# Reproduction and Health of Moose in Southern Sweden

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Doctoral Thesis
Swedish University of Agricultural Sciences
Uppsala 2014

Acta Universitatis agriculturae Sueciae
2014:19

Cover illustration by Jonas Malmsten

ISSN 1652-6880
ISBN (print version) 978-91-576-7986-4
ISBN (electronic version) 978-91-576-7987-1

Print: SLU Service/Repro, Uppsala 2014

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## Abstract

Moose (*Alces alces*) is a highly regarded game species in Fennoscandia, where annual harvest numbers in Sweden, Norway, and Finland together exceed 200,000 animals. For successful management, knowledge about male and female reproduction is essential, as well as the extent to which disease and mortality affect the population.

In 2006, a sub-normal reproductive output (calf per cow ratio) was reported from the island of Öland, and a pilot study in 2007 revealed embryonic mortality and occurrence of the tick-borne pathogen *Anaplasma phagocytophilum*. An expansion of the study (including control areas) was conducted due to the need for updated information on moose reproduction

From 2008 to 2011, reproductive organs, blood, spleens, mandibles, and ectoparasites were collected from moose in three areas in southern Sweden. Reproductive organs were inspected macroscopically, weighed and measured, and sperm samples were taken. Morphology of spermatozoa, chromatin analyses, histological examinations, and pathogen analyses were performed at SLU or SVA in Uppsala.

Male pubertal age varied from 1.5 to 3.5 years, and the proportion of normal spermatozoa increased significantly with increasing body weight, but decreased temporally over the first month of hunting. Male moose had a low testes:body weight ratio compared with other cervids. Cows showed their first oestrus of the season earlier than heifers, and the hunting period appeared to interfere with oestrus in all females. Onset of puberty in females was positively associated with body weight but not with age. Embryonic mortality and unfertilized oocytes accounted for a significant difference ($P<0.01$) between ovulation rates and the proportion of viable embryos found in pregnant females. Moose were competent hosts of *Anapasma phagocytophilum*, and the prevalence of infection, as determined by PCR, varied both temporally and spatially. Moose calf summer survival rates on Öland were significantly lower than in the mainland populations.

The studies performed provide updated information on moose reproductive characteristics, calf survival and moose health. Some changes in population management could potentially improve the reproductive success of moose in southern Sweden. Not all of these parameters might be affected by a change in management, as the surrounding environment and climate play a considerable role in forage availability, the spread of diseases, and calf survival rates.



Keywords: Alces alces, Anaplasma phagocytophilum, corpus luteum, corpus albicans, epididymis, embryonic mortality, female and male reproduction, moose management, sperm morphology, testis

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# Dedication

To my friend Andreas Jonsson (1977-2010). You are always with me.

*The moose will, perhaps one day become extinct; but how naturally then, when it exists as a fossil relic, and unseen as that, may the poet or sculptor invent a fabulous animal with similar branching and leafy horns ... to be the inhabitant of such a forest as this!*

David Henry Thoreau

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# List of Publications

This thesis is based on the following papers, referred to by Roman numerals in the text:

I Malmsten J., Söderquist L., Thulin C.-G., Dalin A.-M. (2014). Characteristics of spermatozoa and reproductive organs in relation to age and body weight in Swedish moose (*Alces alces*). *Submitted*.

II Malmsten J., Söderquist L., Thulin C.-G., Gavier Widén D., Yon L., Hutchings MR., Dalin A.-M. (2014). Reproductive characteristics in female Swedish moose (*Alces alces*) with emphasis on puberty, timing of oestrus, and mating. *Submitted*.

III Malmsten J., Dalin A.-M. (2013). Embryonic mortality and unfertilized oocytes in Scandinavian moose (*Alces alces*). *Acta Theriologica*, DOI: 10.1007/s13364-013-0173-6.

IV Ericsson, G., Malmsten J., Neumann W., Singh N., Dalin A.-M. Summer calf survival of Scandinavian moose along its southern distribution range. *Manuscript*.

V Malmsten J., Gavier Widen D., Rydevik G., Yon L., Hutchings MR., Thulin C.-G., Söderquist L., Aspan A., Stuen S., Dalin A.-M. (2013). Temporal and spatial variation in *Anaplasma phagocytophilum* infection in Swedish moose (*Alces alces*) *Epidemiology and Infection*, DOI: 10.1017/S0950268813002094.

# Abbreviations

| | |
|---|---|
| AP | *Anaplasma phagocytophilum* |
| CL | Corpus luteum |
| DFI | DNA fragmentation index |
| HGA | Human granulocytic anaplasmosis |
| PCR | Polymerase chain reaction |
| PNS | Proportion of normal spermatozoa |
| SLU | Swedish University of Agricultural Sciences |
| SVA | Swedish National Veterinary Institute |

# 1 Introduction

The moose (*Alces alces*) is the largest member of the deer family (*Cervidae*) and is one of the largest ungulates of the northern hemisphere. It inhabits most circumpolar countries and, as a game species, is a highly regarded source of recreation, and meat (Boman *et al.*, 2011). The Eurasian moose (*Alces alces alces)* roams the taiga forests of Fennoscandia, the northeastern parts of continental Europe, Russia, and part of Belarus and Ukraine (Figure 1). North America hosts four subspecies of moose; the tundra-living Alaskan moose (*Alces alces gigas*), the western American moose (*Alces alces andersoni*), the eastern American moose (*Alces alces americana*), and the Shiras moose (*Alces alces shirasi*), which inhabits the middle parts of the Rocky Mountains, (Cronin, 1992).

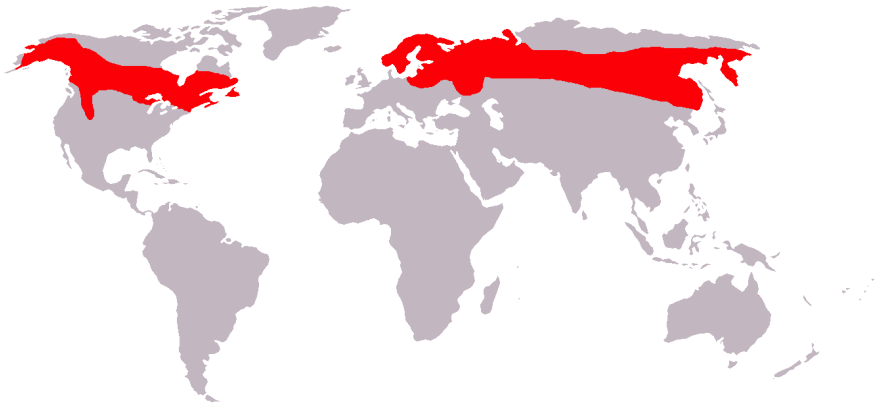

*Figure 1.* Estimated worldwide distribution of moose (Jürgen Gbruiker, Wikipedia®)

Moose are selective browsers and in Fennoscandia they feed mostly on birch (*Betula* sp.), Scots pine (*Pinus sylvestris*), aspen (*Populus tremula*), juniper (*Juniper communis*), willow (*Salix* sp.), and rowan (*Sorbus aucuparia,* Hörnberg, 2001a). In Sweden, moose density is among the highest in the world; local densities can reach 3 moose per $km^2$ in parts of Sweden, but generally range from 0.5 to 2 per $km^2$ (Hörnberg, 2001b). Due to high densities, the high availability of forage, and subsequent successful reproduction, the total harvest of moose in Fennoscandia exceeds the total harvest of all other countries inhabited by moose. From an estimated population of almost 400,000 animals, 90,000-100,000 are harvested each year in Sweden (Kindberg *et al.*, 2010), providing approximately 8.5 million kg of moose meat annually. The value of moose hunting has been estimated at 1.45 billion SEK (Boman *et al.*, 2011).

The moose population in Sweden increased substantially in the 1970s after a systematic change in forest management practices, but also due to a decrease in the number of moose harvested, and low densities of large predators (Cederlund & Markgren, 1987; Hörnberg, 2001b). Current moose habitats are affected by human activities. The single most important aspect of moose browse availability in Fennoscandia is forest management (Andrén & Angelstam, 1993). Modern forest management strategies that involve clear-cutting create large quantities of browse within a few years, and after being replanted with Scots pine or Norwegian spruce (*Pica abies*), browse is available from approximately 5 to 20 years (Kalén, 2012). In areas with low de-foresting activity (and subsequent replanting), moose densities are often low, whereas the opposite is usually seen in areas with high de-foresting activities.

On average one quarter of the population is killed by hunters annually. Harvesting by hunters is therefore the most common cause of death for Fennoscandian moose (Cederlund & Bergström, 1996).

## 1.1 Background to the study

In 2007, the department of Pathology and Wildlife Diseases at the Swedish National Veterinary Institute in Uppsala (SVA), Sweden, was contacted by hunters from the island of Öland in the south-eastern part of Sweden. The hunters reported a lower than expected observed presence of moose calves per observed female (calves per 100 cows), when using the moose observation index method (Ericsson & Wallin, 1999). Calf numbers amounted to 38 per 100 cows in 2007 on the island (Svenska Jägareförbundet, 2014), where large predators such as wolves (*Canis lupus*) and European brown bears (*Ursus*

*arctos*) were, and still are, absent. In comparison, across the Kalmar strait (seven km west of Öland) on the mainland of Sweden, calf numbers per 100 cows generally had reached 70-90 individuals (Svenska Jägareförbundet, 2014). Hunters on the island had refrained from moose hunting from 2001 to 2005, based on mutual agreement. The reason for the agreement was a fear of a moose population collapse after mismanagement of the population prior to 2001. The hunters on the island considered the reason for the observed decline in moose density to be the result of an overharvest of adult moose in general and males in particular. This had resulted in a decrease in the mean age of the population, a low proportion of adult males, and subsequent poor fecundity (Svenska Jägareförbundet, 2014; M. Johansson, personal communication). The five-year break had resulted in a balanced sex ratio of adult moose (Svenska Jägareförbundet, 2014), an increase in population density, a balanced age distribution, and an increased observed presence of prime-aged males (M. Johansson, personal communication), but no increase in the calf:cow ratio.

In 2005, *post mortem* examination of a dead moose calf revealed an infection with the tick-borne bacterium *Anaplasma phagocytophilum*, the causative agent of tick-borne fever. In addition to the finding of the bacterium in splenic tissue, the calf had a severe bacterial pneumonia (*Streptococcous* sp.), which was the main cause of death (Bernodt, 2005). In addition to the reports of low numbers of calves 'at foot', there was thus also a question of the possible effect of tick-borne fever on moose calf health and survival.

Based on the low calf per cow ratio, and the finding of *Anaplasma phagocytophilum* in a moose calf from the island, a pilot study was initiated in order to assess female moose reproduction in harvested moose on Öland, and to investigate the prevalence of *A. phagocytophilum* in all harvested moose during the hunting season in 2007. In the pilot study, examinations of reproductive organs from females were performed, and the results showed ovulation and pregnancy rates comparable to rates reported in other populations, but embryonic mortality in pregnant females was also detected (Malmsten, unpublished). In addition, analyses for *A. phagocytophilum* (serology for the detection of anti-anaplasma antibodies and real-time PCR for the detection of *A. phagocytophilum* DNA in splenic tissue) indicated that the bacterium was present in moose on the island with a PCR-based prevalence of 11.4 % (Malmsten, unpublished). A review of the available scientific literature on moose reproductive characteristics revealed that some basic information on male and female moose reproduction had been overlooked or were not up-to-date. Similarly, knowledge about the possible effects of disease on the reproductive success of moose in Fennoscandia was lacking.

In addition to changes in climate and the surrounding environment (forest management, forage availability), the past 10 years have also seen considerable changes in moose management strategies in parts of Sweden. An increase in calf harvest size (which increases the mean age of a population), and efforts to balance the sex ratio of adults (i.e. increase numbers of bulls by increasing the hunting of females), are common current strategies in moose management. In combination, the changes in the surrounding environment (including vector-borne diseases) may also have affected variables related to reproduction in moose, such as an increase in forage availability after severe storms in southern Sweden in 2005 and 2007.

In order to further investigate and characterize basic moose reproductive physiology (male and female) and assess the presence of *A. phagocytophilum*, in the Swedish moose population, an extended study was conducted in three mainland moose populations and in the Öland population in the hunting seasons from 2008 to 2011.

## 1.2 Moose reproduction

Moose are seasonal polyoestral mammals, with mating taking place during the autumn. During early summer (May - June) females give birth to one or two calves (triplets are rare, Markgren, 1969). There are a number of factors that can influence the reproductive success of moose, and the majority of these are directly or indirectly affected by management strategies (anthropogenic influence), and in moose, the availability of forage is essential for reproduction (Sand *et al.*, 1996).

Moose harvesting is performed according to various strategies, which can have different effects on the reproductive success of a population. For example, Saether *et al*. (2001) reported that a low harvest of calves in exchange for a high harvest of adults caused a long-term decline of the mean age of the population, and the reproductive output of the population decreased.

From the point of view of reproduction, there are some differences between tundra-living moose (Alaskan moose, *Alces alces gigas*) and the taiga-living moose (the remaining North American species, and the Eurasian/Fennoscandian moose). Male tundra moose have a more pronounced semi-polygynous behavior and the females tend to form assemblages in the open landscape, which attracts males of various ages (Schwartz, 2007). Neither polygynous behavior in males nor female assemblages during oestrus seem to occur in the taiga moose. This may be due the less open landscape (boreal forest), where assemblages of moose during the mating season are not necessary in order to increase predator awareness. During the rut,

Fennoscandian males roam large areas in search of available females in, or approaching oestrus, and follow the females until they permit mounting (Cederlund & Sand, 1994). The behavior of male tundra moose is different, as they aggregate around groups consisting of several females.

Research in moose reproduction has been conducted for more than half a century in Fennoscandia (Markgren, 1969), and North America (Edwards & Ritcey, 1958; Pimlott, 1959). Successful reproduction in moose requires a sufficient number of sexually mature males and females, and a suitable habitat with sufficient browse (Solberg *et al.,* 2002; Solberg *et al.*, 2007). In addition, reproductive success is affected by changes in the habitat and forage, the population age distribution, and the sex ratio of adults. Research on reproduction in moose has had, to a large extent, a female-biased focus. Information on age at sexual maturity (Sæther & Heim, 1993; Sand & Cederlund, 1996), timing of oestrus (Markgren, 1969; Haagenrud & Markgren, 1974; Schwartz & Hundertmark, 1993; Garel *et al.*, 2009), duration of pregnancy (Markgren, 1969; Schwartz & Hundertmark, 1993), individual or population-based fecundity (Sæther & Haagenrud, 1983; Solberg *et al.,* 2002), and senescence (Ericsson *et al.*, 2001) have been presented. In conjunction, the effect of different harvesting strategies (Hundertmark *et al.*, 1993; Laurian *et al.*, 2000; Solberg, 2002), climate, and nutrition on reproductive success have been studied (Sand, 1996; Sæther *et al.*, 1996; Bowyer *et al*., 1998). Studies on male reproductive characteristics are few except for minor contributions regarding spermatogenesis (Peek, 1962; Houston, 1968; Bubenik & Timmermann, 1982) and characteristics of the individual spermatozoon (Andersen, 1973). However, the reproductive effect of males on a population basis (male/female ratio, male age structure) has been well documented (e.g. Solberg & Sæther, 1994; Solberg *et al.*, 1999; Mysterud *et al.*, 2002; Mysterud *et al.*, 2005). The birth of calves is vital for long-term management of a population. Subsequently, variables affecting moose calf growth and survival have been described, such as maternal age, predation, and forage availability (Franzmann & Schwartz, 1985; Swenson *et al.*, 1999; Selås *et al.*, 2001; Ericsson *et al.*, 2002; Solberg *et al.*, 2004). There are, however, certain gaps in the knowledge of reproductive characteristics in males and females, embryo survival, moose calf mortality, and moose calf health that need to be addressed.

### 1.2.1 Male moose reproduction

*Puberty*

It is the general perception that males reach puberty during their second autumn in life (at 1.5 years of age), and at that time are able to reproduce (Schwartz, 2007). Yearling male moose in captivity are also reported to breed successfully (Schwartz *et al.*, 1982). However, puberty and breeding ability have been poorly documented in free-ranging moose in general, and in Fennoscandian (Eurasian) moose in particular. Andersen (1973) studied and characterized the ultra-structural and morphological appearance of moose spermatozoa, but the findings were not placed in a general context regarding moose reproductive biology. In North American moose, Peek (1962) and Houston (1968) investigated spermatogenesis in Shiras moose (*Alces alces shirasi*) from Montana and Wyoming by histological examination of testes, but on limited sample sizes (< 20 individuals). Bubenik and Timmerman (1982) conducted a larger study in Ontario, Canada (Eastern American moose, *Alces alces americana*), and concluded that, from a histological point of view, not all yearlings (1-2 years of age) and males aged 2.5 years had sufficient spermiogenic activity to have reached sexual maturity. Clearly, further criteria than histological findings are needed to determine the time of sexual maturity in male moose, such as is the case for domestic cattle where libido, sperm concentration, -morphology, -volume, mating ability, and ability to produce offspring are investigated (Lunstra *et al.*, 1978). Since information on male moose reproduction is limited, it is clear that further studies are needed.

*Rut and mating*

The rut is the period of the year after the shedding of the antler velvet (Hundertmark *et al*., 1989). During the rut, testosterone levels increase, and differences in behavior and movement patterns are observed, as the males actively seek females to mate. Although testosterone levels in moose have not been elucidated, they likely resemble the increased levels observed in another seasonal cervid, the reindeer (*Rangifer tarandus*, Whitehead & McEwan, 1973). The rutting period is considered to begin in September, and has different stages succeeding the shedding of antler velvet. The initial phase consists of the formation of ‘rut-pits’, in which a male moose digs and kicks a shallow depression (pit) in the ground, urinates and kicks the urine-scented dirt on to himself or wallows in it (Markgren, 1969; Miquelle, 1990a). The scent has a strong odor, which probably is stronger in mature males, and is thus more attractive to females (Miquelle, 1991). Females are reported to wallow in these rut-pits as well (Miquelle & Van Ballenberghe, 1985). Miquelle (1991)

suggested that this scent-urination could induce ovulation in females in oestrus. The male then enters the phase of searching an available female in oestrus, courts her and awaits standing oestrus when mating takes place (Markgren, 1969). This phase involves a substantial increase in activity (Cederlund & Sand, 1994).

Fighting among males occurs, and is more common in tundra-living moose than in taiga-living moose. Sparring, which is less intense and less harmful, is a way for males to test their strength and is more common during the pre-rutting period (Peek *et al.*, 1986). As for fighting, the assemblages of females and subsequent aggregations of rutting males on the tundra is an explanation for the common male-to-male interactions as described by Peek *et al* (1986). In Fennoscandia, where moose are intensively harvested, different explanations for the presumed low incidence of fighting have been presented. One suggested explanation is the absence of female assemblages during oestrus (Markgren, 1969); another involves the age structure of male moose in Sweden where, in general, numbers of prime-aged males are low (males aged 6 – 12 years, Mysterud *et al*., 2005) due to an overharvest of males in this age category. Thus, fighting over females is presumably not necessary.

During the last 5-10 years, moose management in most parts of Sweden has changed, partly with an aim to increase the mean age of the male (and female) population, and to balance the adult sex ratio in order to increase the reproductive output and body weights of calves. This increase in the population mean age will also increase the number of prime-aged males, which may lead to more fighting-related mortality in Swedish male moose.

Mating takes place during standing oestrus of the female, and tundra-living male moose can mate different females several times (>12 times), albeit over a 15-day period (Van Ballenberghe & Miquelle, 1993). This indicates that short-term polygyny (several matings in a short period of time) does not occur in moose. The assemblages of females, which occur on the tundra, likely facilitate this mating system (semi-polygynous) and behavior. Similar behavioral studies have not been performed in Eurasian moose, but the scarcity of female assemblages likely does not permit such a semi-polygynous behavior in males. In other species, the size of moose testes may also be a limiting factor. A positive correlation between testes size and level of semen production has been suggested (see Thompson Jr *et al.*, 1979 for example in stallions), i.e. small testes are synonymous with low semen production. Furthermore, Short (1997) suggested that in mammals, the testes size in relation to body weight reflects the mating system used by that species. The testes:body weight ratio in moose, has to my knowledge never been studied.

During the rut, the food intake of males also decreases substantially (hypophagia; Miquelle, 1990b), and this is combined with an increase in movement patterns (e.g. Cederlund & Sand, 1994); a considerable decline in body weight occurs (Miquelle, 1990b; Whittle *et al.*, 2000; Mysterud *et al.*, 2005). This weight loss, in relation to body weight, is most prominent in males aged 6-12 years, according to Mysterud *et al*. (2005), and where such males seek and take part in the mating to a greater extent.

### 1.2.2 Female moose reproduction

*Puberty and sexual maturity*

Puberty in female mammals is the process which results in a female becoming capable of reproducing. Prepubertal development is a gradual process during which several endocrine hormonal processes need to be functional and fully developed (Schillo, 2009). In order to be classified as pubertal, a female must have shown external oestrous symptoms (standing oestrus), have ovulated, and be able to have subsequent cyclicity. After maturation of one or two (rarely three) dominant follicles in an ovary, ovulation occurs during oestrus, which is followed by luteinization of the follicular cavity and development of one or two *corpora lutea* (during metoestrus and dioestrus). If the female is not bred, and if it is still in season (in seasonal polyoestral mammals such as the moose), luteal regression occurs and the cycle is repeated (see below).

A female moose may reach puberty during her second year of life at the age of 1.5 years (Sæther & Heim, 1993). Age at puberty is most likely dependent on body condition and consequently also body weight. Body weight at puberty in moose is correlated to weight at birth (Solberg *et al.*, 2004), and the subsequent weight gain for the following 16-17 months (Sæther & Heim, 1993; Sand & Cederlund, 1996; Ferguson, 2002; Solberg *et al.*, 2004). Moose calves with high birth weights reportedly gained weight faster during the first year in life than calves with low birth weights (Solberg *et al.*, 2004), and this was density- and climate dependent.

If the female reaches a certain weight threshold (which varies spatially), she will enter her first oestrous cycle (i.e. reach puberty) during the second autumn of life, as a 1.5 year old (Sæther & Haagenrud, 1983). If the weight threshold is not reached during the autumn, she will reach puberty during the following autumn (at the age of 2.5 years), or later, depending on body condition.

Puberty and sexual maturity have to be differentiated since sexual maturity in moose reproduction studies has hitherto been used and defined in different ways. It has been defined as the age when a female gives birth to its first calf, but also as the age at mating the year preceding the year of first parturition (e.g.

Sæther & Heim, 1993). When determining sexual maturity in hunter-killed moose females, reproductive organs have been examined *post mortem* for signs of previous pregnancy (determined by the presence of *corpora albicantia* in the ovaries, Markgren, 1969). If they are present, the female is considered to have reached sexual maturity the previous year (Markgren, 1969). Sexual maturity has also been assigned to radio-collared females who give birth for the first time in life (e.g. Sæther et al., 1993). However, applying this approach to reproductive organs (or by field observations of radio-collared females) from female moose estimated to be 3.5 years or older, does not provide information with certainty on whether the female reached puberty the previous year or the year before. Furthermore, a female might have reached puberty at 1.5-years, but may not, for different reasons (lack of available males, *ova*/embryo loss *et cetera*) have become pregnant during her first or subsequent oestrus, and therefore no *corpora albicantia* would be found. Nevertheless, age at sexual maturity (based on detection of signs of previous pregnancy; presence of *corpora albicantia*) in female moose has been studied and reported to range between 1.5 and 6.5 years (Sand & Cederlund, 1996). The range in age at sexual maturity was attributed to different spatial (latitude), temporal and individual variations. Markgren (1969) noted that, depending on year of sampling and region, between 8 and 51 % of 1.5-year old females had reached puberty. The proportion of females aged 1.5 and 2.5 years that became sexually mature, increased with body weight (Sand & Cederlund 1996; Sæther & Haagenrud 1983). Although body weight at puberty has not been studied in North American moose, Schwartz and Hundertmark (1993) stated that puberty generally occurs at 16 months of age, but can be delayed until 28 months of age. In addition, Adams and Pekins (1995) concluded that 63 % of yearling female moose were sexually mature based on *corpora lutea* counts, although the pubertal process was not studied. Most studies of puberty and sexual maturity in Fennoscandian moose have been based on material collected between the 1950s (Markgren, 1969) and the 1980s (e.g. Sand & Cederlund, 1996) and these studies are, to some extent, based on imprecise methods (the sole use of *corpora albicantia*) to assess the age at puberty of the female. Thus, there is a need to investigate whether previous data are applicable to current populations of female moose or not. Moreover, current moose management strategies, as well as the population density, have changed along with the climate and surrounding environment (forest management and forage availability). The effect of these variables on the onset and passing of puberty in moose has not been studied, nor is information on the current reproductive characteristics of Fennoscandian moose available.

*Oestrus, mating, and conception*

The time period immediately preceding oestrus is pro-oestrus, during which the female is often observed in the company of a courting male which is awaiting standing oestrus (Markgren, 1969). During oestrus, one or two dominant follicles (rarely three) reach maturation and ovulation takes place. Whether ovulation occurs during standing oestrus or after (as in cattle) has, to our knowledge, not been determined in moose. Standing oestrus in captive moose is reported to vary from 3 to 36 hours (Schwartz & Hundertmark, 1993).

The seasonal oestrous period has been reported to occur during late September and early October (Markgren, 1969; Haagenrud & Markgren, 1974; Garel *et al.*, 2009) but varies among regions and between years. Some studies used ovulation rate patterns in large samples of ovaries to determine when ovulation had taken place (presence of *corpora lutea*), and then to estimate the timing of oestrus (Edwards & Ritcey, 1958; Simkin, 1965; Markgren, 1969; Garel *et al.*, 2009;). However, the presence of a large mature follicle without the presence of a *corpus luteum* may also be a sign of oestrus. In addition, the size, color and texture of the *corpus luteum* (when present) can give additional information on the point in time when ovulation took place. This way of determining the timing of oestrus has not, to our knowledge, been used previously in moose, but it is more accurate. Another method for estimating the timing of oestrus during the reproductive season of moose is to subtract the duration of pregnancy from the date of calving. By calculating backwards from an observed parturition date and using a fixed number of days (234) for the duration of pregnancy, Markgren (1969), Haagenrud and Markgren (1974), and Broberg (2004), estimated that most females show oestrus and ovulate during the last week of September to the first week of October. However, since the duration of pregnancy varies considerably according to Schwartz and Hundertmark (1993), this calculation procedure is not particularly accurate. Thus, there is a need for updated knowledge regarding the timing of oestrus. The timing of the oestrous period in moose may have changed, when considering the previously mentioned changes in environment, moose management, and population composition, which have taken place in the recent years. In addition, as suggested by Haagenrud and Markgren (1974) and Garel *et al*. (2009), the differences in the timing of oestrus in younger vs. older females need to be further studied.

When the *corpus luteum* has reached its full size at dioestrus the secretion of progesterone reaches its maximum level. If breeding and conception has not occurred, the dioestrus period ends, the *corpus luteum* regresses, and the female will return to oestrus after approximately 24-25 days (Markgren, 1969; Schwartz & Hundertmark, 1993). A discrepancy between the number of

embryos or fetuses and the number of *corpora lutea* in the ovaries (defined as *ova* loss), has been suggested as being an indication of reproductive failure (Markgren, 1969; Crichton, 1992; Schwartz & Hundertmark, 1993). However, if this is due to a lack of fertilization of oocytes (actual loss of *ova*) or to embryonic mortality, and to what extent these occur, have yet to be determined.

*Pregnancy*

The duration of pregnancy has been reported to range from 216 to 240 days in Alaskan captive moose (Schwartz & Hundertmark, 1993). For Swedish moose it is estimated to be around 235 days, but this estimate was based on a study of moose in a zoo in the 1940s (Markgren, 1969). Thus, there is a lack of knowledge about pregnancy duration in free-ranging moose.

In pregnant moose, the embryonic development starts followed by the fetal stage, which occurs after the completion of organogenesis (Schillo, 2009). Embryo mortality has been discussed as a cause of early reproductive failure in moose, but only a single report exists; in this case, a presumed-dead embryo was detected by trans-rectal ultrasonography in an immobilized Alaskan moose (Testa & Adams, 1998). Hence, information on the incidence of embryo mortality in moose is lacking.

There have been some reports describing fetal development in moose, (e.g. Markgren, 1969; Crichton, 1992). The moose fetus (Fennoscandian moose) grows during gestation, reaching a crown-rump length of almost 70 cm and a weight ranging from 7 to 14 kg at parturition (Markgren, 1969). Fetal mortality (resulting in abortion) in moose has not been reported in the literature. However, occasional findings of aborted moose fetuses have been reported to the National Veterinary Institute in Uppsala, Sweden by hunters. These abortions may be due to harsh winters with long periods of temperatures below normal (-30°C) and a high annual accumulation of snow to a significant depth (T. Mörner, personal communication).

*Parturition*

Moose females normally give birth during early summer, and calving dates can be obtained by observational studies of female moose with radio collars (VHF and/or GPS/GSM) (Bowyer *et al.*, 1999; Broberg, 2004;). Parturition dates vary from the beginning of May to mid-June, with a peak around mid- to late May (Broberg, 2004), although later calvings in August have been occasionally reported to occur (Markgren, 1969). Shortly before parturition, the pregnant female locates a secluded area where it can give birth to a calf undisturbed

(Markgren, 1969; Bowyer *et al*., 1998; Bowyer *et al.*, 1999; Keech *et al.*, 2000; Testa *et al.*, 2000; Tremblay *et al.*, 2007).

Obstetric complications are rarely reported, but dystocia has been documented in moose in Sweden, although the cause was unknown (Markgren, 1969).

### 1.2.3 Moose calf health and survival

*Early calfhood*

Similar to other ungulate species, the newborn calf attempts to rise after being cleaned by the mother. On succeeding, the calf tries to find the udder and the teats in order to suck the colostrum, which is rich in energy (Chalyshev & Badlo, 2002) and maternal antibodies, which provides the first immunological protection for neonates (Kruse, 1983; Sokołowska *et al.*, 2008; Stelwagen *et al.*, 2009). Moose calves normally have a high growth rate during their first months of life. In captivity, the milk intake in moose calves increased daily for approximately their first twenty days, at which time it amounted to around five kg of milk per day (Reese & Robbins, 1994; Shochat & Robbins, 1997). Captive calves gain on average 700-800 g of weight per day during their first 100 days of life (Shochat & Robbins, 1997). Providing nutrition for one or two calves requires considerable energy and protein resources from the mother, and if the surrounding environment does not provide sufficient resources, moose calf survival may be affected (Crête & Courtois, 1997).

*Calf summer survival*

The survival rate of moose calves varies considerably depending on the presence of large carnivores. In areas where large carnivores, such as bears (black bears *Ursus americanus*, brown/grizzly bears *Ursus arctos*), or wolves (*Canis lupus*) are present, predation on moose calves during the summer period is common. In some areas, the annual predation rate of one or both of these two main predators on moose calves can exceed 65% (Gasaway *et al.*, 1992; Linnell *et al.*, 1995; Sand *et al.*, 2008).

An unfavorable summer climate also affects moose calf survival, as warm temperatures and low precipitation causes a rapid lignification of forage, which reduces the energy content (Akin, 1989). In single areas without predation, calf mortality rates during the summer have been reported to reach 34 %, by studying calves accompanied by radio-collared females (Stubsjøen *et al.*, 2000), although it was reported to be on average around 10 % (Linnell *et al.*, 1995). In addition, populations under pressure, where summer forage availability is restricted due to high densities of moose and/or other cervids,

may have low calf summer survival rates. There is, however, limited knowledge on calf mortality during the summer in southern Sweden in areas where large carnivores are absent, and where the summer climate is different from that in northern Sweden. Also population density, moose distribution, the presence and effect of vector-borne diseases and environmental factors vary over time, which may affect summer mortality rates.

*Anaplasma phagocytophilim infection*

*Anaplasma phagocytophilum* is a widespread tick-borne pathogen reported to infect various species of wild and domestic animals (Woldehiwet, 2010). It is a Gram-negative, obligate intracellular bacterium of the order Rickettsiales that infects white blood cells (neutrophils, eosinophils, and monocytes). The primary vectors in northern Europe are *Ixodes ricinus* ticks (Woldehiwet, 2010). In domestic ruminants, the infection can cause tick-borne fever (TBF), which is a disease characterized by fever, depression, lethargy, reduced milk yield (in dairy cattle) and abortion (Woldehiwet, 2010). In humans, it may cause human granulocytic anaplasmosis (HGA, Bakken & Dumler, 2008), which is thought to be an under-diagnosed disease, since the symptoms are similar to those of borreliosis/Lyme disease (infection with *Borrelia burgdorferi*). Tick-borne disease and presumed mortality due to infection with *Anaplasma phagocytophilum* have been reported in a moose calf from Norway (Jenkins *et al.*, 2001), and several cases with similar lesions have been identified in moose calves from southern Sweden (Bernodt, 2005; E. Ågren, personal communication). Based on the detected presence of this pathogen in moose from southern Sweden, and its proposed effect on moose health, there is a need to further investigate the occurrence and pathological significance of *A. phagocytophilum* in moose in order to elucidate its possible effect on moose calf health and survival.

# 2 Aims

The overall aim of this study was to describe different factors associated with male and female moose reproduction, and moose calf health and survival in southern Sweden. The specific aims were:

- to investigate and describe characteristics of reproductive organs and spermatozoa, in relation to age and body weight in male moose.
- to investigate and describe reproductive characteristics of female moose with regard to puberty, timing of ovulation, ovulation rates, and mating.
- to determine if pregnancy failure in moose occurs during the embryonic period.
- to determine summer mortality rates in moose calves in southern Sweden.
- to investigate selected aspects of the occurrence and epidemiology of the tick-borne pathogen *Anaplasma phagocytophilum* in moose.

# 3 Materials and Methods

A summary of the methods used in the thesis is provided here. More detailed descriptions are presented in Papers I-V.

## 3.1 Field sampling of shot moose 2008-2011 (Paper I-III, V)

During parts of the moose-hunting period, starting on the second Monday of October each year, samples from recently shot moose were collected in four different provinces [Öland, Småland, Södermanland, Västergötland (only in 2008)] of southern Sweden (Figure 2), from 2008 to 2011. Samples collected during a pilot study 2007 on the island of Öland were also used in papers II and III.

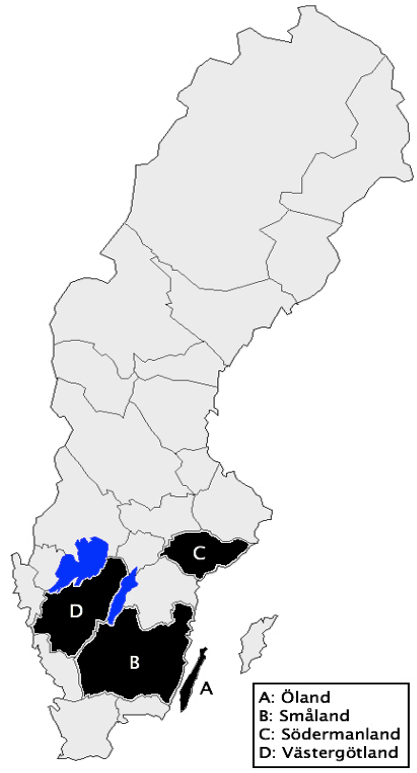


*Figure 2.* Map of Sweden with sampling areas (provinces) highlighted. Illustration: Jonas Malmsten

Field laboratories were set up in the hunting areas during the first week of the hunting period in order to permit direct handling and investigation of fresh samples (see below for investigations performed in the field laboratory). Local hunters in each of the sampling areas were asked to contact trained field personnel immediately after a moose was shot, upon which the field personnel then went to the site of harvest. The field personnel (Figure 3) collected a number of samples from each moose (Table 1), tagged all samples with individual ID-numbers, noted place and time of harvest (and sampling) and transported the samples in coolers (at temperatures < +8°C) to the field laboratory. In addition to the collection of samples, hunters were asked to report the carcass weights of the harvested moose. The carcass weight is the weight recorded after the skin, head, metapodials (lower limbs), and internal organs have been removed (Wallin, 1996). Body weights were estimated from the carcass weights in accordance with Wallin (1996).

*Figure 3.* Field sampling of recently shot female moose. Photo: Bertil Malmsten

Table 1. *Overview of the different samples collected from hunter-harvested moose in southern Sweden, and the objectives of the sampling.*

| Sample | Objective (current project) | Objective (other studies or biobanking) |
|---|---|---|
| Mandible | Age determination | |
| Blood | Pathogen analysis | |
| Spleen | Pathogen analysis | |
| Male reproductive organ | Macroscopic examination[1] and sampling | |
| Female reproductive organ | Macroscopic examination[1] and sampling | |
| Liver | | Trace element analysis |
| Kidney | | Trace element analysis |
| Ticks | | Pathogen analysis |
| Faeces | | Biobanking |
| Deer keds | | Biobanking |
| Abdominal fat | | Fat composition analysis |

[1] See below for full description of investigation performed

During the period when field laboratories were not present, hunters were asked to collect female reproductive organs and mandibles, as well as to report the carcass weight. These samples (22 %, n=55) were given individual ID-numbers, frozen (-20°C) and shipped to the Swedish University of Agricultural Sciences, after the hunting period (Jan 31 the following year) for later investigation.

## 3.2 Field laboratory investigations (Paper I-III, V)

### 3.2.1 Male reproductive organs (Paper I)

Testes and epididymides were removed from the scrotum. Epididymides were separated from the testes (Figure 4), and both specimens were measured and weighed. The texture and color of the cut surface of the testes were recorded, and abnormalities (if present) were noted. From each testis, two tissue samples

(0.5 x 0.5 x 0.2 cm) were taken and placed in Bouin's solution where they remained for 24 hours before being placed in 70 % ethanol. In the cauda of both epididymides, an incision was made and three sperm samples were collected for sperm morphological analyses (dry and wet mount), and chromatin integrity analyses (from left cauda only). The time when the reproductive organ was examined was recorded. Testes and body weight ratios were also calculated.

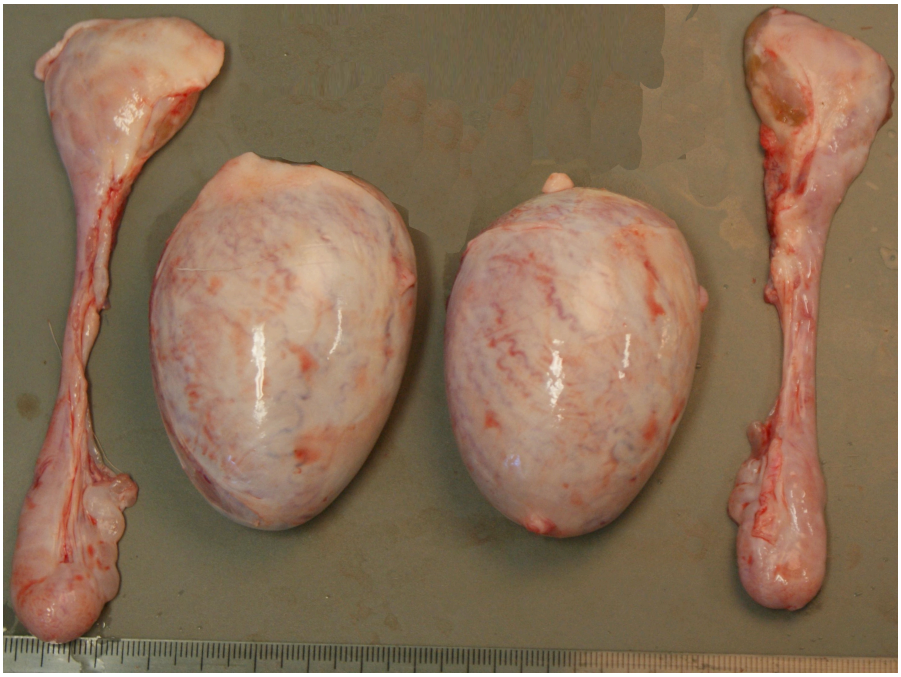

*Figure 4.* Moose testes with separated epididymides from a 2.5-year-old male moose shot in Småland, and with testes weighing 70.1 g (left) and 66.4 g (right). Photo: Jonas Malmsten

### 3.2.2 Female reproductive organs (Paper II-III)

For the female reproductive organs (Fig. 5) the ovaries were removed from the ovarian ligament, measured (length, width, height), and weighed. The presence of follicles was noted (Figure 6) and all visible follicles > 3 mm were counted. If present, *corpora lutea* (Figure 6) were counted and measured (diameter). The ovaries were then placed in 10 % formalin.

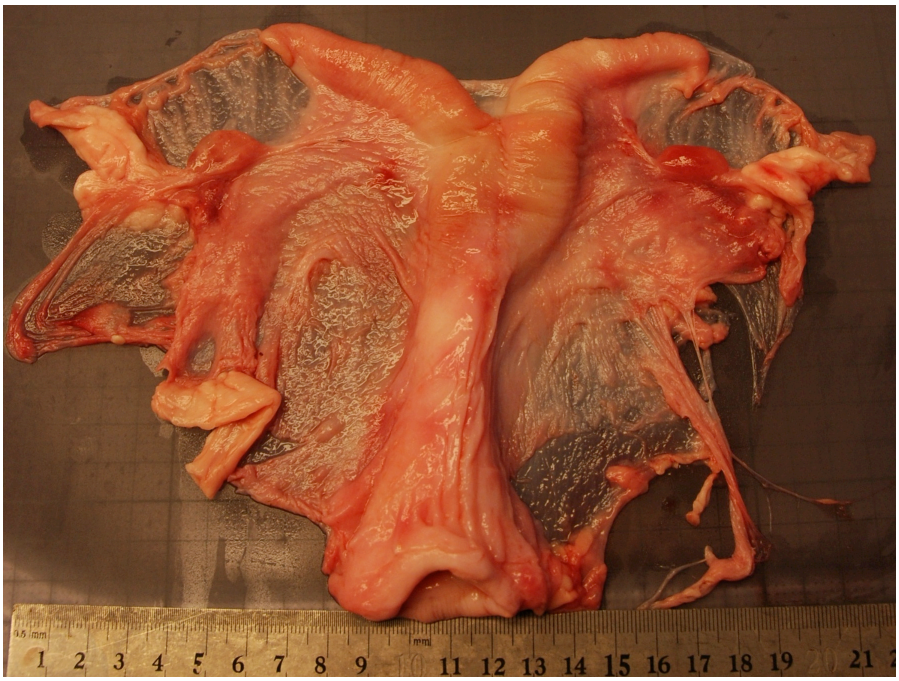

*Figure 5.* Reproductive organ from 3.5-year-old female moose shot in Sörmland. Photo: Anne-Marie Dalin

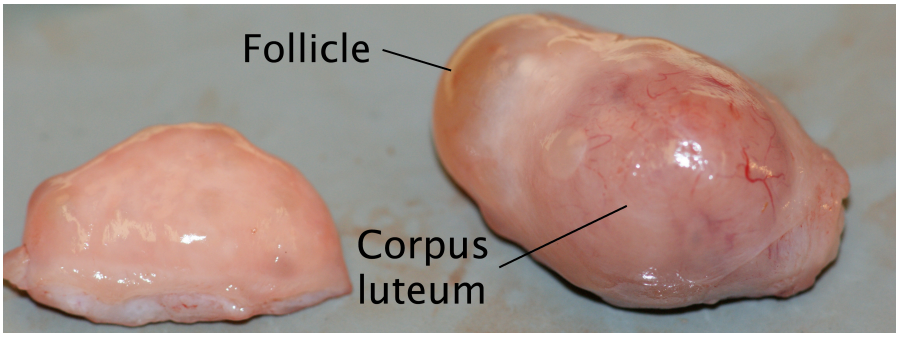


*Figure 6.* Moose ovaries from 5.5-year-old female moose (in dioestrus) shot in Småland. Photo: Jonas Malmsten.

Uteri were measured (horn length), weighed and cut open. The content was noted (allantochorionic membranes, embryos, pus, malformed allantochorionic membranes and/or embryos). The endometrium was inspected (presence and number of caruncles) and any abnormalities noted (color, thickness, texture). The pattern of the blood vessels in the cut surface between the uterus and the broad uterine ligament (*Ligamentum mesometrium*) was inspected and the presence of a pronounced enlargement of the vessel wall was interpreted as a

sign of previous pregnancy (Figure 7). Two tissue samples (each 0.5 x 1.0 cm) from the uterine wall were collected and placed in 4 % formalin. If signs of endometritis (presence of opaque fluid, or discoloration) were observed, a bacteriological swab sample was taken, placed in transport medium (Amies Agar Gel Medium with Charcoal, Copan Venturi Transsystem, Copan Innovation, Brescia, Italy), stored at ambient temperature, and sent to SVA for bacteriological analysis.

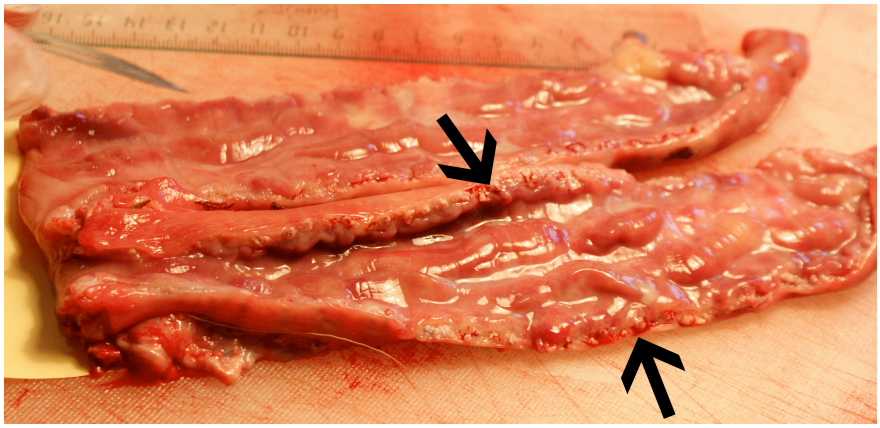

*Figure 7.* Moose uterus (cut open) with enlarged blood vessels in the surface between the uterine wall and the broad uterine ligament (arrows); a sign of a previous pregnancy. Photo: Jonas Malmsten

If the ovaries contained a large follicle (diameter > 10 mm), or one or more *corpora lutea* were present (indicating that ovulation had occurred) but no pregnancy was observed, oestrus was assumed to have taken place less than two weeks previously. The cervix was then investigated for presence of spermatozoa by scraping the cervical folds carefully with a scalpel. The sample was smeared on a glass slide and studied under a light microscope (250-400X, Figure 8). When present, the approximate number of spermatozoa (few, moderate, many) was noted.

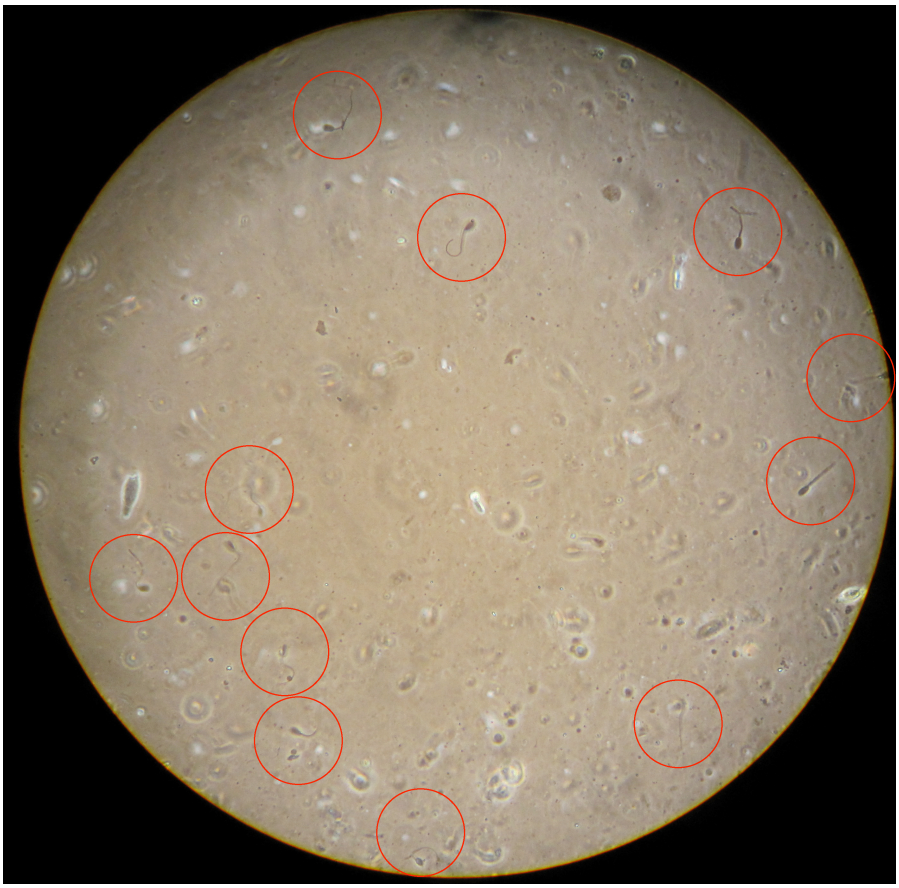

*Figure 8.* Smear from cervical folds of a female moose showing presence of spermatozoa (encircled) indicating that a recent mating had occurred. Photo taken with an ordinary digital camera through the microscope lens. Photo: Bertil Malmsten

In animals in which signs of an on-going pregnancy were found, the uterine content (allanto-chorionic membranes, embryos and/or foetuses) was inspected, and embryos were measured and weighed. Embryonic mortality was determined by the presence of embryos displaying malformations with failure of organogenesis (uneven edges, no visible hind- or forelimbs, misshaped head, absence of visible liver) or pregnancies judged to be older than two weeks (i.e. long and wide allanto-chorionic membranes but without a proper embryo, or the uterus containing only remnants of allanto-chorionic membranes.

It was concluded that there had been unfertilized oocytes when there was a discrepancy between the number of viable, or completed embryonic structures seen, and the number of *corpora lutea* found.

### 3.2.3 Remaining samples (Paper V)

From the blood samples, (taken from the heart or the caudal *vena cava* in hunted moose) were centrifuged (3000 rpm, 10 min), serum was separated and placed in 2 ml plastic tubes and stored at -20°C. The remaining collected samples (mandible, spleen-, liver-, and kidney samples, ectoparasites, and faeces) were also placed in a freezer at -20°C.

## 3.3 Analyses performed at SVA and SLU, Uppsala

After removal of the field laboratories, all samples (fixed and/or frozen) were transported to SLU or SVA in Uppsala for further analysis.

### 3.3.1 Mandibles (Paper I-III, V)

Age determination of harvested moose was performed by sectioning the first molar (M1) of the collected mandible. A crosscut saw with a 4 mm blade was used, and the cementum layers were counted, according to the method described by Wolfe (1969).

### 3.3.2 Blood/serum (Paper V)

One cryovial containing approximately 1 ml of serum was submitted to the Dept. of Bacteriology at SVA for the detection of antibodies against *A. phagocytophilum*. An immunofluorescence assay (IFA) was used to determine the antibody titre. A cut-off titre of 1:40 was set according to previous reports on serological detection of anti-Anaplasma antibodies (Stuen *et al.*, 2002). Titration up to 1:1280 was performed in serum samples of high quality.

### 3.3.3 Spleen (Paper V)

*PCR for detection of A. phagocytophilum DNA*

From the frozen spleen samples, a piece of approximately 2 $cm^3$ was excised and submitted to the Dept. of Bacteriology at SVA for the detection of *A. phagocytophilum* DNA. Briefly, a diagnostic commercial real-time polymerase chain reaction (PCR) test for detection of 16S rDNA using GER3 (TAGATCCTTCTTAACGGAAGGGCG) and GER4 (AAGTGCCCGGCTTAACCCGCTGGC) primers was used as described by Goodman *et al*. (1996), but this was converted to a real-time PCR format by adding a TaqMan probe (Jäderlund *et al.*, 2009).

*DNA sequencing*

In order to investigate the possible presence of different *A. phagocytophilum* genotypes, the 16S rDNA (915 base pairs) and partial *groESL* (1275 base pairs) were sequenced using GER3 and GER4 primers (Franzén *et al.*, 2005).

### 3.3.4 Spermatozoa (Paper I)

*Morphology of spermatozoa*

Sperm smears from both epididymides (left and right) were morphologically analyzed at the semen laboratory at the Dept. of Clinical Sciences, SLU, Uppsala. Head morphology of spermatozoa was studied in dry smears stained with carbol-fuchsine according to the method described by Williams (1920) and modified by Lagerlöf (1934). Five hundred spermatozoa were counted in each dry smear using phase contrast microscopy (x1000). The presence of proximal cytoplasmic droplets, abnormal acrosomes, detached heads and abnormalities of the midpiece and tail were recorded. Two hundred spermatozoa (wet mount preparation) were counted using phase-contrast microscopy (1000X). The abnormalities were classified according to a system developed by Bane (1961). Morphological abnormalities were summarized as proportions of the total number of counted spermatozoa. The proportion (%) of normal spermatozoa (PNS) was calculated and used as a measurement for the morphological quality of spermatozoa. For all the different morphological parameters, a mean value was calculated of the samples from the left and right epididymis.

*Chromatin integrity*

The procedure for chromatin integrity analysis followed the protocol described by Morrell *et al*. (2008) for stallion semen. In brief, abnormal chromatin structure was defined as the susceptibility of sperm DNA to undergo acid-induced denaturation *in situ*. The DNA fragmentation index (%DFI) was calculated and expressed as the percentage of cells with a high ratio of denatured, single stranded DNA (red fluorescence) to total DNA (stable, double stranded DNA [green fluorescence] + single stranded DNA). From each sample a total of 10,000 events was measured at a flow rate of ~200 cells/s. Data collection was carried out using CellQuest, version 3.3 (Becton Dickinson, San José, CA, USA). Further calculations were performed using FCS Express version 2 (De Novo Software, Thornhill, Ontario, Canada).

### 3.3.5 Uteri and ovaries (Paper II-III)

*Fixed uterine samples (Paper III)*

Fixed uterine samples were prepared at the histological laboratory of the Dept. of Clinical Sciences, SLU, Uppsala. The tissue samples were dehydrated, embedded in paraffin, sectioned, and stained with haematoxylin-eosin. A histological verification of endometritis was performed in specimens where this was suspected from the macroscopic examination results in the field laboratories. Endometritis was defined as an increased infiltration of inflammatory cells (neutrophils, macrophages and lymphocytes) in the endometrium. A subset of samples without macroscopic signs of endometritis was used as a negative control.

*Fixed ovarian samples (Paper II-III)*

Ovaries, fixed in 10 % formalin, were sectioned in approximately 1-2 mm thick slices. The slices were macroscopically examined for the presence and number of *corpora albicantia* (i.e. 'scars' from *corpora lutea* present during a pregnancy, Markgren, 1969; Pimlott, 1959) for determination of previous pregnancy.

*Classification of females*

Based on the combined findings of the examination of the uterus and ovaries, the animals were classified into nine different categories (Table 2, Paper II). Puberty was defined as a female having ovulated for the first time; this was determined by the presence of a CL but without signs of a previous pregnancy based on ovarian and uterine findings. The categories ranged from non-pubertal heifers (Category 1), pubertal heifers in different stages of the oestrus cycle (Category 2-3), pregnant heifers (Category 4) to cows in different oestrous cycle stages (Categories 5-7), pregnant (Category 8) or anoestral cows (Category 9).

## 3.4 Moose calf summer survival (Paper IV)

In collaboration with SLU in Umeå, calf summer survival in Södermanland (Öster Malma), Småland (Kronoberg), and Öland (Öland) was studied during the summers of 2012 and 2013. Female moose were immobilized with a combination of etorphine and xylazine from a helicopter, and equipped with GPS/GSM/VHF-collars (Vectronics Aerospace GmbH, Berlin Germany). From April 15$^{th}$ to July 15$^{th}$ (during the calving season), the collars were remotely programmed to record GPS-positions every 30 minutes, which were

sent by SMS every 3.5 hours. A total of 66 adult females were followed for both years (2012 and 2013). Based on positional data, which reflected the movement behavior of the moose, calving sites were identified (clusters of GPS-positions) and the center points of the clusters were approached by field staff at a maximum of three days post-partum with the help of portable VHF-receivers (Model RX8910, Followit AB, Lindesberg, Sweden). The number of observed neonatal calves was recorded. To investigate the effect of handling of calves, the calves of every second site were weighed and ear-tagged with plastic tags with a diameter of 30 mm, printed with individual numbers. When present at the calving site, dead calves were collected and submitted for *post mortem* examination.

## 3.5 Statistical relationships (papers I-V)

In most cases, in order to determine the effect of different explanatory variables such as temporal variables (year and/or time of harvest), spatial variables, and biological variables (age, sex, and carcass weight), on response variables (oestrous category, morphology of spermatozoa/PNS, %DFI, *A. phagocytophilum* serology and PCR results), logistic regression was performed. Logistic bivariate regression was used in order to predict the probability of having passed oestrus for heifers in four different carcass weight categories. All analyses of means were calculated using analysis of variance (ANOVA). In males, associations between carcass weight, testes weight and proportion of normal spermatozoa (Paper I) were calculated using linear regression. All statistics in Paper I-III and V were performed using R (R Core team, 2013). For Paper IV, summer survival (0, 1), year (2012, 2013), area (Kronoberg, Öland, Södermanland), and handling (0, 1) were analyzed using a Generalized linear model (McCullagh, 1984). Multiple comparisons were controlled for using least square means and Tukey-Kramers post-hoc test. The analyses were performed using SAS/STAT® software (SAS Institute Inc., Cary, NC, USA).

# 4 Results

## 4.1 Reproductive characteristics of male moose (Paper I)

### 4.1.1 Samples, age, and carcass weight

Samples were collected from 143 male moose between 2008 and 2011. The mean age of the sampled moose was 3.9 years (SD ±2.3, range 1.5 - 11.5), and the mean carcass weight (n = 96) was 192.0 kg (SD ±32.5, range 75 - 302). The mean elapsed time from death to laboratory handling and investigation was 6.7 hours (SD ±5.1, range 1 - 24)

### 4.1.2 Testicular and epididymal characteristics

In total, 133 pairs of testes and epididymides were collected and examined. The mean weight of the left and right testes were 55.7 g (SD ±13.7, range 8.6 - 87.1) and 56.3 g (SD ±13.3, range 9.3 - 118.6), respectively. For epididymides, the mean weight for the left and right were 11.0 g (SD ±2.2, range 2.0 - 19.0), and 10.7 g (SD ±2.0, range 2.7 - 18.4), respectively. There was a linear relationship ($R^2$ = 0.45 and 0.39, respectively) between testes weight and carcass weight (two subsets of samples depending on time of sampling, Figure 2, Paper I). Cryptorchidism (Figure 9) was observed in three males (2.3 %).

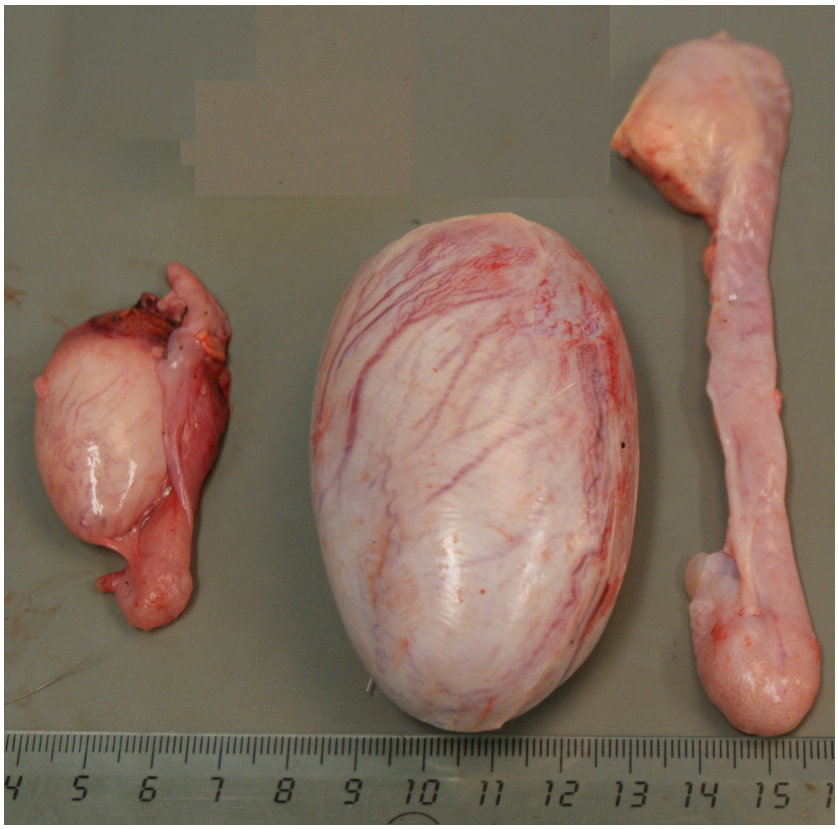


*Figure 9.* A case of cryptorchidism in a 3.5-year-old male moose shot in Småland in 2008, with the left testis weighing 10.7 g and the right testis weighing 87.6 g. Photo: Anne-Marie Dalin

### 4.1.3 Morphology of spermatozoa

The overall mean proportion of normal spermatozoa (PNS) in analyzed male samples (n = 124) was 51.0 % (SD ±21.8, range 1.5 - 82.0, Table 2), and a temporal decline in PNS (regardless of male age) over the course of the sampling period was observed. An overview of sperm morphology results in three different age categories is presented in Table 2. Samples with a proportion of normal spermatozoa exceeding 70 % were observed in 20.9 % (n = 26) of the males and all these samples were collected during the first week of hunting.

Table 2. *Overview of results of chromatin integrity analyses (%DFI) and morphology of spermatozoa (%) in age-determined (years) moose.*

| Age (years) | | %DFI | Abnormal heads | Proximal cytoplasmic droplets | Abnormal acrosomes | Abnormal midpieces | Abnormal sperm tails | Normal spermatozoa |
|---|---|---|---|---|---|---|---|---|
| 1.5 | *n* | *26* | *36* | *36* | *36* | *36* | *36* | *36* |
| | Mean ±SD | 10.0 ±6.7 | 28.9 ±13.5 | 18.8 ±19.1 | 6.3 ±8.9 | 2.1 ±1.8 | 5.5 ±7.4 | 46.6 ±22.4 |
| | Range | 4.4 - 36.7 | 10.5 - 73.9 | 2.8 - 90.3 | 0.5 - 34.3 | 0.3 - 10.8 | 0.8 - 38.0 | 1.5 - 82.0 |
| 2.5-5.5 | *n* | *56* | *65* | *65* | *65* | *65* | *65* | *65* |
| | Mean ±SD | 9.6 ±5.4 | 29.4 ±16.7 | 14.6 ±14.3 | 5.1 ±6.1 | 2.3 ±1.1 | 4.4 ±3.7 | 50.6 ±21.6 |
| | Range | 2.5 - 33.7 | 10.7 - 78.7 | 2.5 - 69.3 | 0.0 - 32.5 | 0.3 - 5.8 | 0.0 - 20.8 | 6.0 - 81.5 |
| 6.5-11.5 | *n* | *22* | *23* | *23* | *23* | *23* | *23* | *23* |
| | Mean ±SD | 8.9 ±2.9 | 24.6 ±16.2 | 9.8 ±5.1 | 6.1 ±9.4 | 2.7 ±1.1 | 3.7 ±1.5 | 59.0 ±19.9 |
| | Range | 5.1 - 16.7 | 11.2 - 88.7 | 3.8 - 22.8 | 0.8 - 43.0 | 1.0 - 5.8 | 1.3 - 7.0 | 1.5 - 79.5 |
| **All** | ***n*** | ***104*** | ***124*** | ***124*** | ***124*** | ***124*** | ***124*** | ***124*** |
| | **Mean ±SD** | **9.5 ±6.2** | **28.3 ±15.7** | **14.9 ±15.0** | **5.6 ±7.6** | **2.3 ±1.3** | **4.6 ±4.8** | **51.0 ±21.8** |
| | **Range** | **2.5 - 36.7** | **10.5 - 88.7** | **2.5 - 90.3** | **0.0 - 43.0** | **0.3 - 10.8** | **0.0 - 38.0** | **1.5 - 82.0** |

n= number of samples/individuals

### 4.1.4 Chromatin integrity

In total, 104 sperm samples were analyzed for chromatin integrity. The mean DNA fragmentation index (%DFI) was 9.5 (SD ±6.2, range 2.5 - 36.7, Table 2).

### 4.1.5 Statistical relationships

Young males (1.5 - 2.5 years of age) had significantly higher proportions of proximal cytoplasmic droplets than males aged >3 years ($p = 0.047$), but no significant effect of age ($p = 0.91$) was observed regarding the proportions of abnormal heads of spermatozoa. There was a positive effect of testes weight on PNS, but no effect of age, year, or elapsed time between field sampling and lab preparation, or %DFI was observed. Since the weight of testes was positively correlated with carcass weight, the latter variable could also have been used as the explanatory variable. A negative effect of date of harvest/death on PNS was noted. The testes:body weight ratio was 0.033 % (SD ±8.0 x $10^{-5}$, range 0.008 - 0.051).

## 4.2 Reproductive characteristics of female moose (Paper II)

### 4.2.1 Samples, age, and carcass weight

From 2007 to 2011, 250 reproductive organs were collected from hunter-harvested female moose (age > 1 year). Age was determined in 94 % (n = 235) of the sampled moose, the mean age being 4.3 (SD ±3.9, range 1.5 - 18.5). The length of the period of sampling was 103 days (starting on the opening day of the hunting period each year). For 13 samples, harvest date was not recorded, but in the remaining 237 samples, the majority (66.7 %, n = 158) was collected during the first week of the hunting period, and 93.7 % (n = 222) during the first month. Most sampled moose (75.3 %, n = 177) were between 1.5 and 4.5 years old, whereas middle-aged (5 - 12 years) and old (> 12 years) females accounted for 16.6 % (n = 39), and 8.1 % (n = 19), respectively. Carcass weights were recorded in 74.8 % (n = 187) of the harvested moose, the mean weight being 151.5 kg (SD ±28.9, range 72 - 220).

Cows accounted for 48.8 % (122/250) of the collected samples. Thus, all of them had experienced one or more pregnancies in previous years (Categories 5, 6, 7, 8 and 9; Figure 2; Paper II). Almost seven percent of the cows (6.6 %, n = 8) were anoestral (Category 9), and of the remaining cows (114 individuals) the majority (94.8 %, n = 110) had passed their first oestrus of the season. Of the cows, 40 individuals were considered to be pregnant.

### 4.2.2 Puberty

Heifers (Categories 1, 2, 3, and 4; Figure 2; Paper II), accounted for 51.2 % (128/250) of all samples, of which 40.6 % (52/128) had no signs of cyclicity in their reproductive organs *post mortem*. These were thus assigned to Category 1 (Table 2, Paper II) and labelled prepubertal. Date of sampling and carcass weight were positively associated with the passing of puberty, but age was not a contributing factor. In brief, the statistical modelling showed that 50 % of heifers with a carcass weight of 120 kg had passed puberty on day zero, whereas a 90 % of heifers with a carcass weight of 180 kg had passed puberty on day zero. The proportions of heifers that had passed puberty also increased with time.

### 4.2.3 Time of oestrus

Based on the findings in the ovaries and uteri, it was concluded that the time of oestrus was different for heifers than for cows. On day zero (the first day of the hunting period each year), > 90 % of the cows had experienced oestrus, whereas the corresponding proportion for heifers was approximately 68 %. In addition, the results showed that approximately 95 % of the cows (with 95 % certainty) had passed oestrus four weeks into the hunting period, whereas 89 % of the sampled heifers had passed oestrus during the same period (Figure 2, Paper II). There was no apparent effect of sampling year or -area on the probability of having experienced oestrus.

*Repeated oestrus*

Of the sampled animals (excluding prepubertal heifers), 7.6 % (15/198) were judged to have returned to a second oestrus, based on the concurrent presence of a regressing *corpus luteum* together with a mature follicle or newly developed *corpus luteum*.

### 4.2.4 Ovulation

Ovulation rates (OR; number of *corpora lutea* per female) differed significantly ($p < 0.01$) among pubertal heifers (mean OR = 1.08, Table 3, Paper II) and cows (mean OR = 1.46). Ovulation rates were higher among females aged 5 – 12 years of age, than among younger (1.5 – 4.5 years of age) and older animals (> 12 years of age, Figure 5, Paper III).

### 4.2.5 Detection of previous mating

The microscopic examination of smears from the folds of the cervix was performed in 53 heifers from categories 2 and 3. Spermatozoa were observed

in 82.5 % (n = 45). In cows (categories 5 and 6, n = 63), spermatozoa were observed in 84.1 % (n = 53).

### 4.2.6 Pregnancy

Pregnancies (categories 4 and 8, for heifers and cows respectively, Table 2, Paper II) were observed in 15 heifers and 40 cows from day zero to day 103. Pregnancies at varying stages ($\geq$ 2 weeks) were detected. Pregnant cows were recorded on day zero, whereas pregnant heifers were recorded from day 19. The earliest estimated mating and conception period was estimated to occur around mid-September. Of the pregnant heifers (n=15), eight were yearlings that were sampled in November (Nov 3 – Nov 11), with a mean weight of 147.4 kg (range 130 – 185 kg).

## 4.3 Reproductive failure in female moose (Paper III)

### 4.3.1 Pregnancy and ovulation rates

Specimens from animals that had ovulated (n = 142) were selected from amongst the female moose. Of these, 37.3 % were considered to be pregnant (n = 53), and had a mean age of 4.3 years (SD ±2.9, range 1.5 - 16.5). Mean carcass weights of the pregnant moose (45 of 53 weighed) were 172 kg (SD ±24.8, range 120 - 220). The mean ovulation rate in the pregnant moose was 1.49. There were no statistical differences regarding the ovulation rate among pregnant moose in the three different sampling areas.

### 4.3.2 Unfertilized oocytes

In the pregnant females, a total of 81 *corpora lutea* and 76 embryonic structures were found, indicating that five oocytes had been unfertilized (6.3 % of all shed oocytes).

### 4.3.3 Embryonic mortality

Of the 76 identified embryonic structures, 25 % (n = 19) of the embryos, belonging to 16 different animals, were judged to be non-viable based on their macroscopic appearance. There was no observed spatial pattern with regards to the presence of embryonic mortality. The risk of finding embryonic mortality increased with the age of the female (p = 0.003).

### 4.3.4 Endometritis

Among the pregnant animals, endometritis was histologically confirmed in 12 individuals (23.1%). In eight of these individuals, embryonic mortality was also found. Thus, 50.0 % (8/16) of all animals with embryonic mortality also

had signs of endometritis, whereas for animals without embryonic mortality, 10.8 % (4/37) were judged to have endometritis. This difference was statistically significant ($p < 0.01$). The culture of samples from uteri with signs of endometritis showed no growth of aerobic bacteria.

## 4.4 Calf summer survival (Paper IV)

In the three study areas, 88 and 66 calves were born in 2012 and 2013, respectively (Table 3). The mean calving dates (range May 10 – May 19) varied significantly between areas ($p = 0.035$) and between years ($p < 0.001$, Table 1, Paper IV). The lowest summer survival rates were observed on Öland in both 2012 (31.8 %) and 2013 (15.8 %) and the highest in Södermanland in 2013 (88.2 %, Table 3). The summer survival rate differed significantly between areas ($p < 0.001$) but not between years ($p = 0.66$). No significant effect of handling was observed ($p = 0.09$), nor was there an interaction effect of handling and area ($p = 0.68$). On Öland and in Småland (Kronoberg), 55 % of the mortalities occurred during the first two weeks after birth, and 71 % of the mortalities had occurred on Öland and in Södermanland after five weeks.

Table 3. *Moose calf summer survival data from three different areas in Sweden in 2012 and 2013.*

| | Kronoberg | | Öland | | Södermanland | |
|---|---|---|---|---|---|---|
| Year | 2012 | 2013 | 2012 | 2013 | 2012 | 2013 |
| Born | 43 | 30 | 22 | 19 | 23 | 17 |
| Alive | 34 | 21 | 7 | 3 | 17 | 15 |
| Survival (%) | 79.1 | 70.0 | 31.8 | 15.8 | 73.9 | 88.2 |

Of all dead calves (31 on Öland, 18 in Småland, and seven in Södermanland), 15 individuals from Öland were retrieved and submitted for *post mortem* examination. The majority (12/15) was found to be in subnormal body condition, had no milk in the abomasum, and with meconium still present in the rectum. The cause of death was determined to be starvation. Of the remaining three calves, two had succumbed to trauma (suspected dog attack, and blunt force to the skull), although the remaining findings resembled those observed in the previous 12 necropsied calves. The cause of death for the last calf was not possible to determine. From Småland, two calves were examined

*post mortem*, and the findings were identical to the majority of the Öland calves. From Södermanland, one calf was examined and found to have died from a congenital heart malformation.

## 4.5 Epidemiology of *Anaplasma phagocytophilum* (Paper V)

In total, 263 moose (of which 81 were calves) were investigated for presence of anti-anaplasma antibodies and *A. phagocytophilum* DNA (detected with PCR). Regardless of sampling area, 100 % of all sampled animals had anti-anaplasma antibodies. In terms of detection of *A. phagocytophilum* DNA, samples from Öland had a higher (36.6 %, n = 131) prevalence than moose from the mainland populations (Småland, Södermanland, Västergötland, 15.9 %, n = 132). There was a significant temporal and spatial variation in the prevalence of *A. phagocytophilum*, but no effect of age, weight, or sex was observed (Table 2, Paper V). The subset of samples selected for genetic sequencing (n = 2) revealed nucleotide differences between the Anaplasma from the mainland and from the sample on Öland. Genetic similarities with the HGA-agent (Human granulocytic anaplasmosis agent), and with sequences reported in red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*) from continental Europe were found.

# 5 General discussion (Papers I-V)

Overall, the results reported in this thesis provide new and valuable information on different aspects of moose reproduction. Paper I indicated the importance of body weight for pubertal development and morphological quality of spermatozoa in male moose; their testes:body weight ratio was among the lowest ever reported in mammals. This suggests that the presence of large, and not necessarily old, males in local moose populations may have a positive effect on reproductive success. This is also true for female moose as body weight was positively associated with pubertal development, and heifers with higher body weights had a higher probability of showing first oestrus (i.e. passing puberty) than heifers with lower body weights (Paper II).

The mating period of moose coincided with the first part of the hunting season, which may have an impact on reproduction. The results imply that current management regimens and hunting principles need to be discussed from an ethical and biological aspect. After mating, moose experienced reproductive failure resulting either from unfertilized oocytes or embryonic mortality (Paper III). If reproductive estimates are based only on ovarian findings (ovulation rates) and such losses are not taken into account, this may lead to an overharvest of the population. Summer survival rates of calves varied among regions (Paper IV), particularly where causes may have involved a combination of inter- or intra-species competition, and climate effects on the availability of suitable forage (population under pressure). Moose may be a host and possible reservoir of *A. phagocytophilum* in southern Sweden (Paper V), and adverse effects of moose calf health and survival cannot be excluded.

Male moose are equally as important as female moose for successful reproduction to occur. This study presents new information on some sperm quality parameters in moose, and their relationships with weights of testes and epdidymides, age, carcass- or body weight and season. The low testes:body weight ratio found (0.033 %) indicated that male moose most likely are not

anatomically or physiologically adapted to cover several females during a short period of time (Paper I), as this ratio can reflect the mating system of a given mammalian species (Short, 1997. This ratio is considerably lower in moose than in other cervids such as red deer and roe deer (Kenagy & Trombulak, 1986). Sperm quality, based on morphological characteristics, was positively correlated with body weight and testes weight, regardless of age (1.5 - 11 years). Moreover, there was an observed temporal decrease in morphological quality of spermatozoa over the hunting period, which suggests that the breeding season of moose in southern Sweden was drawing to an end towards the end of October and beginning of November. Bubenik and Timmerman (1982) showed a similar temporal decrease in sperm production in North American moose, based on histological investigations of moose testes.

More parameters (total number of spermatozoa and sperm motility) are needed to be able to fully evaluate the sperm quality of male moose. Nevertheless, results suggested that the age of pre-pubertal males varied, which may indicate that body weight is more important than age in order to pass puberty. This underscores the importance of having large males in a population in order to ensure reproductive success (Paper I). It is clear that it is important to maintain a population density, which is appropriate for the available levels of forage in order to attain high body weights, which in turn is important for an earlier onset of puberty in male and female moose in the population. Furthermore, a selective harvest of small calves can be a useful approach to avoid cohort effects as small females subsequently give birth to small calves (Solberg *et al.*, 2007). Moreover, avoiding harvesting of large males prior to the end of the mating period may be beneficial for reproductive success.

Paper II revises some statements from previous studies on the timing of oestrus in moose. We found that moose cows pass their first oestrus of the season earlier than heifers, which is similar to the observations of Haagenrud and Markgren (1974). However they also stated that heifers aged 2.5 years passed their first oestrus earlier than heifers aged 1.5 years. The duration of the seasonal oestrus period in our study was, however, longer than reported by Garel et al. (2009), although Haagenrud and Markgren (1974) stated that oestrus and mating took place from early September to late October. In the current study, ovarian activity and stage of pregnancy in pregnant animals indicated that not all cows and heifers had experienced oestrus by the end of October. However, the majority of both heifers and cows had experienced their first oestrus of the season prior to the beginning of the hunting period, indicating that a peak in oestrus activity occurred just prior to this period. It is clear that there is an overlap in timing; several females were just about to commence oestrus or were experiencing oestrus during the first month of the

hunting period in southern Sweden. The effect of hunting during the oestrus period has not been fully evaluated, although Haagenruud and Markgren (1974) suggested that it was unlikely to have a negative effect on reproductive success. However, the ethical implications of hunting during the mating period may need to be addressed by hunters, managers and authorities. Furthermore, neither red deer nor fallow deer (*Dama dama*) in Sweden are hunted during the mating period; it would seem appropriate and consistent to apply the same standards for the timing of moose hunting.

In Paper II we showed that puberty in females (age $\geq$ 1.5 years) was more strongly correlated with body weight than with age, although the weight of an animal generally increases with age. Sexual maturity has been investigated in previous studies (e.g. Sand & Cederlund, 1996) and has been defined as the age when a female gives birth to its first calf, but also as the age at mating the year preceding the year of first parturition (e.g. Sæther & Heim, 1993). Pre-puberty is a maturation process, which culminates in puberty, (i.e. a first oestrus and ovulation), followed by repeated cyclicity, and the animal can then be labeled as having reached sexual maturity. Repeated cyclicity is difficult to assess in free-ranging moose, and thus determination of first ovulation has been used in our studies as the time of puberty. The use of sexual maturity may lead to misinterpretations of the onset and passing of puberty. For example, in a 3.5-year old female that has been determined to be sexually mature based on findings of *corpora albicantia* in the ovaries, it is not possible to state whether it has given birth to a calf at two or three years of age. Similarly, if a female gives birth at three years of age, and is subsequently judged to have become sexually mature the previous autumn (at 2.5 years of age), one cannot rule out that it passed puberty at the age of 1.5 years. Sæther and Haagenrud (1983) used weight as an explanatory variable when studying puberty in Norwegian moose, although that part of their study only included yearlings (females < 2 years of age). Our study showed that not all female moose pass puberty at the age of 1.5 or 2.5 years, since individuals up to 3.5 years old were found with no signs of ovarian activity. In addition, pubertal heifers aged up to 6.5 years were found, where no signs of previous pregnancy were observed. Females older than 3 years that have not passed puberty likely reflect poor environmental conditions (i.e. availability and quality of forage). The presence of such animals in a managed population is not favorable, as these animals do not contribute to the population growth. Furthermore, as shown in our studies, there is a proportion of females with low body weights that still pass puberty and are capable of reproducing. When they reproduce, maternal cohort effects may be a result, as reported by Solberg et al. (2007), where small females give

birth to small calves. These calves become small adults who subsequently give birth to small calves, and this process continues.

The majority of the heifers and cows that had one or more *corpora lutea* present had been mated prior to the time of sampling, which was verified by findings of spermatozoa in smears from the uterine cervix. Nevertheless, there was a proportion of the females that had not been mated. The reason for this could be failure to detect spermatozoa in the cervix, a shortage of males at mating (i.e. a skewed sex ratio due to an overharvest of males), or a disturbance factor present at mating. A skewed sex ratio can be manifested as lower conception rates or delayed births of calves. This effect was, however, reported to be more common in low-density populations (Solberg *et al.*, 2002) and thus likely not valid for the moose population in the south of Sweden. Another reason may be that hunting as such had disturbed the moose (males and females) during mating, although this could not be verified in the present study.

After a successful mating and conception, embryonic development begins. To my knowledge, Paper III is the first moose study that describes the characteristics and occurrence of embryonic mortality in free-ranging moose. There was a significant discrepancy between ovulation rates and signs of pregnancy, which showed that determination of ovulation rates alone might not be a correct measurement for fecundity prediction in a moose population. The incidence of the loss of oocytes (*ova* loss) reported earlier range from 9.3 % (Schwartz & Hundertmark, 1993) to 19 % (Markgren, 1969), but most likely a proportion of the loss (Paper III) can be attributed to embryonic mortality. Macroscopic findings of a non-viable embryo have never been reported previously in moose. However, a presumed dead embryo was earlier observed by transrectal ultrasonography in an immobilized Alaskan moose (Testa & Adams, 1998). There are a number of known causes of embryonic mortality, including the viability and quality of the embryo, quality of the spermatozoa or oocyte at fertilization, inadequate maternal recognition of pregnancy, uterine malformations, chromosomal aberrations, and infectious agents (Diskin *et al.*, 2011). However, it is likely that yet another cause of embryonic mortality in moose could be endometritis (Paper III) although the cause was not identified (Paper III). An anaerobic bacterial or fungal infection may have caused the endometritis in some cases.

The study of moose calf summer survival verifies that moose living along the southern periphery of its distribution in Scandinavia may be subject to some density dependent or density independent environmental stress (Sæther, 1997). Furthermore, the data suggests it is site-specific and may not be a universal pattern for southern Scandinavia. The summer survival rate for

moose calves on the island of Öland (Lat 56 N) is, to our knowledge, among the lowest rate ever reported in areas with no large predators present, whereas Småland and Södermanland moose calf survival rates are in the normal range for Scandinavian predator-free areas (Ericsson, 1999: Swenson *et al.*, 1999; Swenson *et al.*, 2007). Survival rates in populations where predators are absent are usually around 80-90 % (Linnell *et al.*, 1995). We speculate if unfavorable indirect (winter), and direct (spring/summer) climatic conditions may be the ultimate cause of the low survival rates on Öland. Furthermore, a reduced availability of suitable high quality forage may work in concert with unfavorable weather conditions (Crête & Courtois, 1997; Grøtan *et al.*, 2009) and vector-borne pathogens, such as *A. phagocytophilum*. The pregnant female needs to be in good physical condition to supply the rapidly growing fetus with nutrients during the last trimester of pregnancy. Previous studies in areas with and without predation suggest that the highest calf mortality takes place within the first month of life (Swenson *et al.*, 1999; Swenson *et al.*, 2007). The data in our study validate that calves tend to die within the first month, particularly in our two most southern study sites at Lat 56 N; Öland and Småland. Moose on Öland may also be affected by heat stress, in line with increasing global temperatures. Research from North America indicate that summer temperatures above +14°C increase heart, metabolic and respiratory rates, resulting in lower food intake and reduced body weight (McCann *et al.*, 2013). In particular, ambient temperatures above +20°C seem to be critical for moose performance since more energy then go into regulating moose inner body temperature via open-mouth panting than at lower temperatures (Renecker & Hudson, 1986). Öland is regularly above +18°C on average during the summer (SMHI, 2014). Most likely temperatures above the two thresholds +14°C and +20°C lead to increased energy expenditures which may have consequences for the body condition of cows, their ability to produce milk and thereby nurse the calves.

Moose on Öland were more exposed to *A. phagocytophilum* than moose from the mainland, based on a significantly higher presence of *A. phagocytophilum* DNA in samples from Öland compared with those from the mainland (Paper V). Recurring risks of infection might be one reason due to an overall higher infection pressure on the island. Migrating birds that use Öland as a stopover site are reported to carry *A. phagocytophilum* infected ticks (Bjoersdorff *et al.*, 2001). Moreover, other tick hosts such as roe deer are highly abundant on the island.

The effect of an *A. phagocytophilum* infection in moose is hitherto not fully elucidated. However, there is a report of the infection found in a moose calf from Norway, which succumbed to a bacterial pneumonia, but in which *A. phagocytophilum* was considered to have facilitated the lung infection (Jenkins

*et al.*, 2001) and a similar case in a calf from Öland (Bernodt, 2005). Cases of dead moose calves with polyarthritis, and DNA-presence of *A. phagocytophilum* have recently been identified in southern Sweden, including Öland (E. Ågren, personal communication). Since *A. phagocytophilum* has an immunosuppressive effect, such an infection may predispose moose calves for secondary bacterial infections resulting in mortality. Furthermore, a poor body condition, as a result of a sub-optimal milk production in the cow, may increase both the effect of *A. phagocytophilum* infection and subsequently also the risk of secondary (and possibly lethal) infections. Moose should also be considered as a wildlife host of the bacterium, which may affect the ecology of the disease as most mammals are susceptible to infection, including domestic livestock and humans.

# 6 Conclusions

According to the overall aims of the studies, different factors that are associated with male and female moose reproduction, as well as calf health and survival in southern Sweden were described. Specific conclusions from the studies were:

- Different characteristics of male moose reproductive organs, and their relationship to age and body weight:
    - Body weight was positively associated with the weight of male moose testes and epididymides, and also with the proportion of morphologically normal spermatozoa. Age was not associated with any of these factors.
    - The proportion of morphologically normal spermatozoa decreased with time during the sampling/hunting period.
    - The use of chromatin integrity in order to evaluate sperm quality in moose was not indicative of the sperm quality expressed as proportion of morphologically normal spermatozoa.
    - Male moose are not anatomically adapted to cover several females in a short period of time.
    - The abundance of large (body weight) males, rather than having a high mean age of males in a moose population, may positively affect the reproductive output.
    - Male moose with high body weights should preferably not be hunted before the end of the normal oestrous period of females, and a low proportion of adult males in a moose population should be avoided.

- Different reproductive characteristics of female moose with regards to puberty, timing of ovulation, ovulation rates, and mating:
    - The seasonal oestrous period of female moose was longer than previously reported, and differed significantly between cows and heifers. The sampling/hunting period interfered with oestrus in some of the cows and the heifers. Thus, a two- to three-week delay of the opening day of the moose hunt is recommended in southern Sweden, in order to ensure that the majority of heifers have experienced their first oestrus of the season.
    - Microscopic detection of spermatozoa in samples taken from the cervical folds of female moose was a useful method for early identification of recent mating.
    - Ovulation rates were lower in both young (1 – 4 years of age) and old (> 12 years of age) female moose, respectively, compared to in middle-aged females (5 –12 years of age).

- The occurrence of reproductive failure in female moose:
    - Embryonic mortality and unfertilized oocytes accounted for a 25 % discrepancy between ovulation rates and the proportion of viable embryos found in pregnant females. Thus, estimating future offspring rates based only on ovulation rates of harvested females may lead to an overestimate of population growth rate.

- Moose calf summer survival in southern Sweden:
    - Moose calves in Öland, an area where large predators are absent, had significantly lower summer survival rates compared to other predator-free mainland moose populations.
    - The survival rates of moose calves on Öland are the lowest ever reported in moose in areas without the presence of large predators. Inter- and intraspecies competition for forage (population under pressure), together with low precipitation and high temperatures were probable causes of the low survival rates on the island of Öland.

- The occurrence and epidemiology of *Anaplasma phagocytophilum* in moose from southern Sweden:
    - The moose is a natural host of this pathogen in southern Sweden, and results showed that all tested moose had been exposed to the bacterium.
    - PCR analysis to detect *A. phagocytophilum* indicated a significant temporal and spatial variation in the prevalence of this pathogen.
    - *Anaplasma phagocytophilum* infection could pose a risk to moose calf health, and contribute to summer mortality of moose calves in poor body condition.

# 7 Management implications

The study presented in this thesis represents an example of how hunters identified a potential problem in a managed moose population and communicated their concerns to researchers, who then initiated a research project. The pilot study conducted on the island of Öland (in 2007) suggested hypotheses on the decline in moose recruitment rates, and raised questions and hypotheses regarding the general characteristics of male and female moose reproduction resulting in an expanded study (2008-2011).

This project examined a number of factors associated with reproduction in male and female moose in Sweden, and subsequent survival of moose calves. Some of these factors can be affected (negatively or positively) by management strategies, whereas some are more linked to the effect of changes in climate or in the surrounding environment, which need to be considered when making management decisions. The project has also highlighted the importance of investigating several different parameters of moose reproduction in order to achieve a more comprehensive understanding. Several previous studies have focused on female moose, without considering male reproduction or calf health and survival, although these are clearly also crucial to ensure survival and growth of the population.

The forage availability (and quality) is usually reflected in the body weight of moose. Forage availability may in turn be affected by the density of cervid populations, (both inter-species, and intra-species competition). Forage availability is also affected by climate and forest management. Thus, obtaining knowledge of the composition of the cervid population and habitat of different species is essential for making management decisions; the climatic effect also needs to be considered.

The results of this thesis suggest that it is favorable to strive for high body weights in a managed moose population in order to improve the chances of reproductive success. This could be attained by a selective harvest where,

individuals with low body weights are targeted independently of the age of the moose. From a hunter's point-of-view, however, it is easier when only calves are selected because of the difficulty in assessing body size in solitary animals. However, if the forage availability is low, other actions may be required, such as decreasing the density of moose and other cervid species that compete for forage.

Furthermore, the present study shows that it is not advisable to hunt male moose, especially not large males, before most of the mating period has passed. Ethical aspects of hunting during the mating period also ought to be considered. From this perspective, the avoidance of moose hunting during the mating period is recommended, (i.e. during October). Principles related to this have been implemented in the hunting legislation that involves other deer species in Sweden (red deer, fallow deer), where no hunting takes place during the mating season. Thus for moose, either a revision of the legislation is required or the principles regarding hunting during the mating period should be changed.

The spring and summer climate seems to play an important role for moose calf survival rates, e.g. via the ability of the cow to produce milk and thereby feed the calf. The climate impact needs to be considered from a management point-of-view, as moose calves often account for a large proportion of the total harvest. Lastly, the management of forest, agriculture and the overall landscape needs to be taken into account, as this also may affect moose habitat and forage availability and quality.

# 8 Future considerations

In order to further investigate reproduction in Fennoscandian moose, the determination of courting and mating behavior, i.e. the degree of polygyny, needs to be studied in populations of varying density and condition. Large-scale capture of males and females would be required, and the use of GPS-collars followed by intense field observational studies would be beneficial. Furthermore, since the duration of pregnancy (and possible variations in duration) in Fennoscandian moose has not been fully described yet, further field studies (during oestrus and mating, and subsequent parturition) of GPS-collared females would be necessary. In addition, as comprehensive information on the embryonic and fetal development is not available, thorough and repeated investigations (by transrectal ultrasonography) of pregnant females, preferably captive, could be conducted to obtain these data.

There are several parameters such as libido and sperm volume, along with the study of polygyny, that are of interest in order to further characterize male moose reproductive characteristics. Furthermore, studies of possible links between sperm quality and phenotypic attributes (body weight, and antler size and shape) would be of interest for management purposes, since antler size (number of points) currently is one way of selecting males for harvest. By identifying poor sperm quality through phenotypic attributes, some undesirable males (from a reproductive aspect) may be removed from the population.

Other ungulate game species (red deer, fallow deer, wild boar) are increasing in southern Sweden, and the effect of this increase on moose reproduction need to be elucidated, as they may also compete with moose for forage. Thus, careful censuses and habitat (forage) studies, as well as multi-species GPS-collaring and subsequent studies of habitat usage and possible areas of overlap, would be useful. In addition, ethological studies of moose in locations with and without the presence of other ungulate species would enable an investigation of the possible impact of these other species on moose

behavior. An increase in the dispersal and density of large predators may be a reality in southern Sweden in the future. Thus, more complete ecosystems are formed, and the effect of the large predators on moose and other cervid populations in southern Sweden also requires further studies for biological and management reasons.

Further studies are required on the effects of climate (precipitation, temperature) on calf survival, along with studies of possible interspecies competition, density effects, habitat overlap, and forage availability (mentioned previously).

As moose calf health may be affected by infection with *A. phagocytophilum*, experimental studies are needed to determine the impact of infection with this pathogen. A compilation of post-mortem findings and additional microbiological analyses of moose calves submitted for post-mortem examination could further clarify how moose calves are affected by this infection. Furthermore, since little is known about the moose as a possible reservoir or host of other tick-borne diseases, further microbiological analyses of collected moose samples (including detection of *Borrelia burgdorfer*i and tick-borne encephalitis virus, among other pathogens) should be performed in moose. This may be important from a zoonotic, i.e. 'one-health' perspective, but also since an infection with one or several different, previously undetected, pathogens may affect moose health and survival.

# 9 Populärvetenskaplig sammanfattning

Älgen anses vara det viktigaste jaktbara viltet i Fennoskandien. Nästan 200 000 älgar skjuts sammanlagt varje år i Norge, Sverige och Finland. Älgen är även en källa till rekreation, turism och kött, vilket inbringar betydande inkomster för jägare, markägare och turistföretag. För att hållbart kunna förvalta ett jaktbart vilt krävs kännedom om dess reproduktion. Utan en väl fungerande reproduktion sker ingen tillväxt i populationen.

Jägare på Öland kontaktade 2006 SVA i Uppsala med anledning av en oro för den lokala älgstammens utveckling med bland annat ett lågt observerat antal kalvar per ko under jakten föregående säsong. En påföljande pilotstudie visade på förekomst av embryonal död och av den fästingburna bakterien *Anaplama phagocytophilum* i organ från skjutna älgar på Öland. En litteraturgenomgång visade att basal kunskap om älgens reproduktion (hondjur och handjur) behövde uppdateras och att endast få studier hade gjorts av älgreproduktion i södra Sverige. Den svenska älgstammen och dess livsmiljö (klimat, habitat, fodertillgång, populationer av annat klövvilt) har dessutom under de senaste 30-40 åren genomgått stora förändringar. Det kan inte uteslutas att dessa omfattande förändringar av älgstammen och älgens livsmiljö även påverkat reproduktionen, som till stor del styrs av älgstammens sammansättning och tillgången på foder.

Forskning på reproduktion hos älg i Sverige har gjorts sedan mitten av 1900-talet, och då oftast med fokus på honlig reproduktion. Syftet med denna studie var att undersöka och beskriva honlig och hanlig älgreproduktion samt undersöka förekomsten av, och vissa epidemiologiska aspekter på infektion med *Anaplasma phagocytophilum* hos älg i södra Sverige. Vidare undersöktes sommaröverlevnaden hos älgkalvar på Öland och på det syd- och mellansvenska fastlandet.

Under perioden 2008 - 2011 togs prover från älgar på Öland och på fastlandet (Södermanland, Småland, Västergötland). Material (blodprov,

reproduktionsorgan, mjälte, lever, träck, underkäkar, mm) samlades in från ca 400 skjutna vuxna älgar. Ytterligare ca 80 kalvar provtogs för undersökning av förekomst av *Anaplasma phagocytophilum*. Fältlaboratorier sattes upp inom definierade försöksområden i anslutning till älgjaktens start, vilka gjorde det möjligt att på platsen ta prov på ett färskt vävnadsmaterial endast en kort tid efter älgen fällts samt ta ut prover för senare analyser (spermieundersökningar, mikrobiologiska undersökningar mm) vid SLU eller SVA i Uppsala. Utöver detta följdes sammanlagt 66 halsbandsförsedda (GPS/GSM/VHF) älgkor och kvigor i tre olika områden (Öland, Södermanland, Småland) under 2012 och 2013 för att kunna observera när kalvning ägt rum och därefter kunna följa sommaröverlevnaden hos kalvarna.

Det fanns ett signifikant samband mellan handjurens kroppsvikt och andelen normala spermier (i prov från bitestikeln). Testikelvikten och andelen normala spermier var även positivt korrelerade till kroppsvikten. Alla 1,5-åriga tjurar var inte könsmogna och bland de 2,5- och 3,5-åriga tjurarna fanns individer som inte bedömdes vara fullt könsmogna baserat på den spermiemorfologiska bilden. Testiklarnas vikt i förhållande till kroppsvikten, det vill säga kvoten testikelvikt:kroppsvikt, var mycket låg hos älgtjurarna (medelvärde 0,033 %), vilket indikerar att de inte har förutsättningar för att betäcka ett flertal hondjur under en kort tidsperiod. Andelen morfologiskt normala spermier minskade mot slutet av oktober och början av november jämfört med i början av oktober månad, vilket tyder på att den reproduktiva säsongen hos tjurarna sannolikt då befann sig i slutfas.

Hos de undersökta hondjuren var kroppsvikten snarare än åldern avgörande för när en älgkviga blev könsmogen. Älgkorna hade ovulerat (haft ägglossning), dvs. passerat brunst tidigare på säsongen än kvigorna, även om en viss överlappning sågs mellan grupperna. Trettio dagar in på älgjaktsperioden hade dock ett fåtal älgkor inte ovulerat och för kvigor var denna andel högre. Medelålders älgkor (5-12 år gamla) avlossade i genomsnitt ett högre antal ägg än jämfört med kvigor och äldre kor. Hos dräktiga älgkor och kvigor observerades att ca 30 % av de ägg som avlossats inte resulterade i ett normalt embryo. Om slemhinnan i livmodern visade tecken på inflammation (endometrit) ökade risken för embryonal död.

Sommaröverlevnaden hos kalvar från Öland var signifikant lägre än hos kalvarna från Södermanland och Småland, men ingen skillnad sågs mellan de studerade åren. Den låga överlevnaden (32 respektive 16 % under 2012 och 2013) av kalvar på Öland jämfört med fastlandet (medelvärde 77 respektive 79 % under 2012 och 2013), den höga dödligheten kort efter födseln (55 % inom två veckor), samt obduktionsresultaten (att kalvarna inte diat, påvisat hos 16 av 18 obducerade kalvar) tyder på ett dåligt näringstillstånd hos älgkorna under

tiden före och precis efter kalvning. Dåligt näringstillstånd hos älgkorna kan i sin tur ge en otillräcklig mjölkproduktion, vilket kan bero på en otillräcklig tillgång på foder i kombination med negativa effekter av klimatet (låg nederbörd, hög temperatur) under denna kritiska period.

Den fästingburna bakterien *Anaplasma phagocytophilum* påvisades med PCR-metod i mjälte hos i genomsnitt 26 % av alla provtagna älgar, men förekomsten var signifikant högre hos älgar på Öland (37 %) jämfört med de på fastlandet. Samtidigt visade den serologiska analysen att alla provtagna älgar hade blivit infekterade, eftersom de alla hade antikroppar i blodet. Älgen kan därför vara en möjlig reservoar för *A. phagocytophilum*.

Sammanfattningsvis visade studierna av reproduktion att älgens parningssäsong sammanfaller med den nuvarande jakttiden i södra Sverige och att negativa effekter på reproduktionen inte kan uteslutas. Även de etiska aspekterna på jakt under älgens parningssäsong bör beaktas. Studierna visade också att älgens reproduktion är kopplad till kroppsvikten, som i sin tur kan vara beroende av älgstammens (och annat hjortvilts) förvaltning och klimatet. Älgkalvar dör i mycket högre utsträckning på Öland än på fastlandet under sommarperioden. Fodertillgången för korna under sen dräktighet och under digivningsperioden hade sannolikt varit undermålig, vilket kan bero på konkurrens om fodret, samt effekter av klimatet. Infektion med anaplasma-bakterier var vanlig hos älg i södra Sverige. En negativ effekt på älgkalvars hälsa och överlevnad kan därför inte uteslutas.

# Acknowledgements

The funding for these studies was provided by the Swedish Environmental Protection Agency, SVA, the Environmental monitoring and assessment program (FOMA) at SLU, the Faculty of Veterinary Medicine and Animal Science at SLU, and the Wildtech Project (European Union Seventh Framework Programme - FP7/2007-2013, grant agreement no. 222633).

I would like to acknowledge the Department of Clinical Sciences at SLU and the Department of Pathology and Wildlife at SVA for all support provided during my doctoral studies.

The studies performed and the writing of this thesis would not have been possible without the help and support of certain people. I would like to express my sincere and utmost gratitude to:

Professor **Anne-Marie Dalin**, my main supervisor, for your everlasting support, time, effort and care. If more doctoral students had a supervisor like you, the world would see happier, more productive, more tedious, and more competent PhDs. Together, we have conquered obstacles, small and large, and you have always been there for me, no matter the time of day. We do make a great team, and I will miss the early morning hellos, meetings, and joy.

Professor **Lennart Söderquist**, co-supervisor, for so many things. Your scientific advice is always well put, thought through, and bright. And I have the utmost respect for you as a person, colleague, and friend.
Associate professor **Dolores Gavier Widén**, co-supervisor, for your energy, scientific excellence, care, and great advice. You have always been supportive of my ideas and suggestions, and as a person and a researcher, I admire you.

Associate professor **Carl-Gustaf Thulin**, co-supervisor, for always speaking your mind in every situation, may it be professional or from friend to friend. You have been a perfect fit in the supervisors' group, with your experience in a variety of scientific 'jam jars'. I appreciate the many times you have questioned the veterinary scientific reasoning, or when you have planted biological thoughts in my head. Our paths once crossed in a field of hares, and may they forever be connected.
Lecturer **Lisa Yon**, co-supervisor, for your never-ending kindness and valuable scientific advice. Not to mention the way you have improved my scientific writing.
Professor **Mike Hutchings**, co-supervisor, for the interesting cross-disciplinary talks and discussions, and for the way you improved my scientific reasoning from a wildlife ecologists' perspective.

Professor **Göran Ericsson**, my "ghost-supervisor", for always finding the time to answer my range of moose thoughts, questions, and ideas. Your support and competence during my PhD journey has been immensely valuable. And I will never hesitate to start new projects with you, or discuss the latest hockey scores.

Associate professor **Torsten Mörner** for hiring me in 2006, and handing me the initial Öland assignment. Without your open mind and inspiration, I probably would have been somewhere else right now. I am forever grateful.

My beloved colleagues at the Department of Pathology and Wildlife diseases at SVA, and especially the wildlife section; **Erik Ågren** (and an extra thank you for being my boss), **Henrik Uhlhorn**, **Caroline Bröjer**, **Roland Mattsson**, **Ewa Backman**, **Karin Bernodt**, **Aleksija Neimanis**, **Gete Hestvik**, **Arne Söderberg**, **Jessica Åsbrink**, **Axel Sannö**, and **Lena R-C**. You are simply the best and I have missed you loads during the past few years.

The wonderfully helpful **Karin Selin Wretling**, for your time in the field and in the lab, and **Annika Rikberg** for your help with all sorts of moose slides. You both deserve a big raise!

Chief **Carl Hård av Segerstad** for your never-ending positive thinking and support, your open office door, your decisiveness, and your wits.

Division head and professor **Ulf Magnusson** for welcoming me into the Repro-family, for the rapid decision-making, and for the good years at the division. You are worthy of a flannel shirt!

Professor **Jane Morrell** for always finding the time to improve my English in the manuscripts.

**Dr. Mikael Andersson Franko** for all statistical advice and help.

My remaining co-authors **Gustav Rydevik**, **Snorre Stuen**, **Anna Aspán**, **Wiebke Neumann**, and **Navinder Singh**. Thank you!

Det finns ytterligare en uppsjö av mäninskor som jag vill tacka för att denna avhandling blev till. Alla som varit behjälpliga under resans gång (och det är många) skall känna sig delaktiga i detta, vare sig det gäller karvande i döda älgar, ett trevligt samtal över en kopp kaffe eller helikoptertankning:

Kollegorna vid **Avdelning för Reproduktion** för alla trevliga stunder. Ni har förgyllt vardagen vid SLU!

**KV-administrationen** och framför allt **Sussie Pettersson**, **Annika Nyström**, **Micke Rosenius**, **Anette Forsberg** och **Marie Sundberg** för all hjälp och stöd vid allsköns undringar och problem.

**Magnus Johansson** och **Börje Andersson** för er outtömliga energi och ert engagemang för Ölands älgar. Utan er vore projektet ingenting. Och *nota bene*, vi är inte i hamn än…

**Niklas Moberg**, **Tommy Kensing**, **Göran Groth**, och **Anders Hågeryd** för möjliggörande av, och hjälp vid fältarbete. Utan er hjälp hade vi inte lyckats.

**Älgjägare** från när och fjärran. Hade ni vilat på hanen hade vi inte fått ett enda prov…

**Eric Andersson**, **Dr**. **Fredrik Stenbacka**, **Åke Nordström**, **Jimmy Pettersson**, **Kent Nilsson**, **Pilot-Lars-Anders** och **Pilot-Mattias**, samt **Boel**, **Julia**, **Karin**, **Louise**, **Fredrik**, **Annika**, **Örjan**, och **Johanna** för allt slit i fält.

SVAs älgprojektsupportfunktioner i form av **Hasse Strömbom**, **Lillemor Ekström**, **Marianne Wallin**, **IT-gänget**, **Centralförrådet**, **Paketeringen**, och inte minst **Växel-töserna**. Utan er – ingen ordning på undertecknad!

Det gåtfulla salsfolket på SVA i form av **Hasse**, **Johan**, **Lasse**, **Anders** och **Benny**. Tack för roliga och hjälpsamma stunder och för gott kaffe!

Min kära vän, kurskamrat och kollega **Oskar Nilsson**. Du har länge varit en inspiration och nu är jag framme. Men i squash slår du mig aldrig…

Mina kära nuvarande och tidigare doktorandkollegor vid Reprod: **Ola Thomsson**, **Kinna Osbjer**, **Elisabeth Lindahl**, **Denise Laskowski**, **Theo Ntallaris**, **Celina Abraham**, **Metasu Chanrot**, **Panisara Kunkitti**, **Branislav Lakic**, **Johanna Lindahl**, **Kia Nordéus**, och **Sara Persson**. Sådana som ni växer inte på träd!

Mina kära **goda vänner** – ni är många och värdefulla!

Svärmor **Christina** och svärfar **Lars-Helge**. Tack för allt stöd i form av barnpassning, hundpassning, höns-matning, bil-utlåning och utfodring. Ni är bäst!

Min kära mor **Gunilla** och min kära far **Bertil**. Jag har er att tacka för så oändligt mycket.

**Anna**, min älskade hustru och bästa vän. Utan dig är jag ingenting. Och **Vidar**, **Silje** och **Liv** – ni förgyller dygnets alla timmar.

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