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Alternatives for trap monitoring in large rivers and lakes

Camera monitoring and eDNA sampling as alternative for conventional trap monitoring

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Summary

Wageningen Marine Research (WMR) executes a variety of fish monitoring programs in the Dutch rivers and lakes. These programs are often commissioned by the Ministry of Agriculture, Nature and Food Quality (Ministerie van Landbouw, Natuur en Voedselkwaliteit, LNV) and Rijkswaterstaat (RWS). LNV has interest in trend monitoring of silver eel. The interest of RWS is mainly due to the Water Framework Directive. For the Water Framework Directive, data is needed on fish species composition in an specific waterbody. Both interests are monitored with (eel) traps during the migration season (spring and autumn).

To run these monitoring programs, local fishermen are contracted to collect data and maintain the traps according to provided protocols from WMR. As these fishermen catch fish for scientific purposes they have to comply to strict catch regulations and agreements. For example, fish must be quickly released into the same waterbody after measurements. These regulations are to ensure proper handling of catches, data quality and to prevent poaching. When a fisherman fails to comply to these regulations, they will not be able to participate in future collaborations.

In recent years multiple fishermen have violated regulations and were therefore excluded from monitoring programs. WMR has found other fishermen to continue the monitoring in most of these areas. This report will evaluate two potential alternatives: camera monitoring and eDNA sampling. These pilot studies will provide first insights in applicability of both methods as an alternative for conventional trap monitoring.

The objective of this project was to determine how reliable eDNA and video monitoring is in determining fish species and how accurately individuals can be identified and counted. This project was a collaboration with DATURA (eDNA), Visserij Service Nederland and Kroes Brugman Technical Solutions (trap and video).

DATURA took eDNA samples as part of a first exploration in May 2016 at four locations: Belfeld, Lobith, Nieuwe Waterweg and Haringvliet. These results are compared with the trap monitoring at the same locations.

Results show that the eDNA samples have comparable species detection to trap monitoring. eDNA has a high potential for species composition in the large rivers, although more research with multiple samples per site (in time and space) are needed. eDNA and trap monitoring both observe fish that are not observed in the other methods. Trap monitoring has a disadvantage to miss relatively small fish such as *Barbatula barbatula* whereas eDNA may miss species that use the river systems as a corridor (e.g. salmonids). Although a comparison with trap monitoring is carried out, these results should be seen as a first exploration of this methodology in large river systems in the Netherlands, as limited samples were taken as part of a first exploration.

Camera monitoring has a high potential as an alternative for conventional trap monitoring. Fish are recorded by camera's and could be observed without handling of the fish. In the seven weeks of data collection (spring 2018) 19 different species were recorded. Data was analysed based on (1) recordings and catches and (2) based on a species identification test by experts and fisherman. The accuracy of identification varies by species. Flatfish were difficult to identify in the identification test. The accuracy of flatfish in the identification test was 40%. Between flatfish species, sole seemed to be the exception with a perfect score on the test. A top view camera could increase species identification for flatfish. Round fish had an accuracy of 66% on the identification test. Round fish seemed easier to identify from the images.

Of the 19 species caught, 10 of the 19 species were identified with an accuracy higher than 70% and 6 species higher than 90%. In freshwater river systems it is likely that species identification is more accurately having less species which are alike. Moreover, there is only one flatfish (flounder) present in the river systems.

For silver eel trend monitoring, eDNA sampling is not applicable since maturity stage cannot be distinguished. However, eDNA seems promising for species composition. Further research is needed to specify how many water samples in time and space are needed to identify rare diadromous fish species.

Camera monitoring does seem to be applicable for eel trend monitoring. However, further adjustments are needed in the camera set up to distinguish silver eel from yellow eel. Also, citizen science and involvement of fishermen for video analysis are needed to process the large amount of recordings. For species identification camera monitoring may also be applicable. However, flatfish are difficult to identify and a top view camera may solve this problem. Further research is needed to see whether this solves the problem.

1 Introduction

Wageningen Marine Research (WMR) executes a variety of fish monitoring programs in the Dutch rivers and lakes. These programs are often commissioned by the Ministry of Agriculture, Nature and Food Quality (Ministerie van Landbouw, Natuur en Voedselkwaliteit, LNV)[1] and Rijkswaterstaat (RWS)[2]. Some of the activities within the monitoring programs use traps for trend monitoring of diadromous fish which are present in the large lakes and large rivers for a limited amount of time during migration periods (spring and fall).

To run these monitoring programs, local fishermen are contracted to collect data and maintain the traps according to provided protocols from WMR. As these fishermen are contracted to catch fish for scientific purposes they have to comply to strict catch regulations and agreements. For example, fish must be quickly released into the same waterbody after measurements. These regulations are to ensure proper handling of the catches, data quality and to prevent poaching. When a fisherman fails to comply to these regulations, he will not be able to participate in future collaborations.

In recent years multiple fishermen have violated the rules and regulations and were therefore excluded from the monitoring programs. WMR has found other fishermen to continue monitoring in most of these areas. In this project two potential alternative methods are tested and evaluated: 1) camera monitoring and 2) eDNA sampling. These pilot studies will provide first insights in the applicability of both methods as an alternative for conventional trap monitoring.

Method 1: eDNA

The environmental DNA method (eDNA) is used to monitor the distribution of species. The method uses DNA-based identification, also called barcoding, to detect species from extracellular DNA, or cell debris, that species leave behind in the environment (Herder et al. 2014). With this method it is possible to detect species without actually seeing or catching them. eDNA does not require to actually catch fish and with this method fish are 'caught' indirectly by DNA traces in the water. Water samples are analysed on the presence of specific DNA of fish. The use of eDNA for species composition identification is a well-developed method in lakes (Herder et al. 2014). In small brook systems the use of eDNA is more challenging (Herder et al. 2014). The eDNA technique in (large) rivers has not been tested. Moreover, there are several questions whether this technique is applicable as an alternative for traditional trap monitoring. For example: what is the retention time of DNA in the water? How many samples are needed to prevent any false negatives? Where should samples be taken (shores, depth, etc.). Within this project a first exploration will be given with limited eDNA samples compared to trap monitoring. These samples were taken prior this project. Further en thorough research will be needed to answer previous questions.

Method 2: Camera monitoring

Internationally video techniques are available and for fish pass evaluation (VAKI 2016). These techniques are expensive and not always applicable in turbid waters. In the Netherlands a newly developed camera fish detection system (KBTS) may potentially be used as an alternative to count, identify and measure

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[1] LNV has interest in trend monitoring of silver eel. For this purpose, it is important to determine the maturity stage of eel and thus whether silver eel (mature) can be distinguished from yellow eel (immature).

[2] Interest of RWS is mainly due to the Water Framework Directive. For the Water Framework Directive, data is needed on fish species composition in a specific waterbody. In order to determine if camera monitoring is suitable to collect this data, the reliability and accuracy of species identification was tested.

fish. This project explores the possibility to use an 'open'[3] trap with additional video techniques to count and identify fish. Fish will be guided to the camera using a trap but they will be allowed to escape. The advantages of camera boxes are that they can be fixed to a certain location and they register fish without catching them. Since fish will not be caught, this also prevents the possibility of poaching. Furthermore, since fish do not have to be caught or handled which reduces damage and stress. However, camera monitoring also has disadvantages. The need of a power source and less accuracy in identifying species are several problems that camera monitoring could bring. And theft of equipment also becomes an issue. This has never been happened yet and with a 80-100kg submerged equipment seems unlikely to occur.

Overall aim of the project
This project aimed to determine if eDNA and video monitoring can be used as an alternative for conventional trap monitoring. The objective was to determine how reliable eDNA and video monitoring are in capturing fish species and how accurately individuals can be identified and counted. This project was carried out in collaboration with DATURA (eDNA), and Visserij Service Nederland and KBTS (trap and video).

1.1 Quality assurance

CVO is certified to ISO 9001:2015 (certificate number: 268632-2018-AQ-NLD-RvA). This certificate is valid until December 15th, 2021. The certification was issued by DNV GL Business Assurance B.V.

[3] An open trap is defined as a trap that will not catch the fish, since they are allowed to escape the trap.

2 Method 1: eDNA sampling

2.1 Collecting water samples and trap data

The current project uses previous collected data to compare trap monitoring data and eDNA samples. These samples were not taken as part of this project. However, this project was used to report the results of this pilot study (eDNA in large rivers) and compare the results to trap monitoring.

DNA water samples were taken (in May 2016) at locations at which trap monitoring was also present: Lobith, Nieuwe Waterweg, Haringvliet and Belfeld (

Figure *2-1*). Trap monitoring was executed in spring (March – May). Catch is registered by professional fisherman which are yearly tested on their knowledge of fish species. This ongoing monitoring program is funded by LNV and RWS and is used for multiple evaluation programs (e.g. Eel Management Plan, Water Framework Directive).

At each location water samples were taken: 4L and 1L (Table 1). Each sample was analysed using one or two different genetic markers to detect fish species.

Figure 2-1 Locations of trap monitoring (each location has a different colour):

Table 1 Locations with samples taken (4L = 4 litre, 1L = 1 litre) and number of markers used for analysis.

Location	sample	markers
Haringvliet	4L	2
Haringvliet	1L	1
Nieuwe waterweg	4L	2
Nieuwe waterweg	1L	2
Nieuwe waterweg	1L	2
Lobith	1L	2
Belfeld	4L	2
Belfeld	1L	2

2.2 eDNA processing and data analysis

eDNA processing and data analysis protocol can be found in Appendix A (Dutch).

DNA samples and trap monitoring data are compared on species composition. eDNA samples were taken as a first exploration of the use of eDNA in large river systems. This report was to describe the results of the few samples that were taken. At the moment an extended research is executed also by DATURA. Within that research more samples are taken on multiple sites per location, as required for a thorough research. Although a comparison with trap monitoring is done within this report, results should be seen as a first exploration of this methodology in large river systems in the Netherlands.

3 Method 2: Camera monitoring

3.1 Monitoring location

The experiment location was at an existing trap monitoring location in the North Sea Canal near Ijmuiden, at the 'freshwater side' (brackish) of the southern sluice (*Figure 3-1*). This location was chosen due to its accessibility and already present trap setup, but most importantly due to its high diversity in fish species (both fresh water and marine species). The location was situated close to the sluices that separate the North Sea from the North Sea Canal. To determine the accuracy of the trap a location was chosen with a wide range of species, to test how accurately they could be recognized and distinguished from one another.

Figure 3-1 The monitoring location at the sluices near Ijmuiden

3.2 Experimental setup

The camera box was connected to the end of a conventional eel trap (*Figure 3-2*). A net trap was attached to a pole near the shore. The opening of the trap was approximately 4 meters from shore and had a width of 3 meters. The length of the trap was approximately 6 meters and tapers near the end of the trap.

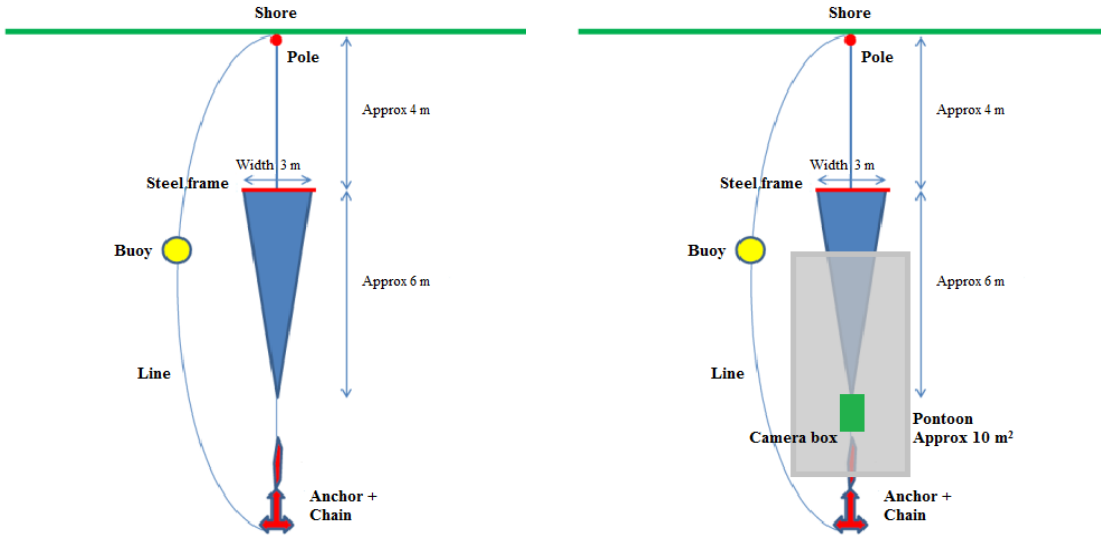

Figure 3-2 Schematic overview of the original trap and the trap with the camera box for this project

The trap was positioned using anchors and buoys. Standard monitoring uses this trap setup to observe fish. For the use of the camera box a few alterations were made to the trap. A 10 m^2 pontoon was positioned using anchors at every corner. The pontoon kept the camera box submerged and the trap in position and afloat (*Figure 3-3*).

The end of the trap was attached to the camera box. The camera is a 3 MP full HD IP camera with build in infrared (IR) LED's. For the experiments however, an external IR lamp was used to reduce the reflection into the camera lens. Fish that reached the end of the trap were guided through the camera box, which started recording once it detected movement. At the other side of the camera box a collection net was attached which collected all fish that had passed the camera box. This collection net was emptied at least once a week. At these moments the camera box (glass) was cleaned.

Can

Figure 3-3 The pontoon with camera that was used at the monitoring location near the sluices of the North Sea canal near Ijmuiden

3.3 Data collection

The monitoring program ran from the beginning of March until May. During this period the trap was deployed continuously over the whole period. Due to the amount of recordings it was chosen to analyse one month: from 5th of April until 14th of May.

The camera system was triggered by movement and started recording when fish entered the camera box. This means that during analyses only relevant time frames at which fish passed the trap needed to be analysed. And no time was lost searching for the passing fish.

The recordings were made using a high-resolution camera. With sufficient light, the camera was able to record in colour. However, as waters in the Netherlands are murky and the camera was shaded by the pontoon, the camera system often automatically switched to infrared recording due to the absence of light. Identification of fish will then be based on grey scaled images.

The data was stored on a server above water and was collected approximately once a week on the same day when the trap was emptied. Data of the actual catches were measured during the emptying of the collection net once or twice a week. Each emptying was considered as a 'period'. In total there were nine periods between 5th of April and 14th of May. During the emptying of the collection net the fish were identified to species, counted, total length measured (cm) before release (Photo 1).

Photo 1 Example of a four-beard rockling caught within the experiment.

3.4 Data analyses

Due to the fact that the availability of software for visual recognition and classification of fish without human observers is limited and expensive, this project used manual species identification for classification and counting.

3.5 Species determination

Species identification was done by observing physiological characteristics of species (appendix A). The analyses was split into two parts:

Part 1
In part 1 the goal was to find and distinguish all individual fish that were caught in the collection net on the camera recordings. There were false detections of murky water, sediment, sunlight etc. Once data was collected, these irrelevant recordings were removed. Every recording was then analysed to identify the species. During this part 1, it was not important to determine accuracy but simply if all caught fish could be recognized and located in the recordings. Once all data was analysed, a selection of 20 videos of individual fish was made, in which compared to the actual catches, 100% were correctly identified.

Part 2
During part 2, these 20 videos were used in an identification test, to determine the potential of species recognition by other people or the potential for citizen science. In this test, experts in the field of marine and fresh water species were asked to identify the species that were shown in the videos. These experts consisted of fishermen, scientists or other specialists in the field of fish species. The experts were not given any knowledge of the actual catches and were only given information on the location of the camera box and in what period the recordings were collected. The goal was to simulate the situation where researchers or fishermen were asked for video analysis if the camera box were ever to be used as a

monitoring method. The experts were asked to identify the species on the recordings as accurately as possible. Experts also had the opportunity to describe characteristics or observations.

Using the results obtained from this test, an accuracy could be determined for classifying fish using video recordings. The results were compared with results from part 1 and also compared between experts. Accuracy of species identification was divided into three categories, round fish, flatfish and eel-like fish, all obtaining their own accuracy.

3.6 Counting of individuals

To test whether fish were missed during the experiment, counting of individual fish was done both from actual catches and video recordings. Each recording was analysed and treated independently. Fish that entered the box from the trap and swam into the collection net were given a "+". Fish that entered the camera box from the collection net and swam back up the trap were given a "-". Fish that swam into the box and returned to the same side were given a "0". Fish that stayed in the box during the whole recording were also given a "0".

Once all recordings were analysed, the amount of fish swimming back (-) were subtracted from the amount of fish swimming into the collection net (+). This total was then compared to the actual catches of that certain period. A total overview of all periods combined was made of the number of individuals found in the collection net and the amount found in the video recordings, per species.

3.7 Distinguishing yellow eel and silver eel

All video recordings of eel were analysed. With the use of physiological indicators (eye size and colour of the fish), an attempt was made to distinguish yellow eel from silver eel. In the identification test performed by external experts, a series of recordings containing eel were shown. It was the experts task to identify if a yellow eel or silver eel was shown in the video. The results between experts were compared to determine if there was any consistency in distinguishing yellow eel and silver eel.

3.8 Practical application

As the project was looking into the potential of camera monitoring as an alternative for conventional trap monitoring, not only species counting and classifying was taken into account, but also practical use. During the deployment there were different circumstances concerning weather, water conditions etc. But also, technical limitations and problems as well as benefits were all taken into account and described in the field.

This resulted in an overview of the possibilities of camera monitoring, its weaknesses and strengths compared to conventional methods, and points for future improvement and possibilities.

4 Results method 1: eDNA

To compare eDNA samples with trap monitoring, the trap monitoring data is divided into two periods: (1) May 2016 and (2) March, April and May 2016 ('spring') (Table 2**Error! Reference source not found.**). Since the samples of the eDNA were taken in May 2016, the best fit with monitoring data is assumed to be with trap monitoring data in May (1). However, the complete 'spring' data is also presented for comparison purposes.

In Belfeld 29 species were observed in the eDNA sample and 29 in the trap monitoring (May 2016). Mackerel and mullet were observed in the eDNA sample, which are highly unlikely species to be present in the river Meuse at this site (consumption fish?). Of the 29 fish, 19 species were observed in both the eDNA sample and the trap monitoring (May 2016). 10 species were only observed in the eDNA sample and 10 only in the trap monitoring.

In Haringvliet 17 species were observed in the eDNA sample and 17 in the trap monitoring (May 2016). In Haringvliet 8 species were observed both in the eDNA sample and the trap monitoring (May 2016). Of all the observed species, 9 species were only observed in the eDNA sample and 9 only in the trap monitoring (May 2016).

At Lobith 27 species were observed in the eDNA sample and 19 in the trap monitoring (May 2016). In Lobith 14 species were observed in both the eDNA sample and the trap monitoring (May 2016). Of all the observed species, 13 species were only observed in the eDNA sample and 5 only in the trap monitoring (May 2016).

At the Nieuwe Waterweg 54 species were observed in the eDNA sample and 32 in the trap monitoring (May 2016). At the Nieuwe Waterweg 21 species were observed in both the eDNA sample and the trap monitoring (May 2016). Of all the observed species, 10 species were only observed in the eDNA sample (including *Pangasius* a consumption fish) and 18 only in the trap monitoring (May 2016).

For the water framework directive (WFD) key indicator species are important for the ecological quality ratio (Eqr) (Van der Molen et al. 2016). In the Netherlands these measures are calibrated based upon trap monitoring. When a key indicator fish is not caught, it is likely that this will negatively influence the Eqr score. The monitoring data from Belfeld and Lobith is used for R7[4] watertype rivers, Haringvliet is used for R8 watertype (Tien et al. in prep). The Nieuwe Waterweg will be used for O2 watertypes (transitional waterbody), but measures ('maatlatten') to evaluate the water body are not finalized yet (Tien et al. in prep).

For R7 watertypes, eDNA samples missed two limnophilic species compared to trap monitoring in May 2016 and an additional two reophilic and one limnophilic species when the eDNA data is compared to data collected in spring 2016 (Table 2, Belfeld). In Lobith eDNA samples registered two extra reophilic species compared to the trap monitoring in May 2016 and only one compared to trap data collected in spring 2016. Also, trap monitoring caught one extra diadromous fish species compared to eDNA and one Limnophilic species (Table 2, Lobith). Haringvliet trap monitoring caught more diadromous and reophilic species compared to eDNA samples. However, trap monitoring missed limnophlic species.

[4]See literature for definition of each water type (Van der Molen et al. 2016).

Table 2 (part 1) Results of eDNA sampling and trap monitoring (May 2016 and March, April and May 2016 'spring'). Table shows eDNA results (black cells) and trap monitoring catches (grey cells).

Dutch name	Scientific name	Belfeld			Haringvliet			Lobith			Nieuwe Waterweg		
		eNDA may 2016	may 2016 trap	spring 2016	eNDA may 2016	may 2016 trap	spring 2016	eNDA spring 2016	may 2016 trap	spring 2016	eNDA may 2016	may 2016 trap	spring 2016
Brasem	Abramis brama												
Harnasmannetjes	Agonus cataphractus												
Alver	Alburnus alburnus												
Fint	Alosa fallax												
Zwarte dwergmeerval	Ameiurus melas												
Bruine dwergmeerval	Ameiurus nebulosus												
Zandspiering/Smelt	Ammodytes tobianus/ Hyperoplus lanceolatus												
Zeewolf	Anarhichas lupus												
Paling	Anguilla anguilla												
Glasgrondel	Aphia minuta												
Schurftvis	Arnoglossus laterna												
Roofblei	Aspius aspius												
Koornaarvis	Atherina sp.												
Bermpje	Barbatula barbatula												
Barbeel	Barbus barbus												
Geep	Belone belone												
Kolblei	Blicca bjoerkna												
lipvissen	Bodianus												
Dwergtong	Buglossidium luteum												
Pitvis	Callionymus lyra												
Goudvis	Carassius auratus												
Giebel	Carassius giebelius												
Kroeskarper	Carassius carassius												
Sneep	Chondrostoma nasus												
Vijfdradige meun	Ciliata mustela												
Haring	Clupea harengus												
Kleine modderkruiper	Cobitis taenia												
Grote marene	Coregonus lavaretus												
Houting	Coregonus oxyrinchus												
Rivierdonderpad	Cottus perifretum												
Rivier- of beekdonderpad	Cottus rhenanus/gobio												
Snotolf	Cyclopterus lumpus												
Karper	Cyprinus carpio												
Zeebaars	Dicentrarchus labrax												
vierdradige meun	Enchelyopus cimbrius												
Ansjovis	Engraulis encrasicolus												
Snoek	Esox lucius												
Grauwe poon	Eutrigla gurnardus												
Kabeljauw	Gadus morhua												
driedradige meun	Gaidropsarus vulgaris												
Driedoornige stekelbaars	Gasterosteus aculeatus												
Riviergrondel	Gobio gobio												
Zwarte grondel	Gobius niger												
Pos	Gymnocephalus cernuus												
kortneusdraakvissen	Hydrolagus mirabilis												
Lipvissen	Labridae												
Rivierprik	Lampetra fluviatilis												
Zonnebaars	Lepomis gibbosus												
Vetje	Leucaspius delineatus												
Kopvoorn	Leuciscus cephalus												
Winde	Leuciscus idus												
Serpeling	Leuciscus leuciscus												

Table 2 (part 2) Results of eDNA sampling and trap monitoring.

Dutch name	Scientific name	Belfeld			Haringvliet			Lobith			Nieuwe Waterweg		
		eNDA may 2016	trap may 2016	spring 2016	eNDA may 2016	trap may 2016	spring 2016	eNDA spring 2016	trap may 2016	spring 2016	eNDA may 2016	trap may 2016	spring 2016
Schar	Limanda limanda												
Slijmvis	Lipophrys pholis												
Wijting	Merlangius merlangus												
Zeedonderpad	Myoxocephalus scorpius												
Pontische stroomgrondel	Neogobius fluviatilis												
Zwartbekgrondel	Neogobius melanostomus												
Regenboogforel	Oncorhynchus mykiss												
Spiering	Osmerus eperlanus												
Pangasius	Pangasius												
Gehoornde slijmvis	Parablennius gattorugine												
Baars	Perca fluviatilis												
Zeeprik	Petromyzon marinus												
Botervis	Pholis gunnellus												
Elrits	Phoxinus phoxinus												
Koolvis	Pollachius pollachius												
Zwarte koolvis	Pollachius virens												
Kleurige/ lozano's grondel	Pomatoschistus lozanoi/pictus*												
Brakwatergrondel	Pomatoschistus microps												
Dikkopje	Pomatoschistus minutus												
Kesslers grondel	Ponticola kessleri												
Marmergrondel	Proterorhinus semilunaris												
Tiendoornige stekelbaars	Pungitius pungitius												
Vorskwab	Raniceps raninus												
Bittervoorn	Rhodeus amarus												
Witvingrondel	Romanogobio belingi												
Blankvoorn	Rutilus rutilus												
Zeeforel/beekforel	Salmo trutta												
Snoekbaars	Sander lucioperca												
Europese sardine	Sardina pilchardus												
Ruisvoorn	Scardinius erythrophthalmus												
Makreel	Scomber scombrus												
Tarbot	Scophthalmus maximus												
Griet	Scophthalmus rhombus												
Meerval	Silurus glanis												
Tong	Solea solea												
Sprot	Sprattus sprattus												
Kopvoorn	Squalius cephalus												
Zwartooglipvis	Symphodus melops												
Grote zeenaald	Syngnathus acus												
Groene zeedonderpad	Taurulus bubalis												
Zeelt	Tinca tinca												
Horsmakreel	Trachurus trachurus												
Steenbolk	Trisopterus luscus												
Puitaal	Zoarces viviparus												
Harders	**Mugilidae**												
Diklipharder	Chelon labrosus												
Dunlipharder	Liza ramada												
Schol/bot	**Pleuronectes platessa/Platichtus flesus**												
bot	Platichtus flesus												
schol	Pleuronectes platessa												
TOTAAL		29	29	32	17	17	24	27	19	22	54	32	49
Number of species caught both in trap and eDNA sample			19	19		8	11		14	16		21	19
Number of species present in only the eDNA sample			10	10		9	6		13	11		10	8
Number of species present in only the trap monitoring			10	13		9	13		5	6		18	23
*pomatoschistus, both species are not present in the eDNA database													

Table 3 Results of key indicator fish species (Water Framework Directive) observed for each method. D = diadromous fish, R = rheophilic fish, L = limnophlic fish according to van der Molen et al. (2016). Nieuwe Waterweg monitoring is not used for river watertypes.

				Belfeld (R7)			Haringvliet (R8)			Lobith (R7)			Nieuwe Waterweg (O2*		
soort	R7	R8	R16	eDNA	trap may 2016	trap spring 2016	eDNA	trap may 2016	trap spring 2016	eDNA	trap may 2016	trap spring 2016	eDNA	trap may 2016	trap spring 2016
aal	D	D	D												
alver	R	R	R												
barbeel	D	D	D												
forel	R	R	R												
bittervoorn	L	L	L												
bot	D	D													
driedoornige stekelbaars	D	D													
elft	RD	RD	RD												
fint	D														
houting	RD	RD	RD												
kleine modderkruiper	R	R	R												
kopvoorn	R	R	R												
kroeskarper	L	L	L												
kwabaal	R	R	R												
rivierdonderpad	R	R	R												
riviergrondel	R	R	R												
rivierprik	RD	RD	RD												
ruisvoorn	L	L	L												
serpeling	R	R	R												
sneep	R	R	R												
spiering	D														
vetje	L	L	L												
winde	R	R	R												
zalm	RD	RD	RD												
zeelt	L	L	L												
zeeprik	RD	RD	RD												
R7 D				3	3	3				3	3	4			
R7 R				5	5	7				7	5	6			
R7 L				1	3	4				1	2	2			
R8 D							2	3	4						
R8 R							1	4	6						
R8 L							1	0	0						

5 Results method 2: camera

5.1 Monitoring period

The results were collected and analysed according to the described periods (*Table 4*). The start date of the experiments was later than expected due to technical issues during the first deployment. Over the course of the second deployment, a logbook was kept to record problems and defects in the system (Table 4). All fish that were caught during these periods were identified, measured and counted (Table 5). A few examples of fish are shown in *Figure 5-4*.

Table 4 Overview of monitoring periods (2018)

Period	Date	Remarks
1	05/04 – 09/04	When setting up for the second deployment, some videos presumably were lost between 5-6 of April.
2	09/04 – 16/04	Sunlight triggered the movement detection and caused a lot of false recordings. This was solved by placing a canvas over the pontoon, blocking off the sunlight.
3	16/04 – 18/04	-
4	18/04 – 21/04	The external IR-lamp broke and constantly turned on and off causing false recordings. Furthermore, there were several blackouts in which fish could have passed undetected.
5	21/04 – 24/04	IR-lamp was still broken. Furthermore, during troubleshooting the camera system was reset to default settings and not updated again. This resulted in a period with very low video quality.
6	24/04 – 30/04	As a temporary solution the IR-lights of the camera were activated. These lights however reflected back into the lens and caused more blind spots on the recordings.
7	30/04 – 03/05	At the beginning of this period, a new external IR-lamp was installed. Remarkably only eel was caught during this period.
8	03/05 – 07/05	-
9	07/05 – 14/05	Murky waters and algae growth and sedimentation in the camera box caused worse visibility than usual.

Table 5 overview of the total catches per Period

Species	Period	1	2	3	4	5	6	7	8	9	Total per species
Silver Eel		8	4	3	6	9	2	4	2	1	39
Yellow Eel				2				1		7	10
Sea Bass		3	5		5	3			3	10	29
Bass		1	2	1					1	2	7
Herring		1	4				1				6
Mullet			3	2	2		93			2	102
Whiting			1						1		2
Cod			1								1
Plaice			1	1							2
Flounder		22	10	4	2	6	2		5	1	52
Sole		1			1	1					3
River Lamprey			1								1
Ruffe		1									1
Rock goby		1					1				2
Five Beard Rockling		1									1
Four Beard Rockling									1		1
Common Rudd					1						1
Silver Bream						1					1
Round goby							1				1
Pouting							1				1
Total per period		39	32	13	17	20	101	5	13	23	

5.2 Accuracy of species determination

Mullet
The thick lip and thin lip mullet were distinguishable from other fish species. Distinguishing between thick lip and thin lip mullet using the camera seemed impossible. However, during conventional monitoring, there is also no distinction made between thin lip and thick lip mullet, since it is time consuming and no target species. In the current monitoring program they are classified as 'mullet sp.'

Flatfish
Out of the flat fish species that were caught, flounder and plaice remained very difficult to classify. Especially the distinction between each other was difficult to impossible, as flounder and plaice share many similar traits. Sole however, was easy to distinguish, mostly due to its body shape.

Additional species: rockling, goby, silver bream
Finally, among the additional species caught, the four-beard rockling and the rock goby were not taken up in the preliminary assessment. The distinguishing traits of the silver bream seemed more apparent than additionally anticipated. The traits were distinctive enough to be determined as silver bream and not normal bream, with which it is often confused. It is however important to mention that there were no bream caught during the experiment period for comparison.

20 videos were shown to experts in fresh water and marine species. In the analysis, no distinction was made between the candidates' backgrounds and data was combined of all participants. Not all videos were observed by all participants.

Most flatfish scored poorly with the exception of sole (100%, Figure 5-2). Plaice, which was shown twice in different videos, scored 0% and 7% (Figure 5-1 and Figure 5-2). Plaice was mainly confused with flounder and dab. Flounder also scored low. Several candidates also added that their identification of the flatfish was often more a guess then a solid answer. The only flatfish species caught that seemed to be easy distinguishable is the sole, scoring a perfect 100% .

The four beard and five beard rocklings both scored low on the test, ~25% of the candidates did manage to recognize the fish as a rockling but were unable to identify the exact species. This was mainly due to the fact that the amount of barbells could not be counted on the videos which is often the key physiological characteristic to distinguish rockling species. The five-beard rockling seemed to be easier to correctly classify (20%) then the four-beard rockling (7%). This could be due to the fact that five beard rocklings are found more often in Dutch coastal waters.

Interestingly, it seems the quality of the video also has an effect on identification. Flounder, which was shown twice, scored differently (Figure 5-1 and Figure 5-2). The first video scored 60% correct as the second video scored much lower with 36% correct. This indicates that aside from physiological characteristics of fish, the quality of the video also effects the ability to classify fish. Possibly due to the position of the fish in de video, water turbidity and swimming speed (and thus time to identify the fish). Overall, flatfish scored low compared to round fish, at 40% correct. This score is substantially increased by the score of the sole. Excluding sole, would results in a score of only 25% correct for the total flatfish. The combined score of all round fish is 66% identified correctly.

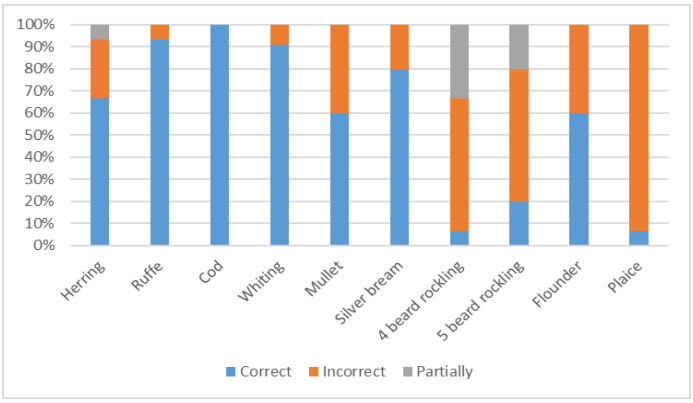

Figure 5-1 Results VIDEO 1-10. Results of identification test by experts. Partially correct means that the name of the species was incorrect but the species characteristics was described correctly. Number of participants n=15.

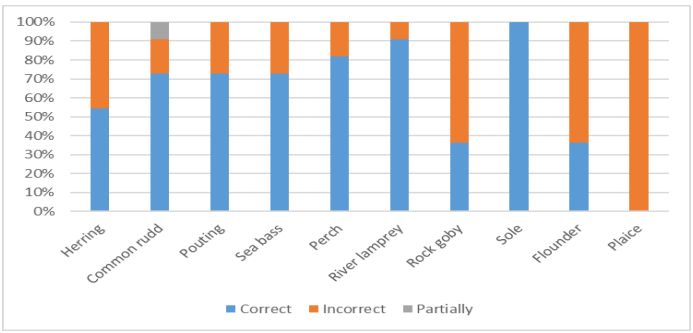

Figure 5-2 Results VIDEO 11-20. Results of identification test by experts. Partially correct means that the name of the species was incorrect but the species characteristics was described correctly. Number of participants n=11.

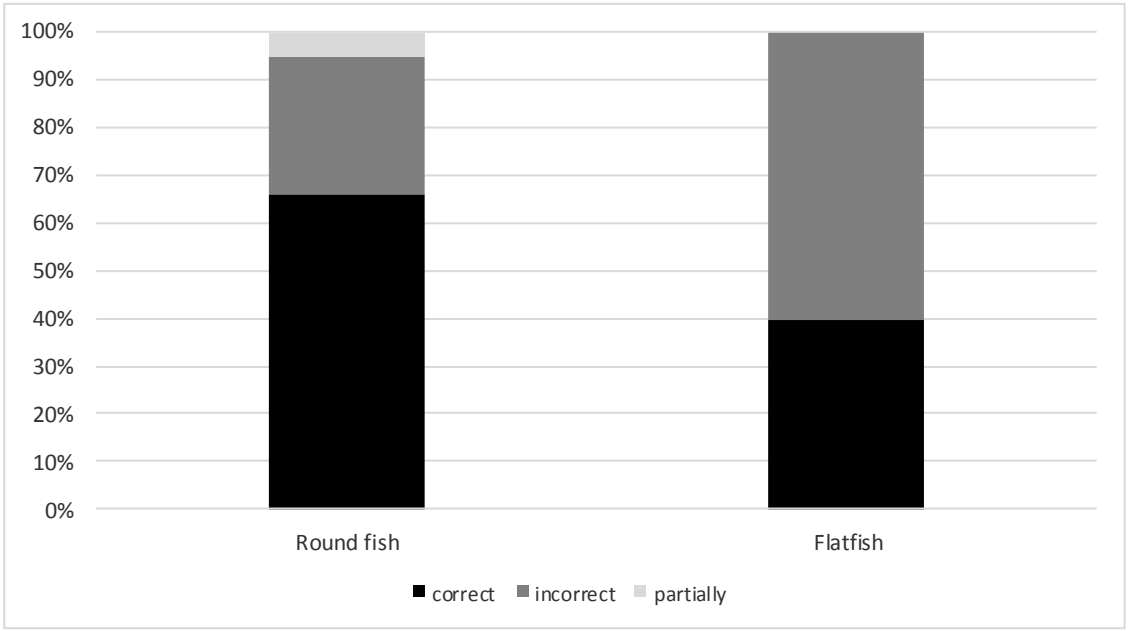

Figure 5-3 Total accuracy of round fish and flatfish in percentages

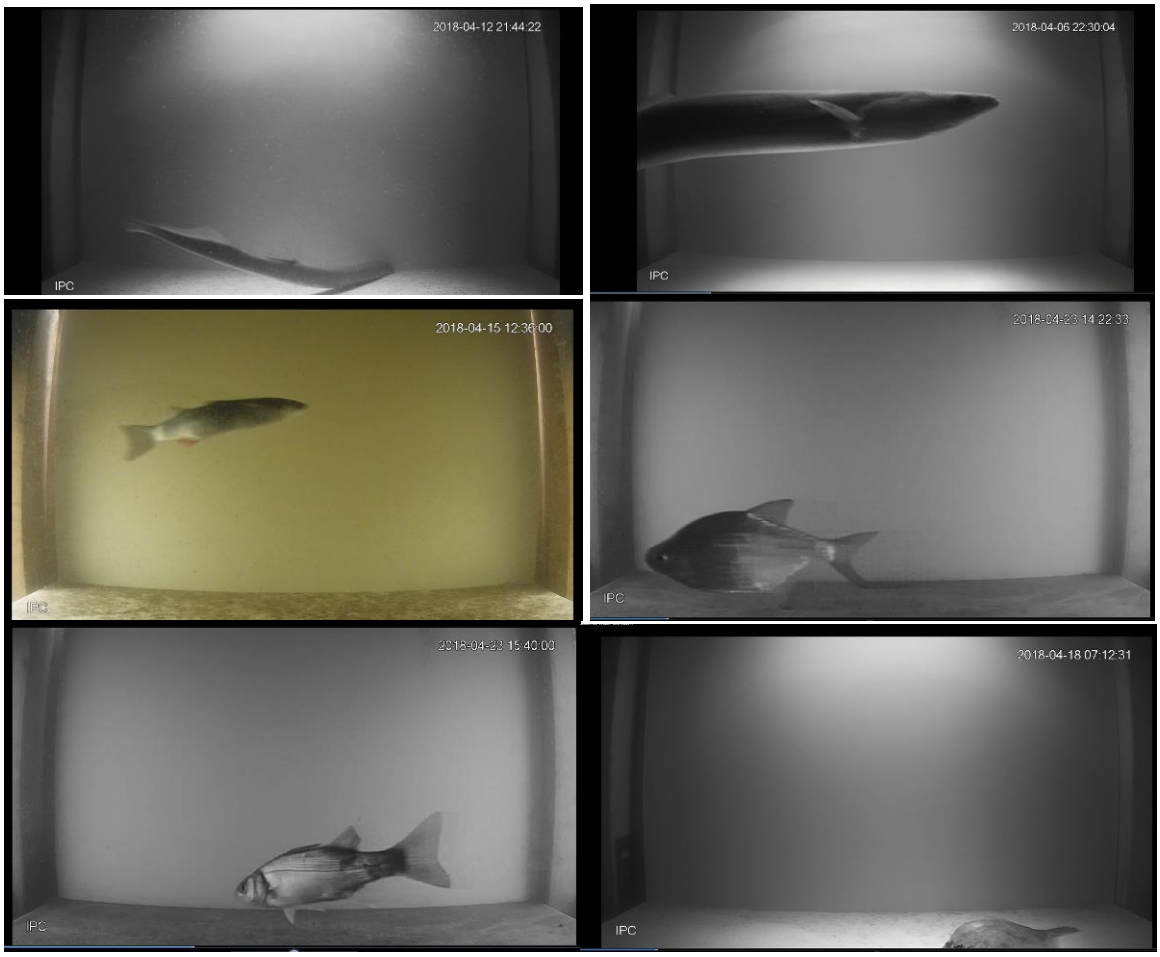

Figure 5-4 Examples of fish observed in the video recordings (left to right, top to bottom): River lamprey, silver eel, mullet (natural light), silver bream, seabass and plaice.

5.3 Accuracy of fish counting

Out of the 226 fish that were caught, 206 of them were found in the recorded videos (Figure 5-5). Individuals passing through the camera box were mostly identifiable and the swimming direction was clearly visible. Period 4 and 5 were excluded from the analyses due to technical issues with the IR lamp which caused blackouts. During these blackouts it was impossible to identify which fish had passed and was therefore useless in the analyses of individual counting.

After analyses of the video recordings, the amount of fish that was seen on video and the amount actually caught were compared (Figure 5-6 and Figure 5-7). Of some species, only one individual was caught during the monitoring period. Except round goby, all of these species were also found on the camera recordings.

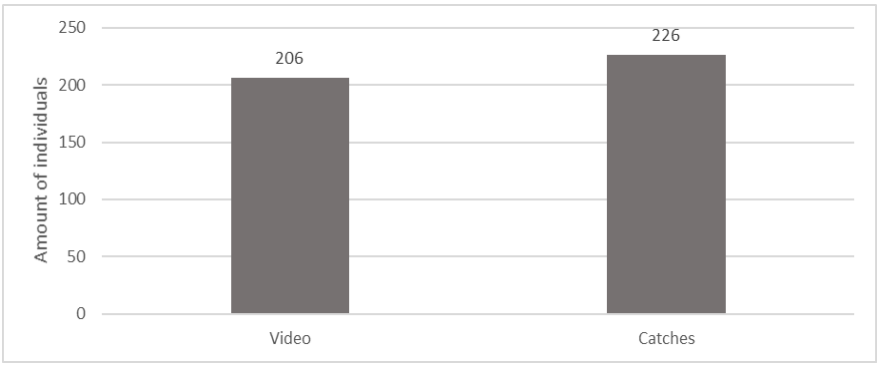

Figure 5-5 Total number of fish caught and counted in videos

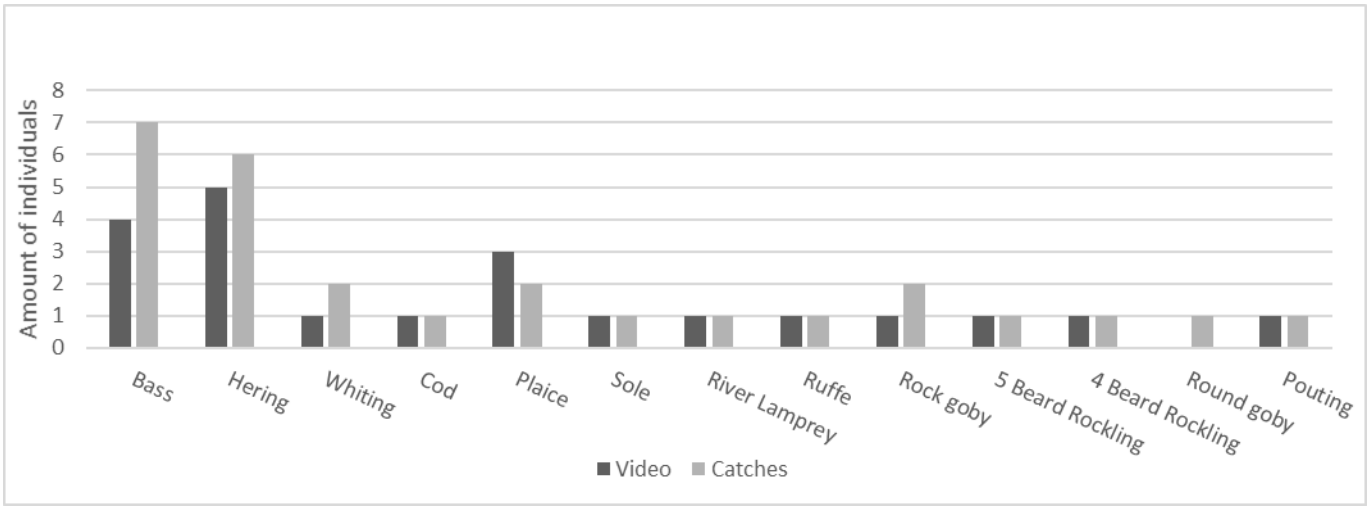

Figure 5-6 Counting results per species

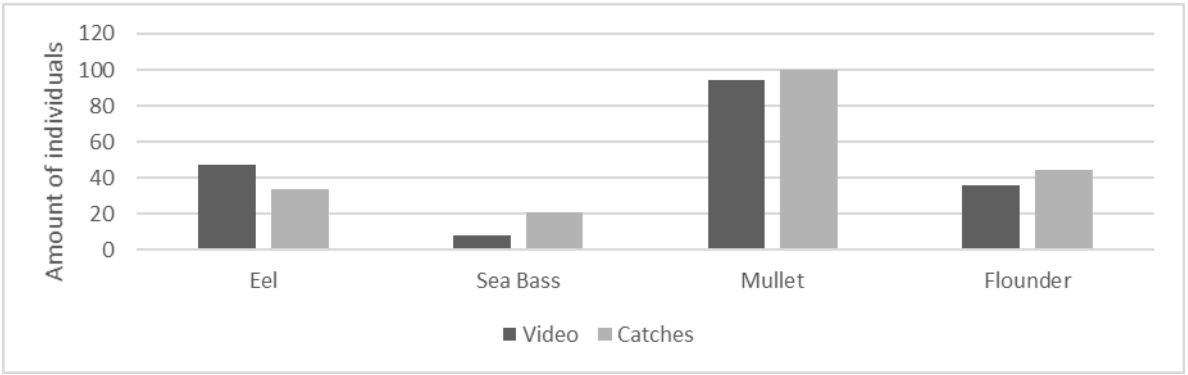

Figure 5-7 Counting results of most abundant caught species

One rock goby and one round goby could both not be found in video recordings. This was during period 6 at which the IR lamp was not working and the internal IR lamp from the camera was used. The internal IR lamp caused large blind spots on the bottom of the camera box due to light reflecting off the back panel into the camera lens. This could explain why these gobies could not be found on the recordings as gobies prefer to swim close to the bottom and therefore out of sight.

During analyses it was observed that perch had the tendency to remain in the camera box instead of continuing into the collection net. This caused a lot of potential double counting of fish or missing other individuals in between consecutive recordings of that particular perch.

It is possible that individuals swam through the camera box via the blind spot at the top of the camera box. Especially smaller fish and flatfish would be able to swim across the camera box this way, undetected or unrecognizable.

Some species were caught in higher abundance then others. There were more eel seen on the camera box then that were actually caught. Looking at the video recordings it seemed eel showed very active behaviour and were constantly looking for a way out. This active behaviour caused many detections and recordings. In several recordings eel would swim close to the bottom or at the top of the camera box which was out of view of the camera. For several recordings this behaviour resulted in inconclusive swimming direction.

The amount of sea bass seen on camera was lower than the actual catches, mostly caused by period 9. In period 9, only two out of ten sea bass were found on camera that were actually caught.

Most of the mullets were caught in period 6. In total 93 of these had passed the camera box over 2 days in period 6. Of these 93 caught, 91 individuals were counted on the video recordings. The remaining mullets (n=7) were caught during other periods.

Finally, the species were categorized by type (Figure 5-8). Round fish showed the largest difference between video counting and catches. Round fish were also the largest group and had the highest diversity. The difference is mainly caused by sea bass and perch.

All though flatfish were difficult to identify, they were easier to count. The main cause of the difference between video counting and catches is likely due to the camera having blind spots at the bottom and top. Flat fish could have passed undetected due to their body shape.

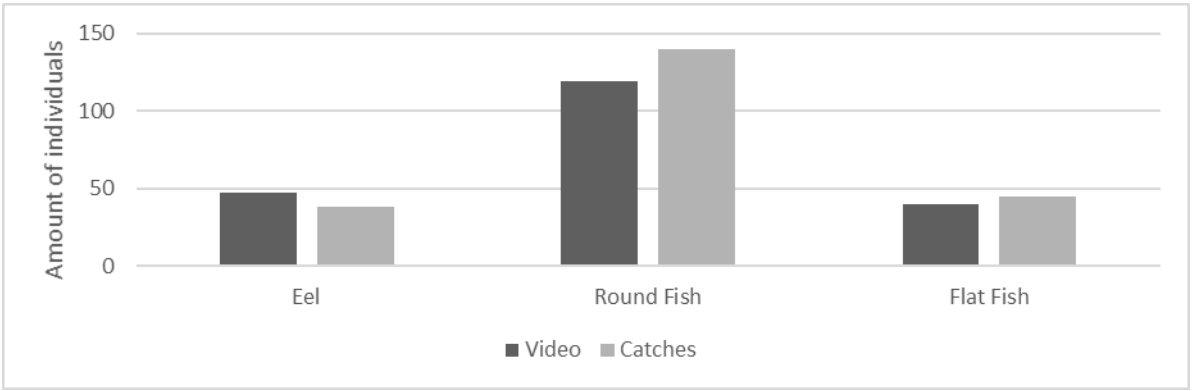

Figure 5-8 Counting results per fish type

6 Accuracy of eel recordings

During the experiment period, there were three periods in which both silver eel and yellow eel were caught and classified based on external characteristics and experience of fishermen. All remaining periods contained only silver eel. To compare video recordings with actual catches, it was necessary to have catches with only yellow eel or only silver eel to have 100% certainty of eel type. Estimating length from video recordings was not possible since most eel exceed the length of the camera box. During the experiment there were no catches with only yellow eel.

In period 9, in which 7 out of 8 eels were yellow eel, it was not possible to isolate the silver eel with 100% certainty.

All video recordings of eel were analysed. With the available recordings it was not possible to distinguish yellow eel from silver eel with a 100% certainty. Because of this, for the identification test by external experts, a period was chosen which contained one yellow eel and four silver eels. Using distinguishing marks or spots, all individual eel were isolated in the video recordings.

One video was shown of each individual eel and two videos of the eel that was presumed to be the yellow eel, to test consistency in the answers. Because during analyses it was not possible to distinguish the two, it was not possible to make a comparison of the catches with the results of the experts.

The results between experts were compared to determine if there was any consistency in distinguishing yellow eel and silver eel. The results of the test varied significantly (Figure 6-1). Between the candidates (n=10) that participated there was a clear difference in answers per individual. The remarks that were given by the candidates furthermore mentioned that most of the answers could not be given with complete certainty.

The presumed yellow eel, Eel 5, obtained different results in both videos. Eel 4 is the only video that seemed to have some consistency between the candidates.

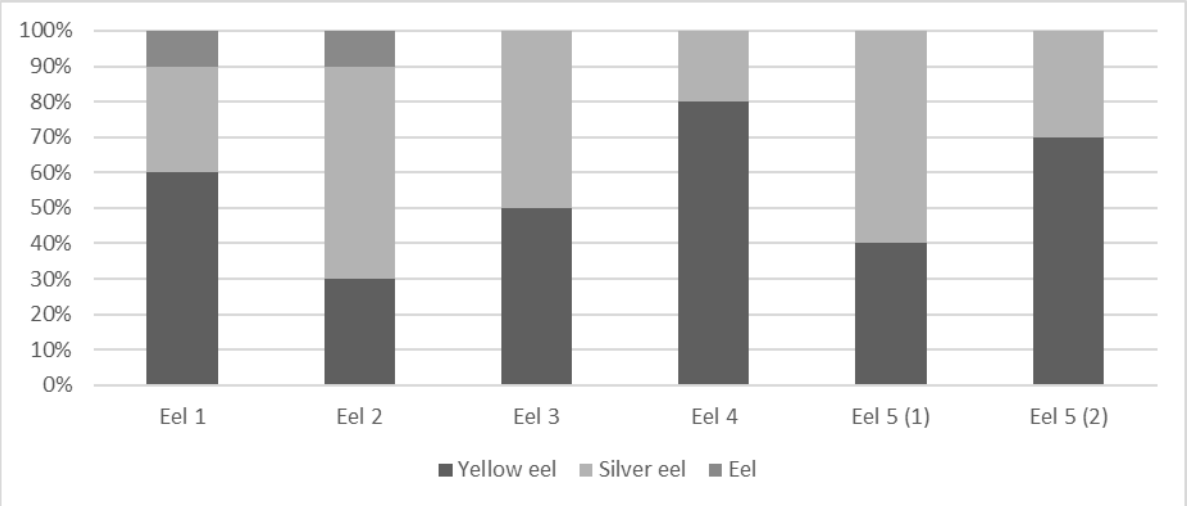

Figure 6-1 Results of the blind test for distinguishing eel (n=10 participants). Eel nr.5 (classified as yellow eel) was shown in two different videos to test consistency.

7 Conclusions and recommendations

7.1 Practical application: method 1 eDNA

Results show that limited eDNA samples has comparable species detection to trap monitoring. eDNA has a high potential for species composition in the large rivers, although more research with multiple samples per site (in time and space) are needed to 'catch' DNA of rare (few DNA) or also diadromous fish species that use the rivers as a corridor during their migration period. They are easily being missed if only one sample is taken. Also, eDNA does not have the ability to quantify the abundance (yet) and determine the trend of the presence of a species. Moreover, the eDNA data also does not allow to produce length frequency data or maturity stage determination. eDNA and trap monitoring both observe fish that are not observed in the other methods. Trap monitoring has a disadvantage to miss relatively small fish such as *Barbatula barbatula*. However, within this pilot study the most relevant species (key indicator species) for WFD monitoring are better caught in trap monitoring. This study showed high potential for eDNA. More water samples (in time and space) and eDNA analysis may have identified more species. More research is needed to decide how many samples should be taken throughout the year to catch more key indicator species. Moreover, it should be taken into account that (WFD) measures to evaluate the eqr score of a waterbody should be calibrated when eDNA samples are used for WFD evaluation. Finally, the results also showed that consumption fish are 'seen' in the waters (mullet, pangasius, mackerel). Especially for key indicator species such as salmon and trout, which are also both consumption fish, could affect eqr scores.

7.2 Practical application: method 2 Camera monitoring

Overall results of fish species
In the seven weeks of data collection, 19 different species were recorded. As was expected, flatfish were difficult to identify both during analyses, and at the identification test. The accuracy of flatfish identification at the test was 40%. Between flatfish species, sole seemed to be the exception with a perfect score. A top view camera could increase species identification in future projects, since the shape of flatfish could be better used for identification.

Round fish had an accuracy of 66% on the test. During analyses round fish seemed easier to identify compared to flatfish. 10 of the 19 species were identified with an accuracy higher than 70% and 6 species higher than 90%. In freshwater river systems it is likely that species identification is more accurately having less species which are alike. Moreover, only one flatfish (flounder) is present.

The accuracy of identification varies per species. With experience the accuracy will likely increase but certain species are likely to remain unidentifiable. Before application it would be important to determine what species are necessary for the monitoring program. The collected data resulted in an accuracy of 91% compared to actual fish counting from the catches. With fine tuning and removing the blind spots, the camera box should be able to approach an accuracy of 100% to monitor all fish. The quality of the video recordings made it possible to determine the swimming direction of the individuals and therefore prevented double counting of the same individual. Even if double counting does occur, it should not pose a problem for trend monitoring as long as it is done consistent for year to year comparison. Behaviour may alter when no trap is attached to the camera box, which is the proposed set up when the method is used for trend monitoring.

Silver eel and yellow eel
For distinguishing yellow eel and silver eel, more research is needed with more accurate video recordings of yellow eel. Although few yellow eel were recorded within the study, videos of both yellow and silver eel were shown to experts. Although IR does provide a clear recording in an otherwise too dark environment, IR recordings did limit the use of colour for identification as recordings were black and white. Recordings using artificial light may have facilitated species identification and distinguishing between silver and yellow eel better.

Practical issues
Even though fish will not have to be caught and measured with camera monitoring, maintenance is still needed. Depending on the local conditions of the monitoring location, the camera box will still require weekly visits to clean the trap and the camera box (glass).

The added benefit of camera monitoring is that it also gives an insight into the behaviour of fish which is not possible in conventional monitoring. However, for trend monitoring this information is not necessary. Also, fish that escape from a trap (e.g. turning around half way the trap) and are missed during trap monitoring could be recorded. Automatic analyses using sophisticated software or highly trained volunteers (citizen science) could increase to potential for being an alternative for trap monitoring. Apart from the technical problems with the external IR-lamp, which was solved easily, there were no major technical issues during the experiment period.

In this pilot study IR illumination was preferred above artificial illumination, as we expect eel might be frightened from artificial lights. This resulted in videoclips with few colours, especially during poor daylight conditions or during the night. Although most of the videos are in greyscale, the quality of the images is high. But the poor colouration might affect the efficiency of species identification or recognition of adult stadium of eel, e.g. silver eel, by citizen scientist. Lately, KBTS has carried out many monitoring projects with the camera box in combination with machine learning analysis (AI) (pers. comm. M. Kroes). The recent findings point out that artificial lighting could increase species identification (unpublished results). Characteristics of species are increasingly visible and thus support the identification (*Figure 7-1*). KBTS claims also that eel did not show hesitation to pass an artificial illuminated camera tunnel (pers. comm. M. Kroes). However, further testing is needed to compare IR and artificial illumination in fish response and species identification.

Figure 7-1 Roach (top) and Gudgeon (bottom) in artificial illumination left and IR illumination right. River Swalm (Belgium) spring 2018.

8 Reference

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Justification

CVO Report: 19015
Project number: 4311300054

The quality of this report has been peer reviewed by a colleague scientist and the head of CVO.

Approved by:	Dr. C van Damme Researcher
Signature:	
Date:	28 October, 2019

Approved by:	Ing. S.W. Verver Head Centre for Fisheries Research
Signature:	
Date:	28 October, 2019

Appendix A eDNA processing and data analysis (Dutch)[5]

Laboratorium analyse

De eDNA samples zijn geanalyseerd op de aanwezigheid van eDNA van vissen door middel van eDNA metabarcoding. Het analyseren van een eDNA sample vindt plaats in drie stappen. Eerst wordt het eDNA op het filter geconcentreerd en gezuiverd. Vervolgens wordt DNA geamplificeerd (vermeerderd) met behulp van PCR. De PCR fragmenten zijn gezuiverd en een DNA library is voorbereid. De library is gesequenced met behulp van Next Generation Sequencing (HiSeq 4000).

1. Het eDNA is geëxtraheerd door middel van een phenol chloroform DNA extractie. Gedurende de extractie lost het filter op waardoor al het DNA vrij komt. Storende stoffen als humuszuren kunnen detectie van het eDNA inhiberen wat kan leiden tot vals negatief resultaat. Gedurende de extractie zijn deze inhiberende stoffen zo veel mogelijk verwijderd.

2. Het DNA van vissen en amfibieën is geamplificeerd middels PCR. Hiervoor zijn specifiek ontwikkelde vissen en amfibieën primers gebruikt die kort fragmenten (~50-110 bp) van het mitochondriaal 12S & 16S DNA vermeerderen.

3. Door middel van gelelektroforese is vastgesteld of de PCR geresulteerd heeft in PCR producten van de juiste lengte. Middels van een tweede PCR zijn Illumina Nextera XT adaptors aan de PCR producten gezet. Vervolgens zijn de PCR producten samengevoegd. De pool van PCR producten van verschillende samples is gezuiverd. Deze pool van PCR producten vormen de zogenaamde DNA library.

4. Door middel van qPCR en een bioanalyzer run is de DNA concentratie van DNA library vastgesteld. De DNA library is verdunt, om optimale clustering op de flow cell van de sequencer te bewerkstelligen.

5. De PCR producten zijn gesequenced met behulp van Next Generation Sequencing (HiSeq 4000 platform, 150 bp paired-end). Hierbij worden miljoenen stukjes (zogenaamde reads) van het DNA uitgelezen. In deze stap wordt het fysieke DNA in het buisje dus vertaald in digitale reads.

Data-analyse

Eerst wordt een standaard verwerking van Illumina paired-end data uitgevoerd. Deze omvat de volgende stappen:

1. FASTQ sequence files zijn gegenereerd met behulp van de Illumina Casava pipeline.

2. Een eerste kwaliteitscheck is uitgevoerd door middel van Illumina Chastity filtering.

3. Vervolgens zijn de reads die PhiX controles bevatten verwijderd.

4. (Restanten van) de sequencing adapters zijn uit de reads geknipt.

5. De kwaliteit van de overgebleven reads is getest met de FastQC tool.

Vervolgens worden de sequenties geanalyseerd met behulp van het software package Obitools. Deze pipeline resulteert uiteindelijk in een tabel waarin voor elk sample aangegeven is hoeveel reads er van elke soort gedetecteerd zijn. Omdat er behoorlijke rekenkracht nodig is voor het verwerken van de sequencing data wordt een workstation gebruikt welke beschikt over 2 six core processoren met hyper-threading en 48 Gb Ram-geheugen. De volgende stappen zijn doorlopen:

1. Illuminapairedend: Genereren van een consensus sequentie op basis van de forward en reverse read.

2. Obigrep: sequenties die slecht alligned werden zijn verwijderd.

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5 Protocol delivered by DATURA

3. NGSfilter: Op basis van de gebruikte primers, en de tags die toegevoegd zijn in de eerste en tweede PCR zijn alle sequenties toegewezen aan het corresponderende sample.

4. Obiuniq: Om de dataset die nu nog bestaat uit miljoenen reads hanteerbaarder te maken zijn alle dubbele sequenties samengevoegd.

5. Obiclean en Obigrep: Filtering van sequenties die hoogst waarschijnlijk afkomstig zijn van PCR- en sequencingfouten.

6. Obigrep: Sequenties die korter zijn dan een marker afhankelijke minimumwaarde, of in de gehele dataset minder vaak dan 10 keer voorkomen zijn verwijderd.

7. Ecotag: De ecotag tool wordt gebruikt om de sequenties te matchen met de referentie database. Deze database bevat alle sequenties van de betreffende marker die aanwezig zijn in de NCBI Genbank en is handmatig gevalideerd op eventuele fouten door Datura.

8. Obigrep: sequenties die voor minder dan 98% overeenkomen met een sequentie van een soort in de referentie database worden verwijderd. Dit betreffen hoogst waarschijnlijk sequencing fouten.

9. Obitab: Tenslotte worden de resultaten geëxporteerd naar een .tab file. Deze file kan geopend worden in Excel.

10. Tenslotte zijn alle detecties, die een aandeel innamen van 0,15% of minder ten opzichte de totale hoeveelheid DNA in het monster, verwijderd uit de dataset. Dit betreffen waarschijnlijk detecties van achtergrond DNA. Bij vissen is zowel getest met behulp van 12S gen als met het 16S gen. De verkregen waardes zijn gemiddeld om zodoende tot een representatief beeld te komen.

Appendix B key physiological characteristics

To distinguish species using video recordings, key physiological characteristics were looked at. An overview of these characteristics that will be used to determine species (Fig. A1).

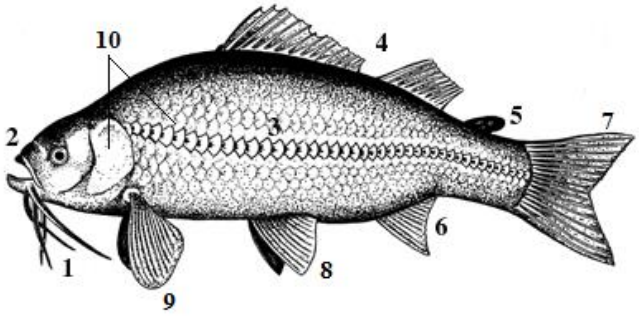

Figure A1 Schematic example fish, showing presence of barbels, position of the mouth.... etc. (Sportvisserij-Nederland 2018)

1- Barbels
Barbels are sensory organs present near the mouth in some species of fish. If present, the amount of barbels and the length can be used to identify the species.

2- Position of the mouth
The position of the mouth can be divided into three categories, superior, inferior and terminal. Superior mouth fish have an upturned mouth opening and a lower jaw longer than the upper jaw. Inferior mouth fish have a down facing mouth opening and a lower jaw shorter than the upper jaw. These types of fish are often bottom feeding fish. Terminal mouth fish have a forward-facing mouth opening and a lower/upper jaw of approximately the same length.

3- Shape, colour and amount of scales on the lateral line
The scales on the lateral line can be easily differentiated from the other scales due to the presence of a horizontal line through these scales. The amount of scales on the lateral line can be specific to certain species as well as the colour or shape.

4- Amount, shape and location of dorsal fin(s)
Fish can have one to three dorsal fins which can be grown together or separate. The front dorsal fin can consist of loose or joint spines. Furthermore, the shape of the dorsal fins can be typical to species as well as its location compared to the ventral or anal fin.

5- Adipose fin
Between the dorsal fin and the tail fin some species have a small adipose fin without fin rays.

6- Amount, shape and location of the anal fin(s)
The anal fin can be cut out or rounded. Some species have very long or multiple anal fins. The position of the anal fin compared to dorsal fin can also be typical for certain species.

7- Tail fin
The shape of the tail fin (e.g. rounded, forked, truncated) or the lack of one can be typical for certain species.

8- Ventral fins
The location of the ventral fins compared to the dorsal fin and anal fin is often used to distinguish certain species from each other. The ventral fins can also absent.

9- Pectoral fins
The stance, size and shape of the pectoral fins can be specific to certain fish species. In some species the lower pectoral fins are separate and thickened into sensory organs. The pectoral fins can also be absent.

10- Spots

Some species have typical spots on the dorsal fins, the body or the gill covers which can be used to distinguish certain species.