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Impact-induced microbial endolithic habitats

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Abstract–Asteroid and comet impacts on Earth are commonly viewed as agents of ecosystem destruction, be it on local or global scales. However, for some microbial communities, impacts may represent an opportunity for habitat formation as some substrates are rendered more suitable for colonization when processed by impacts. We describe how heavily shocked gneissic crystalline basement rocks exposed at the Haughton impact structure, Devon Island, Nunavut, Arctic Canada, are hosts to endolithic photosynthetic microorganisms in significantly greater abundance than lesser-shocked or unshocked gneisses. Two factors contribute to this enhancement: (a) increased porosity due to impact fracturing and differential mineral vaporization, and (b) increased translucence due to the selective vaporization of opaque mineral phases. Using biological ultraviolet radiation dosimetry, and by measuring the concentrations of photoprotective compounds, we demonstrate that a covering of 0.8 mm of shocked gneiss can provide substantial protection from ultraviolet radiation, reducing the inactivation of *Bacillus subtilis* spores by 2 orders of magnitude. The colonisation of the shocked habitat represents a potential mechanism for pioneer microorganisms to invade an impact structure in the earliest stages of post-impact primary succession. The communities are analogous to the endolithic communities associated with sedimentary rocks in Antarctica, but because they occur in shocked crystalline rocks, they illustrate a mechanism for the creation of microbial habitats on planetary surfaces that do not have exposed sedimentary units. This might have been the case on early Earth. The data have implications for the microhabitats in which biological signatures might be sought on Mars.

INTRODUCTION

During the collision of an asteroid or comet, target materials can experience temperatures exceeding several thousand degrees centigrade and pressures of tens of gigapascals (Melosh, 1989). The shock processing of target material in this way is likely to have an important influence on the subsequent nature of biological colonization. This is particularly the case for microbial communities, which usually dominate lithic habitats.

Lithic habitats are important in polar deserts. The extreme physical conditions associated with these environments makes vegetation cover low and makes the lithic habitat one of the few refugia available to life to gain a foothold. An impact structure in a polar region that is dominated by microbial processes and has a low diversity of higher life, therefore, provides an ideal laboratory with which to investigate how impact processing affects the suitability of target rocks for microbial colonization.

In environments that experience extreme temperatures, desiccation, and ultraviolet radiation fluxes sufficient to cause damage to important biomolecules such as DNA, micro-organisms can obtain some degree of protection by colonizing the underside of rocks as "sublithic" organisms (*e.g.*, Berner and Evenari, 1978; Broady, 1981a,b), colonizing cracks in rocks as "chasmolithic" organisms (*e.g.*, Broady, 1981b) or by colonizing the inside of the rock matrix as "endolithic" organisms (*e.g.*, Friedmann and Ocampo, 1976; Friedmann, 1982). These habitats are more protected than the exposed surface of rocks, which are inhabited by "epilithic" organisms.

Because invasion of the interior of the rock matrix depends upon an entry point and available surfaces within the rock, which are primarily determined by inter-grain spaces and micro-fractures,

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the endolithic habitat requires more specific geological conditions compared to the requirements of living in macroscopic cracks or under rocks.

By absorbing solar radiation and buffering against the rapid temperature changes caused by wind, the inside of rocks can provide a thermally more favourable environment than that experienced outside the rock (McKay and Friedmann, 1985). The rock substrate might attenuate damaging ultraviolet radiation. However, it will also attenuate light in the visible region between 400 and 700 nm required for photosynthesis (Nienow *et al.*, 1988), so that many of the organisms, if they are photosynthetic, possess low-light compensation points (the light level at which net photosynthetic production can occur) (Vestal, 1988). The depth of penetration of photosynthetically active radiation (PAR) is therefore an important factor for the success of endolithic colonization. This is the case for colonization of the Beacon sandstones of the Ross Desert in Antarctica (Nienow *et al.*, 1988). The nature of the light and nutrient gradient in the Beacon sandstones results in complex communities with a vertical zonation of layers; in some cases different zones are dominated by pigmented fungi, non-pigmented fungi and algae (Friedmann, 1982; Nienow and Friedmann, 1993).

As well as in the Antarctic, microbial communities have been found inhabiting the underside of quartz rocks in hot deserts, such as those of the Middle East. In hot deserts, living under or within rocks can improve access to moisture from dew (Berner and Evenari, 1978) and, like the Antarctic, it is likely to provide some protection against photoinhibition and DNA damage caused by high visible light and/or ultraviolet radiation.

In this study we describe the colonization by photosynthetic microorganisms of gneisses that were shocked by an asteroid or comet impact ~23 Ma ago in what is today a polar desert region of the Canadian High Arctic.

MATERIALS AND METHODS

Field Site

The Haughton impact structure is a well-preserved complex crater located on Devon Island, Nunavut, Canadian High Arctic, at 75°22' N, 89°41' W (Grieve, 1988). The structure was formed 23.4 ± 1.0 Ma ago near to the Oligocene–Miocene boundary (Jessberger, 1988). The target rocks at Haughton comprise a ~1750 m thick series of Lower Paleozoic sedimentary rocks comprising mostly carbonates (dolomite and limestone), overlying a basement of Precambrian granites and gneisses. By geophysical criteria, the structure has a diameter of ~24 km (Pohl *et al.*, 1988; Scott and Hajnal, 1988). The crater is filled with a series of conspicuous grey-weathering carbonatitic (*i.e.*, carbonate-rich) impact melt rocks (Osinski and Spray, 2001). The presence of basement gneisses in the melt rocks indicates that the excavation depth was >1750 m. Impact melt rocks still cover much of the crater's interior, a notable exception being an ~7 km^2 area made up of paleolacustrine sediments which were laid down on top of the melt rocks in a crater lake(s) which formed following the impact event (Frisch and Thorsteinsson, 1978; Roberston and Sweeney, 1983; Hickey *et al.*, 1988; Whitlock and Dawson, 1990). In the extreme eastern part of the crater, impact melt rocks are found as discrete outcrops separated from the main deposits in the central part of the crater by a complex system of broad (up to ~1 km wide) alluvial terraces associated with meanders of the Haughton River. Fragments of basement gneiss shocked to different levels are common within the impact melt rocks.

Biologically, most of the crater shows typical polar desert characteristics with vegetation cover <5%. The vegetation cover on the impact melt rocks is lower than that of the alluvial terraces (Cockell *et al.*, 2001). Like other sites on Devon Island the soils of the Haughton region are primarily dolomitic and nutrient poor (*e.g.*, Walker and Peters, 1977; Lévesque and Svoboda, 1995; Bliss *et al.*, 1994). The low biological productivity is further exacerbated by the climatic conditions. Devon Island lies within region IVa of Maxwell's (1981) climatic regions of the Canadian Arctic Archipelago and experiences frigid winters with 24 h of darkness and short, cool summers.

Selection of Samples

The gneisses now exposed in the impact melt formation were located at ~1.7 km depth at the time of impact and exhibit a variety of shock levels ranging from negligible to high (>60 GPa). The closest outcrops of gneissic bedrock unaffected by the Haughton impact event occur at present 70 km to the east of the impact structure, in the lower portions of deep glacial trough valleys at Sverdrup Inlet. The unshocked basement gneisses of Devon Island include metatonalitic and metagranitic gneisses with intercalated metasedimentary rafts (Frisch, 1983). These gneisses exhibit pervasive granulite facies metamorphism with local retrogression to amphibolite facies. Unshocked gneisses from southeastern Devon Island yield U-Pb zircon isochron ages of $2518\,^{+56}_{-33}$ Ma (Frisch and Trettin, 1991).

The samples of shocked gneiss used in this study were obtained from two locations in the crater (Fig. 1). The first was an isolated hill of impact melt rocks at 75°24.53' N, 89°49.76' W. At this location, only gneiss that was shocked to pressures greater than ~10 GPa was found. Samples were also collected from an escarpment ("Bruno escarpment", 75°23.9' N, 89°31.6' W) of melt rocks on which gneisses of varying shock were found including gneiss shocked to pressures <10 GPa (referred to herein as "low-shocked" rocks).

Thirty samples of rock shocked to greater than ~20 GPa and 30 samples of rock shocked at less than ~10 GPa were collected. The low-shocked group of rocks were defined by the presence of regular bands of amphiboles and their distinctive

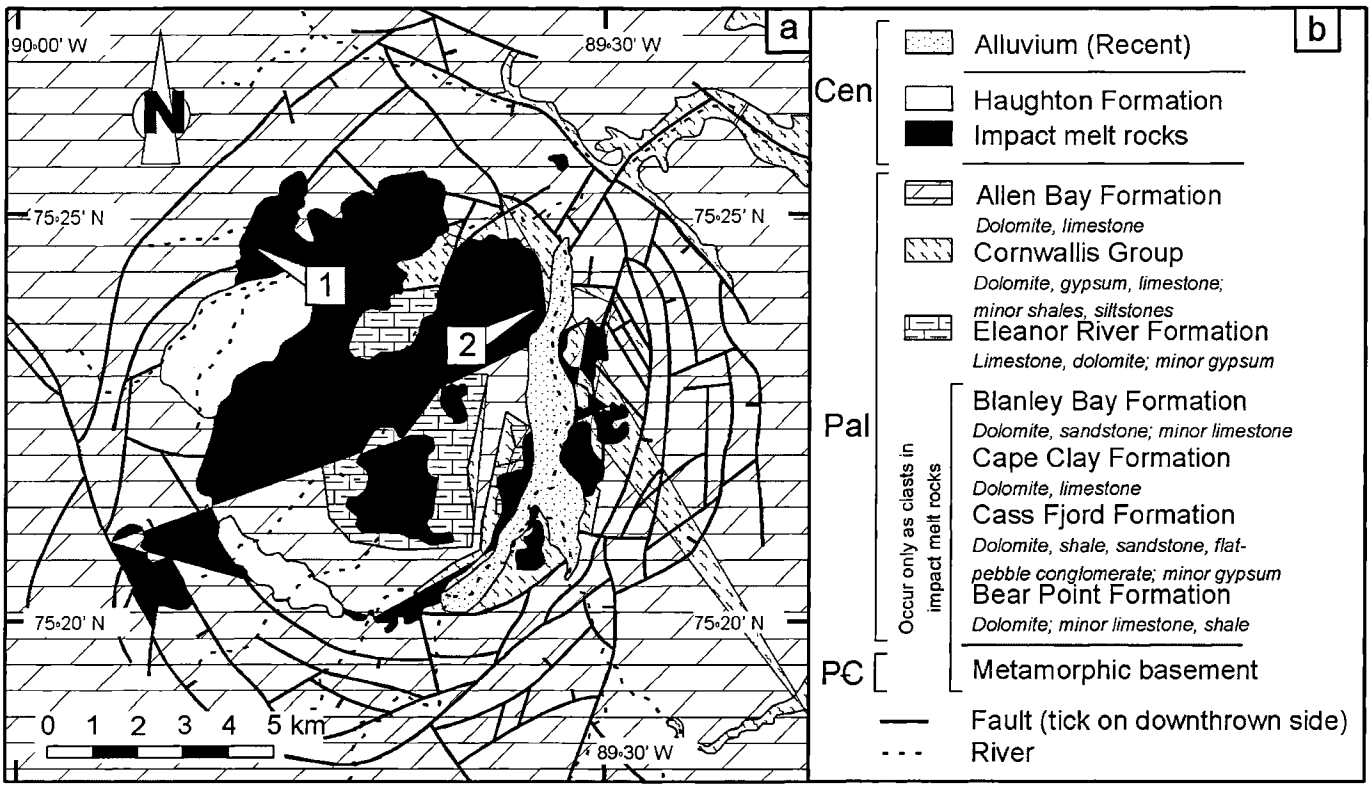

FIG. 1. Map of the Haughton impact structure showing major geologic features. Point "1" is the location of samples collected on the isolated melt-rock outcrop near Trinity Pond. Point "2" is the location of samples collected at "Bruno" escarpment (see "Methods").

black coloration. This is in contrast to the more highly shocked rocks, which have white/grey coloration primarily because of the melting of mafic minerals and the migration of the melts to free surfaces (cf., Bunch *et al.*, 1998) as shown in Fig. 2. These more highly shocked gneisses often have a pumice-like texture. All of these rocks had dimensions less than 15 × 15 × 15 cm. The gneisses were broken open with a rock hammer *in situ* into several pieces or they were cut open using a rock saw (Gryphon Corporation, Burbank, California, USA). The presence of photosynthetic microbial colonization (endolithic or chasmolithic) was recorded for each sample. "Endolithic" colonization was recorded for coherent bands of minimum length 1.0 cm in the subsurface parallel to the surface of the rock. "Chasmolithic" colonization was recorded for colonization of macroscopic cracks into the rock or colonization of a well-developed weathering crust.

Bulk Density and Porosity

The bulk density of the gneisses was measured by preparing small (~1 cm^3) blocks. Each piece was weighed and then wrapped in watertight plastic Saran Wrap™ (S.C. Johnson and Son, Inc., Brantford, Ontario, Canada). The blocks were immersed in a measuring cylinder and the displacement of the water was measured. The bulk density was calculated by dividing the mass by the observed volume displacement. This was repeated for seven samples each of the highly shocked and the low-shocked gneisses.

The size distribution of pores was determined by high-pressure mercury intrusion by MCA Services (Cambridge, U.K.) according to previously described methods (Gregg and Sing, 1982). Four samples of each group were examined. The total pore surface area for pores of different size classes was calculated with the assumption that pores are spherical.

Microscopy

Photosynthetic microorganisms were carefully scraped from the rocks using a sterile blade and deposited onto the surface of a slide. Approximately 20 μL of sterile water was added to the sample. A cover slip was placed over the sample and the slides were examined under visible microscopy using an Olympus BX-51 universal microscope (Olympus America Inc, Melville, New York, USA). The presence of phycocyanin (visualized as an orange-red color) was examined with a mercury-arc lamp epifluoresence assembly attached to the microscope with an excitation filter transmitting at 530–560 nm and an emission filter transmitting at >580 nm. Images were recorded using an E-10 Olympus digital camera.

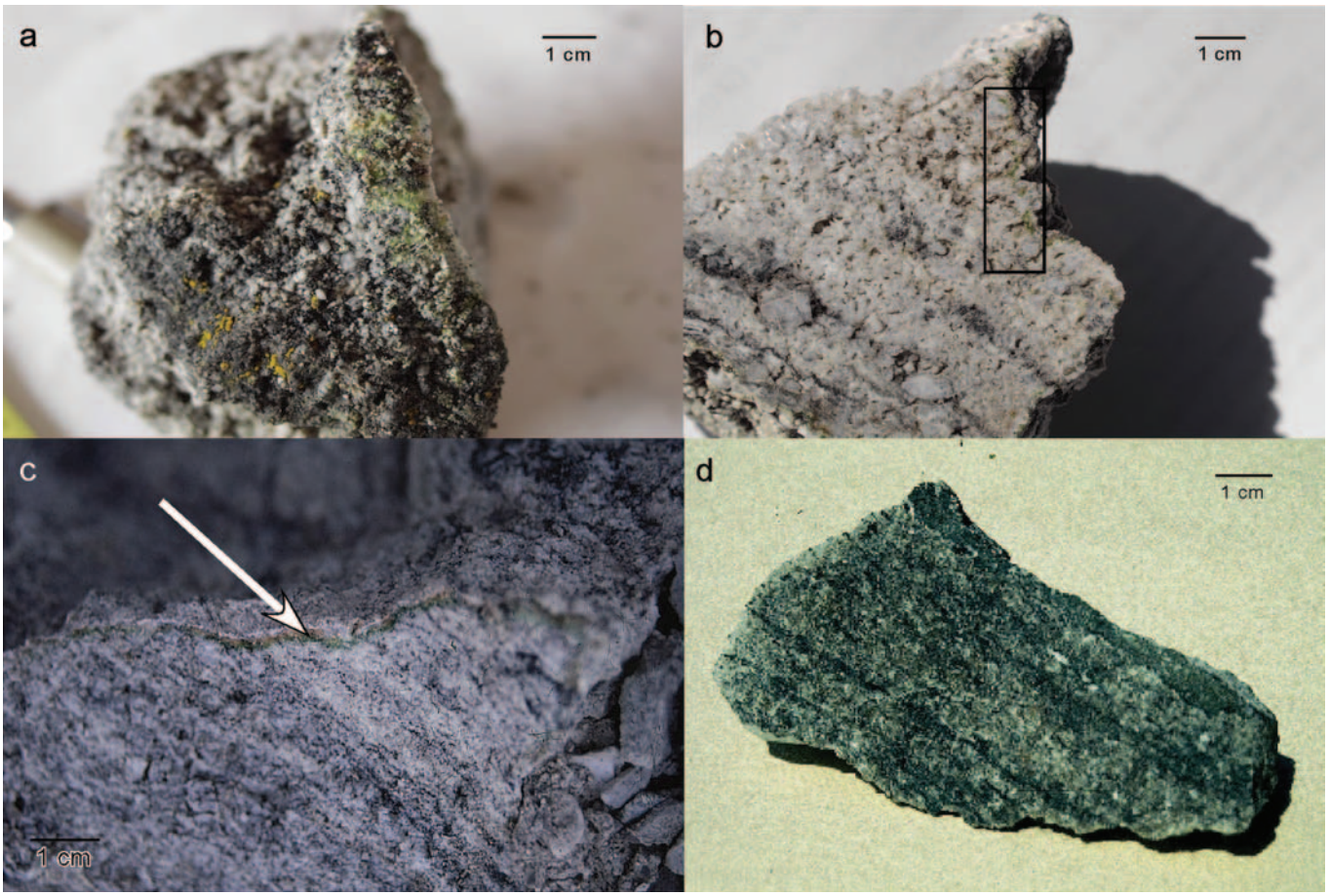

FIG. 2. Cyanobacteria associated with shocked gneiss. (a) Microorganisms colonizing the weathered crust of shocked gneiss. The microbes are found just beneath the crust, which can often be removed by carefully scrapping the rock surface. (b) True endolithic colonization of shocked gneiss shown as the banded region at the top of the rock. The microorganisms have invaded the subsurface and there is evidence of lateral growth through the rock substratum to a depth of ~2 mm. (c) In some rocks colonization is extensive enough to result in a coherent band of microbial growth (white arrow) similar to the bands observed in sedimentary Antarctic rocks such as sandstones. (d) Low-shocked gneiss (<10 GPa) illustrating darker colouration and presence of banded amphiboles.

Samples were prepared for scanning electron microscopy (SEM) by fracturing the rocks so that small (<0.5 cm^3) blocks were made with one surface possessing the community of interest. They were mounted on aluminium plates and gold coated to a thickness of 10–30 nm. The samples were examined under vacuum (<2 × 10^6 Torr) in a Stereoscan 360 scanning electron microscope (Cambridge Instruments, Cambridge, U.K.). Images were digitally stored.

Temperature Measurements

Ground surface temperatures in the Bruno escarpment location were measured over 1 year between 1999 July 25 and 2000 July 28 using a Hobo™ data logger and an external thermistor (Hobo™ TMC6-HB temperature sensor). The measurement location was at the base of the escarpment ~50 m from where the shocked gneisses were collected. Measurements were taken every 2 h.

Measurement of Ultraviolet Radiation and Concentrations of Ultraviolet-Screening Compounds

Penetration of ultraviolet radiation into the shocked rocks was measured using the Deutsches Zentrum für Luft- und Raumfahrt: Biofilm or DLR-Biofilm (Horneck *et al.*, 1996; Quintern *et al.*, 1992; Rettberg *et al.*, 1999). This dosimeter uses the inactivation of a monolayer (~5 × 10^5 spores cm^{-2}) of *Bacillus subtilis* spores to measure the biological effect of ultraviolet radiation. After exposure to ultraviolet radiation the biofilms are incubated in nutrient broth and the protein that is produced by the growing microorganisms (those that were not inactivated) is stained using Coomassie blue dye (Quintern *et al.*, 1992). The absorbance at 590 nm, determined by image analysis, provides a measure of the inactivation of the spores. The inactivation of the spores is expressed as an effective irradiance at 254 nm (*i.e.*, the dose from a laboratory 254 nm ultraviolet radiation source required to produce the same

inactivation as that seen in the field on a calibration biofilm). The sheets of *Bacillus subtilis* are protected by a thin layer of ultraviolet transparent Whirlpak® (Nasco, Ft. Atkinson, Wichigan, USA) plastic and have dimensions 1 × 2 cm. The biofilms conform to the principal of reciprocity, whereby response is dependent upon total dose, not irradiance (Quintern *et al.*, 1992). The method provides data on the integrated ultraviolet exposure over hours or days. The use of this biofilm in estimating the penetration of ultraviolet radiation into ice and snow covered microhabitats has been described previously for Antarctic locations (Cockell *et al.*, 2002).

Sections of shocked gneiss of varying thickness were prepared using a rock saw (Gryphon Corporation, Burbank, California, USA) and the thicknesses were determined by taking four readings of the thickness in different parts of the section using Vernier callipers. DLR-Biofilms were placed under the rock sections and the sections were then placed onto a piece of wood. Black ultraviolet opaque sealant was used to seal the edges of the sections so that no ultraviolet radiation could penetrate to the dosimeters except through the rock sections themselves. The sections were placed outside in an unshaded location for 10 days (2001 July 18 to 28) and then they were retrieved. Fully exposed control dosimeters were run alongside the sections for 4 days. Each day two exposed control dosimeters were retrieved. The dosimeters were processed as described previously (Quintern *et al.*, 1992).

To study the biomolecular characteristics of the organisms, we selected rocks from which they could be scraped from the underside of the weathering crust. It was difficult to remove sufficient biomass from endolithic communities within the rock matrix. We consider the measurements we make with organisms in the weathering crust to be representative of the biomolecular characteristics of the endolithic organisms. Organisms from an area of 1 cm^2 were removed with a sterile blade from four independent communities. We also carried out an identical analysis on four 1 cm^2 samples of epilithic communities on the surface of shocked gneisses. The material was gently homogenized with a glass rod in 4 mL of 90:10 acetone:water (acetone from Sigma Chemicals, St Louis, Missouri, USA) in a 15 mL centrifuge tube. It was left for 2 h in the dark at 4 °C. Further extractions did not increase the absorbance of the supernatant by more than 10%. The samples were centrifuged to remove debris and the supernatant was removed into a new tube.

Three measurements were of importance to our study. (1) Concentrations of the cyanobacterial UV-B (280–315 nm) and UV-A (315–400 nm) screening compound, scytonemin. This compound is synthesized in the sheaths of cyanobacteria in response to ultraviolet radiation exposure. Concentrations of scytonemin in laboratory-grown organisms are known to be correlated to ultraviolet exposure (Garcia-Pichel *et al.*, 1992) and in certain organisms there is field evidence for this correlation as well (Pentecost, 1993). (2) Concentrations of carotenoids, which are synthesized as ultraviolet-quenching compounds that mitigate reactive oxygen species resulting from ultraviolet radiation and high light exposure (Ehling-Schulz *et al.*, 1997) and (3) Concentrations of the light harvesting compound, chlorophyll a (Chl a), required for cyanobacterial photosynthesis.

The absorbance of the supernatant was measured at 384 nm for scytonemin, 490 nm for carotenoids and 663 nm for Chl a in a Unicam UV1 ultraviolet/visible spectrophotometer (Unicam Ltd., Cambridge, U.K.). Absorbances were converted to concentrations ($\mu g/cm^2$) using molar extinction coefficients of 112.6 for scytonemin (Garcia-Pichel *et al.*, 1992), 250 for carotenoids (Britton, 1985) and 102.6 M^{-1} cm^{-1} for Chl a (Büdel *et al.*, 1997). The ratio of Chl a to the combined concentration of scytonemin and ultraviolet-quenching carotenoids (total photoprotective compounds) was also calculated. These techniques are similar to those described previously for polar cyanobacteria (Quesada *et al.*, 1999).

Measurement of Photosynthetically Active Radiation

PAR (400–700 nm) levels in the field were measured using a Quantum meter (Apogee Instruments, Logan, Utah, USA) with the sensor held in the desired orientation to obtain a reading in $\mu mol/m^2/s$.

Light penetration through rock sections was measured by cutting three sections of 0.5 mm thickness from both groups of gneisses. They were mounted on 1 mm thick clear glass slides of dimensions 2.5 × 4.8 cm using Epo-Tek 301 epoxy (Epoxy Technology, Inc, Billerica, Massachusetts, USA), which is transparent to light above 300 nm. The irradiance between 400 and 700 nm at 1 nm intervals was measured through a 4 mm diameter cosine corrected collector at the end of a 1 m fibre optic cable attached to an S2000 Avantes spectroradiometer (Anglia Instruments, Ely, U.K.). The collector was fixed in an upright position in a clamp. Around the edge of the collector a small amount of dried opaque sealant was fixed so that slides could be pressed down onto the sealant to prevent stray light from the sides entering the collector. Thus, only light passing through the slide was measured. On a clear day when incident light levels were constant over an interval of several minutes, three control scans were obtained of the solar spectrum under a glass slide. Immediately afterwards the three rock sections from shocked and low-shocked gneiss were placed over the collector with the rock section facing down on the collector and spectral scans were obtained. A control scan was taken again to verify the constancy of the solar spectrum during the measurements. The means of the scans at 1 nm intervals were calculated.

RESULTS

Density and Porosity

The density of the group of shocked gneiss fragments was 1.17 ± 0.24 g/cm^3. The density of the low-shocked gneisses was 2.61 ± 0.17 g/cm^3. The total pore surface area for pores

with a diameter >1 μm was 0.10 ± 0.057 m^2/g for the highly shocked samples and 0.004 ± 0.0035 m^2/g for the low-shocked samples (Table 1).

Microorganisms in the Lithic Habitat

In 73% of the shocked rocks (22 of the samples), chasmolithic or endolithic colonization was observed. In eight (26%) of these samples a coherent endolithic band (>1 cm in length) of subsurface colonization was observed. In only 3% (one case) of the low-shocked samples was any type of colonization observed and this was found as growth in a weathering crust. Most low-shocked gneisses did not possess a weathering crust that could offer a habitat for colonization (see Fig. 2a). A thin (~1 mm) crust was much more developed on more highly shocked gneiss.

Endolithic colonization was often confined to patches of growth, where invasion and subsequent limited lateral spread into the subsurface occurs (Fig. 2b). However, in some rare cases the spread of the microorganisms in the subsurface environment resulted in a more coherent band of colonization (Fig. 2c), similar to the uninterrupted bands of microbial colonization observed in Beacon sandstones of Antarctica (Friedmann, 1982).

We also observed colonization of the underside and surface of many shocked and unshocked rocks, although we did not quantify the extent because this study was focused on the endolithic habitat generated by the impact. The genera identified on the underside and surface of the rocks, which included *Chroococcidiopsis*, *Aphanothece*, *Gloeocapsa* spp. and some unicellular chlorophytes (Fig. 3), were identical to those observed in the sublithic and epilithic habitat of dolomites and other unshocked rocks near the impact structure. Because this type of colonization is not specific to the shocked rocks, we do not discuss it in further detail here, but we note that it is an important part of the inventory of microbial growth on the shocked rocks.

The microorganisms found in the chasmolithic and endolithic habitat of shocked gneiss were identified as *Chroococcidiopsis* spp. (Fig. 3a,b). Because of the similar morphology of members of this genus, identification of different species or strains is not possible without genetic analysis. We did not observe the same diversity of microorganisms that we found in the sublithic and epilithic habitats described above. Different types of growth form were found, which included solitary cells and colonial growth forms with many cells (over 100 in some instances) contained in a mucilaginous sheath. The morphology of these colonial growth forms was usually spherical, but irregular banded formations were also observed. The organisms were visualized under epifluorescence (Fig. 3a), demonstrating the presence of phycocyanin, expected in cyanobacteria.

Under SEM, the differences between gneisses of different shock levels are more clearly visualized. In Fig. 4 the surface of relatively low-shocked samples is revealed to be coherent with few access points for microbial invasion, whereas extensive fractures and pitting are evident in the more highly shocked gneisses. An example of the growth of a solitary *Chroococcidiopsis* sp. within these impact-induced fractures visualized by SEM is shown in Fig. 4c.

Temperatures in the Shocked Environment

During the period 1999 July 25 and 2000 July 28, when ground temperatures were measured at Bruno escarpment, there were 19 days on which a diurnal freeze–thaw cycle was observed and 17 of these occurred between 1999 August 22 and September 9, at the end of the summer. The other 2 days of freeze–thaw occurred on 2000 June 28 and 29 in the following spring. The lowest temperature recorded was –26.1 °C, which occurred in the first week of March 2000. The highest temperature was 23.6 °C on 1999 August 12. The mean ground temperature over this time period was –11.6 °C.

Penetration of Ultraviolet Radiation and Concentrations of Ultraviolet-Screening Compounds

A total of 14 rock sections had ultraviolet dosimeters sealed underneath them. They ranged from the thickest, 4.1 ± 0.06 mm, to the thinnest, 0.08 ± 0.02 mm, and a range of thicknesses in

TABLE 1. Typical density and light transmission properties of shocked (>20 GPa) and low-shock (<10 GPa) gneisses from the Haughton impact structure.

	Shocked rock (>20 GPa)	Low-shock rock (<10 GPa)
Density ($g\ cm^{-3}$)*	1.17 ± 0.24	2.61 ± 0.17
Pore surface area ($m^2\ g^{-1}$)†	0.10 ± 0.057	0.004 ± 0.0035
Light transmittivity (fraction of incident)‡	0.22 ± 0.02	0.02 ± 0.01

*Three samples of shocked and low-shock gneisses were examined.
†Total pore area (m^2) per g of sample for pores = 1 μm. Determined by mercury intrusion. Values are the mean of three samples.
‡Light transmittivity is the mean fraction of incident light at 680 nm transmitted through a thickness of 0.5 mm of rock.

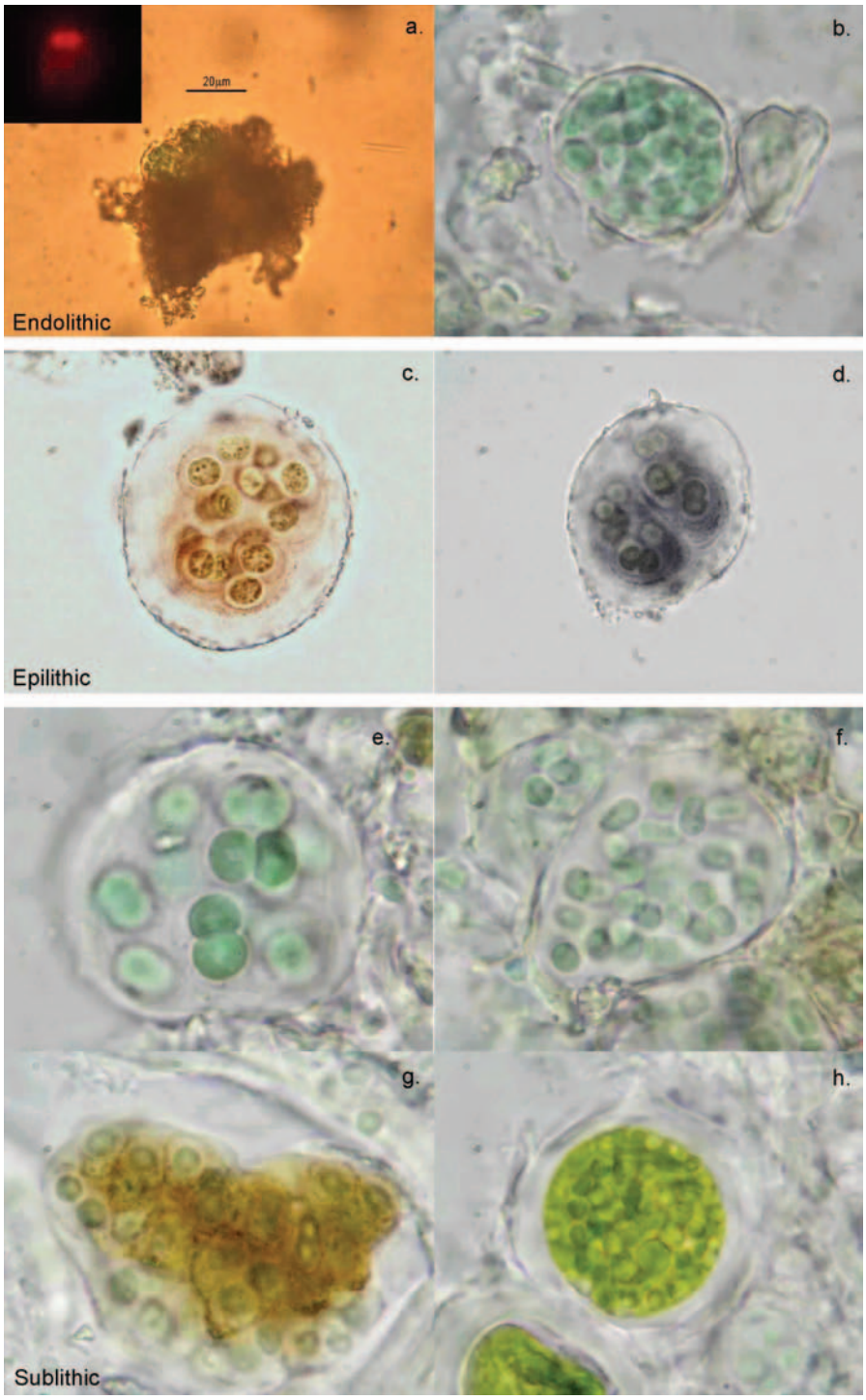

FIG. 3. Microorganisms associated with shocked rocks. (a) Association of two *Chroococcidiopsis* sp. colonial forms with a fragment of shocked gneiss exhibiting endolithic colonization (100×). The inset shows the ultraviolet epifluorescence image of the same fragment of rock. (b) *Chroococcidiopsis* sp. colony from an endolithic community magnified 2000×. Images (c–d) are microorganisms associated with the epilithic habitat of shocked and unshocked rocks in the impact structure. Note brown and black coloration caused by the presence of ultraviolet-screening compounds. (c) *Gloeocapsa sanguinea* (Agardh) Kützing (1000×). (d) *Gloeocapsa alpina* (Nägeli) Brand (1000×). Images (e–h) are microorganisms associated with the sublithic habitat of shocked and unshocked rocks in the impact structure. (e) *Gloeocapsa* cf. *atrata* Kützing (2000×). (f) *Gloeocapsa* cf. *punctata* Nägeli (2000×). (g) *Gloeocapsa* cf. *kuetzingiana* Nägeli (2000×). (h) unicellular chorophyte (2000×). (Image (a), Charles Cockell; images (b–h) Paul Broady).

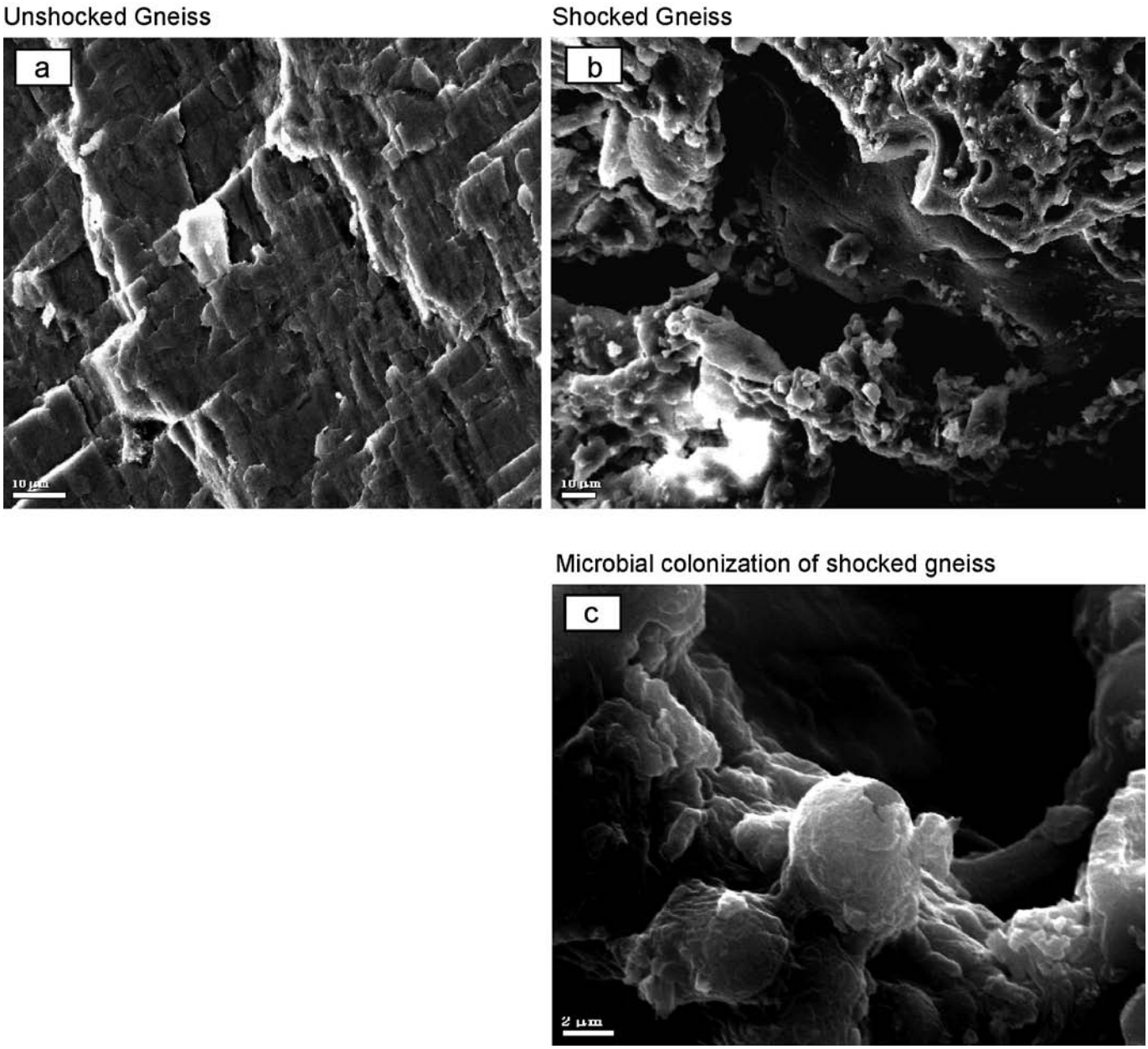

FIG. 4. Scanning electron microscopy of the broken plane of a sample of shocked and low-shocked gneiss. (a) Surface of low-shocked gneiss that exhibits a flat texture through which microbial colonization is impaired. (b) Sample of shocked gneiss. The fractures and cavities induced by shock are evident. (c) SEM micrograph of a solitary *Chroococcidiopsis* growing within an impact-induced fracture.

between. Only the dosimeter under the thinnest section showed spore inactivation to a value of 91 J/m^2 (E_{254}) after 10 days. Under the other sections, including the next thinnest (1.2 ± 0.02 mm) there was no measurable spore inactivation after 10 days. After 6 h the uncovered control dosimeters had a mean exposure of 110 J/m^2 (E_{254}) and after 24 h of exposure the control dosimeters were over-exposed (all spores were killed) as were all control dosimeters left outside beyond the first day of the experiment.

The concentrations of scytonemin, carotenoids and Chl a were 3.3 ± 11%, 0.93 ± 28% and 2.7 ± 3% μg/cm^2, respectively, in the four shocked rock weathering crust communities we examined. The mean ratio of Chl a to the combined concentration of carotenoids and scytonemin was 0.64. In the four epilithic communities, the scytonemin concentration was 27.0 ± 57% μg/cm^2, with a mean value 8.2× greater than the weathering crust communities. The carotenoid concentration was 7.8 ± 55% μg/cm^2, with the mean value 8.4× higher than

in the weathering crust communities. The concentration of Chl a was 2.0 ± 52% μg/cm^2, with the mean value 25% lower than in the weathering crust communities. In the epilithic communities the mean ratio of Chl a to the combined carotenoid and scytonemin concentrations was 0.06. These differences were visualized in the macroscopic appearance of the communities. The epilithic organisms were a black/brown colouration and the endolithic and chasmolithic organisms had a green colouration because of the lower concentrations of ultraviolet-screening compounds (Fig. 3).

Penetration of Photosynthetically Active Radiation

At noon during mid-July we found typical PAR levels to be 1200 μmol/m^2/s on a horizontal surface on a clear day. On a south-facing surface at the same time they were 1600 μmol/m^2/s.

Light penetration through the low-shocked gneiss was more than an order of magnitude lower than the shocked gneiss (Fig. 5) at any given wavelength. At 680 nm, the wavelength of maximum Chl a absorbance, mean transmission in the unshocked gneiss through the 0.5 mm sections was 1.8% of incident and in the shocked gneiss it was 22% that of incident (Table 1). Attenuation was more effective at short wavelengths. Penetration of light at 400 nm was 39% of that at 700 nm in the shocked gneiss.

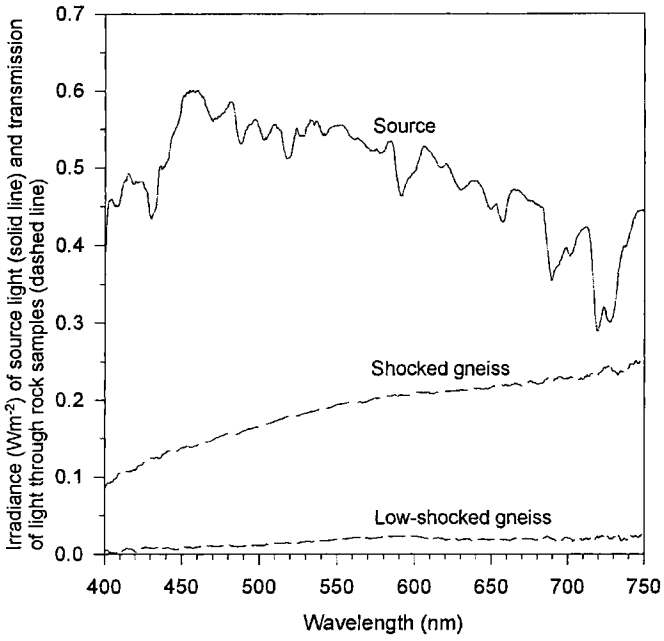

FIG. 5. Mean penetration of light through three 0.5 mm sections of shocked and low-shocked gneiss from Haughton. Upper solid curve shows source light. Dotted lines show the fraction penetration of light through rock sections. Low-shocked gneiss has substantially less penetration of light at all wavelengths examined compared to shocked gneiss.

DISCUSSION

During the asteroid or comet impact ~23 Ma ago on what is now Devon Island, the gneissic target rocks in the vicinity of the impact were shocked up to pressures of ~80 GPa (Bunch *et al.*, 1998), altering their physical characteristics and thus their suitability for subsequent invasion by microbial life.

We found that the cyanobacterial colonization of gneiss shocked to pressures >20 GPa during the impact event was greater than gneiss shocked to <10 GPa. We partly attribute this finding to the fact that the heavily shocked gneiss contains a greater number of fractures, observed under SEM, as well as a greater total pore surface area for pores >1 μm in diameter, both of which provide a habitat for microorganisms and facilitate access into the rock.

However, low-shocked gneiss does contain pore space that should be amenable to colonization. As we demonstrate here, as well as fractures that provide access for microorganisms to the subsurface spaces, another factor of importance for the colonization of the heavily shocked rocks is the higher penetrability of photosynthetically active radiation.

The minimum light required for photosynthesis, based on theoretical loses, is ~20 nmol/m^2/s (Raven *et al.*, 2000), with some variation either side of this value depending upon the organism considered and its mode of photosynthesis. If we take the mean fraction of incident light that penetrated to 0.5 mm depth and at 680 nm (the maximum absorbance wavelength for Chl a) and assume that light decays as a function of natural logarithm, then the diffuse attenuation coefficient at this wavelength is 3.03 mm^{-1} for the shocked gneisses. Using this coefficient we can calculate the depth that microorganisms would receive the minimum light for photosynthesis when the ambient light is 1200 μmol/m^2/s. This depth is calculated to be 3.6 mm, which is consistent with our observations that, except in rare cases, colonization did not occur at depths greater than ~5 mm into the gneisses.

In practice a number of factors vary the depth. Organisms higher in the community shade those lower down. This will reduce this maximum depth of penetration of light. In intact rocks coherent fractures connect to the surface and may help channel the light down (which is less the case in an artificially prepared rock slice) and this factor will increase the depth of penetration. Different shock levels in different rocks and within the same rock will cause light penetration to vary. In the context of these variables, we consider the similarity between the theoretical depth of penetration and the observed depth to suggest that the penetration depth of photosynthetic microorganisms into the more highly shocked gneiss is primarily determined by PAR.

The corresponding theoretical depth at which minimum light levels for photosynthesis occur in the low-shocked gneiss is 1.3 mm, showing that at a very shallow depth into the subsurface the light levels are insufficient to support endolithic colonization, although colonization of a porous weathering crust

is theoretically possible, which explains why we did observe this in a rare case.

The colonization of the rocks was heterogeneous. Although in some samples we did observe a coherent band of microorganisms inhabiting the subsurface as is observed in the sandstones of the Antarctic, these bands were rarely greater than ~1 cm long. This irregularity of colonization is caused by the heterogeneous nature of the substrate. The fractures and pores are not evenly distributed throughout the rock because the effects of the shock caused by the impact are not homogeneous. Bunch *et al.* (1998) showed that the shock characteristics in some gneisses from Haughton varied from ~30 to ~50 GPa over a distance of a centimetre. The microorganisms will colonize the fractures that are physically linked to the surface of the rock, leading to patches of microbial growth beneath the invasion point with some subsequent lateral invasion of the rock subsurface that will be influenced by the extent of shock.

As well as providing a habitat for life, the shocked rocks also provide an ameliorated microenvironment for the organisms compared to the extremes of living outside the shocked habitat. Ultraviolet radiation is known to be an important physical stressor for microorganisms living on the surface of rocks in the arctic (Quesada *et al.*, 1999). It has not previously been measured in the endolithic habitat. As we have shown, the overlying substrate provides substantial protection from ultraviolet radiation for microorganisms in the shocked habitat. Only in a rock section <1 mm thick did we measure *Bacillus subtilis* inactivation after 10 days. Greater inactivation occurred in spores after 6 h of exposure to ambient ultraviolet radiation. The ultraviolet exposure received by an organism with a *Bacillus subtilis*-like inactivation spectrum in the shocked habitat over the summer season (~60 days) might therefore be only slightly greater than the total dose received in 1 day of exposure to unattenuated ultraviolet radiation.

The effective attenuation of ultraviolet radiation, but the penetration of sufficient PAR for photosynthesis is helped by the fact that shorter wavelengths of light in the ultraviolet region are more easily scattered and attenuated than longer wavelengths of light in the PAR region. Even within the PAR region we found a measurably greater attenuation of light in the blue region compared to the red region of the spectrum.

The contention that during the 24 h photoperiod in the summer months microorganisms in these shocked lithic habitats are in an environment where much of the ultraviolet stress is attenuated is further supported by our observations of the lower mean concentrations of the cyanobacterial ultraviolet-screening compound, scytonemin, and ultraviolet-quenching carotenoids in the organisms in the interior of the rocks compared to the epilithic organisms.

The lithophytic communities of the shocked rocks on Devon Island are not as water-limited as the communities associated with the arid environment of the Antarctic Dry Valleys, where annual precipitation is <10 mm/year. Precipitation on Devon Island is approximately an order of magnitude higher than this (Walker and Peters, 1977). Transient water availability from snowmelt and air moisture at relative humidities >70% in the coastal regions of Antarctica has been postulated to be an important factor for the colonization of chasmolithic habitats in quartz stones (Broady, 1981b) and endolithic habitats in sandstones (Friedmann, 1978). On Devon Island during July and August, as well as receiving water from snowmelt, the shocked rocks are often saturated by rain. The greater availability of water during the summer season explains why, in contrast to the lack of epilithic growth in the Dry Valleys of Antarctica, we observe substantial epilithic growth on many rocks in Haughton.

The microorganisms in the lithic habitats at Haughton are less thermally stressed than those in the Antarctic. Gusts of wind were observed to cause rapid freeze–thaw cycles on the surface of rocks in the dry valleys (Ross Desert) of the Antarctic (McKay and Friedmann, 1985). Haughton's shocked rocks suffer freeze–thaw stress during the year, but this is confined to the beginning and end of the light season when metabolic activity would be expected to be low because of low temperatures and the concomitant low-light levels. As we show here, apart from 19 days at the beginning and end of the season (when we measured a diurnal freeze–thaw cycle), during most of the summer light season temperatures are above freezing during the diurnal cycle.

Because the endolith and chasmolith-containing shocked rocks do not constitute a large proportion of the crater surface and they are localised to some specific outcrops, their contribution to photosynthetic productivity in this region of Devon Island is probably not ecologically important. On the impact melt rocks there is between 0.02 and 3% vegetation cover depending upon local topography and location (Cockell *et al.*, 2001). The vegetation is likely to be a more important contributor to the primary productivity of the impact structure than the microorganisms in the shocked rock clasts. This is in contrast to the Ross Desert of the Antarctic, where the lack of vascular plant cover and widespread distribution of lichen-dominated endolithic communities can make them a significant contributor to primary productivity and ecosystem processes (Vestal, 1988; Friedmann *et al.*, 1993).

The climate of Devon has changed substantially since the early Miocene, when the region was below the treeline and inhabited by rabbits and rhinoceroids, as determined from fossil remains in the sediments of the Haughton impact lake(s) (Whitlock and Dawson, 1990). The microorganisms in the shocked rocks today may not represent the same taxa that colonized the rocks after the impact ~23 Ma ago. However, the present-day preferential colonization of the shocked gneiss in comparison to the unshocked or low-shocked gneiss shows how an impact event can create a habitat for microbial life. The *Chroococcidiopsis* spp. within the rocks show how, after an impact event, the sterile habitat generated by the intense heat and pressure of impact can potentially be invaded and

colonized by pioneer microorganisms in the earliest stages of primary succession. These results therefore demonstrate a specific mechanism for post-impact microbial primary succession.

Our findings also have two potential implications for life on the early Earth. First, the impact flux was much higher during the early Archean ~3.8 Ga ago (Chyba *et al.*, 1994) when life had arisen on Earth. Impact-shocked rocks might have provided a habitat for microorganisms on early landmasses long before sedimentary rocks were laid down or exposed. Second, as we show here, the shocked endolithic habitat provides a refuge from ultraviolet radiation. We do not know precisely the ultraviolet radiation climate on the Archean Earth. Before the build up of atmospheric oxygen, ultraviolet radiation might have been attenuated by sulphur, hydrocarbon smogs or other ultraviolet absorbers in the early atmosphere. However, in the absence of these screens ultraviolet radiation might have been much more intense before the formation of an ozone column, with DNA-weighted biologically effective irradiances some three orders of magnitude higher than today (Cockell and Horneck, 2001). Naturally available materials like shocked rocks would have been available to primitive microorganisms to protect themselves.

We recognize the potential exobiological implications of these observations. Because impact events are a universal process, planets that do not have sedimentary deposits might still offer lithic habitats for life in exposed shocked rocks, one such case being the planet Mars. The ability of shocked crystalline rocks to support endolithic communities suggests that a search for biomarkers in similar microhabitats might be a good approach to answering the question of whether life exists or ever existed on that planet.

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REFERENCES

BERNER T. AND EVENARI M. (1978) The influence of temperature and light penetration on the abundance of the hypolithic algae in the Negev Desert of Israel. *Oecologia* **33**, 255–260.

BLISS L. C., HENRY G. H. R., SVOBODA J. AND BLISS D. I. (1994) Patterns of plant distribution within two polar desert landscapes. *Arctic Alpine Res.* **26**, 46–55.

BRITTON G. (1985) General carotenoids methods. *Meth. Enzymol.* **111**, 113–158.

BROADY P. A. (1981a) The ecology of sublithic terrestrial algae at the Vestfold Hills, Antarctica. *Br. Phycol. J.* **16**, 231–240.

BROADY P. A. (1981b) The ecology of chasmolithic algae at coastal locations of Antarctica. *Phycologia* **20**, 259–272.

BÜDEL B., KARSTEN U. AND GARCIA-PICHEL F. (1997) Ultraviolet-absorbing scytonemin and mycosporine-like amino acid derivatives in exposed, rock-inhabiting cyanobacterial lichens. *Oecologia* **112**, 165–172.

BUNCH T. E., GRIEVE R. A. F., LEE P., MCKAY C. P., RICE J. W., SCHUTT J. W. AND ZENT A. (1998) Haughton-Mars 97-II: Preliminary observations on highly shocked crystalline basement rocks on the Haughton impact crater (abstract). *Lunar Planet. Sci.* **29**, #1307, Lunar and Planetary Institute, Houston, Texas, USA (CD-ROM).

CHYBA C. F., OWEN T. C. AND IP W-H. (1994) Impact delivery of volatiles and organic molecules to Earth. In *Hazards Due to Comets and Asteroids* (ed. T. Gehrels), pp. 9–58. Univ. Arizona Press, Tucson, Arizona, USA.

COCKELL C. S. AND HORNECK G. (2001) The history of the UV radiation climate of the Earth—Theoretical and space-based observations. *Photochem. Photobiol.* **73**, 447–451.

COCKELL C. S., LEE P. C., SCHUERGER A. C., HIDALGO L., JONES J. A. AND STOKES M. D. (2001) Microbiology and vegetation of micro-oases and polar desert, Haughton impact crater, Devon Island, Nunavut, Canada. *Arctic, Antarctic Alpine Res.* **33**, 306–318.

COCKELL C. S., WYNN-WILLIAMS D. D., HORNECK G., SCHERER K., RETTBERG P. AND GUGG-HELMINGER A. (2002) Influence of ice and snow covers on the UV exposure of microbial communities—Dosimetric studies. *J. Photochem. Photobiol.* (in press).

EHLING-SHULZ M., BILGER W. AND SCHERER S. (1997) UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. *J. Bacteriol.* **179**, 1940–1945.

FRIEDMANN E. I. (1978) Melting snow in the dry valleys is a source of water for endolithic microorganisms. *Antarctic J. USA* **13**, 162–163.

FRIEDMANN E. I. (1982) Endolithic microorganisms in the Antarctic cold desert. *Science* **215**, 1045–1053.

FRIEDMANN E. I. AND OCAMPO R. (1976) Endolithic blue-green algae in the dry valleys: Primary producers and the Antarctic desert ecosystem. *Science* **193**, 1247–1249.

FRIEDMANN E. I., KAPPEN L., MEYER M. A. AND NIENOW J. A. (1993) Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. *Microb. Ecol.* **25**, 51–69.

FRISCH T. (1983) Reconnaissance geology of the Precambrian shield of Ellesmere, Devon and Coburg Islands, Arctic Archipelago: A preliminary account. *Geol. Surv. Can. Pap.* **82–10**, 11 pp.

FRISCH T. AND THORSTEINSSON R. (1978) Haughton astrobleme: A mid-Cenozoic impact crater, Devon Island, Canadian Arctic Archipelago. *Arctic* **31**, 108–124.

FRISCH T. AND TRETTIN H. P. (1991) Precambrian successions in the northernmost part of the Canadian Shield. In *Geology of the Innuitian Orogen and Arctic Platform of Canada and Greenland* (ed. H. P. Trettin), pp. 179–184. Geological Survey of Canada, Ottawa, Ontario, Canada.

GARCIA-PICHEL F., SHERRY N. D. AND CASTENHOLZ R. W. (1992) Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. *Photochem. Photobiol.* **56**, 17–23.

GREGG S. J. AND SING K. S. W. (1982) *Adsorption, Surface Area and Porosity*. Academic Press, London, U.K. 303 pp.

GRIEVE R. A. F. (1988) The Haughton impact structure: Summary and synthesis of the results of the HISS project. *Meteoritics* **23**, 249–254.

HICKEY L. J., JOHNSON K. R. AND DAWSON M. R. (1988) The stratigraphy, sedimentology, and fossils of the Haughton formation: A post-impact crater-fill, Devon Island, N.W.T., Canada. *Meteoritics* **23**, 221–231.

HORNECK G., RETTBERG P., RABBOW E., STRAUCH W., SECKMEYER G., FACIUS R., REITZ G., STRAUCH K. AND SCHOTT J-U. (1996) Biological UV dosimetry of solar radiation for different simulated ozone column thicknesses. *J. Photochem. Photobiol B: Biol.* **32**, 189–196.

JESSBERGER E. K. (1988) ^{40}Ar-^{39}Ar dating of the Haughton impact structure. *Meteoritics* **23**, 233–234.

LÉVESQUE E. AND SVOBODA J. (1995) Germinable seed bank from polar desert stands, Central Ellesmere Island, Canada. In *Global Change and Arctic Terrestrial Ecosystems* (eds. T. V. Callaghan, U. Molau, M. J. Tyson, J. I. Holten, W. C. Oechel, T. Gilmanov, B. Maxwell and B. Sveinbojornsson), pp. 97–107. Ecosystems Research Report **10**, European Commission, Brussels, Belgium.

MAXWELL J. B. (1981) Climatic regions of the Canadian Arctic islands. *Arctic* **34**, 225–240.

MCKAY C. P. AND FRIEDMANN E. I. (1985) The cryptoendolithic microbial environment in the Antarctic cold desert: Temperature variations in nature. *Polar Biol.* **4**, 19–25.

MELOSH H. J. (1989) *Impact Cratering*. Oxford Univ. Press, Oxford, U.K. 245 pp.

NIENOW J. A. AND FRIEDMANN E. I. (1993) Terrestrial lithophytic (rock) communities. In *Antarctic Microbiology* (ed. E. I. Friedmann), pp. 343–412. Wiley-Liss, New York, New York, USA.

NIENOW J. A., MCKAY C. P. AND FRIEDMANN E. I. (1988) The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Light in the photosynthetically active region. *Microb. Ecol.* **16**, 271–289.

OSINSKI G. R. AND SPRAY J. G. (2001) Impact-generated carbonate melts: Evidence from the Haughton structure, Canada. *Earth Planet. Sci. Lett.* **194**, 17–29.

PENTECOST A. (1993) Field relationships between scytonemin density, growth, and irradiance in cyanobacteria occurring in low illumination regimes. *Microb. Ecol.* **26**, 101–110.

POHL J., ECKSTALLER A. AND ROBERTSON P. B. (1988) Gravity and magnetic investigations in the Haughton impact structure, Devon Island, Canada. *Meteoritics* **23**, 235–238.

QUESADA A., VINCENT W. F. AND LEAN D. R. S. (1999) Community and pigment structure of arctic cyanobacterial assemblages: The occurrence and distribution of UV-absorbing compounds. *FEMS Microbiol. Ecol.* **28**, 315–323.

QUINTERN L. E., HORNECK G., ESCHWEILER U. AND BÜCKER H. (1992) A biofilm used as ultraviolet-dosimeter. *Photochem. Photobiol.* **55**, 389–395.

RAVEN J. A., KUBLER J. E. AND BEARDALL J. (2000) Put out the light, and then put out the light. *J. Mar. Biol. Assoc. U.K.* **80**, 1–25.

RETTBERG P., SIEF R. AND HORNECK G. (1999) The DLR-Biofilm as personal UV dosimeter. In *Fundamentals for the Assessment of Risks from Environmental Radiation* (eds. C. Baumstark-Khan, S. Kozubek and G. Horneck), pp. 367–370. NATO Science Series, Vol **55**. Kluwer Academic Publishers, Dordrecht, The Netherlands.

ROBERSTON P. B. AND SWEENEY J. F. (1983) Haughton impact structure: Structural and morphological aspects. *Canadian J. Earth Sci.* **20**, 1134–1151.

SCOTT D. AND HAJNAL Z. (1988) Seismic signature of the Haughton structure. *Meteoritics* **23**, 239–247.

VESTAL J. R. (1988) Carbon metabolism of the cryptoendolithic microbiota from the Antarctic desert. *Appl. Environ. Microbiol.* **54**, 960–965.

WALKER B. D. AND PETERS T. W. (1977) Soils of the Truelove lowland and plateau. In *Truelove Lowland, Devon Island, Canada: A High Arctic Ecosystem* (ed. L. C. Bliss), pp. 31–62. University of Alberta Press, Edmonton, Canada.

WHITLOCK C. AND DAWSON M. R. (1990) Pollen and vertebrates of the early Neogene Haughton Formation, Devon Island, Arctic Canada. *Arctic* **43**, 324–330.