



**Inland Norway
University**

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

Maurie Hons

Master thesis

**Eradicating the American bullfrog with the
sterile triploid method: methods and
application**

Master in Applied Ecology

2019-2021

Preface

This Master thesis, ‘Eradicating the American bullfrog with the sterile triploid method: methods and application’, has been written to fulfil the graduation requirements of the Master Program ‘Applied Ecology’ at the Inland Norway University of Applied Sciences (Høgskolen Innlandet). I was engaged in researching and writing this thesis from June 2020 to May 2021.

This project was undertaken at the request of Life 3n-bullfrog, a large European subsidised project with the goal to eradicate the bullfrog using an innovative new method, e.g. Sterile triploid method (STM). Together with Alain De Vocht, head of Life 3n-bullfrog, my research goals were formulated. I was completely new in the subject of modelling populations, but by reading tons and tons of literature, I can finally say that I can build a (rather simple) model of a population.

I would like to thank my supervisors for their excellent guidance and support during the process. Karen-Marie Mathisen and Alain De Vocht, who guided me through the first part of my thesis and Olivier Devineau and Sarah Descamps, who supervised me in the last few months. I also wish to thank Natuurwerk, Institute of Nature and Forest Research (INBO) and all the volunteers that helped with data collection. Without all this help, I would not have been able to get to these results. To my fellow classmates at Inland Norway University of Applied Sciences: I would like to thank you as well for the many support. We all had our highs and lows, but together we got through it. I would also like to thank my family and boyfriend. Even though they did not understand much of the subject, they tried to listen when I needed to ventilate, and they motivated me to push through, even at times when I wanted to give up.

I hope you enjoy your reading as much as I enjoyed writing this.

Malaurie Hons

Evenstad, May 28, 2021

Contents

- PREFACE..... 3**
- 1. INTRODUCTION 6**
- 2. BACKGROUND INFORMATION 9**
 - 2.1 STUDY SPECIES..... 9
 - 2.1.1 *Taxonomy*..... 9
 - 2.1.2 *Description and ecology*..... 9
 - 2.1.3 *Problems* 10
 - 2.1.4 *Control measures* 11
 - 2.2 STUDY AREA 12
 - 2.3 STUDY DESIGN 13
- 3. POPULATION SIZE ESTIMATION: EVALUATION OF METHODS 14**
 - 3.1 METHODS 14
 - 3.1.1 *Data collection*..... 15
 - 3.1.2 *Data analysis*..... 16
 - 3.2 RESULTS..... 17
 - 3.2.1 *Catch-depletion*..... 17
 - 3.2.2 *e-DNA analysis*..... 20
 - 3.3 DISCUSSION..... 20
 - 3.3.1 *Catch-depletion*..... 20
 - 3.3.2 *e-DNA analysis*..... 21
- 4. MATRIX POPULATION MODELLING 24**

4.1	CONCEPTUAL MODEL	24
4.2	DESCRIPTION OF THE POPULATION MODEL	27
4.2.1	<i>Parameters</i>	28
4.3	PROPOSED ANALYSIS OF THE MPM.....	32
4.3.1	<i>Asymptotic dynamics</i>	33
4.3.2	<i>Perturbation analysis</i>	34
4.3.3	<i>More advanced analysis</i>	35
5.	CONCLUSIONS AND RECOMMENDATIONS.....	36
	REFERENCE LIST.....	37
	ABSTRACT.....	44

1. Introduction

The American bullfrog, also called *Lithobates catesbeianus* or *Rana catesbeiana*, is a species native to Canada, the USA and Mexico, but now settled in many countries. The population of bullfrogs is currently increasing all over the world. The American bullfrog is understood to be an invasive alien species (IAS) in many countries (IUCN SSC Amphibian Specialist Group, 2015). Alien species are species introduced outside their natural range where they often seem to do better than in their native habitat. Sometimes they thrive so well that they become a risk to native species. In this case they are called invasive alien species. There are many problems that come with IAS (Mcneely, 2001). Mayer et al. showed in 2015 that the cane toad, an invasive alien species in Australia, poses a threat towards the native anurans since they inhibit the activity by inducing avoidance or by reducing activity, such as feeding and breeding (Mayer et al., 2015). Sometimes it concerns a direct threat as in the Cuban treefrog, which predate of Florida's native treefrogs (Mayer et al., 2015). IAS are sometimes capable of disturbing an entire ecosystem and destroy habitats. IAS might also bring diseases that can transfer to other native animals or even humans. Predictions say that climate change will cause invasive species to spread even more (Hoegh-Guldberg O., Jacob, & Taylor, 2018). Drastic measures are needed to eradicate IAS before they cause too much damage. It is very important to act fast and use the correct, specific eradication method for each kind of IAS. The best method to avoid IAS is to prevent them from being introduced. It can be very hard to eradicate some kinds of IAS once they have spread too far. A first step towards eradicating IAS is to gain knowledge about the species' behaviour, ecology and impacts (Mcneely, 2001).

The American bullfrog was introduced in Belgium and other European countries in the 1990s for culinary reasons and was later used for ornamental purposes. The bullfrog escaped from several gardens to nature and also entered Belgium together with live fish transports from other European countries (van Ham et al. 2013). The American bullfrog is listed as 'least concern' on the IUCN red list, because of its great spread, increasing trend and large number of subpopulations and locations (IUCN SSC Amphibian Specialist Group, 2015). The American bullfrog is thriving in Belgium because of the presence of their ideal habitat, their high reproductive rate (up to 20.000 eggs per clutch) and having no predators (van Ham et al., 2013).

The American bullfrog causes many problems in Belgian ecosystems. First of all, they are carriers of the feared chytrid fungus, *Batrachochytrium dendrobatidis*, which causes

chytridiomycosis, a fungal infection. American bullfrogs are immune to this infection and thus do not experience any symptoms, but they can pass it to other species, which are not immune to the disease. *Batrachochytrium dendrobatidis* has already caused hundreds of amphibian species (mostly frog species) to go extinct (Garner et al. 2006). Secondly, American bullfrogs eat anything that fits in their mouth, even their own species, which makes them cannibalistic. As such generalists, they have a very reliable food source and are very competitive towards other species. At last, the bullfrogs can travel great distances which makes it very hard to eradicate the species (Roach, 2004). They are very hard to eradicate because of their complex developmental stages, their abundance, high fertility and the far distances they can cover (Ficetola, Thuiller, & Miaud, 2007). New methods are needed for effective eradication.

This Master thesis study is part of LIFE 3n-bullfrog, a five-year long funded European LIFE project that started in October 2019 (De Vocht, 2019b). LIFE 3n-bullfrog's main purpose is eradicating or controlling isolated American bullfrog populations using a new method based on the sterile insect technique (SIT) (Dyck, Hendrichs, & Robinson, 2005) and the sterile male release technique (SMRT) (Great Lakes Fishery Commission, 2000), namely the sterile triploid method (STM). They want to integrate this method in the existing management plan of the bullfrog in Flanders. The sterile triploid method was created to eventually control invasive aquatic fauna. Within this project, the sterile triploid method is tested using the American bullfrog as a pilot case. Fertile (diploid) females are caught, whereafter the eggs are farmed and fertilized using the extracted sperm from the captured males. The eggs then go into a high-pressure chamber for several minutes that makes the eggs triploid and, in the case of American bullfrog, sterile (Full method: (Descamps & De Vocht, 2017)). When the eggs develop to be larvae, they are moved to an outdoor closed complex to grow further. An initial catch effort in the concerned ponds will be executed before releasing the sterile bullfrogs. After introduction, the population is expected to decline because of infertile egg clutches, which is expected to result in a population decline of 90% (Descamps & De Vocht, 2019). One of the goals of LIFE-3n-bullfrog is to reduce the American bullfrog population by 75% by the end of the project and by 90% three years after the project. It is expected that implementing STM will result in a population decline to a level where ecological effects of the invasive population would not threaten local ecosystems any longer (De Vocht, 2019a). Currently, sexually active and calling triploid males have been created successfully. The STM has been tested in the lab and preliminary assessment of its efficiency in outdoor mesocosms has been carried out. But, more research is needed to gain knowledge about sexually mature triploid bullfrogs their behaviour,

foraging and sexual activity, compared with wild bullfrogs. Also, an accurate estimation of the size of the target population has to be made, as well as an accurate estimation of the population structure to gain insight on how many sterile individuals have to be released in order to have a successful reduction of the population. Mathematical models will clear out what amount of triploid bullfrogs is favourable to release so that survival, population reduction and cost-efficiency is optimal. Population models are often used to assess the effectivity of management actions (Caswell, 2001). The initiation of this step will be carried out in this Master thesis.

The eventual outcome of my Master project contains two parts. In the first part, I will carry out the first steps towards estimating the population size by trying out two different methods and evaluating these methods whilst comparing results. I will evaluate and compare catch-depletion and eDNA analysis as population size estimation techniques. I will also estimate the catch per unit of effort (CPUE) based on the data collected using the catch-depletion method, which can be used as an indication on how to approach control measures other than STM. The second part includes a method for modelling the population of American bullfrogs in Scheps, a small nature reserve in Balen, Flanders. In this part, the first step is to create a conceptual model concluding various parameters such as the life cycle of diploid and triploid individuals, in which sterile individuals will be introduced in a wild population of fertile American bullfrogs whilst and after conducting other control methods. In this conceptual model it is possible to see the expected outcome of the population declining. Hereafter, I will determine and describe the parameters that need to be involved in the population model. The model is supposed to give insights about the amount of triploid bullfrogs that need to be released to reach the goal and to gain information about the amount that the population should decline before implementing STM in order to get a successful result. I will discuss the recommended type of population model as well as in which program the model should be built and describe how to analyse the population model. Using data from other bullfrog populations, I will create a model and discuss the results, serving as an example.

2. Background information

2.1 Study species

2.1.1 Taxonomy

The American bullfrog is part of the kingdom Animalia, the phylum Chordata, the class Amphibia, the order Anura and the family Ranidae (IUCN SSC Amphibian Specialist Group, 2015). The official taxon name is *Lithobates catesbeianus* (Shaw, 1802).

2.1.2 Description and ecology

The American bullfrog is native to North America. It is known to be the largest North American frog with males measuring about 180 mm long and female about 200 mm (B. Bury & Whelan A., 1984). An adult can weigh up to 700 g (National Geographic Society, 2015). The tadpoles are also exceptionally large, reaching lengths up to 178 mm. They can be recognized by their green or olive dorsum with a straw- or maize-coloured belly, and many small dark and fine yellow dots (figure 1). Adults are olive, green or brown on the back, but can vary in colour. The legs of an adult are banded and blotched and there is variable spotting on the back. The underside is white blotchy with grey spots. The head is flat and broad. This frog can be distinguished from other frogs by the lack of dorso-lateral folds. The males' tympanum (i.e. middle ear)



Figure 1: American bullfrog tadpole (Nafis, n.d.)



Figure 2: Adult North American bullfrog (Howes, 2004)

is brown and larger in diameter than the eye (figure 2). The females' tympanum is about the same size as the eye. This characteristic is only visible when they reach sexual maturity (B. Bury & Whelan A., 1984). The American bullfrog reaches its adult size two years after metamorphosis. The year after, in its third year, the bullfrog starts to reproduce. Sometimes they reach sexual maturity after only one year, this depends, among other things, on the climate (R. B. Bury, 1984; Govindarajulu, Altwegg, & Anholt, 2005). The clutch size becomes larger

as the females get older and bigger. An individual clutch size can be as large as 40,000 eggs. Sometimes females lay a second clutch, which contains fewer and smaller eggs than those of the first clutch (Rep, Wingert, & Meshaka, 2015). The average life span of the bullfrog is 7 to 9 years, but some research has shown that they can live up to 16 years or even more (Stoutamire, 1931; Lougheed & Taylor, 2010). A feature that distinguishes the American bullfrog from other frog species is their call. The males' call is very different from other species of frogs. People say that it resembles the mooing of a cow (National Geographic Society, 2015). American bullfrogs are nocturnal predators. They will ambush and eat anything they can fit in their mouths, including insects, rodents, birds, fish and snakes. They wait quietly for their prey to pass and then lunge with their powerful hind legs and mouths wide open (B. Bury & Whelan A., 1984).

2.1.3 Problems

One of the most important threats to biodiversity are invasive species (Richardson et al., 2000), additionally causing many problems in amphibian populations. The invasive bullfrog causes indigenous species to decline because of the bullfrogs' large size, high densities, loud vocalizations high fecundity and broad diet, which makes them very successful regarding competition. The bullfrog is additionally known to have severe effects on native amphibian species through competition (mainly from larvae) and predation (Blaustein & Kiesecker, 2002). Males are assumed to be territorial and can be aggressive when guarding their land (National Geographic Society, 2015). Because of its large body size, the bullfrog often is a very important part of aquatic ecosystems (R. B. Bury, 1984) and can act as a structuring predator in aquatic ecosystems (van Ham et al., 2013). They can survive in a broad range of conditions and thus spread easily (R. B. Bury, 1984). Bullfrog populations are hard to manage once established (Adams, Pearl, & Gherardi, 2007).

In Flanders, the American bullfrog is listed by ISEIA as a black list species (A1-score = 12). Here, the bullfrog mainly has an impact on species level and less on the environment and ecosystem-functioning (De Wavrin et al., 2007). Native species to Flanders that are affected indirectly are *Bufo bufo*, *Epidalae calamita*, *Lissotriton vulgaris*, *Alytes obstetrican* and *Salamandra Salamandra*. They are affected by transmission of pathogens or/and competition. *Pelophylax esculenta* is affected directly through predation (Devisscher et al., 2012; F. Pasmans et al., 2010; Frank Pasmans & Martel, 2011; Anon., 2005; Bovero et al., 2008).

2.1.4 Control measures

There is a general regulation with guidelines for controlling IAS in Europe (European Parliament, 2014). The first step of it is prevention. People need to be aware of the problem so they will not be introducing species consciously. It is very important to communicate the problem towards the people and create a support base. The second step is rapid detection, followed by quick intervention. Creating detection systems for discovering new IAS is very essential in the combat against IAS. Some channels were used to allow people to share their sightings of IAS with others. These channels often include websites or apps where they can report their sightings (van Ham et al., 2013), but this depends on the country. As a last resort, combatting the IAS is the only solution. When combatting IAS, it is important to focus on all developmental stages (adults, metamorphs and larvae). Otherwise, the population will be able to recover in a short time (Ficetola & Miaud, 2008). Over time, several techniques have been invented to eradicate invasive species, which are mostly very specific to a species. Some of the methods that are adequate for controlling a population of bullfrogs are removing the egg clutches and larvae using nets, removing the adults and subadults using funnel traps, nets or by shooting them (Kamoroff et al., 2020). The most successful eradication method currently carried out is to combine number regulation measures (mostly capture with traps) and measures on habitat level, conducted frequently (one or twice a year) for 10-40 years (Ficetola et al., 2007). This method is efficient in a small pond with a closed population, which is often not the case. Bullfrogs are often very wide spread in high numbers (Ficetola et al., 2007).

LIFE 3n-bullfrog is a project that is testing an innovative method for controlling bullfrogs in more open populations, as which is the case in Flanders, Belgium (De Vocht, 2019a). The method is called the sterile triploid method (STM) and is based on the same principles as the already existing sterile insect technique (SIT) (Dyck et al., 2005; Gentile, Rund, & Madey, 2015) and sterile male release technique (SMRT) (Great Lakes Fishery Commission, 2000). SIT is a method that was first invented for combatting screwworm flies (*Cochliomyia ssp.*). Screwworm is a deadly parasite of



Figure 3: Sterile insect technique theory (Yan, 2019)

livestock. By sterilising the screwworm through radiation and then releasing it in big amounts, they were able to eradicate the screwworm (figure 3). SIT is still being used to eradicate different kind of insect species (Dyck et al., 2005; Gentile et al., 2015). SMRT is similar in the way that it sterilizes the male individuals to then release into the wild resulting in a decline of the population. The sterile males will compete with the fertile males and mate with the females. The resulting egg clutches will be unviable. This technique was used on sea lampreys (Great Lakes Fishery Commission, 2000) and large mammals (Gonçalves da Silva, Kolokotronis, & Wharton, 2010). The sterile triploid method (STM) is a recently created method within LIFE 3n-bullfrog, where both sterile male and female larvae are released into the wild population (Descamps & De Vocht, 2017).

2.2 Study area

The American bullfrog is present in five distinct locations in Flanders, which is the northern part of Belgium. One of those areas is a large one from around 200 km² called the valley of the Grote Nete. The bullfrog is known to be very present there with a very high abundance (figure 4) (Descamps & de Vocht, 2016). Flanders consists of many small, shallow, permanent and nutrient-rich ponds, which provide the optimal conditions for reproduction of the American bullfrog such as presence of many algae and the lack of predators in the water bodies for breeding (van Ham et al., 2013). This pilot case is focused on the more or less closed population in Scheps, which is a nature reserve part of the Valley of the Grote Nete, situated in Balen (figure 5).

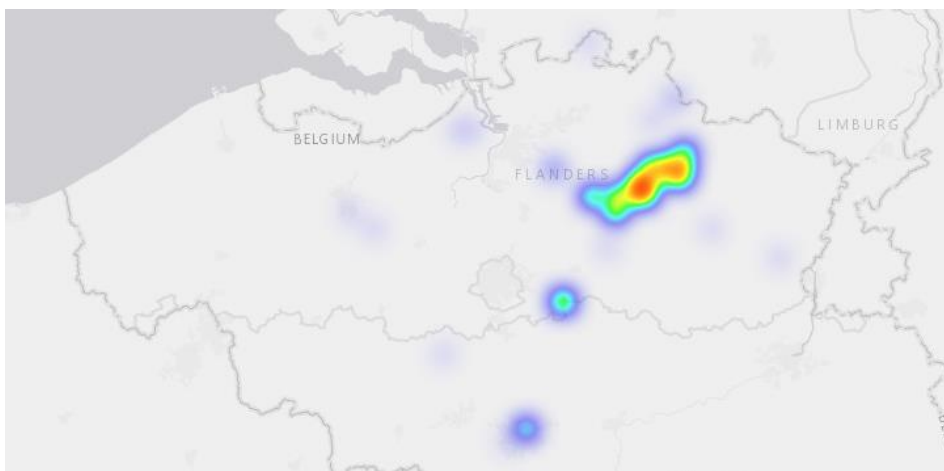


Figure 4: Heat map of the distribution of the American bullfrog in Flanders (www.waarnemingen.be, 2021). The study area Scheps is situated in the largest area with highest density.

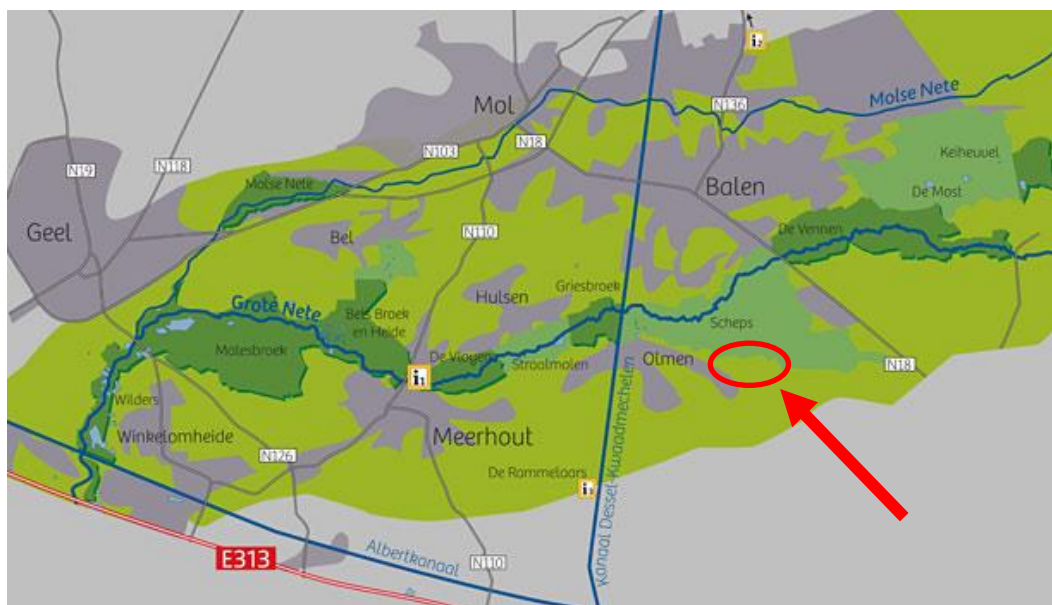


Figure 5: Map of the big Neteforest (Natuurpunt, n.d.), Scheps is indicated with red arrow and circle.

2.3 Study design

The objective of this study was to gain some insights on two elements. First, I wanted to evaluate two different methods of population size estimation to determine which method is the best for this case study, as well make an estimation of the catch per unit of effort (CPUE). These factors are both essential for building a population model and determining how to approach the release of triploid bullfrogs. Secondly, I wanted to gain insight into how a population model for this pilot case, the eradication of the American bullfrog using the sterile triploid method, is best built. The population model is required to give some insights into how to approach the release of sterile larvae into the population, e.g. how to make the eradication method efficient. By knowing the size and growth rate of the population and the life stages that are most sensitive to changes, it is possible to determine how many triploids are best to release when. The eventual population model will also be able to reveal how much the population needs to decline before implementing STM. Specifically in my thesis, I wanted to gain insight on what type of model to choose, what parameters to include, what program to use and which analysis to conduct. There was no data of use collected to build the actual model, so I used data from other bullfrog populations to give insights on how the model should be built and analysed.

3. Population size estimation: evaluation of methods

3.1 Methods

Two methods for estimating the population size were tested, compared, and evaluated, namely catch-depletion and analysing eDNA concentrations. This to determine which method is most efficient for estimating the population size of bullfrogs. Factors that are considered are accuracy, time requirement, expenses, and potential other variables that you can gain from the method. We collected all data for the estimates of population sizes in the valley of the Grote Nete, in different conservation areas. The catch-depletion data also gave insight on the average catch per unit of effort (CPUE) in Scheps, which is essential information in the eradication plan of the American bullfrog. The CPUE can reveal how many funnel traps to place in order to make the population decline the desired amount. By distinguishing trap angles in the calculation of the CPUE, the results indicated in which angle it is best to put the funnel traps in order to catch the desired life stages (tadpoles/metamorphs or adults).

Catch-depletion has successfully been used in the past to estimate the population size of aquatic (invasive) species (Henderson, 2002; Maceina, Rider, & Lowery, 1993). Previous research has shown that catch-depletion can also be used to reduce a population of bullfrogs in Flanders when conducted persistently over several years (Devisscher et al., 2012). But it has also been shown that some populations of bullfrogs in Flanders have grown out of control, as the population in the Valley of the Grote Nete (study area). Therefore, other control methods are needed, like the sterile triploid method (Descamps & de Vocht, 2016).

eDNA analysis is a method used for indicating the presence of bullfrogs that has been tested in Flanders in a wild population of bullfrogs before, with success (Halfmaerten, 2015). This method also showed to be promising in other populations of bullfrogs and other species (Lacoursière-Roussel, Rosabal, & Bernatchez, 2016; Lin, Zhang, & Yao, 2019; Yates, Fraser, & Derry, 2019). In Flanders, the method has also been tested in a mesocosm experiment on a population of bullfrogs very recently to refine the method (INBO, 2020), and has now been conducted on a larger scale. Environmental DNA concentrations can now show the abundance and biomass estimates of bullfrogs (as a signal) whereafter a population estimate can be made per pond (Ficetola & Miaud, 2008; INBO, 2020).

3.1.1 Data collection

The data for catch-depletion was collected in Veerle, Scheps and Nijlen-Lier by Natuurwerk, commissioned by Life 3n-bullfrog. They started to collect data for the area of interest, Scheps, in spring of 2017. The data was not specifically collected for the analyses made in this thesis but in general to keep track of management. Bullfrogs, both adults and tadpoles, were caught using double funnel traps. Double funnel traps were shown to be mostly effective to catch bullfrog larva. On average, 6% of the population is caught with one catch per unit of effort (= one double funnel trap placed during 24 hours) (Devisscher et al., 2012). Every 24 hours the traps were emptied by employees of Natuurwerk. The largest adults caught were used for the triploid breeding program for creating triploid individuals (mostly at the beginning of spring), while other bullfrogs caught were eliminated. The number of traps placed and how they were placed depended on the size, depth, and shape of the pond. In general they tried to place the traps parallel to the shores, which was proven to be the best method with the highest catch rates for larvae (Devisscher et al., 2012). The variables that were collected include timestamp, name of collector, GPS coordinates, number of ponds, number of traps, angle of traps, number of bullfrogs caught per trap for each life stage, bycatches, and notes. The trap angle was not included at the very beginning of the captures (2018). The distinguished life stages are defined in table 1 (Devisscher et al., 2012).

Table 1: Description of distinguished life stages for catch-depletion on the American bullfrog (Devisscher et al., 2012), (Gosner, 1960)

i.CODE	LIFESTAGE	SIZE	CHARACTERISTICS	GOSNER.STAGE
L00	Larva	< 50mm		
L0	Larva	50-100mm	no development of hind legs	23-28
L1	Larva	80-170mm	Commencing development of hind legs	29-37
L2	Larva	80-170mm	Clear development of hind legs	38-41
M1	Metamorph			42-46
M2	Metamorph			> 47 (Kaefer et al., 2007)
AM	Adult male			
AV	Adult female			

Samples of eDNA were taken in Scheps, Veerle, Nijlen-Lier and Molse Nete by the Institute for Nature and Forest Research (INBO, 2020). The selected ponds were all located at the edge of the estimated bullfrog's distribution range to see how far the bullfrogs were spread. They were not randomly selected and thus biased. Only the samples collected in Scheps will be discussed in this thesis. From each water body selected, a large number (20-40, depending on

the size) of at least 0.5 litre water samples were taken. The water was sampled at five meter intervals, just below the water surface, using a long sterile sampling pole. The samples were then pooled into a merged sample and immediately filtered. After filtering, the filters were air dried, capped and stored at -21°C for transportation to the lab, where the samples were analysed. Variables that were collected are ID, period, nr of pond, surface (ha and m^2), filter, litres filtered, copies of DNA, number of bullfrogs, capture (as a control measure) and details regarding the captures (INBO, 2020).

3.1.2 Data analysis

There were two analysing methods considered in this research to estimate the population size out of the collected data using the catch-depletion method. Only tadpoles that were caught were considered for both methods. The first method was the Leslie method. This method requires a minimum of 3 catches. Each catch is plotted against the sum of all previous catches. The catch effort is also considered. If the points lie on a straight line, p is consistent. The abscissa will be cut by the line at a value of x which is the total population (Leslie and Davis, 1939). Another possible method was the Zippin method, also known as the removal method. This method includes the calculation of a goodness of fit test statistic in which n is replaced by its maximum likelihood estimate \tilde{n} (mean population size). If there are less than 5 catches, it is possible to pool some of the captures (Zippin, 1958). Both methods were tested in R (Rstudio Team, 2020), using the FSA-package (Ogle, Wheeler, & Dinno, 2021). Since both methods did not show very adequate results, some plots with the total amount of bullfrogs caught per life stage were created using the package ggplot2 in R (Rstudio Team, 2020; Wickham, 2016)

The catch per unit of effort (CPUE) was defined by estimating the total amount of individuals caught per funnel trap (double trap) per 24 hours. This was done separately for tadpoles/metamorphs and adults. The angle in which the traps were placed was also taken into consideration. Since data from other areas than Scheps were inconsistent (traps were sometimes placed for more than 24 hours), only the data collected in Scheps was included in the estimations. Only the ponds where any bullfrog was caught were included. The CPUE was calculated in Excel (Microsoft Corporation, 2021), whereafter boxplots were created in R, using the ggplot2 package (Rstudio Team, 2020; Wickham, 2016).

The environmental DNA samples were analysed by INBO. The bullfrog abundance can be estimated from the derived eDNA concentrations out of the filter residues of the water samples.

eDNA emission rates do not differ between bullfrog larvae and juveniles and adults only occasionally derive in the water column, which means that the linear regression for larval bullfrogs could serve as a valuable link between eDNA signals and bullfrog abundance estimates. The eDNA concentration per litre filtered water for each water body is first corrected according to the surface area relative to that of the water body. Then, an estimate of the abundance of bullfrogs in the entire water body can be derived from following formula: C_{corr}/r where C_{corr} is the eDNA concentration per filtered litre water corrected for pond surface area, and r is the regression coefficient obtained from the mesocosm experiment (108.888), conducted by INBO before sampling the ponds discussed in this thesis (INBO, 2020). Plots to summarize the collected data were created in R, using the package ggplot2 (Rstudio Team, 2020; Wickham, 2016).

3.2 Results

3.2.1 Catch-depletion

The population size could only be estimated for two ponds in the Scheps area since many of the collected catch-depletion results were biased and because of the small amount of caught individuals. The Zippin method seemed less effective since it does not consider the catch effort and the catch effort sometimes differed between catches. The Leslie method shows a population size estimate of 18,576 (N_0) in Scheps 2 (figure 6) and 82,416 (N_0) in Scheps 20. The slope resulting from the Leslie method is not significantly different from 0, which indicates that the catch unit per effort (CPUE) does not decrease sufficiently through time. Most likely, not enough individuals were removed at each capture to respect the assumptions of the Leslie method. Thus, the results are perhaps doubtful.

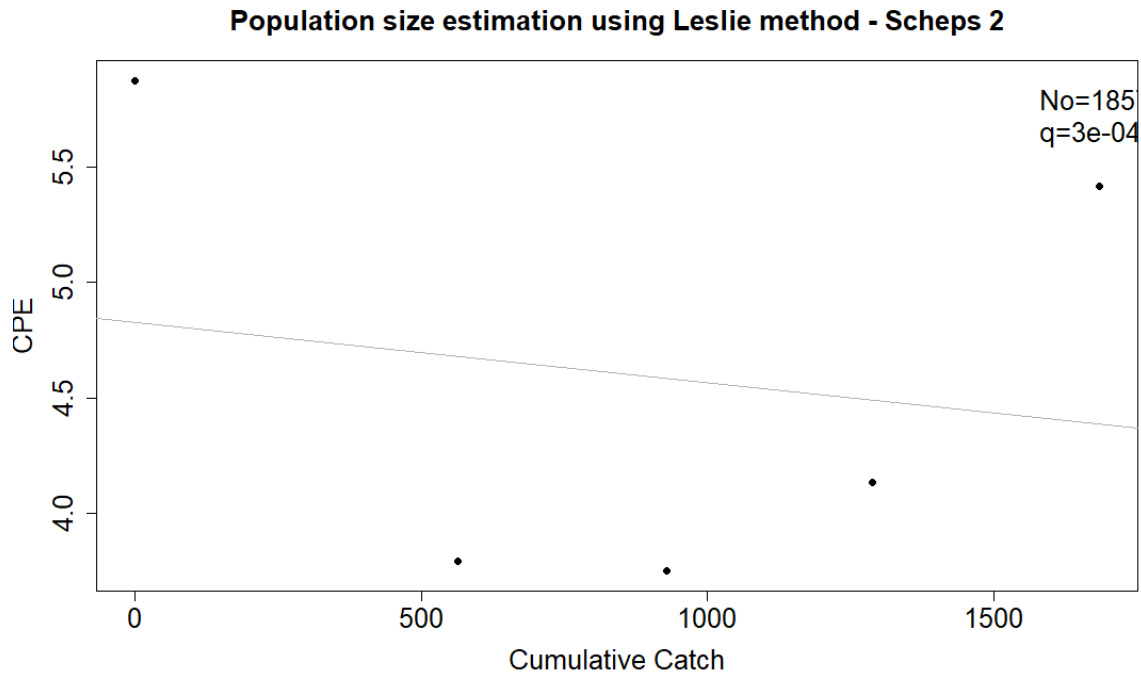


Figure 6: Population size estimation using Leslie method - Scheps 2; the negative slope is insignificant ($p > 0.05$) which makes the estimation unreliable (Rstudio 2020, package = FSA)

We do have some indication of the abundance in bullfrogs per pond in Scheps since they have been catching in these ponds for several years. Scheps 2 and Scheps 20 have by far the highest abundance of bullfrogs. Scheps 6 and Scheps 7 also showed a higher number of bullfrogs in previous years but have not been captured last year (figure 7). The catch effort (nTraps x hours) in Scheps also grew exponentially over the years with a total catch effort of 8088 in 2017 in 10 different ponds, 9952 in 2018 in 10 different ponds, 13992 in 2019 in 8 different ponds and 17304 in 2020 in 10 different ponds.

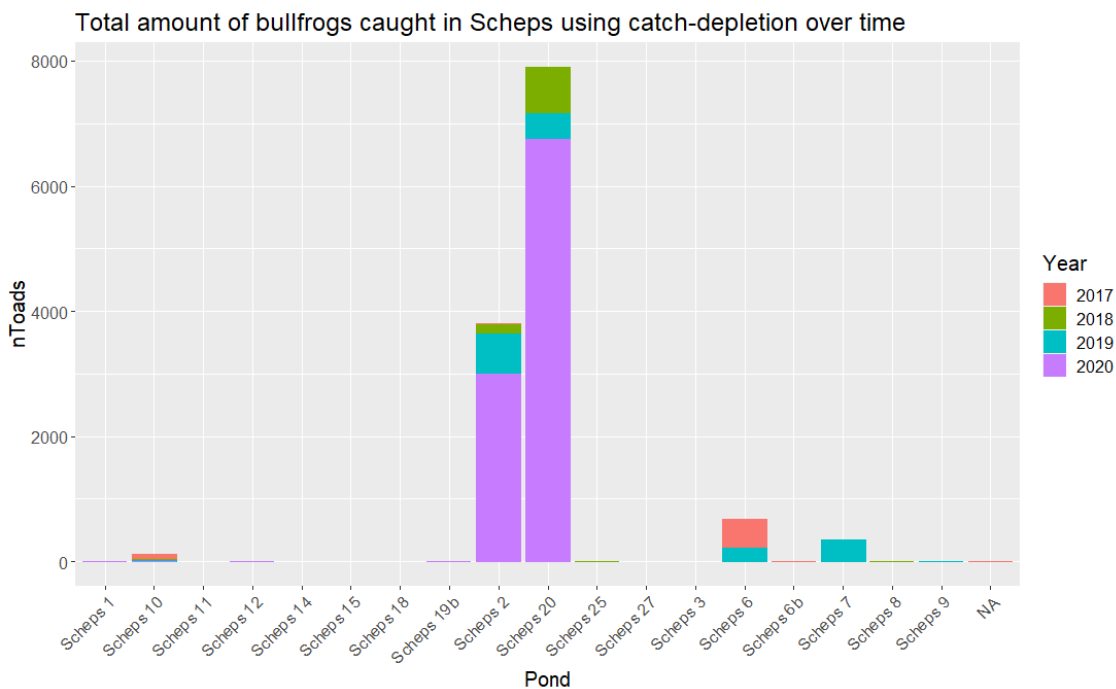


Figure 7: Total amount of bullfrogs caught in Scheps using catch-depletion over time (Rstudio 2020, package = ggplot2) with unequal catch efforts

The mean CPUE considering tadpoles and metamorphs is 233.2 catches when the funnel traps are placed parallel to the shore (0°) and 350.9 when funnel traps are placed under a 45° angle. The mean CPUE considering adults is 0.4 catches when the funnel traps are placed at 0° and 0.3 when the traps are placed under a 45° angle. The median for all mean values is lower than the mean (figure 8).

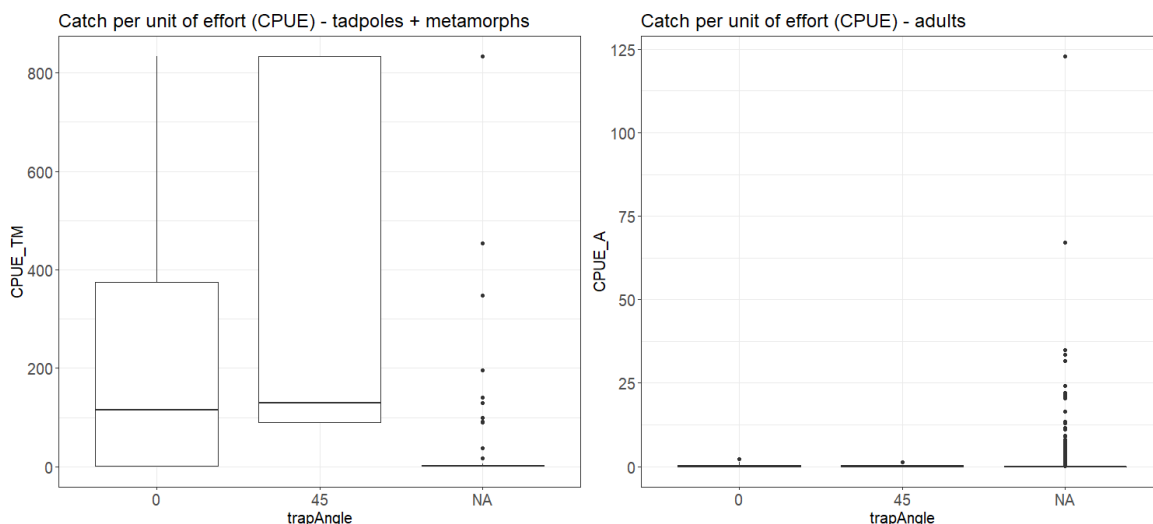


Figure 8: CPUE per trap angle, separating tadpoles/metamorphs and adults. The data applies to the area of Scheps. Ponds where no bullfrogs were caught are not included in the selection. NA's indicate early captures (2018) when the trap angle was not considered

3.2.2 e-DNA analysis

eDNA analysis indicated that some ponds in Scheeps showed a high density of bullfrogs, namely pond 2 and 19B (figure 9). Pond 2 has an average of 3.6 bullfrogs per m² (estimated total abundance of 3967) and pond 19B of 10.7 bullfrogs per m² (estimated total abundance of 6517). The results of catch-depletion also indicated that pond 2 had a very high density in bullfrog, but not pond 19B.

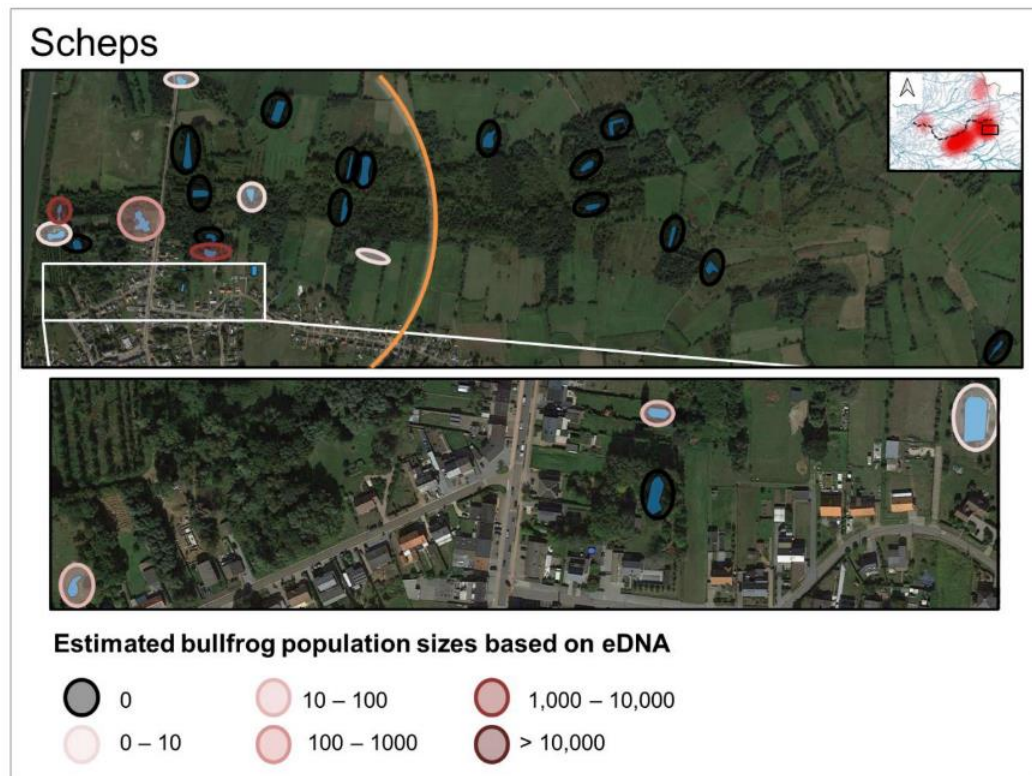


Figure 9: Estimated density of bullfrog per pond in Scheeps based on eDNA. The invasion front, as suggested by the eDNA data, is indicated by the orange line (INBO, 2020).

3.3 Discussion

3.3.1 Catch-depletion

Conclusions that can be made considering the population density based on the catch-depletion results are that pond 2 and pond 20 in Scheeps inhabit by far the highest density in bullfrogs and thus should probably be prioritised in the management plan for the bullfrog. The population seems to be inclining in the last few years, but this cannot be reassured since the capture effort also increased in the past years.

In order to estimate the (sub)population size with reliable results, the catch-depletion data collection needs to be done adequately with more catches. The data for this project was collected by a governmental organisation as a control method for the American bullfrog with, initially, no purpose to use it for research. That is why methods were not always as adequate. It is very important to follow next assumptions to obtain an unbiased estimation of the population size by conducting catch-depletion method.

- Always use same position of funnel traps
- Always use same angle of funnel traps
- Always use same amount of funnel traps (for an equal catch effort)
- Always same amount of time in between captures (for an equal catch effort)
- Captures until there are no or almost no individuals found anymore
- All ponds, located close together, need to be done in a certain time frame
- Data needs to be put in the dataset correctly, without typos, without mistakes

Catch-depletion is a very time-consuming method for estimating the population size. The water body of interest has to be visited several times which also comes with a cost of transport and most often required two employees. The method itself, is rather cheap because funnel nets are reused but the work cost can get high. An advantage of catch-depletion is that it is possible to collect a variety of information about the population such as population structure, sex ratio, reproductive timing, ... Catch-depletion also acts as a control method since the captured bullfrogs are euthanised after every catch.

The CPUE when placing the funnel traps at 0° compared to 45° to the shore are quite similar (estimate of 233 compared to 350). There were almost no adults caught in either angle. It was expected that most tadpoles and metamorphs would be caught when placing the funnel traps parallel to shore (0°) and more adults would be caught in a 45° angle, since adults jump from the shores into the water and would then bump into the nets. Though, results showed that placing the trap in an angle is most efficient for catching tadpoles. The use of funnel traps is not effective for catching adults. Other control measures should be implemented to decrease the number of adult bullfrogs.

3.3.2 e-DNA analysis

All the ponds that were situated in- and right outside the suspected perimeter of the bullfrog distribution area were sampled. Most of the ponds in which bullfrogs are being captured as a

control method, did show a bullfrog signal in the eDNA. Also, most of the ponds that are not being captured showed no bullfrogs at all. There are still some (about 20) ponds in which they are not capturing the bullfrogs even though there are some or many present. It is important to start conducting control measures on these ponds too. There were some outliers in the data indicating a presence of over 200.000 bullfrogs. Previous research has shown that in Flanders, the population densities of the American bullfrog go up to averagely 120.000 tadpoles per ha (Devisscher et al., 2012). Since all ponds that showed such high densities were smaller than 0.4 ha, we can assume that the outliers are real. This indicates that the method is not fully effective for estimating population sizes, especially for large populations.

eDNA concentrations may vary with water temperature and time so it is important to consider this when estimating the abundance of a population (Lacoursière-Roussel et al. 2016). This has not been considered during this data collection. It is recommended that this variable will be added in the future to get an accurate estimation of the population size.

eDNA samples sometimes showed a presence of bullfrogs while there were no captures (figure 10). This information is of great importance since bullfrog eradication programs are focussed on infected ponds. Until now, infected ponds were identified based on the capture data. Overlooking an infected pond could nullify the eradication measures taken (Descamps & de Vocht, 2016; INBO, 2020). It is currently unknown if the signal found in eDNA represents a reliable and accurate source for estimating the population size of wild bullfrogs. The mesocosm experiment conducted beforehand, showed that eDNA concentrations can accurately describe the population size of bullfrogs, but under controlled conditions, in a small population (Devisscher et al., 2012). The results in this project showed that estimations for larger populations of bullfrog might not be correct. Accurate population size estimations using other methods (or proper conduction of catch-depletion) have to be made to compare and assess whether eDNA bullfrog signals of wild populations can be translated to an estimation of the population size.

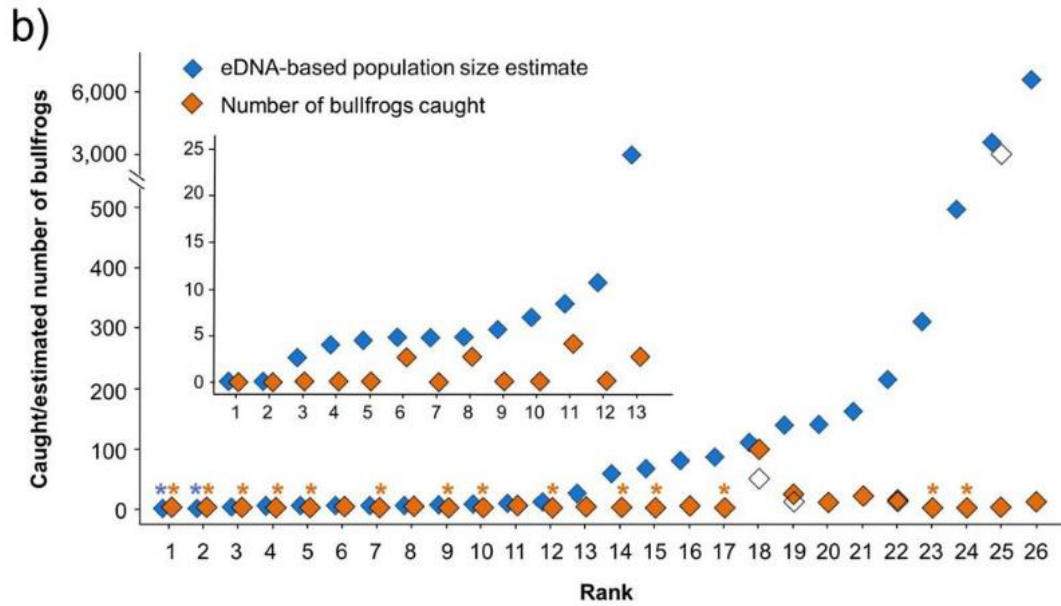


Figure 10: Relation between eDNA-based population size estimates and number of bullfrogs captures (catch-depletion). Ponds where bullfrog capture was performed twice, a distinction between first (filled orange diamonds) and second time (empty diamonds) was made, because bullfrogs were killed after first capture. Blue stars represent the ponds where no bullfrog signal in eDNA was found. Orange stars represent the ponds where no bullfrogs were caught. Note: the y-axis contains a break (INBO, 2020).

eDNA analysis is rather expensive because it requires analysis in a lab. An advantage of eDNA is that it is only required to visit the water body of interest once, by one person, to collect the sample, which reduces the costs. However, it is not possible to collect any other possible variables of interest and the accuracy is questionable. eDNA analysis was shown to be effective as a population size estimation method in other bullfrog populations (Lin et al., 2019), but is also sometimes used only as an indicator for the presence of bullfrogs in order to build a management plan for infected ponds (Kamoroff et al., 2020)

4. Matrix population modelling

4.1 Conceptual model

A first critical step in the modelling approach was constructing a conceptual model. This model was based on an existing model of the American bullfrog made by Govindarajulu et al. in 2005 on a population of bullfrogs on Vancouver Island (Govindarajulu et al., 2005). Other inspirations were the model for *Ae. Albopictus* (Erickson et al. 2010) and the guide for developing amphibian population models (Awkerman et al., 2020). This conceptual model would provide a base for determining what parameters should be included in the population model and what type of model is best to use.

In this model, the life cycle of diploid and triploid bullfrogs was described in developmental stages. In the Canadian bullfrog population, Govindarajulu et al. describe two development tracks, fast and slow track. This indicates the duration of the development from larva to metamorph. Slow track is the 'normal' development which takes averagely 2 years. Some populations showed that it only takes one year to develop to metamorph (Govindarajulu et al., 2005). In 2005, Jooris stated that the development of larva to metamorph in Flanders takes on average 2 years, so that there is no proof of the fast developmental track occurring in Flanders (Jooris, 2005). Looking at the data of recent years, this seems to have changed. Tadpoles are mostly present for only one year before the metamorphosis. Warm and early summers can cause more tadpoles to metamorph in a fast track. Climate warming is causing this to happen more frequently. Ponds warm up faster which speeds up the process of metamorphosis (O'Regan, Palen, & Anderson, 2014). For now, both developmental tracks were included in the conceptual model until there is more information on the percentage of bullfrogs that develop fast track in Flanders. The development from one stage to another was supplemented with the transition probabilities, and for the adult stage, the survival rate.

Another important factor that was included in the conceptual model is the introduction of triploid individuals in the population and the resulting decline. Research has shown that postmetamorphic stages are most vulnerable to changes (i.e. the population is mostly affected when changes take place in postmetamorphic stages) (Govindarajulu et al., 2005), so it would be most beneficial to release metamorphosed triploid bullfrog. It was also discovered that there is a difference in weight between triploid and diploid tadpoles (Descamps & De Vocht, 2017). So again, it would be beneficial to release triploid bullfrogs after metamorphosis to avoid size

differences, which might disadvantage the triploids (e.g. lower chance of mating when small, higher chance of predation). Unfortunately, this is not possible because there is not enough room available to let the triploid bullfrogs develop in a protected area. Thousands of triploids will have to be released at the same time. The only option is to release triploid larvae.

Another factor that was included are other control methods, such as catch-depletion, which is a method that is currently being implemented on a regular basis. Before releasing triploid larvae into the population, the wild bullfrogs in the ponds where they will be released will be captured with funnel nets to make the population decline as much as possible. By focusing on a certain life stage, depending on the period, the population can be reduced drastically using this method (Govindarajulu et al., 2005; Louette, Devisscher, & Adriaens, 2013). Other possible control methods are also considered. The catching mortality of other control measures conducted is currently unknown and thus not added in the figure (figure 11).

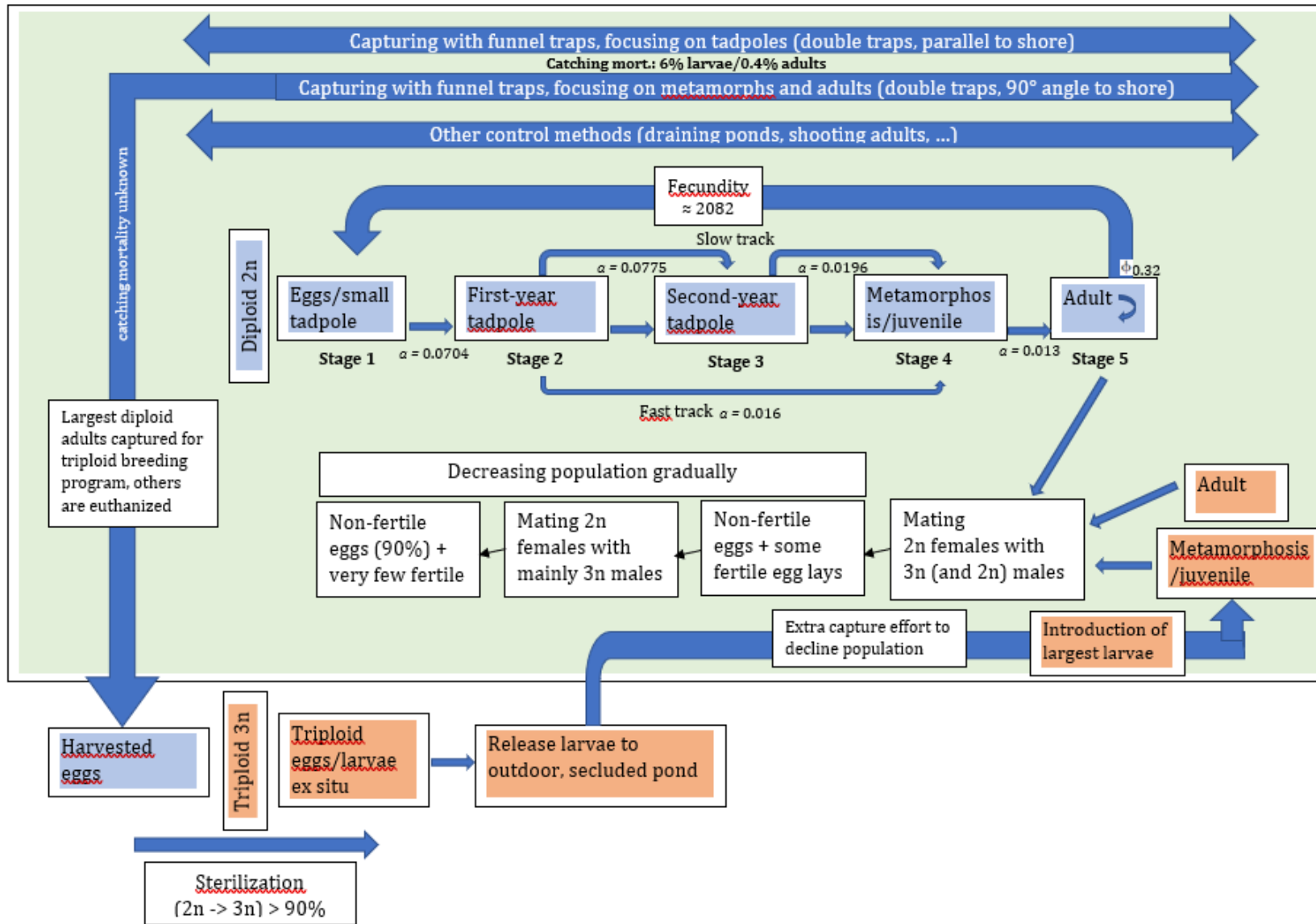


Figure 11: Conceptual model with the life cycle of diploid and triploid (sterilized) American bullfrogs together with the transition probabilities (% of individuals alive after transforming to the next life stage), estimated fecundity, other control method and its estimated catching mortality (Devisscher et al., 2012), and the desired outcome (reduction of 90%) of introducing triploid individuals in a diploid population of American bullfrogs. The green area represents the targeted population. There is no record of the survival rates for triploid males. The life stages of diploid bullfrogs, transition probability and fecundity were based on the conceptual model of Govindarajulu et al., 2005. These might not be accurate for the considering population. Note: 'a' is symbol for transition probability; 'φ' is symbol for survival rate.

4.2 Description of the population model

Matrix population models (MPM) are known to be useful when the life cycle can be described in developmental stages, which is the case for the American Bullfrog (Govindarajulu et al., 2005). They integrate population dynamics and population structure (Caswell, 2001). The data that was collected and will be collected in the future is based on stage-classified life cycles. The population dynamics in the model were simulated with the deterministic model $\mathbf{n}(t + 1) = \mathbf{A}\mathbf{n}(t)$ where $\mathbf{n}_{(t+1)}$ is the vector of proportions for each developmental stage at time $(t) + 1$ and \mathbf{A} is the Leslie matrix (which is constant through time), containing transition probability from one developmental stage to the other (p_{ij}) and fecundity values of each category (F_{ij}) (Caswell, 2001; Zambrano, Vega, Herrera, Prado, & Reynoso, 2007).

Population projections mostly match the duration of one reproduction cycle, which is in most cases one year (Caswell, 2001). In general, the American bullfrog has a standard reproduction cycle of 1 year, although this can differ a bit, depending on temperatures (Devisscher et al., 2012). In this model, a projection interval of 1 year was used.

The assumptions that were made to build the model are:

1. A closed population (no immigration/emigration)
2. A single intermingling breeding population
3. Equal or higher mating success, competition, and probability of predation for triploid individuals compared to diploid individuals (throughout all life stages)
4. Tadpoles in the population develop fast track (in 1 year instead of 2 years)
5. Reproductive cycle of 1 year (sexually mature after 3-4 years)
6. Equal sex ratio in both wild population and introduced triploid population
7. No carrying capacity (infinite number of bullfrogs able to be in an ecosystem without the ecosystem or population collapsing)

The data used for the analyses (chapter 4.3) came from another research (Govindarajulu, Altwegg, & Anholt, 2005). N , the population vector, was unknown for each life stage so a random vector was chosen for the analysis. This is a parameter that does need to be calculated in the future, before implementing the STM, in order to know exactly how many triploid bullfrogs to release.

In the model, six life stages were distinguished as embryo (N_1), hatchling (N_2 ; L00), tadpole (N_3 ; L1/L2), metamorph (N_4 ; M1/M2), juvenile (N_5 ; AM/AV <30g) and adult (N_6 ; AM/AV >30g), based on the distinguishments made for catch-depletion (table 1) and the distinguishments in Govindarajulu's research (Govindarajulu et al., 2005).

The matrix model (A) with transition matrix looks like this for the bullfrog population in Scheps, in which F stands for the fecundity, G stands for transition probability and P stands for probability for surviving and staying in the same life stage. In the model tested in chapter 4.3, F_1 was not included since data is missing for this parameter.

$$A = \begin{pmatrix} 0 & 0 & 0 & 0 & F_1 & F_2 \\ G_1 & 0 & 0 & 0 & 0 & 0 \\ 0 & G_2 & 0 & 0 & 0 & 0 \\ 0 & 0 & G_3 & 0 & 0 & 0 \\ 0 & 0 & 0 & G_4 & 0 & 0 \\ 0 & 0 & 0 & 0 & G_5 & P_6 \end{pmatrix}$$

4.2.1 Parameters

A simple matrix model with the goal of showing the population growth and structure consists of the main demographic data of the population, survival rates, fecundity and sex ratio (Caswell, 2001). Within this project, the effectivity of the sterile triploid method was tested. The STM relates to the fecundity of bullfrogs and the effectivity can thus be analysed by perturbing the fecundity. Extra parameters that can be included are those that are linked to the vulnerability of a population and those that influence inherent species sensitivity, exposure regime, life-history strategy, or extrinsic environmental factors (Awkerman et al., 2020). More parameters would provide a more realistic estimation of the (expected) population growth. All parameters were determined based upon the biology of the introduced bullfrog inspired by the created conceptual model and relevant literature, like chapter 3 from 'Matrix population models', written by Hal Caswell (Caswell, 2001), the article on population matrix model of bullfrogs on Vancouver Island, written by Govindarajulu et al. (Govindarajulu et al., 2005) and other articles considering matrix modelling of invasive (amphibian) species (Andersen, Martin, & Roemer, 2004; Awkerman et al., 2020; Erickson et al., 2010; Gonçalves da Silva et al., 2010; Martelloni, Bagnoli, & Marsili Libelli, n.d.). Another important variable that should be derived from the model is the required catch mortality before implementing the STM. The model is expected to show how much the population has to decline before releasing the sterile larvae in order to gain

the desired results (De Vocht, 2019b), by perturbing the survival rates of different life stages. The catching mortality has not been tested in the model analysed in this thesis because there was no sufficient data available.

Survival rate and transition probability

The survival rate is an essential part of a stage-based matrix population model (Caswell, 2001). First of all, the probability for yearly survival and transitioning to the next life stage needs to be estimated for each life stage. This is called the transition probability (G). The transition probability is calculated by multiplying the survival rate of life stage A by life stage B, to gain the transition probability to life stage B. Besides that, the probability for surviving and staying in the same life stage of the adults needs to be estimated (P), which is simply the survival rate of that life stage (Caswell, 2001; Govindarajulu et al., 2005). Currently, there is no relevant data of the concerned populations to estimate the survival rates. Govindarajulu et al. estimated and collected the survival rates of four populations of bullfrogs in Canada (table 2). It also concerned an introduced population with invasive characteristics but, in this area in Canada, the bullfrog was introduced since the 1960s (Govindarajulu et al., 2005). The bullfrog in Flanders was most likely introduced later, around the 2000s (Devisscher et al., 2012). Habitat and climate are very likely to differ as well, although no ecological or climatical characteristics of the study area were described in Govindarajulu's research. There is absolutely no certainty that the demographics of the bullfrog population in Scheps are similar to the population in Canada. Research has shown that the survival rate may vary among different ponds (Govindarajulu et al., 2005; Turner, 1960) and years (Govindarajulu et al., 2005). Fast track is the main development occurring in Flanders and it is thus decided to leave out slow track, while Govindarajulu made a distinguishment between tadpoles developing fast and slow track (Govindarajulu et al., 2005).

More information is required to make an accurate representation of the survival rates and transition probability of the bullfrog population in Scheps. The survival rates can be estimated for each life stage from recapture data, recovery data or complete follow-up data (including survivors and deaths), of which recapture data is mostly used (Lebreton, Pradel, & Clobert, 1993). In the case of invasive species, with the main goal of estimating survival rates, the recovery method is most interesting. The main difference with recapture data is that the animals are permanently removed from the population after reobservation, thus the method also acts as a control method (Brownie, Anderson, Burnham, & Robson, 1985; Powell, 2017).

Fecundity

Fecundity is a crucial parameter to include in a matrix population model (Caswell, 2001). It can be defined as the measure of an individual's reproductive performance (Bradshaw & McMahon, 2008). When implementing STM, the fecundity of the wild population is expected to lower gradually because of the mating with infertile, triploid bullfrogs. By manipulating the fecundity in the model to lower numbers, the model can show the effects of the sterile triploid method on the population growth.

The fecundity can be estimated by the formula **S x sex ratio x clutch size** in which S is the survival rate over the first interval of a bullfrogs' life (until they reproduce for the first time). Govindarajulu et al. estimated the fecundity of the juvenile American bullfrog in a population in British-Columbia at 2080 surviving eggs/juvenile female (table 2) (Govindarajulu et al., 2005). Fecundity can sometimes differ with age. In previous research, it occurred that an older bullfrog female laid a significantly higher amount of eggs (Kaefer, Boelter, & Cechin, 2007). Including fecundity rates of both juveniles and adults is thus of importance to create a reliable model. There are other factors that influence the fecundity of bullfrogs, such as temperature. In the coldest months, females do not show any sign of fecundity (Kaefer et al., 2007). When temperatures are high, females sometimes lay two egg clutches in one year. It was shown that it is very likely that this is also happening in the concerned population (Jooris, 2005). The second, late clutch contains smaller and less eggs. Because of climate change, it is expected to occur more often (Kaefer, Boelter, & Cechin, 2007). This will probably result in an increase of the population size in the coming years when no management controls are taken. It is thus important to consider temperature, age, and period (to discover the number of cases where two egg clutches are laid) when estimating the females' fecundity of the population bullfrogs in the study area. The fecundity should be estimated over a 1-year interval. More data is needed on the population of bullfrogs in Scheps to estimate accurate fecundity rates.

Sex ratio

The sex ratio in the MPM considers only the sex ratio at birth (Caswell, 2001). We will assume that the sex ratio is 1:1 for both the wild population and the triploid bullfrogs. This parameter is automatically set to 1:1 in the recommended package (see chapter 4.2.1).

Other parameters to include

The previous listed parameters combined, make a simple MPM. There are many other parameters that can be included to make the model more complex, but also more accurate.

Parameters, that affect the population demographics significantly, are valuable to add in the model (Caswell, 2001). Suggested parameters to add are the initial population vector, male competitiveness, proportion fast track/slow track and other control measures. There is no data available for these parameters (table 2).

An important factor that affects the effectivity of the sterile triploid method is the initial population vector. The population vector describes the relative numbers of individuals in each stage. This is especially useful for determining what life stages have to be captured to make the population decline enough before implementing the sterile triploid method. Funnel traps can be placed in a specific angle for catching more tadpoles or focussing more on adults. Different control methods can also be used to focus on a certain life stage. The initial population size of the targeted population should also be calculated in order to be able to calculate actual numbers of triploid bullfrogs that should be released.

Male competitiveness is a parameter that is mostly considered when comparing two different populations. Though, sometimes competitiveness can occur within a population, called intraspecific competition. It is unclear whether the male competitiveness (intraspecific competition) between diploid (wild) and triploid bullfrogs is equal. It is suspected that the competitiveness of triploid males is higher since only the strongest and biggest males will be released to assure that they will survive and compete with the wild males (Descamps & De Vocht, 2017). Thus, it is of importance to look into this parameter. Factors that describe competitiveness are growth, mass at metamorphosis, date of metamorphosis and survival (Griffiths, 1991). The competitiveness can be translated in the model to a greater decline in fecundity when triploid bullfrogs seem to be the most competitive.

As explained earlier, it is suspected that most tadpoles in the population of interest transform in only one year after hatching (fast track). In the model explained in chapter 4.3, only fast track was included. To achieve the most accurate data out of the model, it is best to estimate the ratio fast/slow track in Flanders, since it is possible that there are still some tadpoles transforming slow track.

The effects of other control measures on this population were not included in the test model. There is no sufficient data available that indicate how much the demographics of a population are affected by conducting a control measure. It is also uncertain which control measures are currently conducted at what times since no data has been kept of those

Table 2: Overview of all parameters that are essential to be included in the matrix population model. The survival rates of tadpole and metamorph are calculated and transformed out of the data from Govindarajulu et al. (2005). All values are annually.

Parameters	Life stage	Value	Reference
Survival rate (S)	embryo	0.92	Biek et al. 2002
	hatchling	0.402	Govindarajulu et al. (2004-2005)
	tadpole	0.577	Cecil & Just (1979), Werner (1994)
	metamorph	0.358	Govindarajulu et al. (2004-2005)
	juvenile	0.13	Govindarajulu et al. (2004-2005)
+ probability of staying in life stage (P)	adult	0.32	Govindarajulu et al. (2004-2005)
Transition probability (G)	embryo to hatchling	0.369	Govindarajulu et al. (2004-2005)
	hatchling to tadpole	0.232	Govindarajulu et al. (2004-2005)
	tadpole to metamorph	0.207	Govindarajulu et al. (2004-2005)
	metamorph to juvenile	0.047	Govindarajulu et al. (2004-2005)
	juvenile to adult	0.042	Govindarajulu et al. (2004-2005)
Fecundity (F)	juvenile (female)	2082	Govindarajulu et al. (2004-2005)
	adult (female)	?	
Sex ratio		0.5	Durham & Bennett (1963)
Population vector	embryo	?	
	hatchling	?	
	tadpole	?	
	Metamorph	?	
	juvenile	?	
	adult	?	
Male competitiveness	Diploid juveniles/adults	?	
	Triploid juveniles/adults	?	
Proportion fast track	tadpoles	67.5%	Govindarajulu et al. (2004-2005)
Proportion slow track	tadpoles	32.5%	Govindarajulu et al. (2004-2005)

4.3 Proposed analysis of the MPM

R seemed to be the most sufficient and user-friendly program for this case, using package ‘popdemo’. Popdemo provides tools for creating MPM’s with deterministic and stochastic model implementations (Stott et al. 2012).

4.3.1 Asymptotic dynamics

In management of invasive species, it is useful to know if and how fast the population size is increasing or declining. The asymptotic dynamics of a population can describe the growth rate in a chosen time span. For the bullfrog, it is advised to maintain a time span of 15 years since this represents a bullfrogs' life span (Stoutamire, 1931; Loughheed & Taylor, 2010). The asymptotic population growth rate ($\lambda = \text{lambda}$) can be estimated using the 'project' and 'eigs' function of the package 'popdemo' in R (Stott et al., 2012). When $\lambda < 1$, it means that the population declines. If $\lambda > 1$, it means that the population grows (Caswell, 2001). In the test model, the initial lambda was 0.713596, which indicates an overall declining population. Based on the dynamics of a wild, invasive Canadian population of bullfrogs, the population is expected to increase in the first year, whereafter the decline of the population will initiate (figure 12). When implementing the sterile triploid method in this population, it would result in a less higher increase of the population size in the first year and a faster decrease after the first year.

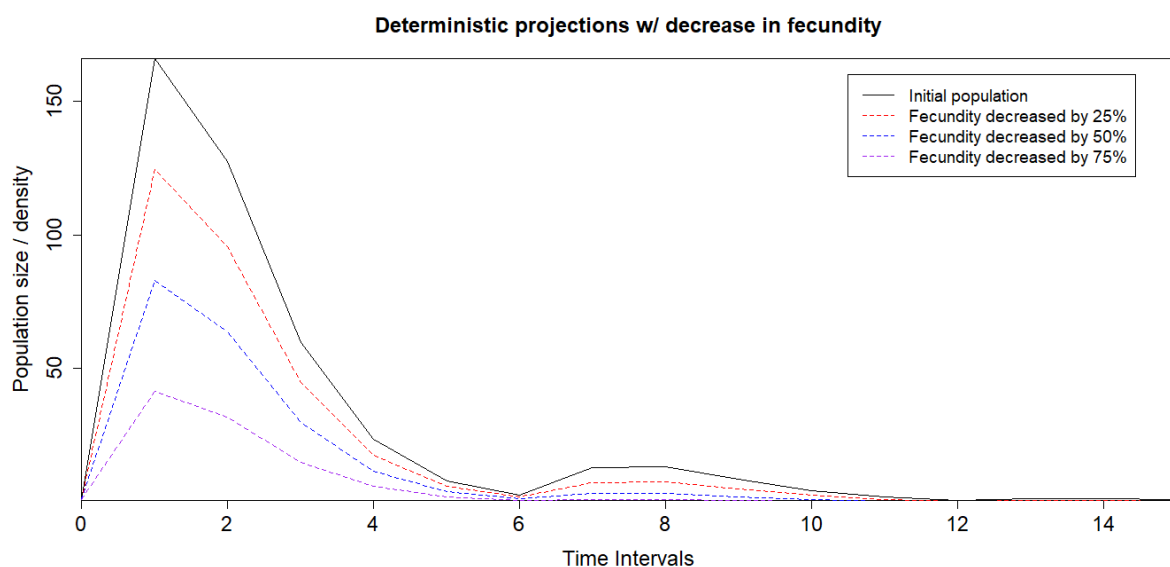


Figure 12: Deterministic projections with decrease in fecundity (initial population, fecundity -25%, fecundity -50%, fecundity -75%, using a random initial population vector, over a time span of 15 years. Based on a population of bullfrogs in Canada (Govindarajulu et al., 2005)

Another value of interest within asymptotic dynamics is the reproductive value. This is the contribution that each stage makes to stable growth (through survival, growth, and reproduction). By discovering the life stages with high reproductive values, management actions can be focussed on this stage so that management becomes more efficient (CRAN,

2018). In this model, a random population vector was used. Thus for this model, the reproductive values would not state anything since it is random.

4.3.2 Perturbation analysis

For managing invasive species, it is essential to know how population dynamics on short and long term may be changed to achieve the goal of eradication. Perturbation analyses show how changes to vital rates such as survival, fecundity, or growth affect population dynamics. By changing a matrix element by some magnitude of perturbation (δ), and calculating a population dynamic (depending on the desired outcome; e.g. asymptotic growth), important information can be revealed (CRAN, 2018). Effects of control measures on population growth rate can be obtained by conducting a sensitivity and/or elasticity analysis of the stable growth rate (Caswell, 2001; Govindarajulu et al., 2005). Sensitivity and elasticity analysis describe and visualise the linear relationships between matrix entries and population dynamics, while transfer function analysis is intended for nonlinear perturbation analysis (CRAN, 2018).

To determine the effectivity of STM, it is essential to perturb the fecundity rates to mimic the introduction of triploid bullfrogs to see what effects this would have on the population structure and growth. This result can be translated to the required number of triploid bullfrogs that have to be released in order to make the population decline.

The first attempts of the transfer function analysis revealed that the population would decline a bit when only conducting STM (figure 13). Other control measures were not implemented in this model yet since no accurate information regarding these measures is available. These would most likely make a big difference on these results since the goal is to first make the population decline as much as possible using other control measures like capture, drainage of pools, shooting of adults, ... These control measures affect the survival rates of bullfrogs and can thus be implemented in the model by perturbing the survival rates of the life stages that are affected. The transfer function analysis indicated that reducing the fecundity by 1000, the lambda would reduce to averagely 0.65, which is a reduction of averagely 0.06 (figure 8).

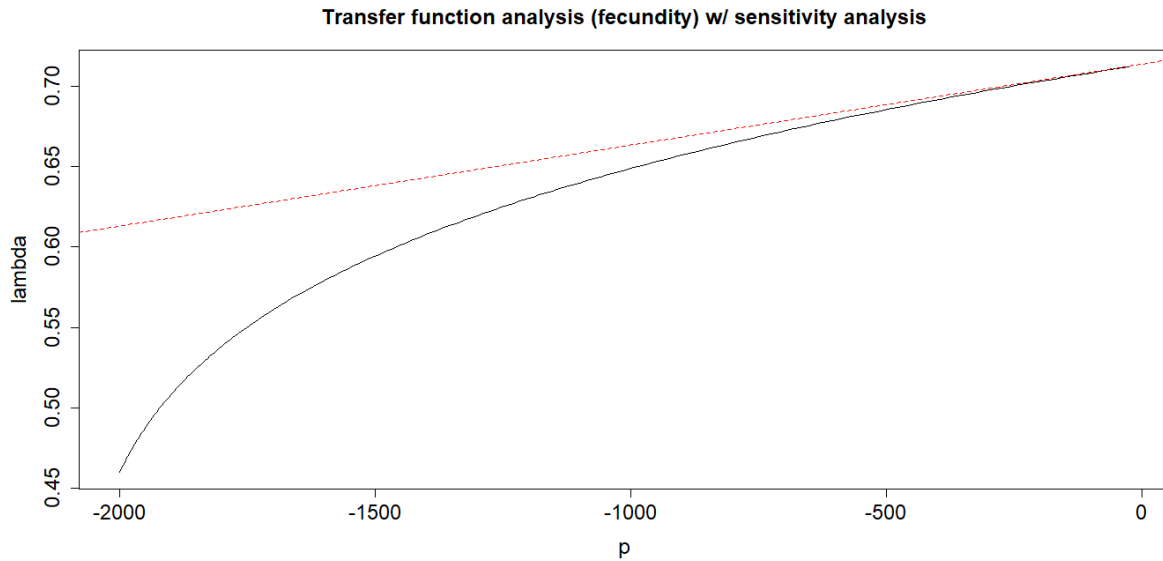


Figure 13: Transfer function analysis, perturbing the fecundity, with sensitivity analysis (red, dashed line). 'p' represents the reduction in fecundity as the reduction of surviving offspring per female per year (Rstudio Team, 2020), package = popdemo).

4.3.3 More advanced analysis

The model that was tested in this thesis was a deterministic model. In deterministic models, the output of the model is completely determined by the parameter's values and the initial conditions. Another type of model is the stochastic model, which possesses some inherent randomness. The same set of parameter values and initial conditions will lead to an ensemble of different outputs (Caswell, 2001). Stochasticity occurs in all populations, but such models are more complicated. Though, they should be considered to gain the most accurate population model.

Asymptotic (perturbation) analyses are sufficient for primitive models where there are no other variables affecting the population growth. In reality, this is (almost) never the case. Asymptotic analyses ignore transient dynamics. But, populations are often subject to environmental disturbances, and thus not in a stable state (Stott, Hodgson, & Townley, 2012). The package 'popdemo' includes functions to calculate three key pairs of indices that measure transient dynamics which are explained in the CRAN sheet (CRAN, 2018). These analyses could also contribute to a more accurate description of the population dynamics.

5. Conclusions and recommendations

Catch-depletion seems to be the most accurate method for estimating the population size of larger populations, which is the case in the Valley of the Grote Nete, and when conducted accurately (Maceina et al., 1993). eDNA is a great method for detecting an even slightly infected ponds, which is very crucial for managing the bullfrog since only a few individuals are needed to begin a new invasion (Halfmaerten, 2015; Kamoroff et al., 2020; Lin et al., 2019). I recommend to look further into the use of eDNA since it is suspected that, for small populations, eDNA analysis could be sufficient. To estimate the population bullfrogs in Scheps, it is recommended to use the catch-depletion method or other methods that were not compared in this thesis (e.g. capture-mark-recapture) (Henderson, 2002).

The main parameters to be included in the matrix population model are the transition probabilities (calculated from the survival rates), the fecundity and the sex ratio (which is automatically set to 1:1 in the model). By perturbing the fecundity, the effects of introducing triploid bullfrogs on the population dynamics can be estimated. So far, it shows that a very large number of bullfrogs would have to be released in order to reach the desired results. But, some parameters of importance were missing in the test model such as the initial population size and vector, the male competitiveness, the ratio fast track/slow track and the effect of other control measures. Moreover, the data in the test model came from a population of bullfrogs in Canada, which might have completely different population dynamics. Also, it is advised to look into the use of stochastic models and transient dynamics since this gives a more realistic image of the population dynamics.

For the next step in Life 3n-bullfrog, it is important to make accurate estimations for the populations in Scheps in order to understand the population dynamics which allows us to accurately estimate the number of triploids that need to be released. This far, the little data that has been collected has not been collected very accurately which makes the results very questionable.

Reference list

- Adams, M. J., Pearl, C. A., & Gherardi, F. (2007). Problems and opportunities managing invasive Bullfrogs: is there any hope? In *Biological invaders in inland waters: Profiles, distribution, and threats* (pp. 679–693). Springer.
- Andersen, M. C., Martin, B. J., & Roemer, G. W. (2004). Use of matrix population models to estimate the efficacy of euthanasia versus trap-neuter-return for management of free-roaming cats. *Javma*, 225(12), 1871–1876.
- Awkerman, J., Raimondo, S., Schmolke, A., Galic, N., Rueda-Cediel, P., Kapo, K., ... Forbes, V. (2020). Guidance for Developing Amphibian Population Models for Ecological Risk Assessment. *Integrated Environmental Assessment and Management*, 16(2), 223–233. doi: 10.1002/ieam.4215
- Blaustein, A. R., & Kiesecker, J. M. (2002). Complexity in conservation: Lessons from the global decline of amphibian populations. *Ecology Letters*, 5(4), 597–608. doi: 10.1046/j.1461-0248.2002.00352.x
- Bradshaw, C. J. A., & McMahon, C. R. (2008). Population Ecology: Fecundity. *Encyclopedia of Ecology*, (December), 1535–1543. doi: 10.1016/B978-008045405-4.00645-5
- Brownie, C., Anderson, D. R., Burnham, K. P., & Robson, D. S. (1985). *Statistical inference from band recovery data - a handbook* (2nd ed.). United States Department of the Interior Fish and Wildlife Service.
- Bury, B., & Whelan A., J. (1984). Ecology and Management of the Bullfrog. *Fish and Wildlife Service*.
- Bury, R. B. (1984). Ecology and Management of the Bullfrog. *United States Department of the Interior Fish and Wildlife Service*.
- Caswell, H. (2001). *Matrix population models* (2nd ed.).
- CRAN. (2018). popdemo: Demographic modelling using projection matrices. Retrieved from CRAN.R website: https://cran.r-project.org/web/packages/popdemo/vignettes/popdemo.html#installing_popdemo

- de Kroon, H., Plaisier, A., van Groenendael, J., & Caswell, H. (1986). Elasticity: The Relative Contribution of Demographic Parameters to Population Growth Rate. *Ecology*, *67*(5), 1427–1431.
- De Vocht, A. (2019a). LIFE 3n-Bullfrog. *LIFE*.
- De Vocht, A. (2019b). LIFE 3n-Bullfrog - The sterile triploid method for population control of aquatic invasive fauna: pilot project on American bullfrog. Retrieved February 5, 2021, from [ec.europa.eu website: https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=7240](https://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=7240)
- Descamps, S., & de Vocht, A. (2016). Movements and habitat use of the invasive species *Lithobates catesbeianus* in the valley of the Grote Nete (Belgium). *Belgian Journal of Zoology*, *146*(2), 90–100. doi: 10.26496/bjz.2016.44
- Descamps, S., & De Vocht, A. (2017). The sterile male release approach as a method to control invasive amphibian populations: A preliminary study on *Lithobates catesbeianus*. *Management of Biological Invasions*, *8*(3), 343–359. doi: 10.3391/mbi.2017.8.3.09
- Descamps, S., & De Vocht, A. (2019). *LIFE Nature and Biodiversity TECHNICAL APPLICATION FORMS Part A – administrative information*. doi: 10.1016/S0014-5793(01)03048-4
- Devisscher, S., Adriaens, T., De Vocht, A., Descamps, S., Hoogewijs, M., Jooris, R., ... Louette, G. (2012). Beheer van de stierkikker in Vlaanderen en Nederland. *Inbo.R*, *52*, 1–209.
- Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2005). Sterile Insect Technique. In *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*. doi: 10.1007/1-4020-4051-2_2
- Erickson, R. A., Presley, S. M., Allen, L. J. S., Long, K. R., & Cox, S. B. (2010). A stage-structured , *Aedes albopictus* population model. *Ecological Modelling*, *221*(9), 1273–1282. doi: 10.1016/j.ecolmodel.2010.01.018
- European Parliament. (2014). Regulation (EU) No 1143/2014 of the European Parliament and

- the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. *Official Journal of the European Union*, 2014(1143), 35–55.
- Ficetola, G. F., & Miaud, C. (2008). Species detection using environmental DNA from water samples. *Biology Letters*, 4(September), 423–425. doi: 10.1098/rsbl.2008.0118
- Ficetola, G. F., Thuiller, W., & Miaud, C. (2007). Prediction and validation of the potential global distribution of a problematic alien invasive species - The American bullfrog. *Diversity and Distributions*, 13(4), 476–485. doi: 10.1111/j.1472-4642.2007.00377.x
- Garner, T. W. J., Perkins, M. W., Govindarajulu, P., Seglie, D., & Fisher, M. C. (2006). The emerging amphibian pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biology Letters*, 2, 455–459. doi: 10.1098/rsbl.2006.0494
- Gentile, J. E., Rund, S. S., & Madey, G. R. (2015). Modelling sterile insect technique to control the population of *Anopheles gambiae*. *Malaria Journal*, 14(1), 1–12. doi: 10.1186/s12936-015-0587-5
- Gonçalves da Silva, A., Kolokotronis, S.-O., & Wharton, D. (2010). Modeling the eradication of invasive mammals using the sterile male technique. *Biological Invasions*, 12, 751–759. doi: 10.1007/s10530-009-9477-5
- Govindarajulu, P., Altwegg, R., & Anholt, B. R. (2005). Matrix model investigation of invasive species control: Bullfrogs on Vancouver island. *Ecological Applications*, 15(6), 2161–2170. doi: 10.1890/05-0486
- Great Lakes Fishery Commission. (2000). STERILE-MALE-RELEASE-TECHNIQUE An Innovative Sea Lamprey Control Method Great Lakes Fishery Commission. *GLFC*.
- Griffiths, R. A. (1991). *Competition between Common Frog, Rana temporaria, and Natterjack Toad, Bufo calamita, Tadpoles: The Effect of Competitor Density and Interaction Level on Tadpole Development*. 61(2), 187–196.
- Halfmaerten, D. (2015). *Application of eDNA detection in the effective management of invasive American bullfrog*. (september).

- Henderson, P. A. (2002). Ecological Methods. *Encyclopedia of Life Sciences*, 1–4. doi: 10.1038/npg.els.0003271
- Hoegh-Guldberg O., Jacob, D., & Taylor, M. (2018). Chapter 3: Impacts of 1.5°C global warming on natural and human systems. In: Global Warming of 1.5 °C. An IPCC special report on the impacts of global warming of 1.5 °C above preindustrial levels and related global greenhouse gas emission pathways [...]. *Special Report, Intergovernmental Panel on Climate Change*, (ISBN 978-92-9169-151-7), 175–311.
- INBO. (2020). *Assessment of the distribution range and estimating population sizes of American bullfrog based on eDNA analyses*.
- IUCN SSC Amphibian Specialist Group. (2015). *Lithobates catesbeianus* (American bullfrog). In *The IUCN Red List of Threatened Species*. doi: 10.2305/IUCN.UK.2015-4.RLTS.T58565A53969770.en
- Jooris, R. (2005). De Stierkikker in Vlaanderen. *Natuur.Focus*, 4(4), 121–127.
- Kaefer, Í. L., Boelter, R. A., & Cechin, S. Z. (2007). Reproductive biology of the invasive bullfrog *Lithobates catesbeianus* in southern Brazil. *Annales Zoologici Fennici*, 44(6), 435–444.
- Kamoroff, C., Daniele, N., Grasso, R. L., Rising, R., Espinoza, T., & Goldberg, C. S. (2020). Effective removal of the American bullfrog (*Lithobates catesbeianus*) on a landscape level: long term monitoring and removal efforts in Yosemite Valley, Yosemite National Park. *Biological Invasions*, 22(2), 617–626. doi: 10.1007/s10530-019-02116-4
- Lacoursière-Roussel, A., Rosabal, M., & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Molecular Ecology Resources*, (March). doi: 10.1111/1755-0998.12522
- Lebreton, J. D., Pradel, R., & Clobert, J. (1993). The statistical analysis of survival in animal populations. *Trends in Ecology and Evolution*, 8(3), 91–95. doi: 10.1016/0169-5347(93)90058-W
- Lin, M., Zhang, S., & Yao, M. (2019). Effective detection of environmental DNA from the

- invasive American bullfrog. *Biological Invasions*, 21(7), 2255–2268. doi: 10.1007/s10530-019-01974-2
- Louette, G., Devisscher, S., & Adriaens, T. (2013). Control of invasive American bullfrog *Lithobates catesbeianus* in small shallow water bodies. *European Journal of Wildlife Research*, 59(1), 105–114. doi: 10.1007/s10344-012-0655-x
- Maceina, M. J., Rider, S. J., & Lowery, D. R. (1993). *Use of a Catch-Depletion Method to Estimate Population Density of Age0 Largemouth Bass in Submersed Vegetation*. 8675(November). doi: 10.1577/1548-8675(1993)0130847
- Martelloni, G., Bagnoli, F., & Marsili Libelli, S. (n.d.). *A dynamical population modeling of invasive species with reference to the crayfish *Procambarus Clarkii**.
- Mayer, M., Brown, G. P., Zimmermann, B., & Greenlees, M. J. (2015). Habitat use of the introduced cane toad (*Rhinella marina*) and native frog species in tropical Australia of Tropical Ecology. *Journal of Tropical Ecology*, (September). doi: 10.1017/S0266467415000474
- Mcneely, J. (2001). Invasive species: a costly catastrophe for native biodiversity. *Land Use and Water Resources Research*, 1(2), 1–10. Retrieved from <http://www.luwrr.com>
- Microsoft Corporation. (2021). *Microsoft Excel*. Retrieved from <https://office.microsoft.com/excel>
- National Geographic Society. (2015). American bullfrog. Retrieved from National Geographic website: <https://www.nationalgeographic.com/animals/amphibians/a/american-bullfrog/#:~:text=The largest of all North,reach 6.75 inches in length.>
- O'Regan, S. M., Palen, W. J., & Anderson, S. C. (2014). Climate warming mediates negative impacts of rapid pond drying for three amphibian species. *Ecology*, 95(4), 845–855. doi: 10.1890/13-0916.1
- Ogle, D. H., Wheeler, P., & Dinno, A. (2021). *FSA: Fisheries Stock Analysis*. Retrieved from <https://github.com/droglenc/FSA>
- Pasmans, F., Muijsers, M., Maes, S., Van Rooij, P., Brutyn, M., Ducatelle, R., ... Martel, A. (2010). Chytridiomycosis related mortality in a midwife toad (*Alytes obstetricans*) in

- Belgium. *Vlaams Diergeneeskundig Tijdschrift*, 79(6), 460–462.
- Pasmans, Frank, & Martel, A. (2011). De schimmelziekte chytridiomycose: nu ook bij de Belgische amfibieën! *ANTenne*, 5(1), 16–17. Retrieved from www.ravon.nl
- Rep, V. R., Wingert, E., & Meshaka, W. E. J. (2015). Clutch size of a large bullfrog, *Lithobates catesbeianus*. *The Center for North American Herpetology*, 4(3), 5.
- Richardson, D. M., Bond, W. J., Dean, W. R. J., Higgins, S. I., Midgley, G. F., Milton, S. J., ... Schulze, R. E. (2000). Invasive alien species and global change: a South African perspective. *Invasive Species in a Changing World*, (May 2016), 303–349.
- Roach, J. (2004). Invading Bullfrogs Appear Nearly Unstoppable. *National Geographic*. Retrieved from <https://www.nationalgeographic.com/animals/2004/09/news-bullfrogs-invading-nearly-unstoppable/>
- Rstudio Team. (2020). *RStudio: Integrated Development for R*. Retrieved from <http://www.rstudio.com/>
- Stott, I., Hodgson, D. J., & Townley, S. (2012). *popdemo: an R package for population demography using projection matrix analysis*. 797–802. doi: 10.1111/j.2041-210X.2012.00222.x
- The MathWorks. (2012). *MATLAB*. United States.
- Turner, F. B. (1960). Postmetamorphic Growth in Anurans. *The American Midland Naturalist*, 64(2), 327–338.
- van Ham, C., Genovesi, P., & Scalera, R. (2013). Invasive alien species: the urban dimension. *IUCN*.
- Vonesh, J. R., & De la Cruz, O. (2002). Complex life cycles and density dependence: Assessing the contribution of egg mortality to amphibian declines. *Oecologia*, 133(3), 325–333. doi: 10.1007/s00442-002-1039-9
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.
- Yates, M. C., Fraser, D. J., & Derry, A. M. (2019). Meta - analysis supports further refinement of eDNA for monitoring aquatic species - specific abundance in nature. *Environmental*

DNA, 1(5), 5–13. doi: 10.1002/edn3.7

Zambrano, L., Vega, E., Herrera, M. L. G., Prado, E., & Reynoso, V. H. (2007). A population matrix model and population viability analysis to predict the fate of endangered species in highly managed water systems. *Animal Conservation*, 10(3), 297–303. doi: 10.1111/j.1469-1795.2007.00105.x

Zippin, C. (1958). The Removal Method of Population Estimation. *The Journal of Wildlife Management*, 22(1), 82–90.

Abstract

The American bullfrog, an invasive species present all around the world, causes many problems in ecosystems. They are very hard to eradicate because of their complex developmental stages, their abundance, high fertility, and the far distances they can cover. Many eradication methods showed to be non-effective, especially in larger and open populations. This Master thesis study is part of LIFE 3n-bullfrog. LIFE 3n-bullfrog's main purpose is eradicating or controlling isolated American bullfrog populations using a new method namely the sterile triploid method (STM). By releasing triploid (sterile) bullfrogs in a wild population, females that mate with the triploid males will produce an infertile egg clutch, which eventually results in a decline of the population. In order to evaluate the effectiveness of the STM in the population of interest, a population model has to be build. This would also reveal how many triploids have to be released to get the desired results. The eventual outcome of this Master project is a method for modelling the population of American bullfrogs in Scheps, a small nature reserve in Balen, Flanders. Another outcome of this thesis is the evaluation of two methods for estimating the population size, catch-depletion and eDNA. The catch-per-unit-of-effort (CPUE) was also estimated for catch-depletion, since this method also acts as a control measure and is of major importance in the management plan of the American bullfrog.

Catch-depletion shows to be non-effective for estimating the population size of bullfrogs in this project. The method was not conducted accurately which made the results biased. When catch-depletion would be conducted following some assumptions, the results would most likely be representative. eDNA concentration analysis is not very effective for population size estimation, especially in larger populations, but is effective for detecting (slightly) infected ponds, which is also of major importance in the management of the bullfrog.

The best model to use in this case is a deterministic matrix population model (MPM). Model analysis were done in R, using the package popdemo. Parameters that are essential to include are transition probability and probability of staying in a life stage (calculated from the survival rates), fecundity and sex ratio. To determine how STM would affect the population, the fecundity is lowered by a certain perturbation rate. Using dynamics from another population, the results were not very promising, showing only a slight decrease in the lambda. Including the population dynamics of the target population, the effects of other control measures, the competitiveness of males, the ratio fast/slow track and the initial population size vector, would increase the accuracy and most likely the effects of STM on the population structure and

growth. It would also be best to treat the model as a stochastic model and to treat the dynamics as transient instead of asymptotic, which is more likely in wild populations.