Influence of oxygen concentration on the macro-invertebrate composition and the WFD rating of the Kleine Dommel

Dennie Riechelman (driechel@hotmail.com)
830827 693020
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Supervisor at Wageningen University: Jeroen de Klein
Department of Environmental Sciences, Wageningen University, Wageningen

Examiner: Jeroen de Klein
Department of Environmental Sciences, Wageningen University, Wageningen

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Abstract

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The Kleine Dommel is a lowland stream located in the south of the Netherlands. Low oxygen concentrations in the Kleine Dommel, due to combined sewer overflows (CSO), lead to an ecosystem that is dominated by undesirable macro-invertebrates. Therefore, the Kleine Dommel scores too low for the water frame directive (WFD). Measures to increase the oxygen concentrations are expensive and depend on the resilience of the receiving water body. This research presents a methodology that predicts the macro-invertebrate composition and the WFD score as a result of annual minimum oxygen concentrations. With this information necessary measures to improve the WFD score can be evaluated on effectiveness. The analysis was based on biological samples of the Kleine Dommel, an oxygen measurement campaign in the Kleine Dommel and biological samples of all the water boards in the Netherlands (Limnodata). The chance of occurrence of macro-invertebrates that depends on the oxygen concentration was assessed by using the cumulative frequency distribution of Limnodata. This resulted in a theoretical macro-invertebrate composition and a WFD score for every annual minimum oxygen concentration from 0 till 15 mg/l. The biggest increase in WFD score can be gained by increasing the richness of species that are classified as indicative by the WFD. This can be achieved by increasing the annual minimum oxygen concentrations. The analysis shows that for a score of 0.6, which is a common target for most water bodies, the oxygen concentration in a R5 water body should stay around 5 mg/l the whole year. The analysis can be extended by including other influences that affect characteristic macro-invertebrate species.

Keywords: WFD, macro-invertebrate, oxygen, anoxia, EQR score, Kallisto

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1 Introduction

In the entire catchment area of the Dommel (including the Kleine Dommel) combined sewer overflow (CSO) events regularly take place as a result of heavy rains. The Kleine Dommel, which is a tributary of the Dommel, flows through several municipalities that have regular CSO events. It is known that this can result in strong ecological impacts such as oxygen depletion due to the biodegradation of the organic matter (Seager et al. ,1990). Continuous oxygen concentration during the year 2010 at the location “de Collse watermolen” in the Kleine Dommel showed several situations with oxygen concentrations of (just above) zero mg/l, which is obviously not desirable.

A CSO event has physical and chemical impacts on the receiving water (Willemsen et al., 1990 ; Seager et al. ,1990). Chemical impacts, besides reducing oxygen concentrations, are an increase of ammonium (NH_4^+) and organic micro-pollutants concentrations and a change of pH, temperature and salinity (Passerat et al., 2011 ; Prat et al., 1981). The influence on the oxygen concentration can result in anoxia, which directly influences the macro-invertebrates, and is therefore one of the most important impacts on the macro-invertebrate community. CSO events have a physical impact by changing the current velocity, turbidity and the sediment organic matter content (Pedersen et al., 1986).

The fact that a CSO event influences multiple physical and chemical properties complicates the analysis of the impact. Often the impact on the macro-invertebrate community is a synergy of all the parameters (Pratt et al., 1981). For example, a well-known synergy is the effect that low oxygen concentrations amplify the toxic impact of unionized ammonia (Gammeter et al., 1991 , Lammersen, 1997). However, oxygen concentrations are a quick responding driving force and can have an instant impact on the ecosystem and therefore are, on their own without regarding other mechanisms, an important research topic.

In 2015 all water bodies that are classified by the water framework directive (WFD) have to meet certain water quality targets (van de Molen, 2003). These targets are based on a scoring system that uses the macro-invertebrate composition, in which some species have a negative influence and others have a positive influence on the score.

Species like *Oligochaetes* and *isopods* that have a negative influence on the WFD score are resilient to low oxygen concentrations and are primarily found in polluted conditions (Polls et al., 1978). According to (Perdersen et al., 1986 ; Willemsen et al. 1990) the dominant species in an environment that regularly has low oxygen concentrations are chironomids and amphipods because of their tolerance to an unstable “oxygen” environment (Polls et al., 1978). Furthermore, *Chironomus spp., Psectrotanypus varius, Tubificidae, Culicidae* and certain leeches are abundant in environments with high organic pollution (and likely low oxygen concentrations). All of these species have a negative influence on the WFD score, whereas groups that have a positive influence on the WFD score, like *Odonata, Trichoptera, Gammaridae and Ephemeroptera* occur in waters that do not receive CSO’s and likely have higher minimum annual oxygen concentration (Willemsen et al., 1990).

The above mentioned indicates that a water body with “bad” oxygen conditions, such as low minimum annual oxygen concentrations, leads to degradation of the ecosystem, which becomes more uniform with only resilient macro-invertebrates. This eventually leads to a lower WFD score for the water body.

The ecological status of the Dommel at this moment does not meet the WFD targets. To reach these targets in 2015 and to maintain this better ecological status in the future, a holistic approach is necessary. This means, that all causes together are treated as one mechanism and the solution is an adaptation of all involved parties (waterboard, municipalities etc).

An extensive project named Kallisto was started with the intention to give advice on improvement of the sewer transport and treatment system to an extent that the receiving water bodies will maintain an acceptable water quality. In this way location specific measures can be taken.

Study Area

In size and discharge the Kleine Dommel is small compared to the Dommel, hence the name prefix Kleine which means small in Dutch. Despite of its relative small size, the Kleine Dommel is an important part of the catchment area of the Dommel and the Kleine Dommel discharges all of its water into the Dommel. Furthermore, the Kleine Dommel is rated as a WFD water body and therefore has to meet the quality requirements in 2015.

The Kleine Dommel is located in the south of the Netherlands flowing in a northerly direction until it combines with the Dommel (Figure 1). It is classified as an R5 water body for the WFD: “slow streaming brook on a sand bottom”. It starts east of the town Heeze and streams east of Eindhoven where it eventually confluences with the Dommel in Eindhoven. The Kleine Dommel is a specific area of interest because there are relative few measurements available compared to the other streams in the basin. Even though the Kleine Dommel receives sewer overflows and is rated mediocre/bad for both the chemical and ecological status.

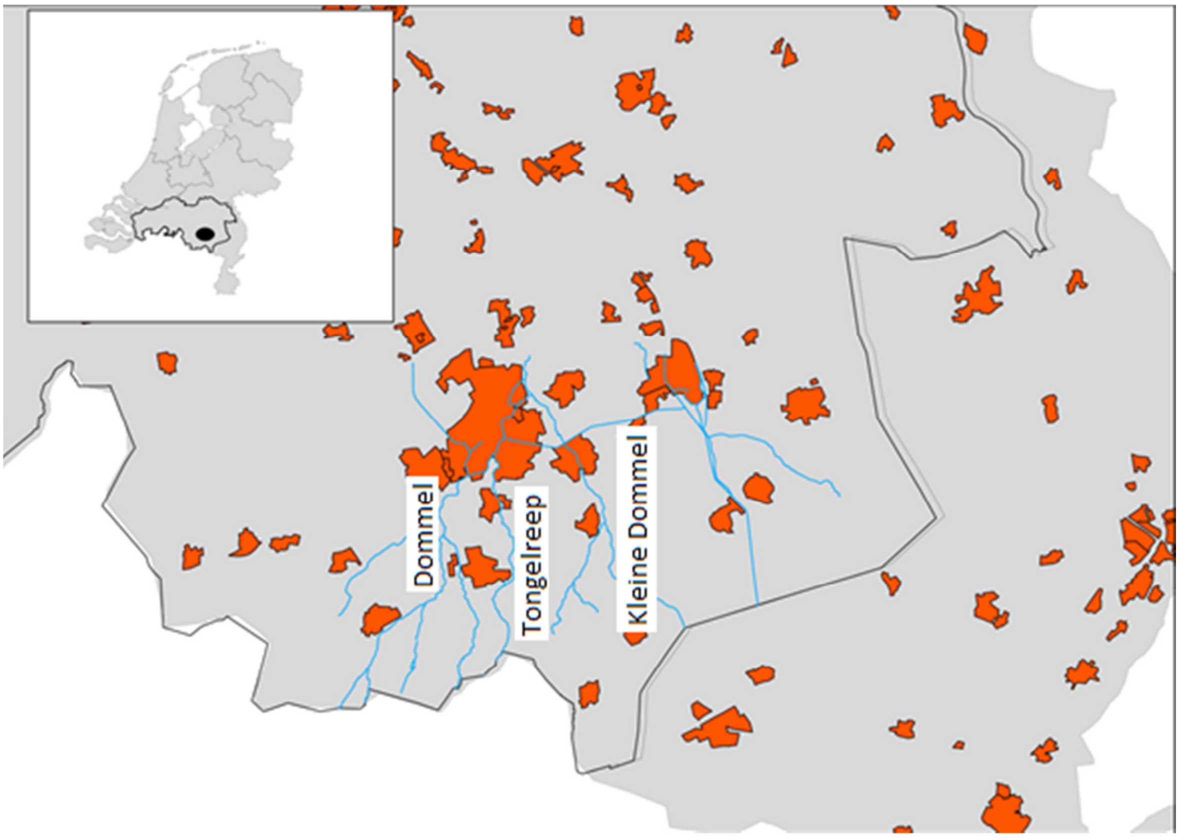


Figure 1, map of the Dommel, the Tongelreep and the Kleine Dommel (left to right)

Objective

The objective of this research is to give insight in the response of the macro-invertebrate composition (and therefore the WFD score) on minimum annual oxygen concentrations. A system to predict the impact of oxygen limitations on the macro-invertebrate composition does not exist yet. Therefore, this research can provide valuable information for policy makers and future research. The prediction about how improvements in the oxygen conditions in a stream will result in a better WFD score corresponds well with the philosophy of the Kallisto project and the results of this research will help to assess the extent of measures to be taken.

Not as much information is known for the Kleine Dommel as for the Dommel and because of time limitations the focus of this research is restricted to the Kleine Dommel. It only focuses on the influence of low oxygen concentrations and no other chemical or physical impacts.

The **main research question** is:

- *What is the influence of an annual minimum oxygen concentration on the macro-invertebrate composition and the WFD score?*

Besides the main research question are the following **sub research questions**:

- What is the temporal and spatial distribution of the macro-invertebrates in the Kleine Dommel compared to a “clean” stream?
- How do oxygen concentrations behave spatially and temporally in the Kleine Dommel?
    - How does the system recover from being anaerobic for a certain period of time?
    - Is there a relation between the depth of an oxygen dip and the time the system needs to recover?
    - Is there a relation between the duration of a “low-oxygen” situation and the time the system needs to recover?
    - How long does an anaerobic situation last after a sewer overflow and how deep is the oxygen dip?
- How sensitive is the rating of the WFD with regard to a change in macro-invertebrate composition?
- Which species are most vulnerable to low oxygen concentrations and which minimum oxygen concentrations are acceptable to maintain enough “sensitive” species and a certain WFD score?

Research structure

The set-up of the research follows the order of the sub research questions. First the biological samples taken by the water board for the Kleine Dommel and a reference stream (the Tongelreep) are analysed on the spatial and temporal distribution of the macro-invertebrates (Chapter 2).

Insight in the oxygen concentration profiles was gained by means of a measurement campaign (Chapter 3).

Better insight in how a WFD score comes about will help to understand where measures to improve macro-invertebrate communities should focus on. This was reached by comparing the scores with a well-known biodiversity index (Shannon Index) and by testing the influence of alterations in the macro-invertebrate composition on the WFD score with a sensitivity analysis (Chapter 4).

The final chapter analyses great amounts of biological samples of all the water boards in the Netherlands (called Limnodata). This chapter connects the chance of occurrence of species with minimum annual oxygen concentrations. Eventually a prediction can be made about a theoretical WFD score as a result of minimum annual oxygen concentrations (Chapter 5).

2 Analysis of macro-invertebrate samples

Waterboard Dommel takes macro-invertebrate samples once or twice a year at different locations throughout its area of operation (Figure 2; Appendix Table 9). The data consists of a list of species and their abundance. This chapter analyses both data of the Tongelreep (a relative natural stream that also connects to the Dommel) and the Kleine Dommel. The Tongelreep is assumed to be the reference because it receives almost no CSO and the water quality is more constant due to the constant inlet of canal water (pers.comm. Mark Scheepens).

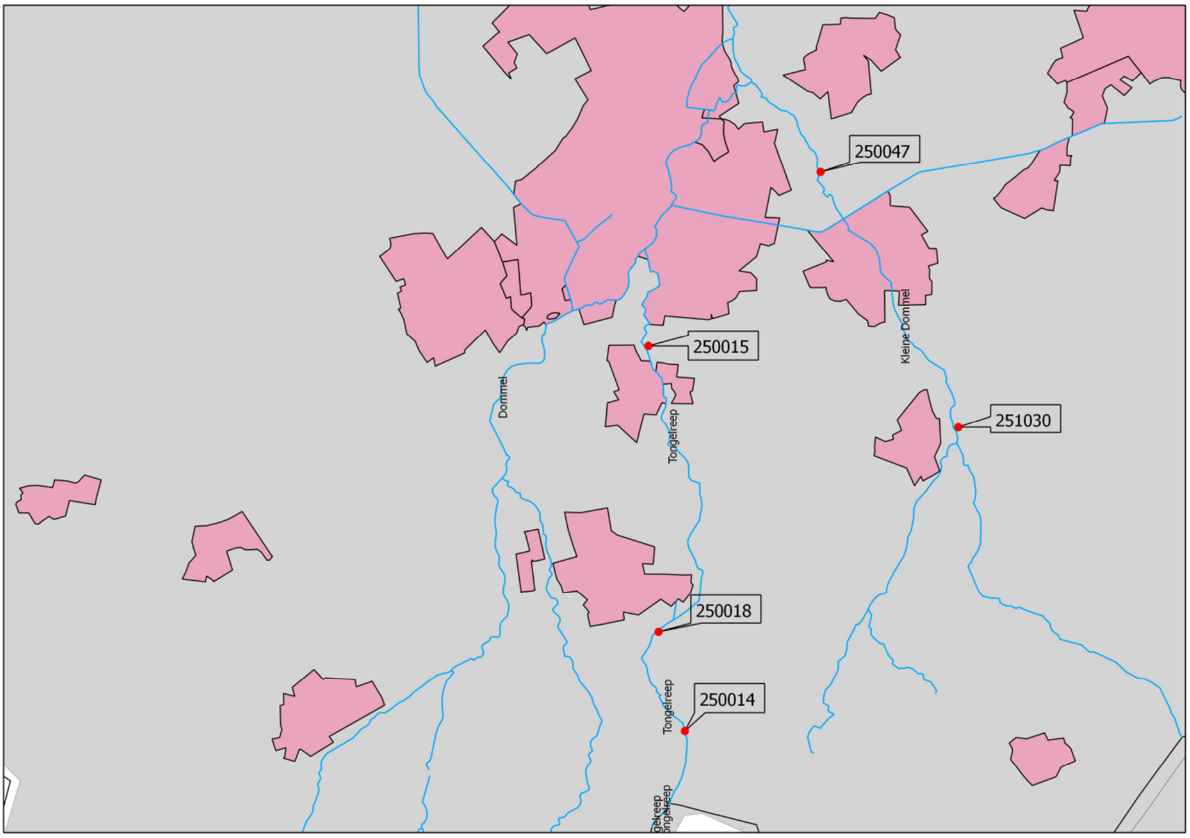


Figure 2, map with macro-invertebrate sample locations of the Tongelreep and the Kleine Dommel

The distribution of species can be analyzed in space or in time. Spatial distribution describes the differences in abundance of species among locations in the same stream. This can provide insight in the spatial preference of species within the same stream.

The temporal distribution shows the variance in time of species on the same location. Species that show a greater variance than others are likely to be more susceptible to environmental influences than species that have a more constant number of individuals.

Both spatial and temporal distribution is analyzed for the Klein Dommel and the Tongelreep (Chapter 2.1 and Chapter 2.2 respectively). Chapter 2.3 highlights the most distinct differences of species composition, total abundance and number of species between the Kleine Dommel and the Tongelreep

2.1 Kleine Dommel

Spatial distribution

Macro-invertebrate samples have been taken on two locations in the Klein Dommel. For every location the 15 most abundant species, based on an average of 1995 till 2008, were used to study the distribution. The fraction of individuals of the 15 most abundant species to the total amount of individuals, ranges from 0.52 to 0.84 and from 0.39 to 0.73 for location 251030 and 250047 respectively. Six species occurred on both locations simultaneously: *Gammarus P.*, *Asellus a., Tubificidae zonder haarborstel, Hydrachnidae, Ologochaeta and Microtendipes.*

Gammarus p. (the only species that is rated positive according to WFD) is much more abundant at location *251030* (Figure 3). The minimum number of individuals at *251030* is almost as much as the maximum at the more downstream location *250047*.

The negative species Asellus a. has a higher abundance downstream, whereas Tubificidae is slightly more abundant upstream.

The remaining three species are not taken into account for the WFD rating. The difference between these species is not significant.

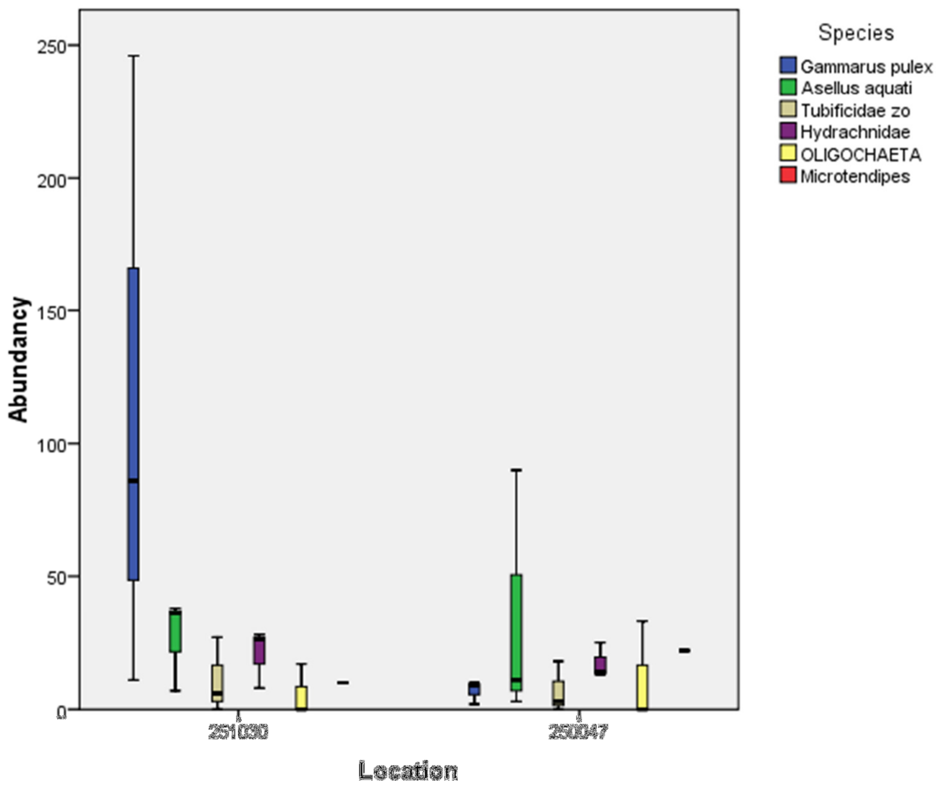


Figure 3, boxplot of 5 most abundant species in the Kleine Dommel for 1995 till 2008

Temporal distribution

The differences in abundance of species can differ strongly between following years (Figure 4 and Figure 5). Even within one year the fluctuation can be strong. At 25-5-2006 the number of individuals of *Gammarus P.* at location 251030 was far above average. Two months later, at 17-8-2006, the population was reduced with a factor of 27. On the contrary, most negative individuals, except for *Asellus a.*, show an increase in individuals in the same period. This population behavior indicates, that some event caused rapid environmental changes (Perdersen et al., 1986). Even species that were not found in other periods appeared at 17-8-2006. This could be because the sample of 17-8-2006 at location 251030 was the only sample taken in August. All other samples were taken earlier in the season. Because it is species dependent when a population reaches its maximum, it is possible that the total species composition differs within time.

Gammarus P. and *Asellus A.* both show a strong increase from May 2005 to May 2006. The other species do not show such an increase. It is interesting to see that both species show an increase. Because *Gammarus P.* is more sensitive for pollution than *Asellus A.,* this increase could be the result of an event that did not stress (organic matter e.g.) the aquatic system by means of pollution.

The sole appearance of some species also happens downstream at location 250047. In 1996, populations of *Asellidae, Cloeon, Sphaeriidae and Gammaridea* are abundant but were not found in other years. Most of the more frequently abundant species are absent in that year. The population of *Gammarus P.* in 2007 behaves opposite to the population in 2006 at location 251030. Instead of decreasing, the population increases from May 2006 to August 2006. The opposite happens with the population of *Asellus A.*

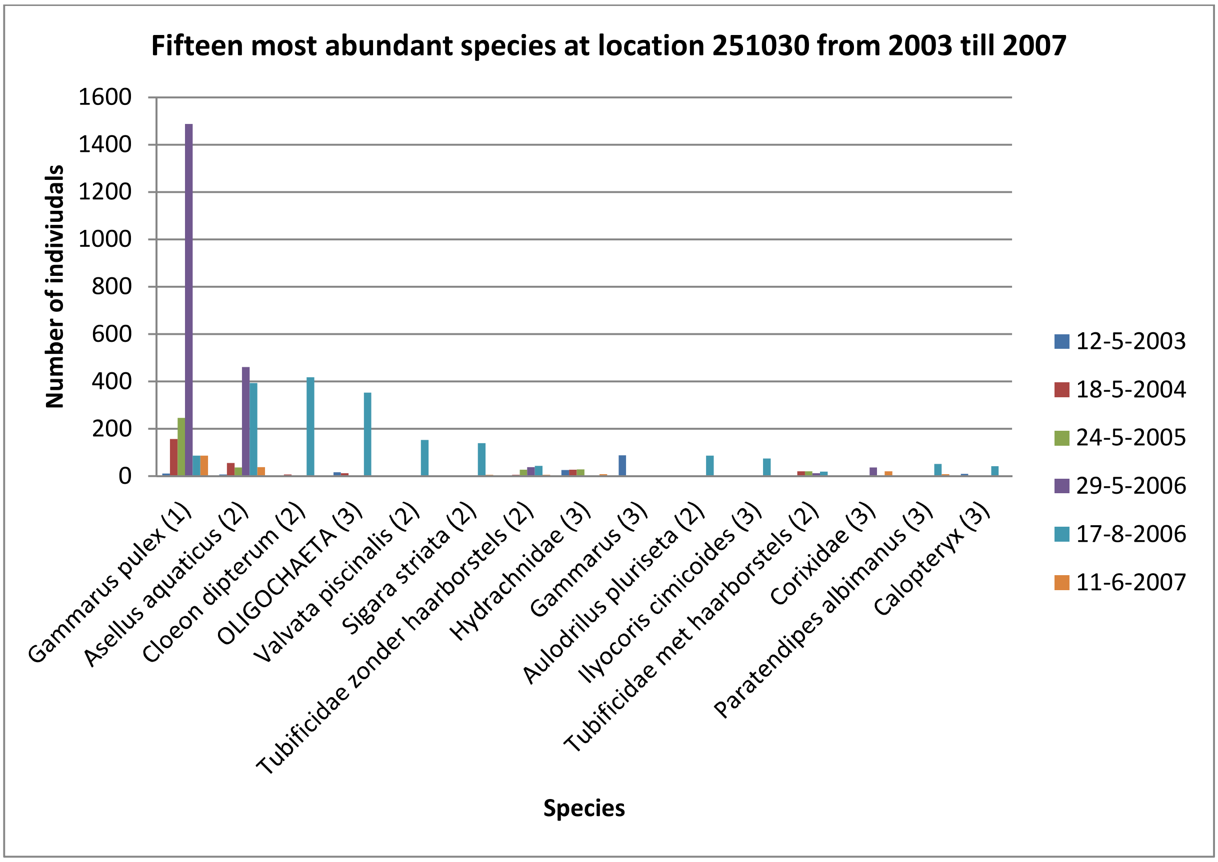


Figure 4, fifteen most abundant species at location 251030

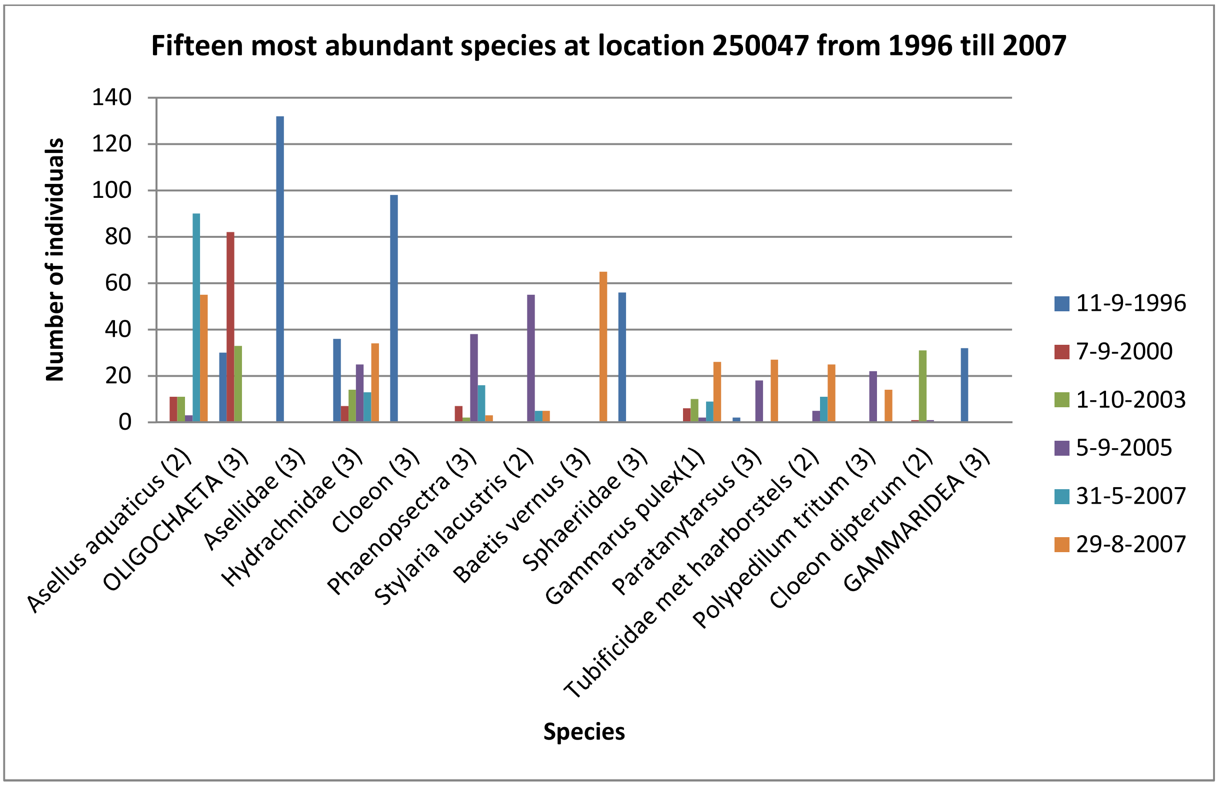


Figure 5, fifteen most abundant species at location 250047

Overall, the total amount of individuals (for the 15 most abundant species) is a factor 2-3 higher for 251030. Only in the year 2007 location 251030 shows a lower count of individuals (Figure 6). The year 2006 shows a peak of total individuals at 251030, whereas downstream no apparent increase is visible. This peak is primarily caused by the high number of *Gammarus P.* The following year (June 2007) the total amount of individuals drops to a minimum (for the entire measuring period), while the most abundant specie remains *Gammarus P.* This indicates that some event resulted in the decline of the total number of individuals and that *Gammarus P.* was either most resistant or fastest to reappear. It cannot be the result of the very high population in May of the previous year because that population collapsed in August of 2006.

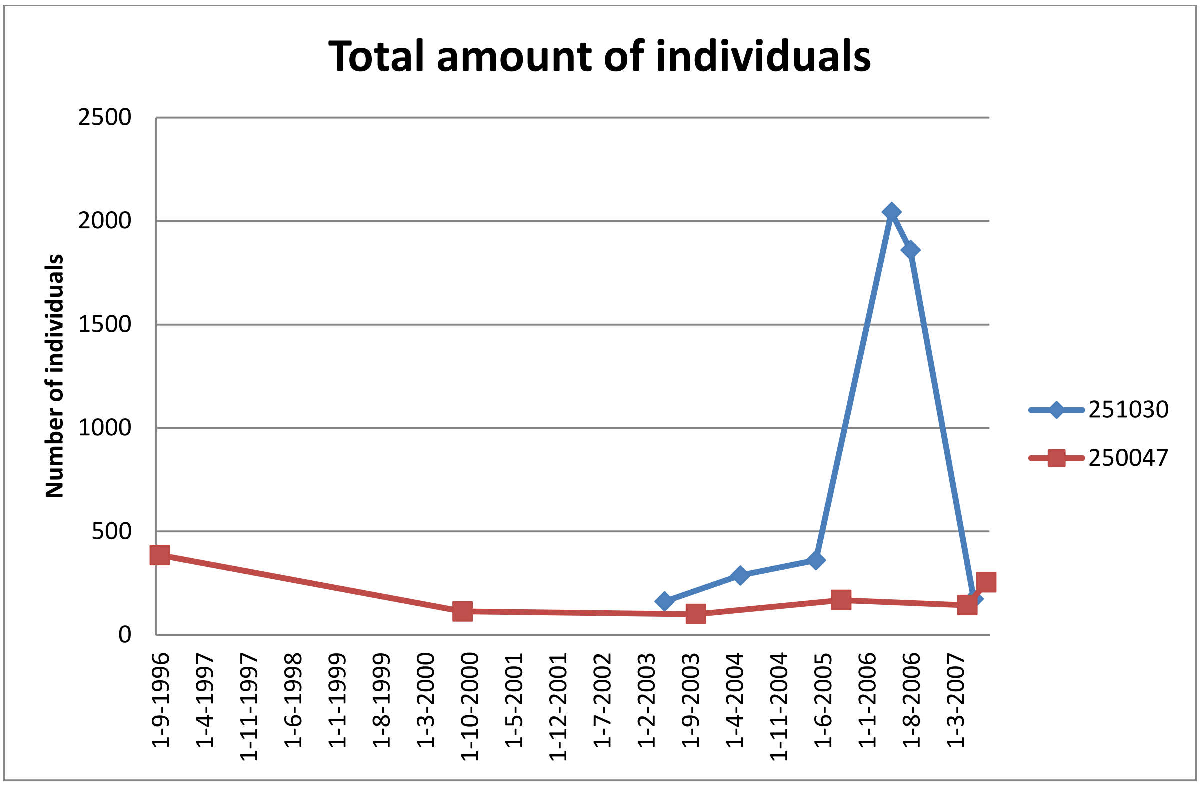


Figure 6, species diversity for the Kleine Dommel

2.2 Tongelreep

There is a decrease in abundance of Gammarus Pulex in the downstream direction (Figure 7). Some species that occur both upstream and downstream are not present in the "center" location (250018).

Oligochaeta shows a very high number of individuals: a maximum of 287 individuals at location 250014 and 328 individuals at location 250015. This could indicate a high level of organic pollution (Perdersen et al., 1986 ; Willemsen et al. 1990) or an unstable environment (Polls et al., 1978). Both are not expected at the Tongelreep. There are no individuals of *Oligochaeta* found at location 250018. The low averages at location 250014 and 250015 (67 and 61 respectively) indicate that the observations with high amounts of individuals are caused by (few) occasional event(s) while other years were very low in abundance. The same distribution, although less obvious, is apparent for both species of Tubificidae.

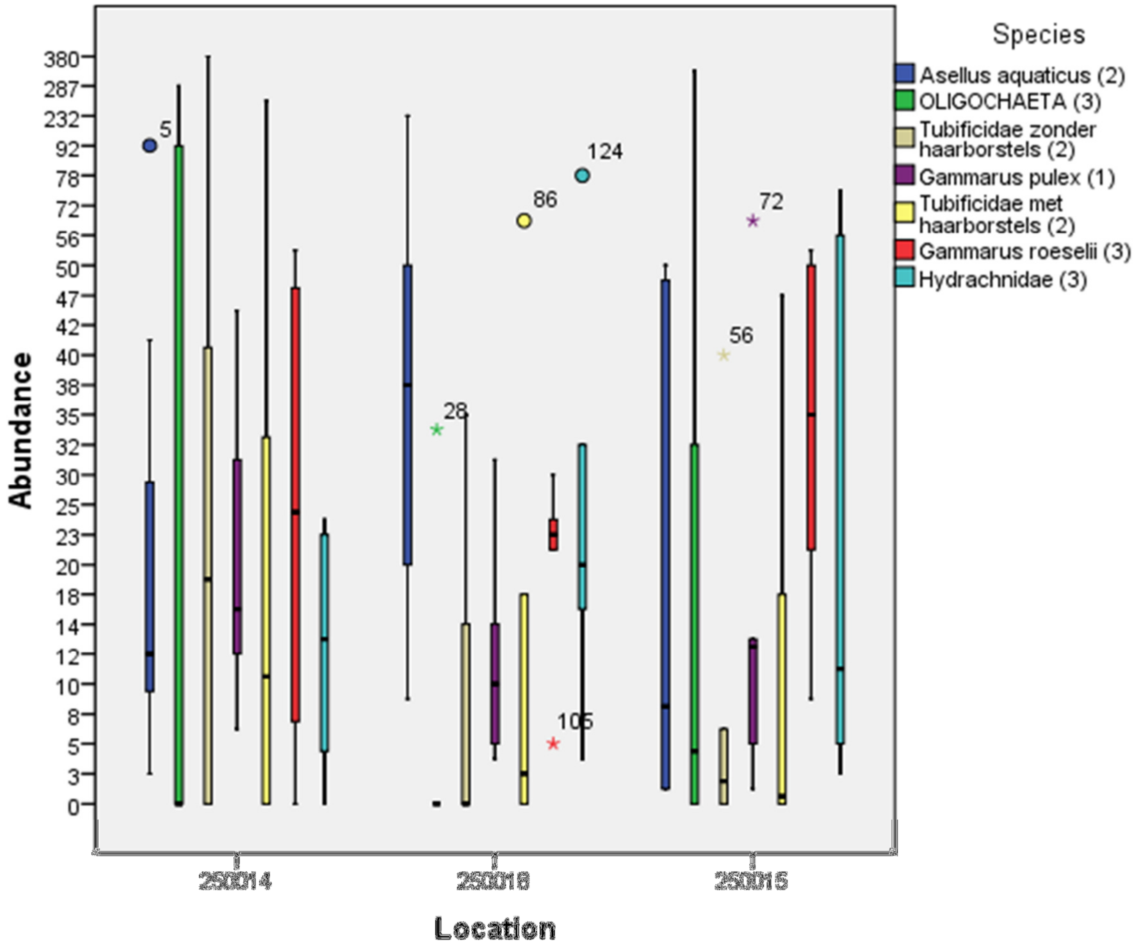
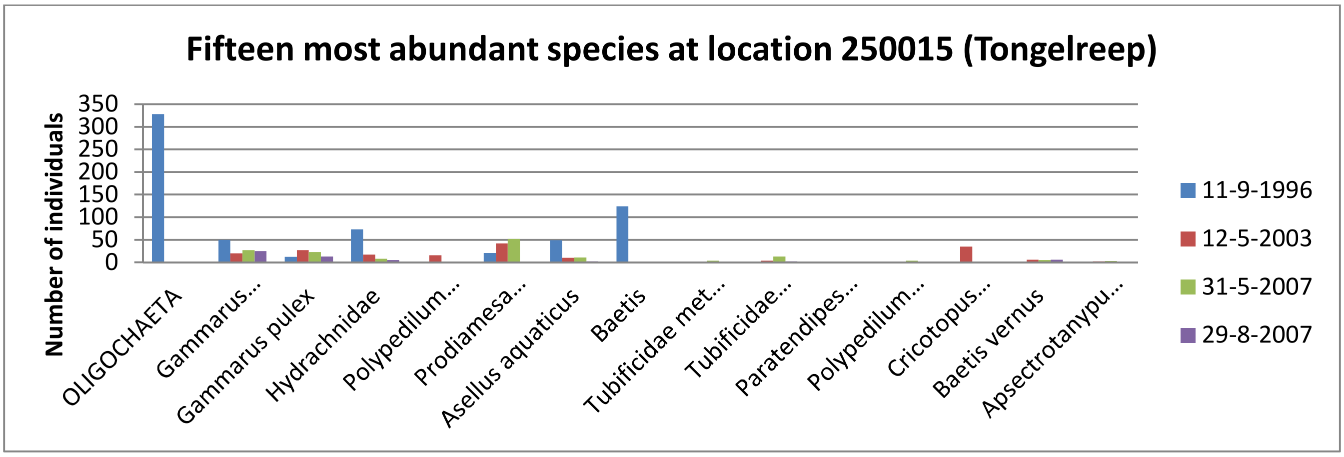


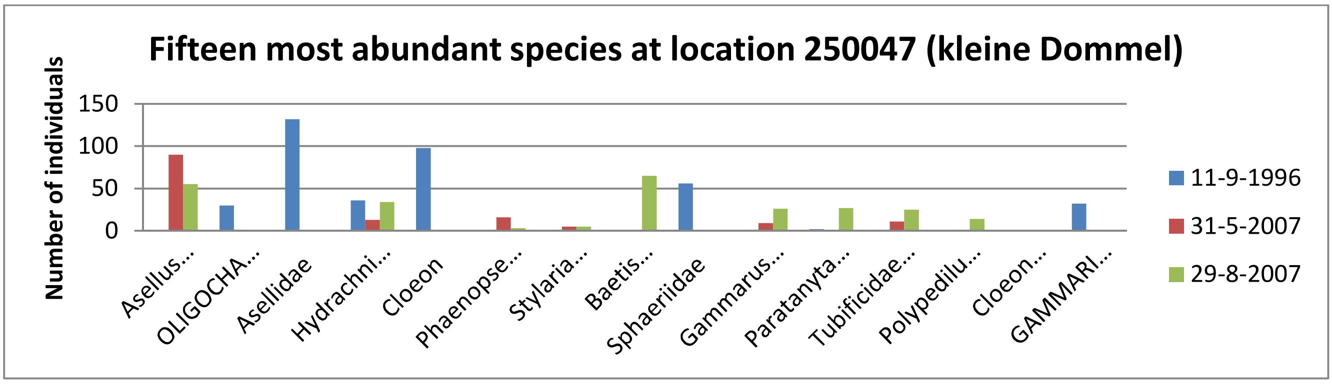
Figure 7, boxplots showing the median, first and third quartile and the minimum and maximum amount of individuals that were found in biological samples that were taken in the Tongelreep from 1995 till 2008.

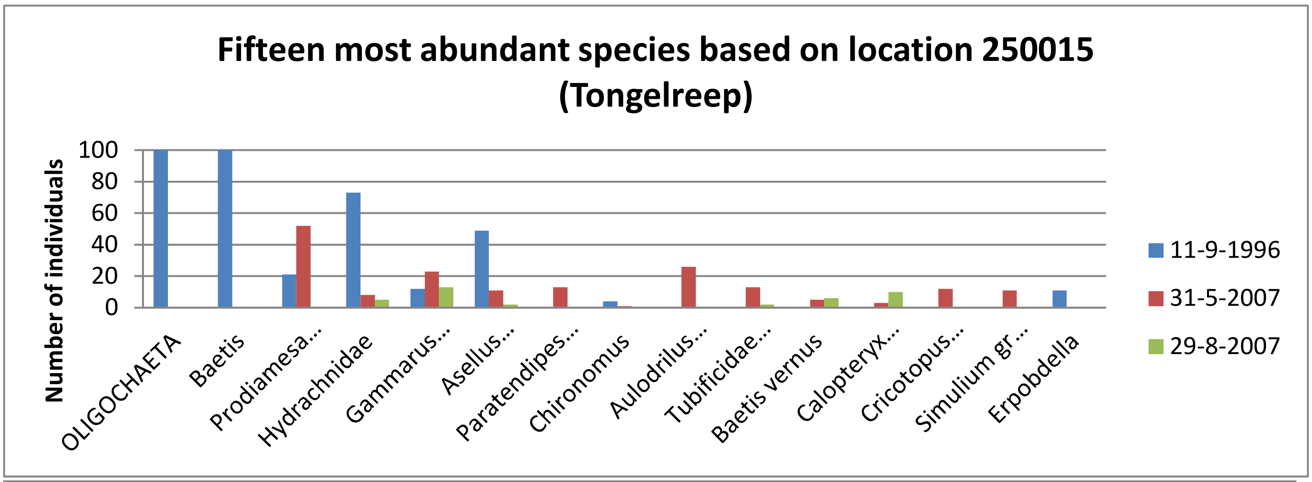
2.3 Difference between Kleine Dommel and Tongelreep

The difference in composition between the Kleine Dommel and the Tongelreep can only be assessed for samples that were taken in the same period, preferably within a few weeks. If the difference in sampling time would be bigger the chance exists that one sample was influenced by a CSO event while the other sample did not receive a CSO event. And therefore a comparison of the two samples would not be representative. Only three samples were taken simultaneously for the most downstream locations of the Tongelreep and the Kleine Dommel. The corresponding dates are 11-9-1996, 31-5-2007 and 29-8-2007. The analyses in this chapter will therefore be carried out on the basis of these dates.

The comparison between the species composition of the Tongelreep and the Kleine Dommel is carried out for the downstream locations (250015 and 250047 respectively; Figure 8). The most abundant species were selected for location 250015 and the same species were selected for location 250047.







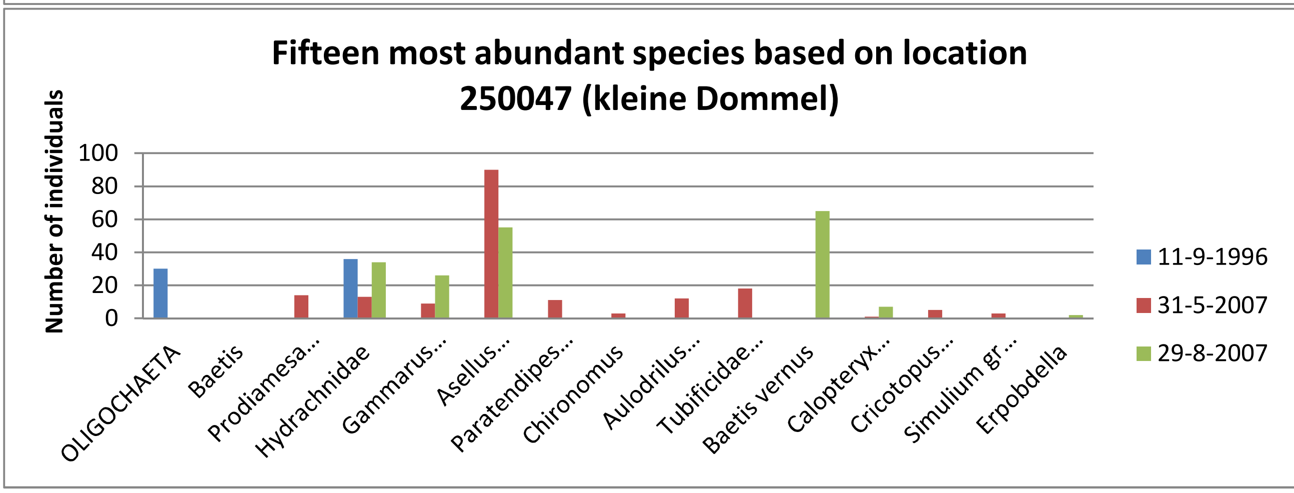


Figure 8, fifteen most abundant species for the Tongelreep and the Kleine Dommel.

The composition of the fifteen most abundant species at location 250015 consists of 2 positive rated species, three negative species and ten undetermined species (Figure 8). The composition of the most abundant species for location 250047 consists of one positive species, four negative species and ten not rated species (Figure 8).

The species grouped by the WFD classification show that for three dates the positive and the indicative species are more abundant at location 250015. The negative and the not defining species are more abundant at location 250047 (Figure 9).

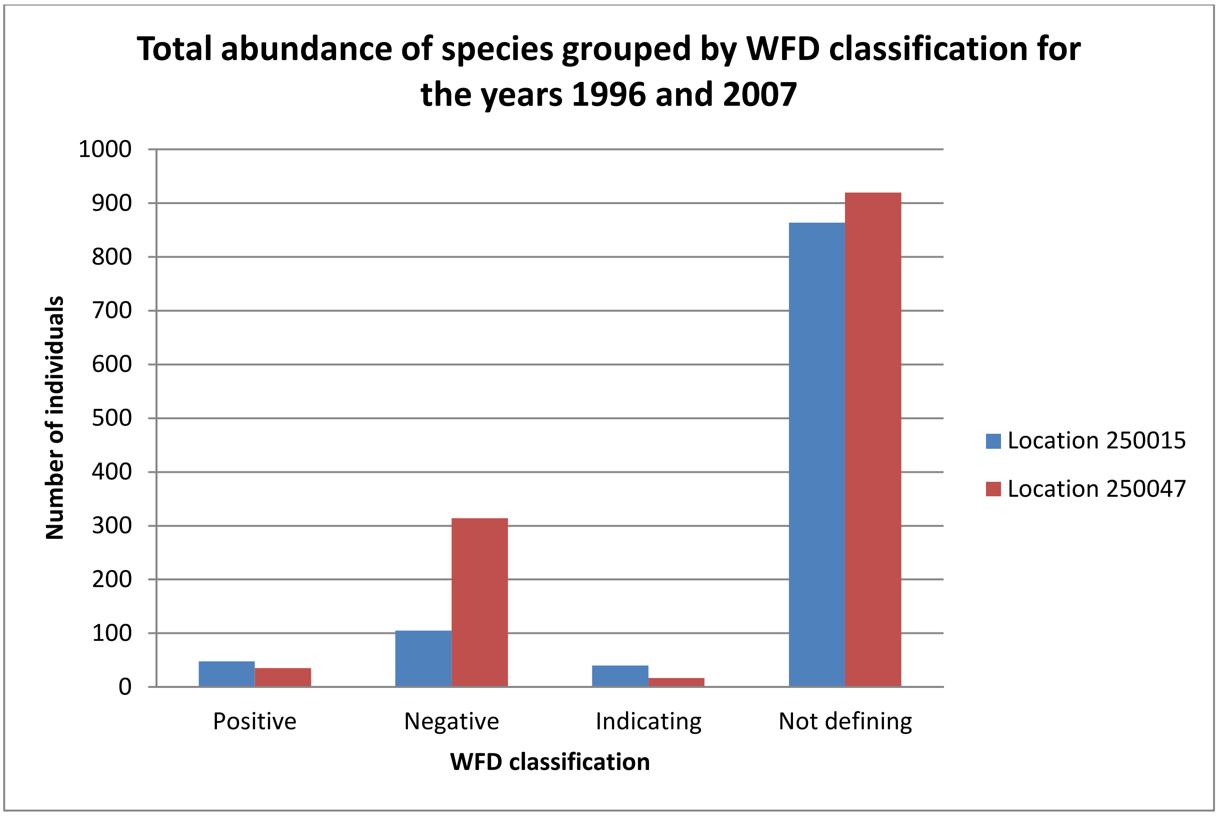


Figure 9, total abundance (species diversity) for the Kleine Dommel and the Tongelreep

The biodiversity (defined as the number of unique species) is the degree of variation of life forms within a given ecosystem. The difference in the biodiversity between location 250047 and location 250015 does not differ for the positive species (except for 1996 where there are none at location 250047; Table 1). The positive group overall consist of only one species; this is the lowest diversity for the four classifications. This is slightly trivial because the EFD only distinguishes 8 positive species.

The negative species are more abundant in the Tongelreep in 1996, while in the other years the number of different negative species is higher in the Kleine Dommel. The indicating species at the Tongelreep are always higher or equal than in the Kleine Dommel. In general the diversity (amount of different species) is smaller for the indicating species than for the negative species. The highest diversity is found within the group of not defining species. The difference between the two locations is only apparent for 29-8-2007 where there are almost twice as much “not defined” species in the Kleine Dommel than in the Tongelreep.

Table 1, biodiversity defined as the number of unique species for location 250047 and 250015.

WFD Classification	11-9-1996		31-5-2007		29-8-2007	
	250047	250015	250047	250015	250047	250015
Positive	*0*	1	*1*	1	*1*	1
Negative	*2*	5	*16*	10	*5*	3
Indicating	*0*	2	*4*	5	*3*	3
Not defining	*37*	36	*28*	25	*24*	13

3 Measuring campaign

The water board has one continuous oxygen measurement station at the location of the Collse watermolen. However, one measurement location does not provide insight in the spatial effects (downstream the Klein Dommel) of a CSO event. Therefore, the design of a measurement campaign that measures oxygen concentrations at a small interval for an extended period at three locations in the Klein Dommel was performed (Chapter 3.1 and chapter 3.2).

3.1 Setup

Three variables were measured: *oxygen, electric conductivity (EC) and temperature*. The measurement interval was chosen at 15 minutes, so that a reasonable measurement resolution could be achieved without the need to change batteries too frequently. With test tube clams four metal rods were attached horizontally to a vertical metal bar that was stuck in the bottom of the stream. The actual sensors were attached, again by test tube clamps, at the end of the horizontal rods.

The oxygen and EC sensors were placed at depths of 30 – 60 centimeter (depending on the water level).

Measurements were carried out at three locations in the Kleine Dommel (Figure 10 ; Appendix Figure 20). Locations are numbered Dommel 1 till Dommel 3 with number 1 being the most downstream location:

1. Dommel 1 is positioned 100 meters upstream of a watermill. The stream meanders relatively strong and it receives at least 50 percent of the day shadow due to a moderately thick tree canopy. Macrophyte growth covers 40 percent of the bottom. The depth in the middle of the stream is 1 meter 30 in dry conditions.
2. Dommel 2 is positioned 20 meter downstream of a confluence with a small stream branch. This part is quite straight for about 100 meters downstream and 400 meters upstream. The location looks very "natural" with wild herbs and much riparian vegetation. The macrophyte growth is moderate with about 20 percent coverage. There is no shading and the depth is about 1 meter 40 in dry conditions in the middle of the stream.
3. Dommel 3 is situated downstream of a confluence with a stream that originates in Belgium. The stream is canalized and does not appear "natural". Macrophyte growth is hard to estimate due to the turbidity, but during the measurement it was mowed several times. The stream is 1 meter 30 deep in the middle in dry conditions. This location is strongly regulated in both water level and mowing management.

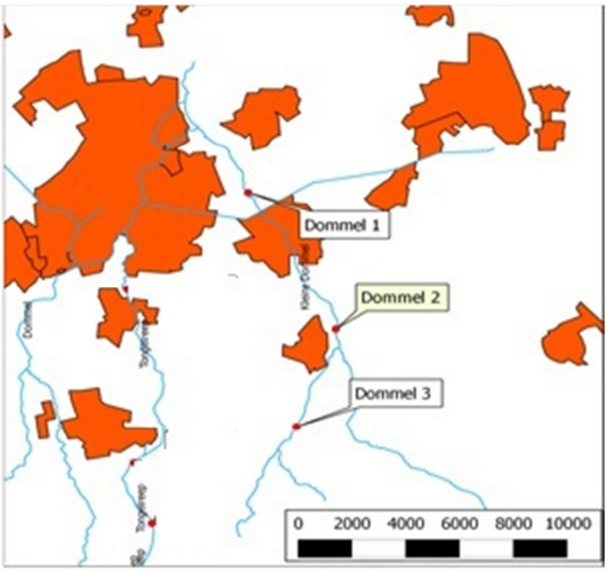


Figure 10, three locations in the Kleine Dommel where oxygen measurements were carried out

Some unforeseen problems occurred during the measuring campaign. Batteries that went dead after 1 day and the flooding of the data loggers at the most downstream location result in some gaps in the data. However, the measurements provided enough data to draw a conclusion about the oxygen, temperature and EGV profiles during July and August.

3.2 Results

Dommel 1

Figure 11 shows the profiles for the location Dommel 1. The missing data in the first week is a result of batteries that had a much lower capacity than expected. The measurements of Dommel 1 should extend to the 15th of August but due to the flooding of the equipment the measurements were ended earlier. Some data was still logged internally. Hence the oxygen profile that starts at the 3th of august.

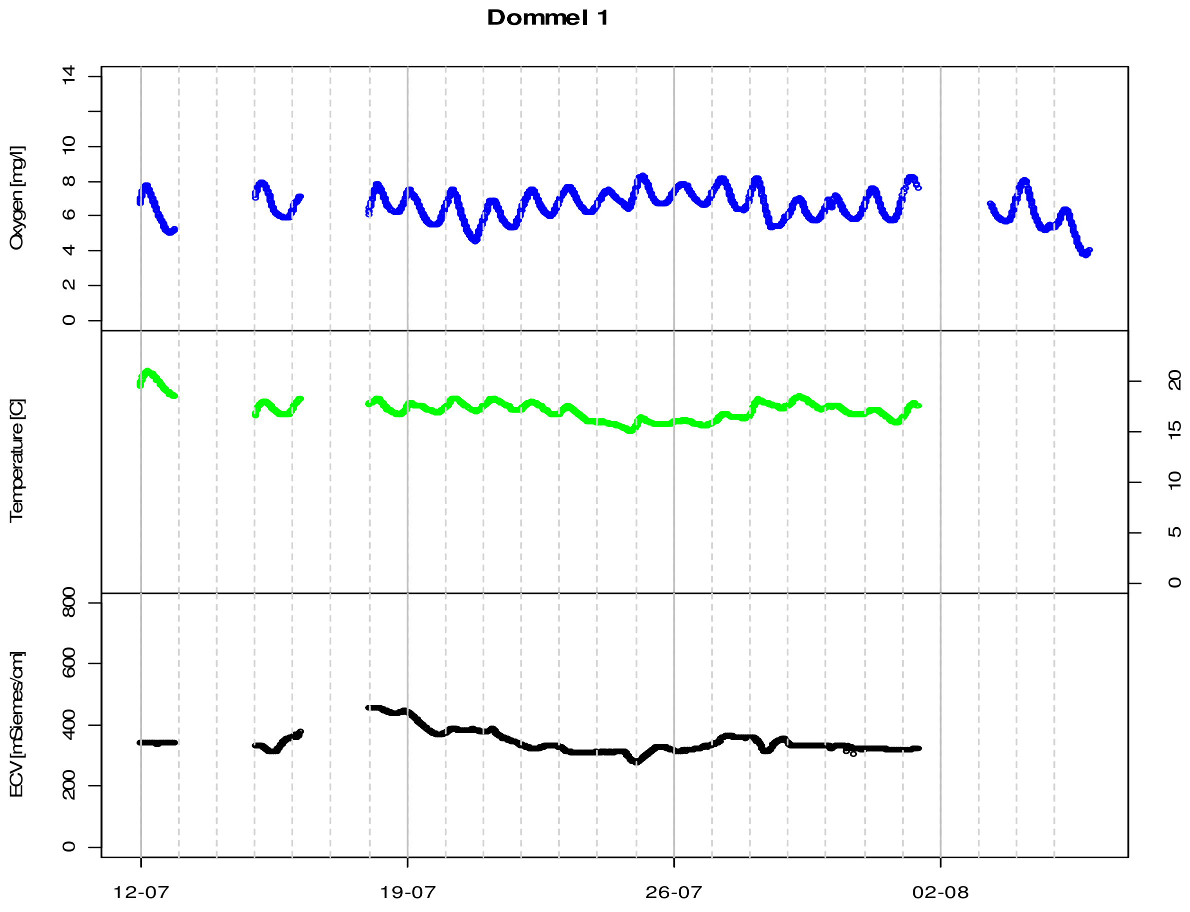


Figure 11, oxygen, temperature and ECV profiles at location Dommel 1

During the entire period of measuring the oxygen concentration has not exceeded 8.3 mg/l and did not fall below 3.7 mg/l. The minimum value was measured at the end, which is very unfortunate because it is not sure whether the oxygen concentration would continue to decrease or had reached its minimum. Oxygen concentrations show a strong day-night pattern which indicates that macrophytes or algae have an impact on the oxygen concentrations. Temperature also shows a day-night pattern but less profound than the oxygen pattern. Therefore, oxygen saturation levels show a truncated pattern with lower values at night.

The ECV shows a relatively long lasting peak that takes several days (with the maximum at 18-7). Even though there have not been any CSO's, ECV can increase with 100 mSiemens/cm.

Dommel 2

Measurement outcomes of the Dommel 2 are presented in Figure 12. Battery failure resulted in missing data in the first two weeks. One CSO occurred (data of municipality) in the end of the measuring period (vertical red line).

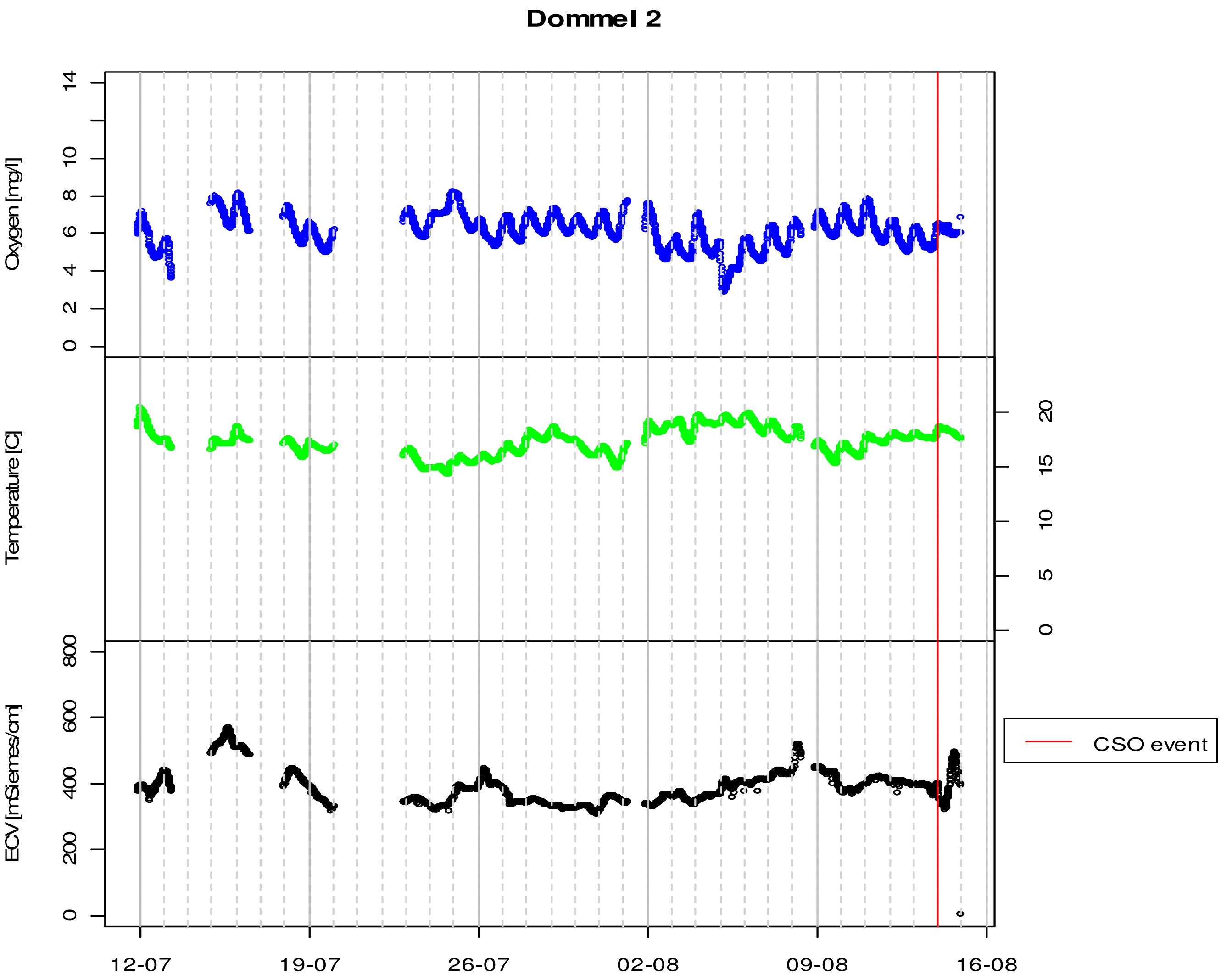


Figure 12, oxygen, temperature and ECV profiles at location Dommel 2

Maximum oxygen concentrations at the Dommel 2 (8.2 mg/l) are equal to the ones found in the Dommel 1 (8.3 mg/l). The minimum oxygen that was reached (2.9 mg/l), is lower than in the Dommel 1 (3.7 mg/l). A higher sensitivity to oxygen depletion of the Dommel 2 also shows from the average oxygen concentrations which is 0.4 mg/l lower at the Dommel 2 (6.5 and 6.1 mg/l for Dommel 1 and Dommel 2 respectively).

There is no apparent difference between temperatures for the Dommel 1 and Dommel 2. Both have an average of 17.3 ^{o}C.

ECV values are higher in the Dommel 2. The peak in the first week is about 200 mSiemens/cm higher and one day earlier than in Dommel 1. It is however not possible to make an exact comparison because of the missing data in the first week. Overall ECV seems to have a slower reaction than the oxygen and that results in more stretched peaks that do not seem to change as rapidly as oxygen does. On August the 5th there is an above average decline in the oxygen concentration while there is no distinct change in either temperature or ECV. After the CSO the ECV values show a sudden increase while the oxygen concentration is not influenced by CSO. There is no direct relation between the ECV and the oxygen at the Dommel 2 ($R^2 < 0.05$).

Dommel 3

Dommel 3 also misses some data in the first week due to battery problems (Figure 13), but the measuring period coincided with the same CSO as Dommel 2. There are two oxygen dips on 25-7 and on 2-8 that are not used in this analysis because they are probably a result of cleaning the equipment. The water board mowed the stream at Dommel 3 somewhere during the week of august the 2nd but the exact time is not known.

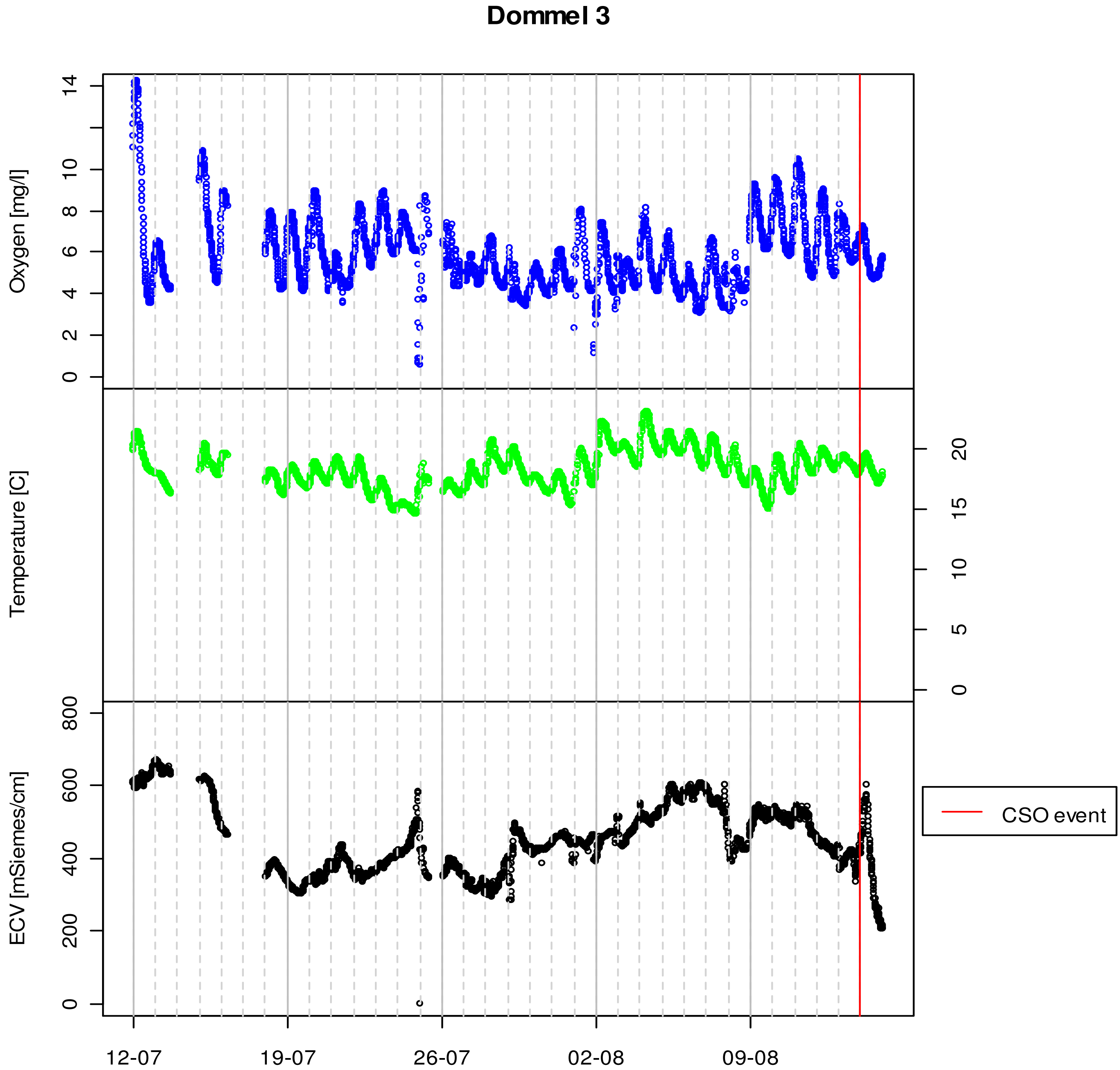


Figure 13, oxygen, temperature and ECV profiles at location Dommel 3

Oxygen concentrations, temperature and ECV show a more extreme changing pattern than in the Dommel 1 and 2. The minimum and maximum oxygen concentrations are 2.3 mg/l and 14.3 mg/l respectively. Average oxygen concentration for the entire period is 5.9 mg/l. Both minimum and average oxygen concentrations are lower than in the Dommel 1 and 2 but the difference with Dommel 2 is not extreme. The big difference between Dommel 3 and the other two locations is the day-night pattern, which is much bigger for Dommel 3. Even though field observations did not result in a high amount of macrophytes, it is possible that this was overseen due to turbidity.

The temperature is on average 1 C^o higher than in the other two locations. The vicinity of the WTP might be an explanation for this.

Also the ECV shows a fickle pattern. The ECV is on average not much higher than in the Dommel 2 but curve has a more extreme pattern.

Duration of an oxygen dip, next to the minimum oxygen concentration that is reached during a dip, has a very strong effect on the macro-invertebrate's survival changes: negative impact on macro-invertebrate increases with the duration of low oxygen concentrations (Lammersen, 1997). Table 2 shows events in which the oxygen concentrations have dropped below two thresholds (3 and 4 mg/l). The table clearly shows that the Dommel 3 has more frequent and longer periods of low oxygen concentrations. At Dommel 1, oxygen level never declined below 3 mg/l and only one concentration below 4 mg/l was measured but this was a relative long period. The maximum duration on concentrations below 4 mg/l is 630 minutes (ten and a half hours).

Table 2, the duration (in minutes) of periods with oxygen concentrations below 3 or 4 mg/l

Measuring location	Event	minutes < 3 mg/l	minutes < 4 mg/l
Dommel 1	1	-	265
Dommel 2	1	90	540
	2		45
Dommel 3	1	135	270
	2	15	15
	3	180	420
	4		180
	5		630
	6		210
	7		15
	8		210

The results in Table 2 correspond with the graphs of the measurement profiles: the more downstream the more the profiles (temperature, oxygen and ECV) are truncated. Peaks and lows are less profound downstream and the duration and frequency is smaller. Although, according to the municipality there was only one CSO, other factors play a role because while no CSO was measured clear dips of oxygen occur that are significantly deeper than the day-night patterns. There are two WTP upstream of the Kleine Dommel, one in Belgium (near the town Dielishoek) and one in the Netherlands (Perkenstraat 1, Seorendonk). Maybe after intensive precipitation there is not yet a CSO but already an increase in discharge of the two WTP upstream.

4 Sensitivity analysis of the WFD scoring system

The WFD score of a water body is an ecological quality ration which is defined as (Brys et al., 2005):

$$EQR = \frac{Observed\ biological\ value}{Reference\ biological\ value}$$

The EQR ranges from 0 – 1. An EQR of 1 resembles the biological value of a water body being as good as the reference and cannot be improved further (according to the WFD). Respectively, an EQR score of 0 means that the observed biological value is much worse than the reference. EQR scores were calculated with Qbwat (Pot, 2010).

The assessment of the ecological status of a water body is based on three criteria (van der Molen, 2003):

- The percentage of individuals that fall within the negative classified species (the higher this percentage the lower the status)
- The percentage of positive classified species (the higher this percentage the better the status)
- The percentage of individuals that fall within the indicative classified species (the higher this percentage the better the status)

Each of these classes have their own weighted influence. Indicative species, for example, have a stronger influence on the score than positive species. Because the macro-invertebrates are divided into three classes and one of these classes has a negative influence on the score, an EQR score can be quite low even though the biodiversity is high (Chapter 4.1). Understanding how the EQR responds on changes of a macro-invertebrate community, contributes to deciding which measurements should be taken to increase an EQR score (Chapter 4.2).

4.1 Measured EQR and biodiversity

Ecosystems are complex systems, in which every part of the system interacts with one another. These properties make it difficult to condense the information about an ecosystem (species composition, biodiversity, landscape diversity etc.) and build a solid indexation of it. There are however many indexes that do so. In fact, the EQR score is also an index that summarizes the "healthiness" of an ecosystem in one single mark. Another index, which is widely used in the ecological field and describes the status of an ecosystem based on the species richness and the abundance of individuals, is the Shannon Index (Maes et al., 2002, Meerman, 2004). In "4.1 Measured EQR and biodiversity" the Shannon Indexes and the EQR scores were calculated with macro-invertebrate samples of the water board for the Tongelreep and the Kleine Dommel and compared with each other.

Theory about Shannon Index

The Shannon indexes (H') were calculated with the R package "vegan" (Oksanen et al., 2011). Coinciding data was used if possible, but this was not often the occasion. The package vegan uses the following definition of the Shannon Index:

$$H' = -\sum_i p_{i \cdot \ln(P_i)}$$

where P_i is the proportional abundance of species i.

The more even the distribution of individuals among the species becomes, the less influence dominating species have on the calculated index (Keylock, 2005 ; Spellerberg, 2003). The index is most sensitive to species diversity and it is, to a lesser extent, sensitive to species richness. Interpretation of the Shannon Index is as follows: the higher the Shannon Index the higher the species diversity.

Results

For the period from 1996 till 2007 (when available) the Shannon Index was calculated for the Tongelreep (three measurement locations; Figure 14) and for the Kleine Dommel (two measurement locations; Figure 14)

There is no correlation between the Shannon Index and the EQR score (R^2 <0.03). It is possible that the same species composition results in a very low Shannon Index but in an average EQR score, as for example at location 250014. In many occasions the Shannon Index increases a following year even though the EQR score decreases. A reason for the dissimilarity could be the divisions in three groups that the EQR score uses. An increase of species richness of the negatives species results in a lower EQR score. But the Shannon Index becomes bigger because the total species richness of the ecosystem increases. Therefore the Shannon Index might not be a good choice for polluted waters where an ecosystem is primarily dominated by negative species. The dissimilarity between the two indexes shows that it can be “dangerous” to draw too much conclusions on a single marking system. Being always a simplification at least some information is left out for every scoring systems.

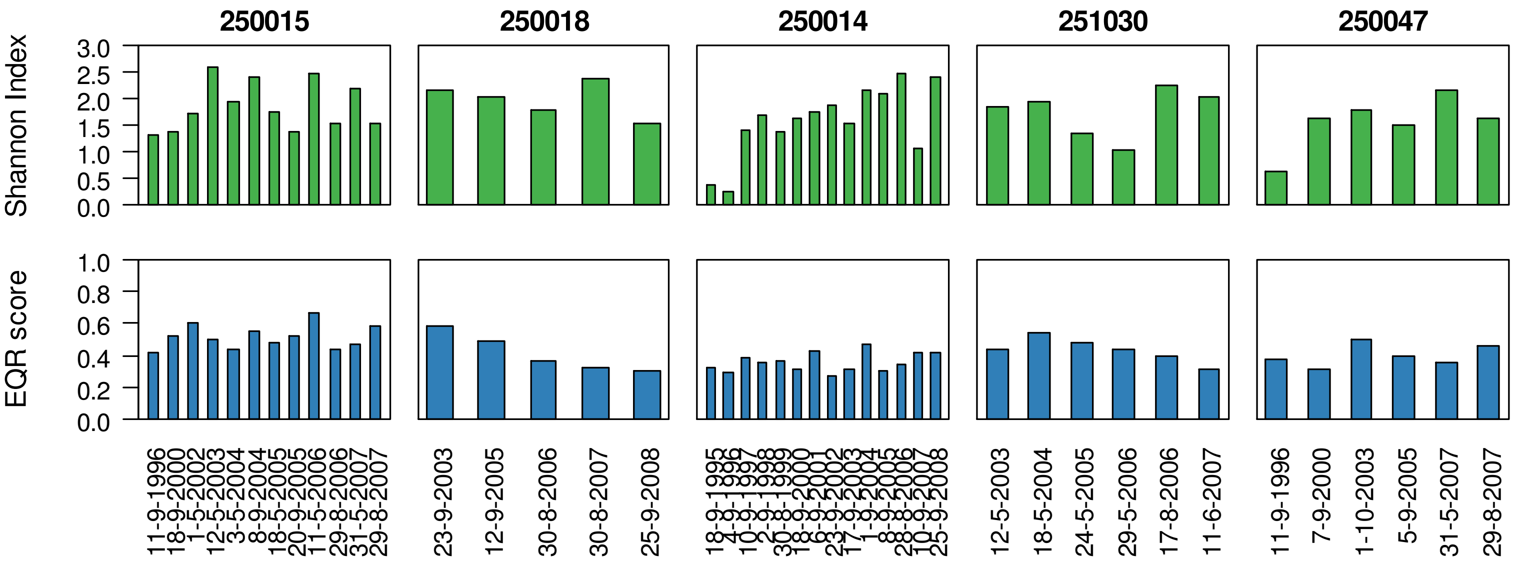


Figure 14, Shannon indexes (above) and EQR scores (below) for all the sample locations of the Tongelreep and the Kleine Dommel.

The water board aims at an EQR score of 0.6. Only the Tongelreep reaches this target in some years. On all locations EQR scores differ significantly from year to year and also the difference among the locations can be great (Figure 14). The next paragraph “Sensitivity analysis” investigates how a chance in the species composition influences the EQR score.

4.2 Sensitivity analysis

The WFD discriminates *positive species, negative species and indicative species classes* (ecological status groups) to which a species can be assigned. Each chance in abundance of species/individuals in a class influences the EQR score. Therefore, the sensitivity analysis comprises per class multiple scenarios with different abundances of species and/or individuals, while the species composition of the other classes stays constant. The field situation functions as a reference value and the changes in abundance of species/individuals are based on the field situation (which is a certain macro-invertebrate sample). For every group the scenarios comprise equal relative changes in species composition. The only difference among the groups is the abundance of species and individuals that were found in the field situation. Table 3 represents the scenarios and their relative changes.

Besides the *positive, negative and indicative class,* the sensitivity analyses includes a *combined class,* which tests the interconnectedness of the three classes. Instead of only changing abundances and species composition for one class at a time, the combined class does this for all three classes simultaneously.

Table 3, scenarios and their description used for the WFD sensitivity analysis

Scenario	Changes made to species composition
Scenario 1 (field)	Field situation
Scenario 2	Individuals of most abundant species X 5
Scenario 3	Individuals of most abundant species X 10
Scenario 4	Individuals of most abundant species X 100
Scenario 5	Individuals of most abundant species X 1000
Scenario 6	Individuals of most abundant species X 10000
Scenario 7	Distribute nr. of individuals of scenario 2 over 3 new species
Scenario 8	Distribute nr. of individuals of scenario 2 over 6 new species
Scenario 9	Distribute nr. of individuals of scenario 2 over 10 new species
Scenario 10	Distribute nr. of individuals of scenario 6 over 3 new species
Scenario 11	Distribute nr. of individuals of scenario 6 over 6 new species
Scenario 12	Distribute nr. of individuals of scenario 6 over 10 new species

Figure 16 presents the 11 (or 12 for the positive and combined group) scenarios for each group. The field situation is of course equal for all the groups. For the positive group, the EQR value for scenario 2 and 3 (an increase in individuals from 45 till 90 for one species) are equal. Scenario 4 (900 individuals) scores 0.009 higher than scenario 3 (90 individuals). Scenario 5 (9000 individuals) and 6 (90000 individuals) are equal and score 0.003 higher than scenario 4. This result is equal to the first 5 scenarios of the indicative group; however, the individual abundance in the indicative group is 9 times less than that of the positive group. The result of the positive species is almost opposite to that of the negative species. A combination of the positive and negative scenario results in an EQR score that is almost an average of the individual positive and negative scores.

The scenarios of the positive species, where the species richness (instead of increasing the amount of individuals of one species) is tested (scenario 7-12), show a different pattern. All these scenarios score higher than scenario 1-6, even though, individual abundances can be much smaller than in scenario 6 (90000 individual for on species). Interesting is that scenario 9 (10 unique species each with abundance of 4 individuals) scores lower than scenario 8 (5 unique species each with abundance of 6). Scenarios 10 – 12 have the highest scores and the biggest increases in score between them. This indicates that the scoring system is sensitive to a combination of both the abundance of individuals and the number of unique species.

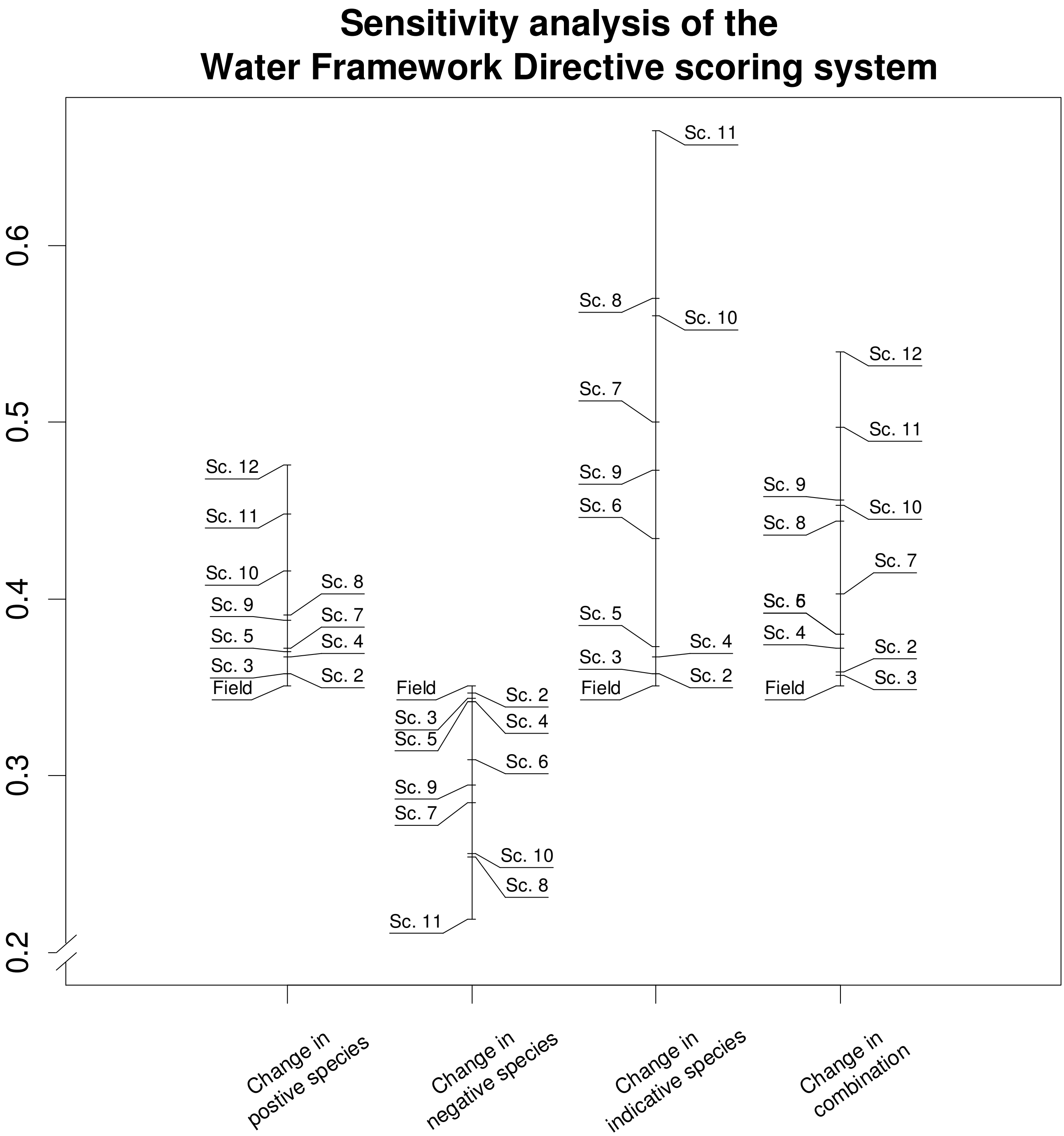


Figure 15, EQR scores of the four scenarios. Dotted lines present the even scenarios.

The pattern that the positive species show, are more or less the same for all four groups (with different scales). There are, however, some differences among the groups that catch attention:

- *Scenarios 8 and 10 of the negative group* have equal scores. Scenario 10 has 5 unique species with abundances of 15000 individuals per species. Scenario 8 with only 10 unique species and an abundance of 36 individuals per species, scores as high.
- *Scenarios 7 scores higher than scenario 9* and *scenario 8 scores higher than scenario 10 of the indicative group*. This means that 6 species with 1-2 individuals score higher than 3 species with 333 individuals and 10 species with 1 individual per species score higher than 6 species with 167 individuals per species.

- *Scenarios 6-8 and 9-11 of the indicative group* show an almost linear increase in score. The increase in score between these scenarios is the biggest of all the scenarios of all four groups.
- *All the scenarios in the combined group* score higher than the field situation. Scenarios 10-12 increase linear but with half the value as in the indicative group.

The scenarios 6-8 and 9-11 for all groups have the same abundance of individuals as scenario 2 and 5 (6) in the same group respectively. The only difference is, that in scenarios 6-11 the individuals are divided over multiple species. The results show, that the scoring is higher when the species richness increases. The highest increase always occurs in scenario 11 (or 12; Table 4). The total abundance combined with the species richness plays an important role for EQR scoring.

Table 4, increase of EQR score for all groups as a result of increased species richness

Group	Increase factor	Scenario (reference scenario)
Positive	1.29	12 (2)
Negative	1.56	11 (2)
Indicative	1.75	11 (2)
Combined	1.42	12 (2)

It can occur that the difference in score is very sensitive, which is for example the case for scenario 9 and 8 of the positive group. This sensitivity is especially distinct for low abundances (<12). The WFD scoring system transforms the abundance of individuals into classes according to Table 5. The classes are narrower in the low range of abundance; therefore, a difference of 1 individual can make a difference in the EQR score. This explains why for the positive group, scenario 9 (10 unique species each with abundance of 4) scores lower than scenario 8 (5 unique species each with abundance of 6).

Table 5, Subdivision of abundance of individuals into abundance classes

Abundance of individuals	Class
1	1
2-4	2
5-12	3
13-33	4
34-90	5
91-244	6
245-665	7
666-1808	8
>1808	9

Source: Stowa

5 Limnodata Neerlandica analysis

An introduction about the origin of Limnodata is explained in Chapter 5.1. The Limnodata is analyzed to find the expected occurrence of a part of the species for an R5 water body (Chapter 5.2). Eventually the EQR scores are predicted for different minimum annual oxygen concentrations (Chapter 5.3).

5.1 Explanation of Limnodata

Limnodata Neerlandica is a Dutch initiative of STOWA to condense great amounts of (reliable) observations of aquatic organisms and macrophytes into two databases (Limnodata Neerlandica en Piscaria). The data is retrieved from chemical and biological samples that were taken by the Dutch water boards. Annually all the water boards together gather great amounts of data. Therefore, the Limnodata database is a sound source for analysis.

For this research only data of species that are used for the WFD scoring of a R5 water body were requested. This data contains a distribution of 5 oxygen concentrations for which there is a 10%, 25%, 50%, 75% and 90 % change that a species occurs (Table 6). The data provides the number of samples on which the distribution is based but there is no information about the abundance of individuals in the samples used.

Table 6, example of data for gammarus pulex with the oxygen concentratiosn at which it was found in the field. The P-10 means that there is a 10 percent change that Gammarus Pulex occurs at 4.1 mg/l or lower.

Species	P-10	P-25	P-50	P-75	P-90
Gammarus pulex	4.1	6.2	8.2	10	11.8

5.2 Analysis of species occurrence

Figure 16 graphically represents *the P-10 value*, *the P-25 value, the P-50 value, the P-75 value* and the *the P-90 value cocentration* of all species that are used in the WFD-scoring system (Table 6). The light blue field represents the oxygen concentrations for which the species chance of occurrence lays between 10 percent and 90 percent. The narrower dark blue field shows a range of oxygen concentrations for which the chance that a species occurs lays between 25 and 75 percent. The dark line shows the 50 percent chance line. The y-axis holds the species sorted on their P-10 values and colored according to their WFD class. Due to space limits the names are not shown.

Comparison of WFD classes

Negative species have on average a chance of 10 percent to occur at oxygen concentrations of 3 mg/l. This concentration is almost double for positive and indicative species (Table 7). At an oxygen concentration of 7.5 mg/l, negative species have a chance to occur that is twice as big (50 percent instead of 25) as the indicative and the positive species. All three species start having the same chance of occurrence with an oxygen concentration of 10 mg/l or more. So if oxygen concentrations drop below 8 or 9 mg/l, negative species will always have a bigger chance of appearance and will therefore probably (abundance is unknown) be more abundant.

Table 7, average chances of occurrence for the three WFD species groups at an oxygen concentration (mg/l)

	P-10	P-25	P-50	P-75	P-90
Indicative	6.3	7.6	9.0	10.2	11.5
Negative	3.0	5.1	7.5	9.9	12.3
Positive	5.6	7.3	9.0	10.5	12.2

Comparison of unique species

There is a wide range (between P-10 and P-90) of oxygen concentrations for which a species can occur. For example *Lasiocephala basalis, Hydropsyche exocellata, Micronecta poweri, Lebertia porosa, Nemoura avicularis, Sperchon turgidus* (all indicative species) have an oxygen range of 3, 4.3, 4.4, 4.5, 4.6, 4.6 mg/l respectively, in which they occur while other species occur in ranges of up to 10 mg/l. The latter species, such as *Tubificidae, Asellus aquaticus, Chironomus, Anisus vortex, Cloeon dipterum* (all negative species), also have a reasonable chance (>10 percent) to occur in low oxygen (<5 mg/l) conditions (Figure 16). This corresponds well with the findings of Willemsen et al., 1990. There are, however, also indicative and positive species that occur at low oxygen concentrations and have a large range. For example *Dryops lutulentus, Sphaerium rivicola, Protzia eximia,* all of which are indicative species, have a chance of more than 10 percent to occur at oxygen concentrations around 2.5 mg/l. For indicative and positive species this is however an exception.

Beside occurrence in oxygen concentrations there is another important difference between the indicative species and the negative species: the number of samples in which they were found is much smaller than for the negative species (Figure 16, right bar plots). This indicates that, even though some indicative species can occur in wide oxygen ranges, they are not very common and negative species occur more often.

There is one exception for the indicative species, namely *Anacaena globulus ,* which occurs relatively more often. The most common positive species are *Potamopyrgus antipodarum* and *Gammarus pulex*, which are the first two green bars in Figure 16.

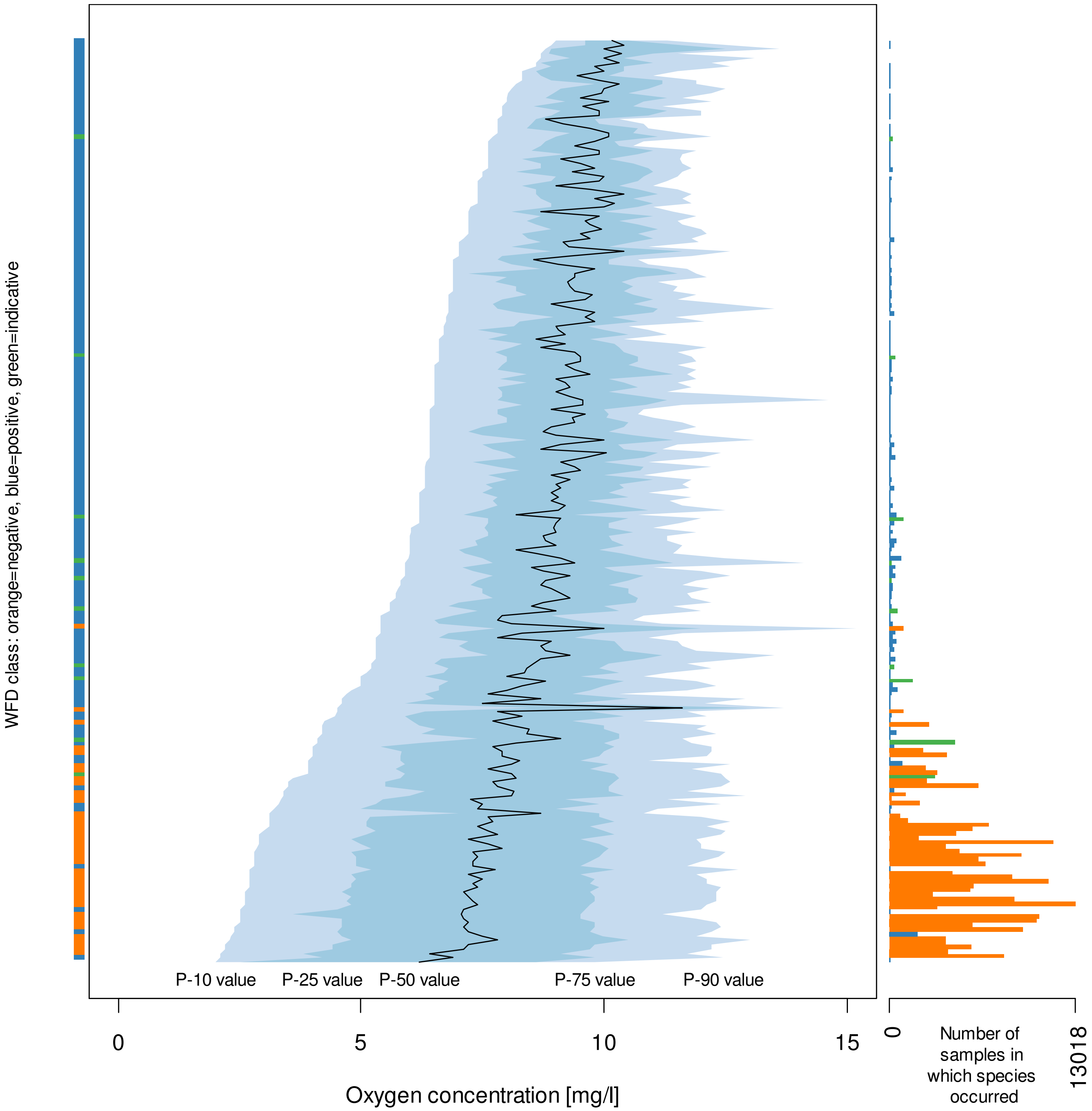


Figure 16, light blue area shows the change between 10 and 90 percent that a species can occur. The dark blue area shows the change between 25 en 75 percent. The vertical black curve shows oxygen concentrations where the occurrence of a species is 50 percent. The bar plot on the right side shows the total of samples that a species occurred in.

5.3 Impact of oxygen concentrations on EQR score

Fixed abundances were assigned to the probability that a species occurs. For example: it was decided that a species occurs with 3 individuals at a certain oxygen concentration, if the chance that it appears lies between 10 % and 50 % (Table 8). With this approach, it was possible to create a range of oxygen concentrations with a corresponding species composition (in this case only with R5 species).

Table 8, abundance classes used for different chances of the appearance of a species. The chance of appearance is dependent on the oxygen concentration.

Chance to appear	Amount of individuals of a species
< 10 %	0
>= 10 % or <= 50 %	3
> 50 %	15

The goal of “Impact of oxygen concentrations on EQR score” is to predict how the species composition (and therefor the EQR score) changes with increasing oxygen concentrations. The chance of appearance (P-value; Table 6) was assessed (by interpolating between two P-value) for a range of oxygen concentrations (from 0.2 till 15.7 with steps of 0.5 mg/l; it starts at 0.2 due to a decision for calculation that was made). Thus for every oxygen concentration in this range, the species that occur as well as the amount of individuals for each of the occurring species is known (Figure 17). With these values the EQR score was calculated with QBwat (Pot, 2007).

The oxygen concentrations are interpreted as minimum annual oxygen concentrations. If the annual oxygen concentration in a stream does not drop below this value, it is assumed that the “potential” species composition in the stream is the same as the theoretical one.

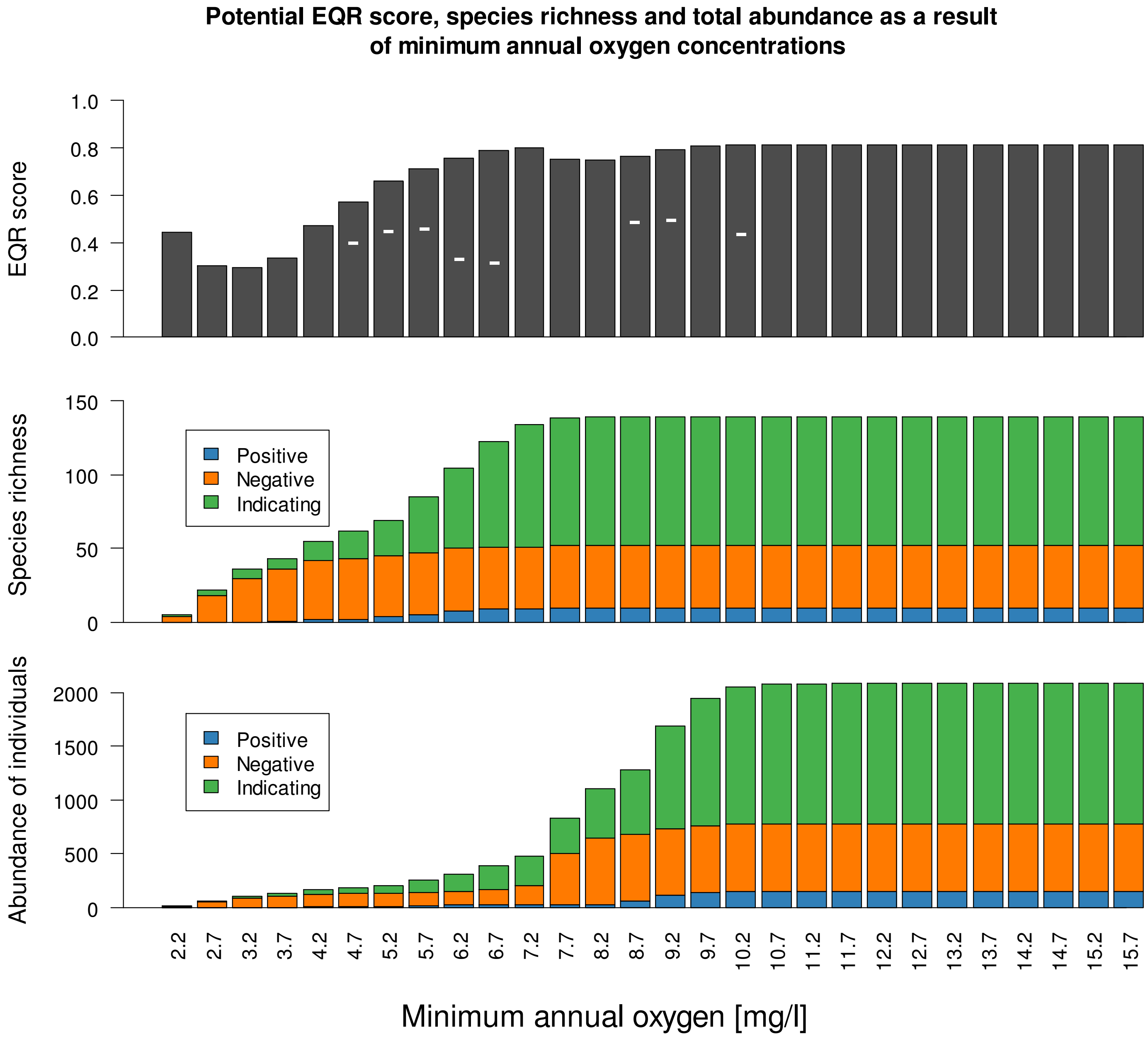


Figure 17, EQR score, species richness and total number of individuals for minimum annual oxygen concentrations. Species richness and the Abundance of individuals are split up in the classes that are used for the WFD scoring. White lines in the uppermost bar plot represent EQR scores for the Kleine Dommel, based on measured minimum annual oxygen concentrations and biological samples.

No species occur at minimum annual oxygen concentrations lower than 2.2 mg/, which is a result of the abundance classes that were chosen (Table 8). Up to 4.2 mg/l, species richness consists primarily of bad species. With increasing oxygen concentrations, the amount of bad species levels off and stays (almost) constant because all the bad species already started occurring at lower oxygen concentrations. However, from 4.2 mg/l and higher, there is an increase in the amount of good and indicating species and this results in an immediate increase of the EQR score. The biggest increase in EQR score can therefore be gained between 4.2 mg/l and 6.7 mg/l. This implies that if a water board wants to improve the score of a stream, it should focus on keeping the annual minimum oxygen concentrations within this range or above 4.7 if the target is an EQR of at least 0.6.

The total number of individuals reacts much later than the species richness. When the chance of appearance of species rises above 50 % the abundance of the individuals starts to grow significantly (due to abundance classes; table 8). This happens at around 7.2 or 7.7 where most negative species fall within the highest abundance class. The indicative species show a more gradual increase. The fact that positive species have such a small abundance is because there are only 10 positive species in the rating system and much more negative and indicative species.

EQR scores that are calculated on the basis of real biological samples of the Kleine Dommel (white line in EQR bar plot), are lower than the EQR scores that were calculated on the basis of theoretical abundances. This indicates that there are other processes that influence the occurrence of species, such as current velocity, organic matter load etc. Another reason could be that the abundance classes that were chosen are not representative because some species are likely to occur in higher abundances than others. This is also shown in Figure 16 where negative species were found in much more samples than the indicative species.

6 Discussion

The macro-invertebrate data that were provided by the water board contained some uncertainties that needed attention during the analyses. Some species appeared double but with slightly different names, it was not sure whether these had to be summed or not. Some species are identified to genus, while in other years they are identified to family level. Therefore it was sometimes necessary to interpret the data with the help of expert knowledge.

At location Dommel 3 the stream has been mowed but it is not clear when exactly this happened. The biggest depression of the oxygen concentration is assumed to be a result of the mowing. But because the equipment was removed and distorted from its place by the mower, this data cannot be used to give an idea about the influence of mowing on the oxygen concentration.

Information about the occurrence of CSO events was delivered by the Municipalities. There was only one sewer overflow at the end of the measuring period while the measurements were taken in a period of intense thunderstorm with excessive precipitation.

Data from the Limnodata database is a big part of this thesis. Because of its size of samples it gives a good representation about the situation in the field. However, it is not sure how the P values should be interpreted. The fact that a species occurs at a certain minimum oxygen concentration does not mean that it cannot occur at lower concentrations. However, due to the size of the dataset the P-values are regarded as reliable.

The Limnodata does contain the number of samples in which a species occurred but no information about the abundance of individuals in these samples was used. Future research could focus on the abundance of individuals in the samples. In that way, for all the species the average amount of individuals at a certain oxygen concentration can be assessed. With this information there would have been no need to create the 3 theoretical abundance classes which were now based on the chance of appearance. This would have been better because these classes are the most sensitive assumption in the analysis because the theoretical abundance classes were chosen equal for all the species. But it is likely that the amount of individuals of bad species is higher if annual minimum oxygen concentrations are low (Perdersen et al., 1986 ; Willemsen et al. 1990). On the other hand, the reported abundances in the Limnodata can have flaws as well, due to differences in sampling and identification methods among researchers.

A drawback of using the approach with the Limnodata is that there is no time component. It is well known that the duration of an oxygen dip does influence the severity of the consequences for the macro-invertebrate (Lammersen, 1997). The analysis of the measurement campaign clearly shows that there is a difference in duration of oxygen dips among the three locations and this would thus influence the macro-invertebrate composition.

The focus of this thesis was on the influence of oxygen limitations on macro-invertebrates. However, the analyses of the data and literature indicate that other variables (salinity or pH) play an, sometimes even more, important role (Seaget et al. ,1990; Passerat et al., 2011 ; Pratt et al., 1981). And synergy of all these variables can be the driving force for macro-invertebrate composition (Pratt et al., 1981; Gammeter et al., 1991). This could be one of the explanations why the calculated EQR scores were smaller than the measured ones. However, the strength of the approach that I have chosen is that it can easily be extended with other variables. It is a simple matter of adding these values (which are also provided in the Limnodata database) and finding the restrictions of macro-invertebrate survival in the same way as was done with the oxygen concentrations.

Another reason that EQR scores based on measured data were lower than the theoretical ones could because the EQR scores based on field data were classified at the minimum annual oxygen concentrations that were based on monthly measurements. It is likely that oxygen concentrations in those years dropped much lower (as was shown in the measuring campaign and by continuous oxygen measurement by the water board). If these scores would be classified at lower concentrations (where they probably belong) they would correspond well with the theoretical scores. The biological samples of the water board support the theory that the minimum annual concentrations probably were lower. The total abundance of the macro-invertebrate community in these samples consisted of only a few species, and the biggest part of these species was negative. Analysis of the Limnodata showed that such a macro-invertebrate distribution is common for streams with low annual minimum oxygen concentrations.

A more basic question is how representative the EQR score really is for an ecosystem. Both the Shannon Index and the literature showed that from an ecological point of view an ecosystem can be relatively rich while the EQR score is low.

7 Conclusion

One and a half month of data did gave inside in the daily oxygen patterns and the spatial difference along the Kleine Dommel. Profiles (temperature, oxygen and ECV) are more truncated downstream. Peaks and lows are less extreme more downstream and the duration and frequency is smaller. This conflicts with the spatial distribution which shows more positive species upstream. This indicates that positive species are not very sensitive to the lower oxygen concentrations, even though the Limnodata analysis conflicts with this conclusion. Apparently other unknown factors play a role.

The analysis of Limnodata and the sensitivity analysis of the WFD both showed that an increase in species richness (of the indicative or the positive species) is the most effective solution to significantly improve the EQR score. The indicative group influences the EQR stronger than the positive group.

An increase of minimum annual oxygen concentrations results in a gradual (almost linear) increase of the species richness, but up to 4.2 mg/l this increase is primarily caused by occurrence of negative species (almost all negative species have >10 % chance of occurrence at 4.2 mg/l). Indicative species start occurring significantly around 4.7 mg/l and the positive species at even higher minimum annual oxygen concentrations. Once the indicative species start occurring (10 percent chance) the EQR score increases significantly, which corresponds well with the findings of the sensitivity analysis.

The EQR scores that were based on measurements (biological samples of water board) in the Kleine Dommel were smaller than the calculated EQR scores. Only taking oxygen into account is a simplification. Other factors play a role and these should be included to get a more precise result.

The end conclusion is that the Kallisto project would be well advised to focus on measures that keep the minimum annual oxygen concentrations as high as possible. If the target is an EQR score of 0.6 than annual minimum oxygen concentrations should be kept around 5.0 mg/l.

8 Acknowledgements

I would like to thank Mark Scheeps for providing his expert knowledge about the Kleine Dommel and the Tongelreep.

Rolf Pot provided the program QBwat, which was very useful for calculating the EQR score. He also provided a list of all the macro-invertebrates that is used in the rating of a R5 water body.

Most graphs were prepared using R (R Development Core Team, 2011) and Komodo Edit interface. The maps were prepared with QGIS (Quantum GIS Development Team ,2011) and geodata from openstreetmaps.com. Thanks to the open source community which makes professional scientific analysis possible without expensive registrations.

Last but not least I would like to thank Frits Gillissen and Jeroen Klein for helping me with the field experiment and preparing the equipment.

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Appendix

Table 9, coordinates of the biological measurements location of the water board. Coordinates are in "rijksdriehoekcoordinaten"

Measurement location	X coordinates	Y coordinates
250014	161903	368186
250018	-	-
250015	161257	381703
250047	165523	384099
251030	168840	378580

Table 10, Alterations of the species compostion for the positive species, indicative species and negative species combined

	Positive species and Indicative species	All species Combined
Scenario 1	Field situation	Field situation
Scenario 2	Individuals of most abundant species X 5	Idem
Scenario 3	Individuals of most abundant species X 10	Idem
Scenario 4	Individuals of most abundant species X 100	Idem
Scenario 5	Individuals of most abundant species X 1000	Idem
Scenario 6	Individuals of most abundant species X 10000	Idem
Scenario 7	Distribute nr. of individuals of scenario 2 over 3 new species	Idem
Scenario 8	Distribute nr. of individuals of scenario 2 over 6 new species	Idem
Scenario 9	Distribute nr. of individuals of scenario 2 over 10 new species	Idem
Scenario 10	Distribute nr. of individuals of scenario 6 over 3 new species	Idem
Scenario 11	Distribute nr. of individuals of scenario 6 over 6 new species	Idem
Scenario 12	Distribute nr. of individuals of scenario 6 over 10 new species	Idem

Figure 18, Measuring location Dommel 1

Figure 19, Measuring location Dommel 2

Figure 20, Measuring location Dommel 3