UNIVERSITY OF CALGARY

Reproductive Behaviour of Vancouver Island Marmots, Marmota vancouverensis:

Conclusions from a Conservation Breeding Program

by

Diane L. Casimir

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Reproductive Behaviour of Vancouver Island Marmots, Marmota vancouverensis: Conclusions from a Conservation Breeding Program" submitted by Diane L. Casimir in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

I examined captive *Marmota vancouverensis* behaviour during the reproductive season, and compared the activity and sociality of captive and wild pairs. Captive pairs exhibited greater activity levels and similar sociality rates compared to wild conspecifics, suggesting that the captive environment has not caused negative behavioural alterations. Captive pairs engaged in social interactions more commonly in nest boxes than in enclosures. Since nest boxes and natural burrow chambers are likely structured and used similarly, I suggest that, in wild animals, social interactions are greater in burrows than above ground. In captive pairs, male dominance, female aggression and copulation duration were positively associated, and female solicitation of male attention was negatively associated, with pup production. Monitoring these behaviours will assist in assessing pairings to increase reproductive success and the number of animals available for reintroduction to the wild. My findings provide considerations for future marmot behaviour studies and the *M. vancouverensis* recovery program.

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CHAPTER 1

General Introduction and Methods

INTRODUCTION

The World Conservation Union estimates that the current species extinction rate is at least two, and possibly three, orders of magnitude higher than the background rate typical of the planet's geological history (Baillie et al. 2004). In response to this crisis, the organization has adopted a policy endorsing conservation breeding as a supportive intervention to avoid the loss of many species, with the ultimate goal of maintenance and/or reestablishment of viable wild populations (Baillie et al. 2004). Conservation breeding and reintroduction programs have contributed to the recovery of a number of threatened species (Baillie et al. 2004; Kleiman 1989).

One commonly encountered difficulty these programs face is the achievement and maintenance of viable captive populations, due to unreliable or low reproductive success (Snyder et al. 1996; Wielebnowski 1998). Behavioural studies can provide information to improve reproductive rates in captivity and aid in the production of animals for reintroduction (Carlstead et al. 1999b; Wielebnowski 1998, 1999). In addition, the preservation of natural behaviours should be a key consideration in conservation breeding and reintroduction programs; the behaviour of animals in the program should be evaluated, and compared to that of wild populations, to help ensure that released animals are behaviourally equipped to interact with conspecifics and survive in the wild (Blumstein et al. 2001; McPhee 2003). Finally, rigorous research within these programs can provide improved knowledge for management of wild populations (Hutchins & Conway 1995).

The squirrel family, Sciuridae, is comprised of some 51 extant genera and 272 species that are found in a wide variety of habitats on all continents, except for Australia, Antarctica and Madagascar (Nowak 1999). Nearctic sciurid species fall into nine genera: flying squirrels (*Glaucomys*), tree squirrels (*Sciurus* and *Tamiasciurus*), chipmunks (*Tamias* and *Eutamias*), prairie dogs (*Cynomys*), ground squirrels (*Spermophilus* and *Ammospermophilus*), and marmots (*Marmota*; Nowak 1999). Of the world's sciurid species evaluated by the World Conservation Union, nine are endangered and 21 are vulnerable. Seven of these species are North American: three that are endangered, the Idaho ground squirrel (*Spermophilus brunneus*), Nelson's antelope squirrel (*Ammospermophilus nelsoni*), and the Vancouver Island marmot (*Marmota vancouverensis*), and four that are vulnerable, Franklin's ground squirrel (*Spermophilus franklinii*), the Mohave ground squirrel (*Spermophilus mohavensis*), the Washington ground squirrel (*Spermophilus washingtoni*), and Palmer's chipmunk (*Tamias palmeri*; IUCN 2004).

North American members of the sciurid family are primarily diurnal, with the exception of flying squirrels, which are nocturnal (Nowak 1999). All North American genera use nests for shelter, refuge from predators, thermoregulation, and parturition and rearing of young (Barash 1989; Layne & Raymond 1994; Michener 1984; Nowak 1999). Tree-dwelling sciurids (flying squirrels and tree squirrels) primarily make nests in hollow trees and branches; these species do not hibernate but, during the winter, animals may stay in their nest for several days and ingest cached food (Nowak 1999). Ground-dwelling sciurids (chipmunks, prairie dogs and marmots) differ from tree-dwelling sciurids in that they nest in ground burrows. Most species in these genera become dormant during the

winter. Some become torpid for short periods of time and then live off of food stored in their burrows (e.g., eastern American chipmunks, *Tamias striatus*, and western American chipmunks, *Eutamias ruficaudus*; Banfield 1974; Nowak 1999) or come above ground to find food (e.g., black-tailed prairie dogs, *Cymonys ludovicianus*; Hoogland 1995). Marmots, ground squirrels, the while-tailed prairie dog (*C. leucurus*), the Utah prairie dog (*C. parvidens*), and Gunninson's prairie dog (*C. gunnisoni*) are true hibernators, living off of their fat reserves during the winter (Barash 1989; Michener 1984; Hoogland 1995). Hibernation duration varies widely both among genera and species, and may even vary within populations of the same species (Barash 1989; Michener 1984).

North American sciurids exhibit a variety of mating systems. Monogamy occurs in some populations (e.g., the Alaskan hoary marmot, *Marmota caligata*; Holmes 1984) and species (*M. vancouverensis*; Bryant 1996; Heard 1977), but polygyny is far more widespread (Barash 1989; Hoogland 1981; Koford 1982; Schwagmeyer 1990). Flying squirrels, tree squirrels and chipmunks may have one to two mating seasons in a year (Banfield 1974; Ferron & Prescott 1977; Goertz et al. 1975; Smith & Smith 1975). In tree squirrels, female estrus is generally asynchronous; adult males generally follow females, assessing their reproductive state, and fight with other adult males over estrous females (Heaney & Thorington 1978; Koford 1982; Thompson 1977). The eastern chipmunk (*Tamias striatus*) appears to have a similar mating system to tree squirrels. Males do not defend a territory or females, and instead follow and fight over estrous females (Yahner 1978).

Most prairie dogs, ground squirrels and marmots have a single mating season each year that commences shortly after emergence from hibernation (Michener 1984), and generally lasts for two to three weeks (Dobson 1984). Mating systems can vary among species, populations, or even across time within populations. Schwagmeyer (1990) classifies the polygynous systems of these genera into three groups: female/harem defense polygyny (sexually active males defend areas in which females reside), male dominance polygyny (males aggregate near female hibernacula, and females solicit matings with dominant males), and scramble competition polygyny (males search extensively for mating opportunities). The most common of these is female/harem defense polygyny, where male dominance is site-related during the breeding season (males tend to win aggressive disputes with competitors when they occur within their home range, and lose them when they take place elsewhere; Dobson 1984). In this system, the number of mates per male tends to be quite small, for example, harems of 2.3 adult females in *M. flaviventris* (Armitage 1986), and 3.7 adult females in *C. ludovicianus* (Hoogland & Foltz 1982).

Above ground reproductive behaviour has been described in some prairie dogs, ground squirrels and marmots (e.g., Armitage 1965, 2003; Barash 1973a, 1989; Hoogland 1995; Michener 1983; Michener & McLean 1996). For example, male Richardson's ground squirrels (*Spermophilus richardsonii*) seem to constantly monitor the estrous status of females, by approaching them and sniffing their genital regions (Michener 1983). Estrous females usually neither flee nor chase the male when he approaches, but pregnant females respond aggressively to approaching males (Michener 1983). However, in many species within these genera, the majority of reproductive behaviour occurs in burrows and, therefore, is difficult to observe (Barash 1989; Schwagmeyer 1990).

Some above ground behaviours that have been identified as being associated with mating in marmots include: approaches, sniffs, chases, play-fights, attempted mounts and apparently successful mounts (e.g., Armitage 1962, 1965; Barash 1973a, 1989; Heard 1977). Above ground descriptions of post-mating behaviour include female nesting behaviour (Armitage 2003), and a decrease in the frequency of social interactions between adult males and adult females (Holmes 1984). However, there exist large gaps in our knowledge of marmot reproductive behaviour, primarily because animals cannot be observed in natural burrows. This not only hinders our general knowledge of marmot species, but also may inhibit conservation efforts aimed at securing the future of these species.

With fewer than 150 animals in existence, *Marmota vancouverensis* (Swarth 1911) is North America's most endangered mammal. Endemic to Vancouver Island, British Columbia (Nagorsen 1987), *M. vancouverensis* is a distinct species that differs from other marmots in skull morphology (Cardini & O'Higgins 2004), pelage (Nagorsen 1987), vocalizations (Blumstein 1999), and behaviour (Heard 1977). Animals are typically found on south to west facing slopes of sub-alpine meadows, ranging from 1040 to 1450m in elevation (Bryant & Janz 1996). These natural meadows are kept clear of trees by avalanches, snow creep (Milko 1984), and fire (Hebda et al. 2005; Milko 1984). Like the other 13 species in the genus, *M. vancouverensis* uses underground burrows (Barash 1989; Heard 1977), consumes a variety of grasses and forbs during the 4-5 month active season (Martell & Milko 1986; Milko 1984), and hibernates for 7-8 months during the winter (Barash 1989; Swarth 1912).

Marmota vancouverensis lives in highly social colonies, consisting on average of 8.3 animals prior to the emergence of young of the year (Heard 1977). Colonies are generally comprised of one or two family groups (Bryant & Janz 1996), and a typical

family group consists of an adult male, one or two adult females, and a variable number of two year olds, yearlings, and young of the year (Heard 1977). The species primarily displays a monogamous mating system (Bryant 1996; Heard 1977), although polygyny may also occur (Bryant 1996). Animals are relatively slow to mature (Bryant 1996); common age of first reproduction is three years in males and four years in females, but animals have reproduced as early as two years of age (Bryant in press). Females often skip one or more years between litters, and, on average, 3.4 pups are weaned per litter (Bryant in press). Offspring generally disperse to new colonies at two years of age (Bryant 1998), although dispersal is more common among males than females (Bryant 1996).

Marmota vancouverensis was first listed as endangered in Canada in 1978 (Shank 1999) and internationally in 1982 (IUCN 2004). Historically (pre 1970), animals were found on a minimum of 25 mountains, but historic population estimates are vague (Bryant & Janz 1996). Population counts starting in 1972 indicate that the population peaked at about 300 – 350 animals in the mid-1980's, due to colonization of regenerating clear cuts, but soon after entered a precipitous decline (Bryant & Janz 1996). At the end of the 2004 active season, population counts estimated 33 wild animals on 10 mountains (unpublished minutes, Vancouver Island Marmot Captive Management Group, Dec 2004).

The behaviour of wild *M. vancouverensis* was studied in the 1970s (Heard 1977), and the ecology of the species and possible reasons for decline were explored in the 1990s (Bryant 1996, 1998; Bryant & Janz 1996). The specific causes of decline are still being elucidated, although current work points to increased predation by wolves (*Canis lupus*), cougars (*Felis concolor*) and golden eagles (*Aquila chrysaetos*) due to habitat alterations

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associated with forestry (Bryant & Page in press), changes in habitat due to a decrease in fire disturbances, and climate change (Hebda et al. 2005).

Between 1997 and 2004, 56 wild-born animals were brought into captivity for a conservation breeding and reintroduction program (Bryant in press). The program currently involves four Canadian facilities: the Toronto Zoo (1997 – present), the Calgary Zoo (1998 – present), Mountain View Conservation and Breeding Centre (1999 – present), and the Tony Barrett Mount Washington Recovery Centre (2001 – present). At the end of 2004, there was a total of 93 animals housed among these facilities (unpublished minutes, Vancouver Island Marmot Captive Management Group, Dec 2004). Animals are generally housed as 'breeding pairs' (one male three years of age or older and one female four years of age or older), or as small groups of immature animals. All animals are owned by the British Columbia Ministry of Environment, and the recovery program is overseen by the Marmot Recovery Foundation.

My study was first developed in the summer of 2001, after successful production of pups by pairs housed at the Calgary Zoo but no pup production by pairs at the Toronto Zoo, and two years of reproductive rates that were lower than expected, despite the presence of copulation in most pairs. At that time, the Vancouver Island Marmot Captive Management Group (comprised of representatives from each breeding facility, the Marmot Recovery Foundation and the Vancouver Island Marmot Recovery Team) recognized that an understanding of reproductive behaviour was critical. However, although my study was endorsed by all of the groups listed above, there were numerous constraints imposed on it. I was required to work within the existing management regime and had no control over how animals were managed, such as which individuals were paired, where and how pairs were housed, when pups were removed from their parents, whether pairs were separated during the reproductive season, and feeding regimes. In addition, while I could, and did, make requests for males to be marked for each season, and cameras and recording systems to be installed in a manner that was conducive to detailed behavioural observations, decisions about my requests were at the discretion of each breeding facility and were only carried out in some cases. In general, experimental approaches are currently not possible in any studies of the captive or wild populations of *M. vancouverensis*. However, even with all of the constraints imposed on my study, I was still in a unique position to be able to examine the reproductive behaviour of this species.

In general, the reproductive behaviour of marmots is not well understood, as much of it occurs in underground burrow systems (Barash 1989). The probability of a pair of producing a litter in the *M. vancouverensis* conservation breeding program has improved and is now generally comparable to that in the wild (Bryant in press), but our understanding of reproductive behaviour in this species and of factors associated with its successful reproduction is still limited. The fact that some individuals who have not produced pups in the past have produced pups after being re-paired suggests that unsuccessful reproduction is not necessarily due to an individual's inability to reproduce, and that the behaviour of individuals in a pair may contribute to successful reproduction. In addition, the artificial burrow chambers provided in this program are in the form of above ground nest boxes, which creates a rare opportunity to study behaviours that normally occur underground and thus are difficult to observe.

The aim of this thesis is to examine the reproductive behaviour of breeding pairs of captive *M. vancouverensis* during the reproductive season. For this study, I defined the

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reproductive season as the period of time from hibernation emergence until parturition for pairs that produced pups, and until 64 days after emergence for pairs that did not produce pups, as this was the longest period of time between emergence from hibernation and parturition for any pair that produced pups during my study. I defined reproductive behaviour as all behaviours that occur during the reproductive season. This study is unique in several ways. It is the first to directly compare the behaviour of captive and wild marmots, and the behaviour of captive marmots in enclosures and artificial burrow chambers. In addition to providing information on the behaviour of animals in the conservation breeding program and how that compares to wild animals, these comparisons provide the first opportunity to make inferences about the behaviour of wild marmots in their burrow chambers. In addition, it is the first study in which examinations of behavioural differences between captive marmots that are subsequently successful and unsuccessful in producing pups throughout the reproductive season in both enclosures and artificial burrow chambers occur. The knowledge gained from this study can be used towards the recovery of this critically endangered species and, I hope, to further our knowledge and help secure the future of all marmot species.

METHODS

Study Sites

Breeding pairs observed in this study were housed either at the Calgary Zoo's Devonian Wildlife Conservation Centre (hereafter referred to as DWCC) in DeWinton, Alberta, Canada (50°54'N, 114°42'W, 1400m) or at the Toronto Zoo in Scarborough, 9

Ontario, Canada (43°49'N, 79°10'W, 143m). At both facilities, the enclosures housing M. *vancouverensis* were in areas not open to the public and were maintained under quarantine protocols.

Calgary Zoo

Breeding pairs were observed at the DWCC during the 2002 to 2004 reproductive seasons. A set of eight enclosures (each consisting of an indoor and outdoor enclosure) was used for housing breeding *M. vancouverensis* pairs (Figure 1.1). In 2002, all enclosures were occupied by marmots, but only three were occupied by breeding pairs. Three breeding pairs each had access to one indoor and one outdoor enclosure, with a nest box in each location. In 2003, five enclosures were occupied by marmots but, once again, only three were occupied by pairs of breeding age (three pairs, each with access to one indoor and one outdoor enclosures were occupied by pairs of breeding age (three pairs, each with access to one indoor and one outdoor and two outdoor enclosures, with a nest box in each had access to two indoor and two outdoor enclosures, with a nest box in each indoor enclosure.

Indoor enclosures were in a building and measured 3.4 x 2.1 x 2.3m (length x width x height), and outdoor enclosures extended from the outside wall of the building and measured 3.7 x 2.1 x 2.3m. Enclosures had a concrete base, covered with straw (and corn cob bedding at latrine sites) in the indoor enclosures and gravel and soil in the outdoor enclosures. All enclosures were completely enclosed with 2.5cm wire cloth mesh with a steel frame. For the 2004 reproductive season, plywood barriers were placed over the mesh separating every other enclosure, so that breeding pairs could not see each other. Straw and/or hay bales, logs and/or tree stumps, and PVC piping were often placed in enclosures



Figure 1.1. *Marmota vancouverensis* enclosures at the Calgary Zoo's Devonian Wildlife Conservation Centre

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as nesting material, resting places and refuge spots, respectively. Nest boxes were constructed of 0.3cm steel and measured $1.2 \ge 0.6 \ge 0.8$ m, with a 25cm (diameter) opening at each end for marmot access. Straw was placed inside the nest boxes as bedding, and the marmots supplemented this with straw and hay provided in the enclosures.

Access to outdoor enclosures was controlled by animal care staff and generally not available on extremely cold or inclement days. Some natural light filtered into indoor enclosures through two windows above the enclosures, but supplemental lighting was also provided. Throughout the study some lighting was provided through fluorescent bulbs in the building's ceiling. Initially these lights were maintained on an electrical timer set to approximate natural light cycles. However, power surges in the building (due to the remote location of the DWCC) consistently altered the timer's program. Therefore, for the majority of the season the timer was de-activated and lights were manually turned on between 07:30 and 10:00 and turned off between 16:00 and 21:00, depending on the schedule of animal care staff. In 2003 and 2004 additional 60 or 100W full-spectrum incandescent bulbs were placed above indoor enclosures and connected to a photocell placed in one of the windows. The photocell activated the lights at sunrise and de-activated them at sunset. Outdoor temperatures influenced indoor enclosure temperatures, but animal care staff regulated them to the best of their ability, aiming to keep them between 10 and 20°C. An alarm was set to alert animal care staff if the building temperature dropped below 0°C or rose above 30°C. During hibernation, the animals were maintained in their respective indoor enclosures and the building temperature was maintained between 5 and 7°C. Pups born in a particular season were housed with their parents throughout the

remainder of the active season and hibernation; they were then moved to a separate enclosure prior to emergence the following active season.

Generally, food was not provided to adult pairs during hibernation. However, in some years, pairs hibernating with their pups were provided with small amounts of rabbit pellets (United Feeds Inc., Sheridan, IN), although the food was rarely consumed. Water was provided ad libitum throughout hibernation but also was rarely consumed. When animals showed signs of emergence in spring, rabbit pellets were provided to all pairs and, as activity levels increased, Mazuri[®] leaf-eater primate diet mini-biscuits (Mazuri, St. Louis, MO) and a selection of vegetables such as spinach, carrots, and yams were added to the diet. Feedings during the active season occurred once per day. In addition, dandelion heads, branches of browse (such as willow, poplar and aspen), and limited amounts of raisins and corn on the cob (in 2002 and 2003 only) were occasionally provided as food enrichment. Water was provided ad libitum throughout the active season.

Animal care staff handled animals approximately once per month during hibernation, when animals were torpid, to monitor the mass of individuals, but animals were never handled during the active season unless a medical emergency necessitated it. In addition, noise levels and entry into enclosures were restricted all year, especially during the reproductive season.

Toronto Zoo

Pairs were observed at the Toronto Zoo during the 2002 and 2003 reproductive seasons. Two sets of two indoor enclosures were located in the quarantine area of the Zoo's Animal Health Center (hereafter referred to as AHC: Figure 1.2). In 2002 both sets housed pairs of breeding age *M. vancouverensis*, but in 2003 only one of the sets housed a

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breeding pair and the other housed a single female and her yearling offspring. Each set of enclosures measured $3.1 \ge 1.6 \ge -2.3$ m (roof sloped from 2.6 to 1.9m) and $3.1 \ge 3.6 \ge 2.6$ m. Two additional sets of enclosures were located in the 'outdoor holding' section of the Zoo (hereafter referred to as OH: Figure 1.2). In both 2002 and 2003, a breeding pair was housed in each set of OH enclosures. These consisted of one indoor enclosure measuring $2.2 \ge 2.2 \ge 2.7$ m and one outdoor enclosure measuring $2.8 \ge 2.8 \ge 1.8$ m.

All enclosures had a concrete base covered in straw (and aspen shavings at latrine sites) in the indoor enclosures, and coarse sand in the outdoor enclosures. All indoor enclosures were enclosed with solid walls. Outdoor enclosures were surrounded with plexiglass from the ground to a height of 1.2m then vinyl-covered 1.3cm chain link fencing. In addition to the provision of straw and hay bales for resting places and nesting material, and sections of PVC piping for refuge spots, shelves, wooden ramps and/or tree stumps were provided in most enclosures. Each pair had access to two or three polypropylene nest boxes that generally measured 0.8 x 0.6 x 0.5m. However, sometimes a larger polypropylene nest box measuring 1.2 x 0.6 x 0.6m was provided. Nest boxes had two 18cm (diameter) openings on two sides for marmot access. In 2002, plywood was placed on the bottom of nest boxes. Primarily straw, but sometimes hay and wood shavings, were placed inside the nest boxes for bedding; the marmots supplemented this with straw and hay provided in their enclosures. In both 2002 and 2003, each pair had access to either one small and one large enclosure in the AHC with one nest box placed in each enclosure, or one indoor and one outdoor enclosure in the OH with two nest boxes placed in the indoor enclosure and one in the outdoor enclosure. Access to outdoor enclosures was controlled by animal care staff and was not available on cold or inclement days.

Lighting for the indoor enclosures in the AHC was provided through skylights and supplemented with fluorescent bulbs on an electrical timer that was adjusted by 15-minute increments throughout the season to approximate natural light cycles. Lighting for the indoor enclosures in the OH was primarily natural light from windows, but animal care staff turned on additional fluorescent lighting when working in the area. Outdoor temperatures influenced indoor enclosure temperatures but animal care staff regulated them to the best of their ability, aiming to keep them from exceeding 20°C. Animals were only maintained in the above enclosures during the active season. During hibernation they were maintained in an environmental chamber. Once animals settled into one of their nest boxes for hibernation the nest box was placed inside a cage in the chamber, and the chamber maintained at 6°C. Pups born in a particular season were housed with their parents throughout the remainder of the active season and hibernation, and then moved to a separate enclosure prior to emergence the following active season.

No food was provided to animals during hibernation. Water was provided ad libitum but was rarely consumed. When animals showed signs of emergence in spring, a rodent-lagomorph pellet diet (Toronto Zoo, Scarborough, ON) was provided to all pairs and, as activity levels increased, Mazuri[®] leaf-eater primate diet mini-biscuits and a selection of vegetables were added to the diet. Vegetables offered consisted mostly of spinach, romaine lettuce, kale and dandelion leaves, with small amounts of broccoli, cauliflower, carrots, yams and beets. Limited amounts of corn on the cob and dandelion heads were occasionally offered as well. Feedings occurred once per day during the active season and water was provided ad libitum. Animals were handled approximately once per month during hibernation to allow animal care staff to monitor their masses but they were never handled during the active season unless necessary due to a medical emergency. In addition, noise levels and entry into enclosures was restricted.

Study Animals

All pairs observed were considered 'breeding pairs', as described above. Using closed-circuit video cameras, I observed three pairs at the DWCC and four pairs at the Toronto Zoo in 2002, three pairs at the DWCC and three pairs at the Toronto Zoo in 2003, and three pairs at the DWCC in 2004. Due to limitations in or challenges of camera and recording equipment at other facilities, observations were limited to animals housed at these two facilities. In addition, because the Vancouver Island Marmot Captive Management Group often decided to maintain some of the same pairs at the same facility in consecutive years, in some cases the same pair was observed for more than one year (see Table 1.1).

Adult male *M. vancouverensis* are larger than adult females (Nagorsen 1987), but size differences were often difficult to discern on camera when the animals were not situated directly next to each other. In addition, while some adult animals have molt patterns (Nagorsen 1987), these often changed over the active season and generally were not visible on enclosure cameras. Consequently, all males in breeding pairs housed at the DWCC were marked so they could be reliably distinguished from their mates. Requests were made to the Toronto Zoo to mark the males in breeding pairs housed at that facility, but this was only done for two males for the 2003 reproductive season. However, in 2002,

							# days	# days start	
			Emergence	Analysis	Parturitio	Analysis	emergence	to end of	# days
Year	Facility	Pair	date	start date	n date	end date	to parturition	observation	observed
2002	DWCC	Gudron/China	2-Apr	2-Apr	Х	3-Jun	X	64	57
2002 .	DWCC	Ivan/Boadecia	24-Mar	24-Mar	Х	27-May	X	64	54
2002	DWCC	Houdini/Judy	7-Apr	7-Apr	20-May	20-May	44	44	44
2002	Toronto	Chase/Babe	12-Mar	14-Mar	5-May	5-May	55	53	34
2002	Toronto	Yang/Naomi	6-Apr	7-Apr	8-Jun	2-Jun	64	57*	30
2002	Toronto	Washington/Stumpy	9-Apr	11-Apr	Х	7-Jun	X	58	57
2002	Toronto	Caruso/Larry	9-Apr	11-Apr	Х	5-Jun	Х	56	56
2003	DWCC	Gudron/China	24-Mar	24-Mar	14-May	14-May	52	52	52
2003	DWCC	Ivan/Boadecia	22-Mar	22-Mar	4-May	4-May	44	44	44
2003	DWCC	Houdini/Judy	22-Mar	18-Apr	Х	24-May	Х	37**	34
2003	Toronto	Yang/Naomi	14-Apr	16-Apr	26-May	26-May	43	43	33
2003	Toronto	Washington/Stumpy	3-Apr	4-Apr	18-May	18-May	46	45	34
2003	Toronto	Caruso/Larry	3-Apr	4-Apr	Х	19-May	Х	46	28
2004	DWCC	Ivan/Boadecia	22-Mar	22-Mar	30-Apr	30-Apr	40	40	23
2004	DWCC	Houdini/Ursula	24-Mar	24-Mar	Х	26-May	Х	64***	25
2004	DWCC	Franklin/Mirabel	24-Mar	24-Mar	Х	26-May	X	64	42

Table 1.1: Emergence from hibernation and parturition dates, and behavioural observation schedule, for adult *M. vancouverensis* pairs at the Calgary Zoo's Devonian Wildlife Conservation Centre (DWCC) and the Toronto Zoo during the 2002 to 2004 reproductive seasons. X replaces data for pairs that did not produce pups.

* VCR broken for the week leading up to parturition

** pups from 2002 were left with pair until 18-April. Impossible to tell female from pups therefore analysis not started until they were removed

*** pair separated from 2-May through 22-May; therefore, no analysis

one of the females housed at Toronto was missing fur on the majority of her rear end, making it easy to distinguish her from her mate.

Marking was conducted during the late summer or fall annual veterinary exams, while animals were anaesthetized. An approximately 2.5cm band of fur across the male's shoulder blades was moistened with 6% hydrogen peroxide and *Absolute Blond* Clairol[®] Hydrience hair dye (Proctor and Gamble, Stamford, CT) was applied to the moistened area. The products rarely came in contact with the marmot's skin. The fur was then dried with a hair dryer on low setting, to avoid the possibility of the marmot getting its eyes, nose or mouth in contact with the products when it awoke. The resultant mark appeared approximately 24 hours after application, was generally rufous in colour, lasted throughout hibernation and the reproductive season, and was molted out towards the end of the summer.

Data Collection

Video Systems

To view marmot behaviours non-invasively, closed-circuit cameras were installed in nest boxes and enclosures and connected to recording systems. Efforts were made to install cameras in every nest box and enclosure available to each pair although this was not always possible. Details of camera coverage for behavioural observations are provided in Chapters 2 and 3.

Calgary Zoo

At the DWCC, most enclosure cameras were colour with 2.8mm lenses (custom manufactured by Future Developments Ltd, Calgary, AB) and placed in Pelco_® DF5-1

dome enclosures (Pelco Canada, Lachine, QC), but in a few cases Sony_® Digital Hyper HAD SSC DC14 Color Video Cameras with 2.8 or 3.5mm lenses (Sony of Canada Ltd, Toronto, ON) were used in indoor enclosures instead. All cameras were mounted in the centre of the enclosure's roof, providing a bird's eye view of the enclosure. Nest box cameras were Extreme CCTVTM EX45N Black and White Night Vision Cameras with 3.6mm lenses and 940nm LEDs (Extreme CCTV Surveillance Systems, Burnaby, BC). These were mounted in a corner of each nest box's roof and the image shot diagonally across and down into the nest box.

Cameras were connected to Everfocus[®] EverPlex 4CQ Color Quad Processors (Everfocus, San Marino, CA), which were in turn connected to Sanyo SRT 2400 or 4040 Time Lapse VCRs (Sanyo Canada Inc., Concord, ON) and Panasonic_® CT-1386YD Color Video Monitors (Panasonic Canada Inc., Mississauga, ON). With this setup, four cameras could be viewed on the same screen simultaneously, the date and time of recording were recorded on the videotape, and animals could be tracked as they moved through their nest boxes and enclosures. VCRs were in use 24 hours per day, seven days per week and tapes were archived. Animal care staff changed tapes daily at approximately 08:30. In 2002, MaxellTM T120 videotapes (Maxwell Technologies Inc., San Diego, CA) were used and VCRs were set to record at 30-hour speed (12 fields/sec). In 2003 and 2004 3M Pro-T162 videotapes (3M, London, ON) were used and VCRs were set to record at 24-hour speed (20 fields/sec). Tapes were played on a Sanyo SRT 2400 or 4040 Time Lapse VCR connected to a Panasonic CT-1386YD Color Video Monitor. Tapes from 2002 were played back at 6hour (60 fields/sec), 18-hour (20 fields/sec) or 30-hour (12 fields/sec) speed during analysis. Tapes from 2003 and 2004 were played back at 8-hour (60 fields/sec) or 24-hour (20 fields/sec) speed during analysis.

Toronto Zoo

At the Toronto Zoo, enclosure cameras were Silent Witness V60N Black and White Video Cameras (Honeywell Video Systems, Louisville, KY) or Sanyo VDC9212 Black and White Night Vision Cameras with 2.8 or 3.5mm lenses. AHC cameras were mounted outside the enclosure and the image shot through a glass window, and OH cameras were mounted in an upper corner of the enclosure. Nest box cameras were Sanyo VDC9212 Black and White Night Vision Cameras with 5.0mm lenses and 880nm LEDs or Extreme CCTV EX45N Black and White Night Vision Cameras with 3.6mm lenses and 940nm LEDs. These were mounted in the centre of the roof of nest boxes and shot straight down into the nest box, but were only installed in nest boxes during the active season (i.e., nest boxes were not recorded when in the environmental chamber but a camera was installed in the upper corner of the chamber). Cameras were connected to Robot Monochrome MV47 Black and White Quad Processors (American Dynamics, San Diego, CA) in the AHC and National Electronics NLMQ4 Black and White Quad Processors (Burtek Systems, Burnaby, BC) in the OH. These were connected to Sanyo TLS924, TLS900 or TLS9072 Time Lapse VCRs and, in the AHC only, Pelco PMM15A or National Electronics M1205 Black and White Video Monitors. Once pairs were moved from the environmental chamber to their enclosures, all available enclosure and nest box cameras and VCRs were in use 24 hours per day, 7 days per week and tapes were archived. Animal care staff changed tapes daily at approximately 07:00. Throughout both seasons, MaxellTM T120 videotapes were used and VCRs were set to record at 24-hour speed (15 fields/sec). Copies of tapes were sent to the Centre for Conservation Research for analysis, and played back on a Sanyo TLS 4072 Time Lapse VCR connected to a Panasonic CT-1386YD Color Video Monitor. Tapes were played back at 2-hour (180 fields/sec), 12-hour (30 fields/sec) or 24hour (15 fields/sec) speed during analysis.

Behavioural Data

I created a mutually exclusive and exhaustive ethogram consisting of 28 behavioural states (see Appendix 1). The ethogram was based on a study of wild *M. vancouverensis* (Heard 1977), studies of other marmot species (Armitage 1962, 1965, 1973, 1974; Barash 1973a, 1989; Concannon et al. 1997b; Lenti Boero 1995; Perrin et al. 1993a; 1993b), and preliminary observations of *M. vancouverensis* videotapes from the DWCC. Where possible, the initiator and recipient of all social interactions were recorded. However, the social behaviour was recorded for the recipient only if they acknowledged the behaviour. For example, if a female was eating and a male sniffed her genital area, the 'genital sniff' was always recorded for the male, but only recorded for the female if she acknowledged it, thereby interrupting her eating session. The location where behaviours occurred (i.e., nest box or enclosure) was also noted. If a behavioural state started in one area and moved to another (e.g. chase/follow) the area in which the behaviour started was recorded as its location.

I conducted the majority of analyses, but research assistants and volunteers assisted in some of the analyses in which the task was simply to document the occurrence and duration of mounts. To ensure high consistency among observers, before allowing them to conduct analyses on their own, I reviewed tapes with them and then tested inter-observer consistency of the detection of mounts and measurement of mount duration.

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Dates of emergence from hibernation were calculated independently for each pair. I defined day zero of the active season as the first day a pair left it's hibernation nest box and then left it every consecutive day throughout the active season (to distinguish hibernation emergence from an arousal from torpor bouts, in which animals may leave their nest box on a particular day but then return to it and become torpid again for a number of days). For pairs housed at the DWCC, videotapes were used to determine the emergence date. All pairs housed at the Toronto Zoo were already leaving their nest box daily when they were transferred from the environmental chamber to their enclosures and, because videotapes from the chamber were not archived after being reviewed by animal care staff, they could not be used to calculate an emergence date. Therefore I used the daily notes of animal care staff to determine when the pairs began to leave their nest boxes consistently.

I analyzed videotapes from a pair's emergence date through to parturition for pairs that produced pups, and to 64 days after emergence for pairs that did not produce pups. I chose 64 days because it was the longest period of time between emergence and parturition for any pair that produced pups over the 2002 through 2004 reproductive seasons. However, in some cases it was possible to observe animals for only a portion of the analysis period. Because videotapes of the environmental chamber at the Toronto Zoo were not archived, analysis of Toronto Zoo animals always started one to two days after the calculated emergence date. In addition, in 2002 a VCR used to record one pair at the Toronto Zoo malfunctioned and therefore it was not possible to analyze tapes for the final week before parturition. At the DWCC in 2003, one pair had its four yearling offspring from the previous year with them for the first 27 days after emergence and it was impossible to distinguish the adult female from her yearling offspring; therefore, analysis was not started until the yearlings were removed. In addition, in 2004 one pair at the DWCC was separated for 21 days because animal care staff felt copulation frequency was unusually high and they were concerned about the appearance of blood in the enclosure; tapes from days when the pair was separated were not analyzed. Table 1.1 outlines emergence from hibernation, parturition and videotape analysis start and end dates.

I collected behavioural data from every tape or every second tape, both within and among pairs. Analysis of every second tape yielded similar estimates of the proportion of time spent in behaviours to those from analyses of every tape, both when all behaviours and when only rare behaviours (those occurring less than one percent of the time) were considered (Figure 1.3a, b).

During the course of the study, I conducted 15,528 hours of behavioural observations. Each pair was observed for an average of 40.4 ± 3.0 24-hour days (Table 1.1). Pairs were included or excluded from different types of behavioural data collection (e.g. all behaviour versus solely the occurrence and duration of mounts) in the nest box and/or enclosure based on: 1) whether individual identification was possible (generally individual identification in the enclosure was possible only when males were marked), 2) how many nest boxes and enclosures available to a pair had cameras in them, 3) the proportion of time individuals were visible on camera (i.e., how well the camera covered the nest box or enclosure) and 4) whether the pair had been observed in previous years. Consequently not all analyses included data from every pair. Specific details of observations of animals are provided in Chapters 2 and 3.



Figure 1.3a. Mean proportion of time *M. vancouverensis* spent in all behaviours when tapes were sampled every day versus every second day (slope = 0.98, $r^2 = 1.0$). Each point represents a different behaviour (n = 28; vertical and horizontal bars represent standard error).


Figure 1.3b. Mean proportion of time *M. vancouverensis* spent in rare behaviours (those that occur <1% of the time) when tapes were sampled every day versus every second day (slope = 1.1, $r^2 = 0.96$). Each point represents a different behaviour (n = 22; vertical and horizontal bars represent standard error).

Statistical Analyses

The use of power analyses prior to beginning a study is generally accepted (Cohen 1969; Hoenig & Heisey 2001; Newton & Rudestam 1999; Thomas 1997). However, the use of them at the conclusion of a study is controversial. Evidence that both the p-value and the power are dependent upon the observed effect size and are inversely related demonstrates that retrospective power analyses do not add to the interpretation of a p-value (Hoenig & Heisey 2001; Thomas 1997). It was not possible to conduct power analyses before starting this study because there was no information on what effect size might be biologically important for *M. vancouverensis* or closely related species. In addition, sample sizes were determined by how many adult pairs were housed at each facility each year, and then by which pairs reproduced successfully.

Although I studied 42% of the world's captive population of adult pairs of *M. vancouverensis* over the three reproductive seasons, the final sample sizes were small (maximum n in a group = 11). These small sample sizes hindered the use of multivariate statistics to examine interactions between dependent variables (for example, mount duration and rate). In the exploratory analyses, such as those examining differences in individual behavioural categories between pairs that produced pups and pairs that did not, it would have been preferable to use discriminant analyses. However, due to the small sample sizes, it was only possible to examine variables separately with univariate analyses. A priori Bonferonni corrections (Newton & Rudestam 1999) were not applied to exploratory analyses of individual behavioural categories, increasing the probability of a Type I error; therefore, results from these analyses were interpreted with caution. I used parametric analyses, such as independent samples or paired samples t-tests, when the raw or transformed data met the test's assumptions. In all other cases the equivalent nonparametric analysis, such as Mann Whitney U, Wilcoxon signed ranks, Friedman's two way analysis of variance by ranks tests and Kolgomorov-Smirnov twosample tests, were used (Newton & Rudestam 1999). The power efficiency of Mann Whitney U and Wilcoxon signed ranks tests can be close to 95%, even with low sample sizes (Siegel & Castellan 1988). The power efficiency of a Friedman two way analysis of variance by ranks test can be greater than 64% for comparisons of two samples and greater than 80% for comparisons of five samples (Siegel & Castellan 1988). Specific details of statistical analyses are provided in Chapters 2 and 3. Finally, when sample sizes are small, Kolmogorov-Smirnov tests can be more powerful than their alternative parametric analysis, the chi-square test.

Data were analyzed using SPSS version 12.0, except for the multiple comparison between groups post-hoc test used on mount rate data in Chapter 3, which was done manually (Siegel & Castellan 1988). Parametric or nonparametric analyses are reported with means \pm standard error or medians, respectively. All tests were two-tailed and pvalues < 0.05 were considered to be significant.

CHAPTER 2

Behaviour of Vancouver Island Marmots in Enclosures and Artificial Burrows: Inferences about Natural Burrow Behaviour and Considerations for Species Recovery

INTRODUCTION

Some of the major considerations for species' recovery programs that involve conservation breeding and reintroduction include the availability of critical habitat, disease transmission, the behavioural repertoire of released animals, and the social interactions within and between released and wild populations (Beck 1994; Kleiman 1989, 1996; McPhee 2003; Price 1999; Snyder et al. 1996). However, wild animals that are housed in captivity are often thought to exhibit altered species-specific behaviour. Activity levels of captive animals can decrease because of a lack of appropriate space or reduced environmental complexity, or because the provisioning of food reduces searching times normally required by wild animals (Price 1999; Stevenson 1983). Housing arrangements in captivity can also alter the social nature of animals, increasing or decreasing the rate of social interactions from natural levels because of crowding or isolation, respectively (Price 1999). Behavioural changes such as these can reduce the success of reintroductions (Carlstead 1996; Kleiman 1989; Mathews et al. 2005; McPhee 2003; Price 1984, 1999). Studies of the behaviour of captive animals and comparisons between the behaviour of captive and wild animals are, therefore, crucial to the success of conservation breeding and reintroduction programs (Mathews et al. 2005; McPhee 2003).

In addition, programs aimed at the recovery of mammals that use burrows need to consider the role of in-burrow behaviour for the factors listed above. Underground burrow systems are a vital resource for many mammals, and may be used for a portion (e.g., ground squirrels and marmots) or all (e.g., mole-rats) of each day. There are few studies on the behaviour of wild mammals within burrows, likely because of the difficulty in accessing burrow chambers, although technology such as endoscopy has aided in conducting limited behavioural observations (Hut & Scharff 1998). Consequently, the vast majority of knowledge on the behaviour of burrowing mammals is based on their above ground activities (e.g., Armitage 1962; Barash 1973b; Heard 1977; Hoogland 1995; Michener & McLean 1996). In-burrow behaviour has been studied in a few captive populations of mammals, but most of these studies are conducted using 'visible burrow systems', which substitute transparent materials such as plexiglass for one or more burrow walls (e.g., Blanchard et al. 2001; Gazit & Terkel 2000; Hodara et al. 1997). However, only a few studies consider whether behaviours exhibited in visible burrow systems are representative of natural in-burrow behaviours (e.g., effects of light; Bennett 1992). A few published studies have observed behaviour in artificial burrows in captive environments through the use of red-light or infrared illumination and video cameras (e.g., Felicioli et al. 1997), but none have compared behaviour between artificial burrows and enclosures.

Prairie dogs, ground squirrels and marmots can spend 50 to 84% of their lives in burrows each year (Armitage 1965; Barash 1989; Hoogland 1995; Michener 2002; Svendsen 1976), and use burrows for hibernation in the winter, and copulation, parturition, rearing of young, escape from predators, and thermoregulation during the active season (Barash 1989; Burns et al. 1989; Michener 1984). Knowledge of in-burrow behaviour, and how it differs from above ground behaviour, is not only important for gaining a comprehensive understanding of the ecology and behaviour of species in these genera, but also may have consequences for hypotheses about sociality in these species. For example, research on ground squirrel and marmot sociality is based, in part, on the frequency and nature of social interactions among individuals within a group (Armitage 1981). However, the measures of social interactions that are used in hypotheses of sociality are based solely on above ground observations (Armitage 1981; Barash 1974a; Blumstein & Armitage 1998; Heard 1977) and ignore the contribution of in-burrow behaviour. Whether knowledge of in-burrow behaviour would lead to more accurate conclusions or facilitate further advances in this field is not clear, but our lack of knowledge on this topic prevents valid assessment of the possible benefits it may bring (King 1984).

There are no published studies of in-burrow behaviour for any wild marmots, or direct comparisons of the behaviour of captive and wild marmots. In addition, apart from a few studies that include limited observations of mating behaviours in cages or nest boxes (Concannon et al. 1997b; Exner et al. 2003; Keeley et al. 2003), the only comprehensive behavioural study of marmots in captivity, conducted on arctic marmots (*Marmota broweri*; Loibl 1983), focused solely on behaviours in enclosures. Vancouver Island marmots (*Marmota vancouverensis*) are the most endangered of the world's marmot species, and the current recovery strategy for the species includes a conservation breeding and reintroduction program (Janz et al. 2000). At all conservation breeding facilities, animals are housed in enclosures, and are provided with artificial burrow chambers, in the form of nest boxes. I studied the behaviour of adult *M. vancouverensis* pairs in the program during the reproductive season. I created behavioural profiles of captive animals, compared the behaviour of captive animals between enclosures and nest boxes, and compared the behaviour of captive animals in enclosures with that of wild animals above ground. In addition, by using the above comparisons, I am in a unique position to make suggestions about the behaviour of wild animals when they share burrow chambers.

Because not much is known about reproductive behaviour in wild M. vancouverensis. I have little basis upon which to pose null hypotheses about the reproductive behaviour of the species in captivity. Within the captive population, behavioural profiles may potentially differ between adult males and adult females, and between enclosures and nest boxes. For example, captive females may spend more time than captive males engaging in behaviours related to burrow maintenance and reproduction, such as gathering nesting materials, as seen in wild yellow-bellied marmots (M.flaviventris) and wild M. vancouverensis (Armitage & Van Vuren 2003; Heard 1977), and each captive male may spend more time pursuing its mate than each captive female, as seen in wild hoary marmots (M. caligata) and wild M. flaviventris (Armitage 1965; Barash 1974b). In addition, captive males may initiate more sexual behaviours than captive females, such as mounts and genital sniffs, and captive females may initiate more playfights than captive males in response to the male's advances, as observed in wild M. flaviventris (Armitage 1965). Finally, certain behaviours (e.g., alert poses and foraging) may occur primarily in enclosures and behaviours that likely reduce an animal's ability to remain vigilant (e.g., mounting or grooming of a conspecific) may occur primarily in nest boxes.

Overall activity levels among captive pairs may differ between enclosures and nest boxes as well. Activity levels may be higher in enclosures because there is more space to explore, and because food, water, and climbing structures (e.g., tree stumps and straw bales) are provided in enclosures. In addition, while enclosures are a relatively protected

environment compared to the wild (Price 1999), enclosures may be perceived as a less safe environment for resting or sleeping compared to nest boxes. Alternatively, activity levels may be similar between enclosures and nest boxes, or even higher in nest boxes than in enclosures, but the nature of their activities may differ between the two locations. For example, active non-social behaviours (e.g., climbing and eating) may be more frequent in enclosures, but active social behaviours (e.g., greeting and play-fighting) may be more frequent in nest boxes. In general, the frequency of social interactions (i.e., physical interactions between individuals) within captive pairs may differ between nest boxes and enclosures. They may be greater in nest boxes than in enclosures because social interactions likely inhibit an animal's ability to remain vigilant and, therefore, nest boxes may be perceived as a safer place to engage in them. In addition, the relatively small size of nest boxes compared to enclosures may increase the rate of social interactions simply because animals in the same nest box will have a higher chance of encountering one another.

The activity levels and rates of sociality may potentially differ between the captive and wild populations as well. Captive animals in enclosures may be less active than wild animals above ground, because there is less space to explore in enclosures than in the wild (Stevenson 1983) and, as food is provided in enclosures, there is less need to search for food (Price 1999). Alternatively, captive animals in enclosures may be more active than wild animals above ground, because they don't spend as much time in non-active alert poses (e.g., standing alert and non standing alert) watching for predators, and/or because they may have more energy to spare than wild animals, possibly because of differences in the digestibility of captive and wild diets. In addition, captive animals in enclosures may be more social than wild animals above ground. This may occur because enclosures are be perceived to be relatively safe when compared to the wild and, therefore, safer places to interact socially. It may also occur because pairs in the conservation breeding program are housed together in the same enclosure, and there is a limit to the physical distance they can put between each other, which may increase social interactions. Alternatively, captive animals in enclosures may be less social than wild animals above ground, because captive animals generally only interact with their mate during the reproductive season and, therefore, may not engage in as many social behaviours relevant to individual recognition (e.g., greeting; Barash 1989) as do wild animals, who likely encounter a greater variety of conspecifics.

METHODS

Data collection

Captive animals

I observed behaviours in nest boxes of six adult females and six adult males, and in enclosures of eight adult females and eight adult males (Table 2.1a, b). Pairs not observed were excluded because they had been observed more than once in previous years, the male was not marked, and/or animals were only visible a small proportion of time. Data on the behaviour of individuals were collected using focal animal sampling and instantaneous sampling (Martin & Bateson 2000) at 5-min intervals. I determined the appropriate interval by first collecting a total of 262 hours of behavioural data on the three adult males and three adult females at the Calgary Zoo in 2002 using focal animal sampling and continuous recording. The data were then sampled at 30-sec, 1-min, 2-min, 5-min, 10-min, and 15-min

<u></u>		Produced					Camera		Proportion of			
) I				N (-1 -					Observat		
			Name		pups (at end	Iviale	coverag	je.	time vi	sible	Obse	rvea
Year	Facility	Pair #	(age in years)	Ovulated	of season)	marked	NB	E	NB	E	NB	E
2002	DWCC	1a	China (7)	No	No	Yes	2 of 2	2 of 2	1.00	0.91	Х	Х
2002	DWCC	2a	Boadecia (8)	Yes	No	Yes	1 of 2	2 of 2	0.01-	0.94		Х
2002	DWCC	3a	Judy (4)	Yes	Yes	Yes	0 of 2	2 of 2	0.00	0.89		Х
2002	Toronto	4	Babe (5)	Yes	Yes	No	2 of 2	2 of 2	0.85	0.65		
2002	Toronto	5a	Naomi (4)	Yes	Yes	No**	2 of 2	2 of 2	0.38	0.48	Х	Х
2002	Toronto	ба	Stumpy (5)	Yes	No	No	2 of 2	1 of 2	0.003	0.37		
2002	Toronto	7a	Larry (12)	No	No	No	2 of 2	1 of 2	0.01	0.27		
2003	DWCC	1b	China (8)	Yes	Yes	Yes	2 of 2	2 of 2	1.00	0.94	Х	Х
2003	DWCC	2b	Boadecia (9)	Yes	Yes	Yes	2 of 2	2 of 2	1.00	0.61	Х	Х
2003	DWCC	3b	Judy (5)	No	No	Yes	2 of 2	2 of 2	1.00	0.92	Х	Х
2003	Toronto	5b	Naomi (5)	Yes	Yes	No]2 of 2	2 of 2	0.81	0.23		
2003	Toronto	6b	Stumpy (6)	'Yes	Yes	Yes	2 of 2	2 of 2	0.01	0.83		
2003	Toronto	7b	Larry (13)	Yes	No	Yes	2 of 2	2 of 2	0.02	0.54		
2004	DWCC	2c	Boadecia (10)	Yes	Yes	Yes	2 of 2	2 of 4	0.99	0.74		
2004	DWCC	8***	Ursula (5)	No	No	Yes	2 of 2	2 of 4	0.97	0.52		
2004	DWCC	9	Mirabel (4)	No	No	Yes	2 of 2	2 of 4	0.98	0.39	Х	Х

Table 2.1a. Female *M. vancouverensis* observed for behaviour profiles at the Calgary Zoo's Devonian Wildlife Conservation Centre (DWCC), and the Toronto Zoo during the 2002 to 2004 reproductive seasons. Shaded areas highlight why individuals were excluded from observations. NB = nest box, E = enclosure

* Indicates the number of locations with cameras installed inside (e.g., NB: 1 of 2 = two nest boxes were provided to the pair but only one had a camera installed inside)

** Male not marked for research purposes, but female missing fur or rear end, facilitating individual

*** Male in pair had been observed twice before

.

					Produced	d		Camera		tion of	•	
			Name	Female	pups (at end	Male	coverage*		time visible		Observed	
Year	Facility	Pair #	(age in years)	ovulated	of season)	marked	NB	Е	NB	E	NB	Е
2002	DWCC	1a	Gudron (7)	No	No	Yes	2 of 2	2 of 2	1.00	0.87	Х	X
2002	DWCC	2a	Ivan (6)	Yes	No	Yes	1 of 2	2 of 2	0.01	0.98		Х
2002	DWCC	3a	Houdini (4)	Yes	Yes	Yes	0 of 2	2 of 2	0.00	0.97		Х
2002	Toronto	4	Chase (6)	Yes	Yes	No	2 of 2	2 of 2	0.88	0.39		
2002	Toronto	5a	Yang (3)	Yes	Yes	No**	2 of 2	2 of 2	0.69	0.38	Х	Х
2002	Toronto	ба	Washington (6)	Yes	No	No	2 of 2	1 of 2	0.08	0.25		
2002	Toronto	7a	Caruso (5)	No	No	No	2 of 2	1 of 2	0.21	0.20		
2003	DWCC	1b	Gudron (8)	Yes	Yes	Yes	2 of 2	2 of 2	1.00	0.92	Х	Х
2003	DWCC	2b	Ivan (7)	Yes	Yes	Yes	2 of 2	2 of 2	1.00	0.74	Х	Х
2003	DWCC	3b	Houdini (5)	No	No	Yes	2 of 2	2 of 2	1.00	0.89	Х	Х
2003	Toronto	5b	Yang (4)	Yes	Yes	No	2 of 2	2 of 2	0.78	0.22		
2003	Toronto	6b	Washington (7)	Yes	Yes	Yes	2 of 2	2 of 2	0.07	0.79		
2003	Toronto	7b	Caruso (6)	Yes	No	Yes	2 of 2	2 of 2	0.30	0.16		
2004	DWCC	2c	Ivan (8)	Yes	Yes	Yes	2 of 2	2 of 4	0.94	0.55		
2004	DWCC	3c	Houdini (6)	No	No	Yes	2 of 2	2 of 4	0.97	0.53		
2004	DWCC	9	Franklin (4)	No	No	Yes	2 of 2	2 of 4	0.97	0.51	Χ	Х

Table 2.1b. Male *M. vancouverensis* observed for behaviour profiles at the Calgary Zoo's Devonian Wildlife Conservation Centre (DWCC), and the Toronto Zoo during the 2002 to 2004 reproductive seasons. Shaded areas highlight why individuals were excluded from observations. NB = nest box, E = enclosure

* Indicates the number of locations with cameras installed inside (e.g., NB: 1 of 2 = two nest boxes were provided to the pair but only one had a camera installed inside)

** Male not marked for research purposes, but female missing fur or rear end, facilitating individual identification

intervals to determine which gave an accurate representation of what was observed with continuous recording (Martin & Bateson 2000). The proportion of time spent in each behavioural category was calculated for the continuously recorded sample, graphed against the proportion of time spent in the same behavioural categories at each of the instantaneous sampling intervals, and a linear regression line was applied to the data. A perfect representation would have yielded a slope and r^2 value of 1.0. When all behaviours were included, I considered the sampling interval acceptable if both the slope and r^2 values were greater than 0.95 and less than 1.05. When only rare behaviours (those occurring less than one percent of the time) were included, I considered the sampling interval acceptable if both the slope and r^2 values were greater than 0.95 and less than 1.05 and the other value was greater than 0.70 and less than 1.30. Analyses using a 5-min instantaneous interval between focal animal samples met these requirements (Figure 2.1a,b).

Wild animals

Data on the above ground behaviour of wild animals were provided by J. Werner and J. Brashares at the Biodiversity Research Centre, University of British Columbia. Data were obtained for six adult females and six adult males over the 2002 through 2004 active seasons. As with the data on captive animals, some of these included animals observed in more than one season. Animals were observed at three sites of natural *M. vancouverensis* habitat: Mount Washington (central Vancouver Island; 49°45'N, 125°17'W), and Heather and Green Mountains in the Nanaimo Lakes area (southern Vancouver Island; Heather: 49°00'N, 124°16'W, Green: 49°03'N, 124°20'W). Behavioural observations were conducted using focal animal sampling and instantaneous sampling at 1-min intervals (J.



Figure 2.1a. Mean proportion of time *M. vancouverensis* spent in behaviours with continuous recording versus instantaneous sampling every five minutes (slope = 1.0, $r^2 = 1.0$). Each point represents a different behaviour (n = 28; vertical and horizontal bars represent standard error).



Figure 2.1b. Mean proportion of time *M. vancouverensis* spent in rare behaviours (those that occur <1% of the time) with continuous recording versus instantaneous sampling every five minutes (slope = 1.1, $r^2 = 0.72$). Each point represents a different behaviour (n = 18; vertical and horizontal bars represent standard error).

Werner pers. comm.). The ethogram used in the study of wild individuals was based on Heard's (1977) study of wild *M. vancouverensis*, as well as observations of current wild *M. vancouverensis* populations (J. Werner pers. comm.). To compare captive and wild individuals, I used the behaviour of the focal animal and the estimated distance to the nearest neighbour (if one was present). For the wild individuals, nearest-neighbour distance estimates generally increased in 1m increments from 0 to 5m, one 5m increment to 10m, and then 5 to 10m increments from 10m upwards.

Statistical Analyses

Behavioural profiles in captivity .

For analyses of the captive population, I included all behavioural data, i.e., 24-hours a day in both nest boxes and enclosures. Because the behaviour of female and male marmots may differ (Armitage 1965, 1998; Heard 1977), and because one animal's involvement in a social behaviour did not necessarily mean that its mate was also coded as being involved, I conducted analyses separately for females and males. For all analyses, except for those comparing the frequency with which females versus males initiated social behaviours, the proportion of time individuals spent in behavioural categories, excluding time spent out of sight, was calculated. When I compared two measures from the same animal during the same year (e.g. proportion of time females spent in social behaviours while in nest boxes versus enclosures within a reproductive season) I used a paired-samples test. As done by Hoogland (1995), I considered any measures from the same animal in different years as independent (hereafter referred to as a 'comprehensive sample'). However, I re-ran analyses excluding subsequent observation years to determine whether the conclusions were the same (hereafter referred to as a 'conservative sample'). When the quality of camera coverage differed between years, the year with the worst camera coverage was excluded first. Parametric analyses were used when the raw or transformed data met the test's assumptions; in all other cases the equivalent nonparametric test was used. In some cases, the distribution of the data changed with the decrease in sample size associated with excluding pairs for conservative samples and, where a parametric statistic had previously been used, a nonparametric statistic was applied.

I used Mann Whitney U tests to compare the proportions of time that females and males spent in individual behaviour categories, and Wilcoxon signed ranks tests to compare the frequencies with which females and males initiated social interactions. Wilcoxon signed ranks tests were also used to test how the proportion of time spent in individual behavioural categories (except for the proportions of time in dig, drink and mesh, as these behaviours by definition could only occur in enclosures) differed between enclosures and nest boxes for both females and males. Behaviours were then categorized as active or nonactive and social or non-social (see ethogram in Appendix 1). I used t-tests and Mann Whitney U tests to compare the proportions of time that females and males spent in 1) active and 2) social behaviours in both enclosures and nest boxes. Finally, I used Wilcoxon signed ranks tests to compare how the proportions of time spent in active and social behavioural groupings differed between enclosures and nest boxes, for both females and males.

Parametric or nonparametric analyses are reported with means \pm standard error or medians, respectively. The only exception is for comparisons of the frequency with which

captive females and males initiated social interactions. Because the relative frequencies are being evaluated rather than raw numbers, only the statistics are reported.

Comparisons of captive and wild animals

A number of steps were taken to ensure that the captive and wild datasets were statistically comparable. The captive data were sub-sampled for observations occurring in the enclosures between 08:00 and 18:00, to match behavioural observations of wild animals, all of which were above ground and between those hours. The wild data were sub-sampled for observations during which an adult female and adult male were within 10m of each other. Data were limited to observations of these age and sex classes because the behaviour of males and females, and of animals in different age groups (e.g. yearlings versus adults) often differs (Armitage 1962, 1974; Johns & Armitage 1979), and my captive observations were of adult female – adult male pairs. I chose 10m as the limit for nearest neighbour distance in the wild dataset because the average maximum possible distance between individuals in the adult female – adult male pairings observed in captivity was approximately 8.7m. I estimated 8.7m by calculating the average of the diagonal distance across adjoining enclosures housing captive pairs observed in my study; pairs observed at the DWCC were housed in adjoining enclosures measuring 3.4 x 2.1 x 2.3m (length, width, height) and 3.7 x 2.1 x 2.3m (Figure 1.1), and the one pair observed in the AHC at the Toronto Zoo was housed in adjoining enclosures measuring 3.1 x 1.6 x 2.3m and 3.1 x 3.6 x 2.6m (Figure 1.2). In addition, I sub-sampled the wild data for observations occurring between emergence and day 64 of the active season, because all observations of captive animals occurred between these times. Emergence dates for nine of the 10 animalyears included in analyses were provided by A. Bryant of the Marmot Recovery

Foundation. In the one case where the emergence date of an individual was not available, I used the average emergence date of other adults that hibernated at the same site within the same year.

Only a small amount of wild data met the above requirements. Therefore, prior to statistical analyses, data from wild individuals were pooled across years. Consequently, all data for wild animals were from observations occurring on an average of 5.0 ± 0.8 days and with an average of 117.8 ± 24.2 point-samples per individual. However, sample sizes decreased to two adult females and three adult males. For consistency, data for individual captive animals were also pooled across years prior to captive – wild comparisons. Therefore, sample sizes decreased to five adult females and five adult males. Consequently, no individuals were observed more than once in captive-wild analyses.

While the ethograms developed for the captive and wild observations were similar, each was tailored for use with video and field observations, respectively. Therefore, behavioural categories were broadly designated as active or non-active and social or nonsocial for statistical comparisons. Analyses were conducted separately for females and males for the same reasons listed above. The proportions of time spent in: 1) active and 2) social behaviours (excluding time spent out of sight) were calculated for captive and wild animals. In contrast to the proportions calculated in the behavioural profiles in captivity, which considered data from both enclosures and nest boxes 24 hours per day, the proportions calculated for comparisons between captive and wild animals reflected the amount of time captive and wild animals spent in active and social behaviours in relation to the amount of time they spent in enclosures and above ground, respectively, between 08:00 and 18:00. I used nonparametric analyses in all comparisons, because the data did not meet the assumptions of parametric tests. Mann Whitney U tests were used to compare the proportion of time in active and social behavioural groupings between wild females and males while above ground. I also used Mann Whitney U tests to compare the proportions of time spent in active and social behaviours between captive females in enclosures and wild females above ground, and captive males in enclosures and wild males above ground. Because all analyses were nonparametric, they are reported with medians.

RESULTS

Behavioural profiles in captivity

All analyses of behavioural profiles consider the time spent in both enclosures and nest boxes, 24 hours a day. Captive animals spent a substantially greater proportion of their time in nest boxes than in enclosures. However, females spent more time in nest boxes $(\text{mean} = 0.91 \pm 0.01)$ than males did (mean = 0.86 ± 0.02 ; t-test: t = 2.3, n_{females} = 8, n_{males} = 8, p = 0.037) when a comprehensive sample was used, or when a conservative sample was used (females: median = 0.90, males: median = 0.84; Mann Whitney U test: U = 3, n_{females} = 8, n_{males} = 8, n_{males} = 8, p = 0.047).

Figures 2.2 and 2.3 illustrate the behaviour profiles of females and males in captivity, grouped as social and non-social behaviours, respectively. In enclosures, males spent a greater proportion time in non-standing alert, standing alert and sitting at the entrance to nest boxes than females did when a comprehensive sample was used (non-standing alert: females: median = 0.00003, males: median = 0.0003; Mann Whitney U test: U = 2, $n_{females} = 6$, $n_{males} = 6$, p = 0.010; standing alert: females: median = 0.0004, males:

median = 0.0009; Mann Whitney U test: U = 5, $n_{females} = 6$, $n_{males} = 6$, p = 0.037; sitting at entrance to nest boxes: females: median = 0.001, males: median = 0.002; Mann Whitney U test: U = 4, $n_{females} = 6$, $n_{males} = 6$, p = 0.025). When a conservative sample was used, the trends for the three behaviours were the same but only significantly different for nonstanding alert (females: median = 0.00007, males: median = 0.0003; Mann Whitney U test: U = 1, $n_{females} = 5$, $n_{males} = 5$, p = 0.015). In addition, when a conservative sample was used, males in nest boxes spent a greater proportion of time autogrooming than females did (females: median = 0.013, males: median = 0.020; Mann Whitney U test: U = 1, $n_{females} = 5$, $n_{males} = 5$, p = 0.016). The trend was the same but not statistically significant when a comprehensive sample was used.

When a comprehensive sample was used, males initiated significantly more attempted mounts (Wilcoxon signed ranks tests: nest boxes Z = 2.2, n = 6, p = 0.027, enclosures Z = 2.1, n = 5, p = 0.038), genital sniffs (nest boxes Z = 2.0, n = 6, p = 0.043, enclosures Z = 2.2, n = 6, p = 0.027), and mounts (nest boxes Z = 2.2, n = 6, p = 0.027, enclosures Z = 2.4, n = 7, p = 0.018) than females did in both enclosures and nest boxes. Males also initiated more approaches than females in nest boxes (Z = 2.0, n = 6, p = 0.042), and females had a strong tendency to initiate more allogrooms and play-fights than males in nest boxes, but the difference was not significant when a comprehensive sample was used. When a conservative sample was used, all trends were similar, but none were significantly different in the enclosures and only attempted mounts and mounts were still initiated significantly more often by males in nest boxes (both attempted mounts and mounts: Z = 2.0, n = 5, p = 0.043).

When a comprehensive sample was used, analyses of each social behavioural category revealed that, in nest boxes, both females and males spent a greater proportion of time allogrooming, attempting mounts, greeting, mounting, and in contact rest, and males also spent more time play-fighting and in 'other social' behaviours (such as lunging), compared to in enclosures (Figure 2.2). Analyses of individual non-social behavioural categories revealed that, in nest boxes, both females and males spent a greater proportion of time autogrooming, nest building, in non-contact rest, in non-standing alert and sitting at the entrance to nest boxes, and less time eating, in standing alert, and in 'other non-social' behaviours (such as walking and exploring), compared to in enclosures. In addition, females spent more time fleeing, and males spent less time defecating and urinating, in nest boxes compared to enclosures (Figure 2.3). When a conservative sample was used, all trends were the same, but the proportion of time both females and males spent in attempted mounts, females spent in non-standing alert and standing alert, and males spent in defecate / urinate, 'other social' and sitting at the entrance of nest boxes were no longer significantly different.

The proportion of time females and males spent in active behaviours was similar in both enclosures (both sexes: median = 0.05; Mann Whitney U test: U = 14, $n_{females} = 6$, $n_{males} = 6$, p = 0.589) and nest boxes (both sexes: median = 0.04; Mann Whitney U test: U = 13, $n_{females} = 6$, $n_{males} = 6$, p = 0.485) when a comprehensive sample was used, and when a conservative sample was used (enclosures: females: median = 0.05, males: median = 0.06; Mann Whitney U test: U = 8, $n_{males} = 5$, $n_{females} = 5$, p = 0.421; nest boxes: females: median = 0.04, males: median = 0.05; Mann Whitney U test: U = 7, $n_{males} = 5$, $n_{females} = 5$, p =0.310). The proportion of time spent in social behaviours was also similar between captive



Figure 2.2. Median proportion of time female and male *M. vancouverensis* spent in social behaviours while in nest boxes (n = 3 females & 3 males) and enclosures (n = 4 females & 4 males). Asterisks indicate, within each sex, where the behaviour constituted a significantly greater proportion of time (i.e., in nest boxes or in enclosures). See Appendix 1 for conversion of behaviour codes.



Figure 2.3. Median proportion of time female and male *M. vancouverensis* spent in nonsocial behaviours while in nest boxes (n = 3 females & 3 males) and enclosures (n = 4females & 4 males). Asterisks indicate, within each sex, where the behaviour constituted a significantly greater proportion of time (i.e., in nest boxes or in enclosures). See Appendix 1 for conversion of behaviour codes.

females and males both in enclosures (both sexes: median = 0.004; Mann Whitney U test: U = 18, $n_{males} = 6$, $n_{females} = 6$, p = 1.0) and nest boxes (both sexes: median = 0.68, Mann Whitney U test: U = 18, $n_{females} = 6$, $n_{males} = 6$, p = 1.0) when a comprehensive sample was used, or when a conservative sample was used (enclosures: both sexes: median = 0.004; Mann Whitney U test: U = 12, $n_{females} = 5$, $n_{males} = 5$, p = 1.0; nest boxes: females: median = 0.70, males: median = 0.71; Mann Whitney U test: U = 12, $n_{females} = 5$, $n_{males} = 5$, p = 1.0).

The proportion of time spent in active behaviours was similar between enclosures and nest boxes for both captive females (enclosures: median = 0.05, nest boxes: median = 0.04; Wilcoxon signed ranks test: Z = 1.6, n = 6, p = 0.116) and captive males (enclosures: median = 0.05, nest boxes: median = 0.04; Wilcoxon signed ranks test: Z = 1.8, n = 6, p =0.075) when a comprehensive sample was used, or when a conservative sample was used (females: enclosures: median = 0.05, nest boxes: median = 0.04; Wilcoxon signed ranks test: Z = 1.2, n = 5, p = 0.225; males: enclosures: median = 0.06, nest boxes: median = 0.05; Wilcoxon signed ranks test: Z = 1.5, n = 5, p = 0.138). However, both captive females and males spent a greater proportion of time in social behaviours in nest boxes compared to enclosures when a comprehensive sample was used (both sexes: enclosures: median = 0.004, nest boxes: median = 0.68; Wilcoxon signed ranks test: Z = 2.2, n = 6, p = 0.028), or when a conservative sample was used (both sexes: enclosures: median = 0.004, nest boxes: median = 0.70; Wilcoxon signed ranks test: Z = 2.0, n = 5, p = 0.043). A large component of the proportion of time spent in social behaviours in nest boxes was time animals spent in contact rest. Therefore, I re-ran analyses considering only the time spent in active social behaviours (i.e., excluding contact rest) to determine whether the relationship was the same. Both females and males still spent more time in active social

behaviours in nest boxes compared to enclosures when a comprehensive sample was used (females: enclosures: median = 0.04, nest boxes: median = 0.16; Wilcoxon signed ranks test: Z = 2.2, n = 6, p = 0.028; males: enclosures: median = 0.04, nest boxes: median = 0.15; Wilcoxon signed ranks test: Z = 2.2, n = 6, p = 0.028), or when a conservative sample was used (females: enclosures: median = 0.04, nest boxes: median = 0.17; Wilcoxon signed ranks test: Z = 2.0, n = 5, p = 0.043; males: enclosures: median = 0.04, nest boxes: median = 0.04, nest boxes: median = 0.16; Wilcoxon signed ranks test: Z = 2.0, n = 5, p = 0.043; males: enclosures: median = 0.043).

Comparisons of captive and wild animals

In all analyses, only the time captive and wild animals spent in enclosures and above ground, respectively, between 08:00 and 18:00 was considered, and data from individuals were grouped across years. Captive females and wild females both tended to spent a greater proportion of time in active behaviours than captive males and wild males, respectively, but the differences were not significant (captive population: females: median = 0.79, males: median = 0.66; Mann Whitney U test: U = 7, $n_{females} = 5$, $n_{males} = 5$, p = 0.310; wild population: females: median = 0.35, males: median = 0.19; Mann Whitney U test: U = 0, $n_{females} = 2$, $n_{males} = 3$, p = 0.083). However, the proportion of time captive and wild females spent in social behaviours compared to captive and wild males, respectively, while above ground was similar (captive population: females: median = 0.06, males: median = 0.05; Mann Whitney U test: U = 8, $n_{females} = 5$, $n_{males} = 5$, p = 0.421; wild population: females: median = 0.10, males: median = 0.06; Mann Whitney U test: U = 1, $n_{males} = 3$, $n_{females} = 2$, p = 0.248)

There was a strong trend for captive females in enclosures to spend a greater proportion of time in active behaviours than wild females above ground did, but this difference was not significant (captive: median = 0.79, wild: median = 0.35; Mann Whitney U test: U = 0, $n_{captive} = 5 n_{wild} = 2$, p = 0.095). The difference was significant when captive males in enclosures and wild males above ground were compared (captive: median = 0.66, wild: median = 0.19; Mann Whitney U test: U = 0, $n_{captive} = 5$, $n_{wild} = 3$, p = 0.036). Unfortunately, the current format of the wild dataset precludes further investigations into how the active behaviours of captive and wild marmots differ. However, there was no difference in the proportion of time spent in social behaviours between captive females in enclosures and wild females above ground (captive: median = 0.06, wild: median = 0.10; Mann Whitney U test: U = 1, $n_{captive} = 5$, $n_{wild} = 2$, p = 0.190), or between captive males in enclosures and wild males above ground (captive: median = 0.05, wild: median = 0.07; Mann Whitney U test: U = 4, $n_{captive} = 5$, $n_{wild} = 3$, p = 0.393).

DISCUSSION

Comprehensive knowledge of the ecology and behaviour of species is important for ensuring their current and future survival. While the above ground behaviour of grounddwelling sciurids is generally well-studied, most of these species spend the majority of their life in underground burrow systems and little is known about their in-burrow behaviour. In addition, in conservation breeding and reintroduction programs, an understanding of the behavioural repertoire of captive animals, and comparisons of behaviour between captive and wild animals, is essential. In this study I sought to elucidate some of this information for one of the largest members of the sciurid family, the critically-endangered *M. vancouverensis*. I found that, during the reproductive season, both females and males spent the majority of their time in nest boxes, the behavioural profiles of females and males differed, and the proportion of time spent in many behavioural categories differed between enclosures and nest boxes. Activity levels were similar between enclosures and nest boxes, but levels of social interactions were significantly greater in nest boxes, both when all behaviours and when only active social behaviours were considered. While in enclosures, captive animals tended to be more active than wild animals above ground, but their levels of social interaction were similar. Based on comparisons between the structure and use of nest boxes in captive *M. vancouverensis* pairs and burrows in wild marmots (using information from the published literature: Barash 1989; Bee & Hall 1956; Beltz & Booth 1952; Bibikow 1996; Blumstein et al. 2001; Bronson 1964; Grizzel 1955; Hamilton 1934; Kapitonov 1960; Svendsen 1976), I suggest that, during the reproductive season, wild *M. vancouverensis* likely engage in greater levels of social interactions in burrow chambers than those exhibited above ground.

Captive males spent a significantly greater proportion of their time in non-standing alert, standing alert, and sitting at the entrance of nest boxes in enclosures than females did. My definitions of standing alert and non-standing alert were created to represent vigilance behaviours (Armitage 1962; Barash 1989; Heard 1977), and sitting at the entrance of burrows is associated with wariness and scanning of the environment for predators and agonistic conspecifics (Armitage & Chiesura Corona 1994; Barash 1989). Wild adult male Olympic marmots (*M. olympus*) also spend more time sitting at burrow entrances than adult females do, watching for predators and non-resident males intruding into their territory (Barash 1973a). Overall, captive females spent less time in enclosures than males did, and directed their activities in enclosures primarily towards feeding, locomotion and nest building. In the wild, reproductive female marmots may spend more time in burrows than

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males (Armitage et al. 1996) and come above ground primarily to meet their increased energetic needs and to gather nesting materials (Armitage et al. 1996; Armitage 2003; Heard 1977). Captive males tended to spend more time autogrooming in nest boxes than females. In addition to grooming behaviours associated with general body maintenance, males were often observed grooming their genital area following copulation, as has been observed in both wild male and wild female alpine marmots (*M. marmota*; King & Allainé 1998).

Captive males initiated more behaviours associated with pursuing reproductive females, such as genital sniffs, attempted mounts, and mounts in both enclosures and nest boxes, and approaches in nest boxes only. Male-initiated genital sniffs likely represent the male's evaluation of the female's reproductive state (Barash 1973a). These behaviours often resulted in play-fights, and females tended to initiate more of these play-fights than males, although not significantly so. Similar dynamics between adult males and females have been observed in wild *M. flaviventris* (Armitage 1965).

Among pairs in the *M. vancouverensis* conservation breeding program, some behavioural categories occurred primarily in enclosures and others in nest boxes. Social behaviours that would likely inhibit an animal's ability to remain vigilant, such as autogrooming, allogrooming, greeting, attempted mounting, mounting, nest building, and play-fighting often comprised a significantly greater proportion of an animal's time in nest boxes than in enclosures. Behaviours often described as constituting a large proportion of the activity budget of wild marmots above ground, such as eating and locomotion (Armitage et al. 1996; Heard 1977), were more prominent in enclosures than in nest boxes, as was the vigilance behaviour, standing alert. However, animals still exhibited vigilance

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behaviours in nest boxes, often freezing in a non-standing alert pose when keepers were inside the enclosures. As observed in above ground studies of wild *M. flaviventris* (Armitage et al. 1996; Armitage & Chiesura Corona 1994), resting constituted a large proportion of animals time in enclosures, but it constituted an even greater proportion of their time in nest boxes.

While enclosures may be a relatively safe place compared to the wild (Price 1999), captive individuals spent more time in social interactions in nest boxes than in enclosures, which may suggest that animals perceive nest boxes as relatively safe places compared to enclosures. The provision of nest boxes in captive environments can provide safe places for social interactions, and refuges from aggressive conspecifics and climatic conditions (Price 1999). For some species, the availability of nest boxes may be critical for successful reproduction (e.g., Millam et al. 1988). In addition, the availability of shelter (e.g., nest boxes or tunnels) can play a large role in the development of behaviours such as avoidance or fleeing (Clark & Galef 1977). Overall activity levels were similar between enclosures and nest boxes, but the nature of these activities differed between the two locations, with social interactions occurring significantly more in nest boxes and non-social interactions occurring significantly more in enclosures. Once again, these results may suggest that nest boxes are perceived as a preferable place for engaging in social interactions, which agrees with the idea that burrows are associated with safety (Price 1999). However, the greater frequency of social interactions in nest boxes compared to enclosures may also be influenced by the greater amount of time animals spent in nest boxes compared to enclosures, and it is impossible for me to discern to extent to which each of these factors

enclosures or nest boxes, is not known. Finally, captive animals may simply have more energy available to dedicate to active behaviours, possibly because of greater energy content of food in captivity compared to the wild. Further study of the diets of captive and wild animals will be necessary to discern whether this is a contributing factor. If the observed wild animals spent more time above ground than the observed captive ones, the relatively higher activity levels in the captive animals may be biased. Regardless, because the differences were fairly large (median proportion of time spent in active behaviours were 79% for captive females and 66% for captive males when in enclosures, compared to 35%for wild females and 19% for wild males when above ground), I suggest that *M. vancouverensis* pairs in the conservation breeding program exhibit similar or higher levels of activity during the reproductive season compared to wild conspecifics.

Adult pairs in the conservation breeding program spent a similar proportion of time in social behaviours in enclosures compared to wild adults when above ground and with 10m of an adult of the opposite sex. Further observations of wild animals will be necessary to conduct comparisons of the specific nature of social interactions in the captive and wild populations. Because the captive pairs live in close proximity year round and, currently, wild *M. vancouverensis* often do not live in close proximity to each other (J. Werner, pers. comm.), the overall yearly rates of social interaction are likely higher in captivity. However, because wild pairs exhibited similar rates of sociality to captive pairs when they were in close proximity to each other, I suggest that any differences in the yearly rate of social interactions between captive and wild pairs may be a function of the distance between the two animals and not differences in the sociality of captive and wild populations.

In addition, because captive and wild pairs in proximity to each other exhibited similar rates of sociality in enclosures and above ground, respectively, I suggest that my results on the differences in the frequency of social interactions between enclosures and nest boxes can be used to make inferences about the behaviour of wild animals that live in proximity to each other while in their burrow chambers. However, the role that the structure and use of nest boxes and burrow chambers may play in social interactions must be considered first.

If animals rarely share burrow systems or chambers in the wild, then there will be few opportunities for in-burrow interactions. In addition, if the distance between animals within a burrow chamber is a primary predictor of the likelihood of social interactions, then a difference in the size or structure of nest boxes and natural burrow chambers may confound my ability to make inferences about behaviour in burrow chambers based on behaviour in nest boxes. Descriptions of excavated burrow systems in the wild vary, and few provide a complete description of burrow use or structure. However, together, these accounts provide us with some useful information. There may be a number of burrow systems within a marmot's home range (Barash 1989; Svendsen 1976), and the number of entrances to each burrow system may vary extensively, from one to two entrances in hibernacula (or winter burrows) to between one and five in summer burrows (Barash 1989; Bibikow 1996; Grizzel 1955). There are no estimates of the density of burrow systems in M. vancouverensis habitat, but one study recorded an average of 148 burrow entrances per hectare (Blumstein et al. 2001). During the active season, animals may switch from their hibernacula and primarily use summer burrows (Barash 1989; Grizzel 1955) or continue using their hibernacula as their primary burrows (Barash 1989). Wild marmots will share

their burrow systems during the active season (Barash 1989; Bibikow 1996; Bronson 1964; Hamilton 1934), but some age-sex classes are more likely to share their systems than others (Barash 1989). Burrow-system sharing is much more frequent in social marmot species than solitary ones and often positively correlates with genetic relatedness, especially early in the active season (Barash 1989). Within burrow systems, there may be a single nesting chamber or multiple chambers (Bibikow 1996; Grizzel 1955; Svendsen 1976). In burrow systems with multiple chambers, the chambers may have different functions (e.g. nesting versus latrine chamber; Barash 1989; Beltz & Booth 1952) or be occupied by a certain animal, for example, the reproductive female (Kapitonov 1960). Most published accounts of burrow structure do not include measurements of nesting chambers. One excavated Brower's marmot (*M. broweri*) burrow chamber was "about three times the size of a marmot" (Bee & Hall 1956). Diagrams of burrows provided by Bibikow (1996) indicate that the average length and height of nesting chambers is 0.67m and 0.29m, respectively.

Captive pairs of *M. vancouverensis* observed in my study had two to three nest boxes in their enclosures, measuring from $0.8 \ge 0.6 \ge 0.5$ m to $1.2 \ge 0.6 \ge 0.8$ m (length \ge width \ge height), with two entrances each. Therefore, the size of nest box chambers is similar to those in natural burrows. In a few cases in 2002 and 2003, during inclement weather, animals housed at the DWCC would not have had access to the nest box in their outdoor enclosure, leaving only one nest box. However, nest boxes at that facility were the largest in the breeding program. Therefore, in most cases, if animals were in the same nest box it was because they chose to be there and, even when in the same nest box, there was enough space that enabled animals to interact or not. This suggests that animals are able to use nest boxes and natural burrow chambers similarly. Based on these similarities between nest boxes and natural burrows, suggestions about in-burrow behaviour based on nest box behaviour are likely justified.

Based on my observations of *M. vancouverensis* pairs in the conservation breeding program, I suggest that a significantly greater proportion of social interactions occur in burrow chambers than above ground, both when all social interactions and when only active social interactions (i.e., excluding resting in contact with another animal) are considered. In wild marmots, burrow-system sharing positively correlates with proximity and social interactions above ground (Barash 1989). Therefore, wild animals that spend time in close proximity and interact socially above ground are likely sharing a burrow system, and their social interactions may be even more pronounced in burrow chambers. In wild *M. vancouverensis*, all age-sex classes use the same social behaviour patterns in approximately the same proportions when above ground (Heard 1977). Therefore, the comparisons and suggestions made in this study may apply to more than just adult female – adult male social interactions in this species.

This knowledge can also be incorporated into discussions and hypotheses regarding marmot sociality. Most studies of sociality in ground squirrels and marmots are based on comparative datasets that evaluate differences in behavioural and ecological parameters among species; one such parameter is the relative frequency of above ground social interactions (e.g., Armitage 1981; Barash 1974a; Blumstein & Armitage 1998, 1999). However, differences in the relative frequency of in-burrow social interactions among species, or differences in the relative frequency of social interactions exhibited in burrow chambers and above ground within species, may influence these hypotheses of sociality. My study suggests that, in *M. vancouverensis*, the frequency of social interactions is likely significantly greater in burrow chambers than above ground. If the trend is similar in other marmots, then hypotheses of sociality based on relative frequency of social interactions among species may be accurate. Conversely, if the trend is not similar in other marmot species, then further study of the relative frequency of social interactions above ground versus in burrows will assist in refining these hypotheses.

Knowledge that social interactions in M. vancouverensis are likely significantly more frequent in burrows than above ground can also help identify possible risks for species recovery. For example, social interactions can provide key opportunities for the spread of pathogens and parasites (Altizer et al. 2003; Corner et al. 2002). High incidences of social interactions in nest boxes in the conservation breeding program, and likely also in burrows in the wild, may facilitate the transmission of pathogens such as Mycoplasma (Raverty & Black 2001) and Yersinia (de With et al. 1999), and parasites such as fleas (Thrassis spenceri; Heard 1977), ticks (Ixodes spp; Heard 1977), mites (possibly Chorioptes spp; de With et al. 1999), roundworms (Baylisascaris laevis; Mace & Shepard 1981), and a tapeworm unique to M. vancouverensis (Diandrya vancouverensis; Mace & Shepard 1981), both within the captive and wild populations, and between released and wild animals. Most of the aforementioned are generally considered non-pathogenic in this species (de With et al. 1999; Raverty & Black 2001), but some may become virulent in stressful situations, for example, extreme temperature, malnutrition, and the capture, transport and release of animals (Lyles & Dobson 1993; S. Black and D. Whiteside pers. comm.). For example, mycoplasmosis has been identified in all captive M. vancouverensis aged two years or older (S. Black and D. Whiteside pers. comm.) and was identified as a

contributing factor in the death of some captive animals following capture from the wild (Raverty & Black 2001).

In addition to providing detailed behavioural profiles of adult pairs in the *M*. *vancouverensis* conservation breeding program, and providing the first opportunity to make educated suggestions about in-burrow behaviour of wild *M*. *vancouverensis* and possibly other social marmot species, results from my study suggests that breeding pairs have not become sedentary and have not changed their social nature as a result of being kept in captivity. Additional study of the behavioural profiles of animals in the conservation breeding program that are slated for release (e.g., Blumstein et al. unpublished data), the proportion of time wild animals spend above ground versus in the burrow, and more detailed comparisons of specific behaviours among the captive, released and wild populations (e.g., Hohn et al. 2000; Kerridge 2005; McPhee 2003; McPhee & Silverman 2004), will prove useful towards further increasing our understanding of *M. vancouverensis* behaviour and achieving a successful recovery program for this species.

CHAPTER 3

Factors Associated with Reproductive Success in the Vancouver Island Marmot Conservation Breeding Program

INTRODUCTION

Conservation breeding and reintroduction programs can be powerful tools in the recovery of endangered species, and have already prevented the extinction of some, for example, the black-footed ferret (*Mustela nigripes*) and the Mallorcan midwife toad (*Alytes muletensis*; Baillie et al. 2004). A solid understanding of the reproductive behaviour of a species, and how environmental and management variables may influence its reproduction, are often essential to the success of these programs (Eisenberg & Kleiman 1977; Wielebnowski 1998). Behavioural studies within conservation breeding programs have helped in understanding factors associated with reproductive success in a number of mammals, for example, cheetahs (*Acinonyx jubatus*; Wielebnowski 1999; Wielebnowski et al. 2002), black rhinoceros (*Diceros bicornis*; Carlstead et al. 1999a; Carlstead et al. 1999b), and maned wolves (*Chrysocyon brachyurus*; Rodden et al. 1996). In addition, these programs often create opportunities to study aspects of behaviour that are difficult to study in the wild.

Of the world's 14 marmot species, four are listed by the World Conservation Union (IUCN 2004): three Eurasian species, the bobac or steppe marmot (*Marmota bobac*; low risk/conservation dependent), the long tailed marmot (*M. caudata*; low risk/near threatened), and Menzbier's marmot (*M. menzbieri*; vulnerable), and one North American species, the Vancouver Island marmot (*M. vancouverensis*; endangered). Most Eurasian
marmot species have been bred in captivity (Rumiantsev & Rymalov 1998), and some have been involved in conservation breeding and reintroduction programs, such as *M. bobac* (Rumiantsev & Rymalov 1998) and the alpine marmot (*M. marmota*; E. Gregorova, pers. comm.; Borgo & Mattedi 2003). Prior to the inception of the *M. vancouverensis* conservation breeding program in 1997 (Janz et al. 2000), the main North American marmot species to be bred in captivity was the woodchuck (*M. monax*), housed primarily under laboratory conditions for research purposes, and not as part of a reintroduction program (e.g., Concannon et al. 1989; Hikim et al. 1991).

Relatively little is known of the reproductive behaviour of marmots, because much of it occurs in burrows that are generally inaccessible in field studies (Barash 1989). Above ground observations of wild marmots have resulted in some generalizations about reproductive behaviour (e.g., Armitage 1965; Barash 1989; King & Allainé 1998). Until now, there have been limited observations of reproductive behaviour within marmot captive breeding programs (e.g., Concannon et al. 1997b; Exner et al. 2003; Keeley et al. 2003). My study thus far has expanded on these observations and has confirmed that in captive pairs, and likely in wild pairs, the vast majority of social interaction during the reproductive season occurs in nest boxes and in burrow chambers, respectively. My study also provided detailed behavioural profiles of captive animals in their enclosures and nest boxes and showed, for example, that, in nest boxes, males initiate more approaches, genital sniffs, attempted mounts, and mounts than females, and females have a tendency to initiate more allogrooms and play-fights than males. However, there is still a gap in knowledge concerning factors associated with reproductive success in marmot captive breeding programs. In fact, there are no published studies detailing the reproductive behaviour of

captive marmots throughout the reproductive season in both enclosures and artificial burrow chambers, and none that identify behavioural correlates of pup production. The remainder of my study addresses this knowledge gap.

In wild *M. vancouverensis*, usually only one adult female and one adult male living within a family group produce pups in any given year (Bryant 1996; Heard 1977). Because of this, and because numbers of animals are limited, adult animals in the *M. vancouverensis* conservation breeding program are usually housed as pairs consisting of one female and one male. *M. vancouverensis* is a seasonal breeder (Heard 1977), and there is evidence that it has a single estrous period during the first month post emergence from hibernation (Spindler, pers. comm.), which is similar to female *M. monax* (Hikim et al. 1991). Gestation in *M. vancouverensis* is approximately 31 - 34 days (Graham, pers. comm.; Keeley et al. 2003), which is similar to the 31-34 day gestation period observed in *M. monax* (Concannon et al. 1997b; Snyder & Christian 1960) and the 30 day gestation period observed in yellow-bellied marmots (*M. flaviventris*; Andersen et al. 1976; Nee 1969).

The probability of producing a litter within the *M. vancouverensis* conservation breeding program is now similar to that in the wild (Bryant in press), but there is limited understanding of why some breeding pairs produce pups while others do not, especially considering that all breeding pairs seem to copulate. Knowledge of factors associated with successful reproduction is important for managing the captive population in a reliable and predictable manner, while meeting the demographic and genetic goals of the program. I studied the behaviour of *M. vancouverensis* pairs during the reproductive season (emergence from hibernation until parturition, or until 64 days after emergence for pairs

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that did not produce pups), both in their enclosures and in their nest boxes, to elucidate factors associated with successful reproduction in the conservation breeding program.

Because behaviours other than copulation may be crucial components of successful reproduction (Dewsbury 1972; Roberts et al. 1999), I compared detailed behavioural profiles of pairs that were successful in producing pups with those that were not. These profiles may differ if, for example, receptive females spend more time approaching males than non-receptive females, as seen in female *M. monax* in captivity (Concannon et al. 1997b). I also examined the proportions of time that pairs spent in social behaviours as the reproductive season progressed. Pairs may decrease the time they spend in social interactions as the reproductive season progresses, as seen in wild *M. flaviventris* (Armitage 1965), hoary marmots (*M. caligata*; Holmes 1984) and Olympic marmots (*M. olympus*; Barash 1973a).

Female *M. monax* are induced ovulators (Concannon et al. 1997b), although spontaneous ovulation may also occur (Amador et al. 1990). Female *M. vancouverensis* may also be induced ovulators (Keeley et al. 2003). Other rodents that are induced ovulators include: meadow voles (*Microtus pennsylvanicus*; Cohen-Parsons & Carter 1987), prairie voles (*Microtus ochrogaster*; Dluzen et al. 1981), the natal mole-rat (*Cryptomys hottentotus natalensis*; Jackson & Bennett 2005), the highveld mole-rat (*Cryptomys hottentotus pretoriae*; Malherbe et al. 2004), and Gambian giant pouched rats (*Cricetomys gambianus*; Malekani et al. 2002). Induced ovulation is different from spontaneous ovulation in that copulation is needed to trigger a luteinizing hormone surge, which results in ovulation (Ramirez & Beyer 1988).

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Among mammals that exhibit induced ovulation, the amount of stimulation required for ovulation to occur varies substantially. For example, rabbits (*Oryctolagus cuniculus*) ovulate after as little as 200 milliseconds of copulatory stimulation (Beyer et al. 1980) whereas in ferrets (*Mustela furo*) intromissions of at least one minute are required to stimulate an ovulatory response (Carroll et al. 1985). There is no information on a minimum amount of stimulation necessary for ovulation in rodents that are induced ovulators. In captive *M. monax*, neither mount frequency nor mount duration are correlated with ovulation (Concannon et al. 1997b).

In addition, in many mammals, copulations must be of a particular character or frequency to initiate the physiological changes necessary for pregnancy in the female (Dewsbury 1972). However, copulations that occur too soon after a prior ejaculation can interfere with establishment of pregnancy (Adler & Zoloth 1970; Yang & Clemens 1998). In captive *M. monax*, both the duration of the longest mount and the number of mounts greater than three and five minutes are positively correlated with fertilization (Concannon et al. 1997b).

Because *M. vancouverensis* is endangered, and breeding facilities have adopted a policy of minimal animal handling, experimental studies similar to that of Concannon et al. (1997b), in which they conducted surgical examinations of females to identify ovulation and fertilization, are not permitted. In this study, I used the birth of pups as my primary success measure. I first determined whether the rate and duration of mounts differed between enclosures and nest boxes, and then compared the rate and duration of mounts between pairs that produced pups and pairs that did not. Using data from analyses of fecal hormones from females observed in this study (reviewed in this chapter's methods section),

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I also compared the rate and duration of mounts between pairs in which the female ovulated and pairs in which she did not. If mount rate and/or duration were positively correlated with both the production of pups and female ovulation, it would indicate that one or both of those factors may be necessary for ovulation, fertilization, or both in *M. vancouverensis*. If mount rate and/or duration were not positively correlated with the successful production of pups, but were positively correlated with ovulation in females, then it is possible that one or both of those factors may be necessary for ovulation in this species.

A number of environmental and management variables can influence reproduction in captive mammals (e.g., Branvold et al. 2003; Concannon et al. 1992; Carlstead et al. 1999a; Wielebnowski et al. 2002). Weather conditions (Schwartz & Armitage 2005), heat stress (e.g., Concannon et al. 1997a), and reproductive suppression (Arnold & Dittami 1997; Hacklander et al. 2003) can influence reproductive rates in wild marmots. Throughout the current *M. vancouverensis* conservation breeding program, breeding pairs have been housed, and have bred successfully, under numerous conditions and management regimes that varied both within and among facilities. However, to ensure that my behavioural comparisons were not confounded by these variables, I also collected data on the environment and management of pairs, and compared these with reproductive success.

METHODS

The mean between-litter interval for captive *M. vancouverensis* is 1.4 years (n = 11), which is significantly shorter than the 1.9-year interval (n = 17) in the wild (Bryant in press). During the 1999 to 2004 reproductive seasons, inclusive, 11 captive females

reproduced more than once, and seven litters produced by those females were the result of consecutive-year breeding. Therefore, I considered that each female had the potential to breed annually, and I treated animal-reproductive seasons as biologically independent among all captive pairs.

Data collection

Environmental and management influences

I obtained data on the age and breeding history of animals brought into captivity from the wild from A. Bryant of the Marmot Recovery Foundation, and data on the management of animals at each facility were obtained from animal care staff at the breeding facilities. I recorded hourly temperatures in the indoor enclosures of pairs observed for behavioural comparisons throughout the reproductive season with DS1921G Thermochron® iButtons (Dallas Semiconductor Corp, Dallas, TX) and hourly outdoor temperatures for those same pairs were obtained from the Environment Canada weather stations closest to the breeding facilities that archived hourly temperature data: the Calgary International Airport (51°6'N, 114°1'W, 1084m) and the Toronto Buttonville Municipal Airport (near Markham; 43°52'N, 79°22'W, 198m). The elevation and latitude of each facility was obtained from the closest Environment Canada weather stations to the breeding facilities: Elbow River South (DWCC), Fort Langley (Mountain View Conservation and Breeding Centre), Mount Washington Resort (Tony Barrett Mount Washington Marmot Recovery Centre) and Toronto Metro Zoo (Toronto Zoo). The use of artificial lights indoors can influence behavioural changes normally associated with seasonal photoperiods

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(Webb 1980). The indoor enclosures at some facilities had natural lighting from windows plus artificial lighting, and others had artificial lighting only. However, where only artificial lighting was provided, the lights were set to mimic natural photoperiods for the area and time of year. Therefore, information on photoperiod was not included in the database.

Behavioural profiles

I observed all behaviours of both individuals in a pair in nest boxes for three pairs that produced pups and three pairs that did not produce pups, and in enclosures for four pairs that produced pups and four pairs that did not produce pups (Table 2.1a, b), using the focal animal and 5-min point sampling methods outlined in Chapter 2. Individuals were excluded from observations when they or their mate had been observed more than once in previous years, the male in the pair was not marked, and/or animals were only visible for small proportions of time. Among pairs for which data on all behaviours were collected, the median proportion of time that individuals were visible on camera was not significantly different between pairs that produced pups and pairs that did not produce pups for females in nest boxes (produced pups: 1.0, did not produce pups: 1.0; Mann Whitney U test, U = 4, $n_{pups} = 3$, $n_{no pups} = 3$, p = 0.80), females in enclosures (produced pups: 0.75, did not produce pups: 0.92; Mann Whitney U test, U = 7, $n_{pups} = 4$, $n_{no pups} = 4$, p = 0.66), males in nest boxes (produced pups: 1.0, did not produce pups: 1.0; Mann Whitney U test, U = 4, n_{pups} = 3, $n_{no pups} = 3$, p = 0.80) and males in enclosures (produced pups: 0.83, did not produce pups: 0.88; Mann Whitney U test, U = 7, $n_{pups} = 4$, $n_{no pups} = 4$, p = 0.77).

Mount rate and duration

I collected detailed data on mounts in nest boxes for seven pairs that produced pups and eight pairs that did not produce pups, and in enclosures for six pairs that produced pups and six pairs that did not produce pups (Table 3.1). Pairs were excluded from observations when the male in the pair was not marked and/or mounts were only visible for small proportions of time. Data on mounts were recorded using focal animal sampling and continuous recording (Martin & Bateson 2000). The goal for each pair was to document the occurrence and location (i.e. nest box or enclosure) of every observable mount and collect duration data on 20 to 50 mounts in each location. However, because some pairs engaged in more mounts than others and camera coverage heavily influenced the number of mounts that could be observed, it was difficult to develop a sampling regime to accomplish the mount-duration sampling goals without reviewing tapes twice (once to count the number of visible mounts and once to gather data on randomly selected mounts). Therefore, the sampling protocol was adjusted as tapes were reviewed. When few mounts were visible, data were collected on every mount and where many were visible all mounts were counted but duration measures were only taken on a sub-sample of mounts (e.g., duration data were collected on every fifth mount). Mount duration data were obtained on a mean of 48.7 ± 11.2 (range 3 - 156) mounts in the nest box and 16.5 ± 4.3 (range 6 - 58) mounts in the enclosure, per pair. It was not possible to accurately discern if or when intromission or ejaculation occurred during mounts and, while one study on M. monax concluded that males tended to ejaculate 2.2 to 7.4 minutes into a mounting and thrusting sequence (Concannon et al. 1997b), there is no information on how long a mount must be before intromission or ejaculation occurs in M. vancouverensis. Therefore, all mounts were

Table 3.1. *Marmota vancouverensis* pairs observed for mount rate and duration at the Calgary Zoo's Devonian Wildlife Conservation Centre (DWCC), and the Toronto Zoo during the 2002 to 2004 reproductive seasons. Shaded areas highlight why individuals were excluded from observations. NB = nest box, E = enclosure

					Produced		Camera		Proportion of			
		Male	Female	Female	pups (at end	Male	coverag	ge*	time mo	ounts	Obse	rved
Year	Facility	(age in years)	(age in years)	ovulated	of season)	marked	NB	Е	NB	E	NB	Е
2002	DWCC	Gudron (7)	China (7)	No	No	Yes	2 of 2	2 of 2	1.00	0.95	Х	Х
2002	DWCC	Ivan (6)	Boadecia (8)	Yes	No	Yes	1 of 2	2 of 2	0.50	1.00	Х	Х
2002	DWCC	Houdini (4)	Judy (4)	Yes	Yes	Yes	0 of 2	2 of 2	0.00	1.00		Х
2002	Toronto	Chase (6)	Babe (5)	Yes	Yes	No	2 of 2	2 of 2	0.85	0.90	Х	
2002	Toronto	Yang (3)	Naomi (4)	Yes	Yes	No**	2 of 2	2 of 2	0.92	0.66	Х	Х
2002	Toronto	Washington (6	Stumpy (5)	Yes	No	No	2 of 2	1 of 2	0.25	0.44	Х	
2002	Toronto	Caruso (5)	Larry (12)	No	No	No	2 of 2	1 of 2	0.50	0.47	Х	
2003	DWCC	Gudron (8)	China (8)	Yes	Yes	Yes	2 of 2	2 of 2	1.00	0.99	Х	Х
2003	DWCC	Ivan (7)	Boadecia (9)	Yes	Yes	Yes	2 of 2	2 of 2	1.00	0.93	Х	Х
2003	DWCC	Houdini (5)	Judy (5)	No	No	Yes	2 of 2	2 of 2	1.00	0.95	Х	Х
2003	Toronto	Yang (4)	Naomi (5)	Yes	Yes	No	2 of 2	2 of 2	0.56	0.51	Х	
2003	Toronto	Washington (7	Stumpy (6)	Yes	Yes	Yes	2 of 2	2 of 2	0.06	0.95	Х	Х
2003	Toronto	Caruso (6)	Larry (13)	Yes	No	Yes	2 of 2	2 of 2	0.47	0.71	Х	Х
2004	DWCC	Ivan (8)	Boadecia (10)	Yes	Yes	Yes	2 of 2	2 of 4	0.99	0.47	Х	Х
2004	DWCC	Houdini (6)	Ursula (5)	No	No	Yes	2 of 2	2 of 4	1.00	0.48	Х	Х
2004	DWCC	Franklin (4)	Mirabel (4)	No	No	Yes	2 of 2	2 of 4	0.98	0.50	Х	X

* Indicates the number of locations with cameras installed inside (e.g., NB: 1 of 2 = two nest boxes were provided to the pair but only one had a camera installed inside)

** Male not marked for research purposes, but female missing fur or rear end, facilitating individual identification

included in my observations regardless of their duration. Where possible, data on mount duration included information on the occurrence of thrusting during the mount, as thrusting may indicate intromission (Nelson 1995). However, thrusting was often difficult to discern in mounts that occurred in enclosures. If a mount started or ended in an area in which the animal was out of sight of the camera it was not included in the mount duration data.

Among pairs for which mount data were collected, the median proportion of time that mounts could have been observed (one minus the proportion of time that both animals were in the same nest box or enclosure and both out of sight, i.e., mounts may have been occurring but, due to limited camera coverage, they could not be observed) was not significantly different between pairs that produced pups and pairs that did not produce pups both for nest boxes (produced pups: 0.92, did not produce pups: 0.98; Mann Whitney U test, U = 26, $n_{pups} = 7$, $n_{no pups} = 7$, p = 0.95) and enclosures (produced pups: 0.94, did not produce pups: 0.83; Mann Whitney U test, U = 17, $n_{pups} = 6$, $n_{no pups} = 6$, p = 0.81).

Endocrine data

Whenever possible, fecal samples from both individuals in pairs observed in this study were collected by animal care staff and sent to the Reproductive Physiology Department at the Toronto Zoo for analysis. Tracers, in the form of food colouring or non-toxic sparkles, mixed with a small amount of peanut butter and placed on a Mazuri[®] leaf-eater primate diet mini-biscuit, were consistently fed to the same animal in a pair (animal care staff chose whether to feed the tracer to the male or female), to facilitate identification of the female's feces. An enzyme immunoassay (Graham et al. 2001) was used to create progestagen (a metabolite of progesterone) profiles for females. Subsequently, an iterative procedure, in which elevations in progestagen concentrations that were greater than the

average concentration over the luteal phase plus 1.75 standard deviations were repeatedly removed, was used to determine the baseline progestagen concentration (Graham et al. 2002). The same person who developed the above procedures supervised the analysis of fecal samples from females observed in my study, and ovulation was assumed to have occurred when fecal progestagen concentrations showed a significant increase from baseline levels (Graham, pers. comm.). The Toronto Zoo provided data on whether females ovulated or not, and ovulation dates, for use in my study. However, data on possible pseudopregnancies or abortions were not available for use in my study.

Statistical analyses

I used paired-sample tests whenever two measures from the same animal or pair during the same time period and year were compared (e.g., duration of mounts with thrusting versus duration of mounts without thrusting across the 2003 reproductive season). Because in many cases an animal produced pups in one year but not the next, or vice versa, any measures from the same animal or pair during the same time period but in different years were considered independent (hereafter referred to as a 'comprehensive sample'). However, I re-ran analyses excluding subsequent observation years to determine whether the conclusions were the same (hereafter referred to as a 'conservative sample'). Where the quality of camera coverage differed between years, the year with the worst camera coverage was excluded first.

I used parametric analyses when the raw or transformed data met the test's assumptions; in all other cases I used nonparametric tests. In some cases the distribution of the raw or transformed data changed with the decrease in sample size associated with excluding pairs for conservative samples, requiring the use of a different test. Parametric or nonparametric analyses are reported with means \pm standard error or medians, respectively.

Behavioural profiles

Based on the results in Chapter 2, that the frequency of behaviours differs between enclosures and nest boxes, I analyzed behavioural profiles separately for these two locations. Because of small sample sizes, these analyses could not be re-run using conservative samples. The proportion of time that individuals spent in each behaviour category, excluding time spent out of sight, was calculated. Individuals were classified as either 'produced pups' (enclosures: n = 4, nest boxes: n = 3) or 'did not produce pups' (enclosures: n = 4, nest boxes: n = 3). Because one animal's involvement in a social behaviour did not necessarily mean that its mate was also coded as being involved, analyses were run for males and females separately.

Behavioural categories were compared between the groups across the entire reproductive season using nonparametric Mann Whitney U tests. I then designated individual behaviour categories as either social or non-social and active or non-active (see ethogram in Appendix 1), and summarized the data by week, to examine broad changes in behavioural patterns in each group as the reproductive season progressed. Mann Whitney U tests were used with the new designations to compare behaviour in each week between animals that produced and did not produce pups in each week. When the results were significant, I used Mann Whitney U tests on the individual behaviour categories to explore what contributed to the differences during those weeks.

Mount rate and duration

Limitations in camera coverage on some pairs prevented analyses of the frequency of mounts; therefore, I estimated mount rates for each pair. The number of mounts observed on videotapes was divided by the proportion of time mounts were potentially visible (Table 3.1) and then by the number of days observed (Table 1.1). The result was the estimated number of mounts per day. I analyzed mean mount durations for each pair, omitting pairs with less than five mount durations.

For both mount duration and mount rate analyses, pairs were classified as either 'produced pups' (n = 8 females and 8 males) or 'did not produce pups' (n = 8 females and 8 males), to test for relationships with the successful production of pups (Table 3.1). Pairs were also classified as 'female ovulated' (n = 11 females and 11 males) or 'female did not ovulate' (n = 5 females and 5 males), to test for relationships with ovulation (Table 3.1). Analyses considered data from the entire reproductive season. I used Wilcoxon signed ranks tests and paired t-tests to compare the duration of mounts with and without thrusting (hereafter referred to as 'thrusting mounts' and 'non-thrusting mounts'). Wilcoxon signed ranks tests were used to compare the rate and duration of mounts occurring in nest boxes and enclosures. Mann Whitney U tests and t-tests were used to compare the rate and duration of mounts between pairs that produced pups and pairs that did not produce pups, or pairs in which the female ovulated and pairs in which the female did not ovulate. Further analyses considered data divided into three time periods: pre-ovulation (emergence until one day before ovulation), ovulation-fertilization (one day before until two days after ovulation), and post-fertilization (two days after ovulation until parturition or, for pairs that did not produce pups, 64 days after emergence). However, it was only possible to assign

the three time periods to pairs in which the female ovulated because the number of days between emergence and ovulation varied greatly (3 to 30 days), making it difficult to estimate expected ovulation dates for females that did not ovulate. I used Friedman's two way analysis of variance (ANOVA) by ranks to compare the rate and duration of mounts across the three time periods, and a multiple comparison post-hoc test (Siegel & Castellan 1988) to identify which periods differed. Finally, two-sample Kolmogorov-Smirnov tests were used to compare the distributions mount durations between pairs that produced pups and those that did not.

RESULTS

Environmental and management influences

Small sample sizes and a large number of potentially important variables restricted the testing of correlations between reproductive success and one or a combination of environmental and management variables. Between 1998 and 2004, two females over the age of 13 were paired with younger males for a total of five reproductive seasons (one female for three seasons and the other for two seasons), and these pairings never resulted in the production of pups. In addition, between 1999 and 2001 pairs at the Toronto Zoo were housed in a single enclosure with mean surface areas between 4.3 and 11.2m² at least six times, and these pairings never resulted in successful reproduction. No other factors measured for my study appeared to promote or hinder the production of pups to a great extent (Table 3.2). The behaviour of one female over the age of 10 was observed twice in my study, but only for mount rate and duration analyses (Tables 2.1a & 3.1), and none of

Table 3.2. Range of environmental and management variables for breeding pairs (males three years of age or older, and females four years of age or older) at all *M. vancouverensis* breeding facilities between 1998 and 2004.

		Pairs that
Variable	All pairs	produced pups
Age (females)	4 to 13	4 to 10*
Age (males)	3 to 8	3 to 8*
Birth place (females)	Wild and captivity	Wild*
Birth place (males)	Wild and captivity	Wild*
Years in captivity (females)	1 to 7	1 to 7
Years in captivity (males)	1 to 7	1 to 7
Years at facility (females)	1 to 7	1 to 7
Years at facility (males)	1 to 7	1 to 7
Years in particular enclosure	<1 to >5	<1 to >5
Years together as pair	<1 to >5	<1 to >5
Where pair established	Captivity (1 Wild)	Captivity (1 Wild)
Bred in wild previously?	Yes and No	Yes and No
Bred in captivity previously?	Yes and No	Yes and No
Overwinter with pups	Yes and No	Yes and No
Marked (males)?	Yes and No	Yes and No
Access to outdoor enclosure?	Yes and No	Yes and No
Mean surface area of enclosures (n	n^2) 4.3 to 123.8	12.7 to 94.7
Number of enclosures available	1 to 4	2 to 4
Average volume of nest boxes (m ³) 0.2 to 0.6	0.2 to 0.6
Number of nest boxes available	1 to 3	1 to 3
Adjacent to another breeding pair?	Yes and No	Yes and No
Sight-line to another breeding pair	? Yes and No	Yes and No
Temperature (indoor)**	4 to 27.5°C	4 to 27.5°C
Temperature (outdoor)**	-20.4 to +28.6°C	-20.4 to +28.6°C
Altitude	~6 to ~1400m	~6 to ~1400m
Latitude	43° 49' to 50° 54"	43° 49' to 50° 54"

* however, in 2004, a two-year-old female in a group of four (two female and two male) two-year-old captive born animals produced pups **only for pairs included in this study the pairs observed were housed in single small enclosures. Therefore, subsequent analyses focused on comparisons between behaviour and reproductive success.

Behavioural profiles

Females and males spent a similar proportion of time in nest boxes regardless of whether they produced pups or not (females: produced pups: median = 0.92, did not produce pups: median = 0.91, Mann Whitney U test: U = 3, $n_{pups} = 3$, $n_{no pups} = 3$, p = 0.513; males: produced pups: 0.89, did not produce pups: 0.87, Mann Whitney U test: U = 2, $n_{pups} = 3$, $n_{no pups} = 3$, p = 0.275). Analyses of behaviour over the entire reproductive season revealed that, while in nest boxes, pairs that produced pups spent a smaller proportion of their time resting in contact with their mate and, consequently, a greater proportion of time resting not in contact with their mate, than pairs that did not produce pups (Table 3.3). In addition, while in nest boxes, females that produced pups spent a smaller proportion of time initiating allogrooms, initiating approaches, and sitting at the entrance of the nest box, and a greater proportion of time nest building, than females that did not produce pups. Males that produced pups spent a greater proportion of time initiating 'other social' behaviours such as lunging at their mate or pinning her down while in nest boxes, and a smaller proportion of time digging while in enclosures, than males that did not produce pups.

Between emergence and week four of the reproductive season, the proportion of time spent in social behaviours while in nest boxes did not differ between pairs that produced pups and did not produce pups, both for males and females (Figure 3.1). However, in weeks five, six, and seven, both males and females that produced pups spent a significantly smaller proportion of time in social behaviours than those that did not produce pups. The difference was still evident in week eight, but was not significant. The reverse

Table 3.3: Significant Mann Whitney U test results showing the median proportion (x 100) of time spent in behaviours by adult female (F) and male (M) *M. vancouverensis* that produced versus did not produce pups in nest boxes (NB; $n_{pups} = 3$, $n_{no pups} = 3$) and enclosures (E; $n_{pups} = 4$, $n_{no pups} = 4$).

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1, Ano pups 1/								
Sex	Behaviour	Location	Produced pups	Did not produce pups	U	р		
F	Initiate AL	NB	0.04	0.37	0	0.0495		
F	Initiate AP	NB	0.00	0.01	0	0.0370		
F	CR	NB	59.80	84.30	0	0.0495		
F	NB	NB	0.77	0.58	0	0.0495		
F	NCR	NB	36.70	10.40	0	0.0495		
F	SN	NB	0.25	0.63	0	0.0495		
М	CR	NB	60.40	87.20	0	0.0495		
М	NCR	NB	35.00	6.10	0	0.0495		
М	Initiate OS	NB	0.02	0.00	0	0.0460		
М	D	Е	2.20	7.00	0	0.0210		

* bold numbers indicate which group spent a greater proportion of time in the behaviour

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Figure 3.1. Median proportion of time *M. vancouverensis* females spent in all social behaviours in nest boxes over the breeding season (\bullet indicates females that produced pups, and \Box indicates females that did not produce pups). Each point represents the preceding seven days (i.e., week 1 = days 0 to 6). Numbers above points indicate sample sizes in the two groups (i.e., 2,3 = 2 in \bullet group and 3 in \Box group). N/A in week 9 represents case where there were data for only one female. Asterisks next to week numbers indicate weeks in which the differences were statistically significant.

relationship existed for all non-social behaviours in nest boxes. This is mainly due to the pregnant female acting aggressively toward the male, and decreasing the amount of time spent in contact rest with him. There were no clear trends over the weeks for social or non-social behaviours in enclosures for males or females.

The proportion of time pairs spent resting in contact and not in contact with their mate while in nest boxes comprised a large component of the social and non-social behaviour groupings, respectively, that I analyzed above. When I analyzed only active social behaviours in nest boxes (excluding resting in contact with the mate) the trend seen for all social behaviour was no longer apparent (Figure 3.2). However, pairs that did not produce pups tended to engage in smaller proportions of social activity in the first week of the reproductive season than pairs that produced pups and, through the rest of the season (excluding weeks two and six), the social activity of pairs that did not produce pups tended to be greater than in pairs that produced pups. This difference was statistically significant in week four. A comparison of the individual behaviour categories that comprised the social activity in week four suggested that pairs that did not produce pups tended to spend a greater proportion of time engaged in allogrooms, attempted mounts, genital sniffs, and play-fights, and a smaller proportion of time in mounts, than pairs that produced pups. However, none of these differences were statistically significant.

Mounts

On average, for pairs in which at least 80% of mounts were visible, the first mounts were observed in nest boxes 2.6 ± 1.4 (n = 8) days after emergence from hibernation and in enclosures 4.8 ± 1.5 (n = 6) days after emergence. In three cases, mounts were observed in the hibernation nest box prior to emergence (one day prior in two cases and four days prior



Figure 3.2. Median proportion of time *M. vancouverensis* females spent in active social behaviours in nest boxes (i.e., excluding contact rest) over the breeding season (• indicates females that produced pups, and \Box indicates females that did not produce pups). Each point represents the preceding seven days (i.e., week 1 = days 0 to 6). Numbers above points indicate sample sizes in the two groups (i.e., 2,3 = 2 in • group and 3 in \Box group). N/A in week 9 represents case where there were data for only one female. Asterisk next to week number indicates the week in which the difference was statistically significant.

in one case). Mounts were observed at all times of the day and night in nest boxes, but primarily between 05:00 and 20:00 in enclosures. There may well have been a higher incidence of mounts in enclosures outside these hours, but most enclosure cameras could not provide images during the night. Most mounts were situations in which males mounted females (mean proportion in nest boxes: 0.96 ± 0.02 , n = 15; mean proportion in enclosures: 0.98 ± 0.01 , n = 12), but there were also a number of observations in which females (mean in nest boxes: 17.3 ± 10.0 mounts, n = 7; mean in enclosures: 2.0 ± 0.9 mounts, n = 4), both exhibiting or not exhibiting thrusting during the mount. All subsequent analyses, unless otherwise indicated, are situations where males mounted females.

Animals were generally in the dorso-ventral position during mounts, as described for other marmot species (Barash 1989). However, during approximately 2% of mounts in both enclosures and nest boxes, animals were in a position other than dorso-ventral (e.g., male lying at an angle to the female in a ventral-ventral position, with genital areas in contact). During dorso-ventral mounts, the male was often observed biting the nape of the female. During thrusting mounts, periods of thrusting often alternated with periods of quiescence.

Mount rate

The rate of mounts in nest boxes (median = 3.1 mounts/day) was significantly greater than the rate of mounts in enclosures when a comprehensive sample was used (median = 0.7 mounts/day; Wilcoxon signed ranks test: Z = 2.8, n = 10, p = 0.01), or when

a conservative sample was used (nest boxes: median = 3.1 mounts/day, enclosures: median = 0.9 mounts/day; Wilcoxon signed ranks test: Z = 2.5, n = 8, p = 0.01). Therefore, I conducted subsequent analyses of mount rate separately for nest boxes and enclosures.

The rate of mounts did not differ between pairs that produced pups and those that did not produce pups, either in nest boxes (produced pups: median = 1.6 mounts/day, did not produce pups: median = 2.2 mounts/day; Mann Whitney U test: U = 23, $n_{pups} = 7$, n_{no} $p_{ups} = 7$, p = 0.85), or in enclosures (produced pups: median = 0.5 mounts/day, did not produce pups: median = 1.3 mounts/day; Mann Whitney U test: U = 11, $n_{pups} = 6$, $n_{no} p_{ups} = 6$, p = 0.26) when a comprehensive sample was used, or when a conservative sample was used for both nest boxes (produced pups: median = 2.2 mounts/day, did not produce pups: median = 3.4 mounts/day; Mann Whitney U test: U = 7, $n_{pups} = 4$, $n_{no} p_{ups} = 5$, p = 0.46) and enclosures (produced pups: median = 0.4 mounts/day, did not produce pups: median = 1.4 mounts/day; Mann Whitney U test: U = 2, $n_{pups} = 3$, $n_{no} p_{ups} = 5$, p = 0.10).

When ovulation was used as a measure of success, rather than the production of pups, the results were similar to those reported above. The rate of mounts in nest boxes in pairs in which the female ovulated (median = 1.6 mounts/day) was not significantly different from the median rate of mounts in nest boxes in pairs in which the female did not ovulate (median = 2.2 mounts/day; Mann Whitney U test: U = 21, $n_{ov} = 9$, $n_{no ov} = 5$, p = 0.84) when a comprehensive sample was used, or when a conservative sample was used (female ovulated: median = 2.9 mounts/day, female did not ovulate: median = 4.8 mounts/day; Mann Whitney U test: U = 8, $n_{ov} = 5$, $n_{no ov} = 4$, p = 0.62). The rate of mounts in enclosures in pairs in which the female ovulated (median = 0.5 mounts/day) was also not

significantly different from the rate of mounts in enclosures in pairs in which the female did not ovulate (median = 1.5 mounts/day; Mann Whitney U test: U = 7, $n_{ov} = 8$, $n_{no ov} = 4$, p = 0.13) when a comprehensive sample was used, or when a conservative sample was used (female ovulated: median = 0.4 mounts/day, female did not ovulate: median = 1.5 mounts/day; Mann Whitney U test: U = 4, $n_{ov} = 4$, $n_{no ov} = 4$, p = 0.25).

When the data were analyzed over three time periods, there was significant variation in the rate of mounts in nest boxes in pairs in which the female ovulated when a comprehensive sample was used (pre-ovulation: median = 2.3 mounts/day, ovulationfertilization: median = 2.8 mounts/day, post-fertilization: median = 0.4 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 6.9$, n = 9, df = 2, p = 0.03). The rate of mounts decreased in the post-fertilization period (multiple comparison post hoc test: z = 10.2, k =3, p < 0.05). The trend was similar in enclosures, but not significantly so, when a comprehensive sample was used (pre-ovulation: median = 0.6 mounts/day, ovulationfertilization: median = 0.6 mounts/day, post-fertilization: median = 0.2 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 2.9$, n = 8, df = 2, p = 0.08). When a conservative sample was used the result for mount rate in nest boxes was similar but not significant (pre-ovulation: median = 3.0 mounts/day, ovulation-fertilization: median = 2.8 mounts/day, post-fertilization: median = 0 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 2.0$, n = 5, df = 2, p = 0.37) and the result for mount rate in enclosures was still not significant (pre-ovulation: median = 0.4 mounts/day, ovulation-fertilization: median = 0.7 mounts/day, post-fertilization: median = 0.3 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 4.5$, n = 4, df = 2, p = 0.55).

When the rate of mounts for pairs in which the female both ovulated and had pups was tested, there was no significant difference across the three time periods in either the nest boxes (pre-ovulation: median = 2.3 mounts/day, ovulation-fertilization: median = 2.2 mounts/day, post-fertilization: median = 0.8 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 5.0$, n = 7, df = 2, p = 0.08) or the enclosures (pre-ovulation: median = 0.6 mounts/day, ovulation-fertilization: median = 0.7 mounts/day, post-fertilization: median = 0.4 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 3.2$, n = 6, df = 2, p = 0.20) when a comprehensive sample was used. However, the trend was similar to analyses of mount rate in pairs in which the female ovulated, and the lack of significance may be due to the decrease in sample size associated with dropping out pairs that ovulated but did not produce pups. Results were similar when a conservative sample was used (nest boxes: preovulation: median = 2.6 mounts/day, ovulation-fertilization: median = 3.4 mounts/day, post-fertilization: median = 1.1 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 1.2$, n = 4, df = 2, p = 0.55; enclosures: pre-ovulation: median = 0.3 mounts/day, ovulationfertilization: median = 0.8 mounts/day, post-fertilization: median = 0.4 mounts/day; Friedman two way ANOVA by ranks: $\chi^2 = 4.7$, n = 3, df = 2, p = 0.10).

Mount Duration

The duration of thrusting mounts (median = 160.4 s) was significantly longer than the duration of non-thrusting mounts when a comprehensive sample was used (median = 17.5 s; Wilcoxon signed ranks test: Z = 2.2, n = 6, p = 0.03), or when a conservative sample was used (thrusting mounts: mean = 166.1 ± 45.0 s, non-thrusting mounts: mean = 27.8 ± 9.7 s; paired sample t-test, t = 7.4, n = 5, p = 0.002). The same relationship was evident when mounts that occurred in nest boxes were tested separately from those that occurred in enclosures. In nest boxes, the duration of thrusting mounts (median = 203.2 s) was significantly longer than the duration of non-thrusting mounts (median = 25.5 s; Wilcoxon signed ranks test: Z = 2.0, n = 5, p = 0.04) when a comprehensive sample was used. The result was in the same direction, but not significantly so, when a conservative sample was used (thrusting: median = 152.5 s, non-thrusting: median = 27.6 s; Wilcoxon signed ranks test, Z = 1.8, n = 4, p = 0.07). It was not possible to test the duration of thrusting versus non-thrusting mounts in enclosures because there were data from only one pair in the non-thrusting group, but the trend was similar to both previous analyses (comprehensive sample: thrusting mounts: mean = 129.9 ± 35.6 s, one pair's non-thrusting mounts: mean = 13.6 s). Therefore, in subsequent analyses, I analyzed thrusting mounts separately from non-thrusting mounts.

The duration of thrusting mounts that occurred in nest boxes (median = 159.5 s) was not significantly different from those that occurred in enclosures (median = 145.2 s; Wilcoxon signed ranks test: Z = 0.5, n = 6, p = 0.60) when a comprehensive sample was used, or when a conservative sample was used (nest boxes: median = 115.7 s, enclosures: median = 139.1 s; Wilcoxon signed ranks test: Z = 1.2, n = 5, p = 0.23). Once again there were data from only one pair in the non-thrusting group in enclosures; therefore, it was not possible to compare the duration of non-thrusting mounts in nest boxes versus in enclosures. However, the trend was similar (comprehensive sample: nest boxes: mean = 31.2 ± 9.5 s, one pair in enclosures: mean = 13.6 s; conservative sample: nest boxes: mean = 35.0 ± 11.2 s, one pair in enclosures: mean = 13.6 s). In subsequent analyses the duration of both thrusting and non-thrusting mounts were pooled for nest boxes and enclosures.

The duration of thrusting mounts was twice as long in pairs that produced pups (mean = 231.7 ± 45.5 s) compared to pairs that did not produce pups (mean = 115.1 ± 23.2 s; t-test: t = 2.3, n_{pups} = 8, n_{no pups} = 8, p = 0.04) when a comprehensive sample was used, or when a conservative sample was used (produced pups: mean = 278.3 ± 69.4 s, did not produce pups: mean = 107.4 ± 26.6 s; t-test: t = 2.5, n_{pups} = 4, n_{no pups} = 5, p = 0.04). However, the duration of non-thrusting mounts did not differ between pairs that produced pups (median = 39.9 s) and those that did not produce pups (median = 17.5 s; Mann Whitney U test: U = 3, n_{pups} = 2, n_{no pups} = 4, p = 0.64) when a comprehensive sample was used. It was not possible to test this using a conservative sample due to the fact that data from only one pair qualified for inclusion in the 'produced pups' group, but the trend was the same (one pair that produced pups: mean = 64.8 s, did not produce pups: mean = 18.6 ± 3.9 s).

Once again, analyses using ovulation as a measure of success rather than the production of pups yielded similar results to those reported above. The duration of thrusting mounts in pairs in which the female ovulated (mean = $209.4 \pm 35.6 \cdot s$) was significantly longer than that in pairs in which the female did not ovulate (mean = $94.1 \pm 27.9 \, s$; t-test: t = 2.2, n_{pups} = 11, n_{no pups} = 5, p = 0.046) when a comprehensive sample was used. The result was similar but not quite significant when a conservative sample was used

(female ovulated: median = 241.7 s, female did not ovulate: median = 90.8 s; Mann Whitney U test: U = 2, $n_{pups} = 5$, $n_{no pups} = 4$, p = 0.05). The duration of non-thrusting mounts did not differ between pairs in which the female ovulated (median = 14.9 s) and pairs in which the female did not ovulate (median = 19.5 s; Mann Whitney U test: U = 3, $n_{pups} = 3$, $n_{no pups} = 3$, p = 0.51) when a comprehensive sample was used, or when a conservative sample was used (female ovulated: median = 37.7 s, female did not ovulate: median = 19.5 s; Mann Whitney U test: U = 3, $n_{pups} = 2$, $n_{no pups} = 3$, p = 1.0).

The only pairs that could be included in the analysis examining mount duration over three time periods were pairs in which the female both ovulated and produced pups. While the trend was that the duration of mounts was greater during the ovulation-fertilization period (median = 545.0 s), this was not significantly different from the duration of mounts that occurred in the pre-ovulation (median = 273.1 s) and post-fertilization (median = 264.1 s) periods when a comprehensive sample was used (Friedman two way ANOVA by ranks: $\chi^2 = 0.7$, n = 3, df = 2, p = 0.71). One pair was observed in more than one season in this statistic, but with such a low sample size it was not feasible to redo the analysis using a conservative sample.

The distributions of the duration of thrusting mounts (durations rounded up to the nearest minute; Figure 3.3) differed between pairs that produced and did not produce pups (two-sample Kolmogorov-Smirnov test: Z = 1.53, $n_{pups} = 26$, $n_{no pups} = 26$, p = 0.019). Further analyses showed that there was no difference in the distributions of thrusting mounts that were between zero and 13 minutes in duration (two-sample Kolmogorov-





Smirnov test: Z = 0.78, $n_{pups} = 13$, $n_{no pups} = 13$, p = 0.570), but there was a highly significant difference in the distributions of thrusting mounts between 14 and 26 minutes in duration (two-sample Kolmogorov-Smirnov test: Z = 0.77, $n_{pups} = 13$, $n_{no pups} = 13$, p =0.001). In fact, 6.7% of thrusting mounts were longer than 13 minutes in pairs that produced pups, whereas only 0.3% of thrusting mounts were longer than 13 minutes in pairs that did not produce pups. In addition, no thrusting mounts greater than 18 minutes in duration were observed in pairs that did not produce pups, whereas thrusting mounts as long as 26 minutes were observed in pairs that did produce pups.

DISCUSSION

A comprehensive understanding of reproductive behaviour, and factors leading to successful reproduction, are crucial components of conservation breeding and reintroduction programs for species at risk. In addition, conservation breeding programs can create opportunities to study features of behaviour that are difficult to study in wild populations. *M. vancouverensis* is the most endangered marmot species and one of the most endangered mammals in the world. Relatively little is known about marmot reproductive behaviour because much of it occurs in burrows, and there is a lack of understanding about factors associated with successful reproduction in the *M. vancouverensis* conservation breeding and reintroduction program. In this study of adult *M. vancouverensis* pairs in the conservation breeding program, I identified behavioural differences during the reproductive season between pairs that produced pups and those that did not. These differences can be used to predict the likelihood of pup production during

the reproductive season, and to assess current, and to plan future, pairings in the conservation breeding program.

Marmota vancouverensis pairs in the conservation program have reproduced successfully under a number of different environmental conditions and management regimes. Apart from the possibility that females over the age of 10, and pairs housed in a single small enclosure with a surface area less than 12.7m² cage do not produce pups, there do not appear to be any environmental or management variables that promote or hinder the production of pups to any great extent. However, a large number of variables, coupled with small sample sizes, precluded statistical analysis of these variables. Data collection should be continued and an analysis conducted that investigates whether one or a combination of these variables is associated with increased or decreased reproductive success.

Behaviours other than copulation may be important in mammalian reproductive systems (Dewsbury 1972). In my study, females that produced pups gathered nesting materials (termed 'nest building') in both enclosures and nest boxes more than females that did not produce pups, but the difference was only significant in nest boxes. This likely reflects the female's preparation for parturition and rearing of her young in the nest box. A similar difference is exhibited above ground by reproductive female yellow-bellied marmots (*M. flaviventris*) compared to non-reproductive females (Armitage 2003). Females that produced pups also spent a smaller proportion of time sitting inside their nest boxes, looking out into enclosures, and males that produced pups spent a smaller proportion of time digging in enclosures. Sitting at the entrance of burrows and looking out has been associated with wariness and scanning the environment for predators and agonistic conspecifics in wild marmots (Armitage & Chiesura Corona 1994; Barash 1989). Females observed in my study that did not produce pups may have spent more time being wary of predators and/or their mates, or they may have been observing their mates' activities in enclosures, such as digging. Alternatively, this difference may be due to the fact that females that did not produce pups had more time available to sit at the entrance of their nest boxes because they were not dedicating time to the preparation of their nest box for parturition and weaning of pups.

In addition, males in pairs that produced pups spent a greater proportion of time initiating 'other social' behaviours, such as lunging at the female or pinning her down while in nest boxes. Lunging was identified by Heard (1977) as a dominance-indicating behaviour in wild *M. vancouverensis*, and was used to create a dominance hierarchy that placed adult males above adult females. In addition, when yearling female *M. flaviventris* fight off adult males that grasp them in a behaviour that resembles a mount, the males pin the female down (Jamieson & Armitage 1987). This pinning behaviour seems to reflect dominance, with the dominant animal pinning the submissive one (Jamieson & Armitage 1987). Therefore, in my study, males in pairs that produced pups may have been more dominant when interacting with females compared to males in pairs that did not produce pups.

Females in pairs that produced pups spent a smaller proportion of time approaching and grooming their mate in nest boxes than did females in pairs that did not produce pups. Female mammals in estrus will seek out males, initiate copulations, and, generally, prefer to remain in close proximity to males (Nelson 1995). In captive *M. monax*, females that were in estrus routinely approached males, whereas anestrous females did not (Concannon et al. 1997b). *M. monax* exhibits a single, prolonged estrous period during their

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reproductive season, lasting an average of 18.1 days and ranging from 12 to 27 days (Hikim et al. 1991). The Belding's ground squirrel (Spermophilus beldingi) also exhibits a single prolonged estrous period, averaging three to four weeks but lasting as long as eight to 10 weeks in some females (Holmes & Landau 1986). It is not clear in either of these species whether females are sexually receptive, or whether copulations can result in pregnancy, throughout the entire period. In addition, there is no information on whether follicles are regressing and re-forming during the estrous period, or whether they are viable for the entire period. In contrast, a single estrous period in Richardson's ground squirrels is relatively short, averaging three days and ranging from one to nine days (Michener 1980). However, females re-enter estrus an average of five days after their previous estrous period ends, and their entire estrous cycle can last three to five weeks (Michener 1980). Hikim (1991) suggests that the adaptive significance of long estrous periods in sciurids who experience short (and environmentally variable) breeding seasons may serve to ensure animals can mate when conditions are optimal and, ultimately, maximize the future survival of their offspring. I could not identify estrus in the females observed in my study, but it is possible that female-initiated approaches and allogrooms were associated with estrus and with the female soliciting attention from the male. The differences in femaleinitiated approaches and allogrooms between females that produced pups and those that did not observed in this study may be due to a decrease in these behaviours once a female becomes pregnant, which terminates estrus (Nelson 1995).

Changes in female behaviour during pregnancy may also explain the observed decrease in the proportion of time spent in social interactions in pairs that produced pups starting in week five and continuing until parturition. This was driven primarily by a decrease in the proportion of time females spent resting in contact with males, resulting from aggressive interactions with the male, such as chasing him out of her preferred nest box. However, it is interesting to note that, although pregnant female *M. vancouverensis* decrease their social tolerance of males, they appear to be very socially tolerant of males throughout the rest of the year, even during pup rearing (pers. obs.) The display of, or increase in, aggressive behaviours by pregnant females may be due to increasing levels of progesterone (Ogawa & Maxson 1987; Payne & Swanson 1972), as has been observed in female *M. monax* (Schoonmaker 1938), *M. olympus* (Barash 1973a), and Richardson's ground squirrels (*Spermophilus richardsonii*; Michener 1983) during pregnancy. However, it may be possible that females that experiencing pseudopregnancies will also exhibit aggressive behaviours towards males. Further analyses that compare the behavioural profiles of non-pregnant, pseudopregnant, and pregnant females will be necessary to help identify whether it is possible to behaviourally distinguish between the three states.

Behaviours such as allogrooms, attempted mounts, genital sniffs, and play-fights are often associated with copulatory behaviour in marmots (Barash 1973a, 1989; Keeley et al. 2003). These behaviours may be necessary to achieve a pair bond or the hormonal processes necessary to initiate copulatory behaviour in *M. vancouverensis*. However, in week four after emergence from hibernation and possibly throughout much of the reproductive season following week two after emergence, these behaviours may have been exhibited more in nest boxes by pairs that did not subsequently produce pups than pairs that did produce pups. In addition, pairs that did not produce pups may spend a smaller proportion of time in male mounts of females during those same weeks. In most North American marmot species, copulations are believed to start shortly after adult females emerge from hibernation (Armitage 1965; Barash 1973a, 1989; Nee 1969; Schwartz & Armitage 1980). Similarly, in the *M. vancouverensis* pairs observed in my study, the first mounts occurred in enclosures and nest boxes 4.8 ± 1.5 days and $2.6 \pm$ 1.4 days after emergence, respectively. However, in some pairs, mounts occurred in nest boxes prior to the emergence of adult females from hibernation. This has yet to be confirmed in wild *M. vancouverensis* but, considering these findings and similar findings in *M. broweri* (Rausch & Rausch 1971) and some Eurasian marmot species (cited in Barash 1989), it seems plausible that mounts do take place in the burrow of wild *M. vancouverensis* prior to emergence as well.

Pairs engaged in mounts in their nest boxes more often than in their enclosures. Occasionally, females were observed mounting males and, in some of those mounts, thrusting was observed. Female-initiated mounting is generally associated with high proceptivity, a term used to describe behaviours exhibited by females to initiate sexual unions (Beach 1968). This behaviour has also been observed in wild female *M. flaviventris* (Armitage 1965) and *M. olympus* (Barash 1973a). Males often reacted to female-initiated mounts by mounting the female, but those male-initiated mounts were usually short. Similar patterns were exhibited in *M. flaviventris* (Armitage 1965). The vast majority of mounts observed in this study were initiated by males, and further discussion pertains only to these mounts.

Comparison of the behavioural profiles of individuals in pairs that did and did not produce pups, suggests that the proportion of time spent in male mounts of females may be positively correlated with successful reproduction during certain weeks of the reproductive season. However, my study showed that there was no significant difference in the rate of mounting between pairs that produced pups and pairs that did not. In fact, pairs that did not produce pups tended to have a greater daily mount rate. The difference in reproductive success lay in the duration of thrusting mounts. Thrusting mounts were significantly longer than non-thrusting mounts, and the duration of both types of mounts did not differ between enclosures and nest boxes. In many species, the presence of thrusting indicates intromission (Nelson 1995). Therefore, mounts with thrusting observed in this study may have indicated copulations with intromission, and there may have been no intromission in mounts without thrusting. If this is the case, it is not surprising that mounts without thrusting were not correlated with the production of pups or ovulation. Because it was not possible to accurately discern any specific behaviours indicating ejaculation, I cannot comment on whether all thrusting mounts culminated in ejaculation or not, as done in a study of captive *M. monax* (Concannon et al. 1997b).

The mean duration of thrusting-mounts was greater for females that ovulated and pairs that produced pups than those that did not. Pairs that produced pups exhibited thrusting mounts averaging 231.7 ± 45.5 seconds, or approximately 3.9 minutes, compared to 115.1 ± 23.2 , or approximately 1.9 minutes, in pairs that did not produce pups. In pairs that produced pups, thrusting mounts tended to be longest during the ovulation and fertilization period, further indicating that the duration of thrusting mounts are likely crucial in achieving ovulation and/or fertilization in this species. To identify the duration of mounts required for ovulation and/or fertilization, it is instructive to look at the distribution of the duration of thrusting mounts of pairs that did and did not produce pups. In pairs that produced pups, 6.7% of thrusting-mounts were greater than 13 minutes. In contrast, only 0.3% of thrusting mounts were greater than 13 minutes in pairs that did not produce pups. In addition, no pairs that did not produce pups were observed engaging in thrusting mounts longer than 18 minutes, whereas thrusting mounts up to 26 minutes were observed in pairs that produced pups.['] Therefore, it is possible that *M. vancouverensis* pairs need to engage in mounts with thrusting that are greater than 13 minutes, and possibly greater than 18 minutes, in duration to accomplish ovulation and/or fertilization. However, further study will be required to identify whether there is a critical duration of thrusting mounts required for ovulation and/or fertilization in this species, and whether there is an interaction between the frequency and duration of mounts. For example, it may be that the probability of ovulation or fertilization increases with the number of thrusting mounts exceeding a certain duration, as was found for the probability of fertilization in *M. monax* (Concannon et al. 1997b).

The range of thrusting-mount durations observed in this study (five seconds to 25.6 minutes) falls within the range of mating durations observed in some other marmot species: one minute to 11.5 minutes in captive *M. monax* (Concannon et al. 1997b), seven to 14 minutes in captive *M. monax* (Hikim et al. 1992), 30 seconds to 23 minutes in wild *M. flaviventris* (Armitage 1965), 30 seconds to 8 minutes in wild *M. Olympus* (Barash 1989), 30 to 50 seconds in wild *M. menzbieri* (Bibikow 1996), and two mounts measuring 65 seconds and 9 minutes in wild alpine marmots, *M. marmota* (King & Allainé 1998). However, in some of these studies, a mating was defined in part by its duration. For example, in Concannon et al (1997b), a mount was not considered a mating unless it was greater than 60 seconds.
While reproductive success was linked to thrusting-mount duration, in my study it was not possible to discern whether the duration of thrusting-mounts was important for ovulation, fertilization or both. In induced ovulators, physical stimulation of the cervix and vagina during mating activates afferent neural pathways to areas of the hypothalamus that produce gonadotrophin-releasing hormones, culminating in a surge of luteinizing hormone and, eventually, ovulation (Ramirez & Beyer 1988). The duration of copulation that is required to complete this process varies. In *M. monax*, minimal cervical and vaginal stimulation is required and mount duration plays no role in inducing ovulation (Concannon et al. 1997b).

The duration of copulations may facilitate fertilization in a number of ways. Most discussions of this topic in rodents refer to the effects of the number of intromissions on fertilization. However, whether the word 'intromission' refers to a mating with penile insertion, or individual penile insertions during mating, varies among studies and not clearly defined in many. Nonetheless, many of the processes discussed are the same. Therefore, hereafter the word 'intromission' refers to multiple penile insertions during matings. It was not possible to accurately count thrusts during my study, but I assume that an increase in the duration of thrusting mounts is likely associated with an increase in the number of intromissions.

Multiple intromissions can facilitate fertilization of eggs by affecting transport of the sperm through the cervix (Adler 1969; Chester & Zucker 1970; Roberts et al. 1999; Schwagmeyer & Foltz 1990). This may be accomplished though mechanical processes, such as distension of the cervix (Chester & Zucker 1970; deCatanzaro 1991), and through neuro-endocrine mechanisms, such as stimulating the release of oxytocin, causing contractions that facilitate sperm transfer (Adler 1969; deCatanzaro 1991; McNeilly & Ducker 1972). However, McNeilly and Ducker (1972), in a study on goats (*Capra aegagrus hircus*), found that the physical stimulus of coitus was a relatively minor component in stimulating the release of oxytocin, and that the smell and physical presence of males stimulated the greatest oxytocin release. Multiple intromissions can also initiate the hormonal conditions in pregnancy that permit implantation and prevent receptivity, by triggering the tonic secretion of progesterone (Adler 1969; Chester & Zucker 1970; Nelson 1995). However, this may not occur in all species. In mice (*Mus musculus*) a large number of pre-ejaculatory thrusts seems neither necessary nor sufficient to induce the secretion of progesterone associated with preparation for pregnancy (Land & McGill 1967) . If the duration of thrusting-mounts is associated with triggering secretions of progesterone in *M. vancouverensis*, this may also explain the observed increase in aggressive behaviours in pregnant females (Ogawa & Maxson 1987; Payne & Swanson 1972).

Further study is required to clarify the role that copulation duration plays in ovulation and fertilization in *M. vancouverensis*. However, these types of investigations routinely involve relatively invasive techniques, such as physical and surgical examinations of animals during the reproductive season. Currently, because the species is so critically endangered, and because it is believed that physical examinations of males and females during the reproductive season may interfere with successful reproduction, all breeding facilities have adopted a policy of minimal disturbance during the reproductive season.

The results from my study have increased our understanding of reproductive behaviour, and behaviours associated with successful reproduction, in the *M*. *vancouverensis* conservation breeding program. In general, when comparing the social

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behaviours of reproductively successful and unsuccessful pairs, males in successfully reproducing pairs initiate more dominance-indicating behaviours towards females, such as lunging at them and pinning them down in nest boxes. Females in successfully reproducing pairs are socially tolerant of the male until approximately week five after emergence, when they become aggressive to males and decrease the amount of time they spend in contact rest with them. Sometimes a pregnant female excludes the male from her preferred nest box, and at other times she allows him into the nest box but does not allow him to rest in contact with her. Females in non-successfully reproducing pairs approach and groom their mate in nest boxes more throughout the reproductive season, possibly in an effort to stimulate copulatory behaviour. While behaviours such as allogrooms, attemped mounts, genital sniffs and play-fights that occur in nest boxes may be associated with copulatory behaviour, these behaviours are not necessarily associated with the long thrusting mounts that are likely necessary to achieve ovulation and/or fertilization. Analyses of the sequences of behaviour surrounding 'long' versus 'short' copulations will help further identify possible behavioural correlates of successful reproduction in this species.

Some of the behaviour patterns described above have been observed above ground in wild populations of *M. vancouverensis* and other social marmot species. The detailed information about reproductive behaviour that has been gained from my study through observations in both enclosures and nest boxes may help in interpreting the above ground behaviours observed in wild marmots during the reproductive season and distinguishing between reproductive and non-reproductive individuals. As is, these findings can be used to predict successful reproduction in the *M. vancouverensis* conservation breeding program, and to assess current and to plan future pairings. It is my hope that these tools can be used to help maximize the number of pups born for reintroduction to the wild, and that the increased understanding of reproductive behaviour in this species will aid both in the recovery of *M. vancouverensis* and in securing the future of all marmot species at risk.

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CHAPTER 4

Management Advances and Areas for Future Research

Management advances

While developing this study, I identified a non-invasive and highly effective technique for marking animals in the *M. vancouverensis* conservation breeding program for individual identification (described in Chapter 1 methods). Other techniques, such as finger paint, had been tried in the past, with disappointing results (unpublished minutes, Vancouver Island Marmot Captive Management Group, August 2001). Details on the technique and products used in my study have been distributed to recovery participants through the captive management group, and continue to be used by some breeding facilities to aid in identification of animals for management and/or research purposes.

Nest box and enclosure cameras purchased with funding obtained during this study have vastly increased the ability of animal care staff at the DWCC to monitor animals in their care. Those cameras remain in place now and continue to be used to monitor animals throughout the year. The detailed behavioural profiles of pairs in both enclosures and nest boxes throughout the reproductive season developed during this study will greatly aid recovery participants at reproductive facilities in understanding the reproductive behaviour of pairs at their facility. In addition, while most facilities are outfitted with enclosure cameras, some facilities do not have cameras in many of their nest boxes or their nest box cameras do not provide an image of the entire nest box. In these cases, the behavioural profiles developed in this study can be used by breeding facilities, along with observations of their animals in enclosures, to infer what is occurring in their nest boxes. When I started this study it was generally presumed among breeding facilities that all mounts were initiated by males. In some situations, these assumptions were even used to tell animals apart during behavioural observations. By marking animals and conducting detailed behavioural observations, my study has shown that females will occasionally mount males and will exhibit thrusting during those mounts. Therefore, when animals are not easily identifiable, it is not safe to assume that the initiator of the mount is the male.

The results of this study can be used by recovery participants and breeding facilities to predict the production of pups and assess pairings in the *M. vancouverensis* conservation breeding program. Some important things to keep in mind while monitoring animals are as follows (the locations, i.e., enclosures or nest boxes, where these behaviours can be observed are indicated in parentheses):

- The rate of mounts tends to be higher in pairs that do not produce pups; therefore, it is not possible to predict success based on the observation of a large number of mounts (enclosures and nest boxes).
- Non-thrusting mounts are not associated with the production of pups; therefore, animals should be monitored for the presence of thrusting mounts (enclosures and nest boxes).
- The average duration of thrusting mounts is greater in pairs that produce pups (enclosures and nest boxes).
- Mounts as long as 13 to 26 minutes may contribute towards successful ovulation and/or fertilization (enclosures and nest boxes).
- 5) In comparison to males in unsuccessful pairs, males in successfully reproducing pairs exhibit behaviours that indicate dominance over the female more often, such as lunging at her and pinning her down (nest boxes).

- 6) While the exhibition of behaviours such as allogrooms, attemped mounts, genital sniffs and play-fights in week four after emergence, and likely in other weeks, may be associated with copulatory behaviour, these behaviours may not be associated with the 'long' thrusting mounts that are likely important for accomplishing ovulation and/or fertilization. If these behaviours are exhibited frequently and the duration of thrusting mounts never seem to exceed 13 to 18 minutes, the pair may not be behaviours other than thrusting mounts; enclosures and nest boxes for thrusting mounts).
- 7) Females that routinely approach and groom the male throughout the reproductive season may be soliciting his attention and are less likely to be pregnant that those that act aggressively towards the male (see below) (nest boxes).
- 8) Females that are pregnant tend to act aggressively toward males, starting around week five after emergence. Each female may exclude the male from her preferred nest box, or allow him in the nest box but not permit him to rest in contact with her (enclosures and nest boxes or, if the female still allows the male in her preferred nest box, nest boxes only).

If behaviours associated with successful reproduction are not exhibited by pairs, breeding facilities may need to consider re-pairing animals either during the reproductive season or for the subsequent season, or explore mate choice options, to encourage the expression of these behaviours. Mating recommendations in conservation breeding programs based solely on genetic and demographic considerations may differ from natural mating strategies (Lindburg & Fitch-Snyder 1994), and some degree of mate choice may be

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advisable because managers may not understand, or be able to identify, traits that animals evaluate when choosing mates (Caro 1998).

More broadly, results from my study indicate that *M. vancouverensis* pairs that hibernate together may copulate prior to emergence. It is not yet clear how important those early copulations are towards reproductive success, but it may be advisable to have adult females and adult males hibernating together in captivity and in the wild in case these copulations are important. In addition, in the wild, there is likely a much higher occurrence of social interactions in burrows than is exhibited above ground, during the reproductive season at least and possibly throughout the entire active season. The social interactions that occur in burrow chambers may be crucial to successful reproduction in this species and, consequently, natural burrows should be protected in the wild not only for hibernation but potentially for successful reproduction as well. Implications of the high levels of social interactions in nest boxes, and likely also in burrow chambers, such as disease transmission within or between captive and wild populations, also need to be considered in the *M. vancouverensis* recovery program. Finally, it appears that captive pairs retain levels of activity and social interactions that are similar to wild conspecifics and, therefore, are likely suitable for release.

Areas for future research

Within the *M. vancouverensis* conservation breeding program, there should be continued data collection and an analysis of how environmental and management variables at breeding facilities relate to reproductive success. In addition, analysis of the sequence of behaviours leading up to, and following, copulations of 'short' and 'long' duration will

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further our understanding of why some pairs do not exhibit the 'long' copulations that seem to be important in accomplishing ovulation and/or fertilization. For example, are 'short' mounts due to males disengaging from the female or females breaking away from the male? Food consumption in pairs housed at the DWCC is currently under analysis, and further study comparing the diets in captivity and in the wild, and on the role of nutrition in the general health and reproductive success of animals, is advisable (e.g., Andersen et al. 1976). Finally, when the population of *M. vancouverensis* becomes stable enough to allow for more hands-on investigations, a study that determines whether the duration of copulations is important for ovulation, fertilization, or both will prove extremely useful in furthering our knowledge of the function of copulation duration in this species.

It appears that adult pairs in the *M. vancouverensis* conservation breeding program are not sedentary and their social nature remains similar to that of wild animals. Because this program is still young and captive-born animals had not yet reached adulthood or were not in enclosures equipped with cameras during my observation years, all of the animals observed in this study were wild-born and, so far, reintroductions have consisted of captiveborn animals. However, wild-born animals may be released back into the wild in the future. My study serves as a baseline measure of whether animals undergo changes in their activity levels and sociality rates when housed in captivity for extended periods of time. Further study and comparisons of the behaviour of captive-born animals slated for release, released animals, and wild animals will prove very useful in evaluating whether captive and release animals behave in a manner that is conducive to interacting appropriately with conspecifics and to survival in the wild, and ensuring the success of the reintroduction program. In addition to these comparisons, estimates of the proportion of time wild and released animals spend above ground versus in the burrow, along with the results from my study, will greatly advance our ability to form hypotheses about in-burrow behaviour in this and other social marmot species.

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APPENDIX 1

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Ethogram of *M. vancouverensis* behaviours S = social, NS = non social, A = active, NA = non active

Behaviour	Code	Definition	S/NS	A/NA
Initiator or Recipient	I or R	Record whether individual was the initiator or the recipient of the	Х	Х
1000,0000		behaviour (record for recipient if		
		initiated behaviour acknowledged)		
Location	E or N	Location where behaviour was	Х	X
		initiated ($E = enclosure; N = nest$		
		box). Animal in specified area if		
		>50% of its body is in that area.		
		and the second		
Allogroom	AL	Manipulating or cleaning mate's fur,	S	A
		includes scratching and excludes C		
		(record I/R)	<u>a</u>	
Attempt to	AM	One animal attempting to get into	S	A
mount		dorso-ventral position with the		
		other, or one animal thrusting		
		against the other but in position		
		where genitals are not in contact		
Approach	AP	(record I/K) Moves to within 1 hody length of	S	Δ
		mate excluding during CF & NB	0	23
		(record I/R)		
Autogroom	ATI	Manipulating or cleaning own fur.	NS	А
1 lutogroom	110	includes scratching		
Arroid	A 37	Moves greater than 1 hody length	S	Δ
Avoid	Av	away from mate's AI AM AP C	D	
		GS M PE SGS (excludes CF and		
		FL) (record I/R)		
Chew ear/neck	С	Chewing the ear/neck region of mate	S	А
Cheve earlieer	Ŭ	(record I/R)	-	
Chase/follow	CF	Both animals walking or running in	S	А
		same direction, one animal		
		following behind the other within 2		
		body lengths, excludes FL, AV &		
		AM, (record I/R)		
		-		

Appendix 1 continued

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Behaviour	Code	Definition	S/NS	A/NA
Contact rest	CR	Stationary - lying, sitting, or crouching, with head positioned up or down, exhibiting minimal movements (includes scanning) with $> 25\%$ of body in contact with mate (excludes SN and NSAT)	S	NA
Dig	D	Digging in enclosure substrate (excludes NB)	NS	A
Drink	DR	Drinking from water dish	NS	А
Defecate/ urinate	DU	Positioned with rump backed into known latrine site	NS	Α
Eat	EA	Consuming food or browse material	NS	А
Flee	FL	Suddenly runs to covered area (nest box, tube), excludes CF, AV and ONS	NS	A
Greet	G	Nose-to-nose or nose-to-face contact with mate (record I/R when possible)	S	A
Genital sniff	GS	Investigating mate's anogenital region (record I/R)	S	А
Behaviour Indeterminable	D	Not possible to definitely define or classify behaviour, because animal is partially out of sight (>25%) or body position prevents proper viewing	X	Х
Mount	Μ	One animal mounting the other dorso- ventrally or in position other than dorso-ventral but genitals are in contact and thrusting is observed (record I/R) *record if thrusting is observed, (Y/N)	S	Α
Mesh	ME	Looking through or interacting through mesh/wall of adjacent enclosure, nose or forelegs touching mesh *record if animal is visible on the other side (Y/N) *record identity of animals in adjacent	NS	Α
Nest build	NB	enclosure in comments section Collecting nesting material (e.g. straw) in enclosure, carrying it in mouth and/or placing material within the nest box (also includes manipulating nesting material within the nest box)	NS	A

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Appendix 1 continued

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Behaviour	Code	Definition	S//NA	A/NA
Non contact rest	NCR	Stationary - lying, sitting, or crouching, with head positioned up or down, exhibiting minimal movements (includes scanning) while not in contact, or $< 25\%$ of body in contact, with mate (excludes SN and NSAT) Suddenly exhibits tenseness of body	NS	NA
alert	140211	with head up and gaze focused (i.e. not scanning; excludes CR and NCR).	110	
Other non social	ONS	Involved in other, non social activity in enclosure or nest box (not in contact with mate), e.g. locomotion (excludes all other non social behaviours)	NS	A
Out of sight	OOS	>75% of animal is in an area of the enclosure or nest box that is not covered with camera, or in area blocked from camera's view	Х	Х
Other social	OS	Involved in other, social activity in enclosure or nest box, in contact with mate or acknowledging contact from mate	S	A
Play-fight	PF	Wrestling, boxing, tumbling and/or sparring in contact with mate, excludes AM, M, G, C, CF (record I/R when possible)	S	A
Standing alert	SAT	Standing on hind legs, forelegs not in contact with mesh, wall, nest box etc.	NS	A
Solicit genital sniff	SGS	Presenting anogenital region to mate (record I/R)	S	A
Sit at nest box entrance	SN	Sitting at nest box entrance; body blocking entrance	NS	NA
Comments		Used for providing further information on any behaviour	Х	X

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