MONITORING JUVENILE RHEOPHILIC FISH COMMUNITIES IN THE LOWER RHINE WITH DIFFERENT SAMPLING TECHNIQUES

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INTERREG Deutschland Nederland

Europäische Union
Europese Unie

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Report number: 20190054/rap03
Report
Version: Final report
Date: 7 juni 2021

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This research was financed by the Interreg project Green Blue Rhine Alliance.

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INDEX

APPENDIXES

1. INTRODUCTION

1.1 JUSTIFICATION

In order to improve the area of suitable spawning and nursery grounds for juvenile rheophilic fish, secondary channels and oxbow lakes have been created in the floodplain areas along the Lower Rhine in Germany and the Netherlands. In order to measure ecological quality and the effects of measures taken, in both countries fish stocks in the river and its floodplain waters are monitored on a regular basis. For this purpose different sampling methods are used by the German and Dutch researchers.

In order to be able to compare the results from the different sampling methods and to obtain a better understanding of the different techniques applied, in July 2020, a joint monitoring program was carried out. For this purpose, two river sections in the Netherlands (Waal) and two comparable sections in Germany (Niederrhein) were monitored using different sampling methods. The monitoring was carried out simultaneously by the German and Dutch research partners. In addition to the more traditional monitoring techniques, water samples for eDNA analysis were collected, using different collection methods. To investigate the presence of (river) lamprey larvae, sediment samples were taken with a venturi sediment dredger.

The research was commissioned by Rijkswaterstaat Oost Nederland in cooperation with the Bezirksregierung Düsseldorf and was carried out by ATKB environmental consultancy in partnership with LimnoPlan and Bureau Waardenburg (BuWa). Analysis of the eDNA samples were carried out by Datura and Sylphium. The research was funded by the Interreg Project Green Blue Rhine Alliance.

1.2 OBJECTIVES

The main objectives of this study are:

- to obtain a better understanding of the different techniques, materials and working methods applied by different partners;
- to collect data on fish stocks in different riverine habitats applying different sampling methods. The focus thereby is on the juveniles of rheophilic fish species;
- to compare the results (i.e. species composition, CPUE, age distributions) from the different sampling methods and to identify most striking differences and similarities;
- to compare the fish stocks (i.e. species composition, CPUE, age distributions) in the different locations.

In order to be able to compare the data collected with different methods, ideally a set of general rules and conversion rates should be established. The limited extent of this investigation however does probably not allow for determining such general rules and conversion rates.

1.3 READER

After this introduction, the methods and materials used in the investigation are set out in Chapter 2. In Chapters 3 the results are presented. Chapter 4 contains a discussion of the results and summarizes most important conclusions. Consulted literature and references are listed in Chapter 5.

2. METHODS & MATERIALS

In this chapter the methods and materials used in this study are described. In Paragraph 2.1 the methodologies and equipment used are explained in further detail. In Paragraph 2.2 the research area and sampling locations are set out. The period in which the study was performed is addressed in Paragraph 2.3.

2.1 METHODOLOGIES AND EQUIPMENT

2.1.1 METHODOLOGY APPLIED BY ATKB (NETHERLANDS)

The methodology applied by ATKB in the Netherlands consists of a combination of seine net fishing and single anode electrofishing. Seine net fishing is used for the monitoring of open water sections with a relatively smooth bottom surface, whereas single anode electrofishing is especially effective in shallow sections rich in structure. Both techniques are explained in further detail in the text below.

Single anode electrofishing

Single anode electrofishing (or one anode electrofishing) from a boat is used for sampling groynes (boulder structures), river banks and sections with wood structures (Figure 1 and Figure 2). Uniform habitat sections ranging between 25 meters and 150 meters in length are sampled. Coordinates of start and end point as well as the length of the section are determined using a handheld GPS device. In case of wood structures, different sides of the structure are sampled and if possible also underneath the structure. This way not only fish that seek shelter within, but also underneath the structure are caught.

Using a gasoline fueled Honda Tench and Subaru generator, an electrical field (200 volts and 6 amps) is generated. Only direct current is used. The effective reach of the electrical field is approximately 1.5 meters. Electrofishing is carried out with one anode net. A stainless steel cathode acts as a negative pole. One extra hand net (not connected to the generator) is handled by a second person to catch any fish missed by the anode net. According to the NEN-EN 14011 standard, this method should be used in situations where current velocity exceeds 1 meter per second.

When the anode touches the water, the electric circuit is completed and any fish within the electrical field are anesthetized and guided towards the anode. This method is suitable for catching fish of any length class. Next, the anesthetized fish are scooped out of the water with the anode net, or with the second net without electricity, and are temporarily stored in round ventilated tubs filled with river water. Directly after measurement, fish are released back into the river.

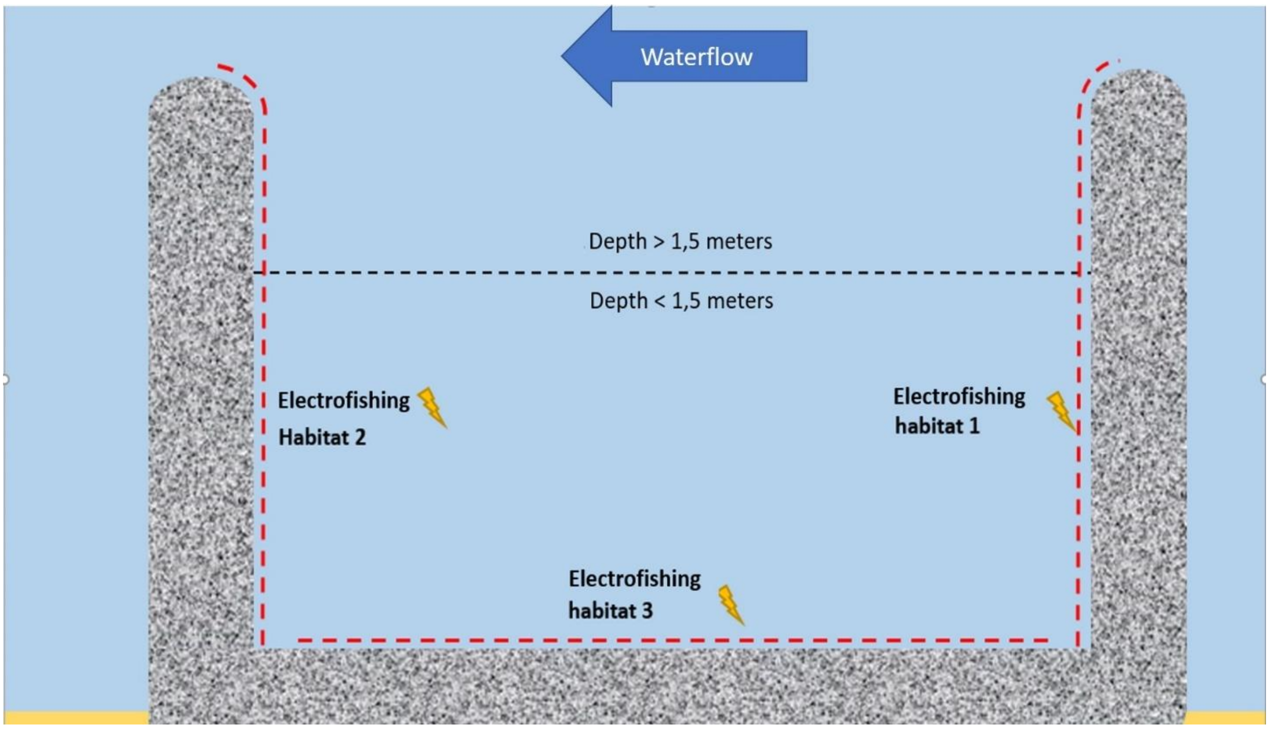

Figure 1 *Sampling of groyne field and a river bank made out of boulders with one anode electrofishing.*

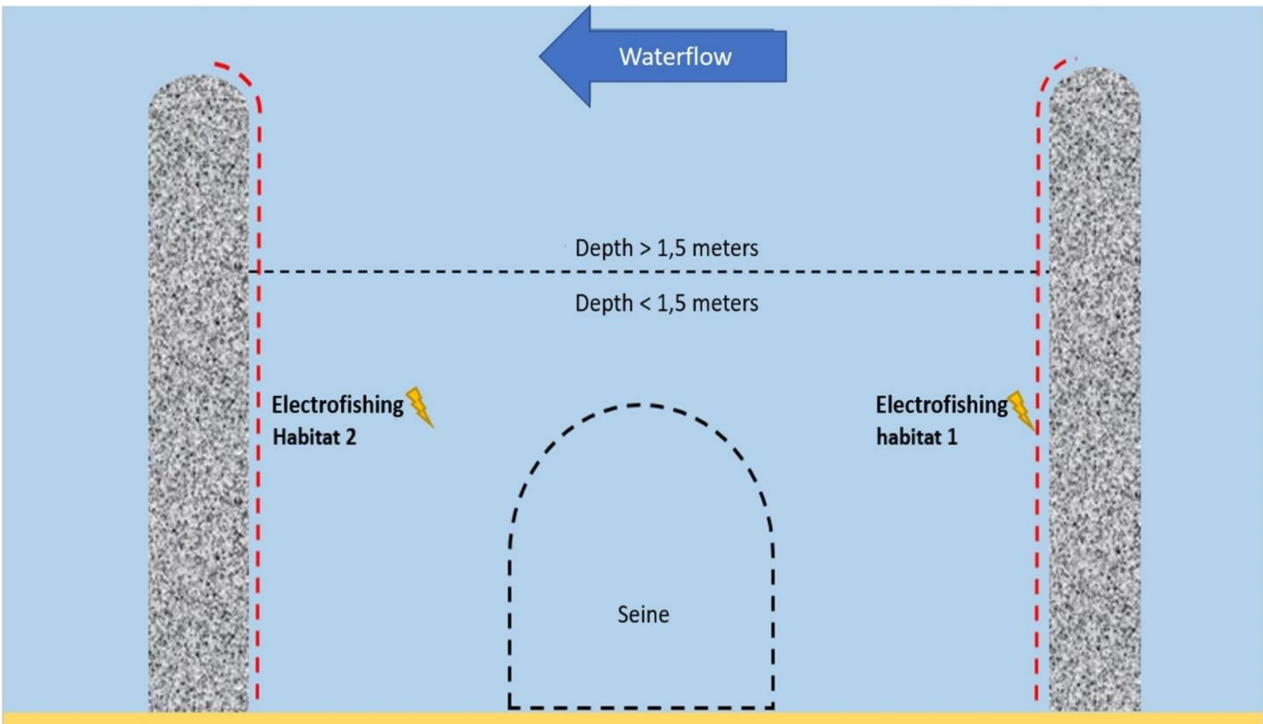

Figure 2 *Sampling of groyne field and sandy river bank using a combination of one anode electrofishing and a seine net.*

Seine net fishing

For monitoring of open water habitat a 75 meter long seine net is used (Figure 3). A seine is a trawl net with two side nets of 25 meter each and a 'pocket' with a length of 25 meters. The length of the line with sinkers (bottom side) is longer than the line with the floaters (surface side). This is to make sure that the sinkers stay at the bottom while pulling in the seine. The sides of the seine have a mesh size of 40 mm. Closer to the pocket the seine has a mesh size of 25 mm. These specifications are in line with the guidelines from the Handbook for Aquatic Ecology (Bijkerk, 2014), which in the Netherlands, dictates the sampling methods to be used in specific situations. Due to the size of the juvenile fish, the pocket of the net is adapted with a fine 12 mm mesh. Depending on water depth, monitoring is carried out with a shallow (maximum water depth of 2.8 meters) or a deep seine (maximum water depth 4.5 meters).

Using a boat, the seine is towed around in a half circle, with a standard 25 meter bank width (Figure 3). One person on the river bank holds the rope on one end of the seine, while the person in the boat tow the seine around in a circle. The area sampled, as well as the location of the sampling, are determined with a handheld GPS device. After towing around the seine, three (wo)men are needed to pull in the seine. Two of them pull in the top line of the seine and one of them pulls in the line with the sinkers at the bottom of the seine and makes sure the sinkers stay on the bottom so fish cannot escape. The enclosed fish are guided into the pocket of the seine. When the pocket is close enough to the shore, the third person pulls in the sinkers rapidly to close the pocket before lifting it up. After opening the pocket, the catch is temporarily stored in tubs filled with river water. Directly after measuring, all fish are released back into the river.

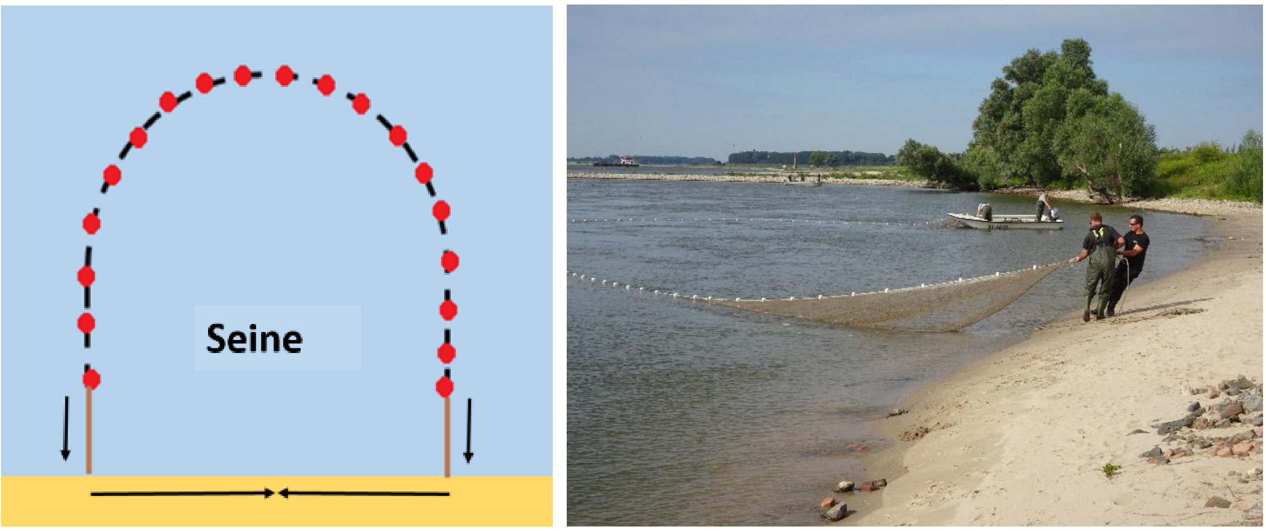

Figure 3 *The Dutch team uses an especially adopted seine net which is towed around by boat and then pulled in from the shore .*

Data collection and processing

The collection of fish data is done in accordance with the work instructions in the Handbook Hydrobiology (Bijkerk, 2018), with an exception of the length measurements: for the purpose of this particular study, total length of (juvenile) fish smaller than 10 cm is measured in millimeters and fish larger than 10 cm is measured in centimeters. In addition to fish data, observations of crayfish and crabs as well as relevant environmental parameters are registered using a tablet and an especially designed "fish app".

Before length measurement, the total number of fish is estimated. In case this total is less than approximately 200 specimen, all fish are measured individually. In case of larger catches, a subsample is taken. Fish that are not part of the subsample are directly released back into the river, while the fish in the subsample are kept in the tub for measurement. This method saves time and prevents unnecessary loss and suffering of the fish, while at the same time the results still provide a reliable indication of the characteristics of the local fish community. Therefore it is important to make sure that the subsample is a good representation of the total catch. First, the largest fish and specimen of species of which only few individuals are present are separated from the rest of the catch and measured individually (also in mm or cm) on a calibrated measuring board. Then a subsample is taken randomly from the rest of the catch. This is done by first weighing the total catch and then dividing it by a factor 2, 4 or 8 (until a workable number of fish is left) or visually.

After measuring 30 individuals of a certain species within a certain length class, instead of measuring, the remaining individuals of this length class of this species (this is called a "group") are counted. The counted individuals are then evenly distributed within this specific length class (group).

After completing the input for a sample/section, the data are send to a central server, where they are stored and readily available. This way a back-up of the data is always available.

Data-analysis and presentation

To calculate fish stock numbers and densities in a certain river section, the catches of separate monitoring locations for each section are summed up per species and length class and are then divided by the corresponding surface area (in hectares) sampled, resulting in an estimated density of n individuals per hectare per section.

For the presentation of the data, fish with similar environmental preferences were grouped into ecological guilds or functional groups based on the classification used by the German partners (Appendix I). A list of fish names in different languages is included in Appendix II. The basis for the grouping of species is the affiliation to ecological guilds (mainly based on flow preferences and general habitat conditions, according to Schiemer & Waidbacher (1992), Schwevers & Adam (2010), Zauner & Eberstaller (1999) and classification in FIBS (Dussling *et al*., 2010), but also the type of relation to floodplain habitats, as empirically determined in large-scale studies in the German Lower Rhine (LANUV, 2019; Scharbert, 2009; Scharbert *et al*., 2019). This guild classification differs from the classification normally used in the Netherlands, i.e. the FAME classification used for the Water Framework Directive (WFD) water types R7 (major rivers).

In coordination with the German team, an allocation to the two age groups AG 0 (Young of the Year / YOY fish, as an indicator of successful reproduction) and AG > 0 (perennial, subadult and adult fish) was made based on empirical values for the growth of juvenile fish in the Rhine. The allocation to age groups AG 0 and AG > 0 is taken into account in some presentations of the dominance distributions.

2.1.2 METHODOLOGY APPLIED BY LIMNOPLAN (GERMANY)

Strip anode (Streifen Anode) electrofishing

The electrofishing was carried out as a standardized stretch fishing by boat using a very powerful motor-driven electrofishing gear of the type EFKO-13000 (13 kW) in direct current operation, whereby a so-called strip anode was used as anode (Figure 4). The strip anode increases the electric field in the water and opens up the option of fishing with continuous current, which means that even larger fish aggregations can be held in the electric field to be netted. The landing of the narcotized fish was carried out by two people. The cathode is a rope cathode dragged over the ground. The fish anesthetized in the electric field are generally taken out of the water, temporarily stored in ventilated tanks, determined and measured at the end of the fishing stretch or recorded in size classes according to the LANUV standard data sheet[1] and released again.

Figure 4 *Strip anode electrofishing as applied by the German team from LimnoPlan.*

The standard length of a fishing transect in the main stream in regular NRW Rhine monitoring is 500 m. In the course of the present study, shorter stretches of 100 m in length were fished in agreement with the Dutch team. All stretches are defined along the shore line, whereby the shore distance and depth ranges were selected in such a way that the effective range of the electric field reached as far as possible down to

[1] https://fischinfo.naturschutzinformationen.nrw.de/fischinfo/de/download

the bottom of the water body (i.e. to a maximum water depth of approx. 1.5 - 2 m). The exact location and length of the sections sampled were recorded with handheld GPS devices.

From the number of fish caught, abundance measures are calculated as CPUE (Catch per Unit of Effort = number of individuals per distance, here 100 m), or per area fished (standard distance x 3 m assumed effective corridor). There is no further extrapolation of real catch results using assumed catch rates. Boat-based stretch fishing with strip anode is usually carried out relatively early in the year (in the May-June time window) as part of the long-term monitoring by LANUV, as it aims at a representative survey of subadult and adult fish (AG > 0) and mass catches of young-of-the-year fish (AG 0) should be avoided as far as possible, as these require a very high processing effort and can only be processed at considerable damage rates. The information on the abundance of young-of-the-year fish (YOY) is usually collected mainly by means of separate monitoring of juvenile fish using point-abundance fishing.

Point-abundance sampling by electrofishing (PAS)
Point Abundance Sampling by electrofishing (according to Persat & Copp, 1989; Copp & Garner, 1995) is a special technique of electrofishing with a special distribution of sampling effort, which is particularly suitable for the recording of YOY fish. In contrast to conventional electrofishing (with more or less continuous current application), in PAS the activated anode is always immersed only at discrete sampling points and not moved, so that only fish at this point within the effective radius of the anode are recorded. The sampling points are distributed absolutely randomly or representatively over the sampling area in order to obtain a realistic picture of the spatial distribution of the fish and the resulting fish density (one has to avoid that particularly promising catch points are sampled selectively).This method has the advantage of largely eliminating shoaling and scare effects, which are a considerable source of error when comparing data of different sampling units and make it difficult to quantify accurately the catch results of regular electrofishing.

From the number of catches, accurate, reproducible density data (individuals per m^2) can be determined using formula that describe the reaction of the fish in the electric field as a function of fish length and equipment characteristics. An important advantage of PAS is that resulting data sets can be analyzed using statistical methods (because the data matrix consists of a large number of small samples).

The shape of the anode used determines the generated field strength and the effective radius, at the same time the effective radius depends on the fish size. The smaller the radius of the anode, the higher the generated field strength, the smaller the fish that can be anesthetized, but the smaller the radius of action. In addition, other technical factors such as type of device, device settings and type of current (direct current or pulsed current), and environmental conditions such as the electrical conductivity of the water, water depth, current conditions and substrate conditions also determine the catch efficiency. The conversion of the catch results (in the raw form as CPUE = number of fish per catch point) into abundance data with area reference (densities) requires device-specific and fish size-dependent effective radius or effective area formulae. This must be empirically determined under representative environmental conditions.

Larger fish of age group > 0 are usually caught in the PAS only accidentally and in small, unrepresentative numbers (as they usually have longer escape distances), the method aims primarily at the representative capture of fish of YOY fish (age group 0).

Within the scope of Rhine monitoring surveys in Nordrhein Westfalen (NRW), the effective area formula determined by Scharbert (2009) has been used to date for the conversion of CPUE into area-related density data (individuals per m^2): **$A = \pi * r^2$** with r = 0,0479 * TL0,5521 (TL = total length of the fish (mm)). This formula was determined for DEKA 3000 portable pulse current devices. The formula has also been used after the change to more modern direct current devices of the type EFGI-650, which was made during the Rhine monitoring some years ago, as a specific effective area formula is not yet available for this type of device (as these devices have different types of current but comparable electrical power, the application of the original effective area formula seems reasonable in order to obtain realistic density data in a first approximation).

Before the Rhine monitoring studies in 2020, a further change of equipment was carried out, as the apparent sharp decline in fish densities in the Rhine requires the use of more powerful electrofishing equipment in order to achieve usable catch results with the point-abundance sampling design. Since 2020, the considerably more powerful, battery-powered stationary gear type EFGI-4000 (4 kW) has been used as the standard gear. The unit and the heavy battery boxes are stationed in a light boat, which is pushed behind the wading anode guide by a helper at a sufficient distance. Also for the EFGI-4000 no device-specific active area formula is available yet. However, the formula used so far cannot be used unchanged, as the device has a considerably better catching effect than the carrying devices. The current active area formulae would therefore lead to a considerable overestimation of actual fish densities.

PAS was executed only in shallow bank-near areas by a wading electrofisher. As in the case of the other types of electrofishing gear, a landing net anode was used on an extended anode rod with a landing net frame diameter of 40 cm and a mesh size of 4 mm. The exact location and length of the sampling areas were recorded with handheld GPS devices. In this report abundance data are shown preliminary as CPUE (number of individuals per fishing point), since the effective areas fished by both teams differ. Normally the density data for each fishing point are calculated by summing up the number of individuals caught per species and the density data related to the sampling area are calculated by averaging the sum of the fishing points (including zero samples). The minimum number of fishing points to represent spatial distribution and fish abundance in a sampling area is 50 fishing points (LimnoPlan, 2015).

Data processing

With point abundance-sampling, all fish individuals are usually measured, since a length specification is required for the formula to calculate the density. For non-measured individuals, an average total length of all fish individuals measured for the respective sample unit was used in the effective area formula. On the basis of empirical values for the growth of juvenile fish in the Rhine and the analysis of length frequency distributions of the measured fish, an allocation to the two age groups AG 0 (YOY fish, as an indicator of successful reproduction) and AG > 0 (perennial, subadult and adult fish) is made. The allocation to age groups AG 0 and AG > 0 is taken into account in some presentations of the dominance distributions.

In the presentation of results, the recorded fish species are grouped into so-called "functional groups". The basis for the grouping of species is the affiliation to ecological guilds (mainly based on flow preferences and general habitat conditions, according to Schiemer & Waidbacher (1992), Schwevers & Adam (2010), Zauner & Eberstaller (1999) and classification in FIBS (Dussling *et al*., 2010), but also the type of relation to floodplain habitats, as empirically determined in large-scale studies in the German Lower Rhine (LANUV, 2016; Scharbert, 2009; Scharbert *et al*., 2019).

2.1.3 LAMPREY LARVAE SAMPLING BY BUREAU WAARDENBURG (BUWA)

In addition to the sampling methods applied by ATKB and LimnoPlan, BuWa applied a venturi sediment dredger for sampling of lamprey larvae (Figure 5). Because these larvae live in the bottom sediment they are generally not caught with more conventional fishing techniques.

Figure 5 *A venturi sediment dredger was used for the sampling of lamprey larvae.*

The operation of the venturi sediment dredger is based on creating a suction effect at the bottom of a tube by injecting the tube with an upward directed flow of water. The created suction effect sucks up sediment (including fauna) at the bottom of the river after which it can be collected. A venturi based dredger, instead of an air based dredger, can be used at very shallow water depths. With each sample of the venturi sediment dredger approximately 1.5 m2 of substrate is sampled to a depth of circa 10 cm. The sampled material is collected in a mesh bag and then placed on a series of two sieves with different mesh sizes, respectively 10 mm and 500 µm (Figure 6). Next, substrate is classified and described and any organisms present in the sediment are collected. Organisms are then taxonomically identified, counted and registered.

Figure 6 *Examples of collected substrate containing fauna, in this case mainly shells of Corbicula fluminea.*

2.1.4 EDNA SAMPLING

In addition to the conventional sampling methods, samples of eDNA (environmental DNA) were also collected. Sampling equipment and protocols were provided by Datura Molecular Solutions BV and Sylphium. These two companies also carried out the analysis of the samples (each company analyzed its own samples). Sampling and filtration on site were performed by ATKB, according to the manuals provided by each company (Figure 7).

For Datura as well as for Sylphium a “small” and a “large” sample were collected on four different locations, making a total of 16 samples. Samples analyzed by Datura were taken with (1) Dead-end filters (0,22 µm, PES – 1 liter) and (2) Cross flow filters (1 µm, PES – 60 liters). Samples analyzed by Sylphium were taken with (1) Sterivex and (2) Dual filters (see Table 1).

A detailed description of the sampling and analysis protocols applied can be found in the documents provided by Datura and Sylphium included in Appendix 3 and 4 of this report.

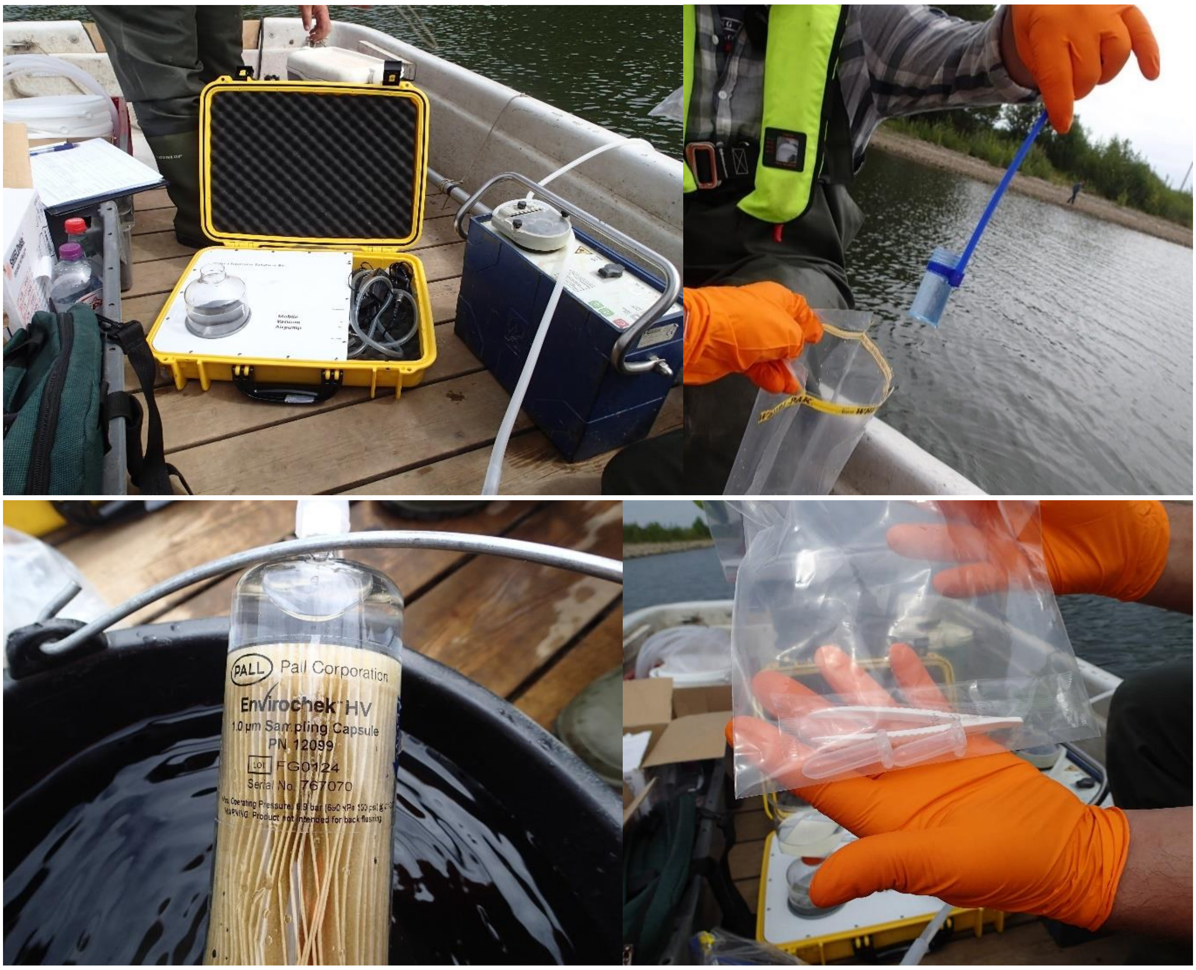

Figure 7 *Collection of eDNA samples in the field with different methods and materials provided by Datura and Sylphium.*

Table 1 *Different filters and sample volumes of eDNA samples taken for analysis by Datura and Sylphium.*

		Datura		Sylphium	
Location	**Habitat**	**Dead-end**	**Cross flow**	**Sterivex**	**Dual filter**
Waal Ophemert	groyne field	1 L	60 L	0,30 L	1,2 L
	secondary channel	1 L	60 L	0,24 L	1,2 L
Niederrhein Walsum	groyne field	1 L	60 L	0,30 L	1,8 L
	secondary channel	1 L	60 L	0,36 L	1,2 L

2.2 RESEARCH AREA AND SAMPLING LOCATIONS

2.2.1 RESEARCH AREA

The research was carried out in two comparable river sections in the Lower Rhine, i.e. "Langsdam Ophemert" in the river Waal in the Netherlands (Figure 8) and "Parallelwerk Walsum" in the Niederrhein in Germany (Figure 9). Both sections are manmade secondary channels located parallel to and on the right side of the main river channel. Under normal conditions both channels are connected to the main channel at the upstream side (inlet) as well as on the downstream side (outlet), having a continuous water flow through the channel. Due to the low water level at the time of visit however, the inlet of the "Parallelwerk Walsum" channel was (just) disconnected from the main channel. There was however a small continuous water flow through the riprap threshold into the channel. In addition to the secondary channels, at each site a nearby groyne field (as part of the main river channel) was monitored as a reference.

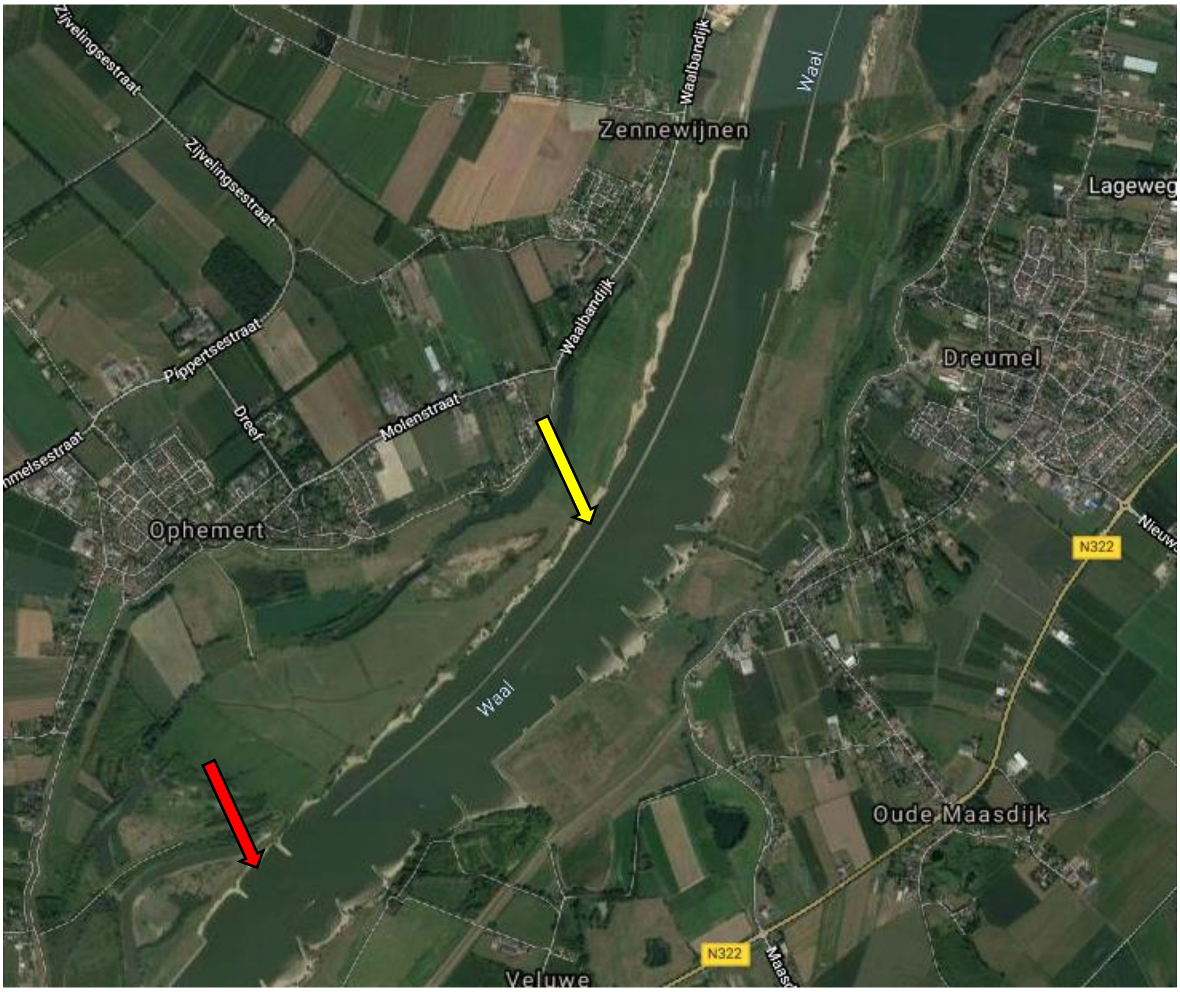

Figure 8 *Research area "Langsdam Ophemert" in the Waal near Ophemert in the Netherlands. Yellow arrow indicates the side channel, the red arrow indicates the groyne field.*

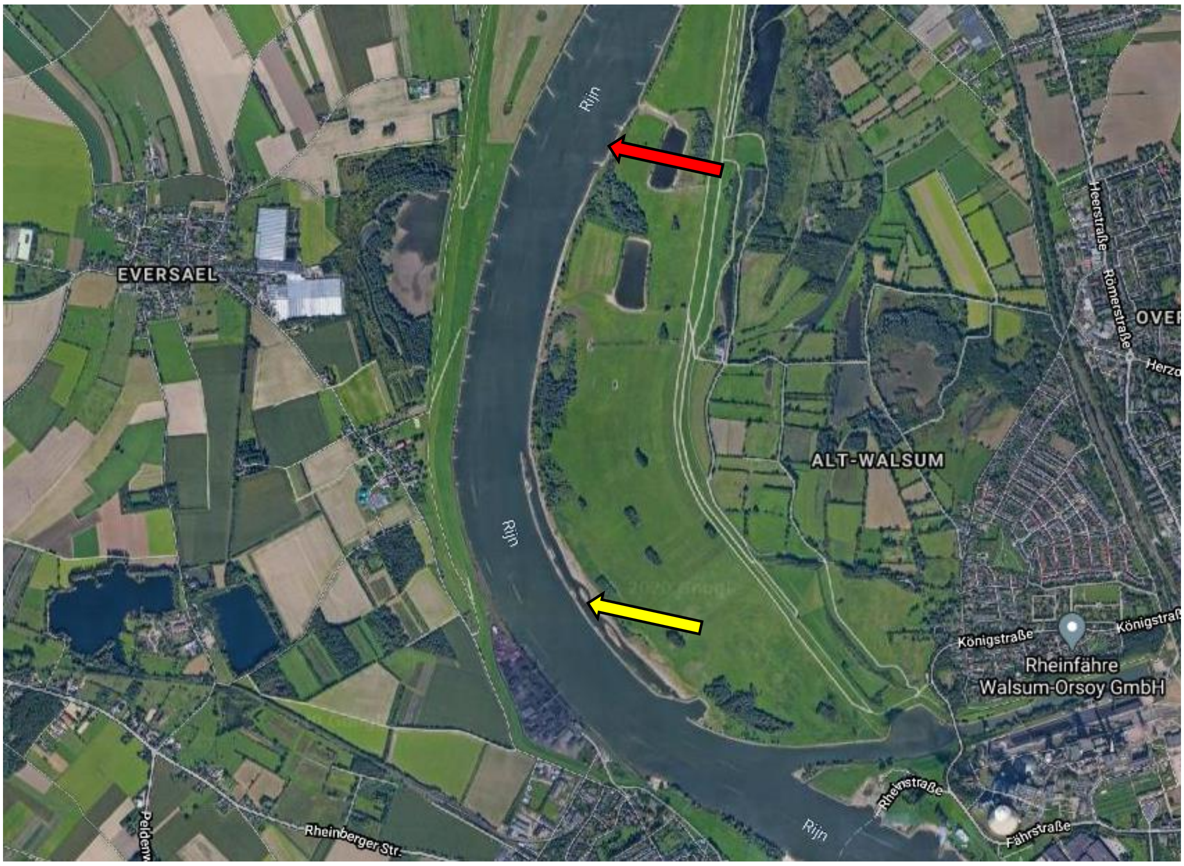

Figure 9 *Research area in the river Rhine near Walsum in Germany. Yellow arrow indicates the side channel, the red arrow indicates the groyne field.*

2.2.2 SAMPLING LOCATIONS

Traditional fishing

In the Waal as well as in the Niederrhein multiple locations/sections within the secondary channel and the groyne field were sampled with the different fishing techniques described in Paragraph 2.1, covering a variety of habitats. In order to avoid sampling the same location twice (once by the German team and then again by the Dutch team), the two research teams agreed on a division of the exact sampling locations/sections prior to the sampling. As the focus of the study is primarily on the stocks of juvenile rheophilic fish within the secondary channels, the areas within these channels were sampled with more effort than the groyne fields. The locations/sections sampled by LimnoPlan are listed in Table 2 and shown on the maps in Figure 10 and Figure 11. The locations/sections sampled by the ATKB are listed in Table 3 and shown on the maps in Figure 13 and Figure 12.

Table 2 Sampling locations/sections and sampling effort by LimnoPlan.

	Stretch electrofishing		Point-abundance-electrofishing	
Main Location	Section code	Section length (m)	Section code	No. of points
Ophemert Secondary channel	S-1	100	PAS-1	50
	S-2	100	PAS-2	50
	S-3	100	PAS-3	50
	S-4	100		
	S-5	100		
	S-6	100		
	S-7	100		
	S-8	100		
	S-9	100		
	S-10	100		
Ophemert Groyne field	S-mc-11	100	-	-
Walsum Secondary channel	S-1	100	PAS-1	50
	S-2	100	PAS-2	50
	S-3	100	PAS-3	50
	S-4	100		
	S-5	100		
	S-6	100		
	S-7	100		
Walsum Groyne field	S-mc-8	100	PAS-mc-4	50

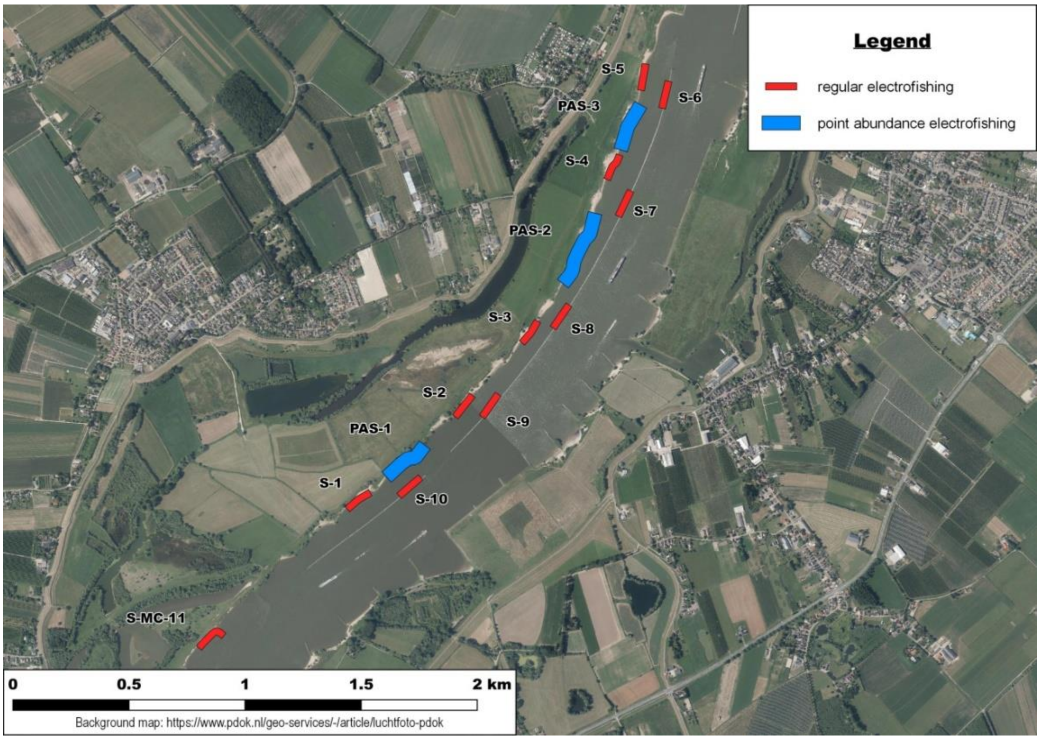

Figure 10 *Map of the Waal near Ophemert (NL) with locations/sections sampled by LimnoPlan.*

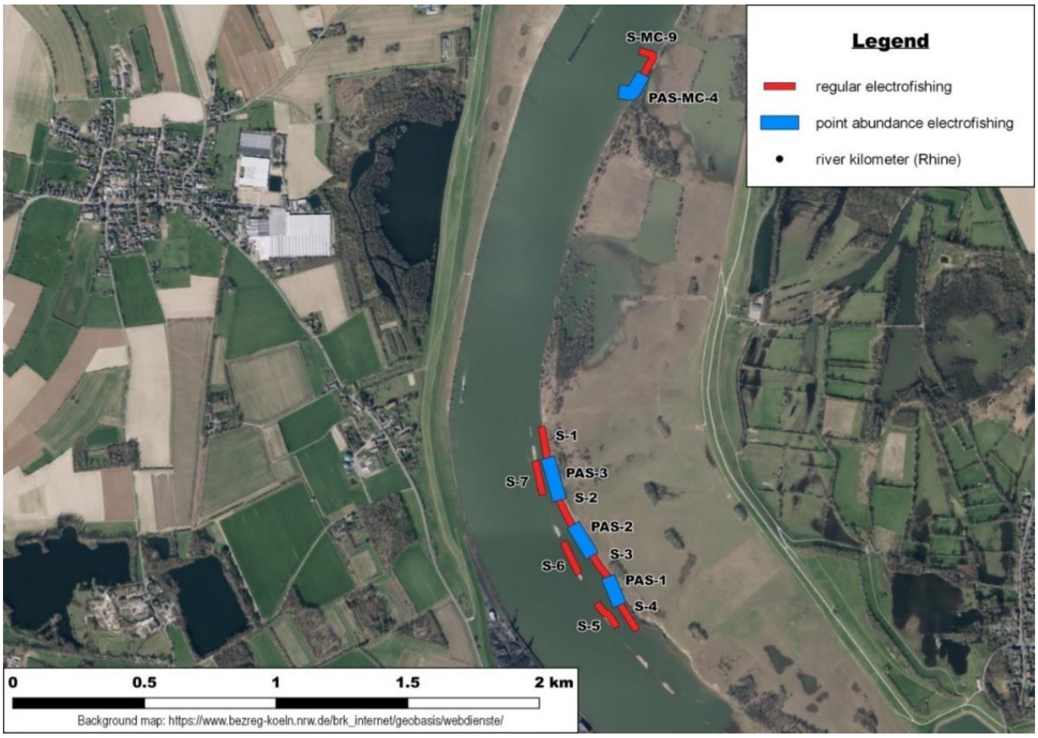

Figure 11 *Map of the river Rhine near Walsum (GER) indicating locations/sections sampled by LimnoPlan.*

Table 3 Sampling locations/sections and sampling effort by ATKB.

Location	Habitat	Code	Method	Surface (m2)	X start	Y start	X end	Y end
Niederrhein Walsum	Groyne field	el5	Electro	38	51,55276	6,68366	51,55275	6,68404
		el4	Electro	45	51,55255	6,68399	51,55270	6,68359
		ze4	Seine	568	51,55186	6,68345		
	Secondary channel	el3	Electro	38	51,54015	6,67792	51,53994	6,67810
		el2	Electro	225	51,53430	6,68199	51,53447	6,68211
		el1	Electro	150	51,53111	6,68632	51,53069	6,68762
		ZE3	Seine	436	51,53959	6,67859		
		ZE2	Seine	825	51,53347	6,68310		
		ZE1	Seine	846	51,53113	6,68687		
Waal Ophemert	Groyne field	713_el	Electro	75	155014	427008	155033	427025
		509_el	Electro	45	154976	426972	154992	426944
		508_el	Electro	60	155144	427106	155109	427123
		507_ze	Seine	839	155021	427056		
	Secondary channel	068_el	Electro	150	155457	427418	155529	427489
		059_ze	Seine	520	155394	427462		
		064_el	Electro	150	156885	429461	156906	429569
		065_el	Electro	45	156640	429020	156624	428981
		056_ze	Seine	366	156909	429876		
		057_ze	Seine	718	156662	429060		
		506_ze	Seine	517	156112	428168		
		066_el	Electro	150	156039	427948	156113	428030
		505_el	Electro	45	155692	427714	155703	427721
		067_el	Electro	45	155948	427984	155912	427933
		504_el	Electro	45	156125	428170	156147	428187
		058_ze	Seine	897	156247	428312		
		061_ze	Seine	614	156000	428042		
		060_ze	Seine	730	155634	427690		

Figure 12 Research area in the river Rhine near Walsum (GER) with sections sampled by ATKB. Left: side channel; Right: Groyne field.

Figure 13 *Research area in the Waal near Ophemert (NL) with locations/sections sampled by ATKB. Upper Left: Secondary Channel North section; Upper Right: Secondary Channel South section. Lower Left: Groyne Field.*

Sampling of lamprey larvae

In order to collect larvae of (river) lamprey, Bureau Waardenburg (BuWa) collected a total of 46 sediments samples with a venturi sediment dredger; 23 samples were taken in the river Waal near Ophemert (Figure 14) and 23 in the Rhine near Walsum (Figure 15). At each site 18 samples were taken in the secondary channel and 5 samples were taken in the groyne field.

Figure 14 Map of the Waal near Ophemert (NL) indicating lamprey larvae sampling locations.

Figure 15 Map of the Niederrhein near Walsum (GER) indicating lamprey larvae sampling locations. Samples 340-344 were taken in the groyne field.

eDNA Sampling

On 4 different locations a total of 16 samples water samples were collected and filtered for eDNA analysis (Table 4 and Figure 12 and Figure 13). Sample collection was performed from a boat by ATKB fieldworkers and according to the protocols of Sylphium and Datura Molecular Solutions BV respectively. At every locations four samples were taken, two samples (one small and one large sample) for analysis by Datura Molecular Solutions BV and two (one small and one large sample) for analysis by Sylphium.

Table 4 Locations of the water samples collected for eDNA analysis.

River	Location	Number of samples	X	Y	longitude	latitude
Rhine	Walsum Groyne field	4	244884	396681	51,55189	6,68321
Rhine	Walsum Sec. Channel	4	244617	395109	51,53781	6,67896
Waal	Ophemert Groyne field	4	155075	427080	51,83232	5,38829
Waal	Ophemert Sec. Channel	4	155687	427676	51,83768	5,39717

2.3 RESEARCH PERIOD

All fieldwork was conducted during the last week of July 2020. Sampling of fish stocks with traditional fishing techniques as well the collection of water samples for eDNA analysis were carried out on July 23th in the Waal near Ophemert in the Netherlands and on July 24th in the river Rhine near Walsum in Germany. Sampling of river lamprey larvae was carried out on July 28th in the Waal and on July 29th 2020 in river Rhine near Walsum.

3. RESULTS

3.1 SPECIES COMPOSITION AND ABUNDANCE

Table 5 shows the different fish species observed at the locations in the Waal and Niederrhein, based on the different sampling methods applied. Appendix 2 contains a list of fish species by their scientific name and their translations in English, German, Dutch and French.

The total number of fish species identified at the different sampling locations using the different methods is approx. 40 species. In some cases, based on eDNA metabarcoding, only genus or family could be determined with certainty. For example, based on eDNA, it is not possible to distinguish between the different members of the genus *Lampreta* or to distinguish between white bream (*Blicca bjoerkna*) from vimba (*Vimba vimba*).

Table 5 *Overview of fish species observed with different sampling methods in the different sampling locations.*

	Waal															Niederrhein															
	secondary channel								groyne field							secondary channel								groyne field							
	fishing				eDNA				fishing			eDNA				fishing				eDNA				fishing				eDNA			
Species	Electro (single anode)	Seine	Electro (strip anode)	PAS	Sylphium_meth1	Sylphium_meth2	Datura_meth1	Datura_meth2	Electro (single anode)	Seine	Electro (strip anode)	Sylphium_meth1	Sylphium_meth2	Datura_meth1	Datura_meth2	Electro (single anode)	Seine	Electro (strip anode)	PAS	Sylphium_meth1	Sylphium_meth2	Datura_meth1	Datura_meth2	Electro (single anode)	Seine	Electro (strip anode)	PAS	Sylphium_meth1	Sylphium_meth2	Datura_meth1	Datura_meth2
Abramis brama		X	X	X	X	X	X	X		X		X	X	X	X		X			X	X	X	X					X	X	X	X
Alburnus alburnus	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X		X	X		X		X	X
Anguilla anguilla	X		X		X	X	X	X	X			X	X	X	X	X		X		X	X	X	X	X		X	X	X	X	X	X
*Ballerus sapa**								X						X	X																
*Barbatula barbatula**																							X								
Barbus barbus		X		X	X	X	X	X				X	X	X	X	X	X		X	X	X	X	X			X	X	X		X	X
Blicca bjoerkna		X					X	X						X	X		X						X								X
Blicca bjoerkna / Vimba vimba					X																X							X		X	
*Carassius auratus / Carassius gibelio**														X																	
Chondrostoma nasus	X	X	X	X			X	X	X					X	X	X	X	X	X			X	X	X	X	X	X			X	X
Chelon sp.			X			X							X																		
*Coregonus sp.**								X																							
*Ctenopharyngodon idella**																												X			
*Cyprinus carpio**							X	X						X	X								X							X	X
*Esox lucius**							X	X						X																	
Gasterosteus aculeatus															X	X			X	X					X						X
*Gobio gobio**																															X
*Gymnocephalus cernua**					X		X															X	X								X
*Lampetra fluviatilis / Lampetra planeri**								X							X																
Leuciscus aspius	X	X	X	X			X	X	X	X				X	X	X	X	X	X			X	X	X	X	X	X				X
Leucaspius delineatus																							X								
Leuciscus idus	X	X	X	X			X	X	X	X	X			X	X	X	X	X	X			X	X			X	X			X	X
Leuciscus leuciscus		X	X	X	X	X					X	X	X	X		X	X	X	X	X	X	X	X			X	X	X		X	X
Liza aurata / Liza ramada																						X	X							X	X
Liza ramada							X	X						X	X																
Neogobius fluviatilis		X					X	X		X				X	X		X					X	X							X	X
Neogobius melanostomus	X	X	X	X			X	X	X					X	X	X	X	X	X			X	X	X	X	X	X			X	X
Perca fluviatilis	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X		X	X	X	X		X	X		X	X	X	X
*Petromyzon marinus**								X																							
Platichthys flesus				X		X	X	X	X			X		X	X																
Ponticola kessleri	X			X	X		X	X	X				X	X	X	X	X		X	X	X	X	X	X			X	X		X	X
*Rhodeus amarus**														X																	
Romanogobio belingi		X	X	X	X	X	X	X		X		X	X	X	X			X				X	X							X	X
Rutilus rutilus	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
*Salmo salar**														X																	X
*Salmo trutta**																					X										
Sander lucioperca		X	X		X	X	X	X	X			X	X	X	X		X			X	X	X	X		X	X		X	X	X	X
*Scardinius erythrophthalmus**								X							X							X	X							X	X
*Silurus glanis**					X	X	X	X					X	X	X							X	X					X		X	X
Squalius cephalus								X					X			X	X	X	X	X	X	X	X	X			X	X		X	X
Vimba vimba																									X	X					
Unknown 1 (Cottidae / Gobiidae)					X	X						X	X							X	X							X	X		
Unknown 2 (Gobiidae)					X							X								X								X			
Number of species	**9**	**14**	**13**	**12**	**15**	**12**	**21**	**26**	**9**	**6**	**5**	**12**	**13**	**25**	**23**	**13**	**15**	**11**	**10**	**13**	**12**	**20**	**24**	**7**	**9**	**12**	**10**	**15**	**4**	**20**	**25**

* species only observed based on eDNA

At least 15 species are only observed based on eDNA metabarcoding of water samples (highlighted and indicated with an asterisk). These include rare species like houting (*Coregonus* sp.), Atlantic salmon (*Salmo salar*), trout (*Salmo trutta*) and river (or brook) lamprey (*Lampreta sp.*), but also more general occurring species like pike (*Esox lucius*) and common rudd (*Scardinius erythrophthalmus*). Based on eDNA, common carp (*Cyprinus carpio*), ruffe (*Silurus glanis*) and common roach (*Scardinius erythropthalmus*) were detected at all of the sampling locations, whilst no specimens of these species were caught with the traditional fishing techniques.

Figure 16 shows the number of species/taxa found at the four different research sites with the different sampling methods used. The highest species abundances are found based on the results from eDNA metabarcoding of the 60 liter samples (method 2) by Datura. Average species abundance for the different locations based on this method is 24.5, varying from 23 species in the groyne field in the Waal to 26 species in the secondary channel in the Waal. The results from eDNA metabarcoding by Datura of the 1 liter samples (method 1) show lower species abundances. Average species abundance for the different sites based on this method is 21.5 species, varying from 20 species in both locations in the Niederrhein to 25 in the groyne field in the Waal.

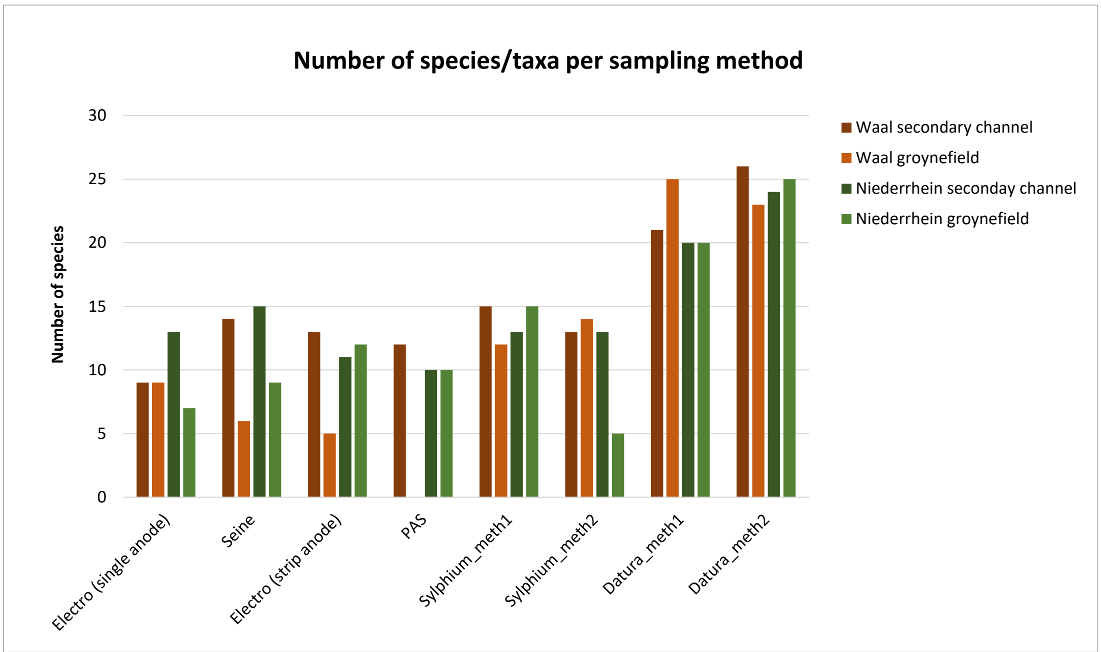

Figure 16 *Number of species/taxa found in different research locations with different research methods .*

The results based on the eDNA metabarcoding by Sylphium show significantly lower species abundances than the results provided by Datura. However, on average they are somewhat higher than the results based on the more traditional fishing methods. The average species abundance for the different sites based on method 1 by Sylphium is 13.8 and 11.3 based on method 2. Surprisingly, if we compare the results from all methods used, the eDNA metabarcoding by Sylphium also shows the lowest species abundance for a specific location. Based on the eDNA sample taken with Sylphium method 2, only 5 species were detected in the groyne field in the Niederrhein. In this case the method did not even detect some of the most commonly detected species like nase, asp and ide, that were observed with all of the traditional fishing methods.

Based on the averages for the different research locations, the more traditional fishing techniques all show lower species abundances than the results from eDNA metabarcoding. In some cases however, species abundance for a specific site is higher based on the results of the traditional techniques than based on the eDNA results provided by Sylphium. Comparison between the different fishing techniques shows highest average species abundance for seine net fishing (11 species) and lowest average species abundance for single anode electrofishing (9.5 species). However, the technique that shows highest species abundance differs between different locations. Strip anode electrofishing for example shows highest species abundance in the groyne field in the Niederrhein and single anode electrofishing shows highest species abundance in the groyne field in the Waal.

3.2 SPECIES ABUNDANCE AND ECOLOGICAL GUILDS

Figure 17 and Figure 18 show the number of fish species per ecological guild based on the results from the different fishing techniques used in the Waal and in the Niederrhein respectively. In case of the Waal, all methods detected a higher or equal (in case of single/one anode electro) number of species in the secondary channel than in the groyne field. PAS was only used in the secondary channel in the Waal and comparison with the groyne field was therefore not possible. In the Niederrhein, only in case of the strip anode the number of species is (just) higher in groyne field (12 species) than in the secondary channel (11 species).

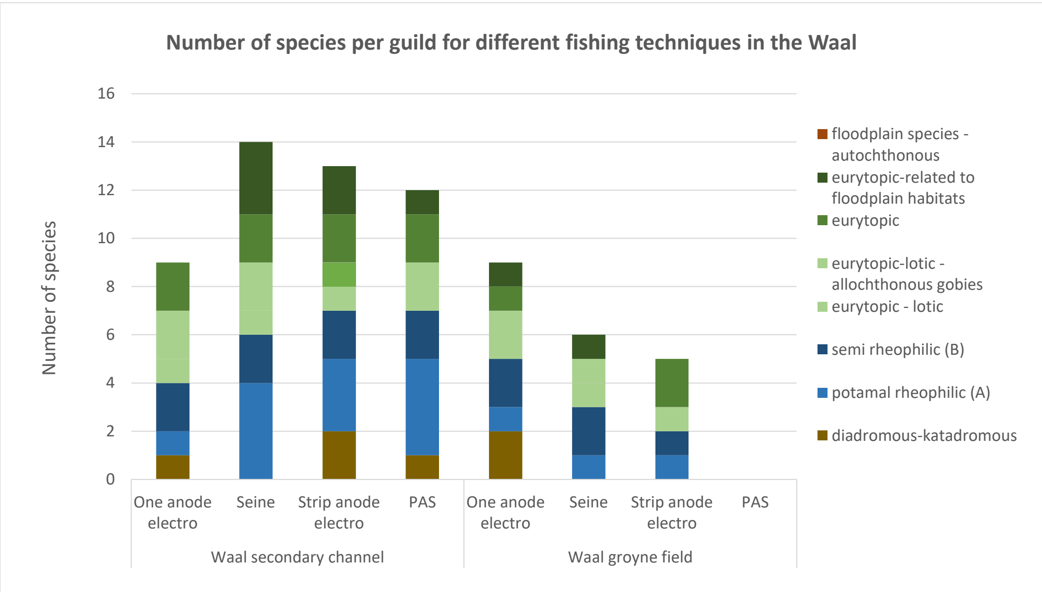

Figure 17 *Number of species per ecological guild with different sampling methods in two locations in the Waal .*

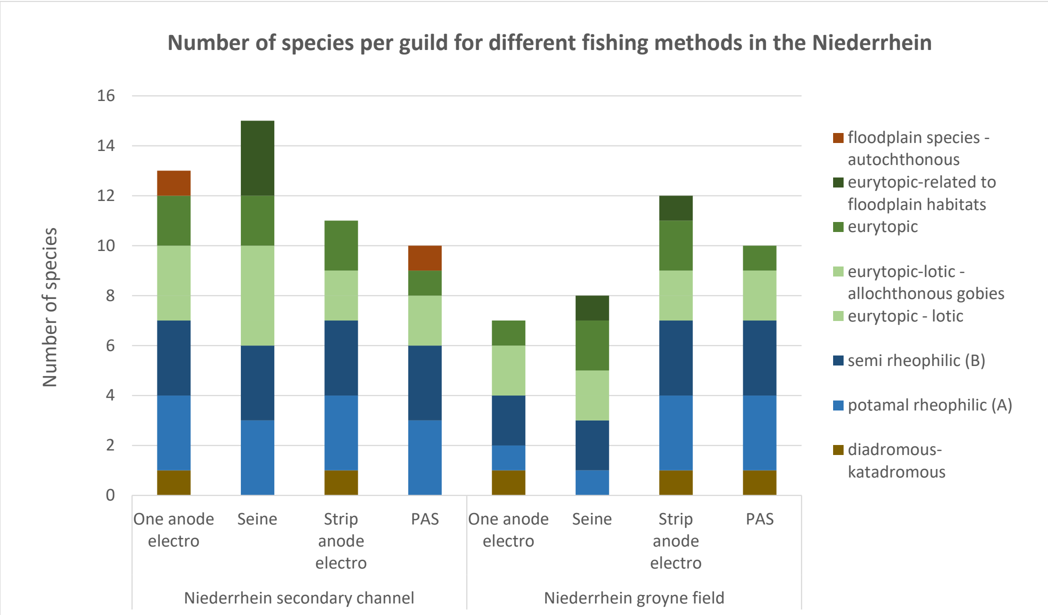

Figure 18 *Number of species per ecological guild with different sampling methods in two locations in the Niederrhein.*

Autochthonous floodplain species were only found in the secondary channel of the Niederrhein, using one anode electrofishing and PAS. Diadromous-catadromous species were caught at all four locations, but not with all methods, never with seine and always with an anode. In the secondary channel of the Waal one or two species of this ecological group were caught with three out of four methods (not with seine). In the groyne field of the Waal (two) diadromous-catadromous species (eel and flounder) were only caught using one anode electrofishing. In the secondary channel of the Niederrhein, diadromous-catadromous species were caught with two out of four methods (not with seine and PAS) and in the groyne field with three out of four methods (not with seine).

Expressed in number of species, eurytopic and rheophilic species make out the largest part of the fish community in all four locations and based on all four methods. In general, the number of eurytopic and the number of rheophilic species per location and method are reasonably comparable. In case of the Niederrhein the numbers of rheophilic A and rheophilic B species are also more or less balanced. In the Waal the number of rheophilic A species in the secondary channel is somewhat higher than the number of rheophilic B species (except for one anode electro), while the results from the groyne field show an opposite image.

Of the eurytopic species, one eurytopic lotic species (Round goby) was only found in the secondary channel of the Waal using strip anode electrofishing. In general, numbers of rheophilic species are higher in the Niederrhein and eurytopic species related to floodplains were found more frequently in the Waal.

3.3 CATCH PER UNIT EFFORT (CPUE)

In Table 5 the CPUE results from the different locations based on the different methods are expressed in number of fish per hectare. In Table 6 the relative CPUEs (%n/ha) for the different species and guilds are shown. Values are given for individual species as well as totals for the different ecological guilds (at the bottom of the tables). A '0' indicates a value less than 0,5 individuals per hectare or less than 0,5% of the total number of fish respectively. The color scale indicates lowest values in green and highest values in red.

The total number of fishes per hectare varies strongly between locations and methods. Lowest CPUE values are found in the groyne field in the Waal with 800 n/ha for strip anode electro and 810 n/ha for seine net. The results from monitoring with one anode electro in the secondary channel and groyne field in the Niederrhein show the highest values: 13,527 n/ha and 10,303 n/ha respectively. These numbers arise mainly from very high numbers of round goby (*Neogobius melanostomus*) caught with this particular method. This selectivity of one anode electro for round goby is also clear from the results in both locations in the Waal.

One anode electrofishing also seems to be more selective for eel (*Anguilla anguilla*) than the other methods. In all four locations total CPUE for this species were highest based on the results from this method. Based on relative CPUE, strip anode electro also seems to be effective for eel. At the same time this species was not caught with seine net and only very occasionally with PAS.

Compared to the other methods used, PAS seems to be more selective for rheophilic species like ide (*Leuciscus leuciscus*) and nase (*Chondrostoma nasus*). Based on the PAS results, semi rheophilic B is the dominant guild in all four research locations and potamal rheophilic A the second most important guild. Based on the other methods eurytopic-lotic or gobies are the most important guilds.

Table 6 *CPUE (number of fish per hectare) for different species and guilds in different research locations with different fishing methods. Color scale indicates relative proportion of total per column.*

	Waal								Niederrhein							
	secondary channel				groyne field				secondary channel				groyne field			
ecological guild / *Species name*	One anode Electro	Seine	strip anode Electro	PAS	One anode Electro	Seine	strip anode Electro	PAS [1)]	One anode Electro	Seine	strip anode Electro	PAS	One anode Electro	Seine	strip anode Electro	PAS
diadromous-katadromous																
Anguilla anguilla	476		137		167				776		157		1.697		300	34
Liza ramada			4													
Platichthys flesus				13	56											
potamal rheophilic (A)																
Barbus barbus		2		39					145	19		281			33	75
Chondrostoma nasus	95	37	47	2.782	278				3.927	1.510	33	1.470	242	18	200	444
Leuciscus leuciscus		2	3	24			33		218	19	14	140			167	151
Romanogobio belingi		248	10	98		24					5					
semi rheophilic (B)																
Leuciscus aspius	63	55	40	194	111	36			121	57	10	67	364	35	233	40
Leuciscus idus	127	168	147	3.821	1.222	24	433		73	104	38	1.275			200	535
Squalius cephalus									315	38	29	828	121			127
Vimba vimba														18	33	
eurytopic - lotic																
Alburnus alburnus	16	248	377			702	267		24	437	14			70	167	
eurytopic-lotic - allochthonous gobies																
Neogobius fluviatilis		5				12				5						
Neogobius melanostomus	825	28	43	314	556				5.358	484	524	1.275	5.939	70	733	370
Ponticola kessleri	63			69	222				412	19		58	121			122
eurytopic																
Perca fluviatilis	79	158	7	45			33		97	503	43			335	33	
Rutilus rutilus	667	491	337	1.539	778		33		1.939	1.771	105	1.361	1.818	158	367	633
eurytopic-related to floodplain habitats																
Abramis brama		78	10	29		12				28						
Blicca bjoerkna		2								9						
Sander lucioperca		41	13		56					43				88	33	
floodplain species -autochthonous																
Gasterosteus aculeatus									121			91				
Sum of guilds																
diadromous-katadromous	476	0	140	13	222	0	0		776	0	157	0	1.697	0	300	34
potamal rheophilic (A)	95	289	60	2.943	278	24	33		4.291	1.548	52	1.892	242	18	400	670
semi rheophilic (B)	190	223	187	4.015	1.333	60	433		509	199	76	2.169	485	53	467	701
eurytopic - lotic	16	248	377	0	0	702	267		24	437	14	0	0	70	167	0
eurytopic-lotic - allochthonous gobies	889	32	43	383	778	12	0		5.770	508	524	1.330	6.061	70	733	493
eurytopic	746	649	343	1.584	778	0	67		2.036	2.274	148	1.361	1.818	493	400	633
eurytopic-related to floodplain habitats	0	122	23	29	56	12	0		0	81	0	0	0	88	33	0
floodplain species -autochthonous	0	0	0	0	0	0	0		121	0	0	91	0	0	0	0
Total	2.413	1.562	1.173	8.966	3.444	810	800		13.527	5.046	971	6.843	10.303	792	2.500	2.531

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Table 7 *Relative abundance (% of number of fish per hectare) for different species and guilds in different research locations with different fishing methods. Color scale indicates relative proportion of total per column.*

	Waal								Niederrhein							
	secondary channel				groyne field				secondary channel				groyne field			
ecological guild / *Species name*	One anode Electro	Seine	strip anode Electro	PAS	One anode Electro	Seine	strip anode Electro	PAS [1)]	One anode Electro	Seine	strip anode Electro	PAS	One anode Electro	Seine	strip anode Electro	PAS
diadromous-katadromous																
Anguilla anguilla	20		12		5				6		16		16		12	3
Liza ramada			0													
Platichthys flesus				0	2											
potamal rheophilic (A)																
Barbus barbus		0		1					1	0		4			1	3
Chondrostoma nasus	4	2	4	30	8				29	30	3	23	2	2	8	17
Leuciscus leuciscus		0	0	0			33		2	0	2	2			7	6
Romanogobio belingi		16	1	1		3					1					
semi rheophilic (B)																
Leuciscus aspius	3	4	3	2	3	4			1	1	1	1	4	4	9	2
Leuciscus idus	5	11	13	45	35	3	433		1	2	4	20			8	23
Squalius cephalus									2	1	3	12	1			5
Vimba vimba														2	1	
eurytopic - lotic																
Alburnus alburnus	1	16	32			87	267		0	9	2			9	7	
eurytopic-lotic - allochthonous gobies																
Neogobius fluviatilis		0				1				0						
Neogobius melanostomus	34	2	4	3	16				40	10	54	16	58	9	29	13
Ponticola kessleri	3			1	6				3	0		1	1			5
eurytopic																
Perca fluviatilis	3	10	1	1			33		1	10	4			42	1	
Rutilus rutilus	28	31	29	17	23		33		14	35	11	19	18	20	15	23
eurytopic-related to floodplain habitats																
Abramis brama		5	1	29		1				1						
Blicca bjoerkna		0								0						
Sander lucioperca		3	1		2					1				11	1	
floodplain species -autochthonous																
Gasterosteus aculeatus									1			1				
Sum of guilds																
diadromous-katadromous	20	0	12	0	6	0	0		6	0	16	0	16	0	12	3
potamal rheophilic (A)	4	18	5	32	8	3	33		32	31	5	29	2	2	16	27
semi rheophilic (B)	8	14	16	47	39	7	433		4	4	8	33	5	7	19	30
eurytopic - lotic	1	16	32	0	0	87	267		0	9	2	0	0	9	7	0
eurytopic-lotic - allochthonous gobies	37	2	4	3	23	1	0		43	10	54	17	59	9	29	17
eurytopic	31	42	29	17	23	0	67		15	45	15	19	18	62	16	23
eurytopic-related to floodplain habitats	0	8	2	0	2	1	0		0	2	0	0	0	11	1	0
floodplain species -autochthonous	0	0	0	0	0	0	0		1	0	0	1	0	0	0	0
Total	**100**	**100**	**100**	**100**	**100**	**100**	**800**		**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

3.4 RELATIVE ABUNDANCE OF ECOLOGICAL GUILDS

In Figure 19 and Figure 20 the relative abundance (% of total number of fishes) of the different ecological guilds based on different fishing methods is shown for the Waal and the Niederrhein respectively.

Again from these graphs it becomes clear that different methods seem to be more or less effective for specific species and/or guilds. In all of three of the locations where PAS was applied, the majority (57% or more) of the fish caught belong to one of the two rheophilic guilds. In case of the seine, 65% or more of all fish belong to the eurytopic species. The seine is also the only method that did not catch any diadromous-catadromous fish species. Fish species from this guild (mainly eel) are more frequently caught with one anode electro and strip anode electro and only incidentally with PAS.

Rheophilic A and rheophilic B species were caught in all locations and with all different methods. The relative abundance of these two specific guilds however differs between locations and methods. In general, the relative abundance of potamal rheophilic A in relation to semi rheophilic B seems to be higher in the Niederrhein than in the Waal.

In general the relative abundance of eurytopic lotic species (*Alburnus alburnus*) seems to be higher in the Waal than in the Niederrhein and the relative abundance of eurytopic lotic allochthonous gobies seems to be higher in the Niederrhein than in the Waal.

The only autochthonous floodplain species caught is the three-spined stickleback (*Gasterosteus aculeatus*). This fish was only caught in the secondary channel of the Niederrhein, both with one anode electro and PAS.

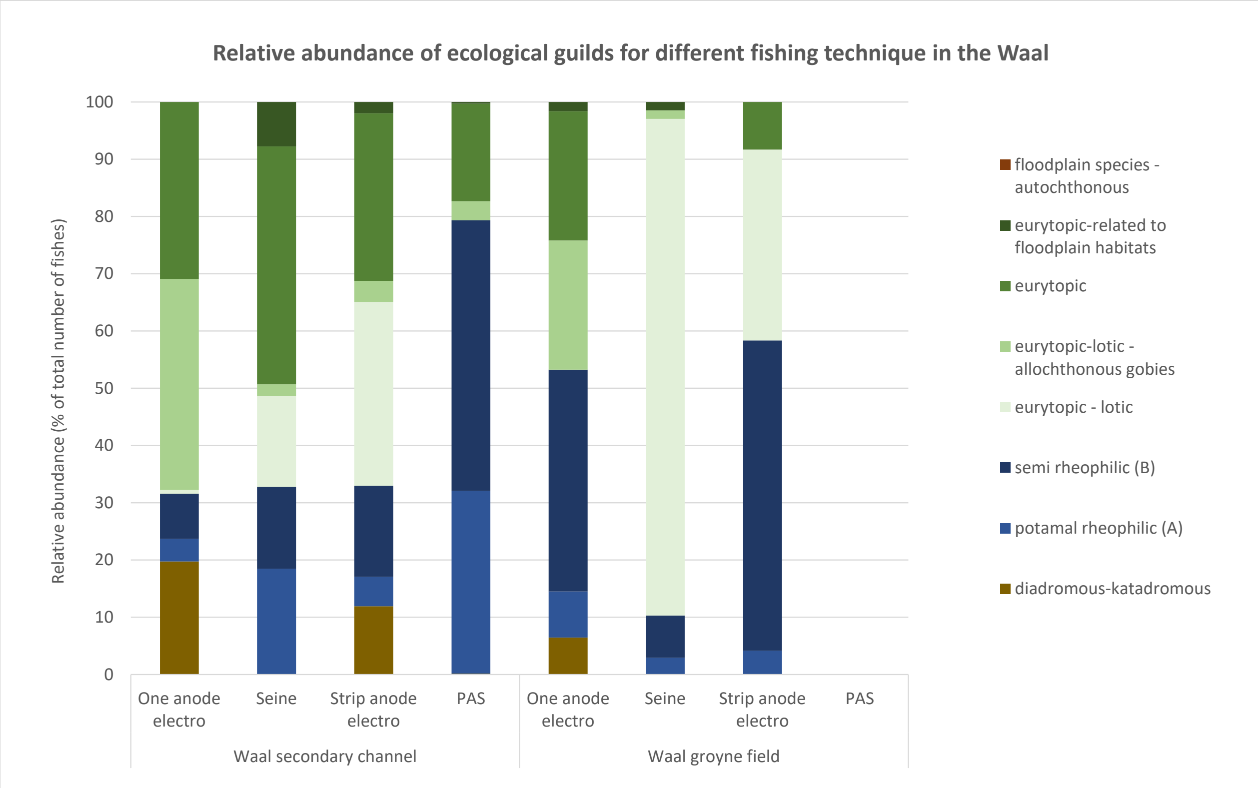


Figure 19 *Relative abundance of ecological guilds in the Waal based on different fishing methods. The PAS method was not performed in the Waal groyne field.*

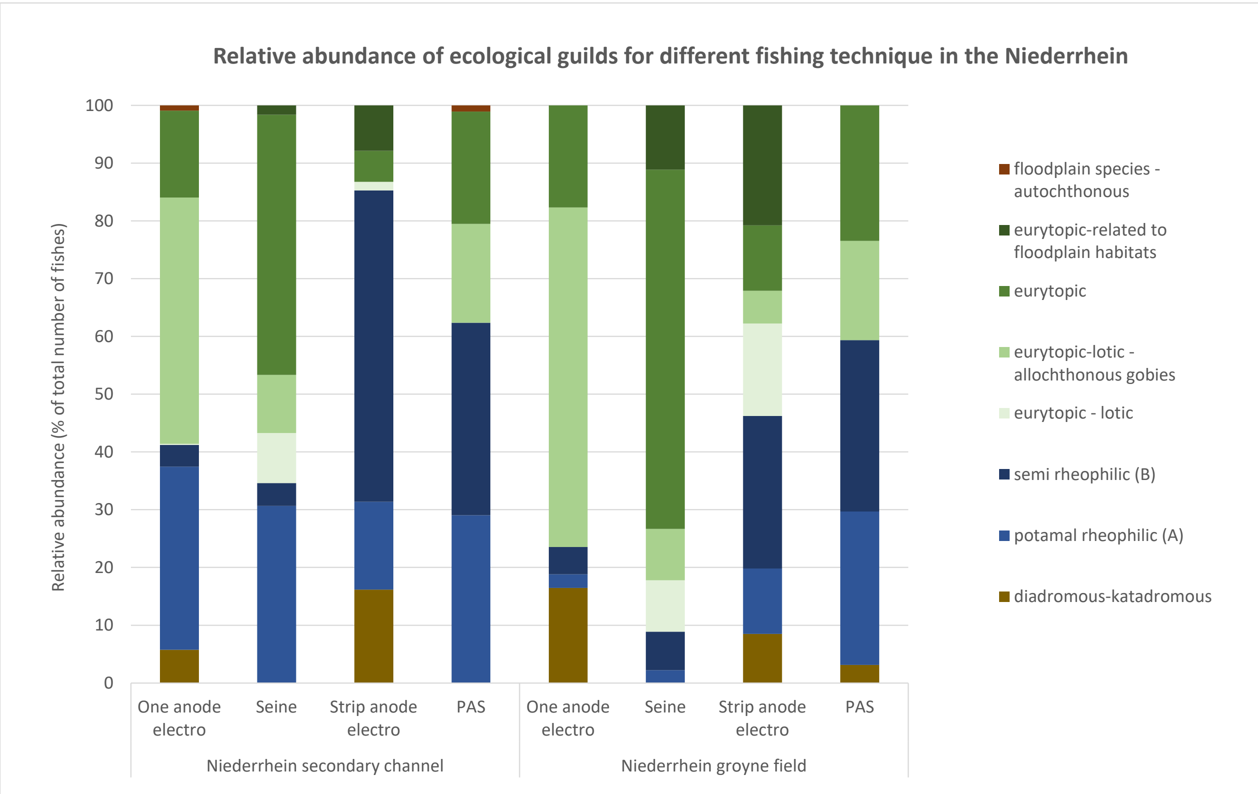
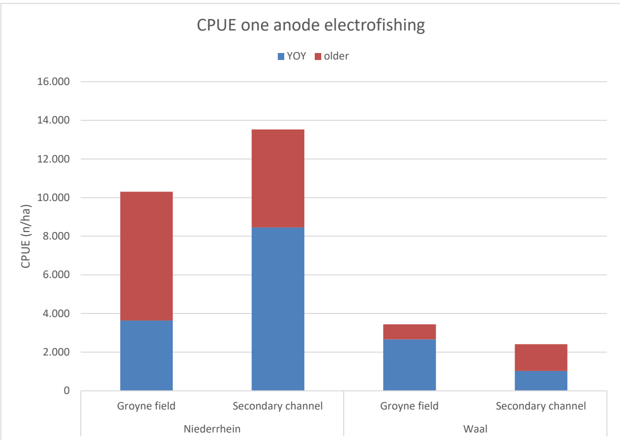


Figure 20 *Relative abundance of ecological guilds in the Niederrhein based on different fishing methods.*

3.5 AGE GROUPS

Figure 21 shows the results in CPUE (n/ha) for two different age groups (Young of the Year and older) at different locations based on the different methods used. In general, CPUE is higher for one anode electro fishing and PAS compared to strip electro and seine net.

With regard to the ratio between YOY and older fish, the most striking difference between the different methods is the fact that in case of PAS the great majority of all the fish caught are YOY and only a very small portion of the fish caught with this method is older. In the secondary channels, seine net fishing also seems to result in a relative high proportion of YOY compared to the one anode and strip anode electrofishing. This is not so for the groyne field. Overall, strip anode seems to result in the highest proportions of older fish. Both one anode and strip anode electric fishing are more effective for larger fish, (older fish) because of the difference in voltage.



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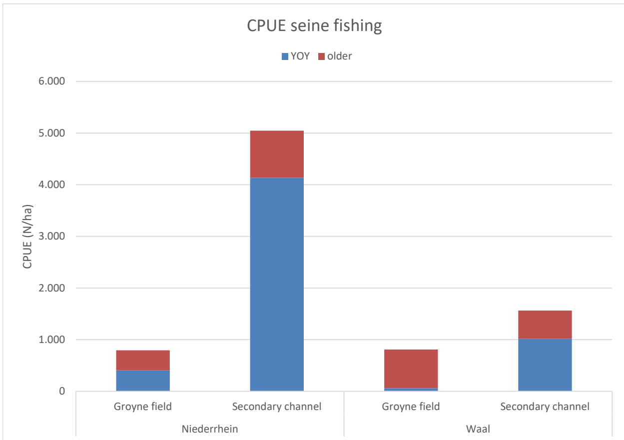

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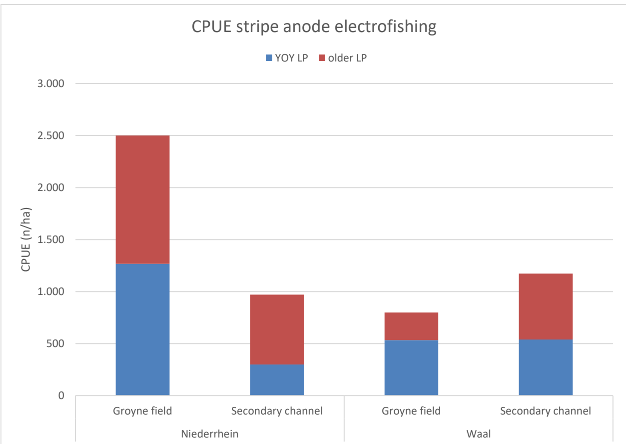

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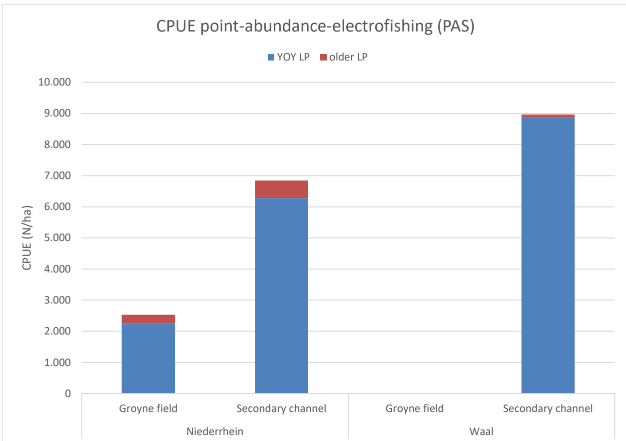

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Figure 21 *CPUE (n/ha) for two different age groups at different locations based on the different methods used.*

3.6 RHEOPHILIC YOUNG OF THE YEAR

In Figure 23 and Figure 22 the number of rheophilic YOY fish caught with different fishing methods is shown for the Waal and for the Niederrhein respectively.

Highest CPUE (approximately 7,000 individuals per hectare) is found with PAS in the secondary channel in the Waal. Other methods used in this site show much lower numbers. Seine and strip anode show the lowest CPUE for rheophilic YOY.

In the Waal, in the secondary channel as well as in the groyne field, ide (*Leuciscus idus*) is the most abundant rheophilic YOY species for all methods used. Second most abundant is nase (*Chondrostoma nasus*). Based on PAS, ide and nase make up for more than 95% of all individuals in the secondary channel of the Waal. Asp (*Leuciscus aspius*) and dace (*Leuciscus leuciscus*) were also observed, but in much smaller numbers. In the Niederrhein the overall diversity of rheophilic YOY is bigger than in the Waal. YOY chub (*Squalius cephalus*) were only caught in the Niederrhein, and the same goes for barb (*Barbus barbus*) with one exception.

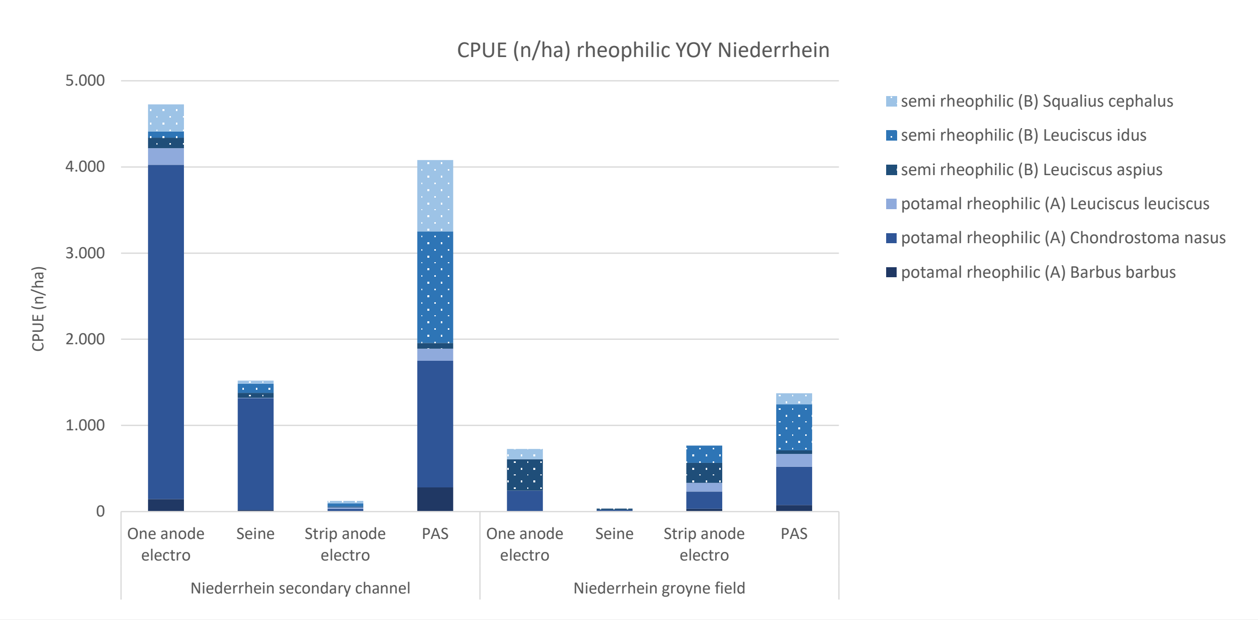
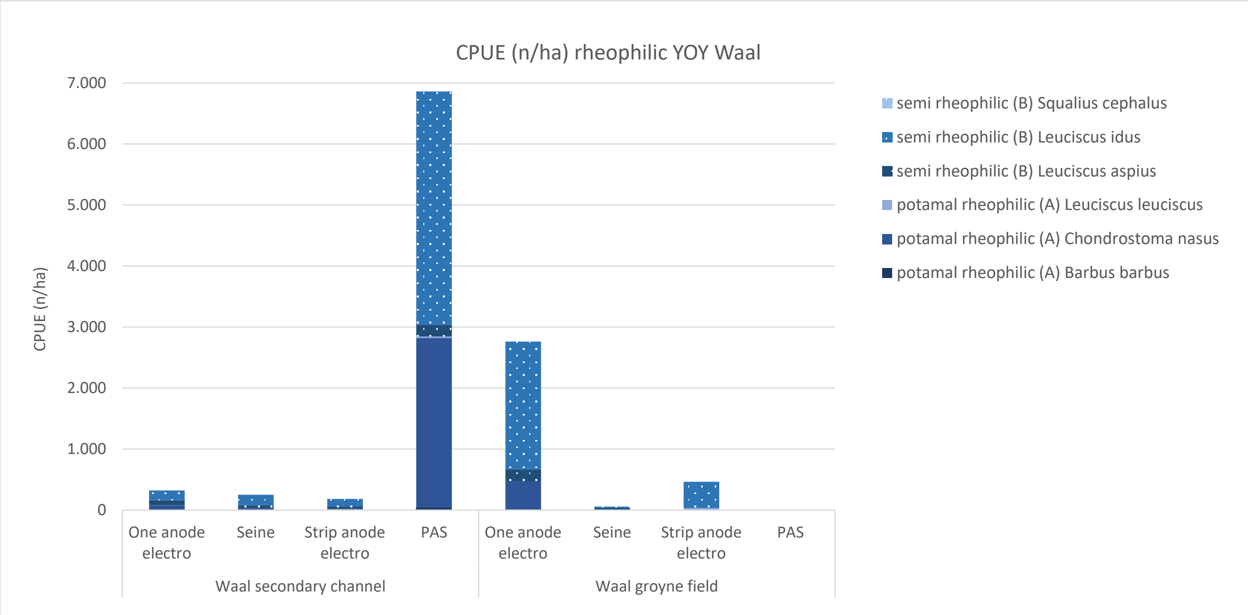


Figure 22 CPUE (n/ha) of rheophilic Young of the Year caught with different methods in the Niederrhein.



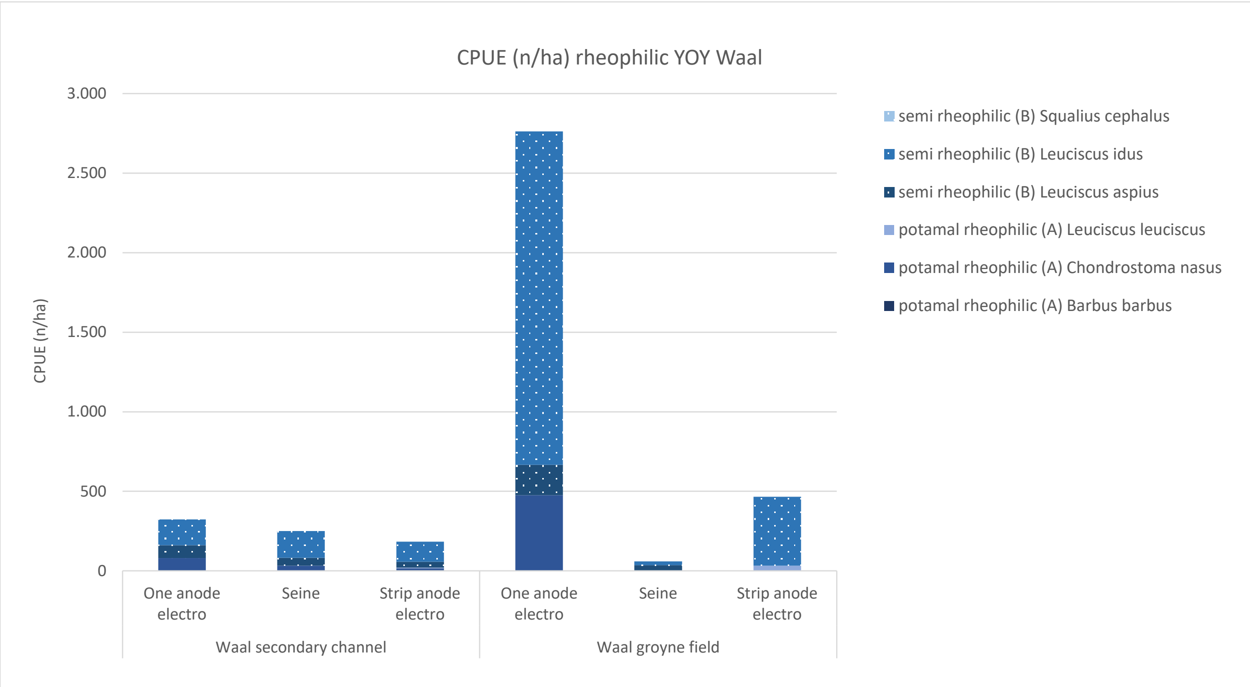


Figure 23 *CPUE (n/ha) of rheophilic Young of the Year caught with different methods in the Waal.*

3.7 LAMPREY LARVAE SAMPLING

In this paragraph the results from the lamprey larvae sampling with the venturi sediment dredger are presented.

3.7.1 SUBSTRATES

The substrate composition in the shore channel at Ophemert consists for the most part of fine gravel, coarse gravel and sand. Pebbles, clay, detritus or dead shell material have also been found at some locations. In the shore channel in the Niederrhein near Walsum, the substrate composition mainly consists of pebbles, coarse gravel, fine gravel and sand, and detritus has occasionally been found. Dead shell material, i.e., Corbicula shells does occur at most of the locations.

Table 8 *Substrate composition at sampling locations in the river Waal near Ophemert.*

		Presence of material (%)										
Location	Depth (m's)	Armour rock	Pebbles	Course grave	Fine gravel	Sand	Silt	Clay	Wood/Leafs	Detritus	Algae weeds	Dead shells
292	3	-	-	10	-	90	-	-	-	-	-	-
293	2,5	-	-	20	40	40	-	-	-	-	-	-
294	3	-	-	-	30	60	-	-	-	-	-	10
295	3,5	-	-	10	40	40	-	-	-	-	-	10
296	4	-	-	-	50	50	-	-	-	-	-	-
298	2,5	-	-	-	40	40	-	10	-	-	-	10
299	2	-	-	-	50	40	-	-	-	-	-	10
301	4	-	-	40	40	10	-	-	-	-	-	10
302	4	-	-	-	100	-	-	-	-	-	-	-
303	2	-	-	10	90	-	-	-	-	-	-	-
304	3	-	-	-	100	-	-	-	-	-	-	-
305	2,5	-	-	40	-	-	-	40	-	10	-	10
306	2	80	20	-	-	-	-	-	-	-	-	-
307	3	-	-	-	80	10	-	10	-	-	-	-
309	2	-	-	-	80	-	-	-	-	20	-	-
310	3	30	-	-	40	-	-	-	-	30	-	-
311	3	-	-	50	50	-	-	-	-	-	-	-
312	2,5	-	-	50	40	10	-	-	-	-	-	-
313	2,5	-	-	50	50	-	-	-	-	-	-	-
314	1	-	-	50	-	-	-	50	-	-	-	-
315	1,5	-	-	50	50	-	-	-	-	-	-	-
316	2	-	-	50	50	-	-	-	-	-	-	-
317	2	-	-	50	50	-	-	-	-	-	-	-

Table 9 *Substrate composition at sampling locations in the Niederrhein near Walsum.*

		Presence of material (%)										
Location	Depth (m's)	Armour rock	Pebbles	Course grave	Fine gravel	Sand	Silt	Clay	Wood/Leafs	Detritus	Algae weeds	Dead shells
322	1,5	-	30	30	35	-	-	-	-	-	-	5
323	1	-	40	40	-	-	-	-	-	-	-	20
324	2	-	60	20	10	-	-	-	-	-	-	10
325	1	-	30	-	40	-	-	-	-	-	-	30
326	1	-	30	-	40	-	-	-	-	-	-	30
327	1,5	-	-	-	50	50	-	-	-	-	-	-
328	1	-	20	-	30	-	-	-	-	10	-	40
329	1,5	-	10	10	60	-	-	-	-	10	-	10
330	0,75	-	40	40	-	-	-	-	-	-	-	20
331	1	-	40	40	-	-	-	-	-	-	-	20
332	0,8	-	40	50	-	-	-	-	-	-	-	10
333	0,5	-	5	40	40	-	-	-	-	-	-	15
334	0,75	-	-	-	10	90	-	-	-	-	-	-
335	1,5	-	50	30	20	-	-	-	-	-	-	-
336	1	-	10	-	90	-	-	-	-	-	-	-
337	1	-	30	20	40	-	-	-	-	-	-	10
338	2,5	-	20	20	60	-	-	-	-	-	-	-
339	1	-	40	20	20	-	-	-	-	-	-	20
340	1,5	-	10	40	40	-	-	-	-	-	-	10
341	2	-	-	-	90	-	-	-	-	-	-	10
342	2,5	-	20	-	60	-	-	-	-	-	-	20
343	4	-	20	30	40	-	-	-	-	-	-	10
344	3,5	-	-	-	75	-	-	-	-	20	-	5

3.7.2 FAUNA

In Table 10 and Table 11 the results from the sampling with the venturi sediment dredger are shown for the Waal and the Niederrhein respectively. In none of the locations lamprey larvae were found. In the Waal, mollusks (*Corbicula fluminea*) and Amphipods (G*ammaridae*) were found in low densities. Mitten crabs (*Eriocheir sinensis*) have been observed at two locations. Considerably more fauna was observed in the Niederrhein than was in the Waal. *Corbicula fluminea* occurs in about half of the sampled locations and in higher densities than in the Waal. In addition, *Gammaridae* and mitten crab are also more common in the Niederrhein. Additionally, gobies have also been caught in some locations in the Niederrhein. Finally, spring moss was found in two places.

Table 10 Macro invertebrates and crabs at different sampling locations in the river Waal near Ophemert.

Location	*Gomphus flavipes*	Lamprey larvae	Bivalvia	Macro fauna	Other
292	-	-	6 Corbicula fluminea	-	-
293	-	-	5 Corbicula fluminea	-	-
294	-	-	3 Corbicula fluminea	-	-
295	-	-	3 Corbicula fluminea	-	-
296	-	-	-	-	-
298	-	-	1 Corbicula fluminea	-	-
299	-	-	-	-	-
301	-	-	-	3 chironomidae	-
302	-	-	-	-	-
303	-	-	1 Corbicula fluminea	-	-
304	-	-	1 Corbicula fluminea	-	-
305	-	-	1 Corbicula fluminea	>10 Gammaridae	-
306	-	-	-	>10 Gammaridae	-
307	-	-	1 Corbicula fluminea	Gammaridae 1, white worm	-
309	-	-	1 Corbicula fluminea	1 chironomidae	1 Chinese mitten crab
310	-	-	-	-	-
311	-	-	-	-	-
312	-	-	-	1 chironomidae	-
313	-	-	-	1 chironomidae	2 Chinese mitten crab
314	-	-	-	-	-
315	-	-	-	-	-
316	-	-	-	1 chironomidae	-
317	-	-	-	>10 Gammaridae	-

Table 11 Macro invertebrates, crabs and fish at different sampling locations in the Niederrhein near Walsum.

Location	*Gomphus flavipes*	Lamprey larvae	Bivalvia	Macro fauna	Other
322	-	-	-	4 Gammaridae	1 Chinese mitten crab
323	-	-	-	5 Gammaridae	-
324	-	-	>30 Corbicula fluminea	>100 Gammaridae	-
325	-	-	>20 Corbicula fluminea	>20 Gammaridae, 1. worm,	-
326	-	-	6 Corbicula fluminea	>10 Gammaridae, 2 worm,	1 Chinese mitten crab
327	-	-	-	>10 Gammaridae, 1 worm	-
328	-	-	> 20 Corbicula flumineas	> Gammaridae	1 round goby
329	-	-	-	3 Gammaridae, 3 worm, 1 chironomidae	-
330	-	-	>15 Corbicula fluminea	5 chiro, 3 worm	-
331	-	-	>30 Corbicula fluminea, 1 painters mussle	>20 Gammaridae	-
332	-	-	4 Corbicula fluminea	>10 Gammaridae, 2 chiro	-
333	-	-	-	>10 Gammaridae, 1 chiro	1 kesslers goby, 1 round goby
334	-	-	-	-	-
335	-	-	>10 Corbicula fluminea	>10 Gammaridae, 1 worm, 1 chironomidae	-
336	-	-	-	>10 Gammaridae, 1 worm, 1 chironomidae	-
337	-	-	2 Corbicula fluminea	>10 Gammaridae, 1 chironomidae	1 Round goby, Fontinalis antipyretica
338	-	-	-	1 worm	Fontinalis antipyretica
339	-	-	-	>10 Gammaridae, 2 chironomidae	-
340	-	-	-	-	-
341	-	-	>100 Corbicula fluminea	>20 Gammaridae	-
342	-	-	>100 Corbicula fluminea	>50 Gammaridae	-
343	-	-	-	>10 Gammaridae	-
344	-	-	-	>10 Gammaridae	-

4. DISCUSSION & CONCLUSIONS

4.1 GENERAL COMMENTS

In this study, data on the fish communities in different types of habitat in the Waal and Niederrhein were collected using different sampling techniques applied by researchers from the Netherlands and Germany. Despite of the different methods applied, coordination between the Dutch and German team and execution of the fieldwork were carried out without problems. The only exception to this was a miscommunication that led to the fact that the groyne field in the Waal was not sampled with the PAS method. More importantly however, the joint monitoring created a unique opportunity for both partners to demonstrate the different monitoring techniques and interchange experiences and information.

4.2 COMPARISON OF METHODS

4.2.1 SEINE NET AND ELECTRO FISHING

Method specific selectivity

When comparing the results of the different fishing techniques, various method-specific aspects must be taken into account, which have a significant influence on the results.

Basically, the two methods used as standard by the two teams should complement each other with their different selectivities and together provide as complete a picture of the fish community as possible. Boat-based electrofishing with single anode (method 1 ATKB) allows efficient fishing of discrete structural elements and thus provides good catch results for fish that reside in the immediate vicinity of cover structures. However, catch success is lower for fish that reside in open water and have greater escape distances. In contrast, due to the magnification of the electric field, boat-based electrofishing with the strip anode (method 1 LP) is more efficient for catching fish in open water with greater escape distances and for larger aggregations of fish (schools). Since the electric field is not interrupted (as in case of single anode electrofishing when the net is taken out of the water to land the fish), larger aggregations of fish can be held in the electric field and detached. However, the boom design of the strip anode limits the maneuverability of the boat and thus impairs efficient fishing of discrete structural elements.

The seine net used (method 2 ATKB) also generally fishes areas farther from shore and deeper than electrofishing conducted close to shore. The results of this method thus represent fish occurrences in a different habitat or staging area. Due to the comparatively large area enclosed by the seine net, fish with a greater escape distance are also efficiently caught. However, the applicability of the method remains limited to obstacle-free substrates; structurally rich and stony substrates cannot be sampled in this way. Due to the relative importance of the substrate factor for fish habitat selection, this results in selectivity of the capture method.

Point-abundance sampling by electrofishing (method 2 LP) is conducted wading by default in juvenile fish monitoring (as this allows for more controlled and efficient shearing off of narcotized fish), so its use is limited to relatively shallow (fordable) areas, usually near shore (note: in principle, pas can also be conducted from a boat and thus in deeper open water areas, but since these areas are of secondary importance to most juvenile fish and accurate area reference is easily lost when fishing from a boat, this is not practiced in standard juvenile fish monitoring). Because the PAS is used explicitly for juvenile fish monitoring, the fact that larger (adult) fish are rarely caught because of the restriction to shallow water areas near the shore and because of the greater escape distances is not relevant. The great advantage of PAS is the structure of resulting data (the result for a sample area consists of numerous small (point) samples that can be analyzed with statistical methods) and the particularly accurate area reference.

Fish Abundance and size of the total catch

The highest catch numbers and total abundances (sum of all species) of all methods used were obtained by electrofishing with single anode with a peak value of approx. 13,530 individuals/ha (in the secondary channel Walsum in the Niederrhein) and an average of approx. 7,420 individuals/ha over all 4 sampled habitats. In three of four sampled habitats, electrofishing with anode nets yielded the highest total abundances in each case (Figure 24).

In contrast, the relatively similar method of electrofishing with strip anode yielded the lowest value of all methods used, averaging about 1,360 individuals/ha across all 4 sampled habitats (maximum 2,500 individuals/ha in the groyne field in the Niederrhein). In three of the four sampled habitats, electrofishing with strip anode yielded the lowest total abundances (Figure 24).

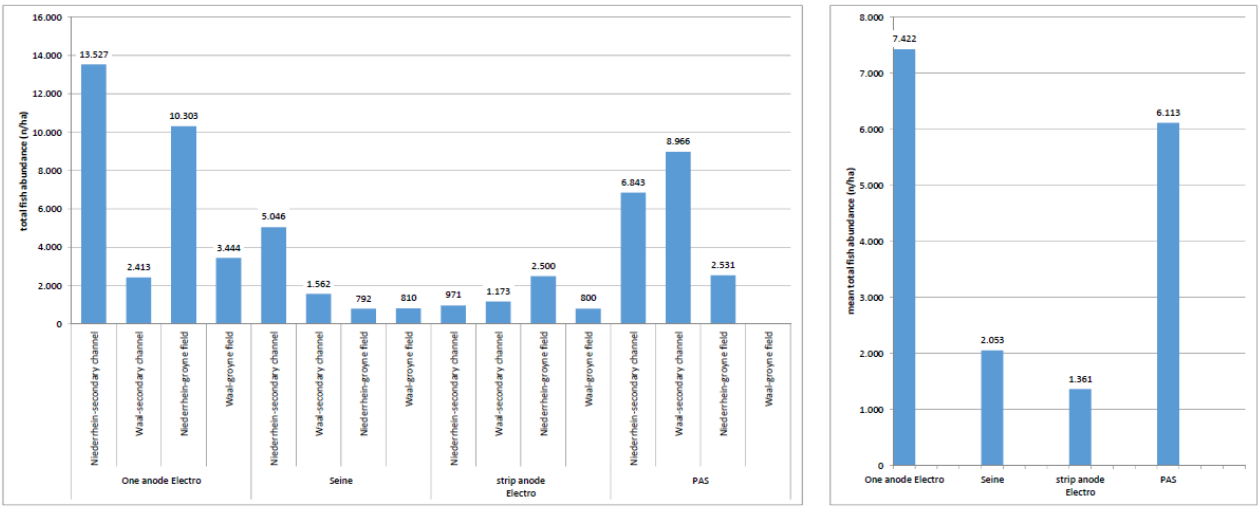


Figure 24 *Total and mean fish abundance (n/ha) based on different fishing techniques.*

These large differences between the relatively similar methods can (at least partly) be explained by the qualitative composition of the catches. The total catch in electrofishing with single anode consisted mainly of gobies, while only few specimen of this species were caught with strip anode. In case of strip anode the total catch consisted mainly of juveniles of rheophilic cyprinids (however, in much lower densities than in case of PAS) (Figure 25). Another possible factor is contained in the different formula and assumptions used for the different methods to correct for the sampled surface area. It is however difficult to indicate precisely how these differences influence the results in relation to the different methods.

The second highest abundances with an average over all 4 sampled habitats of about 6,110 ind./ha and a peak of about 8,970 ind./ha (in the secondary channel in the Waal) were obtained with the PAS. The total catch of this method was comparable to that of electrofishing with single anode, but it represented completely different fish occurrences. PAS captured juveniles of rheophilic cyprinids in high abundance (Figure 25).

With the seine net, with an average over all 4 sampled habitats of approx. 2,050 fish/ha and a single, outstanding peak value of approx. 5,050 fish/ha (in the secondary channel Walsum in the Niederrhein), similarly low abundance values were determined as with the electrofishing with strip anode. The qualitative composition of the seine net catches in the different habitats seems to show a much greater variability than with the other methods, here random events such as the capture of larger shoals of certain species seem to have a greater influence (Figure 25).

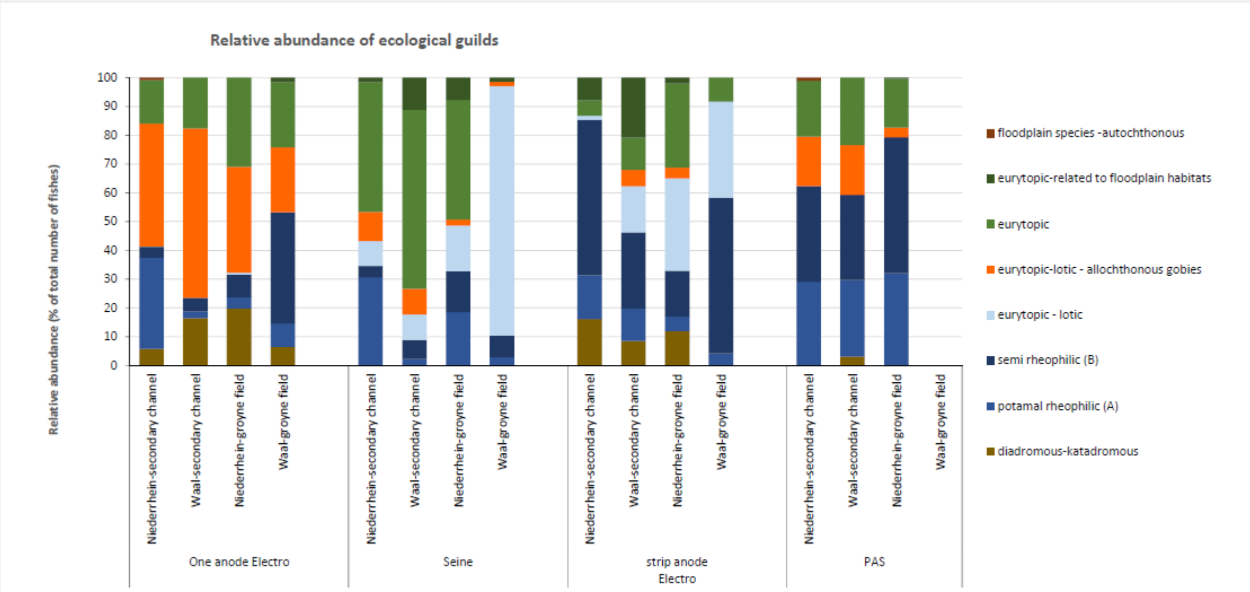


Figure 25 *Relative fish abundance (%n/ha) in different river sections based on different fishing techniques.*

Overall, the four different fishing methods result in highly variable fish stock densities in each of the four habitats sampled, with ratios of minimum and maximum abundances ranging from 1:4 to 1:14.This ratio was significantly higher in the two Niederrhein study areas than in the two Waal study areas (Table 12). These large differences are primarily the result of the fact that each of the techniques is used to sample a specific type of habitat, with its unique fish assemblages and densities. Therefore a real comparison between the techniques is not really possible.

Table 12 Minimum and maximum fish densities in different river sections based on different sampling techniques.

sampling area	minimum (n/ha)	maximum (n/ha)	ratio
Niederrhein – secondary channel	971 (strip anode)	13,527 (one anode)	1 : 13.9
Waal – secondary channel	1.173 (strip anode)	8,966 (PAS)	1 : 7.6
Niederrhein – groyne field	792 (seine)	10,303 (one anode)	1: 13.0
Waal – groyne field	800 (strip anode)	3,444 (one anode)	1 : 4.3

Number of species

Regarding the recorded species numbers, the differences between the fishing methods are relatively small. On average over the 4 sampled study areas, the recorded species numbers per method vary between a minimum of 9.5 (±2.5) species by electrofishing with anode nets and a maximum of 10.8 (±4.4) species by seine fishing. In two locations (secondary channel in the Niederrhein and secondary channel in the Waal), the highest species numbers were caught with seine net fishing. In one location (groyne field in the Niederrhein) the highest species numbers were detected by electrofishing with strip anode and in one case (groyne field in the Waal) the highest species numbers were caught with electrofishing with single anode (Figure 26 and Figure 27).

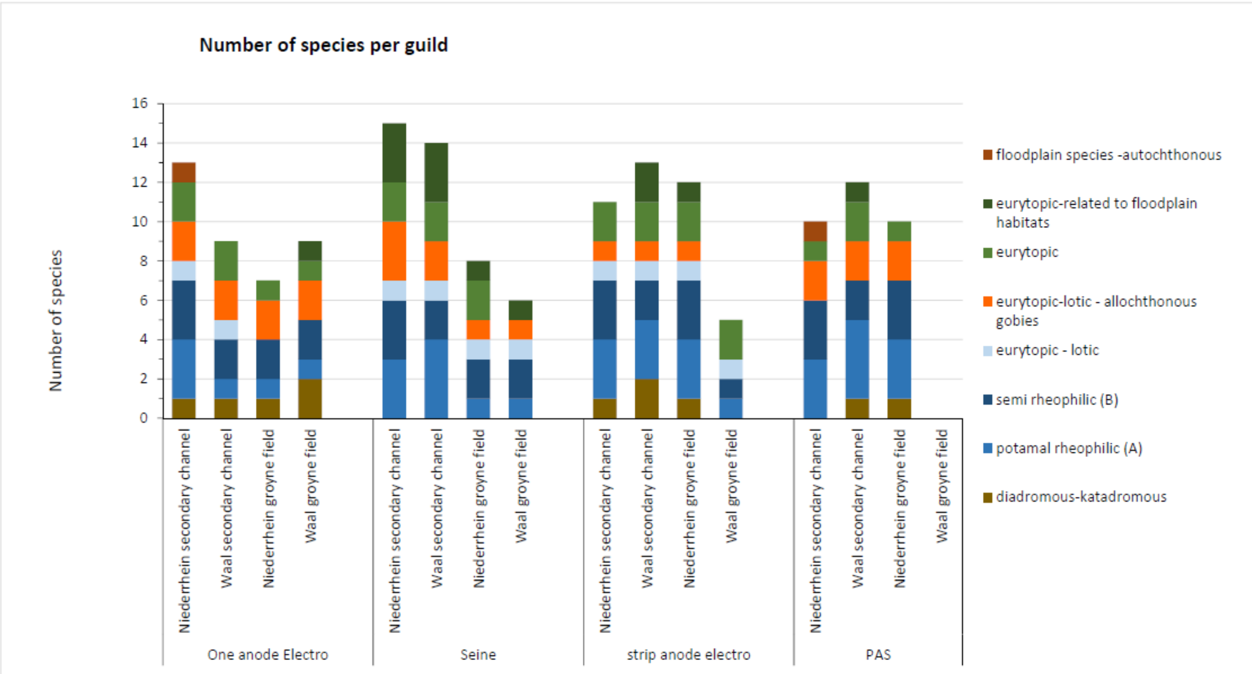


Figure 26 Number of fish species per guild on the different locations based on different fishing techniques.

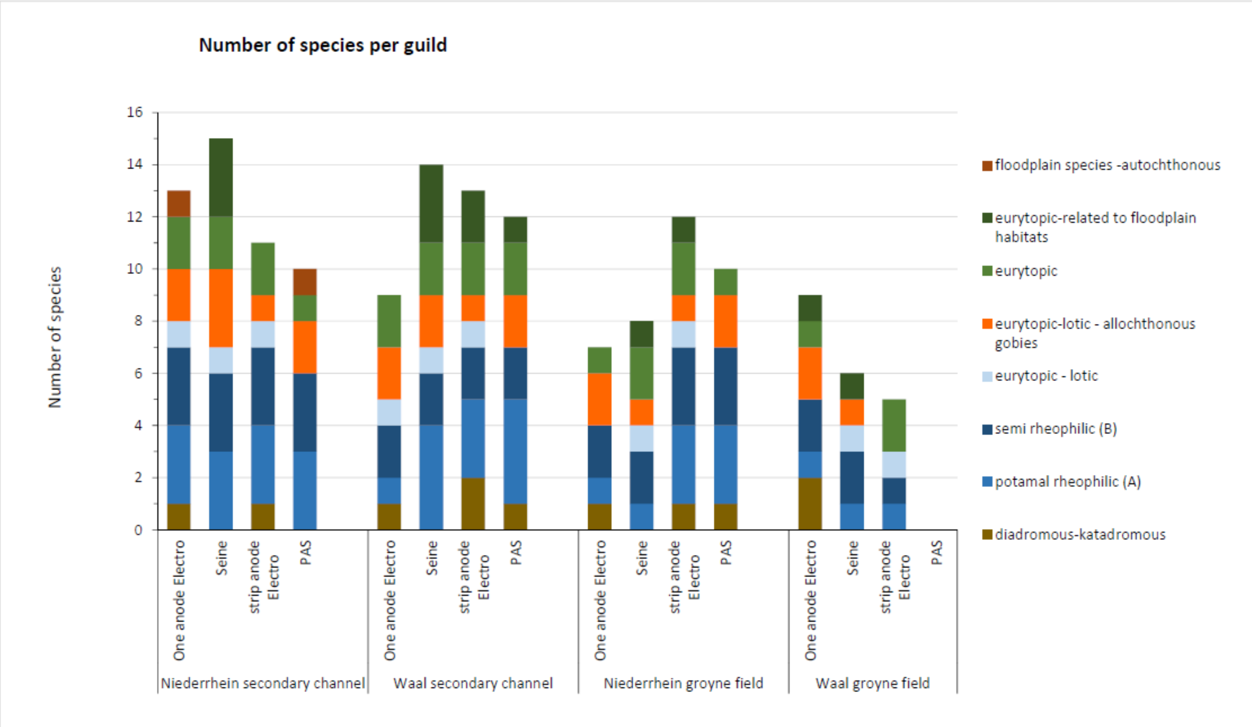


Figure 27 Number of fish species per guild based on different fishing techniques on different locations. N.B. Same data as Figure 26, but in another order.

Qualitative aspects - dominance structure and guild or species abundances

The composition of the total catch of the fishing methods show striking similarities despite a certain variability across the 4 study areas in each case. Thus, the total catches of the boat-based electrofishing with single anode are mainly characterized by a high proportion of allochthonous gobies as well as a relevant proportion of catadromous species (mainly eel). The total catches of boat-based electrofishing with strip anode similarly show a relevant proportion of catadromous species, but are primarily dominated by semi-rheophilic species, with a high proportion of eurytop-lotic species (bleak) also shaping the pattern. In contrast, the total catches of the PAS are dominated by very high proportions of potamal-rheophilous and semi-rheophilous species. Overall, the total catches of the seine net fisheries show less similarity in composition than the other methods; here, random events such as the capture of schools of certain species seem to play a major role. However, the pattern is also characterized by the absence of catadromous species (eel) and low proportions of allochthonous gobies (Figure 28). This is explained by the absence of these species in the specific habitat sampled (the open water habitat with smooth bottom surface), rather than by the technical characteristics of this technique.

The guild structure of the total catches of the different methods in a study area each show much lower similarities than the structure of the total catches of a method in the different study areas. The results are obviously shaped much more by the selectivities of the method than by actual differences in fish population and species abundance in the different study areas (Figure 28 and Figure 29).

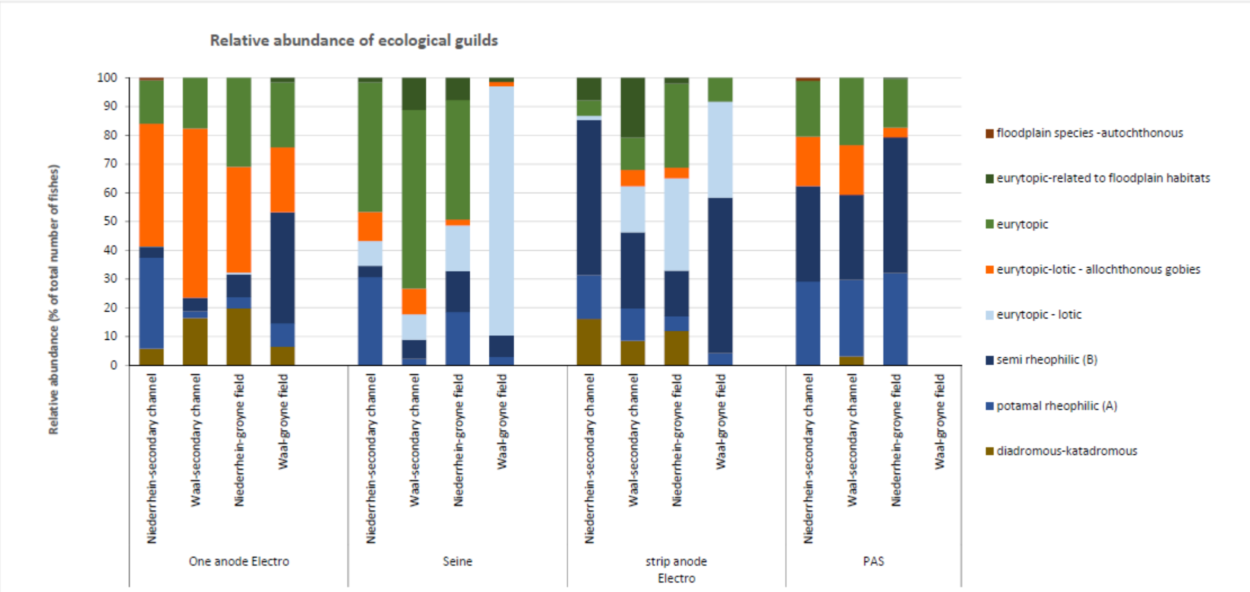


Figure 28 *Relative abundance of fish species per guild on different locations based on different fishing techniques.*

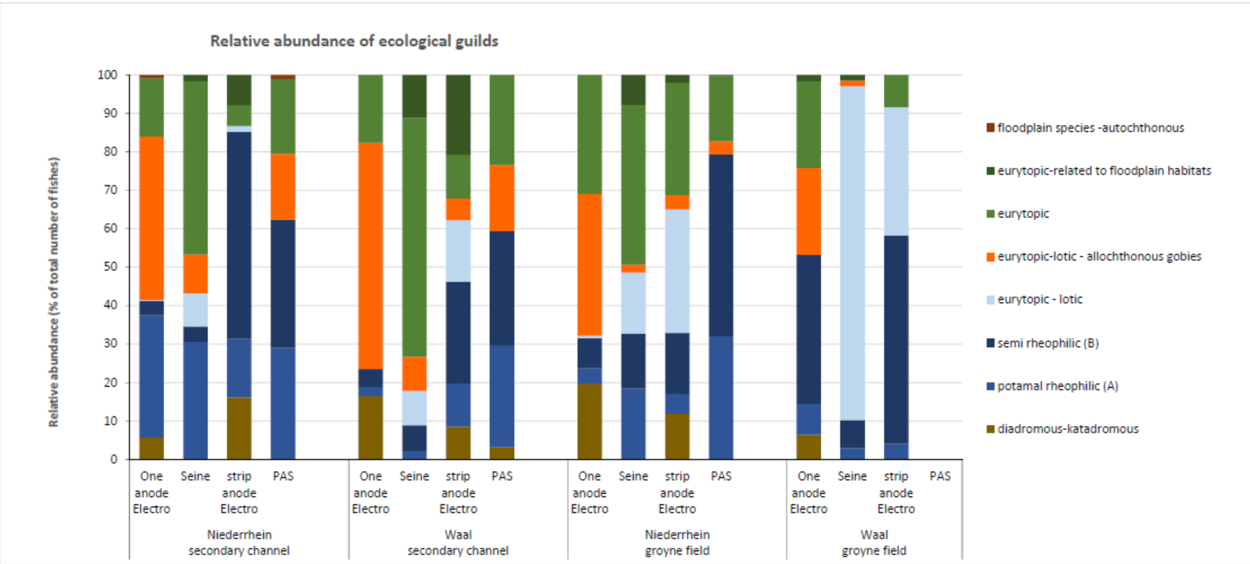


Figure 29 *Relative abundance of fish species per guild based on different fishing techniques on different locations. N.B. Same data as figure 28, but in another order.*

Abundance of juveniles of rheophilic species

The highest abundances of juvenile fish (YOY) of rheophilic species were detected by PAS fishing in the secondary channels in the Niederrhein and Waal (Figure 30). In addition, high abundances were detected by electrofishing with anode nets in the side channel in the Lower Rhine (second highest value = approx. 4,730 individuals/ha) and in the groyne field in the Waal (fourth highest value = approx. 2,760 individuals/ha). Only low abundances of juveniles (YOY) of rheophilic species were detected with the methods of seine net and electrofishing with strip anode (with one exception: an abundance of about 1,520 ind./ha was detected with seine net in the side channel in the Niederrhein, which deviated from the other results of this method). Based on the CPUE, seine net fishing and electrofishing with strip anode show the lowest numbers of (rheophilic) juvenile fish and (Figure 30).

The large differences in CPUE between the different methods are explained by the fact that fact that PAS and One anode electrofishing are used to sample the shallow shore zone, where the majority of the rheophilic YOY reside. Seine net fishing and strip anode on the other hand also sample the areas further from shore (open water), where densities of these fish are much lower. Therefore, the densities expressed in n/ha based on PAS and one anode electrofishing are probably an overestimation of the real situation when translated to the total surface area of the side channel. This makes a it clear that to get a good image of the overall fish assemblage in a certain part of the river, it is necessary to use the different complementary techniques (and to sample the different habitats in the same ratio as they occur in the area under research).

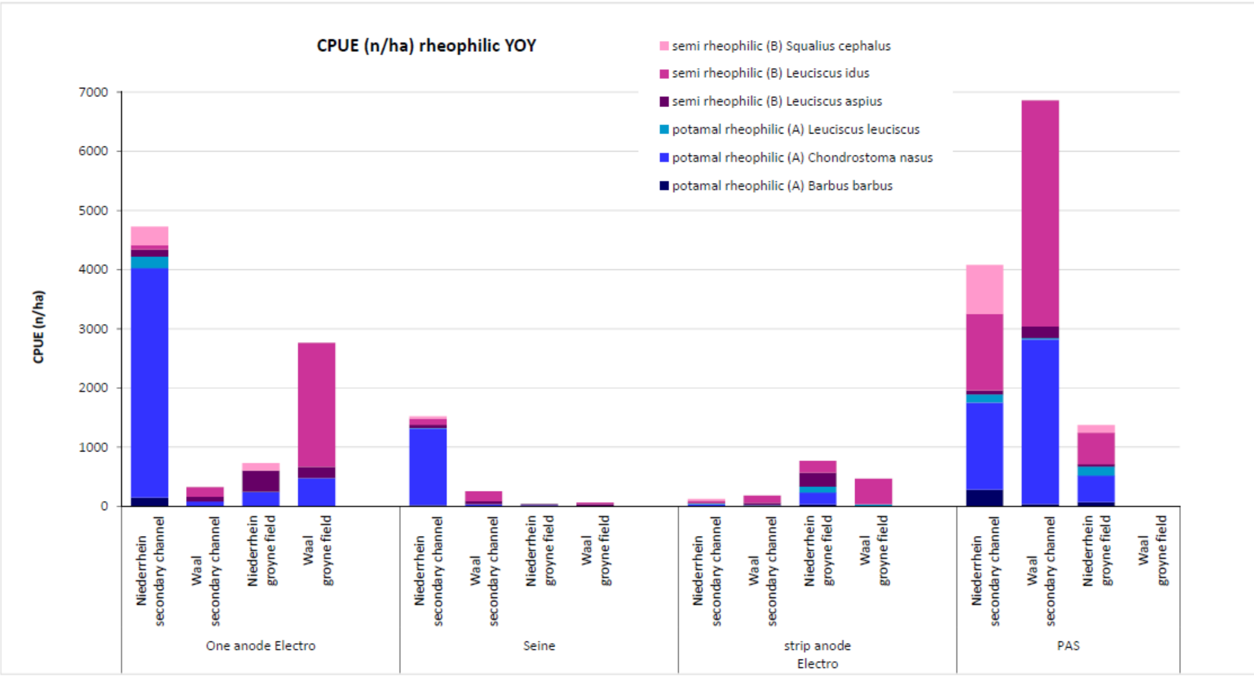


Figure 30   *Number of fish species per guild on different locations based on different fishing techniques.*

4.2.2 EDNA METABARCODING AND LAMPREY LARVAE SAMPLING

Comparison of the results from the different monitoring techniques shows that eDNA metabarcoding results in a higher species diversity than the more traditional fishing techniques for all locations monitored (especially the results based on the method e& analysis by Datura). The other way around, all of the species found with the more traditional fishing techniques were also detected with at least one of the eDNA methods used. The eDNA metabarcoding technique is very sensitive as only a very small amount of eDNA is needed to detect a species. A clear example is the detection of river (or brook) lamprey (*Lampreta sp.*) based on eDNA sampling, while this species was not caught with the venturi sediment dredger (despite of a significant effort). Although a relatively large number of samples per unit of time can be taken using the venturi sediment dredger, the total area sampled remains relatively small. The absence of observations of this species with the venturi sediment dredger may indicate that the species, if present, is only present in low densities. Dorenbosch et al. (2020) did find river lamprey larvae and larvae of river clubtail (*Gomphus flavipes*) in the side channel near Ophemert.

The eDNA technique seems to be a very suitable method to indicate the presence or absence of relatively rare species or species that are difficult to catch with the more traditional techniques. At the same time, there is a clear downside to the use of eDNA, especially in riverine habitats, as eDNA from locations further upstream (and even side streams) can also be detected, as the eDNA of a fish may remain present in the water for several hours or even days[2]. Therefore, in a river the results do not give any certainty whether or not a species that is detected is actually present in a particular location. This makes the technique less suitable for measuring the effects of interventions on fish populations in specific locations or river sections. In addition, the results from eDNA metabarcoding do not provide reliable quantitative information on the numbers or biomass of fishes present or on the age structure of a population (see also Paragraph 7.3.1).

4.3 COMPARISON OF SAMPLING LOCATIONS / RIVER SITES

Due to the methodological variability described above, it is difficult to make reliable statements on actual, real differences of essential characteristics of fish stock composition and abundances in the two river sections (Niederrhein and Waal) and the two habitat types (side channel and groyne field in the main stream).

4.3.1 COMPARISON OF MAIN CHANNEL AND SIDE CHANNEL HABITATS

Comparison of side channels and groyne fields in the main stream

Regardless of the location in Waal or Niederrhein, relatively clear differences between the side channels and the groyne fields in the main stream can be seen. Averaged over the 4 different fishing methods, the number of species in the side channels (12.3 in the Niederrhein and 12.0 species in the Waal) was, significantly higher than in the groyne fields (9.3 in the Niederrhein and 6.7 species in the Waal). It is also evident that the total abundance (population density) in the side channel was significantly higher than in the neighboring groyne field in the main stream. The ratio of population densities (mean of the 4 different trapping methods) in groyne field to side channel was 1:1.6 in the Niederrhein and 1:2.1 in the Waal.
The highest total abundance of about 6,600 n/ha was recorded in the secondary channel in the Niederrhein. With 3,528 n/ha, the third highest abundance was found in the secondary channel in the Waal, only slightly lower than in the groyne field of the Niederrhein (4,032 n/ha). However, with PAS the highest total abundance of juveniles of rheophilic species was documented here (Figure 31).

[2] https://www.environmental-dna.nl/

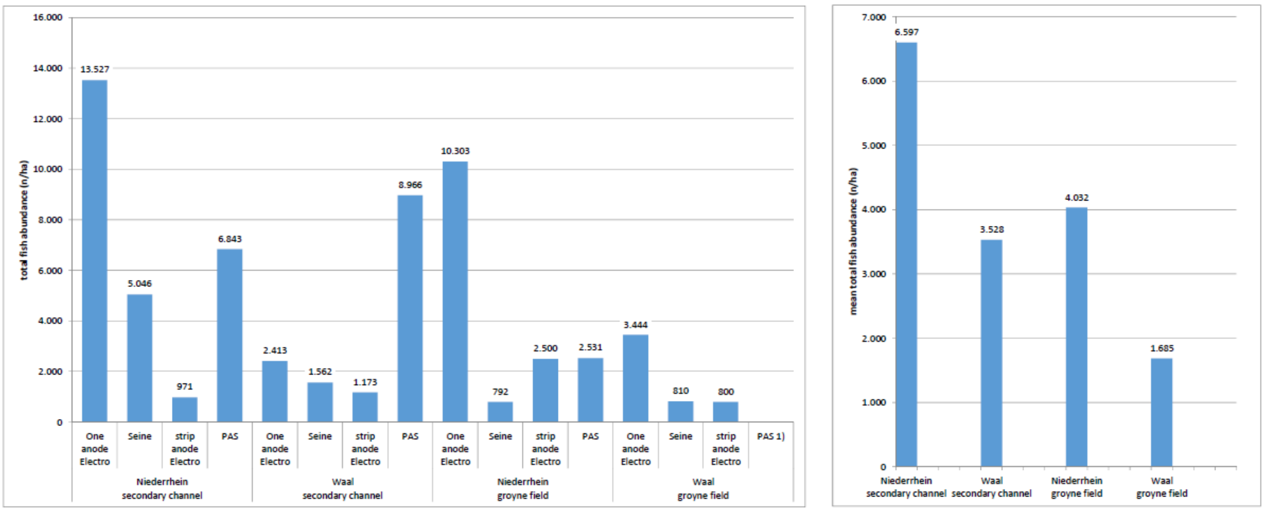


Figure 31 *Total fish abundance at different locations. Left: results per method. Right: Average abundance.*

The results clearly document a special importance of the side channels as fish habitat and the reproduction of rheophilic species by higher total abundances and especially by significantly higher abundances of juveniles of rheophilic species (Figure 32).

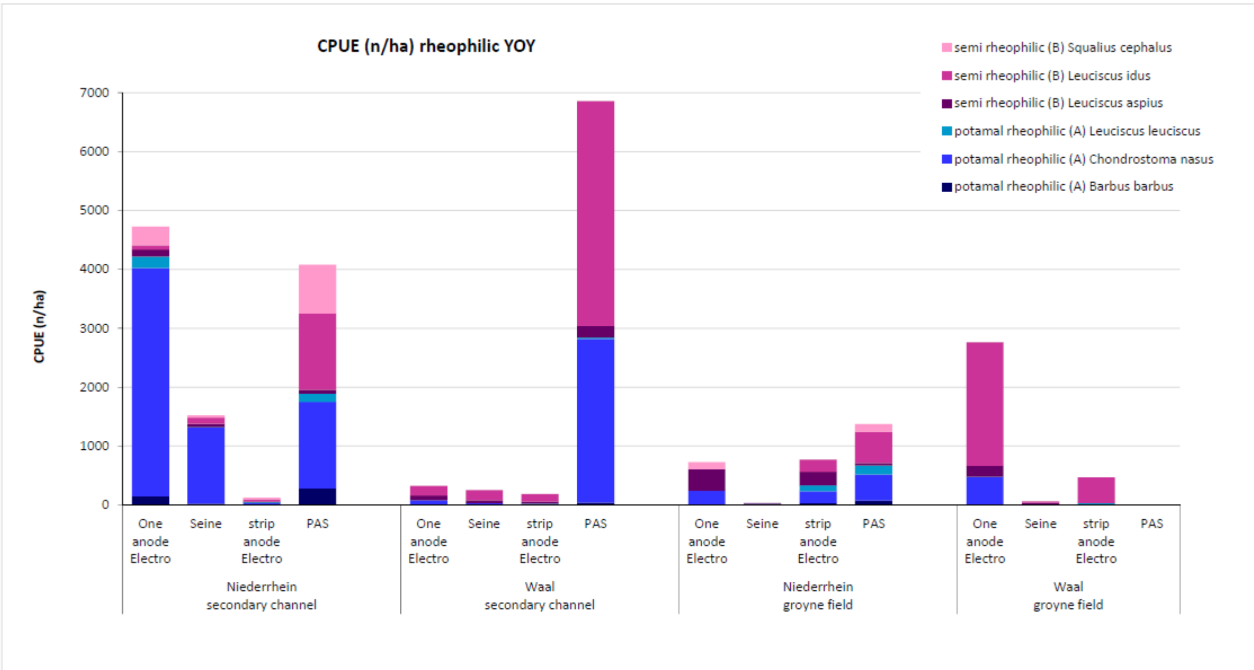


Figure 32 *CPUE of rheophilic YOY per guild at different locations based on different fishing techniques.*

4.3.2 COMPARISON OF WAAL AND NIEDERRHEIN RIVER SECTIONS

In the side channel as well as in the groyne field, the total abundances (as the mean of the four fishing methods) were higher in the Niederrhein than in the Waal (Figure 31). This suggests that fish abundances tend to be higher in the more upstream German section of the Niederrhein than in the more downstream section of the Waal. Although both sections can be classified as metapotamal sections of the Rhine, the

German Niederrhein tends to be characterized by higher flow velocity and especially by higher proportions of coarse substrates (gravel, pebbles). Both factors are of great importance for the occurrence and reproduction of rheophilic and gravel-spawning species. This zonation could well explain a corresponding gradient in fish population densities.

However, specifically through the methodology of the PAS, by far the highest abundances of juvenile rheophilic cyprinids of all habitats studied were found in the side channel in the Waal, with abundances of both the potamal-rheophilic (rheophilic A-) species nase and the semi-rheophilic (rheophilic B-) species ide significantly higher than documented with this methodology in the side channel in the Niederrhein (Figure 32).

Basically, the sample size with one examined river section each is too small to make reliable statements about possible gradients in the fish population, the results could also be influenced by non-representative, local characteristics. Nevertheless, the finding of particularly high juvenile fish densities in the side channel in the Waal, despite less suitable habitat conditions of the downstream section, could be explained by drift or migration of larvae and juveniles from the upstream river sections, which tend to have better reproductive conditions for rheophilic-lithophilic species, which then aggregate in suitable habitats further downstream.

However, due to the small number of samples, no firm conclusions can be drawn from the present study regarding possible quantitative and qualitative differences in fish colonization.

The results of the study program 2020 have to be considered against the background of the general situation and current developments in the River Rhine's fauna. For example, other studies (e.g. Scharbert et al. 2021) show that the study year 2020 was characterized by a mass occurrence of nase fry, which occurred almost everywhere and in all habitat types with extraordinarily high abundances, as is only recorded irregularly in certain years (Scharbert et al. 2019). Under the climatic and hydrological conditions of 2020, the nase (a potamal-rheophilic or rheophilic-A species) apparently had exceptionally good reproductive success in the Rhine. In addition, similarly high juvenile abundances were recorded for the ide (a semi-rheophilic or rheophilic B species).

4.4 CONCLUSIONS

The study offered a unique opportunity to demonstrate the different sampling techniques applied by the German and Dutch partners and to interchange valuable information and experiences. Although the extend of the study was limited, comparison of the results obtained from the different techniques made it possible to draw some general conclusions about the specific possibilities and limitations of the different techniques and about their major differences. In addition, the results from the different techniques also showed some striking similarities that made it possible to draw conclusions on the characteristics of the fish communities in the different locations.

4.4.1 CONCLUSIONS REGARDING THE SAMPLING TECHNIQUES

- Clear differences were observed between the different sampling techniques used. These differences are largely explained by the fact that each of the technique is used to sample a specific type of habitat with its specific fish assemblage and densities (of species and age classes). This emphasize the importance to use complementary techniques to get a good image of the fish assemblage in a river section with different types of habitat.
- Based on the results from the sampling of two river sections in the Lower Rhine in July 2020, single anode electrofishing from a boat showed the highest CPUE of the four different sampling techniques applied. This technique is especially apt for fishing relatively shallow locations rich in structure, like vegetation, groynes and wood structures. Fish of all sizes can be caught, although the technique is less effective in case of very small fish (fry). Compared to the other methods applied, single anode electrofishing shows high numbers of round goby and European eel (mainly as a result of the particular habitat sampled with this method).
- Strip anode electrofishing is apt for catching fish in shore zones and open water. Because there is no interruption of the electrical field, fishes with greater escape distances and larger aggregations of fish can be caught more effectively than with single anode electrofishing. However, because of the boom, the maneuverability is limited. This technique is not very effective in case of smaller fish. Shows lowest CPUE of all techniques applied.
- Seine net fishing is applied to fish in open water. Like strip anode electrofishing this technique is effective in catching fishes with greater escape distances and large aggregations. However, the technique requires a relative smooth bottom surface. Bottom dwelling species like European eel and flounder were not caught with seine net fishing . Although the seine net applied was adapted to also catch smaller fish, CPUE was much lower than in case of single anode electrofishing and PAS. This is partly explained by the fact that fish densities in open water are smaller than in the shore zone.
- PAS as applied in this study (wading) is apt for fishing in shallow shore zone. This technique shows second highest CPUE of all techniques applied. The technique is especially effective for catching small fish (YOY), larger fish are only caught occasionally. The largest advantage of this technique is that it offers for statistical analysis of the data, due to the many points that are fished in a standardized way.
- CPUE between different techniques shows high variations for the same location. Highest CPUE are found with Single anode electrofishing and PAS. This is mainly explained by the fact that both techniques are very effective in catching smaller fish in a relative narrow strip in the shore zone where these fish are concentrated.
- The guild structure based on the total catches in a study area between the different methods show less similarities than the structure of the total catches of a method in the different study areas. The results are obviously shaped much more by the selectivity of the methods than by actual differences in fish population and species abundance in the different study areas.
- No specimens of river lamprey were caught with the venturi sediment dredger. However, the species was detected based on the eDNA samples taken. In order to detect the presence of rare species, eDNA seems to be more effective than other techniques. However, eDNA does not provide information on density, age or exact location of the species.

4.4.2 CONCLUSIONS REGARDING FISH COMMUNITIES AND SAMPLE LOCATIONS

- With the different sampling techniques combined, (a minimum of) 40 fish species were observed in the different locations in the Waal and the Niederrhein; 15 species were "observed" exclusively based on eDNA samples.
- Most of the fish caught in the different locations belong to the rheophilic and eurytopic guilds.
- Based on the results from this study (average of different techniques), rheophilic A make up the largest part of the juvenile rheophilic fish community in the Niederrhein. In the Waal more (semi)rheophilic B were caught than rheophilic A.
- Most abundant rheophilic species in both locations were ide and nase. In addition, asp and dace were also caught in the Waal and the Niederrhein. Barb and chub were only caught in the Niederrhein.
- The results clearly document a special importance of the side channels as fish habitat and as a nursery habitat for juvenile rheophilic fish. In the Waal as well as in the Niederrhein, species diversity was higher in secondary channel compared to the main channel (groyne field). Moreover, the side channels show higher total abundances (population density) and significantly higher abundances of juvenile rheophilic fishes.
- The highest total abundance was observed in the secondary channel in the Niederrhein. However, specifically through the methodology of the PAS, by far the highest abundances of juvenile rheophilic cyprinids were found in the side channel in the Waal, with abundances of both the rheophilic A species nase and the rheophilic B species ide significantly higher than documented with this methodology in the side channel in the Niederrhein.

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APPENDIX I: ECOLOGICAL GUILDS

Name	**Scientific**	**ecolgical grouping * (Scharbert et al. 2019)**	***ölolog. Gilde sensu Schiemer***	**Habitat-Gilde im FIBS (EU-WRRL (D)**
Flounder	*Pleuronectes flesus*	diadromous-katadromous		rheophil
Thin-lipped mullet	*Liza ramada*			nicht klassifiziert
Eel	*Anguilla anguilla*			indifferent
River lamprey	*Lampetra fluviatilis*	diadromous - anadromous		rheophil
Sculpin, Bullhead	*Cottus gobio*	rheophilic-rhithral	rhithral-rheophil	rheophil
Barbel	*Barbus barbus*	rheophilic - potamal	rheophil - A	rheophil
Nase	*Chondrostoma nasus*			rheophil
Dace	*Leuciscus leuciscus*			rheophil
Northern whitefin gudgeon	*Romanogobio belingi*			rheophil
Chub	*Squalius cephalus / Leuciscus cephalus*	rheophilic - semi	rheophil - B	rheophil
Asp	*Aspius aspius*			rheophil
Ide	*Leuciscus idus*			rheophil
Vimba	*Vimba vimba*			rheophil
Gudgeon	*Gobio gobio*			rheophil
Bleak	*Alburnus alburnus*	eurytopic - lotic	eurytop	indifferent
Bighead goby	*Ponticola kessleri*	eurytopic-lotic - allochthonous gobies	nicht-klassifizierte Neozoen	nicht klassifiziert
Round goby	*Neogobius melanostomus*			nicht klassifiziert
Monky goby	*Neogobius fluviatilis*			nicht klassifiziert
Roach	*Rutilus rutilus*	eurytopic	eurytop	indifferent
Perch	*Perca fluviatilis*			indifferent
Ruffe	*Gymnocepalus cernua*			indifferent
Pikeperch	*Sander lucioperca*	eurytopic-related to floodplain habitats	eurytop	indifferent
Bream	*Abramis brama*			indifferent
Silver Bream	*Blicca bjoerkna*			indifferent
Carp	*Cyprinus carpio*			indifferent
Prussian carp	*Carassius gibelio*			indifferent
Pike	*Exos lucius*			indifferent
Catfish	*Siluris glanis*			indifferent
Topmouth gudgeon	*Pseudorasbora parva*	floodplain species - allochthonous	nicht-klassifizierte Neozoen	indifferent
Western tubenose goby	*Proterorhinus semilunaris*			nicht klassifiziert
Sufish	*Lepomis gibbosus*			indifferent
Spined Loach	*Cobitis taenia*	floodplain species - autochthonous	rheophil - B	rheophil
Threespined Stickleback	*Gasterosteus aculeatus*		eurytop	indifferent
Ninespined Stickleback	*Pungitius pungitius*		eurytop	indifferent
Bitterling	*Rhodeus amarus*		eurytop	indifferent
Sun bleak	*Leucaspius delineatus*		stagnophil	stagnophil
Rudd	*Scardinius erythrophthalmus*		stagnophil	stagnophil
Tench	*Tinca tinca*		stagnophil	stagnophil

** classification based predominately on relation to floodplain habitat, empirical data from Rhine-Monitoring*

APPENDIX 2: FISH NAMES DIFFERENT LANGUAGES

Scientific	English	German	Dutch	French
Anguilla anguilla	Eel	Aal	Aal	Anguille
Clarias gariepinus	African catfish	Afrikanischer Waller	Afrikaanse meerval	Silure africain
Alburnus alburnus	Bleak	Ukelei	Alver	Ablette
Umbra pygmaea	Striped mudminnow	Amerikanischer Hundsfisch	Amerikaanse hondsvis	Petit poisson chien
Acipenser sturio	Atlantic Sturgeon	Atlantische Stör	Atlantische Steur	Esturgeon d'europe
Perca fluviatilis	Perch	Barsch	Baars	Perche fluviatile
Barbus barbus	Barbel	Barbe	Barbeel	Barbeau fluviatile
Salmo trutta fario	Brown trout	Bachforelle	Beekforel	Truite de rivière
Lampetra planeri	Brook lamprey	Bachneunauge	Beekprik	Lamproie de planer
Barbatula barbatula	Stone loach	Schmerle	Bermpje	Loche franche
Rhodeus amarus	Bitterling	Bitterling	Bittervoorn	Bouvière
Rutilus rutilus	Roach	Plötze	Blankvoorn	Gardon ordinaire
Pseudorasbora parva	Topmouth gudgeon	Blaubandbärbling	Blauwband	Pseudorasbora
Vimba vimba	Vimba	Zährte	Blauwneus	Vimba/Serte
Platichthys flesus	Flounder	Flunder	Bot	Flet
Abramis brama	Bream	Brachsen	Brasem	Brême
Salvelinus fontinalis	Brook trout	Bachsaibling	Bronforel	Saumon de fontaine
Ameiurus nebulosus	Brown bullhead	Zwergwels	Bruine Am. dwergmeerval	Barbotte brune
Chelon labrosus	Thicklip grey mullet	Meeräsche	Diklipharder	Mulet lippu
Abramis sapa	White-eye bream	Zobel	Donaubrasem	-
Gasterosteus aculeatus aculeatus	Stickleback	Stichling	Driedoornige stekelbaars	Epinoche
Allosa alosa	Allis shad	Maifisch	Elft	Grande alose
Phoxinus phoxinus	Minnow	Elritze	Elrits	Vairon
Alosa fallax fallax	Twaite shad	Finte	Fint	Alose feinte
Alburnoides bipunctatus	Schneider	Schneider	Gestippelde alver	Spirlin
Carassius gibelio	Gibel carp	Giebel	Giebel (wilde goudvis)	Gibèle
Carassius auratus auratus	Goldfish	Goldfisch	Goudvis	Poisson rouge
Ctenopharyngodon idella	Grass carp	Grasfisch	Graskarper	Amour blanc
Aristichthys nobilis	Bighead carp	Marmorkarpfen	Grootkopkarper	Carpe marbré
Coregonus lavaretus	Powan	Blaufelchen	Grote marene	Lavaret du Bourget
Misgurnus fossilis	Wheaterfish	Schlammpeitzger	Grote modderkruiper	Loche d'etang
Poecilia reticulata	Guppy	Guppy	Gup	Guppy
Coregonus oxyrinchus	Houting	Schnäpel	Houting	Bondelle
Cyprinus carpio carpio	Carp	Karpfen	Karper	Carpe
Coregonus albula	Vendace	Kleine Maräne	Kleine marene	Petite Marêne
Cobitis taenia	Spined loach	Steinbeisser	Kleine modderkruiper	Loche de rivière
Blicca bjoerkna	White bream	Güster	Kolblei	Brême bordelière
Leuciscus cephalus	Chub Crucian	Döbel	Kopvoorn	Chevaine
Carassius carassius	carp Burbot	Karausche	Kroeskarper	Carassin
Lota lota	Tubenosed	Quappe	Kwabaal	Lote de rivière
Proterorhinus marmoratus	goby Wels	Marmorierte Grundel	Marmergrondel	Gobie à nez tubulaire
Silurus glanis	Ruffe	Waller	Meerval	Silure glane
Gymnocephalus cernuus	Rainbow	Kaulbarsch	Pos	Grémille
Oncorhynchus mykiss	trout	Regenbogenforelle	Regenboogforel	Truite arc-en-ciel
Cottus gobio	Bullhead	Groppe	Rivierdonderpad	Chabot
Gobio gobio gobio	Gudgeon	Gründling	Riviergrondel	Goujon
Lampetra fluviatilis	Lampern	Flussneunauge	Rivierprik	Lamproie de rivière
Aspius aspius	Asp	Rapfen	Roofblei	L'aspe
Scardinius erythrophthalmus	Rudd	Rotfeder	Ruisvoorn	Rotengle
Acipenser gueldenstaedtii	Russian sturgeon	Donau-Stör	Russische steur	Esturgeon du Danube
Leuciscus leuciscus	Dace	Hasel	Serpeling	Vandoise
Acipenser baerii baerii	Siberian sturgeon	Sibirischer Stör	Siberische steur	Esturgeon sibérien
Chondrostoma nasus	Nose carp	Nase	Sneep	Hotu
Esox lucius	Pike	Hecht	Snoek	Brochet
Sander lucioperca	Pike perch	Zander	Snoekbaars	Sandre
Cyprinus carpio carpio	Mirror carp	Spiegelkarpfen	Spiegelkarper	Carpe miroir
Osmerus eperlanus	Smelt	Stint	Spiering	Eperlan
Acipenser ruthenus	Sterlet	Sterlet	Sterlet	Esturgeon de Sibérie
Pungitius pungitius	Ten-spined stickleback	Zwergstichling	Tiendoornige stekelbaars	Epinochette
Leucaspius delineatus	Moderlieschen	Moderlieschen	Vetje	Able de Heckel
Thymallus thymallus	Grayling	Äsche	Vlagzalm	Ombre commun
Leuciscus idus	Ide	Aland	Winde	Ide mélanote
Romanogobio albipinnatus	White-finned gudgeon	Weissflossengründling	Witvingrondel	/
Salmo salar	Salmon	Lachs	Zalm	Saumon atlantique
Salmo trutta trutta	Sea trout	Meerforelle	Zeeforel	Truite de mer
Tinca tinca	Tench	Schleie	Zeelt	Tanche
Petromyzon marinus	Sea lamprey	Meerneunauge	Zeeprik	Lamproie marine
Hypophthalmichthys molitrix	Silver carp	Silberkarpfen	Zilverkarper	Amour argenté
Lepomis gibbosus	Pumpkinseed	Sonnenbarsch	Zonnebaars	Perche-soleil
Neogobius melanostomus	Round goby	Schwarzmund Grundel	Zwartbekgrondel	Gobie arrondi
Ameiurus melas	Black bullhead	Zwergwels	Zwarte Am. dwergmeerval	Barbotte noire