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Grenspark Kalmthoutse Heide
twee landen - één natuur

INSTITUUT NATUUR- EN BOSONDERZOEK

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The monitoring of Great Crested Newts in Grenspark Kalmthoutse Heide, using traps and eDNA.

Internship report 2022
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In collaboration with: Research Institute for Nature-and Forest (INBO)

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1. Summary

Great Crested Newts are on the Dutch and Flemish Red List and is considered rare, the populations are worldwide decreasing. Decent scientific research is needed in order to conserve this species and to monitor the dynamics of its populations. Each year, the population of Great Crested Newts is monitored at the Dutch side of the Grenspark Kalmthoutse Heide. The newts are trapped in different fens throughout the nature reserve and the population size is estimated. This year, 29 Great Crested Newts were trapped, which results in an estimated population size of 253 individuals due to the Jolly-Seber methodology. Via a picture of the unique pattern of the abdomen, newts can be individually recognized. A dataset with all captured individuals is compiled and possible recaptures are determined. In this study, one male crested newt (#116) was recaptured since 2020, which occurred twice in Leemputten Noord. Recaptures within the same year were not present this year, which makes the population size estimation less reliable. A second study area was added. The Steertse Heide is a Belgian part of the 'Grenspark' and has never been studied on the presence of this study species, except from one fen. eDNA-sampling was carried out to investigate the presence in the Steertse Heide. This method was also used for Dutch fens where no crested newts could be trapped. The results of the eDNA-sampling are not yet available. The second part of this study consists of taking water samples and determining the water quality and its suitability for crested newts in the different fens. The trapped crested newts were present in fens with a high water quality. Five out of 13 fens have an excellent water quality, four fens have a moderate suitability for crested newts, and four fens are not suitable. This results in nine fens where crested newts potentially could reproduce or could be trapped for population monitoring.

2. Introduction

2.1. Objective and problem definition of this study

The Great Crested Newt has a vulnerable status on the Red List of the Netherlands and Flanders (Ravon, 2022; Natuurpunt, 2022). This species is considered rare in Flanders (Natura2000.Vlaanderen, 2022) and is highly protected at a European level (EEA, 2019). This makes it of primary importance in terms of monitoring the population, conserving, and restoring its habitat and following up on the distribution of the species. This research consists of several parts, first of all the population monitoring for the province of North-Brabant, furthermore the fens were examined for water quality and its suitability for crested newts. And finally, in cooperation with the Research Institute for Nature and Forest, it was investigated whether the presence of crested newts can be determined by means of eDNA sampling. The study for the province of North-Brabant is an annual monitoring project and involves searching for newts in the various fens on the Dutch side of the 'Grenspark'. Traps are set up and the caught newts are identified. These data are collected in a database. With the data collected, a prediction is then made of the population size in the Dutch part of the 'Grenspark'. In this way, the 'Grenspark' can monitor the status of the population and whether any measures need to be taken. From my own observations, it appeared that some fens were already dry in early spring, which means that possible breeding fens are lost. By monitoring the fens with traps, it can be determined where the newts still occur and in what numbers. One can hypothesize that the crested newts that normally would reproduce in fens that dried up, will look elsewhere for a suitable place.

As a result, one might expect higher densities in the remaining fens. The opposite hypothesis is that intraspecific competition will increase considerably, and newts will seek out other fens that were not previously part of the distribution within the 'Grenspark'. Via this way, competition can be avoided, and reproductive success is not reduced.
The second part of this study compares the water quality of the undrained fens with suitable values known from literature. By means of this study, one can determine which water parameters the crested newts in the 'Grenspark' prefer. Another goal of this study would be to investigate whether the fens that do not contain newts, show different water parameters compared to the literature and to the other studied fens with crested newts in it. Finally, predictions can be made on the distribution of newts to other suitable fens in the area. For example, when a fen from previous years dries out.

The last part of this study aims at determining the presence of crested newts by an alternative method (eDNA analysis). If there are no crested newts in the traps of a fen, one can conclude that they are absent. However, this assumption is not entirely correct, as it could also be that there were Great Crested Newts present, but that they were simply not caught. In that case, the conclusion was wrong at first. eDNA is a technique that offers a solution to this. According to Biggs et al. (2015), the detection rate of eDNA is 99.3% and that of traps only 76%. This means that in about one out of four cases the conclusion that they are absent, is wrong. In addition, eDNA is more effective because it costs less manpower and only one visit to the fen is sufficient. Contrary to eDNA, traps are always set several times to increase the probability, this is more time-consuming (Biggs et al., 2015). eDNA is used in this study on the Dutch side as a tool to confirm or negate the absence of crested newts in the traps.

A second way in which eDNA is used in this study, is to determine the distribution of the crested newt in a new study area. This area is called the 'Steertse Heide' and is situated on the Belgian side of the 'Grenspark'. At this site, crested newts where only studied in one fen and were found in very low amounts. Due to the new character of the Steertse Heide and the lack of previous research, eDNA is used as a sampling tool for all the fens in this area. This is a very effective way of studying the distribution of the species in the new area. With the knowledge of the crested newt distribution, one can start working on the restoration, creation, and management of the area. The Great Crested Newt receives a lot of attention by managers, ecologists, and nature agencies, due to its protected status in Flanders and Europe and its status as a Habitat Directive species (Natura2000.Vlaanderen, 2022; EEA, 2019). Establishment of the Great Crested Newt means that its habitat, and therefore the area in which it occurs, receives protection by the European Union and funds can be obtained for the restoration or construction of this habitat (European Commission, 2022). For the Steertse Heide, it is an added value to establish the occurrence of this species, funds could be applied for, and possibly new parts can be bought from private owners. These funds will not only help to increase the habitat quality for the crested newt, but also for the other fauna and flora in the area. In order to increase the quality of the area, water samples are also taken from the fens. This will make it possible to check whether the absence of the newt can be traced back to poor water quality. If needed, some restoration actions can be implemented into management and if needed, funds could be applied for. The results of this study on crested newts in the Steertse Heide, can also be further elaborated in the vision document of the area and management can be optimized for the future (ANB, 2019).

2.2. Great Crested Newt (*Triturus cristatus*)

The Great Crested Newt (Triturus cristatus) is the largest native newt, with females reaching a length of 18 cm and males 16 cm (Natura2000.Vlaanderen, 2022). This salamander has a black or sometimes brownish colour, with a yellow to orange-red abdomen which has a unique black spotting pattern. During the mating season, males also develop a black crest and a white to silver coloured stripe on the flanks of their tail (Natura2000.Vlaanderen, 2022). Great Crested Newts can be found in still or semi-current waters, in forests and their edges, scrubland, forest steppes, meadows, gardens and parks (Arntzen et al., 2009). The species has both a terrestrial and an aquatic life cycle, they usually only enter the water during the breeding season, a small percentage remains aquatic throughout the year (Ravon, 2022). This species prefers fens with a neutral to alkaline character, a lot of macrophytes, sufficient depth, fairly nutrient rich, without fish and not completely shaded (Oldham et al., 2000; Gustafson et al., 2009; Natura2000.Vlaanderen, 2022). Reproduction is hampered by low acidity, causing the embryos to die (Gustafson et al., 2009). Crested newts are mostly threatened by changes in water quality, pond degradation and drainage, overgrowth and shallowing of the ponds, eutrophication, and pollution (Arntzen et al., 2009). The introduction of predatory fish and the collection of individuals from the wild are also serious threats to the species. Regular draining of fens can be beneficial, as aquatic predators are lost (Oldham et al., 2000). Great Crested Newts have a 'Least concern' status on the IUCN Red List, globally, in Europe and the EU (Arntzen et al., 2009; EEA, 2019). Despite this status, the population is declining, and the species has a weak to poor conservation status in Belgium and the Netherlands (EEA, 2019). The species is however protected by the EU Habitats Directive: Annex II and Annex IV, as well as the Bern Convention: Annex II and Annex I.

2.3. Study area

This study took place in 'Grenspark Kalmthoutse Heide'. Part of this nature reserve is situated in the north of Belgium, in the province of Antwerp and is part of the Campine region. The Dutch part is located in the south of the country and belongs to the province of Noord-Brabant. The Grenspark covers about 60 km^2 and is part of the Natura 2000 network and consists mainly of woods, polders, dunes, meadows, fens and, of course, heathland (Grenspark Kalmthoutse Heide, 2022a).
In this study, different methods were used. Firstly, traps were used on the Dutch side (Figure 1). Traps were set in the Leemputten (north and south), the Belderven, the Ranonkelven, the Moseven and the Bovenven. Except for the last two, these are all managed by Natuurmonumenten, from whom I obtained access to the grounds and permission to carry out the fieldwork. The Moseven and Bovenven are managed by Evides, from whom I also obtained the same permission. Besides the typical fens of Natuurmonumenten, there was also a ditch next to the Ranonkelven. Because many fens in the area were dry, a trap was set in this ditch as an experiment. Other fens managed by Natuurmonumenten, such as the Granaatven, Leemven and Kleine Meer, were not examined this year because the water level was too low or because they were dry. Furthermore, the Moerven has been transformed into a horse meadow, the Volksabdij is a fishpond, the Wasvennen Zuid are either dry or contain fish, Akkerenven has recently been dug out to combat swamp stonecrop (*Crassula helmsii*) and the Wasven Noord and Bronven are already monitored annually by volunteers from the Province of Noord-Brabant.

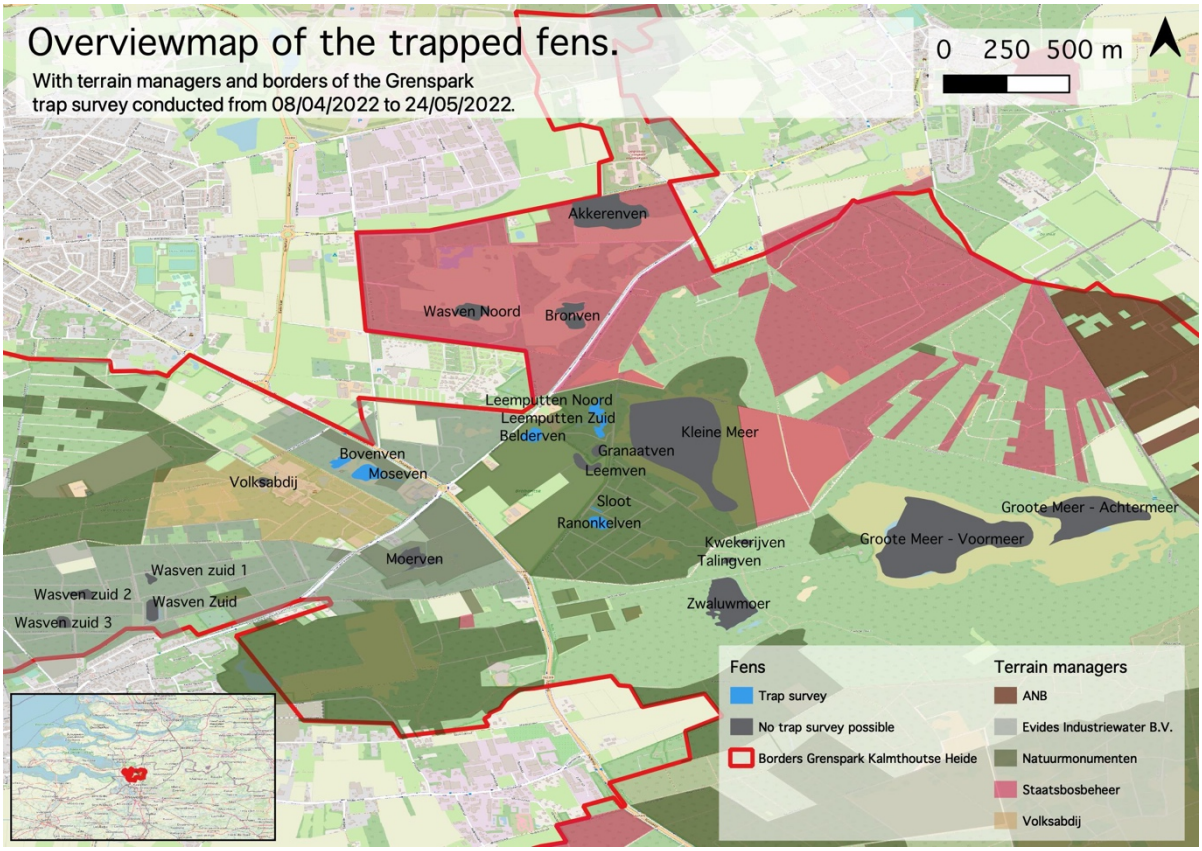

Figure 1: Overview map of the fens that were investigated with traps, of the fens that could not be investigated with traps and of the different terrain managers.
Data obtained via Rudi Delvaux, constructed in QGIS.

The Belgian side of the research took place on the Steertse Heide, a new area (140 ha) that only recently (for the most part) has been under management of the Nature and Forest agency (ANB) (Figure 2). The area consists of agricultural land, wood edges, shrubbery, (wetter) grasslands, fens, and heathland. The goal of the ANB is to own 100% of the area in the future, in 2019 they had already purchased 77%. The other goal is to cease all agricultural activities on the Steertse Heide (ANB, 2019). Because some parts have only been turned into natural areas fairly recent and the remaining agricultural activities in the area, there have been no previous surveys for the presence of the crested newt in this area. Only one fen was already investigated for the presence of crested newts in previous years, here only a few animals were found in the traps. In order to restore, manage and improve this new area, this study was started with eDNA as the sampling method for the fens. On the Steertse Heide, only the eDNA method was used for research and not the traps. In the figure below, the examined fens are shown. Note that the fens on private land were not included in this research. In addition to the fens, a catchment basin was also sampled, which serves as an iron-sand filter for the water flowing into the Groote Meer from the surrounding agricultural land (Grenspark Kalmthoutse Heide, 2022). The water coming from the surrounding agricultural land converges in this basin and will then flow through the iron-sand into the Groote Meer. The iron-sand absorbs phosphates from the water, thus protecting the valuable fauna and flora in the Groote Meer. Because a reasonable amount of water was present, in combination with many macrophytes, this filter was also sampled for eDNA research of crested newts. Water samples were also taken, which could possibly also contribute to the LIFE HELVEX project of this phosphate filter (Grenspark Kalmthoutse Heide, 2022b).

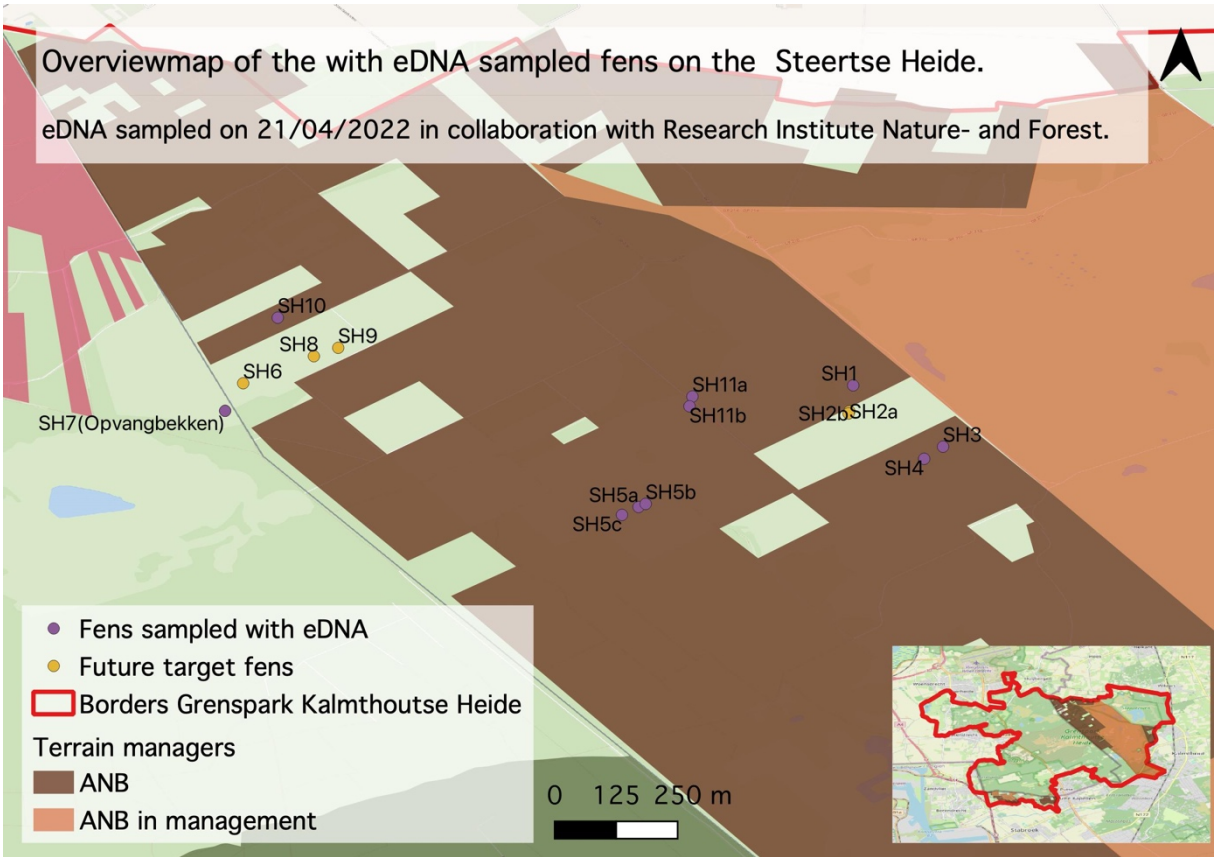

Figure 2: Overview map with the sampled fens for the eDNA research, the future interesting fens for crested newt research and the different managers of the Steertse Heide. Data obtained via Rudi Delvaux and Loïc van Doorn and constructed in QGIS.

The sampled fens were given a name consisting of "SH" (Steertse Heide), followed by a unique number (1-11). On the map it is clearly visible that some parts of the Steertse Heide are still not under management of the Nature and Forest Agency. Because these locations are not under their management, these locations were not included in this study, however, the fens on these sites are interesting to investigate in the future for the presence of crested newts (with or without eDNA), this when they are purchased by the Agency.

3. Material and methods

3.1. Trap-research

Before starting the research, I obtained a permit for exemption from the prohibitions of the Nature Protection Act from RAVON, which allowed me to handle the amphibians. Besides crested newts, other newt species were also caught, of which only the location, sex and number were noted in a notebook. The species that also occur in the 'Grenspark' are: Alpine newt (Ichthyosaura alpestris), Palmate newt (Lissotriton helveticus) and Smooth newt (Lissotriton vulgaris) (Waarnemingen.be, 2022). The monitoring protocol of the Province of Noord-Brabant was used for the trap survey (Appendix 4).

For the trap research, Laar M2 traps were used, which were kept afloat by means of two pieces of pipe insulation. The traps contained at two different heights a conical opening where the newts could enter the trap. The trap was opened by opening the Velcro seams (Fig. 3). In most cases the trap was set vertically, but if the water level in the fen was too low, it could also be set horizontally. In total seven to nine traps were set in different fens in the late afternoon. The traps were placed near water plants, on a southern bank and in a deeper part of the fen. Crested newts emerge from between the water plants at night and go in search of a mating place (often an open, deeper part of the fen), preferring south-facing banks with macrophytes (De Bruyn et al., 2015). The aim was to set the traps during the warmest nights of the study period (8/04-24/05 2022), when the crested newts are most active, and this increases their capture success. Rainy weather was avoided both at night and during the day, as it makes fieldwork more difficult and cools the ambient temperature (especially at night). The morning after the traps were set up, the two to three traps per fen were emptied before noon, so that the animals would not have to remain in the traps for too long. Two buckets were filled with water from the fen, one was used to empty the trap, the other to store the amphibians after examination. The traps were opened over a bucket of water and care was taken to ensure that no animals remained in the trap. In order to disturb the animals as little as possible, the bucket with the examined individuals was released at the same spot as where the trap was located. Vinyl gloves were worn when touching the captured amphibians, to protect the mucous layer of the animals. Each newt was photographed with a smartphone and their number per fen was noted in a notebook. Crested newts were turned on their backs and their unique belly pattern was photographed. In the absence of field assistance, the newts were placed in a transparent plastic container with fen-water and a photograph was taken from below.

Figure 3: Opening a Laar M2 trap.

The invasive aquatic plant: swamp stonecrop (Crassula helmsii), is present in the Belderven. To prevent this plant from spreading to the other fens, all material contaminated with the water of this fen, was disinfected afterwards with a solution of Virkon S (Antec Dupont, Suffolk, UK). A wading suit was worn to enter the fens, which of course also had to be disinfected after use. By examining the Belderven as last and using separate traps, cross-contamination to other fens could be avoided. The Virkon S powder was always dissolved in water inside a large bucket. According to the protocol (see appendix 4), this should be two grams of powder to one litre of water. However, as this was not practical in the field, an arbitrarily higher concentration was prepared. This solution was put into a plant sprayer (Exoterra mister 2L) and could easily be spread over the material, after which the material was rinsed thoroughly with a garden hose. All the material was stored and cleaned in Natuurmonumenten's management shed in Ossendrecht. Each fen is monitored at least twice with traps, fens in which no newts are caught are further examined with eDNA. All caught newts were entered into Waarnemingen.nl per species and per fen, with photographic evidence.

3.2. Calculation of population size

To calculate the population size of the crested newts in the 'Grenspark', the monitoring protocol of the Province of Noord-Brabant was used (Appendix 4). The photographs taken of the abdomen of the newts were used to individualise the animals. First, the different photographs of the caught newts of this year were compared with each other, in order to detect possible recaptures. By looking for a typical shape in the pattern, the individuals could be easily compared with the naked eye. An alternative method is the programme 'Wild-ID'. Because this study only involved a smaller number of individuals, it seemed more efficient to do this without the programme.
The 'Grenspark' has a large database with all the caught individuals and a photo of their abdomen. Via this way, the 'Grenspark' has an idea on how many individuals already have been caught and it is easy to see if any of the newts are recaptured. The spotting pattern of this year's individuals were compared with all the photos of previously captured individuals. Recaptures were noted in the database and individuals that were never captured before were added to the database with the corresponding photo.
Furthermore, it was noted where they were caught, in which year, with which method and in which phase (terrestrial or water) they were in. The Jolly-Seber method was used to calculate the population size; the following method is taken from the sampling protocol (Appendix 4).

The calculation of the Jolly-Seber population size is done by means of the following formulas:

$\hat{a}_t = \frac{m_t+1}{n_t+1}$ $\quad\quad$ $\widehat{M}_t = \frac{(s_t+1)Z_t}{R_t+1} + m_t$ $\quad\quad$ $\widehat{N}_t = \frac{\widehat{M}_t}{\hat{a}_t}$

$\hat{a}_t$: An estimate of the proportion of the population that is marked during sampling t.
mt: The number of marked animals captured in sampling t.
n_t: Total number of animals captured in sampling t.
$\widehat{M}_t$: An estimate of the marked population just before sampling time t.
s_t: Total number of animals released after sampling t ($s_t = n_t$ - accidental kill or disposal)
Z_t: The number of individuals marked before sampling t, not caught in sampling t, but caught in a sampling after sampling t.
R_t: The number of individuals released during sampling t and caught in a subsequent sampling.
$\widehat{N}_t$: An estimate of the total population size at time t.

For Z_t and R_t a value of one was entered if there were no recaptures.

3.3. Water quality

In order to find out which fens have suitable water parameters for newts, a study of water quality was carried out, in addition to the compulsory matters laid down by the Province of Noord-Brabant for monitoring newts. This part of the research was carried out in collaboration with Prof. Jonas Schoelynck of the research group ECOSPHERE within the University of Antwerp. In consultation with Loïc van Doorn and Prof. Schoelynck, the following parameters were measured: oxygen concentration, acidity (pH), electrical conductivity (EC), temperature, phosphate concentration, ammonium concentration, nitrite concentration and nitrate concentration.

These parameters were also studied in Gustafson et al. (2009) and have the greatest influence on the occurrence and reproduction of Great Crested Newts. The influences are discussed in detail later in the discussion.
On arrival at the fen, a multimeter (Type: WTW Multi 3430; 0.1 accuracy) with several probes was immediately placed into the water, this because if the fen is entered or disturbed, water parameters may change (e.g., oxygen concentration) and thus the measurements may be biased. The multimeter was only used once per fen, due to the limited availability and the one-time execution of the eDNA sampling (see further). For smaller, closely located fens, the multimeter was placed in the merged sample of smaller subsamples of each fen. Therefore, these fens will have the same values, however the fens were sampled separately for the nutrient analysis (Loïc van Doorn, personal communication). The multimeter indicated a value for temperature, acidity, oxygen concentration and electrical conductivity. For bigger or more isolated fens, a mixed sample was collected from smaller subsamples taken throughout the fen (again in combination with the eDNA, see below), from which the other nutrient samples could be taken. Three different samples were taken, one sample to examine the phosphate content, one sample to examine the nitrogen compounds mentioned earlier and then a final additional sample that could be used to examine metals or heavy metals if necessary. Before a sample was taken, the syringe was rinsed three times with fen water (Jonas Schoelynck, personal communication). The syringe was then filled with ten millilitres of fen water and a filter was placed on the syringe. The filter makes sure that small organisms, organic material, and sediments do not get into the sample, as these could influence the purity and the concentrations of the sample. With the phosphate sample, the ten millilitres were simply injected through the filter into a test tube, the test tube was closed with a cap and kept cool. First, the samples were kept in a Styrofoam box with ice and then they were stored in the refrigerator. The sample for nitrogen compounds contains a drop of HCl to ensure that the samples can be stored longer (Jonas Schoelynck, personal communication). Again, 10 millilitres of filtered fen water were put into the test tube and the samples were also kept cool. Finally, 30ml of fen water was filtered with a new filter into a larger test tube containing hydrogen nitrate. These test tubes had to be kept in a constantly dark place to prevent the sunlight from converting the hydrogen nitrate into nitrite, water, and oxygen gas. Hydrogen nitrate is also a preservative to keep these samples accurate for longer, for later analysis (Jonas Schoelynck, personal communication). The samples were kept dark and cool. If a filter became saturated during filtering, a new filter was taken, this has no influence on the filtered sample (Jonas Schoelynck, personal communication). The samples were transferred to the lab of ECOSPHERE, the samples for phosphate concentrations and those for nitrogen compounds were analysed by Anne Cools and Anke De Boeck. The samples for additional analyses on metals and other non-nitrogenous compounds were frozen and kept for possible later analysis if required. For the time being, these samples will not be analysed.

3.4. eDNA-research

On Friday 22 April, the selected fens on the Steertse Heide were sampled in order to check the presence of crested newts via eDNA. In consultation with Rudi Delvaux and Loïc van Doorn, the fens that were to be sampled were chosen. The fens where no newts were caught with the traps were also examined with the eDNA method. It could be that the newts were present, but simply not caught with the traps. To confirm the absence, the fens were sampled for Great Crested Newt eDNA.

Given the high cost of sampling and analysing the eDNA samples, this was only done for fens where no Great Crested Newts were caught. As mentioned earlier, on the Steertse Heide, only eDNA sampling was carried out and no trapping was carried out. Smaller fens that were located very close to each other were merged into one sample, in order to reduce costs and because the smaller fens are seen as one entity by the newts (Loïc van Doorn & Rudi Delvaux, personal communication). With each new eDNA sample, new sterile nitrile gloves were worn, and all reusable field material was disinfected with a Virkon S (Antec Dupont, Suffolk, UK) solution of at least 2%, to prevent cross-contamination between different fens (Everts et al., 2022). The DNA analysis is very sensitive and false positive results could be obtained in case of insufficient decontamination. Next, a sterile 0.5L sampling bag was attached to a telescopic sampling pole, with which a subsample was taken from different places in the fen. An attempt was made to obtain the best possible spatial coverage of the entire fen, so that the collection of all subsamples constituted a representative measurement of the entire body of water. The different subsamples (each bag of 0.5L) were collected in a larger sterile bag (placed in a bucket) to form a large homogenised mixed sample. This sample was then filtered on an enclosed disk filter with a 5 µm glass fibre pre-filter and a 0.8 µm PES membrane (NatureMetrics, Surrey, England), through a Vampire sampler pump (Buerkle, Bad Bellingen, Germany) with a disposable silicone tubing. When the filter became clogged or all the sampling fluid was filtered, the disk filter was sealed with plastic caps and stored in a Styrofoam box with ice. New sampling bags, mixing sample bags, silicone hoses and disc filters were used for each fen. The disk filters were stored at -21°C pending analysis (Everts et al., 2022). The further molecular analysis is done by the lab of the INBO.

4. Results

4.1. Trap-research

Table 1: Presentation of the newt species caught per fen and the total number of newts caught.

Fen	Great Crested Newt	Alpine Newt	Palmate Newt	Smooth Newt	Total
Bovenven	0	0	0	2	**2**
Moseven	0	11	0	4	**15**
Ranonkelven	0	0	8	3	**11**
Belderven	0	0	0	2	**2**
Leemputten Zuid	6	11	0	2	**19**
Leemputten Noord	20	2	0	4	**26**
Sloot	3	8	0	4	**15**
Total	**29**	**32**	**8**	**21**	

The trap survey (Table 1) showed that only three fens contained Great Crested Newts (Leemputten N&Z and Sloot), while previous surveys showed that they also occurred in Ranonkelven and Belderven. In the Garnaatven and the Kleine Meer, great crested newts were also caught, but these fens were dry this year (Bastiaens, 2021). In the Leemputten, Great Crested Newts were always caught in recent years, and this year the greatest number was caught here. The largest number (20) of newts was caught this year in the Leemputten Noord, where 18 animals were caught in the first round and then two juvenile newts in the second round. In Leemputten Zuid, six crested newts were caught in the first round and none after that.

The trap set up as an experiment in the ditch next to Ranonkelven proved to be successful. In a single trap, three Great Crested Newts, eight Alpine Newts and four Smooth Newts were caught.
Although no crested newts were caught in Ranonkelven, the species was found in the vicinity. On the other hand, Ranonkelven turned out to be the only fen where Palmate Newts were caught. The Alpine Newt was caught most often, followed by the Great Crested Newt and Smooth Newt, and finally the Palmate Newt (Table 1). In the Leemputten, the most individuals were caught across all newt species, followed by the Sloot and the Moseven and finally the Ranonkelven. In the Belderven and the Bovenven only two newts were caught.
From the database of the 'Grenspark', one can derive how many crested newts have been caught in recent years. Below are graphical representations of the data from this database.

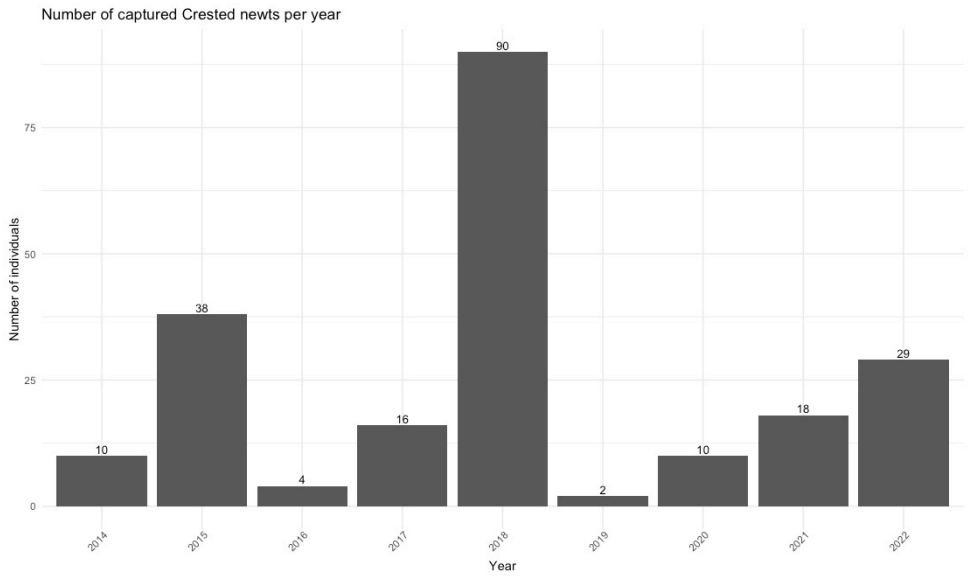

Figure 4: Graphical representation of the number of caught newts per year. Data obtained from the database of the 'Grenspark', graphically represented with RStudio.

Database compiled by Groffen (2014), Kooijman (2015), Leys (2016), Franck (2017), Meijer (2018), van Ooijen (2019), Joustra (2020), Bastiaens (2021) and Ferket (2022).
The number of crested newts caught fluctuates from year to year, with a maximum of 90 individuals in 2018, followed by the lowest number (2) in 2019 (Figure 4). Since 2019, the number of crested newts captured is increasing again, with this year 11 crested newts more than last year. This year 28 male crested newts were caught and 1 female (juvenile).

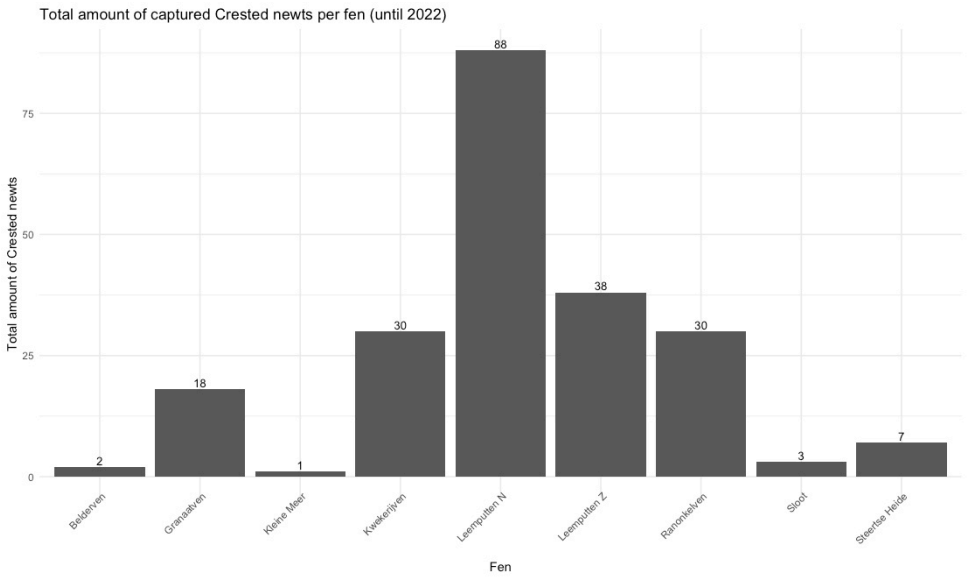

Figure 5: Graphical representation of the total number of caught newts per fen until 2022. Data is from the database of the 'Grenspark' and is graphically represented with RStudio.

Leemputten Noord has the highest number of Great Crested Newts caught, followed by Leemputten Z and the Kwekerij- and Ranonkelven (Figure 5). Only one crested newt was caught in the Kleine Meer and two in the Belderven. De Sloot also has a low number of crested newts caught, but this is due to the single trap that was set this year.
A graphical representation of the number of caught newts per fen and per year is added in Appendix 5.

4.2. Population size

One crested newt was recognised from the database, which was also caught this year. It is male 116 and was caught in both 2020 and 2022 in Leemputten Noord. This is the first time that a crested newt from another year has been recaptured. Within this year, no Great Crested Newts were recaptured, which has a large influence on the population estimate. Using the Jolly-Seber method, the population size was calculated per fen and for the entire 'Grenspark'. Because no newts were caught, Z_t and R_t were filled in with one and m_t with zero. Because there were only two rounds of sampling, the population size was calculated with t as sampling round two.

Table 2: Summary of the estimated population size with the Jolly-Seber method per fen.

Fen	*Population size Jolly-Seber*
Leemputten Zuid	24.5
Leemputten Noord	220.5
Sloot	8
Total	**253**

The estimated total population size in the 'Grenspark' is 253 crested newts, divided among three fens (Table 2).

4.3. Water quality

Table 3: Overview of the different water parameters per fen.

Fen	T(°C)	pH	O2(mg/l)	EC (µS/m)	P (mg/l)	NH4 (mg/l)	NO2 (mg/l)	NO3 (mg/l)
SH1	NA	6.50	6.67	111.0	0.023	0.026	<0.0002	<0.025
SH3	11.1	6.30	1.81	103.6	0.816	0.718	0.0002	<0.025
SH4	17.1	6.90	8.73	111.0	0.184	0.070	0.0002	<0.025
SH5a	14.8	4.60	9.57	67.8	0.004	0.015	<0.0002	<0.025
SH5b	14.8	4.60	9.57	67.8	0.002	0.021	<0.0002	<0.025
SH5c	14.8	4.60	9.57	67.8	0.002	0.039	<0.0002	<0.025
SH7	14.5	5.90	11.41	180.7	0.002	0.074	0.0002	0.041
SH10	15.6	7.10	4.73	335.0	7.623	18.718	0.0006	0.028
SH11a	15.6	6.80	11.95	96.8	0.262	0.052	<0.0002	<0.025
SH11b	15.6	6.80	11.95	96.8	0.717	0.018	<0.0002	<0.025
Bovenven	17.5	4.40	9.49	90.0	0.013	<0.004	<0.0002	<0.025
Moseven	16.3	7.20	9.42	165.0	0.007	0.019	<0.0002	<0.025
Ranonkelven a	17.0	7.50	8.75	188.6	0.003	0.041	<0.0002	<0.025
Ranonkelven b	22.4	7.40	11.03	174.5	0.003	0.064	<0.0002	<0.025
Belderven	15.8	9.10	11.20	446.0	0.002	0.041	<0.0002	<0.025
Leemputten Zuid	26.2	6.70	11.30	245.0	0.132	0.233	<0.0002	1.079
Leemputten Noord	20.4	6.08	11.24	101.2	0.002	0.038	<0.0002	<0.025

Although the temperature measurements were taken on the same day (beside Leemputten and Ranonkelven b), there is a small temperature difference between the different fens, with 11.1°C as a minimum and 17.5°C as a maximum for 22/04/2022 (Table 3). The acidity has some extreme values, with 4.4 and 4.6 as the most acidic values, respectively in the Bovenven and the small fens of SH5. The most alkaline fen is the Belderven with a value of 9.1. The most oxygenated fens were $SH11_{a\&b}$ and SH7, with values of 11.95 and 11.41 mg O_2 per litre. The fens with extremely low oxygen concentrations were SH3 and SH10, with values of 1.81 and 4.73 mg O_2 per litre, respectively. The highest electric conductivity was measured in the Belderven (446 µS/m) and SH10 (335 µS/m) and the lowest in $SH5_{a,b\&c}$ (67.8 µS/m) and the Bovenven (90.0 µS/m). The highest concentration of phosphates was measured in SH10 (7.623 mg/l), SH3 (0.816 mg/l) and $SH11_b$ (0.717 mg/l). With a value of 18.718 mg/l, SH10 has the highest concentration of ammonium, followed by SH3 (0.718 mg/l) and Leemputten Z (0.233 mg/l). No extremely high nitrite concentrations were found. The highest nitrate value was found at Leemputten Z (1.079 mg/l), followed by SH7 (0.041 mg/l) and SH10 (0.028 mg/l).

In order to get a general idea of the nutrient richness in the fens, the fens were classified in the different trophic levels, according to the systematics of nature types for Flanders: Still waters, using different definitions (Haskoning, 2003). The classification of the different fens according to the different definitions can be found in Appendix 6.

4.4. eDNA-research

To date, no results have been received from the INBO lab; when these are known, this report will be partially rewritten.

5. Discussion

5.1. Trap-research

The results of the trap survey were mixed. Due to the low water level of many fens, several fens could not be sampled and the number of ideal spots for a trap placement were greatly reduced. The Laar M2 traps are elongated and very light, which caused many problems due to the low water level. Often, only one opening of the trap was submerged, which reduced the chance of capture. Furthermore, many traps were blown over during the night, which resulted in many failed capture attempts. Given the limited time, it was not always possible to correct this. It was not possible to place more traps per pool to account for these failures, as there were not enough traps. Many of the traps were also damaged and had holes in them, which may have allowed the newts to escape from the trap. For the future, I recommend that the 'Grenspark' invest in Vermandel traps, which are unfortunately more expensive, but in my opinion more worth the investment. This type of trap is also recommended in De Bruyn et al. (2015). Vermandel traps are made of metal and are therefore less likely to blow over, the opening is very wide (which increases the chance of capture), and the chance of holes is much less. A combination of both types of traps seems a good option, the Laar M2 traps can still be used in the deeper fens, as these traps float. In my opinion, this will significantly increase the success of the trap-research.

5.2. Population size

The population size was estimated at 253 individuals, which is a huge number compared to last year's estimate (5-15) (Bastiaens, 2021). Possibly, such a high number was obtained in this study because 20 crested newts were caught in one round of trapping and in one fen. Anyway, this estimate is completely unreliable, as the calculation was done without recaptures, and this is crucial for a correct estimate. Since a recapture within the same year has only occurred once since 2014, it is best to look for a method that does not require this.

5.3. Waterquality

Note that there were two measurements for the Ranonkelven, during the first measurement no filter was used for the phosphate sample by mistake. To limit the influence on the results, an extra measurement was done later. Afterwards, the unfiltered sample turned out to be usable for analysis after all. It provides a very good comparison between the two different measuring moments and was therefore retained. Both Leemputten and the second measurement of the Ranonkelven were only examined for water quality at the end of May. In this period, it was much warmer and there had been very little rain, so the water levels were lower. It is important to take this into account when interpreting the results. Leemputten Zuid probably contains the largest deviation as the fen was reduced to only a small puddle of about one square metre. To obtain reliable measurements, it would be better to take all measurements on the same day.

This was not possible because it was already too late on 22/04/2022 and I had other obligations within my education from 02/05/2022 until 19/05/2022. For SH1, we forgot to note the temperature, this should be avoided and is a point of attention for future fieldwork. A lot of scientific research has been carried out in order to get an overview of the most suitable water parameters for crested newts. Oldham et al. (2000) states that sufficient oxygen levels are more important for the eggs and gill-wearing larvae, than for the adults. As they can take up oxygen from the air. The temperature of the fens was significantly higher with crested newt presence, this due to increased macrophyte- and food resource production in warmer fens (Gustafson et al., 2009). On the other hand, reproduction is inhibited with decreasing pH (Gustafson et al., 2009), all embryos died at a pH of 4.5 (Griffithsand & de Wijer, 1994). Although Gustafson et al. (2009) recorded a successful reproduction at pH 5.2. They state as well, that Crested newt presence increased with a pH around 7 and increasing nutrient levels. High levels of excess or available nutrient levels decreased newt presence, as it lowers the water quality and can be toxic at certain levels due to Gustafson et al. (2009). They state as well that crested newts reproduce more in fens with lower nutrient levels, but non-reproducing adults can occur in fens with higher levels. At an ammonium concentration of 0.5 mg/l, reproduction was no more present due to Gustafson et al. (2009). Suitability of the fens of this study were based on the literal statements above and table 1 (Gustafson et al., 2009, p.303). SH1, Moseven, Ranonkelven and Leemputten Noord have very suitable water parameters for crested newts. Leemputten Zuid is also suitable, but water parameters were a little bit biased due to drought. SH3 is less suitable due to low oxygen levels and higher phosphate- and ammonium levels. SH4 has suitable values except from its polytrophic character due to Leentvaar (P). In $SH5_{a,b\&c}$ and the Bovenven the pH is very low, which is not suitable for reproduction, but adults could be present. SH7 has a moderate suitability due to its pH of 5.9 and a higher nitrate level, but compared to literal values, these values are sufficient for crested newt presence. SH10 is not suitable due to extreme nutrient levels and low oxygen levels. $SH11_{a\&b}$ have a moderate suitability due to their polytrophic (Leentvaar P) character; this might not be too much of an issue as crested newts were trapped here in previous years. Finally, the Belderven is of moderate suitability due to its pH of 9.1. This should not be too much of an issue.

5.4. eDNA-research

Since no results are yet known from this branch of the research, it is not possible to discuss these results. The lab of the INBO recently moved, which causes a strong delay in the sample analysis. Personally, I regret having to submit an incomplete report because of this. On the other hand, it is also to my advantage, since, in addition to the annual obligatory research, I also conducted two additional studies and I must summarise all of this in a 15-page report. With the results of the eDNA survey, this would certainly not have been possible.

6. Conclusion

29 Great Crested Newts were caught in three different fens in the Grenspark Kalmthoutse Heide, of which 28 males and one female. No crested newts were recapture, but one male (#116) of 2020, was captured this year as well. The estimated population size of Great Crested Newts in the study area is 253, this is no reliable estimation. Five fens have an excellent water quality that is suitable for crested newts, four fens have a moderate suitability, and four fens are not suitable. Results of the eDNA sampling are not yet available. The trapped crested newts were all present in fens with a high water quality.

7. Acknowledgements

I would like to thank Rudi Delvaux for the great supervision during the internship, as well as Raoul Van Damme for the guidance before, during and after the internship. I greatly thank the Grenspark Kalmthoutse Heide for financing the eDNA-sampling and providing all the required materials. My sincere gratitude goes to Jonas Schoelynck for lending me a wading suit and a multimeter, as well as financing and providing all the materials for the water quality sampling. Furthermore, I would like to thank Loïc van Doorn for the big help and the guidance during eDNA sampling, as well as Jeroen Speybroeck. I would like to thank Anne Cool and Anke De Boeck of the ECOSPHERE research group, as well as Jesper Berndsen of RAVON, Jeroen Willemsen of Evides and Mickey Nieuwenhout of Natuurmonumenten. A final acknowledgment goes to all the assistants in the field: Romeo Liekens, Dave Van Landeghem, Toon Schoeters, and my mother. Thank you Wout Geudens for proofreading this report.

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9. Appendix 1: Summary of the pre-established goals and methods agreed with the UA

Goal of this research

The great crested newt (*Triturus cristatus*) has been monitored in the Grenspark for many years to get insights into the dynamics of this population. This species is listed in the Natura 2000 annex I and II and receives protection from the European, Dutch, and Flemish legislation. Information on population trends and distribution, obtained through monitoring, can be used for conserving, and managing their habitat. Recently, an area called 'de Steertse Heide' was added to the Grenspark. This area contains many small fens that are a potential habitat for the newts. Due the agricultural history, these areas need some management, and this can be enhanced if there is evidence of the presence of the great crested newt.

Methods used

First of all, the newts will be trapped twice with Laar M2 traps and this with multiple traps per fen. When newts are trapped, their abdomen will be photographed and the unique pattern that will be visible can be used for the identification of the individual. This information will be fed into an existing databank. In fens without any trapped great crested newts, an eDNA sample will be taken. The fens of the Steertse Heide will only be sampled with eDNA and not with traps, due to the lack of data on the presence in these fens. Because the abundance in these fens is expected to be very low, traps are not the most effective sampling method. The sampling for eDNA will be done on 22/04/2022 in collaboration with Loïc van Doorn of the Institute of Nature and Forest Research (INBO). The INBO will analyze the eDNA samples in their lab and will later send the results for further analysis to Arne Ferket. Finally, some parameters of every fen will be taken with a multimeter (pH, temperature, conductivity and 02 content), a sample for nutrient (e.g., PO4, NH4 and NO3) investigation and a sample for (heavy) metals will be taken and analyzed in the lab of Prof. Jonas Schoelynck (ECOSPHERE).

Results of this research

The distribution of the great crested newts in het Grenspark will be reported, the newts will be identified, and the population will be compared with the results of previous years. Fens in which no great crested newts could be trapped will be sampled with eDNA. Via the results of the eDNA sampling, certainty on the presence in the different fens is obtained. The abiotic parameters will be fed into statistical models trying to predict the presence/absence of the great crested newts. The outcome of these models can be compared with the results of the traps and the eDNA, trying to find a correlation. All this information will be useful for drawing management/conservation plans. This can be a handy tool in the restoration and management of the Steertse Heide.

10. Appendix 2: Planning and execution of activities in the field

Day	*# traps*	*Activity*	*Fen*	*Time spend*
08/04/2022	2	Set-up	Leemputten Noord	16h-20h
08/04/2022	2	Set-up	Leemputten Zuid	16h-20h
08/04/2022	2	Set-up	Ranonkelven	16h-20h
09/04/2022	2	Empty	Leemputten Noord	11h-13h30
09/04/2022	2	Empty	Leemputten Zuid	11h-13h30
09/04/2022	2	Empty	Ranonkelven	11h-13h30
11/04/2022	2	Set-up	Belderven	16h30-20h
11/04/2022	2	Set-up	Leemputten Noord	16h30-20h
11/04/2022	3	Set-up	Leemputten Zuid	16h30-20h
11/04/2022	2	Set-up	Ranonkelven	16h30-20h
12/04/2022	2	Empty	Belderven	10h30-14h
12/04/2022	2	Empty	Leemputten Noord	10h30-14h
12/04/2022	3	Empty	Leemputten Zuid	10h30-14h
12/04/2022	2	Empty	Ranonkelven	10h30-14h
12/04/2022	2	Set-up	Belderven	17h-19h30
12/04/2022	4	Set-up	Bovenven	17h-19h30
12/04/2022	2	Set-up	Moseven	17h-19h30
13/04/2022	2	Empty	Belderven	10h-12h
13/04/2022	4	Empty	Bovenven	10h-12h
13/04/2022	2	Empty	Moseven	10h-12h
19/04/2022	1	Set-up	Sloot	18h30-20h30
19/04/2022	6	Set-up	Ranonkelven	18h30-20h30
19/04/2022	2	Set-up	Belderven	18h30-20h30
20/04/2022	1	Empty	Sloot	10h30-14h30
20/04/2022	6	Empty	Ranonkelven	10h30-14h30
20/04/2022	2	Empty	Belderven	10h30-14h30
22/04/2022	/	eDNA	Steertse Heide	10h-19h
27/04/2022	2	Set-up	Belderven	13h-15h30
27/04/2022	3	Set-up	Bovenven	13h-15h30
27/04/2022	6	Set-up	Moseven	13h-15h30
28/04/2022	2	Empty	Belderven	10h-12h30
28/04/2022	3	Empty	Bovenven	10h-12h30
28/04/2022	6	Empty	Moseven	10h-12h30
21/05/2022	4	Set-up	Leemputten Noord	16h-19h
21/05/2022	2	Set-up	Belderven	16h-19h
22/05/2022	4	Empty	Leemputten Noord	9h-13h
22/05/2022	2	Empty	Belderven	9h-13h
24/05/2022	/	Watersamples	Ranonkelven	15h-18h30
24/05/2022	/	Watersamples	Leemputten Noord	15h-18h30
24/05/2022	/	Watersamples	Leemputten Zuid	15h-18h30

11. Appendix 3: Work schedule

WERKROOSTER - academiejaar 2021/2022

vul in/ vink aan

NAAM STUDENT	Arne Ferket	**Rolnummer**	20172132
OPLEIDING	Ma Biology		

1ste semester [ ] **2de semester** [X]

WERKROOSTER STAGE

week	Uurrooster	ma	di	woe	do	vrij	za	zo	Opmerkingen (vb afwijking van uurrooster)	handtekening stagementor
1	van: 12u30 tot: 18u00					M			Meeting op kantoor + Rondleiding Mickey	
2	van: 10u00 tot: 19u00						VW	VW	Fuiken (VW=veldwerk)	
3	van: 10u00 tot: 19u00	VW	VW	VW					Fuiken	
4	van: 10u00 tot: 19u00		VW	VW		VW			Fuiken + eDNA	
5	van: 10u00 tot: 19u00			VW	VW				Fuiken	
6	van: 9u00 tot: 19u00						VW	VW	Fuiken	
7	van: 15u00 tot: 18u00		VW						Waterstalen	
8	van: 8u30 tot: 18u00	DV	DV	DV					Dataverwerking (DV)	
9	van: 8u30 tot: 18u00			DV	DV				Dataverwerking	
10	van: 8u30 tot: 18u00				DV	DV			Dataverwerking	
11	van: 12u30 tot: 18u00	DV							Dataverwerking	
12	van: 13u30 tot: 22u00	V	V	V	V	V	V	V	Schrijven verslag (V)	
13	van: 8u30 tot: 18u00	V	V	V	V	V	V	V	Schrijven verslag	

Handtekening student

12. Appendix 4: Monitoring protocol Noord-Brabant

Monitoring protocol:

- Fens to be monitored: Belderven, Leemven, Granaatven, Leemputten Noord, Leemputten Zuid, Kleine Meer, Ranonkelven, Kwekerijven, Talingven and Steertse Heide.

- Per fen 2 Laar M2 traps should be placed per sampling round, each fen should be sampled three times.

- Each fen should be sampled once before the start of sampling round 2, then each fen should be sampled a second time before the start of sampling round 3.

- During the placement of the traps, it is recommended to wear a wading suit. In addition, you can use a wheelbarrow or similar means to transport the traps to the site (through natural areas, so no paved roads).

- Place the traps with the bottom opening towards the nearest (southern) bank, where possible in openings between the water vegetation. Make sure that the openings are free of water vegetation.

Monitoring period:

- Ideal period is early April to late May.

- Depending on the spring (very cold spring → wait until the temperature rises above five degrees Celsius for at least 7 nights in a row).

- Periods of warmth during April - May increase the chances of finding newts (periods of 3 days during which the temperature remains above 20 degrees Celsius).

- It is recommended to make a planning before the monitoring is carried out, including: Which day you will place the traps (they should be collected and emptied the following day) and in which period you will carry out the monitoring.

Time of monitoring:

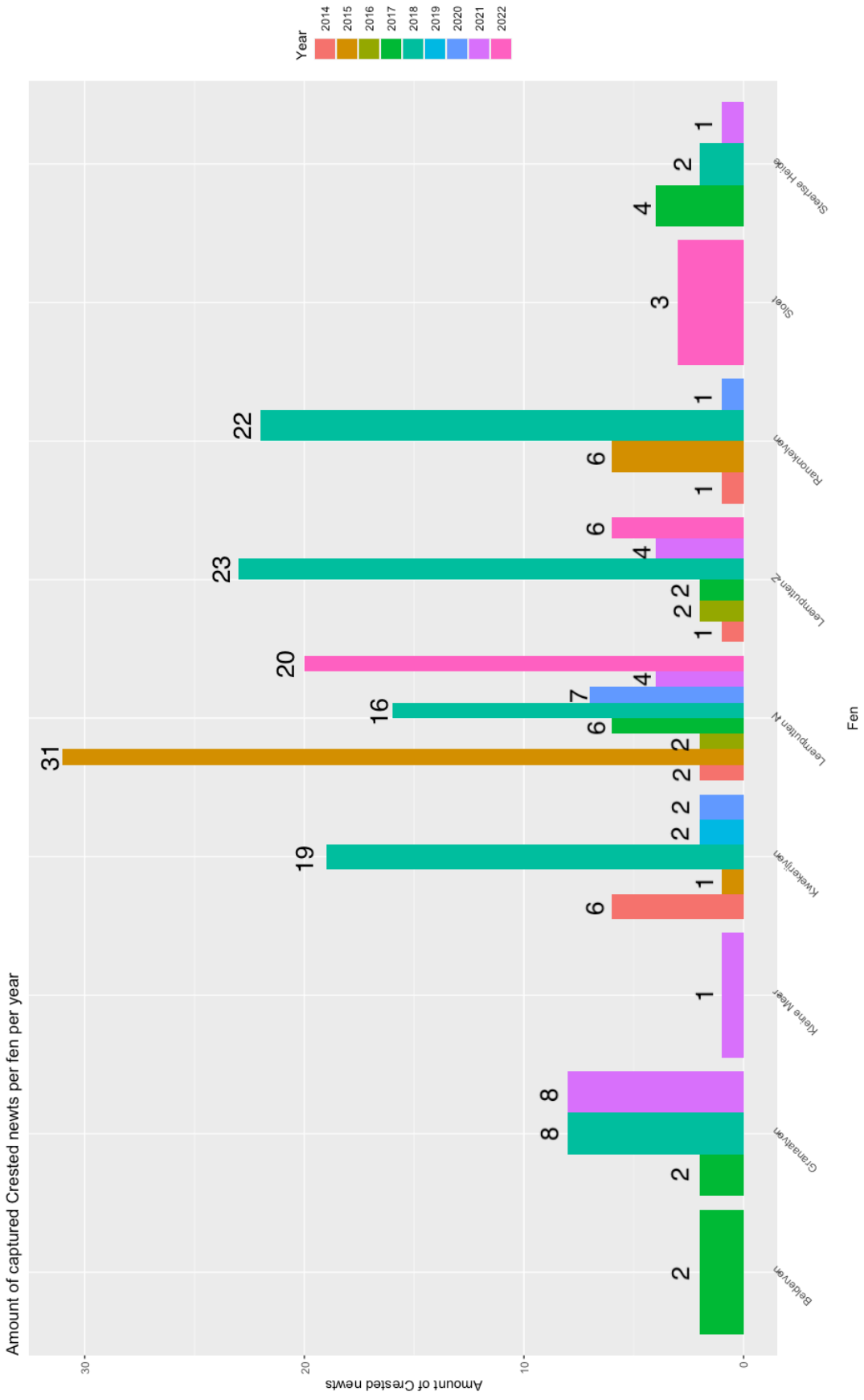
- Place traps in the afternoon between 2 pm - 5 pm.
- Empty the traps in the morning between 9h - 12h.
- Then take the traps to the work shed (Abdijlaan 39, Ossendrecht, the Netherlands) to be cleaned.

Clean the traps:

- 2 grams of Virkon S in a 1 litre plant sprayer or 100 ml ethanol supplemented with 900 ml water. Then spray each trap and your wading suit copiously with the solution. Leave for 5 minutes and then rinse thoroughly with water.

*Beware, Virkon S is toxic, avoid skin contact. If it does come into contact with the skin, rinse with plenty of water.

10. Appendix 5: Graphical representation of the number of crested newts caught per fen, per year.



11. Appendix 6: Table with classification of the different fens into the different trophic levels of stagnant waters.

Table 4: Overview of the trophic levels per fen, classified according to different definitions, indicating the different nutrient states in stagnant waters. Classification according to: Creation of a system of nature types in Flanders: Still waters by Haskoning (2003).

Fen	***Stuijfzand (PO4)***	***Leentvaar (P)***	***Leentvaar (N)***	***Vollenweider (NO3)***
SH1	Weak eutrophic	Meso- to Eutrophic	Ultra- to Oligotrophic	Oligotrophic
SH3	Strong eutrophic	Polytrophic	Eu- to Polytrophic	Oligotrophic
SH4	Mesotrophic	Polytrophic	Ultra- to Oligotrophic	Oligotrophic
SH5a	Oligotrophic	Ultra- to Oligotrophic	Ultra- to Oligotrophic	Oligotrophic
SH5b	Oligotrophic	Ultra- to Oligotrophic	Ultra- to Oligotrophic	Oligotrophic
SH5c	Oligotrophic	Ultra- to Oligotrophic	Ultra- to Oligotrophic	Oligotrophic
SH7	Oligotrophic	Ultra- to Oligotrophic	Ultra- to Oligotrophic	β-Mesotrophic
SH10	Hypertrophic	Polytrophic	Polytrophic	β-Mesotrophic
SH11a	Eutrophic	Polytrophic	Ultra- to oligotrophic	Oligotrophic
SH11b	Strong eutrophic	Polytrophic	Ultra- to oligotrophic	Oligotrophic
Bovenven	Mesotrophic	Meso- to Eutrophic	Ultra- to oligotrophic	Oligotrophic
Moseven	Oligotrophic	Oligo- to Mesotrophic	Ultra- to oligotrophic	Oligotrophic
Ranonkelven a	Oligotrophic	Ultra- to Oligotrophic	Ultra- to oligotrophic	Oligotrophic
Ranonkelven b	Oligotrophic	Ultra- to Oligotrophic	Ultra- to oligotrophic	Oligotrophic
Belderven	Oligotrophic	Ultra- to Oligotrophic	Ultra- to oligotrophic	Oligotrophic
Leemputten Zuid	Eutrophic	Polytrophic	Eu- to polytrophic	α-Mesotrophic
Leemputten Noord	Oligotrophic	Ultra- to Oligotrophic	Ultra- to oligotrophic	Oligotrophic