

Standardized monitoring of *Rangifer* health during International Polar Year

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Abstract: Monitoring of individual animal health indices in wildlife populations can be a powerful tool for evaluation of population health, detecting changes, and informing management decisions. Standardized monitoring allows robust comparisons within and across populations, and over time and vast geographic regions. As an International Polar Year Initiative, the CircumArctic Rangifer Monitoring and Assessment network established field protocols for standardized monitoring of caribou and reindeer (*Rangifer tarandus*) health, which included body condition, contaminants, and pathogen exposure and abundance. To facilitate use of the protocols, training sessions were held, additional resources were developed, and language was translated where needed. From March 2007 to September 2010, at least 1206 animals from 16 circumpolar herds were sampled in the field using the protocols. Four main levels of sampling were done and ranged from basic to comprehensive sampling. Possible sources of sampling error were noted by network members early in the process and protocols were modified or supplemented with additional visual resources to improve clarity when needed. This is the first time that such broad and comprehensive circumpolar sampling of migratory caribou and wild reindeer, using standardized protocols covering both body condition and disease status, has been done.

Key words: body condition; caribou; disease; health; monitoring; parasites; *Rangifer tarandus*; reindeer; standardized protocols.

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Introduction

Understanding and tracking the health status of individual animals provides valuable information for wildlife management (Jean & Lamontagne, 2004; PCMB, 2010; GNWT, 2011). Changes in body condition or infectious disease indices of individuals may reflect shifts in population health and serve as early

warning signals for wildlife managers, wildlife users, and public health officials concerned with zoonotic diseases. Examining interrelationships between various health indicators, as well as their trends over time and across herds and geographic regions, provides new insights into the ecology of a species and functioning of an ecosystem. Foundational to these activities,

however, is securing a comprehensive baseline of the existing health status of the population (Karesh & Cook, 1995), including but not limited to: body condition, genetic diversity, physiological health, contaminants, and pathogen diversity and abundance. Understanding the relationships among various health indicators, and defining what is 'normal' and the variability around that normal within a host population, through population cycles and across the range of a species, is an important first step for identification of 'abnormal' and early detection of and response to changes in health.

Establishing baselines and maintaining monitoring programs for wildlife populations is not easy. Financial constraints, political and/or philosophical differences, transboundary issues, and the general elusive nature of wildlife make it difficult to establish and maintain successful programs (Kofinas *et al.*, 2002; Witmer, 2005). In the Arctic, widely scattered communities, a vast landscape, high costs of field access for research and hunting, and logistical constraints, add to the difficulties of wildlife monitoring. This is particularly true for caribou and reindeer (*Rangifer tarandus* spp.), a very mobile keystone species (Geist, 1998) that is highly valued for cultural and economic reasons across its circumpolar range (Nuttall *et al.*, 2010). Nevertheless, a variety of *Rangifer* monitoring programs of different types have existed over time (Kofinas *et al.*, 2002; Couturier *et al.*, 2004; Gunn *et al.*, 2005; Campbell, 2006; Campbell *et al.*, 2010; Lyver & Nation, 2010).

Monitoring of a sort began with aboriginal people who, for thousands of years, have been keenly aware of the health of caribou or reindeer that they harvested for food, clothing, and tools (Cruikshank, 1981; Ferguson & Messier, 2010). A technical approach to monitoring body condition began in the mid-1900s, with emphasis on describing and validating condition indices which had allometric relations to the status of muscle, bone and fat, and their

chemical constituents: water, protein, lipid, and ash (Dauphiné, 1976; Langvatn, 1977; Ringberg *et al.*, 1981a; b; Reimers & Ringberg, 1983; Huot & Goudreault, 1985; Adamczewski *et al.*, 1987a; Huot & Picard, 1988; Al-layé Chan-McLeod *et al.*, 1995; Gerhart *et al.*, 1996). Monitoring body size (*e.g.*, lower jaw and metatarsal bones) and shape (*e.g.*, heart girth) also provides insight into population trends (Parker, 1981; Crête & Huot, 1993; Mahoney & Schaefer, 2002; Couturier *et al.*, 2010). For the most part, this type of monitoring has been scientist-driven, typically initiated and conducted by wildlife managers or researchers, and often focused on a particular herd intermittently or for a finite length of time.

Opportunistic monitoring of infectious diseases of *Rangifer* by scientists in partnership with aboriginal hunters has also occurred since the mid-1900s, often initiated by hunter reports or submissions of abnormal tissues (*e.g.*, Canadian Cooperative Wildlife Health Centre <http://www.ccwhc.ca/>; Choquette *et al.*, 1967). More recently, targeted programs for contaminants and disease surveillance have developed across many jurisdictions (Elkin & Bethke, 1995; Zarnke *et al.*, 2000; Robillard *et al.*, 2002; Brook *et al.*, 2009; Stieve *et al.*, 2010).

Increasingly, through the wildlife co-management process, *Rangifer* users themselves are driving the context for, and implementation of, monitoring (Lyver & Gunn, 2004; Brook *et al.*, 2009; Lyver & Nation, 2010). People who depend on *Rangifer* for food and income have expressed concerns about how this species, and those who depend on it, will cope with the increasing rate of environmental and political change (Kofinas *et al.*, 2003; Brook *et al.*, 2009). The impacts of climate change, resource development and other stressors on the health of *Rangifer*, and on food safety and security (*i.e.*, population sustainability) as it relates to *Rangifer*, are major concerns for subsistence

hunters (Brotton & Wall, 1997; Brook *et al.*, 2009).

In light of global climate changes and conservation efforts, the Arctic Council launched a number of monitoring programs for Arctic species through the Conservation of Arctic Flora and Fauna Group (CAFF, 2010). The Circum-Arctic *Rangifer* Monitoring and Assessment (CARMA) network, positioned within CAFF, was launched in 2004 in response to increasing concern for *Rangifer* and the need for circumpolar collaboration. The network is a forum to exchange ideas, observations and data, and coordinate *Rangifer* monitoring activities around the Arctic.

A key objective of the CARMA network was to develop and implement standard methods for monitoring *Rangifer* health. Although monitoring activities were already in place for many herds, the methods, frequency, and type of data recorded varied greatly (Kofinas *et al.*, 2002). Integration of data across disciplines (*e.g.*, disease and body condition data), even within a herd, was sometimes lacking. Standardized approaches, both within and among herds, allow comparisons across space and time and, therefore, can provide a much deeper understanding of *Rangifer* health. As standardized baselines become established, links between the various health indicators can be examined and the costs, or benefits, of pathogens and pollutants at the individual, population and community levels can be evaluated. Such information provides the necessary foundation to assess *Rangifer* vulnerabilities and responses to environmental and anthropogenic changes. Importantly, for standardized protocols to be adopted and effective, the procedures need to be clear and without ambiguity, but flexible enough to accommodate differences in monitoring programs and objectives. At the same time, they must include enough indices to allow predictions: for example, to infer the probability of pregnancy from the fat and protein reserves

(Kofinas *et al.*, 2003).

Supported by the International Polar Year (IPY) initiative (2005 – 2011), the CARMA network developed a standardized approach to circumpolar monitoring for *Rangifer*. In this paper we provide an overview of the CARMA protocols and the implementation of these protocols for CARMA-supported sampling of circumpolar caribou herds. We summarize the IPY sampling efforts, and discuss the successes and hurdles to such broad monitoring activities.

Methods

Standardized protocol development and application

Starting in 2006, a sub-group of the CARMA network developed: (i) a manual that described monitoring indicators, the rationale for each indicator, and the relevant literature, and (ii) standardized sampling protocols for collection and measurement of each indicator. The selection of indicators and sampling protocols were developed in consultation with network collaborators. These were based on published and unpublished literature and experiences from previous and ongoing *Rangifer* monitoring programs. Indicators were selected to provide data on age, diseases, physiological condition, short and long-term nutritional status, and maternal investment in reproductive fitness. Some indicators were compatible with, and could be used in energy-protein and body frame size models that CARMA developed to assess and predict *Rangifer* responses to environmental changes (Murphy *et al.*, 2000; Russell *et al.*, 2005).

Recognizing the value and constraints of both hunter and scientist-based monitoring, CARMA initially developed two levels of protocols. The Level 1 protocol was developed for subsistence hunters or community-based sampling and provided basic information on age, frame size, body condition, and a few pathogens. The Level 2 protocol was more com-

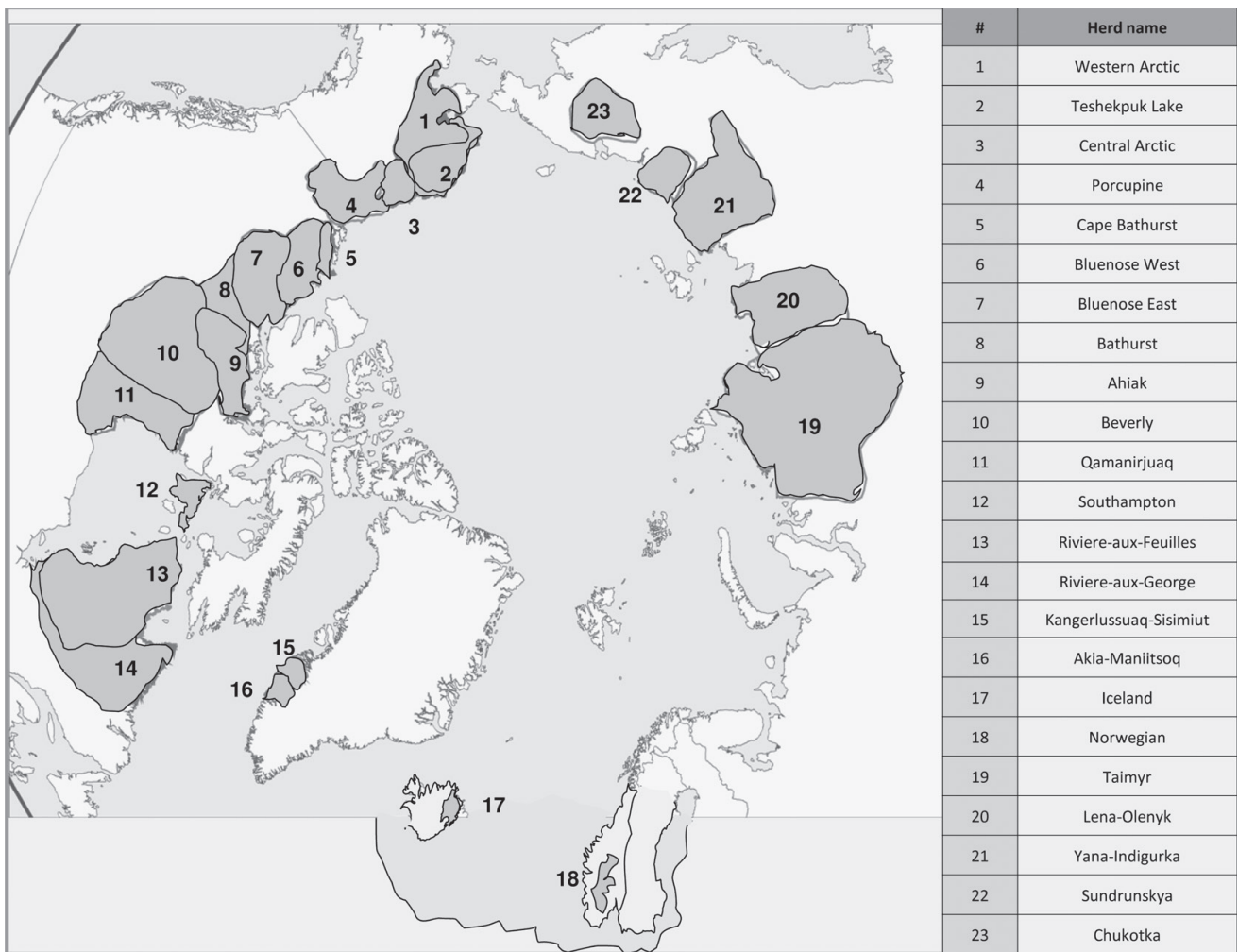


Fig. 1. Distribution of migratory *Rangifer tarandus* herds.

prehensive and typically required at least one well-trained individual to collect and process samples. Additionally, this level required partnerships with commercial or research laboratories to do further analyses on samples. Level 2 included additional indicators for body condition and morphometrics that could be used to quantitatively predict whole body fat and protein reserves, and in-depth sampling methods for assessing contaminants and determining pathogen presence, abundance, and/or exposure.

In the protocols, the sampling procedures and data requirements for each indicator were described and references were provided for further details on more specialized procedures such as tooth sectioning or parasite isolation

from tissues. The manual and protocols included less detail on pathogens and pathogen sampling than was applied in the CARMA-supported field collections so these procedures are described in greater detail in this paper.

CARMA network partners and participants were encouraged to follow the protocols when handling *Rangifer* during IPY-funded collections. The initial selection of herds and the level of sampling was discussed at the annual CARMA meetings and aimed to identify representative reference herds around the Arctic (Fig. 1). The final selection of the reference herds was determined by the priorities and support of management agencies, communities, and co-management boards. Further, the sampling intensity for each individual herd was influenced

by the availability and engagement of regional biologists or managers and by the goals of individual research and monitoring projects.

Formal feedback on protocol implementation was gathered during the 2010 annual CARMA workshop after network members had an opportunity to use the protocols in field collections from 2007 – 2009. Network members (63 people representing 11 government agencies, 12 organizations, eight universities, and six countries) divided into three groups (community people, wildlife managers, and university researchers) and had guided open discussions on whether the protocols were useful and how content and accessibility could be improved.

Data management

All participating herd biologists or managers were asked to submit their data in a Microsoft Excel® datasheet format and all entries were imported into a newly created CARMA Excel datasheet. When possible, an automatic importation function (*e.g.*, “vlookup”) was used to decrease human error. When needed, original datasheets were consulted to validate the data. In the absence of field datasheets, herd biologists or managers were asked to verify and validate their data once it was imported into the common database. For most collections, meta-data records, documenting the date, location, number of animals, and types of data collected, were created by individual researchers and archived with the ArcticNet data portal (www.arcticnet.ulaval.ca).

Results

Standardized protocol development

Draft protocols were discussed with network members before and during IPY at the CARMA annual meetings as well as between meetings as needed (*e.g.*, manager/researcher requiring specific advice on sampling). Protocols were refined periodically based on these discussions

and ongoing feedback and in some cases, based on the results of research studies. For example, the initial protocol for *Besnoitia* included sampling multiple tissues, but based on results from this sampling it was determined that metatarsal skin alone was a suitable index of infection (Ducrocq *et al.*, 2012). The manual and field protocols are available on the CARMA web site (CARMA, 2011a; b; c). An early version of the protocols was also translated into Russian to be tested by CARMA’s Russian collaborators. Indicators and samples collected, the information gained, and some of the potential sources of error associated with collections are summarized in Table 1.

Field collections and application of the protocols

Between March 2007 and September 2010, CARMA collaborators collected body condition and health samples from at least 1206 individual caribou and reindeer from 12 North American, two Greenlandic, and two Russian herds (Fig. 1; Table 2). The Chukotka herd was also sampled as part of the IPY initiative but data from this herd were not available at the time of writing.

Sampling intensity for each herd varied depending on the objectives of the monitoring project. Although only two levels of protocols were initially designed (see methods), in practice, sampling could be categorized into four overlapping, categories.

Level 1 (n = 152 animals)

This was the simplest type of sampling, required the least amount of training and data recording, and was generally done by subsistence hunters. Often hunters were paid for samples and data submission. Hunters were asked to collect information on a minimum number of indicators that were selected to provide important basic data while interfering minimally with meat handling practices (Table 1). Hunters filled in tags (Fig. 2a) to identify

Table 1. Levels of sampling done by CARMA with the samples and data gathered (indicators) at each level, the information that each indicator provides on animal body condition, disease, or contaminants, and observed or anticipated sources of error or sample quality compromise associated with collection or transport of the data or samples [modified from CARMA health and body condition manual (CARMA 2013)].

Level of sampling and information and samples collected	Animal information (condition, morphology, physiology)	Other health measures (pathogens, abnormalities, contaminants)	Possible sources of sampling error in the field or processes that may compromise sample quality during storage and transportation
Level 1 (subsistence hunter collection):			
Animal information recorded on identification tags or data sheets (Fig. 2)	Location Field age Sex Back fat depth Pregnancy Qualitative fatness assessment	Abnormalities	General – Data are not recorded; tags not appropriately attached to samples; data from multiple animals on one tag/datasheet; writing implement smudges when wet. Pregnancy – observer may be unable to detect early pregnancy. Hunter experience may influence their assessment of age and fatness. ¹ Assessment of condition is done in context of the season (<i>i.e.</i> , ‘good’ in spring has less backfat than ‘good’ in fall).
Mandible			
Morphometrics	Body size	Lumpy jaw ³	Measured jaw lengths may differ if measured wet versus dry.
Marrow fat	Body condition		
Tooth eruption and tooth wear	Age class	Dental disease	
Incisor I cementum	Age		Incisor root damaged during extraction.
Molars	Enamel hypoplasia (previous stress ²)		
Metatarsus (+/- foot)			
Hair and skin	Cortisol levels	Skin – <i>Besnoitia tarandi</i> cysts, <i>Filarioidea microfilaria</i> Foot rot (<i>Fusobacterium</i> sp. ⁴)	
Morphometrics	Body size		Small tarsal bone not removed and included in metatarsal bone length measurement. Measured length may differ if measured wet versus dry.
Marrow	Fat		Desiccation of bone may influence results.

Level of sampling and information and samples collected	Animal information (condition, morphology, physiology)	Other health measures (pathogens, abnormalities, contaminants)	Possible sources of sampling error in the field or processes that may compromise sample quality during storage and transportation
Level 2 (trained hunter and community hunts). All of the above plus the following:			
CARMA body size and condition measures (detailed in protocols)	Body condition Size (body, leg, foot lengths, height, chest girth)		Units of measure not recorded in data sheets; tail length included in body length; hoof length may vary due to wear; units of measurement not recorded.
Feces*	Diet Nitrogen balance Pregnancy Hormones	Macro (helminths) and microparasites (protozoa, bacteria, viruses) that are shed in feces Chronic wasting disease ⁵	Freeze/thaw cycles compromise recovery of macro and micro parasites and hormone stability. Deep freeze (-80°C) may reduce recovery of some parasite eggs.
Blood on filter paper	Hormones (e.g., progesterone, cortisol)	Serology for various pathogens Blood-borne pathogens may be detectable by PCR in good quality samples	Incomplete saturation of filter paper strips. Inadequate desiccation of filter papers. Freeze-thaw cycles, excessive heat will compromise antibody, hormone, and DNA quality.
Kidney	Riney kidney fat – body condition	Contaminants ⁶	Riney fat not cut in the field. Storage temperature for contaminants needs to be -80°C
Liver		Contaminants <i>Taenia</i> sp., <i>Fascioloides</i> , <i>Echinococcus</i>	Storage temperature for contaminants needs to be -80°C. Detection varies with search effort, must standardize.
Testicles	Confirmation of sex	<i>Brucella suis</i> ⁷ <i>Besnoitia tarandi</i> ⁸	Repeat freeze-thaw cycles will reduce viability of <i>Brucella</i> .
Hide		<i>Hypoderma tarandi</i>	Inaccurate counts of warbles if heavy infestation where larvae are layered.
Head/Pharyngeal sacs		<i>Cephenemyia trompe</i>	Season of collection influences detectability. Larvae may be overlooked if small or in the nasal turbinates.
Gastrocnemius/Cranial Crural	Protein DNA	<i>Taenia</i> sp., <i>Sarcocystis</i> sp., <i>Toxoplasma</i>	Identification of the correct muscles and associated tendons; search effort for parasite cysts will influence results.

Level of sampling and information and samples collected	Animal information (condition, morphology, physiology)	Other health measures (pathogens, abnormalities, contaminants)	Possible sources of sampling error in the field or processes that may compromise sample quality during storage and transportation
Level 2 detailed (scientific collection). All of the above, plus:			
Heart		<i>Taenia</i> sp., <i>Sarcocystis</i> sp., <i>Toxoplasma</i>	Search effort for parasite cysts will influence results.
Abomasum and first 3 meters of small intestine		Gastrointestinal parasites of abomasum and proximal small intestine	First 3 meters estimated; parasites will migrate in guts post mortem and time since death may influence recovery.
Ileum and ileo-caecal and mesenteric lymph nodes		<i>Mycobacterium avium paratuberculosis</i>	Unable to find lymph nodes; freeze-thaw cycles will reduce organism viability.
Obex and retro-pharyngeal lymph nodes		Chronic wasting disease ⁹	Incomplete removal of brainstem and obex.
Urine	Nitrogen balance		Contamination with blood or other material.
Serum*	Serum chemistry, trace vitamins, and minerals	Pathogen serology	Contamination with rumen contents, hair or dirt if not careful when cutting jugular or other blood vessels. Hemolysis.
Whole blood*	Nitrogen balance DNA	Blood-borne helminths, protozoa, bacteria	Contamination with rumen contents, hair or dirt if not careful when cutting jugular or other blood vessels.
Rumen content	Diet		
Milk sample	Fat and protein content		Inexperience in expressing milk may result in poor recovery.
Ovaries	Current pregnancy status / reproductive history ¹⁰		Difficult to find; lymph nodes mistaken for ovaries.

* Collected during live sampling; ¹(Loison *et al.*, 2001); ²(Wu *et al.*, 2012); ³(Wobeser, 2001); ⁴(Handeland *et al.*, 2010); ⁵(Haley *et al.*, 2009); ⁶(Elkin & Bethke, 1995; Robillard *et al.*, 2002); ⁷(Tessaro & Forbes, 1986); ⁸(Wobeser, 1976); ⁹(Williams, 2005); ¹⁰(Cuyler & Østergaard, 2005)

Figure 2 a

SEX: Male Female

PREGNANT?: Yes or No **NURSING?:** Yes or No

CONDITION: skinny not bad fat very fat

DEPTH of BACKFAT: _____ cm

| | | 2 | | 4 | | 6 | | 8 |

(YOUR HERD) _____ **Jaw Collection**

ID: 001

Date: _____

Location: _____

Return to: (agency/office location, phone number)



Fig. 3 a



Fig. 3b

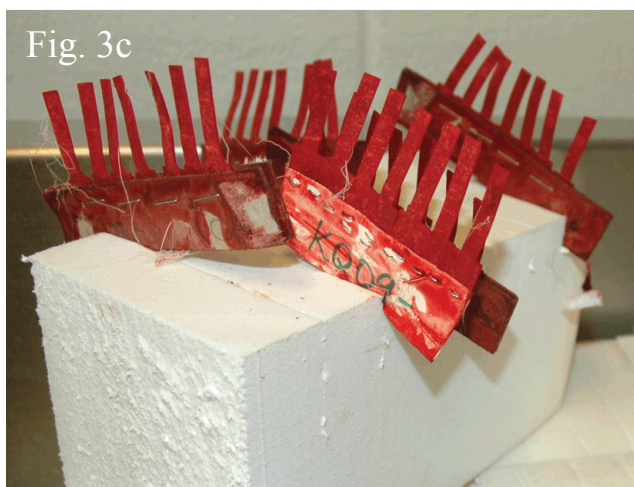


Fig. 3c

Fig. 3 Examples of samples taken and data acquired from caribou sampling activities. (a) Back fat depth is measured at a 45° angle from the base of the tail (see inset) with a measuring tape, a ruler, or the tag (see Fig. 2a). Backfat is measured at the deepest point. (Image credit: Wendy Nixon). (b) Metatarsal bone length measured by calipers (Image credit: Wendy Nixon). (c) Drying of Nobuto® filter paper strips that have been dipped in clean blood (Image credit: Karin Orsel).

location hunted, sex, pregnancy, a subjective assessment of body condition, and observations of any abnormalities. Back fat depth (Parker, 1981; Fig. 3a) was measured using the provided tags or rulers. Samples submitted included the lower jaw and metatarsal bone (Fig. 3b) with skin on, and core data collected included age (using tooth cementum annuli) (Miller, 1974; Matson, 1981), pregnancy, body condition (back fat, marrow fat, and a subjective hunter assessment; Lyver & Gunn, 2004), body size (jaw and metatarsal length; Parker, 1981; Couturier *et al.*, 2010), and presence and intensity of the parasite *Besnoitia tarandi* in metatarsal skin (Ducrocq *et al.*, 2012). *Besnoitia* cysts were quantified in the lab on gross observation using categories of number of cysts per square centimeter [0 = no cysts; 1 = very occasional (< 4 cysts/cm²); 2 = easily observed (4-10 cysts/cm²); 3 = high density (> 10 cysts/cm²)]. Histological examination, however, was determined to be much more reliable both for presence and intensity (Ducrocq *et al.*, 2012).

Level 1 provided key samples and data that could be analyzed to evaluate and compare body condition and size, demographics (recognizing that hunter-based sampling may have strong, but identifiable biases), and *Besnoitia* infection status.

Level 2 sampling (n = 166 animals)

This type of sampling was typically done by trained subsistence hunters or during community organized hunts that were attended by biologists and veterinarians. Samples included those from Level I as well as blood collected on filter paper (Fig. 3c; Curry *et al.*, 2011), the left kidney with fat, liver, and feces (Table 1). Semi-quantitative observations were done for grossly visible parasites and, depending on the collection, some body measurements were recorded (Fig. 2b; CARMA, 2013).

Collection of blood samples allowed for more in-depth examination of pathogen diver-

sity (Curry *et al.*, 2011). Blood was extracted from the filter paper in the lab and tested for exposure to a variety of pathogens using standard serological tests (Curry *et al.*, 2011). Additional testing for *Trypanosoma* spp. and *Setaria* sp. was attempted using polymerase chain reaction-based approaches. DNA was extracted from filter papers using a modified PurGene® (Qiagen, Canada) extraction procedure for compromised blood samples. Primers described by Noyes *et al.* (1999) were used to test for the presence of *Trypanosoma* and the primers described by Laaksonen *et al.* (2009) were used to test for the presence of *Setaria*. Unfortunately, DNA quality was variable and DNA was not reliably extracted from the filter papers (D. Schock & S. Kutz, unpubl. data). Thus, although the presence of *Trypanosoma* and *Setaria* was confirmed from some samples (Kutz *et al.*, 2012), the absence of these parasites could not be confidently established and quantitative assessment was not possible. It was, however, possible to sequence several *Trypanosoma* detected in caribou samples and compare genotypes among caribou herds and among ungulate species (D. Schock & S. Kutz, unpubl. data).

The kidney with surrounding fat was collected and used to establish the Riney kidney fat index (Riney, 1955). The kidney, together with a piece of liver, usually the caudate lobe, was frozen for future analyses. Feces were stored frozen and later tested for helminth and protozoan parasites and the bacteria *Mycobacterium avium paratuberculosis* (Forde *et al.*, 2012).

Observations of grossly visible parasites including: *Echinococcus granulosus* (cysts in lungs; Rausch, 2003), *Fascioloides* (Choquette *et al.*, 1970), *Taenia hydatigena* (cysticerci in liver) and *Taenia* spp. (cysticerci in skeletal or cardiac muscle; Thomas, 1996), *Hypoderma tarandi* (scars or bot larvae on underside of skin; Cuyler *et al.*, 2012), *Cephenemya trompe* (larval bots in the pharynx, examined once head was removed;

Cuyler *et al.*, 2012), *Besnoitia tarandi* (cysts in metatarsal skin and bulbar conjunctiva; Ducrocq *et al.*, 2012), and any other abnormalities were also recorded on the field datasheet. The lungs and heart were examined grossly for parasites, and the gastrocnemius and/or the cranial crural muscles [referred to elsewhere as 'peroneus' (Allaye Chan-McLeod *et al.*, 1995); Fig. 4a, b], were collected and, later in the laboratory, weighed and examined grossly for *Taenia* cysticerci and *Sarcocystis* cysts. The presence of rumen flukes (*e.g.*, *Paramphistomum cervi* or *P. skrjabini*; Nikander & Saari, 2007), were noted only in the Russian Lena-Olenek herd.

This dataset provided substantially more quantitative and semi-quantitative information on body condition and pathogen presence, exposure, and abundance than Level 1. It therefore allowed for more extensive investigations of pathogen/parasite occurrence in relation to body condition and other physiological and demographic parameters.

Level 2 detailed sampling (n = 835 animals)

These were the most comprehensive collections. They were planned hunts done primarily for scientific purposes and/or management monitoring, and in some cases were done in collaboration with community hunts. Typically, these collections involved local hunters and the meat was given to the local communities. In addition to the data and samples listed above, more detailed body size measurements and in-depth pathogen assessments were done (Table 1; CARMA, 2013). Project specific sampling was done during these collections, varied across herds, and samples may have included sampling: the brain stem ventral to the obex and/or lymph nodes for chronic wasting disease (Williams, 2005), conjunctiva and skin from the rostrum, scrotum, and inner thigh for *Besnoitia* research (Ducrocq *et al.*, 2012), abomasum and first three meters of small intestine for gastrointestinal parasite analyses, a 5-10cm section of

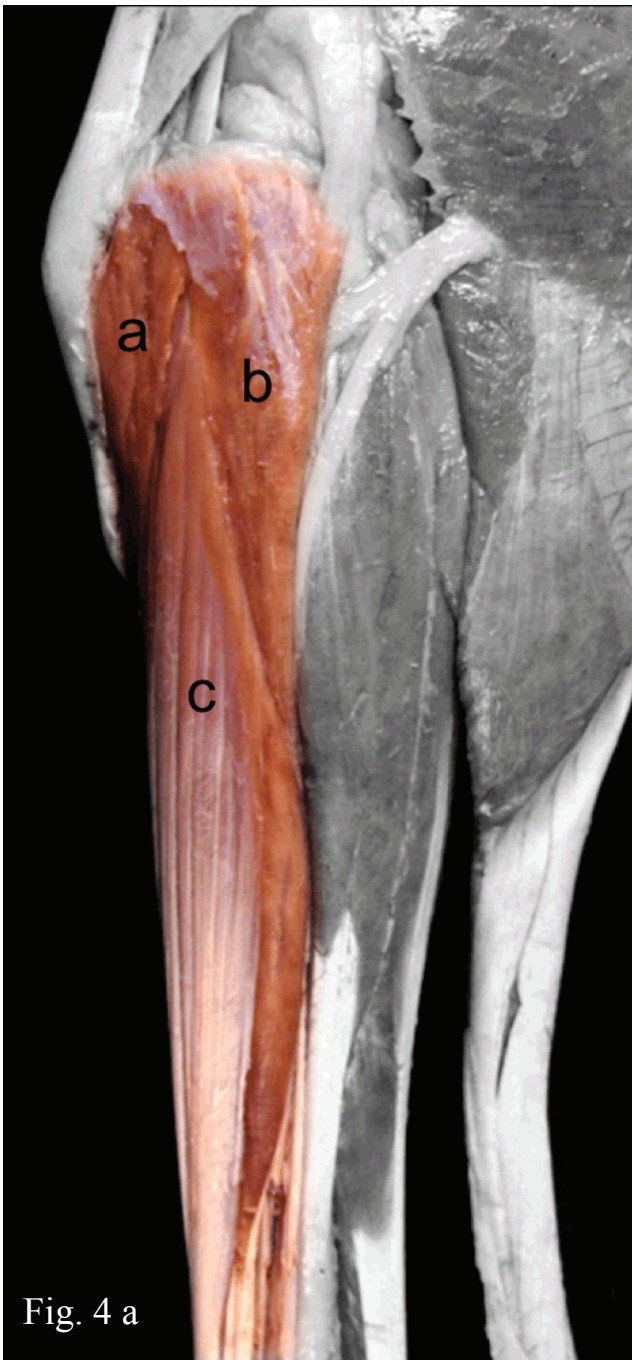


Fig. 4 a



Fig. 4 b

ileum and mesenteric lymph nodes for *Mycobacterium avium paratuberculosis*, ovaries (Dauphiné, 1978), various tissue sections in formalin for describing normal histology, weight of the rumen contents (Huot, 1989), and additional body measurements. Whole (EDTA tubes) and clotted blood for serum were often collected in addition to blood on filter paper. Lungs were dissected along the bronchi and major bronchioles to assess *Dictyocaulus* infection (Anderson & Prestwood, 1981) and livers were cut into 1 cm slices to examine for the liver fluke, *Fascioloides magna*, and *Taenia hydatigena* cysts (Lankester & Luttich, 1988). Transverse cuts through both the heart and the gastrocnemius muscle, and sometimes additional muscles, were done to examine for cysts of *Taenia* spp. Feces, muscle, fur, and urine were also collected for protein/nitrogen balance studies (Barboza & Parker, 2006).

This sampling level allowed for more detailed quantitative analyses of a broader range of pathogens. The broader dataset on body condition indices (back fat, kidney fat, marrow fat, empty and dressed body weight, and cranial crural muscles) provided the data necessary to predict body condition, fat, and protein (Ring-

Fig. 4 The cranial crural muscle group of a caribou leg. (a) Lateral view of the hind leg showing the anatomical position of the cranial crural muscle group (spotlit). The cranial crural muscle group is composed of four muscles: a. the cranial tibial (tibialis cranialis), b. the long fibular (fibularis longus), and c. the fused third fibular (fibularis tertius) and long extensor (extensor digitorum longus). When consulting standard veterinary anatomy texts, the reader should be aware that fibularis and peroneus are synonyms and that both have been widely used; it has recently been agreed that fibularis should be the standard term (b) The cranial crural muscles separated. The long extensor and the third fibular are extensively fused at their upper ends and the two tendons of the long extensor are visible behind the third fibular. There is no need to separate these muscles in the field as they should all be weighed together. (Image credits: Rangifer Anatomy Project, P. Flood and C. Muelling).

Table 2. Collections done by the CARMA network during International Polar Year activities, 2007 – 2010. The types of collections, data, samples, and the number of animals from each herd that were sampled (n) are indicated. Blank cells indicate that no animals were sampled.

	n	Herds ¹															
		WAH	TSH	POR	CB	TUK	BW	BE	BT	B/A	B/Q	RAF	RG	AM	KS	TAY	LO
Type of collection																	
Level 1 (Subsistence)	152			30		44	10		38							30	
Level 2 (Trained hunter/community)	166			25		13	32			56	40						
Level 2 (Scientific)	835	20	21				20		150	73		176	178	47	50	100	
Live-capture	53				15	6	32										
Total	1206	20	21	55	15	19	128	10	150	111	56	216	178	47	50	100	
Body condition indicators																	
Full body mass	474	17	19	20					145	72				41	40	100	20
Dressed body mass	250								67	62				41	40	40	
Total body length	456	18	21	25			19		146	72		35		41	40	39	
Chest girth	440	9	21	15			20		147	73		35		41	40	39	
Hind leg	105	9	20	10						22		19				25	
Metatarsal length	237	18	21	25						73		19		41	40		
Metatarsal bone length	761	14	10	53		9	74		147	27	48	263		46	40	30	
Total jaw bone length	365	9		13			28		49	59	51	39		44	48	25	
Cranial crural m. weight	255			13			19		95	22				41	40	25	
Gastrocnemius weight	103			13					50							40	
Hunter fatness estimate	421	19	20	48		13	12	8	90	12	53	34		27	50	35	
Back fat depth	586	18	17	45		12	81		139	102	25	22		45	50	30	
Kidney fat index ²	451			23			26		139	76	50			47	40	30	20
Metatarsal bone marrow fat	549	1	18	49		13	73		141	36	49	36		47	50	36	
Jaw marrow fat	70		19											41		10	
Femur marrow fat	39	20	19														
Gross parasitism																	
<i>Hypoderma tarandi</i>	721	20	19	11			11		150	68	2	134	145	47	50	34	30
<i>Cephenemyia trompe</i>	444	20	21	11			10		149	73				47	50	34	29
<i>Taenia hydatigena</i>	748	20	20	12			17		150	72		148	144	45	50	40	30
<i>Fascioloides magna</i>	761	20	21	11			19		150	88		148	144	40	50	40	30
<i>Echinococcus granulosus</i>	701	20	20	11			17		149	49		148	145	47	50	15	30
<i>Dictyocaulus</i>	310	20	20	0			17		109	49				45	50		
<i>Taenia</i> spp.	738	20	20	11			20		150	73		148	145	41	50	30	30
<i>Setaria</i> spp.	359			6			19		149	88				47	50		
Fecal parasitism																	
Fecal parasitology (Wisconsin flotation)	572			23	15	6	52		109	36	14	91	90	47	49	40	
Fecals parasitology (Baermann)	566			23	14	6	49		106	35	23	91	89	46	49	35	
<i>Giardia</i> and <i>Cryptosporidium</i> (IFA)	522	10		22	15	6	51		97	35	39	58	58	47	49	35	
Pathogen presence or exposure																	
<i>Besnoitia tarandi</i> ³	1045			13			25		129			636	146	46	50		
<i>Mycobacterium avium paratuberculosis</i> ⁴	544			23	22	28	52	20	109	36	46	61	60	47	40		
<i>Brucella</i> spp ⁵	690			33			80		147			147	144	49	50	40	
<i>Neospora caninum</i> ⁵	647			33			80		145			147	143	49	50		
West Nile Virus ⁵	645			33			79		144			147	143	49	50		
<i>Toxoplasma gondii</i> ⁵	684			33			77		144			148	143	49	50	40	
Bovine Herpes Virus-1 ⁵	636			32			76		143			147	139	49	50		
Bovine Respiratory Syncytial Virus ⁵	636			32			76		143			147	139	49	50		
Para-influenza-3 ⁵	636			32			76		143			147	139	49	50		
Pestivirus ⁵	636			27			57		144			147	144	49	68		

	n	Herds ¹															
		WAH	TSH	POR	CB	TUK	BW	BE	BT	B/A	B/Q	RAF	RG	AM	KS	TAY	LO
Contaminants and heavy metals																	
Kidney ⁶	187								40					47	40	30	30
Liver ⁶	110													40	40	30	
Muscle ⁶	80								10					20	20	30	
Liver – PFOS ⁷	55								5					10	10	30	

¹ Herds: WAH=Western Arctic, TSH=Teshekpuk, POR=Porcupine, CB=Cape Bathurst, TUK= Tuktoyaktuk Peninsula, BW=Bluenose West, BE=Bluenose East, BT=Bathurst, B/A=Beverly/Ahiak, B/Q=Beverly-Qamanirjuaq, RAF=Rivière-aux-Feuilles, RG= Rivière-George, AM= Akia-Maniitsoq, KS= Kangerlussuaq-Sisimiut, TAY=Taymir, and LO=Lena-Olenek; ² Russian collections had potentially an alternate measure of kidney fat that has yet to be calibrated to the KFI in the CARMA protocols; ³ From metatarsal skin sections; ⁴ From ileum and ileo-caecal lymph nodes, and fecal and serum samples; ⁵ From blood sampled by filter papers or serum samples; ⁶ A combination of any of the following heavy metals: Aluminum, Antimony, Arsenic, Barium, Beryllium, Bismuth, Cadmium, Calcium, Cesium, Chromium, Cobalt, Copper, Gallium, Iron, Lanthanum, Lead, Lithium, Magnesium, Manganese, Mercury, Molybdenum, Nickel, Palladium, Platinum, Potassium, Rubidium, Selenium, Silver, Strontium, Thallium, Tin, Uranium, Vanadium, and Zinc; ⁷ Perfluorooctane Sulfonate.

berg *et al.*, 1981b; Huot & Goudreault, 1985; Adamczewski *et al.*, 1987a; b; Taillon *et al.*, 2011) for comparison with reproductive status and individual and group abundance and diversity of pathogens and pathogen exposure.

Live animal sampling (n = 53 animals)

This sampling was done by biologists as a routine component of animal handling during radio-collaring. Data collected included an estimate of age (based on body size and tooth wear), body condition, feces, blood, and hair. Depending on season these animals were allotted in reproductive category (pregnant/non-pregnant, lactating/non-lactating, and in some cases weaning status). Body condition and reproduction status could also be examined relative to status of pathogens that could be grossly observed (*e.g.*, *Besnoitia*), or those that could be assessed serologically or through fecal examination.

CARMA network feedback and protocol evaluation

Overall, the protocols were well received and consistently applied. Formal feedback from

the network, gathered during three breakout groups in 2010, was positive with helpful comments to improve the protocols and their utility (CARMA, 2013). Network members emphasized the importance of the written protocols and visual resources for sampling and laboratory processing (images and directions in the protocols, sampling video, anatomy website), and suggested that these be translated into the languages of the various user groups around the Arctic. Aboriginal members of the network indicated that their view and observations of *Rangifer* ‘health’ may differ from the scientific perspectives and that a community-developed protocol that incorporated this view for health monitoring would be valuable. Additional suggestions included: development of advanced protocols for non-lethal sampling, improved guidance on necessary sample sizes for power analysis, and additional guidance on interpretation of results.

Network members did identify some issues with interpretation of the field protocols (Table 1). One concern was identifying the anatomical limits of the ‘peroneus’ muscle, the weight of which could be used to estimate body pro-

tein. This is actually a complex of four muscles, better referred to as the cranial crural muscles. They are found on the front (cranial aspect) of the shinbone (tibia) in the crus or crural part of the leg; hence the name. They form a coherent functional group that, taken together, flex the hock and extend the digit. The cranial crural muscles are relatively fibrous when compared with other muscles and are not much sought after for food. They can be quickly and consistently removed as a group, and weighed without further dissection. To clarify the muscle group to be sampled, a series of images were produced by dissections of reindeer and caribou by Peter Flood, Christoph Muelling, and others (Fig. 4a, b). A step-by-step instructional Microsoft PowerPoint® presentation describing the appropriate anatomy and sampling process for this muscle group was also produced and is available at <http://www.ucalgary.ca/caribou/Sampling.html>.

Other potential sources of error were identified when datasets were merged into a single database and it became clear through this process that, to avoid errors, complete descriptions of any modifications from the standard protocols needed to be included with all metadata files. For example, the total body length measured for some herds included the tail whereas the tail was excluded for others. Occasionally confusion arose over the units of measurement. In some cases these were not specified on data-sheets. For example, back fat could be measured as 1 mm versus 1 cm, or 1 inch versus 1 cm. This was particularly an issue if measurement tapes included both imperial and metric units. Some datasets contained blank spaces that left ambiguity as to whether an animal had been examined for that specific indicator/pathogen and zero observed, or if the indicator had not been examined at all. Errors, blanks, or unspecified deviations from protocols were rectified by clarification from contributors.

Sampling and data collection for pathogens

were generally standardized and consistent, however, some clarifications to the protocols were also needed to prevent loss of data. For example, quantification for serology requires that filter paper strips are fully saturated. In some cases the blood strips were only partially saturated and results were not quantifiable or comparable. Quantification of *Taenia* cysticerci differed between protocols. Specifically, at level 2, the hunters were only asked if they saw any *Taenia* cysts in the carcass. In contrast, in the detailed level 2 protocol, two horizontal cuts were made through the gastrocnemius and the heart, and more intensive sampling (e.g., more cuts or organs examined) was done for some herds. Once samples for pathogens were collected, standardization in laboratory analyses was achieved by ensuring similar storage of samples among herds and the use of the same laboratory for each pathogen. This limited sources of error and variability among herds.

In response to network member feedback and queries, early in the process two products were developed to supplement the protocols and provide additional visual resources. A DVD on basic and advanced sampling was produced in cooperation with hunters from the communities of Fort Good Hope and Colville Lake, Northwest Territories, Canada (CARMA, 2009). The video was distributed to CARMA network collaborators, participating communities, as well as local and regional wildlife groups and agencies. It is available on the CARMA website (CARMA, 2013). In addition, the *Rangifer* Anatomy Project was initiated to produce high quality anatomical images of *Rangifer* and instructional PowerPoint presentations to supplement the protocols and serve as teaching aids for a variety of audiences. The site is located at the University of Calgary (<http://www.ucalgary.ca/caribou/index.html>), and can be accessed through the CARMA website.

Discussion

CARMA's goal was to bring together the vast knowledge and expertise in the circumarctic so that sampling protocols incorporating local knowledge and science could be developed and provide reliable and comparable information on health and body condition of *Rangifer* across its range. This goal was achieved in that a series of standardized protocols with increasing levels of complexity were developed and implemented in varying degrees across herds. However, as evidenced by feedback in 2010, the CARMA protocols did not adequately incorporate the aboriginal views on health. Protocol development and implementation was a learning process, with challenges and limitations being identified and addressed on an ongoing basis. An advantage of CARMA's web-based approach was that it was responsive to identified needs. The protocols and manual were easily updated and clarified and supplementary resources could be made readily available on the web. This process is ongoing.

An innovative aspect was the partnership with infectious disease specialists, which brought a strong emphasis on monitoring techniques for pathogens and non-infectious diseases. This emphasis was in recognition of two points. Firstly, body condition and disease monitoring has not always been well integrated in the past. Body condition and disease are intricately linked and neither can be well understood in isolation from the other. Secondly, the emergence of new diseases, re-emergence of old, and pathogen spill-over among wildlife, domestic animals, and people has highlighted the need for effective and responsive wildlife disease monitoring and surveillance systems today (Kuiken *et al.*, 2005).

It became clear during IPY that when dealing with a species that is widespread across vast geographical remote regions there is a need to develop flexible yet scientifically robust strategies for data collection. Although the protocols

were originally developed as two defined categories of collection, they were adapted to meet the monitoring, logistical, research, and human resource constraints for each herd. This highlights the importance of providing a prioritized continuum that allows useful and comparable information to be gathered even at the most basic level. A frequent limitation to monitoring is inadequate sample size, which can limit statistical power to detect trends in concomitant variables (Nickerson & Brunell, 1998). Importantly, "required" sample size will vary for each specific pathogen depending on its expected prevalence. Several authors emphasize that working with hunters to monitor health and condition of harvested caribou increases sample sizes (Kofinas *et al.*, 2003; Lyver and Gunn, 2004; Brook *et al.*, 2009; Curry, 2010). The protocols accommodate the trade-off between the greater sample sizes available from hunter-based collections compared to the more detailed dataset from fewer animals collected during intensive monitoring and research projects. For example, a level 1 collection (*i.e.*, the jaw, metatarsal, and other basic data) can provide reliable information on late term pregnancy, age and sex structure, body size, body condition, and abundance of a few select pathogens. Additional information on other physiological parameters can also be gathered from hair (*e.g.*, cortisol and other hormones; Ashley *et al.*, 2011) while teeth can be examined for dental enamel hypoplasia as an indicator of past stress events (Wu *et al.*, 2012). Hunter-based sampling was done prior to IPY across a number of jurisdictions and has, in general, broad acceptance amongst hunters (Gunn *et al.*, 2005; Brook *et al.*, 2009). With widespread hunter involvement, this type of monitoring could provide ongoing, reliable and affordable information on several indices of *Rangifer* health across a broad geographic range and across seasons (Kofinas *et al.*, 2003). As the complexity of data and sample collection increases (*e.g.*, through assisted community hunts

and biologist led collections) more information is acquired and more comparisons are possible. However, such comprehensive monitoring is typically done on fewer herds and over shorter time frames, limiting the number of herds or years that can be compared. Thus, establishing a 'bare minimum' guideline for sample and data collection facilitates ongoing broader (geographically and temporally) comparisons at a basic level. Importantly, all sampling strategies have biases specific to that strategy, for example, subsistence hunters may select for animals in better condition, and these must be considered in the final data analyses.

The additional spatiotemporal sampling and increased sample sizes offered by hunter-based sampling may be particularly valuable for disease surveillance as it can increase detection of infectious diseases that may be present at a low prevalence and/or with a patchy or clumped distribution (Zhang *et al.*, 2011). Working with hunters, however, offers important advantages beyond simply increasing sample size. These include two-way exchange of knowledge, recognition of different ways to assess health and condition, and growing mutual respect and trust developed from working together (Kofinas *et al.*, 2002; Brook *et al.*, 2009; Lyver & Nation, 2010).

Emerging diseases are of increasing global importance, and are also of considerable concern for northern ungulates (Kuiken *et al.*, 2003; Kutz *et al.*, 2004; Laaksonen *et al.*, 2010). Northward range expansion of domestic and wild animals, together with various pathogen vectors, may allow introduction of new pathogens into *Rangifer* range. Concurrently, a warming climate is removing some of the environmental constraints on existing arctic pathogens, resulting in range expansion of pathogens and emergence of disease (Hoberg *et al.*, 2008; Kutz *et al.*, 2009; Laaksonen *et al.*, 2010). Establishing comprehensive baselines for pathogen biodiversity and abundance is necessary if

we are to detect emergence of new pathogens and disease syndromes. Sophisticated molecular diagnostic techniques in combination with the advanced level of collection described here allows, through either physical recovery of the organism or serological evidence of exposure, detection of all known pathogens of *Rangifer* and the quantification/semi-quantification of most.

Monitoring programs must ensure accuracy and precision (*i.e.*, repeatability) of measurements. The CARMA network approached this through the detailed descriptions in the manual and protocols, hosting specific training sessions, and having biologists participate in collections before running collections of their own. Use of the same diagnostic and research laboratories in many cases helped to ensure standardization.

Preservation of biological specimens and data are critical elements of any monitoring program. In particular, appropriately preserved physical specimens allow for investigation of new questions, or re-evaluation of old studies, as new information and techniques emerge (Hoberg *et al.*, 2008). Archiving of CARMA tissues was limited to the capacity of individual researchers and, for those specific caribou populations, provides a rich source of information. Importantly, to ensure appropriate preservation of these materials, and to promote increased rates of archiving in the future, centralized, permanently curated facilities for specimen and DNA archiving are essential.

Data management in large-scale monitoring programs adds complexity at several levels. Data ownership and management is an important issue within any large network. Metadata for CARMA were managed through the Arctic-Net portal, however, mechanisms for long-term storage of full datasets in a central database and subsequent access remains to be established. To date, CARMA has drafted a data policy as well as data submission and request forms. Ideally, in the spirit of open access that IPY promoted,

all data, tissues, and specimens should be made available to the broader scientific community after a reasonable time period. Sharing data also raises questions of format and transcription errors when inputting from data sheets to a database. Exploring the use of a computer program for data mining would reduce transcription errors while transferring data from original databases. CARMA continues to discuss the development and implementation of solutions.

Conclusion

CARMA is a network of *Rangifer* users, biologists, scientists, and managers who have worked together to implement the first broad scale standardized body condition and health assessment of migratory caribou. To our knowledge, this is the most comprehensive sampling effort for a terrestrial mammal across its entire range. Network members were actively and willingly engaged in improving and implementing protocols, and although some challenges were encountered, network members philosophically supported the protocols and applied them. This was a clear reflection of the shared vision of how a comparative approach across space (herds) and time (seasons and years) can substantially improve our current scientific knowledge of *Rangifer* and enables us to monitor and detect changes more rapidly and confidently.

Flexibility and adaptability of sampling regimes are essential to ensure that specific research and monitoring objectives can be addressed. Still, there was consensus that a minimum of standard data and samples should be done for all herds (*e.g.*, Level 1). Easily accessible and up to date protocols provided key resources for field and laboratory personnel. Where needed, these were supplemented with additional visual aids such as the anatomy resources and sampling DVD. Ongoing in-person and practical training and mentorship of hunters and researchers are essential to ensure accurate and consistent sample and data collec-

tion into the future. Engagement of communities and local hunters is critical to promote knowledge sharing and mutually acceptable approaches for long-term health and condition monitoring in caribou.

Data management and ownership, together with authorship on scientific articles, had the potential to become problematic. However, a transparent approach and regular in-person discussions enabled the growth and maintenance of productive collaborations among network members. To be effective, the data and knowledge gained must be transferred back to communities and caribou managers in a timely and effective manner. This has been done directly within the CARMA network as community representatives are key and active network participants, and has also occurred in many regions in the form of co-management meetings. Additional researcher-initiated posters and community meetings presenting preliminary results have occurred. Unfortunately, as the funding from IPY has ended, there are severe financial constraints to returning to communities in person to present final results, many of which will be coming out over the next several years.

Rangifer populations co-exist with increasing human abundance around the Arctic, and like many parts of the world, anthropogenic modifications in parallel with natural phenomenon are influencing their health and sustainability (Balmford *et al.*, 2003). To understand these complex systems, wildlife research needs to focus on large-scale monitoring activities (Pollock *et al.*, 2002; Balmford & Bond, 2005). The CARMA network and the standardized sampling protocols that it has developed, will hopefully enable a more comprehensive understanding of migratory *Rangifer* populations, and provide new insights into the resilience of these animals under the current regime of environmental, social, and political change.

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