          | 43          |
| Rudd                     | 449         | 179          | 277         | 191         | 148         |
| Gudgeon                  | 173         | 1289         | 258         | 446         | 338         |
| Asp                      |             |              |             | 2           | 4           |
| Pike                     | 124         | 99           | 40          | 73          | 49          |
| Nine-spined stickleback  | 30          | 5            | 1           | 1           |             |
| Sunbleak                 | 10          | 1523         |             | 21          | 22          |
| Ide                      | 57          | 2            | 28          | 196         | 113         |
| Tench                    | 401         | 80           | 85          | 256         | 17          |
| Pumpinseed               | 1           |              | 2           |             | 9           |
| <b>Number of fish</b>    | <b>4288</b> | <b>12086</b> | <b>1857</b> | <b>5219</b> | <b>1608</b> |
| <b>Number of species</b> | <b>20</b>   | <b>17</b>    | <b>20</b>   | <b>19</b>   | <b>18</b>   |

Table 22. Total number of collected fishes per sampling event for the Bavelse Leij

| <b>Fish species</b>      | <b>1994</b> | <b>2010</b> | <b>2013</b> |
|--------------------------|-------------|-------------|-------------|
| European Eel             | 3           | 1           | 2           |
| Perch                    | 4           | 53          | 39          |
| Stone loach              | 34          | 20          | 87          |
| Roach                    | 2           | 4           |             |
| Bream                    | 2           | 2           |             |
| Spined loach             |             | 1           |             |
| Rudd                     |             | 14          |             |
| Gudgeon                  | 79          | 1           | 2           |
| Pike                     | 6           | 41          | 21          |
| Nine-spined stickleback  | 1           |             | 1           |
| Ide                      |             | 1           |             |
| <b>Number of fish</b>    | <b>131</b>  | <b>138</b>  | <b>152</b>  |
| <b>Number of species</b> | <b>8</b>    | <b>10</b>   | <b>6</b>    |

Table 23. Total number of collected fishes per sampling event for Bijloop-Turfvaart

| <b>Fish species</b>      | <b>2006</b> | <b>2007</b> | <b>2010</b> | <b>2013</b> | <b>2014</b> |
|--------------------------|-------------|-------------|-------------|-------------|-------------|
| European Eel             | 8           | 3           | 5           | 5           | 2           |
| Perch                    | 422         | 183         | 1340        | 80          | 25          |
| Stone loach              | 2           | 7           | 29          | 427         | 54          |
| Roach                    | 273         | 312         | 49          | 24          |             |
| Bream                    | 38          | 24          | 1           | 32          |             |
| Karper                   |             |             |             | 1           |             |
| Spined loach             | 4           |             |             | 3           |             |
| White Bream              | 37          | 2           |             | 2           | 3           |
| Ruffe                    | 8           | 1           |             | 1           |             |
| Rudd                     | 46          | 2           | 47          | 51          | 13          |
| Gudgeon                  | 20          | 85          | 3           | 5           | 12          |
| Asp                      | 3           | 1           | 2           | 1           |             |
| Pike                     | 30          | 45          | 133         | 52          | 12          |
| Zander                   | 4           | 2           |             |             |             |
| Nine-spined stickleback  |             |             | 37          | 333         | 6           |
| Ide                      | 32          | 15          | 8           | 4           |             |
| Tench                    | 11          |             | 3           | 5           |             |
| <b>Number of fish</b>    | <b>938</b>  | <b>682</b>  | <b>1657</b> | <b>1026</b> | <b>127</b>  |
| <b>Number of species</b> | <b>15</b>   | <b>13</b>   | <b>12</b>   | <b>16</b>   | <b>8</b>    |

Table 24. Total number of collected fishes per sampling event for the Boven Mark

| <b>Fish species</b>      | <b>1994</b> | <b>2004</b> | <b>2007</b> | <b>2010</b>  | <b>2013</b> | <b>2014</b> |
|--------------------------|-------------|-------------|-------------|--------------|-------------|-------------|
| European Eel             | 17          | 1           | 8           | 23           | 19          | 3           |
| Bleak                    | 1           |             | 1           |              | 24          |             |
| Perch                    | 76          | 176         | 328         | 4667         | 433         | 67          |
| Stone loach              | 1           |             | 12          | 9            | 9           | 16          |
| Roach                    | 2448        | 274         | 329         | 8192         | 2252        | 280         |
| Bream                    | 131         | 43          | 232         | 3210         | 895         | 2           |
| Three-spined stickleback | 31          |             | 2           | 4            | 24          | 1           |
| Prussian Carp            |             |             |             | 1            |             |             |
| Grass Carp               | 1           |             |             |              |             |             |
| Common carp              | 6           |             |             | 38           |             | 1           |
| Spined loach             |             |             | 1           |              | 1           |             |
| White Bream              | 4           | 1           |             | 17           | 7           | 6           |
| Chub                     | 1           |             |             |              |             |             |
| Ruffe                    | 8           | 2           | 37          | 99           | 49          |             |
| Rudd                     | 524         | 5           | 37          | 211          | 74          | 30          |
| Gudgeon                  | 15          | 8           | 61          | 1123         | 308         | 53          |
| Asp                      |             |             | 4           | 53           | 25          |             |
| Pike                     | 8           | 45          | 36          | 81           | 34          | 22          |
| Zander                   | 1           | 1           |             | 8            | 4           |             |
| Nine-spined stickleback  |             | 1           |             |              | 5           |             |
| Sunbleak                 | 120         | 5           |             | 301          | 528         | 4           |
| Ide                      | 17          | 20          | 23          | 409          | 39          | 1           |
| Tench                    | 24          | 18          | 8           | 47           | 1           | 3           |
| Pumpinseed               | 1           |             | 21          | 55           | 26          | 21          |
| <b>Number of fish</b>    | <b>3435</b> | <b>600</b>  | <b>1140</b> | <b>18548</b> | <b>4757</b> | <b>510</b>  |
| <b>Number of species</b> | <b>20</b>   | <b>14</b>   | <b>16</b>   | <b>19</b>    | <b>20</b>   | <b>15</b>   |

Table 25. Total number of collected fishes per sampling event for the Galderse Beek

| Fish species             | Aantal gevangen vissen per bemonsteringsjaar |            |           |             |
|--------------------------|--|------------|-----------|-------------|
|                          | 1994   | 2005       | 2010      | 2013        |
| European Eel             | 9  | 5          | 2         | 1           |
| Perch                    | 1  |            | 5         |             |
| Stone loach              | 52   | 114        | 1         | 871         |
| Roach                    | 33   |            |           |             |
| Three-spined stickleback | 58   | 68         | 1         | 27          |
| Common carp              | 3  |            |           |             |
| Rudd                     |  | 3          |           |             |
| Gudgeon                  | 150  | 51         |           | 12          |
| Pike                     | 3  | 26         | 17        | 16          |
| Nine-spined stickleback  | 23   | 672        |           | 680         |
| Tench                    |  | 2          |           |             |
| <b>Number of fish</b>    | <b>332</b>                                   | <b>941</b> | <b>26</b> | <b>1607</b> |
| <b>Number of species</b> | <b>9</b>                                     | <b>8</b>   | <b>5</b>  | <b>6</b>    |

Table 26. Total number of collected fishes per sampling event for the Chaamse Beken

| Fish species             | 1994       | 2006        | 2007       | 2010        | 2013       |
|--------------------------|------------|-------------|------------|-------------|------------|
| European Eel             | 6          | 41          | 30         | 25          | 17         |
| Perch                    | 7          | 204         | 149        | 482         | 43         |
| Stone loach              | 172        | 135         | 85         | 172         | 266        |
| Roach                    | 122        | 130         | 211        | 20          |            |
| Bream                    | 4          | 4           | 5          | 7           |            |
| Three-spined stickleback | 3          |             |            |             |            |
| Common carp              | 1          |             | 1          |             | 1          |
| Chub                     |            |             | 3          | 1           |            |
| Ruffe                    |            | 4           | 12         |             |            |
| Rudd                     | 5          |             | 4          | 1           |            |
| European Bullhead        |            |             |            |             | 3          |
| Gudgeon                  | 184        | 214         | 54         | 119         | 16         |
| Pike                     | 8          | 28          | 20         | 62          | 27         |
| Nine-spined stickleback  | 63         | 3013        |            | 663         | 160        |
| Ide                      | 1          | 12          | 8          | 11          |            |
| Tench                    | 1          | 3           |            |             |            |
| Pumpinseed               |            | 2           |            |             | 13         |
| <b>Number of fish</b>    | <b>577</b> | <b>3790</b> | <b>582</b> | <b>1563</b> | <b>546</b> |
| <b>Number of species</b> | <b>13</b>  | <b>12</b>   | <b>12</b>  | <b>11</b>   | <b>9</b>   |

Table 27. Total number of collected fishes per sampling event for the Kleine Beek

| <b>Fish species</b>      | <b>2009</b> | <b>2013</b> |
|--------------------------|-------------|-------------|
| European Eel             |             | 3           |
| Perch                    | 8           | 12          |
| Stone loach              | 108         | 65          |
| Roach                    | 604         | 217         |
| Bream                    |             | 1           |
| Prussian Carp            | 4           | 7           |
| Common carp              | 2           | 10          |
| Rudd                     | 63          | 279         |
| Gudgeon                  | 1519        | 327         |
| Pike                     | 3           | 4           |
| Nine-spined stickleback  | 244         | 268         |
| Sunbleak                 | 216         | 7           |
| Tench                    | 63          | 14          |
| <b>Number of fish</b>    | <b>2834</b> | <b>1214</b> |
| <b>Number of species</b> | <b>11</b>   | <b>13</b>   |

Table 28. Total number of collected fishes per sampling event for the Strijbeekse Beek

| <b>Fish species</b>      | <b>1994</b> | <b>2006</b> | <b>2010</b> | <b>2013</b> |
|--------------------------|-------------|-------------|-------------|-------------|
| European Eel             |             | 9           | 2           | 2           |
| Perch                    |             |             | 11          | 2           |
| Stone loach              | 22          | 41          | 148         | 410         |
| Roach                    | 10          |             |             | 1           |
| Three-spined stickleback | 12          | 2           |             | 1           |
| Common carp              |             |             |             | 1           |
| Gudgeon                  | 20          | 297         | 807         | 788         |
| Rudd                     | 4           |             |             |             |
| Pike                     | 4           | 32          | 14          | 27          |
| Nine-spined stickleback  | 6           |             | 3           | 8           |
| Tench                    |             |             | 2           |             |
| Pumpinseed               |             | 4           |             | 2           |
| <b>Number of fish</b>    | <b>78</b>   | <b>385</b>  | <b>987</b>  | <b>1242</b> |
| <b>Number of species</b> | <b>7</b>    | <b>6</b>    | <b>7</b>    | <b>10</b>   |

Table 29. Total number of collected fishes per sampling event for the Mark en Vliet

| <b>Fish species</b>      | <b>1994</b>  | <b>2005</b> | <b>2008</b> | <b>2011</b>  | <b>2014</b>  |
|--------------------------|--------------|-------------|-------------|--------------|--------------|
| European Eel             | 76           | 31          | 294         | 792          | 353          |
| Bleak                    | 2780         | 36          |             | 17           | 23           |
| Perch                    | 1662         | 309         | 431         | 7828         | 6187         |
| Bitterling               | 3            | 1           |             |              | 21           |
| Roach                    | 10384        | 465         | 270         | 3596         | 8513         |
| Bream                    | 11849        | 299         | 1277        | 12913        | 8281         |
| Three-spined stickleback | 1            | 2           |             | 29           | 9            |
| Prussian Carp            | 3            |             |             |              |              |
| Common carp              | 8            |             |             | 4            | 1            |
| Spined loach             |              | 1           |             | 2            |              |
| White Bream              | 82           | 4           | 61          | 561          | 614          |
| Tubenosed Goby           |              |             |             |              | 29           |
| Ruffe                    | 827          | 14          | 74          | 7442         | 2112         |
| Crucian carp             | 1            |             |             |              |              |
| Rudd                     | 63           | 29          | 1           | 32           | 103          |
| European Bullhead        |              | 2           | 2           | 46           | 5            |
| Gudgeon                  | 2            |             |             |              |              |
| Asp                      |              | 25          | 3           | 18           | 15           |
| Pike                     | 14           | 8           | 4           | 26           | 36           |
| Zander                   | 156          | 5           | 19          | 357          | 465          |
| Smelt                    | 3            |             |             |              | 7            |
| Nine-spined stickleback  | 2            | 3           | 3           | 3            | 11           |
| Sunbleak                 |              | 21          |             | 11           | 8            |
| Ide                      | 1343         | 28          | 17          | 44           | 25           |
| Tench                    |              | 2           |             | 4            | 30           |
| Pumpinseed               |              |             |             | 2            | 1            |
| Round Goby               |              |             |             |              | 37           |
| <b>Number of fish</b>    | <b>29259</b> | <b>1285</b> | <b>2456</b> | <b>33727</b> | <b>26886</b> |
| <b>Number of species</b> | <b>19</b>    | <b>19</b>   | <b>13</b>   | <b>20</b>    | <b>23</b>    |

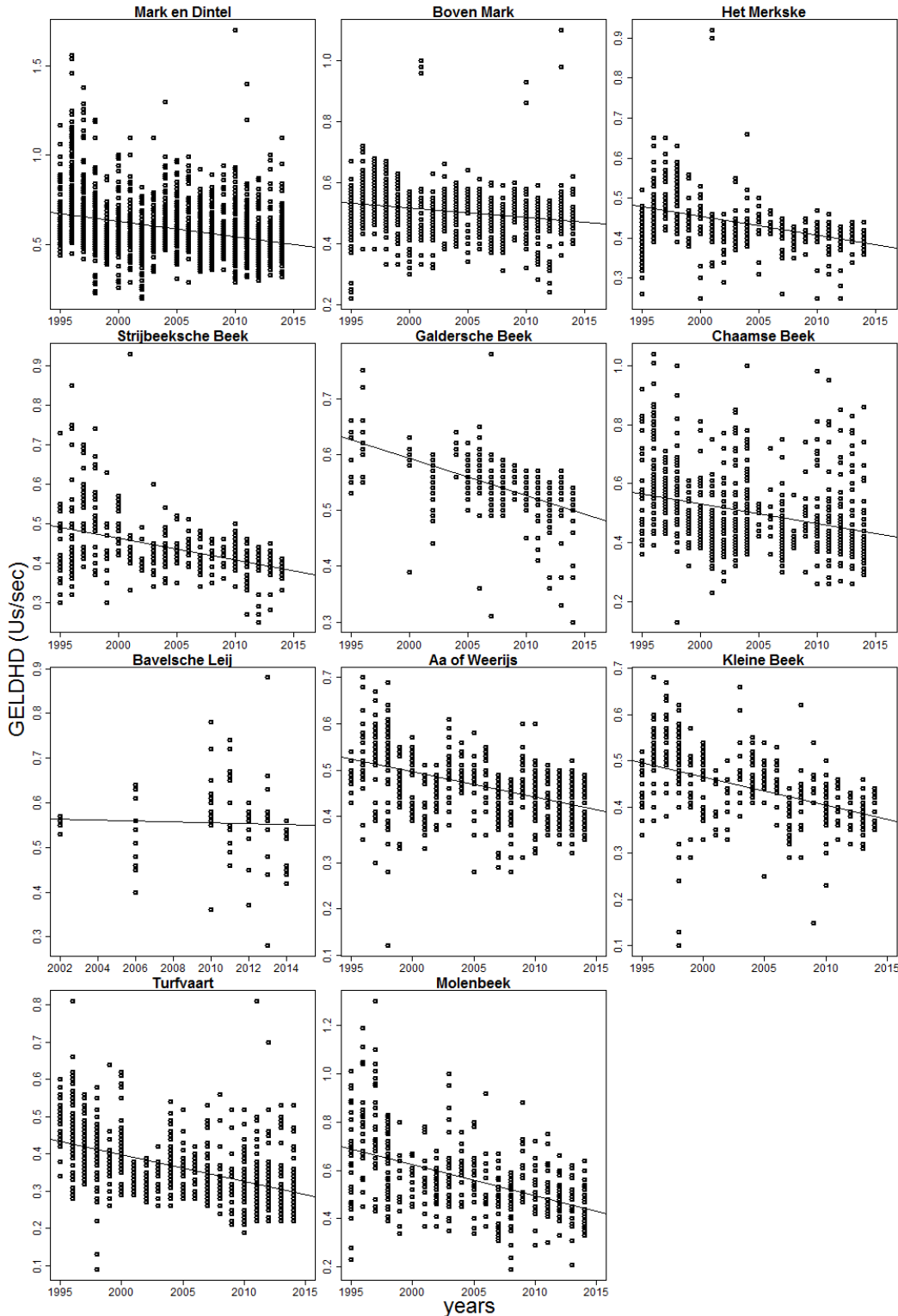
Table 30. Total number of collected fishes per sampling event for the Merkske

| <b>Fish species</b>      | <b>1994</b> | <b>2006</b> | <b>2010</b> | <b>2013</b> |
|--------------------------|-------------|-------------|-------------|-------------|
| European Eel             | 2           |             | 2           |             |
| Perch                    |             | 5           | 55          | 22          |
| Stone loach              | 103         | 110         | 262         | 1458        |
| Roach                    | 1           | 13          | 65          |             |
| Topmouth Gudgeon         |             |             |             | 2           |
| Three-spined stickleback | 110         | 90          | 247         | 513         |
| Prussian Carp            | 1           |             |             |             |
| Spined loach             |             | 1           |             |             |
| Common Carp              |             |             | 1           |             |
| Chub                     | 8           | 16          | 9           | 5           |
| Gudgeon                  | 75          | 148         | 518         | 153         |
| Pike                     |             | 2           | 19          | 6           |
| Nine-spined stickleback  | 3           | 39          | 4           | 217         |
| Tench                    |             | 4           | 1           |             |
| Pumpinseed               |             |             | 6           | 20          |
| <b>Number of fish</b>    | <b>303</b>  | <b>428</b>  | <b>1189</b> | <b>2396</b> |
| <b>Number of species</b> | <b>8</b>    | <b>10</b>   | <b>12</b>   | <b>9</b>    |

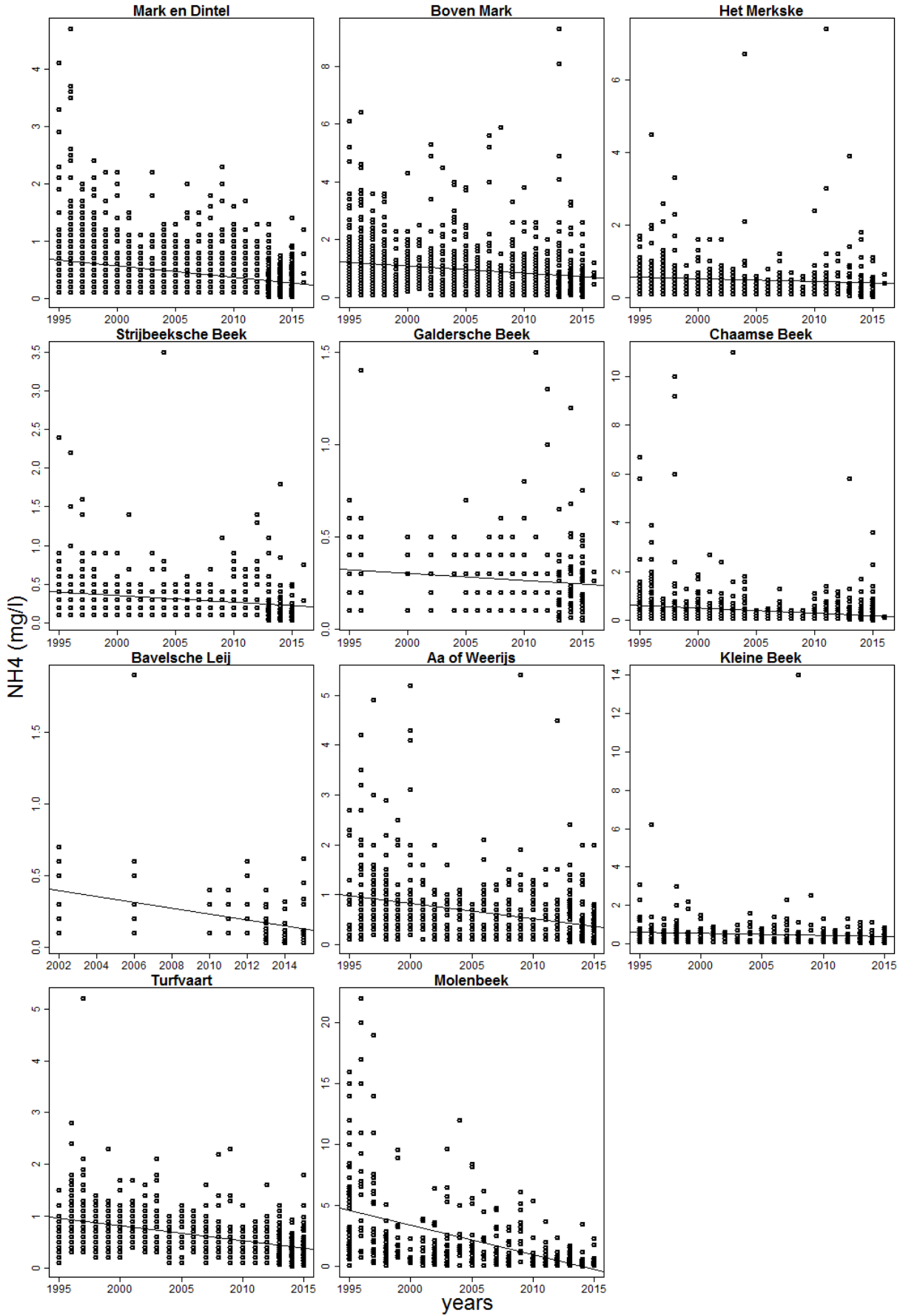
## Appendix 8 Trends in physical-chemical variables of all waterbodies

The linear model for trends of the physical-chemical variables are shown in this appendix. The physical-chemical variables included are conductivity, chloride, oxygen concentration, pH, nitrate, nitrite, ammonium, total nitrogen, temperature, transparency, phosphate and total phosphorus. Abbreviation of the physical-chemical variables are shown in the title of the plots.

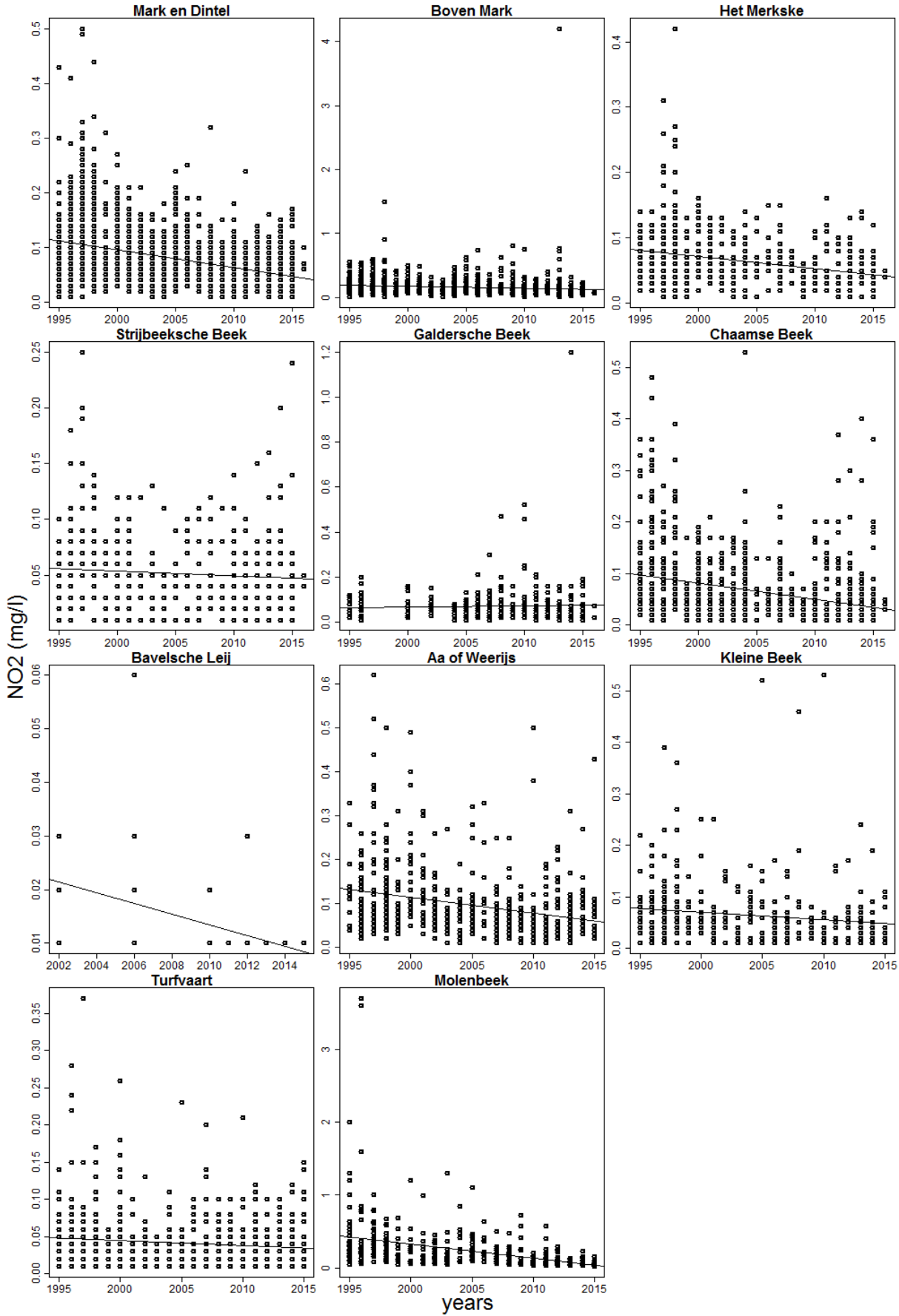
GELDHD concentration of all waterbodies



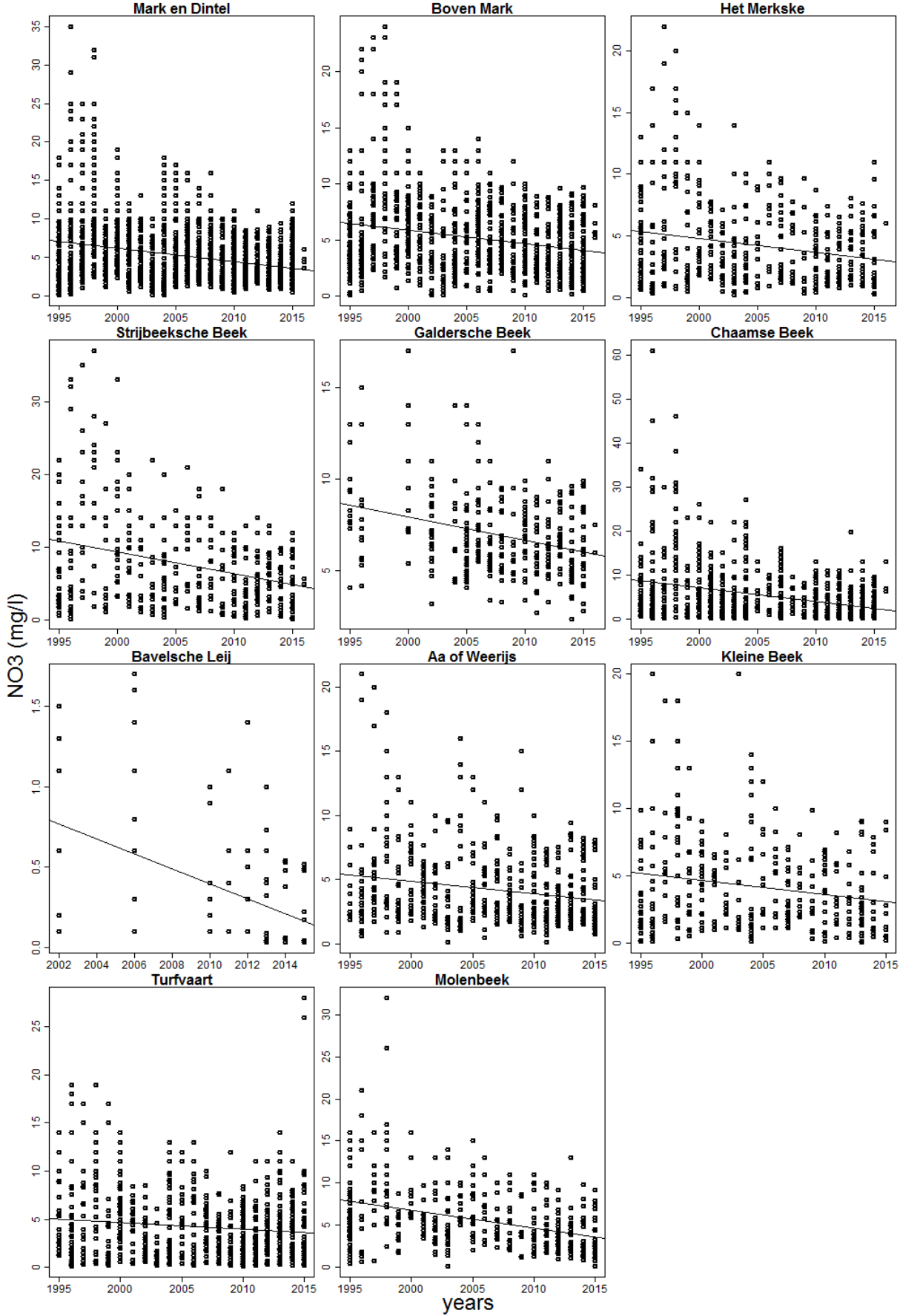
NH4 concentration of all waterbodies



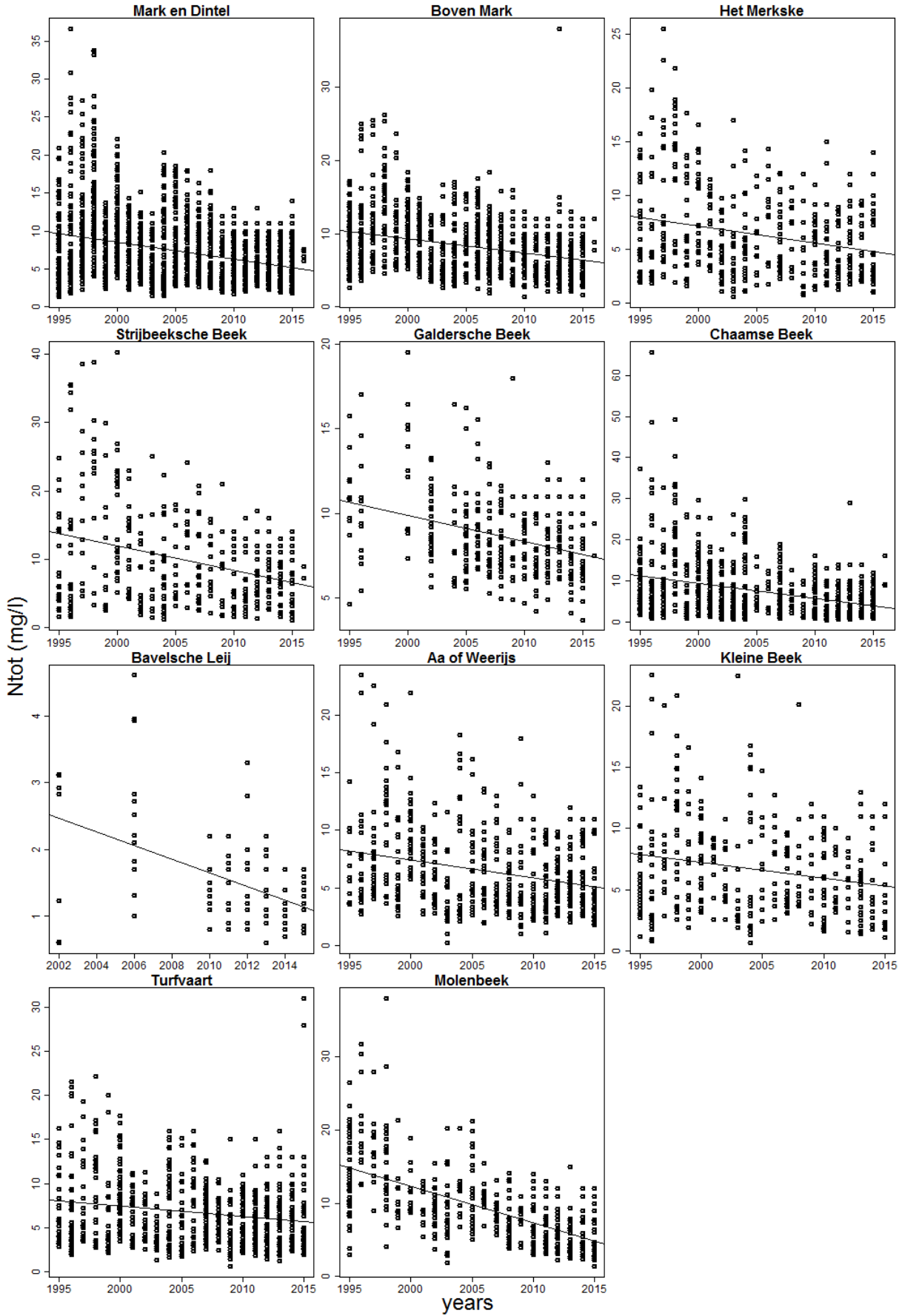
**NO<sub>2</sub> concentration of all waterbodies**



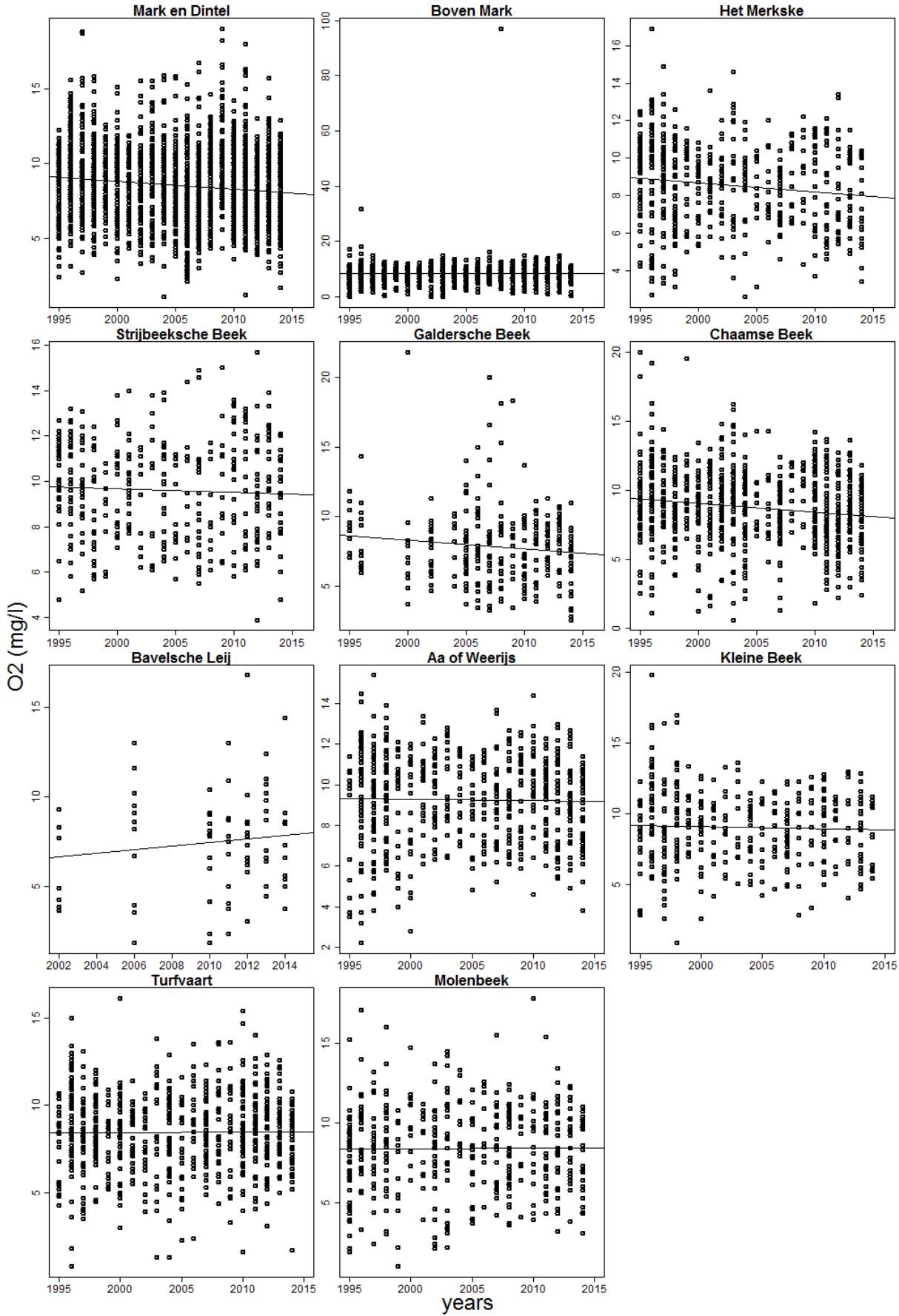
**NO3 concentration of all waterbodies**



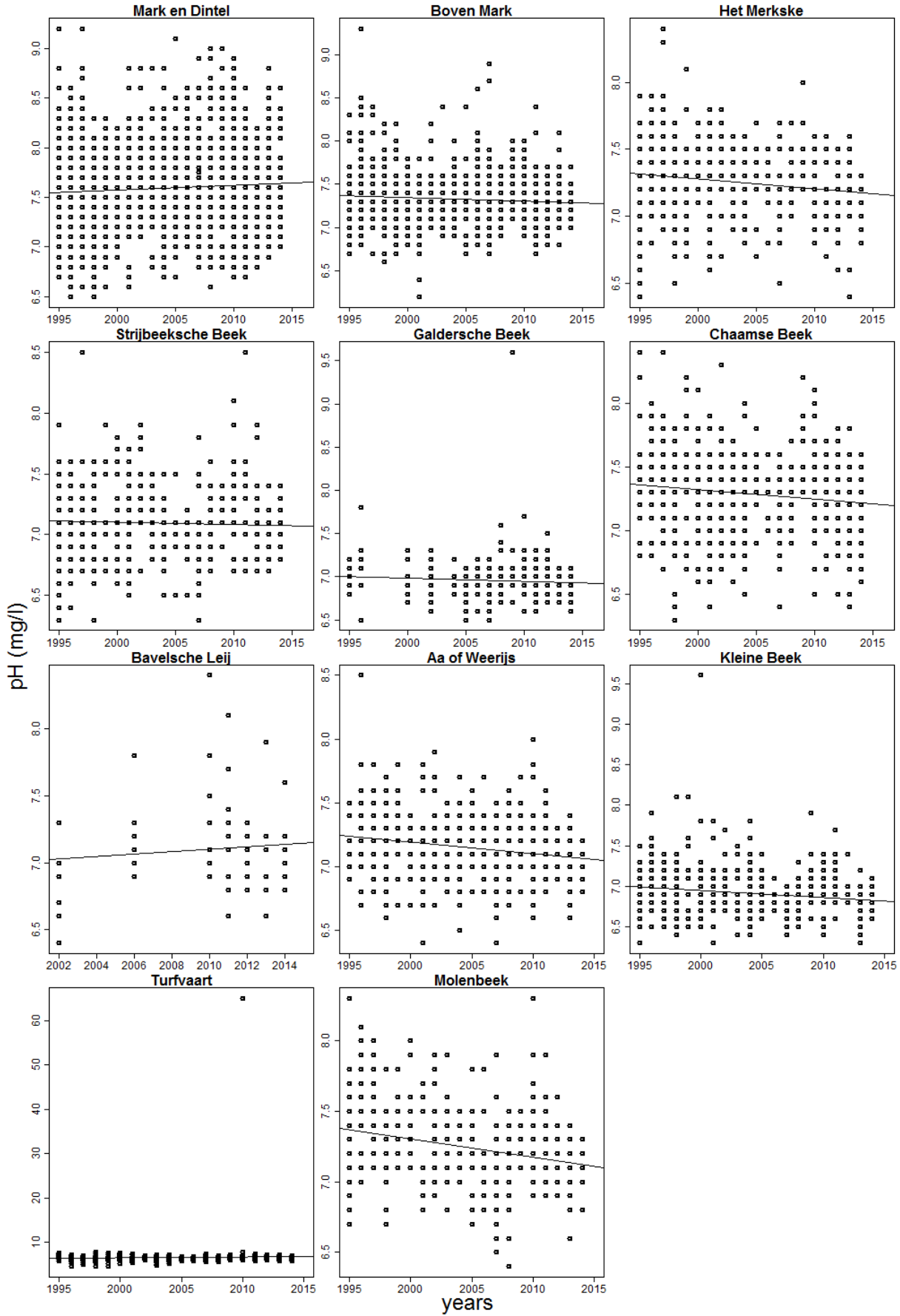
**Ntot concentration of all waterbodies**



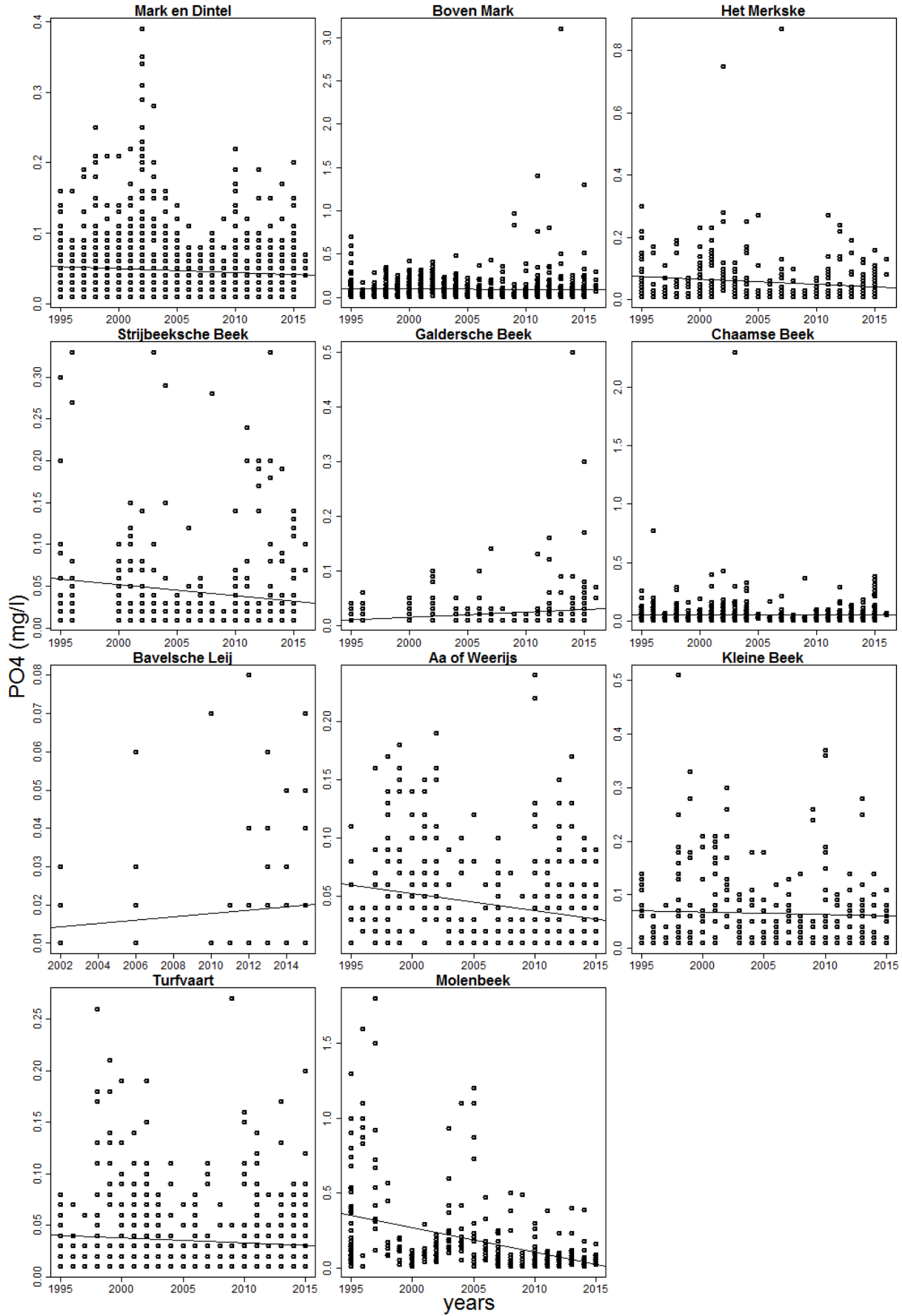
O2 concentration of all waterbodies



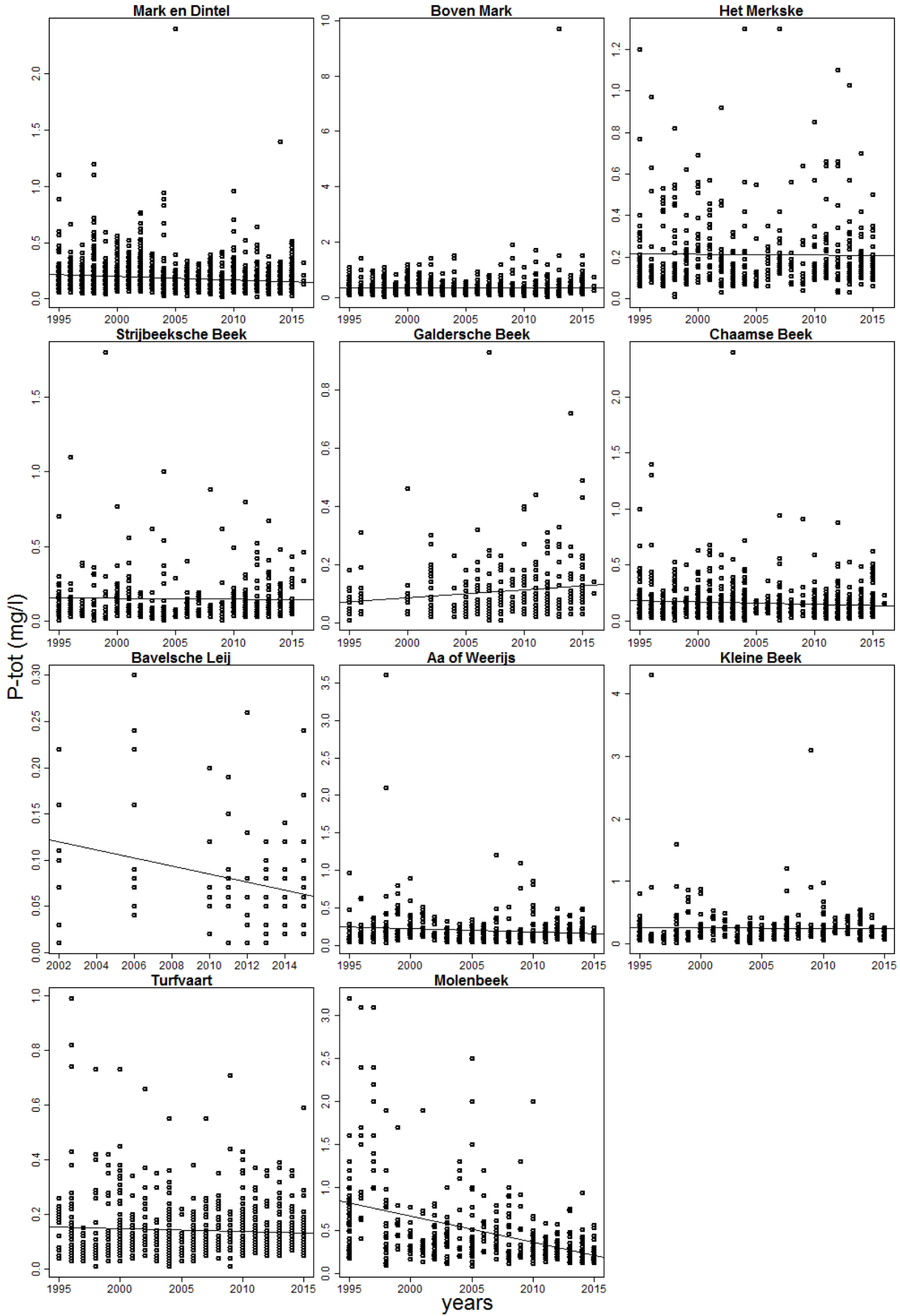
pH concentration of all waterbodies



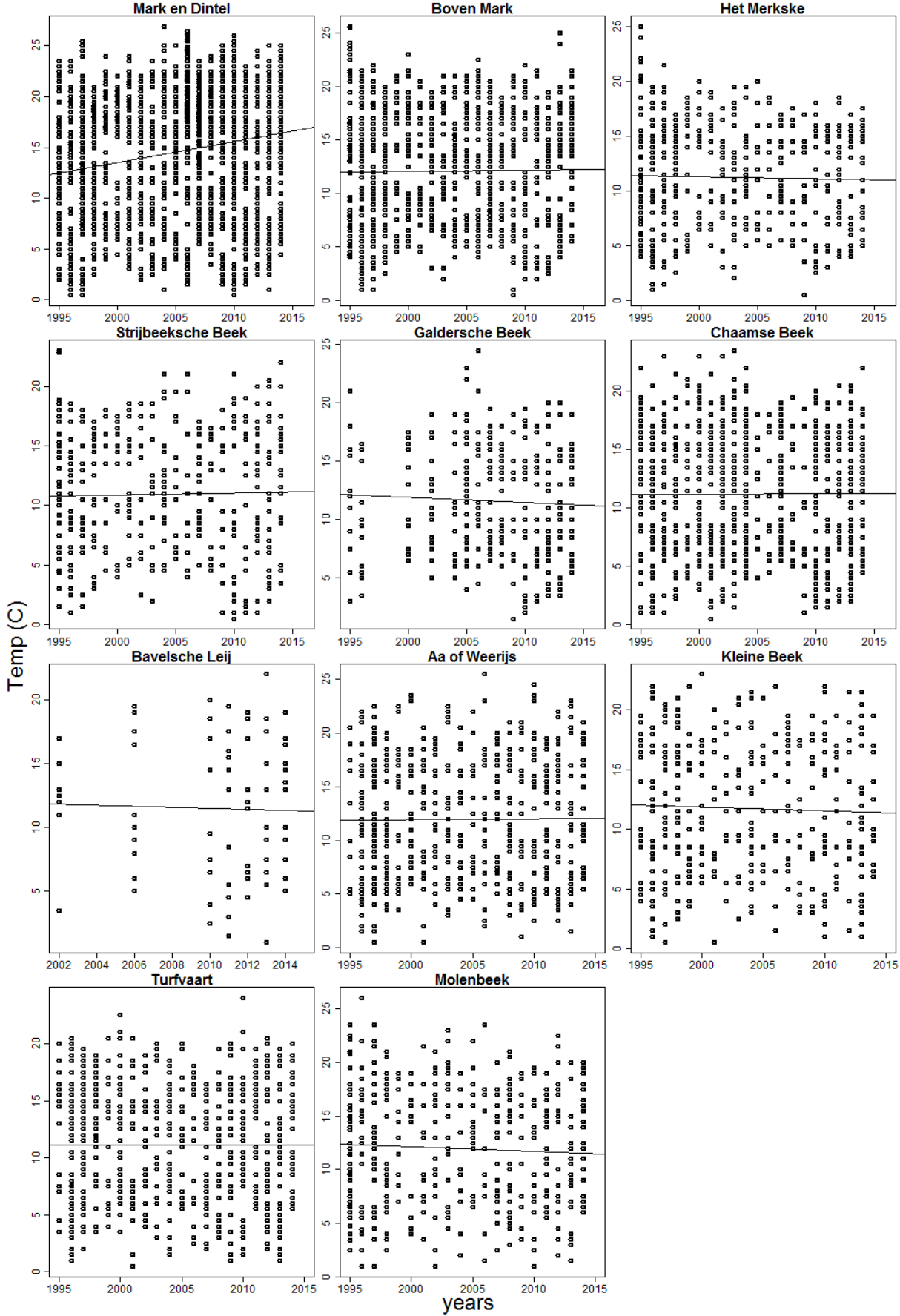
PO4 concentration of all waterbodies



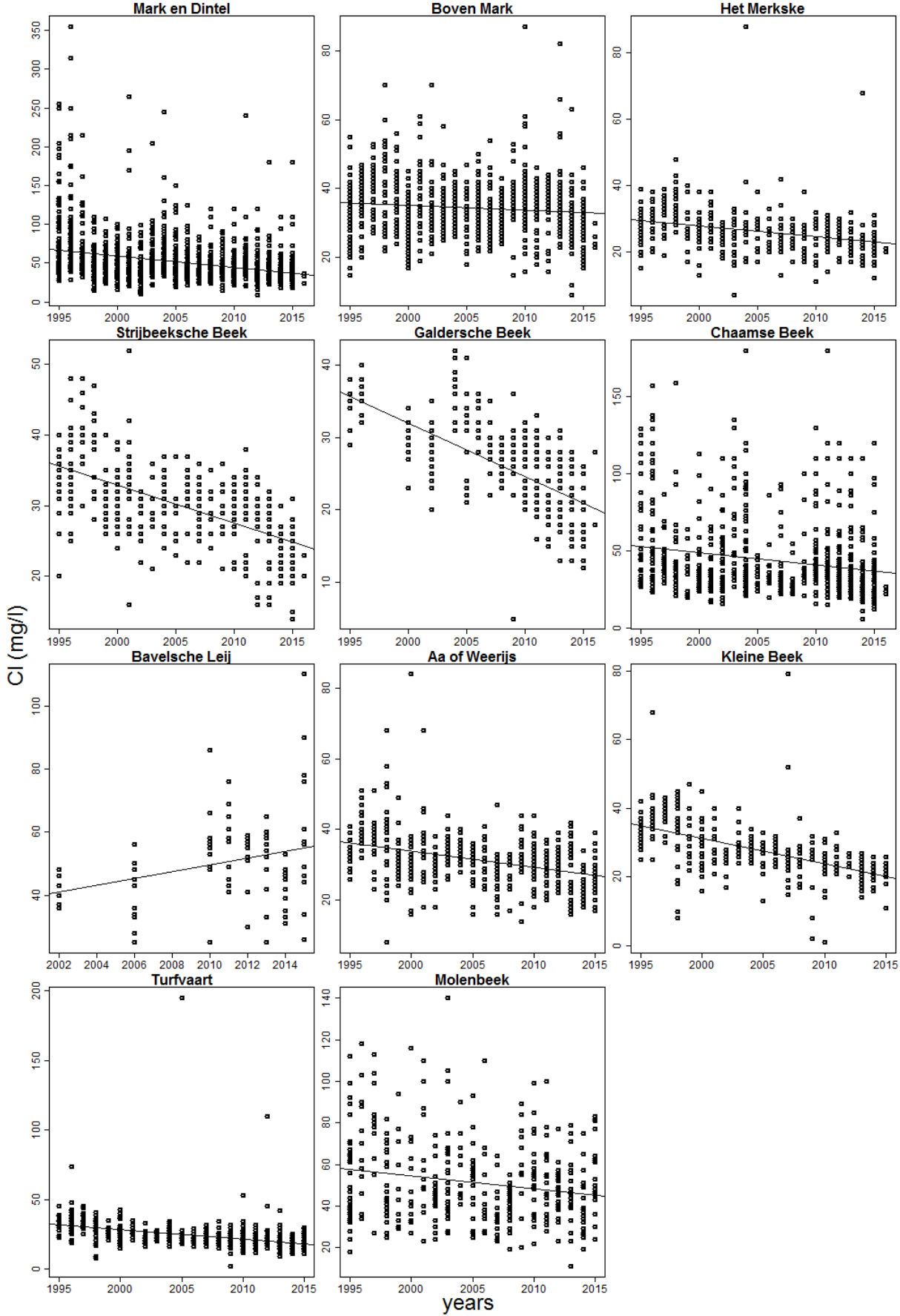
P-total concentration of all waterbodies



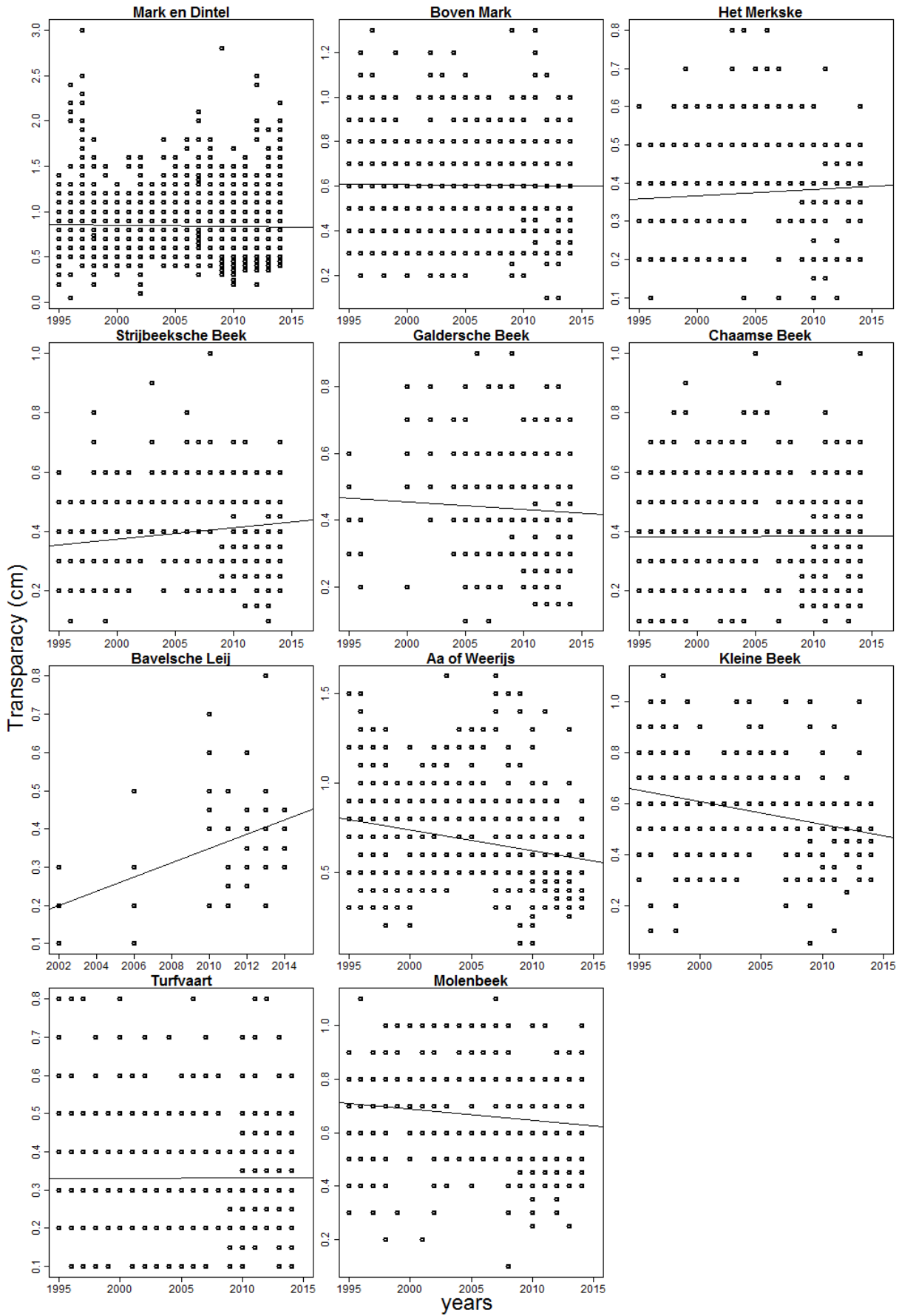
Temp of all waterbodies



Cl concentration of all waterbodies



Transparency of all waterbodies



## Appendix 9 Diversity indices for subareas of Mark en Vliet

An overview of the diversity indices for subareas of Mark en Vliet are shown in this appendix.

### Mark en Dintel

| Year | Species richness | Pielou's evenness | Shannon's diversity |
|------|------------------|-------------------|---------------------|
| 1994 | 7,42             | 0,53              | 1,03                |
| 2005 | 6,83             | 0,61              | 1,15                |
| 2008 | 4,94**           | 0,53              | 0,81**              |
| 2011 | 6,42*            | 0,61              | 1,11**              |
| 2014 | 6,21             | 0,68              | 1,21                |

### Mark-Vliet kanaal

| Year | Species richness | Pielou's evenness | Shannon's diversity |
|------|------------------|-------------------|---------------------|
| 2011 | 6                | 0,56              | 0,97                |
| 2014 | 6,88             | 0,55              | 1,01                |

### Mark kanaal

| Year | Species richness | Pielou's evenness | Shannon's diversity |
|------|------------------|-------------------|---------------------|
| 2011 | 4,5              | 0,81              | 1,22                |
| 2014 | 3,2              | 0,74              | 0,57*               |

### Steenbergse & rosendaalse Vliet

| Year | Species richness | Pielou's evenness | Shannon's diversity |
|------|------------------|-------------------|---------------------|
| 2011 | 7,27             | 0,55              | 0,91                |
| 2014 | 7,55             | 0,57              | 1,09                |

## Appendix 10 Results of simper analysis for gears

The results of the Simper analysis for fishing gear (table 32, 32, 33) and waterbodies (table 34, 35, 36) are shown in this appendix. Simper analysis was used to distinguish the fish species that contributed most for the differences between water types and the used fishing gears. Simper analysis was performed by a Bray-Curtis distance matrix with 999 permutations. Results of the simper analysis are shown by 4 column. The first column shows the fish species per length category, ordered by the species that account for the most difference between groups. The second column shows the amount of contrast per fish species that contributes to the difference between groups. The third and fourth column shows the average density of the fish species in group A and group B..

Table 31. Contrast between Trawler and Seine fisheries.

| specie | contr     | av.a     | av.b    |
|--------|-----------|----------|---------|
| BR_LK1 | 0.1002710 | 15.28423 | 7.73932 |
| BV_LK1 | 0.0998206 | 12.08683 | 12.5832 |
| BA_LK1 | 0.0659755 | 8.43270  | 4.67695 |
| PO_LK1 | 0.0571992 | 8.71247  | 1.45832 |
| BR_LK2 | 0.0530798 | 7.11747  | 2.12500 |
| BR_LK3 | 0.0452224 | 5.32122  | 1.44222 |
| RV_LK1 | 0.0339961 | 0.18927  | 4.59928 |
| RG_LK1 | 0.0257592 | 0.00000  | 3.12076 |
| BV_LK2 | 0.0256214 | 3.07931  | 2.02116 |
| BR_LK4 | 0.0241777 | 2.88468  | 1.38512 |
| KB_LK1 | 0.0210286 | 2.61301  | 0.98854 |
| SB_LK1 | 0.0186609 | 2.35892  | 0.10690 |
| WI_LK1 | 0.0175541 | 1.07163  | 1.41778 |
| VE_LK1 | 0.0162711 | 0.05088  | 2.38353 |
| AL_LK1 | 0.0135635 | 1.68108  | 0.18222 |
| BV_LK3 | 0.0105636 | 1.12538  | 0.14553 |
| SB_LK4 | 0.0095642 | 1.10470  | 0.01667 |
| KB_LK2 | 0.0094237 | 1.16548  | 0.19624 |
| PA_LK4 | 0.0089436 | 1.19941  | 0.02357 |
| SB_LK3 | 0.0083662 | 1.05606  | 0.00000 |
| SK_LK2 | 0.0079277 | 0.05556  | 0.81059 |
| BA_LK2 | 0.0069966 | 0.47339  | 0.46136 |
| RV_LK2 | 0.0068501 | 0.11111  | 0.73055 |
| SK_LK1 | 0.0038791 | 0.00000  | 0.38528 |
| RB_LK1 | 0.0036432 | 0.16112  | 0.26359 |
| SK_LK4 | 0.0032648 | 0.06323  | 0.31624 |
| WI_LK2 | 0.0028276 | 0.05556  | 0.26428 |
| ZE_LK1 | 0.0028243 | 0.00000  | 0.33931 |
| KA_LK4 | 0.0025365 | 0.11878  | 0.22234 |
| SK_LK3 | 0.0023305 | 0.00000  | 0.23982 |
| ZE_LK4 | 0.0022508 | 0.00000  | 0.24326 |
| SK_LK5 | 0.0020614 | 0.10026  | 0.19653 |
| ZE_LK3 | 0.0017630 | 0.04471  | 0.09553 |
| BA_LK3 | 0.0013798 | 0.13730  | 0.04553 |
| WI_LK3 | 0.0013228 | 0.08174  | 0.10000 |
| ZB_LK1 | 0.0012596 | 0.00000  | 0.16464 |
| ZE_LK2 | 0.0011418 | 0.01852  | 0.07887 |
| TD_LK1 | 0.0007405 | 0.08761  | 0.01667 |
| PA_LK3 | 0.0006980 | 0.06323  | 0.01667 |
| DD_LK1 | 0.0003637 | 0.03704  | 0.00000 |
| KM_LK1 | 0.0003538 | 0.03704  | 0.00000 |

Table 32. Contrast between Trawler and Electro fisheries.

|        | contr     | av.a     | av.b     |
|--------|-----------|----------|----------|
| BR_LK1 | 0.1189788 | 15.28423 | 0.346456 |
| BV_LK1 | 0.0933574 | 12.08683 | 3.611473 |
| BA_LK1 | 0.0788310 | 8.43270  | 3.930410 |
| BR_LK2 | 0.0709672 | 7.11747  | 0.058586 |
| PO_LK1 | 0.0666267 | 8.71247  | 0.363467 |
| BR_LK3 | 0.0627332 | 5.32122  | 0.050834 |
| BV_LK2 | 0.0340842 | 3.07931  | 1.056068 |
| BR_LK4 | 0.0312598 | 2.88468  | 0.085207 |
| RG_LK1 | 0.0298601 | 0.00000  | 2.447864 |
| BE_LK1 | 0.0272979 | 0.00000  | 1.987775 |
| KB_LK1 | 0.0225037 | 2.61301  | 0.132377 |
| SB_LK1 | 0.0222727 | 2.35892  | 0.017341 |
| PA_LK4 | 0.0190907 | 1.19941  | 1.122579 |
| TD_LK1 | 0.0175699 | 0.08761  | 1.283933 |
| RV_LK1 | 0.0160548 | 0.18927  | 1.300998 |
| AL_LK1 | 0.0154688 | 1.68108  | 0.009868 |
| BV_LK3 | 0.0133649 | 1.12538  | 0.048036 |
| WI_LK1 | 0.0124031 | 1.07163  | 0.369234 |
| SK_LK2 | 0.0121986 | 0.05556  | 0.947265 |
| SB_LK4 | 0.0118116 | 1.10470  | 0.002890 |
| KB_LK2 | 0.0107745 | 1.16548  | 0.036302 |
| SB_LK3 | 0.0102271 | 1.05606  | 0.000000 |
| BA_LK2 | 0.0101865 | 0.47339  | 0.621753 |
| DD_LK1 | 0.0085779 | 0.03704  | 0.613553 |
| ZE_LK1 | 0.0078616 | 0.00000  | 0.680449 |
| SK_LK1 | 0.0072451 | 0.00000  | 0.493649 |
| PA_LK3 | 0.0067992 | 0.06323  | 0.463226 |
| VE_LK1 | 0.0053510 | 0.05088  | 0.490165 |
| RV_LK2 | 0.0042511 | 0.11111  | 0.180756 |
| RB_LK1 | 0.0030978 | 0.16112  | 0.070691 |
| ZE_LK3 | 0.0027652 | 0.04471  | 0.127021 |
| SK_LK3 | 0.0026007 | 0.00000  | 0.208040 |
| ZE_LK2 | 0.0025067 | 0.01852  | 0.160018 |
| ZB_LK1 | 0.0024361 | 0.00000  | 0.206693 |
| SK_LK5 | 0.0024175 | 0.10026  | 0.159422 |
| SK_LK4 | 0.0022381 | 0.06323  | 0.163602 |
| BA_LK3 | 0.0021495 | 0.13730  | 0.093783 |
| WI_LK2 | 0.0019150 | 0.05556  | 0.105033 |
| KA_LK4 | 0.0017156 | 0.11878  | 0.058505 |
| KM_LK1 | 0.0016539 | 0.03704  | 0.110474 |
| WI_LK3 | 0.0011009 | 0.08174  | 0.052590 |
| ZE_LK4 | 0.0006686 | 0.00000  | 0.061539 |

Table 33. Contrast between Seine and Electro fisheries.

|        | contr    | av.a     | av.b     |
|--------|----------|----------|----------|
| BV_LK1 | 0.127656 | 12.58321 | 3.611473 |
| BR_LK1 | 0.078289 | 7.73932  | 0.346456 |
| BA_LK1 | 0.073732 | 4.67695  | 3.930410 |
| RG_LK1 | 0.056365 | 3.12076  | 2.447864 |
| RV_LK1 | 0.052163 | 4.59928  | 1.300998 |
| BE_LK1 | 0.034266 | 0.00000  | 1.987775 |
| BV_LK2 | 0.029322 | 2.02116  | 1.056068 |
| BR_LK2 | 0.027052 | 2.12500  | 0.058586 |
| VE_LK1 | 0.026893 | 2.38353  | 0.490165 |
| PO_LK1 | 0.024944 | 1.45832  | 0.363467 |
| BR_LK4 | 0.022013 | 1.38512  | 0.085207 |
| TD_LK1 | 0.021712 | 0.01667  | 1.283933 |
| WI_LK1 | 0.021511 | 1.41778  | 0.369234 |
| BR_LK3 | 0.020801 | 1.44222  | 0.050834 |
| PA_LK4 | 0.020037 | 0.02357  | 1.122579 |
| SK_LK2 | 0.017315 | 0.81059  | 0.947265 |
| SK_LK1 | 0.012602 | 0.38528  | 0.493649 |
| ZE_LK1 | 0.012472 | 0.33931  | 0.680449 |
| BA_LK2 | 0.012086 | 0.46136  | 0.621753 |
| RV_LK2 | 0.010509 | 0.73055  | 0.180756 |
| DD_LK1 | 0.010414 | 0.00000  | 0.613553 |
| KB_LK1 | 0.008948 | 0.98854  | 0.132377 |
| PA_LK3 | 0.008344 | 0.01667  | 0.463226 |
| SK_LK4 | 0.006448 | 0.31624  | 0.163602 |
| SK_LK3 | 0.005873 | 0.23982  | 0.208040 |
| WI_LK2 | 0.004624 | 0.26428  | 0.105033 |
| ZB_LK1 | 0.004470 | 0.16464  | 0.206693 |
| SK_LK5 | 0.004211 | 0.19653  | 0.159422 |
| ZE_LK4 | 0.004021 | 0.24326  | 0.061539 |
| RB_LK1 | 0.003841 | 0.26359  | 0.070691 |
| AL_LK1 | 0.003839 | 0.18222  | 0.009868 |
| KA_LK4 | 0.003289 | 0.22234  | 0.058505 |
| ZE_LK2 | 0.003271 | 0.07887  | 0.160018 |
| ZE_LK3 | 0.003017 | 0.09553  | 0.127021 |
| KB_LK2 | 0.002492 | 0.19624  | 0.036302 |
| SB_LK1 | 0.002105 | 0.10690  | 0.017341 |
| WI_LK3 | 0.002088 | 0.10000  | 0.052590 |
| BA_LK3 | 0.002038 | 0.04553  | 0.093783 |
| BV_LK3 | 0.002029 | 0.14553  | 0.048036 |
| KM_LK1 | 0.001556 | 0.00000  | 0.110474 |
| SB_LK4 | 0.000372 | 0.01667  | 0.002890 |

Table 34. Contrast between Mark en Vliet and the small rivers

|        | contr    | av.a     | av.b     |
|--------|----------|----------|----------|
| BV_LK1 | 0.104906 | 6.293462 | 9.354465 |
| BA_LK1 | 0.087660 | 7.243018 | 4.522062 |
| BR_LK1 | 0.063730 | 6.071121 | 3.115584 |
| RV_LK1 | 0.041776 | 0.655697 | 3.414986 |
| RG_LK1 | 0.040807 | 0.028206 | 2.980727 |
| PA_LK4 | 0.037335 | 2.172622 | 0.385605 |
| PO_LK1 | 0.037157 | 3.854923 | 0.671247 |
| BV_LK2 | 0.033631 | 1.408290 | 2.256640 |
| BR_LK2 | 0.030066 | 2.838476 | 0.672161 |
| BR_LK3 | 0.025291 | 2.192606 | 0.359360 |
| ZE_LK1 | 0.021109 | 0.114887 | 1.320730 |
| VE_LK1 | 0.018536 | 0.120477 | 1.615330 |
| SK_LK2 | 0.018431 | 0.305172 | 1.260104 |
| WI_LK1 | 0.017680 | 0.756869 | 0.848466 |
| BR_LK4 | 0.016375 | 1.129641 | 0.554503 |
| PA_LK3 | 0.015521 | 0.769357 | 0.137608 |
| BA_LK2 | 0.014814 | 0.599425 | 0.817878 |
| KB_LK1 | 0.012281 | 0.992073 | 0.590437 |
| SB_LK1 | 0.008147 | 0.900557 | 0.033421 |
| RV_LK2 | 0.007954 | 0.068027 | 0.531320 |
| BE_LK1 | 0.007414 | 0.000000 | 0.398714 |
| SK_LK1 | 0.007138 | 0.020408 | 0.431971 |
| DD_LK1 | 0.006515 | 0.209679 | 0.150326 |
| AL_LK1 | 0.006342 | 0.650388 | 0.058758 |
| BV_LK3 | 0.005785 | 0.461432 | 0.112917 |
| SK_LK4 | 0.005559 | 0.077648 | 0.325633 |
| TD_LK1 | 0.005484 | 0.084782 | 0.211763 |
| SK_LK3 | 0.005190 | 0.084450 | 0.283839 |
| SK_LK5 | 0.004997 | 0.090086 | 0.303233 |
| KB_LK2 | 0.004860 | 0.444560 | 0.129138 |
| ZB_LK1 | 0.004816 | 0.016423 | 0.337771 |
| ZE_LK3 | 0.004561 | 0.013605 | 0.263466 |
| ZE_LK2 | 0.004134 | 0.013605 | 0.238023 |
| SB_LK4 | 0.003950 | 0.412610 | 0.006173 |
| WI_LK2 | 0.003907 | 0.070845 | 0.220488 |
| RB_LK1 | 0.003887 | 0.136833 | 0.153459 |
| KM_LK1 | 0.003470 | 0.020408 | 0.206741 |
| SB_LK3 | 0.003287 | 0.387941 | 0.000000 |
| BA_LK3 | 0.002905 | 0.126262 | 0.096052 |
| KA_LK4 | 0.002790 | 0.050437 | 0.176440 |
| ZE_LK4 | 0.002781 | 0.006803 | 0.196841 |
| WI_LK3 | 0.001758 | 0.057240 | 0.083112 |

Table 35. Contrast in fish species between Mark en Vliet and the small streams

|        | contr     | av.a     | av.b     |
|--------|-----------|----------|----------|
| BA_LK1 | 0.1172241 | 7.243018 | 1.977528 |
| BE_LK1 | 0.0852353 | 0.000000 | 4.127011 |
| BV_LK1 | 0.0830587 | 6.293462 | 1.435027 |
| RG_LK1 | 0.0684615 | 0.028206 | 3.623725 |
| BR_LK1 | 0.0545870 | 6.071121 | 0.082133 |
| TD_LK1 | 0.0530270 | 0.084782 | 2.670219 |
| PA_LK4 | 0.0491445 | 2.172622 | 0.481797 |
| PO_LK1 | 0.0418613 | 3.854923 | 0.055077 |
| BR_LK2 | 0.0308675 | 2.838476 | 0.039524 |
| BR_LK3 | 0.0283601 | 2.192606 | 0.072439 |
| DD_LK1 | 0.0236828 | 0.209679 | 1.053733 |
| BV_LK2 | 0.0228024 | 1.408290 | 0.532171 |
| RV_LK1 | 0.0220705 | 0.655697 | 0.574224 |
| PA_LK3 | 0.0211736 | 0.769357 | 0.194056 |
| SK_LK2 | 0.0203076 | 0.305172 | 0.863524 |
| SK_LK1 | 0.0196714 | 0.020408 | 0.800927 |
| BA_LK2 | 0.0147021 | 0.599425 | 0.316287 |
| WI_LK1 | 0.0140949 | 0.756869 | 0.145550 |
| BR_LK4 | 0.0140711 | 1.129641 | 0.082610 |
| KB_LK1 | 0.0094208 | 0.992073 | 0.031338 |
| SB_LK1 | 0.0092077 | 0.900557 | 0.013245 |
| AL_LK1 | 0.0065755 | 0.650388 | 0.000000 |
| VE_LK1 | 0.0061340 | 0.120477 | 0.238164 |
| BV_LK3 | 0.0058269 | 0.461432 | 0.000000 |
| SK_LK3 | 0.0050986 | 0.084450 | 0.185263 |
| ZE_LK1 | 0.0050898 | 0.114887 | 0.165215 |
| SB_LK4 | 0.0046575 | 0.412610 | 0.000000 |
| KB_LK2 | 0.0043659 | 0.444560 | 0.006623 |
| SB_LK3 | 0.0039266 | 0.387941 | 0.000000 |
| ZB_LK1 | 0.0038396 | 0.016423 | 0.160670 |
| RB_LK1 | 0.0033298 | 0.136833 | 0.026490 |
| BA_LK3 | 0.0032855 | 0.126262 | 0.056120 |
| RV_LK2 | 0.0032682 | 0.068027 | 0.107951 |
| SK_LK4 | 0.0031610 | 0.077648 | 0.098202 |
| SK_LK5 | 0.0027016 | 0.090086 | 0.066225 |
| ZE_LK2 | 0.0026950 | 0.013605 | 0.136018 |
| WI_LK2 | 0.0025564 | 0.070845 | 0.060030 |
| WI_LK3 | 0.0016024 | 0.057240 | 0.044583 |
| ZE_LK3 | 0.0012005 | 0.013605 | 0.049101 |
| KA_LK4 | 0.0010379 | 0.050437 | 0.026490 |
| KM_LK1 | 0.0007485 | 0.020408 | 0.024716 |
| ZE_LK4 | 0.0004830 | 0.006803 | 0.019868 |

Table 36. Contrast between small rivers and small streams

|        | contr     | av.a     | av.b     |
|--------|-----------|----------|----------|
| BV_LK1 | 0.1285987 | 9.354465 | 1.435027 |
| RG_LK1 | 0.0778787 | 2.980727 | 3.623725 |
| BE_LK1 | 0.0765434 | 0.398714 | 4.127011 |
| BA_LK1 | 0.0753854 | 4.522062 | 1.977528 |
| RV_LK1 | 0.0523951 | 3.414986 | 0.574224 |
| TD_LK1 | 0.0508190 | 0.211763 | 2.670219 |
| BV_LK2 | 0.0389811 | 2.256640 | 0.532171 |
| BR_LK1 | 0.0356673 | 3.115584 | 0.082133 |
| ZE_LK1 | 0.0252996 | 1.320730 | 0.165215 |
| SK_LK2 | 0.0236850 | 1.260104 | 0.863524 |
| VE_LK1 | 0.0226909 | 1.615330 | 0.238164 |
| DD_LK1 | 0.0222830 | 0.150326 | 1.053733 |
| SK_LK1 | 0.0203941 | 0.431971 | 0.800927 |
| BA_LK2 | 0.0161781 | 0.817878 | 0.316287 |
| WI_LK1 | 0.0145355 | 0.848466 | 0.145550 |
| PA_LK4 | 0.0130507 | 0.385605 | 0.481797 |
| PO_LK1 | 0.0125281 | 0.671247 | 0.055077 |
| BR_LK4 | 0.0103982 | 0.554503 | 0.082610 |
| RV_LK2 | 0.0099225 | 0.531320 | 0.107951 |
| BR_LK2 | 0.0094211 | 0.672161 | 0.039524 |
| ZB_LK1 | 0.0077846 | 0.337771 | 0.160670 |
| SK_LK3 | 0.0073774 | 0.283839 | 0.185263 |
| SK_LK4 | 0.0070081 | 0.325633 | 0.098202 |
| KB_LK1 | 0.0068167 | 0.590437 | 0.031338 |
| ZE_LK2 | 0.0065664 | 0.238023 | 0.136018 |
| BR_LK3 | 0.0065100 | 0.359360 | 0.072439 |
| ZE_LK3 | 0.0058807 | 0.263466 | 0.049101 |
| PA_LK3 | 0.0058802 | 0.137608 | 0.194056 |
| SK_LK5 | 0.0058506 | 0.303233 | 0.066225 |
| KM_LK1 | 0.0042687 | 0.206741 | 0.024716 |
| WI_LK2 | 0.0040842 | 0.220488 | 0.060030 |
| ZE_LK4 | 0.0034322 | 0.196841 | 0.019868 |
| KA_LK4 | 0.0032414 | 0.176440 | 0.026490 |
| BA_LK3 | 0.0024567 | 0.096052 | 0.056120 |
| RB_LK1 | 0.0024140 | 0.153459 | 0.026490 |
| WI_LK3 | 0.0020583 | 0.083112 | 0.044583 |
| KB_LK2 | 0.0018124 | 0.129138 | 0.006623 |
| BV_LK3 | 0.0016856 | 0.112917 | 0.000000 |
| AL_LK1 | 0.0013018 | 0.058758 | 0.000000 |
| SB_LK1 | 0.0008680 | 0.033421 | 0.013245 |
| SB_LK4 | 0.0001322 | 0.006173 | 0.000000 |

## Appendix 11 Performed NMDS for Mark en Vliet

This appendix shows an overview of the NMDS ordinations that were performed for Mark en Vliet to identify the differences between the used fishing gears and subareas. Furthermore, tables with the total number of individuals and relative occurrence of the fish species in the samples were included. First, all samples of Mark en Vliet are divided into fishing gear and presented in figure 22, where table 37 corresponding to the included data.

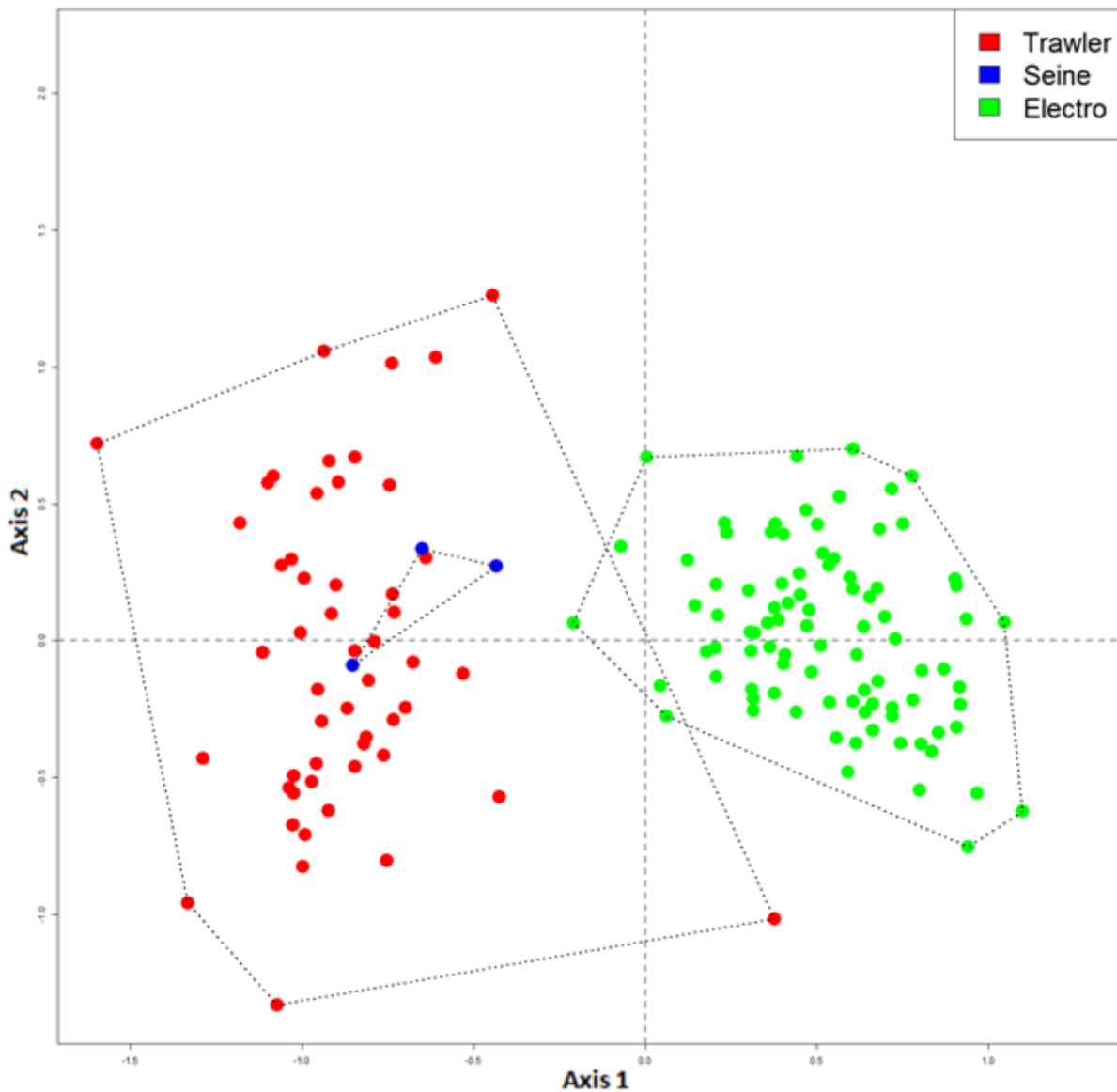


Figure 22. NMDS of all samples (N = 146) from Mark en Vliet, divided into fishing gear (hulled per gear) with relatively comparable community structure of the freshwater fish assemblages. Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted by two dimensions with 0.139 stress. This NMDS corresponds to the data in table 37.

Table 37. Relative occurrence and total number of individuals for fish species in the samples of Mark en Vliet (n = 146). This table corresponds to the data used for the NMDS in figure 22.

| <b>Fish specie abbreviation</b> | <b>Relative occurrence in samples (%)</b> | <b>Total number of individuals</b> |
|---------------------------------|---|------------------------------------|
| Bleak                           | 14.77                                     | 2861                               |
| Perch                           | 91.28                                     | 16846                              |
| Bitterling                      | 4.03                                      | 30                                 |
| Bream                           | 50.34                                     | 35600                              |
| Roach                           | 82.55                                     | 23725                              |
| Three-spined stickleback        | 16.11                                     | 44                                 |
| Prussian carp                   | 2.01                                      | 4                                  |
| Common carp                     | 5.37                                      | 13                                 |
| White bream                     | 29.53                                     | 1328                               |
| Crucian carp                    | 0.67                                      | 2                                  |
| Spined loach                    | 2.01                                      | 3                                  |
| Tubenosed goby                  | 4.70                                      | 29                                 |
| European eel                    | 74.50                                     | 1558                               |
| Ruffe                           | 55.70                                     | 10550                              |
| Asp                             | 17.45                                     | 62                                 |
| European bullhead               | 13.42                                     | 55                                 |
| Gudgeon                         | 1.34                                      | 3                                  |
| Rudd                            | 32.21                                     | 271                                |
| Zander                          | 35.57                                     | 1006                               |
| Pike                            | 32.89                                     | 100                                |
| Smelt                           | 2.01                                      | 10                                 |
| Nine-spined stickleback         | 6.04                                      | 22                                 |
| Sunbleak                        | 6.04                                      | 40                                 |
| Ide                             | 38.93                                     | 1476                               |
| Pumpkinseed                     | 1.34                                      | 3                                  |
| Tench                           | 8.05                                      | 39                                 |
| Round goby                      | 5.37                                      | 37                                 |

Furthermore, the data was separated according to the used fishing gear to understand the relative effect of physical-chemical variables on the freshwater fish assemblages. Only physical-chemical variables and fish species with a significant contribution to the model are shown. Arrows give an indication of the correlations between specific fish species and the physical-chemical variables. The data collected by trawlers is shown in figure 23. and corresponds to the data in table 38. The data collected by the electro fishing gear is shown in figure 24 and corresponds to the data in table 39.

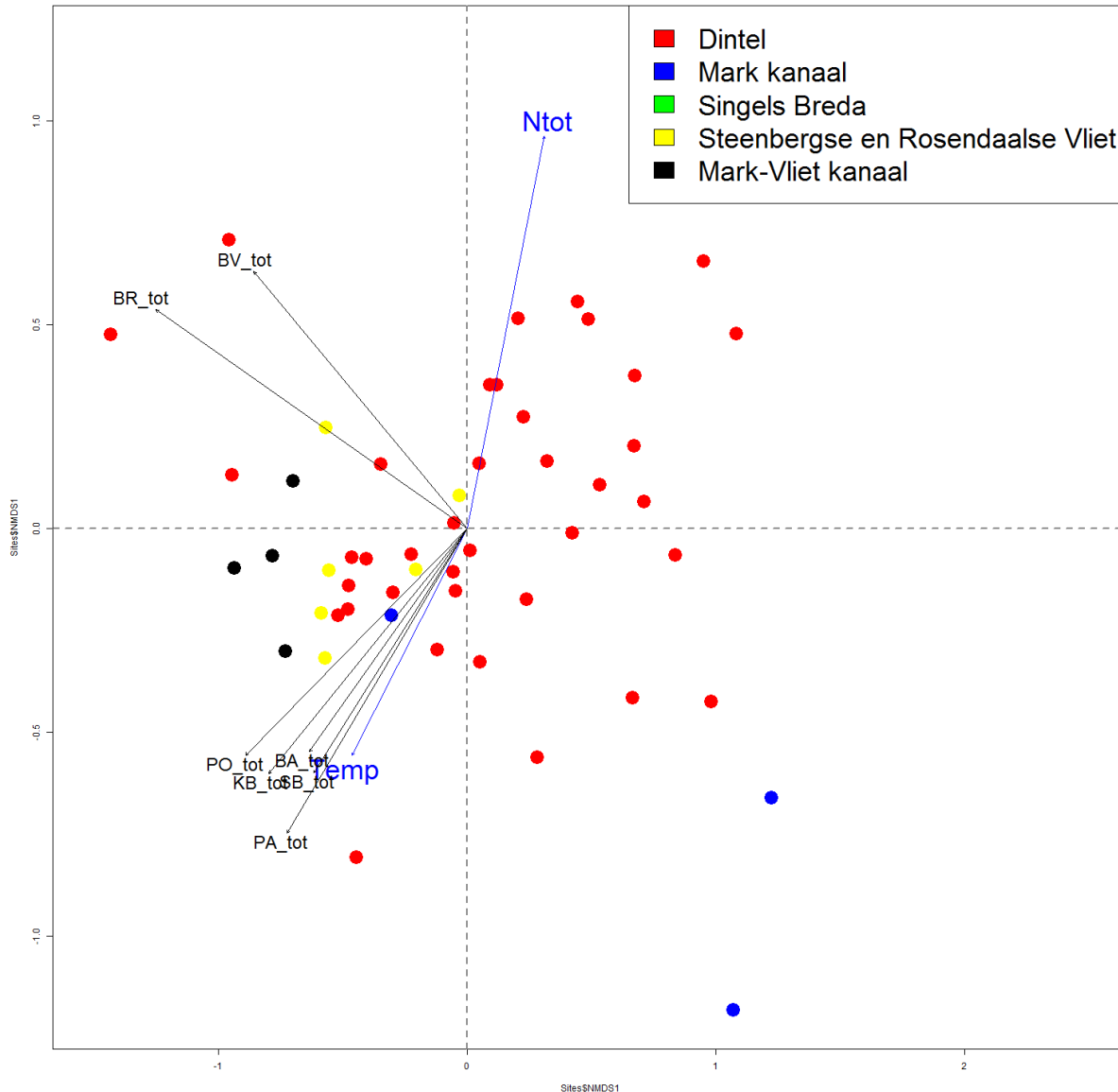


Figure 23. NMDS of the samples collected by trawlers from Mark en Vliet, divided in subareas. Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted by two dimensions with 0.132 stress. This NMDS corresponds to the data in table 38.

Table 38. Relative occurrence and total number of individuals for fish species in the samples collected by trawlers at Mark en Vliet (n = 52). The data in this table corresponds to the NMDS in figure 23.

| <b>Fish specie abbreviation</b> | <b>Relative occurrence in samples (%)</b> | <b>Total number of individuals</b> |
|---------------------------------|---|------------------------------------|
| Bleak                           | 34.62                                     | 2855                               |
| Perch                           | 76.92                                     | 12135                              |
| Bream                           | 100.00                                    | 34606                              |
| Roach                           | 98.08                                     | 22185                              |
| Three-spined stickleback        | 3.85                                      | 2                                  |
| Prussian carp                   | 3.85                                      | 3                                  |
| Common carp                     | 11.54                                     | 8                                  |
| White bream                     | 75.00                                     | 1318                               |
| Spined loach                    | 3.85                                      | 2                                  |
| European eel                    | 53.85                                     | 186                                |
| Ruffe                           | 80.77                                     | 10275                              |
| Asp                             | 30.77                                     | 52                                 |
| Rudd                            | 17.31                                     | 33                                 |
| Zander                          | 90.38                                     | 999                                |
| Pike                            | 19.23                                     | 13                                 |
| Smelt                           | 5.77                                      | 10                                 |
| Nine-spined stickleback         | 3.85                                      | 13                                 |
| Sunbleak                        | 1.92                                      | 7                                  |
| Ide                             | 38.46                                     | 1359                               |

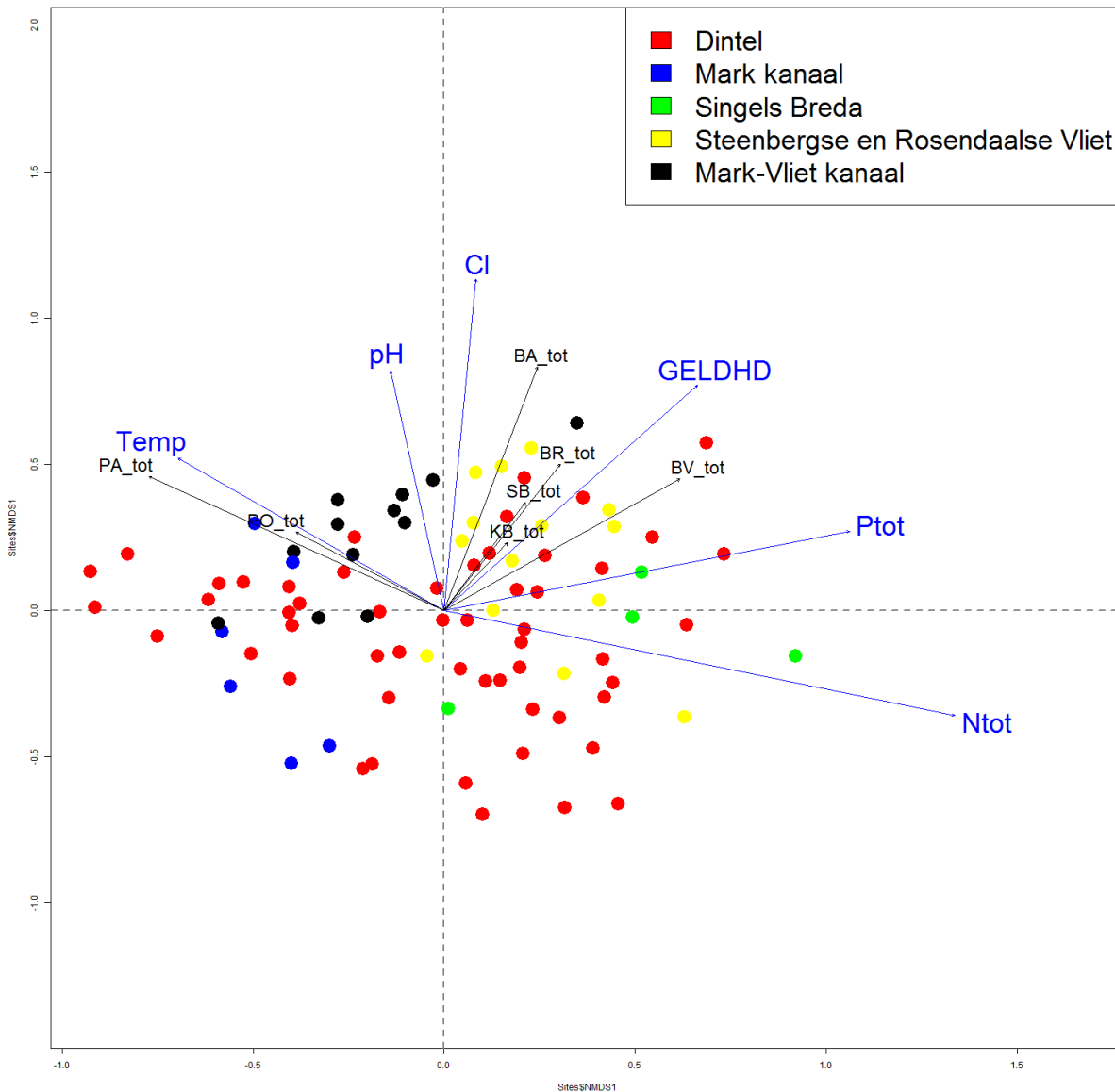


Figure 24. NMDS of the samples collected by electro trajectories from Mark en Vliet ( $n = 94$ ), divided in subareas. Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted by two dimensions with 0.201 stress. This NMDS corresponds to the data in table 39.

Table 39. Relative occurrence and total number of individuals for fish species in the samples collected by electro trajectories at Mark en Vliet (n = 94). The data in this table corresponds to the NMDS in figure 24.

| <b>Fish specie abbreviation</b> | <b>Relative occurrence in samples (%)</b> | <b>Total number of individuals</b> |
|---------------------------------|---|------------------------------------|
| Bleak                           | 2.13                                      | 3.00                               |
| Perch                           | 98.94                                     | 4613.01                            |
| Bitterling                      | 6.38                                      | 30.00                              |
| Bream                           | 21.28                                     | 78.00                              |
| Roach                           | 73.40                                     | 1437.99                            |
| Three-spined stickleback        | 23.40                                     | 42.00                              |
| Prussian carp                   | 1.06                                      | 1.00                               |
| Common carp                     | 2.13                                      | 5.00                               |
| White bream                     | 3.19                                      | 5.00                               |
| Spined loach                    | 1.06                                      | 1.00                               |
| Tobenosed goby                  | 7.45                                      | 29.00                              |
| European eel                    | 88.30                                     | 1372.16                            |
| Ruffe                           | 40.43                                     | 194.00                             |
| Asp                             | 10.64                                     | 10.00                              |
| European bullhead               | 21.28                                     | 55.00                              |
| Gudgeon                         | 2.13                                      | 3.00                               |
| Rudd                            | 39.36                                     | 235.00                             |
| Zander                          | 4.26                                      | 4.00                               |
| Pike                            | 40.43                                     | 85.00                              |
| Nine-spined stickleback         | 7.45                                      | 9.00                               |
| Sunbleak                        | 8.51                                      | 33.00                              |
| Ide                             | 37.23                                     | 112.00                             |
| Pumpkinseed                     | 2.13                                      | 3.00                               |
| Tench                           | 12.77                                     | 39.00                              |
| Round goby                      | 8.51                                      | 37.00                              |

## Appendix 12 NMDS for the Aa of Weerij, Boven Mark en Molenbeek

This appendix shows an overview of the NMDS ordinations that were performed for the Aa of Weerij, Boven Mark and Molenbeek to identify the differences between the used fishing gears and waterbodies. Furthermore, tables with the total number of individuals and relative occurrence of the fish species in the samples were included. First, all samples of the Aa of Weerij, Boven Mark and Molenbeek are divided into fishing gear and presented in figure 25, where table 40 corresponds to the included data.

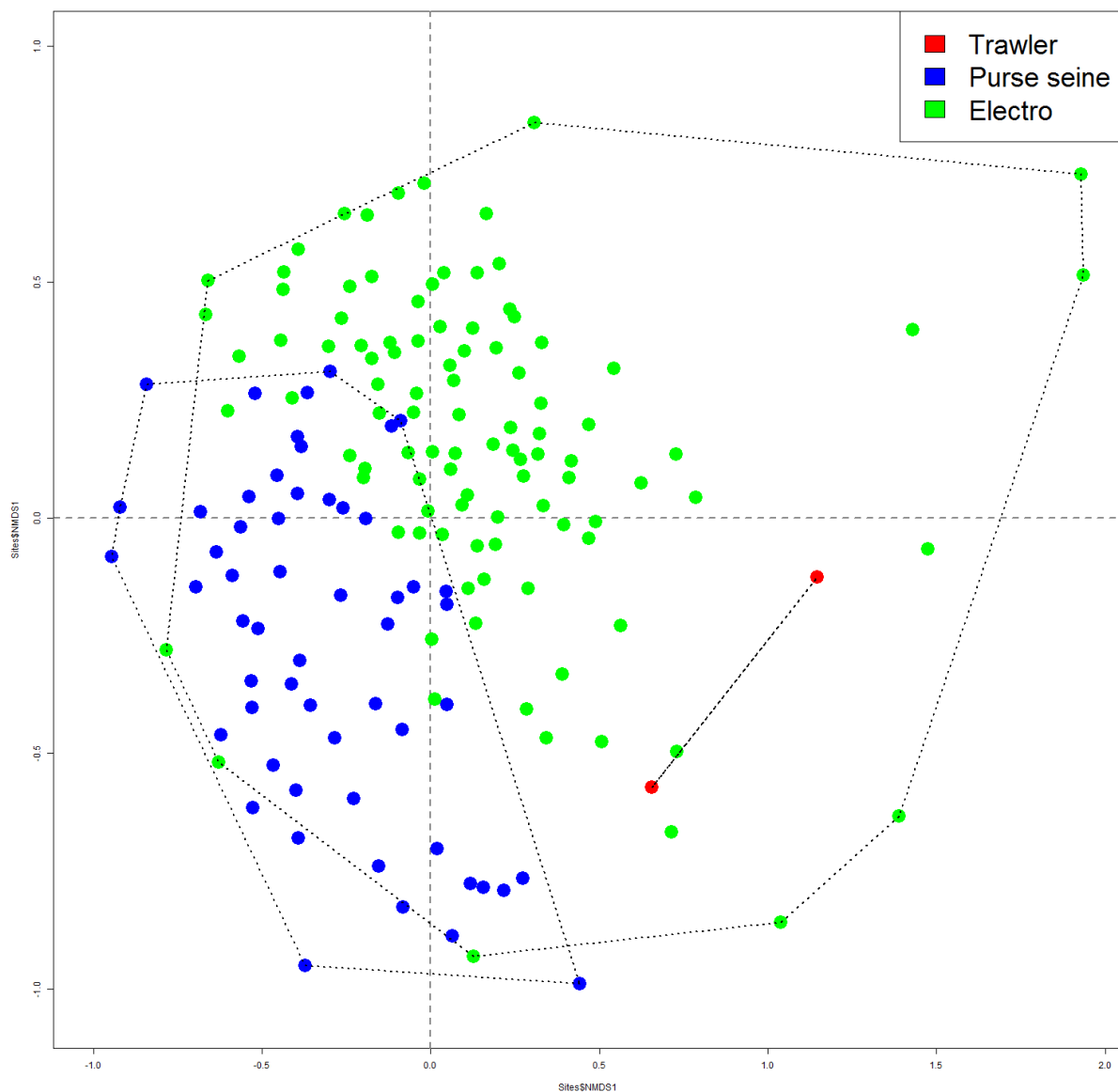


Figure 25. NMDS of all samples (N = 160) from The Aa of Weerij, Boven Mark and Molenbeek, divided into fishing gear (hulled per gear). Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted by two dimensions with 0.252 stress. This NMDS corresponds to the data in table 40.

Table 40. Relative occurrence and total number of individuals for fish species in the samples of the Aa of Weerijs, Boven Mark and Molenbeek (n = 160). This table corresponds to the data used for the NMDS in figure 25.

| Fish specie abbreviation | Relative occurrence in samples (%) | Total number of individuals |
|--------------------------|------------------------------------|-----------------------------|
| Bleak                    | 3.05                               | 37                          |
| Perch                    | 79.88                              | 9622                        |
| Topmouth gudgeon         | 5.49                               | 43                          |
| Stone loach              | 17.07                              | 195                         |
| Bitterling               | 3.66                               | 13                          |
| Bream                    | 55.49                              | 12662                       |
| Roach                    | 93.29                              | 38345                       |
| Three-spined stickleback | 6.71                               | 76                          |
| Prussian carp            | 4.88                               | 24                          |
| Grass carp               | 0.61                               | 1                           |
| Common carp              | 19.51                              | 69                          |
| White bream              | 18.90                              | 763                         |
| Crucian carp             | 1.22                               | 3                           |
| Spined loach             | 12.80                              | 67                          |
| Chub                     | 0.61                               | 1                           |
| European eel             | 28.66                              | 133                         |
| Ruffe                    | 28.66                              | 366                         |
| Asp                      | 8.54                               | 83                          |
| Gudgeon                  | 58.54                              | 6142                        |
| Rudd                     | 75.61                              | 10955                       |
| Zander                   | 4.27                               | 14                          |
| Pike                     | 71.34                              | 809                         |
| Nine-spined stickleback  | 9.76                               | 154                         |
| Sunbleak                 | 31.71                              | 3500                        |
| Ide                      | 35.37                              | 886                         |
| Pumpkinseed              | 18.29                              | 123                         |
| Tench                    | 59.15                              | 1032                        |

Furthermore, the data was separated according to the used fishing gear to understand the relative effect of physical-chemical variables on the freshwater fish assemblages. Only physical-chemical variables and fish species with a significant contribution to the model are shown. Arrows give an indication of the correlations between specific fish species and the physical-chemical variables. The data collected by purse seines is shown in figure 26 and corresponds to the data in table 41.

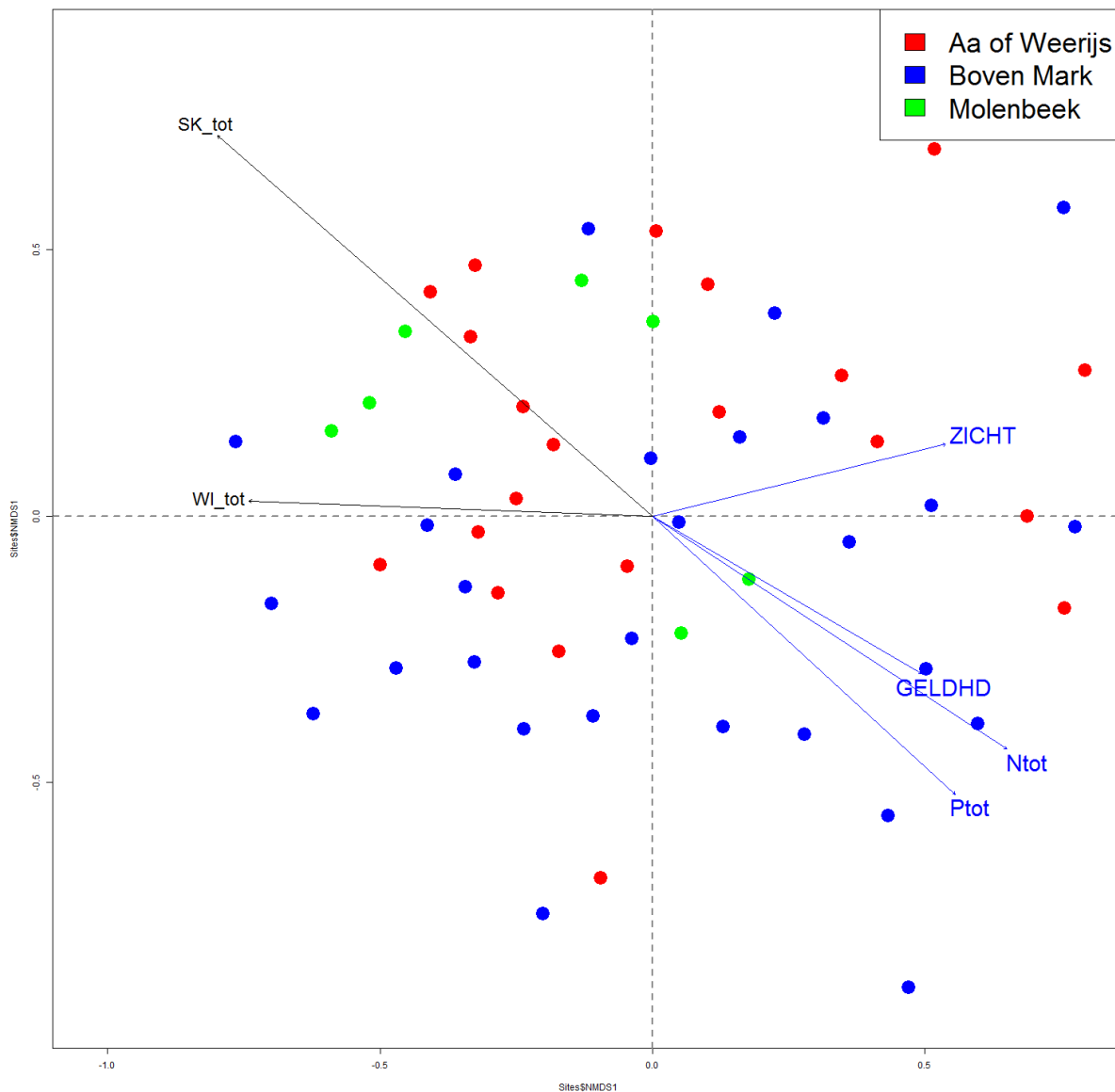


Figure 26. NMDS of the samples collected by purse seine samples from the Aa of Weerijis, Boven Mark and Molenbeek (n = 56). Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted by two dimensions with 0.254 stress. This NMDS corresponds to the data in table 41.

Table 41. Relative occurrence and total number of individuals for fish species in the samples collected by purse seine trajectories from the Aa of Weerij, Boven Mark and Molenbeek (n = 56). The data in this table corresponds to the NMDS in figure 26.

| <b>Fish specie abbreviation</b> | <b>Relative occurrence in samples (%)</b> | <b>Total number of individuals</b> |
|---------------------------------|---|------------------------------------|
| Bleak                           | 8.93                                      | 37                                 |
| Perch                           | 76.79                                     | 3739                               |
| Bitterling                      | 7.14                                      | 9                                  |
| Bream                           | 92.86                                     | 12371                              |
| Roach                           | 100.00                                    | 26897                              |
| Prussian carp                   | 1.79                                      | 1                                  |
| Grass carp                      | 1.79                                      | 1                                  |
| Common carp                     | 17.86                                     | 37                                 |
| White bream                     | 23.21                                     | 578                                |
| European eel                    | 3.57                                      | 3                                  |
| Ruffe                           | 39.29                                     | 307                                |
| Asp                             | 16.07                                     | 61                                 |
| Gudgeon                         | 66.07                                     | 2429                               |
| Rudd                            | 69.64                                     | 9343                               |
| Zander                          | 10.71                                     | 13                                 |
| Pike                            | 66.07                                     | 194                                |
| Nine-spined stickleback         | 1.79                                      | 1                                  |
| Sunbleak                        | 37.50                                     | 2695                               |
| Ide                             | 41.07                                     | 671                                |
| Pumpkinseed                     | 14.29                                     | 13                                 |
| Tench                           | 33.93                                     | 98                                 |

The data collected by the electro fishing gear is shown in figure 27 and corresponds to the data in table 42.

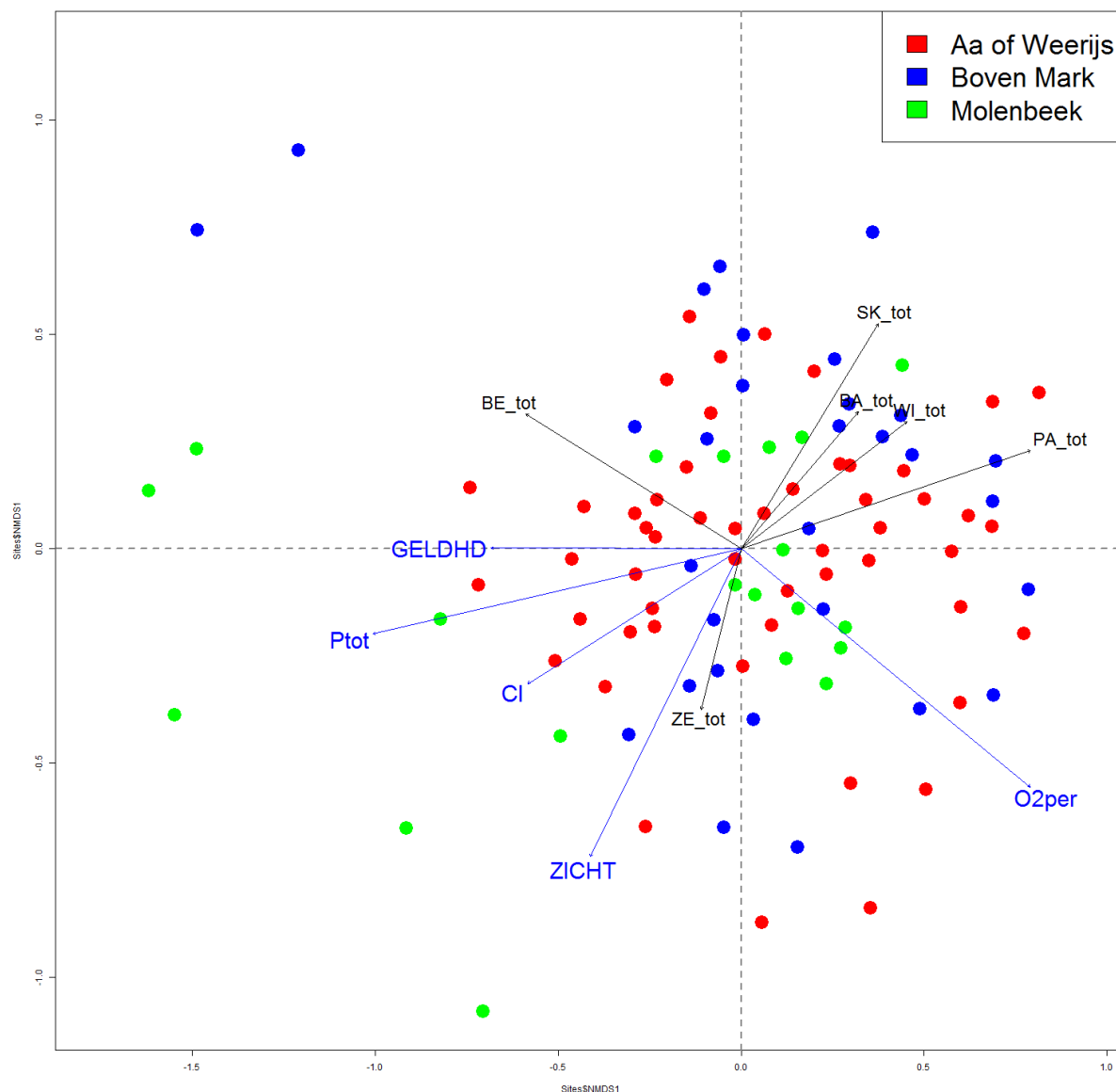


Figure 27. NMDS of the samples collected by electro fishing from the Aa of Weerijis, Boven Mark and Molenbeek (n = 102). Locations of the samples within the NMDS are based on a square root transformation of the numbers of fish species divided into length categories, plotted by two dimensions with 0.251 stress. This NMDS corresponds to the data in table 42.

Table 42. Relative occurrence and total number of individuals for fish species in the samples collected by electro fishing from the Aa of Weerij, Boven Mark and Molenbeek (n = 102). The data in this table corresponds to the NMDS in figure 27.

| <b>Fish specie abbreviation</b> | <b>Relative occurrence in samples (%)</b> | <b>Total number of individuals</b> |
|---------------------------------|---|------------------------------------|
| Perch                           | 83.02                                     | 5883                               |
| Topmouth gudgeon                | 8.49                                      | 43                                 |
| Stone loach                     | 26.42                                     | 195                                |
| Bitterling                      | 1.89                                      | 4                                  |
| Bream                           | 36.79                                     | 291                                |
| Roach                           | 89.62                                     | 11343                              |
| Three-spined stickleback        | 10.38                                     | 76                                 |
| Prussian carp                   | 6.60                                      | 23                                 |
| Common carp                     | 24.53                                     | 32                                 |
| White bream                     | 16.98                                     | 184                                |
| Crucian carp                    | 1.89                                      | 3                                  |
| Spined loach                    | 19.81                                     | 67                                 |
| Chub                            | 0.94                                      | 1                                  |
| European eel                    | 42.45                                     | 130                                |
| Ruffe                           | 23.58                                     | 58                                 |
| Asp                             | 4.72                                      | 21                                 |
| Gudgeon                         | 55.66                                     | 3713                               |
| Rudd                            | 79.25                                     | 1610                               |
| Zander                          | 0.94                                      | 1                                  |
| Pike                            | 75.47                                     | 615                                |
| Nine-spined stickleback         | 14.15                                     | 153                                |
| Sunbleak                        | 29.25                                     | 805                                |
| Ide                             | 33.02                                     | 214                                |
| Pumpkinseed                     | 20.75                                     | 110                                |
| Tench                           | 71.70                                     | 930                                |

## Appendix 13 Abundances of fish communities at the small streams

This appendix shows an overview of developments in abundances of fish communities at the small streams of the Mark watershed between 1994 and 2014. Waterbodies included are: the Galderse Beek (figure 28), Strijbeekse Beek (figure 29), Merkske (figure 30), Bavelse Leij (figure 31), Chaamse Beken (figure 32), Kleine Beek (figure 33) and Bijloop-Turfvaart (figure 34). Four of the seven streams show an increase in the total number of individuals that were collected. Furthermore, the abundant fish communities indicate variable shifts over time for the different streams.

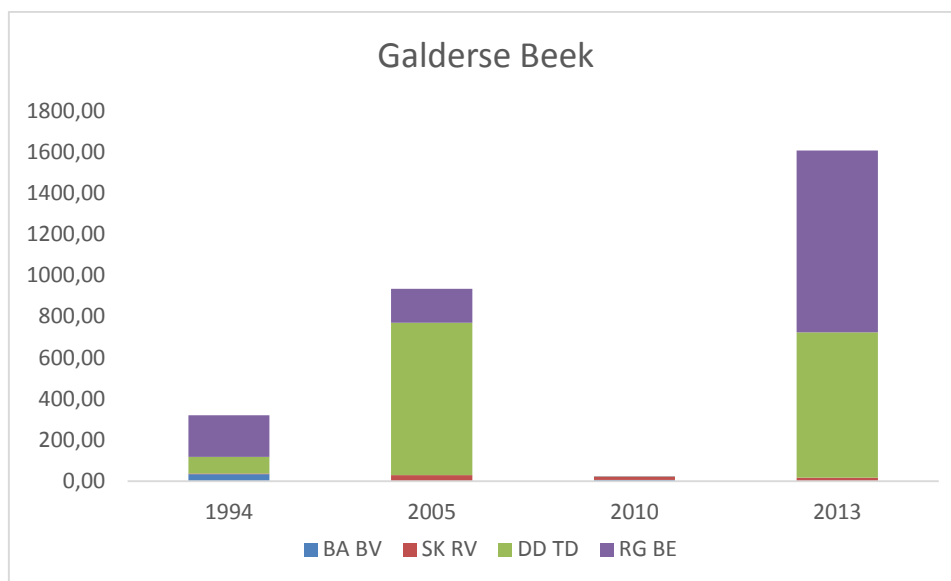


Figure 28. Abundances of the fish communities at the Galderse Beek over time.

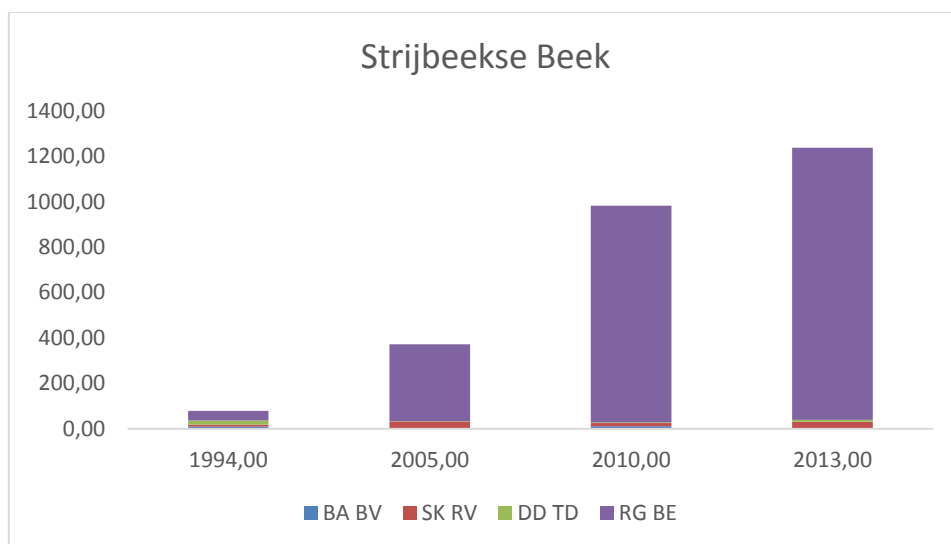


Figure 29. Abundances of the fish communities at the Strijbeekse Beek over time.

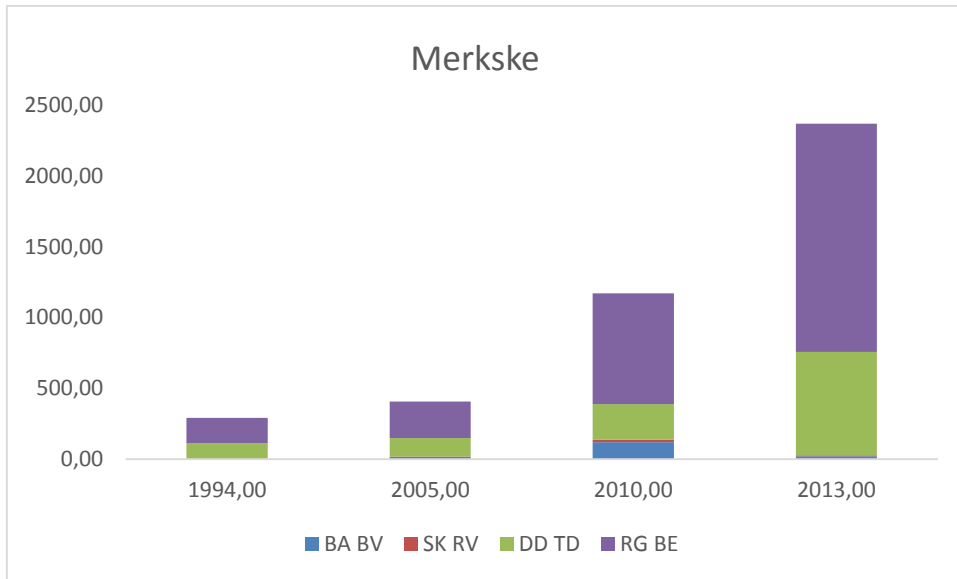


Figure 30. Abundances of the fish communities at Merkske over time.

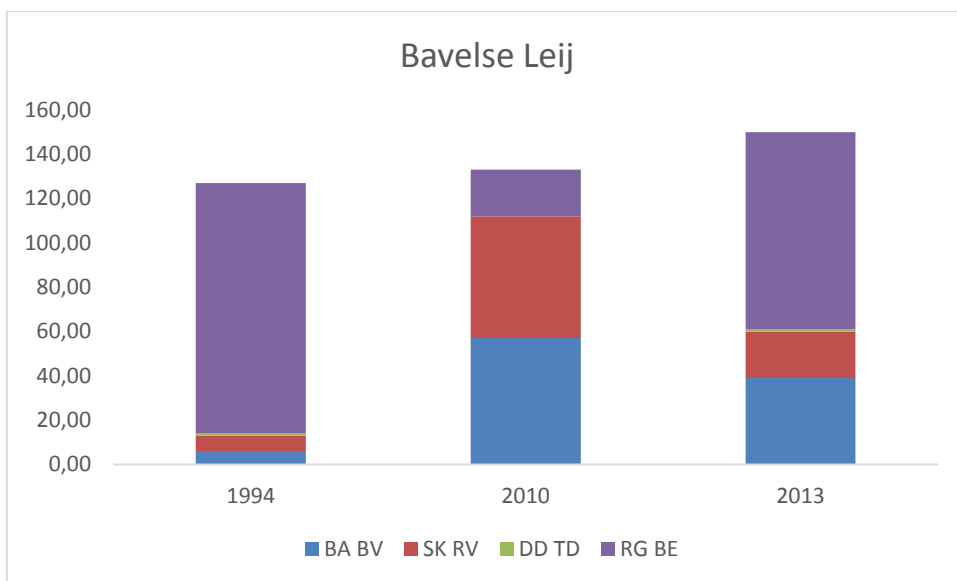


Figure31. Abundances of the fish communities at the Bavelse Leij over time.

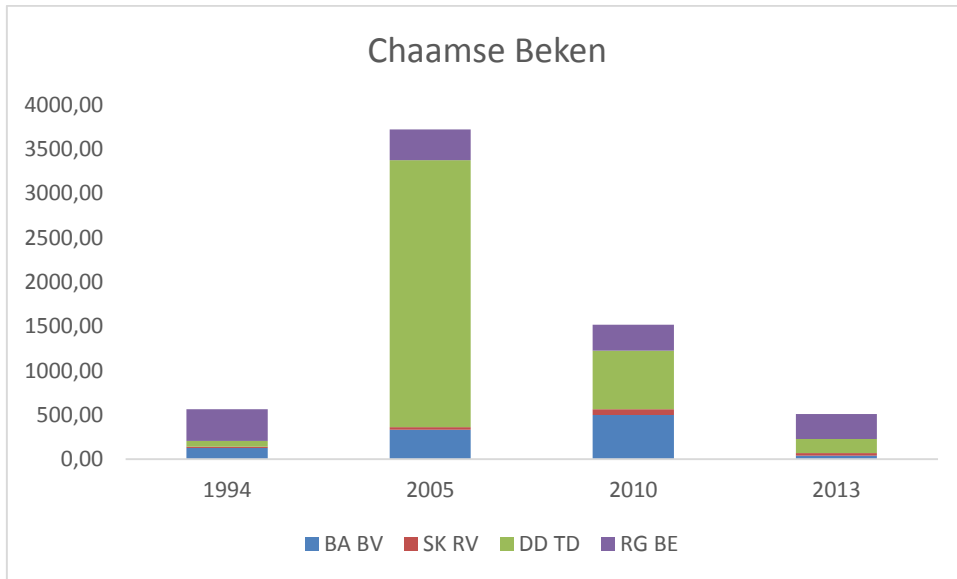


Figure 32. Abundances of the fish communities at the Chaamse Beken over time.

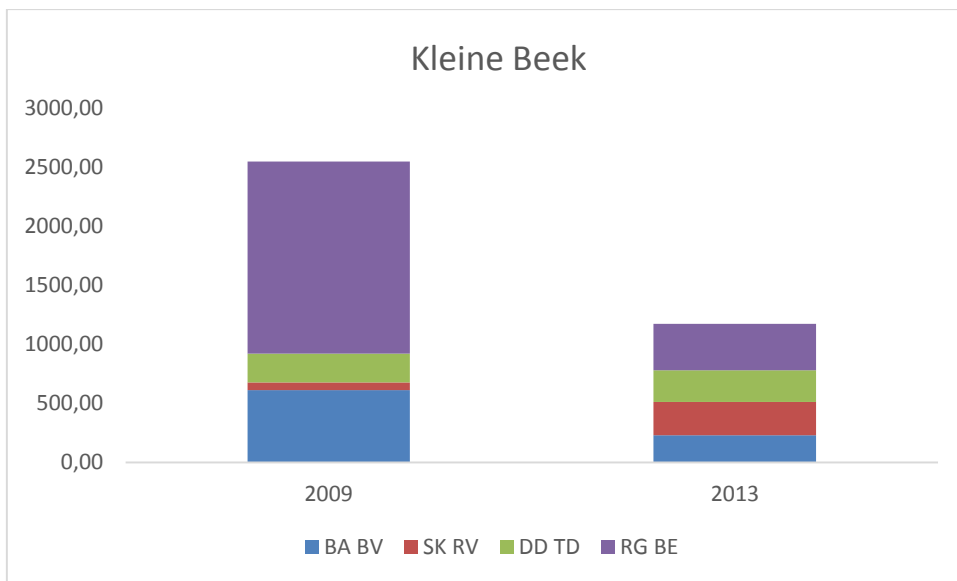


Figure 33. Abundances of the fish communities at the Kleine Beek over time.

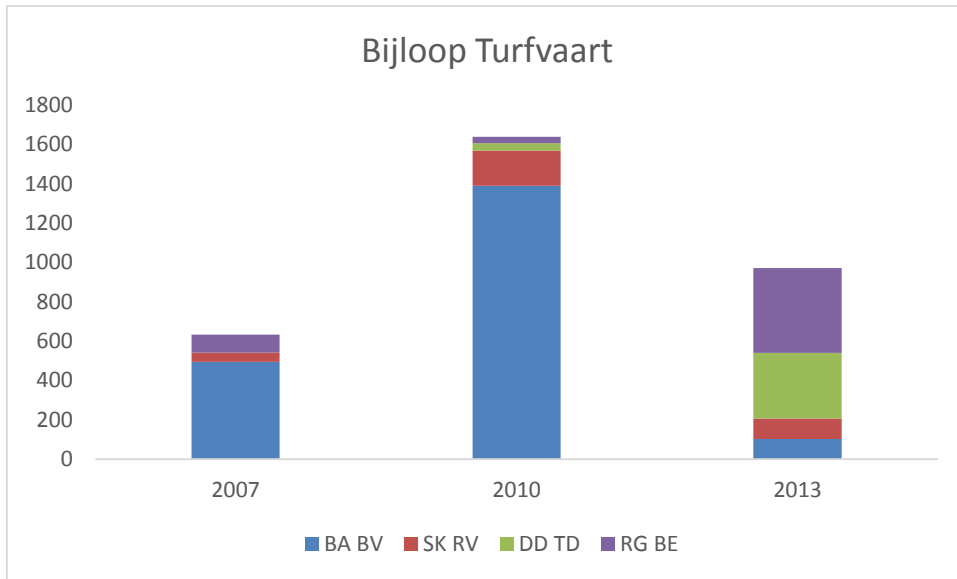


Figure 34. Abundances of the fish communities at Bijloop-Turfvaart over time.

