A diet study of the Eurasian otter (*Lutra lutra)* based on spraint analysis

Concept version, Jan. 2011

Hermsen, J. 850727001
Maarseveen, A., van 880430001

University of Applied Sciences
VAN HALL LARENSTEIN
PART OF WAGENINGEN UR

A diet study of the Eurasian otter (*Lutra lutra)* based on spraint analysis

The development of a standard method on how to do research about the composition of the otter's diet in the Netherlands based on spraint analyses, and a usable reference manual

Leeuwarden
September 2011

This report is written in the scope of Niewold, F. J. J.

Niewold Wildlife Infocentre
The Netherlands

Authors
Hermsen, J. 850727001
Maarseveen, A., van 880430001

Supervisor
Strijkstra, A.
Heijer, M., den

Van Hall institute project number: 594000

Cover drawing: <www.frettcheninfos.de> downloaded 5 September 2011

University of Applied Sciences
VAN HALL LARENSTEIN
PART OF WAGENINGEN UR

Summary

Otters (*Lutra lutra*) have faced a dramatic historic decline in many countries all over the world. The species became extinct in the Netherlands in 1989 until reintroduction took place in 2002. The population is presently growing but still facing threats such as road kills and drowning in fyknets. To get a better understanding of the otter's life style, it is important to know what its diet is.
For this reason a standard method on how to do a diet study was developed in this study. Furthermore a reference manual with guidelines with reference material is given, with the aim to make diet studies easy and feasible for other researchers who want to otter related research.
Otters are widely distributed and the European is the most common sub-species. They live on land as well as in the water where they catch their main prey item; fish. They need to eat often as they have a high metabolic rate, they have no fat layer to isolate their body but their fur is known to be the thickest of all mammal species.
Spraint analysis is a common method to do diet studies since this faecal material contains prey remains and are relatively easy to locate. We used spraints collected in several study areas in the Netherlands (region 1; Wieden, Weerribben, Lindevallei and Rottige Meenthe, region 2; Doesburg and Giesbeek, Doesburg) and Germany, (region 3; Peene, Germany) to develop a standard treatment for spraints. We made an otter diet analysis for these regions to test the method`s usability.
Spraints first need to be found and stored, and later cleaned and then the prey species remains can be examined using a microscope. The remains have to be compared to existing determination material to determine the prey species. Otoliths are special since length and mass may be calculated from measurements.
In our study, 83% of all species found in the spraints were fish. The most common prey is the European Perch, found in 67% of spraint from region 1, in 57% of spraints from region 2 and in 91% of spraints from region 3. From their otoliths, the average mass of Perch was estimated at 90, 14 grams.
The reference manual was designed to include how to find and treat spraints from field work to lab analyses and usable for inexperienced researchers in order to promote more research on the otter diet studies.
In order to reach the goal, two main research questions were developed; **I. What is the diet of the Eurasian otter in the study areas?** And **II; To which preconditions does the reference manual have to cope up?**

Preface

This research report was written as a final thesis project of Hermsen, J. and Maarseveen, A., van for the degree of Animal Management at the Van Hall Larenstein University of applied sciences. The intern supervisors are Mr Strijkstra, A. and Mrs Heijer, M., den. The research was commissioned by the Niewold Wildlife Infocentre with Niewold, F. J. J. as supervisor. The main goal was to produce a reference manual including a standard method for the research of otter diet analysis in the Netherlands based on literature and own experiences while performing a diet study on the otter spraints.

Leeuwarden, September 2011

Jessica Hermsen and Aloïse van Maarseveen

Contents

1. Terminology

Some terms used in this research proposal can be interpreted in several ways. The terms are cleared out in table 1in order to have a clear insight into the intentional interpretation.

Table 1; Terminology, the right way of interpretation of several terms used in this research rapport.

Term	Correct interpretation
Fykenet	A net, used in the fishery industry, to catch the desired fish species
Spraints	A small faecal secretion from an otter which contains prey remains, to mark their territory
Prey remains	The (mostly hard) indigestible parts in a spraint, like bones, fish scales, jawbones, vertebrae, hair, feathers
Determination	The identification of a prey remain, to know from which species it originates
Reference manual	A manual that contains pictures, photographs and drawing of the identifiable parts in the spraints. Prey remain in following research can be compared with these images in the manual.
Detergent	Sodium carbonate, the product that is used to soak the spraints in this research
Vertebrae	The spinal bone structure of the prey
Jawbones	The bones in the upper and lower jaws of the prey
Pharyngeal teeth	A bone that is hooked-shaped with usually a single row of teeth (Conroy *et al.* 2005)
Premaxillae	'Are a pair of bones that form anterior margin of the upper jaw, they consist of a curved part, the ramus, which constitutes the edge of the upper jaw and is generally blunt near its anterior, were it curves medially to its opposite number.' (Conroy *et al.* 2005)
Otoliths	The ear bone of fishes which are specific to a certain species

2. General introduction

Otters have faced a dramatic historic decline in many countries. Populations all over the world declined, and some went extinct, caused by hunting, as their fur was popular in the confection industry and were worth a lot of money. (NCRV, 1994)

Also water pollution in the seventies and eighties caused another threat as the amounts of hydrocarbons in the waters in the Netherlands where to high and fish populations declined. (Niewold, F., 2003)
The last individual of the Eurasian otter (*Lutra lutra)* in the Netherlands drowned in a fykenet in 1989, in Friesland a province in the North of the country. With the death of the last otter, the population was extinct in the Netherlands. (Niewold *et al.*, 2003)

In 2002, when the living conditions for the otter had improved, a five year re-introduction program was set up to bring the otter back to the Netherlands. The waters were cleaner as they contain fewer nutrients, and the hunt on the otter has become illegal. This increases the survival rate to an acceptable amount for re-introduction of the species in the Netherlands. (Niewold, F., 2003)

In 2002, a total of fifteen otters, mainly coming from Eastern Europe were re-introduced in the North of Overijssel. In the following five years another 25 animals were re-introduced in several national parks in the province of Friesland. (Niewold *et al.,* 2003)
The population increased to a minimum of at least 35 animals between 2007 and 2008 (Lammertsma *et al.* 2008).
This resulted in over a 100 youngsters within the following years. The population is still growing but there are still threats that make the population vulnerable. Traffic accidents are the main cause of mortality within the population and drowning in fykenet is the second one. (Wolkers, H., 2011)
Other otter populations in Europe also experience these threats (Brazier M. *et al.* 2001).

Another threat that the otters are facing is inbreed. The reason for this is that some of the dominant males are mating with most of the females, leading to a loss of genes within the DNA. (Wolkers, H., 2011) Here for it is important to secure the future of the otter by protecting the species and its habitat. Knowing what the otter in the Netherlands eat would make it easier to predict their distribution and understand their habitat choice; this can contribute to their protection.

To protect the population in the Netherlands, it is important to understand the otter's ecology. Otters have a very high metabolic rate, and they need to eat often (see chapter 3.1.5).
Here for we need to now of what species their diet consists, as this might influence their behaviour and distribution. When we know of what prey species the otters diet exists it would be easier to protect the otter and its environment. (Niewold, F., 2011)

Otters are territorial animals; which use three methods to mark their territory; 1.) anal gland secretion, 2.) urine and 3.) spraints (BBC, 1998 & Woodroffe, G., 1994). The spraints are interesting for examination of food diet analysis. A spraint is a small fecal secretion which contains prey remains. It is possible to determine the diet of the otter from these spraints as it is possible to assess what species were eaten from examination of the undigested remains, (Niewold, F., 2011) as spraints contain hard, indigestible prey remains which can be used for determination (Jacobsen, L., 2004, Penland, T., 2009 & Woodroffe, G., 1994).

There is a lack of information on the diet of the otter population in the Netherlands and, there is no sufficient standard method on how to do research on the otter's diet in the Netherlands, as has been done in other counties in Europe (Brazier, M., *et al.* 2001, Crimmins, S., 2009, Kloskowski, J., 2005, Guertin, D., *et al.* 2010, de la Hey, D., 2008, McCafferty, D., year unknown, Jacobsen, L., 2004 & Woodroffe, G., 1994), albeit with differing methods.
In this research spraints were examined, collected at six different sites in the Netherlands and one in Germany.

Our research resulted in a diet study on Dutch otters and a reference manual which contain images from the prey remains found during this study.

2.1 Problem description

Finding a sufficient method to do a diet study on the otters diet requires knowledge on how to do this. As there is no standard method this knowledge needs to be gained in advanced of the research and costs a lot of time and effort for new researchers. It would save time and money for researchers when there is a ready standard method and reference manual which can be used for new research.

2.2 Objectives and research questions

The objectives of this research will contribute to the knowledge of the diet of the otter in the Netherlands. Spraint analysis is a common method in order to get a clear insight in the prey preference of the otters in study sites in Europe (Brazier, M., *et al.* 2001, Crimmins, S., 2009, Kloskowski, J., 2005, Guertin, D., *et al.* 2010, de la Hey, D., 2008, McCafferty, D., year unknown, Jacobsen, L., 2004 & Woodroffe, G., 1994).
Spraint analysis will be used in this research, because otters are a difficult species to observe in the wild (Brazier, M., *et al.* 2001). The spraints contain hard, indigestible prey remains which can be used for determination (Jacobsen, L., 2004, Penland, T., 2009 & Woodroffe, G., 1994). At present there is no standardized research method to study their diet, which makes it less attractive for the researchers to study this aspect. A standardized research method in combination with a reference manual, about the recognizable prey remains in the otter spraints, makes the study of the otter's diet easier, more attractive, less expensive and less time consuming. This may leads to more knowledge about their diet, and therefore contributes to the understanding of their way of life. (Brazier, M., *et al.* 2001)
This knowledge can be used to compare differences in the otter diets in the currently investigated study areas but also other areas in new research and to reveal possible seasonal patterns or habitat dependent differences in their diet. These aspects could contribute to nature management and protection of the otters in the study areas, predict potential new habitat and also potentially predict dispersal patterns towards favoured habitats. This protection can be either to support or to control the otter's distribution.

A standardized research method needs to address; how to identify the prey remains (from an otter spraint) and may shed light on prey mass (Jacobsen, L., 2004). The reference manual makes it easy for the target group (new and amateur scientists) to determine from which species the prey remains are. The target group consist out of all kinds of scientists, like professionals, but also students with at least a university-way-of-thinking (for example; students from the Van Hall Larenstein). So, the target group must have some experience with applied science, doing research and some interest in the otter's diet.
The objectives of this research are;

- To determine a sufficient standardized research method for the diet of the otter in the Netherlands
  - Identify the prey remains in otters spraints
  - Calculate the sizes of the otters prey
- Producing a reference manual for the recognizable prey remains in the spraints

Knowledge that is needed to reach the objectives above, are the following; which prey species are present in the study areas? What is the size of these prey species? General determination knowledge is needed and knowledge about the general biology of the otter (how does he live) and knowledge about; how to create a useable reference manual?
To gain this knowledge, some research questions need to be answered. The research questions consist out of two main questions, further specified by sub-questions, which will answer the main questions.

In order to reach the objectives from above, the following main questions are composed;

I. What is the diet of the Eurasian otter in the study areas?

II. How to produce a usable reference manual?

The following sub questions are composed to give a comprehensible and entirety answer to the main question;

I. A. *What is the general biology of the otter?*

I. B. *What kind of prey remains are found in the otter spraints?*

I. C. *What kind of method is used to determine prey species?*

I. D. *Which species (class in % & name) are found in the otter spraints?*

I. E. *How to determine the prey sizes from a prey remain?*

I. F. *What is the size of the prey?*

I. G. *What are the differences of the prey remains in the spraints of the otter in these areas; De Weerribben, de Wieden, Rottige Meenthe, Lindevallei, Doesburg (among de Oude IJssel), Giesbeek and the Peene?*

II. A. *What is the target group for the reference manual?*

II. B. *What is the goal for making a reference manual?*

II. C. *What standards exist for determination reference manuals?*

3. Otter life history: Literature study

Some knowledge about the general biology and ecology of the otter is needed in order to understand his diet and behaviour.

3.1 General information

Otters are geographically widely distributed; the only places where they do not occur are Australia and Antarctica. (Allen, D., 2010)
The Eurasian otter (*Lutra lutra*) is the most widely distributed one (Allen, D., 2010). Their distribution range goes, from East to West, between Ireland to Japan, and from North to South between the Arctic Circle to northern Africa and Indonesia. (Mason, C., 1986)
The species is listed as near threatened in the IUCN list. (IUCN, 2011)

3.1.1 Taxonomy

The Eurasian otter is classified and named as shown in table 1. The family Mustelidae is one of the largest families of the order Carnivora (Mason, C., 1986).
There are thirteen otter species within six genera. They all share similar characteristics such as whiskered muzzle, small round ears, long sinuous body, large webbed feet, short powerful legs and a rudder- like tail. (Allen, D., 2010)

Table 1 Classification of the Eurasian otter by Linnaeus, 1758

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Order	Carnivora
Family	Mustelidae
Genus	*Lutra*
Species	*lutra*
Scientific name	*Lutra lutra*

3.1.2 Habitats

Otters are semi- aquatic animals (Palazón, S., 2008). This means they spend time on land as well as in the water (Chanin, P., 1993). All thirteen otter species live in or close to the water, and prey mainly on aquatic animal species (Allen, D., 2010).
Otters are found in fresh water habitats and rocky shores, like the sea otter. They live at sea level as well as higher altitude such as in the Himalaya Mountains (Allen, D., 2010). The choice of habitat is made by the availability of a suitable place which provides the basic requirements; food and shelter (Woodroffe, G., 1994).

Table 2 shows an overview of the habitat type of the study sites of this research.

Table 2 Habitats and surface of the study sites the Wieden, Weerribben, Lindevallei, Rottige Meenthe, Doesburg, Giesbeek and Peene (Germany), with the source and date were this information came from.

Study site	Habitat	Surface	Sources
Wieden	Meadow Reed Ditches Canals Peat holes Lake	9.412 ha. Together with the 'Weerribben'	np-weerribbenwieden (2011) knnv (2011)
Weerribben	Meadow Reed Ditches Canals	9.412 ha. Together with the 'Wieden'	np-weerribbenwieden (2011) knnv 2011

	Peat holes Lake		
Lindevallei	Diverse swamp Forest Brook valley	847 ha.	Natuurkaart (2011)
Rottige Meenthe	Flowery grassland Peat bog Peat holes Vibrating bogs Sphagnum reed beds Land strips in peat ground	Ca. 1.650 ha. (inclusief De Brandemeer	Natuurkaart (2011)
Doesburg	River Field City	Not applicable	Staatsbosbeheer (2011)
Giesbeek Doesburg	Lake Forest Field City	Not applicable	Staatsbosbeheer (2011)
Peene	River fen	Not applicable	vorpommersche-dorfstrasse (2011)

3.1.3 Ecology

Otters have a streamlined body to swim smoothly and dive through the water; they have whiskers which help them catch their prey. The species is nocturnal, and are not often seen during daytime (Allen, D., 2010). Males can weigh up to thirty per cent more than females; they can reach about eleven kilos (Woodroffe, G., 1994).
They have no fat layer to insulate their body, their fur keeps them warm. Otters are known to have the thickest fur in the animal kingdom. Their fur consists of two layers, an outer layer of guard hairs which are four cm long and a layer of under fur that is extremely dense and about one cm thick. The hair in the under layer is so thick that is seems to be the skin, when examined under the electron microscope, it shows that there are about 20–22 hairs in a bundle and that there are about 20 to 30 bundles per mm², which means 50.000 hairs per cm² (Kruuk, H., 1995). For this reason their fur became very popular in the confection industry (Allen, D., 2010).

3.1.4 Behaviour

Otters are almost non-social animals. There is not much aggression between them when they meet but avoidance will occur. Despite the territorial males who will show some aggression against one another (Kruuk, H., 1995).
Otters use three kinds of scent marking; anal gland secretion, urine and sprainting (BBC, 1998, Brazier, M., *et al.* 2001, Crimmins, S., 2009, Guertin, D., *et al.* 2010, de la Hey, D., 2008, Jacobsen, L., 2004, Kloskowski, J., 2005, McCafferty, D., year unknown, Woodroffe, G., 1994). A spraint is a small faecal secretion, which is used to communicate with other otters. Otters are sprainting all year round, but mainly in the winter when food resources are scarce (Kruuk, H., 1995).
In their sexual behaviour, they are polygamous and polyandrous.
The cubs spend ten to sixteen months with their mother depending on their fishing skills (Kruuk, H., 1995).

3.1.5 General physiology; Physiology of the digestive tract

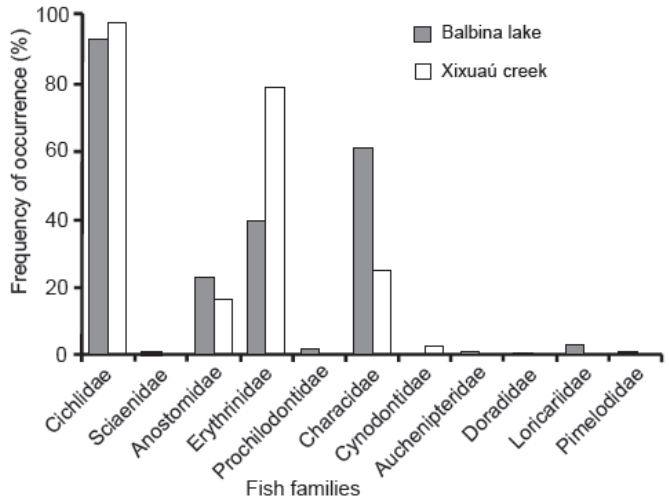
The vision of the otter is very accurate at short distances; otters have a great responding towards the different types of environment when it comes to their sight as they live on land as well as in the water (Chanin, P., 1993). There are some speculations in the world of the otter specialists, that the longer the sight-distance get, the lesser their sight will be (Niewold, F., 2011). No scientific research has proved this to be the case.

Unlike most mammals, otters are adapted to focus and resolve fine details, because their lenses can be modified, to make it more spherical. (Chanin, P., 1993)
The otter's ears are able to detect inaudible sounds for human, when they are on land. This is because they have a quite sensitive hearing system. Sound travels very well under water, but otters are unable to detect the source of a sound in the aquatic environment. In terrestrial environments, hearing, scent and sight together are of great importance. (Chanin, P., 1993)
The shuttling between terrestrial and water environments is one of the short term behavioural options to reduce the energy costs (such as the loss of heat), while foraging in cold water (Dunstone, N., et *al.* 1998). Another option to reduce energy loss is; retreating to their den and taking care of their fur by cleaning it during feeding and rubbing air into their fur to increase the floating ability and to increase insulation (BBC, 1998, Kruuk, H., 1995 & Woodroffe, G., 1994).

Metabolism rates in otter weighing one or more kilos's, is approximately 20% higher than expected from the mammalian standard curve, see appendix II (Iversen, J., 1972). Because of this high metabolic rate, otter spraints are produced after one till three hours after consuming their prey (Niewold, F., 2011). The mean frequency otter sprainting is about fifteen times a day. This makes it one of their majorly behaviour types besides eating, resting, walking, swimming etc. (Niewold, F., 2011). The high frequency may suggest that only a few preys may show up in each spraint.

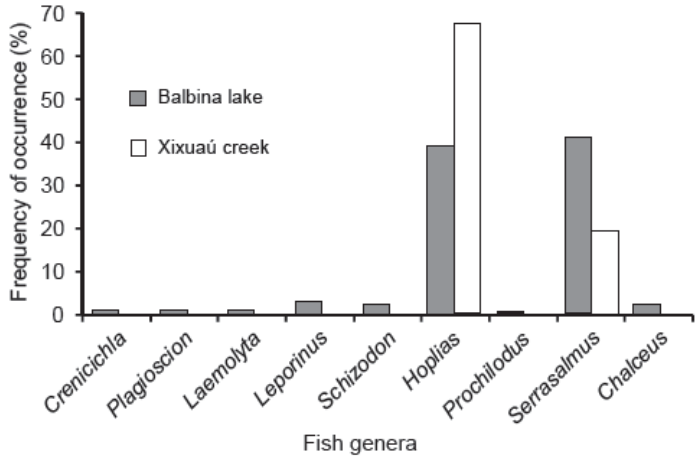
3.1.6 General food composition otter

Otter's food composition consists mainly out of fish but sometimes also amphibians, reptiles, birds, crustaceans, small mammals and insects may be eaten (Carss, D., 1995 & Georgiev, D., 2004). Fish in the otters diet can make up to 70- 95 % (Woodroffe, G., 1994), sometimes even 100 % (Cabral, M., *et al.* 2010) depending on season (de la Hey, D., 2008). In the research of Colares *et al.* (2000) different classes of prey species were presented in three graphs, see appendix I. Frequencies, comparison and seasonal analyses of several fish genera/families are shown in figure 1. A.-C. (Cabral, M., *et al.* 2010).



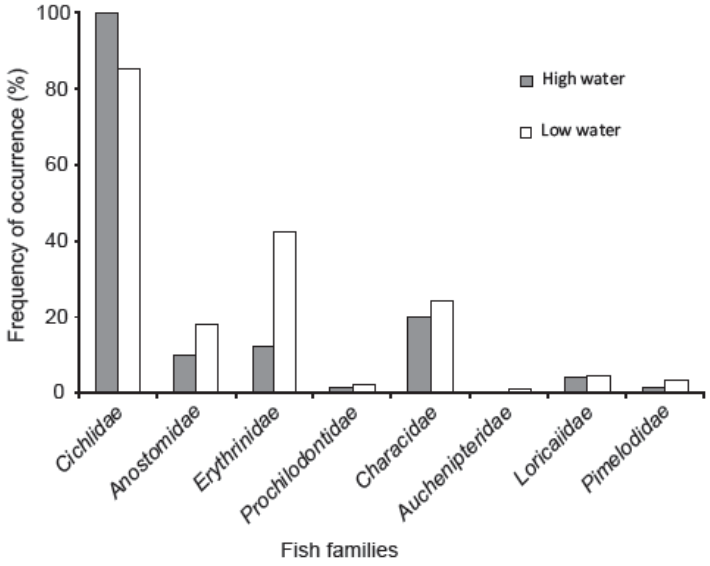
Frequency of occurrence of fish families found in feces samples of giant otters from Balbina hydroelectric reservoir (N = 254 samples) (present study), and in an area without hydroelectric plant influence, Xixuaú creek, N = 37 samples (Rosas *et al.* 1999).

Figure 1.A. Frequency of occurrence of fish families found in the research of Rosas, *et al.* in 1999 on the Giant otter (Cabral, M., *et al.* 2010). These are closely related to the European otter.



Comparison of the frequency of occurrence of fish genera found in feces samples of giant otters from Balbina hydroelectric reservoir (N = 254 samples) (present study), and in an area without hydroelectric plant influence, Xixuaú creek, N = 37 samples (Rosas *et al.* 1999).

Figure 1.B. Comparison of the frequency of occurrence of fish genera found in the research of Rosas, *et al.* in 1999 (Cabral, M., *et al.*, 2010).



Seasonal analyses of the fish families (frequency of occurrence) found in fresh feces samples of giant otters from Balbina hydroelectric reservoir (N = 75 samples) during two entire hydrological cycles (2006-2007).

Figure 1.C. Seasonal analyses of the fish families (Cabral, M., *et al.*, 2010).

These results will be compared with the outcome of this research.
The amount of different fish species in the otter's diet depend on fish abundance as well as swimming speed of the fish. Favourable are the slower moving species, which are the inter-tidal and benthic species (Carss, D., 1995). For any preferences in this aspect in this current research, see chapter 8.
As otters are opportunists, they will consume whatever fish are most readily available (Chanin, P., 1993, Palazón, S., *et al.* 2008 & Woodroffe, G., 1994).

3.1.6.1 Prey species in Europe and the Netherlands

Diet analysis has been done in several European countries, such as Sweden, Denmark and in England between 1967 and 1986 (Bekker *et al.*, 1990), which revealed that the Cyniprids featured severely.

A list of common prey species, found in the otter diet, in Europe are shown in appendix III.

Only one small research has been done in the northern part of the Netherlands (Friesland) in 1990 about the diet of the otter (Bekker, D., *et al.*, 1990). Bekker *et al.* (1990) stated that the dominating fish species in this area were the Bream *(Abramis brama)* and the Pike-perch *(Stizostedion lucioperca)*. Their research also showed that besides the Bream and the pike-perch, some other species, such as the Smelt *(Osmerus eperlanus)*, Common roach *(Rutilus rutilus)*, Silver bream *(Blicca bjoerkna)*, Ruffe *(Gymnocephalus cernuus)*, Perch *(Perca fluvialtilis)* and Eel *(Anguilla anguilla)*, were abundant in the European otter spraints, that were collected in this area, in the period between 1982 and 1987 (Bekker, *et al.*, 1990). Some more up-to-date research is presented by de Nie, (1997), see appendix IV.
Not only fish species were present according to Bekker, *et al.* (1990), but also some species from the Amphibian class, such as Common Toad (Buffo buffo) and European frogs (Rana temporaria) and Edibkle frogs (Rana esculenta). Paragraph 5.2 gives an overview of the classification of the species found in this current research.

4. Otter spraint research methodology

The following chapter describes the methodology that is used during this study. Methodology found, by literature study was combined and used, tested and refined. This final refined method is described in this chapter. All steps were verified with the literature study to make sure it is sufficient.

4.1 Introduction

During this research a pilot study was done in order to determine if the method for cleaning the spraint and examining the prey remains was sufficient. This is explained in appendix V. The method was refined with the results of the pilot study.
The method was used to investigate 78 spraints from different locations in the Netherlands and Germany. The results were used for analysis between locations and habitat type and as a basis for a reference manual.
A flow chart is given in 4.1.2 to indicate the different steps of the current investigation, to clarify chapter 4.

The spraints that were used for this research were collected on seven different study sites.
Figure 2 and 3 show the areas where the spraints for this study were collected.

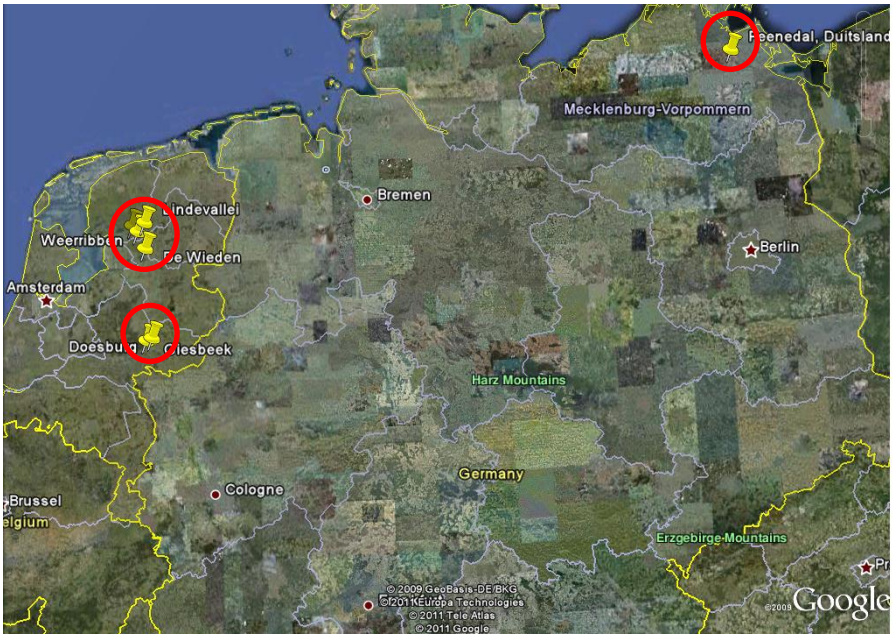


Figure 2 Map with the study areas in the Netherlands; Region 1 (Wieden, Weerribben, Lindevallei and Rottige Meenthe) Region 2 (Doesburg and Giesbeek) and Germany, Region 3 (Peene), where the spraints were collected by the Niewold Wildlife Infocentre.

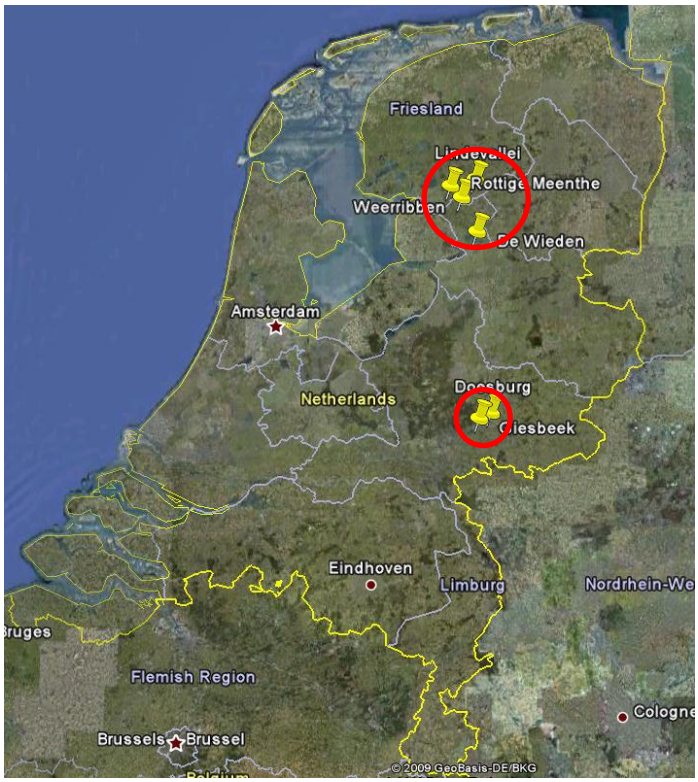
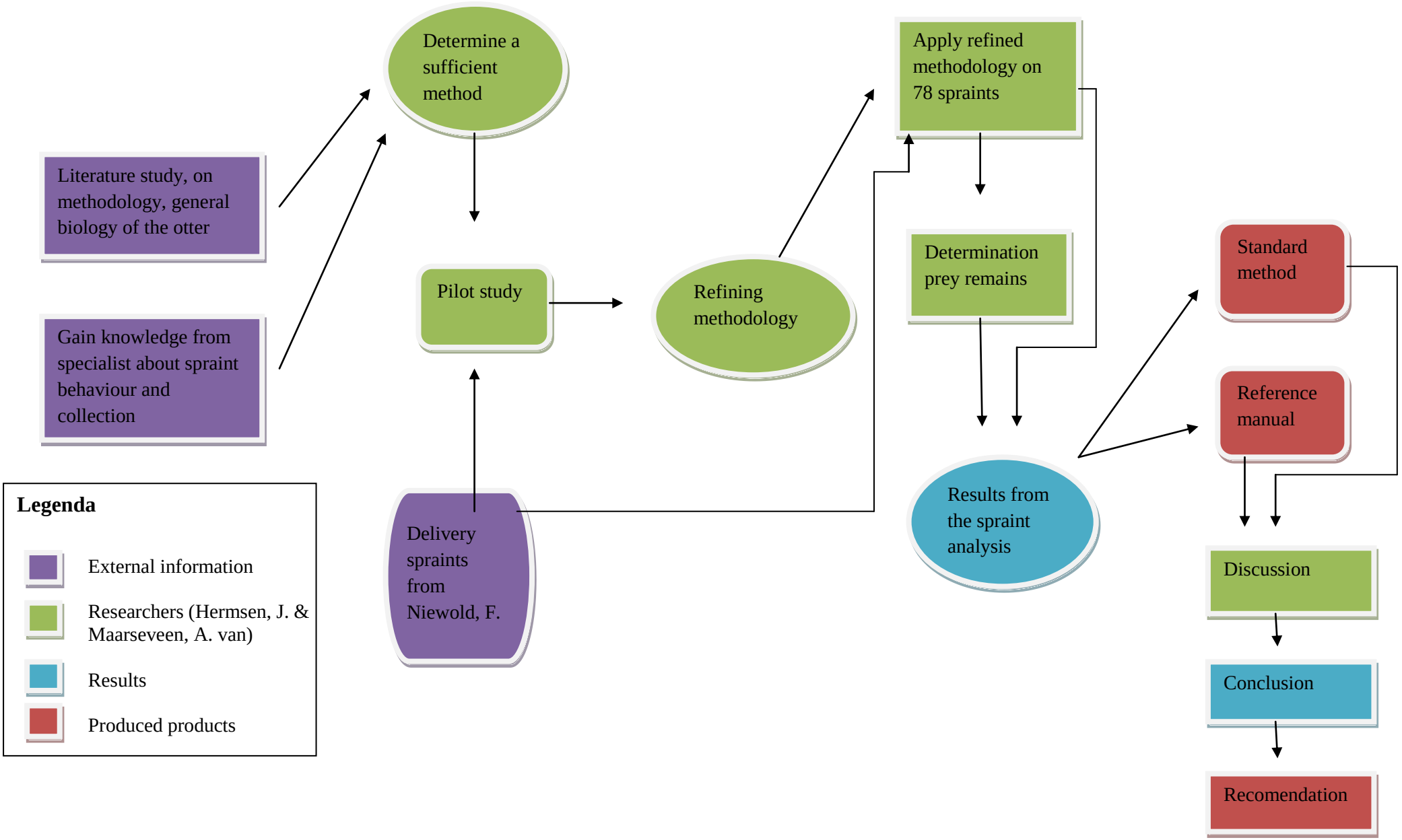


Figure 3 Map with the study areas in the Netherlands; Region 1 (Wieden, Weerribben, Lindevallei and Rottige Meenthe) and Region 2 (Doesburg and Giesbeek), where the spraints were collected by the Niewold Wildlife Infocentre.

4.1.2. Flow chart spraint analyses methodology



4.2 Materials

The used materials and reference literature are listed in tables 3 and 4.

Table 3 Materials needed to perform this research about the diet of the Eurasian otter in several study sites in the Netherlands; Wieden, Weerribben, Lindevallei, Rottige Meenthe, Doesburg, Giesbeek and one in Germany; Peene.

Specific for this research
Sieve 0.6mm
Sodium carbonate Na_2CO_3, 0.07%
Tissues
Petri- dishes
Microscope, Olympus CHS
Microscope slides, cover slips and demineralised water
Measuring scale (with accuracy of 0.1 gram)
Cooking plate
Binocular-camera, Olympus SZ-CTV
Tweezers
Spraints, delivered by F. Niewold

Table 4 Determination books needed to perform this research about the diet of the Eurasian otter in several study sites in the Netherlands; Wieden, Weerribben, Lindevallei, Rottige Meenthe, Doesburg, Giesbeek, and one in Germany; Peene.

Determination books; title	**Author**	**ISBN**
A guide to the identification of prey remains in otter spraint 3rd edition	Conroy, J.W.H. *et al.* 2005	0906282551
Atlas van schubben en andere beenachtige structuren van niet-zalmachtige zoetwatervissen, Organisatie ter verbetering van de Binnenvisserij;	Steinmetz, B. & Muller, R. 1991	9789080012042
The Tailless Batrachians of Europe	Boulenger, G.A. 1897	9780548217207
Voedselecologie van de 'verwilderde' kat	Honstede, B. van & Schut, G. 2010	594000

4.2.1 Recommended sample collection

Spraints can be collected in nature areas where the otter occurs. Some knowledge about the otter's biology is needed in order to find spraints. The otter lives mainly in and around water. Good places to find spraints are water edges such as a pier, a clump of grass or places where animals can cross the water body. Under a bridge is a good location to find spraints, as the spraints are preserved well here because it is less affected by changing weather conditions compared to open air. The otter has routes and passes that it routinely travels. You can find these paths when otters need to cross land between two waters bodies. A small hole, with a section of about 30 cm, can be found in the dense vegetation. The vegetation on the ground has been walked on by the otter, which can clearly been seen.
When a spraint is found, it is important to determine how old the spraint is, this information is important to determine if the spraint is usable for DNA research.
There are also different types of spraints, some are solid and dry (old), and these are easy to collect, but don`t contain much DNA. The fresh spraints contain DNA. Others are jelly like and need to be scraped off the ground with a clean spoon, avoid cross contamination by cleaning the spoon or spatula thoroughly before use. Jellies contain a lot of DNA since it consists of mucus with cell material of the intestinal wall.
The size of spraints differs as well, from very small to the size of a thumb.

Fresh spraints can be used for DNA research and need to be preserved in Ethanol.
An old spraint for diet studies does not need to be preserved in ethanol but can be put in a plastic bag.
For more comprehensive information about spraint collection see appendix VI.
After the spraints are collected they need to be frozen (in this study it is done at -28 degrees Celsius) until examination for subsequent analysis (Colares, E., *et al.* 2000, Georgiev, D., 2004, de la Hey, D., 2008 & McMahon, J., *et al*. 2006).

4.2.2 Sample handling procedure

After the spraints are collected they need to be treated before they can be examined.

The spraints were defrosted at the time of examination.
Mucus was removed by soaking the spraints in a detergent solution (Beja, P., 1996 & de la Hey, D., 2008) sodium carbonate (Na_2CO_3), for a week (Jacobsen, L., 2004). A solution of 0.07% (weight/volume), sodium carbonate with cold tap water was used.

The spraints were then carefully washed in a sieve with a mesh width of 0.6mm until the identifiable parts became clean (Beja, P., 1996, Colares, E., *et al.* 2000, Jacobsen, L., 2004 & Ottino, P., 2004). A result of the pilot study was that a sieve of 0.6mm was the most efficient of sieves with mesh width between 0.5-1.0 mm. This differs from literature which commonly uses 1.0 mm. we checked the accuracy by receiving the wastewater with a sieve with a mesh width of 0.425mm and no prey remains were found. The prey remains in the 0.6mm sieve were washed with running tap water to remove the last mucus. The sieve with the prey remains was dried on the bottom of the sieve using paper towels. To get the prey remains into the petri-dish the sieve was tapped gently on a dry sheet of white A4 printing paper. With another dry sheet of paper the prey remains were then wiped into a petri-dish. After that, the prey remains were air dried (Georgiev, D., 2004 & de la Hey, D., 2008) at room temperature, and individually stored in petri-dishes. The spraints were numbered and details were written on the petri-dish with a waterproof marker and stored until investigation and determination (Crimmins, S., 2009).

4.3 Determination of species

To determine which species are present in the spraint, it was necessary to examine the scales and bones found in the spraint under a microscope with the enlargement of 40x. One spraint however can contain hundreds of fish scales. To examine all scales would take too much time in practice, so an alternative sub-sampling method was chosen.
We examined ten scales, at random from the middle of the petri-dish with tweezers. The material in the petri-dish was shaken again before a new scale was taken from the middle of the petri-dish. This was done for ten scales.
If determination on these scales indicates that they were from the same species the spraint was assumed to contain only one species. If scales from different species were found in the first ten samples, another ten samples were taken and examined. If no additional new species was found in these ten samples, the spraint was assumed to contain two species. If another species was found in the second ten samples then another ten samples were examined, and so on. No more than three species were ever found in this analysis. This time saving method enabled us to examine 78 spraints.

As fish is the main prey, most research time is put in to this. The bones and other prey remains in the spraint were examined and classified as specified as possible.
Only a small percentage of the remains are other than fish. When feathers were found the prey was classified as Aves (bird), when bones were found the group was classified as Chordata.
The determination results were compared with the known living species in the area found by literature study, and dead fish that are collected in the study sites.

4.3.1 Species determination procedures

Literature study
We classify the remains found according to the study area where the spraints were found, a method that is also used in some other otter diet studies like Clavero, M. (2005).

For the identification of fish remains, several items were collected from the spraints, such like pharyngeal teeth (Kloskowski, J., 2005), vertebrae (Palazón, S., 2008, Woodroffe, G., 1994 & Ottino, P., 2004), jawbones/ premaxillae (Conroy, J., *et al.* 2005) and otoliths (McCafferty, D., year unknown).
A manual that contains clear information and photographs of fish scale is the Dutch manual 'Atlas van schubben en andere beenachtige structuren van niet-zalmachtige zoetwatervissen' by Steinmetz, B., *et al.* (1991).
For several other species characteristically features 'A guide to the identification of prey remains in otter spraint' from The Mammal Society is used, which contains very clear drawings (Conroy, J., *et al.* 2005).
The prey remains were investigated with the use of a microscope (Georgiev, D., 2004, Guertin D., *et al.* 2010, de la Hey, D., 2008, Penland, 2009 & Ottino, P., 2004).
Mammal and bird remains were identified by hair/feathers, teeth or bones in the spraints (Kloskowski, J., 2005, Jacobsen, L., 2004 & Palazón, S., 2008). The remains that were classified as Crustaceans (crab and shrimps) were identified by exoskeleton fragments (Guertin D., *et al.* 2010).

<u>This research</u>
The lens of the microscope had the enlargement of 4x and the ocular the enlargement of 10x in this research, sufficient for determination purposes.
All spraints content prey fragments photographed with the use of a binocular microscope with a camera and the 'analySIS' program on a PC. The first 37 spraints where photographed with 1.4x enlargement, spraint 38-78 were enlarged to 3.5x whenever possible.
It was important to have the entire scale in one view because several determination items can be assessed in one picture.
The photos taken were used in the reference manual were the species were described according to the characteristics found, see appendix VI.

For reference, H. Bosma, from Wetterskip Fryslan, delivered several dead fish from Muskrat *(Ondatra zibethicus)* traps, for analysis amoungst which the Bream *(Abramis brama)*, Perch *(Perca fluvialtilis)*, Tench *(Tinca tinca)*, Pike *(Esox lucius)*, Silver Bream *(Blicca bjoerkna)* and *Rudd (Scardinius erythrophthalmus)*.
Their scales were photographed with a binocular-camera with the enlargement 1.4.
These photographs contributed to the determination of the scales found in the spraints, in particular for sub-species accuracy and to add to the reference manual.
Scale removal was done before the fishes were boiled, and were taken from the upper side of the fish, as done in other research (Yamada, 1961). Microscope slides were made from the scales of all the obtained species, photographs were taken with the binocular-camera.
Now, the fishes were individually deposited in a beaker with hot water, and placed on a cooking plate. After the fishes were boiled, they were placed on a cutting board and their heads were cut through with a knife, at the place where the otoliths are located (see figure 2). All useful determination material was removed, boiled again to remove the remaining tissue and photographed with a binocular-camera (see appendix VII). The jaws of the Bream and Tench do not exist of hard material, so they are not good for determination material, as they will be dissolved in the otter's digestive system and have hardly any chance to end up in the spraints.

5. Results

The results and also a small part of the methods are given in this chapter. A small part of the methods does belong in this chapter, for it to be legible and clear.

5.1 Introduction answer to the main and sub-questions

In chapter 2, paragraph 2, two main questions and several sub-questions were developed in order to guide this research and to produce a sufficient product. These questions will be answered in the paragraphs below.

We will frequently use English ore scientific names of species. These are given here together with Dutch names for clarity.

Table 5 gives a translation of the scientific names of the fish species that are used in this research.

Table 5 Translation of the scientific fish names in English and Dutch, for the fish species found in the otter's prey determined by analysing scales and otoliths, collected from seventy-eight otter spraints, which showed six different fish species; the European perch, Pike, Common roach, Gudgeon, European carp and the Tench. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Scientific name	English name	Dutch name
Perca fluviatilis	European perch	Baars
Esox lucius	Pike	Snoek
Rutilus rutilus	Common roach	Blankvoorn
Gobio gobio gobio	Gudgeon	Riviergrondel
Cyprinus carpio	European carp	Europese karper
Tinca tinca	Tench	Zeelt

5.2 Main question I; What is the diet of the Eurasian otter in the wild, in the study areas?

The otter's food composition in several European countries is presented in chapter 3. From our locations in the Netherlands and Germany (Peene), 78 otter spraints were investigated for their prey remains (see chapter 4). 76 spraints out of the 78 contained one or more fish species (table 6). Given in percentage this is 83% of the phylum 'Pisces' (fish) and 17% contained only other phyla, such as Mammalia (mammals), Aves (Birds), Crustacea, Mollusca (Molluscs) and Insecta (Insects), or unknown (could be Mammals, Birds, Reptiles or/and Amphibians).

Only one of the spraints contained a complete paw, with some hairs. Because of the hairs and the bone structure, it certainly had to belong to a mammal species. To identify to which small mammal species it belonged, the paw was compared to reference material and hair comparison (Honstede. B., *et al.* 2010) under the microscope (100x magnification).
The paw was rat-size and the hair structure suggested Common rat *(Rattus norvegicus).*

Table 6 Number and percentage of species (phylum & class) of the otter's prey determined by analysing scales collected from seventy-eight otter spraints. Number of total spraints is one-hundred-six, because some spraints contained more than one species per spraint. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany). The Mammalia and Aves that are presented separately were identified by bones in combination with hairs or feathers. 'Unknown' is a separate class because two spraints did not contained any prey remains and two spraints only contained 'unknown' species (bones from Amphibians, Reptiles, Mammals or Birds).

Phylum	Class	Number of spraints	Percentage
Chordata	Pisces	88	83
Chordata	Mammalia	2	2
Chordata	Aves	2	2
Chordata	Unknown	4	3
Arthropoda	Crustacea	4	4
Arthropoda	Mollusc	3	3
Arthropoda	Insecta	3	3
Total;		106	**100**

The results of to which fish species, the 83% belonged are shown in table 7. 57% of fish species were Perch, followed by Common Roach, 16% Pike and 7% Gudgeon. Common carp and Tench were only found once (1%). Table 7 gives an overview of the fish species that were determined from the prey remains. Mostly found was the European perch.

Table 7 Number and percentage of fish species of the otter's prey, determined by analysing scales collected from seventy-eight otter spraints. Eighty-three per cent of the results consisted out of fish, divided over six different fish species; the European perch, Pike, Common roach, Gudgeon, European carp and the Tench. The total number of spraints is eighty-eight, because some spraints contained more than one fish species per spraint. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Fish species	Number of spraints	Percentage
Perca fluviatilis	50	57
Esox lucius	14	16
Rutilus rutilus	16	18
Gobio gobio gobio	6	7
Cyprinus carpio	1	1
Tinca tinca	1	1
Total;	88	**100**

The fish sizes could be calculated for the main prey item fish. This is done using otoliths collected from the spraints.
Otoliths are part of what is called 'hard prey remains'. They are also called 'ear stones' and consist of aragonite which is a rigid form of Calcium carbonate (Leopold, M., *et al.*, 2001 & Aguilera, O., *et al.* 2008). The remaining part exists out of organic matter, called otoline (Aguilera, O., *et al.* 2008).
Each fish has two otoliths, figure 4 indicates were they are located, this is not species related.

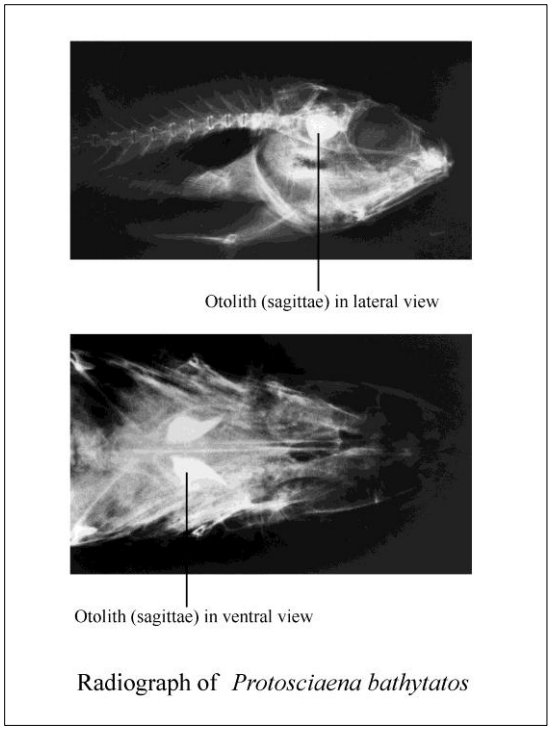


Figure 4 Location of otoliths within a fish *(Protosciaena bathytatos)*; Source; Aguilera, O., *et al.* 2008

Every species has a specific otolith shape (Leopold, M., *et al.* 2001). Different otolith-shapes of several species like the Pike, Common roach, European perch, Gudgeon, Tench and Common carp, are shown in figure 5.

Figure 5.A; Gudgeon *(Gobio gobio gobio)*

Figure 5.B; Perch *(Perca fluviatilis)*

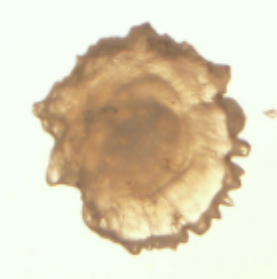


Figure 5.C; Common roach *(Rutilus rutilus)*

Figure 5.D; Pike *(Esox lucius)*

Figure 5.E; Tench *(Tinca tinca)*

Figure 5.F; Common carp *(Cyprinus carpio)*

Figure 5 Otoliths from six fish species; the European perch, Pike, Common roach, Gudgeon, European carp and the Tench, collected from seventy-eight otter spraints, photographed with a binocular-camera, 5.A-5.D with an enlargement of 1.4 and 5.E-5.F with an enlargement of 3.5. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany). (source; authors of this research, 2011)

Otoliths can be retrieved from faecal matter, like spraints (Carrs, D., *et al.* 1994). This also counts for the otoliths in this research, see appendix VIII.

In order to have a direct and clear view about the number and percentage of useful otoliths found in the 78 spraints, see table 8. It is shown that 37% of spraints, contained useful otoliths.

Table 8 Number and percentage of useful otoliths, collected from seventy-eight otter spraints, which contained six different fish species; the European Perch, Pike, Common roach, Gudgeon, European carp and the Tench. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Diet analyses results	Number of otoliths
Spraints including otoliths	29

Spraints without otoliths	49
Total;	**78**

59% of these cases contained one otolith, 34% contained two otoliths, and 7% contained 3 otoliths. This was the case in 59 %. Table 9 gives an overview of the numbers.

Table 9 The amount and percentage of useful otoliths, collected from seventy-eight otter spraints, which contained one, two or three otoliths per spraint. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Spraints with otoliths	Number of useful otoliths	Total otoliths	Percentage
Spraints with 1 otolith	17	17	59
Spraints with 2 otoliths	10	20	34
Spraints with 3 otoliths	2	6	7
Total;	**29**	**43**	**100**

With the use of the CD-ROM with the species specific otoliths from Leopold, M., *et al.* (2001), the different species were determined, (table 10). No different species than the ones already determined with scale determination, were found. It is shown that most of the useful otoliths (35%) belong to the species Common roach, followed by 28% European Perch, 14% European carp, 12% Gudgeon, 9% Tench and 2% Pike. This is a somewhat different pattern compared to the scale determination, because European Perch and the Pike appear less dominant in the otolith findings.

Table 10 Number and percentage of fish species determined with the use of useful otoliths (25+13+5=43 otoliths divided over 29 spraints), collected from seventy-eight otter spraints, which contained six different fish species; the European Perch, Pike, Common roach, Gudgeon, European carp and the Tench. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

	Region 1		Region 2		Region 3	
Fish species	Number of otoliths	Percentage	Number of otoliths	Percentage	Number of otoliths	Percentage
Perca fluviatilis	8	32	1	8	3	60
Esox lucius	1	4	0	0	0	0
Rutilus rutilus	10	40	5	38	0	0
Gobio gobio gobio	2	8	3	23	0	0
Cyprinus carpio	2	8	4	31	0	0
Tinca tinca	2	8	0	0	2	40
Total;	**25**	**100**	**13**	**100**	**5**	**100**

Otoliths are valuable for determination of fish species, and for the calculation individual fish length, width and mass (Carrs, D., *et al.* 1994, Granadeiro, J., 2000 & Leopold, M., *et al.* 2001).
All these aspects were also determined and calculated for the otoliths retrieved from the spraints, in this research, see appendix VIII table 1 and 3. Only the useful otoliths are shown in this appendix. These otoliths were measured and the fish length/width were calculated with the following equations (Leopold, M., *et al.* 2001) for fish length/width and fish mass.

Fish length/width: Y= a+bX, were:

Y= Fish length/width
a= Estimated regression coefficients
b= Estimated regression coefficients
X= Range minimum and maximum numbers of the otolith length

And fishes mass (Leopold, M., *et al.* 2001);

Fish mass: $Y= (aX)^b$

Y= Fish mass
a= Estimated regression coefficients
b= Estimated regression coefficients
X= Range minimum and maximum numbers of the fish length

Table 11 gives an overview of the average prey fish, per species, listing average minimum mass, maximum mass average mass and the number of otoliths on which the averages are based. Appendix VIII, gives an overview of all of the fish's masses.

Table 11 Results of fish mass, determined with the use of forty-three useful otoliths collected from twenty-nine spraints with useful otoliths, which contained six different fish species; the European perch, Pike, Common roach, Gudgeon, European carp and the Tench. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Species	Mass min.	Mass max.	Mean mass	Number of otoliths
Cyprinus carpio	3,3	28,8	12,5	6
Esox lucius	179,2	179,2	179,2	1
Gobio gobio gobio	5,5	14,4	9,7	5
Perca fluviatilis	2,3	537,8	90,1	12
Ritulus ritulus	1,7	39	12,4	15
Tinca tinca	143,9	436,3	324,8	4
Total;				**43**

After an otolith has passed the otters gut, wear can be an influencing factor on the otoliths size, see appendix VIII table 2 for the denotations (Leopold, M., *et al.* 2001). The wear is a kind of erosion of the otolith due to the acid gastric environment (Alexandersson, K., 2006). Table 12 gives an overview of the amount of the useful otoliths and the different wear stages they were in. 30% of the otoliths were in wear stage 'no', which means that they were in a stage without any wear visible.

Table 12 Results of the wear stages of forty-three useful otoliths collected from twenty-nine spraints with useful otoliths, which contained six different fish species; the European perch, Pike, Common roach, Gudgeon, European carp and the Tench. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Wear stage	Number of otoliths	Percentage
Wear .	10	24
Wear no	13	31
Wear 2	10	21
Wear 3 or >3	10	24
Total;	**43**	**100**

The 78 spraints came from seven different study sites, like; the Wieden, Weerribben, Lindevallei, Rottig Meenthe, Doesburg, Giesbeek which are all in the Netherlands and the Peene, Germany. Table 13 shows how many spraints per study site were found. Most of the spraints (22%) were collected in Giesbeek, Doesburg.

Table 13 Number and percentage of otter spraints found per study site. The study sites were divided later on over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

Study site	Number of spraints	Percentage
Wieden	3	4
Weerribben	12	16
Lindevallei	7	9
Rottige Meenthe	15	19
Doesburg	12	15
Giesbeek	17	22
Peene, Germany	12	15
Total;	**78**	**100**

The study sites could be sub-divided into three main regions; region 1 (Wieden, Weerribben, Lindevallei and Rottige Meenthe), region 2 (Doesburg and Giesbeek) and region 3 (Peene, Germany). The numbers and percentage of fish species found in the three regions are shown per region in table 14.

Spraints from region 1 contained 3 fish species, *Perca fluviatilis, Esox lucius and Rutilus rutilus.* The diet in this region mainly consists out of *Perca fluviatilis*, present in 67% of regional spraints. The otter's diet in region 2 contained *Perca fluviatilis, Esox lucius, Gobio gobio gobio, Cyprinus carpio* and *Tinca tinca*. The *Perca fluviatilis* was also in this region the most consumed species (57%). The *Esox lucius* was only consumed ones in region 3 and here also the *Perca fluviatilis* was the most consumed fish species (91%).

Table 14 Number and percentage of fish species of the otter's prey determined by analysing scales collected from seventy-eight otter spraints, which contained eighty-three per cent of fish divided over six different fish species; the European perch, Pike, Common roach, Gudgeon, European carp and the Tench. The total number of spraints of all the regions submitted gives a total of seventy-four spraints, because two spraints did not contained any prey remains and two spraints only contained 'unknown' species. The study sites were divided over three regions; Region 1 (The Netherlands; Wieden, Weerribben, Lindevallei and Rottige Meenthe), Region 2 (The Netherlands; Doesburg and Giesbeek) and Region 3 (Peene, Germany).

	Region 1		**Region 2**		**Region 3**	
Fish species	Number of spraints	Percentage	Number of spraints	Percentage	Number of spraints	Percentage
Perca fluviatilis	28	67	12	57	10	91
Esox lucius	8	19	5	24	1	9
Rutilus rutilus	6	14	0	0	0	0
Gobio gobio gobio	0	0	2	9	0	0
Cyprinus carpio	0	0	1	5	0	0
Tinca tinca	0	0	1	5	0	0
Total;	**42**	**100**	**21**	**100**	**11**	**100**

5.3 Main question II; How to produce a usable reference manual?

The general introduction of this research report (chapter 2), refers to the target group and the goal for the reference manual. Reference manuals have several standards in order to make the reference manual (easy to use for the target group and reaching its goal).

Below some comparing is done with other reference manuals by a small literature study. These standards are *cursively* typed in italic below.

Determination guides used for plants are not an option, because they are ordered by numbering with a description, which is almost impossible to do with scales and otoliths.

Foreyt, W., (2001) developed a very sufficient reference manual for the determination of parasites for the target group 'veterinarians' Boer, H., *et al.* (1974) developed a reference manual on carcass characteristics in cattle for the target group 'animal coroners'. Both reference manuals have standards that makes that any scientist could work with it, which is the target group for this current research about the otter diet. This makes it a priority to use the same type of standards fitting to this current research. The *contents* give a clear overview of what to find in each chapter, so searching for a certain aspect can be done easily and fast.
Having an *abstract*, *terminology*, *preface* and an *introduction* gives a clear overview what to expect in the reference manual. *References* show that the reference manual is supported by research, so it contains proven facts and items. (Boer, H., *et al.* 1974 & Foreyt, W., 2001)
Collection, *preservation*, *preparation* and *behaviour of the wanted items* (samples) were some standards in Foreyt's, W., (2001) reference manual as well. Foreyt, W., (2001) used faecal items as samples, which correspond with the samples (spraints) used in this current research. He clearly described where the samples can be found (collection). The faecal samples were labelled and then preserved for a certain period, which corresponds with this current research (preserving spraints for a certain time in a freezer), (preservation). A microscopy part in the reference manual of this current research is important in order to easily reproduce the research. This part includes explanation and microscopy photographs (including figure numbers and scientific classification and species name giving), (preparation), also done in Foreyt's, W., (2001) reference manual. Boer, H., *et al.* (1974) weighted and measured the carcasses for length, width and mass, which correspondents in this current research with the measurement of otoliths in order to calculate the mass of the prey (fish), (preparation). In this current research the behaviour of the wanted items represent the different spraint types, like jelly, fresh and old. Both Boer *et al.* (1974) and Foreyt, W., (2001) described the behaviours of their wanted items.
For *determination* Foreyt, W., (2001) used *diagnostic techniques* including a *standard method*. This standard method also includes an amount of solution. Both aspects correspond with the current research because one of its goals was to develop a standard method which happens to include a solution as well. Boer, H., *et al.* (1974) also used standard method for the determination and analysis of the carcasses.
Boer, H., *et al.* (1974) included a chapter with *acknowledgements* in their reference manual.

6. General discussion

6.1 Discussion on methodology

Experienced researchers have collected the spraints that were used in this research, they studied the otter for years and know where they spraint. This makes it easy to collect the spraints, but there might be the option of missing new spraint locations (Niewold, F., 2011). When an inexperienced researcher is collecting the spraints this might influence the research as well. An inexperienced researcher might find it difficult to find the spraint spots. This could mean that only the spraints of some otters might be found as only certain spots will be found and used for spraint collection. If individual otters have different prey preference this could influence the results of the diet study.

The samples of the scales were taken at random in the petri-dishes. When taken a scale from a petri-dish it is possible that the preference goes to the bigger scales as these are easier to take, even though the method excludes this preference as the scale in the middle of the petri- dish needs to be taken. Shake the dish after every scale that is taken in order to get a new distribution of the scales in the petri-dish. With this method new scales will be in the middle of the petri- dish after every sample taken. Some scales are very small and difficult to pick up with tweezers. This might have caused for an exclusion of these small scales as they could have been from another species.

The binocular-camera used in this research was last checked in 2009, which means that there is a very small chance that it suffers some lack of accuracy, but still this is possible to be the case.
The microscope used did not have a status which showed when it has last been check-up. This also means that there is a slight possibility that it lacks in accuracy. This could be checked by using another microscope and comparing the results. In the case of the binocular camera it was not possible as there is only one available at the van Hall Larenstein University. In the case of the microscope; different microscopes were used during the laboratory work. Two researchers were working together on two different microscopes each time present in the laboratory. In several uncertain cases the sample was checked under both the microscopes. In none of the cases a difference was found between the two microscopes.

The computer program ‘analySIS’ showed no version type, so if there would be a newer version available, this could expand the possibilities with photographing the prey remains.
The CD-ROM from Leopold, M., *et al.* (2001) could lack in accuracy as the length of a useful otolith from a certain species was in between the ranges of the calculation tables for this species, the length of another otolith from the same species was exactly the same but the width of both otoliths were different. They still had the same fish mass according to the calculation tables for this species. This is an outstanding outcome and therefore questionable for accuracy.

The prey remains found were compared with determination books, to determine the species; these books do not have all the existing fish species in the Netherlands in it. Because not all the species are in it, there is a chance that species might be confused with another look alike species.
It is also possible that the researcher thinks, by a first look at a prey remain, to know the species it belongs to. This can happen when they have experience and seen similar prey remains before. Still, every prey remain need to be examined with caution.

6.2 Discussion Results

<u>This research compared with the literature study</u>
As Allen (2010) and Mason (1986) stated also this research shows clearly that otters do occur in the Netherlands as well as in the North-east of Germany, by the otters spraints found. For the Netherlands it is now even proven that they are also widely distributed in one country. Spraint-research is also according to this research (see chapter 4 & 5), the method to do diet analysis for otters, as shown by

the BBC (1998), Brazier M. *et al.* (2001), Crimmins (2009), Guertin D. A. *et al.* (2010), de la Hey, D.C. (2008), Jacobsen L. (2004), Kloskowski, (2005), McCafferty (year unknown) and by Woodroffe (1994), see paragraph 3.1.4.
Furthermore, literature study showed that otters are semi-aquatic animals (Chanin, 1993 & Palazón, 2008) and that their habitat choice is made by the availability of a suitable place which provides the basic requirements like food (mainly aquatic species according to Allen (2010)) and shelter (Woodroffe, 1994), see paragraph 3.1.2. This is the case according to this current research (see paragraph 3.1.2), because the seven study sites are all 'land combined with water' environments. So food is provided by the rivers, canals and lakes that occur in all of the study sites and different types of shelter are provided by reed, peat holes, canals (with bridges), forest, Sphagnum reed beds and city (contains bridges).

Bekker *et al.* (1990), Carss (1995), Colares *et al.* (2000) and Georgiev, D.G. (2004) stated that the otter's composition contains mainly out of fish but also out of amphibians, reptiles, birds, crustaceans, small mammals and insects. And Woodroffe (1994) even gave numbers about the amount of fish in the otter's diet, 70-95%. This current research shows that this appears close to our results, which showed that several species besides fish, were eaten and that fish made up for 83 per cent of their diet species, see paragraph 5.2.

Colares *et al.* (2000) gave percentages of the amount of fish (between 65-95%), crustacean (25%), mollusc (between 2-45%), insects (between 2-20%), birds (between 2-15%), mammals (between 7-10%) and reptiles (between 1-10%), in the otter's diet in several location in Brazil, see appendix I. In this current research these number were for fish (83%), crustacean (4%), mollusc (3%), insects (3%), birds (2%), mammals (2%) and unknown (Mammals, Birds, Reptiles and/or Amphibians, with a percentage of 3), see paragraph 5.2. So it differs in amount of Crustacean and Mammals, but also in amount of Reptiles as they were placed under 'unknown' together with Amphibians, Mammal and Birds in this current research. Overlap between the species (in percentages) found in literature (Colares *et al.*,2000) and this current research (paragraph 5.2) is seen in Fish, Birds, Mollusc and Insects.

The literature study in paragraph 3.1.6 gives an overview of the results of the research done by Cabral *et al.*, 2010 about the Giant otter's diet in two study sites, Balbina Lake and Xixuau creek. Both study sites are in the Amazons and none of the species in the Giant otter's diet were found in the otter's diet in this research.

The fish species found in our study (paragraph 5.2), were all present in the Netherlands in 1997 according to research done by de Nie in 1997, (paragraph 5.2 compared with appendix IV) which shows that de Nie (1997) did research about possible fish species protection, of fish species that were present in the Netherlands.
The otter diet results in our study (paragraph 5.2) compared with several otter diet studies done in Europe (appendix III), show some differences in the otter's diet. The Perch, Common roach and the Gudgeon were found in both studies, but the Pike, European carp and the Tench, were only found in this current research. Appendix III, shows also several species that were not found in this current research.

Several fish species belonged to the otter's diet in the northern part of the Netherlands (Friesland), in 1990 according to Bekker *et al.* (1990), see paragraph 3.1.6.1. Compared with this current research it showed that only the Perch and the Common roach appear to still belong to the otter's diet, see paragraph 5.2 .

<u>Discussion of the results found</u>
There are differences between the regions and between otolith and scale analysis, these are compared in paragraph 5.2 table 10 compared to table 14.
Otolith-analysis showed different results in the amount of each fish species found in the regions, but no difference was seen in the number of fish species eaten, see paragraph 5.2. The otolith-analysis showed that only the Perch was eaten in all regions. Overall the scale-analysis showed a higher

number of total results than the otolith-analysis. The total result was 74 by scale-analysis where the total results were only 43 by otolith-analysis. The otolith-analysis appeared to be a useful way to calculate fish mass, and showed a more overall spreading of all the fish species than scale-analysis did. Not all fish species were found in all the regions with scale-analysis. While they were found in some regions using otolith-analysis (this occurred five times). When you put it the other way around it was only three times (three fish species were found in some regions with scale-analysis while they were not found with the otolith-analysis).

The study sites per region are all connected with each other by rivers, canals and ditches, so the fishes could travel between them. The study sites per region are in the distribution ranges of the otters, so they could also have travelled between the different study sites. Both aspects above are not relevant for the Peene, Germany.

If the number of spraints found in the study site the 'Wieden' was higher (so more equal distributed), this would probably not affect the results of this research as fish and otters are well travelled species and have the opportunity to do so as mentioned above.

The scale analysis showed seventy-four fish results and not seventy-eight, which was the total number of examined spraints, this is because two spraints did not include any prey remains at all and two spraints only included species from the unknown class, which could not be identified yet, so because of this they were excluded from the results.

Some prey remains from fish, such as pharyngeal teeth, jawbones such as Premaxillae, and vertebrae were not included in the results of this research. This was not possible because a lot of these items were too weak, broken or not even found in the spraints. Many bones from other species than fish, that were found in the spraints were also broken and crushed. These parts were also excluded from examination. The exclusion of these prey remains could have caused different numbers in the results of the different phylum found, in paragraph 5.2.

Also the exclusion could have caused a lack some new prey species, especially mammals and birds as these species have no scales or entire chitin skeleton which is found in the spraints. Species other than fish, were not examined and specified till species name in this research, see paragraph 5.2. In order to get an insight in the second most preferred prey group these prey remains should be further examined. This refers to a literature study about how to do this, and the collection of the determination material needed to perform this examination.

The Common carp and the Tench were both only found ones in this research, see paragraph 5.2. So it could be a coincidence that these species ended up in the diets of the otters to whom these two spraints belonged to, and that both species are not really a part of the otter's diet.

Some fish species could not be determined by the scales collected from the spraints, because there was no reference material for these species, see appendix CD_ROM (database excel). All the unknown fish species were not included in the results. The scales from these fish species were photographed, so they could be determined in a later stage when there is determination material to determine to which fish species these scales belong to, see chapter 8.

Otolith with spraint number 61 and otolith number 1, is a Common roach and has a fish mass of 1.73 kilogram, which is a discussable amount of weight. A fish with this weight, can only be a youngster. The otolith from the Pike was only found ones in the 78 spraints, see paragraph 5.2. This could mean that the head of the Pike is not always a part of the otter's diet, as their scales were found more often. It is also possible that the otolith from the Pike passes the digestive system of the otter easily and fast. So the number of otoliths found in the 78 spraints and the number of otoliths per spraint, could be related to how fast/slow they go through the digestive system. The cause of this may be that otoliths are made of hard indigestible matter and some of them have a jagged exterior (see figure 5. A, 5. C.

and 5. F.), which may cause these otoliths to travel slower through the digestive system, see chapter 8.

The number of otoliths is also related to the number of times the otter eats the head of the fish, because only the fish's head contains otoliths. The otter is an opportunist, so in the wild it could be the case that if otters find sometimes only the fish's head (other animals could leave this behind), they would eat it. When otoliths are found in a spraint they often have signs of wear (Leopold *et al.*, 2001). This can cause problems when comparing the otolith found in the spraint with the reference material. The otolith can be confused with the one of another species of the otolith may not be identifiable at all. This can cause byassed results.

Some otoliths were not included in the results because of lack of accuracy to determine which species they belonged to. These otoliths were photographed, so they could be determined in a later stadium when there is determination material to determine to which fish species these otoliths belong to, see chapter 8.

The sample size was 78 spraints. Mostly the higher the sample size, the more complete the otter's diet overview. However, this research indicates only six different fish species (both scale and otolith determination), which makes up 83% of the diet (scale determination). This appears a pretty good description, also showing potentially meaningful variability between locations and habitats.

It could be that a higher sample size gives more different species of other phylum than fish. In this research the other phyla only represents seventeen per cent of the diet. So it may be questionable if a higher sample size and more information about the other phyla species, (more different species, bone, feather identification etc.) is worthwhile.

6.3 Discussion reference manual

Although the reference manuals from the literature study for the preconditions of the reference manual for this current research (see paragraph 5.3), have whole different target groups and goals, the standards for a sufficient reference manual may be similar as all these reference manuals could be used by the target group (scientists) for this current reference manual.

The conclusion of the standards for a sufficient reference manual according to a small literature study, are that they contain carefully described;

- Contents
- Abstract
- Terminology
- Introduction
- References
- Collection
- Preservation
- Preparation
- Behaviour of the wanted items
- Determination; diagnostic techniques, standard method
- Acknowledgements

7. Conclusion

The conclusion of the otter diet analysis is that their diet exists for 83% of fish species and 17% of other species. The study sites were divided into 3 main regions; region 1 (Wieden, Weerribben, Lindevallei and Rottige Meenthe), region 2 (Doesburg and Giesbeek) and region 3 (Peene, Germany). The main fish species of the otter's diet was the European perch (mean fish mass of 90, 14 gram), who makes up 67% in region 1, 57% in region 2 and 91% in region 3. The remaining part of fish species consists of Pike (mean fish mass of 179, 15), Common roach (mean fish mass of 12, 35 gram) and Gudgeon (mean fish mass of 9, 66 gram). The Common carp (mean fish mass of 12, 46gram) and the Tench (mean fish mass of 324, 78 gram) are the smallest fraction of the fish species found in the otter's diet, as they are both only found once. Otoliths were a useful prey remain for fish mass calculation and can be easily collected from otter spraints. However, there may be otoliths or scale analysis biases since they do not indicate similar representation in the total sample. Another conclusion is that an otter spraint can contain one or more species from one or several phylum. Spraint analysis is a useful method to determine the diet of the otter.

Another conclusion is that scale-analysis and otolith-analysis used together gave the best overall results as scale-analysis gave a higher number of total results and otolith-analysis was a useful way to calculate fish mass, and showed a more overall spreading of all the fish species than scale-analysis.

8. Recommendations

Suggestions for other research

It is possible to do a diet study on otter stomachs. The stomach content can be obtained from deceased or road victim otters in the study areas, if it shows that these are still conserved and usable (Niewold, F., 2011). The stomach content will contain prey remains in an earlier stage than the prey remains in the spraints, which can obtain new information about the diet of the otter. It is recommended to compare any differences between the prey remains collected from spraints with the prey remains collected from stomachs. These road victims will certainly give info on what diet they prefer.
This type of research is done by other Mustelidae species like stoats (de la Hey, D., 2008). Research about how to examine stomach contents need to be done in advance.

Suggestions for refining methods

Nineteen per cent of all the prey remains found in the spraints during this research are not from fish species. Not much literature about determination of Mammalia, Aves, Insecta, Reptilian and Crustacea is found during this study.
In order to determine these species with a high reliability, bones, feathers and hairs need to be collected of Chordata species these can be compared with the prey remains found in the spraints. Research about Crustacea, Mollusca and Insecta species needs to be done in order to be able to determine these species, this can also be done by the collection and comparison of these species with the prey remains.
Hair comparison is done in this research with the paw of the brown rat, which was found in a spraint, this is described in 5.2.

To get an insight in any preferences for slower moving species, like inter-tidal and benthic species, another kind of research is needed. Here for, specialisation in inter-tidal and benthic species, but also knowledge of the natural water system in the study area are needed, which then can be compared with the otter diet in these regions.

A research to gain knowledge about otoliths with a higher wear stage than 3 could expand the set of photographs and calculation tables of the CD-ROM from Leopold, M., *et al.* (2001). This will increase the amount of otoliths from otter spraints that can be investigated to calculate fish mass.

Paragraph 6.2 discusses some possible relation towards the total number of otoliths found in all the otter spraints examined and the number of otoliths found per spraint. Another aspect that is mentioned in this paragraph is that the otolith from the Pike is not a part of the otter's diet and/or passes the digestive system easily and fast. Otoliths are made of hard indigestible matter and some of them have a jagged exterior, which can cause that these otoliths travel slower through the digestive system.
In order to have an insight into these possible relations, this needs to be researched.

It is recommended to use scale-analysis in combination with otolith-analysis, because this gives the best overall results as scale-analysis gives a higher number of total results and otolith-analysis is a useful way to calculate fish mass, and shows a more overall spreading of all the fish species than scale-analysis.

In order to get a more complete reference manual, a follow up project can be designed in which as much fresh water fish species should be examined and parts can be photographed. The more reference material there is the more reliable and specified further research can be.
In this research there was only the opportunity to examine the fish species delivered by H. Bosma. The rest of the comparison of prey remains had to go through existing determination books.
Every time when the target group uses the reference manual produced in this research, new found prey remains and new found species need to be added to this reference manual in order to keep the reference manual up to date by contacting the Niewold Wildlife Infocentre.

Invasive species get chances to travel over time and otters are opportunists so they can also chance their diet over time. So new species need to be added to the manual over the following years. The photographed ‘unknown’ fish scales and otoliths could be determined in a later stadium when there is determination material to determine to which fish species these scales and otoliths belong, see paragraph 6.2.

Suggestions for implementation: how to use the product?
The manual could be updated every five years. All the new data can be added to the reference manual and a new edition of the manual can be published. The administrator of this manual is Niewold, F. as he is the client of this research. New researchers could cooperate with him.

Acknowledgements

For this research, Hermsen, J. and Maarseveen, A., van, would like to thank Stijkstra, A. and Heijer, M., den, for guiding this research. We would like to thank Kuipers, H. and Ende, M., van den, for helping us and sharing their knowledge and skills.
We would like to give out a special thanks to Bosma, H. and Leopold, M. for answering our questions which contributed to the results of this research.
This research is carried out at the van Hall Larenstein University of applied science, Leeuwarden. This University provided space and all the material needed for this research.
This research is done by order of the Niewold Wildlife Infocentre, we would like to thank Niewold, F. for giving us the assignment and sharing information which contributed to the results of this research.

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Appendices

Appendix I Outcome research Colares, E., *et al.* (2000); classification of prey species in percentages

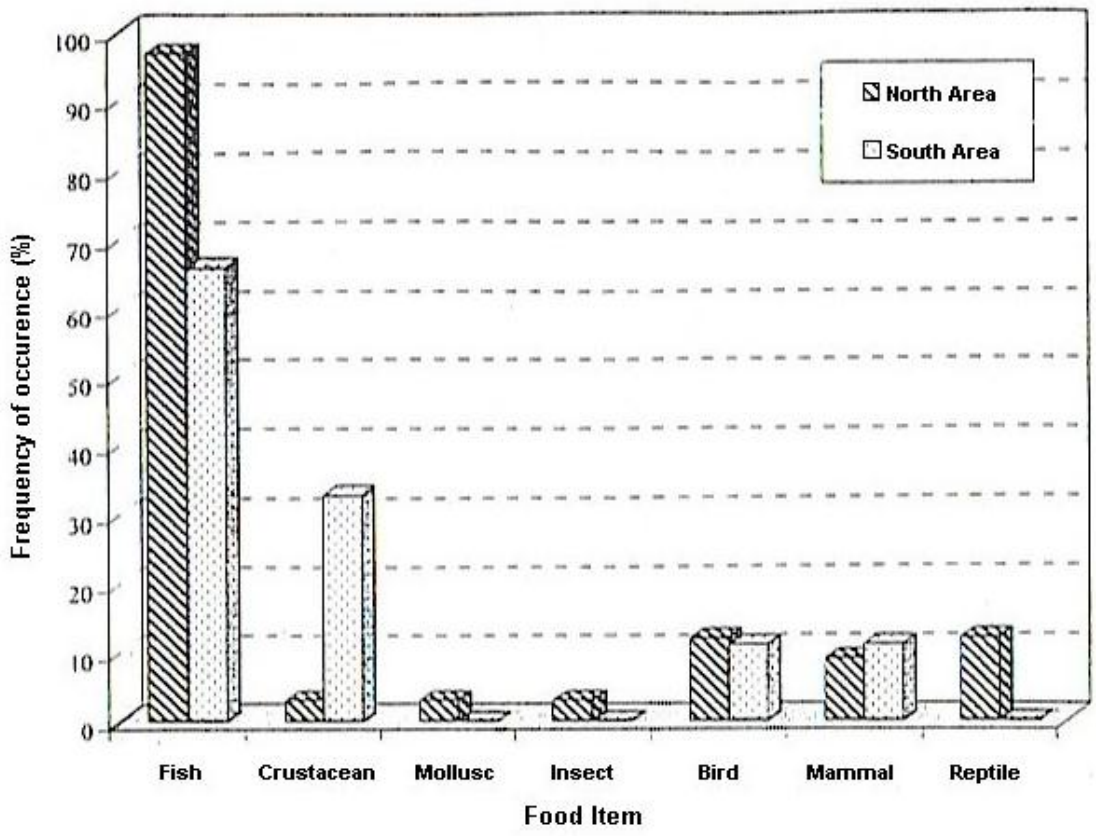


Figure 2. Feeding habits of the otter in the Lagoa do Peixe National Park (click for larger version)

Figure 3; Outcome research Colares, E., *et al.* (2000); classification of prey species in percentages

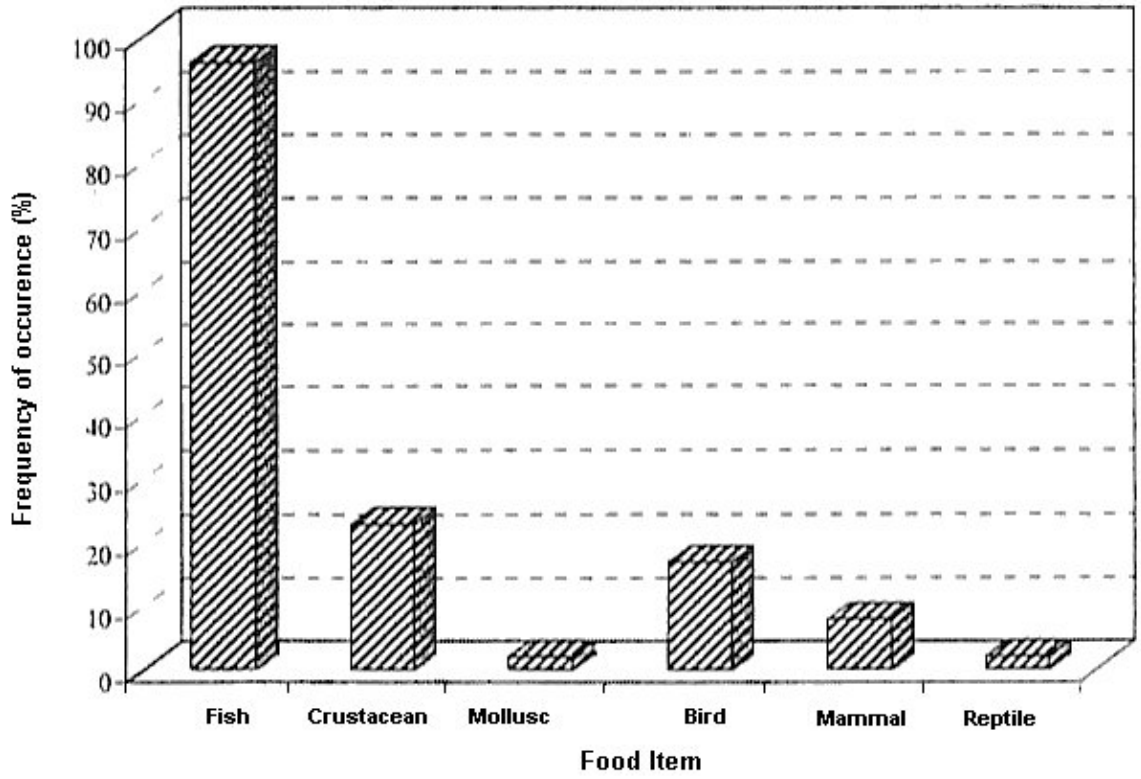


Figure 3. Feeding habits of the otter in the Senandes Creek (click for larger version)

Figure 4; Outcome research Colares, E., *et al.* (2000); classification of prey species in percentages

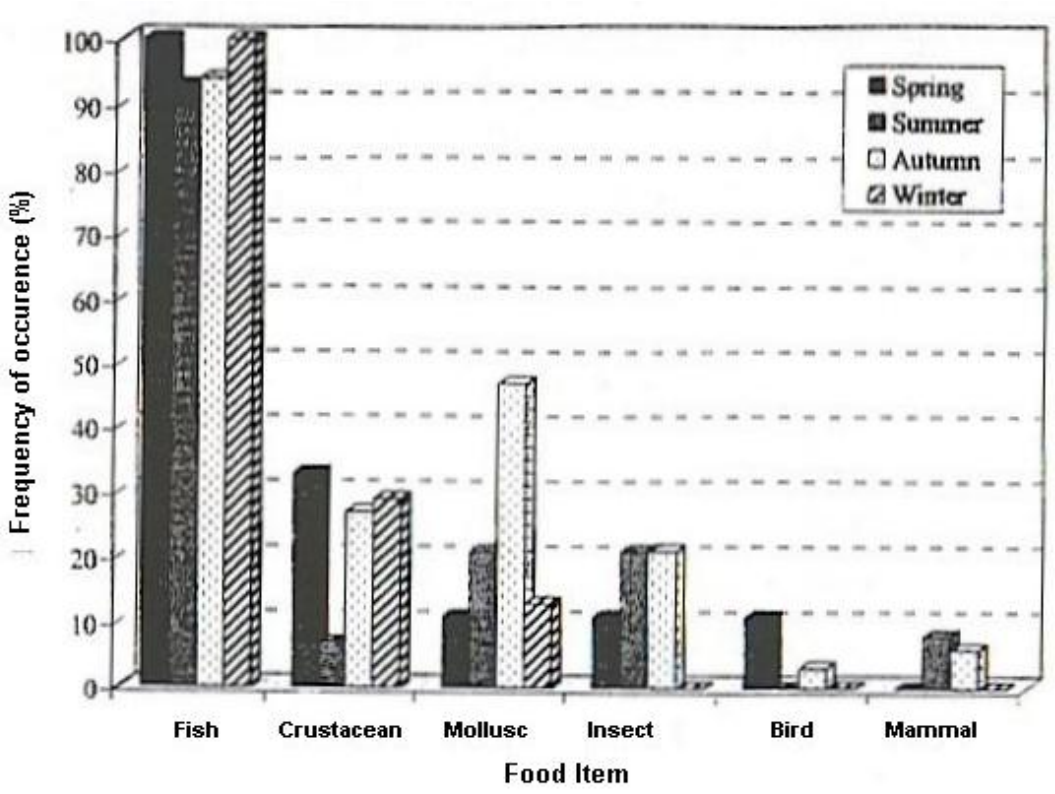


Figure 4. Feeding habits of the otter in the Estiva Creek (click for larger version)

Appendix II Mammalian standard metabolism curve

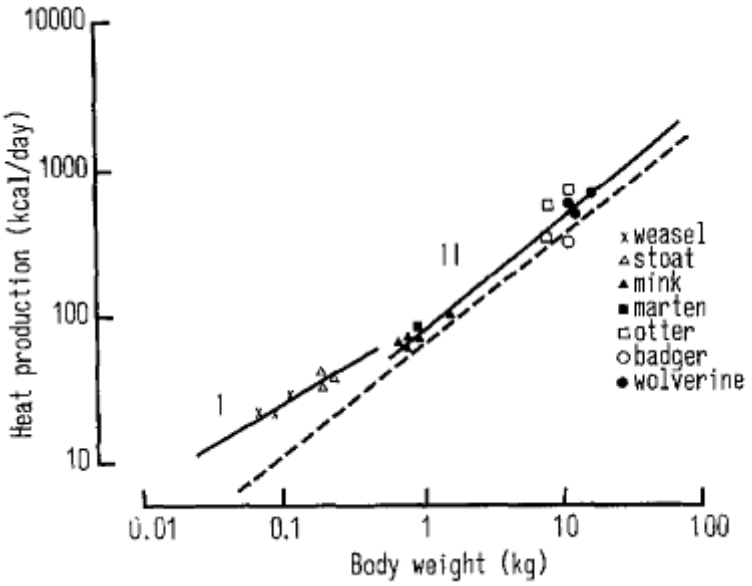


Figure 1 Mammalian standard metabolism curve; 'Log/log plot showing the relationship between heat production and body weight in Mustelids. Dotted line represents the mammalian standard curve and is drawn from the data of Kleiber (1961).' (Iversen, J., 1972)

Appendix III Common prey species by otters in Europe, divided per name (scientific and English), class and collection place with the source and date the information origins from.

Prey species; English name	Prey species; Scientific name	Class	Collecting place	Source
Sticklebacks	*Gasterosteidae*	Fish	-	Chanin, P., 1993
Trout	*Salmoninae*	Fish	Denmark	Chanin, P., 1993 & Jacobsen, L., 2004
Roach	*Rutilus rutilus*	Fish	-	Chanin, P., 1993
Perch	*Perca fluviatilis*	Fish	Denmark	Chanin, P., 1993 & Jacobsen, L., 2004
Eelpout	*Lycodus*	Fish	-	Chanin, P., 1993
Rocklings	*Gadidae*	Fish	-	Chanin, P., 1993
Sea scorpion	*Eurypterids*	Crustacean	-	Chanin, P., 1993
Butterfish	*Stromateidae*	Fish	-	Chanin, P., 1993 & King, J., 1995
Flounder	*Platichthys flesus*	Fish	Denmark	Jacobsen, L., 2004
Rudd	*Scardinius erythropthalmus*	Fish	Denmark	Jacobsen, L., 2004
Gudgeon	*Gobioninae*	Fish	Denmark	Jacobsen, L., 2004
Bream	*Cyprinidae*	Fish	Denmark	Jacobsen, L., 2004
Gunnels	*Polidae*	Fish	Vancouver Island, British Columbia, Canada	Guertin, D., 2010
Sculpins	*Cottidae*	Fish	Vancouver Island, British Columbia, Canada	Guertin, D., 2010
Picklebacks	*Stichaeidae*	Fish	Vancouver Island, British Columbia, Canada	Guertin, D., 2010
High cockscomb	*Anoplarchus purpurescens*	Fish	Vancouver Island, British Columbia, Canada	Guertin, D., 2010
Northern clingfish	*Gobiesox maeandrichs*	Fish	Vancouver Island, British Columbia, Canada	Guertin, 2010
Barbels	*Barbus sp.*	Fish	Northern Spain	Palazón, S., 2008
Brown trout	*Salmo trutta*	Fish	Northern Spain	Palazón, S., 2008
Nase	*Chondrostoma meigii*	Fish	Northern Spain	Palazón, S., 2008
Pike	*Esocidae*	Fish	-	Chanin, P., 1993
Crayfish	*Astacus/Austropotamobius*	Crustacean	-	Chanin, P., 1993 & Woodroffe, G., 1994
Amphibians		Amphibians	-	Woodroffe, G., 1994

Appendix IV Present fish species in the Netherlands in 1997, divided per family name, species, Dutch name, state and proposal for the Red List (source; Nie, H. de, 1997)

Familie	species	Nederlandse naam	Status	%	Voorstel Rode Lijst
Petromyzontidae	Petromyzon marinus	Zeeprik	e	3,5	EN
	Lampetra fluviatilis	Rivierprik	e	7,3	VU
	Lampetra planeri	Beekprik	e	2,1	EN
Acipenseridae	Acipenser sturio	Steur	e	0,30	EX
Anguillidae	Anguilla anguilla	Paling	e	51	SU
Clupeidae	Alosa alosa	Elft	e	0,18	EX
	Alosa fallax	Fint	e	1,3	EX
Cyprinidae	Abramis brama	Brasem	e	59	
	Abramis bjoerkna	Kolblei	e	49	
	Vimba vimba	Blauwneus	exo	0,24	
	Alburnus alburnus	Alver	e	22	
	Alburnoides bipunctatus	Gestippelde alver	e	0,06	SU
	Aspius aspius	Roofblei	exo	>1,8	
	Barbus barbus	Barbeel	e	3,5	EN
	Carassius carassius	Kroeskarper	e	21	VU
	Carassius gibelio	Giebel	est	6,7	
	Chondrostoma nasus	Sneep	e	1,6	EN
	Ctenopharyngodon idella	Graskarper	exo	15	
	Cyprinus carpio	Karper	est	39	
	Gobio gobio	Riviergrondel	e	28	
	Pseudorasbora parva	Blauwband	exo	0,4	
	Leucaspius delineatus	Vetje	e	12	VU
	Leuciscus cephalus	Kopvoorn	e	6,4	VU
	Leuciscus idus	Winde	e	34	SU
	Leuciscus leuciscus	Serpeling	e	6,1	VU
	Phoxinus phoxinus	Elrits	e	0,55	EN
	Rhodeus sericeus	Bittervoorn	e	11	VU
	Rutilus rutilus	Blankvoorn	e	64	
	Rutilus erythrophthalmus	Ruisvoorn	e	47	
	Tinca tinca	Zeelt	e	41	
Cobitiditae	Cobitis taenia	Kleine modderkruiper	e	20	
	Misgurnus fossilis	Grote modderkruiper	e	8,2	VU
	Barbatula barbatulus	Bermpje	e	16	
Ictaluridae	Ameiurus nebulosus	Bruine dwergmeerval	est?	2,2	IK
	Ameiurus melas	Zwarte dwergmeerval	exo	0,18	
Siluridae	Silurus glanis	Meerval	e	4,7	
Esocidae	Esox lucius	Snoek	e	50	
Umbridae	Umbra pygmaea	Amerikaanse hondsvis	exo	4,5	
Osmeridae	Osmerus eperlanus	Spiering	e	20	
Salmonidae	Coregonus l. oxyrinchus	Houting	e	1,7	EX
	Coregonus albula	Kleine marene	exo	0,24	
	Oncorhynchus mykiss	Regenboogforel	exo	5,4	
	Salmo trutta trutta	Zeeforel	e	7,2	VU
	Salmo trutta fario	Beekforel	e	4,4	EX
	Salmo salar	Zalm	e	1,5	EX
	Thymallus thymallus	Vlagzalm	e	0,61	EX
Gadidae	Lota lota	Kwabaal	e	4,4	EN
Poeciliidae	Lebistes reticulatus	Gup	exo	0,36	
Gasterosteidae	Gasterosteus aculeatus	Driedoornige stekelbaars	e	41	
	Pungitius pungitius	Tiendoornige stekelbaars	e	36	
Cottidae	Cottus gobio	Rivierdonderpad	e	18	
Centrarchidae	Lepomis gibbosus	Zonnebaars	est?	3,0	IK
Percidae	Gymnocephalus cernuus	Pos	e	43	
	Perca fluviatilis	Baars	e	60	
	Stizostedion lucioperca	Snoekbaars	est	43	
Pleuronectidae	Platichthys flesus	Bot	e	16	

Appendix V Pilot study for the diet analysis of the Eurasian otter

Pilot study

A pilot study was done before starting the research in the laboratory, to test if the method is sufficient. A form is designed to list and process the collected data in the laboratory (see table 2),
It was tested if sodium carbonate is a sufficient product for cleaning the spraint. Two solutions were used (see table 1) on two separate spraints. There were no apparent differences between the two concentrations, it was decided that during the research 0.07% (mass %) Na_2CO_3 would be used throughout the analysis.

Table 1 Pilot study for the diet analysis of the diet of the Eurasian otter, including the spraint number, date of collection, date of pilot study and percentage of Na_2CO_3 dissolved in cold tap water weight/volume.

Spraint number	**Date of collection**	**Date pilot study**	**Percentage of Na_2CO_3 dissolved in cold tap water weight/ volume**
1	13 October 2010	14 September 2011	0.07%
2	13 October 2010	15 September 2011	0.15%

After the spraint was placed in a measuring cup with the solution of cold tap water with Na_2CO_3 it was stored in a fume cabinet at room temperature.
After 24 hours the spraint was checked if it disintegrated sufficiently to clean it. If not, it was left for another 24 hours.
After soaking, the size of the sieve needed testing, the size of the mesh should not be too big, as prey remains might wash away through it. When the sieve mesh is too narrow the waste particles might not wash away. During this study we tested the sieve with a 0.425 mesh, one with a 0.6 mesh and one with a mesh of 1. The sieve with a mesh of 0.6 had the best results.

After this the prey remains will be air dried at room temperature (Georgiev, D., 2004 & de la Hey, D., 2008). It became clear drying of the spraints took 5 days.

Table 2 Laboratory research table for the analysis of the diet of the Eurasian otter

Laboratory research

Research date: Name researcher:
Collection date: Collection place: Spraint number:
Soaking solution: Soaking time in days: Sieve mesh:

Number researched prey remain	Kind of prey remain	Scale? →number of fingers	Class	Family name	Species	Sub- species	Microscope lens
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

Reference manual

Guidelines for otter spraint based diet analysis: searching, preservation, preparation and determination

Leeuwarden
January 2012

This manual is commissioned by:

Niewold, F.

Niewold Wildlife Infocentre
Van Berchemstraat 4
6981 JB Doesburg
The Netherlands

Authors
Hermsen, J. 850727001
Maarseveen, A., van 880430001

Supervisor
Strijkstra, A.
Heijer, M. den

Van Hall institute project number: 594000

Cover drawing: <www.frettcheninfos.de> downloaded 5 September 2011

University of Applied Sciences
VAN HALL LARENSTEIN
PART OF WAGENINGEN UR

Abstract

This reference manual contains a simple standard method for finding and handling of otter *(Lutra lutra)* spraints for diet related research. The method describes, how to do spraint collection, how to preserve spraints, how to prepare spraints and how to determine to which species the prey remains belong. Also a description of the necessary materials is given. The reference manual also includes photographs of prey remains of several species that can be found in the otter spraints. The method is based on the analysis of 78 spraints from river and marsh habitats in the Netherlands. We anticipate that the manual will support and promote research on otter diet by amateurs interested in public science.

Preface

This reference manual is written as a thesis project of Hermsen, J. and Maarseveen, A., van for the BSc Animal Management, major Wildlife Management at the Van Hall Larenstein University of Applied Sciences in Leeuwarden. The project was supervised by Dr Strijkstra, A., and Mrs Heijer, M., den The research was commissioned by Drs. Niewold, F. J. J. of the Niewold Wildlife Infocentre and aimed for production of a realistic reference manual for prey remains in otter spraints, including a simple but effective standard method for the research on otter diet for amateur research in the Netherlands.

Leeuwarden, January 2012

Jessica Hermsen and Aloïse van Maarseveen

Contents

Introduction

Not many diet studies on the Dutch otter *(Lutra lutra)* population have been done. Here for there is no standard method on how to do this research. The benefit of an easy standard method and reference manual may be that it promotes more research on the diet of this species by amateurs. This is the target group of this reference manual.
The method is based on information from literature study on otter diet studies, done in other countries, which was combined with expert opinions and our own practical experience on spraint analysis.

The manual explains the necessary steps for easy and efficient research on the otter's diet based on spraints.
It starts off with necessary preparation for fieldwork and the materials needed. Furthermore a guide on how to do the field work is given, followed by a full explanation of how to gather, store, prepare and analyse the spraints for prey remains, and how to examine prey remains to determine the species.

The manual includes reference photographs, which can be used to compare prey remains too.
The photographs are all made on prey remains found in otter spraints from otter of river and marsh habitats on several Dutch locations, and from boiled fish.

Terminology

Some terms are used which could be interpreted in different ways. To prevent confusion, some of these terms are defined below.

Table 2; Terminology, the right way of interpretation of several terms used in this reference manual.

Term	Correct interpretation
Reference manual	A manual that contains pictures, photographs and drawing of the identifiable parts in the spraints. Prey remain in following research can be compared with these images in the manual.
Spraint	A small faecal secretion from an otter which contains prey remains, to mark their territory.
Prey remains	The (mostly hard) indigestible parts in a spraint, such as bones, fish scales, jawbones, vertebrae, hair, feathers.
Determination	The identification of a prey part, to its species.
Detergent	Washing fluid with in this case a solution of Sodium Carbonate, the product that is used for cleaning up spraints.

Standard method

This method is set up to make a study on the diet of the otter less time consuming.

3.1 Material

The following material is preferred in order to perform an optimal research (see table 2).

Table 2 Materials needed to perform this research about the diet of the Eurasian otter

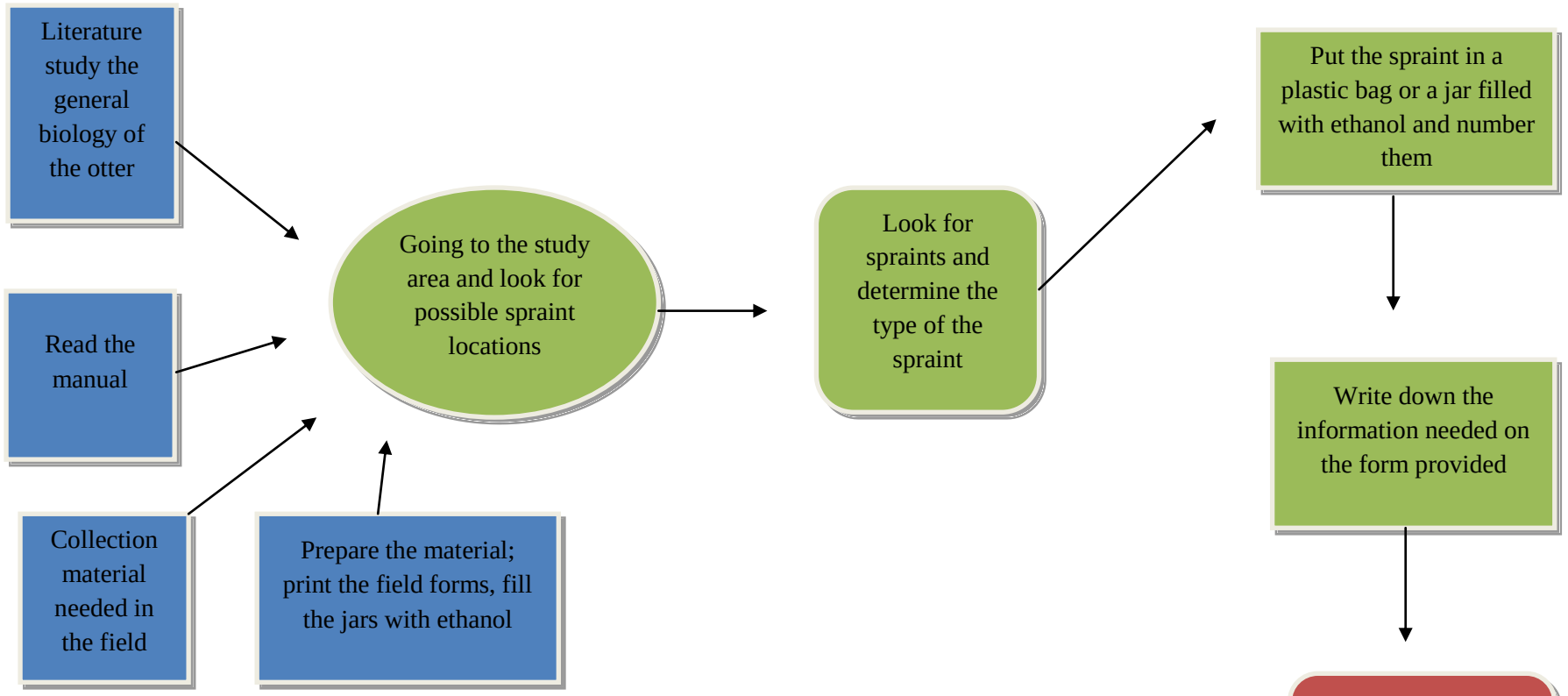
Specific for this research	General materials
Sieve 0.6mm	Measuring cup and stirrer
Sodium carbonate Na_2CO_3, 0.07%	Cutting board
Tissues	Waterproof marker and pen
Petri- dishes	Computer and printer
Microscope, (used for this reference manual; Olympus CHS)	Drawing material; pencil
Microscope slides, cover slips and demineralised water	Disposable gloves
Measuring scale (with accuracy of 0.1 gram)	Beaker
Cooking plate	Knife
Binocular-camera (used for this reference manual; Olympus SZ-CTV)	Stickers, that fit on the jars filled with ethanol
Tweezers	White paper sheets; to empty the sieve with the prey remains on
Spraints	Freezer

The determination books in table 3 were used during this research.

Table 3 Determination books needed to perform this research about the diet of the Eurasian otter in several study sites in the Netherlands; Wieden, Weerribben, Lindevallei, Rottige Meenthe, Doesburg, Giesbeek, and one in Germany; Peene.

Determination book; title	Author	ISBN
A guide to the identification of prey remains in otter spraint 3rd edition	Conroy, J.W.H. *et al.* 2005	0906282551
Atlas van schubben en andere beenachtige structuren van niet-zalmachtige zoetwatervissen, Organisatie ter verbetering van de Binnenvisserij;	Steinmetz, B. & Muller, R. 1991	9789080012042
The Tailless Batrachians of Europe	Boulenger, G.A. 1897	9780548217207
Voedselecologie van de 'verwilderde' kat	Honstede, B. van & Schut, G. 2010	594000

3.2 Flow chart; Field work. The steps to follow when you are going to search for spraints in the field



Field manual
This field manual contains guidance about how to collect spraints.

Preparation for a field trip
Collect all the materials mentioned in table 4. Make sure the stickers will fit on the jars and the jars are pre-filled with ethanol.

Print and cut out the labels designed in appendix 1 according to the amount of spraints that you anticipate to collect. These labels contain all important information that needs to be written down during field work.

Print some extra forms, don`t cut them; these will be used for the fresh spraints that are stored in jars with ethanol. You can number the jars with a pencil; these numbers refer to the ones you write down on your form.

Collect information on the research area and get a detailed map Google Earth, or 1:25000 map). Make an appointment with a specialist in this certain area to get information about the area and the otters that may live here.

Before starting check the weather conditions, this will influence the quality of the spraints. Rain; the spraints might get soak or flushed away. Cold weather close to freezing; DNA may be preserved for a longer time. Hot weather (20C+); the spraint will dry faster.

Table 4 Material list with the materials needed to perform this research about the diet of the Eurasian otter

Kind of material d of
Clipboard
Eraser
GPS
Pencil
Plastic bags to collect the spraints
Plastic jars (with screw caps) 150 ml
Print paper (to make labels)
Stickers to put on the jars
Ethanol 100%, 70%

Fieldwork
Otters have typical places to spraint, where the water is entered or exited and on conspicuous spots around passages where the otter often walks through. Places to look for spraints are indicated in table 5 and illustrated with photographs. You might also invite sprainting by creating a spot.

Table 5 Places to look for otter spraints in the field, with their explanation.

Place	Explanation
Otter passages (figure 1 and 2, appendix 2)	A small hole in the scrubs with a section of about 30 cm
Otter path (figure 1, appendix 2)	A clear path, where the vegetation has often been walked on, comes out/ in these holes and goes to another place. Often from one water place to the other
Water edge	Look for spots at the water edge where the vegetation is flat (walked on) or brown/ yellow (urinated) or where there is an object available to spraint on, like a stone. Drove outs for game are a

	good spot to look.
Create a spot	Create a spot where otters often come, by offering them a tree trunk or another object

Look for traces of the otter at the place where the spraint was found (see figure 3). This will increase the determination reliability that the spraint originates from an otter.
Write down where the spraint was found (shade, sun, under a bridge, etc.) as this might have influenced the quality of the spraint and must be taken into account for further research.

There are different types of spraints, (table 6). The type needs to be determined during collection to know if the spraint contains DNA. Jellies contain most DNA, as a jelly is epithelia mucus from the intestine. Fresh spraints contain usable DNA as well.

Table 6 Spraint types with figure numbers for examples, and their explanation.

Spraint	Figure	Explanation
Jelly	Figure 7 and 8, appendix 2	Mucus, yellow, white or green
Fresh spraint	Figure 6, appendix 2	Wet, strong smell, soft when you poke in it (with a stick)
Old spraint	Figure 5, appendix 2	Dry, smell is not so strong, hard

When the origin of the spraint is unclear the smell may be helpful. Otter spraints have a strong fish smell. This combined with knowledge about the area, the otter and traces found at the location will determine if it safe to assume that it is an otter spraint.

Old spraints are collected in a plastic bag. The information on the label should be filled out as complete as possible and is put in second plastic bag together with plastic bag with the spraint This prevents the label from getting wet and unreadable. Make sure to use capable letters, this makes sure that everyone can read it. Process the data on a computer as soon as possible after the fieldtrip.
Fresh spraints can be used for DNA research and when used for that potential purpose, need to be stored in 70% ethanol. Number the jars with the fresh spraints and link these to a paper or digital archive, i.e. a form or a date file.

Finishing spraint collection
The collected spraints need to be stored frozen at -20C until they are used for further examination.

Date: **Number:**

Place:

Coordinate:

Weather conditions:

Info spraint location:

Amount of spraints found at location:

Info spraint:

Info otter:

Date: **Number:**

Place:

Coordinate:

Weather conditions:

Info spraint location:

Amount of spraints found at location:

Info spraint:

Info otter:

Date: **Number:**

Place:

Coordinate:

Weather conditions:

Info spraint location:

Amount of spraints found at location:

Info spraint:

Info otter:

Date: **Number:**

Place:

Coordinate:

Weather conditions:

Info spraint location:

Amount of spraints found at location:

Info spraint:

Info otter:

Figure 1 Passage and path of the otter (photographed at Wieden/Weerribben on 22 November 2011)

Figure 2 Otters passage (photographed at Wieden/Weerribben on 22 November 2011)

Figure 3 Scratch traces of an otter (photographed at Wieden/Weerribben on 22 November 2011)

Figure 4 Otter spraint (photographed at Wieden/Weerribben on 22 November 2011)

Figure 5 Otter spraint (photographed at Wieden/Weerribben on 22 November 2011)

Figure 6 Otter spraint (photographed at Wieden/Weerribben on 22 November 2011)

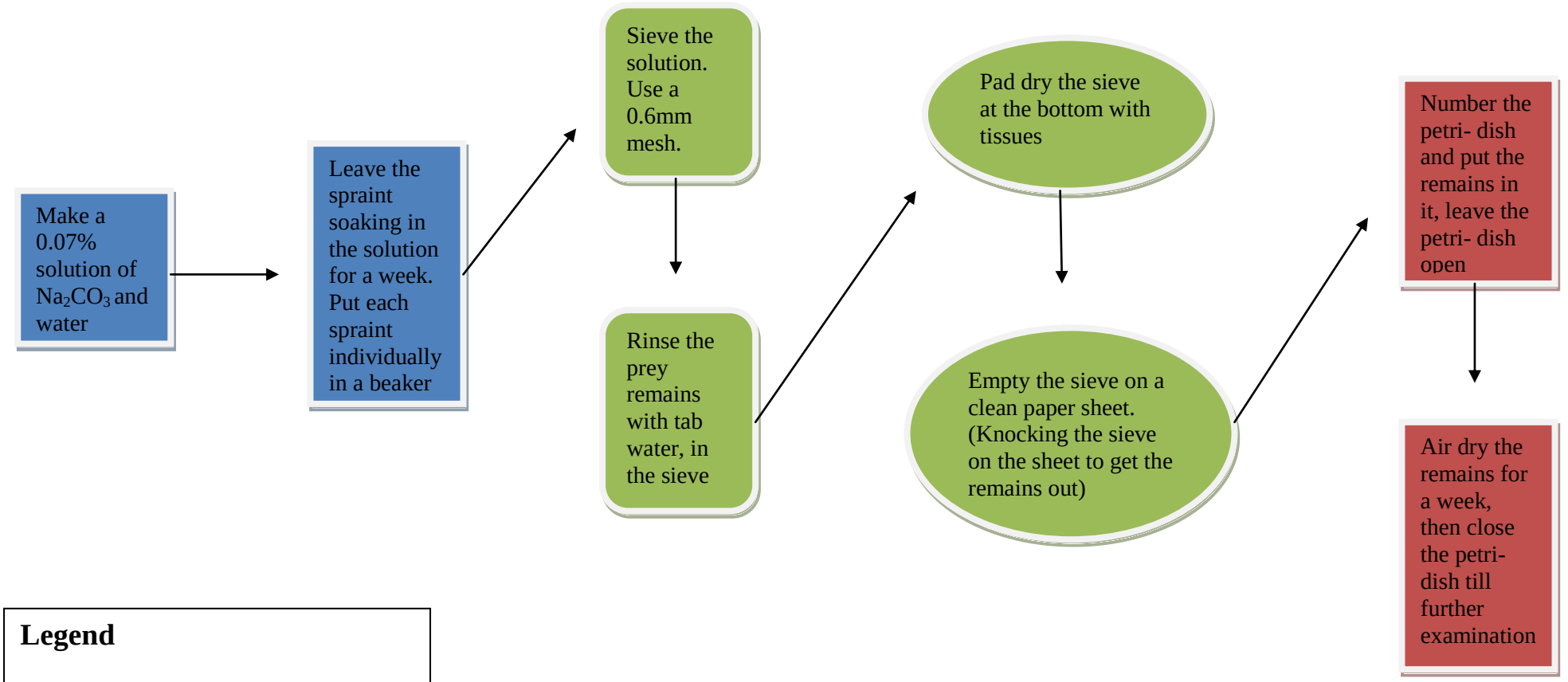
Figure 7 Otter spraint, jelly (photographed at Wieden/Weerribben on 22 November 2011)

Figure 8 Otter spraint, jelly, photographed by Bosma, H. (2011)

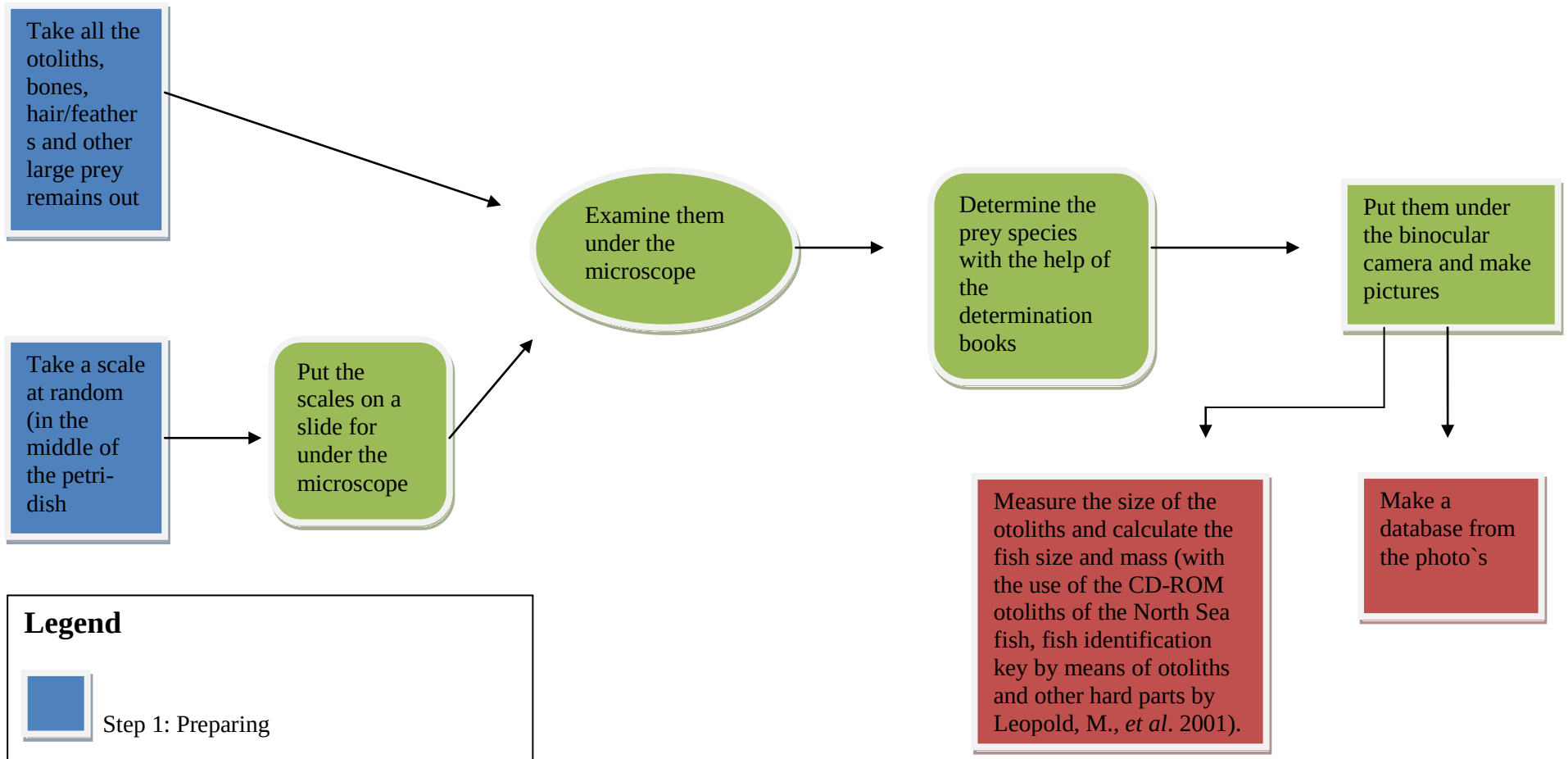
3.3 Spraint preservation

The spraints are collected in plastic bags with tags about the study area and deep frozen at -20 degrees Celsius until examination for subsequent analysis (Colares, E., *et al.* 2000, Georgiev, D., 2004, de la Hey, D., 2008 & McMahon, J., *et al*. 2006).

3.4 Flow chart; spraint preparation, the steps to follow when you clean the spraints (remove waste and mucus from the prey remains)



3.5 Flow chart; determination, the steps to follow when you want to determine the species of the prey remains



Analysis

Determination material for the European perch, Bream, Silver bream, Pike, Tench, Rudd, Common roach, Common carp, Gudgeon, Round goby and the Kessler's goby is given. These species were encouraged after the analysis of 78 spraints of otters in Dutch waters.

4.1 European perch

Table 7 Determination material for the European perch (*Perca fluvialtilis*)

Scientific name	*Perca fluvialtilis*
Exterior	Figure 9 (Sportvisserij Nederland, 2012)
Scale	Figure 10
Otolith	Figure 11
Spine	Figure 12

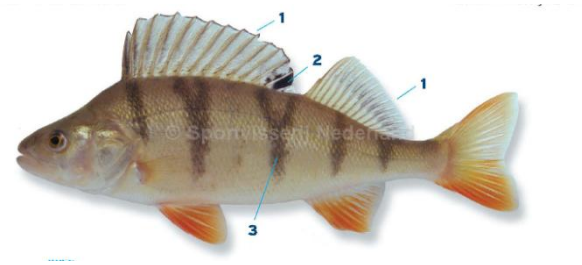


Figure 9 *Perca fluvialtilis* (Sportvisserij, 2012); 1; The Perch has two separated dorsal fins of which only the first one has hard spines. 2; The Perch has a back spot on the back-side of the first dorsal fin. 3; The Perch has vertical dark ties across the body.

Figure 10 *Perca fluvialtilis* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4

Figure 11 *Perca fluvialtilis* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4

Figure 12 *Perca fluvialtilis* spine, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67

4.2 Bream

Table 8 Determination material for the Bream (*Abramis brama*)

Scientific name	*Abramis brama*
Exterior	Figure 13 (Sportvisserij Nederland, 2012)
Scale	Figure 14
Otolith	Figure 15
Jaw	Figure 16
Spine	Figure 17

Figure 13 *Abramis brama* (Sportvisserij, 2012); 1; Number of rows of scales above the side-line, counted from diagonal pointing arrow to the dorsal fin, contains 12-14 scales (excluded the one from the side-line itself). 2; The eye-diameter is smaller than the distance from the eye till the point of its mouth. 3; The mouth is pointing downwards and is far bulging.

Figure 14 *Abramis brama* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 15 *Abramis brama* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 16 *Abramis brama,* jaw, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

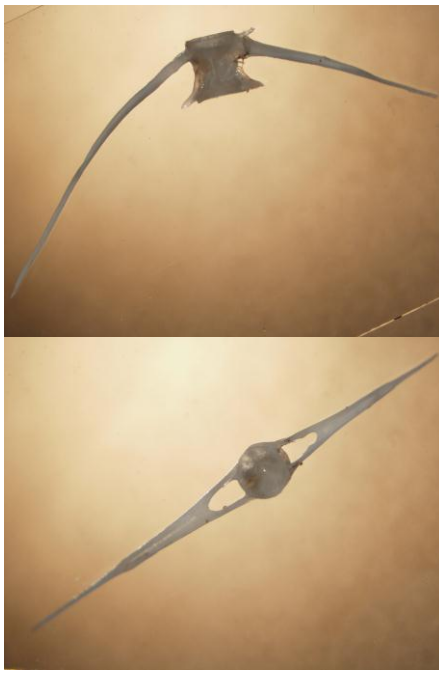


Figure 17 *Abramis brama* spine, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67. Upper photo; side view, bottom photo; front view.

4.3 Silver bream

Table 9 Determination material for the Silver bream (*Blicca bjoerkna*)

Scientific name	*Blicca bjoerkna*
Exterior	Figure 18 (Sportvisserij Nederland, 2012)
Scale	Figure 19
Otolith	Figure 20
Spine	Figure 21
Jaw	Figure 22

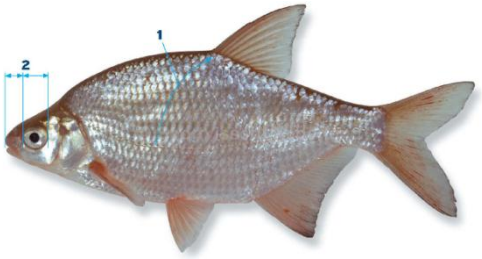


Figure 18 *Blicca bjoerkna* (Sportvisserij Nederland, 2012); 1; Number of rows of scales above the side-line, counted from the oblique pointed arrow to the dorsal fin, counts 8-10 (excluded is the scale on the side-line). 2; The eye-diameter is larger than the distance from the eye to the point of the mouth.

Figure 19 *Blicca bjoerkna* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 20 *Blicca bjoerkna* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 21 *Blicca bjoerkna* spine, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

Figure 22, *Blicca bjoerkna* jaw, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

4.4 Pike

Table 10 Determination material for the Pike (*Esox lucius*)

Scientific name	*Esox lucius*
Exterior	Figure 23 (Sportvisserij Nederland, 2012)
Scale	Figure 24
Otolith	Figure 25
Spine	Figure 26
Jaw	Figure 27

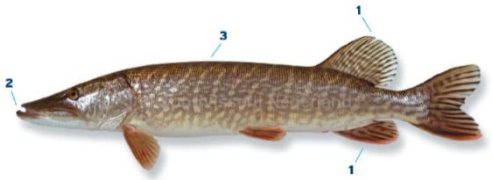


Figure 23 *Esox lucius* (Sportvisserij Nederland, 2012); 1; Anal fin and dorsal fin occurs far backwards on the body. 2; The head runs out into a flat, wide mouth. 3; All over the body there are gold-colored dots and stripes.

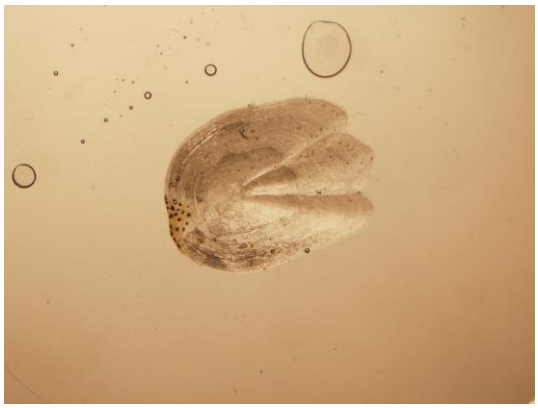


Figure 24 *Esox lucius* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 25 *Esox lucius* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 26 *Esox lucius* spine, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

Figure 27 *Esox lucius* jaw, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

4.5 Tench

Table 11 Determination material for the Tench (*Tinca tinca*)

Scientific name	*Tinca tinca*
Exterior	Figure 28 (Sportvisserij Nederland, 2012)
Scale	Figure 29
Otolith	Figure 30
Spine	Figure 31

Figure 28 *Tinca tinca* (Sportvisserij Nederland, 2012); 1; The iris is orange-colored. 2; The fins (point out is the dorsal fin) have a convex-shape. 3; There are two little mouth-wires present.

Figure 29 *Tinca tinca* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 30 *Tinca tinca* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 31 *Tinca tinca* spine, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

4.6 Rudd

Table 12 Determination material for the Rudd (*Scardinius erythrophthalmus*)

Scientific name	*Scardinius erythrophthalmus*
Exterior	Figure 32 (Sportvisserij Nederland, 2012)
Scale	Figure 33
Otolith	Figure 34
Spine	Figure 35
Jaw	Figure 36

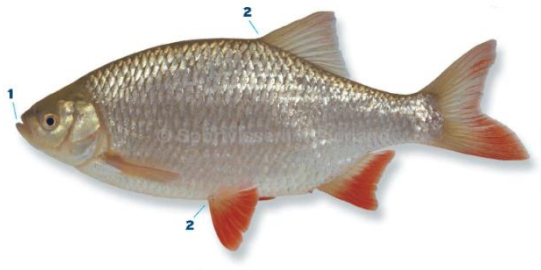


Figure 32 *Scardinius erythrophthalmus* (Sportvisserij Nederland, 2012); 1; The mouth is pointing upwards. 2; The front-side of the dorsal fin is clearly further to the tail of the fish than the pelvic fins.

Figure 33 *Scardinius erythrophthalmus* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

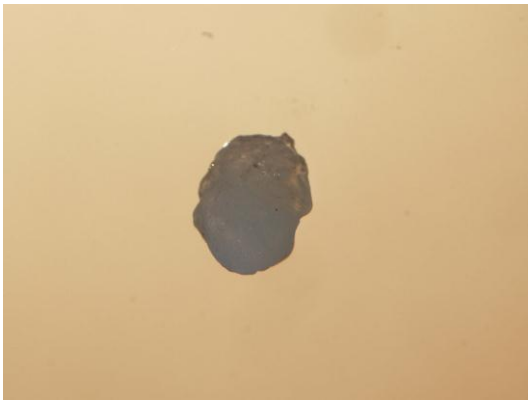


Figure 34 *Scardinius erythrophthalmus* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 35 *Scardinius erythrophthalmus* spine, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

Figure 36 *Scardinius erythrophthalmus* photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 0.67.

4.7 Common roach

Table 13 Determination material for the Common roach (*Rutilus rutilus*)

Scientific name	*Rutilus rutilus*
Exterior	Figure 37(Sportvisserij Nederland, 2012)
Scale	Figure 38
Otolith	Figure 39

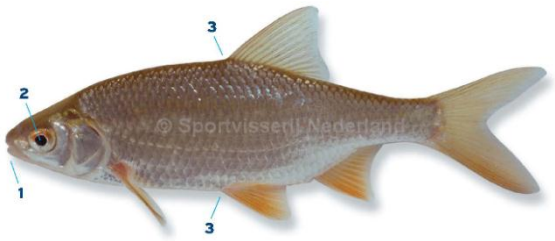


Figure 37 *Rutilus rutilus* (Sportvisserij Nederland, 2012); 1; The mouth is pointing forwards. 2; In the top of the eye, a red spot is shown. 3; The front-side of the dorsal fin is just as far from the tail as the pelvic fins.

Figure 38 *Rutilus rutilus* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

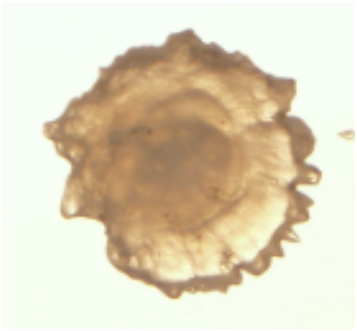


Figure 39 *Rutilus rutilus* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

4.8 Common carp

Table 14 Determination material for the Common carp (*Cyprinus carpio*)

Scientific name	*Cyprinus carpio*
Exterior	Figure 40 (Sportvisserij Nederland, 2012)
Scale	Figure 41
Otolith	Figure 42

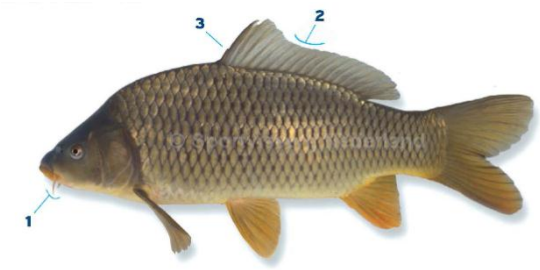


Figure 40 *Cyprinus carpio* (Sportvisserij Nederland, 2012); 1; There are four mouth-wires present, from which two in de corners of the mouth and two shorter ones on top of the upper lip. 2; The edge of the large dorsal fin is hollow incised. 3; The first fin rays of the dorsal fin is firm serrated.

Figure 41 *Cyprinus carpio* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 42 *Cyprinus carpio* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

4.9 Gudgeon

Table 15 Determination material for the Gudgeon (*Gobio gobio gobio*)

Scientific name	*Gobio gobio gobio*
Exterior	Figure 43 (Sportvisserij Nederland, 2012)
Scale	Figure 44
Otolith	Figure 45

Figure 43 *Gobio gobio gobio* (Sportvisserij Nederland, 2012); 1; The mouth is pointing downwards. 2; There are two mouth-wires present, one in each corner of the mouth.

Figure 44 *Gobio gobio gobio* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

Figure 45 *Gobio gobio gobio* otolith, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

4.10 Round goby

Table 16 Determination material for the Round goby (*Neogobius melanostomus*)

Scientific name	*Neogobius melanostomus*
Exterior	Figure 46 (Sportvisserij Nederland, 2012)
Scale	Figure 47

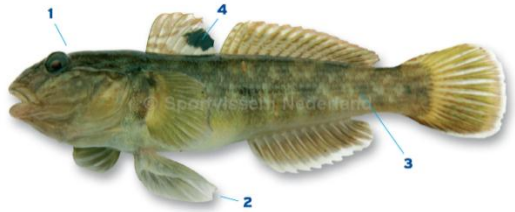


Figure 46 *Neogobius melanostomus* (Sportvisserij Nederland, 2012) 1; The eyes are highly placed into the head. 2; The pelvic fins are fused to a suction-plate, with which the fish can suck itself on a stone-based underground. 3; This species is yellow-grey-colored and has a checker-pattern. 4; In the back of the first dorsal fin, a clear black spot is shown. Males are black of colour during mating season.

Figure 47 *Neogobius melanostomus* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

4.11 Kessler's goby

Table 17 Determination material for the Kessler`s goby (*Neogobius kesslerii)*

Scientific name	*Neogobius kesslerii*
Exterior	Figure 48 (Sportvisserij Nederland, 2012)
Scale	Figure 49

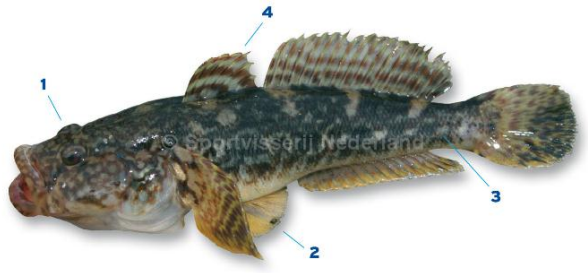


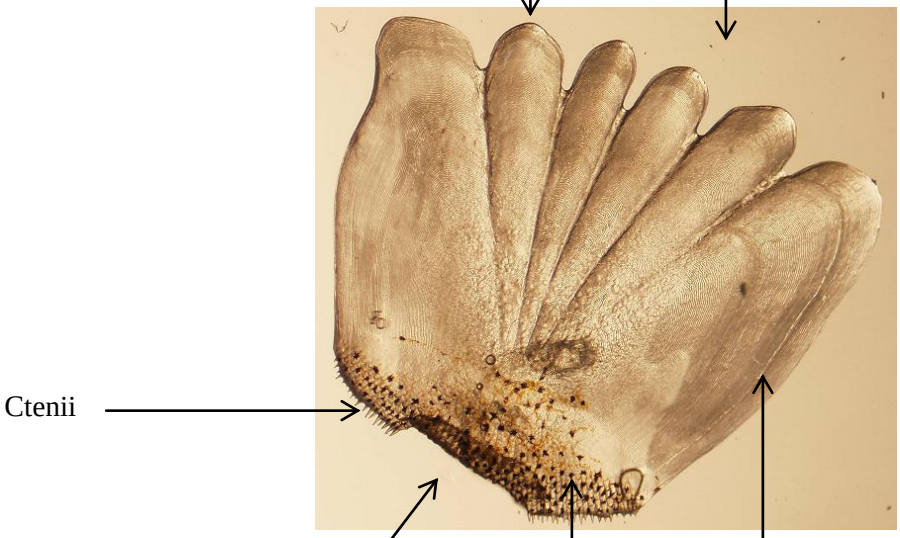
Figure 48 *Neogobius kesslerii* (Sportvisserij Nederland, 2012); 1; The eyes are close together and highly placed into the head. A big, wide head with swollen cheeks and lips, and a mouth pointing upwards. A neck with scales. 2; Pelvic fins are fused with which the fish can suck itself on a hard-based underground. 3; The body and the head are red-brown marbled. 4; The bases of both dorsal fins touch each other. Dorsal fins with horizontal red-brown ties on a lighter underground and without a black spot.

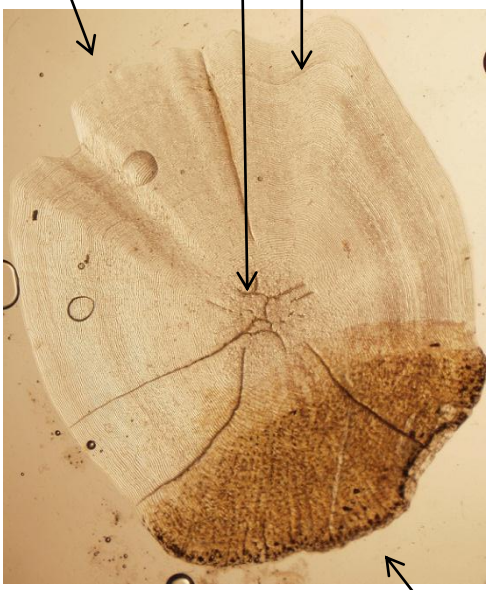
Figure 49 *Neogobius kesslerii* scale, photographed with a binocular-camera (Olympus SZ-CTV) with an enlargement of 1.4.

5. Reference cards

The following cards are developed to help determine the species from an otolith or scale found. By answering the questions you will find the species the scale or otolith belongs to.

5.1 Reference card; scale





Is the scale oval or round?

Oval: Does the scale has Ctenii?

No: Does the scale has Chromatophore in derms?

No: Tench (*Tinca tinca*)

Tench *(Tinca tinca)*

Yes: Pike *(Esox lucius)*

Pike *(Esox lucius)*

Yes: Round goby *(Neogobius melanostomus)*

Round goby *(Neogobius melanostomus)*

Round: Does the scale has Radii?

No: Does the scale has Ctenii?

Yes: Kessler's goby *(Neogobius kesslerii)*

Kessler's goby *(Neogobius kesslerii)*

No: Does the scale has a curvy edge?

No: Round goby *Neogobius melanostomus*

Silver bream *(Blicca bjoerkna)*

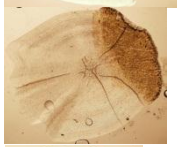
Yes: Does the scale has Chromatophore in derms, in the Focus?

Yes: Bream *(Abramis brama)*

Bream *(Abramis brama)*

No: Rudd *(Scardinius erythrophthalmus)*

Rudd *(Scardinius erythrophthalmus)*



Yes: Does the scale has round Anterior margins?

Yes: Does the scale has Ctenii?

Yes: European perch *(Perca fluvialtilis)*

European perch *(Perca fluvialtilis)*

No: Gudgeon *(Gobio gobio gobio)*

Gudgeon *(Gobio gobio gobio)*

No: Does the scale has Radii at both edges ?

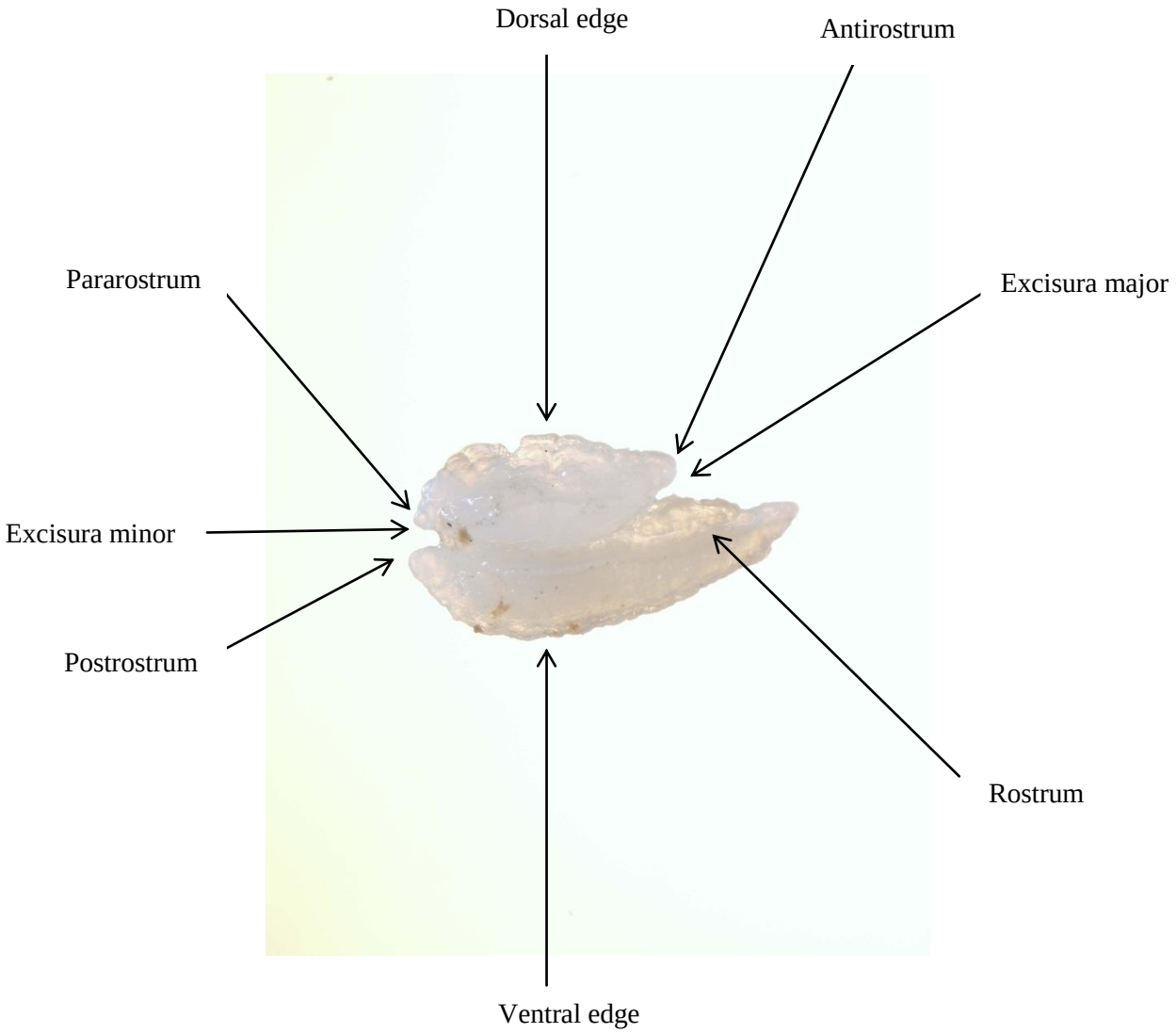
Yes: Common roach *(Rutilus rutilus)*

Common roach *(Rutilus rutilus)*

No: Common carp *(Cyprinus carpio)*

Common carp *(Cyprinus carpio)*

5.2 Reference card; otolith



Is the otolith round or oval?

Oval: Is the outline jagged?

No: Does the otolith have a Excisura minor

Yes: Pike (*Esox lucius*)

Pike (*Esox lucius*)

Yes: Does the otolith have a Excisura major

Yes: European perch (*Perca fluvialtilis*)

European perch (*Perca fluvialtilis*)

No: Silver bream (*Blicca bjoerkna*)

Silver bream (*Blicca bjoerkna*)

Round: Is the outline jagged?

No: Is the outline curvy?

Yes: Tench (*Tinca tinca*)

Tench (*Tinca tinca*)

No: Does the otolith have a weak inlet?

No: Bream (*Abramis brama*)

Bream (*Abramis brama*)

Yes: Rudd (*Scardinius erythrophthalmus*)

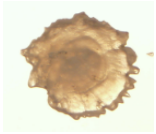
Rudd (*Scardinius erythrophthalmus*)

Yes: Is the rostrum pointy or round

Pointy: Are the Excisura next to the point deep or less deep

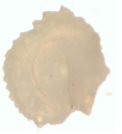
Deep: Common roach (*Rutilus rutilus*)

Common roach (*Rutilus rutilus*)



Less deep: Common carp (*Cyprinus carpio*)

Common carp (*Cyprinus carpio*)



Round: Gudgeon (*Gobio gobio gobio*)

Gudgeon (*Gobio gobio gobio*)

Acknowledgements

For making of this reference manual we would like to thank Stijkstra, A. and Heijer, M., den for their guidance. We would like to thank Kijpers, H. and Ende, M., van den for helping us and sharing their knowledge and skills.
We would like to give out a special thanks to Bosma, H. and Leopold, M. for answering our questions which contributed to this manual.
The making of this reference manual is carried out at the van Hall Larenstein University of applied science, Leeuwarden. This University provided space and all the material needed for the research that needed to be done in advance in order to get the information and material needed to make a reference manual.
This was an assignment in order of the Niewold Wildlife Infocentre, we would like to thank Niewold, F. for giving us the opportunity of doing this, and for sharing information which contributed to the results of the research and this manual.

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CD-ROM;

Leopold, M.F., Damme, C.J.G. van, Philippart, C.J.M. & Winter, C.J.N. (2001) Otoliths of North Sea Fish; Fish identification key by means of otoliths and other hard parts, Expert Centre for Taxonomic Identification, World biodiversity Database CD-ROM Series; ISBN 9075000

Internet

Sportvisserij Nederland (2012) "Fish guide 2012". Available at http://www.sportvisserijnederland.nl (assessed 12 January 2012)

Specialists

Bosma, H. (2011) Wetterskyp Fryslan

Appendix VII Determination materials and their photograph enlargement of the species obtained by Bosma, H., 12 December 2011

Species	**Left column; determination material Right column; its binocular enlargement**	
Bream	- Scale	1.4
	- Otolith	1.4
	- Spine	0.67
	- Jaw	0.67
Perch	- Scale	1.4
	- Otolith	1.4
	- Spine	0.67
Silver Bream	- Scale	1.4
	- Otolith	1.4
	- Spine	0.67
	- Jaw	0.67
Pike	- Scale	1.4
	- Otolith	1.4
	- Spine	0.67
	- Jaw	0.67
Tench	- Scale	1.4
	- Otolith	1.4
	- Spine	0.67
Rudd	- Scale	1.4
	- Otolith	1.4
	- Spine	0.67
	- Jaw	0.67

Appendix VIII Otolith retrieved from otter spraints

Table 1 Denotations and meaning of table 3 in this appendix (source; Leopold, M., *et al.* 2001 & authors of this research, 2011)

Denotation	Meaning
OL	Otolith Length
FL	Fish Length
FW	Fish Width
FM	Fish Mass in kilogram

Table 2 Codes and descriptions of the wear of the otoliths in table 3 in this appendix with their source and date (source; Leopold, M., *et al.* 2001 & authors of this research, 2011)

Codes	Description	Determined by
.	Unidentifiable wear	The authors of this research, 2011
no	no wear	Leopold, M., *et al.* 2001
2	stage 2 of wear	Leopold, M., *et al.* 2001
3	stage 3 of wear	Leopold, M., *et al.* 2001
>3	stage > than 3 of wear	The authors of this research, 2011

Table 3 Otolith information of the useful otoliths (source; authors of this research, 2011). The amount of otoliths found in the spraints, the size (width and length) of the otolith. The fish length and mass calculated with the width and length (Leopold *et al*, 2001). And the amount of wear of the otolith.

Spraint number	Otolith number	Species	OL	OW	FL	FM	Wear
8	2	*Gobio gobio gobio*	1,9	1,5	12,17	14,42	.
11	1	*Perca fluviatilis*	2,6	4,5	6,4	2,34	2
11	3	*Tinca tinca*	2,6	4,5	20,96	143,88	.
12	1	*Perca fluviatilis*	1,4	2,7	16,5	60,77	2
14	1	*Ritulus ritulus*	1,7	1,6	11,73	14,31	no
14	2	*Ritulus ritulus*	1,6	1,3	11,04	11,7	.
15	1	*Cyprinus carpio*	1,6	1,7	8,85	9,79	3
22	1	*Cyprinus carpio*	1,6	2	8,85	9,79	3
25	1	*Ritulus ritulus*	1,7	2	11,73	14,31	no
25	2	*Cyprinus carpio*	2	2,2	12,39	28,83	3
25	3	*Ritulus ritulus*	2,3	2,1	15,87	39,03	no
31	1	*Cyprinus carpio*	1,7	2,1	9,74	13,32	3
39	1	*Cyprinus carpio*	1	1	6,29	3,27	3
40	1	*Ritulus ritulus*	1,8	1,6	12,42	17,3	no
40	2	*Perca fluviatilis*	1	2	11,39	16,98	>3
41	1	*Ritulus ritulus*	1,7	1,5	11,73	14,31	no
41	2	*Esox lucius*	1,3	2,3	29,67	179,15	.
43	1	*Ritulus ritulus*	1,3	1,1	8,97	5,87	no
43	2	*Perca fluviatilis*	1,3	2,4	14,31	37,23	2
44	1	*Gobio gobio gobio*	1,4	1,4	11,26	11,42	no
45	1	*Perca fluviatilis*	1,2	1,7	9,2	8,15	no
47	3	*Ritulus ritulus*	1,3	1,6	8,97	5,87	no
50	1	*Ritulus ritulus*	1,4	1,2	9,66	7,51	no
51	1	*Perca fluviatilis*	1,3	3	18,69	93,3	2
53	1	*Gobio gobio gobio*	1,1	1	8,84	5,53	3
55	1	*Gobio gobio gobio*	1,1	1,2	8,84	5,53	3
56	1	*Perca fluviatilis*	1,3	2,5	15,04	44,19	2
56	2	*Ritulus ritulus*	1,3	1,2	8,97	5,87	.
60	1	*Perca fluviatilis*	1,3	2,2	12,85	25,71	2
60	2	*Tinca tinca*	1,8	3,2	30,34	436,38	.
61	1	*Ritulus ritulus*	0,9	1	6,21	1,73	no
61	2	*Cyprinus carpio*	1,6	1,6	8,85	9,79	.
63	1	*Perca fluviatilis*	1,4	2,5	15,04	44,19	2
63	2	*Perca fluviatilis*	1,4	2,4	11,68	18,52	2
64	1	*Ritulus ritulus*	2	2,1	13,8	24,54	no
64	2	*Gobio gobio gobio*	1,4	1,7	11,26	11,42	3
65	1	*Perca fluviatilis*	2,1	3,6	23,07	192,5	2
66	1	*Tinca tinca*	2	3	28,44	359,43	.
72	1	*Tinca tinca*	2	3	28,44	359,43	.
74	1	*Perca fluviatilis*	2,2	4,7	31,1	537,82	2
76	1	*Ritulus ritulus*	1,3	1,1	8,97	5,87	.
76	2	*Ritulus ritulus*	1,5	1,7	10,35	9,44	no
76	3	*Ritulus ritulus*	1,4	1,2	9,66	7,51	3

Appendix IX CD_ROM (database excel)

See CD-ROM (included in this report)