proposed for the transfer of biomass through marine food webs (*2*). One pathway leads from large phytoplankton by way of a one- to three-step food chain to fish that can be harvested by humans (*1*). The other leads from smaller phytoplankton through about five trophic levels to various gelatinous predators (*2*).

If this hypothesis is valid, then PCB pollution of coastal waters could result not only in PCB-contaminated fish products and diminished primary and secondary production, but also in ecosystems reduced in harvestable fish through a reduction in phytoplankton size and associated alteration of the natural food web.

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Thermal Sensitivity in Lichens

Abstract. *Lichens are believed to be extremely resistant to high-temperature stress when desiccated. Results from a reexamination of this concept indicate that some air-dry lichen thalli can be extremely sensitive to even moderate levels of heat stress whereas others exhibit a considerable degree of heat resistance. These differential levels of thermal resistance correlate exactly with the ecology of these populations.*

It has long been believed that lichens are resistant to high-temperature stress (*1*). This idea is based on evidence presented by Lange (*2*), who examined the heat resistance of 40 species of lichens. The temperature that caused a reduction in the normal respiration rate of air-dry thalli by one-half was accepted as an index of the limit of heat resistance, and these indices ranged from 70°C in *Alectoria sarmentosa* to 101°C in *Cladonia pyxidata*. However, hydrated lichen thalli did not differ from other kinds of plant tissue and their limits of heat resistance ranged from 35° to 46°C. We have reexamined thermal sensitivity in two lichen populations and found that in fact dry lichen thalli can be extremely sensitive to even moderate levels of heat stress. Conversely, other populations, collected from a contrasting environment, exhibited a considerable degree of heat resistance when in the air-dry state. These differential levels of thermal sensitivity correlate exactly with the ecology of the two populations.

Replicate thalli of *Peltigera canina* var. *praetextata* were collected in the air-dry state from deciduous woodland in southern Ontario and *P. canina* var. *rufescens* from an open xeric roadside habitat in central Ontario. The material was

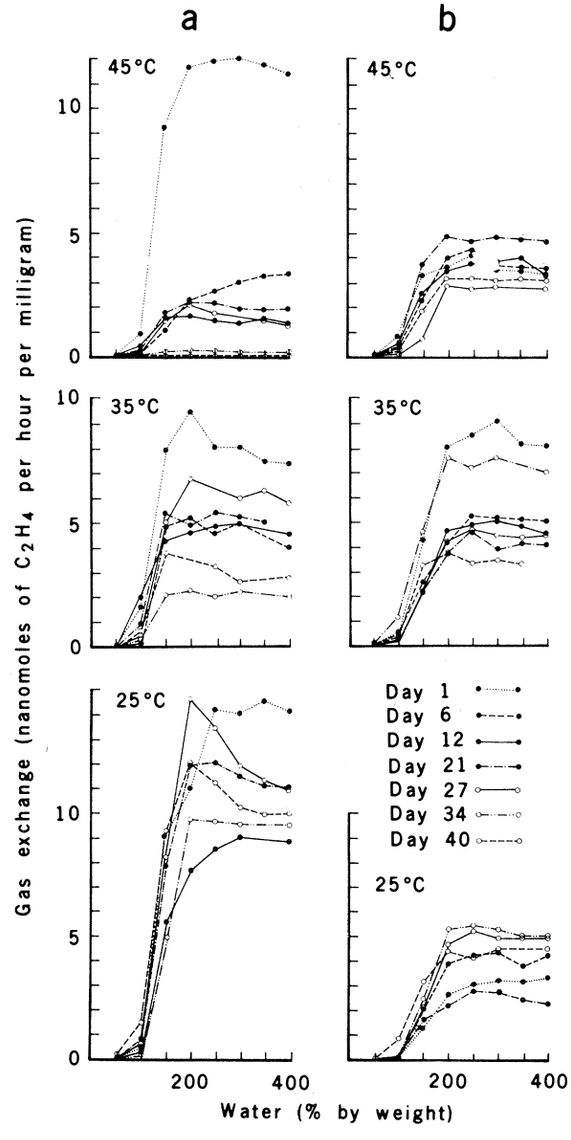

Fig. 1. The nitrogenase activity of (a) *Peltigera canina* var. *praetextata* and of (b) *Peltigera canina* var. *rufescens* after air-dry storage at temperatures of 25°, 35°, and 45°C. Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.

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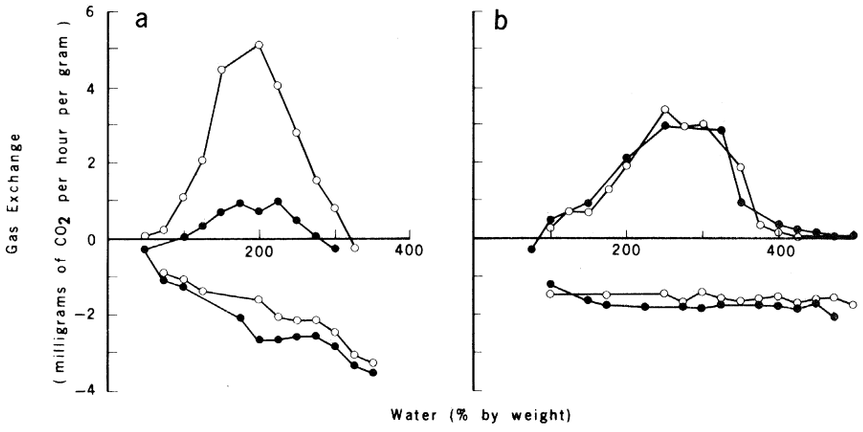

Fig. 2. The respiration (lower pair of curves) and net photosynthetic responses (upper pair of curves) of (a) *P. canina* var. *praetextata* and of (b) *P. canina* var. *rufescens* after 27 days of air-dry storage at 25°C (○——○) and 45°C (●——●). Thallus saturation is expressed as a percentage of the final oven dry weight of each replicate.

stored air-dry at 25°, 35°, and 45°C in growth chambers with a photoperiod of 12 hours of light and 12 hours of darkness and a quantum flux density of 300 μE m^{-2} sec^{-1} for varying experimental periods up to 40 days. Subsequently, we measured the nitrogenase (E.C. 1.7.99.2) activity resulting from these storage temperatures, using acetylene (C_2H_2) reduction with the formation of ethylene (C_2H_4), to assess the level of damage to each population over time. Experiments were conducted at 25°C and a quantum flux density of 300 μE m^{-2} sec^{-1}; we used four replicates for each treatment temperature. All levels of thallus moisture were monitored (*3*). We obtained further evidence of thermal stress by examining net photosynthesis and respiration, using infrared gas analysis (*4, 5*), on both populations after 27 days of storage treatment at 25° and 45°C.

The results (Fig. 1) are clear-cut. Nitrogenase activity is maintained in *P. canina* var. *praetextata* when stored dry at 25°C (Fig. 1a). At 35°C, however, nitrogenase activity gradually declines with time. After 40 days at this storage temperature, *P. canina* var. *praetextata* has lost more than 50 percent of its potential to fix nitrogen. Furthermore, at 45°C, nitrogenase activity declines sharply and by day 40 no activity could be detected (Fig. 1a). Conversely, nitrogenase activity in *P. canina* var. *rufescens* was maintained throughout the experimental storage and time series (Fig. 1b), in marked contrast to the results obtained for the woodland variety.

Net photosynthetic rates in *P. canina* var. *praetextata* are similarly affected by the thermal stress treatment (Fig. 2). After 27 days of treatment at 25°C, *P. canina* var. *praetextata* was unaffected and its rates of net photosynthesis and respiration were consistent with previously reported values (*5*). After 27 days, the 45°C storage treatment has reduced the rate of net photosynthesis to 20 percent of the control but without any concurrent changes in respiration rate (Fig. 2a). The data for *P. canina* var. *rufescens* (Fig. 2b) show clearly that the rates of net photosynthesis and respiration are unaffected by the storage treatment, closely paralleling the nitrogenase response.

The observed field temperature regime to which these two *Peltigera* populations were exposed correlated closely with their contrasting thermal sensitivity. Thallus temperatures in the two diverse habitats, measured simultaneously with embedded microthermocouples, showed marked differences. Thallus temperatures for *P. canina* var. *praetextata* in the woodland under full canopy conditions remained below 30°C for most of the day (Fig. 3a). In the early evening, thallus temperatures were for a brief period slightly in excess of 30°C as a result of sunfleck activity. In contrast, thallus temperatures for *P. canina* var. *rufescens* on the adjacent roadside rose rapidly and tissue temperatures in excess of 60°C were recorded (Fig. 3b). These temperatures are typical of values reported for equivalent habitats in temperate climates (*6*).

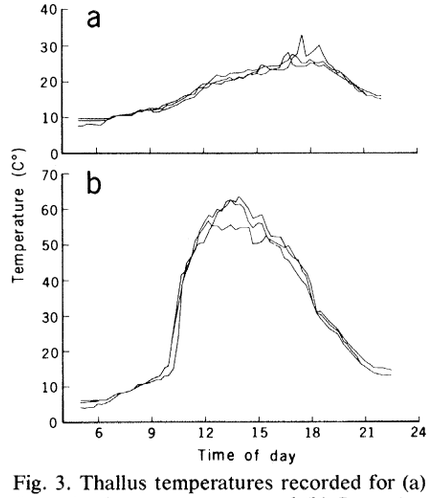

Fig. 3. Thallus temperatures recorded for (a) *P. canina* var. *praetextata* and (b) *P. canina* var. *rufescens* on 22 June 1977 for three replicate thalli.

The idea that all lichens are very resistant to high temperatures when desiccated must be rejected. We have shown that the degree of thermal stress correlates exactly with the ecology of these two *Peltigera* populations, and this correlation would appear to be causal. Certainly, the temperature treatment that was used was relatively moderate in light of the field extremes that were encountered. Lange (*2*) in fact suggested that thermal sensitivity could be a determining factor in the distribution of entire lichen communities (*7*), but his tentative comments have very largely been ignored. The tendency to ignore his remarks may have been due to the unrealistic temperature treatments he used (up to 100°C); these temperatures are never found in a field situation. In the earlier studies, the rate of respiration was used to assess the degree of heat stress. However, the data presented here show that in fact considerable damage can occur to algal photosynthesis and nitrogenase rates without any change in the largely fungal respiration rate. It is probable that more excessive stress treatments would be required before respiration was affected, and this need for greater stress before respiration is affected explains why Lange only demonstrated damage at unrealistic temperatures (*2*). The nature of the damage due to heat stress is obscure but appears to be associated with extensive rupturing of algal cell membranes in *P. canina* var. *praetextata*. Leaching of green pigments occurs immediately during subsequent hydration.

We have reported low summer rates of nitrogenase activity in *Peltigera* (*3*). We interpreted these lower rates as a response to a hot dry summer, and the data presented here adequately confirm this observation. The low summer rates subsequently returned to normal values by early winter; this observation points to a potential for recovery from natural levels of stress. Provisional data for other lichen species of the genera *Peltigera* and *Stereocaulon* (*8*) suggest, however, that arctic and subarctic lichens may be significantly more sensitive to heat stress than temperate varieties. Thus thermal sensitivity may be one of the dominant

parameters in the ecology of lichens and may equally be important for other poikilohydric plants.

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Analysis of Melatonin in Human Plasma by Gas Chromatography Negative Chemical Ionization Mass Spectrometry

Abstract. *Three techniques have been used to measure human plasma melatonin: bioassay, radioimmunoassay, and gas chromatography–mass spectrometry (GC-MS). GC-MS is theoretically capable of the greatest specificity, but in general suffers from insufficient sensitivity. Negative chemical ionization, a new technique, provides a 150-fold increase in GC-MS sensitivity for electron-capturing compounds. Negative chemical ionization GC-MS permits routine measurement in human plasma of melatonin at a concentration as low as 1 picogram per milliliter.*

Measurement of melatonin in human plasma has become possible only within the last few years (*1–10*). Extremely low circulating concentrations (1 to 10 pg/ml or 1 to 10 parts per trillion) permit measurement by only three techniques: bioassay (*4, 5*), radioimmunoassay (RIA) (*3, 6–9*), and gas chromatography–mass spectrometry (GC-MS) (*10*). Quantification at parts per trillion contends with problems of contamination and nonspecific interference. Theoretically, GC-MS offers the greatest specificity since quantified substances are simultaneously identified (by retention time and molecular weight of fragment ions). Newer GC-MS instrumentation offers very high sensitivity for the detection of trace organic compounds; however, when used for quantification of endogenous compounds in a biological matrix, problems of increased chemical background and irreversible adsorption phenomena limit application of these methods to parts per billion (ng/g). A new technique, negative chemical ionization (CI) mass spectrometry, provides the requisite sensitivity and specificity to measure melatonin at concentrations present in human plasma.

The potential sensitivity of negative CI GC-MS has been demonstrated by Hunt (*11*) who showed a 10- to 100-fold sensitivity enhancement for electron-capturing compounds. Appropriately derivatized organic compounds undergo efficient ionization by resonance capture of a near thermal energy electron in an exothermic process. Excess energy is dissipated by fragmentation or collisional stabilization. Cyclic derivatives or polycyclic compounds generally yield structurally specific anions, whereas acyclic derivatives with good anion leaving groups (such as perfluoracyl derivatives of aliphatic alcohols) generate only reagent specific ions.

Quantification of biological compounds in picogram and femtogram amounts required special precautions. Reagents were of high purity (*12*). Glassware was acid washed and silanized with hexamethyldisilazane in a heated vacuum desiccator (*13*).

Blood was collected with the use of silanized glassware and stainless steel needles with heparin or EDTA as anticoagulant and immediately centrifuged at 5000 rev/min at 4°C. Samples of plasma could then be assayed either directly or frozen for subsequent analysis. To 1 ml of plasma was added a tetradeuterated internal standard consisting of 15 to 40 pg of *N*-acetyl-5-methoxy-[$\alpha,\alpha,\beta,\beta$,-$D_4$]tryptamine synthesized by Shaw *et al.* (*14*), which extracts, derivatizes, and chromatographs with nondeuterated melatonin. Deuterated melatonin was also added to saline standards containing 1 to 200 pg of nondeuterated melatonin. The ratio of nondeuterated melatonin (endogenous or saline standard) to deuterated melatonin (internal standard) corrected for recovery (which could vary as much as fivefold).

To 1 ml of plasma or saline standard (containing internal standard) was added an equal volume of 0.5*M* borate buffer

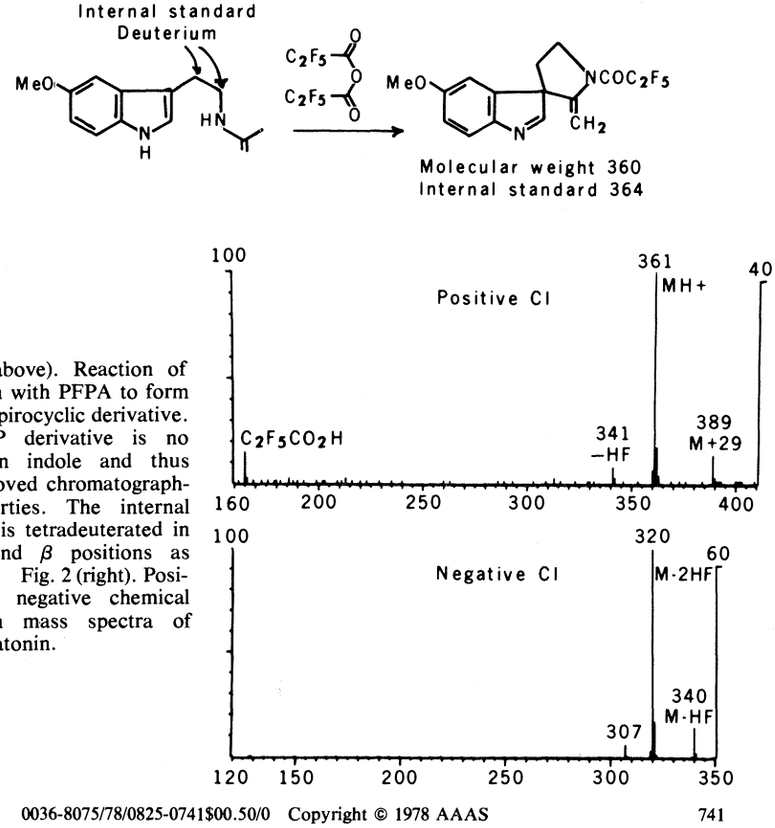

Fig. 1 (above). Reaction of melatonin with PFPA to form a stable spirocyclic derivative. The PFP derivative is no longer an indole and thus has improved chromatographic properties. The internal standard is tetradeuterated in the α and β positions as shown. Fig. 2 (right). Positive and negative chemical ionization mass spectra of PFP-melatonin.

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