Fish sampling campaign in the Rhine – Nort Rhine Westphalia

Seine net fishing and eDNA as a possible survey tool

M. Dorenbosch
N. van Kessel
G. Bonhof

Bureau Waardenburg
Ecologie & Landschap

Fish sampling campaign in the Rhine - North Rhine Westphalia.

Seine net fishing and eDNA as a possible survey tool

M. Dorenbosch
N. van Kessel
G. Bonhof

Bureau Waardenburg
Ecologie & landschap

Fish sampling campaign in the Rhine - North Rhine Westphalia. Seine net fishing and eDNA as a possible survey tool

Dr. M. Dorenbosch, N. van Kessel MSc. & drs. G. Bonhof

Status: final report

Report nr.: 19-037

Date of publication: March, 2019

Photo credits cover page: Bureau Waardenburg

Project nr: 18-0344

Project manager: N. van Kessel

Name & address client: Land Nordrhein-Westfalen, Bezirksregierung Düsseldorf, Dr. N. S. Scheifhacken, Cecilienallee 2, 40474, Düsseldorf, Germany

Signed for publication:

Teamleader, Wendy Liefvelc

Signature:

Please cite as: Dorenbosch, M., N. van Kessel & G. Bonhof, 2019. Fish sampling campaign in the Rhine - North Rhine Westphalia. Seine net fishing and eDNA as a possible survey tool. Bureau Waardenburg Rapportnr. 19-037. Bureau Waardenburg, Culemborg.

Bureau Waardenburg bv
Onderzoek en advies voor ecologie en landschap

Postbus 365 4100 AJ Culemborg
Telefoon 0345 51 27 10
info@buwa.nl www.buwa.nl

Content

Abstract

Seine netting and Environmental DNA metabarcoding (eDNA) in the river Rhine have resulted in the detection of both abundant and rare riverine fish species that occur in the same habitat as allis shad. Allis shad however was not caught by seine netting or detected by eDNA in this study. Seine netting was conducted during day and night. eDNA sampling conducted during the day. Seine netting at night resulted in higher species richness. Total fish density did not differ between day and night. However, density of some species was higher at night. Also detection probability of most species was higher during night sampling.

The observed fish assemblage based on the seine net fishing campaign in the study area shows the method is applicable in habitats with abiotic conditions in the Rhine between Rees and Wesel. eDNA metabarcoding resulted in a large species richness, considerably larger than based on seine netting. Most of the detected species are likely to occur at the sampling sites, however there is a chance of false positives (e.g., eDNA influx from upstream or contamination by passing ships).

Based on the results, seine netting and eDNA metabarcoding are suitable methods to study fish assemblages in the river Rhine.

1 Introduction

1.1 General background

In North Rhine-Westphalia and Rhineland-Palatinate, juveniles of allis shad (*Alosa alosa*) were stocked in the river Rhine in order to reintroduce and establish a self-reproducing shad population in the Rhine river system. As a result of this reintroduction, low numbers of both adults and new recruits have recently been observed in the Rhine. This may indicate a self-reproducing allis shad population is rising in the river Rhine.
Monitoring of the allis shad population in the Rhine is difficult, the population is small and densities are extremely low. Also, allis shad occurs in fast flowing water, a habitat that is difficult to sample with nets. Detection chances of allis shad are therefore low. After reintroduction, the occurrence of adult allis shad in the Rhine has been monitored by the use of trammel nets (three-layered gill nets). Some specimens of adult allis shad have been caught using this technique spring 2018.

1.2 Application of other fish methods

Standardized seine netting
To monitor population development of young of the year (YOY) allis shad, standardized seine netting is proposed in shallow parts of the Rhine where shads are expected. In the Dutch part of the Rhine seine netting has already been applied for more than a decade, the methodology is used for quantitative monitoring of population fluctuations of riverine fish.
Based on data from the Netherlands, the methodology appears to be particularly successful to demonstrate the presence of YOY fish, including species that are present in low densities (in the Netherlands for example barbel, chub, dace, and nase). In the Netherlands, standardized sampling protocols are developed for the application of seine net fisheries (especially with respect tot the European Water Framework Directive). Here, small seine nets (25 m) are applied in shallow riverbanks (up to 1.5 m depths), whereas larger seine nets (75 - 100 m) are applied in shallow river banks with larger water depth (> 1.5 m depth).
To explore whether seine net fishing is a suited method to monitor population development of YOY allis shad, the present study applied a rapid pilot sampling campaign in a shallow river part of the Rhine between Rees and Wesel where YOY shads are expected. Since seine netting can be conducted both during day- and nighttime, the sampling campaign also takes the effect of day and night into account to determine fishing efficiency for detection of low-density species such as allis shad.

Environmental DNA detection
Beside these traditional fishing methods, recent development of environmental DNA (referred to as 'eDNA') detection techniques may be promising to demonstrate the presence of fish species that occur in low densities such allis shad. Aquatic organisms including fish leave small amounts of DNA in the water that can be tracked using the

latest DNA sequencing techniques. Since extremely low quantities of DNA can already be detected, also species that occur in low densities in the river can be observed. Based on next generation metabarcoding, water samples can be screened for an entire fish assemblage, including rare species such as allis shad. Along with the above described sampling campaign in the Rhine between Rees and Wesel, water samples are collected in the present study to determine whether eDNA metabarcoding is a suited technique to determine the presence of allis shad.
A comparable eDNA study has been conducted in the Dutch part of the Rhine in at Lobith in autumn 2018. The presence or absence of allis shad is compared with eDNA data from the Dutch Rhine and provides insight on the presence of allis shad in on a larger geographic scale.

1.3 Research questions

In the present study the follow question are being addressed:

1. How is the fish assemblage obtained by standardized seine netting related to the occurrence of species that occur in low densities in the Rhine (such as allis shad)?
2. Has nocturnal or diurnal seine net fishing a significant effect on the detected fish assemblage structure?
3. Is eDNA detection a useful method in relation to detection chances of species that occur in low densities in the Rhine (such as allis shad)?

2 Methods

2.1 Study area and sample design

The fish sampling campaign was conducted in a shallow river part of the Rhine between Rees and Wesel where YOY allis shads are expected. In a successive day and night sampling campaign, seine net hauls were conducted on eight sample locations. Sample locations are pointed out in figure 1, whereas GPS coordinates are listed in table 1.

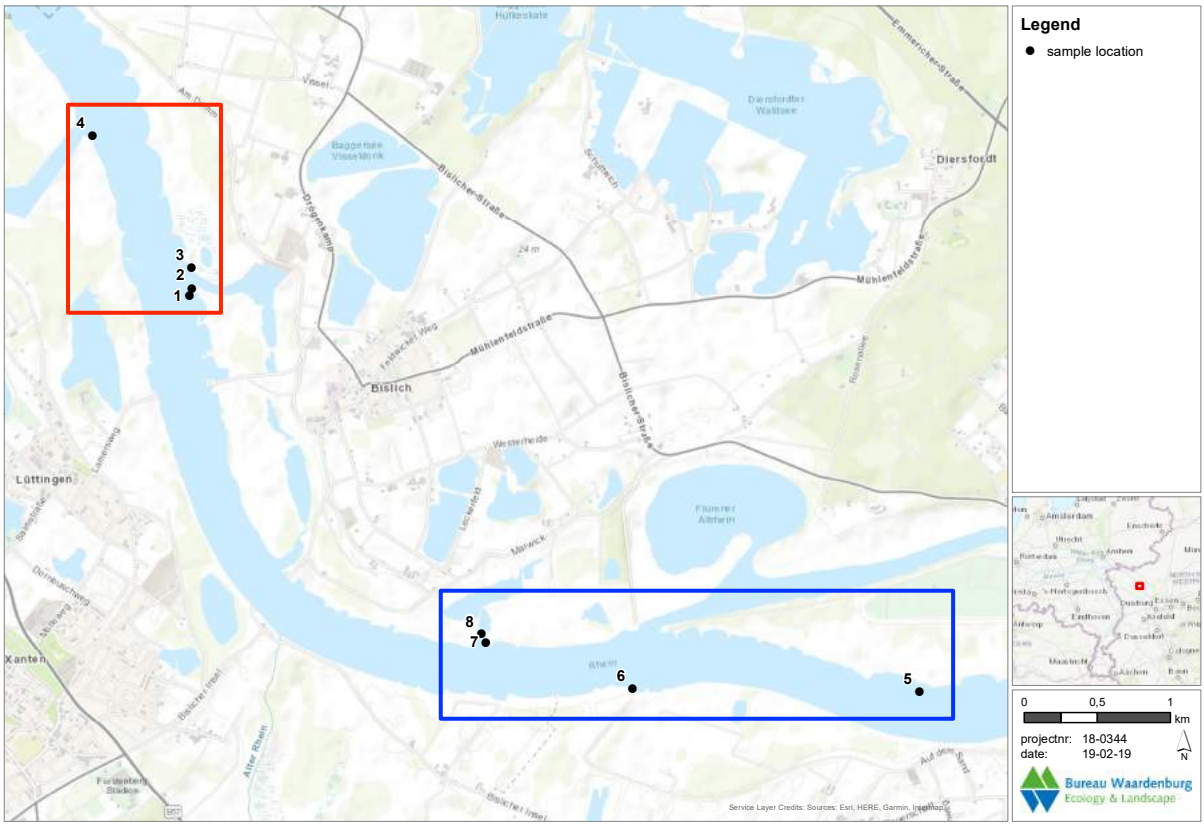


Figure 2.1. Geographic overview of the fish sample locations in the Rhine (numbers refer to table 2.1). Rectangles indicate on which sample locations water has been collected for eDNA survey, red: eDNA survey downstream, blue: eDNA survey upstream.

Study design

With respect to the standardized seine net survey, eight locations have been surveyed during night, six locations during day (table 2.1). Sample locations were evenly distributed either down- or upstream of the village Bislich and encompassed both left and right river shores. Likewise, two eDNA watersamples have been collected downstream of the village Bislich (one on the river shore, one in centre of the river channel), and two eDNA samples upstream of Bislich (river shore, main channel).

Each eDNA watersample consisted out of four subsamples of 250 mL of water of each of the four sample locations down- or upstream of Bislich.

Table 2.1. Coordinates (longitude, latitude, decimal degrees, WGS84) of sample locations (fig.1), including surveyed surface area. -- not sampled at night.

sample location	longitude	latitude	surface day (m^2)	surface night (m^2)
1	51,684912	6,473891	175	--
2	51,685359	6,474178	510	590
3	51,686610	6,474024	625	625
4	51,694808	6,464546	525	562
5	51,659852	6,545551	680	705
6	51,660303	6,517156	495	450
7	51,663295	6,502723	775	720
8	51,663808	6,502324	1800	--

2.2 Standardized seine net survey

Standardized seine net surveys were conducted by hauls of a 75 m long seine net (3 m height, 18 mm stretched mesh size of collector bag).
During each haul, the net is set out in the river, perpendicular to the riverbank by an engine powered small boat, looped back to the rivershore and subsequently pulled to the shore by three field-assistants. After the haul, fish concentrated in the central collecting bag of the seine net are transferred to an 80 L container where fish are identified, measured (cm's, total length) and counted.
Each haul results in a sampled area in the shape of a half-circle, with a surface ranging from 175 = 1800 m^2. The exact surface of each haul is calculated by a handheld GPS during the deployment of the seine net.
The day and night fishing campaign was conducted during one 24 h cycle at October 24, 2018. During daytime (between 9:30 and 15:30 h), all eight locations shown in figure 2.1 and table 2.1 were surveyed once resulting in eight seine net hauls. Subsequently, six locations (table 2.1) were surveyed once during nighttime (between 19:30 and 01:30 h) resulting in six hauls. In total, 14 seine net hauls were conducted.
Figure 2.2 provides a photographic impression of the seine netting.

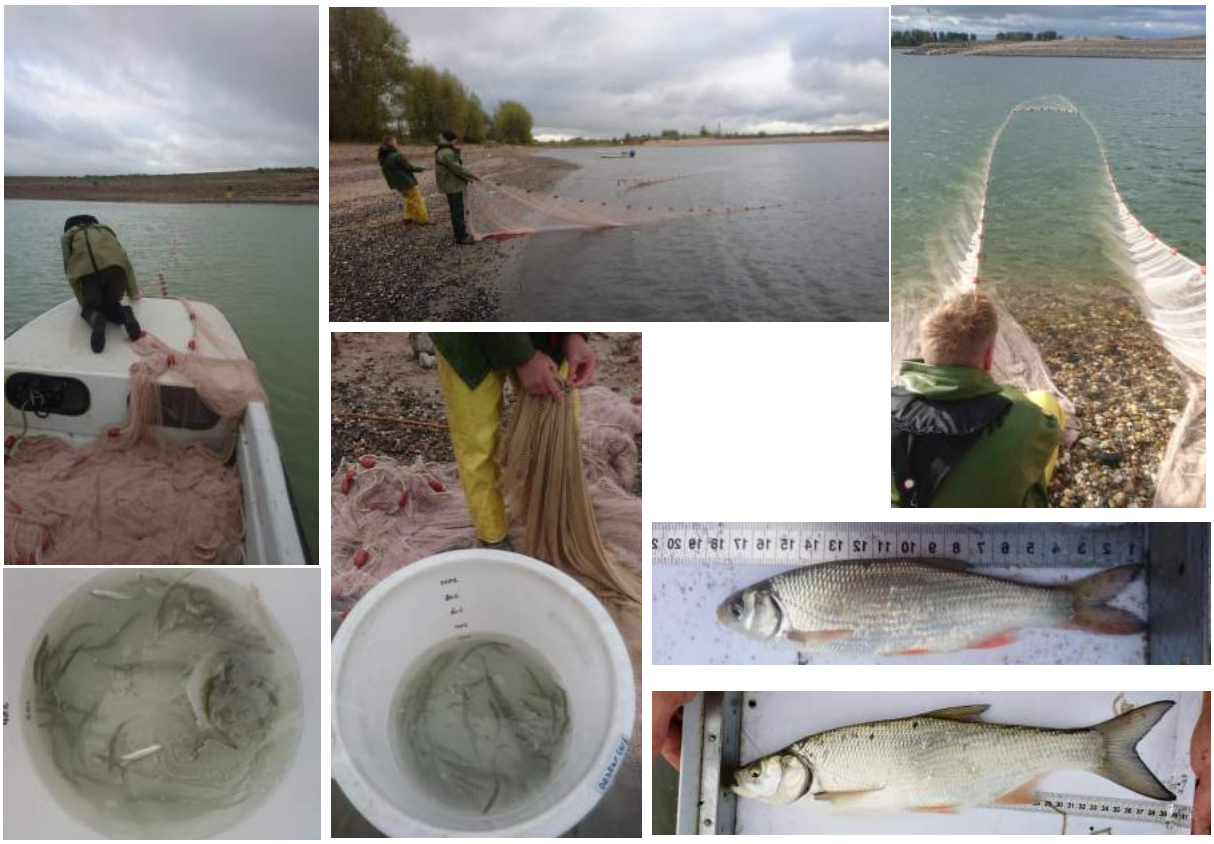


Figure 2.2. Overview of seine net fishing in the study area (setting out the net from a boat, hauling the net to the shore, transferring fish from the collection bag to a reservoir volume, and identifying and measuring fish.

2.3 Environmental DNA detection survey

General description

eDNA metabarcoding is a relatively new fish detection methodology in which fish are detected based on DNA traces fish leave in their environment (figure 2.3). Fish always leave cellular material containing DNA in the water. These organic structures containing DNA can be filtered from the water and isolated from a sample in the laboratory with specific primer combinations unique for fish DNA. By means of PCR the original low concentrations of fish DNA in the sample are amplified into a high concentration of DNA barcodes. This multi-species fish DNA barcode signal eDNA of all fish species that originally were present in the water sample. By means of Next Generation Sequencing all DNA barcodes are translated into their specific nucleotide base sequences that can be compared with a DNA reference database that contains DNA sequences of all fish species that occur in the Rhine (native as well as invasive species). If DNA barcodes that were isolated from the watersample result into a positive genetic match with the DNA reference base, a fish species is considered as a positive match. Based on the number of positive matches, the fish assemblage of the water sample can be reconstructed based on the presence of eDNA of each species in the sample.

The method is very sensitive: extremely low quantities of eDNA can already result into a positive match, rare species that occur in very low densities can be detected. The information of species presence based on eDNA detection can therefore be a valuable contribution with respect to traditional fish survey techniques.
On the same time, the sensitivity of eDNA detection is also a risk. Since even the lowest amount of eDNA can already be picked up, the methodology is very sensitive to contamination that may result in false positive observations of fish species (i.e., the fish is detected in the sample but the eDNA originated from elsewhere).

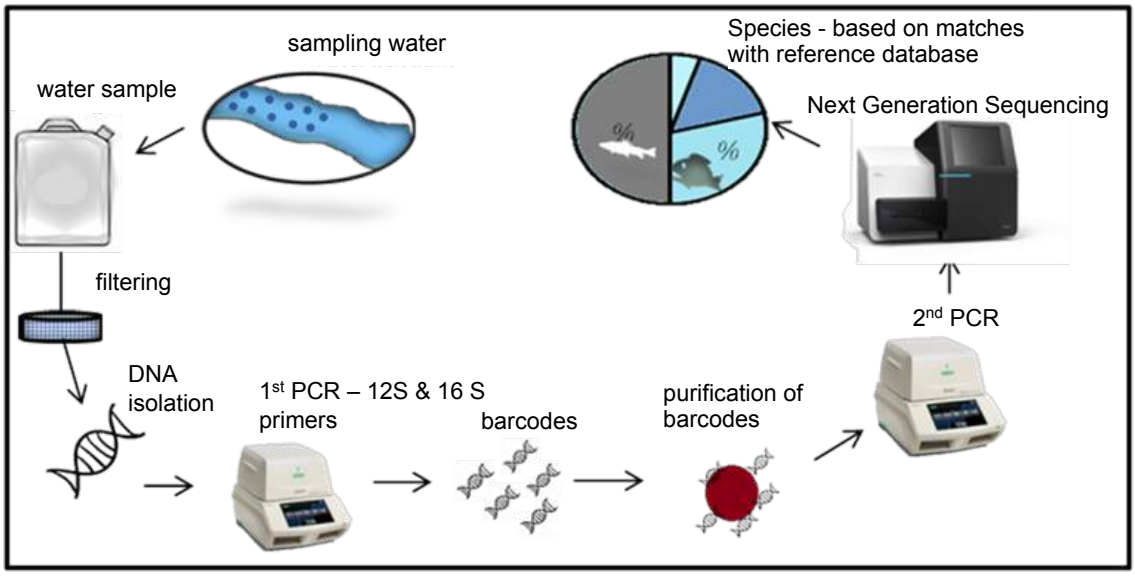


Figure 2.3. *Overview of processes involved with eDNA metabarcoding. The methodology consists out of collection and filtering of water in the field, DNA isolation and PCR amplification with specific 12S and 16S primers, purification of produced barcodes, Next Generation Sequencing of barcodes, and the construction of a species list by cross referencing DNA barcodes from the sample with a DNA reference database.*

Collection of watersamples

Four eDNA watersamples were conducted during the study: two in a stretch of the river downstream of Bislich and two upstream of Bislich (figure 2.1). Of the two samples in each stretch of the river, one was collected in the centre of main channel (referred to as 'river') whereas the second sample was collected near the river shore (1,5 m of the shoreline, referred to as 'shore'). Each eDNA watersample (1 L) consisted out of four 250 mL subsamples of water (collected at sample location in the up- or downstream stretch of the river, figure 2.1). Watersamples were collected in jars from a boat by a field-assistant with sterile gloves at a depth 10 cm below the watersurface. The jars that were used to collect water were sterilized in a DNA free laboratory prior to the sampling campaign.
The watersamples were collected during daytime on the same day of the seine netting campaign, before the seine net hauling campaign to prevent contamination of the boat with fish DNA.

Filtration and storage of watersamples

To prevent rapid breakdown of eDNA in the watersamples, samples were filtered in the field, immediately after collection. The 1 L volume of a watersample was pushed through a 0,2 µm - 64 cm^2 PES membrane (Polyethersulfone) by means of vacuum pump. The PES filter including the eDNA is subsequently stored in a lysis CTAB buffer solution and transported to the laboratory. During filtration and storage of the filter in the buffer solution, the field-assistant wore DNA sterile gloves, prior to transferring the filter into the buffer, sterile gloves were replaced with new sterile gloves to prevent DNA contamination.

Figure 2.4 provides an overview of collection and filtering of water in the field. Water sampling and filtration in the field has been conducted by field-assistants of Bureau Waardenburg.

Laboratory analysis

Each field sample is processed in the laboratory where a specific protocol is followed. The most essential steps are described below. An essential step in eDNA processing is DNA extraction from the filter followed by amplification of the extracted eDNA with specific primers and the Polymerase Chain Reaction (PCR). Two sets of primers are used in the present study, these amplify fish specific DNA fragments of ≈100 basepairs of the mitochondrial 12S rRNA and 16S rRNA gene. Efficiency of these primers has been proved for all native and invasive fish of the Netherlands (which also encompasses all species present in the Rhine). Subsequently 50 PCR cycles have been conducted to amplify DNA fragments. Of each sample, 12 PCR replicates have been run which have been pooled later in the process.

After this first PCR cycle, DNA fragments have been labelled by illumina Nextera XT sequence adapters by a second PCR, purified and pooled to one sample DNA library containing all barcodes of the original water sample.

This DNA library has been sequenced by means of Next Generation Sequencing on a HiSeq 4000 platform. Laboratory analysis have been conducted in a DNA clean lab space where high standard protocols are followed to prevent DNA contamination during the different DNA process steps.

Besides the field samples, also negative control samples are run in the laboratory to determine the degree of DNA contamination of samples either in the field by field-assistants or in the laboratory. Negative control samples consist out of DNA free mineralised water that is filtered similarly to the field samples and should not contain fish DNA.

All laboratory analyses have been conducted by the laboratory of Datura Molecular Solutions BV (www.datura.nl).

Bioinformatica

The next step in the eDNA metabarcoding protocol is the bioinformatica cycle in which the sequenced DNA library containing all DNA metabarcodes of the original sample, is filtered and cross referenced to a DNA references database. To delete PCR and sequence error, the DNA library is filtered through the Obitool pipeline (Boyer *et al.*, 2016), an open source software package. DNA fragments that occur less than 10

times or that are shorter than 30 basepairs are removed from the DNA library. The Obiclean tool is applied to remove DNA fragments that may be the result of PCR and sequence errors.

The purified DNA library is subsequently cross-referenced to a DNA reference database containing 12S and 16S DNA barcodes of all native and invasive fish species that occur or can be expected in the Netherlands. The database has been constructed by Datura Molecular Solutions by sequencing 12S and 16S DNA fragments of actual fish specimens collected in the Netherlands. For some species, DNA barcodes have been completed by DNA sequences published on GENBANK (https://www.ncbi.nlm.nih.gov/genbank). Also, specific 12S and 16S DNA barcodes of allis shad (individuals that have been introduced in the Rhine) have been isolated by Datura and are added to the reference database. DNA barcodes from the original sample that show more than 98% resemblance with barcodes of species in the DNA reference database are considered a positive hit. Finally, a species database is constructed for each water sample where the presence of species is expressed as fraction of the total number of generated DNA fish fragments present in the sample.

Although fish species are expressed as a fraction of the total number of generated DNA fragments, it is important to note that eDNA metabarcoding is not suited to quantify eDNA of each species. The produced number of DNA fragments after sequencing in each sample is merely a result of PCR and sequence efficiency and cannot directly be related to the amount of eDNA originally present in the water sample. The amount of DNA fragments of each species in relation to the total amount of generated fish DNA fragments can be used to distinguish false positive detections from actual detections. If a species occurs in extremely low amount of DNA fragments, the species should carefully evaluated not to be considered as a false positive detection (e.g. as a result of PCR or sequence error or a contamination).

Figure 2.3. Overview of eDNA sample collection and filtration in the study area (collection of water from a boat in sterile jars, filtration in the field of the watersample on 0,2 µm - 64 cm^2 PES membranes by means of a mobile vacuum pump and storage of the filter in CTAB lysis buffer.

2.4 Data analyses

Data preparation

Catches of the seine net data were expressed as fish densities per surface area, as well as species richness of each haul for day and night. Subsequently, mean total fish density, individual species density and total species richness was calculated for day and night based on the number of available hauls (8 hauls during day, 6 at night).

To construct size-frequency plots of each fish species, all observations of a species were pooled together (all hauls, day and night), and the number of individuals was plotted to fish size (in 1 cm size classes).

For the eDNA sampling campaign, a fish assemblage table was constructed for each sample based on the positive detection of fish species in relation to the fish reference DNA database. For each detected species, the presence of the species was expressed as the fraction of DNA fragments of the species in relation tot the total number of fish DNA fragments in the sample.

Data analyses

Statistical differences between mean fish density and species richness in the seine net sampling campaign during day and night were determined by constructing general linear models in which the number of hauls were considered as replicates and day versus night was set as a fixed factor.

For each species, detection chances were calculated for day and night based on the number of hauls a species was observed in, in relation to the total number of hauls.

To determine the efficiency of the sampling procedure (i.e., the number of hauls in relation the observed species richness), species accumulation curves were constructed and Chao total species richness was estimated in the R package VEGAN with the functions 'specaccum' (species accumulation curves) and 'specpool' (estimated species richness) (Oksanen *et al.* 2019). Data analyses were performed in R, version 3.0.

3 Results

3.1 Seine net sampling campaign

Total fish assemblage

In total, ten species have been observed during the seine net fishing campaign (table 3.1, see also appendix for data per haul). Three species were non-native (invasive) fish, i.e., asp, monkey goby and round goby. During day, seven species have been observed whereas at night nine species have been observed. Common bleak dominated the fish assemblage both during day (82%) and night (50%).

Table 3.1. Overview of the fish assemblage (total number of individuals and total species richness) observed with seine net fishing during day (n=8) and at night (n=6).

species		day	night
Abramis brama	common bream	2	20
Alburnus alburnus	common bleak	164	99
Aspius aspius	asp	10	6
Gymnocephalus cernua	ruffe	--	2
Leuciscus idus	ide	6	21
Neogobius fluviatilis	monkey goby	--	15
Neogobius melanostomus	round goby	3	2
Perca fluviatilis	European perch	11	--
Rutilus rutilus	roach	--	18
Sander lucioperca	zander	5	15
total number individuals		**201**	**198**
total species richness		**7**	**9**

Similarity day - night

Six species were observed both during day and night (figure 3.1), whereas one species was only observed during day (European perch) and three species only at night (ruffe, money goby, roach).

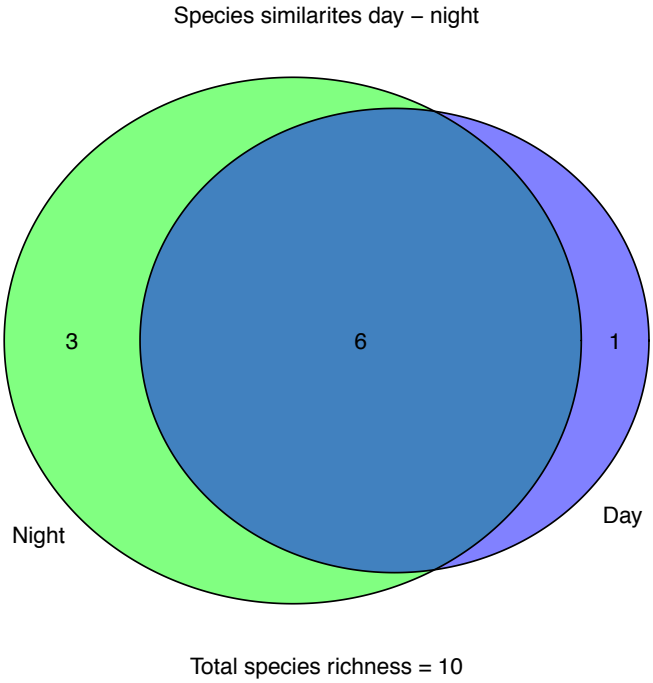


Figure 3.1. Similarity (number of shared species) between the fish assemblage during day and night.

Fish density and species richness day - night

Total fish density does not significantly differ between day and night (figure 3.2a; AIC=10,31; RSS=10,71; df=1, P=0,473) whereas species richness is significantly higher at night than during day (figure 3.2c; AIC=22,33; RSS=51,50; df=1; P<0,001). Although average densities are low, common bream, ide, monkey goby, ruffe, and zander show considerably higher densities at night than during day (figure 3.2b). Asp and European perch showed higher densities during day than at night (figure 3.2b). The most abundant species (common bleak) was observed in comparable densities during day and night (figure 3.2a), likewise was the case for round goby (figure 3.2b).

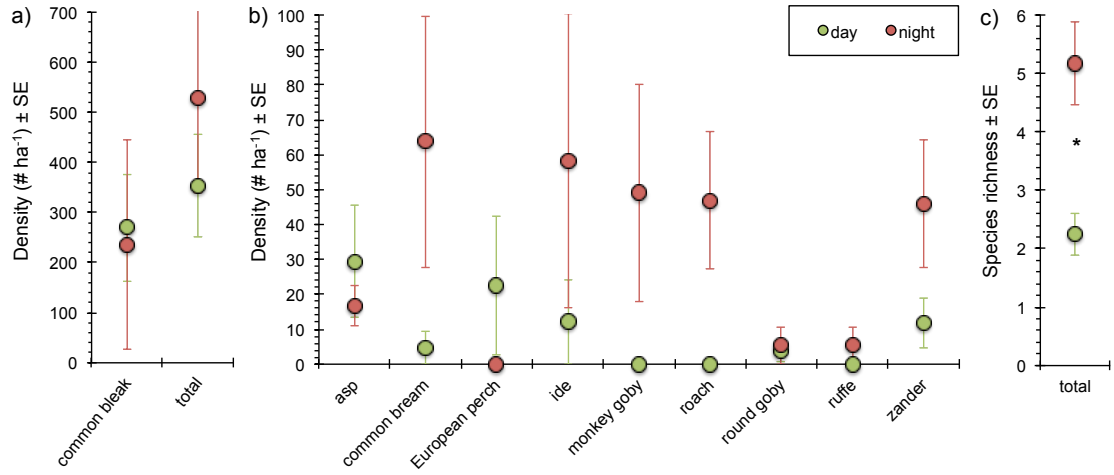


*Figure 3.2. Mean density of the ten observed species and the total fish assemblage (a, b), and mean species richness(c). * Indicate a significant difference (P<0,001).*

Detection probability & species accumulation curves

Based on the occurrence in each seine haul during day and night, detection probability can be calculated for each species (figure 3.3). Highest detection probabilities are observed at night (i.e., common bream, common bleak, asp, monkey goby, roach and zander). Only round goby and European perch have higher detection probabilities during day. The most common fish species (common bleak) showed comparable detection probabilities during day and night.

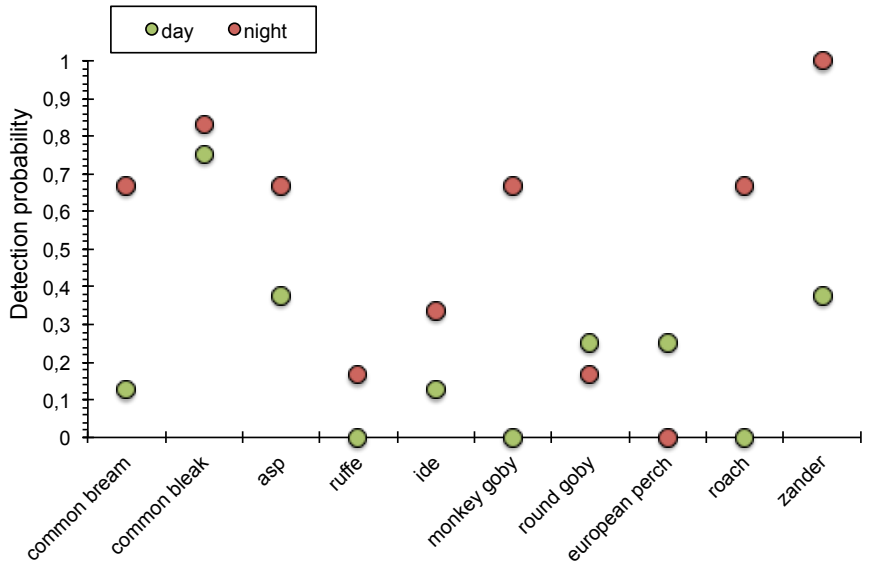


Figure 3.3. Detection probability of the ten observed fish species during day and night.

To estimate whether the number of hauls during day and night was efficient to estimate the species richness, species accumulation curves were constructed and

Chao species richness was estimated. Estimated Chao species richness during day was 7,6 (close to the actual number of observed species during day, n=7), whereas estimated Chao species richness at night was 12,4 (farther away from the actual number of observed species, n=9). This indicates that the number of hauls during day (n=8) provides a representative presentation of the fish assemblage during day (figure 3.4), whereas the number of hauls at night (n=6) most likely underestimates the fish assemblage at night (figure3.4).

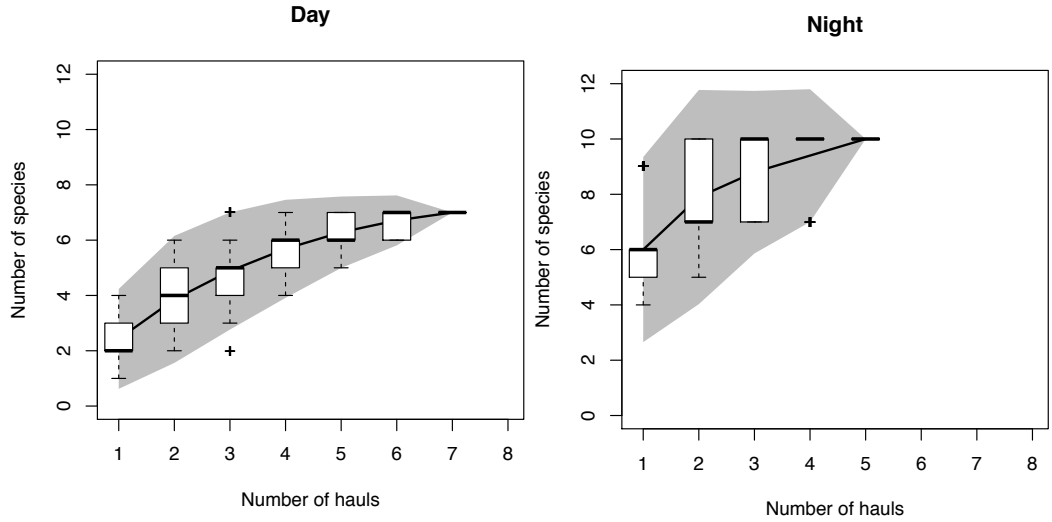


Figure 3.4. Species accumulation curves during day (8 hauls) and night (6 hauls).

Size frequency distribution

For the species asp, roach, zander, ide, common bream and common bleak, size frequencies indicated different age classes (figure 3.5). Both 0^+ fish (individuals recruited in 2018) and older (one or more seasons) fish were present. For common bleak, the density of 0^+ fish was higher than for older fish.

The largest fish that were observed were zander (64 cm) and asp (70 cm).

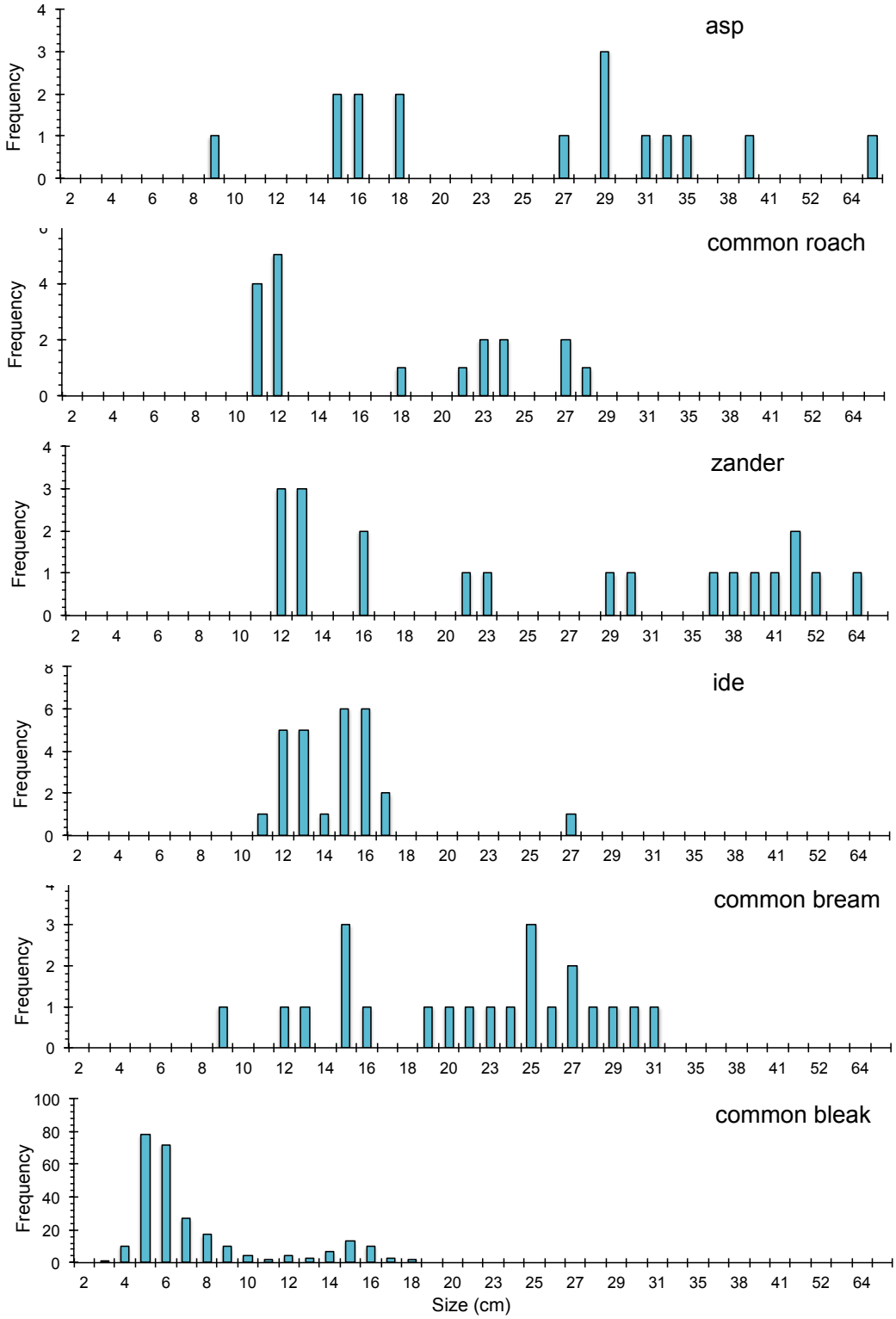


Figure 3.5. Size frequency distribution of fish species where both 0^+ aged fish as well as older fish were observed.

3.2 eDNA detection

Total fish assemblage based on eDNA detection

In total, 36 species have been detected in the four eDNA samples from the study area (table 3.2). eDNA of allis shad was not observed in the samples. Twelve species were observed in all four samples and had a detection chance of 1, whereas six species were observed in three samples (detection chance of 0,75) and five species in two samples (detection chance of 0,5). There was also a large group of species (n=13) that was only observed in one sample (detection chance of 0,25). Subsequently, for three of these 13 species only very low number of DNA barcodes were detected (<0,05 % in relation to the total amount of detected fish DNA barcodes in the sample).
Two sets of species, *i.e. Lampetra fluviatilis/L. planeri* and *Platichthys flesus/Pleuronectus platessa* cannot be distinguished from eachother based on the primers used.

False positives

The interpretation of the latter group should be done with care, i.e., there is considerable risk that the presence of species in a sample is a result of a false positive detection. The presence of eDNA of such a species in a sample is a true fact since the methodology is conducted by a standard protocol. However, a fish species that is both detected in only one water sample as well as in a low number of DNA barcodes, could be the result of eDNA fragments that were present on the sample location while the fish wasn't actually there. This could be the result of contamination by field-assistants, by passing ship traffic that may bring (ballast)water from abroad or by passive downstream transport of eDNA from upstream fish populations. There is also the possibility the species is actually present on the sample location and by chance only an extremely low amount of eDNA was present in the water sample. For species that occur with low amounts of DNA barcodes in a limited group of samples (in this case one sample), it is difficult to distinguish between a 'real' detection and a false positive detection.
This can be illustrated by the detection of yellow sole and European flounder/plaice. Yellow sole and European flounder/plaice are saltwater fishes that frequently occur in the Delta near Rotterdam, the detection of these species in the German Rhine is most likely the result of passive transport of eDNA of the species by the frequent ship traffic between Rotterdam and Germany. The detection of these species and also of Atlantic salmon and river trout could be the result of accidental human transport; the species are frequently consumed and can easily enter the Rhine from watertreatment plants or ships.
Other species that need to be discussed are thinlip mullet, European bitterling and stream bullhead as the species are not or hardly present in the main channel of the river Rhine. Thinlip mullet is known to migrate far upstream and a single adult specimen was caught near Nijmegen (NL) in april 2012 (Van Kessel & Kranenbarg, 2012). Because the species was detected in two water samples, upstream as well as downstream of Bislich, its likely the species was present in the river Rhine at the time of the eDNA sampling. European bitterling is caught on occasion in the main channel of the Dutch river Rhine near the German border, but is mostly present in floodplains.

The species is also present in Germany and the two detections of the species in the river Rhine, also upstream as well as downstream of Bislich, are therefore most likely not false positives. Stream bullhead is present in Germany in tributaries of the river Rhine but has not been caught in the main channel since the nineties of the previous century (pers. comm. N. Scheifhacken). It cannot be excluded that a specimen was present at the sampling location due to downstream migration. But, most likely it is a false positive based on the downstream transport of eDNA of the species.

Table 3.2. Overview of detected fish species in the four eDNA water samples. The presence of a species in a sample is shown as the percentage of DNA barcodes matched to the species in relation to the total number of fish DNA barcodes in the sample. Based on the frequency of occurrence, a detection chance is calculated for each species. 'river' or 'shore' indicate the sample has been collected either in the centre of the river channel or near the shore; 'upstream' or 'downstream' indicate the sample has been collected either up- or downstream of Bislich (see also figure 2.1); -- species is not detected; Notes: A) species could no be distinguished from eachother based on the primers; B) species is only detected with a very low number of DNA barcodes (<0,05% of total fish DNA barcodes); C) species is only detected in one sample.

Species:		river upstream	shore upstream	river downstream	shore downstream	detection chance	note
Alburnus alburnus	common bleak	5,71	8,05	3,22	13,39	1	
Anguilla anguilla	European eel	0,59	1,89	2,72	3,63	1	
Aspius aspius	asp	3,94	5,22	8,52	15,67	1	
Barbus barbus	barbel	2,92	3,06	8,91	3,87	1	
Chondrostoma nasus	nase	0,13	0,20	0,12	0,55	1	
Leuciscus idus	ide	7,71	3,10	3,09	5,26	1	
Neogobius melanostomus	round goby	58,06	31,03	46,71	31,50	1	
Ponticola kessleri	bighead goby	1,88	2,77	4,59	3,09	1	
Romanogobio belingi	whitefin gudgeon	0,61	2,72	1,75	0,75	1	
Rutilus rutilus	roach	12,72	12,79	11,15	12,31	1	
Sander lucioperca	zander	3,88	3,16	1,88	8,26	1	
Vimba vimba	zarte / vimba bream	0,12	0,58	0,08	0,24	1	
Esox lucius	pike	0,10	--	0,67	0,12	0,75	
Gobio gobio	gudgeon	0,05	--	0,19	0,14	0,75	
Gymnocephalus cernua	ruffe	--	0,35	0,12	0,20	0,75	
Leuciscus leuciscus	common dace	0,68	0,75	0,91	--	0,75	
Perca fluviatilis	European perch	--	0,87	2,33	0,56	0,75	
Squalius cephalus	chub	0,32	--	0,17	0,03	0,75	
Blicca bjoerkna	white bream	--	--	0,07	0,12	0,5	
Gasterosteus aculeatus	three-spined stickleback	--	1,55	0,83	--	0,5	
Liza ramada	thinlip mullet	--	0,16	--	0,02	0,5	
Oncorhynchus mykiss	rainbow trout	0,17	--	0,25	--	0,5	
Rhodeus amarus	European bitterling	--	0,03	--	0,03	0,5	B
Abramis brama	common bream	--	21,66	--	--	0,25	C
Ballerus sapa	white-eye bream	--	--	0,13	--	0,25	C
Buglossidium luteum	yellow sole	0,13	--	--	--	0,25	C
Cottus rhenanus	stream bullhead	0,11	--	--	--	0,25	C
Cyprinus carpio	carp	--	--	0,76	--	0,25	C
Lampetra fluviatilis / L. planeri	river / brook lamprey	--	--	0,02	--	0,25	A, B, C
Leucaspius delineatus	white aspe	--	--	0,10	--	0,25	C
Neogobius fluviatilis	monkey goby	--	--	--	0,26	0,25	C
Platichthys flesus / Pleuronectes platessa	European flounder / plaice	--	--	0,52	--	0,25	A, C
Pungitius pungitius	ninespine stickleback	0,13	--	--	--	0,25	C
Salmo salar	Atlantic salmon	0,03	--	--	--	0,25	B, C
Salmo trutta	river trout	--	0,07	--	--	0,25	C
Scardinius erythrophthalmus	rudd	--	--	0,20	--	0,25	C
total species richness		**21**	**20**	**27**	**21**		

Habitat and sample efficiency

eDNA samples have been collected on four locations, either in the centre of the river channel and on the shore. The number of detected species per location was relatively constant (20 - 21 species). Only the water sample from the river channel downstream of Bislich resulted in higher number of detected species (n=27).

Although this may suggest the river channel harbours eDNA of more species than the shore, the results also clearly show that for the maximum richness of detected species (n=36) all samples should be pooled. A species accumulation curve indicates species richness is not yet constant with the four collected water samples (figure 3.6), whereas estimated Chao species richness is 48 species (relatively far away from the 36 species actually observed). This indicates the four eDNA samples is not yet enough to show the entire fish assemblage based on eDNA detection.

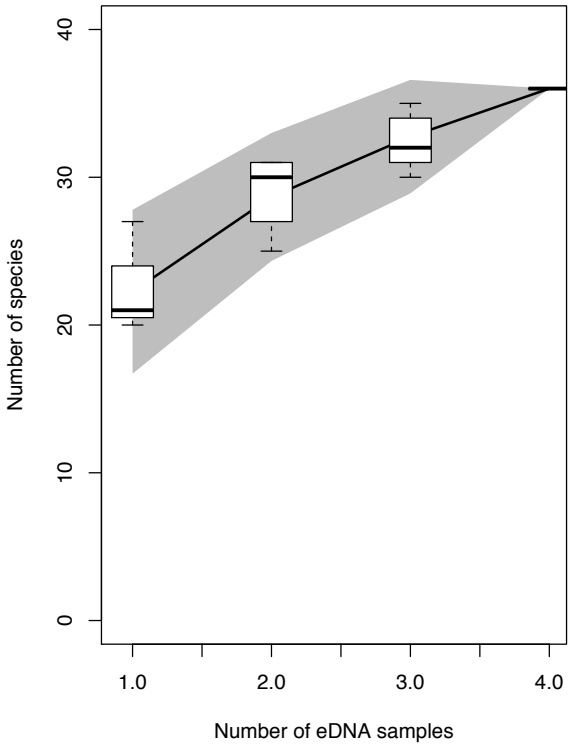


Figure 3.6. Species accumulation curve based on eDNA detection with four water samples.

Comparison between eDNA detection and seine net fishing

All ten fish species that were observed during the seine net fishing campaign were also observed with eDNA detection. The amount of DNA barcodes of these species was relatively high in most samples (table 3.2). Monky goby was an exception. Although this species was detected, the number of DNA barcodes was low and the species was detected in only one sample.

As described above, eDNA detection resulted in a considerably higher species richness (n=36) compared to seine net fishery (n=10) that was conducted on the same time. Although part of the observed species may be considered as false positives

(detected in one sample with an extreme low number of barcodes), eDNA detection resulted in an additional list of species that most likely are present in the study area but were not observed by seine net fishery. This is at least valid for species that occur on more than two sample locations with higher amounts of eDNA barcodes, i.e., European eel, barbel, nase, bighead goby, whitefin gudgeon, vimba bream, pike, gudgeon, common dace and chub (table 3.2).

eDNA detection of allis shad in Lobith - Netherlands

One of the goals of the eDNA pilot in the present study is to test whether allis shad could be detected using eDNA metabarcoding. Unfortunately, the species was not detected, neither based on eDNA of seine net fisheries. Based on the high species richness of the fish assemblage including species that occur in low densities, it may be suggested allis shad was not present in the study area during the study (i.e., Octobre 24, 2018).

In the Netherlands it has been shown that allis shad can actually be detected by means of eDNA metabarcoding. On November 29, 2018, allis shad was detected in one watersample (out of set of nine samples) in the Rhine at Lobith near the Dutch-German border (van Kessel *et al.* in prep). This sample was part of large-scale Dutch eDNA pilot study Netherlands where various waters including the Rhine at Lobith have been monitored in March, April, May and in September, October and November (2018). The positive detection of allis shad is probably the result of YOY migrating downstream. This indicates that despite the low densities of allis shad, it is possible to determine the presence of the species when the number of samples is high enough and samples are collected in the most suitable season.

4 Discussion & conclusions

4.1 Presence of allis shad

One of the goals of the present study was to test whether allis shad could be detected using seine net fisheries and eDNA metabarcoding. The species was not observed on the moment of sampling (October 24, 2018). Since the combination of both methods provides a fair chance of catching YOY allis shad, it is not likely higher number of allis shad were present in the study area on the moment of sampling. The seine net fishing did show the presence of other fish species that occur in allis shad habitat, such as common bleak, ide and asp. This indicates fishing efficiency should be sufficient for catching allis shad.
Additionally, one month after the sampling campaign of the study area, allis shad was detected in the Rhine at the Dutch/German border by means of eDNA metabarcoding (unpublished data). This indicates eDNA metabacoding is indeed a possible method to detect the presence of allis shad in fast flowing river such as the Rhine. Possibly, the downstream migration of YOY allis shad was delayed in 2018 (e.g., a result of extreme low water discharge of the Rhine due to drought).

4.2 Seine net fisheries as a monitoring tool

The seine net fishing campaign showed a fish assemblage of ten species. Although the actual fish assemblage of the study area is higher (see also eDNA results), seine net fisheries results in relatively constant densities of riverine fish such as common bleak. It should be noted that the fishing campaign was conducted relatively late in the season when activity of riverine fishes is lower compared to other seasons.

This is illustrated by a year-round seine net survey (comparable with the method in the present study) of a river stretch of the Rhine/Waal near Tiel (Netherlands; Collas et al., 2018; River Care Project), figure 4.1. Here, there is clear drop in fish densities and species richness in the winter period, starting late autumn.

The observed fish assemblage based on the seine net fishing campaign in the study area also shows the method is applicable in habitats with abiotic conditions in the Rhine between Rees and Wesel (i.e., fast water flow)

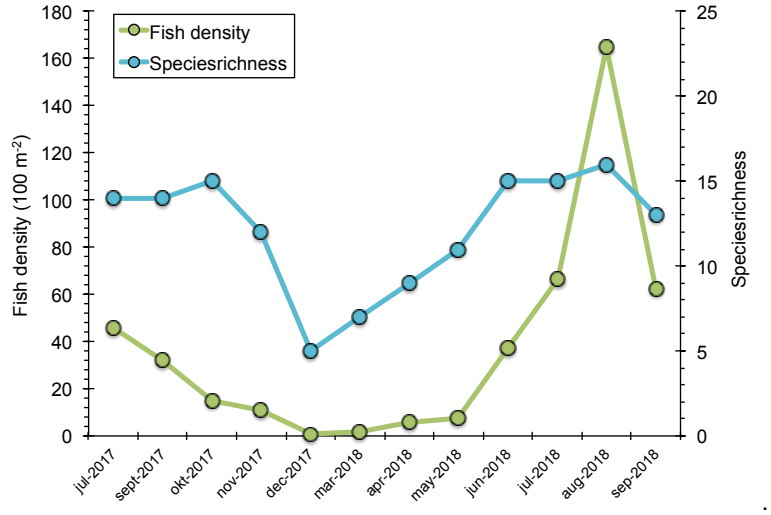


Figure 4.1. Change of fish density and species richness between July 2017 - September 2018 in a year round monitoring survey of the Waal at Tiel (Collas et al., 2018; River Care Project).

Effect of day and night fishing and the number of hauls

The fishing campaign also showed a significant effect of either fishing at night or during day. Despite the lower number of hauls during night, the detection chance of most fish species was higher at night than during day. With respect to monitoring, it is preferable to fish at night rather than during day.

Estimated species richness and species accumulation curves showed the number of hauls (especially at night) is rather low. To ensure a representative species richness it is recommendable to increase the number of hauls to a minimum of ten.

4.3 eDNA metabarcoding as a monitoring tool

As described in the previous chapter, species richness obtained by means of eDNA detection was considerably larger than based on seine net fishing. Detection chances of most fish species are relatively large. Various species that were likely to occur in Rhine were not observed with seine net fishing, but could be detected with eDNA metabarcoding. At the same time, there is a chance on false positive detections. Species that are detected in only one sample and/or in a very low amount of DNA barcodes should be interpreted with care. The detection of allis shad at Lobith in November 2018 indicates eDNA detection can be a valuable addition in monitoring rare riverine fish species such as allis shad.

Number of replicates

In the present study the number of replicate eDNA samples was four. The highest species richness was obtained by combining all samples. Based on estimated species richness and species accumulation curves the number of eDNA samples should be increased to a minimum of eight to provide a representative estimation of the fish assemblage. It should be noted that allis shad at Lobith was also shown in only one of

nine samples, indicating multiple samples (or a larger watervolume) are necessary to detect species that only occur in (extreme) low densities.

4.4 Recommendations for future research

Seine netting and eDNA metabarcoding have resulted in the detection of both abundant and rare riverine fish species that occur in the same habitat as allis shad. Allis shad however was not caught or detected in this study. However, the results of this study and current research (unpublished data) indicate that both methods should be sufficient to catch and/or detect allis shad in the river Rhine. The following recommendations are proposed:

- Seine netting: species richness and detection chance was highest at night. Therefore it is preferable to fish at night. Species accumulation curves show that the number of six hauls is rather low and should increase to at least ten hauls (five up- and five downstream hauls).
- The dispersal of YOY fish is influenced by various parameters, e.g. habitat preferences, time of year, water flow and level and weather conditions. Therefore, at least two seine netting campaigns should be conducted when densities are highest; in June-July (larvae and juveniles) and September (juveniles) (e.g., Dorenbosch et al. 2017).
- Estimated species richness and species accumulation curves indicate that eDNA metabarcoding samples should be increased to a minimum of ten samples. Unpublished data from a current study conducted by Bureau Waardenburg indicates that a larger water volume is necessary to detect rare species. The use of closed filters is likely to decrease the number of false positive detection, due to the prevention of for instance contamination by the researchers.

eDNA sampling by quantitative Polymerase Chain Reaction (qPCR) may be suitable to detect aggregations of rare species during breeding season. qPCR is specifically used to detect a single target species and quantifies the number of DNA molecules of a species in a sample. To increase detection probability 8-12 PCR replicates are recommended (Herder *et al*. 2014). The number of positive replicates per sample may reflect the increase (spawning aggregation and juveniles) and decrease (downstream migration of juveniles) of a species over time. Sampling should be conducted monthly during January – December.

5 References

Boyer F., Mercier C., Bonin A., Taberlet P., LeBras, Y., Taberlet, P., Coissac E., 2016. OBITools: a Unix-inspired software package for DNA metabarcoding. Molecular Ecology Resources 16: 176-82.

Collas, F.P.L., N. van Kessel. A.D. Buijse & R.S.E.W. Leuven, 2018. Maandelijkse vismonitoring oevergeul langsdammen en kribvakken gedurende 2017 – 2018. Reeks verslagen Dierecologie en Fysiologie 2018-4. Department of Animal Ecology and Ecofphysiology, Institute for Water and Wetland Research, Radboud University, Nijmegen, The Netherlands. (*In Dutch*)

Dorenbosch, M. & N. van Kessel (2017). Vismonitoring natuurvriendelijke oevers Maas. Functionaliteit voor vis in de periode 2011 - 2017. Bureau Waardenburg Rapportnr. 17-201. Bureau Waardenburg, Culemborg. (*In Dutch*)

Herder, J.E., A. Valentini, E. Bellemain, T. Dejean, J.J.C.W. van Delft, P.F. Thomsen and P. Taberlet, 2014. Environmental DNA - a review of the possible applications for the detection of (invasive) species. Stichting RAVON, Nijmegen. Report 2013-104

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. Package 'vegan': community ecology package. R Package Version 2.5-5. Retrieved from:. https://cran.r-project.org/web/packages/vegan/vegan.pdf.

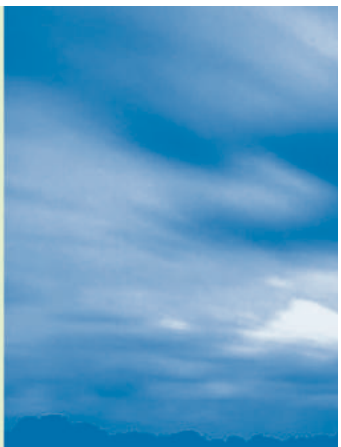
Van Kessel, N. & J. Kranenbarg, 2012. Vissenatlas Gelderland. Ecologie en verspreiding van zoetwatervissen in Gelderland. Uitgeverij Profiel, Bedum. (*In Dutch*)

6 Appendix: Fish data

1/2

Fishspecies	Dutchname	Latinname	Fish length	Count	Day/Night	Date	Locationnr.	Coordinate-x (longitude)	Coordinate-y (latitute)	Surface (m2)	Up- or downstream Bislich
Common bleak	Alver	*Alburnus alburnus*	11	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	12	2	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	12	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Zander	Snoekbaars	*Sander lucioperca*	12	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	13	3	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	14	7	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	15	12	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bream	Brasem	*Abramis brama*	15	2	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	16	8	Day	24-10-18	4	51,6948	6,4646	525	downstream
Zander	Snoekbaars	*Sander lucioperca*	16	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Asp	Roofblei	*Aspius aspius*	16	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	17	2	Day	24-10-18	4	51,6948	6,4646	525	downstream
Common bleak	Alver	*Alburnus alburnus*	18	2	Day	24-10-18	4	51,6948	6,4646	525	downstream
Asp	Roofblei	*Aspius aspius*	18	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Asp	Roofblei	*Aspius aspius*	35	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Zander	Snoekbaars	*Sander lucioperca*	40	1	Day	24-10-18	4	51,6948	6,4646	525	downstream
Ide	Winde	*Leuciscus idus*	17	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	16	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	15	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	12	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	14	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	13	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Zander	Snoekbaars	*Sander lucioperca*	16	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
European perch	Baars	*Perca fluviatilis*	13	2	Day	24-10-18	3	51,6866	6,4742	625	downstream
European perch	Baars	*Perca fluviatilis*	11	2	Day	24-10-18	3	51,6866	6,4742	625	downstream
European perch	Baars	*Perca fluviatilis*	12	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
European perch	Baars	*Perca fluviatilis*	10	3	Day	24-10-18	3	51,6866	6,4742	625	downstream
European perch	Baars	*Perca fluviatilis*	14	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
European perch	Baars	*Perca fluviatilis*	8	1	Day	24-10-18	3	51,6866	6,4742	625	downstream
Asp	Roofblei	*Aspius aspius*	70	1	Day	24-10-18	1	51,6849	6,4739	175	downstream
Common bleak	Alver	*Alburnus alburnus*	8	2	Day	24-10-18	2	51,6854	6,4742	510	downstream
Common bleak	Alver	*Alburnus alburnus*	6	1	Day	24-10-18	2	51,6854	6,4742	510	downstream
Common bleak	Alver	*Alburnus alburnus*	7	4	Day	24-10-18	2	51,6854	6,4742	510	downstream
European perch	Baars	*Perca fluviatilis*	15	1	Day	24-10-18	2	51,6854	6,4742	510	downstream
Common bleak	Alver	*Alburnus alburnus*	7	6	Day	24-10-18	10	51,6633	6,5027	775	upstream
Common bleak	Alver	*Alburnus alburnus*	11	1	Day	24-10-18	10	51,6633	6,5027	775	upstream
Common bleak	Alver	*Alburnus alburnus*	10	1	Day	24-10-18	10	51,6633	6,5027	775	upstream
Common bleak	Alver	*Alburnus alburnus*	6	1	Day	24-10-18	10	51,6633	6,5027	775	upstream
Common bleak	Alver	*Alburnus alburnus*	9	7	Day	24-10-18	10	51,6633	6,5027	775	upstream
Common bleak	Alver	*Alburnus alburnus*	8	7	Day	24-10-18	10	51,6633	6,5027	775	upstream
Round Goby	Zwartbekgrondel	*Neogobius melanostomus*	5	1	Day	24-10-18	10	51,6633	6,5027	775	upstream
Round goby	Zwartbekgrondel	*Neogobius melanostomus*	6	1	Day	24-10-18	10	51,6633	6,5027	775	upstream
Common bleak	Alver	*Alburnus alburnus*	8	1	Day	24-10-18	11	51,6638	6,5023	1800	upstream
Common bleak	Alver	*Alburnus alburnus*	5	24	Day	24-10-18	11	51,6638	6,5023	1800	upstream
Common bleak	Alver	*Alburnus alburnus*	6	18	Day	24-10-18	11	51,6638	6,5023	1800	upstream
Common bleak	Alver	*Alburnus alburnus*	10	1	Day	24-10-18	11	51,6638	6,5023	1800	upstream
Common bleak	Alver	*Alburnus alburnus*	7	2	Day	24-10-18	11	51,6638	6,5023	1800	upstream
Round goby	Zwartbekgrondel	*Neogobius melanostomus*	10	1	Day	24-10-18	11	51,6638	6,5023	1800	upstream
Zander	Snoekbaars	*Sander lucioperca*	52	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Asp	Roofblei	*Aspius aspius*	40	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Asp	Roofblei	*Aspius aspius*	32	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Asp	Roofblei	*Aspius aspius*	29	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Asp	Roofblei	*Aspius aspius*	15	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Asp	Roofblei	*Aspius aspius*	31	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Asp	Roofblei	*Aspius aspius*	27	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Common bleak	Alver	*Alburnus alburnus*	16	1	Day	24-10-18	7	51,6603	6,5172	495	upstream
Common bleak	Alver	*Alburnus alburnus*	6	27	Day	24-10-18	5	51,6599	6,5456	680	upstream
Common bleak	Alver	*Alburnus alburnus*	5	10	Day	24-10-18	5	51,6599	6,5456	680	upstream
Common bleak	Alver	*Alburnus alburnus*	7	8	Day	24-10-18	5	51,6599	6,5456	680	upstream
Common bleak	Alver	*Alburnus alburnus*	8	3	Day	24-10-18	5	51,6599	6,5456	680	upstream
Common bleak	Alver	*Alburnus alburnus*	9	1	Day	24-10-18	5	51,6599	6,5456	680	upstream
Zander	Snoekbaars	*Sander lucioperca*	51	1	Night	24-10-18	4	51,6948	6,4646	562	downstream
Zander	Snoekbaars	*Sander lucioperca*	12	1	Night	24-10-18	4	51,6948	6,4646	562	downstream
Zander	Snoekbaars	*Sander lucioperca*	13	1	Night	24-10-18	4	51,6948	6,4646	562	downstream
Common bleak	Alver	*Alburnus alburnus*	15	1	Night	24-10-18	4	51,6948	6,4646	562	downstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	12	1	Night	24-10-18	4	51,6948	6,4646	562	downstream
Ide	Winde	*Leuciscus idus*	27	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	12	2	Night	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	15	2	Night	24-10-18	3	51,6866	6,4742	625	downstream
Ide	Winde	*Leuciscus idus*	13	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	27	2	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	28	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	24	2	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	23	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	12	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	11	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	10	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	54	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Round goby	Zwartbekgrondel	*Neogobius melanostomus*	7	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Round goby	Zwartbekgrondel	*Neogobius melanostomus*	5	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	25	2	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	16	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	20	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	12	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	15	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	19	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Ruffe	Pos	*Gymnocephalus cernua*	9	1	Night	24-10-18	3	51,6866	6,4742	625	downstream

Fishspecies	Dutchname	Latinname	Fish length	Count	Day/Night	Date	Locationnr.	Coordinate-x (longitude)	Coordinate-y (latitude)	Surface (m2)	Up- or downstream Bislich
Ruffe	Pos	*Gymnocephalus cernua*	10	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Asp	Roofblei	*Aspius aspius*	15	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Zander	Snoekbaars	*Sander lucioperca*	12	1	Night	24-10-18	3	51,6866	6,4742	625	downstream
Common bream	Brasem	*Abramis brama*	22	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Common bream	Brasem	*Abramis brama*	27	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Ide	Winde	*Leuciscus idus*	16	5	Night	24-10-18	2	51,6854	6,4742	590	downstream
Ide	Winde	*Leuciscus idus*	17	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Ide	Winde	*Leuciscus idus*	15	3	Night	24-10-18	2	51,6854	6,4742	590	downstream
Ide	Winde	*Leuciscus idus*	13	3	Night	24-10-18	2	51,6854	6,4742	590	downstream
Ide	Winde	*Leuciscus idus*	12	2	Night	24-10-18	2	51,6854	6,4742	590	downstream
Ide	Winde	*Leuciscus idus*	11	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Asp	Roofblei	*Aspius aspius*	16	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Asp	Roofblei	*Aspius aspius*	18	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Zander	Snoekbaars	*Sander lucioperca*	51	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	18	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	12	2	Night	24-10-18	2	51,6854	6,4742	590	downstream
Common roach	Blankvoorn	*Rutilus rutilus*	11	2	Night	24-10-18	2	51,6854	6,4742	590	downstream
Common bleak	Alver	*Alburnus alburnus*	17	1	Night	24-10-18	2	51,6854	6,4742	590	downstream
Common bleak	Alver	*Alburnus alburnus*	4	10	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	7	7	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	8	4	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	6	23	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	5	43	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	3	1	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	10	2	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bleak	Alver	*Alburnus alburnus*	9	2	Night	24-10-18	10	51,6633	6,5027	720	upstream
Asp	Roofblei	*Aspius aspius*	29	1	Night	24-10-18	10	51,6633	6,5027	720	upstream
Asp	Roofblei	*Aspius aspius*	9	1	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common bream	Brasem	*Abramis brama*	26	1	Night	24-10-18	10	51,6633	6,5027	720	upstream
Zander	Snoekbaars	*Sander lucioperca*	13	2	Night	24-10-18	10	51,6633	6,5027	720	upstream
Common roach	Blankvoorn	*Rutilus rutilus*	12	1	Night	24-10-18	10	51,6633	6,5027	720	upstream
Zander	Snoekbaars	*Sander lucioperca*	41	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Zander	Snoekbaars	*Sander lucioperca*	64	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Zander	Snoekbaars	*Sander lucioperca*	38	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Zander	Snoekbaars	*Sander lucioperca*	29	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Zander	Snoekbaars	*Sander lucioperca*	30	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Zander	Snoekbaars	*Sander lucioperca*	36	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Asp	Roofblei	*Aspius aspius*	29	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	2	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	4	6	Night	24-10-18	7	51,6603	6,5172	450	upstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	3	2	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	27	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	28	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	30	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	23	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	25	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	24	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	29	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	31	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	13	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bream	Brasem	*Abramis brama*	9	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bleak	Alver	*Alburnus alburnus*	6	2	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bleak	Alver	*Alburnus alburnus*	5	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Common bleak	Alver	*Alburnus alburnus*	12	1	Night	24-10-18	7	51,6603	6,5172	450	upstream
Zander	Snoekbaars	*Sander lucioperca*	23	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Zander	Snoekbaars	*Sander lucioperca*	22	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Common roach	Blankvoorn	*Rutilus rutilus*	23	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Common roach	Blankvoorn	*Rutilus rutilus*	12	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Common roach	Blankvoorn	*Rutilus rutilus*	11	2	Night	24-10-18	5	51,6599	6,5456	705	upstream
Common roach	Blankvoorn	*Rutilus rutilus*	22	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	10	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Monkey goby	Pontische Stroomgrondel	*Neogobius fluviatilis*	4	1	Night	24-10-18	5	51,6599	6,5456	705	upstream
Common bleak	Alver	*Alburnus alburnus*	16	1	Night	24-10-18	5	51,6599	6,5456	705	upstream



Bureau Waardenburg bv
Onderzoek en advies voor ecologie en landschap
Postbus 365, 4100 AJ Culemborg
Telefoon 0345-512710, Fax 0345-519849
E-mail info@buwa.nl, www.buwa.nl