# Phenology during pre-diapause in gall-inducing sawflies and the influence of night length

Trond Elling Barstad and Arne C. Nilssen

Department of Natural Sciences, Tromsø University Museum, NO-9037 Tromsø, Norway

Corresponding author:

Trond Elling Barstad

Department of Natural Sciences, Tromsø University Museum, NO-9037 Tromsø, Norway

E-mail: trond.elling.barstad@uit.no

Telephone: +47 776 45 027

**Abstract**

At higher latitudes, photoperiod and temperature are the major environmental cues that act as *token stimuli* regulating diapause onset in gall-inducing sawflies. There is a sensitive period (the induction phase) during pre-diapause in which *token stimuli* signal the seasonal environmental change. Above a critical threshold, behavioural and physiological preparation (the preparation phase) occurs in advance of diapause onset. This study investigates the phenological event during pre-diapause, and the effect *token stimuli*, photoperiod and temperature have on diapause induction for three gall-inducing sawfly species (Tenthredinidae: Nematinae: *Pontania nivalis*, *P. glabrifrons* and *P. arcticornis*) from two regions and over four years. The phenological event during pre-diapause comprising synchronous larval emergence from galls, occurred between 5 and 17 September with a distinct peak on 5 September in all years and for the three species. The photoperiod (night length) at 5 September was 9 hours 7 min for the site at Komagdalen (70 °N) and 9 hours 17 min for the site at Nakkedalen (69 °N). Thus, the night length had to exceed 9 hours to reach the critical photoperiod and the establishment of calendar dates for diapause preparation. The samplings were done in Komagdalen and Ifjordfjellet (2008 and 2009) and Nakkedalen (2012 and 2013), with considerable differences in summer and autumn temperatures between sites and years. Analyses showed that the temperature seemed to have no effect on start of larval emergence from galls. To conclude, gall-inducing sawflies show phenological synchronicity during pre-diapause, in which photoperiod acts as *token stimulus* on diapause induction.

**Keywords:** *Pontania*, phenology, pre-diapause, photoperiod, night length, diapause induction, temperature, Arctic region.

**Introduction**

Seasonal environmental changes are particularly strong at high latitudes, i.e. in the northern hemisphere. Many insect species have successfully adapted to the harsh environmental conditions in the Arctic region (Danks 1981; Downes 1965; Ring and Tesar 1981). The evolutionary adaptation to the rigid cyclic seasonal changes is shown in species-specific phenology, and the species have developed the ability to respond to environmental cues that lead to appropriate timing of seasonal biological events, such as post-diapause development, mating, larval development and diapause-induced dormancy (Danks 1987; Tauber et al. 1986).

Insect survival at high latitudes is mainly ensured through various dormancy mechanisms, including diapause, that are fine tuned to the local environment (Barstad and Nilssen 2012; Danks 1987). Induced environmental cues signal the coming environmental adversity in autumn, often long in advance of the diapause phase itself (Kostál 2006; Saunders 2012; Tauber et al. 1986). The seasonal changes in both photoperiod and temperature are the key environmental cues that act as *token stimuli* to regulate onset of diapause. This occurs during pre-diapause, ensuring that diapause induction happens at the correct time (Danks 1987; Kostál 2006; Saunders 2002, 2011; Tauber et al. 1986). There has, however, been a continuous evolutionary adaptation in photoperiodic response as insects have extended their distribution into higher latitudes with longer winters (Saunders 2012). Under natural environmental conditions, there is a daily cycle of light (i.e. photoperiod) where insects respond to the absolute length of the photoperiod. It is suggested that photoperiod predominantly controls diapause induction in winter-diapausing insects, which occurs when the night length in most cases exceeds a critical threshold. Studies has shown that manipulation of the dark component (i.e. night length) of the circadian cycle has greatest effect on diapause induction (reviewed in Saunders (2013)) (Danks 1987; Goto 2013; Kostál 2006; Saunders 2002, 2010; Tauber et al. 1986).

The critical photoperiod changes regularly with temperature in a way similar to its change with latitude, i.e. critical photoperiod becomes longer as either temperature decreases or latitude increases (Masaki 1999). The light intensity during the photoperiod is of lesser importance, as the threshold value is most commonly very

low (about 17 lux or less). Hence, daily fluctuations in light intensity due to clouds or shades have no effect on photoperiod (Chapman 2013).

Diapause is a common way of surviving long winters in a dormant state at high latitudes (Danks 1987; Downes 1965; Tauber et al. 1986). During the pre-diapause phase, there is a genetically determined sensitive period, known as the *induction* phase. When *token stimuli* reach a certain critical level, the insect starts the preparation phase in which behavioural and physiological preparation for diapause is initiated (Kostál 2006).

In the northern hemisphere, gall-inducing sawflies (Tenthredinidae: Nematinae) are well adapted to the adverse climatic conditions at higher latitudes (Barstad and Nilssen 2012, 2015; Knerer 1993; Kouki et al. 1994). Among those commonly found in northern Norway are the monophagous gall-inducing *Pontania* species within the viminalis-group on their host plants (*Salix* spp.) (Hjältén et al. 2003; Roininen et al. 2002; Zinovjev 1998). These species have an univoltine lifecycle, characterized by synchronous eclosion in spring, often with immediate mating, followed by female ovipositing into young growing leaves on their hosts, turning into pea-shaped galls in which larval development occurs during the summer. Prior to adverse climatic conditions in autumn, the larvae emerge from the galls and pupate in a prepupal state on the ground (Knerer 1993; Kopelke 1999; Price 2003; Viitasaari 2002; Wagner 1993; Zinovjev 1998).

As suggested, photoperiod predominantly controls diapause induction (Danks 1987; Goto 2013; Knerer 1983; Saunders 2002, 2010). It has also been suggested that only mature larvae in sawflies are sensitive to diapause-inducing cues, although so far not shown for gall-inducing sawflies (Knerer 1983, 1993).

In a previous study, we found that the prepupal phase of the three gall-inducing sawfly species (*Pontania* spp.) enter diapause in October (Barstad and Nilssen 2012). The diapause intensity gradually decreased throughout the winter, and terminated mid-winter, and turned into post-diapause quiescence until favourable environmental conditions in spring allow eclosion, mating and oviposition. However, there were some differences between the three species, as a small proportion of *P. nivalis* and *P.*

*glabrifrons* eclosed under laboratory conditions (23 °C) and could eclose as early as October, whereas *P. arcticornis* would not eclose until December (Barstad and Nilssen 2012).

The present study examined the phenological event during pre-diapause (i.e. the diapause preparation phase) for three gall-inducing sawfly species (*Pontania* spp.) in relation to *token stimuli*, photoperiod and temperature. Two geographical regions in northern Norway (approx. 400 km apart) and 4 years are included. Prior to adverse environmental conditions in autumn, during the pre-diapause phase, responses to environmental signals start preparation for the diapause phase in which direct development is arrested (Kostál 2006). In preparation for winter hibernation (i.e. diapause) during pre-diapause, *Pontania* spp. larvae leave their galls in search for suitable overwintering sites on the ground and transform to prepupae. We established the pre-diapause period in which diapause preparation occurs, and we tested the hypothesis that photoperiod is the main *token stimulus* that acts as the diapause-inducing cue in *Pontania* spp..

**Material and methods**

*The focal species and field sites*

Gall-inducing sawflies within the *Pontania*-viminalis group are mostly monophagous. The three species included in this study, *Pontania nivalis* (Vikberg 1970), *P. glabrifrons* (Benson 1960) and *P. arcticornis* (Konow 1904) are strictly monophagous and are commonly found in northern Norway on their willow hosts, *Salix glauca*, *S. lanata* and *S. phylicifolia*, respectively (Kopelke 1999; Zinovjev 1993; Zinovjev 1998). They are obligate univoltines and overwinter as prepupae, having a short pupal phase during early spring with a synchronized eclosion dictated by host phenology, as females prefer to oviposit on young growing leaves during leaf flush. Adults are mostly dedicated to mating, host seeking and reproduction (Viitasaari 2002). Larval development occurs inside galls on the underside of leaves during summer, and the larva exits the galls to spin a cocoon on the ground in autumn prior to adverse climatic conditions and overwinter in a prepupal state (Kopelke 1999; Viitasaari 2002; Zinovjev 1993).

The present study includes three study sites (see Fig. 1), two at 70 °N, in Finnmark, Komagdalen (70 °N, 30 °E) at Varanger Peninsula and 100 km to the west, Ifjordfjellet (70 °N, 27 °E). Komagdalen exhibits arctic climatic conditions (<10 °C arctic isotherm in July), and Ifjordfjellet has similar climatic conditions, although at a higher latitude. These two study sites are classified as erected shrub tundra (Henden et al. 2011; Virtanen et al. 1999; Walker et al. 2005). The third study site was situated approx. 420 km to the south, Nakkedalen (69 °N, 19 °E), in Troms, with a subarctic climate and inhabited by a mixed vegetation of willow shrubs (*Salix* spp.) and birch (*Betula* spp.).

**Data acquisition and analyses**

*Pre-diapause activity*

To determine the degree of synchronous pre-diapause activity among sawfly larvae, shoots with late stage *Pontania*-viminalis galls from *S. glauca*, *S. lanata* and *S. phylicifolia* were randomly collected from different clones in late August (within a few day prior to larval emergence from the gall), from Komagdalen and Ifjordfjellet in 2008, 2009 and Nakkedalen in 2012, 2013 [(Total *n* = 2343, *S. glauca* (n = 574), *S. lanata* (n = 936) and *S. phylicifolia* (n = 833)] (see Online Recource 1 for details). Leaves with one gall were placed in single plastic cups (155 ml, with ventilation holes, stored under natural outdoor conditions) with peat moss (*Sphagnum* spp.). In September, larval pre-diapause activity was monitored once a day until end of September. Pre-diapause activity was classified as present when an emergence hole was found in a gall, accurately reflecting the larval emergence from the gall (Craig et al. 1989).

*Photoperiod and temperature: mean daily temperature and day degree accumulation*

Night length (or day length) and temperature are known to be *the token stimuli* triggering pre-diapause activity and larval emergence from galls in autumn. To assess the amplitude of these factors, night length in September was calculated using the online resource ([http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html](http://www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html)) for the

latitudes of Komagdalen and Nakkedalen (70 °N and 69 °N, respectively). Night length is defined as time (in hours) between sunset and sunrise.

Temperature measurements in Komagdalen (2008 and 2009) and Nakkedalen (2012 and 2013) were registered every second hour using temperature loggers. The loggers were placed in shaded locations at the study sites. Mean daily temperature and day degree accumulation above 0 °C in August and September were calculated using the temperature logger data.

**Results**

*Pre-diapause activity*

The three *Pontania*-species had similar pre-diapause activities (date of larval emergence from the galls) between sites and years. Pre-diapause activity was detected between 1 September and 20 September (total $n = 1684$), although the peak of pre-diapause activity was at 5 September, thereafter rapidly declining and ending 17 September. Combining all species, sites and years, revealed a distinct peak in which 26 % ($n = 438$) emerged from galls on 5 September. The following 12 days (6 – 17 September), 68.1 % ($n = 1147$, max. 230 and min. 10 larvae emerged per day) emerged from galls (Fig. 2a) (see Online Resource 3 and 4 for details).

*Photoperiod and temperature: mean daily temperature and day degree accumulation*

The development of night length in September for Nakkedalen and Komagdalen is graphed in Fig. 2b. The peak of pre-diapause activity on 5 September has night length of 9 hours 7 min. (day length 14 hours 53 min.) for Komagdalen, and night length of 9 hours 17 min (day length of 14 hours 43 min) for Nakkedalen. Larval pre-diapause activity (number of galls with exit holes) found is shown with the same x-axis (date in September) (Fig. 2a) (Online Resource 1 shows details for the *Pontania* species, years and sites, and Online Resource 3 and 4 shows graphically the emergence patterns of the three species). Emergence patterns as a function of date were visually very similar, and the correlations were formally tested with Paired samples t-tests (Online Resource 2) (SPSS version 21). In Online Resource 3 (tests between the species), the

correlations of all three pairwise comparisons were highly significant, whereas in Online Resource 4 (tests between sites and years), seven of the fifteen correlations were significant.

Mean daily temperatures in August and September for sites and years were significantly different ($p < 0.05$) between the sites and years (Fig. 3a and b) (GENLIN in SPSS version 21).

The day degrees accumulation above 0 °C, between 1 August and 5 September, for the years and sites (Komagdalen (2008, 2009) and Nakkedalen (2012, 2013)) was very similar between Komagdalen 2009 and Nakkedalen 2012 (377 and 358 day degrees, respectively), whereas there was a large difference between Komagdalen 2008 and Nakkedalen 2013 (430 and 281 day degrees, respectively) (G-tests in Fig. 4).

**Discussion**

The present study is to our knowledge, the first to show that the date of gall-inducing sawflies entering diapause is caused by night length and has not earlier been studied in regard to diapause-inducing cues, photoperiod and temperature. The distinct peak at the beginning of diapause preparation on 5 September (Fig. 2a) for all the three *Pontania* spp. from the two sites and between the four years was a surprising discovery. Night length on 5 September for Komagdalen was 9 h 17 min. and 9 h 7 min. hours and Nakkedalen (Fig. 2b), respectively. This indicates that 9-9 ½ hours is the critical night length for diapause preparation during pre-diapause. The determination of the critical photoperiod (i.e. night length) is essential in establishing calendar dates at which natural populations start preparing for winter diapause.

Photoperiodism in insects is most commonly found in the diapause induction and maintenance of winter diapause by short days or long nights in autumn. This kind of diapause ensures that insects survive the adverse winter season and that their development is synchronized with seasonally changing environment (Danks 1987; Kostál 2006; Saunders 2002; Shintani et al. 2015; Tauber et al. 1986). Photoperiodic time measurements are strongly connected with the circadian rhythmicity, and among

8

most published insect studies, the response to change in night length, rather than day length, has shown to be essential. In experimentally manipulation of the light and dark component of the circadian cycle, the occurrence of diapause induction is greatest with long nights and close to natural circadian conditions, as manipulation of day length show lower occurrence of diapause induction (Danks 2003; Saunders 2010, 2013, 2014).

At higher latitudes, as in this study, seasonal changes in photoperiod and temperature, or interaction between them, are the major environmental signals regulating diapause onset in insects. Thus, lengthening nights or shorter days, together with decreasing autumnal temperatures, act as *token stimuli* on diapause induction, leading to diapause and winter dormancy (Saunders 2014). Studies show that there are probably a number of different photoreceptors at both the organ and molecular levels in insects, which specifically respond to *token stimulus* such as light (reviewed in Saunders (2012, 2013)

For insects that use photoperiod to programme their diapause, there is a window of sensitivity in advance of the actual diapause stage, the *induction* phase (Kostál 2006) (the photosensitive phase (Saunders 2002)), where photoreceptors in larvae receive photoperiodic signals which act as *token stimulus* distinguishing change in photoperiod. This photoperiodic time measurement is a function of the circadian rhythms (Saunders 2002, 2010), and insects in most cases measure duration or accumulation of absence of light during the dark period of the daily cycle (reviewed in Saunders (2013)), which is determining transition from *induction* phase to *preparation* phase during pre-diapause (Danks 1987; Danks 2003; Saunders 2012, 2013).

In most sawfly studies, photoperiodic response to seasonality has shown to play a widespread role, but only mature larvae have been shown to respond to diapause-inducing cues (Knerer 1983, 1993; Knerer and Marchant 1973; Philogène and Benjamin 1971). For the gall-inducing sawflies in this study, *P. nivalis, P. glabrifrons* and *P. arcticornis*, larval development ceases in late August (Barstad and Nilssen 2015), which implies that there is only a short period (approx. 7-14 days, the diapause induction phase) in which to respond to photoperiodic cues, prior to the diapause

preparation phase which starts on 5 September (only a few individuals started earlier, see Online Resource 3 for details). In the European pine sawfly, *Neodiprion sertifer* there is a shorter critical photoperiodic response along a latitudinal gradient towards south, 17 hours day length in Finland compared to 15 hours in central Europe (Pschorn-Walcher 1970).

With regards to temperature as a *token stimulus*, it has been shown to only have a minor modifying influence on diapause induction in sawflies (Knerer and Marchant 1973). The mean daily temperature (between 1 August and 30 September) (Fig. 3a and b) was significantly different between sites and years. Day-degree accumulation above 0 °C, (between 1 August and 5 September), was significantly different between two of the years and sites. Particularly, the day-degree accumulations difference between Komagdalen (2008) and Nakkedalen (2013) was large (281 and 430 at 5 September, $p < 0.00001$, G-test) (Fig. 4), which implies that the role of temperature is minimal in diapause induction and timing of the following synchronizing activity during diapause preparation.

Phenotypic plasticity in critical photoperiod may vary with latitude within the same species, but response to environmental cues such as temperature may also be subject to phenotypic modification, although the knowledge of inheritance of temperature sensitivity of photoperiodic induction of diapause is in general lacking (Danks 1987; Denlinger 2002; Goncalves et al. 2009; Masaki 1999; Tauber et al. 1986). In their natural environment, insects experience concurrent daily cycles of light (photoperiod) and temperature (thermoperiod) with colder nights than days. The temperature of the cool dark period is shown to be more important for diapause regulation than the warm, light period (Saunders 2014). However, temperature dependence has been shown to only have minimal effect on photoperiodic response during diapause induction among insects at higher latitudes. Among species that exhibit bi- or trivoltinism on a latitudinal gradient, temperature dependence has a more pronounced influence on diapause induction (Danks 2003; Ishihara and Shimada 1999; Saunders 2014; Tauber et al. 1986). Takeda (1996) reported that high temperatures had greater influence in photoperiodic control in southern trivoltines than in northern bivoltine populations of the rice stem maggot, *Chlorops oryzae*. The same result was also shown in the fall webworm, *Hyphantria cunea*, in which southern trivoltines had

greater temperature dependence than northern bivoltine populations on photoperiodic diapause induction (Gomi 1997; Masaki 1968). Among three sympatric sawfly species, however, the bivoltine *Athalia japonica* and multivoltine *A. rosae* and *A. infumata* (repeating 5-6 generations), diapause induction occurs independently of temperature, and summer diapause that only occurs in *A. japonica*, was induced by photoperiod only (i.e. longer day length in spring) (Nagasaka 1992; Nagasaka and Ohsaki 2002; Uchidoi et al. 1993). However, the complexity in life cycle adaptation in bi- and multivoltine insects may not be comparable with the subarctic univoltine species studied in this paper.

The common overwintering stage in sawflies is as a prepupa in a cocoon. The real pupal stage occurs during a short period in spring (Viitasaari 2002). Several studies show that photoperiodism has an extensive role in sawfly larval diapause induction, see Knerer (1993). However, the vast amount of studies of the photoperiodic response in insects, including sawflies, is mostly experimental (Knerer 1983, 1993; Philogène and Benjamin 1971; Pschorn-Walcher 1970; Saunders 2002, 2011; Uchidoi and Takeda 1997; Wallace and Sullivan 1974; Wingfield and Warren 1972). The present observational study has revealed an intriguing synchronous behaviour during pre-diapause in gall-inducing sawflies. Working with gall-inducing sawflies, however, and especially because larval development occurs inside galls may present difficulties in doing a controlled study to test the observational results in this study. In order to assess if temperature acts as a *token stimulus* in diapause induction, experimental studies are needed, and the first step could be to test their ability to induce diapause in the absence of light and using different temperature regimes (Chippendale et al. 1976; Saunders 2014).

The present study, together with our two published studies (Barstad and Nilssen 2012, 2015), gives a clear picture of the life cycle of these three univoltine gall-inducing sawfly species (*Pontania* spp.) (see Fig. 5 for details). There are some differences between the three species, but also striking phenological similarities. All show synchronous pre-diapause activity and enter diapause in early autumn. Their diapause intensity diminishes gradually through the winter, and they enter quiescence in mid-winter and eclose synchronously in early spring. All three species have a quite similar larval development during summer.

These three *Pontania* species, often characterized as subarctic species, are "robust" to cope with global warming (Barstad and Nilssen 2015). If their willow host (*Salix* spp.) can adapt to climate change, their gall-inducing sawfly species will have no problems in surviving. However, change in climatic conditions in autumn may disrupt the time available for diapause preparation and entering the following diapause, which may increase mortality and disrupt population dynamics.

In conclusion, we hypothesize that the phenological synchronicity shown during pre-diapause in this study, is caused by photoperiod time measurement in response to the circadian rhythmicity acting as a *token stimulus*, which is being increasingly supported (Goto 2013; Saunders 2002, 2010).

**References**

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

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

Chapman RF (2013) The insects structure and function. 5th edn. Cambridge University Press, New York

Chippendale GM, Reddy AS, Catt CL (1976) Photoperiodic and thermoperiodic interactions in the regulation of the larval diapause of *Diatraea grandiosella*. J Insect Physiol:823-828

Craig TP, Itami JK, Price PW (1989) A strong relationship between oviposition preference and larval performance in a shoot-galling sawfly. Ecology 70:1691-1699

Danks HV (1981) Arctic arthropods: a review of systematics and ecology with particularly reference to the North American fauna. Entomol Soc Can, Ottawa

Danks HV (1987) Insect dormancy: an ecological perspective. Biol Survey Canada, Natl Mus Nat Sci, Ottawa

Danks HV (2003) Studying insect photoperiodism and rhythmicity: Components, approaches and lessons. Eur J Entomol 100:209-221

Denlinger DL (2002) Regulation of diapause. Annu Rev Entomol 47:93-122

Downes JA (1965) Adaptation of insects in the Arctic. Annu Rev Entomol 10:257-274

Gomi T (1997) Geographic variation in critical photoperiod for diapause induction and its temperature dependence in *Hyphantria cunae* Drury (Lepidoptera: Arctiidae). Oecologia 111:160-165

Goncalves SJMR, Moreira GRP, Isaias RMS (2009) A unique seasonal cycle in a leaf gall-inducing insect: the formation of stem galls for dormancy. J Nat Hist 43:843-854

Goto SG (2013) Roles of circadian clock genes in insect photoperiodism. Entomol Sci 16:1-16

Henden JA, Ims RA, Yoccoz NG, Sorensen R, Killengreen ST (2011) Population dynamics of tundra voles in relation to configuration of willow thickets in southern Arctic tundra. Polar Biol 34:533-540

Hjältén J, Roininen H, Danell K, Price PW (2003) Distribution and oviposition preference of galling sawflies in Arctic Canada. Polar Biol 26:768-773

Ishihara M, Shimada M (1999) Geographical variation in photoperiodic response for diapause induction between univoltine and multivoltine populations of *Kytorhinus sharpianus* (Coleoptera: Bruchidae). Popul Ecol 28:195-200

Knerer G (1983) Diapause strategies in diprionid sawflies. Naturwissenschaften 70:203-205

Knerer G (1993) Life history diversity in sawflies. In: Wagner MR, Raffa KF (eds) Sawfly life history adaptations to woody plants. Academic Press Inc., San Diego, pp 33-59

Knerer G, Marchant R (1973) Diapause induction in the sawfly *Neodiprion rugifrons* Middelton (Hymenoptera: Diprionidae). Can J Zool 51:105-108

Kopelke J-P (1999) Gallenerzeugende Blattwespen Europas − Taxonomische Grundlagen, Biologie und Ökologie (Tenthredinidae: Nematinae: *Euura*, *Phyllocolpa*, *Pontania*). Cour Forsch Senck 212:1-183

Kostál V (2006) Eco-physiological phases of insect diapause. J Insect Physiol 52:113-127

Kouki J, Niemelä P, Viitasaari M (1994) Reversed latitudinal gradient in species richness of sawflies (Hymenoptera: Symphyta). Ann Zool Fenn 31:83-88

Masaki S (1968) Biology of *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) in Japan. Appl Entomol Zool 3:55-66

Masaki S (1999) Seasonal adaptations of insects as revealed by latitudinal diapause clines. Entomol Sci 2:539-549

Nagasaka K (1992) Why does only *Athalia japonica* enter summer diapause among three sympatric *Athalia* sawflies feeding on crucifers? Res Popul Ecol 34:383-395

Nagasaka K, Ohsaki N (2002) Differences in host plant selection among three *Athalia* sawflies feeding on crucifers in Japan. Ecol Entomol 27:326-336

Philogène BJR, Benjamin DM (1971) Diapause in the Swaine jack-pine sawfly, *Neodiprion swainei* as influenced by temperature and photoperiod. J Insect Physiol 17:1711-1716

Price PW (2003) Macroevolutionary theory on macroecological patterns. Cambridge University Press, Cambridge

Pschorn-Walcher H (1970) Studies on the biology and ecology of the alpine form of *Neodiprion sertifer* (Geoff.) (Hym.: Diprionidae) in the Swiss alps. Z Angew Entomol 66:64-83

Ring RA, Tesar D (1981) Adaptation to cold in Canadian Arctic insects. Cryobiology 18:199-211

Roininen H, Danell K, Zinovjev A, Vikberg V, Virtanen R (2002) Community structure, survival and mortality factors in Arctic populations of *Eupontania* leaf gallers. Polar Biol 25:605-611

Saunders DS (2002) Insect clocks. 3rd edn. Elsevier, Amsterdam

Saunders DS (2010) Controversial aspects of photoperiodism in insects and mites. J Insect Physiol:1491-1502

Saunders DS (2011) Unity and diversity in the insect photoperiodic mechanism. Entomol Sci 14:235-244

Saunders DS (2012) Insect photoperiodism: seeing the light. Physiol Entomol 37:207-218

Saunders DS (2013) Insect photoperiodism: measuring the night. J Insect Physiol 59:1-10

Saunders DS (2014) Insect photoperiodism: effect of temperature on the induction of insect diapause and diverse role for the circadian system in the photoperiodic response. Entomol Sci 17:25-40

Shintani Y, Kawazu K, Hirose Y (2015) Photoperiodic induction of prepupal diapause with host phenology in the hibiscus caterpillar, *Xanthodes transversa*. Entomol Sci 18:360-367

Takeda M (1996) Photoperiodic induction, maintenance and termination of winter diapause in two geographic ecotypes of the rice stem maggot, *Chlorops oryzae* Matsumura (Diptera: Chloropidae). Appl Entomol Zool 31:379-388

Tauber MJ, Tauber CA, Masaki S (1986) Seasonal adaptations of insects. Oxford University Press, New York

Uchidoi T, Naito T, Takeda M (1993) Life-Cycle Characteristics of Sympatric *Athalia* Sawflies (Hymenoptera, Tenthredinidae) Sharing Food Resources. Appl Entomol Zool 28:407-415

Uchidoi T, Takeda M (1997) A unidirectional change in daylength: A token for photoperiodic regulation of prepupal diapause in *Athalia japonica* (Tenthredinidae). Appl Entomol Zool 32:471-476

Viitasaari M (2002) Sawflies (Hymenoptera, Symphyta) Vol I: a review of the suborder, the Western Palearctic taxa of Xyeloidae and Pamphiliodae. Tremex Press Ltd, Helsinki

Virtanen R, Oksanen L, Razzhivin V (1999) Topographical and regional patterns of tundra heath vegetation from northern Fennoscandia to the Taimyr Peninsula. Acta Bot Fenn 167:29-83

Wagner MR, Raffa, Kenneth F. (1993) Sawfly lifehistory adaptations to woody plants. Academic Press Inc, San Diego

Walker DA, Raynolds MK, Daniels FJA, Einarsson E, Elvebakk A, Gould WA, Katenin AE, Kholod SS, Markson CJ, Melnikov ES, Moskalenko NG, Talbot SS, Yurtsev BA (2005) The circumpolar Arctic vegetation map. J Veg Sci 16:267-282

Wallace DR, Sullivan CR (1974) Photoperiodism in the early balsam strain of the *Neodiprion abietis* complex (Hymenoptera: Diprionidae). Can J Zool 52:507-513

Wingfield M, Warren LO (1972) The effect of photoperiodism on the development of the sawfly *Neodprion taeda linearis*. J Kansas Entomol Soc 45:1-6

Zinovjev AG (1993) Subgenera and Palaearctic species groups of the genus *Pontania*, with notes on the taxonomy of some European species of the *viminalis*-group (Hymenoptera: Tenthredinidae). Zoosyst Ross 2:145-154

Zinovjev AG (1998) Palearctic sawflies of the genus *Pontania* Costa (Hymenoptera: Tenthredinidae) and their host-plant specificity. In: Csóka G, Matteson WJ, Stone GN, Price PW (eds) Biology of gall-inducing arthropods. USDA For Serv N Central For Res Stat Gen Tech Rep NC 199, pp 204-225

**Figures**
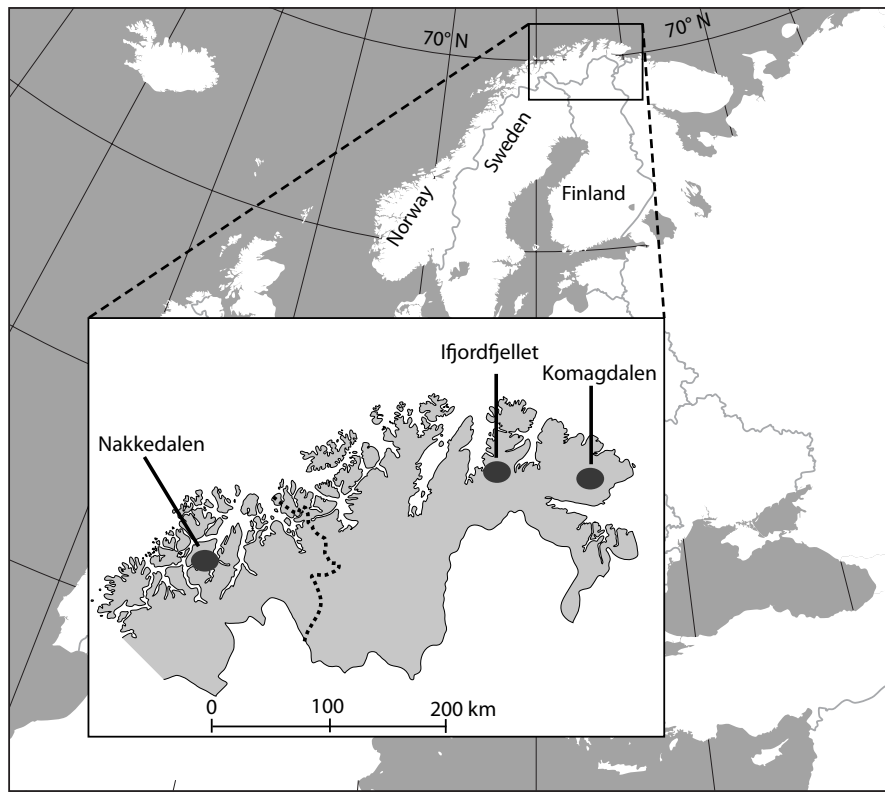


**Fig. 1** Northern Norway showing the study sites Nakkedalen (69 °N) and Ifjordfjellet and Komagdalen (70 °N) in northern Norway.
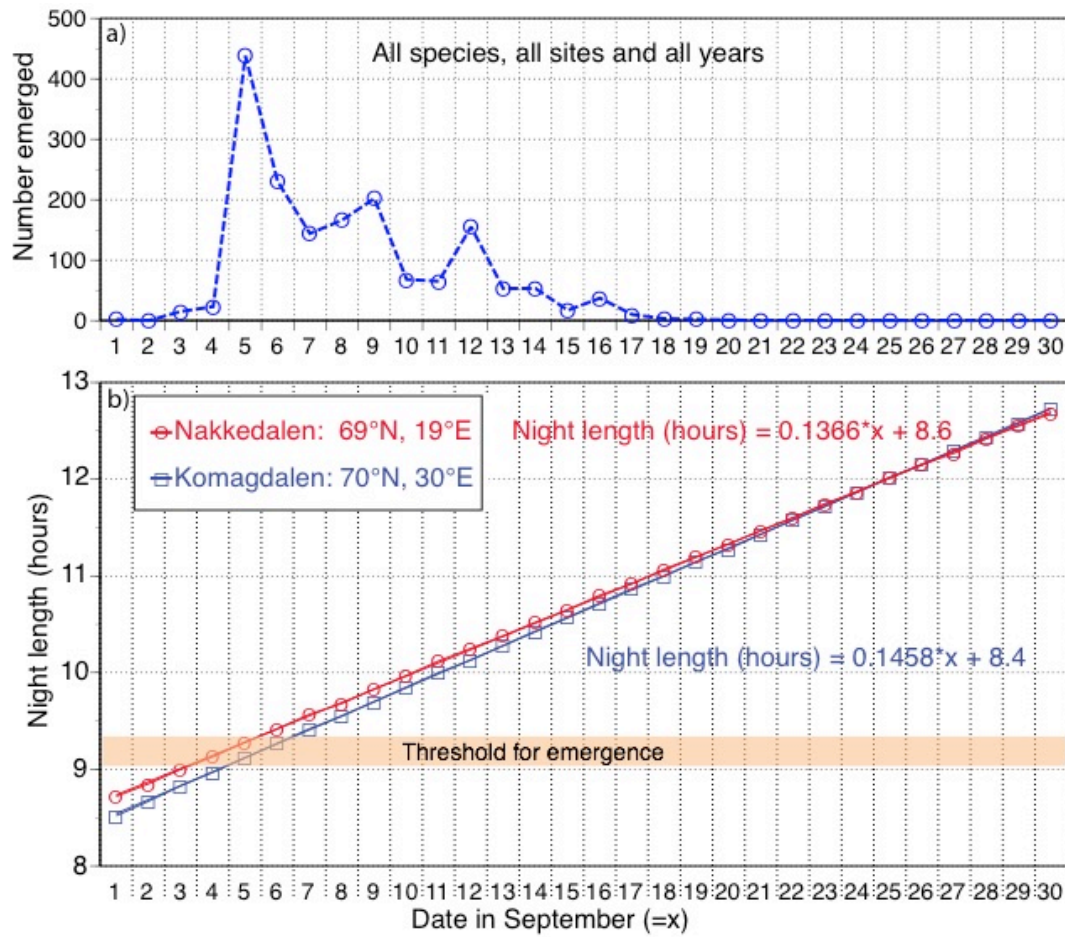
**Fig. 2 a** Larval pre-diapause activity for all species (*Pontania nivalis*, *P. glabrifrons*, *P. arcticornis*), all sites (Nakkedalen, Ifjordfjellet, Komagdalen) and all years (2008, 2009, 2012, 2013), **b** Night length development in September for Nakkedalen (69 °N) and Komagdalen (70 °N).
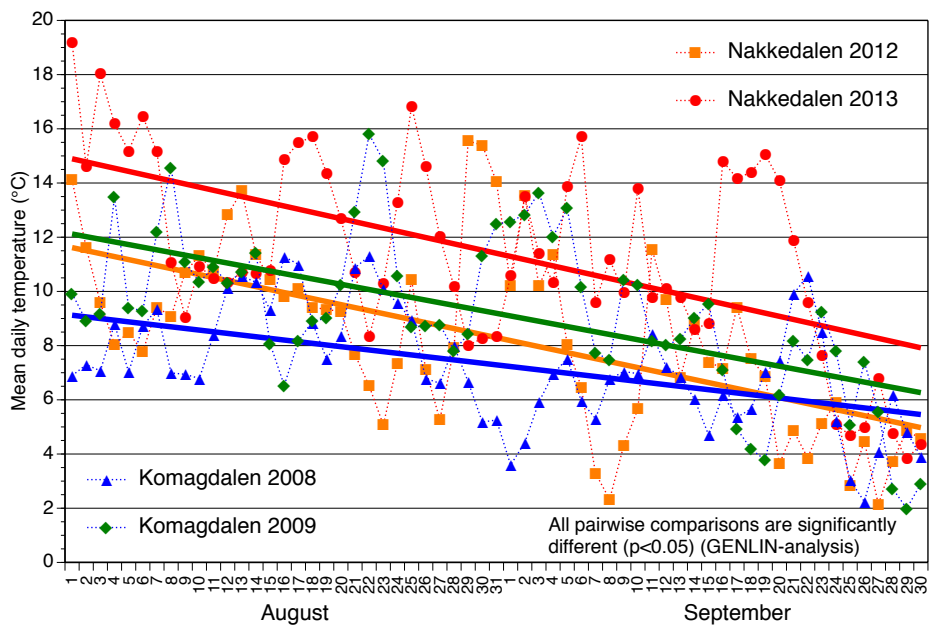
**Fig. 3 a** Mean daily temperature (°C) in August and September, Nakkedalen (2012 and 2013) and Komagdalen (2008 and 2009).
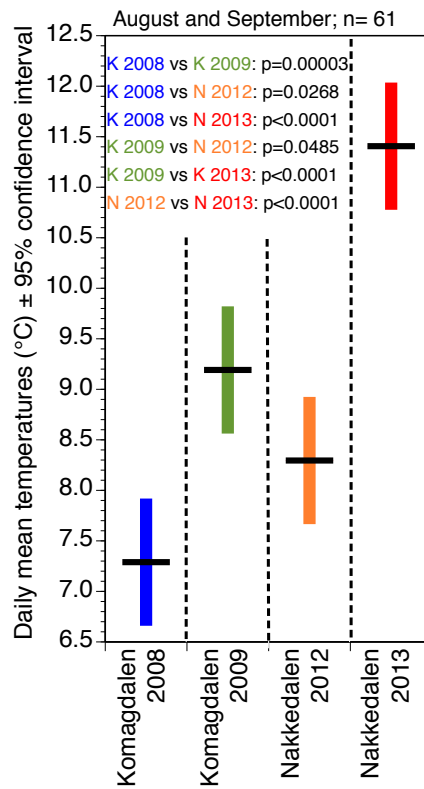
**Fig. 3 b** Mean temperatures ± 95 % confidence intervals for the same sites and years generated in GENLIN-analyses. The means are all significantly different (p < 0.05) from each other.
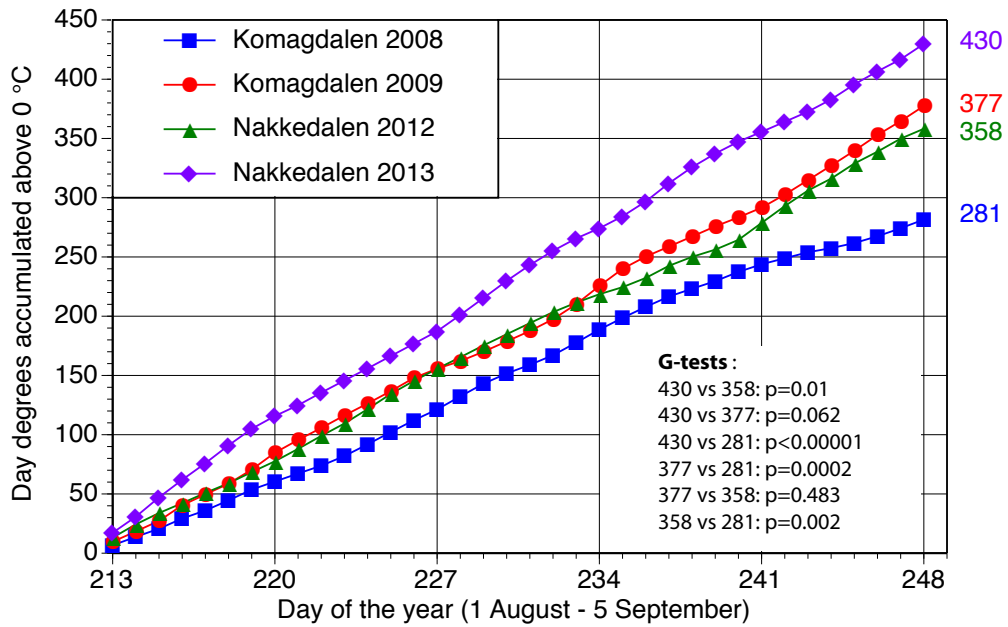
**Fig. 4** Day degree accumulation > 0 °C from 1 August until 5 September, graphically visualized. Four of the pairwise comparisons were significantly different ($p < 0.05$) according to G-tests.
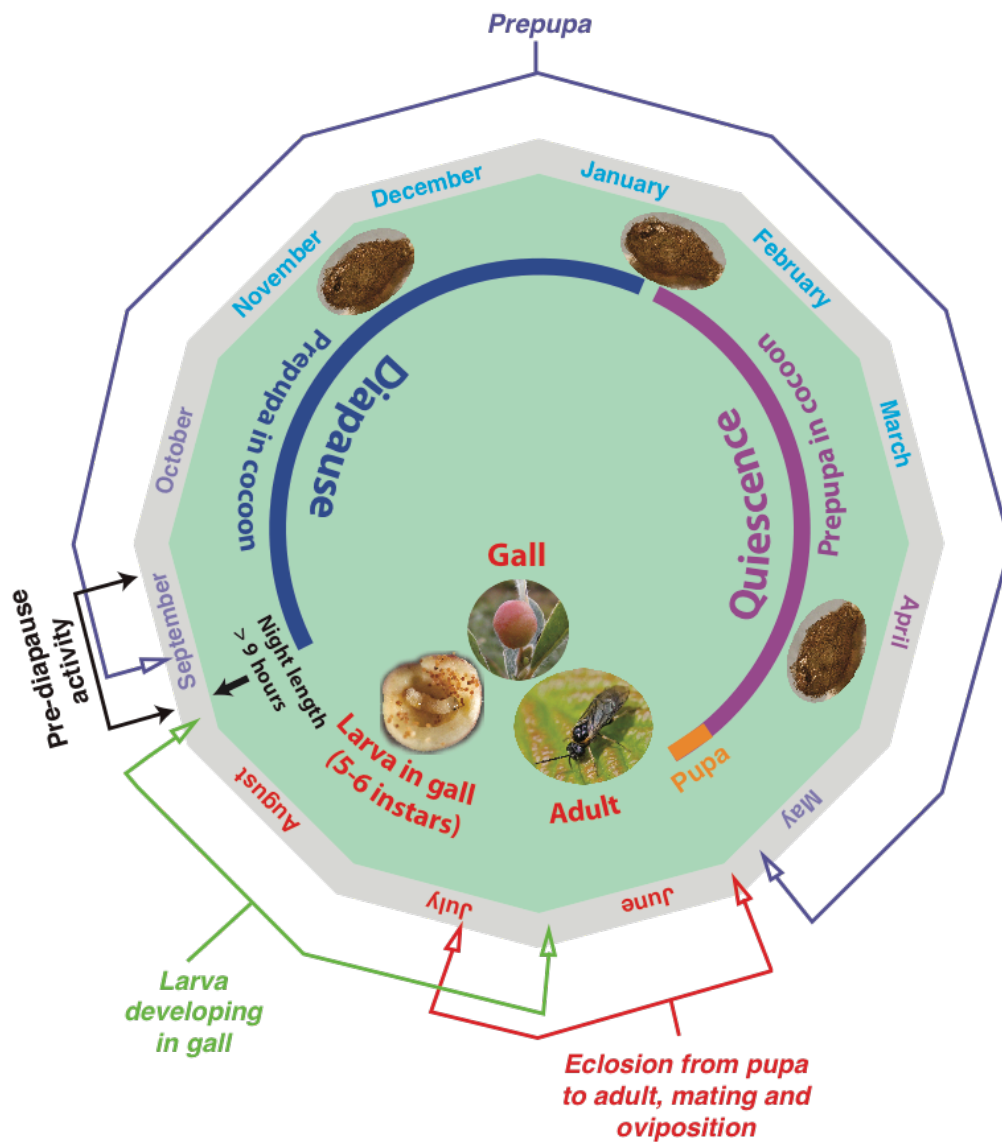
**Fig. 5** Illustration of the phenological events during the life cycle for the three gall-inducing sawfly species, *Pontania nivalis*, *P. glabrifrons* and *P. arcticornis*. They have synchronous pre-diapause activity (larval exit from gall), followed by diapause in early autumn. In mid-winter diapause turns into quiescence, and synchronous eclosion occurs in early spring. After a short period of mating and oviposition, larval development occurs during the summer in growing, enclosed galls.

**Online Resource (Supplementary Tables and Figures text)**

**Online Resource 1** Species and study sites, sample size (total n = 2343, number of galls collected), observed larval activity (total n = 1684) and % larval survival. IF= Ifjordfjellet, KO= Komagdalen and NA= Nakkedalen. Note that there are no data on *P. arcticornis*, IF 2008 and *P. nivalis*, KO 2008.

| Species and study site | Sample size | Observed larvae activity | % larval survival |
|---|---|---|---|
| *Pontania nivalis* 2008 IF | 210 | 140 | 66.7 |
| *Pontania glabrifrons* 2008 IF | 143 | 89 | 62.2 |
| *Pontania arcticornis* 2008 IF | na | | |
| *Pontania nivalis* 2009 IF | 145 | 107 | 73.8 |
| *Pontania glabrifrons* 2009 IF | 151 | 121 | 80.1 |
| *Pontania arcticornis* 2009 IF | 39 | 35 | 89.7 |
| *Pontania nivalis* 2008 KO | na | | |
| *Pontania glabrifrons* 2008 KO | 156 | 127 | 81.4 |
| *Pontania arcticornis* 2008 KO | 399 | 304 | 76.2 |
| *Pontania nivalis* 2009 KO | 142 | 93 | 65.5 |
| *Pontania glabrifrons* 2009 KO | 350 | 260 | 74.3 |
| *Pontania arcticornis* 2009 KO | 250 | 183 | 73.2 |
| *Pontania nivalis* 2012 NA | 60 | 38 | 63.3 |
| *Pontania glabrifrons* 2012 NA | 50 | 27 | 54.0 |
| *Pontania arcticornis* 2012 NA | 53 | 16 | 30.2 |
| *Pontania nivalis* 2013 NA | 17 | 10 | 58.8 |
| *Pontania glabrifrons* 2013 NA | 86 | 68 | 79.1 |
| *Pontania arcticornis* 2013 NA | 92 | 66 | 71.7 |
| SUM | 2343 | 1684 | 71.9 |

**Online Resource 2** Paired sample T-test of the correlation of the occurrence of larval emergence from galls in September between species, sites and year.

**Samples t-test:**
**Species**

| Species | n | Correlation (Pearson's r) | Significance (p) |
|---|---|---|---|
| *P. arcticornis* vs *P.glabrifrons* | 20 | 0.793 | 0.000031 |
| *P. arcticornis* vs *P. nivalis* | 20 | 0.832 | 0.000005 |
| *P. glabrifrons* vs *P. nivalis* | 20 | 0.557 | 0.010696 |

**Paired samples t-test:**
**Sites and years**

| Site and year | n | Correlation (Pearson's r) | Significance (p) |
|---|---|---|---|
| NA2013 vs NA2012 | 20 | 0.605 | 0.004684 |
| NA2013 vs IF2008 | 20 | 0.557 | 0.010819 |
| NA2013 vs KO2008 | 20 | 0.682 | 0.000931 |
| IF2009 vs KO2009 | 20 | 0.976 | 0.000000 |
| NA2012 vs IF2008 | 20 | 0.512 | 0.021100 |
| NA2012 vs KO2008 | 20 | 0.766 | 0.000082 |
| IF2008 vs KO2008 | 20 | 0.724 | 0.000305 |
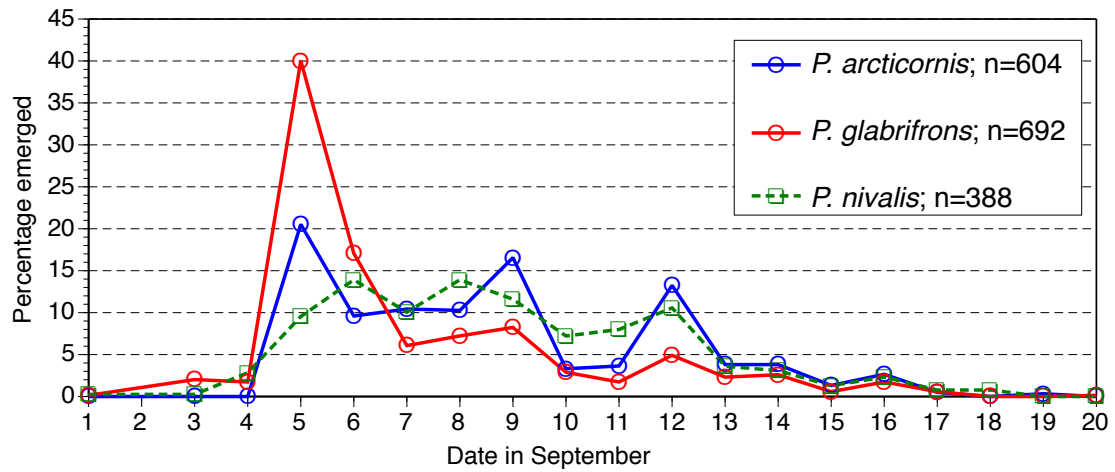
**Online Resource 3** Percentage larval emergence from galls during pre-diapause in September shown for each species, *Pontania nivalis*, *P. glabrifrons* and *P. arcticornis*, sites and years combined.
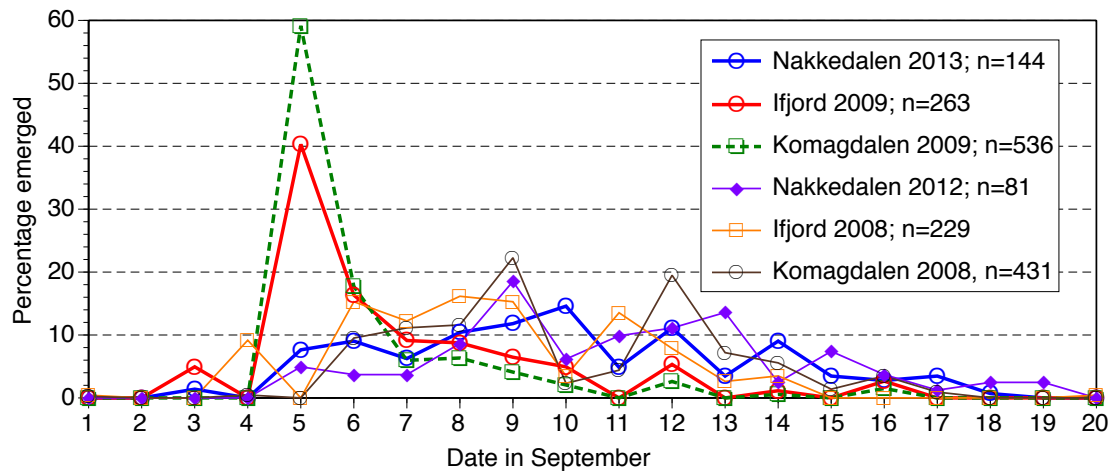


**Online Resource 4** Percentage larval emergence from gall during pre-diapause in September for all sites, species combined.