WHY ARE YOU NOT BREEDING?
A STUDY ON THE REPRODUCTIVE SUCCESS
OF CAPTIVE WOLVERINES (*Gulo g. gulo*)

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ABSTRACT

Conservation biologists have long faced the very challenging task of large carnivore conservation. Both their hunting habits and their very specific ecology make their conservation particularly difficult. Wolverines (*Gulo g. gulo*) are a good example of this. The population of wolverines is close to extinction due to human persecution and habitat loss. The European Association of Zoos and Aquaria (EAZA) introduced the European Endangered Species Program (EEP) for the Wolverine in 1994 but it experiences some irregular breeding. The aim of this study was to identify factors that could be connected to breeding success in these captive wolverines regarding the characteristics of the enclosures, the wolverine biology, the characteristics of the institutions and the influences of the human-animal interactions. To this end, an online survey was developed and sent to all holders of wolverines included in the EEP program. Overall, no main factor or group of factors investigated in this study seemed to be the clear explanation of the differences in breeding success between institutions participating in the Wolverine EEP Program, partly because of the small sample size. However, enclosure size and keeper effect could actually have had an effect on their breeding and further research on these topics is needed. Emphasis has been given to provide the best adequate environment for a wolverine and have good husbandry practices.

INTRODUCTION

Large carnivores are one of the most vulnerable living entities of biodiversity (Balme *et al*., 2014) and conservation biologists have long faced the very challenging task of their conservation (Landa *et al*., 2000; Aronsson & Persson, 2017). Both their hunting habits and their very specific ecology make the conservation of large carnivores particularly difficult (Linnell, 2015). Due to their killing of livestock and wild ungulates, farmers and hunters perceive carnivores as a threat and often have a negative attitude towards them. Moreover, carnivores generally need large home ranges and require a viable prey population. Conservation, therefore, requires extended areas of good quality habitat, which are not easy to find anymore (Landa *et al*., 2000). Additional complexity is added when carnivores affect areas where there are indigenous communities or where traditional practices take place (Aronsson & Persson, 2017). Wolverines (*Gulo g. gulo*) are a good example of this (Landa *et al*., 2000; Aronsson, 2009; Aronsson & Persson, 2017; Aronsson & Persson, 2018).

The wolverine, the largest terrestrial member of the Mustelidae family (Landa *et al*., 2000; Dalerum *et al*., 2006; Aronsson, 2009), is known as one of the rarest and least known large carnivores from the Northern Hemisphere (Landa *et al*., 2000). They are solitary animals occupying a variety of habitats with very harsh environmental conditions, ranging across boreal forests and arctic and alpine tundra in North America and Eurasia (Aronsson, 2009; Copeland *et al*., 2010; Aronsson & Persson, 2018). However, they have lost extended parts of their habitat due to deforestation and human development (Landa *et al*., 2000; Aronsson & Persson, 2017).

Wolverines are opportunistic generalist predators and scavengers (Aronsson, 2009; Mattison *et al*., 2016; Aronsson & Persson, 2018). Ungulates are their main diet component, being able to kill large prey such as reindeer (*Rangifer tarandus*) and moose (*Alces alces*) (Aronsson, 2009). They also hunt livestock, especially domestic sheep (*Ovis aries*) (Ekblom *et al*., 2018) and semi-domesticated reindeer (*R. tarandus*) (Mattison *et al*., 2016), which is why they have been persecuted by humans for a long time (Ekblom *et al*., 2018). This persecution and the above-mentioned habitat loss have brought the population close to extinction (Landa *et al*., 2000; Aronsson & Persson, 2017).

According to Ekblom *et al*. (2018), there are around 850 adult individuals in the wild of Scandinavia, and less than 300 individuals in North America (Defenders of Wildlife, 2018), even though the exact worldwide population of wolverines is unknown (Environment Canada, 2014). The species is listed as 'Least Concern' in the IUCN Red List of Threatened Species, with a decreasing population trend (IUCN, 2018). The European wolverine is considered Vulnerable (VU) (Princée, 2016). Moreover, recent molecular studies have raised concern about the status of the gene pool of the Scandinavian wolverine population (Ekblom *et al*., 2018).

Captive breeding programs are one of the few immediate practical conservation options for species without suitable habitats (Tribe & Booth, 2003; Conde *et al*., 2011). In 1994, the European Endangered Species Program (EEP) for the Wolverine was launched by the European Association of Zoos and Aquaria (EAZA) and the studbook has been maintained by *Nordens Ark* (Hunnebostrand, Sweden) since then (Princée, 2016). The aim of this breeding program is to conserve a healthy captive population to provide a better future to this vulnerable species (EAZA, 2018). There are currently 138 wolverines in captivity under the EEP program, distributed in 42 holders worldwide (from ZIMS for Studbook 2018-12 20). However, as many other captive breeding programs (Kiik *et al*., 2013), the Wolverine EEP program experiences some slight irregular breeding (from ZIMS for Studbook 2018-12 20), probably due to insufficient knowledge of the species-specific requirements (Kiik *et al*., 2013).

Compared with other large carnivores, there is poor knowledge of wolverine reproduction (Persson *et al*., 2006). Even though wolverines have been bred in captivity since 1915 (Blomqvist, 1995), they are still considered difficult to breed (Blomqvist, 2012). Moreover, wolverines have small litters and generally reproduce every two years in the wild (Landa *et al*., 2000; Persson *et al*., 2006). Species with low reproductive rate are more vulnerable than the ones with high reproductive rates (Ruggiero *et al*., 1994). This low reproductive rate in wolverines, and the rarity of their reproductive behavior, makes the breeding of captive wolverines a difficult task (Aronsson & Persson, 2018). Therefore, it is crucial to gain more knowledge about wolverine reproduction and improve their breeding in captivity as soon as possible, before it is too late.

Problems with successful reproduction in captivity are common (Wolf *et al*., 2000; Wielebnowski *et al*., 2002; Zhang *et al*., 2004). Studies show that the characteristics of the environment such as husbandry practices, enclosure complexity and enclosure size influence the breeding success of captive animals (Wall & Hartley, 2017). For example, Miler-Schroeder *and* Paterson (1989) found a positive correlation between breeding success and enclosure volume, enclosure complexity and availability of privacy in lowland gorillas (*Gorilla gorilla*); Roberts (1989) found that large enclosures and availability of several nest boxes were important factors for reproduction success in red pandas (*Ailurus fulgens*); a study on small zoo exotic felids showed that the quality of caretaking by keepers influenced their breeding success (Mellen, 1991). Many more examples can be found in the literature, such as Diez-Leon *et al*. (2013) with captive American Mink (*Neovison vison*), Zhang *et al*. (2004) with captive giant pandas (*Ailuropoda melanoleuca*), Wolf *et al*. (2000) with footed ferrets (*Mustela nigripes*), Wielebnowski *et al*., 2002 with clouded leopards (*Neofelis nebulosa*) and Kiik (2018) with the European mink (*Mustella lutreola*). Other factors such as stress (Price & Stoinski, 2005), familiarity with the breeding partner (Peng *et al*., 2009) and visitor presence (Davey, 2007) are known to have an impact on captive breeding too. Furthermore, physiological, health and behavioral problems, such as hormonal imbalance (Zhang *et al*., 2004; Hermes *et al*., 2006) or inappropriate mating position (Wolf *et al*., 2000), also have an important role in successful reproduction. Moreover, individual and gender differences between animals need to be taken into consideration as well (Carlstead *et al*., 1999).

It is therefore obvious that a study on the wolverine reproduction in captivity considering both the characteristics of the captive environment and the biology of the species would be very beneficial for this threatened species.

Aim of the study

The short-term aim of this study is to identify factors that could be connected to breeding success in captive wolverines regarding the characteristics of the enclosures, the wolverine biology, the characteristics of the institutions and the influences of the human-animal interactions. The long-term aim is to improve the breeding success of the Wolverine EEP program of EAZA, in order to support the conservation of this species. To this end, an online survey was elaborated and sent to all holders of wolverines included in the EEP program.

The following three research questions were addressed: (1) *Are there any factors that are strongly connected to breeding success in captive wolverines? If so, which are they?* (2) *What are the best conditions for a captive wolverine to breed?* (3) *What actions can the zoos take to improve the breeding success of their wolverines?*

MATERIAL AND METHODS
The online survey

The study consisted of the development of an online survey, and the subsequent statistical analysis. The questions were formulated with consideration to identified key factors of successful breeding in wolverines according to literature (such as Magoun & Copeland, 1998; Persson, 2003; Hedmark, 2006; Persson *et al*., 2006; May, 2007; AZA Small Carnivore TAG, 2010; Blomqvist, 2012).

The Wolverine European Endangered Species Program (EEP) of the European Association of Zoos and Aquaria (EAZA) has a total of 56 stakeholders, of which 44 are EAZA members. The online survey was sent to 42[1] EAZA-member holders, which are listed in Table 1. Even though it is a European breeding program, the American Association of Zoo and Aquaria (AZA) partnered in 2013 to help establish a better self-sustaining population of wolverines with a larger amount of gene diversity (Blomqvist & Ness, 2014).

Table 1. List of wolverine stakeholders included in the Wolverine European Endangered Species Program (EEP) of the European Association of Zoos and Aquaria (EAZA) participating in the study

Zoo/Institution	Country	Zoo/Institution	Country
Alaska Zoo	U.S.	Śląski Ogrod Zoologiczny	Poland
Alaska Wildlife Conservation Center	U.S.	Ohrada Zoo	Czech Republic
Ähtäri Zoo	Finland	Orsa Grönklitt Bjornpark	Sweden
Borås Djurpark Zoo	Sweden	Opole Zoo	Poland
Brno Zoo and Environmental Education	Czech Republic	Parc Animalier d'Auvergne	France
Budapest Zool.& Botanical Garden	Hungary	Parc Animalier de Sainte Croix	France
Columbus Zoo and Aquarium	U.S.	Parc Zoologique de Paris (MNHN)	France
Cotswold Wildlife Park and Gardens	England	Ranua Wildlife Park	Finland
Detroit Zoological Society	U.S.	Réserve Zoologique de Calviac	France
GaiaZoo	Netherlands	Salzburg Zoo Hellbrunn	Austria
Han sur Lesse	Belgium	Skansen Foundation	Sweden
Helsinki Zoo	Finland	Skånes Djurpark Resort	Sweden
Highland Wildlife Park	Scotland	Szeged Zoo	Hungary
Järvzoo AB	Sweden	Tierpark Eberswalde	Germany
Kolmårdens Djurpark	Sweden	Tierwelt Herberstein	Austria

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[1] Two EAZA-member institution were not included in the study because the survey was not sent to them by mistake.

Kristiansand Dyrepark	Norway	Wildpark Lüneburger Heide	Germany
Lycksele Djurpark	Sweden	Zoo Duisburg	Germany
Minnesota Zoo	U.S.	ZooMontana	U.S.
Münchener Tierpark	Germany	Zoo Osnabrück	Germany
Namsskogans Familiepark	Norway	Zoo Sauvage de St. Félicien	Canada
Nordens Ark	Sweden	ZSL Whipsnade Zoo	England

The online survey was created with the *Google* survey administration app that is included in the *Google Drive* office suite called *Google Forms* (Version 71.0.3578.98, 2018). An e-mail with a direct link to the survey was sent to all holders listed in Table 1. Before being able to start the survey, a small introduction stating the aims and the functioning of the software was given.

The survey contained a total of 68 questions, split into nine sections: general, enclosure (with three subsections: outdoors, indoor accommodation and other), nest boxes/dens, nutrition, enrichment, training, health, behavior, human-animal interaction and breeding. The questions ranged from multiple choice (only one and more than one options), short answer and paragraph to file upload. All questions were required to be answered, except the upload files questions. A copy of the survey can be found in Appendix I.

The expected time to complete the survey was 15min, but there was no time limit to complete it. Answers were not saved if the user returned to the survey later without having it submitted first. Once it was submitted, answers could not be edited. However, it was possible to go back and forward through the survey, before sending it in, without losing the answers.

Once the respondents submitted their answers, *Google Forms* (Version 71.0.3578.98, 2018) saved them and exported them to a *Microsoft Excel 2010* sheet.

Data handling
All data collected was summarized and processed in *Microsoft Excel 2010*. For more complex statistical analyses, *RStudio* (Version 1.1.463, 2019) was used.

Śląski Ogród Zoologiczny was not included in the data analysis because they had only started keeping wolverines less than two years before the time of the study and they only had one individual at that time. *Namsskogan Familiepark* was not included either as they had been told not to let their individuals breed to avoid future inbreeding. *Parc Animalier d'Auvergne* data was also not included because they moved their wolverines in January of 2019 to a

larger, more complex and enriched enclosure and it was not possible to obtain information about the old enclosure. Even though *Helsinki Zoo* had only one individual at that time, it was included in the data analysis because previous breeding pairs in their institution had had successful breeding. *Orsa Rovsdjurpark* moved their wolverines in the summer of 2018 to a larger enclosure, mostly due to stress caused by a bear (*Ursus sp.*) enclosure next to the wolverine one. However, data on previous enclosure was obtained. Therefore, the size of the older outdoor enclosure was included in the data analysis instead of the size of the new enclosure. *Nordens Ark* submitted two answers as they had two different families in two separate enclosures.

Each question of the online survey was considered a factor, except the file upload questions which were not included in the data analysis. The data consisted of a total of 53 factors, both categorical or numerical, listed in Table 2.

Table 2. List of the categorical and numerical factors of the data collected

Categorical factors		
Breeding Success	Presence of Barriers/Wires	Indoor Accommodations
Country	Substrate Outdoor Enclosure	Indoor Accommodations Public Displayed
Way of Individuals Being Held	Vegetation Outdoor Enclosure	Substrate Indoor Accommodations
Separated or Together	Type of Vegetation Outdoor Enclosure	Off-public Enclosures
Experience Holding Wolverines	Hiding Places	Separation Keepers
Open Top	Possibility to Climb	Night Locking
Material Outdoor Enclosure	Pond	Neighboring Species
Possibility Build Den/Shelter	Special Feeding Routines	Types of Enrichment
Nest Boxes/Dens Provided	Feeding frequency (e.g. every two days)	Change of Enrichment
Bedding Material Provided	Water Resource	Training
Weekly Diet	Food Enriched	Individuals Trained
Health Problems	Type of Stereotypic Behaviors (Current)	Presence Stereotypic Behaviors (Former)
High Season Months	Type of Stereotypic Behaviors (Former)	Presence Stereotypic Behaviors (Current)
Institution Opening	Institutions Always Open	
Numerical factors		
Total Number of Individuals	Age	Total Number of Nest Boxes Provided
Total Number of Females	Pond Size	Total Number of Outdoor Enclosures
Total Number of Males	Number of Indoor Accommodations	Visitors per day during High Season
Outdoor Enclosures Size	Indoor Accommodations Size	Visitors per day during Low Season

The factor Outdoor Enclosure Size was sorted into three categories (Category 1, Category 2 and Category 3; Table 3) with different subcategories. Three different categories were given

to try to give different interpretations of what a "small" or "big" enclosure would be from a wolverine's perspective.

Table 3. Outdoor Enclosure Size

Category 1		Category 2		Category 3	
$0 – 200$ m^2	Very small	$0 – 150$ m^2	Very small	$0 – 500$ m^2	Very small
$201 – 800$ m^2	Small	$150 – 500$ m^2	Small	$501 – 850$ m^2	Small
-		$501 – 1000$ m^2	Medium-Small	-	
$801 – 1500$ m^2	Medium	$1001 – 2000$ m^2	Medium	$851 – 1500$ m^2	Medium
$1501 – 3000$ m^2	Big	$2001 – 3000$ m^2	Big	$1501 - 3000$ m^2	Big
>3000 m^2	Very big	>3000 m^2	Very Big	>3000 m^2	Very big

The factor Neighboring Species was sorted into three categories depending on the type of species: (1) predators, (2) prey-species or (3) both predator and prey-species.

All institutions were sorted into two categories depending on their breeding success: institutions that had never had successful breeding (Non-Successful Breeding Institution, NSBI) or institutions that had had successful breeding (Successful Breeding Institution, SBI). All factors mentioned above (Table 2) were then observed and compared with each other to try to find any patterns related to breeding success.

The average age and the median age of all individuals were calculated, as well as the average age and the median of NSBI and SBI. The age difference between individuals in each institution was calculated and compared. This difference was also compared between institutions that had only two individuals. The different groups of years of experience holding wolverines (2-5 years, 6-10 years, 11-20 years, >20 years) against the number of NSBI and SBI (in %) belonging in each group was plotted.

Wolverines reach sexual maturity around the age of 15 months (Persson *et al*., 2006) but most females do not reproduce until the age of 3-4 years (Blomqvist, 2012). Institutions which had wolverines for only a few years and were in possession of young wolverines might not have had time for breeding to occur. Therefore, the age of the individuals of each institution and their years of experience holding wolverines were examined together. The number of nest boxes and the size of their outdoor enclosure was compared as well.

Eight Fisher's Exact Test and one Pearson's Chi-squared Test were performed with *RStudio (Version 1.1.463, 2019)* to investigate in further detail the factors Outdoor Enclosure Size, Feeding Every Day, Training and Keepers Separated:

- Three Fisher's Exact Tests were run to see if there was a difference between the size of the outdoor enclosures of NSBI and SBI for Category 1, Category 2 and Category 3. For each test, the null hypothesis was that there was no difference between both types of institutions concerning the size of their outdoor enclosures.

- Three Fisher's Exact Test were run to see if there was a difference between the size of the outdoor enclosures of NSBI and SBI with only 2 individuals for Category 1, Category 2 and Category 3. For each test, the null hypothesis was that there was no difference between both types of institutions with only 2 individuals concerning the size of their outdoor enclosures.

- A Pearson's Chi-squared Test was run to see if breeding success and keeping the keepers separated were dependent on each other. The null hypothesis was that breeding success did not depend on the keepers being separated or not from the wolverines.

- A Fisher's Exact Test was run to see if there was a difference in breeding success if wolverine were fed every day or not. The null hypothesis was that breeding success was not dependent on the wolverines being fed every day or not.

- A Fisher's Exact Test was run to see if there was a difference in breeding success when individuals were trained or not. The null hypothesis was that breeding success was not dependent on training.

Institutions with the same Outdoor Enclosure Size subcategory (Very Small, Small, etc.) from Category 1 were grouped together respectively and the factors of NSBI and SBI for each group were compared to find any patterns related to breeding.

Institutions that fed their individuals every day and the ones that did not were grouped together respectively and the factors of NSBI and SBI for each group were compared to find any patterns related to breeding.

All institutions that trained their animals and the ones that did not were grouped together respectively and the factors of NSBI and SBI for each group were compared to find any patterns related to breeding.

All institutions that kept their animals separated from the keepers and the ones that did not were grouped together respectively and the factors of NSBI and SBI for each group were compared to find any patterns related to breeding.

A Hierarchical Cluster Analysis was performed with *RStudio (Version 1.1.463, 2019)* to group together institutions with similar characteristics. All numerical factors were included except Number of Visitors per day during High Season and Number of Visitors per day during Low Season, due to their high distribution. Since the factors were not in the same scale, they were first normalized. To do so, the mean ($\bar{x}$) of all factors was subtracted and divided by their standard deviation (σ), getting normalized factors with $\bar{x} = 0$ and $\sigma \approx 1$. The commands used were (z being the dataset's name) `apply(z, 2, mean)` to calculate the means, `apply(z, 2, sd)` to calculate the standard deviations and `scale(z, m, s)` to normalize the factors. Afterwards, Euclidean distances of all factors were calculated using the command `dist()`. Lastly, a Cluster Dendrogram with Complete Linkage was made with the command `hclust()` and `plot()`.

All institutions were grouped into their cluster according to the result of the Hierarchical Cluster Analysis and differences between NSBI and SBI were searched in each cluster.

RESULTS

37 institutions (88%) out of the 42 wolverine stakeholders included in the Wolverine EEP Program from EAZA submitted the survey. 9 institutions (25%) never had successful breeding in their institution (NSBI) and 26 institutions (75%) had successful breeding (SBI)[2].

Enclosure

Enclosure size
The number of outdoor enclosures for all institutions ranged from 1 to 4 enclosures, with sizes from 120m^2 to 15.000m^2. The number of outdoor enclosures for NSBI ranged from 1

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[2] Excluding *Śląski Ogród Zoologiczny*, *Namsskogan Familiepark* and *Parc Animalier d'Auvergne* and counting *Nordens Ark* as two different institutions, as mentioned in the data handling section.

to 2 enclosures (except one institution with 3 enclosures), with sizes from 314m^2 to 840m^2. Most SBI (80%) also had 1 to 2 enclosures, of which the sizes ranged from 120m^2 to 15.000m^2.

 There were significantly more institutions with non-successful breeding (NSBI) that had small (Category 1 and 3) and medium-small (Category 2) enclosures than institutions with successful breeding (SBI) (Fisher's Exact Tests $p < 0.001$ for Category 1, $p<0.05$ for Category 2 and $p<0.01$ for Category 3) indicating a negative effect on breeding of small enclosures. This was also true for institutions with only two individuals (Fisher's Exact Tests $p< 0.01$ for Category 1, $p<0.05$ for Category 2 and $p<0.01$ for Category 3).

All institutions with the same Outdoor Enclosure Size subcategory (Very Small, Small, etc.) from Category 1 were grouped together respectively and the characteristics of NSBI and SBI for each subcategory group were compared to find any differences. Too few differences were found to see any pattern that could be related to breeding.

Enclosure complexity
All institutions (100%) had vegetation, such as trees, bushes, grass and shrubs. All institutions (100%) provided places to hide and to climb. Most institutions (89%) had a pond in their enclosures, sizes ranging from 1m^2 to 500m^2. Almost all enclosures (95%) were open top, except for two SBI institutions. The outdoor enclosures were mostly made of mesh (43%) with natural ground as substrate (100%). Electric barriers or wires were used in 78% of the institutions. No visible patterns were found in complexity for SBI and NSBI.

Environmental enrichment
All institutions (100%) used enrichment in their enclosures, Sensory (92%) and Manipulative (84%) being the most common types used. Only three SBI did not change their enrichment regularly. No clear pattern was found.

Indoor accommodations
Most institutions (65%) had indoor accommodations, ranging from 1 to 6 indoor accommodations, with sizes from 2m^2 to 275m^2. Only one of these was publicly displayed. The most used substrates were Hay/Straw and Wood shavings/ Woodchips.

The majority of NSBI (67%) had indoor accommodations, ranging from 1 to 4 indoor accommodations, with sizes from 9m^2 to 120m^2. The majority of SBI (62%) also had indoor

accommodations, ranging from 1 to 6 indoor accommodations, with sizes from 3m^2 to 275m^2. No clear pattern was found in this parameter.

Most institutions (70%) had off-public facilities. Only one (NSBI) institution locked their animals indoors at night for safety reasons. No clear pattern was found.

Nest boxes/dens
Almost all institutions (89%) gave wolverines the opportunity to build a shelter/den themselves, only one NSBI and four SBI did not. Almost all institutions (89%) provided nest boxes/dens to their wolverines, only four SBI did not. The number of nest boxes/dens provided differed from 1 to 6 nest boxes/dens. Too few differences were found to see any clear pattern.

67% NSBI provided 1 or 2 nest boxes, just like 57% of SBI. All (100%) SBI with two individuals provided 2 to 4 nest boxes. All NSBI (100%) provided bedding material to their individuals, just like 73% of SBI. Too few differences were found to find a clear pattern.

No visible pattern was found in the comparison of the number of nest boxes and the size of their outdoor enclosure.

Neighboring species
The type of neighboring species differed from predators such as bears, lynx (*Lynx spp.*), wolves (*Canis lupus*) and tigers (*Panthera tigris*) to prey-species such as reindeers, moose and farm animals. Almost half of NSBI (45%) had predators as neighboring species, such as almost half of SBI (46%). No visible pattern was found concerning this factor.

Wolverine biology

Age
The ages of the wolverines ranged from 1 to 14 years. NSBI ages ranged from 3 to 11 years with an average age of 6.7 years, and a median of 6 years. SBI ages ranged from 1 to 14 years with an average age of 6.6 years and a median of 5.5 years. Too few differences were found to find any pattern.

The age difference between individuals of institutions that had only two individuals was compared but no visible pattern was found. 56% of NSBI had one year or less of age difference, like half (50%) of SBI.

Health

Different health problems were presented, mostly non-severe issues such as parasites, lameness and wounds. The main reason of deaths was old age in both groups.

Social behavior

The total number of wolverines in each institution differed between 1 to 5 individuals, having between 1 to 3 females and 0 to 2 males. All NSBI had 2 individuals (one male and one female), as did more than half (54%) of SBI. No clear pattern was found.

Wolverines were held in different ways, the majority (54%) were held in a pair. Practically all individuals (89%) were together all the time. All NSBI (100%) were held in a pair, just like all SBI (100%) that had only two individuals. 78% NSBI kept their individuals together all the time, except for one institution that separated them at night, and another one that separated them during feeding and at night. 88% SBI kept their individuals together all the time as well, except for three institutions that separated them during birth. No clear patterns were found regarding this parameter.

Abnormal/Stereotypic behavior

Most of the individuals living in the institutions at the time of the study (78%) did not present stereotypic behaviors. Most of the former individuals living in the institutions (73%) did not present stereotypic behaviors. The types of stereotypic behaviors in current wolverines performed were Pacing, Somersault and Biting fence. The types of stereotypic behaviors in former wolverines performed were Pacing, Somersault and Head bobbing. 23% of currently living NSBI and 11% of former NSBI presented or may have presented stereotypic behaviors, just like 23% of current SBI and 35% of former SBI. No clear pattern was found.

Institution characteristics

Diet

A variety of diets were described, some in more detail than others. No clear pattern was found. More than half of the institutions (65%) had special feeding routines. Almost half (46%) fed their wolverines once a day and got fresh water from a bowl. Most institutions (60%) presented their food in an enriched way only sometimes.

78% NSBI and 55% of SBI fed their wolverines every day and this was not statistically different (Fisher's Exact Test, p=0.14). Breeding success does not seem to be dependent on if the wolverines were fed every day or not.

Institutions that fed their individuals every day and the ones that did not were grouped together respectively. The factors of NSBI and SBI for each group were compared. No clear patterns were found.

Experience
Almost half of all institutions (46%) had more than 20 years of experience holding wolverines. All NSBI were almost equally distributed in each group of years of experience (Figure 1). Most SBI (58%) had more than 20 years of experience. Too few differences were found to find any pattern.
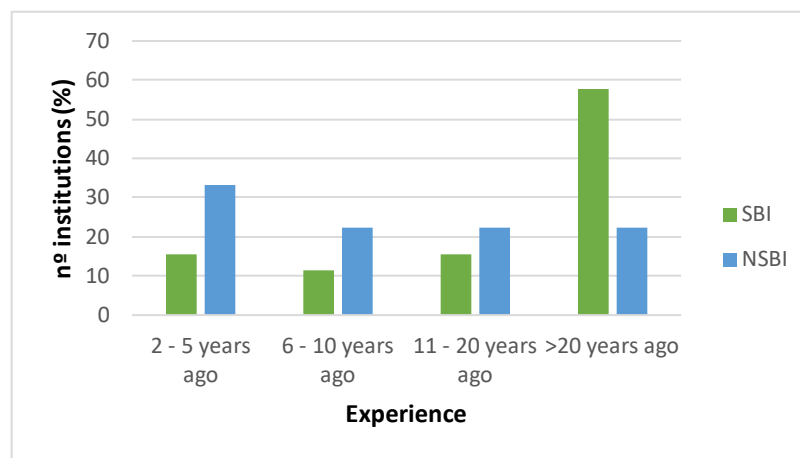
Figure 1. NSBI (%) and SBI (%) years of experience holding wolverines.

To see if any NSBI did not have successful breeding due to biological reasons, the age of the individuals of each institution and their years of experience holding wolverines were compared. One NSBI was found where their wolverines might not have had time to breed: both individuals were three years old, and the institution had been holding wolverines for only 2 -5 years.

Training
More than half of all institutions (57%) trained their animals. Of those that trained their wolverines, most institutions (90%) trained all their animals, except one SBI that trained only males and one SBI that only trained adults.

Most NSBI (78%) trained their animals, just like half (50%) of SBI and this was not statistically different (Fisher's Exact Test p=0.24). Breeding success could not be shown to be dependent on training.

All institutions that trained their animals and the ones that did not were grouped together respectively and then the factors of NSBI and SBI of each group were compared. No clear pattern was found.

Human-animal interaction

Keeper effect

In most institutions (68%), their keepers would enter the enclosure without separating their wolverines into separation facilities. The majority of NSBI (70%) separated their wolverines before the keepers would enter the enclosure, unlike the majority of SBI (81%) which did not keep their wolverines separated from the keepers. This was statistically different (Pearson's Chi-squared Test p<0.05). Breeding success did seem to depend on the keepers being separated or not from the wolverines. Keeping the animals separated from the keepers had a negative effect on their breeding success.

All institutions that kept their animals separated from the keepers and the ones that did not were grouped together respectively. The factors of NSBI and SBI for each group were compared. No visible pattern was found.

Visitor effect

Most institutions (78%) were open all year around, having their high season between the months of April – October. The number of visitors per day during high season ranged from 320 to 300.000 people, and 25 to 200.000 people during low season.

All NSBI institutions (100%) were opened all year around, like most of SBI institutions (77%). The number of visitors per day at NSBI during high season ranged from 500 to 4.500 people, and 25 to 666 people during low season, except for one institution with extremely high number of people (300.000 people for high season, and 200.000 for low season). The number of visitors per day at SBI during high season ranged from 480 to 18.000 people, and 30 to 3.000 people during low season. No clear pattern was found.

Hierarchical cluster analysis

The result of the hierarchical cluster analysis can be seen in Figure 2. Two big clusters were found (yellow marked in Figure 2). 3 NSBI and 17 SBI are in one cluster and 6 NSBI and 9 SBI in the other. No significant differences in factors were found when comparing NSBI and SBI in each cluster.
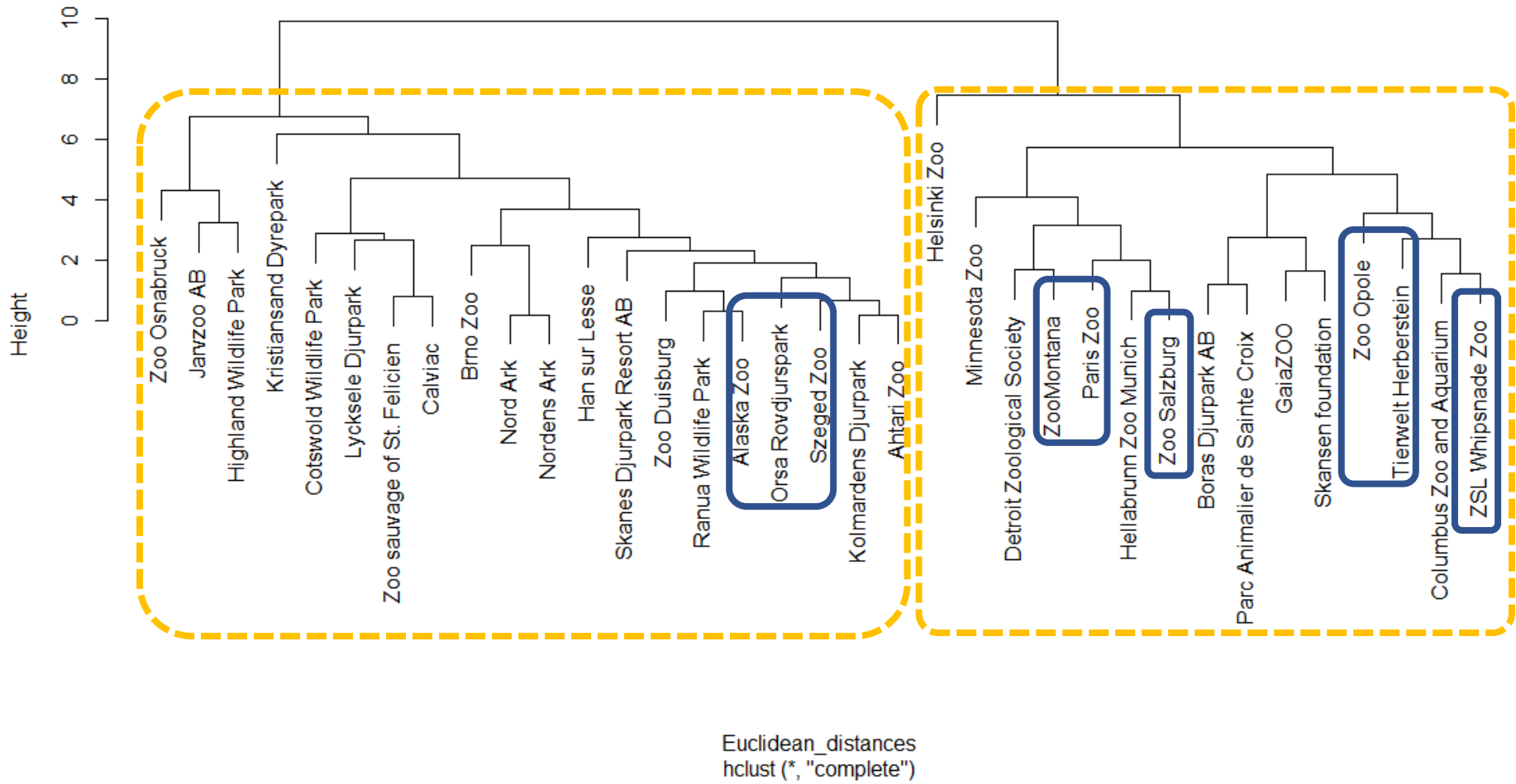
Figure 2. Result of the hierarchical cluster analysis with the factors Total Number of Individuals, Total Number of Females, Total Number of Males, Total Number of Outdoor Enclosures, Outdoor Enclosures Size, Pond Size, Total Number of Indoor Accommodations, Indoor Accommodations Size and Total Number of Nest Boxes Provided. NSBI are marked in blue. Two big clusters are marked in yellow.

DISCUSSION

The aim of this study was to identify factors that could be connected to breeding success in captive wolverines and so improve the breeding success of the Wolverine EEP program of EAZA, in order to support the conservation of this species. To this end, an online survey was elaborated and sent to holders of wolverines included in the EEP program.

Even though almost 90% of the institutions included in the Wolverine EEP program participated in the study, giving a good overview of this wolverine population, the total zoo population was small and the number of zoos having difficulties with breeding was low. The variation between these institutions was probably too large to get conclusive results. Additionally, the fact that there were fewer institutions with no breeding (NSBI) than with successful breeding (SBI) made the comparisons difficult. The variation between both types of institutions was too small to get significant results. Moreover, specific reasons for low breeding success of captive animals are difficult to identify (Taylor & Poole, 1998). There are doubtless many environmental factors that affect breeding success, factors that cancel out each others' effects or factors that are so interrelated that their separate contributions cannot be determined with a small sample size in a cross-institutional zoo study. The results of this study can only pinpoint some general trends across the institutions participating in the Wolverine EEP program that could explain their differences in breeding success.

Nevertheless, a discussion of the results of the online survey is given below in the following order: (1) the characteristics of the enclosure (size, complexity, environmental enrichment, indoor accommodations, nest boxes and neighboring species), (2) the wolverine biology (age, health, social behavior and abnormal/stereotypic behavior), (3) the characteristic of the institutions (experience, diet and training) and (4) human-animal interaction (keeper effect and visitors effect). A discussion of the two main clusters obtained from the hierarchical cluster analysis is also given. A final discussion on other factors that could have been included in the study and future research ideas are given at the end. The best conditions for a captive wolverine to breed are mentioned and suggestions on what actions zoos can take to improve their breeding success are given.

Enclosure

Enclosures and their characteristics play an important role in the breeding success of captive animals (Kiik *et al*., 2013; Marshall *et al*., 2016).

Enclosure size
Results showed that NSBI had a different distribution than SBI concerning their enclosure size: NSBI had smaller enclosures than most SBI. This result is probably due to the fewer number of NSBI compared to SBI. However, despite the small sample size, it could still mean that enclosure size influences the breeding success of captive wolverines. These results go in accordance with several studies that found that enclosure size is correlated with reproductive success, and that increasing the size of the enclosure has a positive effect, such as McCusker (1978) showed in captive felids, Carlstead *and* Shepherdson (1994) in gorillas, Carlstead *et al*. (1999) in black rhinoceros (*Diceros bicornis*) and Peng *et al.* (2007) in giant pandas. Others, however, have demonstrated no such relationship, such as Mellen (1991) in small captive exotic felids and Wall *and* Hartley (2017) in Burmese brow antlered deer (*Rucervus eldii thamin*).

Wolverines are mobile species with large home ranges (May, 2007). The accepted minimum of an enclosure size for a wolverine stated in AZA (2010) is 150m^2, however they recommend housing wolverines in larger spaces to improve breeding success, since small enclosures are quickly worn down by this active species. The minimum enclosure size recommended for a wolverine pair is 500m^2, with an additional 300m^2 enclosure to separate the male or kits when necessary (Blomqvist, 2012). In Sweden, the minimum enclosure size for keeping wolverines is 600 m^2 (Swedish Board of Agriculture, 2009). 3 NSBI had enclosure sizes below the recommended 500m^2. Even though enlarging an exhibit is difficult and expensive, it would be in the wolverines' best interest to try to meet at least the minimum recommended size. If this is not possible, double attention should be put in providing the best environment. Overall, if an enclosure does not have the appropriate environment, animals will not have the physiological or behavioral capacity to breed (Marshall *et al*., 2016). It is important to emphasize that the complexity of the enclosure, rather than just size, must also be considered (Marshall *et al*., 2016), like Stevens *and* Pickett (1994) demonstrated in their study of flamingos and proper housing.

Enclosure complexity
According to this study, all NSBI had a varied topography with natural ground as substrate and plenty of vegetation, with hiding places, climbing surfaces, and a pond, just like AZA (2010) and Blomqvist (2012) - former studbook keeper of the Wolverine EEP Program – recommend to have in a proper wolverine enclosure. This suggests that the complexity of the enclosure does not seem to be the explanatory factor of the differences

in breeding success between NSBI and SBI. However, it was not possible to truly identify the quality of the complexity provided in each institution due to limitations of the survey. Further research is needed to study the quality of the enclosure's complexity of each institution to be able to completely rule it out as an affecting factor in the breeding success of these institutions. One great way to improve enclosure complexity is to use environmental enrichment.

Environmental enrichment
Studies show that environmental enrichment plays a very important role in the wellbeing of captive animals, reducing the presence of abnormal behaviors considerably (Chutchawanjumrut, 2015; Damasceno *et al*., 2017), facilitating normal development and coping mechanisms, increasing activity and behavioral diversity (Carlstead & Shepherdson, 1994), and improving breeding success (von Schmalz, 2003). The quality of the enclosure is very important and should be built by taking the physiological and behavioral needs of each species into consideration (Cain, 2005).

According to this study, all institutions used enrichment in their enclosures, which suggests that environmental enrichment does not seem to be the explanatory factor of the differences in breeding success between NSBI and SBI. However, it was not possible to truly identify the quality of the enrichment provided, as there was no way of knowing if what each institution categorized as an enrichment can actually be considered proper enrichment for wolverines. Olfactory, tactile, searching and hunting behaviors should be stimulated on a regular basis in wolverines, to keep up with their active, exploration, restless energy so typical of the mustelids (Cain, 2005). Good examples of wolverine enrichment are the following: silage and cardboard boxes or paper bags containing meat, hair, skin or feces of other animals, living and fallen trees, hollow logs, tree stumps, small rocks and bushes (Cain, 2005; AZA, 2010; Blomqvist, 2012). Further research is needed to look into the quality of the environmental enrichment of each institution and so be able to completely rule out environmental enrichment as an affecting factor in the breeding success of these institutions.

Furthermore, three SBI did not change their enrichment regularly, which can be discussed if it can be considered enrichment at all, since for environmental enrichment to work, it needs to be changed periodically (Yu *et al.*, 2009). For example, small climbing and horizontal structures need to be replaced every 1-3 months in enclosures holding mustelids (AZA, 2010). All institutions, especially NSBI, need to make sure they are

providing the best enrichment possible and changing it when appropriate. However, institutions need to keep in mind that a lot of change can be stressful and therefore a balance between little to no change and too much change should be found (Wingfield & Kitaysky, 2002; Fairhurst *et al*., 2011).

Indoor accommodations
The majority of NSBI (67%) and SBI (62%) had indoor accommodations. However, wolverines are accustomed to harsh climate and living conditions, therefore indoor accommodations are not strictly necessary if proper protection from heavy rain and sunlight is given (Blomqvist, 2012). Nevertheless, no visible pattern was found concerning indoor accommodations, which suggests that they did not influence the breeding of the wolverines.

Nest boxes/dens
Wolverines in the wild give birth in natal dens (Landa *et al*., 2000). Although captive wolverines also usually dig their own dens, artificial nest boxes made of solid materials having a recommended size of 70 cm x 50 cm x 60 cm with nesting material (such as hay or straw) should be provided, preferably in an off-exhibit facility (Blomqvist, 2012).

The results of this study show that all institutions either gave their animals the possibility to make dens themselves or provided nest boxes, even though the majority did both. One NSBI did not provide any nest boxes and two NSBI provided only one nest box. However, it is recommended that each female should have a minimum of two dens (AZA, 2010; Blomqvist, 2012). These institutions should make sure to provide the proper number of nest boxes in the future to help improve their breeding success. Three SBI did not provide any nest boxes either and one SBI only provided one nest box as well. Even though these institutions had successful breeding, it would be advisable to provide the right amount of nest boxes.

Nevertheless, some NSBI did give the possibility to make dens themselves and provided the correct number of nest boxes, which suggests that nest boxes are not the explanatory factor of the differences found in breeding success, at least in these NSBI. However, it is not only the presence of nest boxes that is important, but their quality too. Bad nest boxes could be considered as not having any at all. Given the questions of this survey, it was not possible to identify the quality of each nest box and see if they follow the recommendations of the literature (such as AZA, 2010 and Blomqvist, 2012). Further studies should try to analyze the quality of the nest boxes and the materials provided to

completely rule out the factor nest boxes/dens as an affecting factor in the breeding success of these institutions. Furthermore, according to Blomqvist *and* Rudbäck (2001), some institutions postulate from empirical information that female wolverines provided with rocky scree in their dens have a higher reproductive success. NSBI should consider trying this to encourage breeding.

Neighboring species
Neighboring species can sometimes be a cause of stress (Hosey, 2008), such as being forced to live close by a predator without the option to escape or being close to prey but not being able to hunt it (Morgan & Tromborg, 2007). A considerate variety of species in proximity were mentioned in the survey with no visible pattern differentiating between NSBI and SBI, which suggest that neighboring species is not a factor influencing the breeding success of these wolverines.

Wolverine biology
The biology of a species has a high influence in successful reproduction (Dalerum *et al*., 2016).

Age
Wolverines reach sexual maturity at about 15 months in females and 14 months in males, but not many 2-year-old females nor 2 to 4-year-old males produce litters (Landa *et al*., 2000). European studbook data confirms that most females do not start reproducing until the age of 3-4 years (Blomqvist, 2012). It is obvious that breeding success in wolverines is influenced by age (Persson, 2005; Aronsson & Persson, 2018). According to Kyle *and* Strobeck (2001), wolverines fecundity decreases after the age of 6; and the probability to successfully reproduce two years in a row slowly declines with age (Persson, 2005).

NSBI ages ranged from 3 to 11 years with an average age of 6.7 years, and a median of 6 years and SBI ages ranged from 1 to 14 years with an average age of 6.6 years and a median of 5.5 years, which suggests that in this case age is not a limiting factor in the breeding success of the wolverines. However, the wolverines of one NSBI might not have had time to breed since both individuals were three years old and the institution had been holding wolverines for only 2 -5 years. In this specific case, the reason for no breeding might be due to the age of the wolverines.

Health
All diseases mentioned in the survey are considered common mustelid diseases according to Fernandez-Moran (2003) and no visible pattern was found when comparing the health

of NSBI and SBI individuals. Health does not seem to be the explanatory factor of the differences in breeding success between NSBI and SBI.

Nevertheless, one could argue that the reason of this unsuccessful breeding is due to genetic factors and further research at the DNA level could support this theory. However, this is unlikely because some wolverines that did not breed in one institution did breed in other institutions (from ZIMS for Studbook 2018-12 20), which shows that the reason of unsuccessful breeding probably does not lie on the genetic factors of the individual.

Social behavior
Even though wolverines are known to be solitary animals, they interact with conspecifics in the wild (Dalerum *et al.*, 2006). In captivity, a compatible pair can co-exist (AZA, 2010; Blomqvsit, 2012) but it is recommended to separate the dam when birth approaches (Blomqvist, 2012).

All NSBI held 2 individuals, which eliminates the group stress factor or any problem with dominance. This suggests that social behavior is not the explanatory factor of the breeding differences between NSBI and SBI. However, due to limitation of the survey, it was not possible to assess the compatibility of these pairs and further research should try to go deeper in this subject. NSBI should try to figure out if the pair that they are holding is compatible and be alert for any signs of the opposite.

Abnormal/stereotypic behavior
Abnormal and aggressive behaviors have been observed in different species belonging to the Mustelidae family, affecting their social and mating behavior (Wolf *et al.*, 2000; Dallaire & Mason, 2017; Kiik, 2018).

All types of stereotypic behaviors mentioned in the survey have been described in other studies with wolverines (Chaudhary *et al.*, 2007), other mustelids (Morabito & Bashaw, 2012; Díez-León & Mason, 2016) and carnivores in general (Clubb & Vickery, 2006). Stereotypic or abnormal behaviors do not seem to be the explanatory factors of the differences in breeding success between NSBI and SBI. However, stereotypic behaviors easily occur among wolverines, especially if they are kept in small enclosures with little possibility to exhibit their natural behaviors (Blomqvist, 2012). Therefore, it is difficult to believe that only 22% of all institutions presented stereotypic behaviors. For example, one institution stated that their current and former wolverines had never presented stereotypic behaviors. However, Chaudhary *et al.* (2007) studied a former wolverine

housed in that same institution, and it showed stereotypic behaviors. Therefore, all institutions should make sure that they are aware of the presence of any stereotypic behaviors. Further research focused on the presence of stereotypic behaviors in the European captive wolverine population would be helpful.

Different studies show that the outcome of a mating attempt depends largely on the male partner, such as Lipschitz *et al*. (2001) with the primate lesser galago (*Galago senegalensis*), Zhang *et al*. (2004) with giant pandas and Kiik (2018) with the European minks. However, other research has shown the opposite: for instance, Poole (1966) reports that the male European polecat (*Mustela putorius*) is aggressive towards a female only if the latter rejects her partner. It is known now that wolverines have a polygamous mating system (Hedmark *et al*., 2007). However, little more is known about the breeding behavior of wolverines (Persson *et al*., 2006) and knowledge of the specific mechanisms of wolverine reproduction is incomplete (Inman *et al*., 2012). Further research to try to find out if the outcome of a mating attempt in wolverines depends mainly on the male or the female could help clarify the differences in breeding between NSBI and SBI.

Institution characteristics
Diet
Providing a high quality and balanced diet for captive animals is essential to maintain high welfare standards (Slight *et al*., 2015). The formulation, elaboration and presentation of all diets must meet the physiological and behavioral needs of each species (AZA, 2010). Moreover, nutrition has a strong influence on reproduction (Taylor & Poole, 1998; Persson, 2003) and wolverine reproduction is highly influenced by food availability in the winter (Persson, 2005).

Unfortunately, the level of detail of each weekly diet description submitted in the survey differed considerably and could therefore not be compared. This impeded to dismiss the factor diet as an explanatory factor of the differences in breeding success of NSBI and SBI. However, NSBI should keep in mind that wild wolverines are opportunistic generalist predators and scavengers (Aronsson, 2009; Mattison *et al*., 2016; Aronsson & Persson, 2018) and that ungulates are their main diet component (Aronsson, 2009; Gallant *et al*., 2016). Reindeer carcasses are the most important food resource for wild wolverines during winter (Blomqvist, 2012), and have a very important role in the diet of breeding females (Koskela *et al*., 2013). NSBI should make sure that their wolverines are offered large pieces of meat or whole carcasses to mimic their feeding behavior in the wild

(Blomqvist & Rudbäck, 2001; AZA, 2010; Blomqvist. 2012). Nevertheless, it has not been determined yet which foods specifically fuel the most energetically demanding periods of reproduction (Iman *et al*., 2012), hence further research on this would be very beneficial.

No significant differences in breeding success were found between institutions that fed their individuals every day and the ones that did not. Even though there is little research on the optimal frequency of feeding in captive wolverines, it has been shown that feeding them large pieces of meat every second day increases their breeding success (Groove, 2001). All institutions, but specially NSBI, should try to do this to enhance their breeding success.

Moreover, most institutions (60%) only presented their food in an enriched way sometimes, suggesting there is room for improvement in this area. All institutions, even though it is time and energy consuming, should try to feed their animals in an enriched way as often as possible. For example, frozen food items are an excellent way to present food in a familiar form for the wolverines (Blomqvist, 2012). Food caching is an integral part of wolverine foraging behavior (May, 2007; Aronsson & Persson, 2018), thus providing meat chunks that can be cached for later consumption is another beneficial enriching way of feeding wolverines (Blomqvist & Rudbäck, 2001).

Experience
Experience in any field is always helpful, and the more experience you get in a field, the more you learn about that specific topic (Henisz & Delios, 2004). Even though no clear pattern was found concerning the number of years that each institution had been holding wolverines, suggesting that experience is not the explanatory factor of the unsuccessful breeding, more than half SBI (58%) had more than 20 years of experience and only two NSBI (22%) had that same experience. Therefore, it cannot be completely ruled out that experience does not have any influence on breeding success. However, a larger sample size would be needed to be sure.

Training
Training animals is a tool that has been increasing in zoos during the last decade (Melfi, 2013). Research shows that it can be very enriching and beneficial for animal welfare if done properly (Brando, 2012; Melfi, 2013; Westlund, 2014; Deane, 2017; Spiezio *et al*., 2017). Furthermore, an animal with high welfare has higher breeding success (von

Schmalz, 2003). Additionally, good training facilitates veterinary interventions and reduces distress during husbandry procedures (AZA, 2010; Westlund, 2014).

No correlation was found between training and breeding success in this case, which suggests that training is not the explanatory factor of the differences in breeding success between NSBI and SBI. However, only 57% of all institutions trained their animals, which might be too few of a sample to be able to say anything conclusive about the effect on reproduction. Moreover, the type and frequency of training might have an impact on its effect or how the wolverines experience it, but that information could not be obtained from the survey.

Considering the benefits of good training, the institutions that do not train their animals, especially NSBI, could try to start to do so to see if it improves the welfare of their animals and so increase their chances of having successful breeding. Regarding the institutions that already train, they should make sure that they are using good techniques and the frequency of the trainings are adequate.

Human-animal interaction
Keeper effect
The majority of NSBI (70%) kept their wolverines separated from the keepers, unlike the majority of SBI (81%) which did not. According to the results of this study, keeping the animals separated from the keepers seems to have a negative effect on their breeding success.

Daily husbandry procedures can raise the levels of stress in carnivores (von Schmalz, 2003) and affect their breeding success (von Schmalz, 2003; Marshall *et al*., 2016). Maybe the husbandry procedures used in NSBI are not ideal for wolverines and it stresses them, affecting their breeding: NSBI need to make sure that the animals suffer the minimum stress possible while being separated and put into separation facilities, and they should try to keep them caged as short as possible to minimize the time of exposure to this stress factor. Moreover, these differences in breeding could also be explained if NSBI do not have an optimal keeper-animal relationship. Therefore, and in accordance with von Schamlz (2003), it would be advisable for NSBI to revise their current husbandry techniques and look more closely the relationship between their keepers and their animals, to make sure that husbandry and keeper effect are not affecting the breeding of their animals. Taking the benefits of training mentioned above into consideration, good

training could be a solution to this issue, by helping to move the animals with little to no stress and have positive effects on the relationships to keepers.

Visitor effect
Visitors and their effect on zoo animals has been broadly studied the past few decades (e.g. O'Donovan *et al*., 1993; Chamove *et al*., 1998; Hosey, 2000; Mallapur & Chellam, 2002; Margulis *et al*., 2003; Mallapur *et al*., 2005; Sellinger & Ha, 2005; Davey, 2007; Hosey, 2008; Hosey, 2013). It seems that depending on the animal and zoo, this effect can be negative (Chamove *et al*., 1998; Sellinger & Ha, 2005; Davey, 2007), positive (Mallapur & Chellam, 2002; Sellinger & Ha, 2005; Davey, 2007) or neutral (O'Donovan *et al*., 1993; Margulis *et al*., 2003; Davey, 2007).

Most institutions (78%) were open all year around, having their high season between the months of April – October, overlapping with the months of wolverine mating period in the wild (April – August; Dalerum *et al*., 2006). SBI had higher numbers of visitors per day (except for one NSBI with an extremely high number of visitors) and still had breeding success. It seems that visitor effect is not the explanatory factor of the differences in breeding success between NSBI and SBI. This goes in accordance with Sears (2011) who demonstrated that visitor effect did not seem to have a negative impact on five species within the Mustelidae family.

Hierarchical cluster analysis
The fact that NSBI were found in both clusters shows that the factors Total Number of Individuals, Total Number of Females, Total Number of Males, Total Number of Outdoor Enclosures, Outdoor Enclosures Size, Pond Size, Total Number of Indoor Accommodations, Indoor Accommodations Size and Total Number of Nest Boxes Provided are probably not the reason why the institutions show differences in breeding and further research considering other factors is needed. However, we see that the 3 NSBI from one cluster are grouped very close together, which suggests that they are indeed similar to each other and might have the same explanatory factor for their unsuccessful breeding. Further research trying to find out (dis)similarities between the institutions grouped closer together would be beneficial.

All factors
Even though these results demonstrate that there is an irregularity of wolverine breeding between zoos, the majority of institutions (75%) did have successful breeding, which shows that the Wolverine EEP Program is working. If we have a look at all the studied

factors together, we see the following pattern: on one hand, more NSBI had smaller enclosure sizes, provided less nest boxes and had less experience holding wolverines than SBI, which one could expect considering their unsuccessful breeding. However, on the other hand, more NSBI provided nest boxes and bedding material, trained their animals more and had less visitors compared to SBI, even though one would expect the contrary based on the benefits of these factors – and non-benefit of visitor presence - mentioned in the literature (such as Sellinger & Ha, 2005; Davey, 2007; AZA, 2010; Blomqvist, 2012; Melfi, 2013; Westlund, 2014). The explanation for this could be that what is negatively affecting the breeding of wolverines is not the presence/absence of these factors, but their quality, as mentioned previously. Another explanation could be that these factors have no effect on wolverine breeding. Nevertheless, we cannot discard the possibility that other factors which were not looked at in this study did have effect on these wolverines.

Missing factors and future research

Other factors, which have not been investigated in this study, may also have confounding effects on the breeding success of the wolverine and should also be considered. For instance, this study did not investigate the effects of translocating animals to new environments or the introduction of new animals, despite the fact that social factors and changes in the environment can have drastic effects on reproduction (Wall & Hartley, 2017). It also did not investigate the origin of the animals, which according to Kiik *et al*. (2013) is more decisive in breeding performance than immediate environmental conditions during breeding. Moreover, this study did not focus on the different personalities each wolverine can have, even though an increasing number of studies report the existence of personality types among animals (Lehmkuhl-Noer *et al*., 2016; Cheng *et al*., 2017; Kanda *et al*., 2017; Kiik, 2018) and individual temperament can be an important factor in their reproduction (Kiik, 2018).

Further researcher considering the factors not included in the study is needed. Furthermore, further research is needed to study the quality of the enclosure's complexity and the environmental enrichment, to get to know the actual presence of stereotypic behaviors in the European captive wolverine population, to try to know more on wolverine reproductive behavior and find out if the outcome of a mating attempt in wolverines depends mainly on the male or the female, to determine which foods specifically fuel the most energetically demanding periods of reproduction and more

research to try finding out other (dis)similarities between the institutions grouped closer together.

Ethical, social and sustainable aspects

The existence of zoos and keeping animals in captivity has been broadly discussed, and it has been looked at from different ethical perspectives. Those with animal welfare and animal rights approaches usually believe that there is no purpose that can justify the captivity of animals; whereas those with environmental ethics typically believe that the existence of captive animals is acceptable, as long as it contributes to the species survival in the wild (Minteer & Collins, 2013). Even if your ethical approach is complete intolerance to the existence of these institutions, it is difficult to deny that zoos play an important role in biodiversity and wildlife conservation (Gray, 2015). Many species have been saved from extinction or brought back from it thanks to the effort of zoos, such as the Arabian Oryx (*Oryx leucoryx*), the Przewalski's Horse (*Equus ferus przewalskii*), the Panamanian Golden Frog (*Atelopus zeteki*) and the Golden Lion Tamarin (*Leontopithecus rosalia*) (Taronga Conservation Society Australia, n.d.).

Furthermore, research in zoos is as important as its existence in terms of helping biodiversity and wildlife conservation. Zoo studies can help contribute in the conservation of endangered species, and thanks to the information gathered, further action can be taken into the right direction. Using this study as an example, we can improve the conditions of captive animals who are in too small enclosures - which is probably seen as ethically wrong no matter what approach one has – and increase their breeding success, contributing so in the conservation of the species.

Conclusions

No main factor or group of factors investigated in this study seems to be the clear explanation of the differences in breeding success between institutions participating in the Wolverine EEP Program. However, enclosure size and keepers' effect could actually have had an effect on their breeding and further research on these topics is needed. Nevertheless, NSBI should pay attention to provide the best adequate environment for a wolverine and have good husbandry practices, particularly focusing on keepers' routine. Special attention should be given to provide the best diet. Furthermore, effort should be put to assess the compatibility of each wolverine pair and be aware of the presence of any stereotypic behavior. Moreover, all institutions should try to train their animals adequately.

POPULAR SCIENCE SUMMARY

The conservation of large carnivores has been very challenging for biologists. The way these carnivores hunt and their very specific needs makes it a particularly difficult task. Wolverines are a good example of this. The number of wolverine individuals in the wild is close to extinction, mainly due to loss of their habitat and the actions of humans. The European Association of Zoos and Aquaria (EAZA) started a program in 1994 called *European Endangered Species Program (EEP)*, to help wolverines reproduce in captivity. The idea of a program like this is to increase the number of individuals in captivity and hopefully get them back to the wild at some point. Unfortunately, some institutions have issues with the reproduction of these animals: they do not seem to have successful breeding, or in other words, their wolverines do not reproduce and/or have offspring.

The goal of this study was to see which could be the reasons why these institutions are struggling compared to other institutions that do have successful breeding. Four main factors were researched: (1) the characteristics of the enclosures, (2) the wolverine's biology, (3) the characteristics of the institutions and (4) the effect of the presence of humans on the wolverines (both the zookeepers and the public).

In order to do this, an online survey with a total of 68 questions was developed and sent to 42 wolverine stakeholders included in the before-mentioned EEP program. 37 institutions (88%) out of the 42 submitted the survey. However, 3 institutions had to be excluded from the survey due to different reasons and one institution was counted twice since they had two separate wolverine enclosures. Out of these 35 institutions, 9 (25%) never had successful breeding in their institution and 26 (75%) had successful breeding.

After looking into the data collected, no main factor or group of factors seemed to give a clear explanation of the differences in institutions regarding their breeding success. Nevertheless, the size of the enclosure and the presence of zookeepers inside the enclosure seem to possibly have had an effect. More research is however needed to look into these two factors more deeply to be able to draw stronger conclusions.

Even though no clear explanation was found, the following guidelines and advise has been given to the institutions struggling with the breeding success of their wolverines: (1) they should pay attention to provide the best adequate environment for wolverines, (2) they should have good husbandry practices, particularly focusing on the keepers' routine,

(3) special attention should be given to provide the best diet, (4) effort should be put to look at the compatibility of each wolverine pair and (5) they should be aware of any abnormal behavior. Moreover, all institutions – no matter their breeding success - should try to train their animals adequately, since research shows that it is beneficial for the animals.

It is important to keep in mind that other factors, which have not been investigated in this study, may also have an effect on the breeding success of the wolverine and should also be considered. For instance, this study did not investigate the effects of moving animals to new environments or the introduction of new animals. It also did not investigate the origin of the animals. Moreover, this study did not focus on the different personalities each wolverine can have. Further research contemplating the factors not included in the study is needed.

One also needs to consider that this study was based on if the factors were present or not, but not their quality. So further research is needed to study the quality of these factors. Moreover, further research is needed in learning more on wolverine reproductive behavior overall.

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APPENDIX I

SURVEY FOR HUSBANDRY PARAMETERS IN CAPTIVE WOLVERINES (*Gulo g. gulo*)

This survey is sent to all holders of wolverines (*Gulo g. gulo*) included in the wolverine EEP Program of EAZA. The results of this survey will help identify factors (such as enclosure design, nutrition, human-animal interaction, health) that lead to breeding success in captive wolverines. Institutional and individual data will be processed anonymously.

The expected time to complete this survey is 15min, but there is no time limit to finish it. Answers are NOT saved if the user returns to the survey later without having it submitted first. Once it is submitted, answers CANNOT be edited. It is therefore advisable to have all the possible useful information available before filling in the survey. However, it is possible to go back and forward through the survey without losing the answers. Unless specified, questions are required an answer.

If you have any questions about the survey or if you have more useful information about your wolverines that you would like to share, please do not hesitate to contact Eva Andersson (EEP Coordinator Wolverine) via eva.andersson@nordensark.se.

Thank you very much for your collaboration, your participation is important!

Please, write the name of your institution:

[*enter short text*]

In which city and country is your institution?

[*enter short text*]

GENERAL

How are the individuals held/kept? *(you can choose more than one option)*

In solitary

In a pair

In separated pairs

In a group or family

Other (specify)

Are the animals/breeding pair together all the time or only during breeding?

Together all the time

Only during breeding

They are never together

Other (specify)

When did your institution begin to hold the species?

Less than two years ago

2 – 5 years ago

6 – 10 years ago

11 – 20 years ago

>20 years ago

ENCLOSURE

Outdoor

How many outdoor enclosures holding wolverines does your institution have?

[*enter a number*]

What is the size of the enclosure/enclosures (m2)? *If more than one enclosure, please specify the size of each enclosure.*

[*enter short text*]

Are the enclosures open top?

Yes

No

Other (specify)

What type of material is the enclosure MOSTLY made of?

Mesh

Fence

Glass

Wall

Other (specify)

Does the enclosure have electric barriers/wires?

Yes

No

What kind of substrate does the outdoor enclosure have?

Natural grown

Grass

Gravel

Sand

Woodchips

Other (specify)

Does the enclosure have vegetation?

Yes

No

If yes, what kind of vegetation?

[*Short answer*]

Do they have places to hide?

Yes

No

Do they have the possibility to climb?

Yes

No

Is there a pond in the enclosure?

Yes

No

If yes, how big is the pond (m2)?

[*enter a number*]

Indoor accommodation

Do the enclosures have indoor accommodations?

Yes

No

If yes, how many indoor accommodations are there?

[*enter a number*]

What size are the indoor accommodations (m2)? *If there is more than one indoor accommodation, please specify the size of each enclosure.*

[*enter a number(s)*]

Are the indoor accommodations publicly displayed?

Yes

No

What kind of substrate do the indoor accommodations have?

Straw/Hay

Dust/Sawdust/Sand

Wood shavings/ Woodchips

Rubber mat/ Rubber floor

Other (specify)

None

Other

Are there off-public enclosures and/or accommodations?

Yes

No

Does your institution separate the animals from the keepers?

Yes

No

Other (specify)

Are the animals locked indoors during nights?

Yes

No

Other (specify)

If yes, what are the reasons for locking them in during nights? *(Not required)*

[*Short answer*]

What species are there in direct proximity to the wolverine enclosure?

[*Short answer*]

If possible, please upload photos or sketches that show if and where the following interior/items are located: *(Not required)*

Climbing apparatus, hiding places, caves, shade, bushes, trees, ponds, water and feeding troughs, dens, partitions, electrical points, service corridors, keeper exits and other furnishing designed to facilitate maintenance.

[*Upload image/document*]

NEST BOXES/DENS

Do they have the possibility to build a shelter/den themselves?

Yes

No

Are there nest boxes/dens provided?

Yes

No

If yes, how many?

[*enter a number*]

How are they designed? *(Not required)*

[*Upload image/document*]

Where are they located in the enclosure? *(Not required)*

[*Upload image/document*]

Is bedding material provided?

Yes

No

NUTRITION

Describe the weekly diet sheet for one individual.

[*Long answer*]

Do you have special feeding routines?

Yes

No

Other (specify)

How often are they fed during a week?

Once a day

Every second day

Every third day

Other (specify)

How do the animals get fresh water?

Bowl

Stream

Pond

Water nipple

Other (specify)

Is the food presented in an enriched way?

Yes, always

No, never

Only sometimes

Other (specify)

ENRICHMENT

Which of the following types of enrichment are used? *(you can choose more than one option)*

Sensory (e.g. visual, olfactory, auditory, taste)

Foods / Feeding (e.g. task-oriented puzzle feeders)

Manipulative / Toys (e.g. balls, boxes, bags, barrels)

Environmental (swings, climbing structures, hiding places)

Behavioral/Social (interact with other animals, artificial decoys)

None

If enrichment is used, is it regularly changed/renewed?

Yes

No

There's never enrichment

If possible, could you upload any photo, video or documents showing the enrichment used? *(Not required)*

[*Upload image/video/document*]

TRAINING

Are the animals trained?

Yes

No

If yes, what are you training? *(Not required)*

[*Short answer*]

If you train, are all individuals trained?

Yes

No, only adults (>2 years old)

No, only juveniles and/or cubs

No, only males

No, only females

Other (specify)

If possible, could you upload a video from a training session? *(Not required)*

[*Upload video*]

HEALTH

What are and have been the most common health problems?

[*Short answer*]

What are and have been the most common causes of death?

[*Short answer*]

Do the individuals or former individuals have or have had ticks?

Yes

No

Maybe

BEHAVIOR

Do the current individuals express any stereotypic behaviors?

Yes

No

Maybe

Other (specify)

If yes, what kind of stereotypic behaviors? *(you can choose more than one option)*

Pacing

Head bobbing

Somersault

Other (specify)

If possible, could you upload a video of the stereotypic behaviors? *(Not required)*

[*Upload video*]

Have former individuals expressed any stereotypic behaviors?

Yes

No

Maybe

If yes, what kind of stereotypic behaviors? *(you can choose more than one option)*

Pacing

Head bobbing

Somersault

Other (specify)

If possible, could you upload a video of the stereotypic behaviors? *(Not required)*

[*Upload video*]

HUMAN-ANIMAL INTERACTION

This section is to determine whether visitors can have effects on mating behavior and birth of the cubs.

Is your institution open all year around?

Yes, all year around

No, only seasonally

Other (specify)

When is the high season for your institution? *(Specify answer in months: e.g. June - September)*

[*Short answer*]

How many visitors a day does your institution have on average during high season?

[*enter a number*]

How many visitors a day does your institution have on average during low season?

[*enter a number*]

BREEDING

Does your institution ever have successful breeding (live cubs born)?

Yes

No

What do you think is the main reason for the successful/not successful breeding?

[*Short answer*]

Have the keepers ever seen courtship and mating behavior on the former and/or current breeding pairs?

Yes

No

Only on the former

Only on the current

Has the institution ever have had cubs that died?

Yes

No

Maybe

If yes, what was the cause of death or what do you think was?

[*Short answer*]

[SEND]

Your response has been recorded. Thank you very much for your collaboration!

If you have any questions or comments, or if you want to send more information about your wolverines, do not hesitate to contact Eva Andersson (EEP Coordinator Wolverine) via eva.andersson@nordensark.se.