Seasonal adaptation, phenology and survival in gall-inducing sawflies

(Tenthredinidae: Nematinae: *Pontania*)

Trond Elling Barstad
A dissertation for the degree of Philosophiae Doctor – July 2016

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The Arctic University of Norway

Tromsø University Museum

Department of Natural Sciences

Faculty of Biosciences, Fisheries and Economics

Department of Arctic and Marine Biology

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Supervisors:

Prof. Rolf A. Ims, Department of Arctic and Marine Biology, UiT - The Arctic University of Norway

Prof. emeritus Arne. C. Nilssen, Tromsø University Museum, UiT – The Arctic University of Norway

Front cover:

Picture of unidentified gall-inducing sawfly (Tenthredinidae: Nematinae) on *Salix phylicifolia* (Photo: Trond Elling Barstad).

Preface

In the beginning of my PhD-project, little did I know about gall-inducing sawflies and their fascinating adaptation to it willow host plants. It is amazing how they are able to make gall in which the larvae live inside. It is when galls appear during the summer that one really can get a picture how common they are, and it seems they are everywhere willows grow. The adults are not easily seen always hiding in the bushes. If one gets a glimpse of them, they seem quite clumsy with their butterfly-like flight. However, during the time I have been working with these small charming creatures, I have come to realize that they are very robust insects highly adapted to the Arctic environment.

always have supported me and given me strength to complete this PhD-project. And to my 4-year old son, Theodor, how always make me happy. I am amazed how patient and understanding hi has been during the recovery periods after the surgeries I have had.

Trond Elling Barstad
July 2016
Tromsø

List of publications

This thesis is based on the articles presented below, referred to by their Roman numerals I-IV.

I. **Barstad TE** and Nilssen AC (2012) Hibernation adaptation and eclosion synchrony in leaf-galling sawflies in subarctic Norway. Polar Biology 35: 1097-1103.

II. **Barstad TE** and Nilssen AC (2015) Spring phenology and the response to global warming in gall-inducing sawflies. Polar Biology 38: 1503-1513.

III. **Barstad TE,** Nilssen AC and Leppänen S (2016) Oviposition preference and larval performance in gall-inducing sawflies. Manuscript.

IV. **Barstad TE** and Nilssen AC (2016) Phenology during pre-diapause in gall-inducing sawflies and the influence of night length. Submitted manuscript to Polar Biology.

Author's contribution

The present author and supervisor Arne C. Nilssen planned study I and II together, whereas I planned studies III and IV. I also carried out the field- and laboratory work. Data analyses were a cooperative work between myself and Arne. C. Nilssen. Sanna Leppänen provided additional data in study III. I was the main writer of the articles I-IV. Supervisor Rolf Ims gave valuable feedback throughout the analyses and the writing process.

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1 Abstract

Arctic insects face harsh Arctic environmental conditions with pronounced seasonality. However, many insect species have been able to adapt to these challenges and survive remarkably well. The phenological adaptation of insects, which is the seasonal timing of biological events during the year, is determined by different environmental cues and is fundamental to their success. Many insects in the Arctic are herbivorous and are temporally restricted to feeding on a certain plant resource or quality, in which temporal disassociation may have detrimental effect on survival. Because current global climate change is most pronounced in the Arctic, insects living in this region may be particularly prone to the negative impact of increasing temporal dissociation with their plant resources, and to their natural enemies such as parasitoids.

The aim of this thesis, that comprise four separate studies, was to increase our knowledge of gall-inducing sawflies (Tenthredinidae: Nematinae: *Pontania nivalis, P. glabrifrons* and *P. arcticornis*) on *Salix* spp., such as that of life history adaptations in the European high north with a particular focus on environmental cues that determine the timing of important life history events.

The first study, an eclosion experiment, investigated diapause adaptation, in which morphogenesis is supressed during dormancy. Subsamples were successively transferred indoors (23 °C) at 14-day intervals from October to April in a test of development time to eclosion and survival ratio. The three *Pontania* spp. had similar eclosion patterns and followed the common outline for overwintering insects. Diapause intensity prevented resumption of morphogenesis in early winter and turned into a post-diapause quiescence mid-winter. During post-diapause quiescence, there is potential for morphogenesis but supressed by hostile environmental conditions (i.e. low temperature). In this study, the three *Pontania* spp. showed increasing synchronous eclosion towards spring. In addition, a few specimens of *P. nivalis* and *P. glabrifrons* did eclose prior to sub-zero temperatures and shortly after diapause induction in autumn. Chilling, a period of sub-zero temperatures needed for eclosion, did not seem to be necessary and the refractory period (the genetically determined period needed to be able to eclose) was short. However, *P. arcticornis* showed slightly different adaptation and eclosed later and after sub-zero temperatures, indicating that chilling may be necessary and that the refractory period may be longer than that of the two other species.

The second study focused on spring phenology and the temporal variation in timing of eclosion in spring was investigated. The three study species showed the same responses. First, the temperature threshold (2.36 °C) and hour-degrees (6909) needed for direct development (i.e. post-diapause) was determined by laboratory-rearing in order to estimate eclosion timing. The results were corroborated by adult presence in field as determined by trapping. Hindcasts of temperature conditions in spring for the last 21 years were performed by using meteorological temperature data that were correlated with field temperature data. This revealed a highly significant advancement in dates of eclosion that was interpreted as evidence of global warming. There were, however, large inter-annual differences in eclosion timing, which was suggested to be correlated with timing of the spring snow melt. These results indicate that *Pontania*

spp. must be adapted to large inter-annual variation in spring climatic conditions and exhibit large phenological plasticity in timing of eclosion.

The third study gave important insight in the behavioural and ecological relationships to adjacent trophic levels for *Pontania* spp., especially female oviposition preferences on the host in relation to larval performance and survival. There is a short phenological window of opportunity for oviposition on suitable host resources, in which female oviposition preference has shown to enhance larval performance for gall-inducing sawflies. However, earlier studies of a few species of *Pontania* spp. have not supported this. Furthermore, the three *Pontania* spp. in this study did not show any oviposition preference that enhanced larval performance. This is probably caused by a brief and early spring eclosion when host plant cues (i.e. difference in plant module size) are lacking, in which the female respond with a non-discriminatory oviposition pattern. Larval survival of *Pontania* spp. was also relatively high, with little mortality caused by parasitoids.

The fourth study focused on diapause preparation during pre-diapause in the autumn and showed that gall-inducing sawflies enter diapause in autumn in response to night length during pre-diapause. The three *Pontania* spp. showed a remarkable phenological synchronous behaviour with a distinct start on 5 September, in which larvae emerge from the galls in search for overwintering sites. The establishment of the critical photoperiod for diapause preparation in relation with autumn temperatures revealed that night length (i.e. photoperiod) acted as a *token stimulus* during pre-diapause.

This thesis provides new information on several aspects of the life history adaptations of *Pontania* spp., showing that they are highly adapted to their high latitude environment and their *Salix* host plants. Although specialist insect species could be expected to be especially prone to the negative impacts of climatic change, in particular in the Arctic, this study does not provide any clear indications that this applies to *Pontania* spp..

2 Introduction

2.1 Seasonal adaptation in Arctic insects

The geographical region north of the Arctic Circle is generally known as the Arctic, the circumpolar region of the northern hemisphere. A more biologically relevant definition, mean July temperature below 10 °C, corresponds well with the northern limit of boreal forest. As from an entomological perspective, the Arctic region includes the transition zone between boreal forest and the Arctic tundra, the sub-Arctic (Downes 1965), in which the northernmost part of Fennoscandia is represented. The climate for this region, especially coastal Norway, is mild for its latitude, and Arctic and sub-Arctic climatic conditions are only present at the highest latitudes, or at high altitudes further south (Danks 1981; Meltofte et al. 2013; Walker et al. 2005).

It is remarkable that insects with their limited ability to regulate body temperature are able to survive in cold climates at higher latitudes, especially when low temperatures are the most important factor insects have to cope with to survive. Insects are, however, the most diverse group of animals in the Arctic, even as the harsh environmental conditions set constraints on insect's ability to survive (Danks 1981, 1986; Hodkinson 2013; Strathdee and Bale 1998). In the northern hemisphere, the pronounced seasonal periodicity in environmental conditions is particularly strong, especially above the Arctic Circle, due to constant daylight in summer and constant darkness in the winter. Insects in the Arctic region must survive short, unpredictable summers and long winters through a variety of physiological and behavioural adaptations (Danks 1987). Low temperatures and small diurnal fluctuations (i.e. small heat budget) set constraints for growth and reproduction during the short summers, and a prolonged dormancy period is needed during the long cold winters (Danks 2004; Downes 1965; Tauber et al. 1986). Insects show a remarkable set of adaptations to survive these environmental conditions. However, insect species diversity, especially among herbivorous insects, decreases into the Arctic (Danks 1981, 1986, 2004; Hodkinson 2013; Strathdee and Bale 1998). Species' geographical origin in relation to glaciation events, evolutionary development and the ability to change ecological requirements are suggested as causes for this pattern (Danks 1981), but our knowledge about biology of insects in the Arctic region is still limited.

Several biological adaptations reflect the requirements needed for survival in an insect's life cycle that is evolutionary perfected to its environment. In the Arctic with strong seasonal changes, the timing of seasonal biological events is of vital importance for survival (Danks 1981, 2004; Downes 1965; Ring and Tesar 1981; Tauber et al. 1986). One of the most fundamental characteristic of an insect's biological adaptation is its phenology, which is seasonal timing of annual cycles in biological events, such as eclosion (i.e. adult emergence), mating (i.e. reproduction), oviposition (i.e. egg laying) and diapause (i.e. dormancy) (Danks 1987, 2004; Tauber et al. 1986). In their preparation for changing environmental conditions, insects are able to perceive and induce environmental cues that signals for seasonal changes, in which specific physiological, behavioural and morphological modifications occur (Danks 1987; Hodgson et al. 2011; Singer and Parmesan 2010; Tauber et al. 1986; van Asch et al. 2007).

For insects living in the short Arctic growing season (i.e. summer), it is an advantage to resume morphogenesis as soon as environmental conditions allow it, in order to prolong development as long as possible. As a result, many species exhibit synchronous early spring eclosion, which maximizes reproduction opportunity and success, and particularly herbivorous insects synchronize eclosion with suitable host plant resources for oviposition and larval development (Danks 2004; Hodek and Hodková 1988; Kostál 2006; Ring and Tesar 1981).

The response to approaching hostile environmental conditions (i.e. winter) is a species-specific dormancy adaptation. During dormancy, insects in the Arctic must survive sub-zero temperatures, either through frost tolerance (i.e. survival of ice formation within the body) or supercooling (i.e. avoiding freezing) (Danks 1981; Humble 2006; Miller 1982; Miller and Werner 1980; Nilssen and Tenow 1990; Ring and Tesar 1981; Tanno 1970). The most common dormancy adaptation among insects in the Arctic is diapause, in which growth and development is arrested (Danks 1987; Saunders 2009; Tauber et al. 1986). Diapause intensity determines the duration of diapause, which is regulated by induced environmental cues such as photoperiod and temperature that acts as *token stimuli* (Danks 1987; Tauber et al. 1986). Diapause initiation occurs before the hostile environmental conditions set in in autumn, and diapause usually terminates mid-winter and what appears to occur spontaneously for many overwintering insects (Denlinger 2002; Masaki 2002). During post-diapause there is a period of quiescence, when there is potential for morphogenesis, but this is supressed by low temperatures. When temperatures rise in spring, the resumption of morphogenesis is often followed by synchronous spring eclosion. Processes regulating diapause initiation, duration and termination may not only be triggered by environmental cues, but also influenced by genetic factors (Danks 1987, 2004; Saunders 2002, 2009; Tauber et al. 1986) (see 3.2 for a detailed description of the eco-physiological phases of diapause). However, the complex physiological processes that initiate, regulate and terminate diapause are still today not fully understood (Danks 2005; Denlinger 2002; Kalushkov et al. 2001; Saunders 2002, 2011, 2012, 2013, 2014).

Hymenopterans make up a significant proportion of the Arctic insect fauna (Danks 1981). Among them are gall-inducing sawflies (Tenthredinidae: Nematinae), that have a circumpolar distribution, are common in the Palearctic and Nearctic and found at high densities as far north as the northern Yamal Peninsula (72 °N), Russia (Roininen et al. 2002). Most are strictly monophagous as a result of a close adaptation with their host plant (*Salix* spp.), limiting their distribution (Hjältén et al. 2003; Kopelke 1991; Nyman et al. 2006a; Nyman et al. 2000; Roininen et al. 2002; Roininen et al. 2005; Zinovjev 1998). Information concerning the distribution and ecology of gall-inducing sawfly species in the Arctic is, however, in general very limited (Danks 1981; Humble 2006; Kopelke 1985b, 1991; Ring and Tesar 1981; Zinovjev 1993; Zinovjev 1998).

The main subjects of gall-inducing sawflies studies have been host association (Kopelke 1985a; Kopelke 1986, 1991; Kopelke 1994; Kopelke 1998, 1999), female preferences and larval performance (Carr et al. 1998; Craig et al. 1989; Ferrier and Price 2004; Fritz et al. 2000, 2003; Hjältén et al. 2003; McGeoch and Price 2004, 2005; Price and Craig 1984; Price 1991, 1992, 1994, 2003; Price et al. 2004; Zinovjev

1998), natural enemies and their parasitoids (Clancy et al. 1986; Humble 2006; Kopelke 1985a, 1994; Kopelke 2003; Nyman et al. 2015; Price and Clancy 1986), phylogeny and evolution (Nyman 2000; Nyman et al. 2007; Nyman et al. 2015; Nyman et al. 1998; Nyman et al. 2000; Nyman et al. 2006b; Price et al. 1998; Price 1994, 2003).

One aspect that has not been comprehensively studied is the phenological adaptation of gall-inducing sawflies. As in many other insect species, gall-inducing sawflies exploit food sources that are restricted in both time and quality, in which their development and eclosion need to be in synchrony to that of the host plant. The short cool summer in the Arctic environment constrains larval development and survival, and hibernation needs to occur before hostile climatic conditions set in in autumn. A few sawfly species have been previously studied with regard to overwintering adaptation and strategies (Humble 2006; Knerer 1983, 1993; Knerer and Marchant 1973; Miller 1982; Miller and Werner 1980; Ring and Tesar 1981), but the focus has been on cold tolerance during winter hibernation, which shows that they are highly adapted to cold environment during dormancy. The larvae synthesize small amounts of sorbitol functioning as an anti-freezing agent (a supercooling point of -9 °C), but being also freezing-tolerant, they overwinter in a frozen state (Humble 2006; Ring and Tesar 1981). They have also been shown to survive extremely low temperatures, at least 20 °C below the supercooling point (Humble 2006; Miller and Werner 1980).

2.2 Host plant interaction in gall-inducing sawflies

Much of the information needed for studying population ecology and environmental adaptation of herbivorous insects is documented in nature (Price 2003). However, knowledge about abundance, population dynamics and adaptation requirements needed to survive in the Arctic is in general poor (Danks 1981, 1986, 2004; Ring and Tesar 1981; Strathdee and Bale 1998), and due to the harsh environmental conditions, population fluctuations are more common compared to insects living at lower latitudes (Danks 1981, 1986, 2004; Downes 1965; Ring and Tesar 1981; Strathdee and Bale 1998). Among herbivorous insects in the Arctic, spatial and temporal variations in suitable host plant resources can severely affect their performance and survival (Singer and Parmesan 2010). As a consequence, spring phenology, i.e. timing of eclosion, mating and oviposition, is an important part of the life cycle and is evolutionary fine-tuned and often in synchrony with host plant leaf flush, when resource quality and accessibility is optimal. Outside this window of opportunity, host plant quality deteriorates and sets constraints on oviposition success and larval performance, and ultimately survival (Hanhimaki et al. 1995; Hunter 2000; Hunter and Elkinton 2000; Martel and Kause 2002; Mopper 2005; Singer and Parmesan 2010).

Comparative studies between species have become increasingly common in the effort to detect adaptations (Clancy and Price 1986; Hjältén et al. 2003; Hunter 1992; Jepsen et al. 2011; Martel and Kause 2002; Price 2003; Price et al. 1994; Price et al. 2004), and among comparative studies involving herbivorous insects, distinct differences in oviposition preference and larval performance have been detected among gall-inducing sawflies (Tenthredinidae: Nematinae) (Craig et al. 1989; Price 2003). It has been shown that female oviposition preferences for certain plant module sizes (e.g.

shoot length, leaf size and leaf position) or growth rates influence gall induction success and larval performance (i.e. fitness) and survival (Cornelissen et al. 2008; Craig et al. 1989; Ferrier and Price 2004; Hjältén et al. 2003; Price 1991). This may be especially evident among gall-inducing sawflies (Craig et al. 1989; Price 2003; Price et al. 2004), but has also been detected among other insect taxa, reviewed by Price (2003).

The plant vigor hypothesis (Price and Craig 1984; Price 1991) suggests that oviposition preference and larval performance pattern is derived from the close host adaptation of female oviposition choice in which there is a feedback from larval performance and that there is a preference-performance correlation based on plant module choice (Price 1991, 2003). In addition, the plant constraint hypothesis suggests that gall-inducing sawflies are phylogenetically constrained by their ovipositor, meaning that the females are restricted to oviposit on matching soft plant resource (Price 1994; Price et al. 2004).

However, even if this preference and performance linkage has been shown to be common among sawflies (summarized in Price (2003)), a few gall-inducing sawfly species show no ovipostion preferences for long, vigorous growing plant modules (i.e. shoots) that enhance larval performance and survival (called non-conformist species) (Price et al. 2004). This is suggested to be due to the timing of oviposition being early in spring, when there are no clues as to what will become fast or large growing plant modules (i.e. shoots) and those that will not (Price et al. 2004). *Pontania* spp. are among the non-conformist species, including *P. arcticornis* in the present study.

2.3 Climatic and environmental change in the Arctic

The global climate is currently changing, and the increase in temperature will be greatest in the Arctic region (Kattsov et al. 2005). Many studies on the response of species to climate change have been published, in which plants and birds dominate. Furthermore, Arctic ecosystems are underrepresented, whereas among insects, certain charismatic groups are overrepresented (Høye 2014; Høye et al. 2007; Parmesan 2006). Due to their intimate adaptations to a cold climate, rapid climate warming may have a negative impact on Arctic insects. There is thus need to increase the knowledge about species' response to climate change (Danks 2004; Høye 2013; Høye and Forchhammer 2008a; Høye et al. 2007). The increasing temperature in the Arctic has been shown to have a large impact on environmental conditions, especially in winter. As a result, changes in snow cover and timing of snowmelt are shown during the advancement of spring. Eclosion phenology in Arctic insects is in general determined by the timing of snowmelt, and has been shown to advance in recent years as a response to earlier snowmelt (Høye et al. 2007) (see also Barstad and Nilssen (2015)). This may disrupt the fine-tuned phenological synchronicity between interacting species, such as herbivorous insects and their host plants (Jones and Despland 2006; Martel and Kause 2002; Singer and Parmesan 2010). Among herbivorous insects, there is a phenological window of opportunity for oviposition and larval development, and beyond this period host plant resources and/or quality deteriorate and set constraints on progeny performance (i.e. lower fitness). As a consequence, temporal disassociation in phenological synchronicity may have a large impact on survival.

2.4 The main objectives

The aim of this thesis was to study various questions involving life cycle adaptations in the Arctic environment for three common congeneric gall-inducing sawfly species, *Pontania* spp. (Tenthredinidae: Nematinae). In particular, we investigated the various stages of dormancy, i.e. diapause initiation, maintenance and post-diapause in relation to *token stimuli*, such as photoperiod and temperature. Phenological adaptation is another interesting subject in which spring phenology may exhibit large temporal disassociation between years in the Arctic, and in which the timing of the host plant leaf flush is essential for oviposition. Phenology during pre-diapause in autumn is another important phase in the life cycle, as preparation for diapause occurs in advance of climatic deterioration. Neither of these subjects have been previously studied for *Pontania* spp., even though they are essential in the understanding of environmental and phenological adaptation. Female oviposition preference in spring and larval performance in summer have been comprehensively studied among sawflies, but not investigated for *P. nivalis* and *P. glabrifrons* that are included in this study. Larval survival was investigated in relation to the preference-performance linkage, but also mortality caused by the major specialist predators, parasitoids, was studied. To summarize, the study addressed how well gall-inducing sawflies are adapted to the Artic environment, and what are the main factors for their success.

The specific aims presented in the articles (I-IV) were:

I.   What are the main factors regulating diapause duration, and when does diapause terminate?

II.   How does climatic variation affect temporal spring phenology?

III. Do the focal *Pontania* species have any ovipostion preference-larval performance correlation enhancing survival during summer?

IV.   To investigate the phenological adaptation during pre-diapause in relation to *token stimuli*, photoperiod and temperature.

3 Study system

3.1 The focal species and its associated host plants

3.1.1. Gall-inducing sawflies

Gall-inducing sawflies belong mainly to the subfamily Nematinae (Hymenoptera: Symphyta: Tenthredinidae) comprising over 200 species that are common in the Arctic and sub-Arctic region on their willow (*Salix* spp.) host plant, although some occurs on poplars (*Populus* spp.) (Price and Roininen 1993). They are among the insect species that have been able to colonise temperate and polar regions in the northern hemisphere, and have a circumpolar distribution suggesting a long evolutionary history that is also reflected in their choice of food plants, mainly willows (*Salix* spp.) (Benson 1950, 1960). Gall-inducing sawflies are closely adapted to their host plants, in which they modify host plant tissue in order to make a gall in which the larvae can live. The evolution of gall-induction among sawflies has occurred several times, as oviposition preferences and gall formation have evolved and shifted towards more central parts of the plant. The result is that most species are strictly host-specific and utilize a short window of opportunity for oviposition on suitable host plant tissue (Nyman et al. 2006a; Nyman et al. 1998; Price 2003; Roininen et al. 2005; Zinovjev 1998).

The focal species in this thesis, *Pontania nivalis* (Vikberg 1970), *P. glabrifrons* (Benson 1960) and *P. arcticornis* (Konow 1904) are Holarctic, common in the Palearctic as well as in the Nearctic, but less known in North America (Hjältén et al. 2003; Roininen et al. 2002; Roininen et al. 2005). They are monophagous and exclusively associated with their willow host, *Salix glauca*, *S. lanata* and *S. phylicifolia*, respectively (Kopelke 1991; Vikberg 1970; Zinovjev and Vikberg 1999), and belong to the *Pontania*- viminalis group that induce pea-shaped galls on the underside of leaves (Kopelke 1999; Zinovjev 1993; Zinovjev 1998) (Fig. 1 a, b, c and d). They have an obligate univoltine life cycle (see Fig. 2 for details), characterized by a short pupal phase in early spring, followed by short period of eclosion synchronized with host plant leaf flush, as there is preference for oviposition on young growing leaves. Host plant phenology greatly influences the life history of associated gall-inducing sawfly species, especially the timing of ovipostion and larval feeding. Adults are short lived and are dedicated to reproduction, host seeking and oviposition. They have a relatively poor flight ability, and consequently, a low capacity to migrate. Mating may therefore take place at the eclosion site or even in the same willow shrub in which they were born (Knerer 1993; Price 1992; Price et al. 2004; Viitasaari 2002). During oviposition, the female injects a secretion together with the egg that initiates gall formation, and gall growth is maintained by secretions during larvae feeding on gall tissue (Kopelke 1994; Viitasaari 2002; Zinovjev 1993; Zinovjev and Vikberg 1998). During the summer, the larvae go through five developmental phases (instars) that together may last up to eight weeks. In autumn, after larval development has ended, the larvae emerge from the gall to hibernate on the ground among the leaf litter (Kopelke 1999; Nuorteva 1971; Zinovjev and Vikberg 1998).

Gall-inducing sawflies are also facultative parthenogenetic, i.e. they exhibit asexual reproduction, in which growth and development occur without fertilization. Unfertilized eggs produce males, whereas fertilized eggs produce females (Knerer 1993; Viitasaari 2002). This adaptation is common among many Arctic insects, an adaptation eliminating the necessity for mating during particularly severe climatic conditions and securing population survival, as females can respond to sudden climatic changes. However, there is limited information concerning which conditions parthenogenesis occurs among gall-inducing sawflies (Craig and Mopper 1993; Danks 2004; Viitasaari 2002).
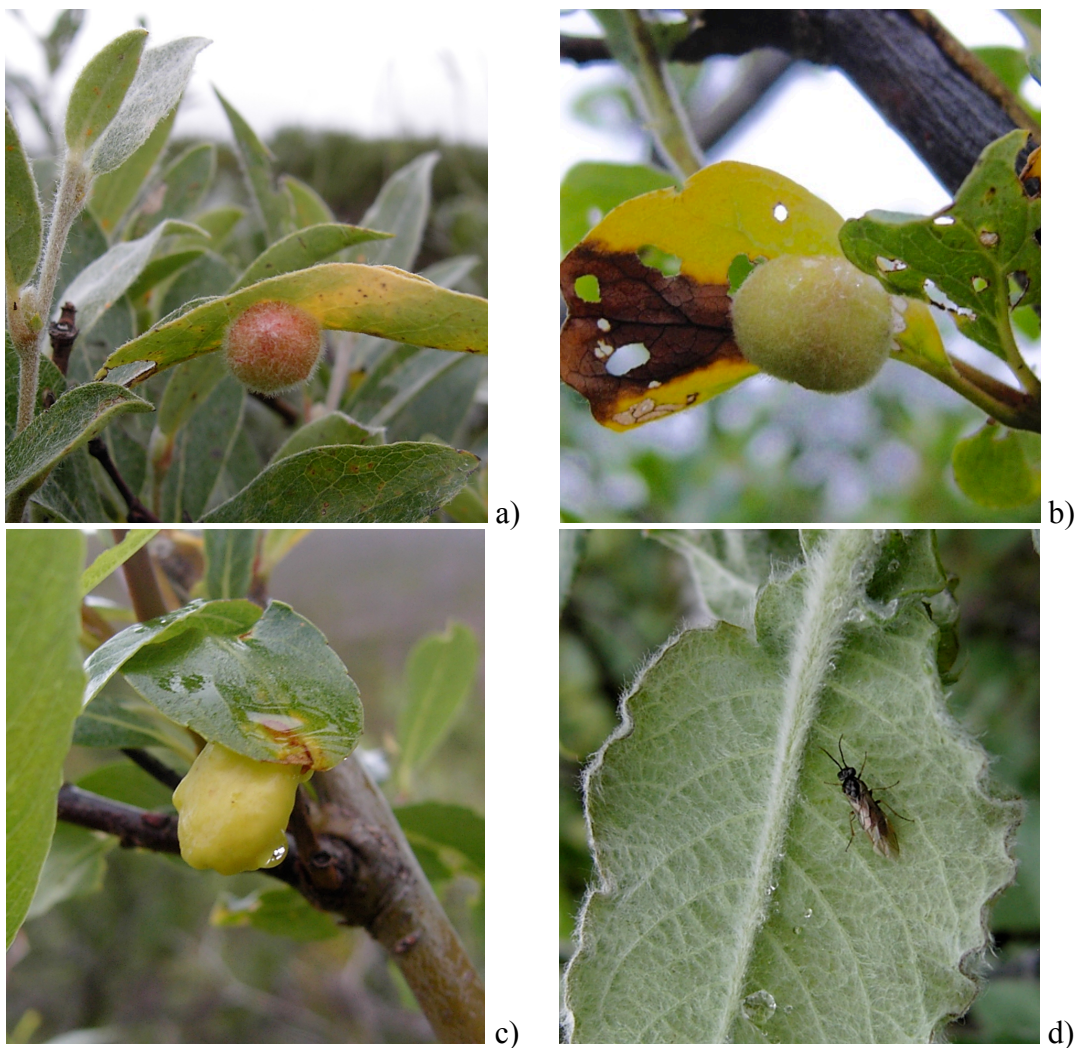
Fig. 1 Species-specific *Pontania*-viminalis galls on a) *S. glauca* b) *S. lanata* c) *S. phylicifolia* and d) unidentified gall-inducing sawfly (Photos: Trond Elling Barstad).
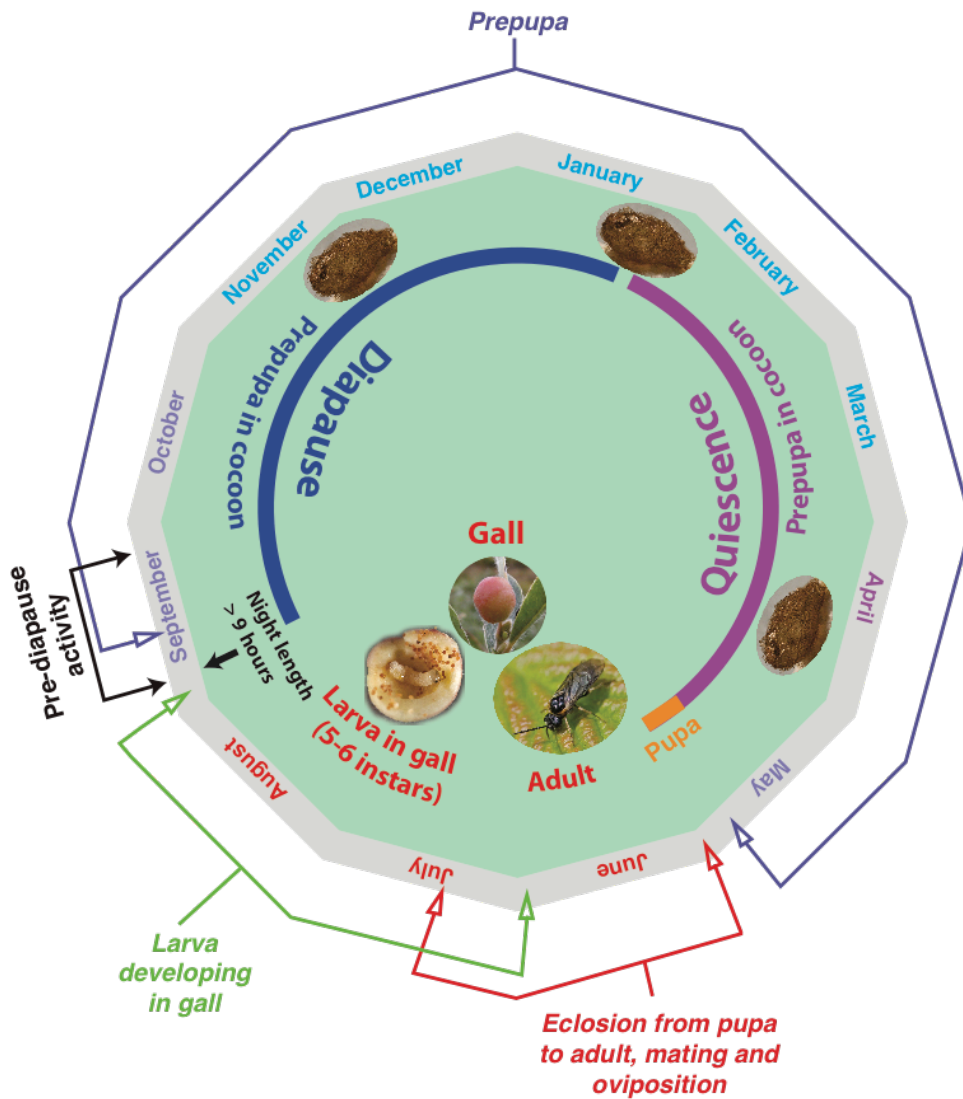
Fig. 2 The life cycle of gall-inducing sawflies (*Pontania* spp.) illustrating the different eco-physiological stages during diapause and the main annual events.

3.1.2 Willows

Willows (*Salix* spp.) comprise over 350 species in the Northern Hemisphere, are both taxonomically and ecologically very diverse and are most common in riparian habitats (Argus 1997). Willows contain a wide range of chemicals that function as feeding or oviposition deterrents, making willows a challenging food source. However, many insect herbivores, including gall-inducing sawflies have adapted to and utilize willows as food (Price et al. 1998; Roininen et al. 2005). Willows are among the earliest leaf flushing deciduous shrubs and trees (Saska and Kuzovkina 2010; Tsarouhas et al. 2003), and temperature acts as the main factor in timing of leaf flush (Hänninen 1990; Lennartson and Ögren 2004). However, there may be large inter-annual variations in the timing of leaf flush due to a strong association between winter and early spring temperatures and dormancy release (Myking and Heide 1995).

3.2 Eco-physiological phases during diapause

Dormancy is the general term that refers to a seasonally reoccurring period (*phenophase)* in which growth, development and reproduction are suppressed in the life cycle of a plant or animal. In most cases among insects, dormancy is diapause-mediated and controlled chiefly by seasonal periodicities at earth's surface or by other periodicities that give seasonal information (Danks 1987).

Dormancy enables insects to survive adverse seasonal periods and to exploit seasonally fluctuating resources (Danks 1987; Kostál 2006). The majority of cases of dormancy are diapause-mediated, which mainly occurs in response to seasonal periodicity. Specific seasonal cues, especially photoperiod and temperature acting as *token stimuli*, inform that seasonal changes are approaching, and allow insects to start specific physiological and behavioural preparations prior to the unfavourable environmental conditions (Danks 1987, 2004; Tauber et al. 1986). It is widely accepted that dormancies can be classified in two broad categories, diapause and quiescence (i.e. morphogenesis is supressed in both categories). The term dormancy should only be used when there is no clear distinction between these two categories. Insect diapause is a process that includes several dynamic phases during dormancy (Danks 1987; Hodek 1996, 2002; Kostál 2006; Tauber et al. 1986). Today this is widely accepted (Kostál 2006) and was pioneered by Andrewartha (1952), who used the term "physiogenesis" to distinguish processes during diapause from direct development that occurs after diapause has ended. The terminology of the processes and successive phases during diapause has been debated. Tauber et al. (1986) and Danks (1987) were the first to discuss and pinpoint the usage of various terms. The need for standardizing the usage of terms has, however, been discussed several times by Hodek (1996, 1999, 2002, 2003). Among insects, there is a wide diversity in diapause expression that complicates the general usage of terms (Danks 1987; Danks 1991a, b; Danks 2002; Hodek 1996, 2002). This thesis mainly follows the outline by Kostál (2006) (Fig. 3), who has simplified and generalized the terminology, which fits well with the processes that occur during dormancy with regard to gall-inducing sawflies. Ontogeny (i.e. morphogenesis) during diapause can be divided into three distinct phases; pre-diapause, diapause and post-diapause, but each phase may include different sub-phases (Danks 1987; Hodek 1996; Kostál 2006; Tauber et al. 1986). These phases are expressed by physiologically changes in relation to environmental conditions, and regulated by both endogenous and exogenous factors, hence the term *eco-physiological phases* (Kostál 2006).

This thesis (Articles I, II and IV) investigates the phenological events during the different diapause phases that occur in autumn, winter and spring in relation to *token stimuli*, photoperiod and temperature, and the following sections give detailed information about each phase and sub-phase.
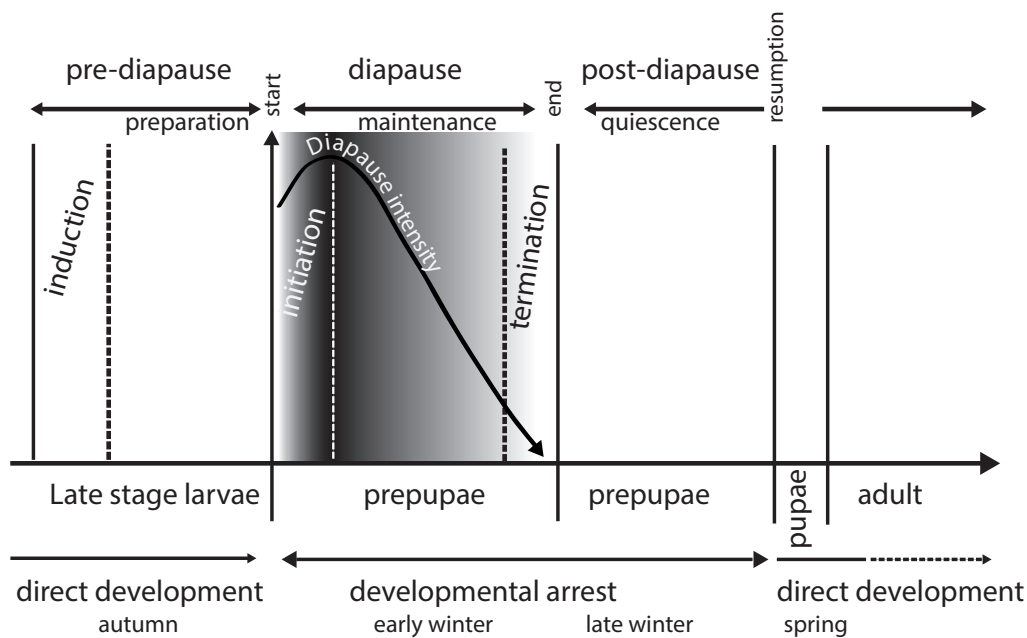
Fig. 3 Eco-physiological phases during diapause for *Pontania* spp.. Modified after Kostal (2006).

3.2.1 Pre-diapause

In advance of harsh environmental conditions in autumn, diapause preparation begins during the pre-diapause, which can be separated into an induction and a preparation phase. During the pre-diapause, direct morphogenesis continues in response to environmental signals and prepares for the diapause phase.

During the induction phase, especially photoperiod and/or in combination with change in daily temperature act as *token stimuli* for behavioural and physiological changes (Danks 1987; Tauber et al. 1986). Photoperiod and temperature may have different influential power as *token stimuli,* and one may even outweigh the other and regulate the response. However, the photoperiodic response has evolved several times during the insects' expansion into higher latitudes, and insects at higher latitudes have been shown mainly to use photoperiod as a diapause-inducing cue. Photoperiod is very reliable as diapause inducing cue, because sunrise and sunset follow the yearly changing circadian rhythmicity, whereas temperatures vary from year to year and therefore have less predictability (Saunders 2009, 2012; Tauber et al. 1986) (see also Barstad and Nilssen (2016)).

Below a critical threshold of photoperiod, possibly in combination with temperature, preparation for induction of diapause will start. In the preparation phase, certain behaviour and physiological changes occur (Kostál 2006). The individuals migrate and search for suitable micro-habitats for dormancy or moult into a morphologically distinct stage. Change in larval colour and a build-up of energy reserves, which are needed for a long period in dormancy, are also among changes seen during pre-diapause (Danks 1987; Denlinger 2002; Saunders 2002).

3.2.2 Diapause

During diapause, direct development (i.e. morphogenesis) is arrested. Other physiological processes, however, still proceed and are regulated by changing environmental conditions. The actual physiological processes that occur during diapause, have proved difficult to study in detail and are still today mostly unknown (Denlinger 2002; Saunders 2002). However, the three distinguishable sub-phases during diapause (initiation, maintenance and termination) respond differently to changes and variability in the environmental conditions (Danks 1987; Kostál 2006). This is shown by differences in diapause intensity that prevent inappropriate timing of eclosion (see section 3.2.3 below for detailed information on diapause intensity). The occurrence of diapause is detected by increasing synchronicity in development time to eclosion through a trial period during dormancy (Danks 1987; Hodek 1996; Kostál 2006; Masaki 2002). In insects that exhibit overwintering dormancy, diapause can be detected by keeping sampled insects at low temperature, either under natural conditions (as done in this thesis) or under experimental indoor conditions. Batches of the sampled insects are then incubated over a period of time at markedly higher experimental temperatures, and the response in time to eclosion and eclosion success is detected (Hodek 1996).

3.2.3 Diapause intensity

Diapause intensity is a physiological trait that can be defined by the relative duration of diapause (developmental arrest) at a given moment under given environmental conditions (Danks 1987; Hodek 1996; Kostál 2006; Masaki 2002), which are shown by duration to eclosion and success in eclosion experiments. Diapause intensity determines the duration of diapause, and high initial diapause intensity prevents eclosion during favourable (i.e. high temperatures) environmental conditions in autumn and early winter. Timing of diapause termination is primarily determined by decreasing diapause intensity and secondly regulated by environmental cues. Seasonal and geographical adaptation in diapause intensity is genetically and phenotypically determined, which is regarded as a programmed process (Masaki 2002). In general, diapause intensity increases during the diapause initiation phase and decreases during the diapause maintenance phase (Kostál 2006) (see Fig. 3).

3.2.4 Initiation phase

Diapause initiation begins when direct development (morphogenesis) ceases. This can be easily recognized as in most cases distinguishable changes happen. The most characteristic change seen is the formation of a cocoon or change in colour and shape (Danks 1987). A detectable decrease in metabolic rate is also characteristic for this phase, and developmental processes are blocked (Danks 1987; Denlinger 2002; Tauber et al. 1986). The termination of morphogenesis during the initiation phase is regulated by several factors at the cellular and molecular levels, which involves a complex "soup" of hormones (Denlinger 2002). Other physiological preparations may also occur, e.g. biochemical changes in which cold-hardiness (i.e. cryoprotection) is needed to survive cold temperatures. However, cold-hardiness in relation to diapause induction may be complex, as both are dynamic processes regulated by internal (i.e. cellular and molecular) and external (i.e. environmental) factors. In gall-inducing

sawflies (*Pontania* spp.), the larvae synthesize small amounts of sorbitol that functions as anti-freezing agent, which makes them capable of surviving extremely low temperatures (Humble 2006; Miller and Werner 1980; Ring and Tesar 1981). Chilling, a minimum duration of cold temperature, or a genetically determined refractory period (or combination of both), may also induce physiological preparation that is a prerequisite for terminating diapause in many insects (Danks 1987; Humble 2006; Ring and Tesar 1981).

Diapause intensity increases during this phase, preventing the resumption of direct development even if favourable environmental conditions (i.e. high temperatures) allow it (Danks 1987; Hodek 1996). However, the detection of the actual transition to diapause is challenging and involves detailed examination.

3.2.5 Maintenance phase

In the maintenance phase, individuals are locked in developmental arrest with low and constant metabolic rate, even if environmental conditions still favour direct development. This phase is regarded as the "true" diapause phase and is maintained for weeks, even months, before resumption of direct development (morphogenesis) can occur (Danks 1981; Kostál 2006). There is, however, still very little known about the physiological processes that retain this diapause phase. Diapause intensity gradually decreases during this phase, and/or in combination with diapause-terminating condition progresses into the termination phase (Denlinger 2002; Sawyer et al. 1993; Tauber et al. 1986)

3.2.6 Termination phase

Diapause may terminate in what appears to be a spontaneous fashion in mid-winter, still during hostile environmental condition. Little is known about the mechanisms that terminate diapause, but are influenced by both internal physiological processes and environmental cues (Danks 1987; Hodek 2002; Hodek and Hodková 1988; Kalushkov et al. 2001; Kostál 2006). During this *eco-physiological* phase, diapause intensity decreases to its minimum level, and the insects lose their ability to maintain diapause in which the potential to resume morphogenesis (i.e. post diapause development) may occur (Danks 1987; Hodek 2002; Hodek and Hodková 1988; Tauber et al. 1986).

3.2.7 Post-diapause

The transition from diapause to post-diapause occurs when *token stimuli* no longer prevent resumption of direct development, but there may be a short transitional period of metabolic change between diapause and post-diapause (Danks 1987; Tauber et al. 1986). The environmental conditions when diapause terminates can greatly differ from those favouring resumption of direct development (morphogenesis), in which a period of post-diapause quiescence supresses the ability to resume direct development (Hodek 1996; Kostál 2006; Tauber et al. 1986). This is particularly true for winter-diapausing insects in which diapause ends mid-winter and turns into post-diapause. During the period of post-diapause quiescence, the characteristics are the same as

during diapause. However, when environmental conditions (i.e. temperature) rise above a threshold, resumption of direct development immediately occurs (Tauber et al. 1986). Because of the absence of *token stimuli* during post-diapause quiescence, there is no delay in the direct development that synchronizes spring eclosion in a population (Danks 2004; Hodek 2002; Hodek and Hodková 1988; Masaki 2002). Under experimental conditions with constant high temperatures, post-diapause development (i.e. no period of post-diapause quiescence) and the following eclosion pattern show increasing eclosion success and eclosion synchronicity as time progresses towards the natural eclosion period of the species (Hodek 2002; Kostál 2006).

4 Materials and methods

4.1 Material sampling

Three of the most common gall-inducing sawfly species in northern Norway are included in this thesis, *Pontania nivalis*, *P. glabrifrons* and *P. arcticornis* that induce pea-shaped galls on underside on leaves on *Salix glauca*, *S. lanata* and *S. phylicifolia*, respectively. This thesis includes three study sites in northern Norway (see Fig. 1, article III). Two were in Finnmark, Komagdalen at Varanger Peninsula (70 °N, 30 °E), and 100 km further west, Ifjordfjellet (70 °N, 27 °E) (article I, II, III and IV). This region belongs to the low Arctic tundra inhabited by erect shrub mainly at low altitudes (Virtanen et al. 1999; Walker et al. 2005). The third study site was 420 km further southwest in Troms, Nakkedalen (69 °N, 19 °E) (article II, III and IV), where the climate is sub-Arctic and is inhabited by a mixed vegetation of willow shrubs (*Salix* spp.) and birch trees (*Betula* spp.). Fieldwork was carried out in Finnmark in 2008-2009, and in Troms in 2012-2013.

The fieldwork was conducted in autumn for all sites where shoots with leaf galls were randomly collected from different clones of the three *Salix* species. Host preference data, shoot length, node position of leaf with gall and total number of nodes (total leaves) were recorded prior to further preparation (article III). Single leaf galls were placed in plastic cups with peat moss (*Sphagnum*) and stored under natural outdoor conditions. Pre-diapause activity (larvae emergence from galls) was recorded in September (article IV) and larvae that emerged from galls (alive larvae) were included in eclosion experiments (article I and II, see below). Death caused by parasitoids was investigated at the Finnmark study sites, and survival was recorded for all sites and years (article III). In Nakkedalen, 2012 and 2013, malaise traps were used to monitor adult flight period, and the occurrence of gall formation and measurement of gall growth during larval development were also monitored (article II).

4.2 Eclosion experiments

4.2.1 Eclosion synchrony and diapause termination (article I)

To investigate diapause adaptation and progression (i.e. when diapause turn into post-diapause quiescence shown by change in eclosion synchronicity) for the three *Pontania* spp., batches of random samples of the three *Pontania* species were placed indoors (mean temperature of 23.12 °C (SD 1.27 °C and relative humidity inside plastic cups 80-90 %) in autumn after winter hibernation had occurred in 2008 and 2009. New batches were taken indoors at 14 day intervals, from October until May, and monitored for adult eclosion every second day to study development time (time to eclosion) and survival (eclosion success).

4.2.2 Development zero and hour degree requirements (article II)

Pontania spp. galls were collected in 2012 using same method as described above (4.1 Material sampling). Galls were stored outdoors through the winter and taken indoors in late March 2013. By this time, diapause should have ended for most specimens and turned into post-diapause quiescence according to results in article I. The larvae are able to monitor temperature, and post-diapause development can occur. By transferring portioned samples into chambers with four different temperatures (9, 15, 13, 28 °C), the rate of development and the following eclosion to adults occur at different times due to the different experimental temperatures. The samples were monitored every day for adult eclosion, and the sex of eclosed specimens was determined.

To calculate development zero (also called threshold temperature (abbreviated as T_0)) and hour-degree requirements, the rate of development (1/days) was linearly regressed on rearing temperatures (9, 15, 13, 28 °C) (see Baker (1980)). The regression can be expressed as $R=aT_0 + b$, where R is rate of development, T=temperature, and a and b are coefficient and constant, respectively.

4.3 Temperature recordings (articles II and IV)

Temperature loggers (inside plastic containers in shaded locations within study sites) were used in Komagdalen (2008 and 2009) and Nakkedalen (2012 and 2013) and recorded temperature every second hour throughout the summer. In article II, temperature data were used in the calculation of eclosion dates in spring and temperature correlation with meteorological weather stations (see section below). In article IV, comparison of mean daily temperature and day degree accumulation above the developmental zero (T_0) between study sites and year were calculated.

Temperatures, recorded four times a day (at 0100, 0700, 1300 and 1900) were obtained from loggers in the field or from nearby official meteorological stations when temperature loggers were not present at the field sites (article II). Following Baker (1980) recommendation (see also Worner (1992)), we selected the meteorological station that gave the best fit (i.e. highest R^2) to the field meteorological data in linear regression analyses. Studies have shown this to work well, as there is

often a strong correlation between field temperatures and nearby meteorological stations (Baker 1980; Nilssen 1997; Worner 1992). The estimated temperatures were then used to calculate eclosion date for selected years for both study areas. This was performed for Komagdalen and Nakkedalen for the years 1993-2013 (hindcasting) (article II).

5 Results and discussion

5.1 Winter: Hibernation adaptation (article I)

The results from the eclosion experiment revealed that the three *Pontania* spp. exhibited increasing survival (Fig. 4, article I) and decreasing development time as winter progressed towards spring eclosion (Fig. 3 article I), following the general pattern shown by Danks (1987, p. 147). The long development time to eclosion and high mortality during the early stage of the experiment is suspected to be due to high diapause intensity. Under natural environmental conditions, high diapause intensity prevents morphogenesis and diapause termination during warmer and unstable climatic periods in late autumn and early winter. Diapause intensity would then progressively decrease with stable sub-zero winter climatic condition, which is common among overwintering insects (Danks 1987; Hodek 2002; Kostál 2006; Masaki 2002).

There may also be a refractory period (i.e. minimum required duration of diapause), genetically programmed "timer" or biological clock that prevents diapause termination in the early phase of diapause (Danks 1987; Hodek 2002; Kalushkov et al. 2001; Kostál 2006; Nunes and Saunders 1999; Saunders 2002). The three *Pontania* spp. showed slightly different first time eclosion. However, the refractory period was quite short as some specimens still succeeded to eclose in autumn, and for most specimens, high initial diapause intensity prevented morphogenesis and diapause termination.

Another factor that prevents too early diapause termination in many insects is chilling, i.e. a period of low temperatures (Danks 1987; Hodek and Hodková 1988). Some specimens of both *P. nivalis* and *P. glabrifrons* exhibit first time eclosion prior to sub-zero temperatures (October and early November), indicating that chilling is not a prerequisite for diapause termination. As for *P. arcticornis,* that did not eclose prior to sub-zero temperatures, we cannot conclude that chilling is not important for diapause termination. However, during the progression of the eclosion experiment, we observed higher survival, earlier eclosion and increasing synchronous eclosion, which indicates that chilling may aid diapause termination (as specimens of the three *Pontania* spp. was stored under natural outdoor winter conditions prior to exposure to experimental indoor condition) (Bartelt et al. 1981; Hodek and Hodková 1988; Humble 2006; Ring and Tesar 1981).

This study is also the first to display the life history adaptation, protandry, in *Pontania* spp., in which males eclose earlier than females (Fagerström and Wiklund 1982; Wiklund and Fagerström 1977). In the eclosion experiment, males had shorter

development time and eclosed 1-2 days earlier than females, although both exhibit the same eclosion rate (Fig. 3, article I). Protandry suggests that earlier eclosion of males maximizes mating and reproduction opportunity. This may be especially important for species that have a short phenological window for reproduction and oviposition on suitable host plant resources. For *Pontania* spp. with a short adult life mostly dedicated to mating and reproduction, and with synchronized eclosion around host plant leaf flush suitable for oviposition, this adaptation may be beneficial (Viitasaari 2002). However, further studies are needed on the topics of mating and reproduction success at the adult stage.

5.2 Spring: Spring phenology (article II)

This study is, to our knowledge, the first to show a trend of earlier eclosion in insects of northern Europe as a result of climate change. Historical temperature data were used to hindcast the time of spring eclosion, in the life cycle of three *Pontania* spp.. Eclosion dates were estimated from the two climatically different sites (Arctic and sub-Arctic), in Northern Norway in the period 1993-2013. Even with great inter-annual differences, there was a significant trend towards earlier eclosion at both sites, indicative of global warming. The same trend has been shown for several insect species in Greenland (Høye et al. 2007).

In spring, there is a short phenological window of opportunity for oviposition during host plant leaf flush, outside of which leaf characteristics set constraints on oviposition success, gall formation and larval performance (Craig et al. 1989; Hunter 1993; Hunter 2000; Hunter and Elkinton 2000; Jones and Despland 2006; Martel and Kause 2002). Like many insects, *Pontania* spp. synchronize eclosion with host plant leaf flush. As shown in article I (Fig. 5), diapause for *Pontania* spp. ends mid-winter and during post-diapause quiescence, eclosion is synchronized towards spring. This is due to time-degree accumulation above a certain threshold temperature that dictates and synchronizes eclosion (Danks 1987; Hodek and Hodková 1988; Kalushkov et al. 2001; Kostál 2006), an event that is of vital importance for mating and oviposition. Plant leaf flush is also predictable due to the same mechanisms of hour-degree accumulation above a certain development threshold (Ahas et al. 2002; Heikinheimo and Lappalainen 1997; Hänninen 1990; Lappalainen et al. 2008). Time of leaf flush has, however, not been investigated for the three *Salix* species used in this study.

To estimate eclosion timing (i.e. eclosion dates) in spring for the *Pontania* spp., article II established the development threshold (also called development zero) needed to calculate hour-degree requirements for estimating eclosion dates (Baker 1980). The *Pontania* spp. were reared in late March at constant temperatures of 9, 15, 23 and 28 °C, when diapause had terminated and turned into post-diapause quiescence (article I). The development threshold was established to be 2.36 °C, which is the minimum temperature requirement for post-diapause morphogenesis, and the calculated hour-degree requirements to eclosion was calculated to be 6909, which is the temperature sum needed to finish morphogenesis (and adult eclosion) (Fig. 2 in article II that shows rate of development time during post-diapause morphogenesis). There were no significant differences in response between the three *Pontania* species or sexes, hence species and sexes were pooled (comparable results, i.e. no significant

differences between the species, were also shown during the eclosion experiment in article I).

Every year, eclosion occurred earlier at the sub-Arctic site, Nakkedalen, than at Komagdalen, the low Arctic study site that inhabiting on of the world's northernmost populations of *Pontania* spp. (Hjältén et al. 2003; Roininen et al. 2002) over the 21-year period (mean difference 16 days). The maximum difference in estimated eclosion dates between the study sites in the study period was almost 2 months (30 July in Komagdalen, 1996, and 4 June in Nakkedalen, 2013). The estimated eclosion events for two years (Nakkedalen 2012 and 2013) were confirmed by comparison with actual eclosion using malaise traps catching adults during their flight period. The peak flight period matched the estimated eclosion dates (Fig. 6 and 7, article II). It is worth remarking that the climatic conditions in spring were extremely different for these two years, thus enhancing the results (see Fig. 6, article II).

An advancement of spring over the last 20 years has been shown in several studies, but was mostly based on mean temperatures (Ahas et al. 2002; Heikinheimo and Lappalainen 1997; Høgda et al. 2013; Høye and Forchhammer 2008b; Høye et al. 2007; Karlsen et al. 2009; Lappalainen et al. 2008; Valtonen et al. 2014). Høgda et al. (2013) showed an earlier start of the growing season for the period 1982-2011, in which mean May and June temperatures increased and correlated significantly with the start of growing season, caused by decreasing snow cover and snow cover length in the period. The above estimated eclosion dates for *Pontania* spp. is the first to show this trend for insects in northern Europe. However, Høye and Forchhammer (2008b); Høye et al. (2007) showed the same trend (i.e. earlier spring phenology) among insects from the high-Arctic site Zackenberg in Greenland. They concluded that this was caused by earlier snowmelt in spring.

5.3 Summer: Oviposition preference and larval performance (article III)

One of the strongest oviposition preference-larval performance linkages for herbivorous insects has been shown among gall-inducing sawflies (Craig et al. 1989; Price 1991, 1994, 2003; Price et al. 2004). However, some leaf galling species (*Pontania*-viminalis group) do not follow this pattern (Price 2003; Price et al. 2004). This study confirms that the leaf gallers, *P. nivalis*, *P. glabrifrons* and *P. arcticornis*, have no oviposition preference that enhances larval performance (Figs. 2 and 3, article III). Hence, two new species can be added to list of non-comformist species; i.e. species that do not follow the plant vigor hypothesis (i.e. having oviposition preferences that enhance larval performance) as presented by Price et al. (2004) (*P. arcticornis* has earlier been added to the list, and functions as a reference species in this study).

Herbivorous insects living in the Arctic show a wide range of adaptations to the severity and seasonality of the climate (Danks 1981, 1986). An important phenological event is timing of spring eclosion and its synchronicity with the host plant leaf flush. Outside the window of opportunity for oviposition, host plant quality changes and sets constraints on larval performance and survival (Hanhimaki et al. 1995; Hunter 2000; Hunter and Elkinton 2000; Martel and Kause 2002; Mopper

2005). The three *Pontania* spp. in this study had similarly high survival rates (Fig. 2 and 3, see also Fig. 5a, 5b and supplementary Tab. 1, article III), and shoot lengths did not differ between the species. There were also no differences in shoot length between living and dead late-stage larvae (Supplementary Fig. 2a, b, c and Fig. 3, see also supplementary Tab. 1, article III). Hence, no shoot length preferences were found.

Furthermore, there are no oviposition cues to distinguish differences in shoot lengths, growth rates or node positions in early spring. Hence, short duration and early spring ovipostion generates into a narrow range of node preference pattern (Price 2003; Price et al. 2004), which was also shown in this study (Fig. 3, article III). In article II, we showed that the adult flight period (i.e. indicative for eclosion), in which mating and oviposition occurs, was brief with duration of the peak flight period was just a few days (max. 20 days) (Fig. 7, article II), which confirm the narrow node preference pattern shown. There was also significant correlation in shoot length and node position (i.e. longer shoots had higher node position) (Fig. 4, article III). This pattern, however, is a consequence of female to oviposition choice, constrained to oviposite on the youngest available. During the oviposition period, as shoots grow, the quality of leaves low on shoots may deteriorate and become unsuitable for oviposition. As a result, only leaves higher on the shoot (i.e. higher node position) become suitable for oviposition as growth season progresses (i.e. in correlation with adult flight period, shown in Fig. 7, article II).

In general, *Pontania* spp. emerge from galls in autumn in search for overwintering sites on the ground to pupate (Fig. 2a, article IV). However, the phenomenon in which larvae do not emerge from the gall and rather pupate inside the gall has been overlooked. This study confirms that this is quite normal for the three *Pontania* spp. (*P. nivalis* 17.8 %, *P. glabrifrons* 17.0 % and *P. arcticornis* 8.9 %), and that they also show no difference in oviposition preference (i.e. shoot length and node position) compared to pupation outside the gall (supplementary Fig. 3, article III, see also supplementary Fig. 2a,b and c, article III). There is no clear explanation for this behaviour, but it could be due to lower larval fitness caused by poor food quality or other unknown factors. This could prolong larval development, and due to the shortness of time in which to find an overwintering site to pupate, they rather pupate inside the gall. However, further studies on this subject are needed.

Mortality caused by parasitoids is common among gall-inducing sawflies. However, parasitoids species assemblages and attacks decrease into higher latitudes, in which parasitoids may have less impact on survival in the Arctic (Kopelke 1985a, 1994; Price and Pschorn-Walcher 1988; Roininen et al. 2002). Results from this study show that *Pontania* spp. survival rates was in accordance with other Palearctic studies (ranged from 7.7 % to 34.5 %, mean = 25.4 %), of which the death caused by parasitoids was low (*P. nivalis*, mean = 12.3 %, *P. glabrifrons*, mean = 10.9 % and *P. arcticornis*, mean = 4.1 %) (details in supplementary Tab. 1 and 2, article III) (Kopelke 2003; Nyman et al. 2015; Roininen et al. 2002).

5.4 Autumn: Pre-diapause (article IV)

This study is the first to show that the *token stimulus* needed for gall-inducing sawflies (*Pontania* spp.) to enter diapause in autumn, is caused by night length (i.e. photoperiod). The phenological event during pre-diapause is larval emergence from the galls in search for an overwintering site to pupate (as prepupae), which is an important event in preparation for diapause. Larval emergence from galls showed a distinct peak on 5 September for all three *Pontania* spp. for two different sites and between four years (Komagdalen 2008, 2009 and Nakkedalen 2012, 2013) (Fig. 2a, article IV). The night length on 5 September for Komagdalen was 9 h 17 min and 9 h 7 min for Nakkedalen, respectively (Fig. 2b, article IV), which indicates that the critical night length is 9-9 ½ hours for diapause preparation during pre-diapause, which is essential in establishing when natural populations starts preparing for winter diapause.

Among most sawflies (Hymenoptera: Symphyta), studies involving diapause induction have shown that larval development needs to end before they can respond to diapause-inducing cues (i.e. mainly photoperiodic response), but this has not previously been shown for *Pontania* spp. (Knerer 1983, 1993; Knerer and Marchant 1973; Philogène and Benjamin 1971). Article II (Fig. 8) showed that larval development for *Pontania* spp. is complete in late August, in which there is a short period to respond to photoperiodic cues (i.e. during pre-diapause induction phase), prior to diapause preparation that starts on 5 September (Fig. 2a, article IV) (only a few individuals started earlier).

The association between the circadian rhythmicity and photoperiodic time measurement in diapause induction among insects is strong, and the response to night length has shown to be of greater importance than day length. The manipulation of the light and dark component of the circadian cycle in experimental studies has shown that diapause induction is highest with long nights and close to natural circadian conditions. Manipulation of day length, on the other hand, resulted in lower occurrence of diapause induction (Saunders 2010, 2013, 2014).

With regard to photoperiod and temperature acting as *token stimuli*, temperature has only a minor influence on diapause induction, in which photoperiod acts as the *token stimulus* (Knerer and Marchant 1973; Nunes and Saunders 1999; Saunders 2012, 2014). The mean temperatures in August and September in this study were significantly different ($p < 0.05$) between sites and years (Fig. 3a and b, article IV). Day-degree accumulation above 0 °C between 1 August and 5 September (i.e. during pre-diapause), were very different between the two sites and years (Komagdalen 2008 and Nakkedalen 2013, 430 and 281 day degrees, respectively) (Fig. 4, article IV). In addition, the synchronous diapause preparation behaviour shown by *Pontania* spp. in September at the sites and years (Fig. 2a, article IV), supported the observation that temperature plays a minimal role in diapause induction and timing of diapause preparation.

Photoperiodic response has probably evolved several times during expansions of species into higher latitudes, but knowledge about the phenotypic modifying effect temperature sensitivity has on photoperiodic induction during diapause preparation is in general still lacking. It has been shown, however, that phenotypic plasticity in

critical photoperiodic responses along a latitudinal gradient may occur within the same species, and that temperature response may also show phenotypic modification. At higher latitudes, however, temperature dependence on photoperiodic response during diapause induction has shown to be minimal (Danks 1986; Danks 2003; Denlinger 2002; Goncalves et al. 2009; Ishihara and Shimada 1999; Masaki 1999; Saunders 2014; Tauber et al. 1986).

6 Conclusion

This thesis presents information on life history adaptations for gall-inducing sawflies that have not previously been studied, and gives a clearer picture of their life cycle at high latitudes. The three *Pontania* spp. studied are very abundant on their host plant (*Salix* spp.) in the Arctic. Increasing our knowledge about their adaptation to the strongly seasonal fluctuating environment in the high north would give important insight into the factors that regulate their abundance and population dynamics. As for ecological studies involving diapause, the aim was to analyse and predict population response to the seasonal changing environment, and to increase precision in the knowledge of responses to environmental factors change.

The diapause adaptations for the three *Pontania* spp. were very similar and followed the common outline for overwintering insects in general. However, chilling was not a prerequisite for diapause completion. In addition, protandry was documented during the eclosion experiment. Development threshold and hour-degree requirements were established in order to estimate eclosion timing, and these estimates were supported by adult presence as documented by trapping in field. Hindcasting climatic conditions in spring for the last 21 years, revealed a highly significant advance in eclosion dates, a consequence of global warming. However, there were great inter-annual differences in eclosion timing. Hence, *Pontania* spp. must be adapted to variation in spring climatic conditions and exhibit large phenological plasticity in timing of eclosion. The three *Pontania* spp. showed a remarkable phenological synchronous behaviour (i.e. preparation for diapause) during pre-diapause with a distinct start on 5 September. The establishment of the critical photoperiod for diapause preparation revealed that night length (i.e. photoperiod) acts as a *token stimulus* during pre-diapause, while autumn temperature played an insignificant role in this respect.

This thesis also give new insights into the behavioural and ecological relationships with adjacent trophic levels for *Pontania* spp.. The three *Pontania* spp. did not show any oviposition preference with regard to larval performance, which is probably caused by a brief and early spring eclosion when host plant cues are lacking for any discriminatory behaviour. *Pontania* spp. also showed a relatively high larval survival, in which mortality caused by parasitoids was low.

In light of the current attention on climate change, there is need for more information on the consequences this has on life history and population dynamics in Arctic insects. Temperature plays an essential factor in several life stages and may cause a strong response and have great effect on phenology and development. Among trophically interacting species that have evolved phenological synchronicity, temporal disassociation at a certain life stage may have a detrimental effect on survival due to

different responses to changing climate (Høye et al. 2013). However, for *Pontania* spp., with their close evolutionary adaptation to its host plants, temporal disassociation in spring phenology is likely to be small. As for temporal disassociation with parasitoids, it may either be beneficial or detrimental for survival. However, due to the relative low impact parasitoids have further south on *Pontania* spp. (Kopelke 1985b, 1999), climate change will probably not have a large effect on mortality caused by changes in parasitoid attack. Furthermore, Arctic willows are likely to expand their distribution with a warmer climate, and there is no indication that gall-inducing sawflies, that are highly adapted to the Arctic, will not follow.

Even though this thesis presents valuable information on life history adaptation for the three *Pontania* species, there are especially two subjects that should be addressed. First, how does the rigidly synchronous behaviour during pre-diapause affect survival during the early stage of diapause, especially in relation to longer and warmer autumns due to climate change. And second, even though article II showed a trend for earlier eclosion due to climate change, there is need for more long-term studies on spring phenology, and especially in relation to *Pontania* spp. host plants.

7 References

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