Astrobiology with Mars'Obot: Identifying Microbial Life Forms Using Ground-based Remote Sensing.

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Summary: Geothermal spring environments are colonised by microbial organisms tolerant of environments otherwise hostile to life. Such environments are analogous to some of the earliest environments for terrestrial life. Hot springs are known to have existed on Mars in the past and they too may once have been home to Martian microbes. Studying these environments present a number of challenges, including researcher safety and protection of ecologically fragile environments. Field robots can potentially assist in managing both challenges. We describe the performance of a teleoperated rover developed by Mars Society Australia to investigate extreme forms of life at two geothermal study sites in New Zealand using a non-contact thermometer and multispectral camera. We found that single and multiple filtered imagery utilising visible and near infrared (NIR) was useful in detecting photosynthetic microbes, and that multiple filtered images provided the best results for life detection. Our study provides insights into the incorporation of robotic methods for astrobiological research.

Keywords: Astrobiology, robotics, remote sensing, Mars analogue research, hot springs, New Zealand.

Introduction

Various types of teleoperated and autonomous robots have been used extensively in underwater scientific research, often in conjunction with direct human presence, e.g. divers and/or crewed submersibles [1]. Both teleoperated and semi-autonomous vehicles have been utilised for surface exploration of the Moon and Mars [2, 3, 4]. However, use of robotics for terrestrial surface science has been limited, other than to test concepts for planetary exploration [5]. Nonetheless, robotic technology has outstanding potential for collecting data from sites where hazards, fragility, or contamination are potential issues. Geothermal areas combine all these factors, exposing researchers to boiling water and noxious fumes and often having delicate structures with unique biotas.

In this paper we investigate the capabilities and limitations of a low-cost (<$1000) teleoperated vehicle in characterising extreme life forms within hot spring environments in the Rotorua area, Taupo Volcanic Zone, New Zealand. The vehicle was developed by Mars Society Australia (MSA) as part of the Marsobot project, and utilises commercial off-the-shelf hardware and open source software [6]. Field work was carried out during the joint New Zealand Astrobiology Initiative (NZAI) and NASA Spaceward Bound New Zealand expedition (http://astrobiology.kiwi/) in January 2015.

Small robots such as these also may have potential for future Mars missions, in that they may complement larger robots being deployed by astronauts to investigate specific sites deemed too hazardous or fragile for direct exploration, or to use in follow-ups of initial site surveys. Previous examples of microrovers evaluated for Mars missions include the Rocky series developed in conjunction with the Sojourner Mars rover [7], and the FIDO vehicle designed to trial concepts employed in MER [8]. Additionally, the Mars Astrobiology Research and Technology Experiment (MARTE), a Mars analogue mission, as conducted in Spain simulated robotic drilling mission to search for subsurface life [9].

Extreme Life Forms

The search for life beyond Earth has been an ongoing quest for humanity since ancient times, although no definitive proof has yet been found [10]. Mars has traditionally been considered as a likely extraterrestrial abode for life, given its similarity to Earth, historically and physically, in relation to the other planets and moons of the Solar System, and because of its location within the outer boundaries of the "habitable zone" [11]. The first direct experiments designed to search for life on Mars were performed during the Viking missions of the mid-1970's [12]. Two robotic landers used four experiments that searched for signs of metabolic activity, as well as the presence of organic compounds. The subsequent results from these experiments showed no evidence for life nor its chemical by-products, and provided important data on the limitations of robotic searches for extraterrestrial life [13]. However, since the Viking missions, life has been found in far more diverse and extreme environments than thought possible in the 1970's, from cold dry deserts to hydrothermal vents in the deep sea, to geysers around hot springs on land [10, 14, 15]. The characterisation of life in these extreme environments has widened the possible settings and physico-chemical conditions under which life could flourish and expanded the criteria for biosignal detection, such as within thermal hot springs [16, 17].

Thermal Springs

Terrestrial hot springs of volcanic terrains are environments characterised by water heated by a geothermal (magmatic) heat source. They are of particular interest in that some hyperthermophilic microbes inhabiting them represent the most primitive forms of life [18, 19]. Siliceous hot spring deposits (sinter), and the nature of their associated microbial life interacting with local mineralogy, play an important role in astrobiological hypotheses related to the search and discovery for life on other planets and moons, as well as in expanding our understanding of early life on Earth [15, 16, 20, 21, 22, 23]. Additionally, evidence of past thermal springs sites also has been observed on Mars, specifically at Gusev Crater as discovered by the *Spirit* rover [24, 25]. Thus characterisation and analysis of active terrestrial hot springs settings will provide further insight into investigations of similar relict environments on other worlds, and allow enhancement of remote sensing and scientific investigation techniques for the detection of past and present life.

Previous research has revealed variations in mineralising biosignatures within many hot springs environments as a function of distance from the thermal vent [22, 23, 26, 27, 28, 29, 30]. This process of deposition of sinter and occurrence of microorganisms form zonal systems, which can act as indicators for temperature and pH conditions [19, 22, 23, 31, 32, 33, 34]. These zones also can be used to infer the location of specific mineralogy or biology, and were used to guide the Mars'Obot rover operations conducted at the Rotorua hot springs sites. We analysed these zones using the Mars O'bot rover, and compared our results with published findings [23]. This was carried out in order to determine the applicability of using a microrover to search for life in an analogous, active extremophile environment, and to identify shortcomings and avenues for future refinement of remote sensing methods in the field of astrobiology.

Study Sites

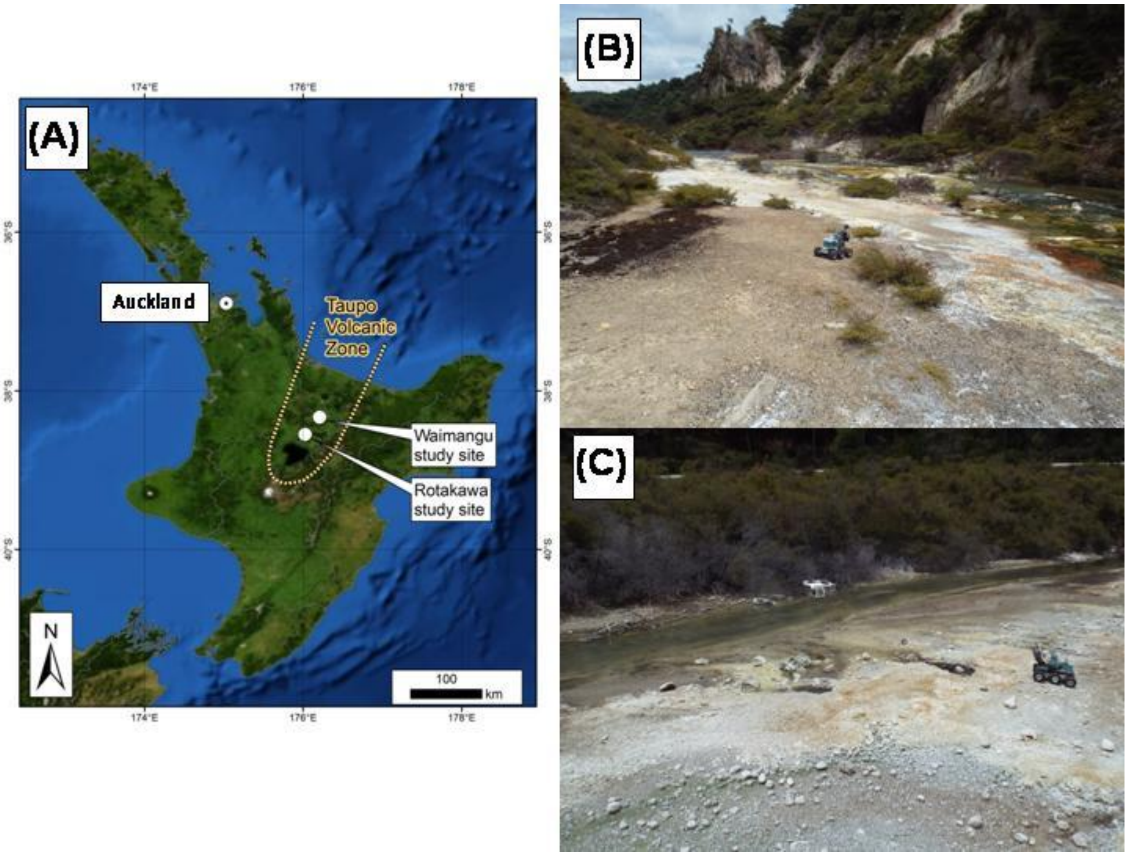

Fig. 1 (A) Overview of study locations in the Taupo Volcanic Zone, New Zealand. (B) Frying Pan Lake outflow stream location, Waimangu Geothermal Vally. (C) Parariki Stream location, Rotokawa geothermal field.

MarsO'bot Junior underwent field trials at two hot spring sites in the Taupo Volcanic Zone, New Zealand, over the period 15-22 January, 2015 (Fig. 1A-C). Both field sites possess an abundance of microorganisms existing in a variety of temperature ranges.

The first field trial was conducted on the bank of the hot water creek and springs at the outlet of Frying Pan Lake, Waimangu Volcanic Valley (Fig. 1B) [35]. This geothermal discharge area possesses water temperatures of approximately 50^o C with stream deposits forming sinters with trace elements of antimony, molybdenum, arsenic and tungsten [22]. The microbes in this stream were found [36] to be predominantly controlled by water acidity, as well as differing water temperature. The overflow stream from Frying Pan Lake supports multiple microbial life forms including cyanobacteria and algae as well as insect life [22].

The second site is located at the acid-sulphate-chloride springs debouching along the pumice-lined banks of Parariki Stream, Rotokawa Geothermal Field (Fig. 1C). These hot springs discharge acid-sulphate-chloride waters, generating acidic surface features [22, 37] and was the subject of a detailed sedimentary facies and biotic study [23]. Parariki Stream drains acidic Lake Rotorua into the Waikato River, with thermal springs appearing on the southern portion of the stream [23, 37, 38]. The Parariki Stream study area in the Rotokawa Geothermal Field covers approximately 130 m^2 and is located on the western floodplain, consisting of well sorted pumice alluvium. Water temperatures for the hot springs at this site were cited to be on the order of 40-91^o C, discharging clear acidic waters [23]. Four distinct types of stromatalitic sinter were identified, including ridge shaped, acid-etched sinter formations close to vents; 1 cm high spicular sinter rims growing on pumice clasts (>2 cm diameter) in somewhat cooler waters (30-85^o C); thin, parallel-laminated sinters forming under very shallow sheet flow areas; and thin, small cup-shaped sinter rims forming on small pumice clasts (<2 cm diameter) located on sandy material saturated with cooled thermal fluids [23]. All sites except for the sinters nearest the spring vents were generally found to be co-located with coccoidal green algal mats. These mats were found within a temperature range of 30–52.5^{o}C, being thickest at ~45^{o}C. The mats belong to the rhodophyte taxon Cyanidiophycease, thereby indicating that they are chlorophyll-based, using photosynthesis as part of its metabolic process [39].

Our aim was to use the instruments onboard our robot to remotely identify and characterise the occurrence of the extensively distributed green mats by exploiting the spectral response of photosynthetic biotic material in the visible and near infrared (NIR) wavelengths. Furthermore, we evaluated whether the microrover could identify the existence of sinter or other biota types at both sites.

Rover Design

Mars'Obot Junior, the rover used at the New Zealand hot springs, is designed around the Dagu Wild thumper six wheel drive chassis. This chassis features independent suspension for each wheel, as well as a 1:75 geared electric motor within each wheel hub. The vehicle is skid steered in a similar manner to the Soviet Marsokhod rover [40]. We chose skid steering owing to the ease at which it can be employed, and because it requires a minimum number of motors for its operation. We have based the control architecture of the rover on the Arduino 8 bit microcontroller. This controller is well supported and has been used in many robotics applications [41, 42]. The Arduino Uno consists of an Atmega 328 chip on a microcontroller development board. It is programmed using the open source Arduino environment that is based on C++. The Arduino Uno possesses 14 digital input/output pins and six analog input pins. We use these pins to operate the non-contact thermometer and operate the filter wheel of the multispectral camera.

In addition to the Arduino Uno we also have incorporated a second Arduino system into our design: The Wild Thumper controller. This controller is also based on the Atmega 368 and was designed specifically for the Dagu chassis we used for Mars'Obot [6]. The pre-installed controller software provides an interface between the 2.4GHZ remote control system and the rover. In addition the Wild Thumper controller provides battery monitoring and will automatically shut down current to the motors if battery voltage drops below a certain level.

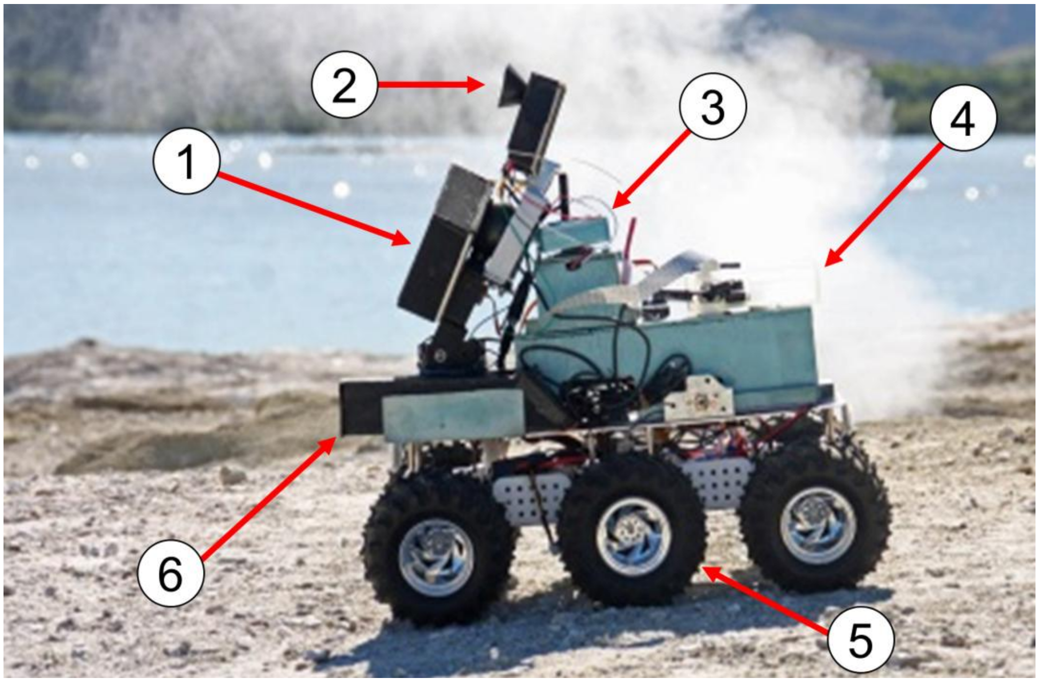

Fig 1. Overview of Junior rover with key components marked. (1) Multispectral imaging system. (2) Non-contact thermometer. (3) Serial modem. (4) Raspberry Pi and wifi link. (5) Electronics box and four wheel drive skid steer mobility. (6) Reflectance spectrometer.

Science Instruments

The instruments aboard Mars'Obot Junior were designed specifically to operate in the hot springs environment in New Zealand, to help characterise these environments and identify photosynthesising microbial life forms present. We aimed to detect the presence of chlorophyll-containing, photosynthesising microbial mats by photographing candidate sites through visible, red and NIR filters, and characterised their context through imaging, thermometry, and spectroscopy.

Multispectral camera

The primary instrument for this purpose is a Logitech Webcam modified to be NIR sensitive. A filter wheel containing an NIR cut filter allowing only visible wavelengths to pass, red (625 nm long bandpass for single-image photosynthesis detection) and 850 nm long bandpass NIR filter is fitted in front of the webcam to provide multispectral imaging in visible, red and NIR wavelengths. Similar filter arrangements have been used in unmanned aerial vehicle (UAV) trials for monitoring vegetation such as crops and have proven useful for characterising vegetation type and health [26].

Table 1: Summary of scientific instruments carried on Junior

Instrument	Characteristics	Purpose
Multispectral camera	Logitech Webcam with visible, red and 850 nm NIR filter wheel.	Identify photosynthetic biota as well as provide visual record of environment.
Non-contact thermometer	Samples ambient plus sample temperature.	Sample temperature readings for hot spring vents
Visible light spectrometer	2592 x 1944 px Raspberry Pi camera with DVD diffraction grating and 1 mm slit.	Provide visible light spectra for photosynthetic biota.

We developed this camera in order to detect photosynthetic biota in the hot springs study sites. The multispectral methodology works by exploiting the high NIR reflectance of vegetation as compared to visible light wavelengths [27]. We thus were able to use the visible, and NIR images to create a false colour three band montage by adding the NIR channel and deleting the blue channel from the visible image. This process highlights the presence of vegetation and other NIR reflective elements within the scene which will display as a red colour in the resulting image. A similar process has been used to create false colour aerial and satellite imagery and serves to highlight photosynthetic biota within an area of interest [43; 44, 45].

We employed band mixing of the red and NIR channels in the false colour image developed as above to create Normalised Difference Vegetation Indices (NDVI). NDVI is a widely used method to quantify amount and vitality of vegetation in a scene by using the difference between the low reflectance of vegetation in red light wavelengths (~600 nm) and the higher reflectance in NIR wavelengths (850 nm) [46, 47]. The resulting index provides dimensionless values that allow inferences on the presence and health of photosynthetic biota in the study area [46, 48].

In addition to using two separate images to create an NDVI we tested the viability of using single-image processing in detecting photosynthetic biota in our study environment. We captured images through the 625 nm red filter and created NDVI from exploiting the blue and red channels of this image. This procedure relies on the theory that the blue channel of the camera contains more NIR than the red channel [45]. Previous application of this method has relied on a custom white balance setting to remove the red cast of the resultant image and to facilitate NDVI processing [45]. We achieved this post image capture by applying histogram equalisation across the red, green and blue bands to neutralise the red colour cast. We then compared the results of NDVI processing through the red filter with visible and NIR filters in order to determine the usefulness of single image capture through a single red filter versus multi image capture through visible and NIR filters. The success of this method would allow for single-image capture of a scene and significantly reduce the amount of time and bandwidth needed to sample a study site.

Spectrometer

Junior carried a visible light reflectance spectrometer based on a Raspberry Pi camera with a pixel resolution of 2592 x 1944, and a diffraction grating derived from a DVD. A version of this method of spectroscopy has been used in previous research, although predominantly for transmission [49]. Our instrument was intended to collect spectra from materials of interest in order to assist in identifying mineralogical or biological composition. Both the multispectral camera and spectrometer were controlled by a Raspberry Pi Model B on a 2.4 GHz wifi link.

Other instruments

Additional instruments carried on the rover included a 1.2 Ghz wireless camera for vehicle navigation and a Melexis non-contact thermometer. The thermometer output was read by the arduino and transmitted via serial link on command from the ground station. The thermometer was used to sample temperature readings around the hot springs in order to assist in assessing the type of environments inhabited by the extremophiles.

Rover control

In order to simplify the overall design and remain within budget we opted for a teleoperated mode of rover control. This mode has been used in previous space missions, such as the Lunakhod rovers sent to the Moon [40]. Teleoperation simulates the operation of the rover in conditions where live or near live communication is feasible, such as on the Moon or at a manned Mars base. Control of the Mars'Obot rover was achieved by a combination of a 2.4 Mhz four channel R/C system and an AP220 2.4 Ghz serial wireless modem. These were chosen for their simplicity, reliability and range, sufficient to control the rover within the specified operational radius of 30 m. The two channels of the R/C system control the forward/reverse motion of the rover and the steering.

Results

The surface of both hot spring sites consisted of loose material of sinter-derived and pumiceous pebbles ~ 1-3 cm in diameter overlying *in situ* sinter deposits (Fig. 2). Local slopes for both sites are less than 5^o, being predominantly creek bed. We found this terrain to be ideal for operating our skid steered rover and we encountered no mobility obstacles in operating in this terrain. The major geological and microbial components of both sites were imaged, spectra obtained and temperature readings collected. The rover traverse of the Waimangu Volcanic Valley site commenced approximately 6 m from the water edge and temperature readings ranged from 35^oC at the start of the traverse to 45^oC at the water's edge. Temperature readings of the water itself from the rover were $\sim 20^o$ lower than those taken from a handheld unit, although readings of the stream bank correlated with those of a handheld device. The discrepancy was likely due to the inability of the rover servo to position the thermometer over the hottest source of the hot spring without risking damage to the rover. We have since designed an extension arm system for the non-contact thermometer to reach inaccessible sites.

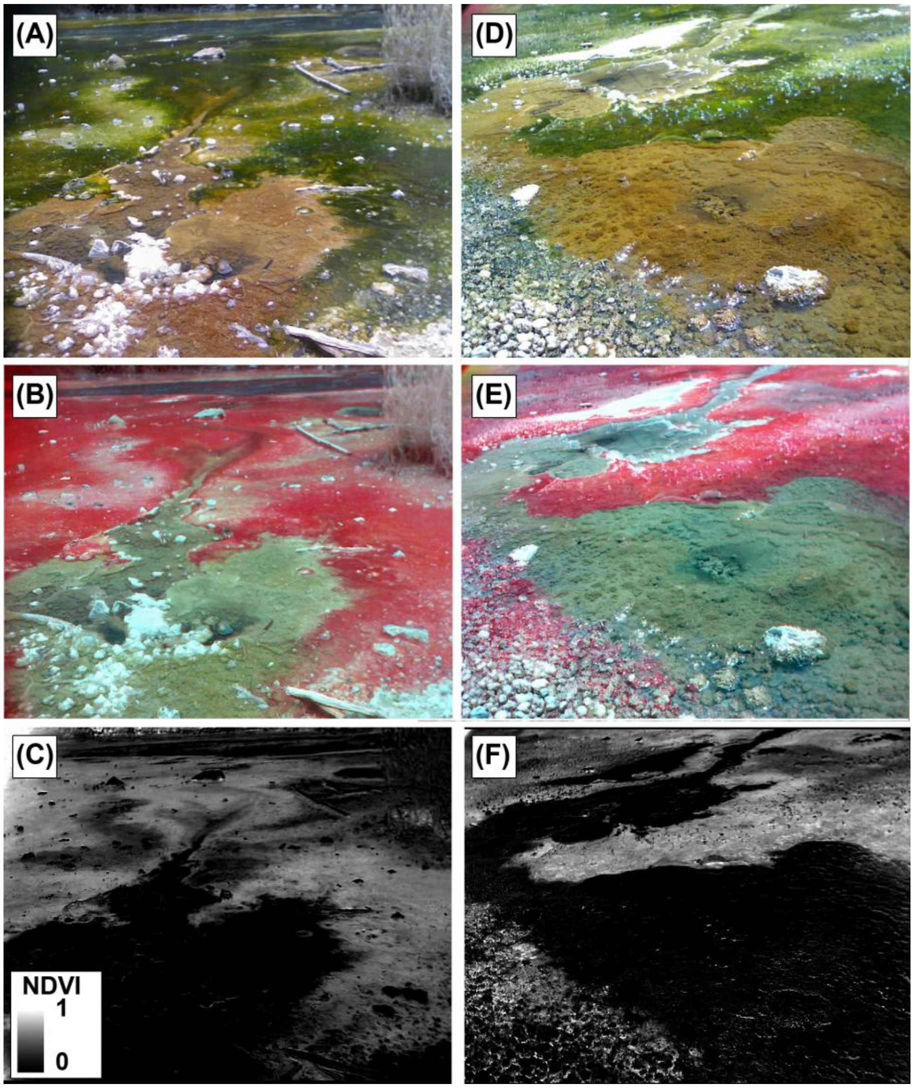

Fig 3. Visible, false colour and NDVI of sample sites derived from Junior's multispectral camera at Waimangu Volcanic Valley, New Zealand. (A) True colour (B) visible/NIR false colour and (C) NDVI images of an outflow vent. (D) True colour (E) visible/NIR false colour and (F) NDVI of microbial deposits.

Figure 3A-F shows multiband analysis as derived from visible and NIR filter photography of the Waimangu Volcanic Valley site as obtained from Junior's multispectral camera. Figure 3A-C were imaged from the site of an outflow vent near the stream shore. Cyanobacterial microbial life in the cooler waters surrounding the vent (30-35^{o}C) (green material, Fig. 3A) was very highly reflective in NIR and displayed as bright red in the false colour image (Fig. 3B). NDVI of this material was also approximating values of 0.5-0.6 (Fig. 2C). We found no evidence of photosynthetic life in the immediate vicinity of the vent itself, as revealed by NDVI values of 0 in this area (Fig. 3C). Temperature readings of the vent were 80-90^{o}C, generally being too high for cyanobacteria to survice. It is thus unlikely the brown material surrounding the vent is cyanobacteria. Additional imaging of sinters near the stream shoreline (Fig. 3D) revealed strong evidence for photosynthetic life forms bounding the cooler waters (30-35^{o}C) of the outflow vent (Fig. 3E-F). We also observed the presence of microbial life at the base of the pebble-like sinter, illustrated in the bottom left of the false colour and NDVI analysis of Figs. 3E-F. This material was absent from the portions of sinter protruding from the stream flow.

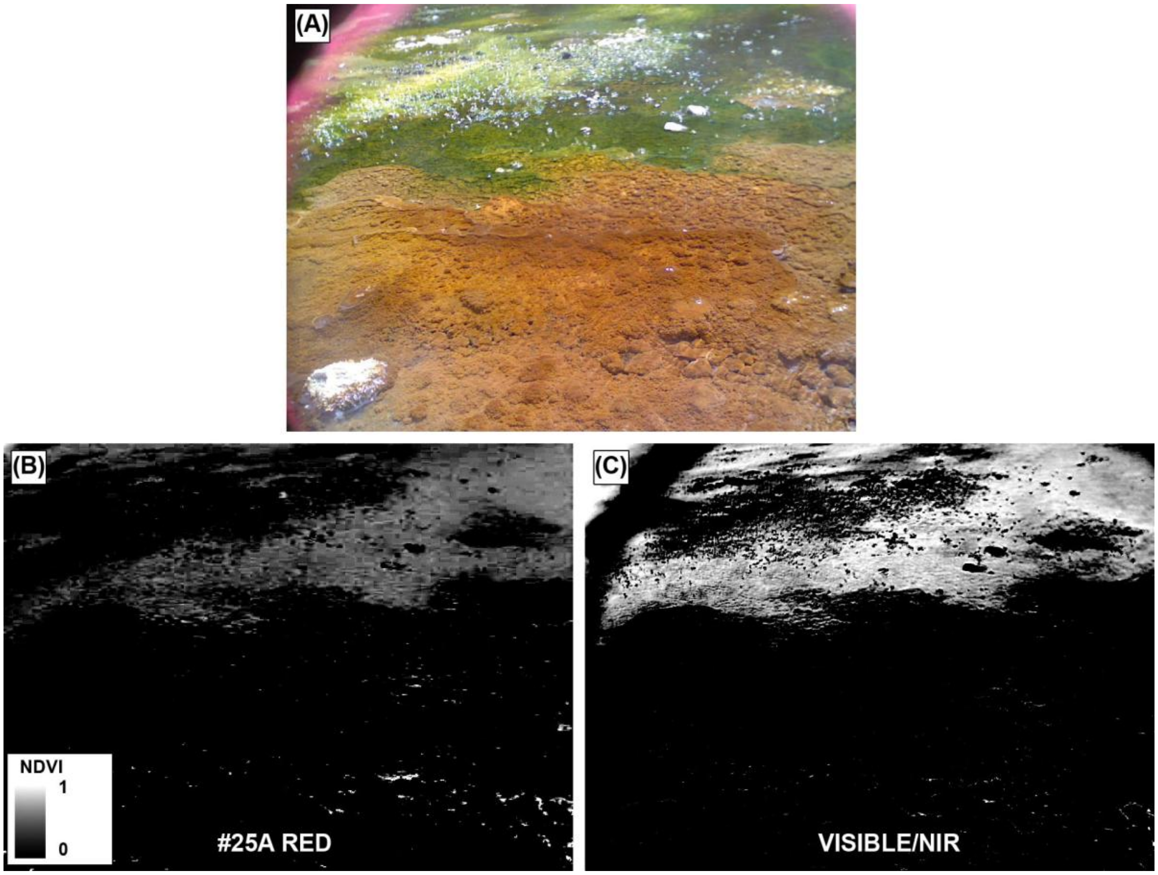

Fig 4. Visible and NDVI of comparison between visible/NIR and red filters from junior's multispectral camera at Waimangu Volcanic Valley. (A) True colour (B) NDVI derived from red filtered image and (C) NDVI derived from visible/NIR images.

We undertook comparative analysis of single filter NDVI and two filter imaging at Waimangu Volcanic Valley, although time constraints allowed us to only capture one image through the red filter at this site (Fig. 4). In order to achieve this we imaged a portion of stream shoreline partially containing algal microbial material (*Cyanidium*, [23]; Fig. 4A). NDVI analysis of the red filtered imagery revealed the presence of the algae (Fig. 4B), though the green algal material was more prominent in the NDVI analysis of the separate visible/NIR images.

We sampled temperatures of the Parariki Stream floodplain and vents at Rotokawa geothermal area using the non-contact thermometer on board Junior and compared them with readings from a hand-held device in a similar manner to the Waimangu site. Although readings of the floodplain for both instruments ranged from 30-40^{o}C, our samples from the actual vents were 52^{o}C, compared with 80^{o}C from the handheld device. Figure 5A-I shows multiband analysis as derived from visible and NIR filter photography of Parariki Stream as obtained from Junior's multispectral camera.

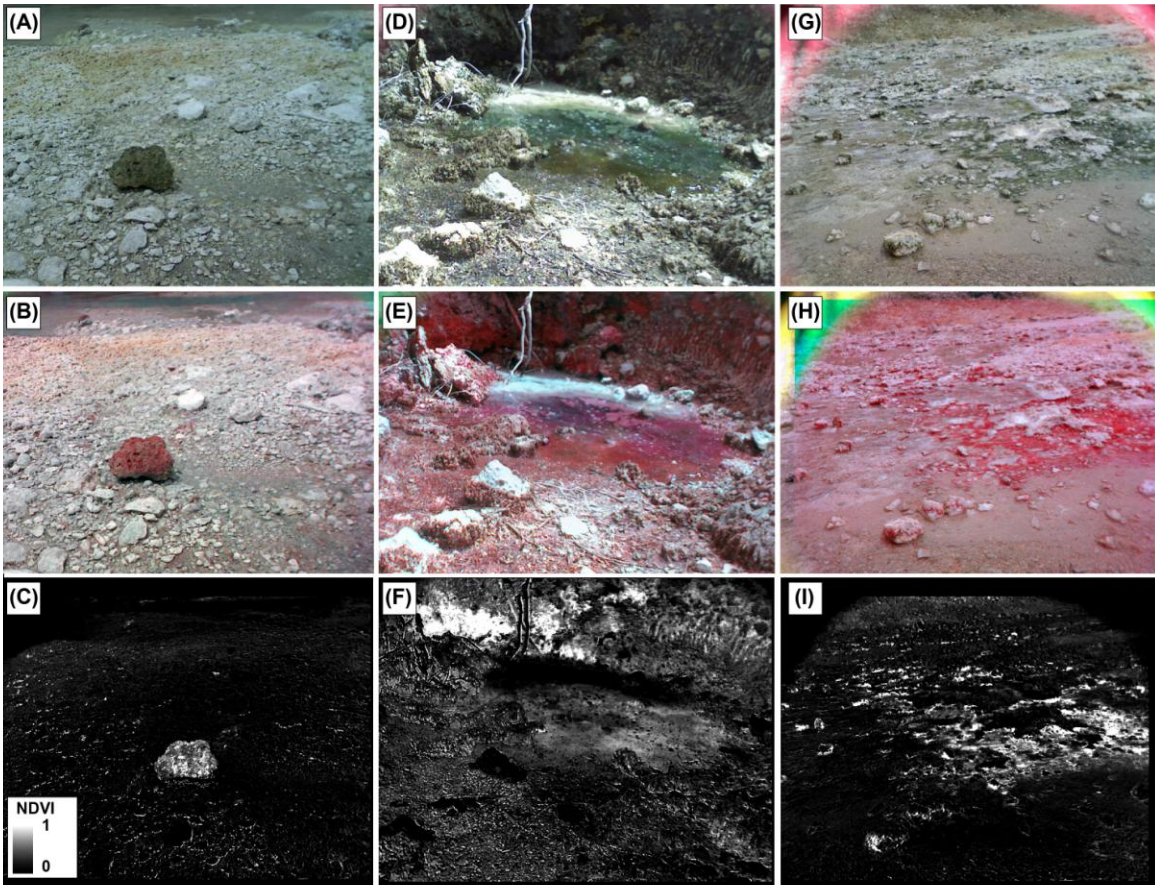

Fig 5. Visible, false colour and NDVI of sites around the Parariki stream from Junior's multispectral camera. (A, D, G) True colour (B, E, H) False colour derived from visible/NIR images. (C, F, I) NDVI derived from visible/NIR images.

We observed that sinters possessing green algal microbial material become prominent in false colour and NDVI analysis whereas other microbial types and mineralogy were not. Fig. 5A-C show analysis of a pumice clast containing algal material that was placed in a region of creek bed where algae was absent. We noted that the clast containing algal material was highly reflective in NIR whereas adjacent clasts embedded within the stream bed were not (Fig. 5B-C). We located a ~1 m diameter thermal pool with an abundance of algalmicrobial material growing around its rim (Fig. 5D). We were able to detect NIR from this pool, despite the green algal material being located underwater, which tends to absorb NIR radiation. We noted that the layer remained visible in false colour and NDVI analysis (Fig. 5E-F) although it was not as prominent as the background vegetation surrounding the pool. It is possible the water may have attenuated the transmission of NIR radiation from the submerged algal material. Fig. 5G-H shows the location of thin layered green algal deposits surrounding rockier material. As with the other sites, the green algae layer was prominent in the false colour and NDVI analysis (Fig. 5 G-H). NDVI values for this layer were high (0.8-1, Fig. 5I) in the densest portions of this green algal layer, which contrasted with lower values present in the imaged clast (0.5-0.8, Fig. 5C) and pool (0.3-0.4).We noted that the green algal layer was absent from the top of the rocky material, probably due to the absence of water in this region. In addition to the algal material being present on the base of the rocky layer, it was also observed mixed within some of the pumice clasts, as evidenced by the high NDVI reading of the large clast in the foreground (Fig. 5H-I).

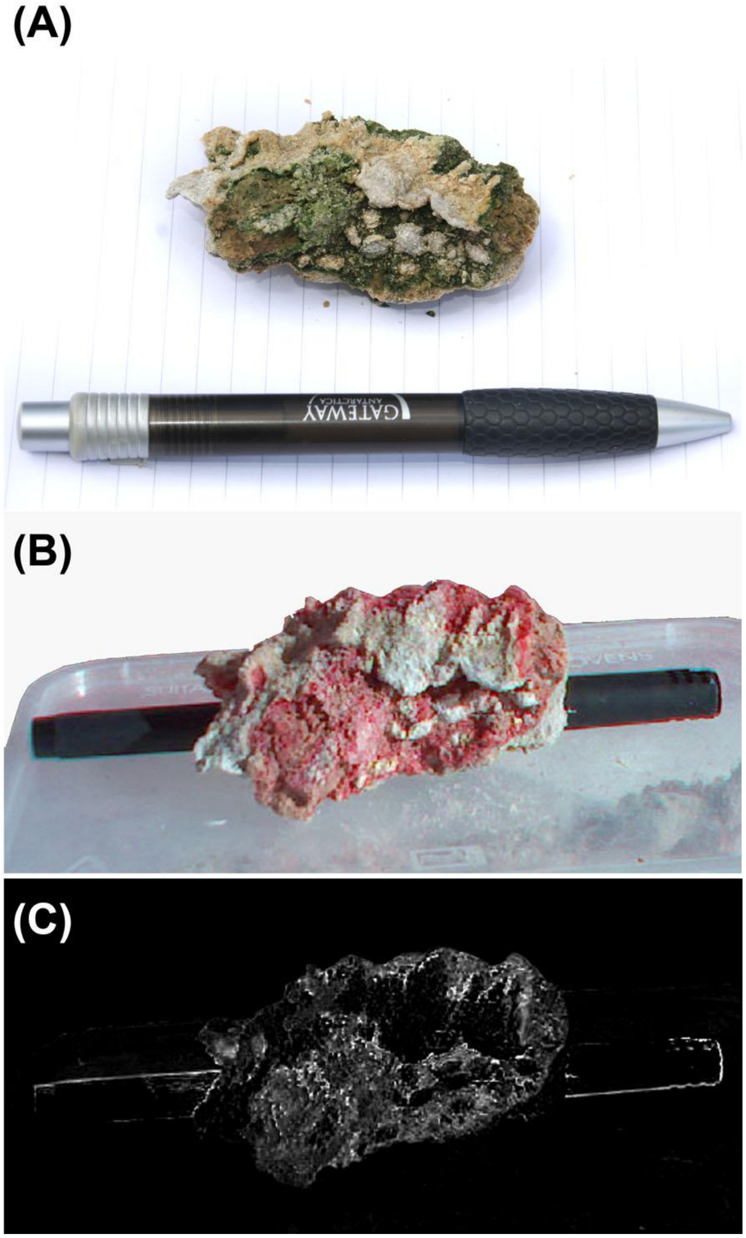

Fig 6. (A) Visible, (B) false colour and (C) NDVI of returned sinter sample taken from the Rotokawa stream containing endolithic green algae. The highlighted area around the scale pen is a product of band mixing the image.

We extracted a sample of sinter from the Rotokawa stream for analysis by Junior in a controlled environment (Fig. 6A-C). The sinter was a portion of a larger pumice clast located ~5 m upstream of the water edge. Unfortunately time constraints precluded context imaging of the sample in situ. Junior imaged the sample in direct sunlight at a distance of 25 cm using the visible and NIR filters. The returned sample clast was impregnated with endolithic green algal material in a similar manner to that observed in the foreground clast in Fig. 5G-I. Visible imagery showed the algae to be green-brown in colour and present within 5 mm of the outer surface of the clast (Fig. 6A). False colour imagery was able to confirm the biological nature of the material, displaying as red in Fig. 6B. NDVI analysis revealed values of 0.3-0.6 (Fig. 6C). There was no discernable difference in NDVI values between the green and brown coloured algal deposits, indicating similar levels of chlorophyll-based photosynthesis for both types of biota. Similar coloured cyanobacteria were analysed by [30] in the outflow of Frying Pan Lake.

Discussion

We were able to successfully operate a small rover in a range of hot springs environments of the Taupo Volcanic Zone near Rotorua, and to return data in regards to some types of extremophiles at two study sites. The rover was able to negotiate the terrain at both sites, with the low slopes and sinter/pebble matrix of the traverse sites proving ideal for the skid-steered mobility of the rover. We found that rover operations were limited to 30 minutes, as constrained by battery life for the vehicle.

Although we were able to successfully obtain temperature readings at both study sites from the non-contact thermometer, we found that readings of the hottest areas in the hot spring vents were underrepresented by 20-30^{o}C. This is probably due to the inability to rotate the instrument platform more than 45^{o}, precluding unobstructed sampling of the vents. It is also possible the non-contact thermometer collection area was too large to obtain unmixed readings of the hottest regions of the study site.

Multispectral imagery of a hot spring site using robotic rovers showed that identification of photosynthetic biological material is possible by using modest budget, off-the-shelf technology. Additionally quantitative assessments on the abundance and health of this biotic material may also be achieved using NDVI analysis. The visible/NIR method was appropriate for this task; however, we were not able to confidently distinguish between other types of extremophiles or mineralogy using this method. Detailed analysis would require the use of narrow pass geologic filters, microscopy and high resolution spectroscopy. These will be included in a future Marsobot rover.

Additionally we found that NDVI on photosynthetic material may be calculated using single imagery collected through a #25A red filter. We note the values derived from this procedure were lower than those sampled through separate images collected through separate visible and NIR filters. It is likely the discrepancy may result from NIR leakage between the red and blue channels of the camera. Thus while this method would be appropriate for healthy vegetation exhibiting high NIR reflectance, it would be less appropriate for diseased or senescent vegetation with lower NIR reflectance.

Obtaining these results has provided useful information on the capabilities and limitations of using robotic sensors to search for extreme forms of life. The results show that it is possible to map the distribution of at least some types of microbial life using low-cost instruments that can be fitted to a small rover. However, these observations are preliminary only, and the rover does not substitute for more sophisticated studies involving sample return, microscopy and other laboratory methods [19, 20, 21], beyond the capabilities of small robots such as Junior. The application of such field robotics in studying fragile and dangerous field sites would be to assist in prioritising the sites for direct sampling by field scientists. Also of importance is ensuring adequate power storage reserves to carry out missions of useful duration, either in the form of larger batteries or batteries that can be rapidly replaced with ones fully charged.

In relation to the robotic astrobiological exploration of Mars, the difficulty in obtaining conclusive evidence for biological, as opposed to mineralogical activity has been acknowledged [11, 20], particularly in the field of paleomicrobiology where return of samples, site revisits and sophisticated laboratory techniques are required to assist in distinguishing life from non-life or on ancient well-preserved hydrothermal sites such as the Warrawoona Group of the Pilbara Region [50]. So far the only specific search for life experiments performed by Viking highlighted the inherent problems of trying to obtain conclusive evidence for life within the limits of robotic methods [11]. Moreover, the size, power and weight constraints of planetary spacecraft limit the carriage of instrumentation to only a few, carefully chosen, experiments. Thus our testing of proposed instruments within a hot springs environment analogous to those extant on early Mars provide useful benchmarks for designing future robotic astrobiological missions targeting such features.

Conclusion

We conducted field trials of Marsobot Junior, a microrover class teleoperated vehicle, within the hot springs environment of Rotorua, New Zealand. We found that multispectral imagery using visible and NIR to be a useful tool in identifying photosynthetic microbial material from mineralogy in extreme environments. We were also able to characterise biological material within a pumice clast sampled from the Parariki Stream study site. Although single-filter multispectral imagery also provided useful data we suggest that radiation leakage between channels limit this technique to sampling healthy vegetation. Time constraints derived from rover energy requirements restricted the amount of data that could be gathered by the rover and highlights some of the difficulties in using robots for astrobiological research. Results and lessons learned from Junior’s mission, especially with respect to instrument selection, design and on power reserves, will be used to further develop instrumentation for astrobiological and extremophile research.

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