DISSERTATIONS IN
FORESTRY AND
NATURAL SCIENCES

Tommi Paakkonen

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Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences

UNIVERSITY OF
EASTERN FINLAND

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No 66

Academic Dissertation
To be presented by permission of the Faculty of Science and Forestry for public examination in the Auditorium N100 in Natura Building at the University of Eastern Finland, Joensuu, on May, 25, 2012 at 12 o'clock noon.

Department of Biology

Kopijyvä Oy
Joensuu, 2012
Editors: Prof. Pertti Pasanen
Prof. Matti Vornanen, Prof. Kai Peiponen
Distribution:
Eastern Finland University Library / Sales of publications
julkaisumyynti@uef.fi; http://www.uef.fi/kirjasto
tel. +358-50-3058396

ISBN: 978-952-61-0771-4 (printed)
ISSN: 1798-5668
ISSNL: 1798-5668
ISBN: 978-952-61-0772-1 (PDF)
ISSN: 1798-5676 (PDF)

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ABSTRACT

The deer ked, *Lipoptena cervi* L. (Diptera: Hippoboscidae), is an ectoparasitic fly that spread to Finland from the southeast in the early 1960's. Presently its northern distribution limit lies at approximately 65°N and it is gradually spreading northwards. The principal host species is the moose, *Alces alces*, but the deer ked is about to establish contact with another potential host, the semi-domesticated reindeer, *Rangifer tarandus tarandus*, causing possible threats to reindeer husbandry.

To study how the intensity of deer ked parasitism varies with the age and gender of the moose, and whether parasite densities differ between anatomical regions of the host, the skins of 23 moose were examined in autumn 2006 for the presence of deer keds, which were extracted and their total numbers estimated. All inspected moose in eastern Finland were heavily parasitised. The bulls had the highest parasite intensity (10616 ± 1375 keds) and density (35.7 ± 4.4 keds/dm^2 of skin), the cows had a higher number of keds than the calves (3549 ± 587 *vs.* 1730 ± 191 keds), but the densities were close to equal (11.8 ± 1.7 *vs.* 9.4 ± 1.1 keds/dm^2). Anatomically, the anterior back with approximately half of all keds had the highest density (54.1 ± 8.9 keds/dm^2), possibly because of the longest fur in that area providing shelter for the parasites, and due to the negative geotaxis and phototaxis displayed by the deer ked. The posterior back harboured approximately one fourth of all keds, followed by the front limbs, abdomen, head and hind limbs.

Moose blood samples (n = 78) were collected in autumn 2006 from deer ked-infested and deer ked-free regions in Finland (62–68°N) to investigate whether intensive parasitism affects the health of the host. Also, tissue samples (n = 23) were collected from a deer ked-infested region (62°N) to determine, how the parasite load correlates with physiological variables of the moose. The differences in blood and plasma values between the deer ked-free and deer ked-infested animals were minor. In the infested regions, the moose had higher mean corpuscular haemoglobin concentrations unlikely to have been caused by the

parasitism. With the exception of hepatic n-3 polyunsaturated fatty acids, the intensities of deer keds did not show consistent correlations with the values of plasma clinical chemistry, endocrinology, amino acids, body energy stores, tissue fatty acids or enzyme activities. Thus, the moose in eastern Finland seemed to tolerate intensive deer ked parasitism relatively well.

To investigate whether the deer ked had an influence on the welfare of the reindeer, 18 enclosure-housed animals were divided into three experimental groups: a control group and two infected groups inoculated with 300 deer keds per host in August–September 2007. One of the infected groups was medicated with antiparasitic ivermectin in November. Similar to the moose, the keds caused no clear changes in the wide array of physiological variables measured during and at the end of the study in December. The survival of the deer keds was very low (2.1%), suggesting that the reindeer may not be an ideal host species for the parasite. Ivermectin seemed to be an efficient antiparasitic agent against deer keds.

The deer ked imago can encounter subfreezing ambient temperatures (T_a) during a short autumnal period between emergence and host location. The cold-tolerance of the imago was investigated by determining its lower lethal temperature (LLT_{100}, 100% mortality) during faster and slower cold-acclimation, by measuring the supercooling point (SCP) and by analysing the levels of potential low-molecular-weight cryoprotectants. The LLT_{100} of the deer ked imago was approximately –16°C, which would enable it to survive freezing nighttime T_a north of its current area of distribution. The SCP was –7.8°C, higher than the LLT_{100}, suggesting that the deer ked could be freeze-tolerant. The concentrations of free amino acids, especially nonessential, were higher in the cold-acclimated deer keds, possibly contributing to cold-tolerance.

Universal Decimal Classification: 591.557.8, 595.773, 599.735.31

CAB Thesaurus: Alces alces; antiparasitic agents; blood analysis; body regions; cold tolerance; ectoparasites; health; hosts; ivermectin; Lipoptena cervi; parasitism; physiology; reindeer; tissues

Yleinen suomalainen asiasanasto: ekofysiologia; fysiologia; hirvi; hirvikärpänen; kudokset; kylmänkestävyys; loiset; parasitismi; poro; terveys; verikokeet

Acknowledgements

This thesis was carried out at the Department of Biology of the University of Eastern Finland. I wish to express my gratitude to the present and former Heads of the Department for providing the excellent working facilities. This thesis was financially supported by the Finnish Cultural Foundation, the Finnish Cultural Foundation North Karelia Regional Fund, the University of Eastern Finland, the Ministry of Agriculture and Forestry, the Oskar Öflund Foundation, the Finnish Game Foundation and Vetcare Oy.

Foremost, I want to thank Petteri Nieminen and Anne-Mari Mustonen that have been my supervisors and mentors ever since my MSc thesis. Without your endless encouragement, great support, constructive criticism and valuable comments (and our frequent research sessions) this thesis would not have been possible. I have always appreciated you professionally and during all these years we have become friends, of which I am very glad. I also thank my third supervisor, Heikki Roininen especially for his crucial help to arrange the financial support at the end of the project.

I sincerely thank the official reviewers of this thesis, Juhani Itämies and Antti Oksanen for their comments and statements, and Rosemary Mackenzie, who kindly revised the language. I also thank my co-authors Reijo Käkelä, Sauli Laaksonen, Jari Aho, Harri Eerilä, Laura Härkönen, Arja Kaitala, Teemu Kiljander, Raine Kortet, Sanna-Mari Kynkäänniemi, Vesa-Pekka Lehto, Pekka Niemelä, Katri Puukka, Joakim Riikonen, Vesa Ruusila, Seppo Saarela, Milla Solismaa and Hannu Ylönen for their valuable contributions in the original papers.

I acknowledge Heikki Hyvärinen for his valuable comments on paper IV and for assistance with the moose age determinations. I wish to express my gratitude to Tatiana Hirvonen, Anna-Liisa Karttunen, Anita Kervinen, Rauni Kojo,

Leena Mattila, Maini Mononen, Jarmo Pennala, Jukka Sormunen and Pekka Tynjälä for their assistance with the laboratory work. I also thank Juha Asikainen, Sauli Härkönen, Johanna Laine, Marja-Liisa Martimo-Halmetoja and Tapani Repo for technical assistance and the employees of the Zoological Gardens of the University of Oulu for their help during the reindeer experiment. Pekka Karhu and the hunting clubs Kaatamon Erä and Ristinpohjan Metsästysseura gave me valuable help with the moose samples.

I thank my parents Hilkka and Aaro for all possible support they have given me all these years. Furthermore, I thank my siblings, Kimmo, Janne, Juha and Jaana and their families for sharing their lives with me. I am grateful to my godfathers Ahti and Timo as well as all other relatives that have supported me. I am also sincerely grateful to all my friends for providing important counterbalance to science. Special thanks belong to Pekka, Henri and Matti for their friendship. I also acknowledge my father-in-law, Tapio for his support. Unfortunately, two very close persons passed away during my studies, Annikki and Jarmo, I miss you every day.

Finally, I wish to thank the most important person in my life, my dear wife Jenni, for her love, support and encouragement. Our companionship is the rock solid foundation for everything else in life. I dedicate this thesis to you with all my love.

LIST OF ABBREVIATIONS

AA	Amino acid
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
BM	Body mass
Chol	Cholesterol
CK	Creatine kinase
CV	Coefficient of variation
DSC	Differential scanning calorimetry
EDTA	Ethylenediaminetetraacetic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FID	Flame ionisation detector
G-6-Pase	Glucose-6-phosphatase
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LLT_{100}	Lower lethal temperature (100% mortality)
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
RIA	Radioimmunoassay
RP	Retroperitoneal
SC	Subcutaneous
SCP	Supercooling point
SFA	Saturated fatty acid
T_3	Triiodothyronine
T_4	Thyroxine
T_a	Ambient temperature
T_{acc}	Acclimation temperature
TGA	Thermogravimetric analysis
UFA	Unsaturated fatty acid

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I-IV.

I Paakkonen T, Mustonen A-M, Roininen H, Niemelä P, Ruusila V, Nieminen P. Parasitism of the deer ked, *Lipoptena cervi*, on the moose, *Alces alces*, in eastern Finland. *Medical and Veterinary Entomology 24:411–417, 2010.*

II Paakkonen T, Mustonen A-M, Käkelä R, Laaksonen S, Solismaa M, Aho J, Puukka K, Nieminen P. The effects of an abundant ectoparasite, the deer ked (*Lipoptena cervi*), on the health of moose (*Alces alces*) in Finland. *Parasitology Research, in revision*.

III Paakkonen T, Mustonen A-M, Käkelä R, Kiljander T, Kynkäänniemi S-M, Laaksonen S, Solismaa M, Aho J, Kortet R, Puukka K, Saarela S, Härkönen L, Kaitala A, Ylönen H, Nieminen P. Experimental infection of the deer ked (*Lipoptena cervi*) has no negative effects on the physiology of the captive reindeer (*Rangifer tarandus tarandus*). *Veterinary Parasitology 179:180–188, 2011.*

IV Nieminen P, Paakkonen T, Eerilä H, Puukka K, Riikonen J, Lehto V-P, Mustonen A-M. Freezing tolerance and low molecular weight cryoprotectants in an invasive parasitic fly, the deer ked (*Lipoptena cervi*). *Journal of Experimental Zoology 317A:1–8, 2012.*

In addition, some unpublished results are presented.

AUTHOR'S CONTRIBUTION

In papers I–III, the author participated in the planning of the experiments and was responsible for the main part of sample and data collection, data analyses and writing of the manuscripts. For experiment IV, the author participated in designing the research protocol and was the person responsible for collection of samples and data, analyses and data analysis and took part in writing the manuscript.

Contents

1 Introduction

1.1 PARASITISM

Parasites are organisms that live directly at the expense of other organisms, known as hosts (Wall and Shearer 2001). This relationship is considered harmful for the host, but normally the parasite is not lethal, as that would also be destructive for the parasite itself. The harm caused by parasitism varies with parasite species and parasitism intensity as well as with the physiological condition of the host. The harm may be defined as a reduction in host-related factors, such as general condition and growth, or as a reduction in fitness, i.e., the ability of the individual to pass its genes on to the next generation. Parasites can have *i*) direct and *ii*) indirect effects on the hosts. *i*) Blood-feeding parasites are usually small compared to their hosts but, when present in large numbers, the blood loss of the host may be severe and cause anaemia (O'Brien *et al.* 1995; Pérez *et al.* 1999). Ectoparasites can cause itching (pruritus), hair loss (alopecia) and cutaneous inflammation, and the saliva of haematophagous parasites may cause toxic or allergic responses (Wall and Shearer 2001). *ii*) The presence of parasites may increase patterns of avoidance behaviour, such as head shaking, stamping, tail switching or scratching, and these activities may reduce the time allocated to feeding and resting and, thus, parasitism may indirectly reduce the growth and well-being of the host. Severe disturbance can cause stereotypical avoidance responses, such as running and raging, which increase energy expenditure and could even cause injuries (Mullens 2003).

Ectoparasites live on or burrow into the surface of the host's epidermis while endoparasites live inside their hosts (Wall and Shearer 2001). Most eukaryotic endoparasites are worms (Helminthes), which are classified as tapeworms (Cestoda) and flukes (Trematoda), belonging to Platyhelminthes, and

roundworms (Nematoda; Samuel *et al.* 2001). Most ectoparasites are arthropods (Arthropoda), such as ticks (Ixodidae) and lice (Phthiraptera). Dipterous insects (Diptera) are the most diverse group of arthropod parasites, including, e.g., mosquitoes (Culicidae), black flies (Simuliidae), biting midges (Ceratopogonidae), tabanids (Tabanidae) and snipe flies (Rhagionidae). The interrelationships between ectoparasites and hosts may take a variety of forms. When a parasite is totally dependent on its host, parasitism is called obligatory, whereas facultative parasites live or feed on their hosts only occasionally and are not totally dependent on them (Wall and Shearer 2001).

Cervids are parasitised by several eumetazoans, of which platyhelminths and nematodes are principally endoparasites, while most arthropods are ectoparasites (Samuel *et al.* 2001). For example, *Paramphistomum cervi* is a rumen trematode observed in North American moose (*Alces alces*; Hoeve *et al.* 1988), while *Dictyocaulus capreolus* is a nematode lungworm of the moose and other cervids (Gibbons and Höglund 2002). *Elaphostrongylus* spp. parasitise the nervous system of wild cervids and, for instance, *E. alces* was documented to infect Swedish moose (Stéen *et al.* 1997). *Setaria tundra* is a filaroid nematode parasitising especially the reindeer (*Rangifer tarandus tarandus*) and causing, for example, peritonitis (Laaksonen *et al.* 2009). The winter tick (*Dermacentor albipictus*) is a common ectoparasite of moose in North America with a documented intensity of over 30000 ticks per animal (Samuel and Welch 1991). The moose infected by the winter tick can display alopecia (Glines and Samuel 1989; Mooring and Samuel 1999), similar to the mule deer (*Odocoileus hemionus hemionus*), which is parasitised by tiny mites (*Demodex* sp.) that live in or near hair follicles (Gentes *et al.* 2007). Many haematophagous insects of the order Diptera visit their hosts only briefly to consume blood (Samuel *et al.* 2001). Dipterans that utilise cervids include various mosquitoes, black flies, biting midges and tabanids. The larvae of the moose throat bot (*Cephenemyia ulrichii*) mature in the pharyngeal cavity of moose (Nilssen *et al.* 2008; Angulo-Valadez *et al.* 2010). The

warble (*Hypoderma tarandi*) and the throat bot (*C. trompe*) are common parasites of reindeer (Laaksonen *et al.* 2008).

1.2 BLOOD-FEEDING ARTHROPODS

It has been suggested that blood-feeding arthropods evolved simultaneously with the first nest-breeding or communally living vertebrates 65–225 million years ago, and there are two theories about the evolution of this feeding habit (Lehane 2003). The first one includes species that sought shelter in the nests of vertebrates or species that tried to utilise some vertebrate-associated resources; the second theory includes species with morphological preadaptations to blood-feeding (Wall and Shearer 2001). According to the first hypothesis, some arthropods that took shelter in the nests of vertebrates began to utilise dead skin or feathers that dropped on the floor of the nest, and the subsequent step to begin feeding directly on the host was short. These ectoparasites occasionally consumed blood, for instance, from wounds, which could have proceeded to blood-feeding, as blood is of higher nutritional value compared to dead skin and feathers. Generally, there is strong competition for resources, such as dung, and usually the first one to arrive has an advantage over others; for example, the horn fly (*Haematobia irritans*) lays eggs on dung within 15 s after defecation (Lehane 2003). Competition favoured species that could feed on vertebrates and, thus, were always close to these restricted resources. The second theory suggests that predators of other arthropods and plant liquid feeders had already developed mouthparts adapted to piercing, biting or sucking (Wall and Shearer 2001). After occasional blood meals from vertebrates, some species may have eventually evolved blood-feeding habits.

All vertebrates are potential hosts to ectoparasites but the parasites prefer some species over others and, generally, large herbivores have the highest risk to become parasitised (Lehane 2003). Host selection is a complex and still poorly known

process but, apparently, one of the most important factors is host availability. Compared to solitary carnivores that have often wide home ranges, large herbivores are usually social and have fairly steady population dynamics. Thus, parasites can easily reach them year after year. The locomotion of the parasite is also an important factor in host selection, and permanent or specialised ectoparasites are usually small with relatively low mobility (Wall and Shearer 2001), while species with good flying ability are more often generalists that visit their hosts only briefly to consume blood (Lehane 2003).

Ectoparasites have several morphological adaptations enhancing their parasitic life style: piercing or cutting mouthparts ensure feeding, while the laterally or dorsoventrally flattened body facilitates movement through the fur or feathers of the host (Lehane 2003). Many periodic and permanent ectoparasites are wingless, which also assists their locomotion on the host. The hard exoskeleton and cuticular combs protect the body and the soft joints between the body segments, while claws and spines enhance attachment and prevent detachment of the parasite. In relation to their body size, blood-feeding ectoparasites consume large amounts of blood during a single meal, as finding a host can be uncertain and consuming blood is risky. To ensure feeding as quickly as possible, haematophagous parasites salivate, e.g., anti-coagulation agents and vasodilatory substances to maximise blood flow, and antihistamines to minimise inflammation and itching. The feeding patterns of haematophagous ectoparasites make them significant medical and veterinary concerns, as they can act as vectors for pathogens (Edman 2003).

1.3 THE DEER KED

The deer ked (*Lipoptena cervi* L., Diptera: Hippoboscidae) is a dorsoventrally flattened parasite with a hard exoskeleton (Metcalf and Metcalf 1993), and it has large claws enhancing attachment and preventing detachment (Haarløv 1964). The

deer ked drops its wings upon attachment on the host, making any subsequent host switch difficult or impossible (Hackman *et al.* 1983). Both sexes live on the host and are haematophagous. In contrast to capillary-feeding mosquitoes, the deer ked is presumably a pool feeder: it cuts the skin and consumes the blood from the dermal haemorrhage (Haarløv 1964). The reproductive strategy is viviparous (Figure 1); the egg hatches in the reproductive tract and the developing larva is fed by maternal secretions (Meier *et al.* 1999). The female can produce 20–32 pupae, one at a time, which fall to the forest floor or snow and the imagines emerge during the next autumn (Popov 1965; Ivanov 1981). There is one generation per year, which flies from the end of July till early November (Ivanov 1981). Observations on the maximum life span of imagines without food vary significantly from 14–16 days (Popov 1965) to 44–51 days (Välimäki *et al.* 2011), after which they perish unless they find a suitable host. According to Popov (1965), the optimum ambient temperature (T_a) for survival at this life stage is 2–5°C with ≥70% air humidity. Deer keds stay close to their emergence sites waiting for a potential host to arrive (Ivanov 1981). Imagines can fly approximately 50 m at the T_a of 14–24°C and *ad* 15 m at 7–11°C. According to Ivanov (1981), the deer ked lives for 120–180 days after settling on a host.

In Finland, the deer ked has been reported to parasitise the wild forest reindeer (*Rangifer tarandus fennicus*; Kaunisto *et al*. 2009), and the semi-domesticated reindeer is also suggested as a potential host for this parasite (Kynkäänniemi *et al.* 2010). However, the principal host species is the moose (Välimäki *et al.* 2011). This is similar to Soviet Belarus, where the number of deer keds correlated with the population size of the moose (Ivanov 1981). The prevalence of deer keds was 100% with 1144–5082 keds per animal. In the Leningrad region of the former Soviet Union, the intensity of parasitism was 200–300 keds per moose with a maximum number of approximately 1000 flies (Popov 1965). Recently, Madslien *et al*. (2011) reported high deer ked numbers on Norwegian moose—up to 16500 keds per host.

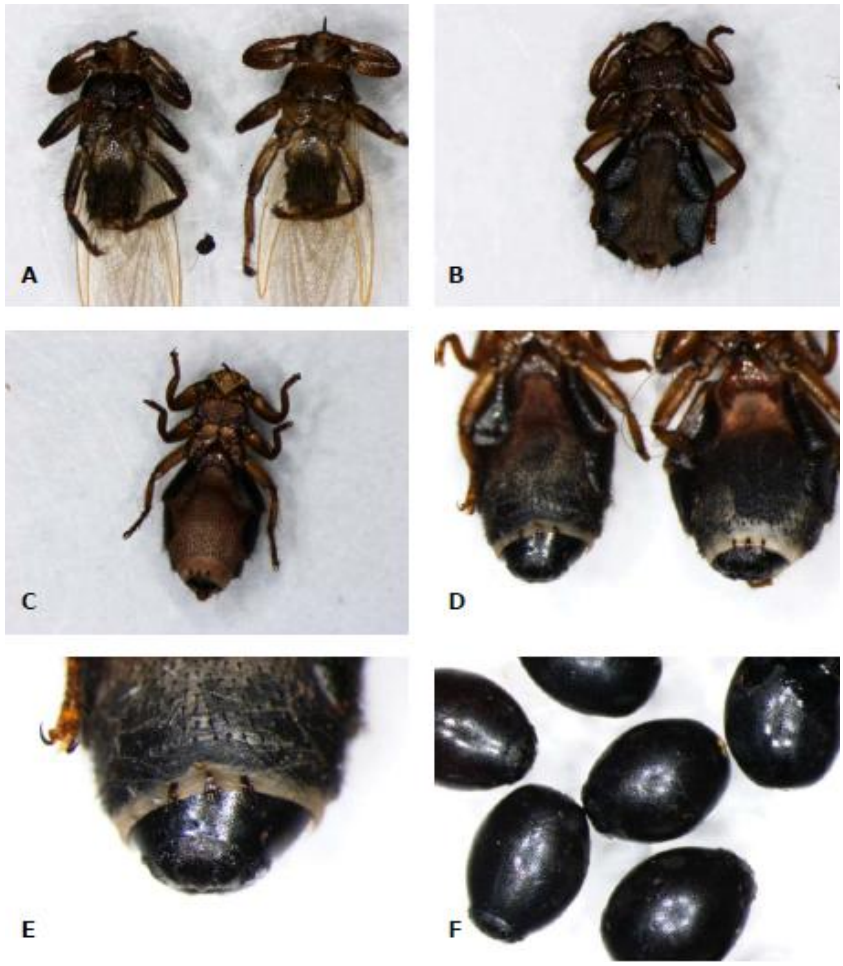

***Figure 1**. Female deer keds at different stages of reproduction, A. recently attached winged females, B. a blood-fed female, C.–E. females at different stages of giving birth. F. Pupae. Photographs by Tommi Paakkonen.*

Prevalences based on wintertime bedding site examinations varied between 67 and 96% in Finland (western parts of the country and northern Ostrobothnia and Kainuu regions) and between 24 and 100% in southern Norway (Välimäki *et al.* 2011). On other cervid hosts, infection intensities were lower and prevalences within the same range as for the moose. For instance, the deer ked could be found on 78% of red deer (*Cervus elaphus*), 64% of roe deer (*Capreolus capreolus*; Kadulski 1996) and

76% of fallow deer (*Dama dama*) in Poland (Szczurek and Kadulski 2004). In addition to cervids, deer keds have been observed on the grey wolf (*Canis lupus*; Itämies 1979), European bison (*Bison bonasus*; Izdebska 2001) and some domestic animals, such as the cattle (*Bos taurus*), the sheep (*Ovis aries*) and the horse (*Equus ferus caballus;* Ivanov 1981; Mehlhorn *et al.* 2010).

After the last glaciation, the moose spread from the west through Sweden to northern Finland and from the east to all parts of the country (Nygren 2009), and it can be speculated that the deer ked also entered Finland simultanously. More recently, the Finnish moose population was hunted almost to extinction by the 1920's (Nygren 2009) and probably for that reason, the deer ked also vanished from Finland. After the Finnish moose population had re-established itself and become sufficiently dense, the deer ked spread to the country from the southeast in the early 1960's, across the Soviet border (Hackman *et al.* 1983). Recently, its northern limit of distribution has been spreading northwards at a rate of 11 km per year and it is currently located at approximately 65°N (Välimäki *et al.* 2010). Thus, the deer ked is establishing contact with a potential host species, the semi-domesticated reindeer.

Surviving subzero T_a is crucial for insects, e.g., at high latitudes and altitudes, where they often encounter persistent freezing T_a. Species inhabiting cold regions have evolved two survival mechanisms: freeze-tolerance and freeze-avoidance (Lee 2010). Freeze-tolerance is mediated by ice-nucleating agents allowing safe extracellular freezing, supplemented by the presence of polyols and antifreeze proteins (Bale 1996). Freeze-avoidance is accomplished by the removal of potential nucleating agents, by antifreeze proteins and low-molecular-weight cryoprotectants leading to supercooling. The most common cryoprotectants are polyols, sugars and amino acids (AA; Fields *et al.* 1998; Renault *et al.* 2006; Clark and Worland 2008). Polyols and sugars can be derived from the breakdown of glycogen stores (Muise and Storey 1997; Worland *et al.* 1998) and AA from protein degradation or as left-overs from reduced protein synthesis (Renault *et al.* 2006).

Deer ked pupae have been documented to survive wintertime T_a north of the present distribution area (Härkönen *et al.* 2010), but the survival of imagines at subzero T_a remains uninvestigated. After attaching on a host, deer ked imagines are protected from freezing by staying close to the skin and, thus, they are vulnerable to cold only during the short autumnal period required for host location. Nighttime frost is common in autumn in the distribution area of the species, and there exist observations of deer keds attaching on moose and humans after nights with subzero T_a (T. Paakkonen, unpubl. obs.). Determining the adult survival of the deer ked would be beneficial for predicting the potential of this parasite to spread further north.

1.4 THE MOOSE

The moose (*Alces alces* L., Artiodactyla: Cervidae) is the largest of all cervid species (Bjärvall and Ullström 1996). It inhabits boreal forests all over the Northern Hemisphere, but in Scandinavia the average population densities are the highest, 0.7–0.8 animals per km^2 (Lavsund *et al.* 2003). The grey wolf and the European brown bear (*Ursus arctos arctos*) are the principal predators of the Finnish moose, but human activities, such as hunting, are at present the most important factors affecting the size of the moose population (Bjärvall and Ullström 1996; Nygren 2009). The moose is an economically important game animal and, in Finland, hunting produces 10 million kg meat annually (Finnish Game and Fisheries Research Institute 2012a).

The moose consumes a wide array of terrestrial plants, for example, pine shoots and leaves of willows and birches, but also aquatic plants, for instance water lilies (Bjärvall and Ullström 1996). In summer, an adult moose may consume 30 kg of feed per day, while the daily intake in winter is only 10 kg or less. Ruminants, such as the moose, have a unique four-compartment stomach containing microorganisms: bacteria, protozoa and fungi (Forsberg *et al.* 2000; Vaughan *et al.* 2000). This enables

fermentation of polysaccharides, e.g., cellulose, hemicellulose, starch and pectin into readily utilisable sources of energy (Committee on Nutrient Requirements of Small Ruminants 2007). As a result, the primary contributors of energy for the ruminant are by-products of microbial fermentation, i.e., short-chain volatile fatty acids (FA; predominantly acetate, propionate and butyrate), which are absorbed directly from the rumen into the circulation. Most of the glucose is synthesised in the liver by gluconeogenesis from propionate originating from the rumen.

Due to microbial hydrolysis, ingested lipids are degraded into glycerol and free FA in the rumen (Committee on Nutrient Requirements of Small Ruminants 2007). Glycerol is further metabolised into volatile FA, and free unsaturated FA (UFA) are biohydrogenated as follows: complete biohydrogenation results in the formation of the end-product, 18:0, while incomplete hydrogenation yields conjugated isomers of 18:2n-6 and 18:3n-3 as well as various isomeric forms of 18:1n-9. Due to these modifications, the proportions of FA absorbed in the gut may diverge significantly from those of the feed, and the proportions of saturated FA (SFA) in tissues are high compared to those in monogastric animals (Jenkins 1993; Seal and Parker 2000; Committee on Nutrient Requirements of Small Ruminants 2007).

The microorganisms use dietary proteins and nitrogen of nonprotein origin to synthesise microbial proteins in the rumen (Committee on Nutrient Requirements of Small Ruminants 2007). From the viewpoint of the host animal, metabolisable protein consists of a combination of these sources and is digested postruminally. Liver synthesises urea, which is recycled into the rumen to be hydrolysed into ammonia, which is then utilised for microbial protein synthesis. Thus, the total protein intake of ruminants diverges from the amount and quality of proteins in the feed.

Moose bulls have antlers that start to grow in April, in August–September they are full-grown, just before the mating season, and in December–January the antlers are shed (Bjärvall and Ullström 1996). The mating season is in September–October,

during which period the behaviour of the bulls becomes more aggressive when they compete for cows. The calves are born in spring after a gestation period of approximately 235 days and the cow usually gives birth to twins.

The moose is active year-round and well adapted to boreal winter conditions with its large body size, long legs (Lundmark 2008) and insulative pelage (Scholander *et al.* 1950). The lower critical temperature of the species is <–30°C, but the moose is sensitive to heat stress (Renecker and Hudson 1986). Outside the mating season, moose are usually solitary, but in winter, when the animals save energy and use the same paths in deep snow, small groups may occasionally form (Bjärvall and Ullström 1996). As a result, population densities may be locally as high as 5–6 moose per km^2 (Lavsund *et al.* 2003). The species is considered harmful for the forest industry, as overwintering moose can prefer areas with sapling stands and forage on shoots, causing reductions in forest growth (Siivonen and Sulkava 1994).

1.5 THE REINDEER

The reindeer (*Rangifer tarandus tarandus* L., Artiodactyla: Cervidae) is a semi-domesticated circumpolar cervid that inhabits arctic and subarctic regions (Bjärvall and Ullström 1996). The Fennoscandian reindeer is surmised to be descended from the wild Eurasian mountain reindeer that colonised, e.g., Finland after the last glaciation 9000 years ago (Ukkonen 2001). It has been herded by the Sami people in northern Fennoscandia for approximately 1000–1500 years (Banfield 1961). Artificial selection has differentiated the reindeer from its wild ancestor, and colour and body size vary substantially among semi-domesticated reindeer.

Both genders have antlers that are full-grown in September, but they are larger in males than in females (Bjärvall and Ullström 1996). The antlers of males are shed in December–January, while gestating females may keep their antlers until

late May. Barren females, on the other hand, may cast their antlers 1–2 months earlier (Epsmark 1971). The size of the antlers is the most important factor in establishing hierarchy within the herd (Bjärvall and Ullström 1996). The mating season starts at the end of September, lasts till October, and after approximately 220 days of gestation, a single calf is born.

The reindeer, too, is a ruminant and in summer it feeds on grasses, sprigs and leaves of willows and birches (Bjärvall and Ullström 1996). In winter, the main food items are lichens, of which the reindeer lichen (*Cladonia rangiferina*) is the most important. Due to the low protein content of the feed, reindeer are in negative nitrogen balance in winter (Hyvärinen *et al.* 1975; Pösö 2005), which they can partly compensate by recycling urea into the rumen, where the microflora metabolise it into proteins (Committee on Nutrient Requirements of Small Ruminants 2007). At present, supplemental feeding has become important in winter, as the size of the reindeer population has increased considerably during the last decades (Helle and Kojola 2006). There are 0.7 million semi-domesticated reindeer in Fennoscandia, while Russia has approximately 1.5 million semi-domesticated and 1.3 million wild reindeer (Syroechkovski 2000).

The reindeer is finely adapted to life in cold climates with the lower critical temperature at ≤–30°C (Nilssen *et al.* 1984). Its highly insulative winter fur consists of hollow, air-filled guard hairs and dense, woollen underfur (Scholander *et al.* 1950; Timisjärvi *et al.* 1984). Extremities are heterothermic with a counter-current heat exchange mechanism (Irving *et al.* 1957; Johnsen *et al.* 1985). Unlike adult reindeer, newborn calves depend on non-shivering thermogenesis of brown adipose tissue for survival (Soppela 2000).

1.6 PREVENTION OF PARASITES

Medication against metazoan parasites has been used routinely in reindeer husbandry since the 1970's (Helle and Kojola 2006).

One of these pharmaceuticals is ivermectin, a synthetic antiparasitic agent without antibacterial activity (Dourmishev *et al.* 2005). It prevents the conduction of nerve impulses in synapses that use glutamate or γ-aminobutyric acid as neurotransmitters and leads to eventual paralysis of the parasites. It does not affect the hosts, as in mammals these synapses are located only in the central nervous system, and ivermectin cannot cross the blood–brain barrier.

In Finland, most semi-domesticated reindeer are treated annually with ivermectin, either during round-ups of breeding animals or later in winter when the animals are gathered in corrals for feeding (Laaksonen *et al.* 2008). Originally, the treatment was targeted at the warble and the throat bot, subsequently also at gastrointestinal nematodes. The half-life of ivermectin in ruminant plasma is 3 days, and after approximately 3 weeks its plasma concentrations are very low or undetectable (Oksanen *et al.* 1995; Cerkvenik *et al.* 2002).

2 Aims of the study

The present thesis was undertaken to investigate the characteristics of deer ked parasitism and the potential health effects of this parasite on its hosts (Table 1). In Finland, the principal host of the deer ked is the moose (Välimäki *et al.* 2011) and, due to the northward spread, the semi-domesticated reindeer could also be exposed to deer keds in the future (Kynkäänniemi *et al.* 2010).

The specific aims of this thesis were:

1. To study how the intensity of deer ked parasitism varies with the age and gender of the moose, and whether the densities of deer keds differ between anatomical regions of the moose hide (I).

2. To investigate whether selected physiological variables of the moose vary between deer ked-infested and deer ked-free regions in Finland, and how the intensity of deer ked parasitism or the gender and maturity of the moose affect these parameters (II).

3. To determine whether an experimental deer ked infection has effects on the health of enclosure-housed reindeer by measuring a wide array of haematological, endocrinological and biochemical variables (III).

4. To assess the survival of deer ked imagines at subzero T_a by determining the lower lethal temperature (LLT_{100}) and the supercooling point (SCP) of the species and by examining deer keds for the presence of potential low-molecular-weight cryoprotectants (IV).

Table 1. *Summary of the main aims and results of the studies in this thesis.*

Research questions	**Principal results**
I: What are the basic characteristics of deer ked parasitism on moose in eastern Finland?	All the examined moose in eastern Finland were highly parasitised by deer keds, and the intensity of parasites varied depending on the sex and maturity of the host, with the anterior back as the most preferred anatomical region for the parasites.
II: How does deer ked parasitism affect the health and well-being of wild moose?	The haematological and clinical chemistry variables of the moose did not show consistent variation between the deer ked-infested and -free regions in Finland.
III: How does the experimental deer ked infection (300 parasites inoculated per host) affect the health and well-being of captive reindeer?	There were no consistent effects of deer ked parasitism on the measured physiological variables of the reindeer at the intensity of infection employed.
IV: How does the deer ked cope with subzero T_a determined by SCP, LLT_{100} and the presence of selected cryoprotectants in the species?	The deer ked could be a freeze-tolerant species with the SCP at −7.8°C and the LLT_{100} at −16°C. It displayed increased concentrations of free AA after cold-acclimation.

3 Materials and methods

3.1 EXPERIMENTAL ANIMALS

Sixteen adult moose (8 bulls, 8 cows) and seven calves (6 males, 1 female) were hunted from the deer ked-infested region of Liperi commune, eastern Finland (62°31'N, 29°08'E) between October 7 and November 26, 2006 (I–II). Based on annual rings of the incisor teeth (modified from Rolandsen *et al.* 2008), the average ages of the bulls and cows were estimated to be 3.1 ± 0.4 and 4.4 ± 0.6 years. Moose younger than 1 year of age were classified as calves. In addition to this group I (n = 23), moose from other deer ked-infested regions in western and central Finland (group II; n = 34; 63°40'–65°47'N, 24°31'–25°57'E) and from deer ked-free regions in northern Finland (group III; n = 24; 65°01'–68°54'N, 24°43'–27°01'E) were also hunted between October 1 and December 12, 2006 (II).

Eighteen adult reindeer (7 males, 11 females) with an average age of 2.8 ± 0.6 years were kept at the Zoological Gardens of the University of Oulu, Finland (65°03'N, 25°27'E) between May 29 and December 13, 2007 (III). The experiment was approved by the Committee on Animal Experiments of the University of Oulu (STH378A; May 16 2007/ESLH-2007-03532/Ym-23). For identification, all the reindeer were provided with coloured collars and numbered ear tags. The males had been castrated to enable easier handling. The reindeer were fed *ad libitum* with a commercial diet (Poron-Herkku, Rehuraisio, Espoo, Finland; 10.5% raw protein, 3.8% raw fat, 12.5% raw fibre, energy content 11.7 MJ metabolisable energy/kg dry matter) supplemented with lichen, hay and birch and willow leaves.

The deer keds used for the experimental infection of the reindeer were either reared (from wild-collected pupae from various parts of Finland, 60–65°N, n = 1260) at the University of Oulu, Department of Biology, or collected as imagines in the

communes of Rantsila (64°30′N, 25°39′E) and Liperi in August–September, 2007 (n = 2340; III). Before being used for infecting the reindeer, the keds were maintained in plastic containers with moist moss to retain humidity. The deer ked imagines tested for cold-tolerance (IV) were collected in Liperi on September 10, 16 and 30, 2008 and October 10, 2010.

3.2 STUDY PROTOCOLS

The reindeer were divided into three experimental groups (control, infected and medicated; n = 6 per group) with an equal sex ratio and average age (III). On May 29 and June 13, 2007, the reindeer were treated to eliminate any pre-existing endo- and ectoparasites with subcutaneous (SC) ivermectin (Oksanen *et al.* 1993: 0.2 mg/kg body mass (BM); Vetpharma AB, Lund, Sweden), and on May 29 with topical deltamethrin (75 mg per reindeer; Schering Plough, Ballerup, Denmark). Each group was kept in its own outdoor enclosure (570 m^2) at natural T_a and photoperiod. The reindeer in the infected and medicated groups were inoculated on 6 occasions between August 16 and September 27 with an equal total number of parasites (300 per reindeer). All the animals were immobilised in a handling crib, and the deer keds were placed on the anterior back of the reindeer in the infected and medicated groups. On November 6, the medicated group was treated with SC ivermectin (0.2 mg/kg) and the other two groups were given equivolume 0.85% saline injections. On December 10–13, the reindeer were stunned with a captive bolt pistol and killed by exsanguination. Some individuals from each experimental group were selected for sampling each day.

The cold-tolerance of the deer keds (IV) was tested in the natural light–dark cycle using programmable clima chambers (ARC-300/–55+20, Arctest, Espoo). The keds were maintained in transparent plastic vials with damp moss and a gauze covering placed in the clima chambers. The deer keds fasted during the study, but the durations of the acclimation periods were shorter

than the expected survival of deer ked imagines without food (Popov 1965; Välimäki *et al.* 2011).

To determine cold-tolerance (IV), 175 deer keds collected on September 16, 2008 were subjected to either faster or slower cold-acclimation *ad* –20°C as follows: during the first 24 h the acclimation temperature (T_{acc}) in the clima chambers was reduced from a T_a of +10 to 0°C. The faster acclimation group (T_{acc} reduced by 5.0°C/day) consisted of 92 flies divided into 5 vials, and the slower acclimation group (2.5°C/day) of 83 flies in 4 vials. One vial of keds from both groups was removed from the chambers at 5°C intervals, i.e., every 1–2 days. The keds were observed at room temperature *ad* 30 min for signs of coordinated movement, after which the numbers of dead and live keds were calculated. Subsequently, 100 deer keds collected on September 30, 2008 were placed in a clima chamber in 5 vials. The T_{acc} was reduced from a T_a of +10 to –10°C within 48 h, followed by a subsequent decrease of 2°C/day *ad* –20°C. One vial was removed from the chamber each day and the keds were observed for signs of recovery. The data were utilised to determine the LLT_{100}, the T_a at which no specimens survived.

The possible low-molecular-weight cryoprotectants were examined at the T_a and 2 different T_{acc} (IV). On September 10, 2008, 106 deer keds were randomly divided into 3 groups, outside T_a (+13°C), 0°C and –4°C. The group in the T_a was sampled immediately after collection. The others were acclimated during 24 h to their target T_{acc} in the clima chambers and sampled after an additional 4 days. The SCP and water content were determined from deer keds collected on October 10, 2010. The keds were transferred into a clima chamber and acclimated from the prevalent T_a (+4°C) to –4°C in 48 h. The keds were observed for signs of coordinated movement before selecting the specimens for the SCP measurement (n = 6).

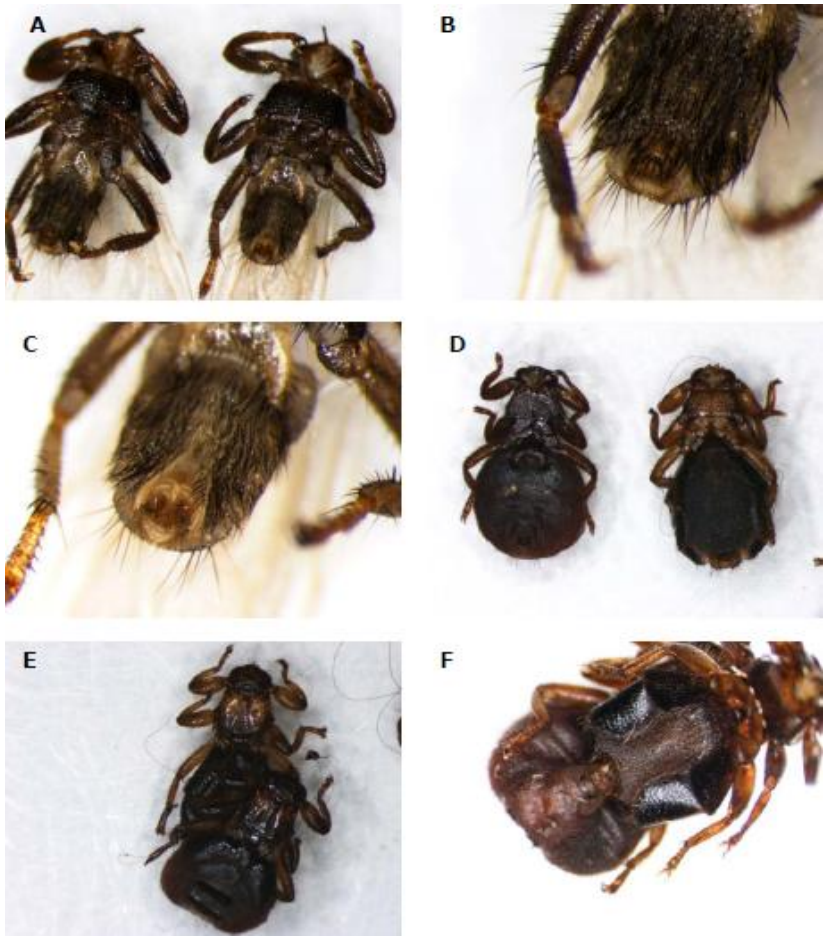

***Figure 2**. Categories of deer ked imagines, A. a recently attached winged female and male ked, B. abdomen of a winged female ked, C. abdomen of a winged male ked, D. a blood-fed male and female ked, E.–F. copulating pairs. Photographs by Tommi Paakkonen.*

3.3 SAMPLING

After skinning, the pelts of the moose and reindeer were weighed (II–III). The moose pelts were divided into six sections (head, anterior back, posterior back, front limbs, hind limbs and abdomen) based on the anatomical region and the length of the fur (Sokolov and Chernova 1987; I). Each section was sealed in a plastic bag and frozen at –20°C. Later, in the laboratory, the hair was cut with scissors and all deer keds and other visible

ectoparasites were collected. The keds were divided into categories as follows: recently attached winged, recently attached wingless and non-blood-fed, blood-fed, copulating pairs and pupae (Figures 1–2). A random subsample of keds (n = 200 unless the total number per section was less) from all skin sections was divided by sex. Based on the sex ratio and the mean weights of these male and female keds, the total numbers of flies of both sexes on each skin section were estimated. The pelts of the reindeer were examined similarly, except that all live and dead deer keds were calculated immediately after skinning (III). The skin areas were determined by placing a metal grid (square size 20 × 20 mm) on the skin sections and calculating the number of squares covering them (I) or by drawing the outlines of each pelt on paper and subsequently placing the metal grid on the paper and calculating the number of squares covering the area (III).

The BM of the reindeer were measured on August 15, November 6 and at the end of the study on December 10–13 (III), while the BM of the moose were estimated from the weights of the carcasses (Wallin *et al*. 1996; I–II). The livers of the moose and reindeer were dissected and samples were also taken from *musculus rectus abdominis* as well as from SC (rump) and retroperitoneal (RP) adipose tissues, frozen with liquid nitrogen and stored at –80°C (II–III). The weights of the liver, kidneys and omentum, the lengths of the carcass and pelt and the weights of the testes (moose only) were also measured. The thickness of the SC fat layer in the rump correlating with the body fat stores of the moose (Stephenson *et al.* 1998) was measured at an incision in the gluteal-sacral region (Finnish Game and Fisheries Research Institute 2011; I–III). The adrenal glands of the reindeer were dissected and weighed and one of them was preserved in 5% formalin, processed conventionally into thin sections and stained with hematoxylin-eosin (III). The absolute and relative thicknesses of the layers of the adrenal cortex were measured microscopically by two independent observers. The other adrenal gland was stored frozen at –80°C for subsequent biochemical analyses.

The moose blood samples were collected from cut jugular blood vessels immediately after killing, into test tubes containing ethylenediaminetetraacetic acid (EDTA; II). The reindeer were immobilised in the handling crib and their blood samples were taken from the left external jugular vein with aseptic needles into test tubes containing EDTA on seven occasions (May 29, June 13, August 15, October 2 and 16, November 6, December 10; III). The blood samples were centrifuged at 2000 × *g* for 20 min to obtain plasma, which was frozen with liquid nitrogen and stored at –80°C (II–III). One ml of whole blood was refrigerated at 4°C for the complete blood count analysis.

The low-molecular-weight cryoprotectants were examined from 20 samples (n = 6–7/group), each containing 4–6 deer ked individuals based on preliminary biochemical analyses performed in order to determine the requirements of sample mass for reliable analyses (IV). The keds were snap-frozen, weighed and subsequently crushed and pulverised in liquid nitrogen. Distilled water was added to the pulverised keds and the samples were centrifuged at 2000 × *g* for 15 min and the water-soluble fraction was extracted and used for the analyses.

3.4 ANALYTICAL METHODS

3.4.1 Haematology

The complete blood count was determined with the Vet abc Animal Blood Counter (ABX Hematologie, Montpellier, France) at the Municipal Veterinary Clinic (Joensuu, Finland) within 48 h of collecting the blood (II–III). Equine calibration was used for the analyses, and comparisons with existing moose (Adolfsson 1993) and reindeer data (Nieminen 1980; Rehbinder and Edqvist 1981; Catley *et al.* 1990) showed that the obtained haematological values were mostly similar to these previous measurements.

3.4.2 Clinical chemistry and nitrogenous compounds

The plasma clinical chemistry variables (II–III) were analysed using reagents purchased from Randox Laboratories Ltd (Crumlin, UK). The total cholesterol (Chol) was determined by the Cholesterol Enzymatic Endpoint Method. The low-density lipoprotein (LDL) Chol and high-density lipoprotein (HDL) Chol levels were measured with the Direct LDL- and HDL-Cholesterol reagents. The triacylglycerol and glucose concentrations were measured by the Triglycerides GPO-PAP and Glucose Liquid Reagent Hexokinase Methods, and the creatinine concentrations by the Creatinine Colorimetric Method. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined with the ALT (GPT) Alanine Aminotransferase EC 2.6.1.2 ECCLS and AST (GOT) Aspartate Aminotransferase EC 2.6.1.2. ECCLS reagents. The Bilirubin DCA Method, Total Protein Biuret Method, Urea Enzymatic Kinetic Method, Ammonia Enzymatic UV-Method, Uric Acid Enzymatic Colorimetric Method, CK NAC-activated Creatine Kinase EC 2.7.3.2 reagents and Total Antioxidant Status reagents were also utilised. For the actual measurements, the Technicon RA-XT™ analyser (Technicon Ltd, Swords, Ireland) was used.

The plasma concentrations of free AA and other nitrogenous compounds (II–III) were determined by ion-exchange chromatography (Biochrom 30 Amino Acid analyser, Biochrom Ltd, Cambridge, UK) at the Oulu University Hospital.

3.4.3 Endocrinology

The plasma insulin concentrations were measured with the Human Insulin Specific radioimmunoassay (RIA) kit (Linco Research, St. Charles, MO, USA; intraassay and interassay variations, 2.2–4.4% and 2.9–6.0% coefficient of variation [CV], respectively; II–III). The plasma leptin concentrations were measured with the Multi-Species Leptin RIA kit of Linco Research (2.8–3.6% and 6.5–8.7% CV; II–III), also used previously in cervids (Soppela *et al.* 2008; Scott 2011), and the plasma ghrelin concentrations with the Ghrelin (Human) RIA

kit (Phoenix Pharmaceuticals, Belmont, CA, USA; <5% and <14% CV; II–III). The plasma glucagon concentrations were determined with the Double Antibody Glucagon kit from Diagnostic Products Corporation (Los Angeles, CA; 3.2–6.5% and 6.0–11.9% CV; III) and the plasma adiponectin levels with the Human Adiponectin RIA kit from Linco Research (1.78–6.21% and 6.90–9.25% CV; III). The plasma cortisol (II–III) and triiodothyronine (T_3; III) levels were measured with the Spectria Cortisol- and T3- [^{125}I] Coated Tube Radioimmunoassay kits from Orion Diagnostica (Espoo; cortisol: 2.6–5.4% and 6.5–7.3% CV; T_3: 3.8–7.5 and 4.8–7.0% CV) and the plasma thyroxine (T_4; III) levels with the Coat-A-Count Total T4 kit (Siemens Medical Solutions Diagnostics, Los Angeles; 2.7–3.8% and 4.2–14.5% CV). For the actual measurements, the 1480 Wizard™ 3'' Gamma Counter (Wallac Oy, Turku, Finland) was used (II–III). The hormone assays were validated in such a way that serial dilutions of the plasma samples showed linear changes in sample binding/maximum binding values that were parallel with the standard binding/maximum binding curves produced by using the standards of the manufacturers.

The adrenal gland samples were weighed and homogenised, and the adrenal catecholamine concentrations (noradrenaline, adrenaline, dopamine) were measured by Agilent 1100-type high-performance liquid chromatography (Decade II, Antec Leyden, Zoeterwoude, the Netherlands; III) at the Department of Biology, University of Oulu. The Agilent ChemStation software (Agilent Technologies Inc, Palo Alto, CA) was used for device control, sample injection and chromatogram analysis.

3.4.4 Tissue enzyme activities and other biochemistry

The liver and muscle samples were weighed and homogenised (II–III). The homogenisation was carried out in cold citrate buffer for the glucose-6-phosphatase (G-6-Pase; pH 6.5) and glycogen phosphorylase measurements (pH 6.1). The activity of G-6-Pase was measured according to Hers and van Hoof (1966) using glucose-6-phosphate as the substrate in the presence of EDTA, after an incubation period of 30 min at 37.5°C. The

glycogen phosphorylase activity was determined in the presence of glucose-1-phosphate, glycogen, sodium fluoride and AMP (Hers and van Hoof 1966). The homogenisation of the muscle, liver and fat tissue samples was carried out in cold 0.85% NaCl for the lipase measurement. The lipase activity was measured using 2-naphthyl laurate as the substrate according to Seligman and Nachlas (1962). The activities of plasma alkaline phosphatase were determined with *p*-nitrophenyl phosphate as the substrate (pH 10.5 at 37.5°C). The glycogen and total protein concentrations of the muscle and liver samples were measured according to Lo *et al.* (1970) and Lowry *et al.* (1951), respectively. All the analyses were performed with the Hitachi U-2000 spectrophotometer (Hitachi Ltd, Tokyo, Japan). The hepatic lipids were extracted according to Folch *et al.* (1957) to determine the liver fat-% (III).

3.4.5 Fatty acid profiles of tissues and diet

The FA profiles were determined, as FA and their derivatives can be important mediators of inflammatory reactions and participate in host immunity (Anderson and Fritsche 2002; Muturi *et al*. 2005), and FA profiles also reflect the nutritional state of the host (Rouvinen-Watt *et al*. 2010). The samples of adipose tissues (SC and RP), liver, muscle, plasma (II–III) and the commercial diet of the reindeer (III) were transmethylated (Christie 1993) by heating with 1% H_2SO_4 in methanol in nitrogen atmosphere. The FA methyl esters (FAME) formed were extracted with hexane. The dried and concentrated FAME were analysed with a gas–liquid chromatograph equipped with two injectors and flame ionisation (FID) and mass selective detectors (6890N network gas chromatograph system with an autosampler, a FID and a 5973 mass selective detector, Agilent Technologies Inc). The peaks were reintegrated manually and the mass spectra extracted using the Agilent ChemStation software. The FAME were identified based on the retention time, mass spectrum and comparisons with authentic (Sigma-Aldrich Inc, St. Louis, MO) and natural standards of a known composition and published reference spectra (American Oil

Chemists' Society 2012). The quantifications were based on the FID responses. The peak areas of the FID chromatograms were converted to mol-% by using the theoretical response factors (Ackman 1992). The FA were marked by using the abbreviations: (carbon number):(number of double bonds) n-(position of the first double bond calculated from the methyl end). The fractionation coefficients (III) were calculated as follows: (mol-% in tissue)/(average mol-% in diet).

3.4.6 Potential cryoprotectants

The selection of cryoprotectants to be analysed from deer ked homogenate was based on the most common cryoprotective agents usually present in insects (Zachariassen 1985; IV). The glucose concentrations were determined spectrophotometrically using the Glucose Liquid Reagent Hexokinase Method kit from Randox Laboratories Ltd with the Technicon RA-XT™ analyser. The glycerol concentrations were analysed using the Glycerol UV method kit and the D-sorbitol/xylitol levels with the UV method kit, which determines the sum of sorbitol and xylitol (R-Biopharm, Darmstadt, Germany). The trehalose concentrations were determined with the Trehalose K-TREH kit from Megazyme International (Bray, Ireland) and the fructose concentrations with the Fructose assay kit (Sigma-Aldrich Inc). All these assays were performed with the Hitachi U-2000 spectrophotometer. The concentrations of free AA and other nitrogenous compounds were determined by ion-exchange chromatography (Biochrom 30 Amino Acid analyser) at the Oulu University Hospital. The results were calculated per mg fresh weight.

3.4.7 Supercooling point and water content

The SCP and the enthalpy of freezing were determined by differential scanning calorimetry (DSC) using the DSC 823e equipment (Mettler Toledo, Greifensee, Switzerland; IV). The deer keds were stunned with nitrogen and sealed hermetically in aluminum crucibles. The specimens were balanced for 5 min at 25°C, followed by cooling at a rate of 1°C per min *ad* –30°C.

Ice crystal formation was observed as an exothermic peak in the heat flow curve, and the onset temperature of ice crystal formation represented the SCP. To determine the water content of the specimens, thermogravimetric analysis (TGA) was utilised (Q50 TGA, TA Instruments, New Castle, DE, USA). The keds were kept in the crucibles, which had been punctured immediately prior to the TGA. The specimens were balanced for 5 min at 30°C and heated at a rate of 10°C per min *ad* 150°C. The vaporisation of water was detected as the decrease in mass during the heating procedure.

3.5 STATISTICAL ANALYSES

Comparisons between multiple experimental groups were performed by the one-way analysis of variance (ANOVA) and the Duncan's *post hoc* test using the SPSS program (*v*.15.0 or 17.0, SPSS Inc, Chicago, IL, USA; I–IV). The normality of distribution and the homogeneity of variances were tested by the Kolmogorov-Smirnov and Levene tests, respectively. If the assumptions were not met after standard transformations, the nonparametric Kruskal-Wallis ANOVA on ranks and the Dunn's *post hoc* test were performed using the SigmaPlot program (*v*.11.0, Systat Software Inc, San Jose, CA). When comparing two groups, the Student's *t*-test for parametric data (II–III) and the Mann-Whitney U test for nonparametric data (II) were performed using the SPSS program. Differences in the time-series were analysed using the general linear model for repeated measures (repeated measures ANOVA; III). The χ^2-test was performed to analyse the distribution of live and dead keds in the acclimation groups (IV).

To analyse the relationships in the FA composition according to different study groups and tissues, the data were subjected to the multivariate principal component analysis using the SIRIUS *v*.6.5 software package (Pattern Recognition Systems AS, Bergen, Norway; Kvalheim and Karstang 1987; II–III). The data were standardised and the relative positions of the samples and

variables were plotted using 2 new coordinates, the principal components PC1 and PC2, describing the largest and the second largest variance among the samples. Correlations were calculated by the Spearman Correlation Coefficient (r_S; I–II, IV). A *p*-value <0.05 was considered statistically significant (I–IV). The results are presented as the mean ± SE.

4 Results

4.1 DEER KED PARASITISM INTENSITY AND DENSITY ON THE MOOSE IN EASTERN FINLAND (I)

All the examined moose (n = 23) were parasitised with deer keds. The bulls had on average 10616 ± 1375 (range 7594–17491) deer keds, the cows 3549 ± 587 (817–5130) keds and the calves 1730 ± 191 (852–2309) keds, all the values differing significantly from each other (Figure 3; Table 1). Also the density of deer keds was the highest on the bulls (35.7 ± 4.4 keds/dm^2 of skin) and, anatomically, on the anterior back (54.1 ± 8.9), where approximately half of all the keds were located. The posterior back (32.8 ± 6.2) harboured approximately one fourth of all the keds, followed by the front limbs (11.9 ± 2.5), abdomen (9.2 ± 1.9), head (5.0 ± 0.8) and hind limbs (1.9 ± 0.5).

The sex ratio of the deer keds was close to equal. The recently attached winged and wingless keds had rather similar weights, but the blood-fed keds were expectably heavier, and the blood-fed males were heavier than the females.

4.2 SURVIVAL OF DEER KEDS ON THE REINDEER AFTER THE EXPERIMENTAL INFECTION (III)

At the end of the experiment in December, the reindeer in the control group had no deer keds on their pelts, the reindeer in the infected group had both live (6 ± 3) and dead (5 ± 1) deer keds, while the reindeer in the medicated group had only dead keds (17 ± 3). The survival of the keds on the infected group was 2.1 ± 0.9% and the average recovery of the dead and live parasites on the infected and medicated groups 4.7 ± 0.8%. The densities (live and dead keds) on these two groups were relatively similar (infected: 0.09 ± 0.02; medicated: 0.13 ± 0.03

keds/dm² of skin). There was a single pupa in the infected group.

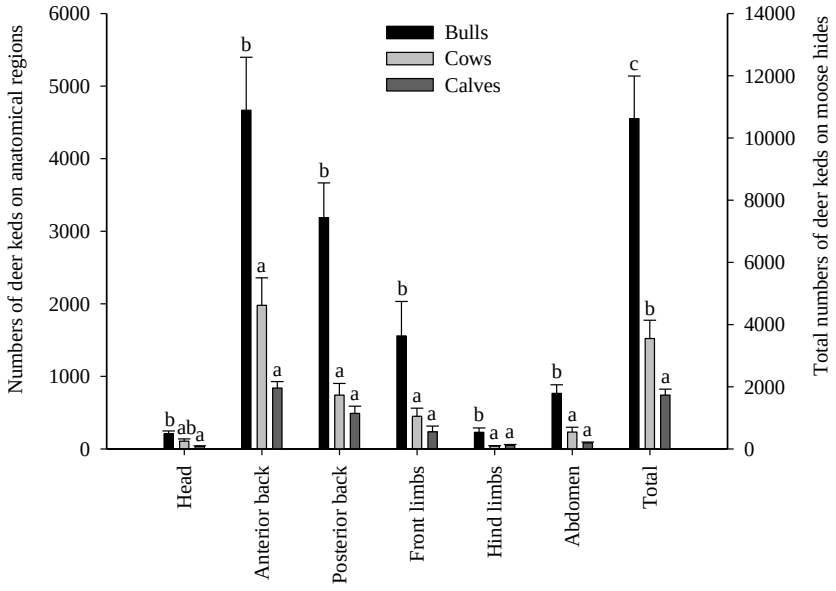

***Figure 3**. Intensities of deer ked parasitism on different anatomical regions of the moose in eastern Finland (I), mean + SE. Means with no common letter differ at p <0.05.*

4.3 PHYSIOLOGICAL EFFECTS OF DEER KED PARASITISM ON THE MOOSE AND REINDEER (II–III)

The total number and density of deer keds correlated positively with the BM, the masses of pelts, kidneys and testes and with the length of pelts of the moose (II). In the reindeer, neither the BM, organ masses, weights of the omentum and RP fat, the thickness of the SC fat layer nor the adrenal cortex histology differed according to the treatments (III).

In the moose of experiment II, the mean corpuscular haemoglobin concentrations (MCHC) were lower in the deer ked-free regions (group III: 33.8 ± 0.8 g/dl) than in the deer ked-

infected regions (group I: 36.1 ± 0.3; group II: 35.3 ± 0.2 g/dl). In the reindeer, the haematological variables were not affected by the infection (III; Table 1). The clinical chemistry of the moose showed some regional variation, e.g., groups II–III had higher total Chol and LDL-Chol levels and lower ALT activities than those of group I, but no clear effects of deer ked parasitism were documented (II). Likewise, the clinical chemistry values of the reindeer showed no consistent differences between the study groups (III).

Neither the plasma and adrenal endocrinological variables nor the tissue glycogen, protein and lipid concentrations varied consistently between the groups (II–III). The intensity of parasitism correlated with the liver G-6-Pase activities of the moose (II), while the enzyme activities of the reindeer were unaffected by the deer ked infection (III). The intensity and density of deer keds correlated positively with the moose plasma lysine and threonine concentrations and inversely with the alanine levels (II). The reindeer in the control group had lower valine concentrations than the animals in the infected group, but there were no differences in the other AA at the end of the experiment (III).

4.4 AGE- AND GENDER-RELATED DIFFERENCES IN THE PHYSIOLOGICAL PARAMETERS OF THE MOOSE (II)

In the material collected from eastern Finland (group I), the cows had heavier livers, omental and RP fat masses and thicker SC fat layers than the bulls and calves. The adult moose had higher haemoglobin and mean corpuscular haemoglobin (MCH) values than the juveniles, and the cows had higher mean corpuscular volumes (MCV) than the bulls and calves. The juveniles displayed lower plasma creatinine concentrations than the adults. The bulls had higher liver G-6-Pase activities than the other groups while the cows had lower RP fat lipase activities than the bulls and calves. The adult moose had higher plasma 1- and 3-methylhistidine concentrations than the juveniles.

4.5 SEASONALITY OF THE REINDEER PHYSIOLOGY (III)

The magnitude of BM changes of the reindeer was minor during the follow-up between August and December (control: 4.9 ± 4.8; infected: 3.4 ± 3.7; medicated: –2.4 ± 2.4%). The blood haemoglobin, haematocrit and MCH values increased towards the winter, while the response of lymphocytes was an increase with peaks in October–November, followed by a decrease in November–December. The plasma total Chol, urea, T_3 and ghrelin concentrations and urea/creatinine ratios decreased in the autumn, while the creatinine concentrations increased towards the winter. The plasma leptin concentrations correlated positively with the thickness of SC fat ($r_s = 0.518$, $p < 0.05$).

4.6 TISSUE FATTY ACID COMPOSITION OF THE MOOSE AND REINDEER (II–III)

4.6.1 Effects of deer ked parasitism (II–III)

The proportions of several individual n-3 polyunsaturated FA (PUFA) and the n-3 PUFA sum in livers of the moose correlated inversely with the intensity and density of deer keds (Figure 4), as did the 18:3n-3 percentage in SC fat and the 16:0 proportions in liver and fat tissues (II). The percentages of 18:0 in moose adipose tissues correlated positively with the intensity and density of parasites. Generally, the differences in the FA proportions of the reindeer were minor and rarely significant between the experimental groups (III). To give some examples of these scattered findings, it can be mentioned that livers of the infected reindeer had higher percentages of total SFA and lower UFA/SFA ratios than those of the other groups.

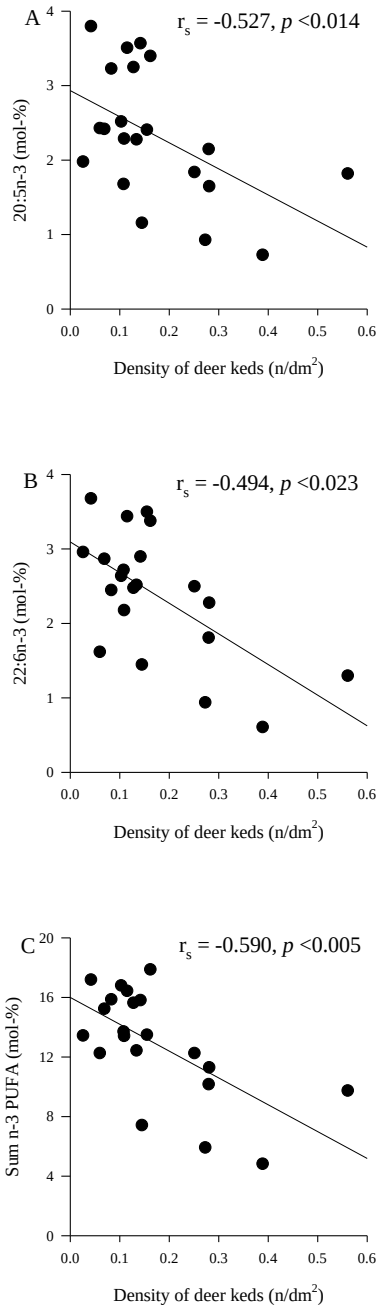

***Figure 4**. Correlations of average deer ked densities with proportions of A. 20:5n-3, B. 22:6n-3 and C. total n-3 polyunsaturated fatty acids (PUFA) in liver total lipids of the moose in eastern Finland (II).*

4.6.2 Effects of age and gender of the moose (II)

SC fat of the bulls contained lower percentages of 16:0 and higher percentages of 18:0 than those of the cows and calves, while the cows had higher 18:1n-9 proportions, monounsaturated FA (MUFA) sums and UFA/SFA ratios than the other groups. In RP fat, the percentages of 16:0 increased according to the sequence: bulls < cows < calves, while the proportions of 18:0 increased in the opposite order. The bulls had lower hepatic n-3 PUFA sums than the cows and calves (Figure 5), and the calves had lower hepatic 18:1n-9 proportions than the adults. The age and gender of the moose had only minor effects on the muscle and plasma FA profiles.

4.6.3 Tissue-specific differences in the moose and reindeer (II–III)

Moose liver, muscle and plasma contained lower percentages of 16:0, 18:0 and total SFA than RP fat (II; Figure 5). The percentages of total SFA in the reindeer increased according to the sequence: muscle ≤ plasma ≤ liver ≤ SC fat ≤ RP fat (III; Figure 5). Reindeer liver tissue contained the lowest proportions of 16:0, while the highest percentages were found in the fat depots. RP fat and liver contained more 18:0 than the other tissues. The proportions of total MUFA of the moose were the lowest in plasma and the highest in SC fat (II) and, in a similar manner, the total MUFA percentages were lower in liver and plasma than in the other tissues of the reindeer (III; Figure 5). Plasma and livers contained less 18:1n-9 than the other tissues of both species (II–III).

The n-3 and n-6 PUFA sums and total PUFA percentages in the moose were the lowest in fat tissues (Figure 5), and a similar trend was also observed for several individual n-3 PUFA (II). The UFA/SFA ratios in the moose were also the lowest in the fat depots. The PUFA totals increased in reindeer tissues as follows: SC fat ≤ RP fat ≤ muscle ≤ plasma ≤ liver (III; Figure 5). The percentages of 22:5n-3, 22:6n-3 and total n-3 PUFA in the reindeer were the highest in liver and plasma. Muscle and

plasma contained more 18:3n-3, and SC and RP fats less 20:5n-3 than the other tissues. The n-6 PUFA sums were the highest in liver and plasma (Figure 5), and the percentages of 18:2n-6 increased according to the sequence: SC and RP fat < muscle and liver < plasma. With 20:4n-6, the sequence was as follows: RP and SC fat < muscle and plasma < liver.

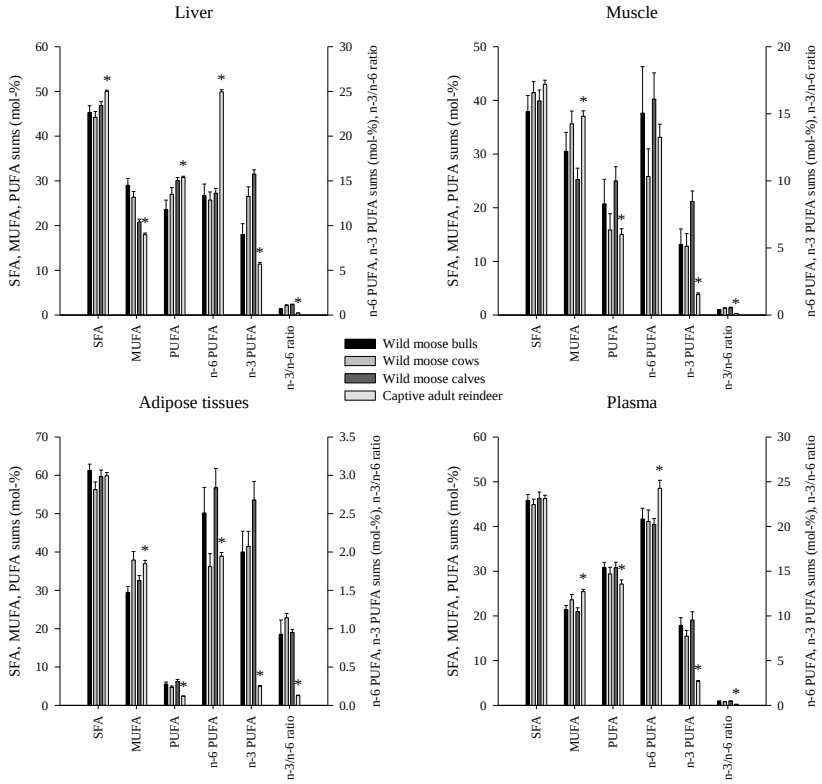

Figure 5. *Fatty acid sums and n-3/n-6 polyunsaturated fatty acid (PUFA) ratios in different tissues of the moose and reindeer (II–III), mean + SE. SFA = saturated fatty acid, MUFA = monounsaturated fatty acid. The adipose tissues represent the pooled results of subcutaneous and retroperitoneal fats. * = Significant difference between the reindeer and moose (p <0.05; all moose groups pooled for statistical analyses). Statistical differences between the moose groups can be found in the original publication (II).*

4.6.4 Species-specific differences and comparison to the diet (II–III)

Compared to the dietary profile, reindeer tissues contained more C14–19 SFA (III). An exception to this was 16:0, with tissue proportions quite similar to that of the commercial diet. The feed contained more C20–24 SFA than most of the reindeer tissues, but liver had higher percentages of C22–24 SFA than the diet. Generally, tissues had more C14–19 MUFA than the feed, with the exception of 18:1n-9 in all the studied tissues. Reindeer liver contained more 24:1n-9 than the diet, while the proportions of C20–22 MUFA were higher in the feed. The percentages of 18:3n-3 were higher in the diet than in tissues, and the same was observed for total n-3 PUFA, with the exception of liver. Liver and muscle of the reindeer had more C20–22 n-3 PUFA than the feed. The diet contained more 18:2n-6 and total n-6 PUFA than tissues, which had higher proportions of 20:4n-6. The diet had more total PUFA than tissues, but the proportions of total *trans* FA were higher in tissues compared to the dietary profile.

The reindeer had higher hepatic SFA totals and lower sums of MUFA than the moose (II–III; Figure 5). The n-3 PUFA sums of the moose were higher in all tissues compared to those of the reindeer, while n-6 PUFA totals were higher in reindeer livers, and the same was also observed in plasma. The n-3/n-6 PUFA ratios were lower in the reindeer in all the studied tissues.

4.7 SURVIVAL OF THE DEER KED IMAGINES AT SUBZERO TEMPERATURES (IV)

The survival-% of the keds did not vary according to the rate of cold-acclimation. In the faster acclimation group (5.0°C/day), 86% of the keds could be revived after being exposed to –5°C and all keds in the slower acclimation group (2.5°C/day) survived this T_{acc}. The numbers of live deer keds decreased significantly between –10 and –15°C in the faster acclimation group, while in the slower acclimation group the difference in survival was significant both between –5 and –10°C and

between –10 and –15°C. When exposed to a decrease of 2°C/day, no keds survived at –16°C, the LLT_{100} for the deer ked, and the difference in mortality became significant between –14°C and –16°C (Table 1). The SCP of the specimens was determined to be –7.8 ± 0.2°C and the water content 54 ± 1%.

The glucose and glycerol concentrations of the deer keds did not vary according to cold exposure, but the concentrations of several AA and related molecules were higher in the cold-exposed keds (Figure 6; Table 1). Usually, the increases became significant at –4°C. In addition, the alanine, γ-aminobutyrate, ammonia, arginine, asparagine, glutamate, glutamine, proline and serine concentrations correlated negatively with the T_{acc}. The trehalose, sorbitol/xylitol and fructose concentrations were below the detection limits with the methods employed.

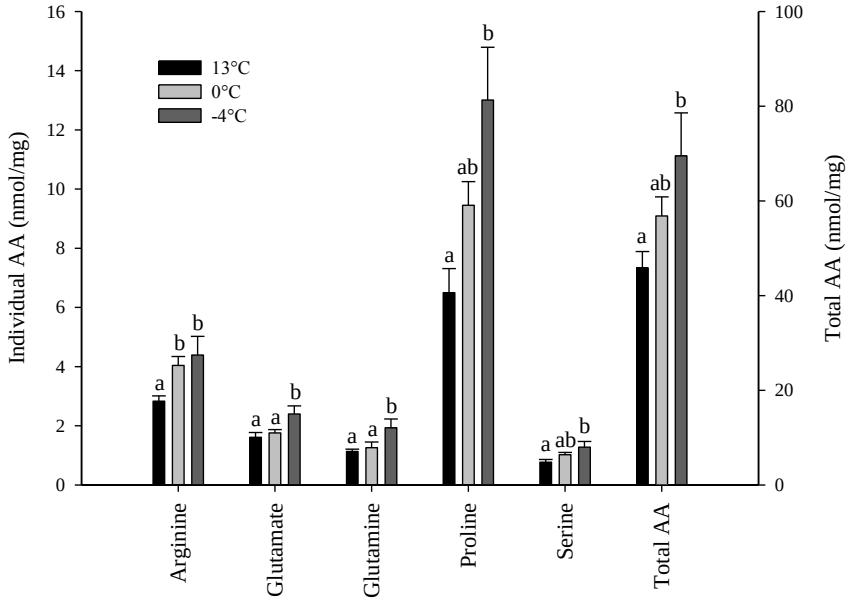

***Figure 6**. Free amino acid (AA) concentrations of the deer keds at different acclimation temperatures (IV), mean + SE. Means with no common letter differ at p <0.05.*

5 Discussion

5.1 DEER KED PARASITISM ON THE MOOSE (I)

The prevalence of deer ked parasitism on the moose in eastern Finland was 100% (I), higher than the values of 67–96% determined by Välimäki *et al.* (2011) using the wintertime bedding site examination method in western parts of the country and in northern Ostrobothnia and Kainuu regions. The total numbers and densities of deer keds were very high in eastern Finland (I; Figure 3). The numbers of keds were 1.5–2.1 times higher than reported for moose in Soviet Belarus (Ivanov 1981), 9–35 times more than on moose in the Leningrad region of the former Soviet Union (Popov 1965) and 115–708 times more than on Danish red deer (Haarløv 1964). Madslien *et al.* (2011) reported recently equally high deer ked intensities on moose in Norway (up to 16500 keds per host). These numbers are much higher than reported previously for other louse fly species. The maximum number of sheep keds (*Melophagus ovinus*) was 300–400 per sheep (Legg *et al.* 1991), while the number of louse flies on birds seems to be smaller, a few dozen (Hutson 1981).

While there are no reports on other louse fly species on the moose as numerous as the deer ked, the winter tick, a one-host parasite of wild cervids (Mooring and Samuel 1999), had intensities of ≥30000 ticks per moose in North America (Samuel and Welch 1991), and the ticks preferred the shoulders, neck and withers (Mooring and Samuel 1999), not dissimilar to the deer keds in our study (I; Figure 3). The distribution of deer keds on the pelts of the Norwegian moose was also relatively similar (Madslien *et al.* 2011). In the case of other ectoparasites, the average density of cattle biting lice (*Bovicola bovis*) on steers was 26.5 lice per skin dm^2 (Watson *et al.* 1997), less than the average deer ked density on the moose bulls in eastern Finland

(I). The highest *B. bovis* densities were on the poll and shoulder regions (88.6 and 79.5 lice/dm^2), higher than the densities of deer keds on the anterior back of the moose cows and calves (35.3 and 26.0 keds/dm^2), but lower than on the bulls (97.6 keds/dm^2). Regarding other mammalian hosts, arthropod ectoparasites were found in 44% of dogs (*Canis familiaris*; Aldemir 2007), and 82% of cats (*Felis catus*) had the flea *Ctenocephalides felis,* with approximately 16 parasites found per host when using a random sampling method of minor areas (Hsu *et al.* 2002). Over 45% of the fleas were located on the head and neck, followed by the dorsal region with over 27% of the fleas. The sheep ked preferred the rib and shoulder regions (Legg *et al.* 1991), and animals with an open, long and fairly greasy fleece were the most ideal hosts (Evans 1950). The deer ked was reported to display negative geotaxis and phototaxis (Ivanov 1981), which may explain why most of them were attached to the withers of the moose (I). The fur is the longest (up to 200 mm) in this region (Sokolov and Chernova 1987), which could provide the best shelter for the parasites that are situated close to moose skin.

Previously, the intensity of deer ked parasitism correlated positively with the body size of the host (Kadulski 1974). The adult moose had higher parasite intensities than the calves, which supports this finding, but the bulls harboured more keds than the cows, despite similar skin areas (I; Figure 3). Based on the natural history of the deer ked, the moose with large home ranges and high levels of activity could be at the highest risk to become parasitised. During the mating season in September–October, moose bulls in rut wander over large areas searching for mates (Valste 2001). Moreover, 64% of bulls in Finland move more than 15 km between seasonal home ranges, while only 30% of cows travel as far (Heikkinen 2000). The moose travel several times between summer and winter home ranges before settling in their winter habitats. Thus, bulls could gather deer keds from larger areas in autumn, which may partly explain the higher numbers of deer keds on their pelts (I). This is supported by the results of Cederlund and Sand (1994), who noted that the autumnal home ranges of Swedish moose bulls were larger than

those of cows. Similar results have also been obtained in Finland, particularly in the southern parts of the country (J. Pusenius *et al.*, unpubl. data). In the present study, the cows had more keds than the calves but equal densities (I). Ivanov (1975) observed that if two potential hosts of a different body size were available, the deer keds chose the bigger one. On this basis, the calves that are still following the cows may attract fewer keds.

The population size of deer keds in Finland can be preliminarily estimated at approximately 400–500 million keds, which could produce 4–8 billion pupae per generation (I). Ivanov (1981) calculated that in 1972 the size of the deer ked population in Belarus was approximately 36 million keds, significantly less than the hypothetical estimate in Finland. The number of deer keds correlated positively with the size of Belarusian moose population, while the numbers of roe deer and red deer had no correlation with the population size of the deer ked. In eastern Finland, the sizes of other cervid populations are notably smaller than that of the moose (Finnish Game and Fisheries Research Institute 2012b). This, together with the results obtained by Ivanov (1981), suggests that the moose could be the principal host species of the deer ked in the study region, which was also the conclusion arrived at by Välimäki *et al.* (2011) for Finland. For this reason, reducing the number of moose could be the most efficient way to control the size of the deer ked population.

5.2 PHYSIOLOGICAL EFFECTS OF DEER KED PARASITISM ON THE MOOSE AND REINDEER (II–III)

Arthropod ectoparasites have previously influenced the red blood cell variables of their hosts (Williams *et al.* 1978; Stromberg *et al.* 1986; O'Brien *et al.* 1995; Pérez *et al.* 1999), and due to the intensive parasitism of deer keds in eastern Finland (I), it was hypothesised that the deer ked could have deleterious effects on the haematological values of its hosts. However, the influence was difficult to detect (II–III). The moose in the deer

ked-infected regions had higher MCHC values than the moose in the deer ked-free areas, but this was probably of minor physiological significance and not caused by deer ked parasitism (II). The survival of the deer keds was very low on the reindeer (2.1% at the end of the experiment) and, also in this case, the parasites did not induce anaemia (III). In fact, the blood haemoglobin, haematocrit and MCH values increased towards the winter in all reindeer groups, independent of infection (see also Nieminen 1980; DelGiudice *et al.* 1992).

Based on previous data on the rate and volume of blood consumption by the deer ked (Ivanov 1981) and the estimated total blood volume (Leighton 2000) and life span of erythrocytes (Röhme 1981) in the moose, it was calculated that bulls with a mean number of 10000 keds would have to replace their total erythrocyte population every 25–80 days—1.8–5.6 times more often than normally (II). Blood loss at this level would probably induce anaemia, but the analyses did not support this. Nor could decreased haemoglobin values be observed in three deer ked-parasitised Norwegian moose individuals (Madslien *et al.* 2011). Severe blood loss would increase the degree of anisocytosis of erythrocytes, and, subsequently, the red blood cell distribution width (Davidsohn and Nelson 1974; Weksler and Moore 1990). Eventually, decreases in MCV, MCH and MCHC would ensue. However, these effects were not present and, thus, the blood volume consumed by the wild deer ked or its blood consumption rate may be lower than those documented in previous experiments by Ivanov (1981). Furthermore, the host species used by Ivanov (1981) are not thoroughly explained in his thesis, but it can be presumed that he utilised conventional laboratory rodents rather than cervids, which could have affected the results. There may also be geographical differences in the feeding habits of deer keds between Finland and Belarus.

Parasitism may also cause leukocytosis on the hosts (Losson *et al.* 1988; Catley *et al.* 1990; O'Brien *et al.* 1995), implying, e.g., microbial infection, but in the present studies there were no parasitism-induced differences in the white blood cell counts of

the moose or the reindeer (II–III). The moose in all regions, including the deer ked-free areas, had equal percentages of eosinophils, which were higher than those measured in the reindeer, suggesting that all these moose could have had high intensities of various eukaryotic parasites. This can complicate attempts to distinguish the effects of a single parasite species. The difference between the moose and the reindeer is understandable, as the reindeer were captive animals and they had been pre-medicated with antiparasitic agents before the actual experiment. In most cases, the eosinophil-% of the reindeer were below the detection limit, also in individuals inoculated with deer keds. Generally, the current findings were quite similar to other studies, showing no statistically significant or physiologically relevant effects of ectoparasitism on white blood cell values (Schwinghammer *et al.* 1986ab, 1987).

The lack of consistent correlations between the intensity and density of parasitism and the body energy stores, enzyme activities, clinical chemistry, endocrinology and AA values gives further support for the finding that, although intense, the parasitism did not seem to induce detectable physiological changes on the moose (II). The same phenomenon was also observed in the experimentally infected reindeer, albeit their parasite intensities were lower (III). Interesting physiological variables in this respect would be stress-related hormones and parameters indicating catabolism and, thus, negative energy balance due to insect harassment. Increased plasma cortisol concentrations have previously indicated stress in parasitised cattle (Schwinghammer *et al.* 1986ab, 1987), but no correlation between the cortisol values and parasitism intensities could be observed in the moose (II). In a similar manner, there were no differences in the plasma cortisol concentrations of the reindeer, nor in the adrenal catecholamine levels and histological findings between the groups at the end of the study (III). Cortisol is excreted in a pulsatile manner (Genuth 1998), and stress associated with the chase of the moose and handling of the reindeer may have caused interindividual variation in the rhythms of cortisol excretion (Rehbinder and Edqvist 1981;

Säkkinen *et al.* 2004). Any possible subclinical differences between the experimental groups caused by parasitism could thus have been masked by these phenomena.

Potential deleterious effects of harassment and skin irritation by ectoparasites include increased duration of time allocated to various energy-consuming activities, such as standing, walking and running (Schwinghammer *et al.* 1986ab, 1987; Mörschel and Klein 1997; Hagemoen and Reimers 2002). Reduced time for eating and resting may impair the weight gain of the hosts (Williams *et al.* 1977; Stacey *et al.* 1978), and this could have been reflected in the variables of nitrogen and fat metabolism or in the levels of weight-regulatory hormones. For instance, negative energy balance in reindeer is characterised by decreased serum lipid and urea levels and plasma leptin and insulin concentrations (Soppela 2000; Soppela *et al*. 2000, 2008). However, the physiological values did not correlate with the intensity or density of parasites on the moose (II), and neither were there any clear differences in these variables between the study groups of the reindeer (III). Intensive muscle work is often reflected as elevated plasma creatine kinase (CK) activities (Adlercreutz *et al.* 1983), and harassment by deer keds could have hypothetically increased the physical activity of the moose and reindeer, and hence also their CK levels. Again, the current results did not support this, as only the duration of chase of the moose correlated with the plasma CK activities (II). Moose are generally in a good physical condition after the summertime food abundance (Schwartz *et al.* 1987ab), similarly to captive reindeer with *ad libitum* feeding. This may reduce the negative effects of deer keds, as the nutritional state of the host can affect its responses to parasitism (Nelson 1984). It is also possible that seasonal changes in, e.g., haematology, clinical chemistry and thyroid axis could have obscured some of the physiological effects of parasitism on the reindeer (III).

It is known that n-3 PUFA can have beneficial effects on, e.g., cardiovascular health (Ackman and Cunnane 1992), but their influence on the host immune response to bacterial infections has been contradictory (Anderson and Fritsche 2002). However,

Muturi *et al.* (2005) suggested that an increased n-3/n-6 PUFA ratio in gut mucosa could enhance host resistance to eukaryotic endoparasites. Especially important FA regarding immunity against parasitism could be 20:4n-6, 20:5n-3 and 22:6n-3 (Kumaratilake *et al.* 1997; Arun Kumar and Das 1999). With the exception of hepatic n-3 PUFA (Figure 4), the intensity and density of deer keds had no consistent correlations with the tissue FA percentages of the moose (II). Also, the differences in the FA profiles between the reindeer groups were minor and inconsistent (III). It is intriguing that the highest deer ked intensities and densities were observed on the moose bulls (I) with the lowest hepatic 20:5n-3 and 22:6n-3 proportions and n-3/n-6 PUFA ratios in liver and muscle (II; Figure 5). The lower n-3 PUFA proportions and sums could indicate negative energy balance, as the mobilisation of n-3 PUFA is more efficient than that of n-6 PUFA during calorie restriction, and this may lead to an unfavourable n-3/n-6 PUFA ratio (Nieminen *et al.* 2009; Rouvinen-Watt *et al.* 2010). Finnish moose bulls were documented to be more active in autumn than cows (Heikkinen 2000), and September–October is the period of rut (Bjärvall and Ullström 1996), which reduces the time allocated to feeding (Schwartz *et al.* 1987a) and could be one explanation to the lower n-3 PUFA proportions (II).

The calves generally had the highest n-3 PUFA percentages but the lowest parasite intensities, and this might explain why the values of deer ked intensity and density correlated inversely with several hepatic n-3 PUFA (Figure 4). Thus, it remains to be determined whether this observation between higher n-3 PUFA proportions and lower parasite numbers actually represents a causal relationship in the moose. There were no indications of FA alterations caused by deer ked infection of the reindeer that could have elicited effects on the immune response to eukaryotic parasitism (III). Possible effects of deer keds on the nutritional state of the reindeer could have been reflected in their FA profiles, as it has previously been observed that reduced percentages of 18:3n-3 and 18:2n-6 in serum and bone marrow could have potential as biomarkers of undernutrition in

the species (Soppela 2000; Soppela and Nieminen 2002). However, no signs of this phenomenon were detected in the tissues analysed for the present thesis (III).

When discussing the differences between these two ruminant species, the sums of hepatic SFA were higher and those of total MUFA lower in the reindeer than in the moose (II–III; Figure 5). The n-6 PUFA sum was clearly higher in the livers of the reindeer, and a similar trend was also observed in plasma. The livers of both species had the highest n-3 PUFA totals, but these sums and the n-3/n-6 PUFA ratios were lower in the reindeer in all the studied tissues. The species-specific features of the FA profiles may derive from different FA composition in the commercial feed of the captive reindeer and the natural diet of the moose (Chernobrovkina *et al.* 2008; Vedernikov and Roshchin 2010). The adipose tissues contained high proportions of total SFA and MUFA and low PUFA sums, independent of the species (see also Tanhuanpää and Pulliainen 1975; Soppela and Nieminen 2002). Previous studies have documented similar incorporation of MUFA in adipose tissues of monogastric mammals (Cochet *et al.* 1999; Mustonen *et al.* 2007). In ruminants, the proportions of dietary FA are not reflected in a straightforward manner in the adipose tissue FA profiles due to the biohydrogenation of UFA by rumen microorganisms (Jenkins 1993; Bessa *et al*. 2007). The relative enrichment of longer-chain n-3 and n-6 PUFA in liver and muscle is presumably due to their higher proportions of structural membrane phospholipids compared to white adipose tissues.

With the exception of one individual with minor hair loss of unknown aetiology, the majority of the moose examined in eastern Finland showed no signs of alopecia (I). Previously, the chewing louse (*Damalinia* (*Cervicola*) sp.) has been documented as causing the hair-loss syndrome for Columbian black-tailed deer (*Odocoileus hemionus columbianus*; Bildfell *et al.* 2004), and North American moose infected by the winter tick also displayed alopecia (Glines and Samuel 1989; Mooring and Samuel 1999). Moreover, deer ked parasitism was suggested to be a causative factor for the hair loss observed in Norwegian

moose, although the parasite intensities were no higher than on the moose in eastern Finland (I; Madslien *et al.* 2011). Besides hair loss, the moose in Norway also manifested skin lesions similar to previously documented findings in the outer ear canals of moose infected by *Chorioptes* sp. (Hestvik *et al.* 2007). It is noteworthy that 27% of the Norwegian moose with documented abnormalities had no deer keds on their pelts (Madslien *et al.* 2011). The contradictory findings on the Finnish and Norwegian moose indicate that the possible causality between deer keds and the observed alopecia will have to be determined in future studies. In some reindeer, the experimental deer ked infection induced grooming, and bare patches were observed on a few pelts, indicating that deer ked parasitism may cause localised hair loss for reindeer (III; Kynkäänniemi *et al.* 2010). This could lead to potentially detrimental effects, such as increased heat loss in wintertime, and might reduce the well-being of the host. Although the hairless areas may be small, the cumulative effect of increased energy demand could be significant. It was estimated by McLaughlin and Addison (1986) that the daily energy requirements of the moose could double at –20°C if it lost 30% of its winter pelage.

Ivermectin is commonly used against a wide range of endo- and ectoparasites of animals and humans (Oksanen *et al.* 1995; Oksanen and Nieminen 1996; Dourmishev *et al.* 2005), and in Finnish Lapland, most reindeer are treated annually with ivermectin against metazoan parasites (mainly arthropods and nematodes; Laaksonen *et al.* 2008). The reindeer in the medicated group had only dead deer keds on their pelts (III; Kynkäänniemi *et al*. 2010), suggesting that ivermectin is also effective against deer ked parasitism. However, the efficacy of ivermectin could not be verified with accuracy, as the survival-% of the deer keds was very low and the numbers of parasites could not be determined at the time of ivermectin treatment but only at the end of the experiment 5 weeks after the medication.

It seems that the moose in eastern Finland tolerate deer ked parasitism relatively well (I–II). While the deer ked is a recent newcomer to Finland (Hackman *et al.* 1983), there are reports of

high deer ked intensities in the former Soviet Union (Popov 1965; Ivanov 1981). The Finnish moose population had been hunted to near extinction by the 1920's and was partly replenished by Soviet stock after World War I (Nygren 2009)—presumably together with its parasites. It could be surmised that the co-existence of the moose and the deer ked has endured for long periods of time and allowed the moose to adapt to this parasite (II). In the case of the reindeer, the survival of deer ked imagines and the numbers of pupae produced were at low levels, indicating that the reindeer is not an optimal host species for deer keds (III; Kynkäänniemi *et al.* 2010). One explanation of this may be the higher density of the reindeer winter pelage compared to that of the moose (Timisjärvi *et al.* 1984; Sokolov and Chernova 1987), which does not allow the parasites easy access to the skin. However, pupae observed on wintertime bedding sites suggest that the deer ked could be capable of reproducing on reindeer (Kaunisto *et al.* 2009). In contrast to the moose, other cervids might rather be auxiliary host species of lower quality for the deer ked (see also Välimäki *et al.* 2011).

5.3 SURVIVAL OF THE DEER KED IMAGINES AT SUBZERO TEMPERATURES (IV)

Unlike free-living insects, the actual period of cold exposure for deer ked imagines is the short time frame in autumn, which they have to spend near their emergence sites, waiting for a potential host. This takes place in August–October (Hackman *et al.* 1983), when the average T_a is usually above 0°C and frosts are not nearly as severe as in winter (Finnish Meteorological Institute 2012). The obtained values of SCP and LLT_{100} were understandable in the context of the natural history of the deer ked. Almost all keds survived at –5°C, and most of them recovered from –10°C (IV). The LLT_{100} was determined to be approximately –16°C and the SCP –7.8°C, suggesting that the deer ked could be freeze-tolerant.

The obtained LLT_{100} is rather similar to those of some insect species that are not freeze-tolerant (*Episyrphus balteatus*: LLT_{90} –10 to –15°C; Hart and Bale 1997; *Antarctopsocus jeanneli*: LLT_{100} –12 to –15°C; Slabber and Chown 2004). The *Nemoura arctica* nymph (LLT_{50} <–15°C; Walters *et al.* 2009), which is considered freeze-tolerant, also survived at least similar T_a to those that the deer keds were able to survive. Yet, freeze-tolerant dipteran species, such as *Mycetophila* sp. adults (LLT –40°C) or *Chymomyza costata* diapausing larvae (LLT_{50} –80°C; Sinclair 1999), can survive T_a that are substantially lower than those the keds were exposed to. The benefits gained by the deer keds from the LLT_{100} of –16°C include the possibility of extending their flight season further into late autumn by surviving the freezing nighttime T_a and still being able to locate a host successfully during daytime (IV). $T_a \leq$–16°C are seldom encountered in the study area when flying deer ked imagines are present (Finnish Meteorological Institute 2012).

Free AA concentrations often increase in insects during cold-acclimation and diapause (Mansingh 1967; Zachariassen 1985). The same was observed in the deer keds, as especially the nonessential AA, such as proline, increased in concentration in the cold-acclimated keds (IV; Figure 6). The increases in AA levels could be derived from both protein degradation and reduced synthesis (Renault *et al*. 2006). The present results cannot ascertain the origin of the AA, but in the winged imagines nutritional sources are excluded, and the keds thus had to provide them from existing resources. Up to two-fold increases in free AA concentrations were previously observed in diapausing insect larvae (Mansingh 1967), and the increase in the keds was also significant (52%). Proline and other AA can contribute to cryoprotection, as they are able to lower the freezing point by concentrating the haemolymph (Fields *et al.* 1998). AA stabilise enzymes by protecting them from damage and enabling the recovery of their functions after cold exposure (Carpenter and Crowe 1988). They can also prevent low-temperature damage in artificial lipid membranes (Anchordoguy *et al.* 1988), and these effects could explain the

increases in concentrations of AA during cold exposure in insects.

As cryoprotective sugars and polyols are mostly derived from body glycogen stores (Muise and Storey 1997; Worland *et al.* 1998), the natural history of the deer ked could explain why increased concentrations of these potential cryoprotectants could not be observed after cold-acclimation (IV). The deer ked feeds only after it has attained a host, and thus its energy stores are determined before it is deposited as a pupa. For the deer ked, it could be useful to utilise AA for cryoprotection and to preserve glycogen for the location of a host, as insects with short-duration flights are known to utilise carbohydrates to power flight (Yuval *et al.* 1994). This fits the behavioural patterns of deer ked imagines: a short-distance flight to the host followed by one-host parasitism (Ivanov 1981; Kaitala *et al.* 2009).

It has been observed that the emergence success of deer ked imagines decreases significantly at latitudes north of the present distribution area (Härkönen *et al.* 2010). Nevertheless, some imagines emerged at 70°N, indicating that the colder climate does not necessarily hinder the spread of this parasite further north, where it might cause hazards to reindeer husbandry (Kynkäänniemi *et al.* 2010). The present results indicate that also imagines would probably survive the autumn and be able to locate a host in regions at higher latitudes (IV). They would presumably emerge later and within a shorter period of time in northern Lapland than in the area of the present experiments (Härkönen *et al.* 2010). According to climate statistics issued by the Finnish Meteorological Institute (2012), the average min T_a in September are usually slightly above 0°C, the average T_a is 5.8°C and the absolute min –11.4°C in Sodankylä, Lapland. These conditions would probably allow the survival of deer ked imagines and, thus, range expansion of the species further north would be feasible. Weather patterns can be of importance, as it was suggested by Madslien *et al.* (2011) that the exceptional Norwegian deer ked infestation and the possibly related alopecia of moose in 2006–2007 could have been partly induced by the unusually warm summer and autumn T_a, accompanied

by the late occurrence of frosts, enhancing the emergence and host location of winged keds. The spread can be further assisted by global warming (see also Härkönen *et al.* 2010), which could prolong the flight season by reducing the occurrence and severity of autumnal nighttime frosts.

6 Conclusions

1. The moose in eastern Finland were heavily parasitised by deer keds. The calves (852–2309 keds per individual) had fewer keds than the adults, possibly because deer keds prefer a host of a larger body size, and the bulls (7594–17491) had more keds than the cows (817–5130) as they are more mobile in autumn and could thus gather deer keds from larger areas. The density of deer keds varied between different anatomical regions. It was the highest on the anterior back (26.0–97.6 keds/dm^2 of skin), where half of all keds were located. This could be explained by the long hair on the withers and by the negative geotaxis and phototaxis displayed by the deer ked.

2. The haematological and clinical chemistry variables of the moose did not show significant differences between the studied deer ked-infested and deer ked-free regions in Finland. An exception to this was the MCHC, which was lower in the moose in the deer ked-free regions. Deer ked parasitism did not correlate consistently with the measured variables, but the proportions of liver n-3 PUFA correlated inversely with parasite intensity and density. It remains to be determined whether this is a causal relationship. Apart from minor differences in haematology and tissue FA profiles, the bulls, cows and calves were fairly similar in their physiological variables.

3. There were no clear effects of deer ked parasitism on the measured physiological variables of the enclosure-housed reindeer at the intensity of infection employed (inoculum: 300 keds per reindeer). As the survival of the deer keds was very low (2.1%), it is unlikely that a longer follow-up period would have produced significant effects later in the winter. Ivermectin seemed to be an efficient antiparasitic agent against deer keds. The seasonal changes in the haematological, clinical chemistry

and endocrinological variables of the reindeer mostly conformed to previously reported patterns.

4. The LLT_{100} of the deer ked was approximately –16°C and the species could be freeze-tolerant with a SCP of –7.8°C. The deer ked does not seem to accumulate high concentrations of low-molecular-weight cryoprotectants, but the free AA concentrations were higher in the cold-acclimated keds, possibly contributing to cold-tolerance. The LLT_{100} would be compatible with the survival of deer ked imagines in regions north of the present area of distribution.

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UNIVERSITY OF
EASTERN FINLAND

Publications of the University of Eastern Finland
Dissertations in Forestry and Natural Sciences

ISBN: 978-952-61-0771-4 (printed)
ISSN: 1798-5668
ISSNL: 1798-5668
ISBN: 978-952-61-0772-1 (PDF)
ISSN: 1798-5676 (PDF)