Available online at www.sciencedirect.com
ScienceDirect
Polar Science 4 (2010) 275–283
NiPR National Institute of Polar Research
http://ees.elsevier.com/polar/

# Monitoring and identification of airborne fungi at historic locations on Ross Island, Antarctica

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Received 27 October 2009; revised 10 February 2010; accepted 15 March 2010
Available online 30 March 2010

**Abstract**

Air sampling in the 'Heroic Era' historic huts on Ross Island, Antarctica confirmed fungal presence, viability and winter survival. Cultivation and consensus sequence-based identification of *Cladosporium cladosporioides*, *Pseudeurotium desertorum*, *Geomyces* sp. and *Antarctomyces psychrotrophicus* demonstrated that they dominated the air environment within the huts. *Cadophora* sp. and *Thebolus* sp. were also isolated from the air and identified by morphological characteristics. Viable fungal colony forming units generally dropped in winter 2007 samplings from levels recorded in summer 2006 but were still substantial and greater than observed in summer 2008 and summer 2009 sampling at some locations. Comparing interior to exterior sampling, at the Hut Point and Cape Evans sites, there were more fungi recovered from the air in the interiors but at Cape Royds location, more fungi were recovered from the outside environment, possibly due to the impact of large amounts of organic material from the nearby Adélie penguin rookery. This research reveals airborne fungal biodiversity in summer and winter and demonstrates spores are widespread particularly in the interiors of the huts. Completed conservation efforts appear to have reduced fungal blooms and spores, which should reduce future adverse impacts to wood, textiles, paper and other artefacts so that this important polar heritage can be preserved.


*Keywords:* Fungi; Polar biology; 'Heroic Era'

## 1. Introduction

Three historic huts were constructed on Ross Island, Antarctica in the early 20th century by the 'Heroic Era' explorers Robert Falcon Scott and Ernest Shackleton. *Discovery* hut was built in 1902 at Hut Point and used by Scott during his first expedition, the second hut was built in 1908 for Shackleton's *Nimrod* expedition at Cape Royds, and the third and final hut was built at Cape Evans in 1911 for Scott's *Terra Nova* expedition to the South Pole. The huts were all prefabricated timber and contain large quantities of supplies for both the humans and animals, which were part of the expeditions. The three huts were subsequently occupied by Shackleton's Ross Sea party (under Aeneas Mackintosh) in 1915–17. The huts were abandoned when the last expeditions left in 1917 and were not

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doi:10.1016/j.polar.2010.03.008

revisited until 1947. Then after establishment of the United States (later named) McMurdo Station in 1955, the 'Heroic Era' huts were visited periodically until the early 1960's. Since then, the historic huts have been visited yearly by conservators, tourists, base staff and scientists. All three huts provide unique environments different from the outside environment. Held et al. (2005) reported that the average temperature and relative humidity in the Cape Evans hut over a three year period (December 1999 to December 2002) was −14.7 °C and ranged from +9.4 to −35.1 °C with relative humidity averaging 74.6% and the range from 59 to 87.3 %. At Hut Point, the temperature range over the same three year period was from +6.6 to −39.0 °C and relative humidity averaged 73.5% with the range from 49.2 to 91.3%. At the Cape Royds hut, the temperature range over the same three year period was from +2.5 to −35.1 °C and the relative humidity averaged 71.6% with the range from 53.6 to 89.3%. All of these conditions indicated that for fungi to be viable from one year to another, the microorganisms must be cold tolerant, and capable of withstanding extreme cold.

Airborne microorganisms have been studied in Antarctica as an indication of robustness of the organisms, their mechanisms of spreading, and association with human impact in this environment (Cameron et al., 1973, 1974, 1977; Sun et al., 1978; Marshall, 1997; Upton et al., 1997; Marshall, 1998). Meyer (1962) and Corte and Daglio (1964) allowed propagules to settle onto exposed agar plates at different field locations and their studies targeted bacteria although fungi were noted as being present. Since then a variety of methods have been used to sample for airborne microorganisms in Antarctica (Marshall, 1997).

Many theories have been proposed as possible mechanisms used by fungi to survive unfavourable conditions in Polar Regions. Robinson (2001) reported that Antarctic and Arctic fungi used physiological mechanisms to survive including becoming cold tolerant, accumulating stress protectants such as trehalose and cryoprotectant sugars, and producing polyol. Others have suggested that changes in the cell membrane composition (Finotti et al., 1993; Onofri et al., 1994), secretion of antifreeze proteins (Snider et al., 2000), secretion of exopolysaccharides (Selbmann et al., 2002), and biochemical adaptation (Fenice et al., 1997; Fenice et al., 1998) are important attributes needed for survival under polar conditions. Along with physiological adaptation, there are morphological characteristics to ensure survival such as cold avoidance rather than tolerance with reestablishment, or possibly reactivation, in spring/summer from spores produced before winter (Marshall, 1997), re-colonisation from fungal material from outside Antarctica (Marshall, 1998), acclimation of fungal material due to slow cooling of environment, abbreviated life cycles, dominance of sterile fungi in the cold environments and dominance of dark hyphae due to melanin production (Onofri et al., 2004). The one major conclusion from studies of cold fungal communities is that there is not one specific adaptation that confirms survival in adverse conditions. Marshall (1997) used air spore sampling to investigate the seasonality of fungal spores over a 13 ½ month period at 3 sites on Signy Island, South Orkney Islands, Antarctica. The fungal counts were greater in summer than winter and the highest concentration of fungal spores were detected in late spring and early summer (with the maximum daily catch at any of the three sites being 0.77 spores/$m^3$).

In order to identify potential fungi with propagules in the air and to understand whether fungal material can overwinter within the huts, we conducted air sampling and determined viability by culturing and quantified as total colony forming units generated at 2 degrees Centigrade (°C) per cubic meter (CFU/$m^3$). Locations at *Discovery* hut and *Terra Nova* hut were sampled at the end of Austral summer, January 2006, 2008 and 2009, and at the end of winter, August 2007. Aerial fungal material sampling was also conducted at the *Nimrod* hut sites in January 2006 and 2008.

## 2. Methods

### 2.1. Location of sampling sites

The three historic huts sampled are located on Ross Island, Antarctica with *Discovery* Historic Hut at Hut Point (77° 50′ 50″S, 166° 38′ 30″E, Antarctic Specially Protected Area (ASPA) 158*)*, *Terra Nova* Historic Hut at Cape Evans (78° 38′ 10″S, 116° 25′ 04″E, ASPA 155), and *Nimrod* Historic Hut at Cape Royds (77°33′10.7″S, 166°10′6.5″E, ASPA 121).

In January 2006, the following locations were sampled, chosen for either being near to an organic source of material, or for being in a specific location, either enclosed or relatively open in the interior of one of the historic huts or exterior:

- At *Discovery* hut, a total of 15 locations, 4 outside the hut and 11 inside the hut.
- At *Terra Nova* hut, a total of 24 locations, 11 outside the hut and 13 inside the hut.

- At *Nimrod* hut, a total of 19 locations, 13 outside the hut and 6 inside the hut.

The sampling in August 2007 was a repeat of a selection of the same interior locations conducted in January 2006 but only at *Discovery* hut and *Terra Nova* hut. Both huts had been officially closed for 5 months previous to sampling, although *Discovery* hut may have been visited infrequently. *Terra Nova* hut had not been visited and the doors had not been opened for six months prior to the aerial testing. Nimrod hut could not be safely reached in August 2007. The samplings of January 2008 and 2009 were a repeat of a selection of the locations sampled in January 2006.

### 2.2. Air sample, quantitation of colony forming units, and viability testing

Air samples were collected in Antarctica in January 2006, August 2007, January 2008 and January 2009 using a surface air sampler (Merck MAS-100 Eco®, Whitehouse Station, NJ, U.S.A.). This volumetric sampler aspirates air at a fixed speed for variable periods of time through a perforated plate containing 400-holes positioned over a Petri plate containing agar growth medium, consisting of 0.2% yeast extract, 1.5% malt extract and 1.8 % agar (Difco, Becton, Dickinson & Company, Franklin Lakes, New Jersey, USA). The sampler was operated at calibrated flow rates, either 200 or 500 litres of air taken per sample. After completion of air sampling, the plates were removed from the sampler, sealed with Parafilm (Penchiney Plastic Packaging, Menaha, WI, U.S.A.) and kept cold (0–4 °C) while in Antarctica and travelling to Hamilton, New Zealand.

Upon arrival at The University of Waikato in Hamilton, the agar growth medium plates were incubated at 2 °C. The plates were examined every week for up to six weeks and the number of colonies on the agar plates were counted after three and after six weeks. The calculations to quantify viable microorganisms from the air were done in two manners: first, determining total colony forming units per cubic metre (CFU/$m^3$), which was a total count of all colonies seen on the agar plates which represented filamentous fungi, yeast and bacteria and second, fungal colony forming units per cubic metre (CFU/$m^3$) which were the count of filamentous fungi only. Quantitation was made from the total number of colonies per plate after 6 weeks and adjusted using the positive hole conversion table provided by the manufacturer of the MAS-100; the conversion is based upon the principle that as the number of viable particles being impinged on a given plate increases, the probability of the next particle going into an empty hole decreases.

### 2.3. Identification of fungi

Fungi were identified by morphological features and also a selection of colonies of the dominant fungal isolates were identified by molecular DNA techniques. For the latter, fungal material was scraped from pure cultures and DNA extracted using a bead beating technique, method described by Miller et al. (1999). The rDNA gene (sequences encoding ribosomal RNA) internal transcribed spacer (ITS) regions 1 and 2 and 5.8S regions were amplified using primers ITS1 and ITS4 (Gardes and Bruns, 1993). PCR amplification was done in a MJ Research PTC Mini-cycler (Watertown, MA, U.S.A.), with the following protocol: 94 °C for 5 minutes; 35 cycles of 94 °C for 1 minute, 50 °C for 1 minute, 72 °C for 1 minute followed by a final extension step of 72 °C for 5 minutes.

Sequencing reactions were performed at the Waikato DNA Sequencing Facility, Department of Biological Sciences, the University of Waikato. Separate sequences were performed with both the ITS1 and ITS4 primers and were compared for similarities then combined using the CAP3 sequence assembly program (Huang and Madan 1999) to form a consensus sequence, which was compared to sequences in GenBank using BLASTn to find the best match.

## 3. Results

### 3.1. Airborne fungi outside the three Historic huts on Ross Island, Antarctica, 2006–2008

In January 2006, outside *Discovery* hut, the mean number of fungal spores was 2.5 CFU/$m^3$; there were no fungi found at three of the four sites tested and 10 fungal CFU/$m^3$ were recorded next to the hut's main door underneath the porch area. For the 11 sites at *Terra Nova* hut sampled in 2006, the mean value outside the hut was 8 fungal CFU/$m^3$ with a range of 0–60 with no airborne fungi detected at 5 of the 11 sites outside. At *Nimrod* hut, in January 2006, the mean value for exterior sites sampled was 72 fungal CFU/$m^3$ with a range of 0–560. At 6 of 13 sites sampled outside, no fungal material was detected; the highest level recorded was 610 CFU/$m^3$ in the stables area next to the hut. The mean outside *Nimrod* hut was 39 fungal CFU/$m^3$ in the summer of 2008 sampling with one site having no fungal material detected and the highest level again recorded in the stables area with 112 CFU/$m^3$.

### 3.2. Airborne fungi inside Discovery hut

Table 1 gives the results in the interior of *Discovery* hut in summers of 2006, 2008, 2009 and winter 2007; the mean value was 3348 fungal CFU/$m^3$ with a range collected at the sites of 1 to >26,280 CFU/$m^3$. The latter was recorded in summer 2006 from the sample taken approximately one metre in front of hay fodder (Fig. 1a) across from the main entrance and was at least 100 times greater than any of the other 10 sites tested inside *Discovery* hut and the highest of any sample tested at any time in this study.

In August 2007 (winter), the sampling in front of the hay fodder in the entrance of *Discovery* hut resulted in the total colony forming units at 8800 CFU/$m^3$ and the fungal colony forming units at 8360 CFU/$m^3$; these values were about one-third of the counts from the summer 2006 sampling but show that there was a tremendous amount of viable fungal material in winter time present in the air in front of the fodder. The levels of colony forming units both total and fungal in the main room and near the cooking area increased from summer to winter but decreased in the meat room (Table 1). These 4 sites were re-sampled again at the end of summer 2008 and 2009 and the fungal CFU/$m^3$ had dropped to levels lower than the results from the summer 2006 and winter 2007 sampling. The fungal CFU/$m^3$ in January 2009 had risen from summer 2008 levels at all four sites sampled.

### 3.3. Airborne fungi in Terra Nova hut

The values were considerable inside *Terra Nova* hut in summer 2006, as given in Table 2, with an average of 1838 fungal CFU/$m^3$ ranging from 8- >13,140. Fungal colony forming units were recorded at all sites sampled inside *Terra Nova* hut, the highest level observed, >13,140 CFU/$m^3$, was in the laboratory area where there was a visibly mouldy boot as well as fungal blooms on the wood walls and on crates in the kitchen area (Fig. 1b, c and d). The crates and wood walls were original to the *Terra Nova* party and the mouldy boot was made by a Ross Sea party member in 1915 from a *Terra Nova* expedition sleeping bag abandoned in 1913 (personal communication, D. Harrowfield). At *Terra Nova* hut in August 2007, both the total colony forming units and the fungal colony forming units of all 5 inside sampled locations were less than the summer 2006 sampling results (Table 2). In January 2008, 13 sites inside *Terra Nova* hut were re-sampled including the same 5 sites that were sampled in winter 2007 and overall the mean fungal colony forming units inside *Terra Nova* hut were more than the winter sampling. The fungal CFU levels in January 2009 were less at all five sites when compared with the levels in January 2006 but higher than winter 2007 and when compared to January 2007 they were higher at 3 sites (floor of main room, bunk area next to galley and under bunk lab area near mouldy boot and fungal blooms on walls) and lower at 2 sites (top of darkroom and inside dark room).

### 3.4. Airborne fungi inside Nimrod hut

Fungi were recorded at all sites sampled inside *Nimrod* hut, as given in Table 3, and the highest level was recorded from a shelf in the biology laboratory area, though this value, 60 CFU/$m^3$, was significantly less than the highest recordings at *Discovery* and *Terra Nova* huts. In January 2008, 13 of the 19 sites tested in January 2006 were re-sampled, 7 outside the hut and 6 inside the hut (Table 3). Inside *Nimrod* hut during the January 2006 sampling, there was an average 19 CFU/$m^3$ with a range of 8–32 while in the January 2008 sampling levels had dropped to average 9 CFU/$m^3$ with a range of 4–16. Extensive restoration occurred at *Nimrod* hut in the summers of 2007 and 2008.

### 3.5. Identification of fungi

Fungal isolates were tentatively identified based on morphological characteristics and sixteen of the

Table 1
Colony forming units/$m^3$ in summer (2006, 2008 and 2009) and winter (2007) at 4 locations inside *Discovery* Hut.

| *Discovery* hut sample location | Summer 2006 total CFU/$m^3$ | Winter total CFU/$m^3$ | Summer 2008 total CFU/$m^3$ | Summer 2006 fungal CFU/$m^3$ | Winter 2007 fungal CFU/$m^3$ | Summer 2008 fungal CFU/$m^3$ | Summer 2009 Fungal CFU/ $m^3$ |
|---|---|---|---|---|---|---|---|
| Meat room | 320 | 180 | 24 | 170 | 148 | 4 | 30 |
| Main room | 80 | 362 | 24.8 | 30 | 339 | 1.4 | 46 |
| Cooking area | 20 | 205 | 19 | 20 | 187 | 9 | 20 |
| Entrance way in front of hay | >26,280 | 8800 | 10 | >26,280 | 8360 | 1 | 15 |

Colony forming units per cubic metre of air abbreviated as CFU/$m^3$.


Fig. 1. a. Fodder in Discovery hut. b. Mouldy boot from under bunk in *Terra Nova* hut. c. Crates in kitchen area in *Terra Nova* hut. d. Mould on crates in kitchen area in *Terra Nova* hut.

dominant fungal species isolated from specific locations in *Discovery* and *Terra Nova* huts in January 2006 and August 2007, as given in Table 4, were also identified using DNA sequence analyses of the two internal transcribed spacer regions of rDNA, ITS1 and ITS2. When the sequences from the 16 chosen isolates were combined to give consensus sequence, they were identified as four fungi, *Cladosporium cladosporioides* strain ATCC 58991 (Genbank accession number AY361966, sequence match 546/546), *Pseudeurotium*

Table 2
Colony forming units/$m^3$ in summer (2006,2008 and 2009), winter (2007) at 5 locations inside *Terra Nova* hut.

| *Terra Nova* hut sample location | Summer 2006 total CFU/$m^3$ | Winter total CFU/$m^3$ | Summer 2008 total CFU/$m^3$ | Summer 2006 fungal CFU/$m^3$ | Winter fungal CFU/$m^3$ | Summer 2008 fungal CFU/$m^3$ | Summer 2009 Fungal CFU/ $m^3$ |
|---|---|---|---|---|---|---|---|
| On floor of main room | >5256 | 0 | 99 | 1448 | 0 | 97 | 164 |
| Top of darkroom | 1745 | 18 | >1114 | 440 | 14 | >1114 | 295 |
| Bunk area next to galley | 3260 | 15 | 81 | 955 | 14 | 79 | 260 |
| Dark room | 2210 | 1 | 187 | 675 | 1 | 183 | 40 |
| Lab area under bunk near mouldy boot | >13,140 | 20 | 56 | >13,140 | 20 | 52 | 170 |

Colony forming units per cubic metre of air abbreviated as CFU/$m^3$.

Table 3
Colony forming units/$m^3$ in summer (2006 and 2008) at 6 locations inside *Nimrod* hut.

| *Nimrod* hut sample location | Summer 2006 total CFU/$m^3$ | Summer 2008 total CFU/$m^3$ | Summer 2006 Fungal CFU/$m^3$ | Summer 2008 Fungal CFU/$m^3$ |
|---|---|---|---|---|
| Inside hut main area on high shelf above the window | 30 | 15 | 16 | 8 |
| Inside on shelf in biology area above open biscuit box | 60 | 30 | 32 | 16 |
| Inside hut on shelf inside Shackleton's room | 66 | 33 | 30 | 15 |
| Inside Mawson's laboratory | 30 | 15 | 8 | 4 |
| Inside hut main area between canvas divide and stove | 26 | 13 | 18 | 9 |
| Inside hut main area between canvas divide and door | 30 | 15 | 8 | 4 |

Colony forming units per cubic metre of air abbreviated as CFU/$m^3$.

*desertorum CBS 986.72* (Genbank accession number AY129288.1, sequence match 481/507), *Geomyces* sp. BC9 (DQ317339, sequence match 561/561) and *Antarctomyces psychrotrophicus* (Genbank accession number AM489755, sequence match 526/528) as given in Table 4. Additionally, as identified by morphological features, the genera *Cadophora* and *Thelebolus* were cultured from the air samples as well as some unidentified white filamentous fungi.

## 4. Discussion

The ability of fungi to develop in a variety of structural forms depending on environmental conditions or nutrient availability is well known. The production of spores is reported as both a method to survive unfavourable conditions and for dispersal of fungal material to new nutrient sources. Inside the historic huts, the fungi present could be producing spores for both reasons. A proposed survival mechanism for fungi is to become dormant in winter and then re-establish when conditions are more favourable; this strategy favours spore forming fungi since spores are considered to be more resilient to unfavourable conditions than fungal mycelium (Cameron et al., 1973). The key findings of our study are as follows: a) there is year-to-year viability of propagules; b) there is diversity of said fungi and c) our findings suggest that human impact is not a threat to the historic huts.

The presence of the three historic huts on Ross Island has altered the natural microbial biota in the area they were built, with the first introduction of 'aliens', foreign microorganisms, to Antarctica occurring in the early 20th century. These microorganisms could have had origins from the initial locations of the sourced organic materials as well as the ports where the ships docked en route to Ross Island. It is speculated that the historic sites provide environments for both endemic and fungal species introduced by human activities, albeit all being capable of adaptation to the environment (Farrell et al., 2004; Farrell et al., 2008). The most frequently isolated fungal genera from historic woods and artifacts

Table 4
Consensus sequence fungal identification group, isolate number, season sampled and locations of sample.

| Fungal identification group by consensus sequence | Fungal Isolate number | Season sampled | Location collected from |
|---|---|---|---|
| *Antarctomyces psychrotrophicus* | 6011 | Summer | *Discovery* Hut, Inside in meat room |
| *Pseudeurotium desertorum CBS 986.72* | 6020 | Summer | *Discovery* Hut, Inside in front of hay in entrance way |
| *Pseudeurotium desertorum CBS 986.72* | 6022 | Summer | *Discovery* Hut, Inside main room |
| *Cladosporium cladosporioides* strain ATCC 58991 | 6035 | Summer | *Terra Nova* Hut, Inside on floor of main room |
| *Pseudeurotium desertorum CBS 986.72* | 6051 | Summer | *Terra Nova* Hut, Inside dark room |
| *Pseudeurotium desertorum CBS 986.72* | 6078 | Summer | *Terra Nova* Hut, Under bunk lab area near mouldy boot |
| *Cladosporium cladosporioides* strain ATCC 58991 | 7001 | Winter | *Discovery* Hut, Inside in meat room |
| *Pseudeurotium desertorum CBS 986.72* | 7003 | Winter | *Discovery* Hut, Inside in meat room |
| *Geomyces* sp. BC9 | 7004 | Winter | *Discovery* Hut, Inside main room |
| *Pseudeurotium desertorum CBS 986.72* | 7007 | Winter | *Discovery* Hut, Inside near cooking area |
| *Geomyces* sp. BC9 | 7008 | Winter | *Discovery* Hut, Inside near cooking area |
| *Cladosporium cladosporioides* strain ATCC 58991 | 7011 | Winter | *Discovery* Hut, Inside in front of hay in entrance way |
| *Pseudeurotium desertorum CBS 986.72* | 7012 | Winter | *Discovery* Hut, Inside in front of hay in entrance way |
| *Pseudeurotium desertorum CBS 986.72* | 7013 | Winter | *Terra Nova* Hut, Inside on floor of main room |
| *Geomyces* sp. BC9 | 7014 | Winter | *Terra Nova* Hut, Inside on top of darkroom |
| *Cladosporium cladosporioides* strain ATCC 58991 | 7018 | Winter | *Terra Nova* Hut Under bunk lab area near mouldy boot |

identified by Arenz et al. (2006) were *Cadophora* (21%), *Cladosporium* (18%), *Geomyces* (17%), *Cryptococcus* (8%), *Hormonema* (6%), *Rhoturula* (3%) and *Fusarium* (3%). Duncan et al. (2008) identified in the interior samples of the historic huts additionally *Penicillium roquefortii*, *Penicillium expansum*, and other *Penicillium* sp.

All three historic huts could be considered to offer a more favourable environment for fungal growth than outside the huts as there is a ready supply of nutrients and protection from the weather conditions. Fungi have been identified outside the historic huts, though, as Blanchette et al. (2004) isolated and identified several species of *Cadophora*, a wood destroying soft rot fungus from wood in contact with soil from the exterior of *Terra Nova* and *Nimrod* huts, speculating that due to the great diversity of *Cadophora* species found in the historic woods, and their presence in soils and dead moss thalli that *Cadophora* spp. were endemic to Antarctica and not an introduced species.

Held et al. (2005) reported the number of hours when conditions were favourable for fungal growth by the criteria of hours per year when temperatures were above 0 °C and relative humidity was above 80%, *Discovery* hut had the least number of hours of the three huts within these conditions. Interestingly, the largest average number of fungal colony forming units isolated from the air recorded from this study of the historic huts was in *Discovery* hut. This hut is the most visited of the three but *Discovery* hut also has a readily colonized food source, hay fodder, which appears to be the primary origin of the high total cell and fungal counts. When studying the relationship between organic material and fungal colonisation in Antarctica, Fletcher et al. (1985) noted that many areas containing no organic material contained fungi and concluded that these fungi were airspora which were not active until put onto agar plates and incubated in warmer conditions. At *Discovery* hut, the main source of fungal spores were bales of hay fodder in the entrance, which in summer 2006 and winter 2007 the area near them had airborne fungi measured at >26,280 and 8360 CFU/$m^3$, respectively, though both were higher than the amount of fungal material recorded in the summer 2008 and 2009 when the fungal CFU had decreased to 1 and 15, respectively. The reason for the reduction of spores near the hay after the winter of 2007 is not clear.

There are a number of locations inside *Terra Nova* hut where there are visible fungal blooms, and observations indicate the fungi are proliferating and the problem is becoming more extensive (Blanchette et al., 2004; Farrell et al., 2008; Arenz et al., 2006). Unlike *Discovery* hut, fungal material in the *Terra Nova* samples increased in the summer 2008 sampling. In 2008, restoration of this hut began, as conducted by Antarctic Heritage Trust, which included removing artefacts, the southern, eastern and western walls weatherproofed, snow and ice removed from underneath the floor of the building and vortex generators installed about 30 metres south of the hut to create turbulent air flow to reduce the snow build up on the southern and eastern aspects of the hut. The sampling in the summer of 2009 was done during the restoration work, with the centre third of the timber floor and linoleum having been lifted to expose the wooden joists, and it could be that this activity increased the amount of viable aerial fungal material detected inside the hut.

At *Nimrod* hut, the fungal CFU was the greatest outside rather than inside the hut; this could possibly be due to a number of exterior locations being high in organic material including the stables area, and/or impact of wind dispersing fungi. This area originally housed the horses and has become a popular nesting site for the Adélie penguins from the nearby rookery, and a site where bottles, wood, rope and food scrapes were dumped during the 'Heroic Era'. From 2004–2008, the Nimrod hut was restored by Antarctic Heritage Trust and the roof was reclad, repairs were made to the timber cladding, the stables area was cleaned, Mawson's lab was relined, ice was removed from underneath the structure, waterproof cladding fitted and melt water flows redirected around the site rather than underneath it. It can be assumed from the results from *Nimrod* hut (CFU/$m^3$ reducing over time) that the conservation work has removed surface fungi and decreased the amount of viable aerial fungal material. At the earlier stages of restoration, the opposite is occurring as can be seen at *Terra Nova* hut where on-going repairs and conservation works are increasing CFU/$m^3$, perhaps fungal material from beneath the floors and behind materials are being disturbed due to the work-in-progress. When the restoration is complete, these fungi may be removed and continual monitoring will confirm in the future whether restoration efforts result in a continued decrease in counts of fungi in the historic huts.

All sixteen dominant fungal isolates from this study of the air samplings were grouped by similarity of the DNA sequence of the two internal transcribed spacer regions of rDNA, ITS1 and ITS2, and the four groups were identified to belong to 3 genera and one family, *Cladosporium cladosporioides*, *Pseudeurotium desertorum*, *Geomyces* sp. and *Antarctomyces psychrotrophicus*.

The dominant genus in Arenz et al., 2006 study of biodiversity on wood and artefacts, *Cadophora* sp.,

was detected in this present aerial sampling study but not in significant numbers at any location. The Arenz et al. (2006) study measured presence at a site as being positive whether that be one colony or many colonies, while the aerial fungal study counted every isolate obtained as a colony. *Cladosporium* and *Geomyces* were found in both studies and were most dominant as cultured from air samples likely because of their prolific production of spores. It may be speculated that *Cadophora* sp. was not found in large numbers in this study since it produces fewer aerial spores as compared to *Cladosporium* and *Geomyces*. Although fewer aerial spores are produced by *Cadophora* sp., they apparently are found extensively in wood in contact with the ground.

We expected that the level of fungal material collected by air sampling would be greatest at the end of summer (January) as the fungi would have experienced the longest period of warmer temperatures allowing the microorganisms to grow and produce reproductive structures. This would also be consistent with increased human impact as the frequency and numbers of visitors increases during October−January with visitors peaking in January (Harrowfield, 1989). However, we found that both summer and winter viability and quantity of fungal material were significant and that human visitation impact appears to a negligible factor considering the amount of fungi. These results show that fungal material are present in the air of the historic huts in both summer and winter, are viable and capable of producing colonies indicating a significant amount of fungal adaptation and robustness.

## Acknowledgements

We thank David Harrowfield for helpful comments and insights, Nigel Watson, Al Fastier and conservators of the Antarctic Heritage Trust for their support and cooperation during this study, support personnel of Scott Base for their assistance in conducting this research in Antarctica and Antarctica New Zealand for logistic support. This research was supported in part by the 2004 Vice Chancellor Fund of The University of Waikato and by the National Science Foundation Grants 0229570 and 0537143 to R. A. Blanchette. We also sincerely thank Lisa Robson and Joanne Thwaites Kelly, formerly from The University of Waikato and Benjamin Held and Brett Arenz from the University of Minnesota for assistance with sampling in Antarctica and the identification of fungi.

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