Field Test of Terrestrial Arthropod (Springtails and Mites) Data Collection Methods and Protocols Proposed for the ABMP

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Disclaimer
The views, statements, and conclusions expressed in this report are those of the authors and should not be construed as conclusions or opinions of the ABMP. Development of the ABMP has continued since this report was produced. Thus, the report may not accurately reflect current ideas.

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Executive Summary

During the 2005 arthropod (springtail and mite) field test, ten locations (3 in the boreal, 4 in the foothills, 2 in the parkland, and 1 in the grassland) were visited to test the arthropod sampling protocols. In addition, prior to conducting the field tests, a 2-day field tour was conducted at 9 locations (6 in the boreal, and 3 in the parkland) to refine the field methods.

Field samples were collected very effectively and efficiently (<1 hour/site) in all habitat types and sampling regions. Only slight modifications are suggested on the collection method initially proposed by the arthropod expert.

Sample processing and specimen identification required the most time and effort by both ABMP staff and experts. Arthropods were extracted from the soil samples using berlase funnels. The specimens collected were then put through a 300μm sieve to separate debris from the sample. Specimens collected from the sieve were processed into morphospecies groups at a rate of 6.7 hrs/site. Results indicated mites to be a more specious group than springtails, as 47 mite and 12 springtail species were detected from the 10 sites surveyed during the field trials. On average, the number of mite species per site (14.2 ± 3.8) was more than double that of springtails (4.9 ± 1.7) across all site types.

Since the ABMP relies on undergraduate students to conduct the field data collection, these staff will not have time during the summer to sort the specimens in the lab as their time is already allocated to other taxonomic ID. Either staff that can work during the fall will need to be hired, or specimen processing will need to be contracted out. The final stage of species identification can only be accomplished by an expert.

We end the report with a description of the arthropod protocols recommended for the ABMP, including the data sheets that are required for field data collection.

Background

The rate, diversity, and magnitude of industrial development in the forested regions of Alberta have increased over the past several decades, and are projected to grow substantially over the next 20 years, as are human populations and related infrastructure grow. This growth has already produced considerable impact upon forested landscapes in the province, and careful management is required to ensure that the cumulative impacts of developments do not adversely affect biodiversity, and the environment in general. Addressing this concern is important to the competitiveness of Alberta economies because market forces are increasingly demanding that protection of ecosystems and biodiversity be clearly demonstrated as conditions of product sales. Industrial players in Alberta, especially the forestry and energy sectors, have demonstrated strong commitments to using adaptive management processes in the conduct of their businesses. Systems and processes that can provide early warning of changes in biodiversity and their relationships to specific industrial and other human activities are urgently required. The Alberta and Canadian governments are similarly committed to conserving biodiversity (Canadian Forest Accord 1998; Canadian Biodiversity Strategy 1995; Alberta Forest Legacy 1998; Alberta's Commitment to Sustainable Resource and Environmental Management 1999). The provincial government is a proponent of adaptive management as a mechanism to ensure that the diverse and rapidly expanding human activities within Alberta's forests are sustainable.

ABMP Phase I: Program Development

Although the need for a comprehensive provincial biodiversity monitoring program was recognized during the 1990s, Alberta did not have the capacity to conduct such a program. As a result, between 1997 and 2002 a partnership (including Alberta industry, government, and research institutes) developed the Alberta Biodiversity Monitoring Program (ABMP). This program was designed to measure and report on the status and temporal changes in biodiversity, habitats and anthropogenic (man-made) disturbances in Alberta at the regional and provincial scales. The ABMP:

- Supports existing commitments for biodiversity monitoring.
- Developed common, standardized methodology that will be applied across all jurisdictions within Alberta.
- Includes monitoring that:
  - occurs in both aquatic and terrestrial systems,
  - occurs across a hierarchy of spatial scales,
  - occurs in locations having a wide range of land use histories, including those with limited human influence and,
  - includes life forms from diverse taxonomic groups and trophic levels.
- Will estimate natural variability to assist interpretations of the significance of any changes observed.
- Will be transparent and subject to rigorous technical review.
- Will provide data and information freely to everyone.

A team of more that 20 scientists identified protocols to survey a broad diversity of biota, habitat structures, vegetation communities, and landscape patterns within Alberta (Table 1). These protocols were reviewed by other scientists from across North America and then amalgamated into an integrated design that will effectively and efficiently survey all elements. The ABMP is designed to monitor long-term, broad-scale changes in biodiversity, many of which are anticipated to be small and occur slowly over time. The basic survey design consists of 1656 sites, evenly spaced on a grid pattern throughout Alberta. Once the ABMP is fully operational, approximately 375 ABMP sites will be surveyed each year. The program will facilitate comparisons among geographic regions, and will feed information into an adaptive management process for use by land managers and regulators interested in evaluating long-term sustainability of biodiversity and resources. The ABMP was designed to detect, with at least 90% certainty, i) a change of 3% per year within a region after 3 visits to all sites in the region, ii) a two-fold

difference between regions after one complete set of surveys, and iii) to have less than a 10% probability of declaring a difference when there really was none.

Table 1. Types of elements that are monitored as part of the ABMP.

Terrestrial	Aquatic
Species Taxonomic Groups	
Mammals	Fish
Birds	Benthic Macroinvertebrates
Springtails	Zooplankton
Mites	Phytoplankton
Vascular Plants	Benthic algae
Bryophytes	
Lichens	
Fungi	
Habitats	
At the Local Patch Scale	
Live trees by species and size class	Basin characteristics
Dead tree & log by size and decay class	Channel characteristics
Shrub cover by height	Submergent and emergent vegetation
Cover and composition of low ground vegetation	Vegetation at edge of water body
Cover and composition of litter	Amount of down wood
Soil amount and composition	Water physiochemistry
Vegetation diversity	
At the Landscape Scale	
Diversity of habitat types	Length by stream type/size
Area of >200 habitat types	Area by basin type/size
Patch size, shape, and connectivity for five major habitat types	Connectivity and sinuosity of aquatic elements
Human Footprints	
Urban and/or industrial habitats	Bridges, culverts, and stream crossings
Rural residential	Residential and cottage
Roads	Agricultural habitats
Vegetation covered trails and seismic lines	Harvested habitat
Agricultural habitats	
Harvested habitat	

Information and analyses generated from the program will be used to delineate correlations between development events and changes in biodiversity, that in turn can be pursued by experimental research. Above all, the ABMP is designed to be scientifically credible, transparent, and to satisfy the biodiversity monitoring needs of government, industry, and the public. This program will provide early warning of biodiversity change, and reduce the cost of sustaining biodiversity by avoiding expensive species recovery programs. See http://www.abmp.arc.ab.ca/ for additional details about the ABMP.

ABMP Phase II: Prototype Project

Many of the scientific and technical aspects of the ABMP were completed by 2002. A group of senior managers from government, industry, and non-government organizations reviewed the ABMP and decided that a Prototype Project would be the best method to move the ABMP from the developmental phase into the operational phase. As such, the ABMP Prototype Project was initiated in January 2003. The ABMP Prototype will:

1) Implement a communications plan that highlights the value of the ABMP to secure long-term support from government, industry, and non-government organizations.
2) Collect data from approximately 8% of the ABMP sites as a cautious rollout of the program.

3) Develop, test, and refine the protocols that have not been completed (aquatic, fungi, and terrestrial arthropod protocols).
4) Refine and test the remote sensing protocols.
5) Test the statistical adequacy of the ABMP protocols and sampling design.
6) Develop a platform for data management that will effectively store, handle, retrieve, and distribute data.
7) Develop standard analyses, reports, and other products and services that will empower end-users to draw reliable inferences from the ABMP data.
8) Development a long-term business plan addressing governance, funding and other essential items.

Objectives

In 1999 an initial set of terrestrial arthropod sampling protocols were developed for the ABMP and subsequently field tested during the 2002 pilot year. See the document "Test of field procedures for terrestrial ground plots" http://www.abmp.arc.ab.ca/18_Chapter_Compendium/Chapter05.pdf. The methodologies tested were deemed not effective in complying with the overall goals and objectives of the ABMP.

In 2003 the ABMP Science Committee recommended that scientific experts be contracted to develop new terrestrial arthropod protocols for the ABMP. During 2004, Jeff Battigelli produced a report describing potential arthropod protocols for the ABMP. This report was reviewed by external peers and the ABMP Science Committee. As a consequence of those reviews, the protocols were revised and the report was then posted on the ABMP web site http://www.abmp.arc.ab.ca/Documents/ABMP%20arthropods%20revised%20Sampling%20Protocols.pdf. A field test of the proposed arthropod protocols was suggested for 2005.

The present report describes field testing that occurred, the problems that were encountered, the solutions that were developed, and a summary of the data that were collected during 2005. We end the report with an Appendix describing the arthropod protocols recommended for the ABMP, including the data sheets that are required for field data collection.

Methods

Overview

Two field staff were hired to conduct the arthropod field test. To ensure the field staff implemented the protocols effectively, the author of the arthropod report (Dr. Jeff Battigelli) as well as Dr. Heather Proctor from the University of Alberta, participated in a two day pre-trial field trip. In addition, the ABMP Field Coordinator and Scientist participated to ensure that appropriate solutions were developed for problems as they were encountered during the data collection and specimen processing. Two types of testing were conducted:

1) Test of Arthropod Data Collection The arthropod expert (Jeff Battigelli) suggested focusing the ABMP arthropod collection to Springtails (*Collembola*) and Mites (*Acari*), specifically the suborder of *Orbatid* mites, as these are the most dominant groups which can be found within the organic soil layer (L – Litter, F – Fermentation, and H – Humus), and can be sampled during a single visit to a site. See the report "Revised terrestrial arthropod sampling protocols for the ABMP: Examining the biodiversity of soil mesofauna (Acari and Collembola) in Alberta" for further details http://www.abmp.arc.ab.ca/Documents/ABMP%20arthropods%20revised%20Sampling%20Protocols.pdf. These data collection methods were field tested to determine whether they could be conducted effectively in the variety of sites that would be encountered in the ABMP.
2) Test of Laboratory Sample Processing The arthropod experts refined the extraction techniques and specimen processing methods that had been outlined in the report "Revised terrestrial arthropod

sampling protocols for the ABMP: Examining the biodiversity of soil mesofauna (Acari and Collembola) in Alberta" for further details http://www.abmp.arc.ab.ca/Documents/ABMP%20arthropods%20revised%20Sampling%20Protocols.pdf. These refined protocols were tested during the trial.

Ten locations (3 in the boreal, 4 in the foothills/mountains, and 3 in the parkland/grassland) were visited to test the arthropod protocols in the variety of habitats that will be sampled by ABMP. Prior to conducting the field tests, 9 locations (6 in the boreal, and 3 in the parkland) were visited, and some alterations to the field tests were made. Results from the field trip and the field test are described below.

Test of Arthropod Data Collection

Sample Collection Method – It was initially recommended that the ABMP take specimen collections from both leaf litter and the organic soil horizon at each site. During the 2-day field trip, it became obvious that only one of the collection methods would be necessary to meet ABMP goals and objectives. Although each collection method would yield slightly different mesofaunal communities, the combination of the two methods would be twice as costly to process while resulting in a large degree of overlap in the species of *collembola* and *orabitid* mites collected. Thus only one sample was collected. It was decided that the soil core method of collecting springtails and mites would be the preferred technique for the ABMP due to its simplistic nature and its repeatability across habitat types.

Soil Core Design – The corer was fabricated out of 5 cm diameter stainless steel pipe that was 40 cm long with handles on the upper portion (Figure 1). In addition, the tip of the corer was tapered to 4.5 cm (allowing the soil core to expand once inside the corer), and had a serrated edge for cutting through the organic substrate by rotating the corer like a cork screw (or twisting back and forth).

Figure 1: Picture of corer designed to collect soil cores for ABMP arthropod collection.

Sample Location – Four locations were sampled at each site we visited. Locations were 80 m from site centre along the sub-ordinal transects (corresponding to the NE, NW, SW, and SE quadrants). Due to the destructive nature of repeatedly taking soil cores, these locations are outside of the 50 x 50 m quadrants that the majority of ABMP data would be collected (Figure 2).

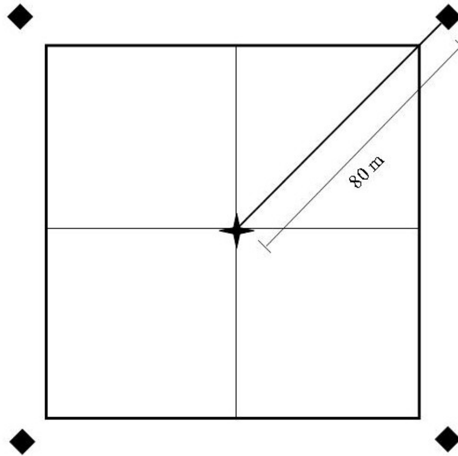

Figure 2: Locations of arthropod sampling at ABMP sites.

Coring Collection Method – After the 2-day field trip, and examining various habitat types that would be encountered throughout the ABMP sampling, the following methods were employed for the field trials:

- Only the organic component of the soil profile was collected. This consisted of the LFH horizon (litter, fermentation, and humus layers) and excluded the mineral soil. The distinction between organic and mineral was easily made in all soil conditions we surveyed based on the colour, texture, and natural breaking point of the soil core. In addition, the soil corer could not penetrate far into the mineral layer. After extracting the core, it was grasped above and below where colour and texture changed, and bent until the core broke. The layer above the break was called the LFH layer.
- In some instances (bogs) the organic layer was deeper than the corer and mineral soil was not reached. In these instances, the entire 40 cm profile of organic material that the corer extracted was collected from each core.
- The goal was to collect 500 ml of organic material from each sample location (quadrant), resulting in a total of 2 L (four 500 ml samples) per site.
- A minimum of 4 cores were taken from each sample location.
- In many cases, less than 500 ml of organic material was collected in the first 4 cores. In these cases, additional cores were taken in a systematic fashion (Figure 3) until 500 ml was collected. The number of cores needed to collect 500 ml was recorded.
- All cores that were collected at a location were mixed together in a sampling bowl and a 500 ml sub-sample was placed into a cloth bag for storage and processing. The volume of soil remaining was recorded and then returned to the site.
- If total volume of organic material was less than 500 ml after taking 24 cores, the total volume of soil was recorded, all organic soil that had been collected was placed into a cloth bag for storage and processing, and sampling ceased for that location.
- Where no organic soil was present, or organic soil was mixed with the inorganic soil (eg, cultivated agricultural fields), only the L (litter) was collected. This included taking just the leaves, grass, and/or other debris on the top of the core.
- For each location, organic material from the corer was described to help the interpretation of results. Descriptions included, but were not limited to: animal material, stumps/logs that could not be cored through, stumps/logs that could be cored through, water, rocks, bare mineral soil, etc.

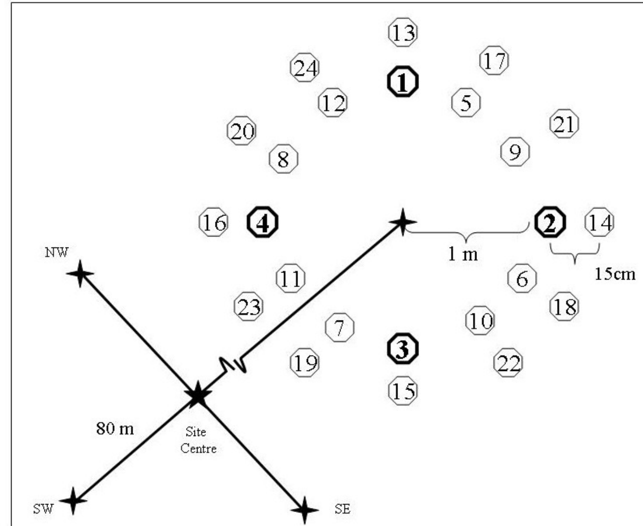

Figure 3: Systematic location of coring sites around a sampling point.

Preserving Samples/Specimens – Samples were collected and placed in cloth bags which contained standardized labels. Labels included the Date, Site, Quadrant, and Collectors initials. While in the field, samples were then kept cool and dry by placing them inside a plastic bag, and placing the plastic bag inside a cooler containing ice. The ice was surrounded by cardboard and newspaper to ensure that samples did not directly contact the ice. Samples were kept in the cooler for a maximum of 6 days to minimize the number of soil organisms that died.

Tests of Laboratory Sample Processing

Extracting Specimens – Extraction of springtails and mites from the organic material started between 4 and 6 days after they were collected. Arthropods were extracted in the laboratory using the berlase funnel technique. Each 500 ml sample was placed onto the mesh screen (that had been covered with cheese cloth) inside a separate berlase funnel. The original sample bag containing the label was attached to the berlase funnel and a new label containing the same information was placed into the specimen cup for continuity of further processing. A 25 watt light bulb was used to provide a constant heat source to each funnel. This heat dried the organic material from the top, stimulating the arthropods to migrate to the bottom and then to drop into a specimen cup that was attached to the funnel (Figure 4). The specimen cup was filled with a 50:50 mixture of antifreeze and water to kill and temporarily preserve the specimens. The extraction process continued for 7 days (168 hours), by which time all the samples were completely dried and no further organisms were being collected.

Sorting to Morphospecies – The arthropod experts (Jeff Battigelli and Heather Proctor) spent one full day instructing 2 field staff on how to remove specimens from the antifreeze, sieve the specimens, sort the samples into collembola and orbatid mites, and to further sort the specimens into morphospecies groups. After 5 days of sorting, Jeff Battigelli spent an additional day with the field staff to refine their techniques and answer questions and concerns that arose over that time.

Each sample was poured through a 300µm sieve. All springtails and mites collected by the sieve were sorted into morphospecies and placed into multi-well culture plates containing 70% ethanol. A brief description of the morphospecies and the number of individuals found was then recorded on data sheets with reference to the specific well number. All microorganisms other than springtails and mites were poured into a separate storage vial containing 70% ethanol and labeled with the original collection date, site, quadrant, and collector's initials.

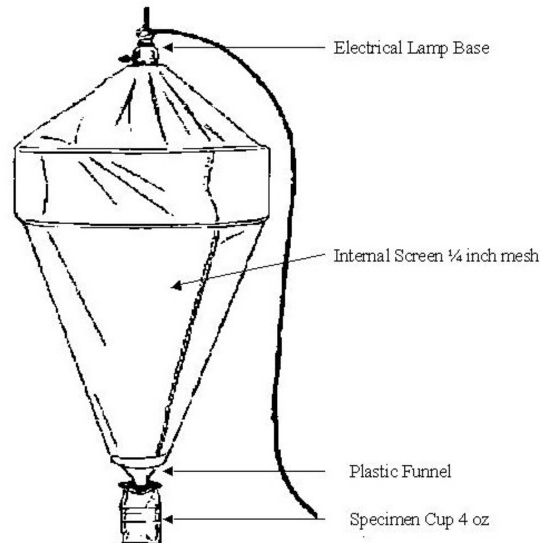

Figure 4: Picture of berlase funnel extractor used in ABMP field trials.

Species Identification – Identification of springtails and mites to species required considerable knowledge - the ABMP summer staff could not acquire that expertise within the time allowed. Thus, after the samples were sorted into morphospecies groups within the lab, they were sent to Jeff Battigelli for him to identify.

Results

Sample Collection

Time Required – Collection of arthropod samples in the field was reasonably quick under all habitat conditions. It required an average of 12 mins (n=39) for 1 person to collect a sample which resulted in an average total of 48 mins to collect all 4 samples per site. These times include all aspects of the sampling collection: locating the sampling point, coring the soil, mixing the sample, measuring the sample, labeling the sample, and recording the data. Sample times varied from 10-15 mins per sample (40-60 mins per site) for the sites included in the field trial.

Preserving and Shipping the Samples

Time Required – Very little time was required to preserve samples while in the field. Samples were placed in coolers when the crew returned to camp and left untouched until the extraction process. The crews brought the coolers to the lab for extraction at the end of the field shift.

Once arthropod sampling is incorporated into the ABMP, however, more time will be required to ship specimens. To meet the constraint of a maximum amount of time in the cooler of 6 days, samples will need to be shipped to the lab periodically during a regular 10-day ABMP field shift. Field crews will need to locate a shipping source (Courier, Bus, etc) every 3-4 days throughout a field shift, to ensure the samples arrive at the laboratory in time for extraction. Depending on the location of the field camp, shipping could require 1-4 hours to be completed.

Specimen Extraction

Time Required – It requires one person approximately 6 mins to assemble and position one berlase funnel, and an additional 5 mins to insert the sample and start the extraction process. These times include all aspects of the assembly and extraction process: assembly, running power cords, cutting cheese cloth, pouring antifreeze mixture, placing the sample into the funnel, hanging the funnel, and writing a new label for the specimen cup. Once the extraction process began, it required one person 10 mins per day, for 7 days, to inspect the berlase funnels to ensure they were functioning properly (i.e., bulbs had not burned out, etc.) and slightly longer if repairs were needed. It required one person approximately 2 mins per sample to remove the specimen cup and discard the remaining soil sample after the 7 day extraction process.

Specimen Processing by Field Staff

Time Required – Due to the variability in sorting samples during the instructional phase (and finding appropriate methods to do the processing) and then processing the samples after the staff felt adequate in the methods, few samples remained to accurately record sorting times. Once field staff were confident in their sorting abilities, it required an average of 100 ± 42 mins (n=16) per sample to sort both springtail and mite specimens into morphospecies retained from the 300µm sieve. Times tended to vary greatly depending on site type and cleanliness of the sample; sites with more species, and sites with lots of debris (sand), obviously took longer to sort. Table 2 shows the "per site" breakdown in approximate times and costs for each taxonomic group.

Table 2: Breakdown of time and cost for sorting and identifying springtails and mites per site.

Taxonomic Groups	Sorting To Morphospecies (ABMP Staff) [1]				Expert ID [2]
	Ave. Time Spent/Site (Min)	**Min. Time Spent/Site (Min.)**	**Max. Time Spent/Site (Min)**	**Approx. Cost ($$) Per Site**	**Approx. Cost ($$) Per Site**
Mites	239 ± 56	165	100	$66.00	$150.00
Springtails	161 ± 50	290	220	$44.00	$100.00
Springtails and Mites (Combined)	400 ± 100	265	495	$110.00	$250.00

[1] Cost based on an average student salary of $120/day @ 7.25 hrs/day

[2] Cost based on field trial costs: $500/day @ 5 days for 10 sites (These are approximate break downs)

Specimen Identification by Experts

Time Required – After the samples were stripped of all debris and sorted into morphospecies groups by the ABMP field staff, it required the arthropod expert (Jeff Battigelli) an average of 8 ± 4(SD) mins to further examine each sample to ensure morphospecies groups were accurate and make changes if needed; approximately 29.2 ± 13.1 mins per site. However, the majority of time is required to clear and mount example species onto microscope slides to finalize the species determinations. The expert did not provide precise times, but needed a total of 5 man-days to complete the identification of 10 sites.

Arthropods Found at the Site Level – During the field trial a total of 12 springtail *(collembolan)* and 47 mite (*orbatida)* species were detected. The boreal and foothills sites had higher diversity than the parkland and grassland sites (Table 3). A total of 960 individuals were detected, of those 368 were springtails and 592 were mites.

Table 3: Number of springtail (*collembola*) and mite (*orbatida*) species retained from a 300µm sieve after collections obtained during the field trials.

Site Types	Total # Species Mites	Total # Species Springtails	# Individuals (Mites)	# Individuals (Springtail)
Boreal Sites (3 sites)	30	9	309	82
Foothills Sites (4 sites)	32	9	148	113
Parkland Sites (2 sites)	21	6	56	65
Grassland Sites (1 site)	12	3	29	100

During the actual field test slightly different results were found. Boreal forest sites averaged higher mite species numbers than the foothills and parkland/grassland sites (Table 4). Sites in all natural regions had between 4 and 6 springtail species (Table 4). The number of mite species present across all site types was more than double that of the springtails.

Table 4: Number of springtail (*collembola*) and mite (*orbatida*) species retained from a 300µm sieve, per site, after collections obtained during the field trials. Values are mean ± 1 standard deviation.

Site Types	# Species (Mites)	# Species (Springtails)	# Individuals (Mites)	# Individuals (Springtails)
Boreal Sites (n=3)	18.0 ± 3.6	4.7 ± 1.5	103.0 ± 64.4	27.3 ± 4.9
Foothills Sites (n=4)	12.3 ± 3.5	5.8 ± 2.2	37.0 ± 25.1	28.3 ± 21.7
Parkland/Grassland Sites (n=3)	13.0 ± 1.7	4.0 ± 1.0	28.3 ± 11.0	55.0 ± 39.5
Total Sites (n=11)	14.2 ± 3.8	4.9 ± 1.7	54.2 ± 48.0	36.0 ± 26.1

Arthropods Found at the Sample Level – Four 500 ml samples were collected at each site. On average, each 500 ml soil sample yielded 5.2 ± 3.3 mite species and 2.3 ± 1.5 springtail species per sample (n=39).. Samples collected from the boreal had the most mite species and individuals per sample with comparable numbers found in the other natural regions (Table 5).

Table 5: Number of springtail (*collembola*) and mite (*orbatida*) species and individuals retained from a 300µm sieve, per 500 ml soil sample collected during the field trials. Values are mean ± 1 standard deviation.

Samples	# Species (Mites)	# Species (Springtails)	# Individuals (Mites)	# Individuals (Springtails)
Boreal Samples (n=11)	7.7 ± 4.1	2.1 ± 1.6	32.9 ± 40.0	7.5 ± 6.8
Foothills Samples (n=16)	4.3 ± 2.7	2.8 ± 1.7	10.5 ± 8.9	7.6 ± 7.0
Parkland/Grassland Samples (n=12)	4.3 ± 2.0	1.8 ± 0.8	7.4 ± 5.5	13.8 ± 19.3
Total Samples (n=39)	5.2 ± 3.3	2.3 ± 1.5	15.2 ± 23.1	9.4 ± 12.2

A complete list of all springtail and mite species detected during the field trials, including which habitat type they were located, can be found in Table 6

Table 6: Springtail (*Collembola*) and mite (*Orbatida*) species detected during 2005 field trials.

Taxon	Species	Boreal	Foothills	Grassland	Parkland	Total
Collembola	*Folsomia nivalis*	41	39		7	87
Collembola	*Folsomia sp.nr. fimetaria*	3	10			13
Collembola	*Folsomides parvulus*	2		1	29	32
Collembola	*Hypogastrura H. nivicola*	1	19	96		116
Collembola	*Isotoma (Desoria) sp.nr. nigrifrons*	5	7		1	13
Collembola	*Isotoma (Desoria) sp.nr. nigrifrons 2*	1	5			6
Collembola	*Metisotoma grandiceps (juv)*	13	3	2	6	24
Collembola	*Morulina multatuberculata*	12				12
Collembola	*Onychiurus (Protaphorura) parviconis*	4	29		17	50
Collembola	*Sminthurides Sphaeridia pumilis*				6	6
Collembola	*Tomocerus sp. (juv.)*		1			1
Collembola	*Tricanthella sp.*		1			1
Orbitada	*Achiptera sp.nr. nitens*	6				6
Orbitada	*Ametroproctus (Coropoculia) sp.*		1		1	2
Orbitada	*Banksinoma spinifera (Hammer 1952)*		1			1
Orbitada	*Camisia biverrucata (CL Koch 1893)*		2	1	1	4
Orbitada	*Camisiid juv*		20		5	25
Orbitada	*Cepheus corae (Jacot 1928)*	3	3			6
Orbitada	*Ceratoppia quadridentata (Haller 1882)*	3	3		1	7
Orbitada	*Ceratozetes gracilis*	105	1	1		107
Orbitada	*Ceratozetes gracilis (Michael)*	2		2	1	5
Orbitada	*Ceratozetes virrinicus (Banks)*		2			2
Orbitada	*Cultroribula bicultrata (Berlese 1908)*	1		1		2
Orbitada	*Damaeid juv*		2			2
Orbitada	*Dentizetes nudentiger*	3	9	1	6	19
Orbitada	*Dentizetes rudentifer*		2		4	6
Orbitada	*Diapterobates sp.*	1	4		6	11
Orbitada	*Diapterobates sp. 2*				1	1
Orbitada	*Eobrachychthonious borealis*	3				3
Orbitada	*Eobrachychthonious borealis (DN)*	9	1			10
Orbitada	*Epidamaeus gibbermoratus (Nicolet 1855)*	6	26	2		34
Orbitada	*Epidamaeus sp. A*		1		3	4
Orbitada	*Eremaeus translamellatus (Hammer)*		9	3		12
Orbitada	*Euphthiracarus alazon (Wakkes 1964)*	28				28
Orbitada	*Geohypochthonius sp.*	20	1			21
Orbitada	*Hermaniella granulata (Nicolet 1855)*	2	2			4
Orbitada	*Jornadia sp.*	10	5			15
Orbitada	*Moritzoppia clavigera (Hammer 1952)*	8	8			16
Orbitada	*Mycobates sp. D*				5	5
Orbitada	*Neonothrus humicola (Forrslund 1955)*		6	1		7
Orbitada	*Nortonella gildersleeveae (Hammer 1952)*		2		1	3
Orbitada	*Oppiella nova*	41	27		1	69
Orbitada	*Orb Juv.*	10	8		1	19
Orbitada	*Oribatodes sp. (Damaged)*	1				1

Table 8 Continued

Taxon	Species	Boreal	Foothills	Grassland	Parkland	Total
Orbitada	*Oribatula tibialis (Nicolet 1855)*	10	3		2	15
Orbitada	*Peloptulus americanus (Ewing 1907)*				10	10
Orbitada	*Phthiracarus ligneus*	6				6
Orbitada	*Pilogalumna sp. A*	7	1	10	1	19
Orbitada	*Pilogalumna sp. B*	6		1		7
Orbitada	*Platynothrus peltifer (CL Koch 1839)*		10		2	12
Orbitada	*Quadropppia quadrcarinata*	8	3			11
Orbitada	*Scheloribates sp.nr. Latipes*	1		2	3	6
Orbitada	*Suctobelbella acutidens (Forrslund 1944)*		2			2
Orbitada	*Suctobelbella sp.nr.subtrigona*	9	1			10
Orbitada	*Tectocepheus velatus*	3			4	7
Orbitada	*Tegoribates americanus (Hammer 1958)*				2	2
Orbitada	*Tryhypochthonious tectorum (Berlese 1896)*	11	2	1		14
Orbitada	*UNKN sp. (Damaged)*	1				1
Orbitada	*UNKN sp. 2 (Damaged)*	1	2			3

Discussion and Conclusions

Test of Arthropod Data Collection

Collection Samples – The methods for collecting organic soil at the sites worked well during the trials. Most issues were resolved during the 2-day field trip prior to the field trial. Sampling from forested areas was easiest since these habitats contained distinctive organic and mineral layers. Grassland, and to a certain extend Parkland sites, had soil conditions quite different from forested areas. The distinction between organic and mineral layers in these soils was often less pronounced and much less organic material was present. Thus, relatively few cores were needed in the forest, whereas many cores were needed in the parkland and grassland. Human modified soils (agriculture, cutblocks, etc.) posed additional concerns. Organic materials were rare in some of these sites, and in other sites the organic and mineral soils were mixed. The revised method of using a systematic design to collect cores, along with restrictions to the material collected, provided crew members with consistent methods across all sampling locations.

By restricting sampling to the LFH layer, consistency was maintained by all crews in all habitats. To ensure unbiased samples were collected, restrictions on sampling were formalized:

- Cores were always taken in sequential order (see Figure 3) irrespective of the condition of each location.
- When a core was taken it was noted whether it contained unique features such as animal material (scat), was modified by humans (i.e., soil mixed), corer pushed into the water table, or contained downed woody material (i.e., corer went through a decayed log).
- If a core location had a restrictive feature that did not allow a core to be taken it was also noted. Cores were not collected if locations were in standing water (see below), had impenetrable rock(s), or impenetrable stumps/logs.

Under some conditions, consistent sampling was not possible. In these cases, additional criteria were used:

- Where the LFH layer was indistinct (eg, grasslands), the rooted vegetative zone where the core naturally brakes away from the remaining mineral soil was collected. Under these scenarios, the core was held at the top by one hand and bent with the other hand until the

rooted portion pulled free of the remaining soil. If needed, a knife was used to cut some of the longest roots away from the organic mass before the entire core disintegrated (soils are very powdery throughout).

- Where no organic soil was present, or it was mixed with the inorganic soil (eg, cultivated agricultural fields), only the L (litter) was collected. This was also the case for cutblocks where modification to the soil may have overturned the organics (mounding) and there was bare mineral soil at the surface with litter (included leaves, grass, and/or other debris) on the top of the core. Under these scenarios, many cores (core scrapings) were required and 500 ml of material was not always gathered.
- If a core location was in standing water, no core was taken because the arthropod community would be much different under the water, introducing unwanted variability between samples. If a vegetative mat was present above the water table, a core was taken. The unique nature of all core locations were documented to help interpretation of the results.

Preserving and Shipping Samples – The time required by the field crews to store samples and ensure the arthropod samples are safely maintained was minimal (2-5 mins/day). However, in the ABMP to ensure the sample extraction begins within the 4-6 days of collection, a person may need to spend up to 4 hours periodically throughout a field shift, shipping the samples back to the laboratory. Shipping was not tested during the field trials. With careful planning it may be possible to schedule ABMP sites so that sampling occurs near towns every 3-4 days, but other logistic factors also influence the order that sites will be sampled. As a minimum, additional planning by the field coordinator will be required to ensure that specimens are shipped regularly.

Arthropods Found – A total of 12 springtail and 47 mite species were detected across habitat types and ecoregions. These results are not surprising; current knowledge indicate that mites are more specious than springtails. More species were detected in the forested regions (boreal and foothills) than found in the parkland and grassland. Interestingly, fewer springtail and mite species were detected per site than for birds, vascular plants, mosses, or lichens – all of which have approximately 30 species per site. Sampling effort (field time plus lab time) is approximately 4 hours per site for vascular plants, springtails, and mites, whereas it is approximately 8 hours for birds, mosses, and lichens.

Tests of Laboratory Sample Processing

Extracting Specimens – Extraction of the arthropods using the berlase funnels worked well. After the initial set up and insertion of samples, very little effort was required to monitor the equipment until the process was finished and the samples removed. Extraction, however, required a considerable amount of space, and will need capacity to run 1500 funnels (each with a 25 watt light bulb) when samples are being collected for the complete ABMP (approx. 375 site/year). This extraction space will require a large electrical source because a maximum of 40 light bulbs can be connected to each 20 Amp electrical circuit (i.e, 40 circuits will be required). We found that distributing the funnels evenly in rows allowed the person checking the equipment to quickly move up and down the rows and monitor the samples.

Sorting to Morphospecies – Most of the time required for arthropod sampling occurs during the processing of samples and identification of species. It required approximately 1.7 hours to sort, describe, and preserve each sample (6.7 person/hours per site). In addition, the field staff require approximately 5 days training before they become proficient and consistent in processing the springtails and mites. A detailed training and instructional program, including at least one day with an expert, needs to be developed.

It will require approximately 2.5 weeks for two field staff to sort the springtails and mites into morphospecies from all the sites that they sample during a year (approximately 17 ABMP sites). Sorting arthropods however, must be integrated with specimen identification for other taxonomic groups (mosses,

lichens, fungi, and vascular plants). Under the scenario presently used by the ABMP, the entire month of August (4 weeks) has been allocated to processing moss, lichen, and vascular plant specimens. It may be necessary to employ some of the summer staff for longer periods (i.e., through September and October) to process the arthropods, or to outsource the entire sorting and identification process of springtails and mites.

Species Identification – Identification of springtails and mites to species requires a unique expertise. To accomplish this, specimens that are sorted into morphospecies by ABMP field staff, must be further processed by an expert. During the field trial the ABMP Field Coordinator sent the sorted specimens to Jeff Battigelli for identification. This is a process consistent with ABMP methods for other taxa (Mosses, Lichens, Vascular Plants). Once the ABMP is fully implemented, it will be important to develop more "outside" expertise so that processing can occur quickly. Developing this capacity will need to be addressed for long term success.

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Appendix 1

Suggested Arthropod Data Collection Protocols

We suggest that collection of arthropod samples be integrated into the "spring suite" of the ABMP terrestrial protocols, since this integrates well with existing data collection. In addition, higher arthropod diversity can be found in the litter layer during this period as the soils and litter are generally moist at this time of year. The coring method we tested work well in all conditions we attempted. The following is a detailed breakdown of the coring method proposed:

- Only the organic component of the soil profile is collected. This consists of the LFH horizon (litter, fermentation, and humus layers) and excludes the mineral soil. Determining the LFH horizon is usually straight forward in most soil conditions based on the colour and texture (natural breaking point) of the soil and resistance of the soil corer to penetrate too far into the mineral layer. The organic layer is typically dark in colour, coarse and fibrous (containing rooting systems) whereas the mineral soil is typically lighter in colour, finely particulate, and lacking the bulk of most rooted plants. After distinguishing the transition from LFH to mineral horizon, grasp the LFH by one hand and gently break apart (or cut with a knife) the mineral horizon with the other hand, placing only the LFH into a mixing bowl.
- If the LFH is indistinct (eg, grasslands), the core above and including the plant rooting zone (where the core naturally brakes) is collected. These soils can appear all one colour and have similar textures throughout. These cores will naturally "disintegrate" below the plant roots. Grab the core at the top (vegetative part) and the bottom (mineral soil) will likely fall off.
- Where no organic soil is present (eg, agriculture), only the litter is collected. This may only included some leaves, grass, or other debris on the top of the core location.
- If a core location has standing water at the surface, no core is taken. If a vegetative mat is present above the water table (this is judged prior to a person standing on the mat) a core is taken.
- If the organic layer is deeper than the corer can penetrate (i.e., corer is not long enough to reach the mineral soil). The entire 40 cm profile of organic material the corer extracts is collected from the core. This can be common in lowland areas such as Black spruce/Tamarack bogs.
- A total of 500 ml of LFH is attained from each sample location (quadrant) equaling 2 L of sample material per site.
- A minimum of 4 cores are taken from each sample location (quadrant), and additional cores are collected (up to 24) if needed to make up the total volume of 500 ml required per sample.
- If more than 4 cores are required to accumulate 500 ml of LFH, additional cores are taken in a clockwise fashion (see Figure 3) until 500 ml is attained. The number of cores required to get 500 ml of LFH is recorded.
- A maximum of 24 cores will be attempted per sample location (quadrant). If the total volume of organic material is less than 500 ml after taking the maximum 24 cores, the total volume of organic material is documented and sampling ceased for that location.
- Cores are taken in sequential order and each core location is given a descriptive code whether it was taken or not. Descriptions include: If cores can not be taken – SW=Standing Water, R=Rocks, SL=Stumps/Logs; If cores could be taken – AM=Animal Material, HD=Human Disturbance (i.e., mineral soil), WT=Water Table, DWM=Downed Woody Material (i.e., decayed logs, roots, etc).
- After 500 ml of LFH has been obtained, LFH from all cores are mixed together in a sampling bowl and a random 500 ml sample is extracted and placed into a cloth bag for storage and processing. The remaining LFH (if any) is measured for volume and returned to the site.
- A general description of the sample (litter) condition is taken to assist with interpretation of results if needed. One or more of following are circled on the data sheet: Dry, Wet, Fresh, Old.
- Any additional comments can be put down describing the process or sample taken.

Sample Preservation

- Samples are placed into cloth bags and labeled with the Collector's Initials, Date, ABMP Site, and ABMP Quadrant.
- Samples are then put into a plastic bag (to be kept dry) and placed into a cooler with ice. Samples are separated by cardboard from the ice.
- Arthropods extraction must be started within 6 days of the samples being collected to minimize the loss of organisms within the sample. For consistency of results among collections, no extraction will take place until at least the 4th day of collection (i.e., extraction will occur between 4-6 days of collection).

<u>Suggested Laboratory Sample Processing</u>

Extracting Specimens

Specimen extraction requires a large room with enough 20 Amp electrical circuits to run 40 circuits of 40 berlase funnels (each funnel has a 25 watt light bulb).

- Extraction equipment should be assembled and in working condition before samples are collected. Equipment can be run in series with the use of power bars and an extension cord.
- One person will be required to receive all samples as they are shipped/transported from the field to the laboratory.
- Samples will be placed into the extractors between the 4th and 6th day of being collected to standardize the loss of organisms.
- Place a piece of moistened cheese cloth onto the wire mesh of the berlase funnel prior to inserting sample material to minimize the amount of fine particulate matter and dry debris that passes through into the specimen cup.
- Place sample collection on top of cheese cloth and hang funnel to light bulb assembly.
- Screw on specimen cup, containing 50:50 antifreeze and water mixture and sample label, to bottom of assembly.
- Labels should be small, written on write-in-the-rain paper, and placed directly into the specimen cup. Labels should include: Collector's Initials, Date of collection, ABMP Site, and Quadrant. Labels should be small enough so they can be directly transferred from the specimen cup to long-term collection vials when sorting and identification takes place.
- Turn on light bulb and leave for 7 days. It is important not to bump or otherwise disturb the extractors during this process to minimize fine debris from falling into the specimen cups (i.e., the dirtier the sample the more effort required to sort the specimens).
- Check the extractors each day during the process to ensure they continue to function properly (i.e., power has not failed, light bulbs burned out, etc).
- On the 7th day, remove specimen cups and discard the sample material into the garbage. Specimens are stored in the antifreeze mixture until further processing (sorting and identification) takes place.

Sorting to Morphospecies

Specimens collected in the antifreeze from the extraction process are poured through a 2-sieve stacked array. The top sieve is 300µm in size and the bottom sieve is 50µm. Specimens collected in the 300µm sieve are processed further by ABMP staff by separating all specimens into springtails and mites and further sorting them into morphospecies groups. All different morphospecies groups/sample are placed into separate wells (containing 70% ethanol) on a multi-well culture plate. Data sheets are filled out describing each morphospecies group including the number of individuals detected. The culture plate is then covered with parafilm and sealed until it is shipped to an expert for species determinations. All

remaining organisms/site from the 300µm sieve, which are not springtails and mites, are placed into a separate storage vial containing 70% ethanol. The 50µm sieve is used to capture the soil arthropods and debris that pass through the 300µm sieve. These materials from the 50µm sieve is placed into a separate vial containing 70% ethanol and stored. After being stored for 2 years by the ABMP, these collections will be transferred to the Royal Alberta Museum for their use and/or disposal. Any organisms that pass through the 50µm sieve are disposed of with the left over antifreeze.

It will be difficult to integrate specimen sorting into the existing suite of ABMP "laboratory" protocols if field staff are only hired for 4 months. Thus, 2 options are suggested along with their associated trade-offs of implementation.

<u>Option 1:</u> Contract Sorting to an Outside Source.

Due to the final step of species identification already requiring expert identification, all processing after the extraction can be (1) packaged into a single contract and become the responsibility of the expert, (2) the expert sub-contracts the sorting to cheaper personnel which is then performed under direct requirement to the expert, or (3) the ABMP contracts the sorting to morphospecies before delivery to an expert for identification.

Benefits

- No loss of data/information from other ABMP protocols by having to reallocate manpower in the laboratory to process the arthropod samples, if the status quo is maintained. This in turn could save money if field staff fails to process all specimens (if effort is spread out between protocols) within the allotted timeframe, therefore requiring additional outsourcing to complete the processing.
- Effort and training of field staff remains focused on existing duties and quality of data is maintained.

Trade-offs

- Increased costs to the ABMP, although this is an inevitable occurrence of adding any new data element, but more so if implementing the arthropod protocols without any downsizing of other protocols.
- Decrease in quality control without direct supervision of sample processing (conversely, it can be argued quality may increase if a consistent source of expertise is established and maintained annually as the ABMP will likely experience high turnover of undergraduate field staff that are hired to collect data during the summer).

<u>Option 2:</u> Hire at least some field staff for longer than four months and sort to morphospecies in the laboratory

Currently, it requires the entire month of August to process, vascular plants, mosses, lichens, and tree cores, after dividing duties among field staff and providing a detailed training regiment. If some field staff were hired for 5 or 6 months (these could not be undergraduates because undergraduates return to school in September after a 4-month work period) they could process the samples during September and October.

Benefits

- Quality of data can be monitored. Similar to other laboratory tasks, a detailed training regime can be established and monitored to ensure goals and expectations for the sorting process can be met.

Trade-offs

- There are many undergraduates that wish to work for the 4-month summer period. Thus the ABMP can select only the most qualified/motivated for their summer field staff. It will be more difficult to find staff that wish to work for 5 or 6 months. These staff will not be returning to school, and thus probably are looking for long-term (permanent) work. To hire

field staff for 5 or 6 months, the ABMP may be forced to hire less qualified people.

- Increase ABMP staff costs to maintain status quo of current laboratory tasks as well as adding the duties of sorting arthropods to morphospecies.

Specimen Identification

No changes are suggested for this aspect of the protocol. Specimens are to be sent to experts for species identification. Experts will provide the ABMP with a database pertaining to the number of springtail and mite species per sample provided.

Long Term Specimen Preservation

After species identification has been confirmed from the samples, specimens will be transferred from the culture plates to storage vials containing each separate species (preserved in 70% ethanol), and will be labelled and stored by the ABMP for 2-years. In addition, the unsorted (remaining) samples containing specimens other than springtails and mites will be retained for 2 years. At least 1 representative specimen of each species will be retained by the ABMP for reference and credibility purposes. After 2-years, the ABMP will donate the sorted specimens and the non-sorted specimens to the Royal Alberta Museum. The Royal Alberta Museum will assume the responsibility of storing or discarding this material.

Suggested Data Sheets
Alberta Biodiversity Monitoring Program

Spring Protocols Arthropod (Soil Core) Collection

Page____of____.

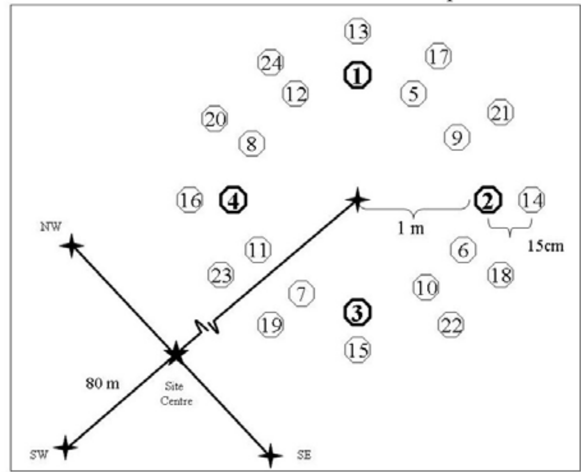
Nearest Town (Direction and Distance) ____________________

____

____

Date:______________________

Crew:______________________



Cores Collected

Quadrant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
NE [1]																								
Sample Condition [2]			Dry							Wet						Fresh						Old		
Comments																								
NW [1]																								
Sample Condition [2]			Dry							Wet						Fresh						Old		
Comments																								
SW [1]																								
Sample Condition [2]			Dry							Wet						Fresh						Old		
Comments																								
SE [1]																								
Sample Condition [2]			Dry							Wet						Fresh						Old		
Comments																								

Take a minimum of 4 cores from each quadrant and collect only the L (Litter), F (Fermented), and H (Humus) layer from each core. Mix samples together and collect a random 500 ml sample. Take additional cores in sequential order (up to 24) if more material is required to make up 500 ml.

[1] Tick each core location cell up to the number of cores required to make a 500 ml sample. If a core can not be taken from a location note the reason: SW=Standing Water, R=Rocks, SL=Stumps/Logs. If a core can be taken but the sample is unique, note the reason: AM=Animal Material, HD=Human Disturbance (i.e., mineral soil), WT=Water Table, DWM=Downed Woody Material (i.e., decayed logs, roots, etc).

[2] Circle (at least one) the general description of the aggregated sample collected.

Additional Soil Volume

Quadrant	Soil Volume
NE	
NW	
SW	
SE	

Record the volume of all additional soil collected if more than 500 mls was collected from the 4 primary cores or soil in excess of 500 mls is collected from additional cores taken.

ABMP Site:_______ Year:________

Alberta Biodiversity Monitoring Program

Arthropods (Springtails and Mites)
Laboratory – Sorting to Morphospecies

Sorting Technician:_____________________
Date:_________________________________

Quadrant	Springtail or Mite	Morphospecies ID *(Sorting Name)*	Well #	Number of Individuals (Tally)	Species Name (Entered by Expert)

ABMP Site:_______ Year:________

Alberta Biodiversity Monitoring Program

Arthropods – *Collembola* (Springtails) Laboratory Morphospecies Description

Determined By:___________________________

Morphospecies ID:________________________

Date:______________________

Family:__________________________________

Genus:__________________________________

Species:_________________________________

ABMP Sites Found:______ ______ ______ ______

______ ______ ______ ______ ______ ______

______ ______ ______ ______ ______ ______

______ ______ ______ ______ ______ ______

______ ______ ______ ______ ______ ______

Defining Morphospecies Character(s):

Overall Body Colour:_____________________

Colour Pattern:____________________

Setae:________ **Scales:**______________

Eye Patch: **Present** **Absent**

Colour:___________________________

Antennae:_______________________________

Shape:___________________________

Size:____________________________

Colour:__________________________

Furcula: **Present** **Absent**

Length:__________________________

Notes/Comments:

Sketch/Photo:

Arthropods – *Orbatids* (Mites)
Laboratory Morphospecies Description

Determined By:________________________

Morphospecies ID:______________________

Date:____________________

Family:______________________________

ABMP Sites Found:_____ _____ _____ _____
_____ _____ _____ _____ _____ _____
_____ _____ _____ _____ _____ _____
_____ _____ _____ _____ _____ _____
_____ _____ _____ _____ _____ _____

Genus:______________________________

Species:_____________________________

Defining Morphospecies Character(s):

Overall Body Colour:__________________

Colour Pattern:__________________

Ornamental Pattern:______________

Setae Shape:__________________________

Size:______________________________

Sensillus Shape:_______________________

Size:___________________________

Body Size:____________________________

Notes/Comments:

Sketch/Photo: