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# **Effects of Repeated Captures on Svalbard Reindeer (*Rangifer tarandus platyrhynchus*) – Implications for Animal Welfare and Scientific Bias**

Cassandra Ugland

Master of Science in General Ecology

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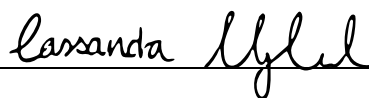
## Preface

This thesis is submitted as an integral part of the General Ecology MSc degree at the Norwegian University of Life Sciences, Ås, Norway, under the supervision of Professor Leif Egil Loe, Professor Erik Ropstad, and Liv Monica Trondrud. The data used in this thesis was supplied by the Svalbard Reindeer Capture Mark Re-capture project.

I would like to thank Professor Leif Egil Loe for his excellent guidance and support during this process, and for providing me with the opportunity to undertake fieldwork on Svalbard. Both Professor Erik Ropstad and Liv Monica Trondrud have been instrumental in my understanding of reindeer physiology, and I thank them for innumerable discussions that have fueled my work. In addition, I would like to thank Gabriel Pigeon and Liv Monica Trondrud for their work that provided me with my return to baseline data. Finally, I thank all my friends and family for their constant optimism and support when I needed it the most. It has been an incredible experience and I am extremely grateful to everyone that has taken part in this journey.

Norwegian University of Life Sciences

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Cassandra Ugland

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## Abstract

Capturing and marking animals is an essential component of wildlife research. However, data gathered using invasive methods may lead to scientific bias and compromise animal welfare. It is therefore important to evaluate the effects that handling may have on the physiology, behavior, and fitness of captured individuals. Studies with access to both long-term behavioral and physiological data are rare, and so provide an important insight into the full scope of effects handling may have on wildlife. Importantly, multiple metrics should be evaluated because previous studies have found a disconnect between behavioral and physiological responses to capture and handling, where individuals can habituate to one metric, but sensitize to another. A previous study established that there were no fitness effects associated with a single capture event per year for Svalbard reindeer (*Rangifer tarandus platyrhynchus*). This study therefore examines the effects of repeated captures on individuals' ability to return to baseline (RTB) values of behavior (activity) and physiology (heart rate), and subsequent fitness of 36 adult, known-aged female Svalbard reindeer. Individuals were captured by use of snowmobiles in March 2018. At the first capture, individuals were sedated and fitted with GPS-collars containing activity sensors and subcutaneous heart rate loggers. As part of a winter energetics study, individuals were recaptured up to 3 times over the next 5 weeks and restrained for blood sampling without sedation. In August of the same year calf-at-heel status was recorded. Generalized linear models and linear mixed-effects models were used to analyze the data with the aim of quantifying how long time the animals used to return to their pre-capture baselines. Capture was found to perturb the return time of both activity and heart rate, but the effect of repeated captures differed between the two metrics. For each subsequent capture, activity took less time to return to baseline, indicating individuals became more tolerant to capture. Heart rate on the other hand took longer to return to baseline with each successive capture, indicating individuals became less tolerant to capture. Individuals captured repeatedly did not significantly differ in body-condition nor reproductive success compared to individuals captured only once, although there was a biologically important effect on daily mass loss of repeated captures. These findings suggest that Svalbard reindeer are physiologically sensitive to repeated captures, despite appearing undisturbed when using behavior in isolation. Repeated capture and handling therefore arguably negatively impacts the welfare of Svalbard reindeer by causing individuals to remain stressed for up to a full day following release. This study highlights the importance of physiological data when examining researcher effects on wildlife and cautions against repeated handling events over short time periods.

## List of Abbreviations

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<b>Abbreviation</b>	<b>Meaning</b>
<b>CI</b>	Confidence Intervals
<b>CMR</b>	Capture Mark Re-capture
<b>ECG</b>	Electrocardiogram
<b>GAM</b>	Generalized Additive Model
<b>GLM</b>	Generalized Linear Model
<b>HR</b>	Heart Rate
<b>IQR</b>	Inter-quartile Range
<b>LME</b>	Linear Mixed Effects
<b>RTB</b>	Return to Baseline
<b>SD</b>	Standard Deviation
<b>SE</b>	Standard Error

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# 1 Introduction

Data collection by means of capturing and handling wild animals is a cornerstone in numerous conservation and management programs. Individual-level data, such as repeated reproductive and survival observations of known-aged individuals, is vital for investigating population dynamics (Clutton-Brock and Sheldon, 2010). Physiological and behavioral studies may also depend on recaptures to affix and later retrieve logger data stored in various devices (Wilmers *et al.*, 2015). Thus, to study wildlife biology, direct interaction is often essential. However, the handling of wild animals can lead to two distinct problems. First, it is possible for invasive methods to cause subjects to deviate from the norm, affecting the validity of results. For example, deployment of telemetry equipment could affect behavior (Dennis and Shah, 2012; Stabach *et al.*, 2020) and/or fitness traits (Putman, 1995), which could bias the key biological processes under study. Secondly, animal welfare can be compromised when individuals are subjected to invasive methods (Dennis and Shah, 2012; Jewell, 2013; Zemanova, 2019). Public opinion on invasive techniques in animal research also affect which methods can be employed in future studies (McMahon *et al.*, 2012; Cattet, 2013). As such, methods that might cause unnecessary harm to individuals require thorough review. Both for scientific accuracy and animal welfare concerns it is crucially important to document and understand the short and long-term effects researcher interaction with wildlife may have on study populations.

Direct interaction with wildlife can lead to scientific bias by altering the behavior or long-term fitness of study animals (Jewell, 2013). Researcher effects on subjects may therefore reduce the validity of assumptions drawn from smaller, handled sample populations as they may not accurately represent the larger, unhandled population. It is therefore important to explore if, and how, methodology causes the study population to differ from the control. Changes can range from minor to severe and from short-term to long-term, affecting the degree to which results are likely to be biased. Though some effects of capture and handling may be short-term their repercussions can be serious. A study by Ordiz *et al.* (2013) that found European brown bears (*Ursus arctos*) fitted with GPS collars reacted to mere human presence (~50m) by changing their daily rhythm and spending more time hiding than foraging for up to two days following capture. Studies on white-tailed deer (*Odocoileus virginianus*) have found significant post-capture changes to movement that led to biased results for up to 14 days following capture (Dechen Quinn *et al.*, 2012). Their recommendation was to discount captured individuals from analyses in this period and suggested other studies with marked animals thoroughly investigate potential effects that could bias results. Other capture and



handling events may have longer-term effects for wildlife. In addition to behavioral changes, researcher interaction can lead to fitness costs in studied individuals. Another study on bears found that repeated disruption of normal activities may accumulate to affect fitness traits. Cattet *et al.* (2008) found a long-term relationship between multiple captures and body condition of both grizzly bears (*U. arctos*) and American black bears (*U. americanus*), where individuals captured more frequently had poorer body condition than those captured only once. In severe cases, capture and handling may affect survival rates of studied individuals. Capture myopathy (a condition brought on by capture and handling with marked morbidity and mortality) is known to affect a wide range of wildlife species (Breed *et al.*, 2019), with death occurring up to 30 days succeeding capture (Montané *et al.*, 2002; Dechen Quinn *et al.*, 2014). Individuals suffering from capture myopathy are likely not behaving naturally and should be excluded to avoid bias (Dechen Quinn *et al.*, 2014).

Quantifying animal welfare is a challenging task as there is no universal consensus on the definition of, nor how to measure, animal welfare. It is generally agreed, however, that body-condition, mental welfare, and ability to behave naturally as specified for that species all play into good overall welfare status (Hewson, 2003). During capture and handling some individuals sustain injuries or may even be killed, incurring significant individual welfare costs. Data collection using external fixtures and internal surgical procedures also necessitate repeated interaction to remove and retrieve the technology, subjecting the same individual to potentially stressful or harmful handling. For example, one study's use of intraperitoneal radio transmitters in European brown bears were not biocompatible and led to internal injuries to studied individuals, with some cases resulting in death (Arnemo *et al.*, 2018). While studies that lead to substantial wildlife mortality are largely condemned as having too high welfare costs, as welfare costs become increasingly minor, the limit of acceptable interaction becomes harder to define. Individual welfare may therefore be compromised for short periods in pursuit of gathering information needed for management and conservation (Cattet, 2013). For instance, a study by Stabach *et al.* (2020) found short-term effects on scimitar-horned oryx (*Oryx dammah*), with increased stress and headshaking after fitting GPS collars, though as these effects dissipated within a few days the welfare concerns were considered negligible. Though many practitioners accept some short-term discomfort or distress for long-term gain, suitable methods to measure the welfare of an individual are less established. Attempts to quantify animal welfare have traditionally relied on physical measures such as body condition or overall health (Hewson, 2003). However, this approach does not capture the effects on welfare at finer temporal scales. Stress responses should therefore also be considered alongside body condition

to investigate short-term and cumulative effects. Heart rate (HR) is often used as a proxy for chronic stress responses, and correlated reductions in health and reproductive success (Tarlow and Blumstein, 2007). Similarly, activity levels can be used to remotely investigate behavioral stress responses, such as changes to time spent foraging (Arlettaz *et al.*, 2015). A handful of studies have found that individuals can have elevated HR and therefore energy expenditure beyond distances and times where they are visually disturbed (Ellenberg *et al.*, 2013; Ditmer *et al.*, 2015; Hammond *et al.*, 2019; Charuvi *et al.*, 2020). By extension, it is possible that the physiological metrics of handled animals need longer to return to baseline than the behavioral metrics, although this relationship lacks thorough study. While there is considerable uncertainty about the definition of animal welfare and how it should be measured, most scientists and practitioners would advocate that multiple effects should be monitored and that physiological traits are particularly relevant as they are directly related to elevated stress and may be more sensitive than behavioral metrics.

When negative stimuli are long-term or recurring, wild animals can tolerate, habituate, or sensitize to the disturbance. Habituation refers to reduced individual responses to repeated stimuli, while sensitization refers to increased individual responses to repeated stimuli (Reimers and Colman, 2006). Habituation can have both positive and negative outcomes for the habituated individual. Individuals that are habituated will likely experience less stress in the presence of humans than those that are not but might also be less wary of potentially dangerous situations (Bejder *et al.*, 2009). Instances where individuals habituate to, or are tolerant of, human activities might represent cases where animal welfare is not being compromised, while activities that lead to sensitization due to repeated interaction could point to potential welfare concerns.

Biologging and biotelemetry technologies present opportunities to study wild animals without human presence for extended periods, assuming wild animals can sustain, or return to, natural behavior and physiology after being instrumented with such technology. This is especially beneficial for studying elusive and shy species, as well as those that inhabit inaccessible regions. In recent decades biologging and biotelemetry technology have advanced rapidly, with smaller sensors, longer battery capacity, and increased range, allowing researchers to collect novel information about wildlife (Rutz and Hays, 2009; Wilmers *et al.*, 2015). Prior to this technology, ecological studies were limited to in-field data collection, where researcher presence could be biasing results. Furthermore, physiological studies were limited to captive and laboratory settings, where the nature of the situation (e.g., forced behaviors, limited movements) could bias results. At present some biologgers can collect data

on non-visible traits, such as running HR and body temperature from individuals. This physiological data, in combination with spatial and activity data from GPS technology, can aid researchers in understanding the temporal extent of capture and handling events.

The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) is endemic to the Svalbard archipelago, where it has no natural predators (Colman *et al.*, 2001), although a few incidents of polar bear predation have been reported, including within the study area (Derocher *et al.*, 2000). Svalbard reindeer exist in smaller groups (Reimers and Eftestøl, 2012) and have reduced vigilance in comparison with other subspecies (Reimers *et al.*, 2011), both likely due to lack of predation. To date there have been a handful of studies dedicated to investigating the effects of stress (Omsjø *et al.*, 2009) and potential habituation of Svalbard reindeer (Colman *et al.*, 2001; Reimers *et al.*, 2011). Reimers and Colman (2006) note that habituation to human activities in ungulates appears to be common, which agrees with studies on Svalbard reindeer habituation. Both Colman *et al.* (2001) and Reimers *et al.* (2011) found that Svalbard reindeer had lower flight initiation distances (the distance from humans at which individuals initiate flight responses) in areas with higher human activity than areas with few humans. Colman *et al.* (2001) also found no significant relationship with hunting and increased vigilance. The aforementioned habituation studies investigated the relationship with human presence and vigilance or avoidance in Svalbard reindeer, but did not study if behavioral changes occur in individuals that have been captured and handled.

The Svalbard reindeer Capture Mark Re-capture (CMR) project has been ongoing since 1995 and generates long-term, individual-level data (Albon *et al.*, 2017). The Svalbard reindeer CMR project is involved in studying Svalbard reindeer captures around April-May, with a census undertaken in July-August (Paulsen *et al.*, 2019). Reindeer are captured in a net held between two snowmobiles, whereby they are restrained and various data is collected, including body mass and pregnancy status (Omsjø *et al.*, 2009). The Svalbard reindeer CMR project is one of few animal studies that has access to continuous long-term individual level data. Continuous behavioral data (e.g., activity levels) has been collected since 2009 by use of GPS collars that transmit satellite data, and in the past 4 years physiological data (e.g., HR) has also been collected by internal biologgers that require retrieval. In 2017 and 2018, a subset of the marked population was subjected to multiple captures during each capture season. In particular, between March-April 2018, 36 animals were subjected to surgical procedures during which biologgers were implanted subcutaneously, and the animals were subsequently captured up to 3 times (i.e., up to 4 captures in total) as part of a study on energy expenditure (Trondrud *et al.*, 2021).

A previous study by Omsjø *et al.* (2009), used capture data from multiple years to investigate the effects of one capture per year on the reproduction and body condition of individuals within the same population used in this study. Though Omsjø *et al.* (2009) suggested capture time should be kept to a minimum to reduce short-term stress associated with capture, they found no evidence of long-term effects of capture and handling on Svalbard reindeer reproductive fitness. On the other hand, a recent, unpublished study found a significant effect of intensity of capture and handling on flight and comfort distances (the distance from humans at which individuals return to normal behavior) for Svalbard reindeer, where handled individuals had longer flight and comfort distances than unhandled individuals (Glesser, Unpublished data). Although Omsjø *et al.* (2009) found no long-term effects of one capture per season, the effects of repeated capture and handling are less understood and requires further study.

The aim of the present study was to assess the physiological, behavioral, and fitness effects of repeated captures on Svalbard reindeer. Specifically, the impact of repeated captures on the time taken to return to baseline (RTB) of HR and activity levels after capture, and subsequent body condition and reproductive success were investigated. Quantifying the potential effects of repeated handling is vital for evaluating if, and how, animal welfare may be compromised, and to determine if the results are affected by scientific bias. Based on the hypotheses that capture events cause behavioral and physiological perturbations from normal values, and that repeated capture events may cause fitness disadvantages and reproductive costs the following predictions were addressed in this thesis:

### **H1 – Repeated capture events cause perturbations to activity levels and heart rate**

1. Individuals are expected to sensitize to repeated captures, with incremental increases in the time taken for activity levels and heart rate to return to baseline values with each additional capture event.
2. Heart rate is expected to take longer to return to baseline values than activity levels (Charuvi *et al.*, 2020).

### **H2 – Repeated capture events incur fitness costs**

1. Individuals caught multiple times are predicted to have a higher body mass loss through the intensive capture period than individuals caught once.
2. One capture per year has no effect on reproduction (Omsjø *et al.*, 2009), however the increased degree of invasiveness (surgery followed by repeated captures) is predicted to be associated with lower calving probability.

## 2 Materials and Methods

### 2.1 Study Area

The data used in this thesis is based on a free-living population of Svalbard reindeer and was collected between March 2018 and March 2019 on Nordenskiöld Land, Svalbard (77°50'-78°20' N, 15°00'-17°30' E) (Figure 1). The 150 km<sup>2</sup> study area contains the Colesdalen and Semmeldalen valleys, as well as sections of Reindalen valley (Albon *et al.*, 2017). Nordenskiöld Land has the largest population of reindeer on Svalbard, with an estimated 9,455 ( $\pm$  268) individuals (Le Moullec *et al.*, 2019). The study area is covered by snow for the majority of the months October through mid-June (Loe *et al.*, 2007), although winter conditions can be highly variable between years. There is little human activity throughout the field area, especially during the summer months (Loe *et al.*, 2007), aside from limited hunting by residents from the middle of August to the end of September (Governor of Svalbard, 2021).



**Figure 1** Regional (top left) and local map showing the location of the study area, Nordenskiöld Land, Svalbard. The study area is dashed in red containing the Colesdalen, Semmeldalen, and Reindalen valleys. Map sourced from: Norwegian Polar Institute (2021)

## 2.2 *Study Species*

The Svalbard reindeer is known for having shorter ears, legs, and snout than their more temperate relatives (Vrolik, 1829). The Svalbard reindeer is the only large herbivore on the archipelago, and so is unaffected by intraspecies competition (Loe *et al.*, 2016). Out of an estimated population of 22,400 (Le Moullec *et al.*, 2019), around 200 are harvested annually (MOSJ, 2020). Hunting pressure is localized to a few hotspots around the main human settlement of Longyearbyen. In the absence of natural predators the majority of reindeer mortality on Svalbard is linked to starvation in late winter (Albon *et al.*, 2017) in interaction with high population density (Hansen *et al.*, 2019). Reindeer have multiple adaptations to living in cold climates, including thick pelts, large fat reserves, and selective brain cooling to name a few (Blix, 2016). Svalbard reindeer are non-territorial (Loe *et al.*, 2016), non-migratory (Tyler and Øritsland, 1989), and exist in small social groups (Reimers and Eftestøl, 2012). This is in contrast to reindeer found elsewhere that exist in sizable social groups and undergo large seasonal migrations (Tyler and Øritsland, 1989).

## 2.3 *Capture and Handling Methodology*

The individuals included in this study were captured at 10 months of age and marked with collars and ear-tags that have unique color and number combinations (Albon *et al.*, 2017). The Svalbard reindeer CMR project attempts to recapture marked individuals annually, but with a variable success rate. Adult females and adjourning calves are first observed with binoculars at distances up to 1 km, whereby marked individuals or groups are shepherded by 2 or 3 snowmobiles into areas where they can be caught. Following this, the capture snowmobiles speed up to close the gap. As the snowmobiles pass the focal animal, the two passengers drop a handheld net (7 x 5 meters; Figure 2). Once caught, the animals are removed from the net in the shortest time possible and restrained using ropes to tie their legs together.

Captured individual reindeer are weighed with a spring balance, and a blood sample is taken from the jugular vein. Body temperature is measured using a rectal thermometer twice per individual, first immediately succeeding capture and a second measurement just before release. In addition to these measurements the reproductive status of adult females is recorded using ultrasound checks. Reproductive success is determined by calf-at-heel, which is the presence or absence of a calf the following summer.

During the 2018 winter field season, a subset of the marked population were subject to repeated captures (up to 4 captures per individual) to study daily energy expenditure (Trondrud

*et al.*, 2021). A total of 36 females had HR- and temperature loggers (DST centi-HRT, Star-Oddi, Iceland) implanted subcutaneously during the first capture. Individuals were anesthetized during logger implantation, but not during subsequent captures. Activity and movement data were collected from GPS collars (Vectronic Aerospace GmbH, Berlin, Germany, ~750 g) fitted to the same 36 females. Of the 36 females, 7 of them were captured once, 9 were captured twice, 5 were captured three times, and 15 were captured four times in total. The average number of days between captures 1 and 2, 2 and 3, and 3 and 4 were 16, 2, and 14 days respectively. Individuals with both subcutaneous loggers and GPS collars were classified as focus animals. Known-age individuals marked with plastic collars, but no subcutaneous loggers, caught once were classified as control animals.



**Figure 2** Showing part of the capture process, where individuals are chased and captured in a hand-held net (7 x 5 meters) between two snowmobiles. Taken by Erik Ropstad, 2014.

#### **2.4** *Biologger Programming and Retrieval*

HR (in beats per minute) was recorded every 15 minutes by the HR- and temperature loggers. HR was recorded by converting 4 sec. electrocardiogram (ECG) signals. Quality assessment (0-3, where 0 is the highest quality) of each ECG signal was also recorded for each

HR reading. Only HR recordings with the highest level were used for analyses, and only HR recordings between 175 bpm and 20 bpm were kept, as reliability of recordings with HR above or below this was substantially decreased. The loggers were retrieved during the winter field season of 2019 for all individuals that could be re-captured. In total 25 of the 36 loggers were retrieved and included in the statistical analysis. HR calculations were visually validated using raw ECG signals taken every 6 hours.

Activity sensors inside the GPS unit of the collars recorded gravitational acceleration along back-forth (x-axis) and left-right (y-axis) axes. Each axis recorded the mean difference in acceleration over a 5-minute period of the x- and y-axes movements respectively, giving a value in the range of 0-255.

## *2.5 Data Handling*

All data handling was undertaken in R version 4.0.3 (R Core Team, 2020), using RStudio version 1.3.1093 for Mac (RStudio Team, 2020). In order to investigate the effects of repeated captures on Svalbard reindeer the following metrics were used: raw activity, percent activity, active HR, and combined HR (herein collectively referred to as metrics). In addition, calf-at-heel and capture-weight values were used to address predictions concerning reproductive success and body-condition. Raw activity was calculated as the sum of each 5-min record in the x- and y-axes from the GPS collars, giving a value in the range of 0-510. The raw activity data was then classified as inactive (0) or active (1), where an individual was considered to be inactive if the sum of the x- and y- axes was below 50. Percent activity was calculated by averaging the 0/1 values for 9-hour running mean windows. Active HR was filtered by the 0/1 values to exclude inactive individuals. Activity data was included as an explanatory variable for the combined HR values, which included both active and inactive HR values.

As body mass naturally declines during the winter season (Cuyler and Øritsland, 1993), mass loss data was prepped before analyzing the effects of captures on fitness by calculating actual daily mass loss (total mass loss divided by days between first and last capture) and by calculating the change in body mass over time using a linear mixed effects (LME) model. To determine if reproductive success was affected by changes in elevation following capture, individuals were defined as ‘elevation fleers’ if they gained more than 150 m following a capture event and remained at elevated heights for more than 5 days. All the individuals fitted with subcutaneous HR- and temperature loggers (focus animals) were between 5-8 years old. Only individuals with known pregnancy status were used to investigate reproductive success.



## 2.6 Statistical Analysis

All statistical analysis was undertaken in R version 4.0.3 (R Core Team, 2020), using RStudio version 1.3.1093 for Mac (RStudio Team, 2020). Significant results are defined as  $p < 0.05$ , while near-significant results are defined as  $p < 0.1$ .

### 2.6.1 Return to Baseline (RTB)

Return to baseline served as the response variable in subsequent statistical analyses, and all RTB values were quantified by Gabriel Pigeon, Université de Sherbrooke. RTB is an estimate of the time taken (in hours) for values of a given metric to no longer be significantly different ( $p > 0.05$ ) from baseline levels (Appendix 1). As activity and HR both change according to season (Arnold *et al.*, 2018), RTB was not defined as the return to pre-capture levels, but rather the return to a baseline that changes with time. The following methodology was used to estimate RTB for each individual metric and capture event (from first to fourth capture). Firstly, hourly moving averages were calculated with 9-hour windows for each metric across the whole period. The benefit of using moving averages instead of hourly computed averages was that it accounted for daily cycles in activity and HR values. A 9-hour window was chosen based on visual verification of cycle-length in activity and HR. The response variables were then fitted in separate generalized additive models (GAM) against time (date and time of day) using a thin plate regression spline (Wood, 2017), and an autoregressive function (AR1) was used to account for temporal autocorrelation. For the model using all HR data (combined HR), activity was added as a continuous explanatory variable to account for the effect of activity on HR. Predictions from the GAMs were used to quantify a baseline value with standard error (SE) for each response variable per individual over the entire period. The return to baseline times for each individual at each capture event were extracted in the following way: the deviation from the baseline (residuals of the model) were quantified as a function of time since capture (in hours) per capture event, with the same thin plate regression and autoregressive structure as the baseline models. By fitting the residuals to a new GAM, uncertainty (SE) in the predictions could be accounted for. For each prediction, a t-value was calculated by dividing the fitted residual ( $y$ ) by the square root of the sum of the variance of the residual and the baseline (Johnson, Kotz and Balakrishnan, 1995). P-values were then obtained from the Student t distribution with 1000 degrees of freedom. Finally, RTB was calculated as the difference between capture time and time at which the residual and baseline were no longer significantly different ( $p > 0.05$ ).

### 2.6.2 *RTB as a function of metrics and capture number*

To investigate causes of variation in time taken to return to baseline, LME models for each metric were constructed using the `lmer` function in the `lme4` package (Bates *et al.*, 2015). The first model ( $n = 25$ ) included captures 1 through 4, while the second model ( $n = 20$ ) discounted capture 1 to avoid confounding capture effects with surgery effects. Both of these models had capture number as the explanatory variable. In a third model return time was modeled with capture number and metrics as interactive explanatory variables. Individual identity was included as a random effect in all three models to account for individual differences and repeated observations. 95% confidence intervals (CI) were calculated for both models using the `bootMer` function from the `lme4` package (Bates *et al.*, 2015), by bootstrapping the model 1000 times.

### 2.6.3 *Effect of repeated capture on body-condition*

To investigate the effect of repeated captures on body-condition an LME model was used to determine the background mass loss trend (grams lost per day) by modeling weight observations with Julian day and capture count as interactive explanatory variables, again using the `lmer` function in the `lme4` package (Bates *et al.*, 2015). Capture count was grouped into two categories by captures = 1 and captures  $\geq 2$ . Individuals caught only once ( $n = 21$ ) included both focus and control animals, while individuals caught multiple times ( $n = 29$ ) were limited to focus animals. Individual identity was included as a random effect in this model.

### 2.6.4 *Effect of repeated capture on reproductive success*

In order to test the effects of repeated captures on reproductive success three generalized linear models (GLM) were constructed using the `glm` function from the base package (R Core Team, 2020). Reproductive success was determined by calf-at-heel (calf = 1, no calf = 0), and each model was fitted with a binomial distribution. Reproductive success was modeled with capture number ( $n_1 = 1, n_2 = 4, n_3 = 4, n_4 = 7$ ), daily mass loss ( $n = 16$ ), and elevation gain post capture ( $n_{flee} = 7, n_{stay} = 28$ ) as explanatory variables in the three different models (to avoid overparameterization due to small sample size). Only focus animals were used in these models. An unpaired t-test was also used to examine the difference in daily mass loss of individuals without a calf-at-heel ( $n = 3$ ) and individuals with a calf-at-heel ( $n = 13$ ), where all individuals had been confirmed pregnant in March.

### 3 Results

#### 3.1 Behavioral and physiological responses to repeated capture events

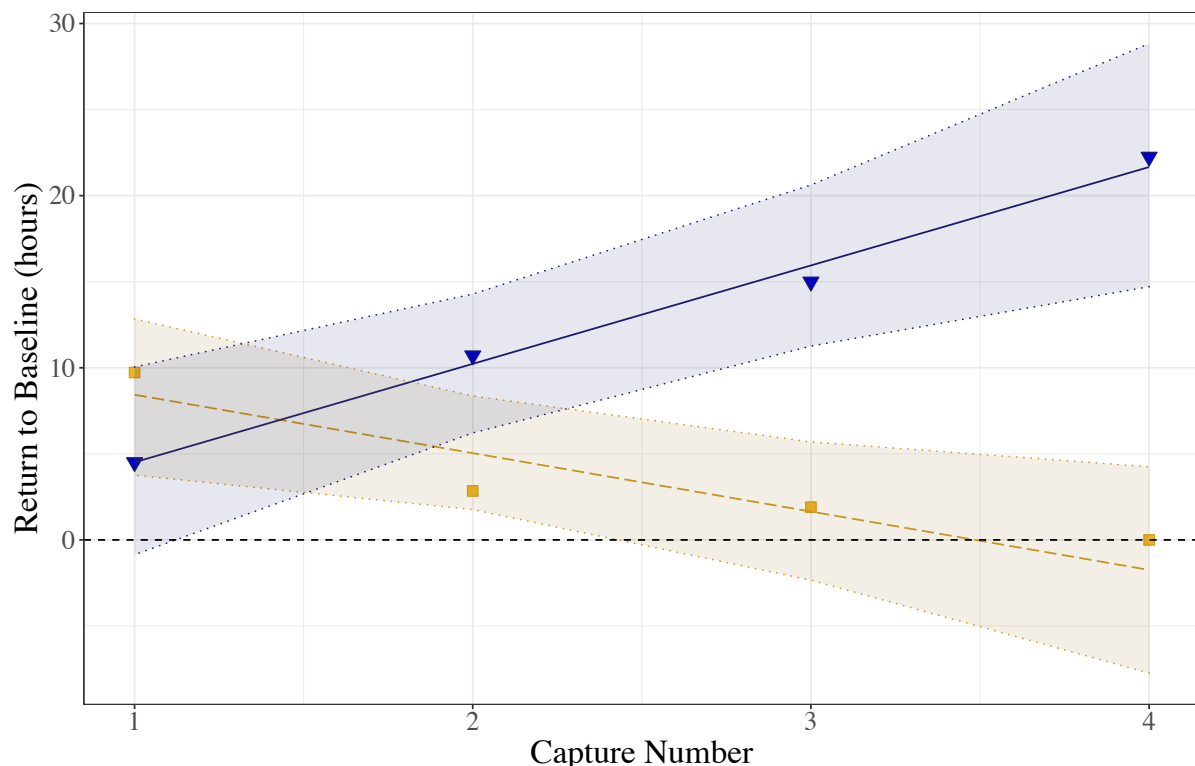
The interaction between capture sequence and metric type (HR and activity level) was significant (Table 1; Figure 3), irrespective of inclusion of the first capture event (Appendix 2; Appendix 5). The HR results show support for hypothesis H1.1, with incremental increases in the time taken to return to the expected HR baseline with each additional capture. The opposite was true for activity level results, where the time taken to return to the expected activity baseline was reduced with each additional capture, which is in line with hypothesis H1.2. Activity levels fell below the baseline immediately after the first capture (involving sedation), meaning individuals were moving less following capture one. Conversely, individuals were more active after the second, third, and fourth captures. On average, individuals had HR values that were higher than the expected baseline immediately after capture for all four captures.

Individual reindeer had activity levels after the first capture that were significantly different from the expected baseline activity levels (Table 2), and raw activity levels had a significant negative relationship with number of captures. Activity levels took on average 9.7 (standard deviation in Appendix 3) hours after the first capture to return to the expected baseline when sedation was involved, though this average was based on 6 of 25 individuals that deviated from the baseline. The average return time for activity levels at the second capture was 2.8 hours, with 12 of 20 individuals deviating from the baseline. By the third and fourth captures activity levels no longer significantly deviated from the expected baseline, and only 1 of 15, and none of the 13 individuals caught 3 and 4 times respectively deviated from the baseline. Although activity levels took less time to return to the expected activity baseline with each additional capture, 7 individuals showed clear responses to capture in their movement patterns by fleeing to higher elevation (visually assessed in Appendix 4). Seven individuals all moved to higher elevations (between 150-200 m increases) following their second captures and remained at higher elevations for several days.

HR values were not significantly different from the expected baseline HR levels at first capture, and only 5 of 21 individuals deviated from the baseline. By the second, third, and fourth captures HR values were significantly different from the expected baseline and took on average 10.7, 15.0, and 22.3 hours to return to the expected HR baseline for each additional capture (Appendix 6), with 12 out of 18, 6 out of 15, and 11 out of 13 individuals captured 2, 3, and 4 times respectively deviating from the baseline.

**Table 1** Estimated return to baseline times (hours) based on an individual linear mixed effects model. Return time is modeled against capture number in interaction with model (raw activity and combined heart rate, HR). Presented are predictor variables of the model with parameter estimates, 95% confidence intervals and  $p$ -value for each estimate. Marginal  $R^2$  represents the variation explained by the fixed effects and conditional  $R^2$  represents the total variation explained by the model (fixed + random effects). Significant values ( $p < 0.05$ ) are highlighted in bold.

<b>Return to Baseline Time</b>			
<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Activity	11.87	4.63 – 19.10	<b>0.001</b>
Capture Number	-3.45	-6.32 – -0.58	<b>0.018</b>
Heart Rate	-13.17	-23.47 – -2.87	<b>0.012</b>
HR * Capture Number	9.04	4.96 – 13.13	<b>&lt;0.001</b>
$N_{id}$	25		
Observations	140		
Marginal $R^2$ / Conditional $R^2$	0.163 / 0.242		



**Figure 3** The predicted relationship between the time taken to return to baseline activity levels (dashed yellow line) and heart rate values (solid blue line), and the capture sequence for 25 female Svalbard reindeer. Shaded 95% confidence intervals are included for both models. Average return times (from raw data) are included for activity levels (yellow squares) and heart rate (inverted blue triangles).

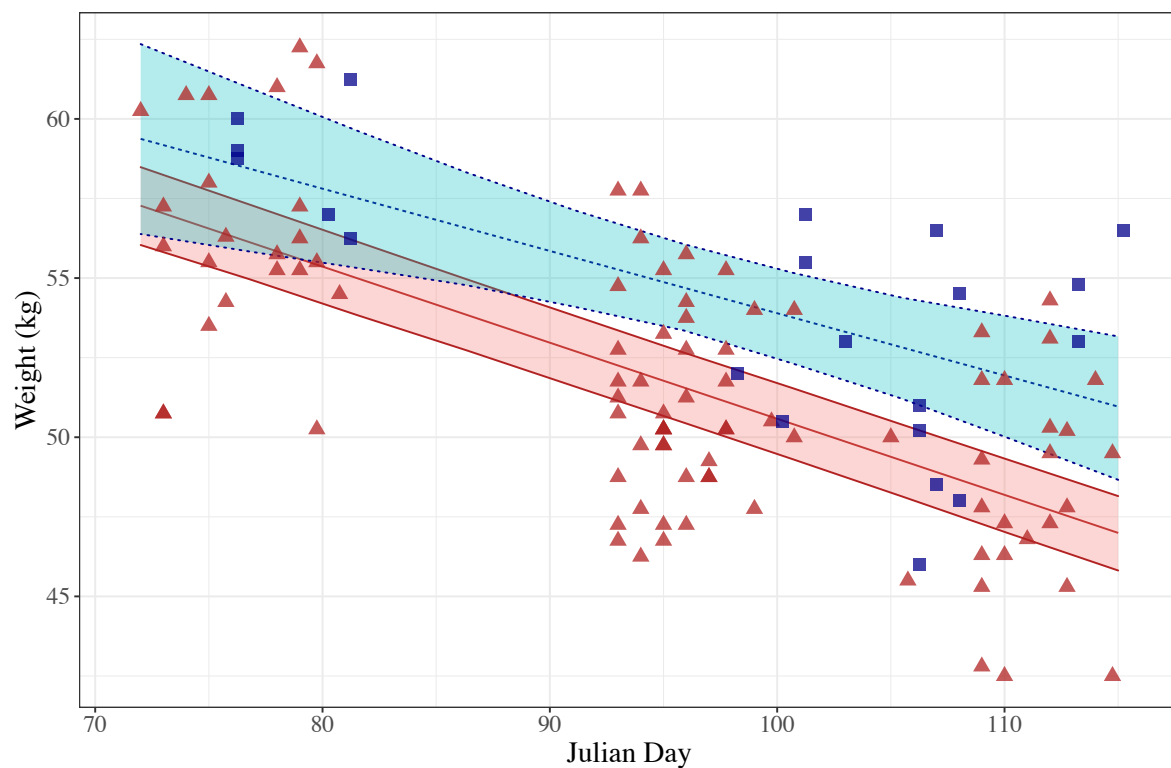
**Table 2** Estimated return to baseline times (hours) for the four different metrics (raw activity, percent activity, active heart rate, and combined heart rate) based on individual linear mixed effects models. The value for the intercept indicates how many hours each metric remained perturbed and the *p*-value indicates if the perturbation was significant at the first capture. Capture number indicates how much (in hours), and in which direction, each metric changed with each additional capture. The capture number *p*-value indicates if the relationship between each metric and capture sequence was significant. Marginal R<sup>2</sup> represents the variation explained by the fixed effects and conditional R<sup>2</sup> represents the total variation explained by the model (fixed + random effects). Significant values (*p* < 0.05) are highlighted in bold.

<i>Predictors</i>	<b>Raw Activity</b>			<b>Percent Activity</b>			<b>Active HR</b>			<b>Combined HR</b>		
	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Intercept	11.83	5.17 – 18.49	<b>0.001</b>	9.70	2.78 – 16.63	<b>0.006</b>	7.68	-4.31 – 19.66	0.209	-1.21	-9.70 – 7.29	0.781
Capture Number	-3.39	-6.05 – -0.74	<b>0.012</b>	-2.12	-4.90 – 0.66	0.135	4.36	-0.26 – 8.98	0.065	5.72	2.41 – 9.03	<b>0.001</b>
N	25 <sub>id</sub>			25 <sub>id</sub>			22 <sub>id</sub>			22 <sub>id</sub>		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.076 / 0.147			0.030 / 0.062			0.052 / NA			0.145 / 0.171		

## 3.2 Body-Condition and Reproductive Success

### 3.2.1 Body-Condition

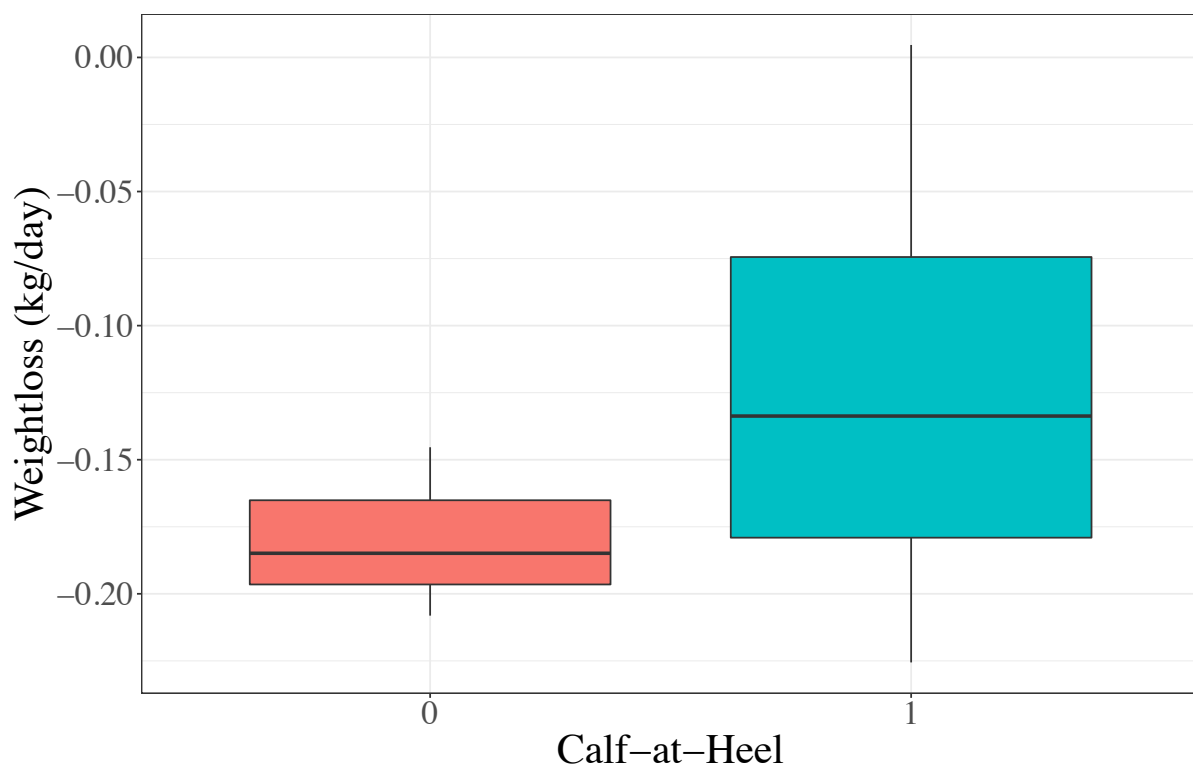
The mean weight at first capture for all individuals across the capture period was  $54.3 \pm 4.2$  kg. The background mass loss over time based on weight of individuals captured only once was 196 grams per day (SE: 54g;  $p < 0.01$ ). Comparatively, individuals captured multiple times were found to lose 239 grams per day (SE: 11g;  $p < 0.0001$ ) over the same time period. Although a biologically large difference, hypothesis H2.1 was not supported statistically as there was no significant difference found between the mass loss trend of individuals caught multiple times compared with the background mass loss during the capture period (Figure 4).



**Figure 4** Predicted daily mass loss trend for individuals captured multiple times (red and solid; 239 g/day;  $n = 29$ ) plotted against the background mass loss trend for individuals captured once (blue and dashed; 196 g/day;  $n = 21$ ). Daily mass loss for the two groups was modeled using a linear mixed effects model with time (Julian day) with group (caught once or multiple times) used as the explanatory variable. The standard deviation of the random intercept, capturing individual variation, was 3.1 kg. Shaded areas represent the 95% confidence intervals. Raw weight data for each capture was included as extra point information, with individuals caught once represented with blue squares, and individuals caught multiple times represented with red triangles.

### 3.2.2 Reproductive Success

Thirty-three of the 36 focus animals had known pregnancy status, of which 28 were pregnant and 5 (15.2%) were not. Of the 28 pregnant focus individuals, 16 were re-sighted the following summer. Of these 16 individuals 1 had been caught once, 4 had been caught twice, 4 had been caught three times, and 7 had been caught four times. Three (18.8%) of the 16 re-sighted individuals were without a calf at heel, and all had been captured a total of four times each. Still, there was a no significant relationship ( $p = 0.9$ ) between the probability of calf-at-heel and capture count, despite 43% of individuals caught 4 times losing a calf. There was a near-significant trend between daily mass loss and loss of calf ( $p = 0.084$ ; Figure 5), where the three pregnant individuals observed without a calf-at-heel the following summer had a higher daily mass loss than pregnant individuals observed with a calf-at-heel. Hypothesis H2.2 was therefore inconclusive due to limited sample size.



**Figure 5** The daily mass loss (kg/day) for individuals known to be pregnant that were either observed without a calf-at-heel (red;  $n = 3$ ), or with a calf-at-heel (green;  $n = 13$ ). Median values for each group are represented with a bold line through the box. The inter-quartile range (the 25<sup>th</sup> and 75<sup>th</sup> percentiles; IQR) is represented by the colored boxes, with the whiskers showing the range of the data.

## 4 Discussion

Studies involving wild animals require methodology reviews to avoid scientific bias and to ensure that the individual well-being of study animals is not being compromised. The findings of the present study provide an insight into the effects of repeated captures on Svalbard reindeer and their implications. This study found a mismatch between activity levels and heart rate responses to repeated captures, where individuals are behaviorally tolerant but sensitize physiologically with increasing number of captures. There was a non-significant, though biologically important effect of repeated captures on body mass loss and reproductive success of the individuals involved, but sample sizes were too small for firm conclusions. This study cautions against undertaking studies with a similar degree of invasiveness, both from an animal welfare and scientific bias point of view. In particular, fitness metrics from intensively handled individuals may be lower than the population mean and may therefore not represent the total population.

### 4.1 *Differential behavioral and physiological responses to repeated captures*

In this study, activity levels and HR responses of Svalbard reindeer differed with repeated captures. For every capture, animals took less time to return to baseline activity levels but more time to return to baseline HR. These findings lend partial support for the hypothesis that repeated capture events cause perturbations to activity levels and heart rate. This indicates the Svalbard reindeer became less physiologically tolerant of repeated handling events in 2018 despite appearing behaviorally undisturbed.

#### 4.1.1 *Behavioral tolerance*

Previous studies on the behavioral responses of Svalbard reindeer to human activities have concluded that reindeer habituate to humans by comparing areas with varying amounts of human disturbance (Colman *et al.*, 2001; Reimers *et al.*, 2011). Reimers *et al.* (2011) found reindeer in Colesdalen and Reindalen (both within this study area) had lower vigilance, but longer alert, flight, and escape distances than areas with more frequent human interaction. They suggested this difference may in part be due to the live capturing undertaken by the Svalbard reindeer CMR project. In the present study, however, repeatedly handled individuals appeared behaviorally tolerant, and many individuals did not deviate from expected activity baseline at all. Despite this, a few individuals fled to higher elevations following capture. Evasive behavior in response to captures have been observed in several species, including roe deer (*Capreolus*



*capreolus*; Morellet *et al.*, 2009) and pygmy rabbits (*Brachylagus idahoensis*; Rachlow *et al.*, 2014). In both these studies the focal animals were observed to avoid capture areas for several weeks. Svalbard reindeer have very low activity levels during the winter compared with other reindeer subspecies, which is a survival strategy to conserve energy (Cuyler and Øritsland, 1993). For the Svalbard reindeer behavioral tolerance to repeated capture and handling could therefore be a response to mitigate the costs associated with traversing difficult terrain or expending unnecessary energy in a snow-covered landscape with high cost of locomotion (Parker *et al.*, 1984). In the summer months behavioral tolerance of human activities has been theorized to be a way of maximizing the time spent on building up energy reserves needed to survive the winter (Colman *et al.*, 2001).

Habituation and behavioral tolerance are sometimes portrayed as a positive outcome for wildlife (Bejder *et al.*, 2009), however habituation may incur significant costs for a number of different species (Geffroy *et al.*, 2015; Charuvi *et al.*, 2020). Most notably, habituation can lead to increased risk of sublethal and lethal encounters if individuals habituate to unpredictable predators (Frid and Dill, 2002). This cost of habituation is less likely to adversely affect Svalbard reindeer, as they lack natural predators and are subject to low hunting pressure (Colman *et al.*, 2001). While the individuals in this study are not significantly altering their activity levels in response to repeated captures, the behavioral data available only describes how much individuals are moving and does not capture changes to fine-scale behaviors, such as vigilance. As such, it is possible individuals are remaining more vigilant, consuming less, as well as maintaining higher HR levels following capture.

#### 4.1.2 *Physiological sensitization*

This is the first study to investigate physiological responses to repeated handling of Svalbard reindeer. Although few individuals deviated from baseline activity values after each capture, the majority of individuals displayed an increase in return times to baseline HR values with each capture event. Many individuals had higher than expected HR for almost a full day after the fourth capture. This indicates that individuals remember negative handling experiences, which translates to elevated stress associated with repeated captures. While there are few studies that have similar methodologies, a study on impala (*Aepyceros melampus melampus*) used blood cortisol values and found individuals caught multiple times had a learnt stress response to approaching humans (Hattingh *et al.*, 1988). Responses to repeated capture and handling, such as increased time taken to return to baseline HR values and elevated blood

cortisol values, reveal physiological distress and can lead to significant lethal and non-lethal costs for affected individuals (Ellenberg *et al.*, 2013).

Though it was predicted that both activity levels and HR would take incrementally longer per capture to return to baseline values, mismatches between behavioral and physiological responses are not uncommon. A handful of other studies have found similar mismatches, where wildlife behavioral responses do not convey corresponding stress-responses to disturbance (Ellenberg *et al.*, 2013; Ditmer *et al.*, 2015; Hammond *et al.*, 2019; Charuvi *et al.*, 2020). HR and other stress-responses have been found to be elevated despite little or no change to behavioral responses in yellow-eyed penguins (*Megadyptes antipodes*; Ellenberg *et al.*, 2013), American black bears (Ditmer *et al.*, 2015), California ground squirrels (*Otospermophilus beecheyi*; Hammond *et al.*, 2019), and masked lapwings (*Vanellus miles*; Charuvi *et al.*, 2020). Reduced or absent behavioral responses coupled with significant physiological responses may be due to prior experience, differences in the costs or benefits associated with reacting behaviorally, or simply that the study animal has cryptic or lacks behavioral responses (Charuvi *et al.*, 2020). In the case of the Svalbard reindeer behavioral habituation and tolerance to repeated captures may come at the expense of longer RTB times for physiological responses, as an evasive response might have mitigated the stress of remaining in the capture area (Morellet *et al.*, 2009; Rachlow *et al.*, 2014; Charuvi *et al.*, 2020).

#### **4.2** *Tendencies for higher body mass loss and lower calving rates for repeatedly captured individuals*

Repeated handling has been shown to affect individual fitness, including reduced body condition (Suazo *et al.*, 2005; Cattet *et al.*, 2008) and reproductive success (Ellenberg *et al.*, 2013; Mortensen and Rosell, 2020). Cattet *et al.* (2008) found lasting effects on body condition of both grizzly bears and American black bears that were directly proportional to the number of times bears were caught. Similarly, repeated captures have been found to lead to reductions in body mass for two subspecies of beach mice (*Peromyscus polionotus phasma* and *P. p. niveiventris*; Suazo *et al.*, 2005) and Eurasian beavers (*Castor fiber*; Mortensen and Rosell, 2020). The present study found no statistically significant effect of repeated captures on body mass, despite there being a biologically large difference between individuals caught once and individuals caught multiple times. Individuals caught multiple times were found to lose 22% more mass per day compared to the baseline trend. As individuals caught multiple times no longer deviated from baseline activity levels by the third and fourth captures this elevated mass

loss might be linked to extra energy-consumption related to elevated HR (Cuyler and Øritsland, 1993), or, for some individuals that they had moved to high-elevation areas with less accessible forage. As body mass was not recorded when researchers returned the following summer it is unknown if there were any long-term effects of this elevated mass loss.

The reproductive success of Svalbard reindeer is strongly associated with April body mass (Albon *et al.*, 2017), as such the elevated daily mass loss of individuals captured multiple times could be cause for concern. Although capture events and human disturbance have been linked to lower reproductive success in different species (Ellenberg *et al.*, 2013; Mortensen and Rosell, 2020), Omsjø *et al.* (2009) found no effect of a single capture event per year on reproductive success of Svalbard reindeer. This study provides support for the findings in Omsjø *et al.* (2009), as no significant relationships between reproductive success and repeated capture were uncovered. However, examining the effects of repeated captures on the reproductive success of individuals was limited by the number of re-sighted pregnant individuals the following summer, as only 48% of the pregnant focus individuals were re-sighted. It is worth noting that all the individuals that had lost their fetus/calf between April and August had been captured 4 times each. Those three individuals also had higher daily mass losses than individuals who kept their calves. Despite the indication that repeated captures were affecting reproductive success firm conclusions could not be made due to the small sample sizes. These indications are supported by other studies examining reproductive success of species with high anthropogenic disturbances. In yellow-eyed penguins capture and handling elicited the strongest stress-responses, compared with approaches and prolonged presence, and areas with frequent human disturbance have significantly lower breeding success (Ellenberg *et al.*, 2013). Additionally, Mortensen and Rosell (2020) found long-term effects of repeated captures on reproductive success in Eurasian beavers, where individuals caught over multiple years had smaller litters. Non-lethal effects of capture and handling, such as reduced body condition and reproductive success, should be of concern as cumulative individual responses could be influencing population-level processes.

### **4.3 Scientific Bias and Animal Welfare**

There is considerable overlap between animal welfare concerns and scientific bias; if enough individuals suffer the same welfare issue the result could translate to population-level changes (Cattet, 2013). As such, it is important to investigate potential animal welfare concerns with scientific bias in mind, and vice versa.

#### 4.3.1 Potential for Scientific Bias

Repeated captures have been shown to affect a number of different behavioral, physiological, and fitness traits. Cumulations of these effects can lead to reduced validity of data gathered. A study by Camacho *et al.* (2017) found individual pied flycatchers (*Ficedula hypoleuca*) caught over multiple years became more resistant to trapping with age. They suggested learned negative associations with prior trapping were driving experienced individuals to evade capture, biasing data collected by age. Reimers *et al.* (2011) found the population used in this study had longer alert, flight, and escape distances than elsewhere on the archipelago, which was suggested to be due to researcher interaction. This, coupled with HR sensitization to repeated captures found in this study, suggests learned stress could be affecting the capture probability of handled Svalbard reindeer. Differences in capture methodology between studies may help avoid bias related to capture probability, but studies that repeatedly expose the same individuals to stressful experiences should be wary of potential bias.

This study found individuals were less active than expected after the first capture, which involved sedation. However, the subsequent captures all had higher activity levels and HRs than expected. When the first capture was discounted the significant effects of repeated capture became near significant effects ( $p < 0.1$ ), though the interaction between activity levels and HR remained significant. This may have been due to prolonged effects of sedation despite being given antidotes (Montané *et al.*, 2003). It is unclear if this difference is related to the effects of sedation or if it is simply a consequence of reducing the number of captures.

While this study didn't find a significant effect of repeated captures on fitness nor reproduction, there was a biologically significant effect on daily mass loss. The small sample size of this study reduces the potential for having caused population-level effects, but it also makes it difficult to determine the extent to which the methodology in this study could lead to scientific bias. The Svalbard reindeer CMR project, or any study with similar methodology, should therefore caution against conclusions drawn from repeatedly handled parts of the population. Because few studies have both behavioral and physiological data, researchers risk falsely concluding that human activities have little effect on study animals when using one response in isolation (Charuvi *et al.*, 2020). This potential issue can be circumvented by increasing the number of metrics investigated, though this generally involves more extensive researcher interaction, which can lead to increased stress and affect individual welfare.

#### 4.3.2 *Costs to Individual Welfare*

Individual capture events can present welfare challenges, though if these are short-term, minor, or affect a small portion of the sample population they are largely overlooked in favor of gathering important information (Cattet, 2013). This study found that by the 4<sup>th</sup> capture individual Svalbard reindeer were taking nearly a full day to return to expected baseline HR values. While this is categorized as a short-term effect, there is a substantial welfare cost for the individuals involved of being stressed for this period of time (Moberg, 2000). Fortunately the data used in this study was collected for a specific purpose (Trondrud *et al.*, 2021), and it is unlikely the Svalbard reindeer CMR project will carry out such a rigorous capture regime again. In the case of this study, without the subcutaneous loggers, the escalating effect on HR would have remained undetected. The use of bio-logging can thus give an insight into cryptic responses and inform researchers about the full effect they have on their subjects. Studies that report behavioral tolerance or habituation to researcher interaction, and therefore declare no welfare conflicts, might be overlooking physiological welfare issues.

## **5 Conclusion**

Capture and handling are important methods in animal studies, but may lead to behavioral, physiological, and/or fitness consequences for focus animals. The present study is one of few with access to long-term individual data on behavior, physiology, body-condition, and reproductive success. It was therefore well placed to investigate how repeated captures affect these different parameters in a social ungulate. This study demonstrated the dichotomy between behavior and physiology, where Svalbard reindeer showed signs of behavioral tolerance, but physiological sensitization to repeated capture and handling. Individuals caught multiple times had biologically important increases in daily mass loss, although small sample sizes limited conclusions about the full effects of repeated capture on Svalbard reindeer fitness. The findings of this study complement a growing body of literature that suggest behavior alone may not be an adequate way of measuring disturbance. This further emphasizes the importance of including physiological responses to understand the full impacts of researcher interaction on wildlife. As human-wildlife interactions are increasing globally, such findings are important to conserve and manage wild populations.

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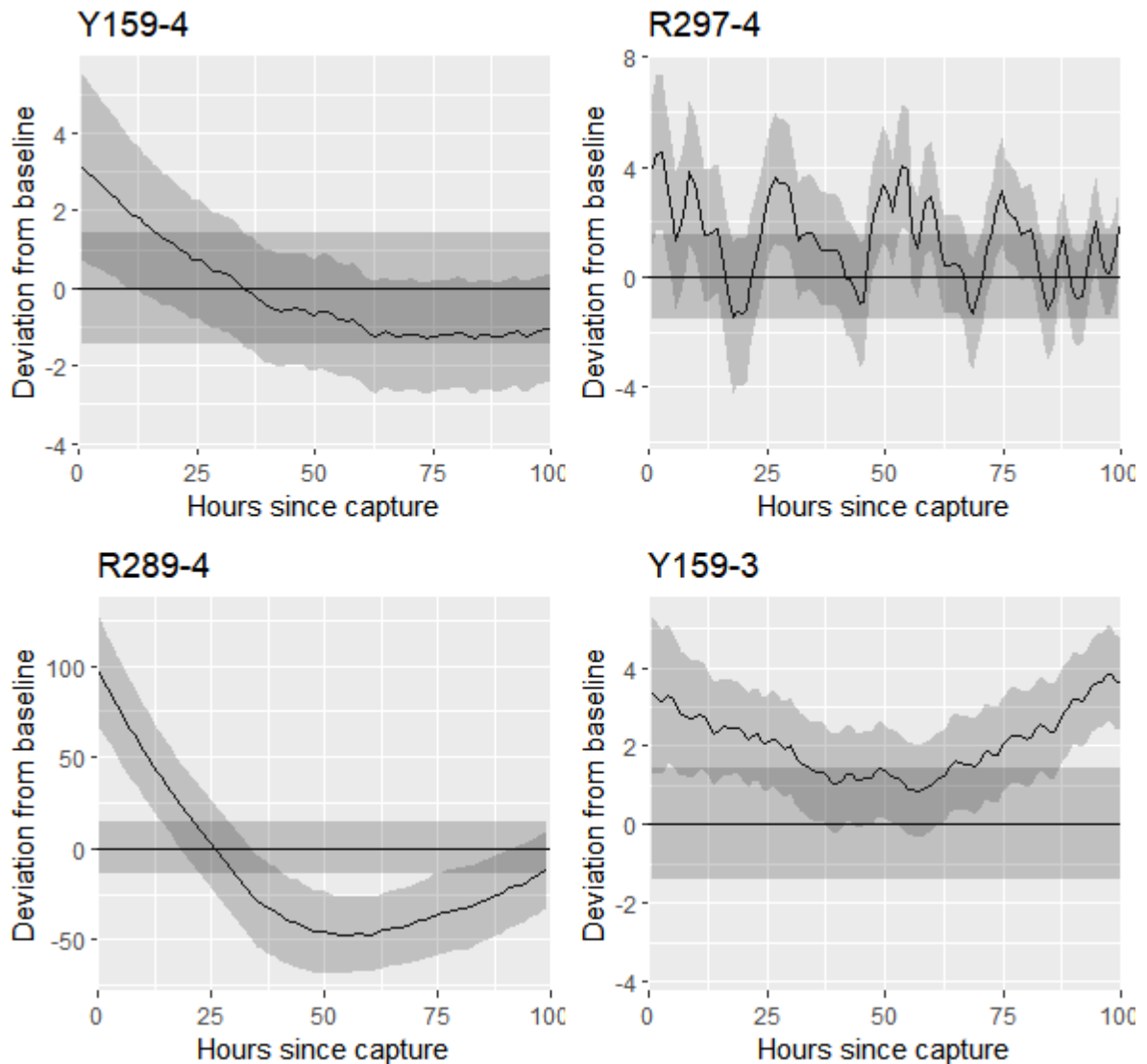
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## 7 Appendices



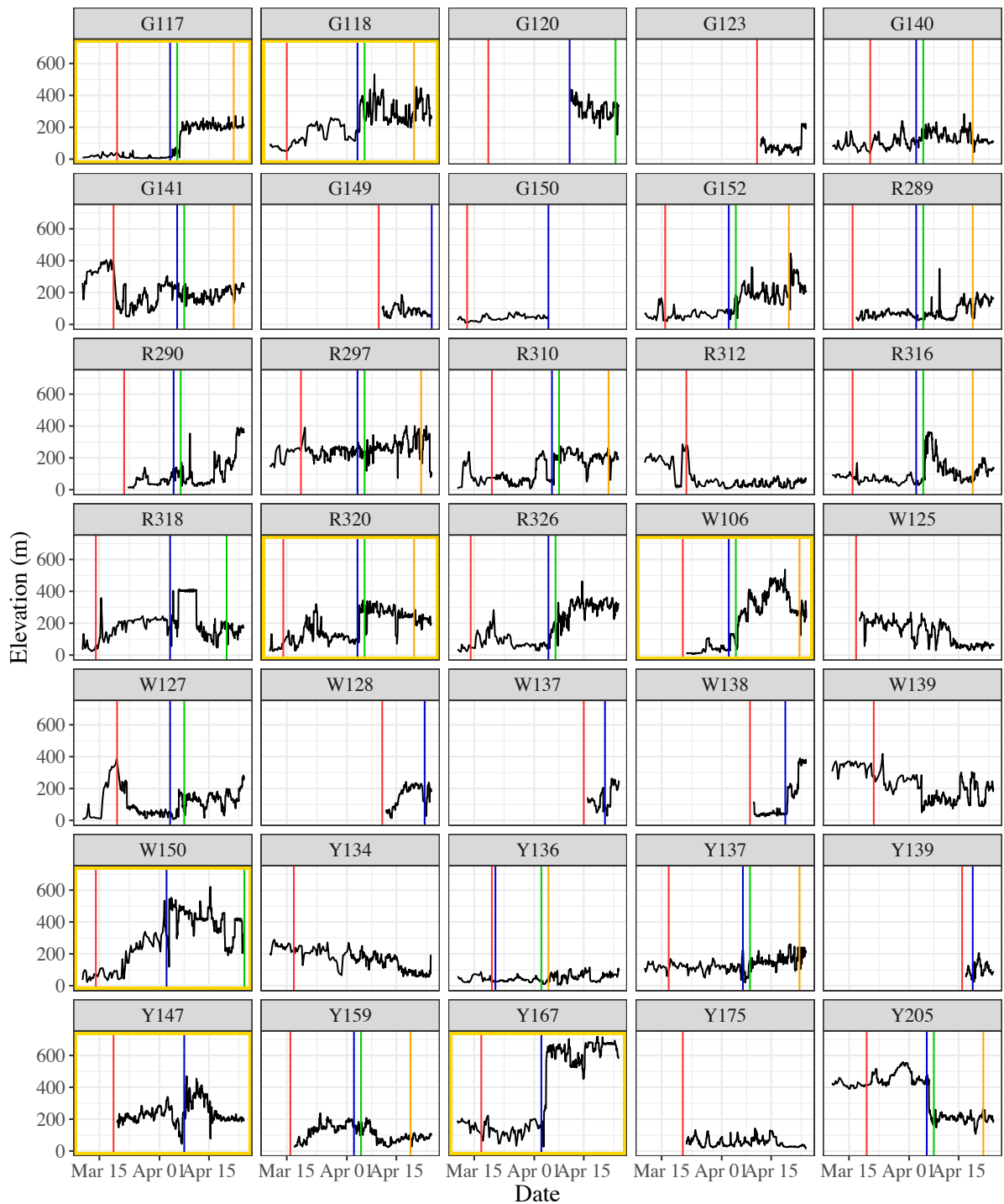
**Appendix 1** Examples of return to baseline predictions using combined heart rate (HR). Predicted deviation from baseline of combined HR (in beats per minute) is plotted against hours since capture. This figure shows a random selection of four individual- and capture combinations (ID-X represents individual and capture number in title). As a number of captures occurred within short timeframes (less than two days apart) some plots only show up to 40 hours since capture.

**Appendix 2** Estimated return to baseline times (hours) based on an interactive individual linear mixed effects model, with the first capture removed for effects of sedation. Return time is modeled against capture number in interaction with model (raw activity and combined heart rate). Presented are predictor variables of the model with parameter estimates, 95% confidence intervals (CI) and *p*-value for each estimate. Marginal R<sup>2</sup> represents the variation explained by the fixed effects and conditional R<sup>2</sup> represents the total variation explained by the model (fixed + random effects). Significant values (*p* < 0.05) are highlighted in bold.

<b>Return to Baseline Time</b>			
<i>Predictors</i>	<i>Estimates</i>	<i>CI</i>	<i>p</i>
Activity	5.89	-6.91 – 18.69	0.367
Capture Number	-1.46	-5.76 – 2.84	0.506
Heart Rate	-6.87	-25.16 – 11.42	0.461
HR * Capture Number	7.09	0.97 – 13.21	<b>0.023</b>
N <sub>id</sub>	20		
Observations	94		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.262 / 0.310		

**Appendix 3** The average time (hours) taken to return to the estimated baseline per capture for raw activity levels and combined heart rate, calculated with raw data. SD indicates the standard deviation from the mean.

Cap. No.	<b>Activity</b>		<b>Heart Rate</b>	
	Mean	SD	Mean	SD
1	9.7	21.4	4.5	9.8
2	2.8	3.5	10.7	11.9
3	1.9	7.4	15.0	19.6
4	0.0	0.0	22.3	22.0



**Appendix 4** Elevation profiles (meters) for individuals are illustrated with a black line and the date of each capture event with colored vertical lines. Capture one is red, capture two is blue, capture three is green, and capture four is orange. The seven individuals that fled to higher elevation (more than 150m for more than 5 days) following one of their captures are highlighted with a yellow box.

**Appendix 5** Estimated return to baseline times (hours) for the four different metrics (raw activity, percent activity, active heart rate, and combined heart rate) based on individual linear mixed effects models, with the first capture removed for effects of sedation. The value for the intercept indicates how many hours each metric remained perturbed and the *p*-value indicates if the perturbation was significant at the first capture. Capture number indicates how much (in hours), and in which direction, each metric changed with each additional capture. The capture number *p*-value indicates if the relationship between each metric and capture sequence was significant. Marginal R<sup>2</sup> represents the variation explained by the fixed effects and conditional R<sup>2</sup> represents the total variation explained by the model (fixed + random effects). Significant values (*p* < 0.05) are highlighted in bold.

Predictors	Raw Activity			Percent Activity			Active HR			Combined HR		
	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>	Estimates	CI	<i>p</i>
Intercept	5.75	0.97 – 10.52	<b>0.018</b>	11.15	0.67 – 21.63	<b>0.037</b>	-1.09	-24.92 – 22.74	0.928	-1.02	-19.30 – 17.25	0.913
Capture Number	-1.39	-3.00 – 0.22	0.091	-2.53	-6.06 – 1.00	0.160	7.03	-0.76 – 14.81	0.077	5.67	-0.40 – 11.73	0.067
N	20 <sub>id</sub>			20 <sub>id</sub>			18 <sub>id</sub>			18 <sub>id</sub>		
Marginal R <sup>2</sup> / Conditional R <sup>2</sup>	0.057 / NA			0.040 / NA			0.069 / NA			0.065 / 0.132		

**Appendix 6** The predicted time required to return to baseline values for the four different metrics (active heart rate, combined heart rate, percent activity, and raw activity). Prediction indicates how many hours each metric used to return to the baseline for each capture number, with 95% confidence intervals included.

Cap. No.	Active HR			Combined HR			Percent Activity			Raw Activity		
	Prediction	2.5%	97.5%	Prediction	2.5%	97.5%	Prediction	2.5%	97.5%	Prediction	2.5%	97.5%
1	12.04	4.16	20.24	4.51	-0.86	10.05	7.58	3.39	12.27	8.43	3.76	12.84
2	16.39	11.03	21.65	10.23	6.21	14.28	5.46	2.49	8.73	5.04	1.76	8.36
3	20.75	14.69	26.81	15.95	11.26	20.62	3.33	-0.35	7.26	1.65	-2.34	5.69
4	25.11	15.96	34.23	21.66	14.71	28.83	1.21	-4.75	7.19	-1.74	-7.74	4.25





**Norges miljø- og biovitenskapelige universitet**  
Noregs miljø- og biovitenskapelige universitet  
Norwegian University of Life Sciences

Postboks 5003  
NO-1432 Ås  
Norway