COMNAP Fellowship Report 2016

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Project title

Title: 100 years on: a re-evaluation of the first discovery of limno-terrestrial microfauna of the McMurdo Sound region.

Home Institute

South Australian Museum, Adelaide, Australia

Host Institute

Gateway Antarctica (University of Canterbury, NZ)

International project collaborators

Professor Ian Hawes (Gateway Antarctica)
Associate Professor Mark Stevens (South Australian Museum)

I. BACKGROUND & RATIONALE:

It is a little over 100 years since James Murray, biologist with Shackleton's 1907-09 British Antarctic Expedition, published his account of microscopic life in ponds at Cape Royds, Ross Island (see Murray, 1910). Murray's monographs on rotifer and tardigrades (and less so for nematodes) are perhaps the most comprehensive early accounts of terrestrial biology from Antarctica. Their existence provides a unique opportunity to address two topical issues in Antarctic terrestrial biology (*Species Introduction* and *Biogeography*) that have important management implications.

Species introductions

The threat of exotic species introductions to Antarctic communities has a high profile amongst managers, but while introduced species are known for the Maritime Antarctic, no naturalised terrestrial exotics are known from the extreme McMurdo Sound region. Perhaps this is because of the difficulty in identifying taxa in a region where most organisms are inconspicuous, including the tardigrades and rotifers of Murray's research. These are, however, amongst the most likely of invaders, being widespread, microscopic, easily dispersed and mostly tolerant of desiccation and other extreme conditions. Furthermore, Cape Royds is a prime location for exotic introductions, having high visitation, including at times when quarantine management was not prioritised. A re-evaluation of these microfauna will provide an indication of whether 100 years of discontinuous human occupation of Antarctica may have had an impact on local biota and thus provide a baseline for risk assessment of invasive taxa in continental Antarctica.

Biogeography

The current paradigm of Antarctic terrestrial biodiversity is that numerous refugia persisted through glaciations, where organisms survived and later expanded. Genetic differences between populations, and in some cases apparent speciation of new taxa, have resulted (Stevens et al., 2006; Hawes et al.,

2010). For managers, this highlights a need to consider protection of populations, rather than species, with movement of organisms between locations in Antarctica potentially as risky to regional diversity as new introductions. Taxonomic groups with different dispersal capacities show regionality to different extents, and comparisons of genetic diversity between populations of microfauna from the Ross Sea and Indian Ocean sectors of Antarctica will provide an opportunity to evaluate the extent of regional isolation within what should be a readily-distributing group of organisms.

II. OBJECTIVES OF THE PROJECT

Main Objective

To resample ponds at Cape Royds where rotifers, tardigrades and nematodes were collected and described by James Murray during the 1907-09 British Antarctic Expedition. Comparison of existing fauna with that of 100 years ago will (a) allow molecular methods to be applied to material from the type localities of several endemic Antarctic species and (b) determine whether any new taxa have been introduced over 100 years of discontinuous human presence.

Secondary objective

To collect and characterise microfauna from a range of other shallow water habitats in the McMurdo Dry Valleys to determine local distributions, examine links to water quality and to allow comparison with samples already collected from other parts of the Antarctic to develop a multi-scale picture of their biogeography in Antarctica.

III. METHODOLOGY

Field sampling and microfauna collection

Sampling at Cape Royds

The five largest lakes (Pony Lake, Green Lake, Coast Lake, Clear Lake and Blue Lake; Fig 1) from Cape Royds were sampled for microfauna present in cyanobacteria and water column (Fig 2. Sampling in Cape Royds). Cyanobacteria (three squares of 6.5 cm^2) was collected from within the lake and placed in a bucket with 2 L of water. Cyanobacteria was broken down into small pieces by the use of a spatula, the content was poured onto a 400 µm mesh sieve and the flow through was kept in another bucket. Cyanobacteria retained in the 400 µm mesh sieve were photographed and latter discarded. The filtrate from the 400 µm mesh sieve was poured through a 38 um sieve, and the retained material (small sediment and microfauna) was transferred into a 50 ml Falcom tube for later analysis. Microfauna were also collected from the water column. A 20 L bucket was used to collect 60 L of water and passed them through the 38 µm sieve where the microinvertebrates were retained (when present). Water chemistry (pH, salinity and temperature) was also recorder for each lake, and water samples taken to be analysed for major ions using standard techniques (Table 1).

Figure 1. Map of lakes from Cape Royds where sampling took place (inserted from James Murray's manuscripts)

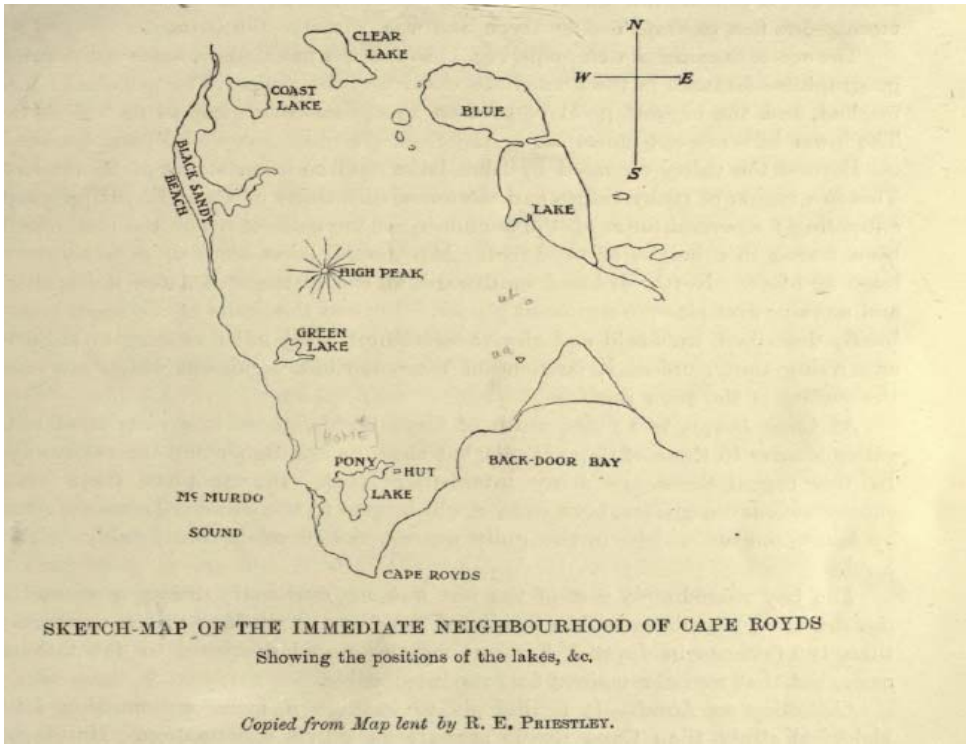

Figure 2. Sampling at Cape Royds

<u>Sampling at the Dry Valleys</u>

Our sampling took place in the Wright and the Miers Valley. Cyanobacteria was sampled (following the same methodology used at Cape Royds) and water chemistry recorded (Tables 2, 3). Sediment samples were also collected at each of the ponds along three equidistant 10 m transects. Sampling took place at the pond shore, at 5 m and at 10 m away from pond (Fig. 3; tree samples per transect and nine samples per pond). Sediment samples (10 cm deep and 8 cm diameter) were placed in

individual plastic bags which were latter analysed for microfauna presence. In order to extract microfauna we followed a modified sugar centrifugation protocol (Velasco-Castrillón et al. 2014).

Figure 3. Lake transect

Table 1a. Cape Royds' Lakes locations and field measurements

Lake	Date	Coordinates pond		pH	salinity (parts/1000)	Temp (°C)
		South	East			
Pony Lake	29/01/2016	77° 33.176'	166° 09.952'	9.93	10.1	0.6
Green Lake	29/01/2016	77° 32.982'	166° 09.358'	10.42	8.73	1.2
Coast Lake	29/01/2016	77° 32.549'	166° 09.085'	9.23	0.73	1.4
Clear Lake	29/01/2016	77° 32.514'	166° 09.804'	9.3	1.86	0
Blue Lake	29/01/2016	77° 32.618'	166° 10.363'	10.35	0.27	0.6

Table 1b. Cape Royds' Lakes locations and water chemistry. All values g m^{-3}

Lake	Ca	Mg	K	Na	Cl	SO_4
Pony Lake	102	301	283	3180	4400	2300
Green Lake	74.6	290	376	2580	4200	650
Coast Lake	10.8	26.2	18.6	207	390	47
Clear Lake	23.3	54.5	119	554	700	159
Blue Lake	302	495	67.9	1500	152	27

Table 2. Wright's ponds

Site	Date	Coordinates		Pond features		water chemistry	
		South	East	Elevation	diameter (m)	pH	salinity (parts/1000)
Wright 01	22/01/2016	77° 33.980'	161° 24.867'	584 ft	80 x 70	8.31	7.31
Wright 02	22/01/2016	77° 34.212'	161° 21.807'	1108 ft	90 x 70	9.89	0.09
Wright 03	22/01/2016	77° 34.113'	161° 20.961'	1271 ft	70 x 50	8.13	2.79
Wright 04	22/01/2016	77° 34.103'	161° 20.567'	1089 ft	50 x 25	8.36	3.77
Wright 05	22/01/2016	77° 34.069'	161° 20.203'	973 ft	25 x 25	8.45	6.65
Wright 06	22/01/2016	77° 34.094'	161° 19.746'	1081 ft	40 x 30	8.06	2.48
Wright 07	25/01/2016	77° 33.453'	160° 57.171'	2113 ft	100 x 90	9.39	0.51
Wright 08	25/01/2016	77° 33.734'	160° 55.246'	2459 ft	90 x 60	8.55	1.69
Wright 09	25/01/2016	77° 33.457'	160° 54.563'	2330 ft	100 x 90	9.87	0.1

Table 3. Miers' ponds

		Coordinates pond		Pond features		Water chemistry	
Site	Date	South	East	Elevation	diameter (m)	pH	salinity (parts/1000)
Miers 01	14/01/2016	78° 06.078'	163° 47.921'	584 ft	50 x 70		
Miers 02	15/01/2016	78° 05.622'	163° 49.931'	534 ft	2000 x 800	9.12	0.04
Miers 03	16/01/2016	78° 03.254'	163° 44.063'	1369 ft	70 x 30	8.49	0.34
Miers 04	16/01/2016	78° 03.311'	163° 44.398'	1360 ft	20 x 60	8.59	0.48
Miers 05	16/01/2016	78° 03.547'	163° 45.767'	439 ft	40 x 30	8.48	0.35
Miers 06	16/01/2016	78° 03.629'	163° 46.001'	1490 ft	180 x 400		
Miers 07	17/01/2016	78° 07.475'	164° 09.883'	178 ft	15 x 10	10.07	0.89
Miers 08	17/01/2016	78° 07.45'	164° 09.997'	204ft	16 x 10		
Miers 09	16/01/2016	78° 03.839'	163° 46.003'	1587 ft	200 x 300		
Miers 10	16/01/2016	78° 04.070'	163° 48.593'	1786 ft	12 x 100	8.49	0.63
Miers 11	17/01/2016	78° 07.565'	164° 11.494'	162 ft	10 x 8	10.74	1.78
Miers 12	17/01/2016	78° 07.637'	164° 12.241'	168 ft	30 x 20		
Miers 13	17/01/2016	78° 07.569'	164° 11.212'	171 ft	10 x 6		
Miers 14	17/01/2016	78° 07.435'	164° 11.440'	123 ft	12 x 8		

Microinvertebrate isolation

Specimens were poured into a petri-dish to be examined under a dissecting stereo microscope at magnification 10X and 40X. Taxa were divided into glass blocks using micro-syringes and morphotypes were selected for morphological inspection and subsequent molecular analysis. Specimens were carefully transferred with an Irwin loop into a water droplet on a slide and imaged under microscope (Celestron- LCD Digital Microscope, USA) at 100X before placing in separate 1.5 ml Eppendorf tubes. The remaining microfauna not selected for imaging were stored at -20 °C.

Molecular work

Total DNA was extracted from the whole specimen and amplified using LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG -3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). PCR products of the mitochondrial *COI* gene were amplified on a thermocycler with the following conditions: 94 °C for 10 min for initial denaturation, followed by 35 cycles at 94 °C for 1 min, 50 °C for 1.5 min, and 1 min at 72 °C, with a final extension of 72 °C for 10 min. The 25 µl PCR reaction mix for one reaction included: 5 µl of DNA template, 0.1 µl of Immolase DNA Polymerase (5u/µl), 1 µl of each 5 µM primer (Invitrogen^{TM}), 12.9 µl of PCR water and 5 µl of 5X MRT buffer (5X immolase buffer, 4 mM dNTP mix, 0.25 mg/ml BSA). PCR products were run in a 1.5 % agarose gel, and subsequently stained in a red-gel solution for 30 min and visualised in a transilluminator. PCR products with positive amplification were sent for sequencing to *Macrogen* or *AGRF* (Australian Genome Research Facility).

Sequence analysis

Mitochondrial COI sequences gene were checked visually and chromatograms were inspected to resolve unclear base calls; short sequences were disregarded and longer ones (~ 658 bp) used during the Geneious alignment carried out with Geneious Bioinformatics package v3.8 (Biomatters, Ltd, Auckland, NZ). All sequences were compared with those on GenBank using the BlastN algorithm to

confirm that they belonged to the targeted specimens. Contaminated or misidentified sequences were removed.

IV. PRELIMINARY RESULTS

A total of 169 specimens were isolated and classified to different taxonomic levels. DNA was extracted from those 169 specimens (96 rotifers, 43 tardigrades, 30 nematodes) and 100 of them have been sequenced (sequence success rate of targeted specimens: 22%). Species found in our 2016 field trip were compared to those reported by Murray from the 1907 – 1909 Antarctic expedition (see Tables 4, 5 & 6). Only the bdelloid rotifer *Adineta grandis*, the tardigrades *Acutuncus antarcticus* and *Diphascon* sp., and the nematode *Plectus frigophilus* were confirmed by molecular data (> 99% sequence similarity). Two unidentified bdelloid rotifers had no close sequence match with those publicly available in GenBank; and for three unidentified tardigrades their closest match correspond to *Minibiotus* (78% sequence similarity). When comparing our findings with those reported by Murray, we noticed that no new species have been recorded for Cape Royds lakes'. Although, their distribution amongst lakes has slightly changed (see Table 4 & 5). Nematodes were not of particular interest to Murray, he only mentioned that "two kinds of nematodes" were found in the lakes, most likely one of those corresponds to *P. frigophilus* (judging by image shown at Murray's manuscript). No microinvertebrates were found in Pony Lake during Murray's expedition or during our 2016 field trip.

Table 4. Rotifer diversity in Cape Royds' lakes

taxon/species	Green Lake		Coast Lake		Clear Lake		Blue Lake	
	Murray	2016 field trip	Murray	2016 field trip	Murray	2016 field trip	Murray	2016 field trip
***Adineta gracilis* Janson, 1893**					x		x	
***Adineta grandis* Murray, 1910**	x	x		x		x		
Adineta vaga vaga* (Davis, 1873)			x		x		x	
***Adineta* (unidentified)**				x		x		
***Habrotrocha constricta* (Dujardin, 1841)**	x							
***Habrotrocha* (unidentified)**		x		x				
***Macrotrachela habita* (Bryce, 1894)**							x	x?
***Philodina alata* Murray, 1910**				x			x	x
***Philodina gregaria* Murray, 1910**			x	x	x	x	x	
***Philodina* sp.**							x	x
***Epiphanes senta* Müller, 1773**		x	x					
***Encentrum* sp.**							x	

Table 5. Tardigrade diversity in Cape Royds' lakes

species	Green Lake		Coast Lake		Clear Lake		Blue Lake	
	Murray	2016 field trip	Murray	2016 field trip	Murray	2016 field trip	Murray	2016 field trip
Acutuncus antarcticus			x	x		x	x	
Macrobiotus oberhauseri								
Macrobiotus polaris								
Diphascon alpinum							x	
Diphascon sp.								x

Table 4. Nematode diversity in Cape Royds' lakes

taxon/species	Green Lake		Coast Lake		Clear Lake		Blue Lake	
	Murray	2016 field trip	Murray	2016 field trip	Murray	2016 field trip	Murray	2016 field trip
Plectus frigophilus				x				x
Plectus murray				x?				
Nematodes (unspecified)			x				x	

V. FUTURE WORK

- Isolate and identify microfauna from the Dry Valleys.
- Extract DNA from more specimens, including those collected in in the Dry Valleys.
- Optimise PCR protocols in order to increase the amplification rate success.
- Send for sequencing PCR products.
- Generate dendrograms based on the COI gene for rotifers, nematodes and tardigrades.
- Correlate species presence with water chemistry.

VI. BUDGET EXPENSES (estimates)

A total of US$ 12000 received from COMNAP has been spent as following:

- Airfares (two return flights Adelaide – Christchurch): US$ 1200
- Living expenses in NZ: US$ 300
- Terrestrial transport: US$ 400
- Molecular lab work: US$ 600
- Sequencing: US$ 1600
- Computer software/hardware: US$ 1200

Some of this budget has also been used as contribution to salary and expenses for the work performed during this 7 months of project. The rest of the money will be used for molecular lab work, sequencing and salary.

VII. ACKNOWLEDGEMENTS

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