PROJECT # 1009-008-06 GIS # HB-23-168 July 30 2012

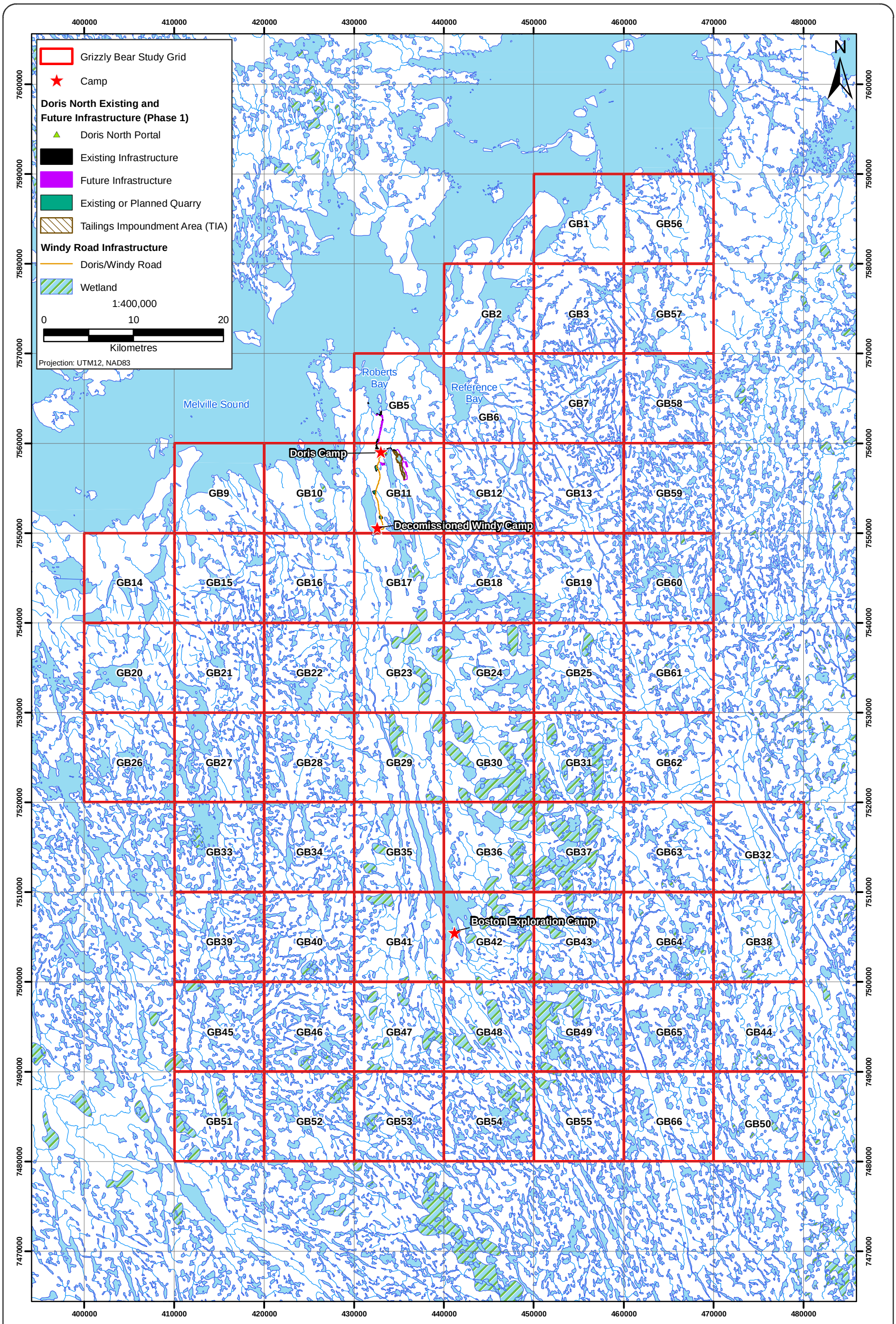

Figure 2.2-1

NEWMONT NORTH AMERICA

Grizzly Bear DNA Survey Grid, Doris North Project, 2011

Figure 2.2-1

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Plate 2.2-1. Fold-up tripod design used during the 2011 grizzly bear DNA study.

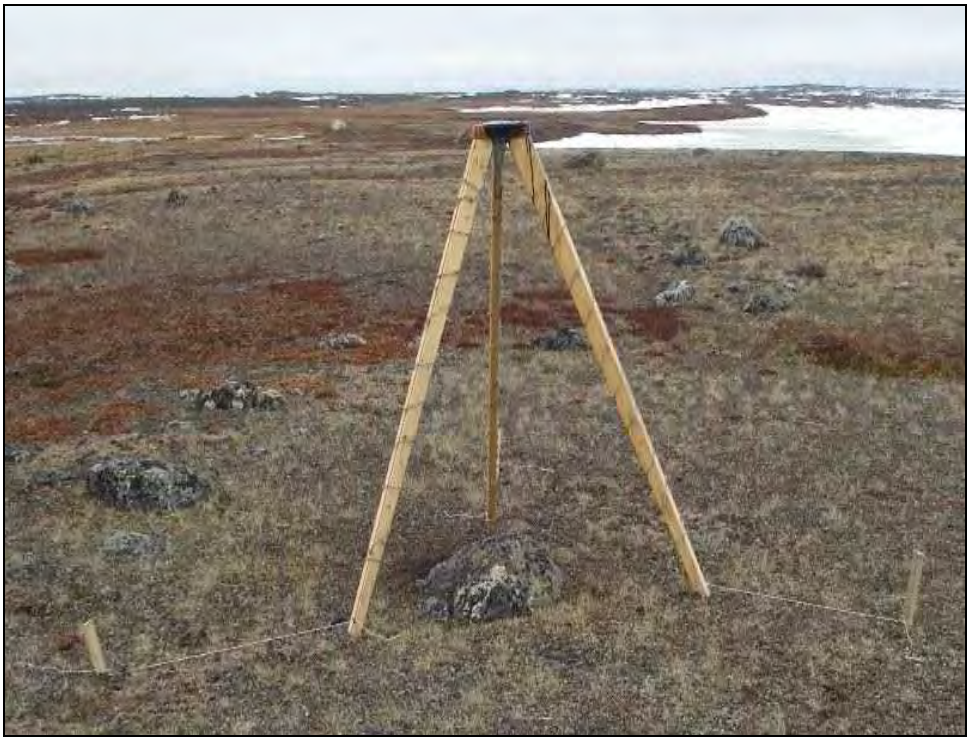

Plate 2.2-2. Fold-up hair snagging tripod anchored with wooden stakes.

2.3.2 Microsatellite Genotyping

A highly variable 8-locus marker system — including gender — was used during microsatellite genotyping. This process was established during previous work with GN-DOE in the same region. The analysis followed a 3-phase approach, starting with a first pass of all 8 markers on all extracted samples. After the first pass, mixed and bombed samples were set aside, with 'bombed' being defined as having produced high-confidence data scores for < 4 of 8 markers during the first pass.

The first pass was followed by a cleanup phase in which data points that were weak or difficult to read the first time were re-analyzed. In some cases multiple rounds of re-analysis were used when it appeared that there was potential to upgrade a sample to a high-confidence 8-locus score. Several more samples may be excluded following this clean up phase.

Samples that produce incomplete data are normally rejected, but in 2010, nine of the 163 samples were missing data for marker *G10B*. These were not weak samples of the sort that might produce unreliable data, but rather strong samples that were affected by an episode of contamination. Despite efforts to keep pre- and post-PCR DNA in separate buildings, a procedural breakdown at the lab resulted in *G10B* alleles 156 and 158 appearing in samples that should not have those alleles.

Some samples appear to have been contaminated at the extraction phase, so whenever possible leftover hair was used to repeat the extraction after the lab and equipment were cleaned.

Unfortunately, nine samples remained that had low-confidence scores at *G10B*, including two samples that were the only samples from their given individuals. In most of these cases, the true genotype can be deduced, since the contamination involved specific alleles, but the samples were scored as they appeared on the runs rather than adding an extra, subjective layer to the data interpretation.

The last phase of analysis was error-checking, following published protocol of selective data re-analysis (Paetkau 2003). Some routine scoring errors in the data were found, typical of what might be encountered whenever working with sparse DNA sources like hair follicles. Once these errors were corrected, the most similar pair of genotypes in the dataset mismatched at 3 of 8 markers. This error checking process has been validated through extensive blind testing, and found to effectively prevent the recognition of false individuals through genotyping error (Kendall et al. 2009).

In 2011, marker *CXX110 (Ho* = 0.83) was added to the 8-locus system used in 2010 so that the Hope Bay marker system now matches the 9-locus setup used in the GN-DOE grizzly bear database.

2.4 POPULATION ANALYSES

The total number of grizzly bears identified during the two years of the program are reported; however, because the study area grid changed near the end of 2010 and again in 2011, there is no way to accurately reflect capture and recapture probabilities across all 66 cells. As a result, a population estimate is derived only for the northern portion of the study area that remained consistent over the two years of the program (i.e., cells 1 – 37).

Pollock's Robust Design model in program MARK (© G.White; available online at http://www.phidot.org/software/mark/download/index.html) was used to estimate the number of grizzly bears present on the Doris North study area. The robust design model is a combination of the Cormack-Jolly-Seber (CJS) (Cormack 1964, Jolly 1965, Seber 1965) live recapture model and the closed capture models. The model is described in detail by Kendall et al. (1995, 1997) and Kendall and Nichols (1995). The key difference from the CJS model is that instead of just one capture occasion between survival intervals, multiple (> 1) capture occasions are used. These occasions are close together in time, allowing the

assumption that no mortality or emigration occurs during these short time intervals. The power of this model is derived from the fact that the probability that an animal is captured at least once in a trapping session can be estimated from just the data collected during the session using capture-recapture models developed for closed populations (Cooch and White, 2012). The longer intervals between trapping sessions allows estimation of survival, temporary emigration from the trapping area, and immigration of marked animals back to the trapping area.

Kendall et al. (1995, 1997) term the intervals between trapping sessions the primary sampling periods, where gains (birth and immigration) and losses (death and emigration) to the population can occur. Secondary sampling periods are the shorter intervals where the population is effectively closed to gains and losses. In this study, the primary sampling periods are 2010 and 2011, and the secondary sampling periods are the six two-week intervals between hair collections in a given year.

For each trapping session (i), the probability of first capture [$p(ij)$] and the probability of recapture [$c(ij)$] are estimated (where j indexes the number of trapping occasions within the session), along with the number of animals in the population that are on the trapping area [$N(i)$]. For the intervals between trapping sessions, the probability of survival [$S(i)$], the probability of emigration from the study area [*gamma"* (i)], and the probability of staying away from the study area given that the animal has left the trapping area [*gamma'* (i)] are estimated. With two primary sampling periods, *gamma"* applies to the interval before the second trapping session, and *gamma'* is not estimated because there are no marked animals outside the study area at that time.

Several models were selected *a priori* that were anticipated to best estimate population parameters of grizzly bears in the Doris North study area. The top model was selected from the suite of models based on an information theoretic approach that utilizes the Akaike Information Criterion (AICc) corrected for small sample sizes (Burnham and Anderson 2002). AICc provides an unbiased approach to model selection as models are weighted relative to each other based on a log-likelihood distribution. This approach also enables model parameters to be averaged across models that are closely ranked. AICc seeks the most parsimonious model, i.e., explaining the greatest amount of variation with the fewest parameters.

3. Results

3. Results

3.1 LAB RESULTS

3.1.1 2010

A total of 411 samples were collected during 2010 and submitted for DNA analysis. The detailed field hair collection and genetic marker data are recorded in Appendix 1. A summary of individual bear genotypes and capture/recapture rates for 2010 is included in Appendix 2.

The 411 records in the 2010 bear database were classified as follows:

1. *Successful samples* (40%): 163 samples that were assigned to individuals.
2. *Inadequate samples* (5%): 20 samples that lacked sufficient hair follicles for DNA extraction.
3. *Subselected samples* (27%): 111 samples that were excluded due to subselection rules.
4. *Bombed samples* (28%): 115 samples that failed during microsatellite analysis.
5. *Mixed samples* (0%): 2 samples that showed evidence of > 2 alleles per marker.

WGI reported that the quantity of hair in the samples was excellent, with a mean of 7.0 guard hair roots per extracted hair sample (treating underfur as equivalent to 0.2 guard hairs) and 85% of extracted samples having > 2 guard hair roots. However, the microsatellite analysis success rate was lower than expected, with 58% of extracted samples producing viable genotypes. It may be that exposure to the sun is causing more rapid DNA degradation on the barren grounds relative to similar projects in forested habitat. In contrast to grizzly bears, late-winter wolverine hair collections on the barren grounds have typically performed better than average in the lab. Colder temperatures, drier conditions, and less exposure to UV radiation combined, likely accounts for the better performance of wolverine hair samples in the lab.

The success rate of samples was not uniform, varying from ≤ 45% for sessions 1 and 5, to ≥ 64% for sessions 0 and 2. Once again, this relationship between success rate and collection date suggests a potential environmental influence that will have to be addressed in future field sampling.

From 163 successfully analyzed hair samples, a total of 31 individuals were identified, including 17 males and 14 females. While there were some cases where multiple samples from a single sampling period were tied to the same bear, there were also many bears that were detected across multiple sampling sessions. Nine individuals were recaptured at least once, three individuals recaptured twice, and eight individuals were new captures during the last sampling session.

These 31 individuals were compared to 278 grizzly bears identified by a GN-DOE study west of Bathurst Inlet. One bear was common to both datasets, identified as individual 152 in this dataset and 68813N1 in the GN-DOE dataset. The chance of encountering two individuals with the same genotype goes up when comparing large datasets, so in order to lower the match probability in this case, three additional markers were analyzed on sample 152, which also matched the data on record for GN-DOE 68813N1. With the match now being based on 11 markers (including gender), the only realistic conclusion is that the samples in question came from the same bear.

In addition to analyzing extra markers to confirm the single match between datasets, the three extra markers were also analyzed in cases where bears in the two datasets matched six or seven of the original eight markers (1MM and 2MM-pairs). Given that all of the bears in the GN-DOE dataset already had genotypes for these three extra markers, this approach was more efficient than the traditional error-checking strategy, which would require searching the archives for old samples, and then re-analyzing the mismatching markers in those samples. In each of these cases ≥ 1 of the 3 extra markers mismatched, such that all pairs now differed at ≥ 3 markers, confirming that the genotypes in question came from different bears.

There are two main types of errors that can occur with individual ID: identifying more individuals than are actually present because of genotyping error (addressed above), and identifying fewer individuals than are actually present because of identical genotypes. Calculated match probabilities vary by orders of magnitude depending on what assumptions are made about the degree of relatedness between the sampled animals, and thus provide no practical means of assessing the risk of sampling two individuals with the same multilocus genotype (0MM-pairs). For this reason, empirical data from observed mismatch distributions is used to predict this risk.

With 31 individuals, the observed mismatch distribution would not be based on enough data to provide a reliable estimate of match probability. Therefore, these 31 bears were combined with the 277 that were unique to the GN-DOE dataset, and the new file of 308 barren ground grizzly bears was used to create an 8-locus mismatch distribution. Extrapolation from this 8-locus mismatch curve, in which there are 5 1MM-pairs and 68 2MM-pairs, suggests that ~0.5 pairs of individuals are expected to be encountered with the same 8-locus genotype if ~308 animals are sampled from this study population.

Given only 31 animals were sampled, the chance of having sampled two with the same genotype is too low to be of practical relevance. However, the ~0.5 probability of encountering a matching pair in the larger group of 308 animals indicates that it was appropriate to confirm the match between sample 152 and GN-DOE's 68813N1 using three extra markers.

While the majority of the samples in the combined barren ground grizzly bear file had data for all eight markers, it was necessary to test how much the missing *G10B* data would impact the match probability for the nine samples affected by contamination issues. For this reason, a 7-locus mismatch distribution was created, excluding *G10B* from the analysis. The 7-locus mismatch distribution predicts a 6-fold higher match probability than with eight markers (31 1MM-pairs and 327 2MM-pairs).

Consistent with this prediction, two pairs of individuals from the GN-DOE dataset (known to be different individuals based on data from other markers) matched at all seven markers other than *G10B*. However, in the Doris North dataset, the removal of *G10B* data had no impact on individual identifications. This indicates that the 8th marker was not strictly necessary for accurate individual identification in the Doris North dataset, even if it would be needed to prevent false matches in a project that sampled hundreds of animals.

3.1.2 2011

A total of 1,623 samples were collected and submitted for DNA analysis during 2011. The detailed field hair collection and genetic marker data are recorded in Appendix 3. A summary of individual bear genotypes and capture/recapture rates for 2010 is included in Appendix 4. Between 2010 and 2011, the number of samples increased by four times. The study area roughly doubled, from 3,700 km^2 to 6,500 km^2, which led to some of this increase. The increase was also due to methodological lessons learned from the 2010 season, such as the type of bait used and the positioning of posts on the landscape.

The 1,623 records in the 2011 database were classified as follows:

1. *Successful samples* (15%): 241 samples that were assigned to individuals.
2. *Inadequate samples* (30%): 485 samples that lacked sufficient hair follicles for DNA extraction.
3. *Subselected samples* (43%): 703 samples that were excluded due to subselection rules.
4. *Bombed samples* (12%): 187 samples that failed during microsatellite analysis.
5. *Non-bear samples* (0%): 7 samples that did not appear to be from bears.

Based on the poor success rates in 2010, the sample quality threshold was increased in 2011, extracting only those samples that had ≥ 20 underfur or ≥ 2 guard hairs with roots. The strict threshold for sample quality caused a marked increase in the number of inadequate samples (30% compared to 5% in 2010). Combined with stricter subselection rules, the amount of material used per extraction also increased to 7.4 guard hair roots per extracted sample.

There was no obvious pattern relating success rate with check number. The check with the shortest time interval (13 days for session 2) had a 50% success rate, whereas success rates in excess of 70% were observed for sessions 1 and 4 where the collection interval was one day longer. Similarly, there was no relationship between success rate and extraction rate, which varied from 22% for session 5 to 67% for session 3. There was also no obvious pattern in terms of progression with season, with the worst success rates observed in sessions 2, 5, and 6.

The 241 samples in 2011 were assigned to 39 individuals (17M; 22F), 18 of which were recaptures from 2010 (7M; 11F). Eleven of 14 females detected in 2010 were again detected in 2011. Over two years of sampling, a total of 52 individual bears (27M; 25F) have been identified. Individuals were once again compared to the 278 grizzly bears in the GN-DOE database, and no new matches were identified in 2011.

3.2 POPULATION ANALYSES

The top ranked model parameterized capture probability equal to recapture probability, which varied between trapping occasions within secondary sessions and between primary sessions, and no difference between males and females (Table 3.2-1). Capture/recapture probabilities ranged from 0.03 to 0.46 (Table 3.2-2). The probability of survival was lower for males (S = 0.53) than for females (S = 0.96), and the estimated probability of emigration from the study area was higher for males (*gamma"* = 0.43) than females (*gamma"* = 0.04).

A total of 41 individual grizzly bears (23M; 18F) were identified in the northern portion of the study area (cells 1–37). Based on parameters of the top ranked model, there was an estimated 25 males (95% Confidence Interval (CI): 19–38) and 20 females (95% CI: 16-31) present on the reduced trapping grid in 2010. The following year, there was an apparent decrease in both males (13; 95% CI: 12–15) and females (18; 95% CI: 16–26) on the trapping grid.

There were several cases in both 2010 (Figure 3.2-1) and 2011 (Figure 3.2-2) where an individual grizzly bear was captured in more than one cell during a single trapping occasion. Over both years, 11 females were caught twice during a single session, and two females were caught three times. Additionally, five males were caught more than once during a single session; three were captured three times, and two were captured twice. These capture events represent straight line distance movements ranging from 10 to 64 km for females, and 22 to 60 km for males. Additionally, multiple grizzly bears were detected at a sampling post during a single trapping occasion eight times in 2010, and 10 times in 2011. Cell 29 had the most detections with 10 over two years. With the exception of cell 29, grizzly bears

appeared more concentrated along the coast in 2010, whereas they appeared more evenly distributed across the study area in 2011.

Table 3.2-1. Model Rankings for Grizzly Bear Population Estimation in the Doris North Study Area (Cells 1-37), 2010-2011

Model	AICc	Delta AICc	AICc Weight	Model Likelihood	Parameters
p=c=diff within secondary; M=F; primary 1 ≠ primary 2	209.59	0.00	0.87	1.00	20
p=c=constant within secondary; M=F; primary 1 ≠ primary 2	214.21	4.62	0.09	0.09	12
p=c=constant; M≠F; primary 1 ≠ primary 2	215.84	6.26	0.04	0.04	12
p constant; c constant; p≠c; M≠F; primary 1 ≠ primary 2	220.07	10.48	0.004	0.005	16
p=c=diff within secondary; M≠F; primary 1 ≠ primary 2	250.37	40.78	0.00	0.00	32
All different	397.44	187.85	0.00	0.00	53

Table 3.2-2. Parameter Estimates Derived from the Top Model Describing Grizzly Bear Population Dynamics in the Doris North Study Area (Cells 1-37), 2010-2011

Parameter	Estimate	Standard Error	95% Confidence Interval	
			Lower	Upper
Probability of Survival (S) – males	0.53	0.03	0.48	0.58
Probability of Survival (S) – females	0.96	0.01	0.92	0.98
Probability of Emigration (*gamma"*) - males	0.43	0.05	0.33	0.53
Probability of Emigration (*gamma"*) – females	0.04	0.06	0.002	0.47
*$p=c_{(1,6)}$ – primary 1	0.13	0.04	0.07	0.23
*$p=c_{(2,6)}$ – primary 1	0.18	0.03	0.13	0.24
*$p=c_{(3,6)}$ – primary 1	0.07	0.03	0.02	0.17
*$p=c_{(4,6)}$ – primary 1	0.13	0.04	0.07	0.25
*$p=c_{(5,6)}$ – primary 1	0.18	0.05	0.10	0.29
*$p=c_{(6,6)}$ – primary 1	0.22	0.06	0.13	0.35
*$P=c_{(1,6)}$ – primary 2	0.07	0.03	0.02	0.17
*$P=c_{(2,6)}$ – primary 2	0.13	0.05	0.06	0.28
*$P=c_{(3,6)}$ – primary 2	0.03	0.02	0.01	0.10
*$P=c_{(4,6)}$ – primary 2	0.33	0.06	0.22	0.47
*$p=c_{(5,6)}$ – primary 2	0.40	0.08	0.26	0.55
*$p=c_{(6,6)}$ – primary 2	0.46	0.09	0.30	0.63
**N (males) – primary 1	25	4	19	38
**N (females) – primary 1	20	4	16	31
**N (males) – primary 2	13	1	12	15
**N (females) – primary 2	18	2	16	26

** $p=c_{(ij)}$ = probability of capture (p) and recapture (c) during the i^{th} trapping occasion of j occasions.*
*** N = the number of animals estimated to be on the study area during the primary session.*

PROJECT # **1009-008-07** GIS # **HB-23-165** *July 27 2012*

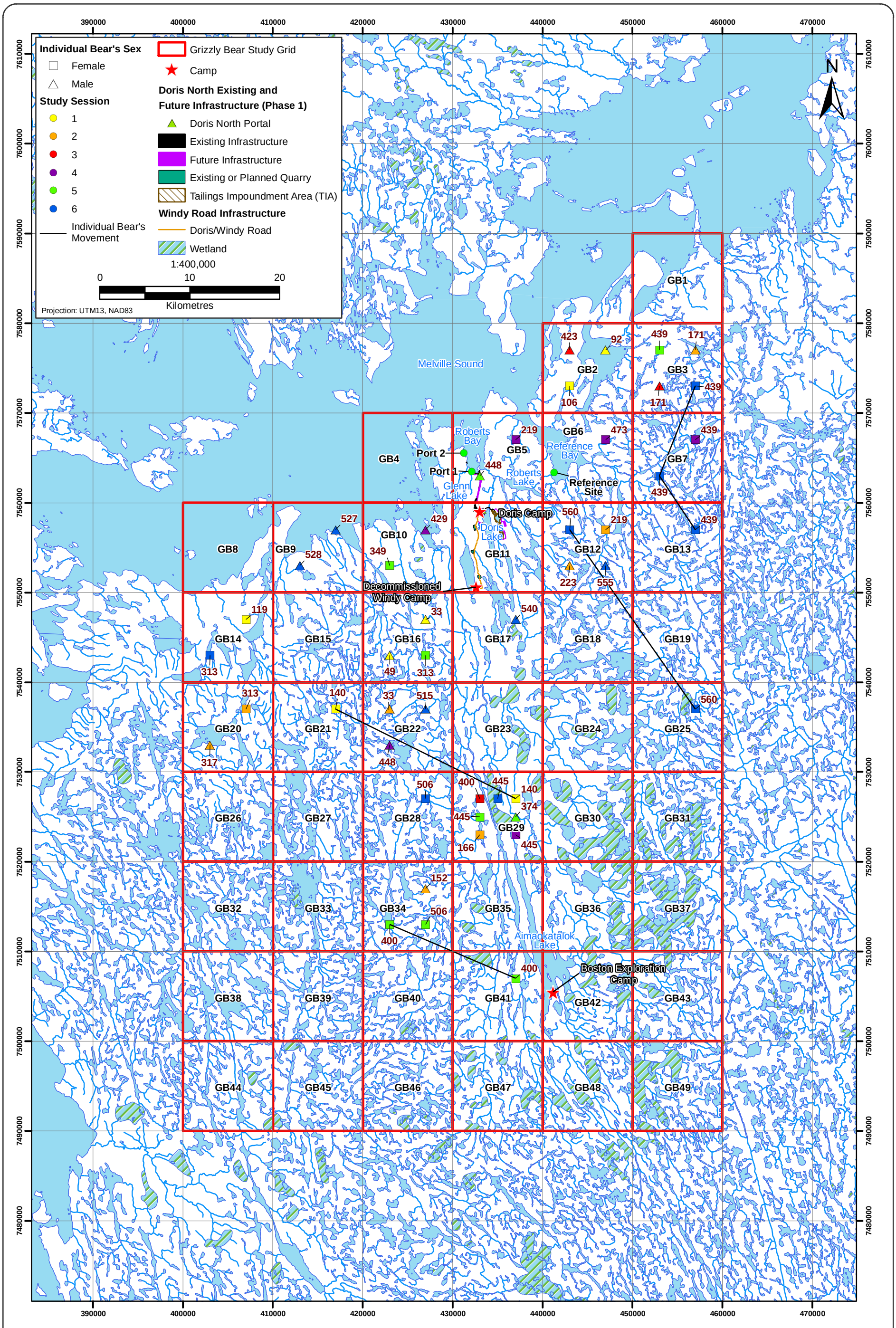

Figure 3.2-1

NEWMONT NORTH AMERICA

Grizzly Bear DNA Hair Capture Locations, Doris North Project, 2010

Figure 3.2-1

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