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ORIGINAL PAPER

J. W. Bjerke · D. Joly · L. Nilsen · T. Brossard

# Spatial trends in usnic acid concentrations of the lichen *Flavocetraria nivalis* along local climatic gradients in the Arctic (Kongsfjorden, Svalbard)

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**Abstract** The dibenzofuran usnic acid is an important secondary lichen metabolite, having many postulated biological roles, but evidence from field surveys is scanty. Trends in usnic acid concentrations and lobe width in the arctic lichen *Flavocetraria nivalis* were analysed along local longitudinal and altitudinal gradients in the Kongsfjorden area, northwestern Spitsbergen. Temperature was measured along the same gradients during 1 year. Other environmental variables were also estimated. The model that best explains the variability in usnic-acid levels includes the parameters effective temperature sum, frost sum and temperature range. Temperature range indicates a relationship between high usnic acid levels and humidity, whereas the two first parameters indicate a relationship with low temperatures, which could be direct or indirect. Much of the variability in usnic acid levels and lobe width could not be explained by the selected models. Thus, the secondary metabolism and lobe growth in this lichen is a complex matter, involving numerous environmental and possibly also intrinsic factors.

## Introduction

Lichens constitute a prominent part of terrestrial arctic ecosystems and contribute considerably to carbon sequestration, nitrogen assimilation and nutrient turnover. Many arctic lichens are coloured yellow, orange, brown or black by secondary metabolites that accumulate in the upper cortex. One of the most common lichen pigments is the yellow-green phenolic usnic acid, belonging to the dibenzofurans (Huneck and Yoshimura 1996), which is found in species from a wide range of genera and families. It is a highly interesting metabolite, because it accumulates in high quantities, normally making up from 1% to 9% of the total dry weight of the lichen (Huovinen et al. 1985; Quilhot et al. 1987; 1989; Bjerke et al. 2002; Bjerke 2003). Other lichen metabolites are also often found in such quantities. It is therefore reasonable to believe that usnic acid, and other secondary metabolites, serve important biological functions. Usnic acid is known to have high activity against viruses, microbes, protozoans, insects and fungi (Ingólfsdóttir 2002). It also absorbs efficiently in the ultraviolet (UV) range of the spectrum (Rancan et al. 2002), and therefore probably renders some protection against UV-B radiation (Bjerke et al. 2002; Buffoni Hall et al. 2002).

Lichen metabolites vary in concentration within and between individual thalli, and between populations of the same species. Usnic acid is generally found with the highest concentrations in the youngest thallus zones (Fahselt 1984; Quilhot et al. 1987; Bjerke et al. 2002; Buffoni Hall et al. 2002; Hyvärinen et al. 2002). In some cases, usnic acid concentrations of young thallus zones show high variation between thalli of the same uniform population (Mirando and Fahselt 1978; Fahselt 1984), whereas in other cases, intra-population variability is low in comparison with the variability between populations growing under different climatic conditions (Rundel 1969; Quilhot et al. 1998; Bjerke et al. 2002). Spatial variability in concentration has been explained in various ways. Intrathalline variability is mostly explained by differences in metabolic activity and exposure to stress factors, especially solar radiation (Mirando and Fahselt 1978; Quilhot et al. 1998; Buffoni Hall et al. 2002). Variation between thalli and between populations is found to correlate with variation in some climatic factors, such as solar radiation (Rundel 1969), temperature (Hamada 1991; under laboratory conditions) and altitude (Fernández et al. 1998; Quilhot et al.

J. W. Bjerke (✉) · L. Nilsen
Department of Biology, Faculty of Science,
University of Tromsø, 9037 Tromsø, Norway
E-mail: jarle.bjerke@ib.uit.no
Tel.: +47-77644439
Fax: +47-77646333

D. Joly · T. Brossard
Laboratoire ThéMA,
CNRS & Université de Franche-Comté,
Besançon, France

1998; Bjerke et al. 2002). When concentrations increase with altitude, UV-B radiation is often mentioned as a possible causal factor, since it is more strongly correlated with altitude than visible light is (Blumthaler et al. 1997), but other factors such as temperature and humidity certainly also vary along altitudinal gradients. Experiments with various bands of UV and visible radiation have shown that UV-B and/or UV-A radiation may in some cases induce the synthesis of usnic acid, but it has also been shown that usnic acid is degraded by shortwave radiation, so results are equivocal (Solhaug and Gauslaa 1996; BeGora and Fahselt 2001a, 2001b; Bjerke et al. 2002; Bjerke 2003; Buffoni Hall et al. 2002).

Thus, there are many uncertainties concerning which environmental variables regulate the biosynthesis and thereby the concentration of usnic acid in nature. Despite these uncertainties, it has been suggested that chemical signatures in lichens, in particular the concentration of usnic acid, may prove useful as a tool for monitoring spatial or temporal variations in UV-B fluxes (Galloway 1993; Rikkinen 1995; Quilhot et al. 1998; Bjerke and Dahl 2002). If this was possible, usnic acid concentrations should be closely correlated with incident radiation, but since lichen metabolites may be altered in response to a number of different stimuli and often do not show any correlation with solar radiation, this potential may be limited (BeGora and Fahselt 2001a; Bjerke 2003).

The objective of this study was to evaluate in detail how the concentrations of usnic acid vary along local climatic gradients in the Arctic, and assess whether there are trends that could be related to any climatic parameters, in particular, various temperature-related variables. Spatial variations in solar radiation and humidity were estimated from temperature-based indices. The results are of importance for evaluating the bioindicator values of chemical signatures in lichens and for explaining the impact of single climatic factors on the synthesis of usnic acid. A coast-inland gradient in the Kongsfjorden area, Svalbard, was selected, because here the same usnic acid-producing species are found in quantities from sea level to mountain summits and from the westernmost cape to the inland glaciers. The Kongsfjorden area offers an almost 1,000-m-high altitudinal gradient of continuous distribution of the studied species, undisturbed by a forest canopy, coupled with a strong oceanity-continentality gradient, from the west to the east. The Arctic is also of interest from an ecoclimatic point of view, since large climatic changes are expected to take place in polar regions (Weller 2000), which could affect lichen viability, distribution and physiology (see, for example, Cornelissen et al. 2001; Solheim et al. 2002; Bjerke et al. 2003), and thereby also usnic acid synthesis.

## Materials and methods

### Study area

The fiord Kongsfjorden is situated in northwestern Spitsbergen (Fig. 1). The peninsula Brøggerhalvøya delimits the fiord to the south. In the northern side of the fiord, the large island Blomstrandhalvøya is situated close to the south-facing slopes of the major island. In the eastern parts of Kongsfjorden, several arms of the inland glacier reach the sea, but a relatively large and floristically rich ice-free hilly terrain, Ossian Sars-fjellet, is found between the glacier arms. The community Ny-Ålesund is situated halfway between the northwestern cape of Brøggerhalvøya (Kvadehuken) and the glacier Kongsvegen. It has a mean July temperature of 5.1°C and a mean annual precipitation of 385 mm (Førland et al.

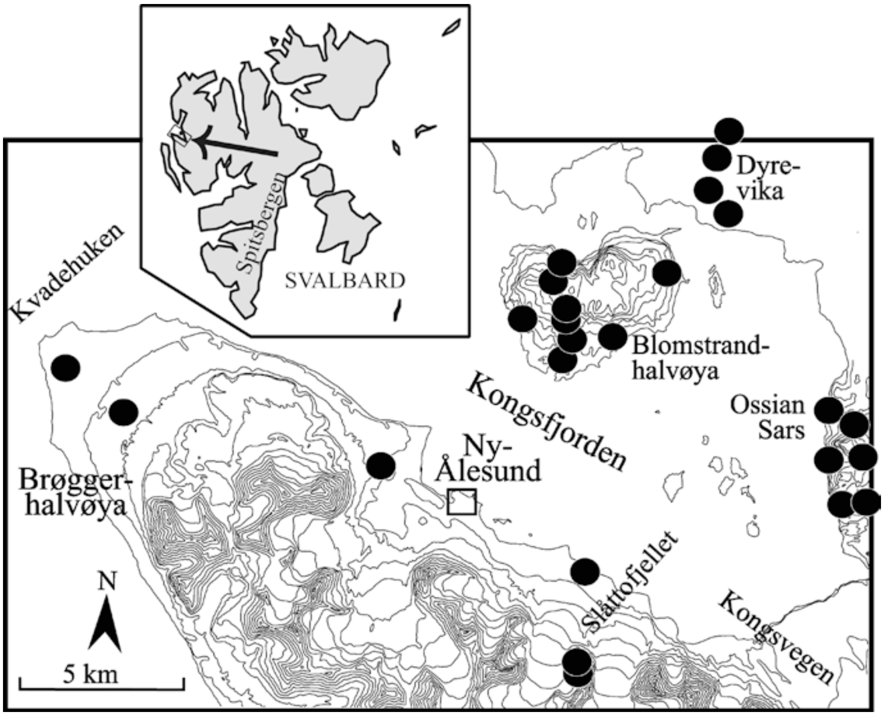

**Fig. 1** The study area on northwestern Spitsbergen showing the locations of the 25 sites. The study area is indicated on the small Svalbard map (*rectangle + arrow*). The Kongsfjorden map lacks contour lines in the Dyrevika area

1997), and is in the northern arctic tundra zone, but very close to the boundary with the middle arctic tundra zone, which is found to the east and in south-facing areas north of the fiord (Elvebakk 1997).

## Temperature monitoring and interpolation

A network of temperature loggers (HOBO H8 Pro Temp/external logger, 64 Kb memory, with an external thermocouple sensor, Onset Computing, Bourne, Mass.) was established in the Kongsfjorden area in July 2000. The values obtained from the loggers were compared with the ones from standard meteorological sensors, and the standard deviation of the differences never exceeded 0.3°C (Météo-France 2000).

As the study focuses on the vegetation-temperature relationship, the sensors were positioned 10 cm above the ground. For that reason, the size of the shelters had to be small. A preliminary test was carried out to define their technical characteristics: white plastic cylinders, 20 cm long and 5 cm in diameter, in oblique positions with their upper ends facing to the north. The structure of the shelter allows air to circulate. The accuracy of the system was tested both in France and in Svalbard with satisfactory results (Météo-France, unpublished report).

The loggers were placed according to two major sampling purposes: an optimal regular sampling of the study area, and an optimal sampling of local environment variation in terms of morphotopography, altitudinal transects on both sides of the fiord from west to east and land cover (Joly et al. 2003). The exact position (longitude, latitude, altitude) of each logger was obtained by using Geographical Position Systems (GPS) (Differential bifrequence GPS, Leica Geosystems Heerbrugg, Switzerland). The primary objective with the temperature monitoring is to use the data for modelling climatic variation in the area (see Brossard et al. 2002), but the data are useful in other ecoclimatic studies as well. The temperature data were downloaded from the loggers in July 2001.

Because the values were observed in punctual field stations, an interpolation process was carried out to allow a cartographical presentation of temperatures in squares of 50 m×50 m resolution. The statistical correlations at meso- and microscales between temperature measurements and variables describing the environmental conditions are systematically explored for recognition of the most significant combination of factors for the spatial model of temperature variation (Joly et al. 2003). The interpolation procedure involves processing temperature values at each pixel, restoring the thermic field with a high accuracy in the entire area. The calculations are made both for the daily maximum and minimum temperatures of the complete observation period to calculate the thermic values that are used as predictor variables in the present study (see below).

## Lichen sampling

The lichen cover in this area is extensive, although the reintroduction of reindeer in 1979 led to rapid reductions of lichen mats in certain areas, especially in lower-lying strandflats on Brøggerhalvøya (Wegener et al. 1992; Elvebakk 1997). The usnic acid-producing fruticose species *Flavocetraria nivalis* (L.) Kärnefelt and Thell is one of the most abundant species in the area (Lynge 1938; Elvebakk 1997). It typically grows on exposed sites that have a discontinuous snow cover in winter. This species was selected for this study, since previous vegetation analyses had shown that it was the most abundant and widespread usnic acid-producing species in the area (A. Elvebakk, personal communication).

From each site where temperature loggers were placed out, ten thalli of *F. nivalis* were collected during a 2-week period in July 2000. They were randomly sampled from a circular area of 10 m diameter with the logger in the centre of the circle. At a few sites where *F. nivalis* was sparser, the diameter of the collection area was increased to 20 m. All sampled thalli grew in highly exposed microsites. Thalli growing in shade (e.g. hidden between rocks) were avoided. In total, lichens were collected from 25 sites from sea level to 970 m altitude (Fig. 1). *F. nivalis* was scattered in the westernmost part, and around one logger placed in a rocky scree at ca. 500 m altitude at Kvadehuken, no thalli of *F. nivalis* were found. In the remaining area, *F. nivalis* was abundant. The sites are divided into five geographical groups: western Brøggerhalvøya (sites A–C), Slåttofjellet (sites D–F), Blomstrandhalvøya (sites G–O), Dyrevika (sites P–S) and Ossian Sars-fjellet (sites T–Y). The predominant slope aspect and slope angle of each site were measured. The thalli were air-dried, kept in darkness and transported to the University of Tromsø within 2 weeks after sampling.

## Quantitative analyses of usnic acid

Since previous analyses of *F. nivalis* from other areas had shown that the highest concentrations of usnic acid are found in the lobe tips (Bjerke et al. 2002), and since preliminary tests showed that this was the case for *F. nivalis* from Kongsfjorden as well, only the outermost 0.5 cm of the thalli were used. Usnic acid was extracted in three series, first in excess acetone for 24 h, then in a 1:1 mixture of acetone and methanol for another 24 h, and finally in pure methanol for 24 h. This was necessary to obtain a complete extraction. The combined extracts were mixed, filtered and diluted to 25 ml. Extracts were analysed quantitatively by reversed-phased high performance liquid chromatography (RP-HPLC) with a mobile phase consisting of methanol and 1% orthophosphoric acid in ultra-pure water, as described by Bjerke et al. (2002).

## Measurements of lobe width

*F. nivalis* has a rather variable morphology, especially concerning height of thalli and cushions and width of single lobes. In areas with high grazing pressure, the lichen tends to be considerably smaller than in other areas. Microclimatic conditions can also affect size. We therefore measured the width of the lobes, which we considered as a more accurate estimate of size than thallus height. The width was measured on air-dried thalli under a stereo-microscope to the nearest ocular unit (0.06 mm), and the narrowest part right below the uppermost branching node was chosen, since this part showed little intrathalline variation.

## Environmental variables

The temperature data were used to derive various parameters that contribute in different ways to describe the climatic properties of the studied sites. The following parameters were derived, the first four chiefly in accordance with Tuhkanen (1984):

1. Length of the growing season. It is defined as the period of the year during which growth and other physiological functions in general are possible. Days with daily mean temperatures above 0°C were included here.
2. Effective temperature sum. It is the cumulative number of degrees above a certain threshold (0°C selected here) recorded during a year. Daily mean temperatures were used.
3. Frost sum. It is the “coldness sum” analogous to the effective temperature sum, and is here defined as the cumulative number of daily mean degrees below 0°C recorded during a year.
4. The Conrad index of continentality ($C$). It is a conventional thermic index that is based on the fact that the annual temperature range is small in an oceanic climate and large in a continental one. For a delimited area as Kongsfjorden with negligible latitudinal differences, the index is practically identical ($r = 0.990$) to the temperature range, i.e. the difference in mean temperature between the warmest and coldest months of the year. We therefore used the temperature range, and not $C$, in

the analyses. Temperature range gives an indication of relative differences in water availability, since oceanic sites are characterised by low ranges and a humid climate, whereas sites that are more continental are characterised by larger ranges and a drier climate.

5. Model-based index of thermophily. Botany-based indices of thermophily are generally used to detect areas on local and regional scales with a high number of temperature-demanding plants (including bryophytes and lichens) and to estimate temperature regimes based on botanical data (Elvebakk 1990; Karlsen and Elvebakk 2003). This approach was taken a step further by Brossard et al. (2002), who developed a model-based index of thermophily primarily used for the Kongsfjorden area. Model-based index values were estimated for each site according to Brossard et al. (2002).
6. Radiation index. Temperature is often closely associated with fluxes of solar radiation. However, other factors such as wind, shading, proximity to cooling water bodies etc. contribute to reduce the correlation between temperature and solar radiation. An index for estimating solar radiation was developed. This parameter is a theoretical index calculated for a cloudless day. For estimating the most potential conditions of incoming energy, global solar radiation is given for the summer solstice (21 June). One value is calculated for each hour of the day, using both solar characteristics (height on the skyline and azimuth) and land characteristics (gradient and aspect) according to the equations given by Dumoulin and Parizet (1987). The mask effect due to the topography is also integrated. Finally, the 24 values are added to obtain a theoretical estimation of global radiation.

In addition, mean temperatures of the months and of the growing season were calculated and used as stand-alone variables in preliminary analyses, but later excluded due to apparent lack of informative data and a need for a reduction of the number of predictor variables included in the model selection analyses.

Longitude, latitude and altitude were also used as predictor variables. The same is the case for slope angle and aspect, but these two parameters were also combined to calculate the heat load index (Geiger 1966), which takes into account that southwest-facing slopes experience the greatest diurnal heat load because of delayed ground heating.

An estimate of grazing and trampling pressure was necessary in order to assess whether the presence of reindeer affects usnic acid concentrations. A five-level scale (0–4) was used, where 0 indicates no observed reindeer and the level 4 indicates high density of reindeer. Little published information on the number of reindeer in other areas than Brøggerhalvøya is available, but our own observations and personal communications indicate that very few reindeer graze in the northern and eastern parts of the study area. Collection date was included as the last predictor variable. Thus, no direct measurements of humidity and solar irradiance were made, but these factors can indirectly be derived from some of the various temperature-based parameters, e.g. effective temperature sum, radiation index and temperature range.

### Statistical analyses

Usnic acid concentrations and lobe width data were explored graphically by means of boxplots showing the median, the interquartile distance, 1.5 times the interquartile distance and outliers. The ecological questions we attempted to answer were whether usnic acid concentrations are correlated to any environmental variables, and if so, which are the most important variables. Thus, to find the linear models that best fit the variation in response variables, Akaike's information criterion (AIC) was used. AIC incorporates the log-likelihood with a penalty for added parameters, and thus aims at finding the best model(s) among the candidate models by making a compromise that few parameters may lead to high bias and that a complex model with many parameters may result in large sampling variance (Akaike 1973; Burnham and Anderson 2002). Since the ratio between number of observations and number of parameters is less than 40, the second-order variant of AIC ($AIC_c$) was used. Analyses were done by using S-PLUS 6.1 for Windows (Insightful, Seattle, Wash.) with the library MASS enabled (Venables and Ripley 2002). Interaction terms were not included in the analyses since no a-priori support exists for any particular interactions. $AIC_c$ values and maximised log-likelihood were subsequently calculated according to Burnham and Anderson (2002).

## Results

### Usnic acid concentrations

The overall mean concentration of usnic acid from the 25 sites is 6.03% of dry weight lichen. Mean site values range from 4.48% to 9.07%, whereas median values range from 4.25% to 9.03% (Fig. 2). The highest values were found at the summit of the mountain Slåttofjellet (Fig. 2, site F) and near sea level at Kvadehuken (site A). The loftiest site at 970 m (site S) has the third highest mean and median value. No sites have exceptionally low values. Scattered sites from the entire coast-inland gradient have mean and median concentrations between 4.25% and 5.16%. Remarkably, almost all of the easternmost sites (Ossian Sars-fjellet; sites T–Y) have values at the lower end of the range.

As the interquartile ranges of the boxplots in Fig. 2 show, some sites experience high variability, despite apparently uniform habitat properties within each site. In particular, site P, a south-facing site near sea level, has high intrasite variability with individual values ranging from the lowest to the highest on the overall range.

Models with the lowest $AIC_c$ values are given in Table 1. They explained between 34% and 35% of the variance in usnic acid concentrations. The least complex model of those with almost identical values includes effective temperature sum, temperature range (≈C) and frost sum. The slightly more complex models include the heat load index and inclination. The explanatory variables included in the simplest model indicate that usnic acid concentrations are related to areas that experience low temperatures in both winter and summer (Table 2). In fact, the three sites with the lowest temperature sums (sites S, F and A) have the highest mean usnic acid levels, whereas the three sites with the highest temperature sums (sites T, U and V; all in Ossian Sars-fjellet) have the lowest, the second lowest and the fifth lowest mean usnic acid values. Site F, the one with the highest usnic acid concentration, is also the coldest site in winter (i.e. it has the lowest frost sum). The summit of Blomstrandhalvøya (site K) has the second lowest frost sum, and has the highest mean usnic acid level of the sites on Blomstrandhalvøya. The negative estimate of temperature range (Table 2) indicates a tendency towards higher usnic acid levels in the oceanic sites than in the more continental sites. Most sites have a nonnormal (close to lognormal) distribution of usnic acid levels (Fig. 2). The use of log-transformed data resulted in the same selection of models. The maximised likelihood differs only slightly between the models (Table 1).

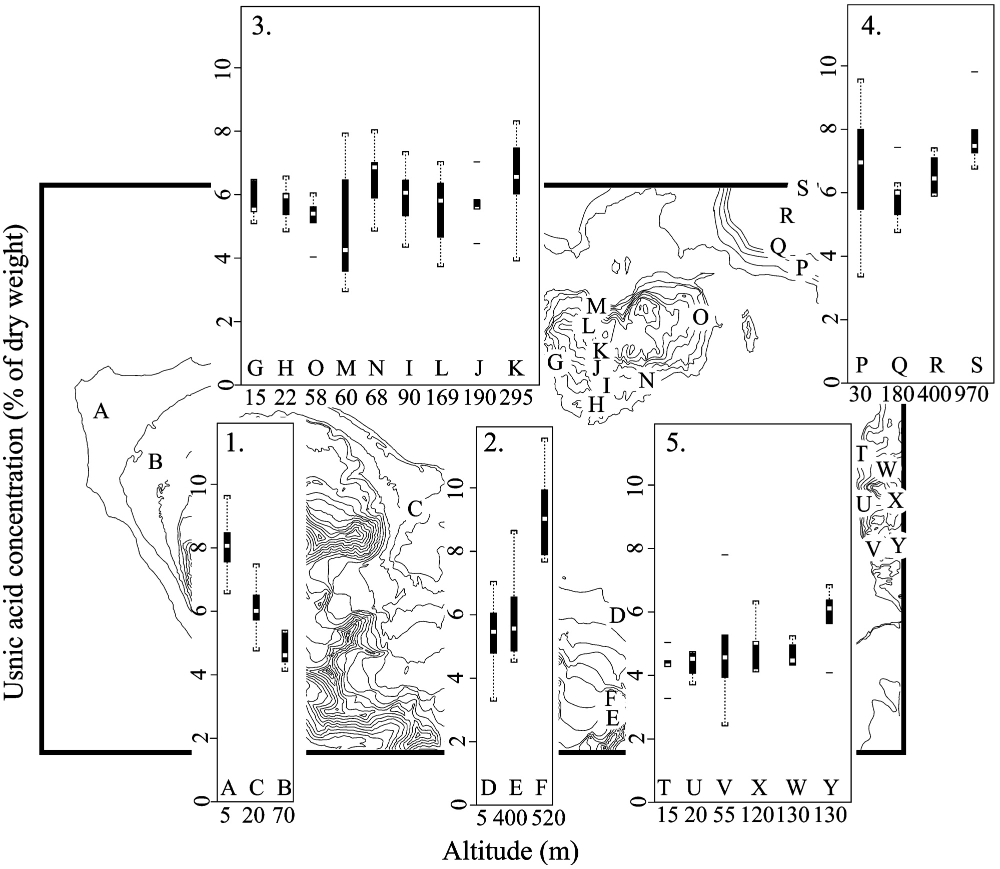

**Fig. 2** Usnic acid concentrations at the 25 sites in Kongsfjorden. The sites are sorted for each geographical group according to altitude. The five groups are: *1* western Brøggerhalvøya, *2* Slåttofjellet, *3* Blomstrandhalvøya, *4* Dyrevika, *5* Ossian Sars-fjellet. Altitude is shown as *numbers* on the *horizontal axis*, whereas usnic acid concentrations are shown as percentage of dry weight on the *vertical axis*. The *boxplots* show the median (*white square* within *box*), the interquartile distance (*box*), 1.5 times the interquartile distance (*whiskers*) and outliers (*individual horizontal lines*). The locations of the sites A–Y are shown on the map behind the boxplots

**Table 1** AIC$_c$ values, maximised log-likelihood [log(L)] and adjusted multiple correlation coefficients ($R^2$) for the four models that best explain the variation in usnic acid concentration

| Model | Explanatory variables | AIC$_c$ | log(L) | $R^2$ |
|---|---|---|---|---|
| 1 | Temperature sum + temperature range + frost sum | 91.17 | −150.32 | 0.341 |
| 2 | Model 1 + heat load index | 91.44 | −149.94 | 0.349 |
| 3 | Model 1 + inclination | 92.88 | −150.26 | 0.342 |
| 4 | Model 1 + heat load index + inclination | 92.98 | −149.84 | 0.351 |

**Table 2** The estimated parameter values and their standard errors for model 2 of the usnic acid model selection analysis, which includes four variables. Estimated values are almost identical for models 1 and 2

| Variables | Estimate | St. error |
|---|---|---|
| Temperature sum | −0.1016 | 0.0541 |
| Temperature range | −0.2246 | 0.0829 |
| Frost sum | −0.0522 | 0.0169 |
| Heat load index | −0.1144 | 0.0844 |

### Lobe width

The overall mean and median lobe width values are 1.79 mm and 1.70 mm, respectively. Most sites have median values not far from the grand mean (Fig. 3). However, site R and to a lesser extent site Q, differ from the other sites by having broader lobes than elsewhere. In this southwest-facing slope at Dyrevika, *F. nivalis* is the dominating species, being very abundant from sea level to 1,000 m altitude. Close to site R was also the only place where fertile specimens of *F. nivalis* were observed. Thus, it appears that the conditions there are very suitable for the growth of this species.

The model with the lowest AIC$_c$ value includes 10 explanatory variables, and models with similar values have either 9 or 11 variables (Table 3). Thus, a simple relationship between lobe width and the measured environmental variables was not found. Being such complex models, they do not explain much of the variability in lobe width, only between 36 and 38%. The correlation between usnic acid concentration (response variable) and lobe width (predictor variable) was very low ($R^2 < 0.01$ both when all sites are included and when sites Q and R are left out), and not significantly different from 0 (not shown).

## Discussion

We have shown here that usnic acid concentrations vary considerably between sites along local longitudinal and altitudinal transects, and that the variability is correlated, at least in part, with climatic factors, with the coldest sites having the highest concentrations. This is

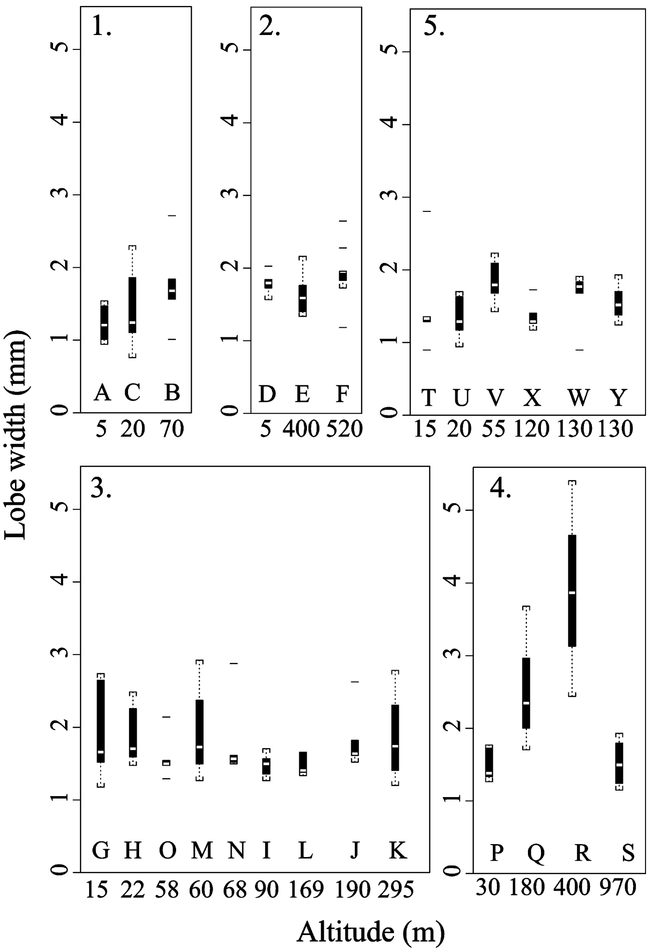

**Fig. 3** Lobe width measurements from the 25 sites in Kongsfjorden. Sites, boxplots and geographical groups as for Fig. 2

**Table 3** AIC$_c$ values, maximised log-likelihood [log(L)] and adjusted multiple correlation coefficients ($R^2$) for the three models that best explain the variation in lobe width

| Model | Explanatory variables | AIC$_c$ | log(L) | $R^2$ |
|---|---|---|---|---|
| 1 | Altitude + latitude + slope angle + sampling date + frost sum + index of thermophily + radiation index + slope aspect + longitude + length of growing season | −181.41 | −88.09 | 0.378 |
| 2 | Model 1 + temperature sum | −180.64 | −87.82 | 0.383 |
| 3 | Model 1−length of growing season | −179.89 | −86.96 | 0.363 |

the first time that a detailed survey of the spatial variability of usnic acid levels, and levels of secondary lichen metabolites in general, is reported and therefore the first time that such a relationship has been demonstrated in nature. Previous studies along spatial gradients in the field have included comparatively fewer sites with lower control of environmental variables, and have therefore not been able to detect such relationships. The positive correlations with altitude found in some lichen studies, and considered an effect of higher UV-B radiation fluxes at high altitudes (e.g. Fernández et al. 1998; Quilhot et al. 1998; Bjerke et al. 2002; Rubio et al. 2002) may instead be related to the lower temperatures at higher altitudes. No direct relationships with solar radiation were found in the present study, as both altitude and the radiation index were among the first variables to be excluded during the selection analyses. The present results correspond to the results from some recent field experiments where the effects of enhanced UV-B radiation on secondary metabolism were shown to be very modest or absent (Bjerke et al. 2003, Bjerke 2003).

The physiological aspects concerning the impact of low temperatures on secondary metabolism in lichens are not much studied. Lichens from cold environments are generally very tolerant to freezing stress (Kappen et al. 1996). It may be that secondary metabolites contribute to the high tolerance, for example, by reducing ice formation within the cells, either because of hydrophobic properties, or because they can be suspended in the cell sap and thereby lower the freezing point (Avalos and Vicente 1987; Longton 1988; Rikkinen 1995; Huneck 1999). Thus, the trend towards higher concentrations of usnic acid in colder environments may be a protective response against freezing effects. Laboratory experiments have shown that usnic acid accumulates when nutrient availability is low, and degrades under high photosynthetic activity (Vicente et al. 1980; Herrero-Yudego et al. 1989). Another interpretation is therefore that usnic acid may accumulate under low temperatures because of lower photosynthetic rates. However, this interpretation is not supported by BeGora and Fahselt (2001a) whose results indicate that usnic acid is synthesised during periods with a positive carbon balance.

The coldest sites are all situated in the areas most regularly covered by sea fog or clouds (cf. Svendsen et al. 2002). Thus, the observed relationship between coldness and usnic acid concentrations could be indirect, and instead caused by increased dew formation (i.e. increased water availability) due to fog and clouds at the coldest sites. Lichens need to be wet and metabolically active over long periods to be able to synthesise large quantities of secondary metabolites as a response to a regulatory factor, such as solar radiation (Solhaug et al. 2003). Thus, the inclusion of temperature range in the model is probably a reflection of the direct and indirect impact humidity has on usnic acid synthesis, since oceanic (i.e. more humid) sites tend to have higher concentrations than more continental (i.e. drier) sites. Bjerke (2003) also indicated that water availability plays an important role for the usnic acid synthesis in *F. nivalis*.

If we, despite the results of the model selection analysis, still hold to the hypothesis that usnic acid has a photoprotective role, some support to this hypothesis should be given. It is known that UV repair mechanisms are enzymatic and therefore become increasingly inefficient with decreasing temperatures (Björn et al. 1999;

Buffoni Hall et al. 2003). Polar lichens such as *F. nivalis* are metabolically active even at near-zero or subzero temperatures (Schipperges 1992; Kappen et al. 1996). Thus, *F. nivalis* is probably often metabolically active under conditions of low temperatures and relatively strong solar radiation. Excessive irradiation could then lead to irreversible damage on molecular structures, for instance, in algal thylakoids. The mycobiont response would therefore be to increase concentrations of usnic acid under low temperatures to compensate for the concurrent decrease in UV repair efficiency of the photobiont. Due to slower desiccation rates, lichens growing in oceanic habitats may be metabolically active more often than lichens from more continental habitats during periods of low temperatures and at least moderate levels of solar radiation. By using the same argument as above, one would therefore expect higher levels of usnic acid in the most oceanic sites. This situation is equivalent to open-shade conditions (see Rikkinen 1995), in which lichens stay metabolically active for much longer periods than in adjacent habitats with higher exposure to direct sun.

The selected models explain only a minor part of the variability in usnic acid concentrations. A large part of the overall variation is caused by intrasite variability, which could not be explained by the models, as we assumed that all thalli within each site grew under identical climatic conditions. Thus, we can only speculate on what causes the high variability within some sites. Fahselt (1984) suspected that high variability within a uniform population was because of innate, genetic differences among thalli, and not a reflection of microclimatic conditions. Biotic factors such as infection of pathogenic fungi and insect herbivory (Ingólfsdóttir 2002) would possibly also result in intrasite variability if the distribution and activity of the attackers were not spatially uniform. Although the collected lichen samples bear extremely few indications of biotic attacks, we should not exclude this possibility, as initial establishment of parasitic fungi and grazing insects are often difficult to detect.

The variability in lobe width was not much explained by the environmental variables included in the analysis. The best models include between 9 and 11 explanatory variables, and they explain not more than 38% of the variability. Site R, which has remarkably wider lobes than the other sites, is probably a site having a good combination of environmental variables, facilitating extensive thallus growth. It has very little grazing pressure, but lichens from other areas with little grazing pressure do not have exceptionally wide lobes. It is one of the sites with the lowest temperature sum, the lowest frost sum and the shortest growing season. Thus, the large thallus sizes at this site may be related to slower desiccation rates, and thereby longer periods of metabolic activity.

The relatively high intrasite variability in lobe width is an important factor for explaining the low correlation coefficients. As for usnic acid, there may be some abiotic or biotic differences on microscale causing the high variability. The lack of correlation between usnic acid concentration and lobe width implies that usnic acid is not highest in small thalli with a thin medullary layer and a larger proportion of the thallus made up by the usnic acid-containing outer cortex. Thus, the variability in usnic acid concentrations cannot be explained by morphological differences between and within sites.

Altogether, we conclude that usnic acid levels are altered by numerous stimuli, among which low temperatures and humidity are of some importance for usnic acid synthesis. It is therefore not likely that usnic acid levels can be used to biomonitor UV-B radiation fluxes as suggested by some authors (see Introduction). There is still much unexplained intrasite and between-site variability in usnic acid concentrations and lobe width that one should attempt to assess in detail through controlled field and laboratory experiments.

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