

**Survival and recruitment in a
population of critically endangered
northern pool frog (*Pelophylax
lessonae*) in Norway**

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PREFACE

My master's degree in coastal ecology at the University of Agder is with this thesis completed and marks the end of my study period. This project has been exciting from start to finish and I am grateful to have been a part of it. I have been able to work with important management methods and received a small peek into the interesting world that is conservation and management.

A huge thank you is directed to Lars Korslund, my supervisor, for excellent guidance throughout this thesis and for every valuable discussion, I appreciate that every question has been welcomed, no matter how big or small. Thank you for the great interest you have had and the contagious excitement you have shown towards the project. It has been thrilling to be a part of such an important project and I have learned so much during this time. Your help during this time has been invaluable, from field trips and creation of models, to reviewing my dissertation.

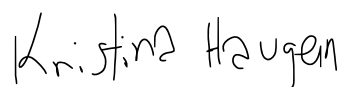
Thank you to Dag Dolmen (NTNU), for the effort you have put into the data collection throughout the years, which have been used in this thesis.

Thank you to Eline Gourinel and Silje Halvorsen for helping me with figure appearances and assisting with language, grammar and ideas.

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Kristiansand 22.05.20

Kristina Haugen

A handwritten signature in black ink that reads "Kristina Haugen". The script is cursive and fluid, with the first name and last name clearly distinguishable.

ABSTRACT

Amphibians are the most threatened vertebrate group in the world today, and some of the species' major threats are introduced predatory fish and habitat destruction. This combined with low conservation - and varying management efforts have put the group in a dire situation, where actions are often made under high levels of uncertainty. The northern pool frog *Pelophylax lessonae* Camerano 1882 is the most endangered vertebrate species in Norway today and the population has experienced frequent reproductive failure and declines in the last 12 years. There is limited knowledge about the northern pool frog which needs to be improved, in terms of management and conservation to prevent species extinction.

A photo-identification and capture-mark-recapture method was used to collect data on the population from 3 primary ponds, and several temporary inhabited peripheral ponds, in Arendal Municipality, Norway between 2007-2019. Every individual encountered was photographed and included in a photo-identification software, I3S Classic. As each individual has a distinct pattern on their abdomen, this was used to identify the individuals and to create a capture history for each individual. Apparent survival (ϕ) and recapture probability (p) was estimated in an open population Cormack-Jolly-Seber model in program MARK. A total of 277 individuals from these ponds were included in the analysis. The focus was to see the effects different factors has had on survival and recapture probability, and model selection was based on AICc. In addition, the effects of climatic conditions on recruitment was also investigated, as well was the rate of migration between different ponds.

The Norwegian pool frog population has, since the beginning of the monitoring period experienced a decline of 85 % (from estimated 115 to 17 adult individuals). Adult survival for this 12-year period has been estimated to be 0.62 (0.58-0.66) with a recapture probability of 0.75 (0.68-0.80). Models have shown a link between increased adult survival and increased ground temperature in winter, and that increased summer temperature is significantly linked to increased reproductive success. The presence of fish did not seem to have a significant effect on recruitment. Dispersal has been registered between 4 of the examined ponds. Of the individuals included in the database 6.1 % have been registered to disperse between two or more ponds. The rate of dispersal may be higher as new of the year frogs, but these individuals was not included as they are difficult to identify due to an underdeveloped abdominal pattern. This study has contributed to fill some of the knowledge gaps regarding the northern pool frog. However, more data is needed to better be able to estimate the effect of temperature and fish on survival and reproduction of the species at its northern boundary.

SAMMENDRAG

Amfibier er den mest truede vertebratgruppen i verden, hvor de største truslene er introduserte fiskearter og habitatødeleggelse. Dette, i kombinasjon med liten grad av bevaring og forvaltning, hvor forvaltningstiltak ofte skjer under usikre forhold, er gruppen nå i en sårbar situasjon. Den nordlige damfrosken *Pelophylax lessonae* Camerano 1882 er den mest truede vertebraten i Norge, og har opplevd kontinuerlig reproduksjonssvikt og nedgang i populasjonen de siste 12 årene. Vi har kontinuerlig opplevd store kunnskapshull angående denne truede arten, som derav kan gjøre forvaltning utfordrende.

En bildegjenkjenning- og fangst-gjenfangst-metode har blitt brukt for å samle inn data om populasjonen fra 3 hoved lokaliteter, og flere midlertidige dammer i Agder, Norge, i perioden 2007-2019. Hvert individ ble fanget, fotografert og inkludert i en bildegjenkjenningsdatabase, I3S Classic. Hvert individ har individspesifikke markeringer på mageregionen, som ble brukt for å gjenkjenne individer å lage en fangsthistorie for hvert enkelt individ. Overlevelse (ϕ) og sannsynlighet for gjenfangst (p) ble estimert i en åpen populasjon Cormack-Jolly-Seber modell i program MARK. Totalt 277 individer fra disse dammene ble inkludert i dataanalysene, hvor hensikten var å se effekten ulike faktorer har hatt på overlevelse og sannsynligheten for gjenfangst. Modellseleksjonen ble basert på AICc-verdier og en parsimoni-framgangsmåte. I tillegg ble effekten av hvordan klimatiske forhold påvirker reproduksjonen, samt spredningsraten mellom de ulike dammene undersøkt.

I løpet av overvåkningsperioden har damfroskbestanden hatt en drastisk nedgang på 85% (fra estimer 115 til 17 voksne individer). Overlevelse hos voksne individer har under denne 12-årige perioden blitt estimert til 0.62 (0.58-0.66) med en gjenfangst på 0.75 (0.68-0.80). Modeller har vist en sammenheng mellom økt voksen overlevelse og økt bakketemperatur under vinter, og økt sommer temperatur har en signifikant sammenheng med økt reproduksjon suksess. Tilstedeværelsen av fisk så ikke ut til å ha noen signifikant effekt på rekruttering. Spredning har blitt registrert mellom 4 av de undersøkte dammene, og av individene inkludert i databasen ble 6.14 % registrert til å forflytte seg mellom to eller flere av dammene. Spredningsgraden kan likevel være høyere enn dette, da årets nye frosker ikke ble inkludert i analysen pga vanskeligheten med å identifisere dem pga et underutviklet mønster på mageregionen. Dette studiet har bidratt til å tette noen kunnskapshull angående den nordlige damfrosken. Men mer informasjon er nødvendig for å bedre estimere effekten temperatur og fisk har på overlevelse og reproduksjon på en art som lever på sin nordlige grense.

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1 INTRODUCTION

1.1 THE AMPHIBIAN BIOLOGY

Amphibians have existed for 350 million years (Collins, 2010) and there are 6 824 described species of salamanders (order *Caudata*), frogs (Order *Anura*) and caecilians (Order *Gymnophiona*) (IUCN, 2020). The term amphibian originates from the word *amphibious* meaning “both ways of life”. It refers to the life phases most amphibians go through, aquatic larvae and terrestrial adult (Campbell et al., 2011a; Duellman & Trueb, 1994). Amphibians have a few common traits: glandular skin (both mucous and granular (poison) glands), lack of epidermal structures as feathers or scales (although dermal scales are present in most caecilians), a quadrupedal movement and lack of impermeable eggs (Duellman & Trueb, 1994). Multiple different reproduction and life strategies have been observed in amphibians, e.g. external and internal fertilization, some species lay several hundred eggs while others lay a few eggs and display parental care (Duellman & Trueb, 1994; Stebbins & Cohen, 1997). Due to a thin and permeable skin (Campbell et al., 2011a), amphibians are usually found in humid habitats (Stebbins & Cohen, 1997). As the skin allows for exchange of water, gas and electrolyte between the individual and the environment, in environmental monitoring, amphibians are often thought of as an indicator for the current state of the ecosystem (Brühl, Schmidt, Pieper, & Alscher, 2013; Campos, Lourenço-de-Moraes, Llorente, & Solé, 2017; Collins & Storfer, 2003; Dolmen, 1997).

In the 1980s, reports of amphibian declines started coming in, species were disappearing at an alarming rate (Dolmen, 1997; Duellman, 1999; Stuart et al., 2004). Today, more amphibian species are threatened with extinction than any other vertebrate group (IUCN, 2020) and the number of species are declining faster than that of mammals or birds (Stuart et al., 2004). According to IUCN (2020) 41% of amphibian species are threatened with extinction and Alroy (2015) has estimated around 200 extinctions have occurred since the 70s and 80s. The extinction rate for amphibians is now estimated to be approx. 200 times higher than the historical background extinction rate (Collins, 2010). Based on estimations from Alroy (2015), even with no increased environmental threat, at least 6.9 % of all anuran species may become extinct within the next century.

1.1.1 THREATS TO THE AMPHIBIAN COMMUNITY

The main threats and reasons for biodiversity loss and species extinction today is habitat loss, contaminants, alien species, commercial use, diseases and climate change (Campbell et al.,

2011a; Collins, 2010; Semlitsch, 2000; Stuart et al., 2004). Either acting together or separately to drive the decline and/ or extinction of amphibian populations worldwide (Collins, 2010). The greatest threat is the *alteration and destruction of habitats* (Campbell et al., 2011b; Campos et al., 2017; Collins & Storfer, 2003; Semlitsch, 2000). As amphibians inhabit both aquatic and terrestrial habitats, alteration to any of these habitats can lead to a decline (Semlitsch, 2000). Several amphibian populations have been ruined due to removal of spawning sites in Europe and the Middle East (Duellman & Trueb, 1994). Amphibians are exposed to *contaminants* from both aquatic and terrestrial habitat (Brühl et al., 2013; Campbell et al., 2011a; Gray, Miller, & Hoverman, 2009) that can be toxic to different life stages and lead to mortality, reduced growth (Semlitsch, 2000) and endocrine disruptions (Brühl et al., 2013). *Introduced species* is a serious threat where fish is the biggest threat as they compete with and prey on amphibians (Semlitsch, 2000). Native amphibian populations has often been dramatically reduced (Collins, 2010), as they are associated with declines (Semlitsch, 2000), anuran extinctions (Duellman, 1999), reduced species richness (Martinez-Solano, Barbadillo, & Lapena, 2003) and lower abundance (Pilliod & Peterson, 2001).

Commercial use and capture is an international problem because of (1) removal of breeding adults, (2) exposure of alien pathogens to native populations and (3) disruption of populations genetics by reintroductions of amphibians from other parts of the world (Semlitsch, 2000). *Diseases* are responsible for massive die-offs and widespread declines (Gray et al., 2009; Picco & Collins, 2008; Scheele et al., 2019). *Climate change* has also been listed as a potential cause of decline in amphibian populations (Stuart et al., 2004) where the temperature change can contribute to alterations in amphibian populations directly as well as triggering habitat alteration (McMenamin, Hadly, & Wright, 2008; Semlitsch, 2000). Temperature change may also increase disease outbreaks (McMenamin et al., 2008), alter behavior (e.g. changes in phenology; (T. J. C. Beebee, 2014; Li, Cohen, & Rohr, 2013; McMenamin et al., 2008), and reduce recruitment success (Li et al., 2013; McMenamin et al., 2008; Semlitsch, 2000).

1.1.2 CONSERVATION AND MANAGEMENT

Conservation efforts have long focused on “flag-ship” species, commonly associated with large mammals, e.g. elephants *Loxodonta africana* Blumenbach 1797. Flag-ship species often generate sympathy in humans as they often have appealing appearances, e.g. the giant panda *Ailuropoda melanoleuca* David 1869. Amphibians were long neglected until the decline were noticed and after this, the group received massive attention, and even made the cover story at New York Times Magazine (Duellman, 1999).

As the declines the amphibian populations are experiencing today is so new, conservation and management options are only starting to be identified (Hoffmann et al., 2010). Captive breeding is a debated option as it is controversial but could potentially be a necessary alternative (Griffiths & Pavajeau, 2008; Hoffmann et al., 2010) as their life-strategies make them ideal for breeding and reintroduction programs (Griffiths & Pavajeau, 2008). Some *ex situ* conservational efforts have had positive impact. But many have had limited success, as well as been criticized for being too simple and too expensive for the success rate (Sterrett et al., 2019). Regardless, amphibians are experiencing high levels of threat combined with low levels of conservation efforts (Hoffmann et al., 2010).

There are two approaches to management, a reactive (wait till declines are detected) and proactive approach (develop a plan before declines) (Grant, Zipkin, Nichols, & Campbell, 2013). The proactive approach is encouraged as a way to prevent species from attaining protected status, as well as giving the option of no action, although the management plan has been developed (Sterrett et al., 2019). When creating a management plan, the population(s) and environment must be included (Semlitsch, 2000), but the challenge with management actions is that they are often made under high levels of uncertainty (Sterrett et al., 2019). Therefore, a rational and transparent approach is needed, as the structured decision-making approach multiple conservation organizations have implemented (Grant et al., 2013). The best way to improve amphibian conservation is to set clear goals and objectives for decisionmakers (Sterrett et al., 2019).

1.2 THE EUROPEAN POPULATION OF POOL FROG

The pool frog *Pelophylax lessonae* Camerano 1882 is common in much of central Europe, from the central and northern part of France, around Donau and the Black sea, down south to northern Italy and northeast to Russia (Figure 1.) (Direktoratet for Naturforvaltning, 2006; Tegelstrom & Sjogren-Gulve, 2004; Zeisset & Beebee, 2001). A few populations are



Figure 1. The distribution area for the pool frog *Pelophylax lessonae*. The orange parts display the range for the continental pool frog, while the black arrows display the locations for the Swedish and Norwegian populations. Map taken from the IUCN Redlist for pool frog (Kuzmin et al., 2016).

found north of the main distribution area, making up a northern clade. These populations are found in a few sites in Agder, Norway (Dolmen, 1997) approx. 100 localities in Uppsala, Sweden (Sjögren, 1991), and one, now extinct, population in Norfolk, Britain (T. Beebee et al., 2005) .

The pool frog as a species is listed as least concern (LC) in the IUCN Red list (Kuzmin et al., 2016), because of the large distribution area and presumably high population numbers. However, the pool frog is considered to be declining because of threats such as habitat loss (Söderman, Nilsson, & Lindgren, 2015) and introduced fish species (Kuzmin et al., 2016). The pool frog is not very adaptable and can therefore be found in similar habitats all over its range, preferring stagnant waterbodies (lakes, swamps) and mixed forests and meadows (Kuzmin et al., 2016).

1.3 THE CONTINENTAL AND NORTHERN POOL FROG AND THEIR DIFFERENCES

Isolated populations of pool frogs have been discovered in Britain (T. Beebee et al., 2005), Norway (Dolmen, 1997) and Sweden (Sjögren, 1991). What made them stand out was the similarities between three populations and the differences from the pool frog population in Central Europe (T. Beebee et al., 2005; Snell, Tetteh, & Evans, 2005). The Swedish population is the largest with distribution in approx. 100 ponds (Söderman et al., 2015), the Norwegian is the smallest where reproduction occurs in three ponds (Direktoratet for Naturforvaltning, 2006), unfortunately the British population went extinct in the 1990s (T. Beebee et al., 2005).



Photo 1. Two individuals of the northern pool frog clade. A distinct sexual color dimorphism between a female and a male. Most individuals have less distinct differences than seen here. (Photo: K. Haugen)

The continental and northern pool frog are morphologically different. The continental type is bright – or grass green while the northern clade is mainly brown with a dorsal stripe (light green, yellow or white color). Individuals in the continental population have no distinct sexual color dimorphism, in contrast to the northern clade where the dorsal color of males are golden brown while females are almost black (Photo 1.) (T. Beebee et al., 2005; Hoogesteger, Rahkonen, & Karhilahti, 2013; Snell et al., 2005; Tegelstrom & Sjogren-Gulve, 2004). Sjögren (1991) suggested these color differences could have thermal benefits in the north. Bioacoustics analysis have also been used to provide additional evidence of a northern clade as singing is

inherited and not learned (Wycherley, Doran, & Beebee, 2002). Dolmen (1997) described their mating call as “a loud, crispy laugh, about the same sound as when one drags their nail across the teeth of a comb”.

Genetic testing of individuals from Norway, Sweden and Britain revealed homozygosity at all six examined loci and fixation of the same alleles in five of them (T. Beebee et al., 2005). Phylograms and cluster analysis based on RAPD data showed strong groupings (Snell et al., 2005), low genetic variety at microsatellite loci from samples from Britain, Norway and Sweden, as well many shared alleles which can be explained by a common descent (T. Beebee et al., 2005). These results demonstrate that the northern populations are genetically different from the main population in Central Europe and belong to a northern clade (T. Beebee et al., 2005; Snell et al., 2005; Tegelstrom & Sjogren-Gulve, 2004; Wycherley et al., 2002; Zeisset & Beebee, 2001). Because of the differences in the morphology and genetics, and the small and isolated populations, the clade is of high conservational value (T. Beebee et al., 2005; Tegelstrom & Sjogren-Gulve, 2004).

1.4 THE NORWEGIAN POPULATION OF POOL FROG

The pool frog was discovered in 1986 and are now one of the most threatened vertebrates in Norway (Reinkind & Engemyr, 2019). The species is classified as critically endangered (CR) in the Norwegian Red List due to their limited range and small population size (Artsdatabanken, 2015).



Photo 2. The Norwegian pool frog from one of the breeding sites in Agder, Norway. (Photo: D. Dolmen)

1.4.1 LIFE HISTORY AND REPRODUCTION

The northern pool frogs live at their lower thermal limit in the north and are a thermophilic species (Reinkind & Engemyr, 2019). Because of this, reproduction is highly dependent on season and does not begin until mid - May to late June, later than both the common – and the moor frog in the same region (Reinkind & Engemyr, 2019; Söderman et al., 2015). Since breeding rarely happens before early June, Swedish studies have suggested mating does not occur until the water temperature has reached 16 °C (Reinkind & Engemyr, 2019). The pool frogs spend most of its time in and around waterbodies, both during and after breeding. Several frogs remain in the pond after breeding while a few others

move towards other ponds, streams (Reinkind & Engemyr, 2019) or adjacent marshes for the remaining of the summer (Sjögren-Gulve, 1998b).

The pool frogs reach sexual maturity around age 3 (Dolmen, 2012), where the female lays approx. 1500-2000 eggs in different clusters beneath aquatic vegetation (Reinkind & Engemyr, 2019). The larvae survive mostly on dead organic matter, bacteria and microscopic animals (Reinkind & Engemyr, 2019), and after metamorphosis, occurs usually mid-August, spiders and insects are potential prey, including other frogs as they can be cannibalistic (Dolmen, 2012; Reinkind & Engemyr, 2019). If the summer is too cold metamorphosis might not happen in time for hibernation and reproduction can fail (Reinkind & Engemyr, 2019; Söderman et al., 2015). The migration to overwintering sites begins late August – early September, but some individuals can still be found in and around the pond mid-September (Reinkind & Engemyr, 2019). Holenweg and Reyer (2000) saw pool frogs hibernating in woodlands, 3-7 centimeters below the surface in soil, under moss and other favorable spots (e.g. roots). Swedish studies have shown similar hibernating spots approx. 50-100 meters from the breeding pond (Sjögren-Gulve, 1998b), but no studies on hibernating spots have so far been conducted in Norway (Reinkind & Engemyr, 2019).

1.4.2 THREATS TO THE NORWEGIAN POOL FROG

ABIOTIC THREATS

During the last century, extensive forestry and farming has strongly reduced the amount of breeding ponds and natural habitat (Dolmen, 1997; Kauri, 1981a). Much of the habitat surrounding the pool frog sites is subjected to forestry and in combination with drainage ditches being dug up, the pool frogs spreading potential has been impacted. Beside the direct impact of *habitat alteration*, the heavy machinery used in forestry has potential to destroy the overwintering sites as they hibernate on land, but at currently unknown locations (Reinkind & Engemyr, 2019). Another potential growing threat is *climate change* and the temperature change it can cause (details on effect of temperature in 1.1.1).

BIOTIC THREATS

The devastating *disease* caused by Bd have been detected in in the north. With the use of environmental-DNA from water samples, five ponds in Akershus, Norway have tested positive for the fungi Bd (Taugbøl et al., 2017). However, the fungi have not been detected at the pool frog sites. The pool frog have several natural *predators*, where the main predators are the grass snake *Natrix natrix* Linnaeus 1758, who feed almost exclusively on amphibians (Kauri, 1981b;

Söderman et al., 2015) and predatory fish (Dolmen, 1997, 2012) (details in 1.1.1). A threat the population may face in the future is the possibility of *genetic pollution by continental pool frogs*. A group of continental pool frogs and their hybrid version *Pelophylax kl. esculentus* Linnaeus 1758, were released in Finnøy, Rogaland from Poland. Since the release and till now, the population has increased and spread throughout almost the entire island. The fear is that this population will spread to the mainland, toward the northern population and cause genetic pollution (Dolmen, 2009, 2012). However, Finnøy is approx. 180 km away from the northern pool frog locations, therefore the threat is currently on the lower end. Aside from direct threat from outside sources, the pool frog population can experience threat from within the population due to population size. The Norwegian pool frogs have low genetic variation and a small population size which can make it vulnerable to environmental changes (Reinkind & Engemyr, 2019), as well as inbreeding and genetic drift may be a threat (Campbell et al., 2011b).

1.4.3 MANAGEMENT

LAWS AND CONVENTIONS

Norway has a duty and obligation through international conventions (e.g. Bern Convention and Convention on Biological Diversity) and Norwegian laws (Biodiversity Act) to protect the European flora and fauna, especially species of high conservational value (Reinkind & Engemyr, 2019).

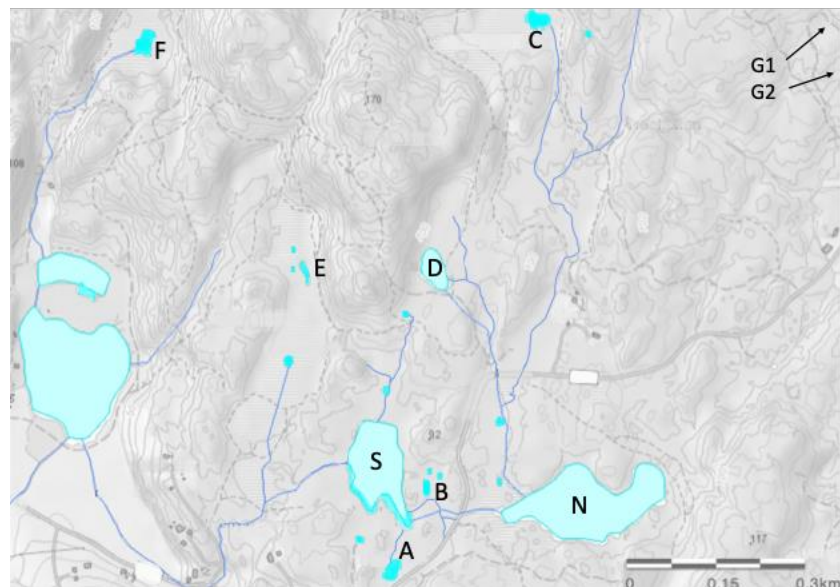


Figure 2. Map over the different locations where viable pool frog populations exists as well as locations that is deemed as appropriate habitat for pool frogs. In these sites, action and different measures to preserve the pool frog populations have been carried out. The map is taken from The Norwegian Water Resources and Energy Directorates (NVE) map services.

MANAGEMENT PLAN 2006-2011

An action plan for the period 2006-2011 was created for the pool frog with the aim to “secure the long-term survival for the northern pool frog in Norway”. The goal is to secure existing spawning sites and strengthen the species through restoration and improvements of habitats and increase the current distribution area through relocation of individuals (eggs, adults or larvae).

Biotope improvements, e.g. new ponds, relocations or predator removal, have been made at the breeding sites, A, B and C (see Figure 2.) to make the habitats more suitable. The additional sites around the breeding sites could potentially support a pool frog population. Therefore, improvements have also been made at those sites with the intent to increase their natural range (Direktoratet for Naturforvaltning, 2006).

MANAGEMENT PLAN 2019-2023

During the period of 2013-2018, no management plan was created. Nonetheless, some measures were carried out, primarily monitoring and fish removal. Predatory fish has been the biggest threat to the population, and brown trout *Salmo trutta*, Linnaeus 1758, has been detected several years in a row (Reinkind & Engemyr, 2019). Over the years, multiple measures have been implemented to improve habitat and population size but with little long-term success. The goal for the upcoming years is to secure the breeding population and to try to facilitate satisfactory habitats for the species. As a way to achieve these goals, management will implement different actions, e.g. a breeding program in Kristiansand Zoo (Reinkind & Engemyr, 2019).

1.5 AIM OF STUDY

There exist knowledge gaps regarding the Norwegian pool frog population, as this is a “newly” discovered species. Therefore, during the last 12 years the pool frog population has been subjected to annual monitoring where every captured individual was photographed *in situ*. The objective is to use photo-identification and identification software, program Interactive Individual Identification System (IIS) as a tool, which recognizes individuals based on the *locations* of markings. The aim of this study is to estimate winter survival of adult pool frog in the population, and document rate and direction of dispersal between the different sites. In addition, environmental factors affecting the recruitment within the population will be investigated.

2 METHODS

2.1 DATA COLLECTION

2.1.1 STUDY AREA

The monitoring of the northern pool frog population took place in Arendal Municipality, Agder County, Norway. The population is mainly limited to three breeding ponds, but one or few individuals are infrequently observed at some additional sites surrounding the main distribution (Figure 2). Due to the status of the species in Norway, and the vulnerability of the population, the Norwegian Environment Agency has decided that detailed information regarding the location of some sites should not be made public (Reinkind pers. comm.). Therefore, a more precise description of the location of the sites will not be given. The population has been monitored twice a year, early summer (late May to early June) and late summer (late August to early September) every year for a 12-year period (2007-2019), except in 2015 and 2016 when monitoring only was carried out in early summer (due to lack of funding). During the monitoring, the focus areas were the three main breeding ponds, from here on named A, B and C (Figure 2), but some of the additional sites surrounding the main distribution area were also included when possible. A capture-mark-recapture method (CMR) (Lettink & Armstrong, 2003) was performed as each individual pool frog can be separated by distinct markings on their abdomen. Each individual discovered at each site was captured using a hand net, before the abdomen and side was photographed by the researcher. The efforts to gather this data has been performed by Dag Dolmen, from the University Museum of the Norwegian University of Science and Technology (NTNU).

2.1.2 CAPTURE-MARK-RECAPTURE

A capture-mark-recapture (CMR) (Figure 3) approach is a method to identify individuals within a population (Lindberg & Rexstad, 2006; Thanchira & Ivana, 2018). The method is used in situations where animals can be marked, e.g. toe clipping, or identified

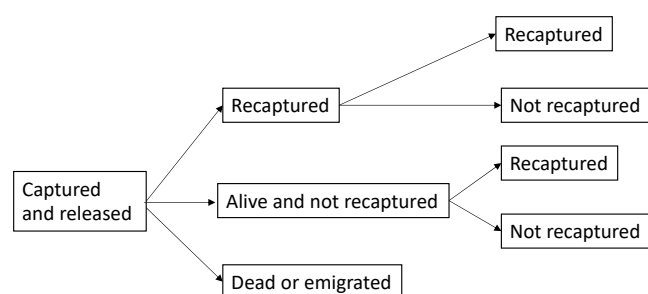


Figure 3. A simple illustration on mark-recapture methodology with a two-sampling period.

in other ways and later detected by either capture or sighting (Lettink & Armstrong, 2003), e.g. bird rings. At the beginning of the study, the researcher will go out and capture a part of the population, marking or photograph individuals, before releasing it back to the population. On

the first sampling event, the researcher will capture a part of the population again, some will be recaptured, some will not, and others may be caught for the first time. During the second sampling event, the same procedure was repeated, recaptured or not recaptured, the more sampling events, the higher quality the data will have (Figure 3).

CMR is a valuable tool for managers as the population size can be estimated (Petit & Valiere, 2006; Thanchira & Ivana, 2018) as well as survival rates and population trends (Langtimm et al., 2004; Lettink & Armstrong, 2003). However, most techniques of marking are considered invasive, therefore photo-identification have been presented as a non-invasive alternative. This approach recognizes the individuals by distinct natural markings (e.g. spots, stripes, fin contours) and have been used on a range of different animals (Hillman et al., 2003; Holmberg, Norman, & Arzoumanian, 2009; Langtimm et al., 2004; Pellitteri-Rosa et al., 2010; Thanchira & Ivana, 2018).

The northern pool frog has developed camouflage well fitted to the environment it lives in and finding them can prove difficult. Therefore, when arriving at the pool frog location, it is important to be quiet and have slow movements as the frogs are easily scared. To find the frogs, binoculars was used as the frogs are small, shy and hard to see at a distance, focusing on “lagoons” or other shallow areas. When a frog was spotted, slow movements was important when moving toward where it was positioned. The frogs were captured using “bait” on a fishing rod and a hand net. The procedure was to place the hand net underwater, before luring the frog in it by the use of “bait” placed on the end of a fishing line, moving up and down in the water, mimicking the movements of an insect. As soon as the frog was lured inside the net, the net was quickly lifted and closed to prevent escape. Every individual captured were photographed on the abdomen and the right side before it was released back to where it was caught. During mating season, it can be easier to find the males due to their mating calls. The croak bags will inflate and can be easier to spot, including the sound the males make, can make it easier to locate the spot where it sits. As this is time-consuming efforts, a “quicker” way to find the frogs are to use a scare tactic. Walk along the water edge, paying close attention to every movement, any movement or ripple can be a frog jumping back into the water. Stop where the movement was, and wait for it to resurface, hopefully close to where it disappeared. Then the same procedure to capture it is used, as previously explained.

2.2 PHOTO-IDENTIFICATION

To aid with the extensive data collection gathered a photo-identification, program Interactive Individual Identification System (I3S Classic 4.02 <https://reijns.com/i3s/>) (Tienhoven, Hartog, Reijns, & Peddemors, 2007) was applied. The identification system creates a database of different individuals which assist the user to identify new or re-discovered individuals. I3S Classic uses the placement of the markings on the individual, usually dots, to recognize the individual. The program needs no information in regard to size or shape, only the location of the marking. Photo-identification software's has been developed and used on a variety of different species (Chaves, Hall, Feitosa, & Côté, 2016; Hillman et al., 2003; Sannolo, Gatti, Mangiacotti, Scali, & Sacchi, 2016), and provides better estimates which improves management and conservation (Tienhoven et al., 2007).

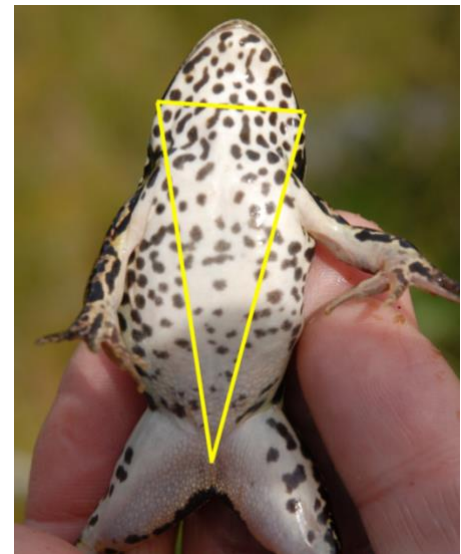


Photo 3.. A preview of the triangle the three reference points create and an e.g. of markings on the abdomen of a pool frog. (Photo: D. Dolmen)

2.2.1 CREATING A NEW DATABASE

When creating a new database, some parameters must first be defined, such as reference points and metadata. In this database, three reference points were made, these must be clearly visible in every photo (and preferably form a triangle, see photo 3.) and are the first areas to be marked and marked in a specific order. The reference points and order of markings chosen in this database are left jaw, right jaw and groin. For every photo, metadata was added, providing additional information about the individual, are recorded in the database. The metadata categories are defined when the database is created. In the given database the information required is gender (M/F), age (0+/1+/ adult), location (A, B, C, D, E, F, S & N), season (spring/ fall) and year (2007-2019). Location, season and year is known, but age and sex need to be decided by the user.



Photo 4. An additional abdomen photo to illustrate different markings on different individuals. Photo taken from the breeding program in Kristiansand Zoo by K. Haugen.

2.2.2 DIFFERENTIATING BETWEEN AGES AND SEXES

As age and sex are information that needs to be included in the metadata, it is important to know how to separate the two. The two sexes are separated based on morphology as the pool frog displays sexual color dimorphism, see details in 1.3.3 and Appendix I. Age can be trickier, as different individuals have different growth rate (see e.g. photo of two different 1+ in Appendix I). Age was decided based on time of year it was found as some 1+ individuals are very similar to 0+ age class in size but are found at different seasons (see Appendix I (photo 4 and 5 in Appendix)). The size of the individual also impact age decision as larger individuals most often are older, this is especially seen in females. Males can be more difficult to age determine as they do not increase their body size to the same extent as females. In the case with males, age decision was made easier by the CMR method, which provides more reliable data on age (Halliday & Verrell, 1988). This is because we can then go back into the dataset to see how many times and in what years that individual was caught and recaptured. From that information, we see the minimum age that ind. has to be, e.g. an ind. captured in 4 consecutive years is minimum 4 years old, it could be older, but not younger.

2.2.3 PHOTO IDENTIFICATION

As seen in photo 4, the pool frog has markings on their abdomen, and this pattern is distinct and different for every individual (see photo 3 and 4)(Dolmen, 2012), which is why this method can be used to separate individuals. To achieve best accuracy, it is important to be precise and provide the program with enough markings on each photo. The program uses an algorithm to calculate similarities between positions of spots recorded on each photo, and to be most accurate the program requires a sufficient amount of positions, between 12-30. If there were more than 30 obvious spots on the frog, the biggest and most prominent was marked first, where the focus was on round spots. It was important to place the mark in the center of the spot for the highest accuracy. Marks with different shapes were avoided as the center is more difficult to establish in a line mark than a round mark. After metadata had been filled in and the required dots had been marked, the next step was to search through the database to find the best match. When the search was complete, the program provides a list of the top matches based on the pattern made on each photo. Every photo is given a score, lower score, better match. The rating of possible matches is provided by the program; however, the final match is up to the user. If the individual in the photo was not encountered before, it was recorded as the first entry of a new individual in the database received a unique reference number specific to that individual. If the user

determined that it was a recaptured individual the photo and metadata was included in the database folder for that individual.

2.3 DATA ANALYSIS

2.3.1 STATISTICAL ANALYSIS IN PROGRAM MARK

Apparent survival (ϕ) and recapture probability (p) in the Norwegian pool frog population was estimated in an open population Cormack-Jolly-Seber model (CSJ) (Lebreton, Burnham, Clobert, & Anderson, 1992) in program MARK (White & Burnham, 1999). The focus was to estimate winter survival for the adult part of the northern pool frog population in Norway. The model selection was based on AIC_c (the modified Akaike's information criterion) and the parsimonious approach (Burnham & Anderson, 2002). CMR studies of open populations are more complicated as they include births, deaths, immigration and/ or emigration. Lettink and Armstrong (2003) explained this as if an individual is not captured in the beginning of a study, it can mistakenly assume it came as a result of immigration or birth. Similar as when an individual has not been detected, it may mistakenly be presumed dead or that it has emigrated to another site.

To find the best model for the data, a model selection was carried out. The first step was to find the optimal model structure for recapture. The different effects on recapture probability that was tested was; interaction between sex and time and an additive effect of sex and time, both separately and together. Different recapture probabilities were also tested in the years 2015 and 2016 as those years had less monitoring efforts than other years. Different recapture probability models were tested as there may be some variations in the sampling time e.g. due to time spent at a site or differences in weather at sampling time. When the best model for recapture probability was found, it was used in every model thereafter when the best model for survival was found (Appendix VI.). The same procedure was used to find the best model for survival, including; average winter temperature, average yearly snow depth, snow model, estimated winter ground temperature and variance ground winter temperature (All models are presented in Table 1.). The snow model is based on estimated winter ground temperature, snow days and average snow depth (For more details, see Appendix V).

Of the 413 individuals captured and registered in the database, only adult (classified as 2+ and older) individuals (and 1+, that was recaptured later in the sampling period) from site A, B and C (Including ind. captured at site N before recaptured at A, B or C) was included in the data analysis, a total of 277 individuals. The individuals caught at other locations, e.g. F or D, which did not receive the same monitoring efforts, were not included as they would

contribute to incorrect data. The 0+ age class was not included in this analysis due to the often-underdeveloped pattern on their abdomen. This feature makes it difficult to follow individuals from age 0+ to 1+ as the pattern changes with age and would therefore most likely contribute to a false mortality. This has also been seen in adult pool frogs as some markings can “melt” together with another, creating e.g. a line or some other distinct mark. Photos where the individual twisted or otherwise made the photo poor was also excluded.

Population size was estimated for the different years (2008-2019) in program Microsoft Excel (Version 16.37), with the use of recapture probability and number of captured individuals in the following formula: $\hat{N}_i = n_i / p_i$ where n_i is the number of individuals caught in year i and p_i is the recapture probability for year i .

3.2.2 STATISTICAL ANALYSIS IN PROGRAM R

Statistical analysis of recruitment were performed in R software, version 3.6.1 (R Core Team, 2020) by L. Korslund. Recruitment was defined as number of 1+ in year n / number females in year $n-1$) in the Norwegian pool frog population was estimated in program R where the model selection was based on AIC_c (the modified Akaike’s information criterion).

A visual inspection showed that the data was not normally distributed but rather with many small and a few high values. The recruitment numbers were therefore log-transformed (1 was added to all recruitment numbers before transformation to avoid the problem of log-transforming zero). To account for lack of independence between observations from different sites the same year linear mixed models, with years as a random intercept, using the lme function from the nlme library (J, D, S, D, & R Core Team, 2020) was applied. To find the best model explaining the variation in recruitment between years and sites several models were investigated (All models are included in Table 2.). The highest parameterized model included an interaction between average summer (June, July and August) temperature, to account for any temperature effects on larva growth, and known presence of fish in the pond (factor: Yes/No) to account for potential predation of larvae, and an additive effect of modelled ground winter temperature, to account for any effect on juvenile winter survival. All nested and less parameterized models, down to a null model, was also tested. AIC was used to select the best among the models. The ggplot2 library plots was used to visualize the result (Wickham, 2016).

3 RESULTS

The database contained 1040 photos of 413 individuals from all investigated sites, but only 277 of these individuals was included in the data analysis. The remaining individuals was caught at other sites than A, B or C, or was in the age class 0+ or 1+.

3.1 SURVIVAL

Although some variation in recapture between years was seen, the models based on constant recapture probability was most parsimonious (Appendix VI). This indicates that the variation in the recapture rate was too small to justify including these extra parameters in the best models. Therefore, the model structure with constant recapture probability was implemented in every model for annual adult survival hereafter, and only models with different survival structure is presented here.

The model that was selected to be the best was supported by the AIC_c values and the most parsimonious, was model 1. This model is based on constant annual survival, where the estimated annual winter survival in the adult population were 0.62 (95 % confidence interval 0.58-0.66) with a 0.75 (95 % confidence interval 0.68-0.80) recapture probability. The model based on constant survival (Model 1.) performed much better than the model where adult survival was allowed to vary freely between years (ΔAIC_c 14.91), and this suggests that the variation in survival between years were relatively small (Model 14, Figure 4.). Although the constant model was selected the best, the next best model $\phi_{\text{var.ground}}$ (Model 2.) showed some support and included variance in ground winter temperature. This indicates that some of the between-year variation in adult winter survival can be explained by variance in winter ground temperature, but as Model 1. was the best model, it suggests that the variation between the years are relatively small.

Model no.	Models	No. of parameters	AIC _c	Δ AIC _c	Deviance
1	ϕ	2	1110.25	0.00	310.37
2	$\phi_{\text{var.ground}}$	3	1111.21	0.96	309.30
3	ϕ_{vt}	3	1111.62	1.39	309.72
4	$\phi_{\text{est.ground}}$	3	1111.78	1.53	309.88
5	ϕ_g	3	1112.25	2.00	310.35
6	$\phi_{\text{vt}+g}$	4	1113.59	3.35	309.66
7	$\phi_{\text{sd}+g}$	4	1114.18	3.93	310.24
8	ϕ_{sd^*g}	4	1114.49	4.25	308.53
9	$\phi_{\text{sd}+g(\text{vt}+g)}$	5	1114.55	4.29	308.58
10	ϕ_{SM^*t}	5	1115.44	5.19	309.47
11	ϕ_{vt^*g}	5	1115.45	5.20	309.49
12	$\phi_{(\text{sd}^*t)(\text{vt}^*t)}$	7	1116.32	6.06	306.26
13	ϕ_{SM^*g}	9	1121.82	11.57	307.65
14	ϕ_t	13	1125.16	14.91	302.65
15	ϕ_{t+g}	14	1127.25	17.00	302.65
16	ϕ_{t^*g}	25	1141.19	30.95	289.79
Global	$\phi_{t^*g} p_{t^*g}$	48	1164.96	54.70	264.22

Table 1. Survival (ϕ) models tested. Model ranking of CJS CMR models estimating apparent survival (ϕ) and recapture probability (p) for the pool frog population in Agder Municipality, Norway from 2007-2019. Based on constant recapture probability. Sex effect (male vs. female) g ; time effect, t ; snow model, sm ; average temperature winter, vt ; average yearly snow depth, sd ; estimated ground winter temperature, $est.ground$; variance winter ground temperature, $var.ground$.

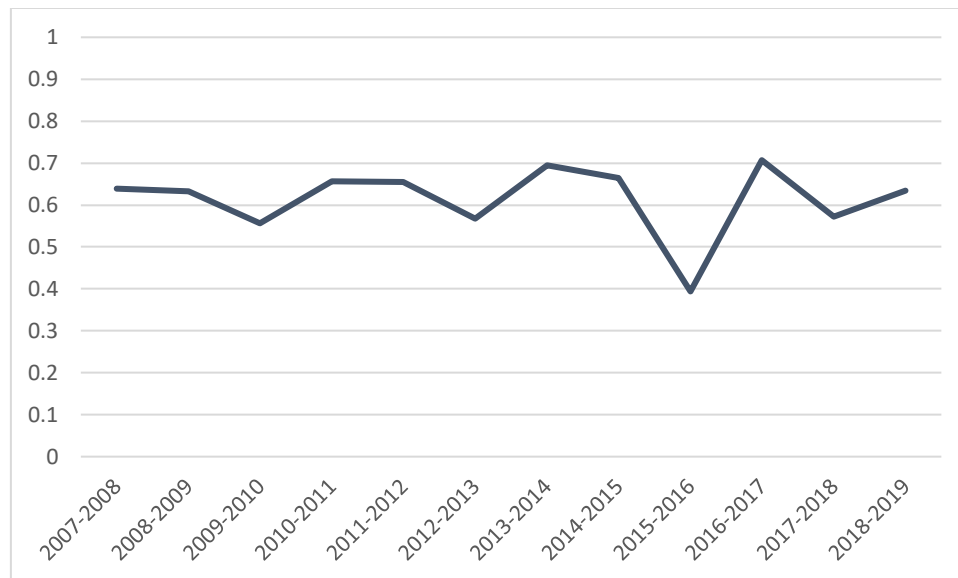


Figure 4. Based on estimates from model 12. Estimated annual survival for the adult population at site A, B and C from 2007-2019. The y-axis displays estimated annual adult survival and the x-axis displays the winter year in question.

Estimated annual survival based on time effect (Figure 4. Model 14.), have been included to show the small variations in the model, expect for the 2015-2016 winter. The decrease seen in 2015-2016 has been tried to be explained by environmental factors in models, but Model 1 was the best, indicating that the variations seen are not enough to support adding extra parameters.

Estimated ground winter temperature (Figure 5.) has shown some variations throughout the years, with some peaks and some lows, where the ground winter temperature and estimated

adult survival (Model 4. Figure 6.) appear to have a small connection. Increased winter ground temperature leads to an increase in adult survival, yet the differences in survival, from the highest to lowest survival rate are extremely low, 0.044.

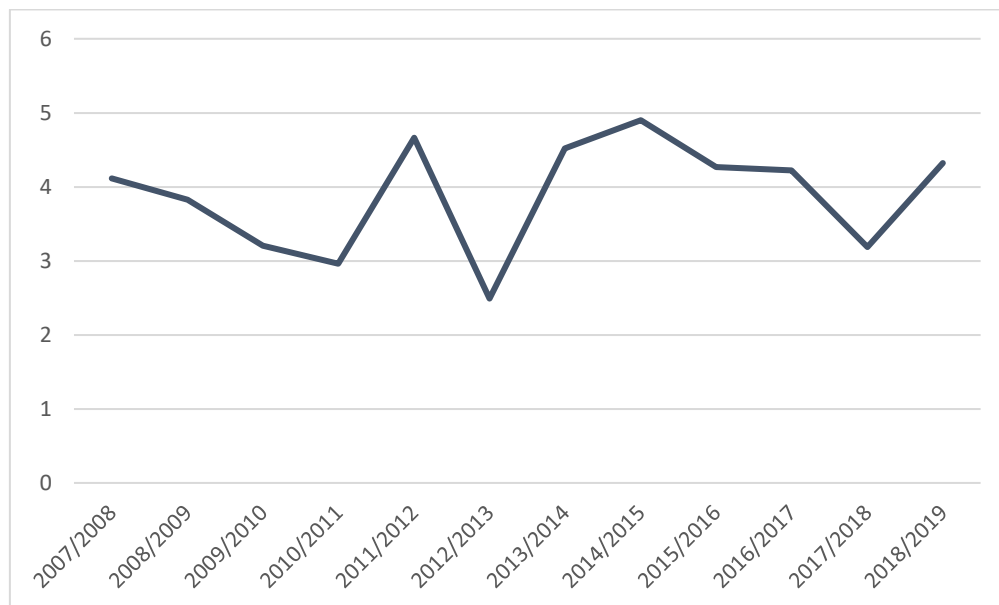


Figure 5. Estimated ground winter temperature used in the snow model and model 4.

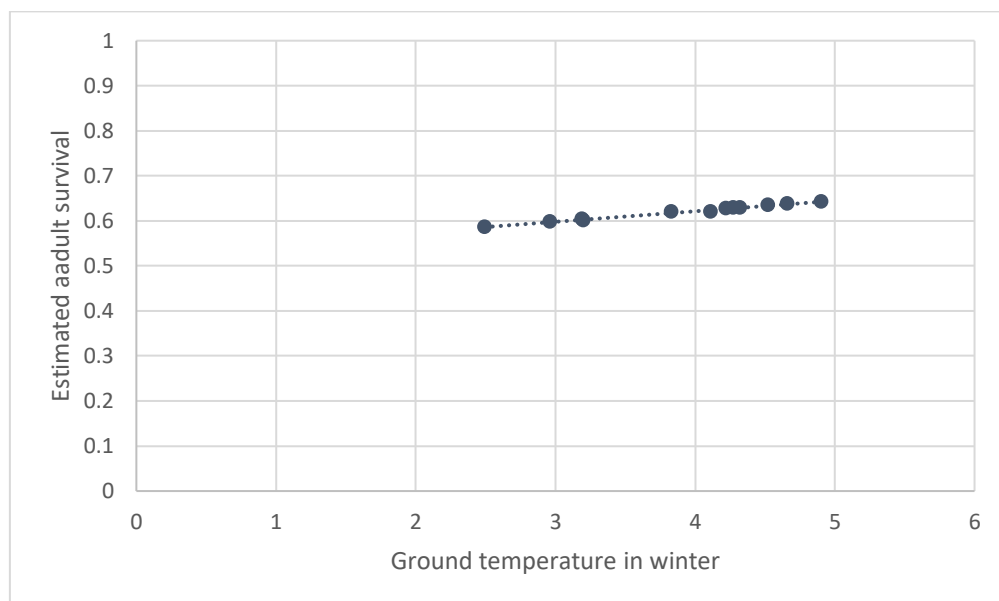


Figure 6. Estimates of adult survival from model 4, and estimated ground winter temperature.

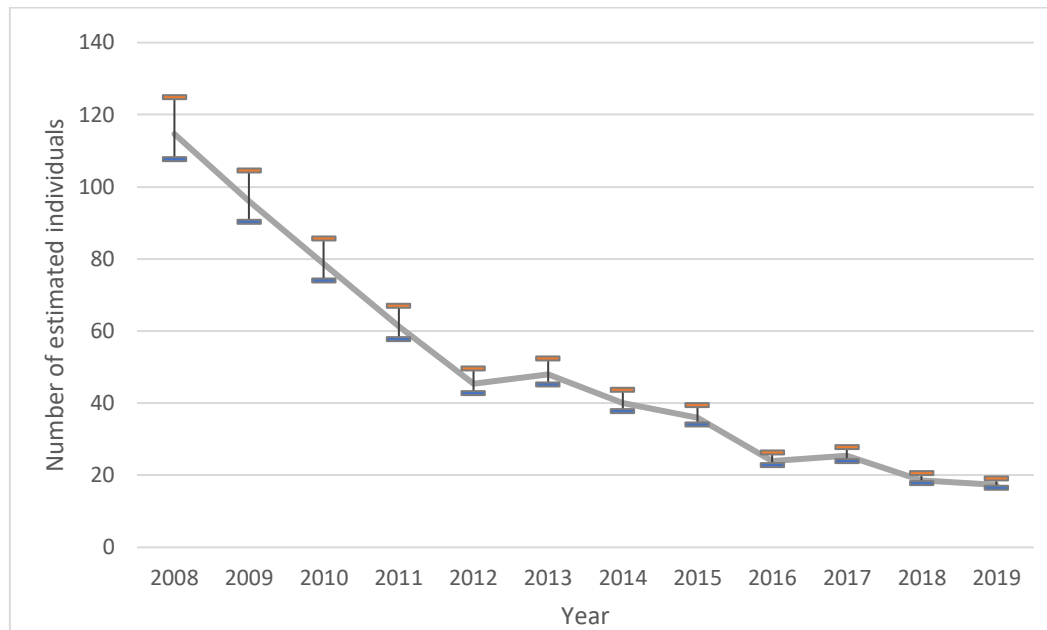


Figure 7. Estimated adult population size and development in the adult pool frog population at location A, B and C in Agder Municipality, Norway with a 95 % confidence interval. The population size was estimated based on number of observed adults from each year and a constant recapture probability ($p = 0.75$).

The CJS model in program MARK estimated recapture probabilities and survival rates from the best model. Based on the best model, model 1, the population size estimations are based on constant recapture probability = 0.75. The overall trend for the adult population is, except for few variations in some years, negative (Figure 7). The strongest decline was from 2008 to 2012, where the population experienced a 60.9 % decline in size. From 2008 to 2019, the adult pool frog population have declined a total of 85 % (from estimated 115 to 17 adult individuals) based on the estimated adult population size. Based on the 95 % confidence interval, the most uncertain estimates are in the beginning of the monitoring period, and the closer to today we come, the less uncertainty there are.

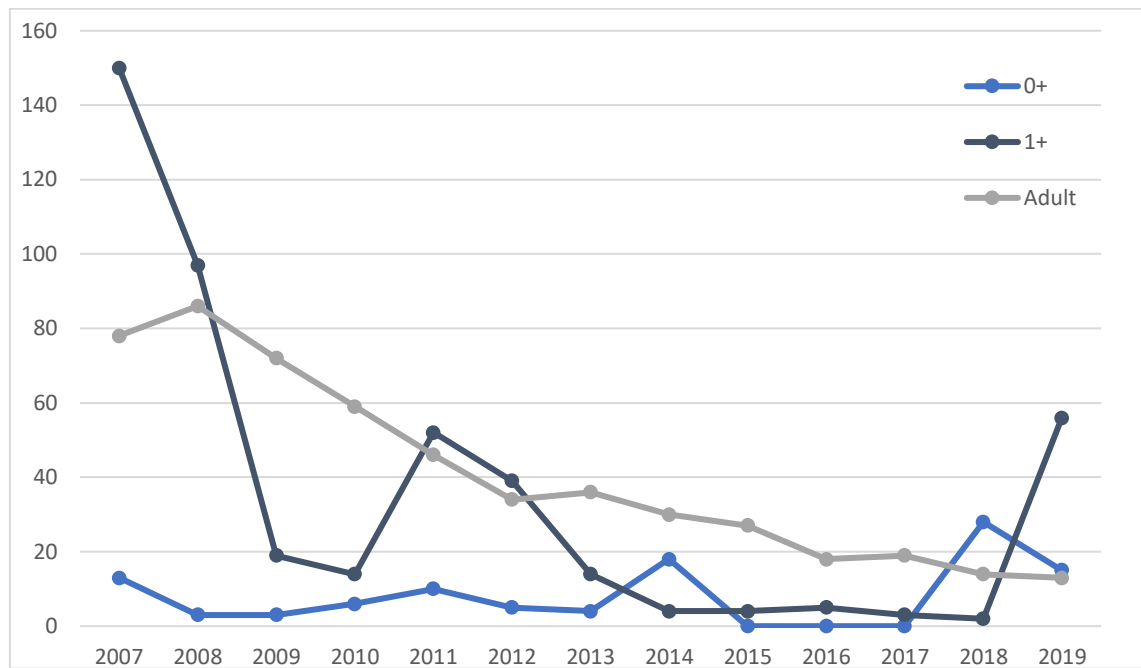


Figure 8. Observed pool frogs in the age class 0+, 1+ and adults at location A, B and C in Arendal Municipality, Norway during a twelve-year period. The graph displays observed number of individuals in the Norwegian population. 0+ is defined as recruitment for that year (blue), 1+ is juveniles/subadults (last year's recruitment) (black) and adults are individuals age 2 or older (grey), defined as adults. The y-axis corresponds with the number of individuals captured in the sampling events (spring and fall) from each year.

There are strong variations in the subadult (1+) part of the population where the highest recruitment events occurred prior to 2011, until 2018 (Figure 8). 2018 had the highest number of captured subadults since 2008. The highest recruitment period registered was 2006, displayed by the high numbers of subadults from 2007. As a result, the adult population had an increase in 2008 but has since experienced a negative trend. Although small variations occur in some years, such as the tiny increase in the adult population in 2013, the overall trend is negative. There are small variations in captured 0+ except for 2014, 2018 and 2019 which shows increases in the recruitment, but 0+ are not included in further analysis as subadults have shown to be a better choice in regard to recruitment and reproductive success analysis. The 0+ age class is difficult to find and are most likely highly underrepresented in the dataset. In the 2014-2017 period, there is low to none recruitment registered. Due to no sampling events in fall of 2015 or 2016, no 0+ has been registered these two years.

3.2 RECRUITMENT

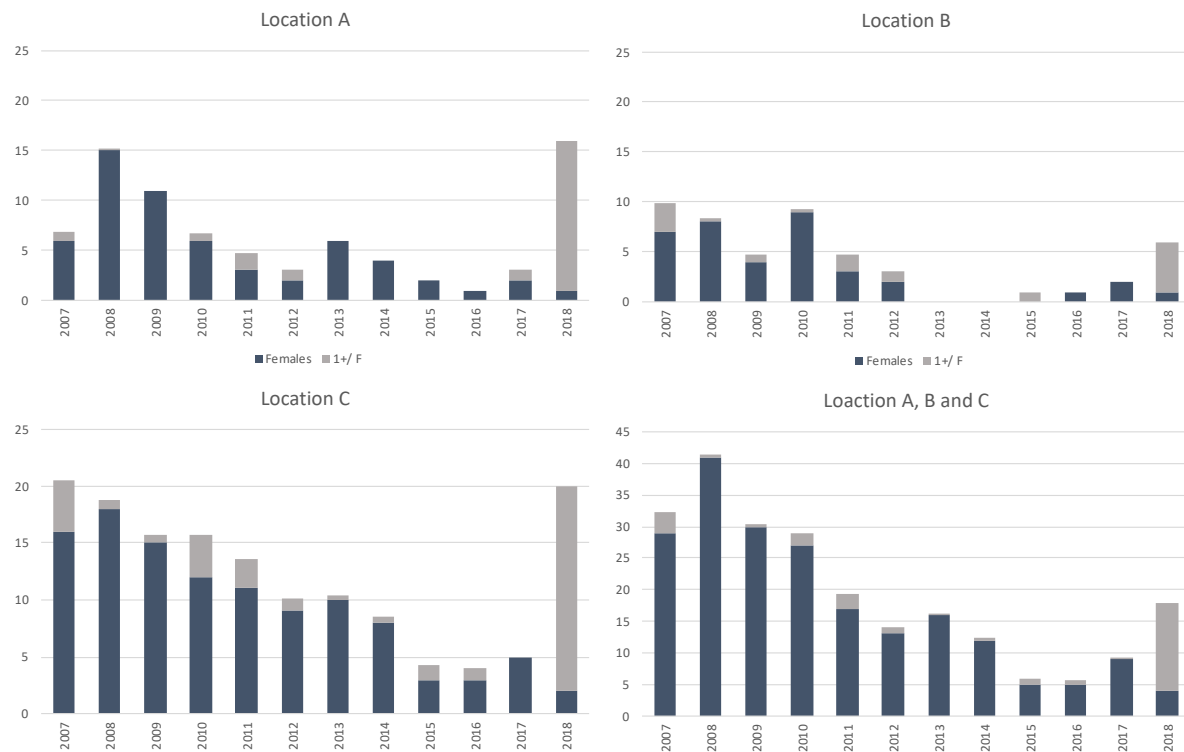


Figure 9. Recruitment figure based on 1+ one year/ observed females next year, from each of the three breeding locations including one of the entire recruitment population (A, B and C) in Agder, Norway. e.g. site C in 2011: We found 45 1+ at site C in 2011, and 12 females in 2010. Recruitment are therefore 3.8 (=45/12). The y-axis displays 1+/females and females each year. The total height of a bar corresponds with observed females (black) and 1+/females (grey).

Recruitment (here referring to 1+ found the next year) was calculated as 1+ one year/observed females the year before (Now referred to as 1+/females) (Appendix II). Location A has had low 1+/females in almost every year except 2018 where recruitment was the highest (Figure 9). The number of observed females at site A have varied much through the years, where the lowest observed females was in 2016 and 2018. 2018 is one of the two years where only 1 female was observed at site A, but this is also the year with highest 1+/ female. Other than the high recruitment per female in 2018, 2007 and 2011 has been one of the more successful years at location A, but with little success the remaining years. The overall trend is low recruitment per female, but site A has had higher population of observed females compared to site B.

Site B have been subjected to fluctuations in observed females, but not as much as site A has been. Compared to location A and C, B has the lowest number of females in its population, but 1+/ female is better than in location A, except in 2018. There is a three-year gap period (2013-2015) with no observed females at site B, corresponding with no recruitment in the first two years, but one 1+ was observed in the spring of 2016. A few females were registered in 2016 and 2017, but no recruitment was observed until 2018. 2018 had the highest

1+/ female throughout the monitoring period, identical to what was seen at site A and C. In the monitoring period, the population at site B had its best 1+/ female in 2007, 2011 and 2018, although low.

Based on 1+/ female and number of observed females, location C is the best out of the three sites. Recruitment per female has been decent at site C in 2007, 2010 and 2011, but in the period of 2013-2017 recruitment and observed females have decreased. 2018 was the best year based on 1+/ female, identical to what was seen at site A and B. Site C has not been subjected to the same variations in observed females as site A and B has been. In relation to number of observed females, site C has been consistent in the number of observed females, in comparison to site A and B which have had fluctuations through the years. But the trend for observed females at site C are negative.

The stacked bar chart for all three locations show some fluctuations in observed females but display an overall negative trend, similar to the bar chart for location C. Recruitment per female for the three sites added together have been poor except for 2007, 2011 and 2018. The year 2018 display a big difference from previous years as this is the year with lowest number of females but highest number of recruitments per female. A common trend in the figures is the inconsistency of number of females compared to survived and captured 1+/ female.

After carrying out a model selection (Table 2.), the best model, based on AIC_c values was model 1, which included average summer air temperature, as summer temp. and reproduction is significant. With increasing summer temperature, reproduction increases with it, but there are not enough data points to help explain the large difference between reproduction and summer temperature (Figure 9.).

Model no.	Models	λ	K	AIC _c	Δ AIC _c	w
1	R _{s.temp}	-28.86	4	65.71	0.00	0.46
2	R _{s.temp+fish}	-28.79	5	67.58	1.87	0.18
3	R	-31.27	3	68.54	2.83	0.11
4	R _{s.temp*fish}	-28.28	6	68.56	2.85	0.11
5	R _{s.temp+fish+w.temp}	-28.55	6	69.09	3.38	0.084
Global	R _{s.temp*fish+w.temp}	-28.02	7	70.05	4.34	0.054

Table 2. Reproduction (R) models tested. Model ranking of CJS CMR models. For 1+/females in Agder Municipality, Norway from 2008-2019. λ is log likelihood, K is no. of parameters in the model and w is model probability. Average summer temperature, $s.temp$; effect of fish, $fish$; estimated winter ground temperature, $w.temp$.

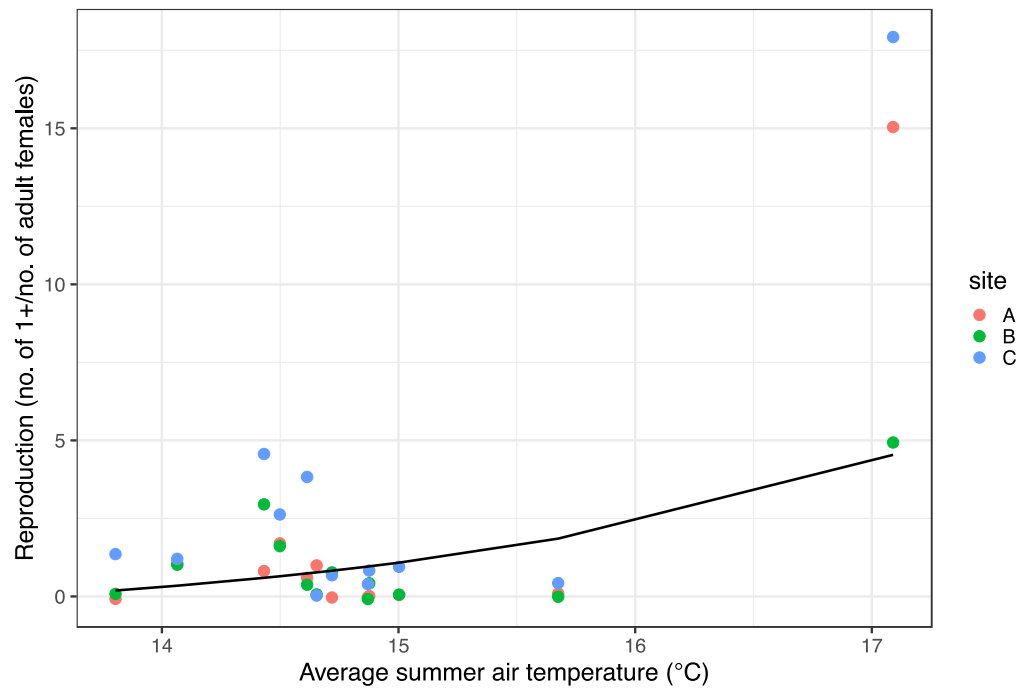


Figure 10. Figure based on model 1 in Table 2. Summer temperature and reproduction (1+/females) at the three breeding sites A, B and C. Model created in program R Software by L. Korslund. The geom_jitter function was included to add a small variation to the location of each point, so no mark was lost behind another.

3.3 DISPERSAL

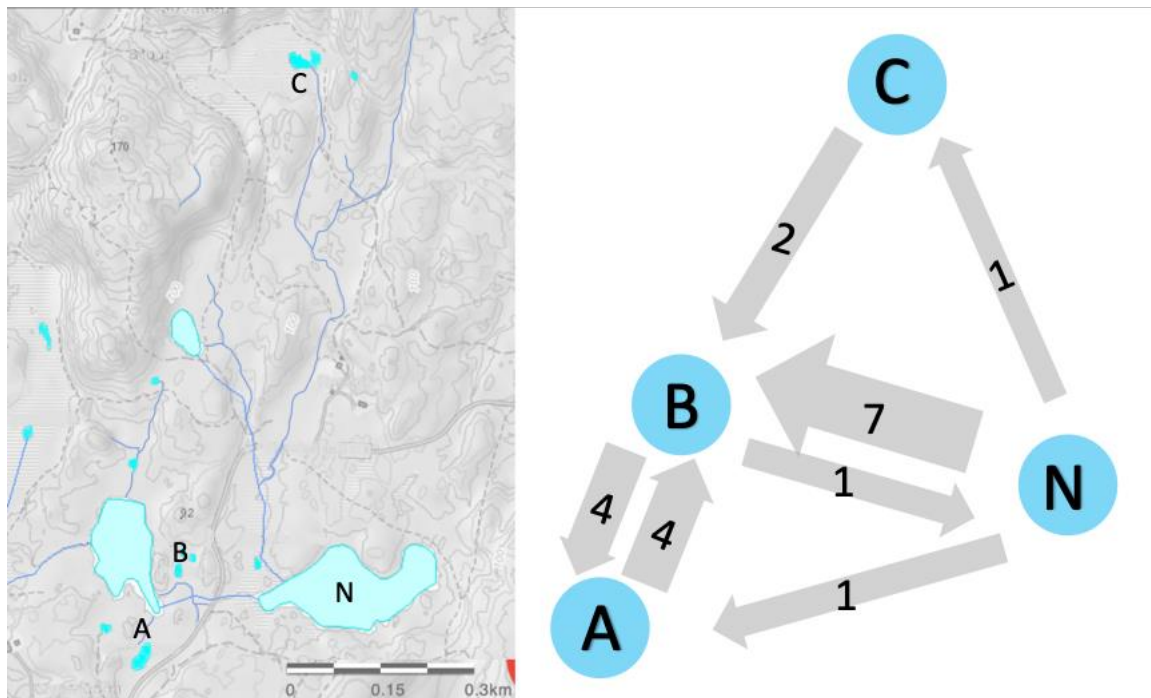


Figure 11. Dispersal movements within the Norwegian pool frog population. The thickness of the arrows, as well as the number inside, illustrates the degree of dispersal (counted as number of movements from one site to another between the different locations in Agder Municipality, Norway).

Migration from	Migration to	2008	2009	2010	2011	2012	2017	2018
A	B	3	1					
C	B							2
B	A	2			1		1	
	N			1				
N	A				1			
	B	1	2	3		1		
	C				1			
Sum		6	3	4	3	1	1	2

Table 3. The number of dispersal movements from site x to site y and the year those movements occurred.

During a 12-year period, 17 registered individuals have been documented to have dispersed between different locations (Figure 10.). The highest dispersal rate has been from location N to B, with a total of 7 dispersal movements. The second highest movements have been from A to B and vice versa, while the remaining dispersal events have been minimal with only a few other individuals moving toward the remaining sites (Table 3. See movements in Appendix III).

The longest route taken has been by one individual who moved from N to C, which is connected through a system of small streams (Figure 11), and two other individuals from C to B. The two routes have the approx. same distance, 1.1 km as the crow flies, but following the waterways this distance is much longer, especially when moving from C to B. The majority of movements occurred in the beginning of the monitoring period, from 2008-2011, while the population size was relatively large, with only a few movements in the later years (Table 3). No movements have been registered in the period 2013-2016. Every individual who dispersed permanently established at the site they moved to, except for two individuals, one who moved from site N to B, back to N where it stayed for a couple years before coming back to B where it stayed (minimum 5 years – dispersed at adult age, see Appendix III). The second showed a similar movement, dispersal from site A to B, before being recaptured at A 3 years later (minimum 6 years – dispersed at adult age). The fraction of individuals who have dispersed in the population is low, only 6.1 % (17 registered individuals in a population of 277 registered individuals) of the adult population have shown to relocate from the area they were first captured in, to a second or third location.

4 DISCUSSION

In this thesis, the aim was to estimate winter survival in the adult pool frog population, look at factors affecting recruitment and document dispersal rate and direction between the different sites. Estimated winter survival for the adult population has been surprisingly consistent, without much variation, where it appears climate has a lower impact than previously believed. Much of the dispersal events has been directed towards one location, site B, but there has overall been a low degree of dispersal within the population. Recruitment has been significantly impacted by summer temperature, but the presence of fish has not shown any significant effect, according to the available data.

4.1 ADULT SURVIVAL

The annual winter survival rates for the adult part of the Norwegian pool frog population calculated from capture-mark-recapture data was 0.62 (0.58-0.66). This is lower than estimates from one continental pool frog study (0.72-0.84) (Peter, 2001b), but higher than another (average 0.379) (Anholt, Hotz, Guex, & Semlitsch, 2003). However, Anholt et al. (2003) did not take into account emigration as they only investigated two ponds with ~ 35 km distance, and emigrating individuals can give lower survival rate because of false mortality (Peter, 2001b). They also noted there were differences in recapture probabilities and recapture efforts from each sampling event. The annual survival estimated in the Norwegian population also was higher than that of the Swedish population (0.11-0.45) (Sjögren, 1991), however, only one site was included in the Swedish study, and it did not appear to include emigration. Emigration has been, to a certain degree, included in this study, however, I cannot rule out that some individuals have migrated out of the investigated areas to other uninvestigated locations. Regardless, to estimate survival rates is difficult as time of death is often unknown and survival varies with a range of different factors e.g. time or environment (Peter, 2001b).

One of the biggest limitations to this study is the photo-identification, as the markings on the pool frog abdomen are not static but evolve to a certain degree with age. Some new markings appear as the individual becomes older, while closely positioned spots have been seen to “melt” together, e.g. and thus forming a line. As the photo-identification software uses a pattern based on the position of markings to find the best match, a change in the pattern may lead to a missed match. Although the final matching is up to the user, the program supplies the user with the most likely matches, and if the individual in question is not found among the suggested candidates, the user may mistakenly create a new individual and not include the observation as a recaptured individual. When some markings have fused together, the user is

forced to make judgement call as to whether this is the same individual and the markings have “melted” together, or if it is indeed a new individual. Therefore, some discretion must be shown. What has been observed when using this program is that the program has given a high rate (high rate is low match, and low rate is high match) on photos that are of the same individuals. This has been seen on some occasions, leading the user to believe the individual investigated has not been captured previously. I have gone through the database on several occasions, seeing that this has occurred from time to time and corrected it, hopefully most of these instances have been fixed. Some frogs have quite distinct markings, therefore recognizing some of them are easier than others. I have seen that the program has failed to detect the correct individual within the top 30 or 40 matches, although I know for sure that I have seen the same individual previously. If the user is capable to correct for this it is possible to add the observation to the right id, but this might have occurred with other, less distinguishable individuals, resulting in inflating the number of unique individuals in the database. The consequence is a wrongly increased population size, and perhaps a higher mortality rate, however, as the survival rate has been so consistent throughout, I do not expect this to have caused a too big of an impact. Also, a study Tienhoven et al. (2007) did on spotted raggedtooth shark, *Carcharias taurus* Rafinesque 1810, with the same photo-identification software used here, saw that even if 40 % of the individuals in the database only had one reference image, the search option gave a 72 % likelihood of a positive match. Therefore, it is not expected to have impacted the result too much as the database has been reviewed on more than one occasion. Other limitations might be photo quality. Thanchira and Ivana (2018) saw that photo quality significantly impacted the software’s effectiveness, and although a few of the photos in my study was out of focus, this was not a common problem.

An assumption was that there would be higher recapture probabilities for males than females, as seen in Anholt et al. (2003) and Peter (2001b), as males have more high risk strategies than females (Shine, 1979). However, the results were not consistent with what was assumed as there was not enough difference between recapture probability in males and females to defend a model with extra parameters (males 0.71 and females 0.81). Even if the model that included differences in recapture probability in males and females was supported, it would still contradict what has been seen in the continental species as females actually had higher recapture probability than males in this study.

Adult winter survival has shown some variations, as model 3, which included winter temperature, showed lower survival in a few winters, but the variations between years have been low. The variations seen can also be a result of a weakness, as this is a prediction based

on the model. It has previously been stated that the northern pool frog benefits from warm summers and mild winters (Dolmen, 2012), yet, hibernating amphibians use less energy in colder than milder winters (Holenweg & Reyer, 2000; Reading, 2007). Energy use is seen in weight loss where Holenweg and Reyer (2000) reported that weight loss is higher in warmer winters than in cold ones. The 2009-2010 winter was extremely cold (Appendix V.) as a cold wave hit Europe, and a population decline was expected (Dolmen, 2012). Survival has been shown to decline in extremely cold winters as well as when there are strong variations in winter temperature (Anholt et al., 2003), as they change hibernation sites during winter, and often more than once (Holenweg & Reyer, 2000). However, this phenomenon was observed in Switzerland, and the likelihood that the northern winters are warm enough for this to occur is unknown, but doubtful. I could not detect any sign of low mortality this winter, 2009-2010, but the impact of variation in ground winter temperatures was tested in Model 2, which had some support as it was the second-best model. Ground winter temperature showed a small link between increased ground winter temperature and increased adult survival, although extremely small. Li et al. (2013) has suggested that amphibians that currently live at their thermal limit could benefit from milder winters, which are expected to become more common due to climate change. However, we know little to nothing of what the northern pool frogs do during winter, therefore, I will not speculate too much as this requires more research.

As the survival rate has been surprisingly consistent throughout the years, this indicates that mortality is lower than previously presumed. It was assumed that climate had a bigger impact on adult survival than what it seems to have, therefore it will be interesting to include age and life expectancy. Previous studies and statements have concluded that the pool frogs become ± 6 years old. (Dolmen, 2012; Smirina, 1994), but as previously stated, CMR provides reliably data on age (Halliday & Verrell, 1988). Therefore, based on the CMR method used in this study, the Norwegian pool frogs can become at least 9 years old. Several individuals (7,6 %) have been captured between 6 to 9 years, indicating that these individuals are at least 6-9 years old. If including individuals that managed to become at least 5 years old, the proportion is 15,5 % (captured between 5-9 years). The average life expectancy, for individuals from 2 years and older was calculated to be 3.1 years. When individuals who only lived to be at least 2 years old was removed from the calculation, and only included individuals that had reached sexual maturity (minimum 3 years old), life expectancy grew to 4.3 years. This can indicate that mortality is high when young, but that survival increases with age, similar to what is suggested by Elmberg (1990) in *R. temporaria*. This is consistent with a r-selection strategy, high output of offspring and survival increases with age (Pianka, 1970).

As previously stated, age determination can prove difficult, and be a limitation in this study as individuals of the same age class can differ in size quite extensively. This could lead to errors in the data as some individuals may have been classified as adults, although being subadults, and vice versa. This was especially seen in males, as they are the smallest of the two sexes, and age determination mostly have been based on body size. However, most of these errors are believed to have been avoided by the use of the CMR method, as it gives more reliable data on age (Halliday & Verrell, 1988).

4.2 DISPERSAL BETWEEN DIFFERENT LOCATIONS IN THE POPULATION

A knowledge gap in the Norwegian pool frog population has for long been dispersals. Observations have been made of pool frogs dispersing to new ponds naturally (Direktoratet for Naturforvaltning, 2006), but how many who disperse and where they move to specifically have been unknown. Some of the individuals registered in the database (1+ and older) have displayed dispersal movements, although only a small percentage, 6.14 %. The low dispersal movements can be attributed to that many amphibians demonstrate genuine philopatry (Waldman & McKinnon, 1993). The majority of the dispersal movements occurred in the beginning of the monitoring period, but since then, dispersal have been sparse. This can potentially be due to population size and/ or density as the stock was higher in the past then what it is today, seeing as the population has been experiencing a negative trend. Therefore, based on the registered movements, one hypothesis is that dispersal is influenced by density, as the data shows higher dispersal rate when the population size are higher. Sjögren-Gulve (1998b) had a similar assumption as juveniles are reported to disperse more than adults “probably because of their higher population density”.

Another possible reason for the high dispersal rate in 2007-2010 could be related to precipitation. In a continental population of pool frog in Switzerland higher dispersal rate was observed in a year with more rain per day than the one with less (Peter, 2001a). A similar phenomenon was observed by Sjögren-Gulve (1998b), the majority of movements was registered during warm and rainy nights as the rain prompts a synchronized emigration behavior. Most of the individuals who moved away from the pond returned within few days, while some did not (Sjögren-Gulve, 1998b). In this study, the 2007-2009 period had the wettest summers (June, July and August) in the monitoring period, and the month of July in 2007-2009 was extremely wet (101.5 mm to 244.5 mm) compared to the 30-year average of July months (92 mm) (historical rain data taken from the Norwegian Meteorological Institute). This may

have provided a window of favorable conditions for dispersal, as these summers had high precipitation combined with adequate air temperatures (Appendix IV).

The majority of dispersal events have been from site N to B, with 7 registered movements, where the second highest have been from A to B and vice versa, with 4 registered movements each way, while the remaining movements have been minimal. A reason to why the majority of dispersal movements have been between A, B and N can be because these three locations are within close proximity to one another (150-250 meters in air distance). Especially A and B are close to one another, 160 meters apart and with an almost continuous water passage connecting the two ponds. This distance is within the dispersal range observed in juvenile pool frogs in Sweden by Sjögren-Gulve (1998b). Another contributing factor, beside the vicinity, is that some individuals move away from the pond after breeding season to adjacent marshes where they spend the remaining of the summer (Dolmen, 2012). As these sites are so close, and since there are ample marshes surrounding both these sites, the time spent in the adjacent marshes can work as a springboard to other locations (Sjögren-Gulve, 1998b).

Almost every individual who dispersed, was caught at one site before being caught at second site, where the data indicates the individual stayed until it was not recaptured again, showing only one movement. However, two individuals displayed a different pattern. The first individual dispersed from site A to B before being recaptured at site A again a few years later. While the majority of the small group who disperse appears to move only once, this individual moved twice. The second individual displayed three dispersal events; moving from site N to B, before going back to N within the same year, and staying there, before proceeding to move back again to site B. The same pattern, moving between different sites and back again, has also been observed in the continental pool frog (Peter, 2001a).

Regardless of these two individuals, the data reveal few dispersal movements, and those who relocate from one location to another stays at the second location until it is not recaptured again, presumed dead or wandered to a location which is not inspected. An interesting feature observed in the dispersal model is the high level of emigration from site N, with only one movement back. It is unknown where the individuals who disperse from site N come from, especially as no movements have been registered to site N, only one occasion has been observed. An important notion to mention is the limited monitoring at the other sites in the area, surrounding site A, B and C. Site N is the location, except from A, B and C, that has been most often investigated, although sampling events at this site appear random and sparse. The results from this study does not show dispersal to other sites than these four sites, but that does not mean it does not occur, only that I have no data to document such events. Therefore, there is a

possibility that more dispersal than what has been registered here has occurred. Such permanent dispersal from A, B, C or N to other nearby sites will have been interpreted as mortality, and thus might have negatively affected the estimated survival.

Another important point is that the individuals who have been registered to present with a dispersal pattern are the ones registered in the database, respectively subadults and adults. Therefore, a limitation to the study in the part of dispersal is that only a part of the population has been included. The 0+ age class and a few subadults (with little or no markings) was not included in the CMR models due to the inability to distinguish them from one another. Although dispersal has been seen to occur in every life stage, and not just limited to a specific life stage (Peter (2001a), juveniles seem to be more prone to disperse than adults (Dolmen, 2012; Sjögren, 1991; Sjögren-Gulve, 1998b). Unfortunately, my data makes it impossible to study juvenile dispersal. Dolmen (2012) have reported observations of dispersal in the 0+ age class, and my dispersal results are only based on registered subadults and adults, therefore, this study, and previous observations, fit with what is seen in Peter (2001a), dispersal is not limited to a specific life stage. Peter (2001a) argue that females disperse more when young while males disperse more when older. This is not represented in my result. However, a similarity between what was seen by Peter (2001a) and my study was that males seem to have a slightly higher relocation rate than females, as 10 males and 7 females (Appendix III) have been registered to disperse between different locations.

In regards to the high movements from site N, Dolmen (2012) observed that the new ponds which was dug up at site N as a biotope improvement measure, appears to serve as a nursery area for juveniles. This could explain the amount of dispersal events *from* site N compared to other sites, as 0+ and unregistered subadults might use site N as a nursery before moving away as they get older and/ or reaches maturity. At most, 0+ have been registered to move up to 750 meters, as the crow flies, and older frogs up to 400 m (Dolmen, 2012). Results from my study included three individuals who moved up to 1.1 kilometers, as the crow flies, from site N to C and from C to B. This corresponds with the findings of Sjögren-Gulve (1998a), who concluded that individual pool frogs in Sweden move < 1 km to other ponds. However, site N and C are connected through a system of tiny streams, which can provide an easier migration route for the individuals to take. Since lengthy dispersal were only seen in 3 out of 17, and since the remaining individuals moved at shorter distances (up to 300 m), such distances are presumed to be unusual. Dispersal rate will decrease with increasing distance (Peter, 2001a) and dispersal distance depends much on the environment (Söderman et al., 2015). As numerous streams are connecting the multiple sites in this study area some movement was expected.

Sjögren (1991) monitored a population of pool frogs in Uppsala from 1984-1987 which showed 3.5 % of juveniles migrating to a neighboring population, where only 1 % of adults exhibited the same behavior. In Peter (2001a), water frog dispersal between ponds can be up to 12.2 % per year, where the pool frog displayed the highest dispersal rate. My results are in-between of what has been reported in other studies, higher than the Swedish study, but lower than the Swiss study. Both of these studies has been conducted in a shorter time period than my study, therefore this may impact the results, as well as other environmental factors, e.g. precipitation has been implicated in dispersal rate (Peter, 2001a; Sjögren-Gulve, 1998b).

There were several noticeable dispersal events made from site C to E and F in 2011, however, multiple individuals were relocated to and from these specific locations (Direktoratet for Naturforvaltning, 2006) during the same period those events were registered. Therefore, those movements were after all likelihood man-made and have therefore not been included in the dispersal data. An interesting feature however was the low number of dispersals from site C, which is curious as the population at location C is the largest. I expected to see that the rate of dispersal would be higher at sites with a higher population, but according to the data this does not appear to be the case. However, this may be contributed to habitat, as site C has a more suitable habitat with more edges and lagoons than e.g. site A. Perhaps dispersal occurs due to limited space and increased competition. Therefore, a hypothesis may be that dispersal from site C has been sparse as there has been less need to relocate. Another possibility may be that the areas surrounding site C has been underexamined, although the sites around C are few to none, beside A, B and N (1.1 kilometer as the crow flies), as well as they are less than optimal (Lydersen et al., 2020). A possibility is that there is little to no dispersal within this population or the majority of the dispersal events is done by individuals who is a) not recaptured, or b) too young to be included and registered in the database.

Another thought has been how the pool frogs orientate themselves in the environment and find their way to other pool frog locations. Perhaps they can smell their conspecifics or maybe sound impacts or contributes and lead them to the correct sites. Dispersal movements in the Swedish population indicates that the movements observed are target-oriented movements (Sjögren-Gulve, 1998b). The data so far, or lack thereof, points to no dispersal to or from either site D, E, F, or S, although some 0+ dispersal has been observed at a few of these sites, prior to the study period (Dolmen, 2012). However, there is a possibility of more dispersal within the population than what has been registered.

4.3 RECRUITMENT

The common trend seen in the recruitment figure is the inconsistency of number of females compared to 1+/ female. This was especially noticed in 2018, when a total of only 4 females were observed, but recruitment was higher than it had been for years. This can indicate that there is no apparent correlation between the number of females in the population and the number of 1+/ females, but that other factors, e.g. climate or predators, may have a higher impact. However, this is only the subadults that survived the winter at that same location, and who was captured, this does not necessarily match the reality, although it appears to fit as the trend is quite clear and the dataset is over so many years. Regardless, reproduction have occurred but to a varying degree, and most often unsuccessful. There is overall low survival of number of 1+/ females in almost every year for the three breeding sites, except for 2018 and 2007. There are some variations within the different sites, but the overall trend is clear. Neither site A nor B has had any good recruitment years since 2007, while C has had low recruitment since 2010, consistent with previous observations (Reinkind & Engemyr, 2019).

After 2010, recruitment has been low or close to none existent, and only a few years have showed decent recruitment. Site A has consistently showed low or no recruitment, same as site B, while site C has had the best recruitment in the last 12 years. However, 2018 have shown to be the best recruitment year since 2010, at all sites, which most likely can be contributed to the unusually warm and long summer (Reinkind & Engemyr, 2019)(Appendix IV).

Based on the number of subadults found in 2007, 2006 has been, and are still the best reproductive year based on data from this study, for all three breeding sites. This was also the year with the highest 1+ recorded in the 12-year period. Since 2007, the number of juveniles surviving their first winter has decreased for almost every year, with a few exceptions. Based on number of subadults observed, 2007, 2010 and 2018 has been the best years, but when including the 1+/female results, 2010 is removed and replaced by 2011. Although the result from 2010 is not far from 2011, 2011 was still a better year based on 1+/ females.

A limitation is linked to the recruitment results, as some subadults has only been counted, and not included in the database due to some may have underdeveloped pattern, similar to what is seen in 0+. Hence, there may be a possibility that some subadults have been counted twice, leading to an over-estimation of recruitment. However, it was not usual to observe under-developed pattern in subadults.

During the monitoring period, site A has consistently had low recruitment of subadults, where the highest number of subadults was found in 2007 and 2019, most likely linked to the

favorable summer temperatures experienced these years (Dolmen, 2012; Reinkind & Engemyr, 2019). Site A experienced a doubling of its female stock from 2007 to 2008, this may be linked to the high recruitment from 2006, as well as some individuals dispersed to site A that year. The 2009-2010 winter was extremely cold, and Dolmen (2012) stated that that winter probably decimated the pool frog population, especially the 0+ age class, however, analysis of subadults and winter temperature did not show much, if any, support. Therefore, it does not appear the survival of subadults are particularly impacted by winter temperature. While my results show no decrease in adult survival this winter, site A experienced a decrease in females from 09-10, only a few individuals lost at site C, while site B experienced an increase in its female population during that same period. The decrease observed at A and C might be attributed to natural mortality. The increase in females at site B is most likely linked to dispersal, as 2010 was a year where site B received some dispersing individuals from e.g. site N. Site A experienced some subadult survival after winter, but remained low until 2014, when the summer temperatures increased and the highest number of 0+ were observed, but unfortunately, none were rediscovered the next year.

An important factor that is known to contribute to declines and are the biggest threat to frogs is predatory fish (Collins, 2010; Reinkind & Engemyr, 2019). Predatory fish has been observed in the pond at site A and C (2015-2018) (Reinkind & Engemyr, 2019), and based on the documented effect fish has on anurans (Duellman & Trueb, 1994), an impact was expected. However, data analysis based on recruitment and the effect fish has had on reproduction at the three breeding sites had little support. Based on model selection of recruitment, the model that included the effect of fish was not supported, the best model included only summer temperature, which was significant. Indicating that reproduction is more dependent on summer temperature than the impact of fish. Although the results indicate this, it does not mean fish does not have a negative impact, it simply means that I have no data that can document the effect. Perhaps reproduction was low these years due to colder summers, therefore no significant effect was observed. Luckily the fish was mostly removed from the sites by the 2018 summer (L. Korslund, personal communication, 2020).

Site A had no registered subadults in the period 2014-2017, including a decrease in observed females, where only 1-2 females were registered in 2015-2018. A similar feature was seen at site C, although a few subadults was registered as this population is larger than site A. As there were no sampling events in the fall of 2015 or 2016, there is little to know or be said about recruitment from these years, where the only information available are from the spring monitoring. There were no 1+ registered, which suggests that either winter survival was

extremely low, little recruitment occurred or that the recruitment from these years were hit hard by predation from fish, or all three.

Site B has had the lowest number of observed females of the three breeding sites. Aside from this, the site has had a higher number of 1+ female than site A, except for in 2018. This can be linked to dispersals as mentioned, especially as the recruitment model is based on subadults found the following year, and 0+ are too small to include in the database due to the high source of error. Or, it can possibly be linked to female fecundity and link to body size, (described in more detail later), as the females at site B perhaps have higher quality offspring than those at site A. Another possibility may be the improvements made at site B during the first management plan, where new ponds were dug up to expand and improve the habitat. It might also be a mix of the three. As a part of the biotope improvements from the management plan, new ponds were dug up at site B, but location A was not subjected to the same measures (Dolmen, 2012). The expansion of the habitat might have increased the survival at site B. Dolmen (2012) observed that the excavated ponds at site B have been used by both juveniles and adults throughout the summer seasons. He noted that the enlarged habitat has provided the frogs with more space and less competition, which can minimize the intraspecific predation, as the pool frog can be cannibalistic. Therefore, it is likely that the new ponds have increased juvenile survival (Dolmen, 2012). The question that remains is, why has there been such a steady decline if the improvements made have been sufficient. This is most likely contributed to the low recruitment experienced, low recruitment leads to low population input.

There were no registered females at site B from 2013 to 2015, despite that, one individual in the age class 1+ was caught in 2016. Due to the absent sampling event in 2015, there is uncertainty in whether reproduction occurred, or if the observed 1+ is an immigrant from another site. Beside a few occurrences, no recruitment has been registered at site B in the 2013 to 2017 period. The best 1+/female at site B has been in 2007 and 2018, equal to what has been seen at the other sites, although the best recruitment period, based on number of captured subadults was in the beginning of the monitoring period (2007-2008). However, of the three breeding sites, site B has been the poorer one based on counted females and recruitment, and has, like site A, experienced low recruitments of subadult in the monitoring period.

Site C has, throughout the monitoring period had higher number of observed females and recruitment than A and B, where the best years, equal to A and B, been in the beginning of the sampling period. Also here was there a decline in 1+ captured in 09 and 10, which have been assumed to be linked to winter condition, as previously mentioned. 2010 was a decent recruitment year at site C, both with number of 1+ found the following year, but also in regard

to 1+/ female. This was the last good year before the warm summer of 2018. The female part of the population experienced a 50% decline in 14-15, one of the two years with lowest survival rate. This could be linked to winter temperature as weight loss is higher in warmer winters and females have higher loss than males (Holenweg & Reyer, 2000), but this was also the year where fish arrived in the pond. The combination of, increased predation, decreased prey available and lower body weight may have been too high. However, this might also be the case of natural mortality as the frogs get older. Reproduction took a hit in the years with fish predation as there has not been observed such a low number of recruitments at site C as in 2014-2017. Fortunately, the breeding population saw a peak in reproduction in 2018, the best reproductive year of all, based on 1+/ female. Site C has consistently been the best of the three sites, which may be explained by abiotic factors, e.g. pH, as site C are the better of the three sites (Lydersen et al., 2020).

An expectation was that the number of females was connected to recruitment. However, it appears that recruitment is not linked to number of observed females. It could rather potentially be linked to the females age and/ or body size. Fecundity (clutch weight) in females have been positively correlated with body size in multiple amphibian species (Halliday & Verrell, 1988; Kuramoto, 1978). Body size and age have also been linked, though that link is usually quite weak, especially when including the typical size differences seen in body size within an age class (Halliday & Verrell, 1988). However, outside factors which also impact the recruitment per year must be included, e.g. predators and climate. The grass snake, who prey primarily on amphibians (Kauri, 1981b), have been observed at all three breeding sites, but any potential impact has not been tested. Brown trout have also been registered in the pond at site A and C from 2015 to 2018 (Reinkind & Engemyr, 2019). As previously mentioned, to have fish in the ponds was expected to have an impact on recruitment, however, my results do not support this.

Environmental factors is also expected to contribute to a more or less favorable climate (see 1.4.1 for details) as the northern pool frogs have high requirements for habitat (Dolmen, 2012) and climate for the species to have a successful breeding season (Reinkind & Engemyr, 2019). Since the beginning of the monitoring period (1996), the years with the best reproductive success have been 1997 and 2006, both of these years' hallmarks were high summer and winter temperatures (Reinkind & Engemyr, 2019). These are the only two years, in addition to 2018, that have had this combination, but decent summers with reproduction could be compensated with milder winters according to Reinkind and Engemyr (2019). Reproduction is significantly impacted by summer temperature, and experiences reproductive failures in cold summers, equal

to what Sjögren (1991) have reported seeing in the Swedish population. However, even though the population experiences failure in cold summers, climatic adaptation has reported in pool frog larvae, as those from higher latitudes (northern) develop faster than those from low latitude (southern) (Orizaola, Quintela, & Laurila, 2010). Similar to *R. temporaria*, where the northern larvae have a faster development than the southern, although this was assumed to be influenced by climate rather than genetics (Laugen, Laurila, Rasanen, & Merila, 2003).

An idea was whether fertility could be impacted as the population has low genetic diversity (Reinkind & Engemyr, 2019) and the genetic diversity will continue to decrease with each generation and smaller population size (Browne & Zippel, 2007). However, as this has not been tested in the Norwegian population, the closest comparison I can draw is to the Swedish population. Although this breeding population is higher than the Norwegian (Reinkind & Engemyr, 2019; Tegelstrom & Sjogren-Gulve, 2004), lower genetic variability was not associated with lower fertility or reduced viability (Sjögren, 1991). However, an important notion to point out, the Swedish population is larger than the Norwegian, and although this is not a threat to the Swedish population does not automatically mean it is not a threat to the Norwegian population. Therefore, it would be interesting to look at this in the Norwegian population as well, to see whether the findings are the same or if there is a difference between the two.

4.4 MANAGEMENT

The population has, in the monitoring period (2007-2019), experienced a 85% decline in size (from estimated 115 to 17 adult individuals). The species has consistently experienced reduced reproduction, and therefore had little recruitment to the breeding population (Reinkind & Engemyr, 2019), which is seen 2 to 3 years later in the population (Sjögren, 1991). The population is now at a critical low and the extinction risk is high if the population does not recover (Reinkind & Engemyr, 2019). Although, the population do show large fluctuations in population size (Dolmen, 2012), similar to the Swedish populations (Sjögren, 1991). Earlier data (found in management plan 2019-2023 (Reinkind & Engemyr, 2019)) shows that the population was around the same size as it is now, back in 1996, but increased drastically the next years, thanks to a very good reproductive year. This suggests that the population only requires a few good years with decent survival before an increase can be seen in the population. Which is why 2018 was such a valuable year as the population experienced high recruitment and several subadults was captured the following year, which suggests good winter survival

among the subadults as well. It appears the 2018-2019 winter conditions was decent as multiple 2+ have been observed in 2020 so far (L. Korslund, personal communication, May, 2020). This may indicate that we can be cautiously optimistic. It appears the high summer temperature and successful reproduction experienced in 2018, combined with good winter survival, might lead to a much-needed increase in the population. As well as a relief for the breeding population, with an increased input of individuals, who next summer might contribute to reproduction.

An important notion to point out, the discovered 2+ may not only be recruitment from 2018, but perhaps some are a result of the breeding program in Kristiansand Zoo. Twenty-five 1+ were released to site A and B in the summer of 2019, where the hope is that the program can provide some success and much needed release to the small population. Therefore, it will be interesting to see if the released individuals from the Zoo has survived the winter and can contribute to the population when they reach sexual maturity. It will likely benefit the population to keep the breeding program in the Zoo and have a “reproduction backup” for when reproduction fails or explore other options either *in* or *ex situ*.

Climatic adaptations have been seen in the pool frog larvae, as they grow and develop quicker than what has been observed for the continental species (Orizaola et al., 2010), and this can be of high importance to management. This might mean that measures and improvements can be of higher importance to species survival than previously thought. However, failed reproduction still occurs in cold summers, and my results indicate that successful reproduction is highly dependent on summer temperature. Although many biotope improvements have been proposed and implemented in the area, the species have high habitat requirements. It seems that most of the improvements have not had the desired effect as no long-term success have been achieved (Dolmen, 2012; Reinkind & Engemyr, 2019). A proposed measure is to use the experience from the 15-year period to improve the measures already implemented that was not good enough. Fish removal from surrounding lakes, could create an ideal situation for pool frogs to expand and to form new breeding grounds, however, as this is in direct and significant conflict with recreational fishing, it has not gone further than an implied measure (Dolmen, 2012). Further, the results from this study suggest that a cold summer alone is enough to effectively limit recruitment, regardless of whether there is fish present or not.

From before, knowledge on dispersal in the system was minimal. We now know more about the rate and direction of adult dispersal, but we do not know whether dispersal occurs via waterways or not. It is likely that the individuals use streams which partly connects many of the sites, or marshes when the water level is higher, as no pool frogs have been discovered to be

killed on the road (Direktoratet for Naturforvaltning, 2006). However, this is also an area in need of more research.

As the pool frogs hibernate below the ground (Sjögren-Gulve, 1998b), and since the survival model that included estimated ground winter temperature had some support, it would be beneficial for management to investigate location of hibernation habitat. It might lead to better management actions if we knew more about the location of hibernation spots and their quality. Heavy machinery are used in the forestry in the area, and such traffic might impact hibernation sites (Reinkind & Engemyr, 2019).

A very controversial suggestion is looking into adding new genetic material to the population. Reinkind and Engemyr (2019) has stated that a population with higher genetic variation would be positive in regard to vulnerability to internal and external factors. Since the British pool frogs went extinct (T. Beebee et al., 2005), Swedish pool frogs has been released in Britain (2005-208), counting approx. 1500 individuals (Reinkind & Engemyr, 2019). This might be a potential solution to the low population size and low genetic diversity, to release a few individuals from the Swedish populations into the Norwegian to increase population size and genetic variation. This suggestion is made on the basis that it has been done in Britain, and appear successful, however, any introduction from another population should not be made lightly. Although I am cautiously optimistic about the population size today, due to recent observations, this might be a topic to discuss, with a critical eye, in the future, if the population are dangerously close to extinction.

Management actions can be challenging as they are often made under high levels of uncertainty (Sterrett et al., 2019). This is especially noted in the Norwegian pool frog population as this species have, since the discovery, had a significant knowledge gap. The goal is that some of these knowledge gaps will be one step closer to being filled by the results from this study.

5 CONCLUSIONS

Adult survival, in this study, has been higher, and more consistent than what was anticipated, and less impacted by temperature than previously believed. There seem to be a small positive relationship between survival and ground temperature in winter but does this does not appear to have a strong effect on survival as the survival is relatively constant between years. Recruitment has remained low almost throughout the entire study period, where the number of females and subadults per females has remained inconsistent. Indicating that other factors than the number of females affect reproduction. Recruitment is strongly and positively affected by summer temperature. Adult dispersal occurs within the population, although at a low percentage, and one site received the majority of the dispersing individuals. A few individuals have been seen to disperse over a large distance, although this is probably rare. The majority of dispersals occurred in the beginning of the monitoring period and may be connected to precipitation or to a higher population size. Questions remain whether dispersals are impacted by density or environmental factors, therefore more research is necessary.

There is still much to learn about this secretive species that is part of our fauna. Therefore, further research is vital to provide updated information to management. With more information, management authorities can make well-informed decisions on how to rightfully manage and preserve this threatened population of high conservational value.

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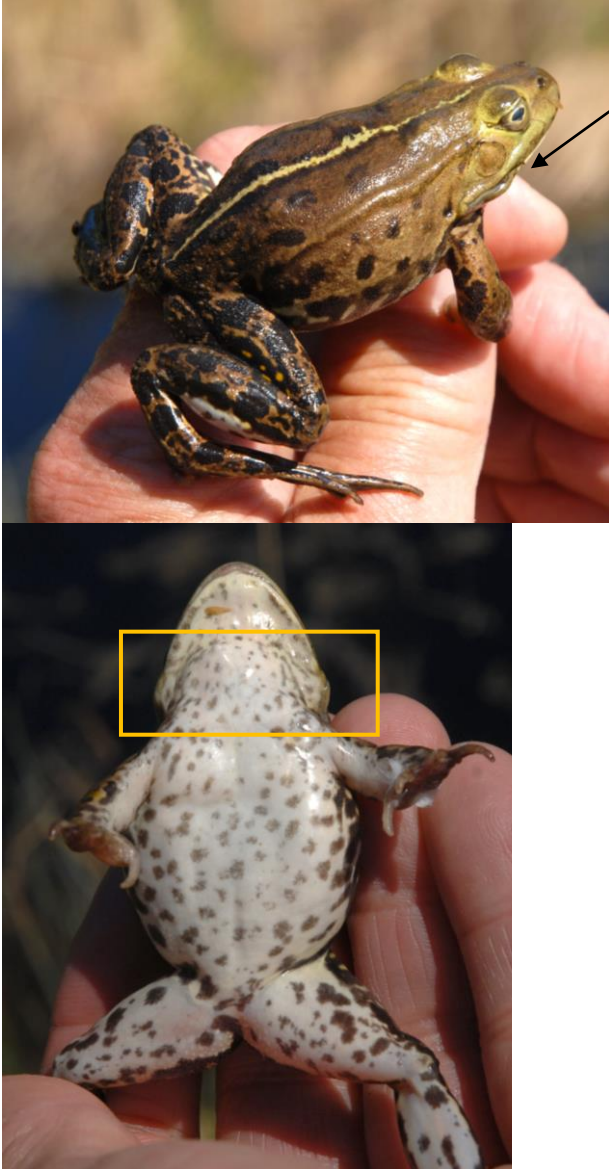

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APPENDIX I: SEX AND AGE DETERMINATION

How to differentiate between gender and age.

	<p>1. Male adult</p> <p>Smaller than the female (Dolmen, 1997).</p> <p>Croak bags by the jaw</p> <p>Males have a broader and more distinct jawline, see marked yellow. Where the jaw begins, it goes a bit outward, characterized by males, as females lack this (L. Korslund, personal communication, August ,2019).</p> <p>Sexual color dimorphism – see details in 1.3.3</p> <p>Categorized as an adult due to size, but also because this is a recaptured ind. and we can say something about the minimum age based on recapture history. This specific ind. is minimum 4 years old as it has been recaptured in 4 consecutive years.</p>
	<p>2. Female adult</p> <p>Bigger and ticker than the males (Dolmen, 1997).</p> <p>No distinct jaw line (see yellow marking) (L. Korslund, personal communication, august, 2019).</p> <p>Sexual color dimorphism – see details in 1.3.3</p> <p>Categorized as an adult due to size, but also because this is a recaptured ind. and we can say something about the minimum age based on</p>

	<p>recapture history. This specific ind. is minimum 4 years old as it has been recaptured in 4 consecutive years.</p>
	<p>3. 1 +</p> <p>1+ can be hard to distinguish between as they vary significantly in size. Both individuals in photo 3 and 4 are 1+. We know this individual (Photo 3) is 1+ as this was an individual breed in Kristiansand Zoo and released in spring 2019 which was later recaptured at the same location it was released.</p>

	<p>4. 1 +</p> <p>This individual is very similar to photo 5 in size but is categorized as 1+ as it was found during spring (May). Due to reproduction time for pool frogs, details in 1.4.1, this ind. cannot have been born the year it was captured (D. Dolmen, personal communication, April, 2020).</p>
	<p>5. 0 +</p> <p>This ind. was found during the fall sampling event and is categorized as a 0+ based on size and time of year it was caught.</p>

APPENDIX II: RECRUITMENT TABLES FOR FEMALES AND OFFSPRING

The column with "1+ female" is from the year recruitment occurred, not the year 1+ was found. E.g. females from year 2007 is 6, the following year, 5 1+ was captured. These are included in recruitment from year 2007, therefore the column with 1+ female is in row 2007 ($5/6 = 0.83$).

A	Females	0+	1+	1+ female
2007	6	0	42	0,83
2008	15	1	5	0,067
2009	11	1	1	0
2010	6	4	0	0,67
2011	3	2	4	1,67
2012	2	2	5	1
2013	6	1	2	0
2014	4	11	0	0
2015	2	0	0	0
2016	1	0	0	0
2017	2	0	0	1
2018	1	10	2	15
2019	3	8	15	

Table 1. Number of observed females, 0+, 1+ and 1+ female from each year at location A

B	Females	0+	1+	1+ female
2007	7	0	38	2,86
2008	8	1	20	0,38
2009	4	0	3	0,75
2010	9	0	3	0,33
2011	3	3	3	1,67
2012	2	0	5	1,00
2013	0	0	2	0,00
2014	0	2	0	0,00
2015	0	0	0	1,00
2016	1	0	1	0,00
2017	2	0	0	0,00
2018	1	2	0	5,00
2019	3	0	5	

Table 2. Number of observed females, 0+, 1+ and 1+ female from each year at location B

C	Females	0+	1+	1+/ female
2007	16	13	70	4,5
2008	18	1	72	0,8
2009	15	2	15	0,7
2010	12	2	11	3,8
2011	11	5	45	2,6
2012	9	3	29	1,1
2013	10	3	10	0,4
2014	8	5	4	0,5
2015	3	0	4	1,3
2016	3	0	4	1,0
2017	5	0	3	0,0
2018	2	16	0	18,0
2019	2	7	36	

Table 3. Number of observed females, 0+, 1+ and 1+/ female from each year at location C

A, B, C	Females	0+	1+	1+/ female
2007	29	13		3,3
2008	41	3	97	0,5
2009	30	3	19	0,5
2010	27	6	14	1,9
2011	17	10	52	2,3
2012	13	5	39	1,1
2013	16	4	14	0,3
2014	12	18	4	0,3
2015	5	0	4	1,0
2016	5	0	5	0,6
2017	9	0	3	0,2
2018	4	28	2	14,0
2019	3	15	56	

Table 4. Number of observed females, 0+, 1+ and 1+/ female from each year at location ABC.

APPENDIX III: DISPERSAL MOVEMENTS WITHIN THE POPULATION

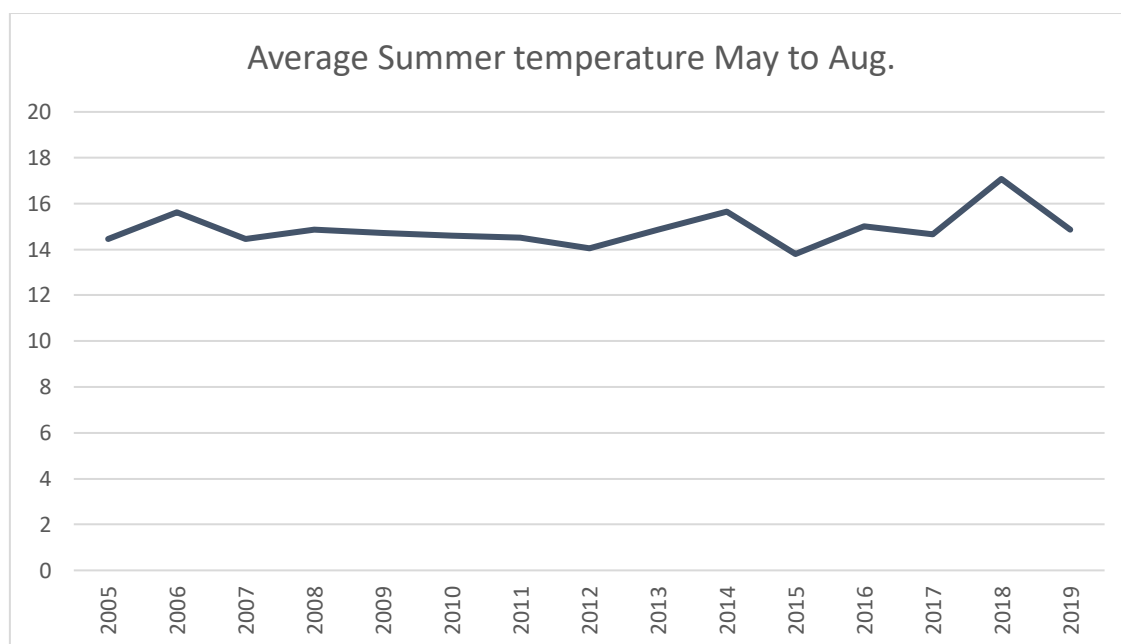
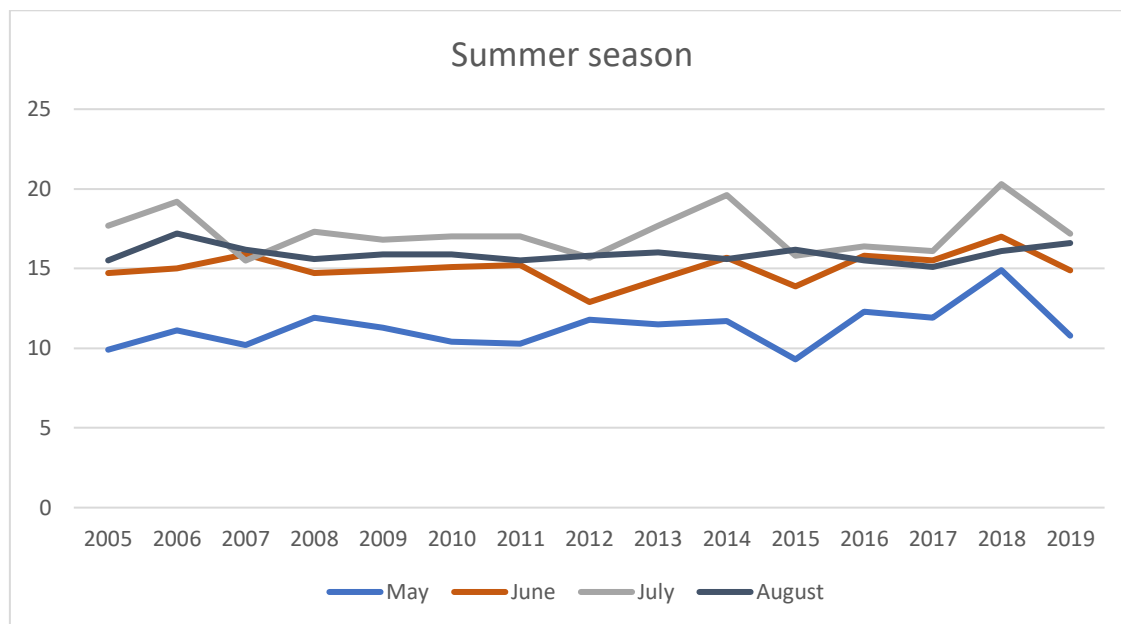
Dispersal movements within the Norwegian pool frog population at each site movements have been registered in the database created. The dispersal figure is based on these data.

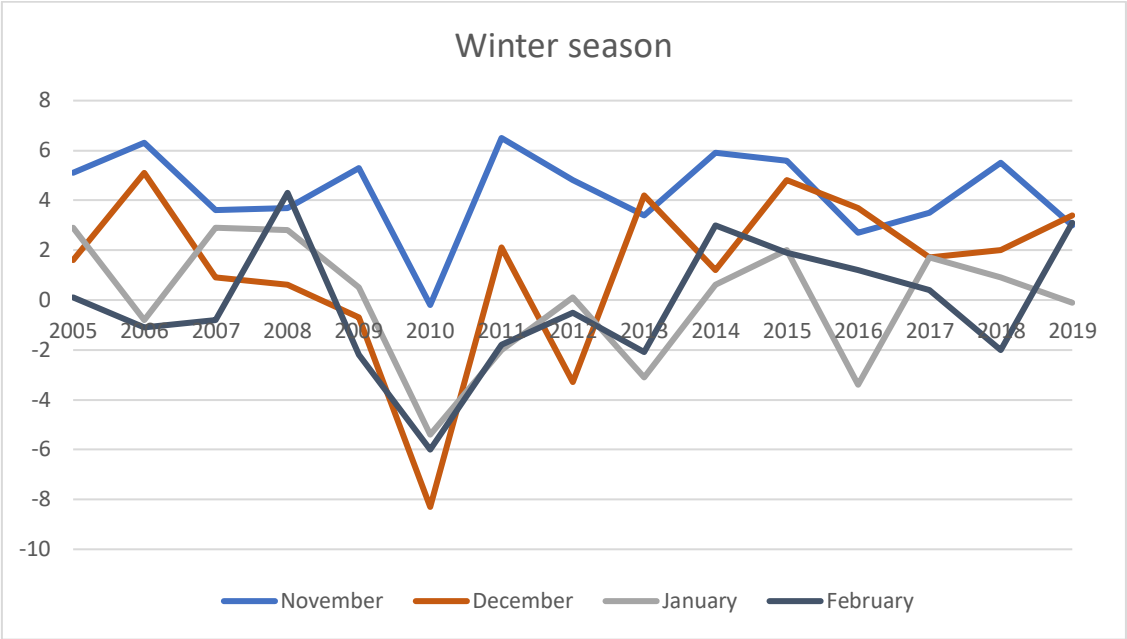
Ind.	Sex	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
003	M										B	A	A	
024	M	B	A	A		A	A	A						
035	M				N	A	A	A						
045	M	A	B			A								
062	F	B	B/A	A										
066	M		A	B										
083	M	A	B											
091	M	A	B											
156	F		N/B	B										
164	M		N	B										
171	F		N	N/B	N	N	B							
181	F		N	N	B									
185	F			N	N/B	B	B							
186	F		N		B									
258	F			N	N	C	C							
416	M										C	C	B	B
436	M											C	B	B

Table 5. Dispersal movements for each individual who relocated from the place they were first captured to a second or third location. The letter in each route is the location where each ind. was captured and the year. Where the / is used, this is to illustrate that the individual was found at both locations in the same year.

APPENDIX IV: TEMPERATURES

Average air temperature in °C for each month. Data from Landvik weather station (13 km away) and the meteorological institute.





APPENDIX V: SNOW AND TEMPERATURE MODEL

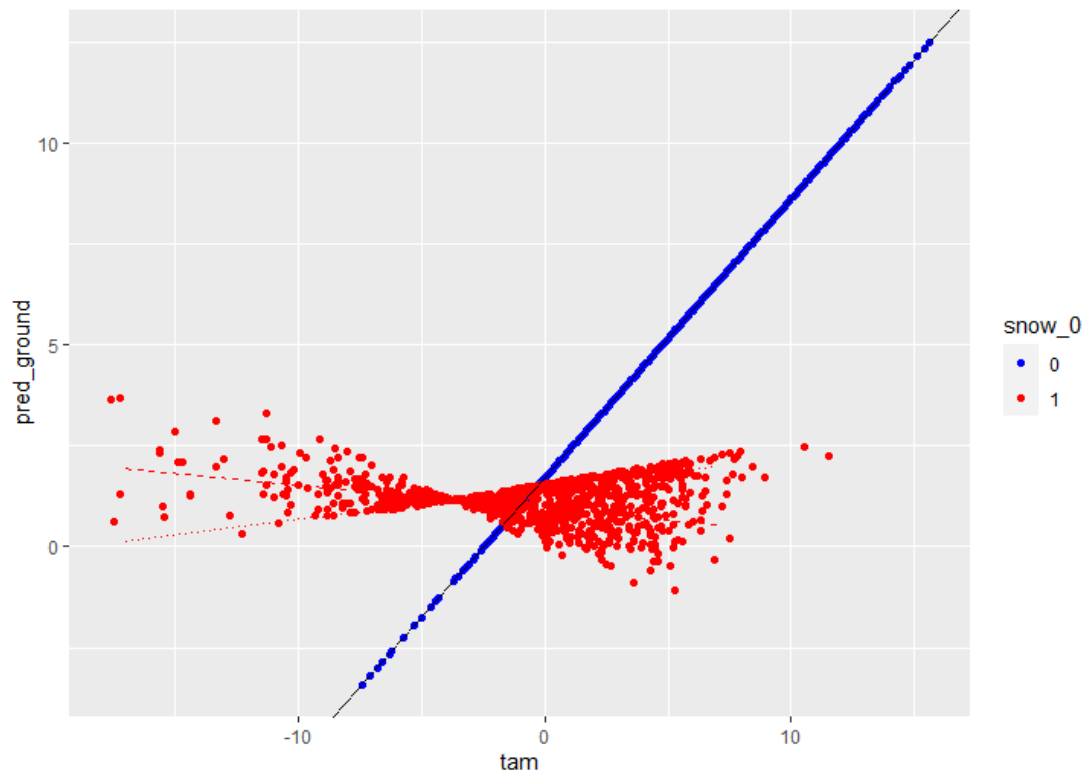


Figure 1. Predicted data from site A in the period 2007 to 2019. This model is used when estimating ground temperature in winter for the same period, with low and high temperature and snow (red). Model created in program R. x-axis represents air temperature and y-axis predicted ground temperature. Model created by L.Korslund.

Winter	Ground temperature °C	Snow depth	Days with snow
2007/2008	4.11	2.92	40
2008/2009	3.83	4.18	73
2009/2010	3.20	22.87	117
2010/2011	2.96	14.74	126
2011/2012	4.66	3.71	49
2012/2013	2.49	21.33	142
2013/2014	4.52	10.01	61
2014/2015	4.90	1.85	41
2015/2016	4.27	6.87	72
2016/2017	4.22	2.48	44
2017/2018	3.19	17.06	119
2018/2019	4.32	6.20	67

Snow depth: Average snow depth from October to April (Based on modeled data from xgeo)

Days with snow: Every day with at least 0.1 cm snow from October to April (based on modeled data from xgeo).

Ground temperature: Modeled average ground temperature based on snow depth data from xgeo and air temperature data from the Landvik weather station (13 km away).

APPENDIX VI: RECAPTURE PROBABILITY

Model selection for recapture probability.

Model no.	Models	No. of parameters	AIC _c	Δ AIC _c	Deviance
1	$\varphi_{t^*g} p^{15+16}$	26	1140.19	29.94	289.78
2	$\varphi_{t^*g} p_g$	26	1140.19	29.95	289.79
3	$\varphi_{t^*g} p$	25	1141.08	30.83	292.87
4	$\varphi_{t^*g} p_t$	36	1152.85	42.60	280.08
Global	$\varphi_{t^*g} p_{t^*g}$	48	1164.96	54.70	264.22