Identifying benefits and relative effectiveness of two early-life enrichment strategies for American mink (*Neogale vison*)

by

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Dalhousie University is located in Mi'kma'ki, the ancestral and unceded territory of the Mi'kmaq. We are all Treaty people.

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Abstract

The husbandry and housing of captive American mink (*Neogale vison*) farmed for their fur have been adapted in recent decades to better facilitate the expression of motivated, species-specific behaviours and promote improved welfare. For example, separate nesting areas with bedding are provided to allow for privacy and warmth at whelping (wild mink will nest in underground dens), and one manipulable environmental enrichment is now required in each pen to prevent abnormal behaviours and negative affective states like boredom (mink are skilled hunters and do not have the opportunity to chase or chew 'prey' in captivity). These interventions have been somewhat successful in improving mink health, welfare, and productivity, but issues such as stereotypic behaviour (SB) and kit mortality remain widespread. In the present study, two early-life enrichment strategies were implemented in captive mink and their relative effects on various measures of welfare were compared to that of a standard-housed group. Enrichment at whelping (EW; dams provided with extra, high-quality nest building materials prior to whelping) was predicted to positively impact nest environments, dam behaviour, and dam welfare in the peri-whelping period, indirectly conferring long-term benefits for kit stress responsiveness; it has been demonstrated in other species that maternal welfare can influence the quality of maternal care delivered to offspring, which in turn modulates offspring stress response development and behaviour. Enrichment once kits were mobile (EK; kits provided with extra physical enrichment items from 3-15 weeks post-whelping) was predicted to benefit kits directly through increased behavioural opportunities in the juvenile phase, a critical period for the prevention of SB. Our results demonstrated that EW housing positively affected several behavioural indicators of dam welfare in the peri-whelping period (e.g., SB), facilitated improvements in nest structure, and resulted in a trend towards decreased kit mortality compared to other treatments; however, dams' basal faecal cortisol and kit-directed maternal care behaviours were not affected. EW kits also did not exhibit the expected faecal cortisol response to a stressor, and spleen weights of EW kits as adults (an indicator of chronic stress) did not differ from those of other treatments. EK housing significantly increased kits' enrichment use compared to EW and standard-housed kits in the juvenile period, however, this enrichment strategy did not appear to confer long-term protective effects for the development of SB in EK kits as adults. Kit behaviour and temperament were also largely unaffected by EK housing, aside from a positive effect towards reduced inactivity in the nest box (a potential indicator of fear) and a negative effect towards increased lying awake (a potential indicator of boredom) in the late juvenile period. Interestingly, EW kits also exhibited increased social play compared to EK kits in this period. Overall, we conclude that the EW enrichment strategy was relatively more beneficial for mink, emphasizing the role of the perinatal environment in modulating kit development and facilitating the expression of motivated behaviours in dams. The EK intervention was effective at increasing enrichment use, which may have practical applications for enrichment provision in farmed mink; however, critical periods of enrichment provision for the prevention of SB in mink should be further investigated.

List of Abbreviations Used

EE	Environmental enrichment
SB	Stereotypic behaviour
HPA axis	Hypothalamic-pituitary-adrenal axis
EW	Enriched at whelping
EK	Enriched kits
SH	Standard housed

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1. General Introduction

1.1 Introduction to Animal Welfare

The term 'animal welfare’ is frequently used throughout the present thesis, but it is a dynamic and multifaceted term which is differently understood by different researchers. Conceptualizations of animal welfare, and prescribed treatments or interventions to improve welfare in turn, have progressed and adapted since the earliest days of animal welfare science to reflect changes in understanding of animal sentience or capacity for mental and physical suffering (Browning & Veit, 2022). For example, early welfare researchers like Barnett & Hemsworth (1990) defined welfare solely as an animal’s biological fitness (i.e., welfare is only reduced if the animal’s ability to survive and reproduce is impacted). Similarly, in biology, ‘good welfare’ generally means that animals are free from debilitating diseases, injury, or malnutrition and are not kept in conditions that cause physical deformities (Fraser, 1995). Those who support these conceptualisations of animal welfare do not always consider the animal’s subjective experience of pain or discomfort due to these injuries to be of importance (e.g., Broom 1991). Moreover, such physical ailments have long been monitored as part of regular farm husbandry because it is plausible that poor physical condition may lead to impaired reproduction, greater likelihood of mortality, and reduced or lesser quality product (eggs, meat, milk, fur, leather, etc.); this has been demonstrated for several farmed species (Cockram & Dulal, 2018; Cornelison et al., 2018; Granquist et al., 2019; Green et al., 2002; Kowalczyk et al., 2019; Lescourret & Coulon, 1994; Rajala-Schultz et al., 1999; Warnick et al., 2001), and thus there are practical reasons for farms to regularly evaluate this type of welfare in their animals.

Another similar, somewhat dated animal welfare concept refers to an animal’s ability to regain physiological balance (i.e., reach homeostasis), and inability to do so is thought to be evidenced by changes in their physiology and behaviour (National Board of Agricultural Research, 1975 as cited in Hagen et al., 2011). A refined definition was put forward which referred to animal welfare as the state of an animal as they attempt to ‘cope’ with adversity in their environment at a particular point in time (Fraser & Broom, 1990). Later clarifications to this definition were made, including an animal’s ability to cope being synonymous with their ability to control their mental and bodily stability, and prolonged failure to cope resulting in failure to grow, failure to reproduce, or death (Broom, 1999). This concept considers the strategies that animals may implement to help them cope with stressful environments or situations (Koolhaas et al., 1999), including modifying their behaviour (e.g., hiding, freezing, fleeing, or aggression; Forrester, 1980; Wechsler, 1995) or redistributing energetic resources within the body (e.g., suppression of parasympathetic nervous system processes in favour of sympathetic nervous system or hypothalamic-pituitary-adrenal [HPA] axis activation; de Bruijn & Romero, 2018; Koolhaas et al., 2010) and the potential for these strategies to become maladaptive after persistent failure to alter the animal’s situation (e.g., Odberg, 1989), leading to compromised welfare. In fact, much of early animal welfare science was related to chronic stress (i.e., persistence or consistency of stress over a long period) and symptomology of chronic stress that indicated an animal could not reach their homeostatic condition.

Some researchers at this time, however, began to propose that animals may have poor welfare if they are experiencing negative affective states such as frustration, fear, boredom, or stress, even if they are in good physical health (Dawkins, 1988; Duncan, 1993). Early welfare scientists were reluctant to infer feelings, emotions, or affective states from animal behaviour or physiology (de Waal, 2011; Duncan, 2005; Panksepp, 2005; Wemelsfelder, 1997), but advancements in research related to animal emotion (e.g., affective neuroscience) have led to emotions being recognized as mental and bodily states that can and should be evaluated when determining animal welfare (Anderson & Adolphs, 2014; Boissy & Lee, 2014; Mendl et al., 2010; Ross & Mason, 2017). Concepts of welfare that include reference to animal emotions consider animals' subjective perceptions of their environments rather than relying solely on objective assessments of the safety or comfort of their conditions; thus, these concepts of feelings, emotions, sentiment, or mood are used to infer an animal's own experience of their welfare state. Although complex phenomena, emotions are measurable via demonstrated neuronal, physiological, behavioural, and cognitive correlates (reviewed in Jirkof et al., 2019) and are often strong motivators of behaviour (Dawkins, 1990; Fraser & Duncan, 1998). They may also be described as corresponding with different levels of arousal, or as having positive or negative valence (e.g., pleasure or happiness and fear or frustration, respectively; Boissy et al., 2007; Webb et al., 2019). Negative affective states (e.g., fear) have been studied more extensively than positive affective states, but there has been increasing interest in determining biological and behavioural tools for measuring emotions such as contentedness or pleasure in animals (Boissy et al., 2007; further discussed in Section 1.2). Animal emotions have also been more recently incorporated into the idea of coping (i.e., regaining physiological balance) in response to adverse internal or external events, with special attention given to an animal’s ability to reach a state which they perceive as positive (Arndt et al., 2022)

As evident from these progressions in animal welfare concepts, the goal of welfare research historically has been to prevent or reduce physical suffering and the experience of negative affective states rather than to promote opportunities for positive experiences. For example, the Five Freedoms proposed by the Farm Animal Welfare Council in the 1960s were a foundation for animal welfare research and largely focused on the prevention of suffering (i.e., freedom from hunger and thirst, discomfort, pain, and injury, disease, fear, and distress). One of the five freedoms related to promotion of a positive outcome (i.e., freedom to express normal behaviour; Farm Animal Welfare Council, 2009), yet this outcome does not explicitly value positive experiences. More recently, welfare science has shifted towards the promotion of ‘positive’ welfare rather than only preventing ‘negative’ welfare. This distinction began with discussion of whether ‘happy’ states in animals were possible (Webster, 2008), or whether animals have ‘likes and wants’ (Dawkins, 2006). Positive welfare is sometimes thought to mean fulfillment of needs that are ‘luxurious’, in that they are extraneous of basic needs, but the experience of positive mental and physical states beyond what is necessary for immediate survival is considered by many to be integral to welfare (Rault et al., 2020). In positive welfare research, there is often less use of negatively biased terms like “lack,” “prevention,”, or “freedom from” and more use of terms like “provision,”, “fulfillment” and “freedom to” (Rault et al., 2020). There are two main diverging conceptualizations of positive welfare: first, that positive welfare arises from providing animals with what they like and want (Yeates & Main, 2008), and second, that positive welfare arises when the effects of positive experiences outweigh the effects of negative experiences (Vigors & Lawrence, 2019).

The somewhat standard definition of animal welfare used today is ‘the physical and mental state of an animal in relation to the conditions in which it lives and dies’ (World Organization for Animal Health, 2019). This definition considers both health-related and affective aspects of welfare, and additionally incorporates humane end-of-life conditions. An animal is generally presumed to be in a ‘good’ welfare state if they are “healthy, comfortable, well-nourished, safe, [are] not suffering from unpleasant states such as pain, fear and distress, and [are] able to express behaviors that are important for [their] physical and mental state” (World Organization for Animal Health, 2019, p. 333). A similar animal welfare concept will be applied in the present research. We additionally incorporate the goal of promoting positive welfare states, as parts of this thesis address providing mink with stimuli that they may like and which may fulfill behavioural motivations; however, we do not implement the term ‘positive welfare’ due to its inconsistent use in the literature (Rault et al., 2020). Given the dynamic nature of animal welfare (i.e., that animals may fluctuate between good and poor welfare states), the present research also considers an animal’s ability to effectively respond to environmental adversity by adapting their behaviour (e.g., avoidance) or physiology (e.g., HPA axis responses) to be an important component of welfare, as performance of such ‘coping’ strategies facilitates the maintenance of balanced affective and biological states.

1.2 Measures of Animal Welfare

Welfare indicators are complex in that for many proposed measures, there is mixed evidence for their validity (i.e., whether a measure reflects the intended target), accuracy (i.e., whether the scale of the measured values reflect actual degrees of welfare), completeness (i.e., whether a measure provides a comprehensive assessment of the welfare state of the animal), or reliability (i.e., whether a measurement produces consistent results when repeated by the same observer, different observers, at different times, or under different conditions; Browning, 2022). For example, some measures associated with poor welfare are highly variable between members of the same species: restlessness and inactivity are each potential behavioural responses to short-term (i.e., acute) or chronic stressors and are considered to vary by individual coping style (Ferreira et al., 2016; Mason, 1991b), which undermines the reliability and validity of these measures. Moreover, certain measures are designed to target either physical or mental aspects of welfare and separately do not offer a comprehensive assessment of welfare states or potential routes of intervention (e.g., undernutrition may arise due to a variety of environmental or internal causal factors, and alone does not indicate the nature of the welfare issue; Leliveld & Provolo, 2020). It is recognized today that no single measure of welfare is adequate on its own (Mason & Mendl, 1993), and thus multiple behavioural, physiological, and/or physical health-related measures used in conjunction are considered ideal for evaluating animal welfare.

1.2.1 Detecting poor welfare states

Physical symptoms of poor health or biological functioning have long been used as preliminary indicators of compromised welfare (Morton & Griffiths, 1985), even if they do not provide a comprehensive view of the animal’s overall welfare state. Deviations from an animal’s normal appearance or demeanor, signs of injury or disease, and poor body condition are often physically or behaviourally diagnosable by animal caretakers and thus offer feasible and practical welfare monitoring tools. However, sources of pain or discomfort in animals are sometimes only detectable after death (e.g., bone breakages: Gregory et al., 1991; Gregory & Wilkins, 1989; skeletal asymmetry: Díez-León et al., 2016; Sørensen et al., 2005; Tuyttens et al., 2005), and some animals may be especially good at concealing physical pain or discomfort from observers (Carbone, 2020). Moreover, animal biological fitness may not appear to be impaired because factors monitored for productivity (e.g., reproduction) will sometimes appear unaffected in individuals under farmed conditions, but many animals with good productivity are found to have severe welfare problems (Nimon & Broom, 1999).

Alternatively, physiological indicators can help in assessing aspects of welfare. Such indicators can be used while the animal is living and may be more sensitive to welfare problems than measures such as productivity or allow detection before visible signs of poor health and fitness. Physiological indicators of poor welfare that have been validated for use in conjunction with others include measures like increased heart rate, heart rate variability, increased epinephrine or norepinephrine, high basal glucocorticoid levels, sustained or impaired HPA axis activity following a challenge, or reduced immune system response following a challenge (Kovács et al., 2014; Mormède et al., 2007; O'Neill, 2019; Ralph & Tilbrook, 2016). Each of these physiological signs can be measured relatively noninvasively (e.g., via blood, saliva, urinary, or faecal samples, or by wearable heart rate monitoring devices) and are commonly used to infer animals' subjective perceptions of stressors in the environment or compromised ability to cope with adversity (Broom, 1991, 1999).

However, to ensure accuracy of these measures, it is necessary to control for individual factors which can affect basal activation of these systems (e.g., sex, age, reproductive status, and activity level; Blanca & Simon, 2023; Reeder & Kramer, 2005). Moreover, certain physiological measures can be affected by arousal of either positive or negative valence. For example, changes in heart rate or heart rate variability can reflect excitement or anticipation of positive stimuli (Amaya et al., 2020; Zupan et al., 2016), so the valence of the situation must be understood before these measures can be interpreted. The validity of glucocorticoids in reflecting welfare is similarly debated: glucocorticoids have many beneficial or non-stress-related functions in the body (e.g., memory formation; Finsterwald & Alberini, 2014) and their levels may increase in response to emotions of positive valence such as pleasure (e.g., comfort derived from allogrooming; Tamminen et al., 2021) or sexual arousal (Borg et al., 1991). There is also high individual variability in glucocorticoid responding based on personality and cognition, and changes in glucocorticoid levels are inconsistently paralleled by changes in behaviour, morphology, or physiology in the literature (Tiemann et al., 2023). Moreover, it has been argued that concentrations of blood, salivary, or faecal glucocorticoids, even in conjunction with behavioural measures, are insufficient to infer whether that animal is demonstrating HPA axis responses to stress, and that concentrations and actions of these hormones in target tissues are preferable when measuring the animal's welfare state (Ralph & Tilbrook, 2016).

Similarly, several behavioural measures have been validated as poor welfare indicators while others are subject to debate and require further review in research. Avoidance behaviour, aggression, and self-mutilation behaviours are generally accepted to reflect attempts to cope with physical pain or negative affective states (Broom, 1991, 1999; Kubo et al., 2015; McDonnell, 2008; Wechsler, 1995). Avoidance behaviour, for instance, may increase after previous negative handling experiences or negative interactions with humans as well as in situations of pain or poor health (Bak & Malmkvist, 2020; Sharma & Phillips, 2019), though this behaviour has been shown to vary within species (Slayi, 2023). Similar to avoidance, freezing or hiding in response to fear is common in many species (Riemer et al., 2021). Animals may eventually or alternatively attempt to flee in the presence of fear-inducing stimuli, or may show aggression if the stressor cannot be escaped (Steimer, 2002). Aggression is a highly variable as a response to fear, as it depends on factors like individual coping style (Coppens et al., 2010; Elizabeth Bolhuis et al., 2005; Twiss et al., 2020) or sex (Sabbi et al., 2021; Scandurra et al., 2018), though in individuals where aggression is not exhibited, freezing behaviour or behavioural passivity (e.g., inactivity) have been identified as alternative responses to fear or pain (Ferreira et al., 2016). Situational context must also be known in order to infer welfare state from aggressive behaviour and correctly implement welfare interventions, as aggression may be motivated by territoriality, resource defense, or frustration in addition to pain or fear (Kleszcz et al., 2022); each of these motivational states, however, indicates that one or more of the animal's needs are not met and would thus be welfare concerns.

Stereotypic behaviour (defined in welfare research as invariant, repetitive, and apparently functionless patterns of motor behaviour typically only present in captive members of a species; Mason, 1991a) is also sometimes used as a behavioural measure of poor welfare since it often occurs under conditions that are independently determined to cause poor welfare (Broom, 1991, 1999; Mason, 1991b). However, use of this measure has been debated since blocking animals' performance of stereotypic behaviour can induce stress responses (Würbel & Stauffacher, 1996) and therefore it may be an effective way for animals to cope with environmental adversity. Animals that exhibit stereotypic behaviour in a given environment may also be 'better off' compared to non-stereotyping animals when other welfare metrics are considered (e.g., stereotypic behaviour was most often linked with good welfare in a review of publications investigating the welfare of animals within sub-optimal environments; Mason & Latham, 2004). Aiming to reduce stereotypic behaviour via methods like selection (McGreevy & Nicol, 1998b, 1998a) may therefore reduce animals' ability to regulate their arousal or may cause different coping behaviours to be exhibited rather than dealing with the root welfare issue. Some also suggest that stereotypic behaviour may initially be performed in response to the stressors or behavioural constraints of captivity, but over time can develop into habitual motor patterns along with changes to neuroanatomy (e.g., Tatemoto et al., 2022), at which point they are no longer indicative of poor welfare. Stereotypic behaviour can also be exhibited during high arousal in situations of positive or negative valence (Poirier & Bateson, 2017), so in this way it is an unreliable welfare indicator. Conversely, absence of stereotypic behaviour in captive populations may not be indicative of 'good' welfare; aversive environments are not always shown to elicit stereotypies, which calls into question whether stereotypic behaviour can be relied upon as a sole index of welfare (Mason & Latham, 2004). In fact, high levels of inactivity may be equally as likely as stereotypic behaviour in situations of environmental adversity but may present differently based on individual coping style (Ferreira et al., 2016; Mason, 1991a).

Despite these caveats, it is generally accepted that stereotypic behaviour in captive animals develops in response to suboptimal conditions, and even if stereotypers are better equipped to cope with these conditions (or possibly, become less sensitive to their environment over the course of stereotypy development; Cooper & Nicol, 1991; Fentress, 1976), the presence of stereotypic behaviour still indicates a group-level welfare concern. Often developed in stimulus-poor or monotonous environments, stereotypic behaviours are thought to be frustration-induced (i.e., due to limited opportunities to perform motivated behaviours; Mason et al., 2001, 2007) or result from boredom (hypothesized because extremely under-stimulated animals show impaired neural pathway development, leading to inflexible and limited behavioural repertoires, i.e. highly stereotypic and perseverative behaviour; Burn, 2017; Mason & Rushen, 2006). Thus, either in the early stages of their performance or throughout the lifetime of their performance, stereotypic behaviours may be indicative of negative affective states. High stereotypers also spend a large portion of their daily time budget performing stereotypies (Dantzer, 1986), which limits the time available to perform other behaviours believed to be more functional or beneficial for welfare and can thus reduce body condition and productivity (e.g., body fat, reproductive success, and growth/survival of offspring; summarized in Díez-León et al., 2016). Moreover, maternal expression of stereotypic behaviour in pigs has been found to affect the amygdalae of offspring through epigenetic effects, and thus maternal stereotypy may impact amygdala-related functions like threat detection, fear response, social behaviour, and emotional memory (Tatemoto et al., 2020, 2023). Although these findings are more recent and further research is required to elucidate the relationship between parental stereotypic behaviour and offspring emotionality, this further implicates stereotypic behaviour in the welfare of captive animals.

1.2.2 Detecting good welfare states

Accepted measures of good welfare are often behavioural rather than physiological. As posited by Volpato et al. (2009), there would be less selective pressure for the evolution of physiological pathways involved in states of positive affect and physical comfort since these states do not impair survival or reproduction, and thus, there are fewer measurable changes in the animals' systems that reflect attempts to reach homeostasis (as in, for instance, the stress response or immune response). There have been some attempts, however, to measure sensations of wanting and reward in animals through activity of the mesolimbic dopaminergic and opioid systems, respectively (Spruijt et al., 2001); these systems are argued to be important for animal welfare since disruptions in mesolimbic dopamine activity can induce anhedonia and reduced wanting/motivation (e.g., Der-Avakian & Markou, 2012; Moreau, 2002), and endogenous opioids are responsible for opposing stress, fear, and pain and promoting feelings of relaxation and comfort (Henry et al., 2017; Valentino & Van Bockstaele, 2014). However, such experiences of pleasure or reward are transient and cannot be used independently to assess welfare. Moreover, the measure of dopamine in the reward centre of the brain (i.e., the nucleus accumbens) is a delicate, invasive procedure and can only be conducted post-mortem or by using microdialysis techniques in controlled laboratory conditions (Serra et al., 2018); thus, this measure is not widely implemented in farm or captive animal settings. The effects of endogenous opioid activity have been indirectly measured through hypo- or hyper-algesia tests or administration of opioid antagonists like naloxone (Cronin et al., 1985; Dodman et al., 1987; Kennes et al., 1988; Rushen, 1985), however, opioid systems interact with and can be modulated by many other systems (e.g., the HPA axis). Thus, the role of opioids in modulating welfare is difficult to isolate.

Reward-seeking behaviours can alternatively be measured as indicators of good welfare. Expression of active and positive engagement with the environment through activities such as exploration, foraging, and hunting are characterized as goal-directed behaviours and appear to be motivated by experiences of liking or wanting (Mellor, 2015). Elective interaction with the environment has also been associated with animal agency and choice, which is thought to be important for welfare (Špinka, 2019). Preferential consumption of rewarding foods or interaction with rewarding objects is also considered to reflect capacity for wanting (and therefore absence of depression-like states like anhedonia; Lecorps et al., 2019, 2020; Luna et al., 2020). Other behaviours associated with good welfare include resting (Fureix & Meagher, 2015), social play (Vinke & van Leeuwen, 2005; though see Dallaire et al., 2018), and affiliative social interactions like allogrooming (Mellor, 2015); these activities generally occur in conditions of low stress and good fitness, and may even be rewarding for animals (Burghardt, 2005). Recently, certain types of vocalizations have also been suggested to indicate good welfare (Laurijs et al., 2021).

It is also considered a sign of good welfare if captive animals spend similar amounts of their time budget performing certain behaviours as they would in the wild (Auer et al., 2021; Veasey et al., 1996) or perform a similar diversity of behaviours in captivity as they would in the wild (e.g., Rabin, 2003; Sohel Khan et al., 2022; Veasey et al., 1996). This brings us to the assessment of good welfare through ‘natural living’ – along with affective states and physical health, an animal's ability to lead a 'natural' life is often considered a main component of animal welfare (Fraser et al., 1997). Natural behaviour is commonly defined as behaviours that animals or their ancestors have evolved to exhibit in wild-living conditions, or tend to exhibit in simulated naturalistic environments (Bracke & Hopster, 2006). The behaviours must have evolved during the evolution of a species or during its domestication, and should be adaptive in that they improve the animal's fitness (Špinka, 2006). They are considered hard-wired to some extent (i.e., innate), but can be subject to plasticity (e.g., behaviours that young would normally acquire from their parents through social learning are also included under this definition; Špinka, 2006). The welfare of a captive animal can then be evaluated based whether they have opportunities to perform behaviours that wild-living members of their species would display.

However, this idea has faced scientific criticism as a measure of animal welfare because not all behaviours categorized as 'natural' have the same impact on welfare when prevented or restricted (reviewed in Dawkins, 2023); in fact, some natural behaviours can indicate poor welfare (e.g., hiding or fleeing from predators). Much of an animal's natural behavioural repertoire is indeed composed of attempts to avoid death, predation, disease, malnutrition, exposure to adverse weather, and so on (Yeates, 2018). Thus, a suggested method of refining our understanding of how natural behaviours relate to welfare is to determine what animals value, as shown by what they will approach, interact with, avoid, or work for (Dawkins, 2023). This research is ongoing, but some information about preferences in captive species is already available in the literature (e.g., swimming has been validated a highly motivated natural behaviour in mink; reviewed in Kornum et al., 2017). Certain 'natural' behaviours have also been deemed to be important for animals based on studies that investigate whether blocking or preventing these behaviours has consequences for welfare (e.g., mink showed signs of distress when deprived of a nest box, which function similarly to the dens they would use in the wild; Hansen & Jeppesen, 2000). Thus, most welfare assessments in farmed animals are now based on their ability to express species-specific behaviours, with an emphasis on behavioural needs. 'Naturalness' of these behaviours is not necessary for this evaluation, but behaviours naturally performed by a species (i.e., their behavioural biology) can provide support for why some behavioural needs exist.

There has been pressure on animal farming systems from a social sustainability standpoint to modify captive environments to better permit the expression of these natural behaviours and to prevent poor welfare in the form of mental or physical suffering. The fur industry in particular is a target of this societal pressure; many countries have begun to phase out the farming of American mink and other furbearers due to issues of animal rights and animal welfare which have not been sufficiently addressed, in addition to concerns related to disease transmission and environmental impacts of fur farming ("Global Fur Farm Bans," 2015; *The End of the Dutch Mink Farming Industry - One of the Largest in the World*, n.d.). For the fur industry and farming of mink to be sustainable, it is imperative that husbandry methods and cage environments be adapted to prevent suffering (e.g., disease) and promote behaviours associated with good welfare. In the present thesis, there is an emphasis on fulfilling such behavioural needs in American mink, a species farmed for their fur, as a method of promoting opportunities for good welfare; however, we base our conception of these needs on aspects of minks' behavioural biology and on previous research which validates these behaviours as important to mink welfare. The following literature review discusses the behaviours of wild-living mink and provides a brief introduction to the origins of mink farming. The typical housing and husbandry standards of farmed mink are then discussed, along with existing research assessing the effects of these housing aspects on welfare and the adaptations made to these practices in previous years to promote good health, productivity, and welfare in mink.

2. Literature Review

2.1 Introduction to American Mink (*Neogale vison*) and their Natural Histories

American mink are a mustelid species native to North America, though they have been introduced in parts of Europe, Asia, and South America (of note: they are typically larger and more aggressive than their European counterparts, *Mustela lutreola*, and are considered an invasive species where they have established feral populations as a result of escapes and/or intentional release from fur farms; Bonesi & Palazón, 2007; Sidorovich et al., 1999). Their North American ranges extend from the Southern United States as far north as Alaska (Dunstone, 1993), so they are able to cope with both hot and cold climates. They prefer to live near rivers, lakes, wetlands, or coastlines, with most mink activity occurring within 100-200m of bodies of water (Dunstone, 1993).

Like most other mustelids, mink are opportunistic carnivores. They are skilled hunters both on land and in water (with aquatic prey constituting up to 89% of their diet; Gerell, 1967). Mink will often swim as a means of travelling (Harrington et al., 2012; Williams, 1983) and some individuals will dive over 100 times per day (Harrington et al., 2012; Hays et al., 2007), despite being semi-aquatic mammals with few specialized adaptations for swimming. They produce an oil to aid in 'waterproofing' of fur, but lack webbed feet, are poorly insulated against heat loss when wet, and have poor underwater vison (Bagniewska et al., 2015). Mink are also skilled climbers and can climb trees (Lariviere, 1995). They have long, thin bodies to aid in pursuing burrow-living prey such as rodents and lagomorphs and will often take over the dens of such species rather than dig their own.

Home ranges of wild mink are relatively small compared to those of other carnivores (when measured as linear distances along waterways, approximately 3.2-8.4 km on average in males and 1.7-6.5 km in females; Haan & Halbrook, 2015), perhaps making them better-suited to captive environments with limited space allowance compared to other carnivores with larger home ranges and/or daily travel distances (Clubb & Mason, 2007). They will often cross their entire territory on a daily basis (distances of up to 3.1 km recorded in one day; Haan & Halbrook, 2015), making use of multiple underground dens dispersed throughout this range and changing dens on successive nights (Dunstone, 1993; Gerell, 1970).

Mink are generally a solitary, territorial species; male-male interactions in adults are highly aggressive and typically occur during the mating season (European Commission, 2001). Adult males and females do not interact with the exception of late winter when males will leave their territories in search of females for mating (Dunstone, 1993; Macdonald et al., 2015). Mink display sexual dimorphism including differences in body and skull dimensions, body weight (males up to 2x heavier than females), and canine/upper and lower carnassial sizes (wider/longer in males; Thom et al., 2004); these differences are thought to arise from intra-species competition, given that male territories never overlap (Dunstone, 1993), and specialization for certain prey and/or ecological niches (Thom et al., 2004).

Both males and females become reproductively mature in their first year. The mating season lasts about four weeks (beginning in early March; Dunstone, 1993). Mink have gestation lengths of 51 days and, like many mustelids, show delayed implantation of 1-14 days, so young are typically born in late spring (April-May). Females exhibit superfecundation (multiple ova from a single ovulation) and superfoetation (multiple ovulations within a single mating season), resulting in the potential for multiple matings with males and multiple paternity of litters (Macdonald & Harrington, 2003). Wild-born mink give birth to litters of 7.6 kits on average (ranging from 5-11 kits; Pagh et al., 2021). Mothers raise their young alone and perform extensive rearing of kits; kits are nutritionally reliant on the mother until 5 or 6 weeks of age but do not leave to establish their own territories until at least 3 months of age (Gerell, 1970). Kits are born altricial, but have demonstrated immediate olfactory abilities (Jonasen, 1987) and ability to vocalize (Brandt et al., 2013) at birth. Eye-opening begins gradually at postnatal day 27 in concurrence with development of hearing (Lassen, 2007 as cited in Brandt et al., 2013; Brandt et al., 2013). In farm settings, gradual weaning from milk has been observed without intervention around 34-40 days along with the emergence of motor abilities such as gait and running (Brink & Jeppesen, 2005). In the wild, a maximum lifespan of 10 years has been observed, with a breeding life of 6-7 years.

The behavioural biology of mink is becoming increasingly well understood, and this knowledge is essential for evaluating mink welfare in captive conditions; as conceptualized in the present thesis, animals are in a good state of welfare when they are not suffering from physical pain, discomfort or negative affective states, can effectively modify their behaviour or physiology to cope with stressors in their environment, and are provided with opportunities for situations of positive affect (i.e., able to fulfill highly motivated behavioural needs or interact with environmental stimuli which they like or want; summarized in Section 1.1). Moreover, as stated by Dunstone (1993) and others, the ecology of wild animal species must be taken into consideration when designing husbandry protocols and housing under farmed conditions. Swimming, diving, climbing, hunting, travelling, and use of several den sites are clearly activities of importance for mink, but opportunities for these behaviours have historically been limited in captivity. Moreover, suppression of promiscuous/ polygamous mating behaviours, early separation of the dam and her litter or early placement of kits in solitary housing, and constraints affecting maternal behaviour in the peri-whelping period are still common welfare issues faced by mink in farm settings. The historical origins of farming mink under commercial conditions and problems historically faced with mink husbandry are further discussed below.

2.2 Farming of Mink for their Fur

Farms raising mink and other fur-bearing animals were first established as a conservation effort to prevent depletion of wild populations of fur-bearing species. There was concern from government, conservationists, and trappers alike about poor trapping practices; traditional trapping methods often damaged pelts or left pelts vulnerable to damage by scavenging species, and trappers often over-harvested or harvested before furs were primed out of desire for revenue, so this change also allowed for improved pelt quality (Colpitts, 1997). Moreover, the development of extensive new road and rail systems, human settlements, clearing of forests, and draining of swamps across North America from 1892 to 1911 resulted in severe habitat fragmentation, decreases in numbers of wild-trapped pelts, and consequent increases in fur prices (Colpitts, 1997). New road systems also made it possible for greater numbers of trappers to travel further north, encroaching on traditional Indigenous trapping areas. By 1926, white trappers constituted 50% of the ranks of trappers in northern areas where Indigenous people had previously been the sole harvesters, and there were greater numbers of trappers congregating than the region could sustain (Colpitts, 1997).

The origins of commercial fur farming are difficult to isolate due to poor record keeping and lack of a refined definition of what ‘fur farming’ entails (for example, early trappers in the fur trade era were known to keep fox captive until their pelts primed; Colpitts, 1997). The first recorded ‘fur farms’ raised mink at Lake Casadacka, New York in the 1860s (*About Mink Farming | Fur Commission USA*, n.d.). Attempts at mink farming in Canada were conducted shortly thereafter in Richmond Hill, Ontario from 1866-1887 (Fur Institute of Canada, 2015; *History of the Fur Trade – CMBA*, n.d.), though fur farming is considered to have officially become established in Canada in the 1880s (Fur Institute of Canada, 2015; *History of the Fur Trade – CMBA*, n.d.). The most accurate records were kept for farms raising fox (e.g., successful fox breeding was reported in Quebec in 1898, in Ontario in 1905, and in Nova Scotia in 1906; Dominion Bureau of Statistics Fur Statistics Branch, 1929), though farms labelled as ‘fox’ farms were also known to raise mink, raccoon, skunk, marten, fisher, coyote, and badger. Fur farms in Canada were mainly limited to the Atlantic provinces leading up to the 1910s, but a small number of farms were also established in the Western provinces at this time (Fur Institute of Canada, 2015; *History of the Fur Trade – CMBA*, n.d.).

Early farms were mostly experimental and struggled with problems such as poor breeding, parasites, disease, infanticide among nervous dams, inadequate housing (including restriction of monogamy and family housing, preferred by foxes), and escape attempts via digging or climbing (Balcom, 1916; Colpitts, 1997). In the 1890s, however, woven wire fencing became available, and fur farming ventures became more feasible.

By 1896, fox were being raised commercially using wire fencing and large, outdoor pens, producing a strain (the Dalton-Oulton strain) that won high profits and was used as foundation stock on other farms (Balcom, 1916; Colpitts, 1997). Silver fox in particular was the most popular and profitable fur-bearing animal farmed for many years, and farmers were relatively successful in breeding this pelt colour, though understanding of genetic strains and colour inheritance was limited (Ashbrook, 1937; Balcom, 1916). Canadian fox farms peaked in number in 1938 before beginning to decline, largely due to the resurgence of trapping and consumers' desires for 'wild' fur products.

Smaller species like mink and muskrat replaced fox as the most-farmed furbearer during and after World War II, at which point mink farming became more widespread across Europe as well (European Commission, 2001). Farmed mink were originally dark brown in colour, as are wild mink, but selection and genetic mutation made a variety of colour types possible (e.g., black, albino, cream, light brown, gray, and silver-blue), with high predictability of offspring phenotypes based on colour types of mated pairs (Thapa et al., 2023). These available colour types, as well as ideal fur length and density, made farmed mink ideal for fur products; the black and albino colour types are most appealing as black can be worn with any colour of clothing and white furs can be dyed in any colour (Wang et al., 2022). From 1953 to 1989, world production of mink grew from 4.2 to 34 million kits annually (European Commission, 2001; Jørgensen, 1985). Farms at this time still raised fox in lesser number, as well as beaver, badger, lynx, fitch, marten, fisher, coyote, chinchilla, rabbit, Siberian hare, and karakul sheep.

Promoters of fur farming described farming practices as being based on rigorous science (e.g., a federal government publication in 1939 displaying a photograph of a Quebec mink farm with the caption “Mink are raised here with modern, scientific methods”; DBS Report for the Year 1939, pp. 7-8, as cited in Colpitts, 1997). However, widespread mortality and morbidity occurred due to unsanitary housing conditions and the spread of diseases and parasites like ear mites, distemper, and hookworm (e.g., the causes of death for 1500 fox and 500 mink on Alberta farms in 1931; Colpitts, 1997). Inadequate pen sizes, poor diet and/or portioning of feed, and penning on the same patches of ground for years contributed to very poor environments. Moreover, farmers in Canada reported losses due to cold and exposure. Early research related to mink farming was primarily concerned with mink biology (i.e., nutrition, reproduction, and pelt condition), with the goal of preventing losses and optimizing production and economic gain (e.g., Enders, 1952; Shackelford, 1950; Rice, 1967 as cited in Nimon and Broom, 1999). It was not until recent decades that research on the welfare of farmed mink began (see Braastad, 1992 for a review of research on nest box and platform use, stress, stereotypic behaviour, and effects of management on behaviour). In years since, modifications to the cage environment and husbandry of mink have been implemented to optimize both their productivity and welfare. These modifications, and the research supporting their benefits, are further discussed below.

2.3 Welfare of Mink in Relation to Social Aspects of Housing

2.3.1 Weaning Age and Dam-Kit Interactions

On commercial mink farms, young are commonly weaned at 6-8 weeks of age, though in Scandinavian countries weaning is often delayed until 8 weeks. As previously discussed in Section 2.1, mink are nutritionally dependent on the mother until 5-6 weeks of age, but in the wild do not leave their natal territory until 11-12 weeks of age. In a farm setting, Gimpel (1997, as cited in Nimon & Broom, 1999) investigated the role of the mother as a 'secure base' for exploratory behaviours in kits. Between 8.5 and 13.5 weeks of age, kits made more attempts to return to the rest of the litter and performed more distress vocalizations when the mother was absent. Additionally, there was a trend towards decreased latency to explore when the mother was present. These results together suggest that the mother's presence during the first several months of life is instrumental to kits' development of exploration behaviours and learning to interact with their environment, and later weaning in farmed settings may be beneficial to kit welfare.

In Mason (1996, as cited in Nimon & Broom, 1999), weaning was delayed until 11 weeks in a late-weaning group while another group was weaned at 7 weeks in accordance with standard farm procedures. The standard-weaning group performed significantly more stereotypic behaviour than the late-weaning group as adults, and their stereotypies also presented as more established patterns and sequences ('established' usually referring to stereotypies with decreased variability, more predictable timings or durations, and often greater resistance to intervention or disruption; Mason & Rushen, 2006). Moreover, prevalence of tail biting (a form of self-mutilation in mink) was higher in kits weaned at 7 weeks of age compared to kits who remained with the mother for 6 months (de Jonge 1988, 1989 as cited in Mason, 1994); tail biting was also shown to be reduced by delaying weaning until 11 weeks of age (Mason, 1994). Kit behaviours in the peri-weaning period also show effects of weaning age: kits' distress calls were increased when weaned at 6 weeks compared to weaning at 8 to 10 weeks, and numbers of eosinophil leukocytes – a white blood cell type used to measure immune system function (Davis et al., 2008) – were lower (Houbak and Jeppesen, 1987 as reported in Nimon & Broom, 1999).

While delayed weaning appears to be beneficial for kits, there may be short-term consequences for dams of extended presence of kits, particularly when housed in a way that does not facilitate temporary escape from kits. In dams of litters weaned at 6, 8, and 10 weeks of age, respectively, circulating eosinophil leukocyte levels increased with the weaning age of kits (Heller et al., 1988). However, mothers demonstrated recovery from this inferred stress shortly after weaning in the 8- and 10-week groups compared to evidence of sustained stress after weaning in the 6-week group. This was thought to indicate that early separation from kits is a source of stress for dams (as supported by weight loss in dams following weaning; Sørensen et al., 2001 as cited in Mink Code of Practice Scientists' Committee, 2012), but longer-term presence of kits in the pen also appeared to be a stressor. This was speculated to be lessened if dams have the option to avoid or escape kits in situations of late weaning (Heller et al., 1988), as they would in the wild (Dunstone, 1993). This stress may also be a by-product of less space availability in the cage as kits grow, creating problems with stocking density (Mink Code of Practice Scientists' Committee, 2012).

When provided with 'get-away bunks', i.e., elevated shelves or platform-like structures in the cage that kits cannot reach, dams did in fact use them increasingly as kits aged (Buob et al., 2013; Dawson et al., 2013) and spent increased time resting (Dawson et al., 2013), though kits' opportunities for nursing were not affected, nor were kits' growth rates or mortality (Buob et al., 2013). Dams also suffer less from symptoms of mastitis when provided with these escape opportunities (Buob et al., 2013), and similarly, dams who remain with kits for longer in cage areas with increased space have been found to remain healthy (de Jonge and van Iwaarden, 1995 as cited in Nimon & Broom, 1999). Stereotypic behaviour in dams typically also increases the longer kits are kept with them (Hansen, 1990), and indeed, this can be reduced by providing dams with get-away bunks (Buob et al., 2013; Dawson et al., 2013; Hansen, 1990; Jeppesen, 2004). However, incidence of nursing sickness (a metabolic disease common in older dams, dams with large litters, or who are experiencing stress and characterized by weight loss, emaciation, dehydration, and potential mortality; Rouvinen-Watt, 2003) has not been improved by these bunks thus far (Buob et al., 2013; Dawson et al., 2013), nor have dam stress levels in the form of decreased cortisol (Buob et al., 2013).

2.3.2 Social or Solitary Housing

Following weaning, kits are typically kept in their litters until 10-11 weeks and then divided into individual or pair-housing, or removed from the whelping pen and housed in pairs directly at weaning (typically consisting of male-female sibling pairs; European Commission, 2001). In the Netherlands, mink may alternatively be separated into groups of three at weaning (typically three females), while in some parts of Europe the entire litter may be housed together, with or without the dam, until maturity (European Commission, 2001; Mink Code of Practice Scientists' Committee, 2012). Thus, multiple social housing scenarios are possible: family housing (littermates together with the dam), group housing (multiple juvenile littermates), or pair housing (typically two juvenile littermates). Single housing is often implemented at 10-11 weeks of age, but placement in single housing immediately following weaning is not recommended as kits have shown signs of increased stress when weaned into isolation (i.e., increased vocalizations;

Jeppesen et al., 2000). Family housing does not generally show increased benefits for mink above pair-housing, and pelt quality is typically best in pair housing (Hansen et al., 1998) as aggression and food competition can occur in family-housed mink (however, this may be affected by factors such as temperament, number of nest boxes, or feeding levels; European Commission, 2001).

Outside of North America, families or groups are typically housed in multiple conjoined cages. This was first explored by de Jonge (1996a, b, as cited in Nimon & Broom, 1999) and de Jonge and van Iwaarden (1995, as cited in Nimon & Broom, 1999); rearing mink in horizontally conjoined cages with multiple siblings or without weaning (i.e., mothers remain in the interconnected pens with kits for at least 30 weeks until pelting) was demonstrated to be economically beneficial as pelt quality was not negatively affected, aggression was mitigated, and mothers remained healthy. About 15% of Dutch mink were raised this way as of 2001 (European Commission, 2001). Vertical stacking of interconnected cages rather than linking of adjacent cages also became popular around this time; about 30% of mink kits in the Netherlands were raised in two vertically stacked cages by 2001 ('climbing cages'; Scientific Community on Animal Health and Welfare, 2001). However, groups in stacked cages have been found to have a higher proportion of individuals with severe tail-biting and fur damage compared to group housing in horizontal row cages or traditional cages, as well as more agonistic (i.e., aggressive and defensive) behaviour and reduced occurrences of resting, eating, or drinking (Pedersen et al., 2004). This housing system may have greater welfare consequences than row housing since there is only one nest box and one feeding area accessible, whereas there are multiple of each in row housing; though, morbidity and mortality were higher in both stacked and row cage systems compared to traditional housing (Pedersen et al., 2004). In Canada, group housing of littermates or separation of littermates into pairs at weaning is most common; in either scenario, juveniles are typically housed in one whelping cage ($0.225m^2$) with one nest box and feeding area.

As adult mink are solitary in the wild (Dunstone, 1993), farmed mink are typically housed individually as adults. In Canada, this may begin at 10-11 weeks of age, or at seven months of age following pelting if individuals are chosen to be kept for breeding. Although there has been some success housing adult mink together if reared together from birth or reared without weaning (de Jonge and van Iwaarden, 1995 as cited in Pedersen et al., 2004), severe aggression has been reported when multiple unfamiliar adults are housed together (Dunstone, 1993; Rice 1967 as cited in Nimon and Broom, 1999). Moreover, mink housed in groups from 2-10 months of age have demonstrated the establishment of dominance hierarchies, with mortality and morbidity occurring among subordinate mink (Heller & Jeppesen, 1986).

Housing of four or more adults in one cage was common in the 1980s, but the prevalence of this in more recent years is unknown (European Commission, 2001). Housing mink individually allows for easier control of feed rationing and supports weight gain, in addition to eliminating aggression between individuals (e.g., Hansen et al., 2014); pair-housed mink often have more fur-chewing damage on their backs and necks than single-housed mink, indicating occurrence of aggression (Damgaard and Hansen, 1996 as cited in Mink Code of Practice Scientists' Committee, 2012; Hansen et al., 1998). Despite this, single-housed mink can show reduced weight gain and lower pelt quality compared to pair-housed mink, and there was no difference found in tail-biting between single- and pair-housed groups in some studies (Damgaard and Hansen, 1996 and Møller, 1991 as cited in Mink Code of Practice Scientists' Committee, 2012). Kits raised in single housing may also have more fearful temperaments, demonstrating fear of human contact, conspecifics, and unfamiliar objects, than kits raised in pair housing (Hansen et al., 1997 as cited in Mink Code of Practice Scientists' Committee, 2012). Conversely, family-housed females show more curious temperaments than single-housed females (Pedersen & Jeppesen, 2001). Single housing may also affect mating success: mink raised in single housing sometimes show difficulty mating with other mink raised in single housing, whereas mink raised in family groups are much more successful (Hansen et al., 1997 as cited in Mink Code of Practice Scientists' Committee, 2012).

2.4 Welfare of Farmed Mink in Relation to Cage Environments

2.4.1 Cage Size

Farmed mink are typically housed in indoor/sheltered wire mesh pens in a side-by-side arrangement. Research on the effects of cage size is somewhat limited, though it has been concluded based on existing data that adjusting cage size alone does not significantly improve welfare unless cage sizes are extremely small (e.g., 15cm W x 45cm L x 15cm H, or floor areas of 0.0675m^2; European Commission, 2001). Thus, pens worldwide typically have a floor space of 0.14-0.27m^2 and a height of 30-45cm (e.g., minimum floor space of 0.255m^2 in Italy, Argentina, and Norway, with new Norwegian cages meeting a minimum of 0.270m^2 [European Commission, 2001; Mink Code of Practice Scientists' Committee, 2012]; overall minimum floor space of 0.27m^2 with main cage area of 0.18m^2 in China [Chinese State Forestry Administration, 2006 as cited in Mink Code of Practice Scientists' Committee, 2012]).

Farms in most countries use the same cage design for single males and females, females and their litters, or group- or pair-housed juveniles. In Canada, however, there are specific requirements for pen dimension according to the sex, stocking density, and life stage of mink: all newly built or purchased pens in Canada must have a minimum floor space of $0.145m^2$ for single adult females or single juveniles of either sex and $0.225m^2$ for single adult males, single females with their litters, and pair-housed juveniles (see National Farm Animal Care Council, 2013 for details of height, width, and depth requirements). For pens housing more than two juvenile mink, floor spaces must be $0.2225m^2$ at minimum, with an extra $0.0438m^2$ or $0.0645m^2$ for every additional female or male, respectively. One justification for these varying pen requirements is that pen height is known to affect minks' ability to access feed. Mink are fed on the mesh top of the cage, and stretch up on their hind legs to eat; thus, if they cannot easily reach the cage top due to sex or age differences in body length, welfare may be impacted (Díez-León et al., 2017).

Studies of cage size often modify cage complexity (i.e., by connecting multiple cages, or adding structures/objects) rather than actual cage dimensions, and this is often conducted in parallel with manipulations of the number of animals housed. For example, females housed with their entire litter in three conjoined cages showed reduced stereotypic behaviour compared to females housed with one male sibling in a standard cage (Jeppesen et al., 2000). Vertical stacking of interconnected cages rather than linking of adjacent cages is also common ('climbing cages'); housing of females in vertically connected climbing cages has been found to significantly reduce stereotypic behaviour compared to housing in standard cages (Lidfors et al., 2012). There are many other studies, however, which show no effect of cage size manipulations on behaviour (summarized in Mink Code of Practice Scientists' Committee, 2012), and when two cages are conjoined, mink will work for access to the extra compartment but it is not as valuable to them as a compartment containing enrichments (Cooper & Mason, 2000; Mason et al., 2001; later discussed in Section 2.5). In terms of physiological effects, there was no difference in fecal cortisol metabolites between adult females given access to one cage with 0.27m^2 of floor space or two cages of this size (Hansen et al., 2007), and cage size did not significantly affect measures of chronic stress (cortisol concentrations or the number of eosinophil leucocytes) in mink kits housed in male-female pairs across three cage sizes (1.05m^2, 0.27m^2, and 0.10m^2; Hansen & Damgaard, 1991). Raising mink in large (0.4648m^2) or small (0.232m^2) cages also had no effect on neutrophil/lymphocyte ratio, eosinophils or cortisol concentrations in Aulerich et al. (1991, as cited in Mink Code of Practice Scientists' Committee, 2012). Small changes in cage size do not significantly affect the weight gain of single-housed kits, unless the cages are extremely small (European Commission, 2001).

There is even some evidence to suggest that increased cage sizes can have negative impacts on welfare. In Hansen et al. (1992), stereotypies were found to be increased in mink housed in male-female pairs in large cages compared to male-female pairs in standard or small cages. However, stereotypies on their own are not an accepted indicator of poor welfare for reasons discussed in Section 1.2.1, and may also be explained by general increases in activity in large cages (European Commission, 2001). One study also found that eosinophil counts were reduced in mink housed in large cages compared to standard or small cages (1.05m^2, 0.27m^2, and 0.10m^2, respectively; Hansen et al., 1992), which suggests that mink in larger cages were experiencing increased stress. However, eosinophil counts have not been thoroughly validated as an indicator of welfare in mink since they can be influenced by a variety of factors.

2.4.2 Nest Boxes

As discussed in Section 2.1, wild mink use multiple underground dens spread across their home range and tend to sleep in different dens nightly. This indicated to farmers and researchers alike that access to a separate, private nesting area would benefit mink. In fact, when provided with a nest box, mink typically spend 70-75% of their time in them (European Commission, 2001). Mink will also make use of several nest boxes when provided, but in cages with multiple mink, a 1:1 ratio of nest boxes for juvenile mink was not deemed necessary by Schwarzer et al. (2017) as juveniles tended to sleep in groups of two or more, and also seemed to designate some nest boxes as latrine areas rather than sleeping areas. It is standard in many countries to provide a single nest box mounted outside or inside the cage; nest boxes are often made of wood and match the width of the cage, with a depth of 15-30cm (0.03-0.09m^2 of floor space). Nest boxes are provided throughout breeding and whelping for the purpose of nesting, and throughout the rest of the year to provide more insulated, private shelter spaces; some farms have differentiated requirements for nesting and sleeping boxes (European Commission, 2001). In Canada, a single nest box must be provided for each cage for the majority of the year (i.e., whelping, lactation, furring, and winter months) with the exception of temporary blocking from the nest box as needed to retrain mink not to soil it (up to 1 week) or to facilitate conditioning during exceptionally warm weather (up to 2 weeks; National Farm Animal Care Council, 2013). There are no required dimensions for nest boxes, but they must hold ‘adequate bedding’, allow for ‘good’ nesting behaviour, and be of adequate size for all individuals to fit comfortably at the same time while still keeping kits in proximity to the dam, if housing a dam and her litter (National Farm Animal Care Council, 2013).

Before it was mandated that nest boxes be provided on farms (mandated in the Netherlands in 2003, though year of requirement in other countries is unknown; Mink Code of Practice Scientists’ Committee, 2012), multiple studies in mink suggested severe welfare consequences of limited nest box access. Fur quality was found to be poorer in male and female mink without a nest box; this was thought to be due to increased time spent lying against the wire mesh of the cage and increased heat loss from time spent exposed in the cage, in turn impacting blood circulation and nourishment of hair follicles (Hansen, 1988 as cited in Nimon & Broom, 1999). Lack of access to a nest box was also suggested to lead to increased metabolic rates and adrenal gland weights (Handen, 1998 as cited in Nimon & Broom, 1999), the latter of which may indicate chronic stress (as demonstrated in rats; Rostamkhani et al., 2012; Ulrich-Lai et al., 2006). Mink without nest boxes during the growing period are also found to consume more feed without gaining weight (Hansen et al., 1994), which supports the suggested effect on metabolic rates (Handen, 1998 as cited in Nimon & Broom, 1999) and may be due to increased energy expenditures required for thermoregulation. Moreover, mink housed in pens without nest boxes had lower levels of circulating eosinophil leucocytes than mink with nest boxes (Hansen & Damgaard, 1991), effects which are comparable to that of 30-min sessions of immobilization stress (Heller & Jeppesen, 1985). Stereotypic behaviour in female mink is also affected by nest box access; more stereotypic behaviour was performed in females without a nest box compared to those with them (Hansen et al., 1992), and juvenile female mink raised with a nest box performed fewer stereotypies in the fall of their first year than those raised without a nest box (Hansen et al., 1994). Furthermore, deprivation of nest boxes at whelping is shown to increase kit mortality and reduce kit growth (Møller, 1990).

Given the body of research that supports the benefits of nest box provision, it is no longer necessary nor ethical to conduct studies which limit access to a nest box. However, there are several outstanding issues on the topic of nest box provision not addressed in current literature (Mink Code of Practice Scientists' Committee, 2012); for example, it remains unknown which style of nest box dams prefer and/or is most effective in reducing kit mortality. There are also different styles of nest boxes used on different farms (e.g., 'penthouse'; 'drop-in'; whelping/European style; and open/covered top), yet the relative effects of each of these types of physiological and behavioural measures of welfare in mink are unknown. It would furthermore be beneficial to determine which material is best for nest box construction, or which bedding material is best to provide for insulation of the nest box, in terms of degree of heat retention offered in the winter and during whelping (Mink Code of Practice Scientists' Committee, 2012); this is further discussed below.

2.4.3 Nest Building Material and Facilitation of Good Maternal Care

Bedding material to insulate the nest box, often barley straw, rye straw, or wood shavings, has historically been provided to mink year-round in countries with colder climates (e.g., Scandinavia), but for only part of the year on farms in countries with warm and cold seasons (European Commission, 2001). Straw provision in summertime is not common because straw is costly and requires effort from farmers to distribute; moreover, mink will often rid the nest box of straw themselves in hot weather (European Commission, 2001). In Canada, nest building material provision is mandated during furring and winter months, as well as during whelping and lactation (National Farm Animal Care Council, 2013); bedding is generally provided across all countries prior to whelping to facilitate the building of nests for kits, though pine or cedar shavings cannot be used during this period as the resins can irritate kits' skin (National Farm Animal Care Council, 2013). Straw or wood shavings help to insulate and darken the nest, protecting against drafts and increasing privacy. Most pregnant mink instinctively make a bowl shape out of this material, and if not, farmers assist in shaping the nest (European Commission, 2001).

The types of bedding provided have been shown to differentially modulate kit mortality and reproductive success of dams. For example, supplying dams with Easy Strø (a heat-treated wheat straw) increased the number of live kits born and decreased kit mortality at 3-4 weeks post-whelping compared to supplying dams with wood shavings (Sønderup et al., 2009). Barley straw has also been suggested to be beneficial, as dams with barley straw had decreased variation in inter-birth intervals at parturition (Malmkvist & Palme, 2008). Artificial plastic nests are also thought to maintain higher, less variable nest temperatures; kits raised in artificial plastic nests with straw gained more weight on average than kits raised with only standard wood shavings (Malmkvist & Palme, 2008). Provision of artificial nests with straw may moreover improve maternal care (measured via kit retrieval testing, which demonstrates dams' reaction times to kits being removed from the nest); dams with an artificial nest and access to straw retrieved their kits faster than dams with an artificial nest or straw alone, or wood shavings alone (Malmkvist &

Houbak, 2000). Additionally functioning as an opportunity for mink to perform highly motivated nest building behaviours, access to bedding material and related substrates may reduce stereotypic behaviour (Nieuwenhuis and de Jonge 1989 as cited in European Commission, 2001) and prolonged access to bedding materials has also been shown to be beneficial for the reproductive success of pregnant dams (Schou et al., 2018).

Provision of nest boxes and adequate nest building materials can thus contribute to subjective perceptions of privacy and comfort in the peri-whelping period, which is not only essential for promoting reproductive success but also for the prevention of abnormal maternal behaviours such as cannibalism or rejection of the offspring. High prepartum and postpartum stress levels (Bahr et al., 1998; Culot et al., 2011; Poley, 1974), pain (usually associated with mastitis), and environmental stressors such as large litters or overcrowding (Kustritz, 2005) have all been found to contribute to behaviours such as cannibalism in other mammalian species. In mink, large litter sizes have in fact been shown to reduce maternal motivation to perform maternal care (Malmkvist et al., 2016), and farms will often cross-foster kits of large litters to control litter sizes (National Farm Animal Care Council, 2013). Cross-fostering of kits has been found to increase infanticide in other species (e.g., in cats; Kustritz, 2005), however, this has not been directly studied in mink.

In other mammalian species, anxious dams who leave the nest frequently or who perceive stressors in the whelping environment may also be more likely to reject or kill their litters (Lezama-García et al., 2019). Early fox farms, for example, reported issues with dams destroying their litters after becoming disturbed by farm activity, and this caused farmers to restrict visitation of their facilities around whelping (Colpitts, 1997). A study of infanticide in fox dams (Braastad & Bakken, 1993) also concluded that primiparous dams were more likely to kill their offspring, though they did not find that environmental disturbances were a causal factor (also supported by Pyykönen, 2008); rather, cub-killing correlated with individual variations in self-grooming (higher), standing when grooming of cubs (higher), and resting inside the nest box during the first postpartum day (lower). Infanticide may also be a trait related to personality or genetic factors, as cub-killing fox dams tend to repeat this behaviour across whelping seasons (European Commission, 2001). However, infanticide and rejection of litters has not been thoroughly studied in mink, nor have differential tendencies in maternal behaviours like nursing or grooming as they relate to factors within the nest box, cage, or greater farm environment; most reproductive research in mink to this point has focused on litter size at birth, kit growth, and general kit mortality (e.g., Malmkvist et al., 1997; Meagher et al., 2012; Schou & Malmkvist, 2017; Sønderup et al., 2009).

There has thus been limited research regarding overall improvement of the whelping environment and/or how bedding materials provided to dams might be enhanced. Bedding materials for nest building prior to whelping would ideally facilitate a bowl shape to keep kits close together and improve nest temperatures, given that kits cannot thermoregulate and often die due to hypothermia during early postnatal weeks (National Farm Animal Care Council, 2013). However, the standard bedding materials provided on farms may be insufficient to form well-insulated nests. There has also been limited research regarding how the provision of alternative nest building materials may influence the behaviour and stress physiology of dams and their kits; for example, Campbell et al. (2013) found that nests incorporated with wood shavings were better constructed than nests made of chopped straw, but these authors did not include measures of dam welfare or long-term effects on kits when each of these bedding materials were used. It may also benefit dam productivity and welfare to determine how stressors in the peri-whelping environment may be minimized, or how opportunities for stress-reducing behaviours may be better facilitated, to improve quality of maternal care and reduce mortality in litters.

2.5 Welfare of Mink in Relation to Environmental Enrichment

Environmental enrichment (EE) is an interdisciplinary term generally referring to ‘enhancements’ (e.g., changes or additions) to animals’ environments or husbandry, but in welfare research, these enhancements are evaluated based on biological relevance to the species and ability to improve welfare (Newberry, 1995). This may include environmental modifications that increase the behavioural choices available to an animal or enable the expression of species-specific behaviours to a greater extent than would be possible in a barren environment (Shepherdson, 2003). EE interventions commonly used in captive animals include increasing the complexity of the cage environment, providing mobile, manipulable enrichments within the cage, offering greater opportunity for social interaction (i.e., social enrichment), or offering more naturalistic or complex foraging/feeding opportunities (i.e., feed enrichment; Maple & Perdue, 2013). In solitary species like mink, social enrichment via access to conspecifics is beneficial early in life, but not once mink are mature (e.g., there are positive impacts of late weaning and family/group housing but only for a limited time after nutritional independence from the mother, whereas housing in pairs is beneficial until maturity; previously discussed in sections 2.4.1 and 2.4.2). Feed enrichment strategies such as providing diets of varying textures or tastes, introduction of novel edible enrichments, or stimulation via foraging tasks (i.e., ‘hiding’ feed around enclosures or presenting obstacles to attaining feed) have demonstrated benefits in other captive carnivores (e.g., Bashaw et al., 2003; Riggio et al., 2019; Ruskell et al., 2015; Watters et al., 2011), but this has not yet been explored in mink since commercial farm diets are extremely regulated and the enclosures of farmed mink are not compatible with these types of foraging tasks. Thus, the former two enrichment strategies will be the focus of this review.

2.5.1 Structural Enrichments

Two tested methods of increasing cage complexity in mink include connecting multiple cage compartments (e.g., Hansen et al., 2007) and providing swimming water (e.g., Ahola et al., 2011; Hansen & Jeppesen, 2001; Schwarzer et al., 2016; Vinke & van Leeuwen, 2005). As discussed in Section 2.4.1, mink appear to value access to interconnected cage spaces, but the appeal of added cage compartments is limited when provided without other forms of enrichment. For example, Cooper & Mason (1997) reared kits in standard cages connected to six other compartments containing resources that mink were hypothesized to value (a hay box meant to offer an alternative den site, a water bath, novel objects which were rotated daily, a prey-like toy, and a raised platform and tunnel), and one empty compartment. The mink visited and spent time in each compartment but demonstrated the highest motivation to access the novel object and water bath, followed by the toy and platform/tunnel compartments (measured via maximum weights of weighted doors pushed open to enter these compartments). These results are in alignment with other studies showing that mink are highly motivated to access swimming water as a form of cage enrichment Cooper & Mason, 2000; Mason et al., 2001) and show motivation to access water baths regardless of whether their presence is indicated by resource cues (i.e., swimming may be internally rather than externally motivated; Warburton & Mason, 2003). Moreover, when deprived of swimming water for 24h, mink have shown increased stereotypic behaviour and a urinary cortisol increase similar to that observed in 24h food deprivation scenarios (Mason et al., 2001). The relative incentive value of a swimming basin, a running wheel, and an empty compartment has also been investigated (Hansen & Jensen, 2006); mink demonstrated equal willingness to work for access to both the running wheel and swimming water, while reduced effort was exerted to reach the empty compartment. Interestingly, despite being equally valued, the running wheel and swimming basin did not seem interchangeable as they were used at different times of day and therefore seemed to satisfy different underlying behavioural motivations (Hansen & Jensen, 2006). Access to a running wheel has been shown to prevent the development of stereotypic behaviour, however, there is some indication that wheel-running is in itself a form of stereotypic behaviour (Hansen & Damgaard, 2009); thus, running wheels have not yet been provided in commercial farm settings.

Similarly, swimming basins are not often provided on commercial farms due to limited evidence of their benefits for mink welfare and productivity. For instance, mink will exhibit similar levels of frustration when their access to a swimming basin is blocked versus when they are blocked from an empty cage (Hansen & Jeppesen, 2000). Moreover, similar levels of stereotypic behaviour (Hansen & Jeppesen, 2001) and tail-biting (European Commission, 2001) were found in mink raised with and without free access to swimming water. Another study found no difference in anticipatory behaviour or stereotypic behaviour between mink housed with and without swimming water after deprivation of swimming water for 2.5 weeks, indicating that there may be no consequences of absence of swimming opportunities in mink who have never been provided with swimming water (Vinke et al., 2006). Moreover, there appear to be individual differences in use of water basins that may be dependent on genetic factors or variations in individual activity levels (Mononen et al., 2008; Vinke et al., 2008). Swimming water is also a difficult method of enrichment for farms to implement for practical reasons: they are costly to install and maintain and may even pose risks for health (i.e., issues with hygiene could arise if not regularly cleaned and refilled) and kit survival (primiparous dams given swimming water had increased kit mortality, possibly because their nests became wet and dirty; Møller, 1991 and Skovgaard et al. 1997, as cited in European Commission, 2001). It has been suggested that future work aim to investigate methods of water-based enrichment that do not involve full basins for swimming; for instance, some Dutch farmers use water sprinkling systems when temperatures are high, and mink will approach these jets of water (European Commission, 2001). Cooling pens with water vaporisers or sprinklers was also suggested as a measure of protection from heat exhaustion in the WelFur protocols, an on-farm welfare assessment tool for fur-bearing species in European countries (Møller et al., 2015; Mononen et al., 2012), while swimming water itself is currently noted in the protocol as a ‘medium certainty’ enrichment regarding its effectiveness (Møller et al., 2015).

Alternatively, inclusion of raised platforms for climbing and elevated resting opportunities can serve as a feasible method of increasing environmental complexity for mink (e.g., Ahola et al., 2011; Axelsson et al., 2009; Buob et al., 2013; Hansen et al., 2007). When mink are not using a nest box, it has been demonstrated that they prefer a resting place that is above floor level, such as a shelf (Hansen et al., 1994). Female mink especially show a tendency to use elevated shelves or tubes fixed to the cage ceiling when pair-housed with males, inferring that they may use this space as a refuge from their larger male siblings (Hansen et al., 2007; Hansen et al., 2011); moreover, shelves are often large enough to fit two animals, but mink are often observed using them one at a time, indicating they may fulfill a need to be alone (Jeppesen, 2004). Lactating dams may also use elevated platforms to escape kits later in the nursing period, as previously discussed in Section 2.3.1 (e.g., Buob et al., 2013; Dawson et al., 2013; Jeppesen, 2004). Even mink who are singly housed will make use of elevated wire shelves, potentially to gain a better vantage point of their surroundings (Hansen et al., 1994). Incorporation of wire shelves in the cage has been found to reduce stereotypic behaviour in females when provided beginning at seven months of age (Axelsson et al., 2009; though, mink on two different farms were used in this study, and this effect was only found on one farm). Although these shelves or platforms are often used for resting, welfare benefits may also be conferred through greater opportunities for climbing; for example, females housed in climbing cages after weaning performed significantly less stereotypic behaviour during the winter than those kept in standard cages, though both housing types included a net shelf and plastic cylinder enrichment (Lidfors et al., 2012). Based on these studies evidencing their benefits, all mink pens in Canada are required to have a hammock, shelf, or platform enrichment if housing multiple mink (mandated as of 2014), however, an elevated nest box (i.e., ‘jump up’ or ‘penthouse’ cages) can substitute for these structural enrichments since mink must climb or jump to access them. About 25% of pens on Dutch farms had already included a wire mesh platform or plastic cylinder in pens by 2001 (European Commission, 2001).

2.5.2 Mobile, Manipulable Enrichments

Adding mobile items to the cage environment is also an effective and feasible form of EE in farmed mink. Objects such as play balls and loose tunnels or cylinders are thought to offer behavioural outlets for activities such as hunting and foraging, and are shown to reduce cortisol levels, fur chewing, and stereotypic behaviour as well as negative affective states like boredom (Buob et al., 2013; Dallaire, 2012; Dallaire et al., 2012; Hansen et al., 2007; Jeppesen & Falkenberg, 1990; Meagher et al., 2014; Polanco et al., 2021). Although somewhat less manipulable, ropes, chains, or lengths of rubber hose fixed to the cage ceiling also have welfare benefits in mink and are often interacted with through pulling or chewing (Buob et al., 2013; Hansen et al., 2007; Meagher et al., 2014; Polanco et al., 2021); these enrichments are often referred to as 'fixed' enrichments rather than mobile enrichments, along with the previously mentioned items like hanging shelves, platforms, hammocks, or tunnels.

In studies investigating minks' preference for the categories of items highlighted here (i.e., mobile enrichments) and in the previous section (i.e., structural, fixed enrichments), fixed enrichments seemed to be used more by mink than mobile enrichments (Axelsson et al., 2009; Dallaire & Mason, 2016; Hansen et al., 2007). These conclusions were based on comparisons between structural, fixed enrichments like hanging shelves, platforms, and lengths of rope/hose and mobile enrichments like loose play balls or tunnels on the cage floor. However, since shelf- and platform-like structures are often used by mink for resting, it is understandable that a larger portion of their daily time budgets may be dedicated to use of these enrichments than to use of mobile items. Mobile enrichments may also be subject to a habituation effect, where kits' interest in fixed items is not impacted in the same way: for example, in Hansen et al. (2007), fixed hanging rope enrichments were being regularly replenished as they were torn down by mink, potentially causing a novelty effect which attracted more attention than permanently present mobile items. Furthermore, use of play ball enrichments was observed to subside within one month of their introduction to the cage environment, demonstrating that mobile enrichments may become less useful in improving mink welfare after relatively short periods of presence in the pen (Jeppesen & Falkenberg, 1990).

From 2008 onwards, mink in Europe were required to be housed with environmental enrichment, though 88% of mink in Norway were already provided with 'activity objects' by 2001; data from other countries was not available at this time (European Commission, 2001). In Canada, farms were required to provide a minimum of one mobile enrichment in each pen as of December 31, 2013 (National Farm Animal Care Council, 2013). There is no mandated age of introduction, but once an enrichment introduced, it must remain in the pen for the duration of the animal's lifetime. EE is primarily implemented on commercial farms as an intervention to reduce or prevent stereotypic behaviour and self-mutilation; however, it has been widely demonstrated in captive carnivores that EE does not abolish stereotypic behaviour altogether but rather reduces it (Shyne, 2006; Swaisgood & Shepherdson, 2006). Moreover, many of the ways in which EE is speculated to mediate stereotypic behaviour and self-mutilation require their direct use (Mason et al., 2007), and as discussed, enrichment use declines over time when items are permanently present in the cage. Enrichment use in carnivores is also known to be dependent on the age of the animal (Ward et al., 2023), and in mink specifically, has been linked to the age at which enrichment is introduced (Ahola et al., 2011; Campbell et al., 2013; Mason, 1993). Although enrichment protocols have contributed to partial improvements in the occurrence of self-mutilation behaviours on mink farms (e.g., tail chewing; tail tip lesions were found in 5.4% of mink in a study assessing lesions at pelting; Hansen & Møller, 2012), stereotypic behaviour remains widespread (current prevalence is unknown but was estimated to occur in 35-85% of adult females based on a large-scale Netherlands study; reported by European Commission, 2001). Thus, further research is needed regarding how enrichment provision in mink may be improved.

2.6 Conclusions and Aims of Thesis

The previous chapters highlight how farm environments and husbandry practices have historically constrained behavioural opportunities for mink and/or negatively impacted mink welfare, and how animal welfare research has helped to improve housing practices by identifying behavioural needs of mink and means of satisfying them in farm housing. However, there remain areas of limited or conflicting research, particularly regarding optimization of the whelping environment and provision of effective environmental enrichment for mink. Although farmed mink reproduce relatively well in Canada, there is limited research regarding which nest building materials may be most conducive for nest building and kit survival, and how whelping environments can be ameliorated to promote improvements in maternal behaviour and welfare. Furthermore, past research regarding which physical enrichments are most beneficial for mink are confounded by habituation effects impacting the mobile items used, as well as lack of consideration for the different uses of ‘fixed’ and ‘mobile’ items. It also remains unknown when or for how long enrichments should be provided in farm settings to confer optimal welfare benefits.

The objective of this thesis was therefore to determine how various enrichment strategies implemented from whelping through the juvenile phase affect the long-term welfare and success of farmed mink. Specifically, we aimed to answer the following questions:

1. How do greater nest building opportunities for dams in the peri-whelping period affect dam behaviour and stress physiology, offspring survival, and long-term welfare in kits? To what extent are the latter mediated by maternal care?
2. How does early-life provision of diverse and novel physical enrichments affect kit behaviour in the juvenile phase, and to what extent does juvenile enrichment mediate stereotypic behaviour development later in life? Which enrichment type(s) are used most by kits?

2.6.1 Thesis Hypotheses

The study cohorts for this thesis included one group of dams given access to more numerous, greater-quality nest building materials and additional enrichment (hanging rope) in the peri-whelping period (enriched at whelping; EW), while other dams received only standard nest building materials and enrichment (i.e., a single mobile enrichment). Kits in a second group were supplied with numerous physical enrichments of varying properties and degrees of novelty from approximately 1-4 months of age, beginning when they first became mobile (enriched kits; EK). The behaviours and stress physiology of these groups were then compared to a standard housed group who whelped in standard conditions and were housed with one standard, unchanging enrichment item as juveniles.

My hypotheses were as follows:

1. Dams provided with enriched nest building materials were hypothesized to experience reduced stress in the peri-whelping period and deliver improved maternal care to kits compared to dams provided with standard nest building materials. If this hypothesis was supported, it was specifically predicted that:
    a. Enriched dams would exhibit reduced basal cortisol, reduced stereotypic behaviour, and increased performance of behaviours related to good welfare in the peri-whelping period compared to standard-housed dams.
    b. Enriched dams would build better nests and perform increased kit-directed maternal care behaviours compared to standard-housed dams.
        i. In turn, reductions in mortality, increases in growth, and improvements stress response development in offspring of enriched dams were predicted.
2. Provision of more diverse, novel enrichment for kits was hypothesized to improve kit welfare in the juvenile period and deliver long-term effects on behaviours of kits as adults. Specifically, it was predicted that:
    a. Enriched kits would demonstrate increased enrichment use compared to kits with standard enrichment.
        i. Further, kits were predicted to preferentially interact with fixed enrichments compared to mobile enrichments (however, it was considered whether renewal of novelty of both EE types and inclusion of only hanging chains or ropes in the 'fixed' enrichment category would affect this outcome).

b. Enriched kits would show reductions in behaviours associated with poor welfare and increases in behaviours associated with good welfare in the juvenile period compared to kits with standard enrichment.
c. Lastly, enriched kits were predicted to exhibit reduced stereotypic behaviour development later in life compared to kits housed in standard enrichment as juveniles.

2.6.2 Thesis Outline

The study outlined in Chapter 3 discusses the enrichment use of kits enriched as juveniles compared to that of juveniles in standard conditions (i.e., corresponding with hypothesis 2a in the above list). Changes in enrichment use as kits aged, and as familiar items were exchanged for novel ones, were investigated in addition to relative levels of interaction with each enrichment type.

The study outlined in Chapter 4 discusses the effects of each enrichment strategy (i.e., enrichment at whelping and enrichment as juveniles) on behavioural and physiological measures in dams and/or kits compared to mink in control housing (i.e., corresponding with hypotheses 1, 2b, and 2c). Mink were observed from the peri-whelping period through pelting in the late fall. The behaviours of the kits as adults were then investigated after all individuals had been in standard housing for one year (see Figure 2.1 for timeline of housing interventions and experimental measures). The relative benefits of each enrichment strategy for mink welfare were compared.

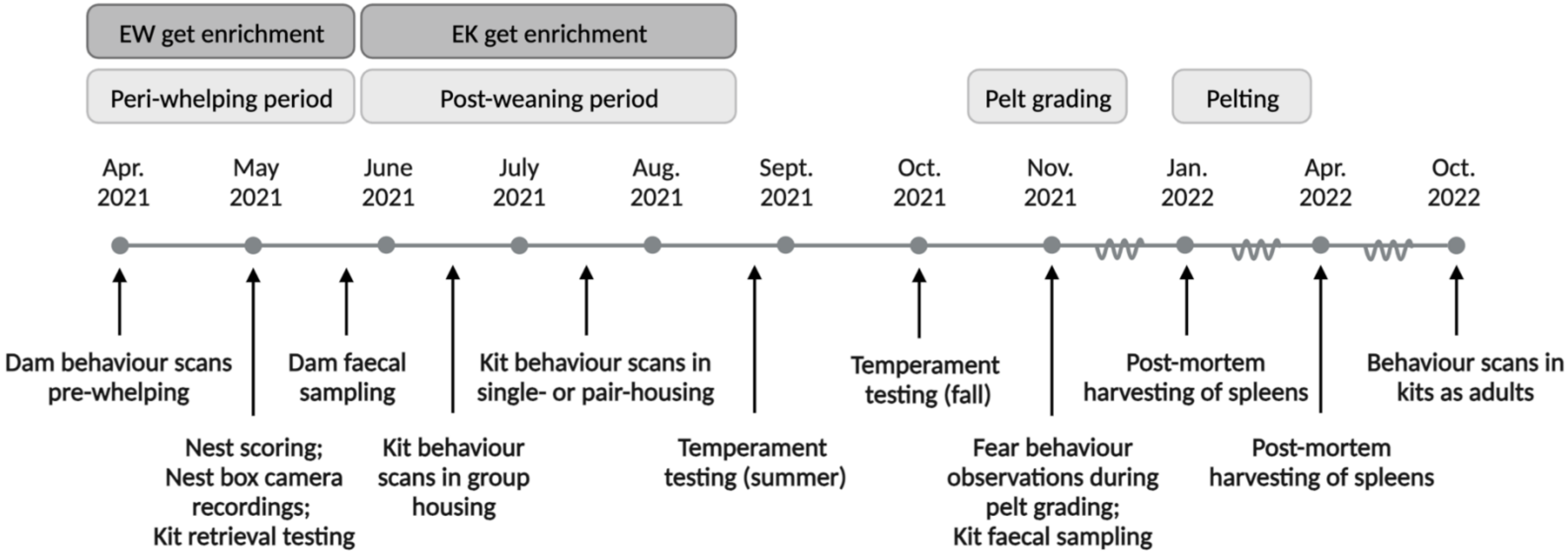

Figure 2.1 Timeline of interventions for experimental groups and typical events in farm husbandry (top) and data collection for various tests (bottom). Line squiggles represent time jumps.

3. Diversity and Novelty in Environmental Enrichment Increases Enrichment Use in Juvenile American Mink (*Neogale vison*)*

3.1 Abstract

It is standard practice on mink farms in Canada to provide one manipulable environmental enrichment (EE) in each cage to benefit animal health and welfare, and once an enrichment is introduced, it must remain in the cage for the duration of that animal's life. However, mink might habituate to permanently present enrichments, which reduces interaction with these items over time. In this study, juvenile mink were provided with multiple EEs (mobile and hanging items) that were regularly exchanged to maintain novelty in addition to a standard, permanently present EE (enriched kits; EK). EE use over time, as well as interactions based on EE type, were compared to that of two groups which were standard-housed as juveniles (standard housed, SH; enriched at whelping, EW) with access to a permanently available standard enrichment. EK housing was found to significantly increase kits' EE use compared to standard housing ($p < 0.001$). Despite some evidence of rapid habituation to novel objects (e.g., decreased use of hanging rope EE in second week of access: $p = 0.019$), item exchange appeared to have a sustaining effect on exploration of all EEs in the cage, including familiar objects. Thus, it may be beneficial to provide farmed mink with multiple EEs, both novel and familiar, to promote their optimal use and facilitate welfare benefits. Objects with greater manipulability or malleability were also used more by kits than those with fewer possible uses (e.g., pig's ear versus other, less malleable mobile EEs: $p < 0.001$; rope versus less malleable hanging EE: $p < 0.001$). Although this effect was persistent across all periods of observation, it is possible that kit age at time of object introduction or differences in

novelty may have contributed to these differences in their use. Further research is required to determine kits' preference for different EE types when all items are equivalently novel, and when differences in developmental stage are fully accounted for.

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Gabrielle B. Clark contributed to the planning of experimental methods and conducted data collection, statistical analysis, and the majority of writing for this co-authored manuscript.

3.2 Introduction

The welfare of captive animals can be improved by providing environmental enrichment (EE) that enables the expression of natural behaviours to a greater extent than would be possible in a barren environment. In applied ethology and animal husbandry, EE refers specifically to biologically relevant stimuli that function to improve animal welfare (Newberry, 1995). Methods of EE can include incorporating fixed or structural elements to the cage environment that add complexity or stimulation for the animals, or inclusion of mobile, manipulable enrichments within the cage (Maple & Perdue, 2013). It is important that enrichments be selected based on their ability to satisfy specific behavioural needs for the target species. For instance, the provision of manipulable objects in the cage that can be chased or chewed has been shown to reduce cortisol levels, fur-chewing, and stereotypic behaviour as well as negative affective states like boredom in American mink (*Neogale vison*), a mustelid species farmed for their fur (Hansen et al., 2007; Meagher et al., 2014, 2017; Meagher & Mason, 2012; Polanco et al., 2021). Mink are opportunistic predators who in the wild will mostly consume fish, amphibians, and crustaceans, but will also hunt rabbits and other prey on land (Gerell, 1967). In captivity, mink do not have the same opportunities for hunting; commercial fur farms typically feed their mink a meat-based paste that is placed directly on the mesh wire on top of the cage (National Farm Animal Care Council, 2013). Therefore, it is thought that the provision of mobile EEs may offer an outlet for these unfulfilled behavioural needs (e.g., chasing or chewing) and in this way confer their positive effects on abnormal behaviour, boredom, and stress in mink (Hansen et al., 2007; Meagher et al., 2017, 2014; Meagher and Mason, 2012; Polanco et al., 2021).

Despite this theorized benefit of mobile EEs, existing research suggests that fixed and unmovable EEs are used by mink more often than those that are mobile and chaseable (Axelsson et al., 2009; Dallaire & Mason, 2017; Hansen et al., 2007). In these studies, fixed enrichments seem to be used by mink as additional resting places, which may facilitate escape from the reach of newborn kits and larger males or allow them to obtain a greater vantage point from which to monitor their surroundings (Axelsson et al., 2009; Buob et al., 2013; Hansen et al., 2007; Jeppesen, 2004). It is also possible that fixed enrichments may fulfill a behavioural need for mink to be alone, as only one individual was typically observed using the structure at a given time despite having the capacity for two mink (Jeppesen, 2004). These potential benefits are thought to account for the greater use of fixed EEs (shelves or hanging tunnels and fixed lengths of rope) compared to mobile enrichment types (play balls and plastic tunnels or tennis balls placed on the floor of the cage; Axelsson et al., 2009 and Hansen et al., 2007, respectively).

The results of previous studies, however, may be impacted by several confounding factors. Since mink are inactive for a large portion of their daily time budget, even in the wild (Clubb & Mason, 2007; Dunstone, 1993), it is understandable that they may dedicate more time to the use of shelves or hanging tunnels than to items which require more active use. The mobile enrichments used in previous studies may also have been subject to a habituation effect while fixed items were not impacted to the same extent. In some instances where mobile enrichments are permanently present in the cage, other fixed object types such as hanging rope are being regularly replenished, potentially causing a novelty effect (Hansen et al., 2007). A study measuring the effect of play ball enrichments on mink welfare observed that interaction with play balls subsided within one month of introduction (Jeppesen & Falkenberg, 1990). The novelty of mobile EEs should therefore be renewed regularly by replacing familiarized objects in the cage with novel objects. Moreover, certain mobile EEs such as play balls have only one use (e.g., to be rolled) and therefore seem to attract less interaction over time, whereas other mobile EEs like loose tubes in the cage may offer multiple uses (e.g., rolling, climbing, or hiding), which may increase opportunities for object novelty (Axelsson et al., 2009). A logical next step in this area of research would be to provide mink with a variety of fixed and mobile enrichments and quantify their relative use based on how often they are exchanged and the number and/or nature of possible uses they have.

Despite the potential benefits outlined above, the continuous exchange of all items in the cage could negatively impact welfare as well as research validity, since interruptions in access to familiar items may be a source of stress for mink. Many studies in rodents support that once EE is supplied, access to the items should not be interrupted or revoked because this can induce anxiety or depression-like behaviours in the animal (e.g., Morano et al., 2019; Smith et al., 2017). To prevent this, and its potential confounding effect on further experimentation, studies investigating novelty effects on EE use must be designed with a strategy of object novelty renewal that allows one or more familiar items to remain in the cage while others are continuously exchanged. Correspondingly, it is standard practice on mink farms in Canada to provide at least one manipulable enrichment in each pen, and once an enrichment is introduced, it must remain accessible for the duration of that animal's life (National Farm Animal Care Council, 2013). Maintenance of this practice may act to prevent the negative effects of enrichment removal on behavioural and physiological measures of stress.

It is also recommended, but not mandated, by the Mink Code of Practice in Canada that enrichments be provided in early life, because access to enrichments during early development is known to have an important role in the prevention of abnormal behaviours such as stereotypic behaviour (National Farm Animal Care Council, 2013). Supplying EE once animals have matured is shown to be less effective at reducing the performance of stereotypic behaviour (Mason 1993, Ahola et al. 2011, Campbell et al. 2013), and animals show less motivation to gain access to enrichments when introduced to them as adults (Tilly et al., 2010). Animals raised in stimulus-poor conditions may also perceive enrichment introduction later in life as a stressor (i.e., an unpredictable environmental change; Fairhurst et al., 2011) or may have greater novelty-induced fear responses to enrichment that are resistant to habituation (e.g., Cooper et al., 1996; Jones, 2001). However, enrichment introduction typically does not begin on mink farms while false bottoms are in place (i.e., plastic net-like material placed on the floor of the cage to cover gaps between wires, for the duration of whelping, until kits become mobile) due to concerns for safety – for example, play objects may become soiled during this time due to the accumulation of faeces in the cage and pose a health issue (National Farm Animal Care Council, 2013). Interestingly, if EE is provided too early, it can also interfere with maternal care (e.g., a reduction in time spent nursing and increases in non-maternal behaviour were demonstrated in enriched rodent dams; Li et al., 2016). Thus, when provided too early in the postnatal period, EE may in fact have negative consequences for offspring development and welfare. Kits may also not develop the motor skills necessary to interact with enrichments until four weeks of age, and object play was not observed to emerge until seven weeks of age in a previous study (Jonasen, 1987), so it is unclear exactly when these items become useful in mink; there have been limited attempts thus far to provide enrichments to kits this early in development.

Few studies have been conducted to determine the preference of mink for enrichment items with various properties (e.g., fixed or mobile) as well as various degrees of novelty. In the present study, the enrichment-directed behaviours of mink kits with access to a multitude of play objects, some of which were exchanged at regular intervals to maintain novelty (enriched kits; EK), was compared to that of two standard-housed groups with access to one standard enrichment that remained permanently present in the cage (standard housed kits [SH] and kits who were enriched at whelping [EW]; these groups were kept distinct due to differential nest environments in their first week of life, which are the focus of a separate chapter). It was hypothesized that the EK group would demonstrate greater EE use than standard-housed groups (SH and EW) due to the novelty and variety of enrichments available. EE use was also hypothesized to be maintained at a higher level over time in the EK condition than in the standard-housed conditions due to the regular renewal of object novelty. This data may also contribute to identifying trends in EE use as kits age or as object types are exchanged. Moreover, we aimed to replicate previous research findings regarding minks' increased use of hanging enrichments compared to mobile enrichments, although it was considered whether renewal of novelty of both enrichment types in this study would result in a change in use. There was no hypothesis regarding which of the individual hanging and mobile enrichments provided to EK kits would attract the most use, but these results will be discussed.

3.3 Methods

3.3.1 Research Ethics Statement

This research was approved by the Dalhousie University Faculty of Agriculture Animal Care & Use Committee (#1033575) and the Clinical Research Ethics Review Board of the Royal Veterinary College (URN 2021 2034-3). All housing conditions and husbandry practices in effect were standard for fur farms in Canada (National Farm Animal Care Council, 2013), aside from the extra enrichments provided to some treatments.

3.3.2 Subjects And Housing

242 female mink were bred to account for potential unsuccessful copulations and/or poor litter health (desired sample size of ≥30 litters in each treatment, each with ≥1 male-female pair, as indicated by sample size calculations prior to commencement of the study). Litters were excluded from the study if fewer than four kits survived. Male and female mink selected at breeding for use in the study were balanced across Dark, Mahogany, Pastel, Demi, and Stardust colour types (strains). All dams had been housed individually (American mink are solitary in the wild, Dunstone 1993) indoors at the Canadian Centre for Fur Animal Research (Nova Scotia, Canada) in 75 (L) × 60 (W) × 45 (H) or 40 (H) cm wire-mesh cages with wire shelf, external wooden nest boxes, and a single physical enrichment (plastic ring) prior to inclusion in the study. Adult mink received daily afternoon feedings and juvenile mink (i.e., from weaning to maturity) were fed twice daily in the morning and afternoon with a meat-based paste (Sea Crest Fisheries Ltd., Saulnierville, NS). All mink had access to drinking water *ad libitum*.

Females that bred successfully were assigned to one of three treatment groups via blocked pseudo-randomization. These included two standard-housed conditions in which kits received a standard plastic ring enrichment (standard-housed [SH] and enriched at whelping [EW]; litters in the latter group were enriched through different environmental modifications than those used in this study, which were in effect from the pre-whelping period to postnatal day eight for the purpose of a separate chapter), and one enriched condition (enriched kits; EK) where dams and kits received a standard plastic ring enrichment in addition to a plastic chain fixed to the cage ceiling at 28±7 days post-whelping, when kits were expected to become mobile (reported at approximately four weeks post-whelping; Jonasen, 1987). One additional mobile EE was provided to EK kits at weaning (either a plastic wiffle ball or golf ball); enrichment type was randomly assigned such that approximately half of the EK litters in each whelping cohort (dams who whelped within three days of each other) received a golf ball and half received a wiffle ball. These enrichment items were selected based on proven welfare and/or production benefits (Díez-León et al., 2013; Meagher et al., 2014; Díez-León & Mason, 2016). Groups were counterbalanced for colour type and parity, and cages for each were evenly distributed throughout the barn.

3.3.3 Post-Weaning Kit Observations

Group Housing

Our desired sample size was maintained up to and including the post-weaning period (n_{SH}, n_{EW}, and n_{EK} = 45, 40, and 37 litters following exclusions and losses). Kits were weaned at six weeks post-whelping, at which point the dam was removed from the whelping pen and placed in a separate standard cage. Kits remained in the whelping pen and were housed in groups of four to six (n_{SH}, n_{EW}, and n_{EK} = 218, 207, 183 kits, respectively); if there were more than eight kits in a litter, they were split across two pens (applied to ten SH pens, three EW pens, and seven EK pens). Kits in EK continued to have access to prior enrichments (i.e., hanging plastic chain and standard ring enrichment in addition to a second mobile EE introduced at this stage), while standard-housed kits (SH and EW) continued to have access to a standard plastic ring. Scan sampling observations were conducted three consecutive days per week for the following four weeks (from weaning to ten weeks post-whelping). Cages were observed three times per day (two morning rounds at approximately 8:30am and 10am and one afternoon round at approximately 1pm; all rounds occurred between an 8:00am morning feeding and a 3:00pm afternoon feeding). Behaviours were recorded through scan samples every 20s over a 2-minute period before moving to the next cage. Kits' interactions with enrichments were recorded (enrichment use defined as head in contact, licking, or sniffing the EE within 1cm, excluding being inactive with the enrichment), as well as other behaviours not relevant to this chapter. The order in which cages were observed was reversed each scoring day to prevent the systematic scoring of some cages earlier than others. A schedule was implemented during this observation phase for the exchange of EK enrichments (see Figure 3.1). This schedule entailed exchanging golf balls for wiffle balls and vice versa at post-weaning Week 3 (eight weeks post-whelping), while the hanging chain remained present in the cage.

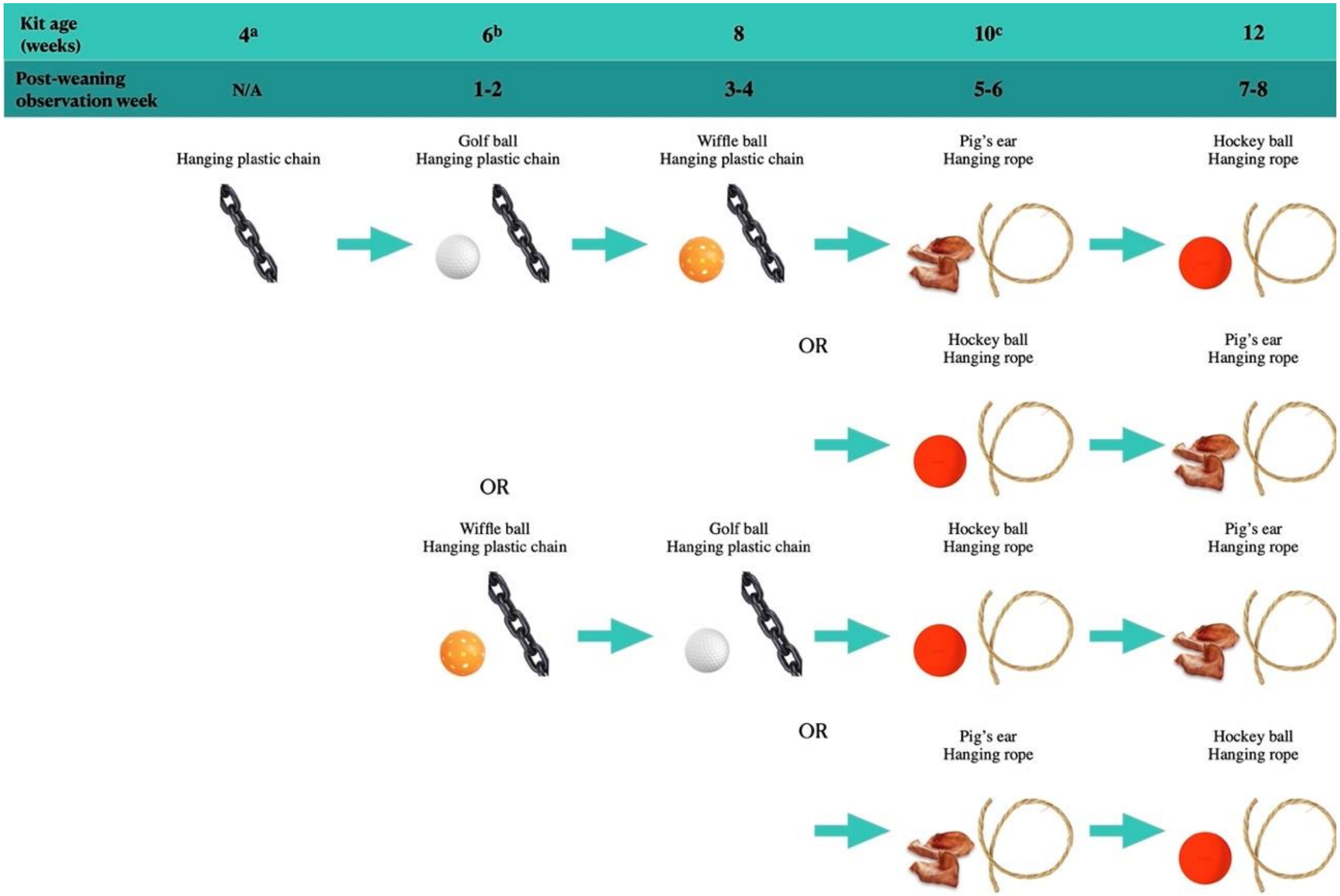

Figure 3.1 Schedule of enrichments for kits in EK. Enrichment type was randomly assigned such that approximately half of the litters in each whelping cohort (dams who whelped within 3 days of each other) received a golf ball and half received a wiffle ball. Exchange to pig's ears or hockey balls at 10 weeks was counterbalanced by whelping cohort to account for the effect of age on enrichment use. [a]Kits become mobile. EK kits begin to receive enrichments while dam is still in the pen. [b]Weaning. Dams moved to separate pens. Enrichments (previous and novel) remain in pen with kits. [c]Kits separated into single- and pair-housing. One male-female pair and one single female from each litter continue to have access to the enrichments for their treatment.

Single- or Pair-Housing

At ten weeks of age (five weeks post-weaning), kits were moved to single- or pair-housing pens according to standard farm protocol. One male and one female from each litter were chosen for pair-housing and remained in the whelping cage. These male-female pairs (n_{SH}, n_{EW}, and n_{EK} = 55, 43, and 44 pairs, respectively) continued to be observed according to the group-housing observation protocol and enrichments for EK continued to be exchanged according to the schedule in Figure 3.1. This included the golf or wiffle balls being exchanged either for street hockey balls or pig's ears such that approximately half of the EK pens received each object. Hanging chains were exchanged for ropes fixed to the cage ceiling (43 cm or 38 cm long, according to cage height; see Figure 3.2 for an example of enrichment layout). Since the rope and pig's ears were depletable enrichments (i.e., kits were able to chew or otherwise deplete them until they were no longer visible in the cage), these objects were replenished as needed at the beginning of each week's observations (i.e., once weekly). This schedule of enrichment exchange continued until post-weaning Week 8 (thirteen weeks post-whelping), such that mobile enrichments were exchanged for novel enrichments every two weeks or as depletable enrichments (pig's ears and hanging rope) needed replenishing (see Figure 3.1). After the conclusion of the post-weaning observation period, EK enrichments remained in the cage for two additional weeks (fifteen weeks post-whelping) until removal, at which point all kits had a standard ring for enrichment.

Figure 3.2 Example of enrichment for the EK housing condition in Week 5-6 or 7-8 of observation (hanging rope and hockey ball in addition to standard ring enrichment).

3.3.4 Statistical Analyses

Data were formatted for analysis by calculating the average proportion of observations where EEs were in use per kit (henceforth referred to as average EE use) based on the total number of observations (see equation below).

$$\text{Average proportion of observations where EEs were in use} = \frac{\#\ Ocurrences}{(\#\ Kits\ per\ pen)(\#\ Scans\ per\ day)(\#\ Days\ observation)}$$

Statistical analyses were conducted with jamovi statistical software (The jamovi project, 2022, v. 2.3.18.0). Significance level was set at $p < 0.05$. Results were defined as trending when $0.05 < p < 0.10$. The details of the analytical methods used for each test are as follows:

To assess levels of enrichment use across treatments, average EE use was analysed using Welch's One-Way ANOVA in the post-weaning Week 1-4 period, Week 5-8 period, and Week 1-8 period (all observation weeks) with treatment as a factor. Data were transformed to fit the normal distribution using the square root transformation; normality was assessed visually using Q-Q plots and Levene's test was used to assess homogeneity of variance. Post-hoc comparisons were performed using the Games-Howell correction to account for unequal variances. Group means and 95% confidence intervals were back-transformed for presentation.

To assess EE use over time within treatments, average EE use for various enrichment categories (hanging EEs, the ring EE, mobile EEs with and without the ring included, and all EEs collectively) were analysed using linear mixed model analyses with week of observation as a factor and pen number as a cluster variable. EK pens were analysed separately from SH and EW pens since trends in use of EK enrichments specifically were of interest; however, treatment was an additional factor for the analysis of ring use over time in standard-housed groups (SH and EW). Data were transformed using the square root transformation and visually analysed for normality using Q-Q plots. The Bonferroni correction was used for post-hoc tests to account for the number of comparisons. The Satterwaite method was applied for degrees of freedom to account for violations of homogeneity of variance. Due to emerging differences in EE use between Week 1-4 and Week 5-8 of observation, paired Student's t-tests were performed for each enrichment category to compare EE use between these periods; these differences were presented when applicable to simplify reporting of results. Means and 95% confidence intervals were back-transformed for presentation.

EE use by item type was analysed using non-parametric Kruskal-Wallis ANOVAs and Dwass-Steel-Critchlow-Flinger pairwise comparisons as this data could not be transformed to fit the normal distribution. EE use in EK pens alone was investigated, as other treatments only had access to the ring enrichment. Average use of hanging vs. mobile EEs and individual hanging and mobile EE sub-types were compared with object type as a factor.

3.4 Results

3.4.1 Enrichment Use Across Treatments

Treatment significantly affected the average EE use observed in both the Week 1-4 ($F_{2,65.5} = 26.6$, $p < 0.001$) and Week 5-8 periods ($F_{2,63.6} = 92.0$, $p < 0.001$; see Figure 3.3). Post-hoc tests revealed that, on average, EE use was observed more often in EK pens than in SH and EW pens in Week 1-4 ($t_{60.9} = -6.41$, $p < 0.001$ and $t_{61.3} = -6.85$, $p < 0.001$, respectively) and Week 5-8 ($t_{48.6} = -13.0$, $p < 0.001$ and $t_{48.1} = -13.0$, $p < 0.001$, respectively; see Table 3.1). This effect persisted across all weeks combined (Week 1-8; $F_{2,63.3} = 112.0$, $p < 0.001$) towards more EE use on average in EK pens compared to SH and EW pens ($t_{46.2} = -14.4$, $p < 0.001$ and $t_{49.8} = -14.4$, $p < 0.001$, respectively; see Table 3.1). There was no difference in EE use between SH and EW pens during any of these periods ($t_{67.0} = 0.512$, $p = 0.866$, $t_{66.9} = 0.063$, $p = 0.998$, and $t_{66.0} = 0.578$, $p = 0.832$, respectively).

Table 3.1 Back-transformed average enrichment use (mean with 95% CI in parentheses) across observation periods and housing conditions.

EE use by period	SH	EW	EK
Week 1-4	0.007 (0.005, 0.008)	0.006 (0.005, 0.008)	0.018 (0.015, 0.023)
Week 5-8	0.004 (0.003, 0.006)	0.004 (0.002, 0.006)	0.065 (0.052, 0.080)
Week 1-8	0.006 (0.005, 0.007)	0.005 (0.004, 0.007)	0.043 (0.036, 0.065)

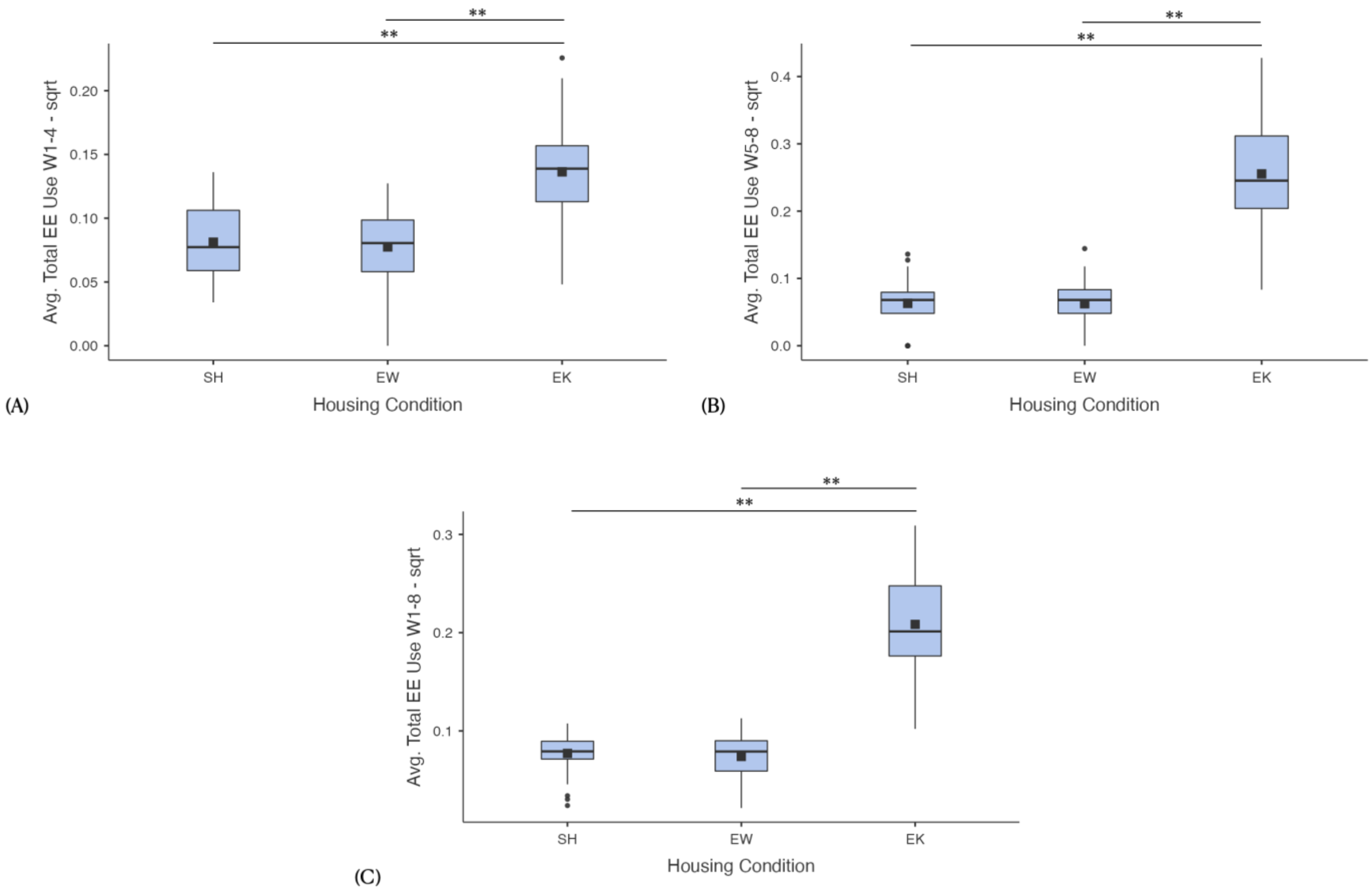

Figure 3.3 Square root transformed average proportions of observations where enrichments were in use across **(A)** Week 1-4 of observation, **(B)** Week 5-8 of observation, and **(C)** Week 1-8 of observation (all weeks). Black squares show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by jamovi software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. n = 31, 29, and 32 sample points, respectively. Bars indicate a significant difference with ** indicating p<0.001.

3.4.2 Enrichment Use Over Time

EK Enrichment Use Over Time

Average use of hanging EEs significantly differed by week of observation ($F_{7,262} = 15.9$, $p < 0.001$; see Figure 3.4A). Generally, hanging EE use was higher in the Week 5-8 period than in the Week 1-4 period ($t_{33} = -3.553$, $d = -0.609$, $p = 0.001$; see Table 3.2). In addition to differences between Week 1-4 and Week 5-8 collectively, hanging EEs were used more in Week 5 than in Week 6 ($t_{256} = 3.435$, $p = 0.019$), Week 7 ($t_{256} = 8.238$, $p < 0.001$), and Week 8 ($t_{256} = 7.917$, $p < 0.001$), respectively. Use of hanging EEs was also significantly higher in Week 6 than in Week 7 ($t_{256} = 4.803$, $p < 0.001$) and Week 8 ($t_{256} = 4.482$, $p < 0.001$; see Table 3.2 or Table A1.1).

Average ring use in EK pens also differed by week of observation ($F_{7,260} = 3.61$, $p < 0.001$; see Figure 3.4B). However, the only differences occurred between Week 1 and Week 2 towards more use in Week 2 ($t_{257} = -3.375$, $p = 0.024$), and between Week 1 and Week 3 towards more use in Week 3 ($t_{257} = -3.403$, $p = 0.022$; see Table 3.2). There were trending differences in ring use between Week 2 and Week 7 towards more use in Week 2 ($t_{264} = 3.092$, $p = 0.062$) and between Week 3 and Week 7 towards more use in Week 3 ($t_{264} = 3.122$, $p = 0.056$; see Table 3.2 or Table A1.2). There was no difference in the EK group's ring use between Week 1-4 and Week 5-8 collectively ($t_{33.0} = 0.869$, $d = 0.149$, $p = 0.391$).

Use of additional mobile EEs in EK pens (i.e., excluding ring use) also varied by week of observation ($F_{7,296} = 6.45$, $p < 0.001$; see Figure 3.4C). However, aside from higher use in Week 5-8 collectively compared to Week 1-4 collectively ($t_{33.0} = -6.690$, $d = -1.147$, $p <$

0.001; see Table 3.2), average use of additional mobile EEs did not vary significantly within these periods (see Table A1.3 for individual week comparisons). Similarly, with ring use included, mobile EE use in EK pens varied significantly by week of observation ($F_{7,296} = 3.42$, $p = 0.002$; Figure 3.4D), but these differences occurred primarily between Week 1-4 and Week 5-8 towards higher use in Week 5-8 ($t_{33.0} = -6.256$, $d = -1.073$, $p < 0.001$; Table 3.2). There were no significant differences in average mobile EE use within Week 1-4 and Week 5-8 (see Table A1.4 for individual week comparisons).

Total average EE use in EK pens, which includes all hanging and mobile enrichments, also differed by week of observation ($F_{7,261} = 6.45$, $p < 0.001$) but similarly only varied between weeks in the Week 1-4 period compared to the Week 5-8 period and not within these periods (Figure 3.4E; see Table A1.5 for individual week comparisons). EEs were generally in use more in Week 5-8 collectively than in Week 1-4 collectively ($t_{33.0} = -7.102$, $d = -1.218$, $p < 0.001$; see Table 3.2).

Standard-Housed Enrichment Use Over Time

There was no effect of treatment (consisting of SH and EW; $F_{1,84.4} = 1.219$, $p = 0.273$) or interaction effect between treatment and week of observation ($F_{7,561.1} = 0.667$, $p = 0.701$) on average EE use in standard-housed pens, i.e., use of the standard ring enrichment (Figure 3.5A, B). However, there was a significant effect of week of observation on EE use in these treatments collectively ($F_{7,561.1} = 10.480$, $p < 0.001$; Figure 3.5B): average ring use was higher in Week 2, Week 3, and Week 4 compared to Week 1 ($t_{552} = -5.234$, $p < 0.001$, $t_{552} = -6.289$, $p < 0.001$, and $t_{552} = -4.439$, $p < 0.001$, respectively; see Table 3.3). There were no further differences in ring use within the Week 1-4 and Week 5-8 periods (see Table A1.6 for remaining comparisons), though there were significant decreases in ring use in select weeks in the Week 5-8 period compared to the Week 1-4 period; this is represented as lower collective average ring use in Week 5-8 compared to Week 1-4 for both EW pens ($t_{35.0} = 2.46$, $p = 0.019$) and SH pens ($t_{33.0} = 2.07$, $p = 0.046$; see Table 3.3).

3.4.3 EK Enrichment Use by Object Type

Enrichment Use Between Mobile and Hanging Items

EE use varied significantly by category (hanging or mobile; $x^2 = 42.9$, $e^2 = 0.622$, $p < 0.001$; Figure 3.6). Mobile enrichments (0.037±0.019) were observed in use more often on average than hanging enrichments (0.009±0.006).

Enrichment Use Within Mobile and Hanging Items

EE use also varied by sub-type of mobile enrichment ($x^2_4 = 99.5$, $e^2 = 0.510$, $p < 0.001$; Figure 3.7A, B) and hanging enrichment ($x^2 = 15.1$, $e^2 = 0.199$, $p < 0.001$; Figure 3.8). The ring (0.006±0.004) and the wiffle ball (0.006±0.006) were used significantly more on average than the golf ball (0.0.002±0.003; $W = -6.95$, $p < 0.001$ and $W = 4.33$, $p = 0.012$, respectively) and the hockey ball (0.002±0.005; $W = -7.35$, $p < 0.001$ and $W = -5.35$, $p < 0.001$, respectively). Use of the ring and wiffle ball did not differ ($W = -1.82$, $p = 0.699$), nor did use of the golf ball and hockey ball ($W = -1.81$, $p = 0.703$). The pig's ear (0.048±0.033) was used more often on average than all other mobile EEs (compared to ring: $W = 9.29$, $p < 0.001$; compared to golf ball: $W = 9.43$, $p < 0.001$; compared to wiffle ball; $W = 8.84$, $p < 0.001$; and compared to hockey ball: $W = 10.21$, $p < 0.001$; Figure 3.7A). The rope was used more on average than the chain (0.014±0.012 and 0.005±0.004, respectively; $W = 5.50$, $p < 0.001$; Figure 3.8).

Table 3.2 Back-transformed EK enrichment use (mean with 95% CI in parentheses) by item type and week of observation.

<table>
<tr><th>EE use by week</th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th></tr>
<tr><td>Hanging EE</td><td>0.001</td><td>0.004</td><td>0.003</td><td>0.002</td><td>0.020</td><td>0.009</td><td>0.001</td><td>0.001</td></tr>
<tr><td></td><td>(0.000, 0.002)</td><td>(0.002, 0.007)</td><td>(0.002, 0.006)</td><td>(0.001, 0.004)</td><td>(0.013, 0.028)</td><td>(0.005, 0.015)</td><td>(0.000, 0.002)</td><td>(0.000, 0.003)</td></tr>
<tr><td></td><td colspan="4">0.004</td><td colspan="4">0.011</td></tr>
<tr><td></td><td colspan="4">(0.003, 0.006)</td><td colspan="4">(0.008, 0.014)</td></tr>
<tr><td>Ring EE</td><td>0.001</td><td>0.006</td><td>0.006</td><td>0.004</td><td>0.002</td><td>0.002</td><td>0.001</td><td>0.004</td></tr>
<tr><td></td><td>(0.000, 0.002)</td><td>(0.003, 0.010)</td><td>(0.003, 0.009)</td><td>(0.002, 0.007)</td><td>(0.001, 0.005)</td><td>(0.001, 0.004)</td><td>(0.000, 0.003)</td><td>(0.002, 0.007)</td></tr>
<tr><td></td><td colspan="4">0.006</td><td colspan="4">0.005</td></tr>
<tr><td></td><td colspan="4">(0.005, 0.008)</td><td colspan="4">(0.004, 0.007)</td></tr>
<tr><td>Mobile EE without ring</td><td>0.000</td><td>0.002</td><td>0.005</td><td>0.001</td><td>0.008</td><td>0.009</td><td>0.015</td><td>0.013</td></tr>
<tr><td></td><td>(0.000, 0.001)</td><td>(0.001, 0.003)</td><td>(0.002, 0.008)</td><td>(0.000, 0.001)</td><td>(0.003, 0.014)</td><td>(0.004, 0.018)</td><td>(0.007, 0.027)</td><td>(0.005, 0.024)</td></tr>
<tr><td></td><td colspan="4">0.003</td><td colspan="4">0.022</td></tr>
<tr><td></td><td colspan="4">(0.002, 0.004)</td><td colspan="4">(0.016, 0.029)</td></tr>
<tr><td>Mobile EE with ring</td><td>0.004</td><td>0.009</td><td>0.021</td><td>0.006</td><td>0.013</td><td>0.015</td><td>0.020</td><td>0.023</td></tr>
<tr><td></td><td>(0.002, 0.008)</td><td>(0.005, 0.014)</td><td>(0.008, 0.019)</td><td>(0.004, 0.009)</td><td>(0.007, 0.022)</td><td>(0.008, 0.025)</td><td>(0.010, 0.033)</td><td>(0.013, 0.036)</td></tr>
<tr><td></td><td colspan="4">0.010</td><td colspan="4">0.030</td></tr>
<tr><td></td><td colspan="4">(0.008, 0.013)</td><td colspan="4">(0.024, 0.036)</td></tr>
<tr><td>Total (all EEs)</td><td>0.009</td><td>0.026</td><td>0.032</td><td>0.017</td><td>0.063</td><td>0.048</td><td>0.047</td><td>0.052</td></tr>
<tr><td></td><td>(0.004, 0.015)</td><td>(0.016, 0.037)</td><td>(0.022, 0.044)</td><td>(0.011, 0.024)</td><td>(0.046, 0.083)</td><td>(0.030, 0.072)</td><td>(0.026, 0.073)</td><td>(0.031, 0.080)</td></tr>
<tr><td></td><td colspan="4">0.026</td><td colspan="4">0.073</td></tr>
<tr><td></td><td colspan="4">(0.021, 0.031)</td><td colspan="4">(0.061, 0.086)</td></tr>
</table>

Table 3.3 Back-transformed standard-housed enrichment use (mean with 95% CI in parentheses) by week of observation.

EE use by week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Ring EE	0.001 (0.000, 0.001)	0.005 (0.003, 0.007)	0.007 (0.005, 0.009)	0.004 (0.003, 0.004)	0.003 (0.001, 0.004)	0.002 (0.001, 0.003)	0.002 (0.001, 0.004)	0.001 (0.000, 0.002)
Ring EE, SH	0.007 (0.005, 0.008)				0.004 (0.003, 0.006)			
Ring EE, EW	0.006 (0.005, 0.007)				0.003 (0.002, 0.005)			

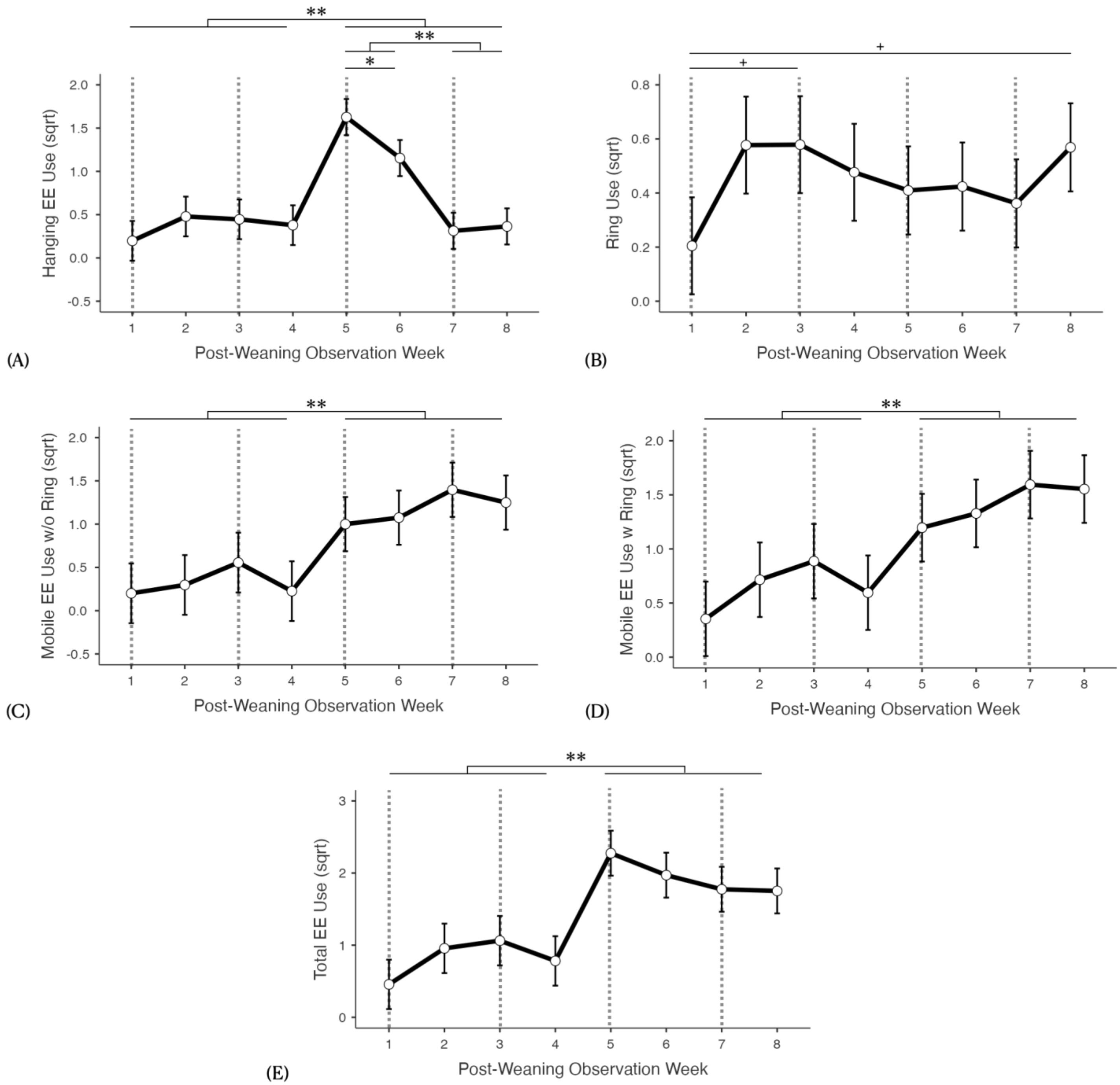

Figure 3.4 Square root transformed average proportions of observations where the following enrichments were in use in EK pens across weeks of post-weaning observations: **(A)** hanging EE, **(B)** ring EE, **(C)** mobile EEs without the ring, **(D)** mobile EEs with the ring, and **(E)** all EEs. Dashed lines indicate the exchanging of enrichments according to the schedule in Figure 3.1. Bars around means represent 95% CIs. By week, **n** = 34, 34, 34, 34, 42, 42, 42, and 42 sample points, respectively. Bars indicate a significant difference with ** indicating $p<0.001$, * indicating $p<0.05$, and + indicating $0.05<p<0.10$. Differences indicated within W1-W4 and W5-W8 represent results from linear mixed model analyses, while differences indicated between W1-W4 and W5-W8 collectively represent results from paired Student's t-tests.

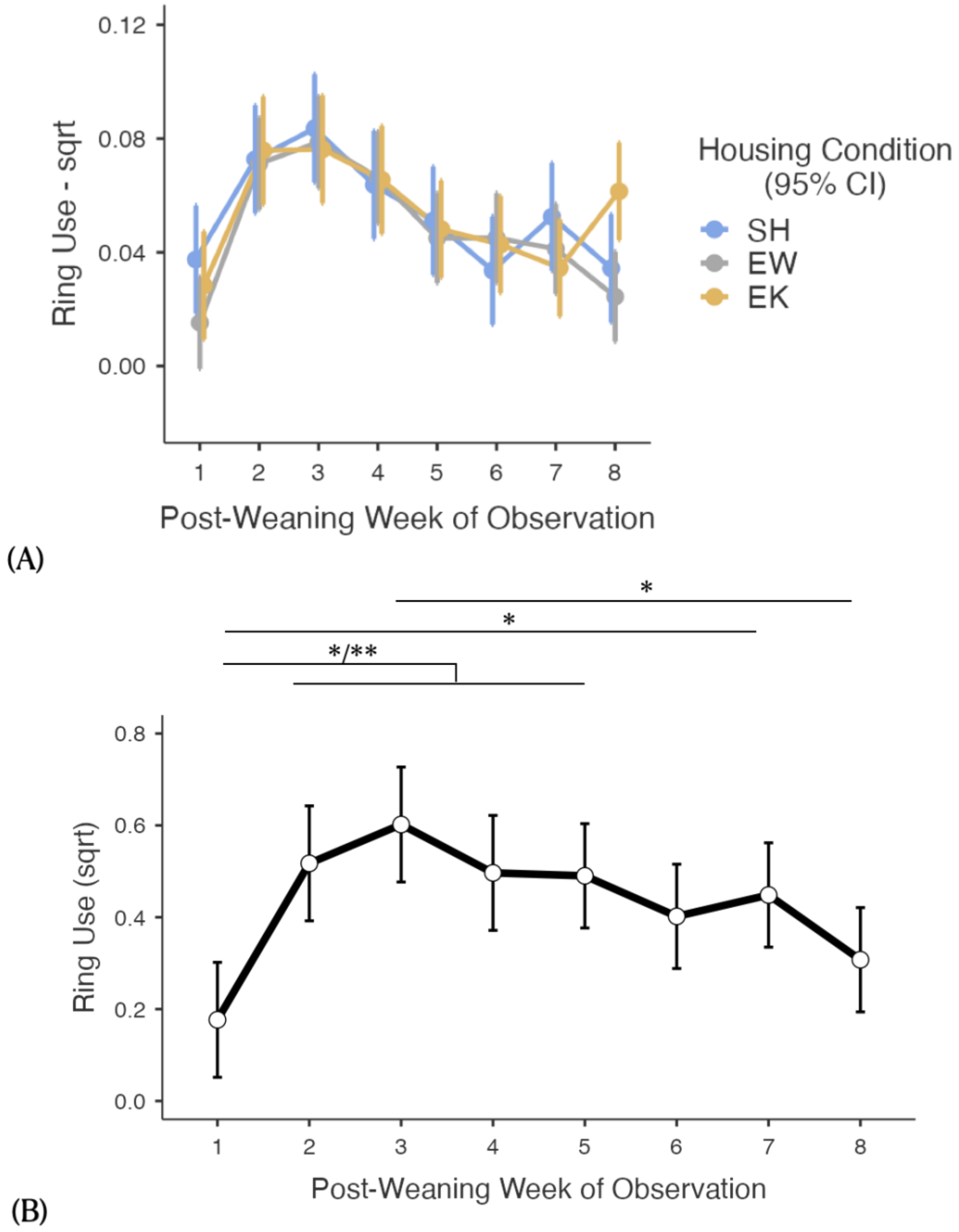

Figure 3.5 Square root transformed average proportions of observations where the ring EE was in use across weeks of post-weaning observations for **(A)** each treatment separately and **(B)** SH and EW (standard-housed treatments) combined. Bars around means represent 95% CIs. By week, data points consist of an average across **n** = 34, 34, 34, 34, 34, 34, 34, and 34 pens for SH, **n** = 47, 47, 47, 47, 49, 49, 49, and 49 pens for EW, and **n** = 34, 34, 34, 34, 42, 42, 42, and 42 pens for EK, respectively. Bars indicate a significant difference with * indicating $p<0.05$ and ** indicating $p<0.001$. Differences indicated within W1-W4 and W5-W8 represent results from linear mixed model analyses, while differences indicated between W1-W4 and W5-W8 collectively represent results from paired Student's t-tests.

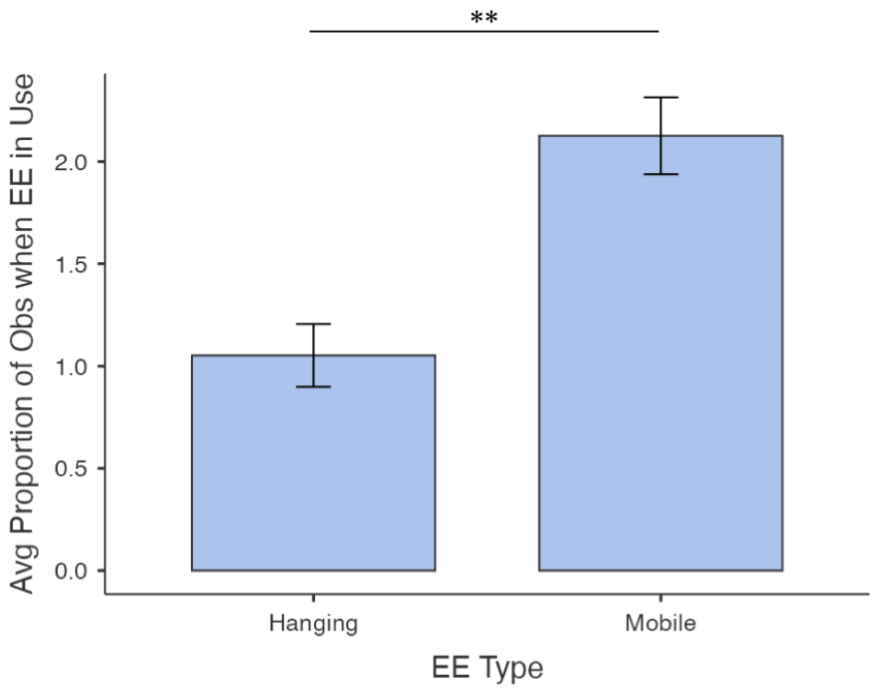

Figure 3.6 Average proportions of observations where hanging or mobile enrichments were in use (calculated per pen across all weeks). Data is for EK kits only (other conditions had only standard ring enrichment). Black squares show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by jamovi software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. n = 35 and 35 sample points, respectively. Bars indicate a significant difference with ** indicating $p<0.001$.

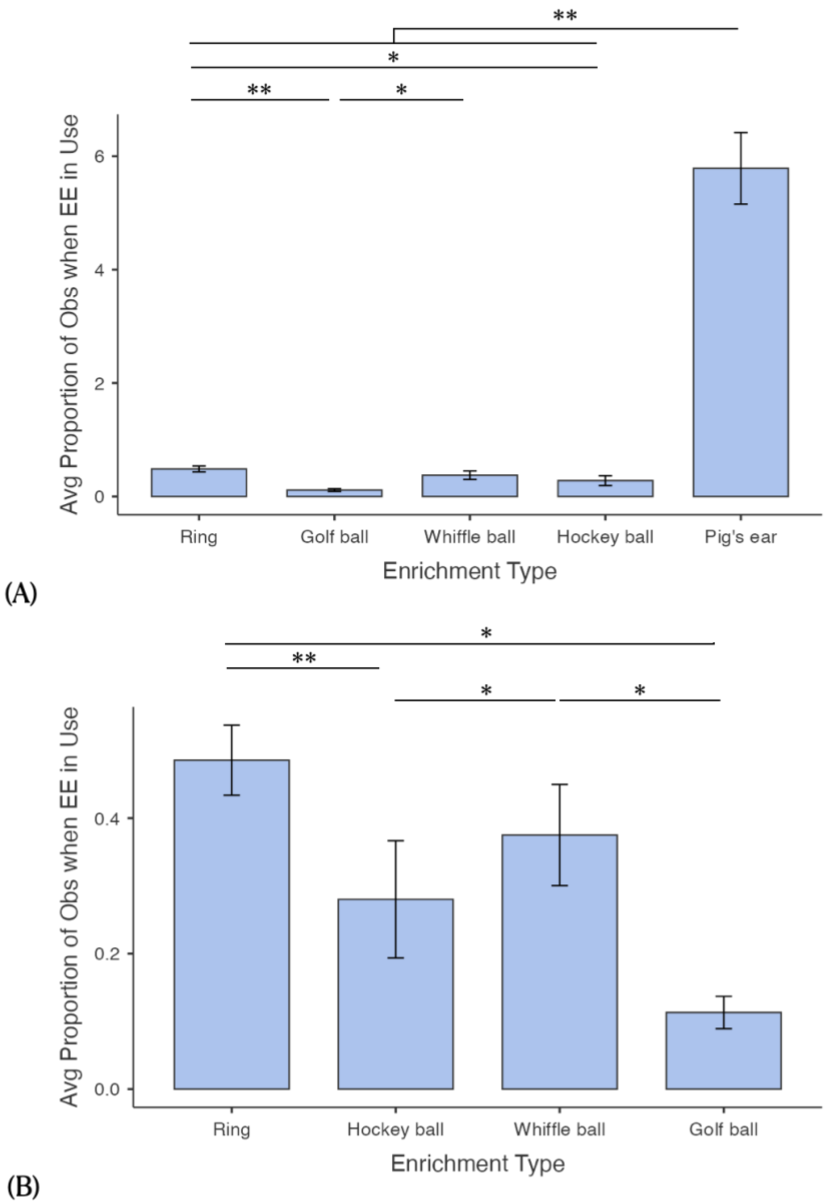

Figure 3.7 Average proportions of observations where enrichments were in use according to **(A)** enrichment type, including all mobile enrichments (Ring = standard ring enrichment, GB = golf ball, WB = wiffle ball, HB = hockey ball, and PE = pig's ear) and **(B)** mobile enrichment type excluding the pig's ear. Data is for EK kits only (other conditions had only standard ring enrichment). Error bars represent standard error. n = 42, 35, 35, 42, and 42 sample points, respectively. Bars indicate a significant difference with * indicating $p<0.05$ and ** indicating $p<0.001$.

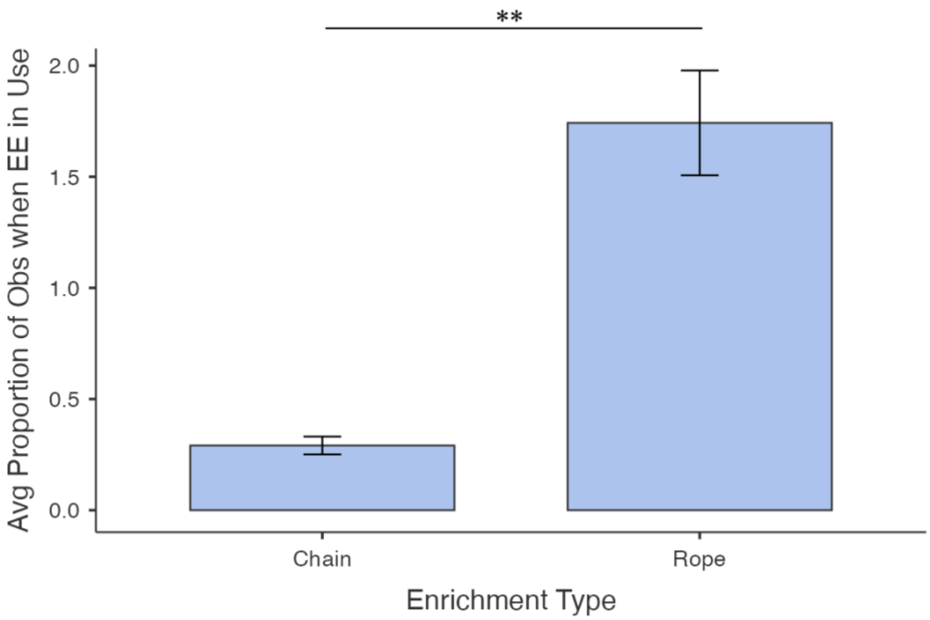

Figure 3.8 Average proportions of observations where enrichments were in use (calculated per pen across all weeks) according to hanging enrichment type. Data is for EK kits only (other conditions had only standard ring enrichment). Error bars represent standard error. n = 35 and 42 sample points, respectively. Bars indicate a significant difference with ** indicating p<0.001.

3.5 Discussion

Overall, the findings of this study support our hypothesis that kits in enriched housing (i.e., with access to multiple EEs, both novel and familiar) would demonstrate greater enrichment use than kits in standard housing (i.e., with access to only one familiar EE). There was significantly more enrichment use observed in the EK group compared to the SH and EW groups across the entire post-weaning period; therefore, the enrichment provision strategy applied in this study was successful in promoting greater interaction with enrichments. Based on these results, it may be advisable that at minimum, more than one enrichment be provided in mink pens to increase their use. This concept is supported by previous studies in rodents. Although EE for rodents is typically comprised of more than one item per cage due to the antagonistic interactions that can occur when only one enrichment is provided for group-housed rodents (i.e., fighting for resources/EE devices; McQuaid et al., 2012, 2013, 2018, Van Loo et al., 2002, Weber et al., 2017), it was previously unclear whether access to multiple of the same enrichment is more beneficial than access to multiple different items. In a study where cages were either supplied several of the same EE or a variety of EEs, greater enrichment use in addition to more pronounced effects on welfare measures were observed in cages with a diverse selection of enrichment objects (Abou-Ismail, 2011). This finding may be especially relevant if the enrichments provided have different properties and can be interacted with in different ways, as was the case in the present study. It was proposed by Zhang et al. (2022) in a study with dairy calves that provision of multiple items that can be interacted with in different ways (e.g., stationary brushes, plastic chains, dry teats, and hay nets) may satisfy different intrinsic behavioural motivations of calves (e.g., grooming, suckling, and feed intake), thus increasing overall item interaction in a cumulative way rather than specifically motivated interaction with one type of object.

As such, the simultaneous provision of both hanging and mobile enrichments may have contributed to item interaction in this study since these categories of items serve different uses. We did not expect these categories to be used equally, but contrary to our predictions, mobile enrichments were used significantly more than hanging enrichments. This may be because mobile enrichments were exchanged more frequently than hanging enrichments (every two weeks as opposed to every four weeks), and kits' habituation to each may have differed accordingly. An alternate explanation may be that there were a greater number of mobile EEs available in the cage to interact with; since there were two mobile enrichments present in enriched cages, more than one kit could easily play with one at a given time, whereas there was only one hanging enrichment provided at a time. However, multiple kits were sometimes observed interacting with the rope or chain (e.g., climbing, tugging, or chewing) at once, since the items were large enough to allow this. This explanation would also only apply to the group-housing phase, since there were more enrichments present in the pair cages than there were kits, yet the preference for mobile enrichments persisted. Alternatively, it is possible that the hanging enrichments provided did not share the same functional advantage as enrichments categorized this way in previous studies. The hanging items in such studies (e.g., shelves and tunnels) were used extensively due to their functionality as additional resting places, or opportunities to reach a higher vantage point and/or be alone in the cage, whereas mobile enrichments such as play balls and loose tubes were used to a lesser extent and were primarily chased or chewed (Hansen et al., 2007; Meagher et al., 2017; Meagher & Mason, 2012; Polanco et al., 2021). The hanging lengths of rope or chain used in this study more closely resemble the latter category of enrichments based on their use for climbing and/or chewing, but they are not mobile to the same extent as loose items on the cage floor. These results demonstrate that it may be appropriate in future studies to further subdivide the category of ‘fixed’ or ‘hanging’ enrichments into fully fixed structural enrichments, which provide an additional resting space, versus fixed but manipulable objects which can only be climbed, chewed, etc., when investigating the effects of enrichment properties on their use.

Within mobile enrichments, the pig’s ear was used most often, followed by the standard ring enrichment and the wiffle ball. The hockey ball and golf ball received the lowest amounts of use and were interacted with at similar levels despite being provided in different post-weaning periods. Although specific methods of enrichment interaction were not recorded, anecdotally, we observed that the ring was used in as many as four different ways by kits; these include being rolled, rested in (with the ring in either a vertical or horizontal position; rings were large enough to prevent mink from getting stuck), chewed, or climbed on. In contrast, the golf ball and hockey ball were only observed being rolled and/or climbed. The wiffle ball similarly had more possibilities for interaction compared to other play balls, but fewer uses than the ring; in addition to being rolled and/or climbed, the wiffle balls were often seen being dragged or carried in kits' teeth due to their light weight and the holes perforating their surface. Both the golf ball and hockey ball were heavier than the wiffle ball and did not have surfaces that facilitated being carried in this way. A similar effect was observed within hanging enrichments: the rope was observed in use more often than the chain, potentially because the rope could be climbed, chewed, unraveled, and torn down whereas the chain could only be climbed and chewed.

The relative popularity of the standard ring enrichment is interesting because it was present in the cage the most consistently and for the longest duration of time in this study, yet its use in EK pens remained relatively consistent across all weeks of observation. This suggests that despite not being regularly exchanged, greater opportunities for object novelty may have been offered by the ring due to its more numerous uses (Axelsson et al., 2009) than by novel objects with fewer uses. This finding is similar to that of a previous study regarding enrichment novelty versus enrichment complexity in rats (Abou-Ismail & Mendl, 2016). The authors found that novelty, in the sense that multiple replicates of the same object were exchanged for multiple of another object weekly, was less influential than the characteristics of the objects available at any given time, even if one or more of these objects remained accessible throughout the entire study period (Abou-Ismail & Mendl, 2016).

These authors also proposed that increased use of EE in the condition with more diverse, permanently present objects may be due to the lack of control and predictability imposed by frequent exchange of enrichments in the cage environment, which can adversely affect welfare (Abou-Ismail & Mendl, 2016). It is important to consider that, firstly, rotating of enrichments could be considered enrichment removal in the sense that the animal loses access to an item they have come to find rewarding, even though another enrichment arrives in its place. Such removal may be perceived as a negative event or environmental challenge over which the animal has no control, similar to other routine changes to the animal's environment such as cage cleaning that evoke physiological and behavioural responses of stress (Morgan & Tromborg, 2006). There is evidence that uncontrollable events made predictable by signaling with a cue or temporal consistency are less aversive than uncontrollable, unpredictable events, suggesting that predictability allows a perception of control over the event and thus enhances the animals ability to cope (Bassett & Buchanan-Smith, 2007). Complete enrichment removal evokes a depression-like phenotype in captive animals, including helplessness behaviour (Smith et al., 2017), which has been linked to uncontrollability and/or unpredictability of events that directly or indirectly impact the animal (Burger & Arkin, 1980; Morgan & Tromborg, 2006). Meanwhile, events or stimuli perceived as positive (such as food presentation and other food-related rewards) appear to have enhanced welfare benefits when somewhat unpredictable, potentially reflecting the probabilistic nature of food acquisition in the wild (de Oliveira & Vasconcellos, 2022). As far as we are aware, little research has been conducted regarding whether control or predictability of enrichment access or removal has benefits for item use and measures of welfare. While the schedule of EE exchange used in this study may have offered some predictability over a longer period, it is unlikely that four bi-weekly enrichment rotations were sufficient for mink to learn this schedule, especially as there were no obvious cues or signals associated with the exchange; though, some uncertainty may have arisen about the constancy of these enrichments as the study progressed. When objects are permanently present, as the ring was in this study, Abou-Ismail and Mendl (2016) suggest that opportunities for the animal to exert control over their environment may be increased, thus improving their welfare and positively impacting other factors associated with enrichment use (Abou-Ismail & Mendl, 2016). In fact, it has been proposed that controllability may be a more important aspect of EE than the complexity of the enrichment objects (Sambrook & Buchanan-Smith, 1997). Either of these hypotheses (i.e., number of item uses or degree of controllability) are plausible regarding kits' use of the ring enrichment.

However, these hypotheses do not explain why the pig's ear was used significantly more than all other mobile enrichments. The pig's ear could be chewed but was not able to roll, and therefore be chased, as effectively as the ring or play ball EEs. In this sense, it had a limited number of uses compared to the ring and wiffle ball enrichments. Moreover, it was replaced/exchanged more frequently than all other mobile items. Replacements were made once per week within the two-week period of access to the pig's ear if a pen had depleted theirs entirely, which may have contributed an additional source of novelty, since although the enrichment's properties would not have been novel to the kits there would have been a brief period of absence before its re-introduction. The same applies to the rope, as it was replaced on a weekly basis if a pen had torn theirs down from its fixture. As such, the additional novelty component of the pig's ear compared to other mobile enrichments, and of the rope compared to the chain, may have promoted their greater use. However, it is also possible that the pig's ear may have satisfied an appetitive or food-related motivation for the kits, as their gradual disappearance from the cage infers that some or most of the enrichment may have been ingested. It is well-established that diets varying in textures or tastes, introduction of novel edible enrichments, or stimulation via foraging tasks can be a source of enrichment for captive carnivores (Bashaw et al., 2003; Riggio et al., 2019; Ruskell et al., 2015; Watters et al., 2011). If the pig's ear can be considered feed enrichment, interactions with the item may have been reinforced by its incentive value as food. Studies have also found that species-specific edible enrichments were used more than nonedible enrichments by captive animals that forage for their food rather than hunt (Elmore et al., 2012; Roy et al., 2019; Zhang et al., 2022).

The previously mentioned concept of controllability may also apply to the observed differences in pig's ear and rope use compared to other enrichments: there are suggested to be different 'grades' of enrichment controllability according to the sophistication of their cause-effect relationships, or ways in which they can be interacted with, even in contexts where all enrichments are novel to the cage environment (Sambrook & Buchanan-Smith, 1997). According to this concept, fixed objects (defined as the animal being able to move only in relation to the object) are claimed to have less controllability than moveable objects (defined as the animal and object moving with respect to each other and the enclosure), which in turn both have less controllability than malleable objects (defined as an action being applied to a point on an object resulting in an effect or change to that same point on the object; Sambrook and Buchanan-Smith, 1997). By this logic, the malleable enrichments provided in this study (i.e., the pig's ear and rope, which were both destructible) may have been appealing to kits in offering a greater ability to exert control or physically manipulate the environment. Destructible or deformable enrichments have similarly been found to be preferred in other species (in chimpanzees: Videan et al., 2005; in pigs: Van de Weerd et al., 2003; Perre et al., 2011; Courboulay, 2014). Like pig's ears, other destructible animal products (e.g., hide strips) have been shown to be very attractive to mink in pilot studies (e.g., informal pilot data from Meagher et al., 2014), thus this nature of enrichment may have potential if robust and inexpensive versions are identified. Cost and durability are important considerations for edible or easily destructible enrichments since informal observations indicated that some pens in the present study depleted their pig's ears and/or hanging ropes in as little as two days, although other pens maintained the same pig's ear and rope for 14+ days without markedly depleting them.

Regarding trends in enrichment use over time, it can be qualitatively observed that average use of available enrichments was higher in each week of observation in EK pens compared to standard-housed pens, and there was more evidence of habituation to enrichment in standard-housed pens compared to EK pens: ring use was lower in Week 5-8 collectively compared to Week 1-4 collectively in standard-housed pens, while ring use in EK did not differ significantly across these periods (in fact, use of other enrichment categories was increased compared to prior weeks). This aligns with our hypothesis that enrichment use would be maintained at a higher level over time in the EK condition. The twelve-week period of access to the ring in this study may therefore have been sufficient to observe habituation in housing conditions where it was the only object available in the cage, which reflects previously demonstrated habituation to permanently present mobile items within one month of introduction (e.g., Jeppesen and Falkenberg, 1990). However, despite less evidence of habituation in EK pens, average weekly use of the ring enrichment occurred in similar amounts between standard- and enriched-housed kits and followed similar trends when looking at individual weeks in the early and late observation period (e.g., significant or trending differences towards lower use in later weeks compared to earlier weeks). Notably, ring use in standard and enriched housing followed divergent trends in Week 8 of observation where an unexplained increase in ring use occurred in EK pens (though non-significant), which is likely why EK ring use did not differ between Week 1-4 and Week 5-8 collectively. It is possible that with a prolonged observation period, habituation to the ring may have become evident in the EK condition as well.

Use of other EK enrichments was more variable over time. Most of the significant variation in enrichment use for EK pens occurred according to the properties of the enrichments provided in different periods; EE use in Week 5-8 was generally greater than use in Week 1-4, which is likely due to the provision of the rope and pig's ear in the latter period, though it should be noted that housing changes occurred during this phase as well (i.e., split into pair-housing) and this may have had some effect. Within Week 1-4 and 5-8, there were slight, but non-significant, increases in mobile EE use in weeks where mobile EEs were exchanged compared to the following and preceding weeks. This may demonstrate a positive effect of object novelty on EE use, but this novelty effect appeared to diminish rather quickly depending on the object(s) introduced. In some cases, EE use declined within one week of novel object introduction; for instance, average use of hanging EEs declined significantly between the first and second week of access to the hanging rope enrichment. Interestingly, the same was not demonstrated for mobile EEs in the same week: when wiffle balls or golf balls were exchanged for hockey balls or pig's ears, mobile EE use remained elevated in the week following the exchange.

These trends in EE use around times of object exchange may reflect an initial increase in object exploration due to novelty, followed by a return to interaction with familiar objects if the properties of the novel object are not sufficiently appealing to kits. A similar effect was seen in a recent study where rat cages were modified either by the addition of new objects, an increase in size to existing objects, or added complexity to existing objects (Pisula et al., 2021). In both the object size modification and object complexity modification conditions, there was more initial exploration of the changed object followed by a shift to exploration of unchanged objects. The authors theorized that if an environmental modification is not sufficiently complex, all necessary information about the object will be incorporated rather quickly without satisfying the animal's need for sensory and informational stimulation, resulting in lingering arousal and redirection of exploratory behaviour to other aspects of the environment (Pisula et al., 2021). This may demonstrate that certain objects provided in this study (namely, the pig's ear) offered sufficient complexity to activate more advanced, long-term exploration and prevent a redirection of exploration to familiar objects. This may also explain the slightly increased use of the ring enrichment in EK pens in Week 8 and its correspondence to somewhat decreased use of other EEs, which may reflect a return to interaction with familiar objects when novel objects have been sufficiently explored. That said, it can be concluded that maintenance of object novelty was effective at maintaining kits' interest in the items present since EE use generally showed little fluctuation *within* Week 1-4 and 5-8 in EK pens, even if this attention was not always directed towards novel objects.

We also aimed to identify a time at which enrichment provision becomes useful in kits, or whether there are changes in enrichment use as kits age. The increase in use of enrichments from Week 5-8 (age 10-13 weeks) relative to Week 1-4 may reflect changes in play style preferences as mink age; social play in mink is known to emerge around five weeks of age and increase across subsequent weeks (Brink & Jeppesen, 2005; Jonasen, 1987), while object play, similar to enrichment use as defined here, emerges later. This may explain a transition to greater object play later in development. However, a previous study regarding ontogeny of play in mink found that all forms of play, including object play, peaked between eight-ten weeks of age and declined thereafter (Vinke & van Leeuwen, 2005). Although trends in other forms of play (e.g., social, locomotor) were not evaluated in this study, a decline in object play after eight to ten weeks was not observed. Rather, overall use of enrichments in EK was increased at ten weeks of age compared to prior weeks and remained consistent until thirteen weeks of age. This aligns more with the finding of Dallaire and Mason (2016) that object play did not decrease to the same extent as social play between ten-eleven weeks and sixteen-twenty weeks.
However, any potential age effects on EE use in this study are difficult to disentangle from effects of enrichment properties on EE use. For example, it could be speculated that

there was an age effect in EK kits regarding the pig's ears' relative popularity compared to other mobile enrichments, since it was provided in either Week 5-6 or Week 7-8 of the post-weaning period (when kits were ten-eleven or twelve-thirteen weeks old), and there was significantly more enrichment use seen during these times compared to the Week 1-2 and Week 3-4 observation period (when kits were six-seven or eight-nine weeks old). However, the hockey ball was also an enrichment provided in either Week 5-6 or Week 7-8, and use of the hockey ball was not found to be significantly different than that of the golf ball, while the wiffle ball was used significantly more than the hockey ball; these were both enrichments provided in earlier weeks. Similarly, levels of hanging enrichment use were roughly equivalent between Week 1, 2, 3, and 4 and Week 7 and 8, which may reflect a return to 'normal' levels of use following habituation to the hanging rope introduced in Week 5. With that said, it cannot be definitively concluded from these results whether EE use was affected by kit age; future studies should provide enrichments with similar properties (and therefore similar appeal to kits) according to a similar schedule of enrichment exchange across all post-whelping weeks.

The standard-housed groups may serve as a better representation of potential age effects in enrichment use, as there was no renewal of object novelty or provision of enrichments with varying properties across time periods. Ring use in the standard-housed groups was significantly low in post-weaning Week 1 compared to Week 2, 3, and 4, and then began to decline across subsequent weeks. This reduced EE use in post-weaning Week 1 is interesting because the same trend was demonstrated for all EEs, regardless of housing condition. It is possible that object play was not yet fully developed in kits by this age since it was not observed until seven weeks of age in a previous study (Jonasen, 1987), though enrichments were presently used by kits to some extent at six weeks of age. Alternatively, increased stress due to weaning may have influenced kits' use of enrichment items until post-weaning Week 2, at which point there may have been some recovery. Stress is generally known to decrease exploratory behaviour , though intervention with EE is also shown to mediate these stress effects and promote explorative behaviour, particularly in early life (Bak & Malmkvist, 2020; Mkwanazi et al., 2018). It is possible that EEs were able to mediate weaning stress to some extent but were not able to influence kit behaviour in the immediate post-weaning week. However, we collected no physiological stress measures from kits around the time of weaning, so this is speculative. Such benefits of EE in the immediate post-weaning period could also be validated by determining whether kits value access to enrichments at this time (e.g., by conducting consumer-demand studies which assess 'costs' paid to access enrichments; Cooper and Mason, 1997, 2000).

3.6 Conclusion

The results of this study demonstrate that enrichments with more numerous uses, greater malleability, or greater controllability were most effective at promoting EE interaction. Although the ring enrichment, which is standard to provide across all Canadian mink farms, was one of the most-used enrichments, the presence of multiple EEs in the cage was able to significantly increase overall enrichment use compared to provision of the ring alone. There also seemed to be a positive effect of object novelty on enrichment use in weeks where familiar EEs were exchanged for novel ones, however, these increases were not statistically significant, and there appeared to be rapid habituation to these items depending on their properties. More 'complex' novel enrichments may remain cognitively stimulating across a longer period and therefore warrant more exploration, prolonging the positive effects of novelty on EE use. It is unclear whether kit age influenced EE use in this study; further research is required in which the enrichments supplied are equivalently appealing to kits and follow a similar schedule of enrichment exchange across all post-whelping weeks. In such a study, it may also be beneficial to provide the same number of fully fixed structural enrichments, fixed but manipulable enrichments, and mobile enrichments to provide more information about kit preference for different enrichment types. Consumer-demand studies could also be used to determine whether kits value access to enrichments during certain stages of development or housing (e.g., following weaning). Measures of physiological stress should also be collected from kits around the time of weaning to investigate the relationship between EE use and weaning stress.

4. Enrichment of the Perinatal Environment in American Mink (*Neogale vison*) Confers More Benefits than Enrichment in the Juvenile Period*

4.1 Abstract

Farmed mink pens commonly include separate nesting areas to provide privacy and warmth in the perinatal period, and one manipulable enrichment is introduced for juveniles to prevent stereotypic behaviour (SB) development. Here, potential enhancements for each of these housing practices were implemented (enrichment in the perinatal/peri-whelping period [EW]: extra, high-quality perinatal nest building materials for dams; and enrichment once kits were mobile [EK]: kits provided with extra physical enrichment items from approximately postnatal month 1-4) and their relative effects on various measures of welfare were compared to that of standard-housed mink (SH). EW dams performed less SB, rested more, built higher-quality nests, and tended to have lower kit mortality than dams of other conditions. Offspring stress responsiveness was assessed by sampling faecal cortisol before and after a handling event, but this event appeared insufficient to induce a measurable stress response. Inactivity in the nest box was decreased in EK kits as juveniles relative to other groups, while social play was reduced and lying awake was increased compared to EW and SH juveniles, respectively. SB development was not prevented by EK interventions; in fact, EK kits may have developed more diverse SB sub-types than EW and SH kits. Overall, EW housing conferred more benefits than EK housing, which emphasizes the role of the perinatal environment in modulating kit development. EK enrichment may have been ineffective in improving kit welfare due to the timing of its removal; further research is required regarding critical periods of physical enrichment to improve welfare.

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Clark, G. B., Díez-León, M., & Meagher, R. K. (in prep). Enrichment of the perinatal environment in American mink (*Neogale vison*) confers more benefits than enrichment in the juvenile period.

Gabrielle B. Clark contributed to the planning of experimental methods and conducted data collection, statistical analysis, and the majority of writing for this co-authored manuscript.

4.2 Introduction

Farm animal welfare has been linked to aspects of housing such as feeding regimens, access to social partners (or separation from others, if a solitary species), and adequate space for movement or travel according to what the species has evolved to do in the wild (Clubb & Mason, 2007; Lewis et al., 2022; Mason et al., 2001; Nimon & Broom, 1999; Rushen, 1985). Likewise, aspects of housing and husbandry for American mink (*Neogale vison*), a species farmed for their fur, have been adapted to better allow the expression of natural behaviours, in turn improving animal health and productivity. The Code of Practice in Canada dictates that commercial mink must be provided access to a separate nesting area in the form of a nest box to allow for privacy and warmth (National Farm Animal Care Council, 2013); in the wild, mink will often make use of multiple underground dens scattered throughout their territory (Dunstone, 1993). Although nest boxes are generally provided year-round (see National Farm Animal Care Council, 2013 for exceptions regarding temporary blocking or removal), bedding material for nest building and insulation must additionally be provided during whelping, lactation, furring, and winter months. This is especially important in the peri-whelping period because mink are altricial and born without the ability to thermoregulate (developed at 29 days of age), therefore hypothermia is one of the most common causes of kit mortality in the early postnatal period (Martino & Villar, 1990). Provision of a nest box is shown to reduce kit mortality and increase kit growth rate compared to litters raised without a nest box (Møller, 1990). Nest box provision can also have stress-reducing effects for females prior to whelping and decrease their performance of stereotypic behaviour, i.e., invariant, repetitive, and apparently functionless patterns of motor behaviour (Hansen et al. 1994, Nimon & Broom 1999, Hansen & Jeppesen 2000). In turn, reduced physiological stress and stereotypic behaviour are known to correlate with improved kit-directed maternal care behaviour and nest construction (Malmkvist & Palme, 2008; Schou et al., 2018).

However, there is a need for research regarding ways in which bedding materials and/or general nest environments might be improved at whelping. Nest boxes and bedding are standard provisions in most mink-farming countries prior to whelping (e.g., China Fur Commission of the China Leather Industry Association, 2016; European Commission, 2001; National Farm Animal Care Council, 2013). European guidelines additionally recommend providing enough bedding material to build a closed nest in the box and to ensure the nest box is protected from draughts (Møller et al., 2015); the bedding materials provided in Europe may include hay, straw, flax, shredded straw/paper, wood shavings, wool, or other materials with insulating properties. In Canada, standard nest building materials include wood shavings, chopped straw, and hay, and current recommendations for farms specifically include packing nest box corners with bedding and providing a nest building material that facilitates a bowl shape to keep kits close together and improve nest temperature (National Farm Animal Care Council, 2013). However, these guidelines do not specify which materials are favourable for insulation or construction of an enclosed nest, and standard bedding materials (particularly those provided in Canada) may be insufficient to form a nest capable of maintaining optimal temperatures; kit mortality in farmed mink is considered similar to that of wild-living mink (estimated between 20 and 35% and 22 and 35%, respectively; European Commission, 2001), but could be improved. It is also possible that lack of adequate nesting material may constrain behavioural opportunities for females, thus affecting their stress levels, stereotypic behaviour, and maternal care behaviour (Malmkvist & Palme, 2008).

Limited access to straw for nest building has been shown to significantly reduce offspring weights, increase mortality of live-born kits, and increase maternal cortisol compared to groups with a pre-made plastic nest or plastic nest with straw (Malmkvist & Palme, 2008). Motivation to perform maternal care behaviours may also be improved by increased nest building opportunities, since dams with access to a plastic nest with straw were quicker to retrieve their kits in a kit retrieval test than those without these materials (Malmkvist & Palme, 2008). Prolonged access to standard nest building materials has also been shown to be beneficial: dams provided with nest building materials in January had greater reproductive success, measured by litter size and offspring survival, and reduced basal stress levels compared to dams who received materials in March (Schou et al., 2018). Moreover, Campbell et al. (2013) found that nests incorporated with wood shavings were better constructed than nests made of chopped straw; however, they did not include measures of dam welfare or long-term effects on kits when each of these bedding materials were used. From this study, it was also concluded that chopped straw may be an ideal nest bottom substrate if additional materials such as uncut straw or wood shavings are also provided to fortify the nest (Campbell et al., 2016; Sønderup et al., 2009). Thus, there is an opportunity for researchers to examine dams' welfare and reproductive success when multiple standard and high-quality substrates are provided, including the quality of nest construction facilitated by these materials.

There has also been little investigation regarding the effects of prenatal or early postnatal stress, maternal care, and nest quality on development of stress responsiveness in farmed mink. It has been established in other mammalian species that prenatal stress and maternal stress during lactation can directly impact offspring stress responsiveness and long-term health, namely via actions of maternal stress hormones on the developing fetus (reviewed in van Bodegom et al., 2017; Weinstock, 2017) or by passage of maternal stress hormones through maternal milk (Stead et al., 2022). This is in addition to behavioural effects of maternal stress on quality of maternal care and resulting implications for offspring stress responsivity. It has been documented in many species (including rodents, pigs, non-human primates, humans, and dogs; summarized in Lezama-García et al., 2019) that increased quality of nursing, licking, and grooming of offspring can improve stress resilience and mitigate anxiety- or depression-like phenotypes in the offspring as adults. For altricial dams (including mink) that also build nests leading up to parturition as a component of maternal care, nest building behaviours also play a role in promoting long-term kit health since nest temperature is known to modulate offspring stress response development (Jans et al., 1985; Jans & Woodside, 1990).

Another adaptation to the husbandry of farmed mink in the most recent Code of Practice is the requirement of providing one manipulable environmental enrichment (EE; defined in welfare science as changes or additions to animals' environments or husbandry that are biologically relevant to the species; Newberry 1995) in each pen to prevent or reduce stereotypic behaviour, physiological stress, self-harm behaviours like tail chewing, and negative affective states such as fear or boredom (e.g., Hansen & Jeppesen 2000, Hansen et al. 2007, Dallaire et al. 2011, Meagher & Mason 2012, Meagher et al. 2014). EE may also have applications for modulating mink temperament (Bak & Malmkvist, 2020;

Meagher et al., 2014). The EE provided is typically a mobile object within the pen that mink can chase and chew, which provides an outlet for innate behaviours (e.g., hunting) that are otherwise restricted in captivity and reduces underlying behavioural frustrations (Maple & Perdue, 2013). EE is often provided to target stereotypic behaviour performance by reducing stress or arousal arising from aversive environments, offering greater opportunity to exert control over the environment, and/or by occupying the animal's time with other behaviours (Mason et al., 2007). Stereotypic behaviour may alternatively arise from central nervous system dysfunction or neuroanatomical changes occurring after time spent in captivity, namely in reward-sensitive areas or areas responsible for inhibiting repetitive, habit-like behaviours, in which case EE can be used to protect against these changes (Díez-León et al., 2019; Tatemoto et al., 2022).

The ontogeny of stereotypic behaviours in captive animals remains poorly understood, but they are estimated to become fully developed by seven months of age in mink (Jeppesen et al., 2000). EE is relatively unsuccessful at reducing stereotypic behaviour if provided only after animals have reached maturity (Mason 1993, Ahola et al. 2011, Campbell et al. 2013), and animals can show novelty-induced fear responses or reduced motivation to gain access to enrichments when introduced to them as adults (Cooper et al., 1996; Fairhurst et al., 2011; Tilly et al., 2010). It is therefore recommended that EE for farmed mink be provided early in life. Although these practices have contributed to partial improvements in the occurrence of self-mutilation behaviours (e.g., tail chewing), stereotypic behaviour in the form of unvarying and apparently functionless motor patterns remains widespread (current prevalence is unknown but was estimated to occur in 35-85% of adult females based on a large-scale Netherlands study; reported by

European Commission, 2001). Moreover, there is no mandated age of EE introduction on commercial mink farms in Canada, and optimal durations and/or timing of EE provision have not yet been identified.

The objectives of the present study were twofold. Firstly, we aimed to determine if enrichment of the whelping environment could positively modulate dam behaviour, welfare, and reproductive success, and deliver long-term benefits to kit stress responsiveness. We provided one group of dams (enriched at whelping; EW) with more numerous, greater quality nest building materials and additional enrichment (hanging rope) in the perinatal/peri-whelping period, while other dams received only standard nest building materials and enrichment. It was hypothesized that dams provided with enriched nest building materials would demonstrate reduced basal faecal cortisol levels and reduced stereotypic behaviour compared to dams with standard nest building materials due to greater opportunities to express natural nest building behaviour and access to a physical, chewable enrichment. Consequently, kits in enriched whelping environments were hypothesized to receive a higher quality of maternal care than kits of non-enriched dams as an indirect result of decreased maternal stress and greater motivation to perform kit-directed maternal care behaviours. Improved nest construction in enriched whelping conditions was also predicted, and this factor in combination with potential increases in maternal care and reductions in prenatal stress were predicted to benefit kit survival and stress responsiveness later in life (assessed via faecal cortisol responses following a stressor and post-mortem spleen weights as an indicator of chronic stress effects; Díez-León et al. 2016).

Secondly, we investigated whether supplying multiple, more diverse enrichment items that allow for the expression of motivated behaviours like chasing, chewing, etc. for kits in the early juvenile phase is more effective than standard enrichment in preventing the development of stereotypic behaviour and promoting behaviours associated with good welfare. Kits in a second group (enriched kits; EK) were supplied with numerous physical enrichments of varying properties and degrees of novelty from approximately 1-4 months of age, beginning when they first became mobile. This intervention was hypothesized to positively impact behaviours related to poor welfare in kits (e.g., boredom, stress, fear, and aggression) and modulate stereotypic behaviour development later in life by providing greater behavioural opportunities to kits. It was demonstrated in Chapter 3 that the variety and novelty of EEs provided to kits in this condition were successful at increasing and sustaining kits' use of enrichments compared to kits housed in standard conditions; thus, the juvenile enrichment condition was predicted to deliver a greater impact on kit behaviour and stereotypic behaviour than standard juvenile conditions.

4.3 Methods

4.3.1 Subjects And Housing

242 female mink were bred to account for potential unsuccessful copulations and/or poor litter health (desired sample size of ≥30 litters in each treatment with ≥1 male-female pair in each litter, as per sample size calculations conducted prior to the study). Male and female mink selected at breeding for use in the study were balanced across Dark, Mahogany, Pastel, Demi, and Stardust colour types (strains). All dams were housed individually (American mink are solitary in the wild; Dunstone, 1993) indoors at the

Canadian Centre for Fur Animal Research (Nova Scotia, Canada) in 75 (L) × 60 (W) × 45 (H) or 40 (H) cm wire-mesh pens with wire shelf, external wooden nest boxes, and a single plastic ring enrichment prior to assignment of their respective conditions. Mink were fed with a meat-based paste (Sea Crest Fisheries Ltd., Saulnierville, NS) placed on the mesh roof of the pen; adults were fed once daily in the afternoon and kits were fed twice daily (morning and afternoon) beginning at weaning. All mink had access to drinking water *ad libitum* via automatic drinkers. The research was approved by the Dalhousie University Faculty of Agriculture Animal Care & Use Committee (#1033575) and the Clinical Research Ethics Review Board of the Royal Veterinary College (URN 2021 2034-3).

Housing in the Peri-Whelping Period

Dams who bred successfully were assigned to one of three experimental groups via block pseudo-randomization: standard housing (SH; n = 59), enriched at whelping (EW; n = 119) or enriched once kits were mobile (EK; n = 64). The group size for EW was approximately twice as large as that of other groups to ensure an adequate sample was maintained in the perinatal period (whereas SH and EK litters could later be supplemented from the farm population if necessary). Groups were balanced for colour type and parity and pens were evenly distributed throughout the barn to account for potential effects of variable lighting, temperatures, noise levels, etc. Dams assigned to the SH and EK housing conditions were given standard nest building materials (chopped straw with wood chip bedding) in the period leading up to whelping. Dams in EW were given standard nest building materials in addition to a handful (~ 7.7g) of crumpled tissue (Kaytee Clean & Cosy bedding) to soften/insulate their nests, and a handful (~ 3.9g) of curled aspen shavings (excelsior) to fortify the structure of their nests. These materials were provided on the same date for all EW pens, thus access lasted a minimum of 7 days and a maximum of 16 days prior to whelping due to variable whelping dates. A length of rope (43 cm or 38 cm long, according to pen height) was also fixed to the ceiling in EW pens (provided a minimum of 5 days and a maximum of 14 days prior to whelping); this rope functioned as a hanging enrichment but could also be unwound and incorporated into nests by the dams, serving as an additional nest building material. These materials were replenished if the observer deemed that they had been soiled or dropped through the pen bottom. At approximately four weeks of age (28±7 days post-whelping, when kits were expected to become mobile; Jonasen, 1987), EK pens were provided with a hanging plastic chain in addition to a standard ring enrichment. Dams and litters in SH and EW had continued access to a standard ring enrichment (see Figure 2.1 for a timeline of housing interventions for each group). Litters were excluded from further testing if fewer than four kits survived to this stage.

Housing in the Post-Weaning Period: Group Housing

Kits were weaned at six weeks of age, at which point the dam was removed from the whelping pen and housed in a separate standard pen (number of litters surviving to this stage after exclusions: n_{SH}, n_{EK}, and n_{EW} = 45, 37, and 40, respectively). Kits remained in the whelping pen and were housed in groups of four to six (n_{SH}, n_{EK}, and n_{EW} = 218, 183, and 207 kits, respectively); if there were more than eight kits in a litter, they were split across two pens (applied to 10 SH pens, 3 EW pens, and 7 EK pens). Enrichment provision for EK kits through this period is detailed in Chapter 3, but in brief, access to the hanging chain and standard EE was maintained in addition to introduction of a second mobile enrichment (a golf ball or wiffle ball; Figure 3.1). A schedule of enrichment exchange was implemented for EK kits such that mobile EEs were exchanged bi-weekly and hanging EEs were exchanged monthly to maintain object novelty. Access to a standard ring enrichment was maintained for SH and EW kits with no enrichment exchange.

Housing in the Post-Weaning Period: Pair-Housing

At ten weeks of age (five weeks post-weaning), kits were moved to single- or pair-housing pens according to standard farm protocol. One male and one female from each litter were chosen for pair-housing and remained in the whelping pen (n_{SH}, n_{EK}, and n_{EW} = 55, 44, and 43 pairs, respectively). A single female from each litter, where possible, was moved to a drop-in cage (dimensions of 18 x 20 x 30; n_{SH}, n_{EK}, and n_{EW} = 21, 23, and 27 females, respectively). Male-female pairs and single-housed females in EK continued to have access to rotating enrichments (a standard ring, a hanging EE in the form of a hanging rope at this stage, and a second mobile EE in the form of a pig's ear or hockey ball at this stage; detailed methods in Chapter 3) until 15 weeks of age, at which point these were removed and only a standard enrichment remained. Male-female pairs and single-housed females in SH and EW maintained access to a standard ring enrichment throughout this period.

4.3.2 Maternal Behaviour Observations

Dam Behaviour Scans Pre-Whelping

Scan sampling observations of all dams were conducted by RM three to five times per day for five consecutive days prior to whelping (within 6-22 days of dams' whelp dates).

Table 4.1 Ethogram for adult and kit behaviour scans.

Behaviour		Description
Adult females only	Nest construction	Manipulating bedding materials with head/limbs or circling in nest forming deeper bowl (circling must be repeated). Often performed in a scooping motion using the chin. Relevant in peri-whelping period only
	Stereotypic behaviour (SB)	Locomotor SB – Movement of whole body including directed movement of hind legs; translocation. Performed as three or more consecutive repetitions (adapted from Díez-León et al., 2016, Polanco et al., 2017)
		Whole-body SB - Movement of upper body with no directed movement of hind legs; stationary. Performed as three or more consecutive repetitions (adapted from Díez-León et al., 2016, Polanco et al., 2017)
		Head-based SB – Movement of head only. Performed as three or more consecutive repetitions (adapted from Polanco et al., 2017, 2018)
		Wire gnawing – Chewing bars of pen. Persists for at least 3 seconds
		Scrabbling – Digging at nest box bedding or pen floors/walls. Both forepaws must be engaged in the digging motion. Persists for at least 3 seconds (adapted from Meagher et al., 2013)
	Borderline SB	Movement pattern interrupted before three repetitions, or switching occurs between elements of common stereotypies without repeating a sequence 3 times. Not analysed as stereotypies but instead included in overall activity (e.g., Díez-León et al., 2016)
Kits only	Social play	Biting, pushing with nose, hitting with forepaws, chasing, pouncing on, or wrestling with another mink, without signs of aggression (see below) (Dallaire and Mason, 2016)
	Aggression	Resembles social play but with audible hissing, screaming and/or persistent escape attempts by one mink (Dallaire and Mason, 2016); behaviour code is assigned to perpetrating member of the interaction
	Defensiveness	As above, but behaviour is assigned to non-perpetrating member of the interaction (i.e., hissing, screaming, or attempting to escape)
All ages	Resting	Inactivity with head down and eyes closed or hidden (adapted from Meagher et al., 2013)
	Lying awake	Inactivity with eyes open (adapted from Meagher et al., 2013)
	Inactivity	Lying motionless other than slight postural adjustments; category used if observer can’t tell if resting vs lying awake (adapted from Meagher et al., 2013)
	General activity	Engaged in activity not in any of the above categories; includes eating, drinking and grooming self
	Enrichment use	Head in contact, licking, or sniffing within 1cm; excludes being inactive with the enrichment

All rounds began after the 8am morning feeding and ended by 12pm (afternoon feedings were at approximately 3pm). The occurrence of stereotypic behaviour, lying awake, use of the rope enrichment (EW only), and resting were noted (see Table 4.1 for ethogram). The first behaviour to occur upon observation was recorded before moving to the next pen, with a 30-s habituation period if necessary (i.e., if mink appeared vigilant of the observer and/or if a behaviour could not be classified without prolonged observation).

Nest Scoring

Nest construction was scored visually for all dams approximately five days per week throughout the whelping period (from April 22 to May 5). Scores were collected by RM and undergraduate research assistant MH (note: neither observer could be blind to housing conditions, or the hypotheses being tested). Inter-rater reliability was assessed through percent agreement calculations (minimum agreement of 80%) and Cohen's kappa (minimum score of 0.61-0.80). Nests were given a score on a scale from 1-7 (rating system described in Table 4.2). Nest scores on postnatal day (PND) -1 and PND 7 were identified for analysis with respect to whelping dates for each pen; pens were excluded from analyses if no scores were obtained within ±1 day of these dates. A rating for the incorporation of premium materials into the nest structure was also recorded for dams in EW (rating system described in Table 4.2).

Nest Box Camera Recordings

Once dams had whelped, nest box cameras were installed in a random selection of pens balanced across conditions and evenly distributed throughout the barn (n_{SH}, n_{EW}, and n_{EK} = 9, 14, and 13, respectively). Activity was recorded for eight consecutive days post-whelping. Continuous video analysis was conducted *post hoc* to quantify time spent performing kit-directed maternal care behaviours, stereotypic behaviours (only scrabbling and wire gnawing were observed in video footage since camera views were restricted to the nest box), nest construction, and time spent out of the nest box; see Tables 4.1 and 4.3 for ethogram. Due to time constraints of research personnel and occasional equipment errors in the processing/recording of nest box video, a sample of 6h per day in 30-min periods across the odd hours of the day (1-1:30am, 3-3:30am, 5:5-30am, etc.) were scored for a selection of four postnatal days (two days within PND 1-4 and two days within PND 5-8; preliminary descriptive analyses showed little difference in the quantity of maternal care behaviour performed within PND 1-4 and 5-8). Selection of postnatal days for scoring was randomized using the online randomizer www.random.org when possible, i.e., when complete footage for all postnatal days was available. Scoring order by postnatal day and time of day was also randomized using www.random.org to account for observer fatigue and other observer effects. Scoring was conducted by GC and undergraduate research assistant CAW (both blinded to housing condition where possible, i.e., where EW bedding materials could not be seen); inter-observer reliability was calculated on a subset of four videos with a minimum threshold of 80% agreement for each behaviour code. Percent agreement was calculated as # agreements/# total observations*100; criteria for an ‘agreement’ included use of the same behavioural code, the same behaviour start time within 5s, and the same behaviour end time within 5s according to the video time stamp. Footage for several pens could not be analyzed due to missing or non-continuous footage on the required days of observation (i.e., equipment and/or internet connectivity issues), resulting in a final sample of 5 SH pens, 9 EW pens, and 9 EK pens used in analyses.

Table 4.2 Nest shape and nest material use scores (Modified from Malmkvist and Palme, 2008; Meagher et al., 2012).

Nest Shape Score	**Description**
1	No nesting material
2	Some nesting materials; flat, unstructured
3	All nesting material; flat, unstructured
4	Saucer-shaped indentation
5	Round hole with a side higher than the dam when lying down (covering at least one side of box or 25% of circumference of nest)
6	Round hole with sides mostly higher than the dam when lying down
7	Round hole with high sides and a 'ceiling' or overhang on at least one section; may have fur incorporated
Nest Material Use Score (EW)	**Description**
1	None of extra material taken into nest.
2	Some material used.
3	Most of material placed on top of nest box and/or at least two types of enriched nesting material (paper, shredded aspen fibre, or rope) used in nest.
4	All or most (>1/2) of two types of nesting material in addition to some (<1/2) of a third type used and incorporated into the nest.

Table 4.3 Ethogram for maternal care in nest box camera recordings.

Behaviour	**Description**
Kit retrieval	Bringing a kit from outside the nest area back into the nest, whether by using head/mouth or limbs
Nursing	Kits head/mouth in contact with teats. If view of kit's head is obstructed, can be evident from suckling motion in belly and pawing at the teat with forepaws. Bout marked as finished if behaviour is obscured for more than 5 seconds
Licking and grooming	Licking or gentle biting of the kits' anogenital region, head, or body. Bout marked as finished if behaviour is halted for more than 1 second. If target area of grooming is obscured but grooming motions are clear, list as body grooming (rather than anogenital or head)
Out of nest box	Dam leaves the nest box, behaviour not visible. Re-entry occurs when all four paws are across the threshold of the nest box

Kit Retrieval

Kit retrieval tests were conducted with a random subset of dams balanced across treatment, colour type, and parity (n_{SH}, n_{EK}, and n_{EW} = 34, 32, and 45, respectively) at 7±2 days post-whelping by RM and MH. This test involved removing one kit from the nest and placing them in the main pen area facing the nest box entrance (see Malmkvist & Houbak, 2000; Meagher et al., 2012). Male and female kits were selected for removal on an alternating basis whenever possible to control for sex effects on retrieval latency. The latency of the dam to touch (i.e., come within 1cm of the kit) and retrieve the kit (i.e., return them to the nest) was recorded. Dams were given a maximum of 180s to retrieve kits, and if not retrieved by this time, they were recorded as not retrieving the kit and excluded from analysis.

Kit Mortality and Growth Data Collection

Kits were counted and weighed by farm staff at PND 1 ("first weight"; used to account for kits not born live), three weeks of age, and at weaning. Using this data, kit mortality across litters of different housing conditions was assessed from first weight to three weeks and from first weight to weaning. Litters were excluded from analysis if foster kits were added to the litter at any point (fostering was avoided in trial litters whenever possible, though 5 EW litters and 3 EK litters were excluded for this reason). Average kit weights at three weeks and at weaning were also assessed using this data. Likewise, causes for exclusion included kits being fostered into the litter or loss of all kits in the litter by the time of weight recordings.

4.3.3 Kit Behaviour Observations

Kit Behaviour Scans in Group Housing

Following weaning, scan sampling observations of all pens (n_{SH}, n_{EK}, and n_{EW} = 45, 37, and 40, respectively) were conducted three consecutive days per week for the following four weeks (from weaning to 10 weeks of age) by GC, RM, and MH (note: observers were not blind to housing conditions or hypotheses being tested). Inter-rater reliability was assessed using Cohen's kappa (minimum score of 0.61-0.80; for scores in the moderate reliability range [e.g., a score of 0.57 for EE use], discrepancies were reviewed to improve reliability going forward). Scan sampling observation methods are detailed in Chapter 3. Kits' interactions with enrichments were recorded in addition to social play and resting (serving as 'good' welfare behaviours), aggression, defensiveness, lying awake, or general inactivity (serving as 'poor' welfare behaviours; locations in the pen were noted for the latter two behaviours to assess degree of 'hiding' in nest box; Meagher et al., 2013), and activity, which served as a control behaviour (see Table 4.1 for ethogram). Pen observation order was reversed each scoring day to prevent the systematic scoring of some pens earlier than others.

Kit Behaviour Scans in Single- or Pair-Housing

All male-female pairs remaining in the study (n_{SH}, n_{EW}, and n_{EK} = 55, 43, and 44 pairs, respectively) continued to be observed according to the group-housing observation protocol until 13 weeks post-whelping. Females placed in single housing were not observed, but EE rotation for EK females continued.

4.3.4 Behavioural Observations in Kits as Adults

Temperament Testing

Following the juvenile observation period (and after additional EEs had been removed from EK pens), all single- and pair-housed kits remaining in the trial were tested for temperament by GC and MH using the stick test; both observers were blind to the previous housing conditions of the kits. This test involves inserting a popsicle stick into the pen and recording kits' behavioural responses (Meagher et al., 2011; Mononen et al., 2012). Reaction categories included curious, aggressive, fearful, or unresponsive (see Table 4.4 for ethogram). Tests were repeated over two consecutive days to determine the reliability of temperament scores for each subject (i.e., whether kits' response to the stick test was consistent across tests), and two rounds were conducted per day to revisit any kits who were sleeping in the first round. Kits with conflicting responses across testing days were excluded from analysis; responses were considered conflicting if kits responded as 'curious' on the first test and 'fearful' or 'aggressive' on the second test (Meagher et al., 2011). However, a fearful reaction followed by a curious reaction, an aggressive reaction followed by a curious reaction, or a curious reaction followed by an unresponsive reaction were accepted, as some decrease in fear/aggression or interest in the stick due to habituation was expected over repeated tests. In these cases, only the subject's initial response was kept. Stick testing was conducted in the late summer when kits were 14-16 weeks old (n_{SH}, n_{EK}, and n_{EW} = 113, 101, and 107 kits, respectively) and repeated in the fall at 27-29 weeks (n_{SH}, n_{EK}, and n_{EW} = 111, 100, and 106 kits, respectively; some kits were lost between tests due to mortality or morbidity) to account for potential changes in kit temperament with age.

Table 4.4 Stick test response categories (modified from Meagher et al., 2011).

Score	**Description**
Fearful (F)	The mink moves away from the stick or, if initially standing as far from the stimulus as possible, remains at that distance while attending to the stimulus for at least 30 seconds
Curious (C)	The mink approaches and sniffs the stimulus; it can make tooth contact without a hard bite, i.e., without closing its teeth- a gentle exploratory nibble
Aggressive (A)	The mink rapidly delivers a hard and sustained bite (clamping its teeth together fully) to the stimulus
Unresponsive/ other (N)	The mink is alert and faces the stimulus but does not respond in one of the specified ways.

Fear Behaviour Observations During Pelt Grading

As part of the standard farm pelt grading process in the late fall (30-32 weeks of age), all pair- and single-housed trial kits were captured and restrained on a pelt grading table to assess their pelt quality (n_{SH}, n_{EK}, and n_{EW} = 79, 83, and 91 kits, respectively). During this process, number of discrete vocalizations were counted and other fear behaviours including physical struggling, attempts to bite handler, and urination were recorded using 1-0 sampling as additional measures of temperament and fear behaviour (Zieliński et al., 2019).

Behaviour Scans in Kits as Adults

One year following the conclusion of juvenile observations and removal of extra EEs for the EK group (at approximately 16 months of age), stereotypic behaviour (SB) of various subtypes (i.e., locomotor, whole-body stationary, head-based, scrabbling, and wire-gnawing) were scored in the remaining trial females (n_{SH}, n_{EK}, and n_{EW} = 31, 37, and 36, respectively). Behaviours such as activity, resting, lying awake, or inactivity, and relevant locations of the latter two behaviours in the pen (Meagher et al., 2013), were also recorded (Table 4.1). All mink were single housed by this stage, with access to a single standard ring enrichment. Observations were conducted by GC over five non-consecutive days in a two-week period from October to November (note: locations of inactivity sub-types in the pen were only noted during the latter three days of analysis). GC was blinded to the females' previous housing conditions. Pens were observed four times per day; all rounds were conducted between 12pm and 3pm (morning feeding occurred at 8am and afternoon feedings were at approximately 3pm). The first behaviour observed was recorded before moving to the next pen, with a 30-s habituation period if the subject appeared vigilant of the observer and/or if a behaviour could not be classified without prolonged observation.

4.3.5 Physiological Measures

Dam Faecal Sampling and Cortisol Extraction

To compare basal cortisol levels of dams across groups, faecal samples were collected from a random subset of dams balanced across housing conditions and locations in the barn (n_{SH}, n_{EK}, and n_{EW} = 44, 36, and 57 dams, respectively). Sample dates were adjusted according to each dam's whelp date to ensure sampling during similar biological states (approximately 20 days post-whelping); screens were put out beginning at 10am and retrieved within two hours of that time the next day. Samples were frozen until later processing to assess levels of faecal cortisol metabolites (FCM) with a mink-validated 11ß-hydroxyaetiocholanolone enzyme immunoassay (adapted methods from Malmkvist et al., 2011). The loss of several samples during transport for processing resulted in a final sample of 29 SH dams, 27 EK dams, and 41 EW dams for analysis.

Kit Faecal Sampling and Cortisol Extraction

To assess differences in stress physiology of kits across groups, faecal samples were collected from a subset of male-female pairs (n_{SH}, n_{EK}, and n_{EW} = 29, 29, and 32 pairs, respectively) preceding and following pelt grading. Pelt grading was used as a stress event since it is practiced annually on commercial farms, and by substituting this event for experimental restraint stress in carrying cages (typically used to induce stress in mink; Malmkvist et al., 2011), we aimed to avoid subjecting mink to additional stress. Screens were put in place for pre-test faecal sample collection two days prior to pelt grading at approximately 2pm and collected the following day between 10am-1pm. On the testing day, pelt grading took place from approximately 8am-10am; screens were put in place for post-test faecal sample collection four hours following pelt grading at approximately 2pm and collected the next day between 9:30am-12:30pm. Faecal samples were frozen for later cortisol metabolite extraction using a mink-validated 11ß-hydroxyaetiocholanolone enzyme immunoassay (adapted methods from Malmkvist et al., 2011).

Post-Mortem Harvesting and Weighing of Spleens

The spleens of pair-housed males were harvested upon pelting (n_{SH}, n_{EK}, and n_{EW} = 17, 18, and 16 males in January pelting; an additional sample of n_{SH}, n_{EK}, and n_{EW} = 8, 8, and 10 males were added in April pelting). Spleens were trimmed of fat and weighed; weights were then compared across groups. Body weights and lengths of mink were also recorded to use as controls in analyses if needed.

4.3.6 Statistical Analyses

All statistical analyses were conducted with jamovi statistical software (The jamovi project, 2023; v. 2.3.18.0 for Mac). Figures were generated using Prism (GraphPad Software, 2023; v. 10.02 for Mac). Significance level was set at $p < 0.05$. Results were defined as trending when $0.05 < p < 0.10$. Assumptions of normality and homogeneity of variances for parametric analyses were assessed using Shapiro-Wilk and Levene's tests, respectively. Transformations were performed as necessary (either square-root transformations or $\log_{10}$ transformations, as appropriate) with mean and 95% confidence interval (CI) subsequently back-transformed for presentation. Where parametric analyses were not appropriate, non-parametric alternatives were used.

Analysis of Housing Condition Effects on Dam Welfare Indicators

For these tests occurring prior to postnatal week three, SH and EK housing conditions were pooled for analysis (henceforth referred to as SH&EK) as they were in equivalent housing at this time. Behavioural scan data pertaining to dam stereotypic behaviour, resting, lying awake, and interaction with the rope EE were formatted for analysis by calculating the average proportion of observations where a behaviour occurred based on the total number of observations (see equation below).

$$\text{Average proportion of observations where behaviour occurred} = \frac{\#\ Ocurrences}{(\#\ Scans\ per\ day)(\#\ Days\ observation)}$$

Average performance of these behaviours was compared across EW and SH&EK dams using Student's t-tests when assumptions of normality and homogeneity of variances were met; rope use was assessed qualitatively as no other groups had this enrichment. Where data were not normally distributed and transformations were not successful, analyses were conducted using non-parametric Mann-Whitney U-tests. Basal FCM in ng/g of SH&EK dams and EW dams were compared using a Student's t-test.

Analysis of Housing Condition Effects on Maternal Care and Nest Building

Maternal care behaviours were formatted as a percentage of time for analysis (total cumulative time spent performing behaviour / total time observed * 100). Average bout durations of each behaviour were also analysed (total cumulative time spent performing behaviour/total number of occurrences); these bout durations were presented in minutes or seconds as appropriate. Average percentages of time and average bout durations were then compared across EW and SH&EK dams using Student's t-tests when assumptions of normality and homogeneity of variances were met, or non-parametric Mann-Whitney U-tests when data were not normally distributed or when transformations were not successful. Latency of SH&EK dams to touch and retrieve kits (in seconds) was compared to that of EW dams using Student's t-tests; a two-way ANOVA with housing condition and dam colour type as factors was also conducted to determine effects of dam colour type (Clausen et al., 2008); it was determined in preliminary analyses that kit touch and retrieval latencies did not differ by kit sex (overall average of 23.6 and 42.7s for females and 24.7 and 43.4s for males, respectively), so we did not control for this in analyses. Nest scores for SH&EK dams and EW dams were compared using non-parametric Mann-Whitney U-tests as subjects were assigned scores on an ordinal ranking system. Nest scores on PND -1 and PND 7 were assessed in separate models. Frequencies of material use scores and combinations of materials used in EW dams were assessed using descriptive statistics.

Analysis of Housing Condition Effects on Kit Mortality and Growth

Percent mortality at three weeks and at weaning were compared across housing conditions using a non-parametric Mann-Whitney U-test and Kruskal-Wallis one-way ANOVA, respectively. Average kit weights at three weeks and at weaning were compared using a Student's t-test and one-way ANOVA (Welch's) with housing condition as a factor.

Analysis of Housing Condition Effects on Kit Behaviours as Juveniles

Behavioural scan data pertaining to kit activity, social play, aggression, resting, inactivity, and lying awake were formatted for analysis by calculating the average proportion of observations where a behaviour occurred based on the total number of observations (see equation below).

$$\text{Average proportion of observations where behaviour occurred} = \frac{\#\ Ocurrences}{(\#\ subjects\ per\ pen)(\#\ Scans\ per\ day)(\#\ Days\ observation)}$$

Average proportions of observations where behaviours occurred were analysed using one-way ANOVA (Welch's, to account for unequal variances) and Tukey's post-hoc comparisons with housing condition as a factor when assumptions of normality and homogeneity of variances were met. Where data were not normally distributed and transformations were not successful, analyses were conducted using non-parametric one-way ANOVA and Dwass-Steel-Critchlow-Flinger pairwise comparisons.

Analysis of Housing Condition Effects on Kit Temperament as Adults

Counts of temperament categorizations from summer and fall stick tests were compared across pair-housed kits and single-housed female kits using separate Chi-Square tests of association, with housing condition across rows and response categories across columns. Fisher’s exact test was used as needed to account for cells with counts below five. Regarding fear behaviours during pelt grading, number of vocalizations across pair- and single-housed kits of different housing conditions were compared using non-parametric Kruskal-Wallis one-way ANOVA. Occurrences (yes/no) of attempts to bite handler, physical struggling, and urination were compared using separate Chi-Square tests of association, with housing condition across rows and occurrence categories across columns. Fisher’s exact test was used as needed to account for cells with counts below five.

Analysis of Housing Condition Effects on Kit Stress Responsiveness and Chronic Stress Effects

Kit pre- and post-test FCM in ng/g for each housing condition were analysed using paired Student’s t-tests. Correlation of male spleen weights with body weight and body length were assessed using descriptive scatterplots. When it was determined that these did not correlate positively for all males and body weight/length were not needed as covariates in the analysis, spleen weights were analysed using one-way ANOVA (Welch’s) with housing condition as a factor.

Analysis of Housing Condition Effects on Kit Behaviours as Adults

Behavioural scan data pertaining to female kits' stereotypic behaviour performance, resting, lying awake, or inactivity as adults, as well as locations of inactivity subtypes, were formatted for analysis by calculating the average proportion of observations where a behaviour occurred based on the total number of observations (see equation below).

$$\textit{Average proportion of observations where behaviour occurred} = \frac{\#\ Ocurrences}{(\#\ Scans\ per\ day)(\#\ Days\ observation)}$$

Average proportions of observations where behaviours occurred were analysed using one-way ANOVA (Welch's, to account for unequal variances) and Tukey's post-hoc comparisons with housing condition as a factor when assumptions of normality and homogeneity of variances were met. Where data were not normally distributed and transformations were not successful, analyses were conducted using non-parametric one-way ANOVA and Dwass-Steel-Critchlow-Flinger pairwise comparisons.

4.4 Results

4.4.1 Housing condition effects on dam welfare indicators

EW dams performed significantly less SB than SH&EK dams (0.181±0.015 and 0.244±0.018, respectively; $U = 5953$, $p = 0.012$). EW dams also rested more (back-transformed proportion of observations: 0.371, 95% CI [0.334, 0.410]) compared to SH&EK dams (0.317, 95% CI [0.281, 0.354]; $t_{240} = -2.05$, $p = 0.041$; Figure 4.1). Lying awake was not affected by housing ($U = 7279$, $p = 0.937$; Figure 4.1). Dams in the EW condition used the hanging rope enrichment in a proportion of 0.034±0.033 observations on average, though this behaviour was quite variable (range of 0.150). Dams in SH&EK and EW housing had similar basal FCM (back-transformed mean: 25.70 ng/g, 95% CI

[21.88, 29.51] and 28.18 ng/g, 95% CI [23.44, 34.67], respectively), thus there was no effect of housing on this measure ($t_{95.0} = 0.904$, $p = 0.368$).

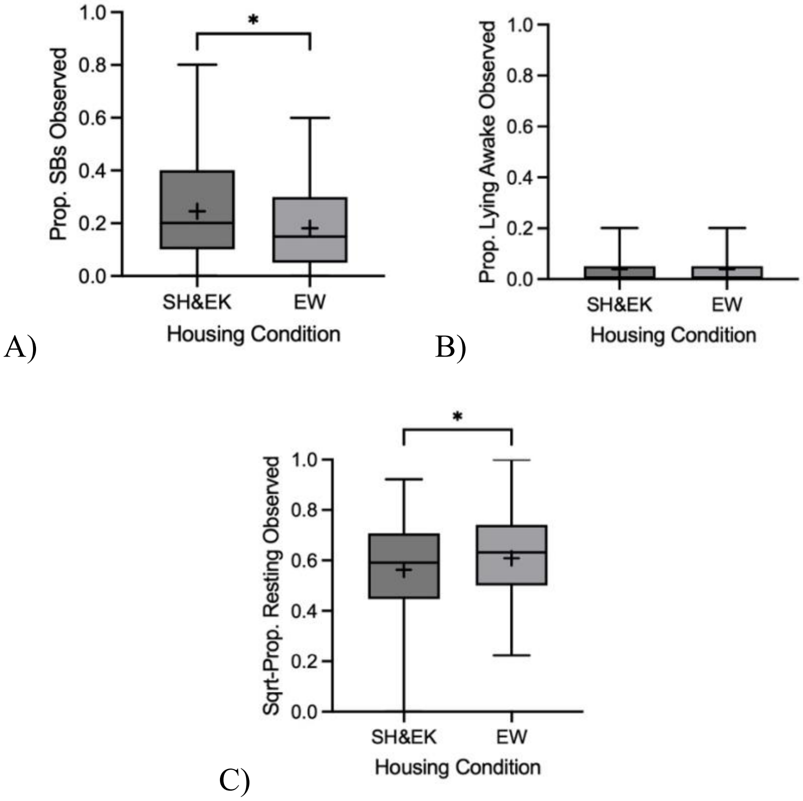


Figure 4.1 Average proportion of observations where the following behaviours occurred in dams of the SH and EK conditions pooled compared to dams in EW: **A)** stereotypic behaviour, **B)** lying awake, **C)** resting (square root transformed). Black + signs show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value. n = 123 and 119 sample points, respectively, for each figure. Bars indicate a significant difference with * indicating p<0.05.

4.4.2 Housing condition effects on maternal care and nest building

Time spent nursing, anogenital grooming, body grooming, and head grooming were not affected by housing condition, nor were average bout durations of these behaviours (p-values ranged from $p = 0.117$ for $t_{21.0} = -1.634$ to $p = 100$ for $U = 62.5$; Figures 4.2 and

4.3). Time spent performing nest construction behaviours and average nest construction bout durations were similar across groups (*U* = 63.0, p = 1.000 and $t_{21.0}$ = -0.448, p = 0.659, respectively). Time spent scrabbling and average scrabbling bout duration also did not differ by housing (*U* = 0.943, p = 0.896 and $t_{3.00}$ = -2.173, p = 0.118, respectively); measures of wire-gnawing behaviour could not be analysed due to a lack of this behaviour in SH and EW dams. However, EW dams spent greater time out of the nest box compared to SH&EK dams (back-transformed percentage of time: 6.18%, 95% CI [4.42, 8.65] and 4.43%, 95% CI [3.71, 5.28], respectively; $t_{21.0}$ = 2.167, p = 0.042; Figure 4.2). Average out-of-nest box bout duration was not affected ($t_{21.0}$ = 1.561, p = 0.133; Figure 4.3).

Latency to touch and retrieve kits did not differ by housing condition ($t_{95.0}$ = 0.265, p = 0.792 and $t_{92.0}$ = -0.509, p = 0.612, respectively); SH&EK dams had latencies of 13.49s (95% CI [10.00, 17.78]) to touch and 32.36s (95% CI [25.70, 40.74]) to retrieve their kits (back-transformed), while EW dams had latencies of 14.13s (95% CI [10.00, 19.95]) to touch and 25.70s (95% CI [22.39, 38.90]) to retrieve their kits (back-transformed). Moreover, neither measure differed by dam colour type ($F_{3,89}$ = 0.575, p = 0.633 and $F_{3,86}$ = 0.841, p = 0.475), and there was no interaction effect between dam colour type and housing ($F_{3,89}$ = 0.470, p = 0.704 and $F_{3,86}$ = 0.329, p = 0.804).

Nests of EW dams were rated significantly higher than those of SH&EK dams on PND-1 (4.98±0.096 and 4.42±0.090, respectively; *U* = 5126, $p < 0.001$) and on PND 7 (5.11±0.109 and 4.55±0.095, respectively; *U* = 5185, $p < 0.001$; Figure 4.4). A material use score of 3 (n = 40 dams) was the most common in EW by PND 7, with a score of 4

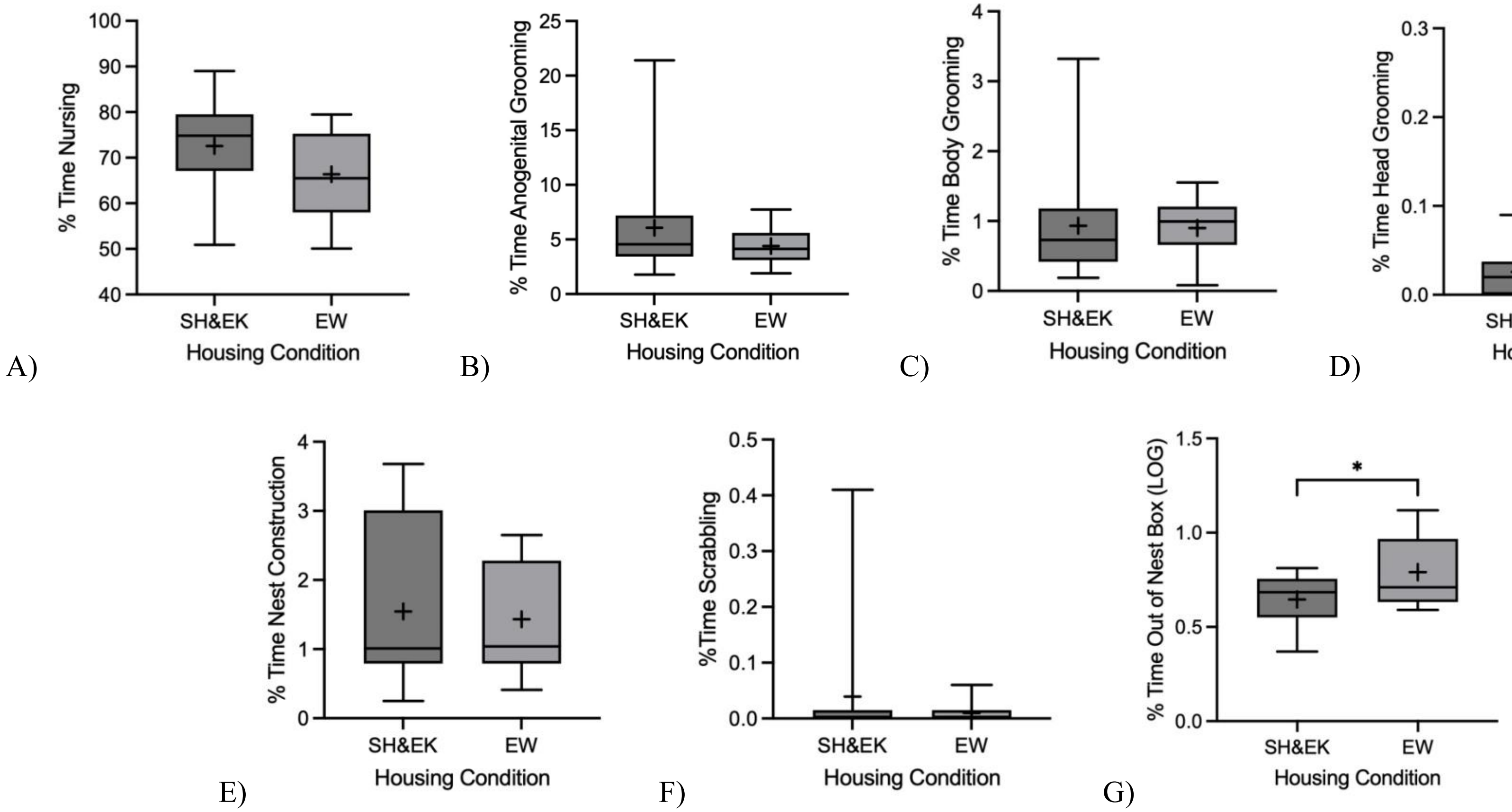


Figure 4.2 Percentage of time dams in the SH&EK conditions and EW condition were observed performing the following behaviours: **A)** Nursing kits, **B)** licking and grooming kits' anogenital region, **C)** licking and grooming kits' body region, **D)** licking and grooming kits' head region, **E)** nest construction, **F)** scrabbling, and **G)** out of the nest box. n = 14 and 9 sample points, respectively for all figures. Black + signs show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value. Bars indicate a significant difference with * indicating $p<0.05$.

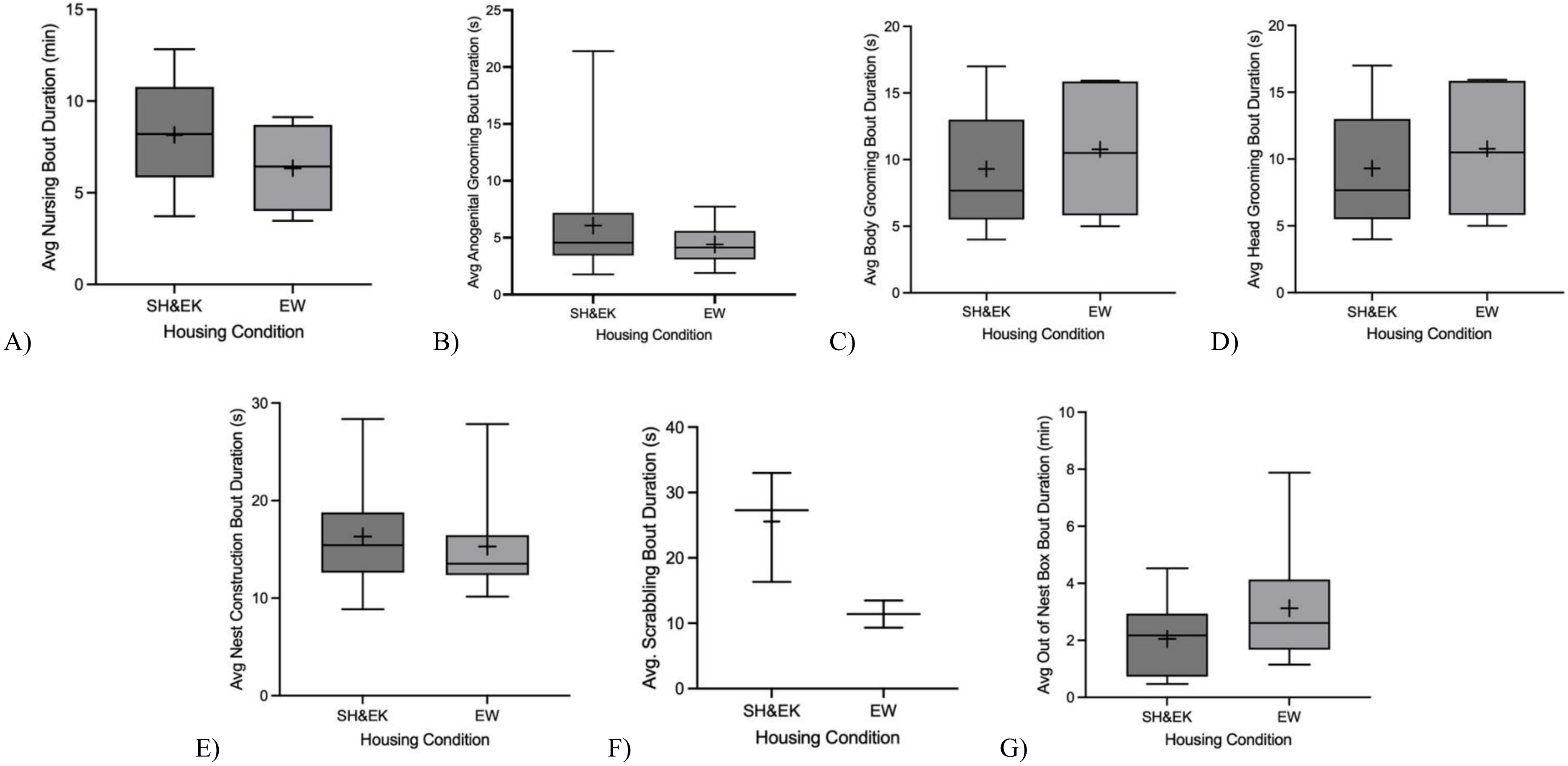
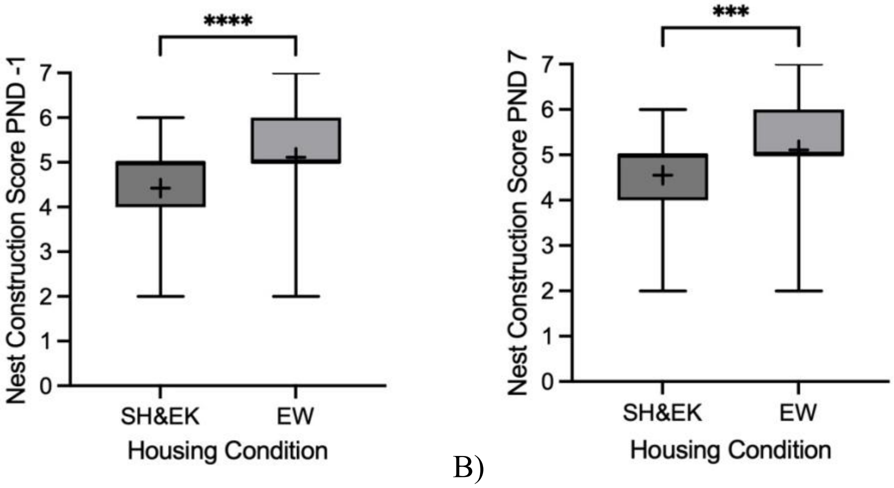


Figure 4.3 Average duration of bouts of the following behaviours across dams in the SH&EK conditions and EW condition: **A)** Nursing kits, **B)** licking and grooming kits' anogenital region, **C)** licking and grooming kits' body region, **D)** licking and grooming kits' head region, **E)** nest construction, **F)** scrabbling, and **G)** out of the nest box. n = 14 and 9 sample points, respectively for Figures a-c, e, and g; n = 11 and 5 sample points for Figure d, and n = 3 and 2 sample points, respectively for Figure f. Black + signs show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value.

being the second most common (meaning all materials were incorporated into the nest; n = 37 dams). The entirety of the crumpled paper, curled aspen shavings, and rope were used in combination most often (n = 43 dams), followed by crumpled paper and aspen shavings (n = 34 dams) and crumpled paper and rope (n = 16 dams). Only one dam used a combination of curled aspen shavings and rope. In dams that made use of only one EW material, the crumpled paper was used most often (n = 12 dams).



A) B)

Figure 4.4 Average nest construction scores across dams in the SH&EK conditions and EW condition on **A)** PND -1 (n = 123 and 118 sample points, respectively) and **B)** PND 7 (n = 121 and 118 sample points, respectively). Black + signs show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value. Bars indicate a significant difference with *** indicating p<0.001.

4.4.3 Housing condition effects on kit mortality and growth

There was a trending difference between EW (7.68±2.28) and SH&EK (9.67±1.98) litters in the direction of lower percent mortality for EW litters (U = 3421, p = 0.075; Figure 4.5). There was no difference in percent mortality from first weight to weaning (x^2_2 = 1.67, p = 0.435). Average kit weights at three weeks were 119±2.57g and 118±2.75g in

SH&EK litters and EW litters, respectively, thus there was no effect of housing condition ($t_{171} = 0.197$, $p = 0.844$). Likewise, average kit weight at weaning did not differ by housing condition ($F_{2,98.9} = 0.490$, $p = 0.614$); kits in SH, EK, and EW had average weights of 395±10.95g, 401±13.05g, and 386±9.31g, respectively.

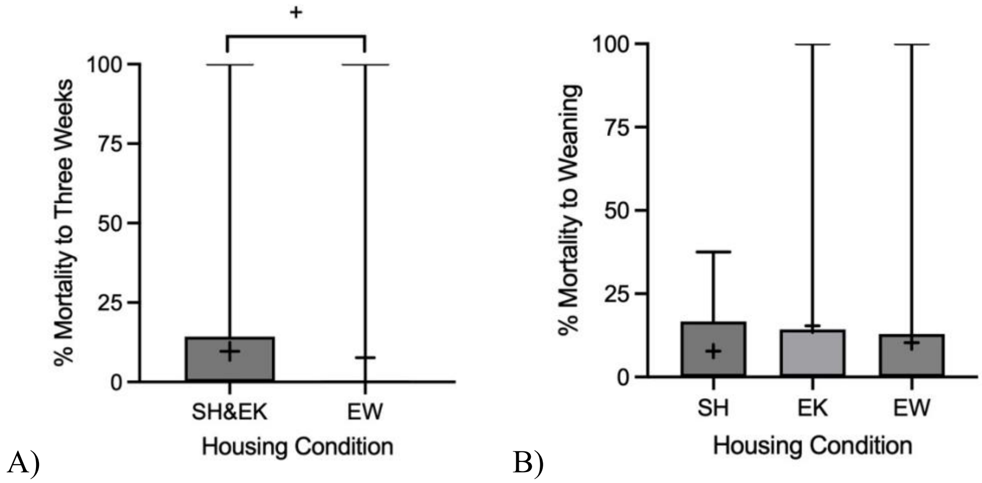


Figure 4.5 Percent mortality of kits from **A)** first weight to three weeks between SH&EK litters and EW litters (n = 95 and 82 sample points, respectively), and **B)** first weight to weaning across litters of different conditions (n = 48, 47, and 82 sample points, respectively). Black + signs show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value. Bars indicate significant relationships with + indicating $0.05<p<0.10$.

4.4.4 Housing condition effects on kit behaviours as juveniles

Group housing

There was no effect of housing on any measures of kit behaviour in the group-housing phase (p-values ranged from $p = 0.184$ for $x^2_2 = 3.391$ to $p = 0.923$ for $x^2_2 = 0.159$; see Table 4.5).

Table 4.5 Treatment effects on proportions of observations where juvenile behaviours were observed in the group-housing period. SE given for measures analysed parametrically, SD given for measures analysed non-parametrically. n_{SH}, n_{EK}, and n_{EW} = 36, 33, and 47 pens, respectively.

Measure	SH	EK	EW	Statistic	P-value
	Mean ± SE	**Mean ± SE**	**Mean ± SE**		
Social play	0.042 ± 0.003	0.040 ± 0.003	0.0044 ± 0.002	$F_{2,70.3} = 0.741$	0.480
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Aggression	0.001 ± 0.002	0.001 ± 0.001	0.001 ± 0.002	$X^2_2 = 1.627$	0.443
Defensiveness	0.001 ± 0.001	0.000 ± 0.001	0.000 ± 0.001	$X^2_2 = 3.391$	0.184
Activity	0.124 ± 0.026	0.121 ± 0.030	0.123 ± 0.030	$X^2_2 = 1.142$	0.565
Resting	0.112 ± 0.195	0.076 ± 0.024	0.080 ± 0.026	$X^2_2 = 1.333$	0.514
Lying awake	0.002 ± 0.002	0.003 ± 0.002	0.002 ± 0.002	$X^2_2 = 1.585$	0.453
Lying awake in NB	0.016 ± 0.010	0.016 ± 0.010	0.015 ± 0.009	$X^2_2 = 0.159$	0.923
Inactive in NB	0.169 ± 0.041	0.161 ± 0.037	0.186 ± 0.162	$X^2_2 = 1.173$	0.556

Table 4.6 Treatment effects on proportions of observations where juvenile behaviours were observed in the pair-housing period. SE given for measures analysed parametrically, SD given for measures analysed non-parametrically. Significant results are in bold. n_{SH}, n_{EK}, and n_{EW} = 46, 37, and 42 pens, respectively.

Measure	SH	EK	EW	Statistic	P-value
	Mean ± SE	**Mean ± SE**	**Mean ± SE**		
Social play	0.021 ± 0.002	0.017 ± 0.002	0.024 ± 0.002	$F_{2,80.9} = 3.560$	**0.033**
Activity	0.231 ± 0.006	0.242 ± 0.007	0.239 ± 0.006	$F_{2,79.2} = 0.913$	0.406
Resting	0.257 ± 0.008	0.253 ± 0.008	0.248 ± 0.009	$F_{2,80.9} = 0.305$	0.738
Inactive in NB	0.137 ± 0.008	0.089 ± 0.008	0.141 ± 0.010	$F_{2,80.6} = 10.840$	**< 0.001**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Aggression	0.000 ± 0.001	0.001 ± 0.002	0.000 ± 0.001	$X^2_2 = 0.333$	0.846
Defensiveness	0.000 ± 0.001	0.001 ± 0.000	0.000 ± 0.001	$X^2_2 = 1.117$	0.572
Lying awake	0.001 ± 0.006	0.014 ± 0.010	0.011 ± 0.008	$X^2_2 = 6.167$	**0.046**
Lying awake in NB	0.005 ± 0.006	0.004 ± 0.005	0.006 ± 0.007	$X^2_2 = 1.772$	0.412

Pair-housing

Social play significantly differed across housing conditions in the pair-housing phase; EW kits were observed performing social play more often on average than EK kits (t_{122} = -2.54, $p = 0.033$; Table 4.6), though this behaviour did not differ between EW kits and SH kits (t_{122} = -1.11, $p = 0.508$) or between SH and EK kits (t_{122} = -1.52, $p = 0.285$). Aggressive and defensive behaviours remained similar across housing conditions in pair-housing, as did activity levels, resting, and lying awake in the nest box (Table 4.6). However, lying awake in the cage showed effects of housing condition towards more lying awake in EK kits than in SH kits (W = 3.35, $p = 0.047$); lying awake did not differ between EK and EW kits (W = -1.96, $p = 0.348$) or SH and EW kits (W = 1.87, $p = 0.382$). Inactivity in the nest box also differed by housing condition in the pair-housing phase: EK kits were inactive in the nest box less often on average compared to SH kits (t_{122} = 3.61, $p = 0.001$) and EW kits (t_{122} = -3.859, $p < 0.001$), while SH and EW kits did not differ in this behaviour (t_{122} = -0.337, $p = 0.939$; Table 4.6).

4.4.5 Housing condition effects on kit temperament

There was no effect of housing condition on the temperaments of pair-housed kits as determined by the stick test in the summer (X^2_4 = 2.31, $p = 0.679$) or fall months (X^2_6 = 4.68, $p = 0.620$; Table 4.7). Likewise, temperaments of single-housed females did not differ across conditions in either season (X^2_6 = 5.09, $p = 0.552$ and X^2_6 = 3.95, $p = 0.729$, respectively; Table 4.7). Number of vocalizations during handling for pelt grading did not differ across conditions in pair-housed (x^2_2 = 3.95, $p = 0.832$) or single-housed kits (x^2_2 = 2.21, $p = 0.332$; Table 4.8). Attempts to bite the handler occurred in similar levels between kits of each condition in pair- and single-housing (X^2_2 = 0.490, $p = 0.783$ and

Table 4.7 Summary of temperament test statistics for pair-housed kits in summer ($n_{SH} = 92$; $n_{EK} = 78$; $n_{EW} = 80$) and fall ($n_{SH} = 88$; $n_{EK} = 74$; $n_{EW} = 75$) and single-housed kits in summer ($n_{SH} = 21$; $n_{EK} = 23$; $n_{EW} = 27$) and fall ($n_{SH} = 21$; $n_{EK} = 23$; $n_{EW} = 26$) across treatments. 'S' denotes summer test results and 'F' denotes fall test results.

Response	**Pair-Housed Summer Stick Tests**					**Single-Housed Summer Stick Tests**			
	Treatment	**Counts**		**% Within Treatment**		**Counts**		**% Within Treatment**	
		S	F	S	F	S	F	S	F
Curious	SH	66	77	71.7 %	87.5 %	6	10	28.6 %	47.6 %
	EK	58	62	74.4 %	83.8 %	10	14	43.5 %	60.9 %
	EW	52	61	65.0 %	81.3 %	13	14	48.1 %	53.8 %
Fearful	SH	9	4	9.8 %	4.5 %	12	1	57.1 %	4.8 %
	EK	7	2	9.0 %	2.7 %	11	2	47.8 %	8.7 %
	EW	12	4	15.0 %	5.3 %	11	2	40.7 %	7.7 %
Aggressive	SH	0	4	0%	4.5 %	0	5	0.0 %	23.8 %
	EK	0	8	0%	10.8 %	1	6	4.3 %	26.1%
	EW	0	5	0%	6.8 %	0	7	0.0 %	26.9 %
Unresponsive	SH	17	3	18.5 %	3.4 %	3	5	14.3 %	23.8 %
	EK	13	2	16.7 %	2.7 %	1	1	4.3 %	4.3 %
	EW	16	5	20.0 %	6.7 %	3	3	11.1 %	11.5 %

Table 4.8 Summary of pelt grading fear behaviour statistics for pair (n_{SH} = 58; n_{EK} = 60; n_{EW} = 64) and single-housed kits (n_{SH} = 21; n_{EK} = 23; n_{EW} = 27) across treatments.

		Pair-housed kits		**Single-housed kits**	
Measure	**Treatment**	**Mean ± SD**		**Mean ± SD**	
Vocalizations	SH	1.31 ± 6.23		3.05 ± 6.72	
	EK	1.30 ± 3.79		0.74 ± 3.33	
	EW	0.58 ± 2.58		4.04 ± 10.2	
	Treatment	**Counts (yes)**	**% Within Treatment**	**Counts (yes)**	**% Within Treatment**
Attempts to bite handler	SH	14	24.1 %	16	76.2 %
	EK	12	20.0 %	13	56.5 %
	EW	16	25.0 %	21	77.8 %
Physical struggling	SH	14	24.1 %	5	23.8 %
	EK	12	20.0 %	6	26.1 %
	EW	23	35.9 %	4	14.8 %
Urination	SH	2	3.4 %	1	4.8 %
	EK	2	3.3 %	5	21.7 %
	EW	1	1.6 %	6	22.2 %

$X^2_2 = 3.17$, $p = 0.205$), as did occurrences of physical struggling ($X^2_2 = 4.33$, $p = 0.115$ and $X^2_2 = 1.08$, $p = 0.582$) and urination ($X^2_2 = 0.520$, $p = 0.742$ and $X^2_2 = 3.13$, $p = 0.194$, respectively; Table 4.8).

4.4.6 Housing condition effects on kit stress responsiveness and chronic stress effects

There was no change in pre- vs. post-test FCM in SH kits ($t_{27.0} = 0.714$, $p = 0.481$) or EK kits ($t_{28.0} = 0.935$, $p = 0.358$; Figure 4.6). However, EW kits had significantly decreased FCM in the post-test period (back-transformed mean: 33.11 ng/g, 95% CI [19.50, 56.23]) compared to the pre-test period (70.79 ng/g, 95% CI [53.70, 95.50]; $t_{31.0} = 2.655$, $p = 0.012$; Figure 4.6). There was no difference in pre-test (i.e., basal) FCM levels across kits of different housing conditions ($x^2_2 = 1.813$, $p = 0.404$). Spleen weights did not differ between SH males (back-transformed mean: 7.14g, 95% CI [5.97, 8.53]), EK males (7.04g, 95% CI [5.85, 8.49], or EW males (7.40g, 95% CI [6.32, 8.65]; $F_{2,49.0} = 0.097$, $p = 0.908$).

4.4.7 Housing condition effects on kit behaviour as adults

Performance of locomotor SB and whole-body SB did not differ across females reared in different housing conditions ($x^2_2 = 3.878$, $p = 0.144$ and $x^2_2 = 1.770$, respectively; Figure 4.7); there were no observations of head-based SB, scrabbling, or wire gnawing. There was also no difference in observed activity, resting, lying awake, or inactivity across housing conditions (Table 4.9).

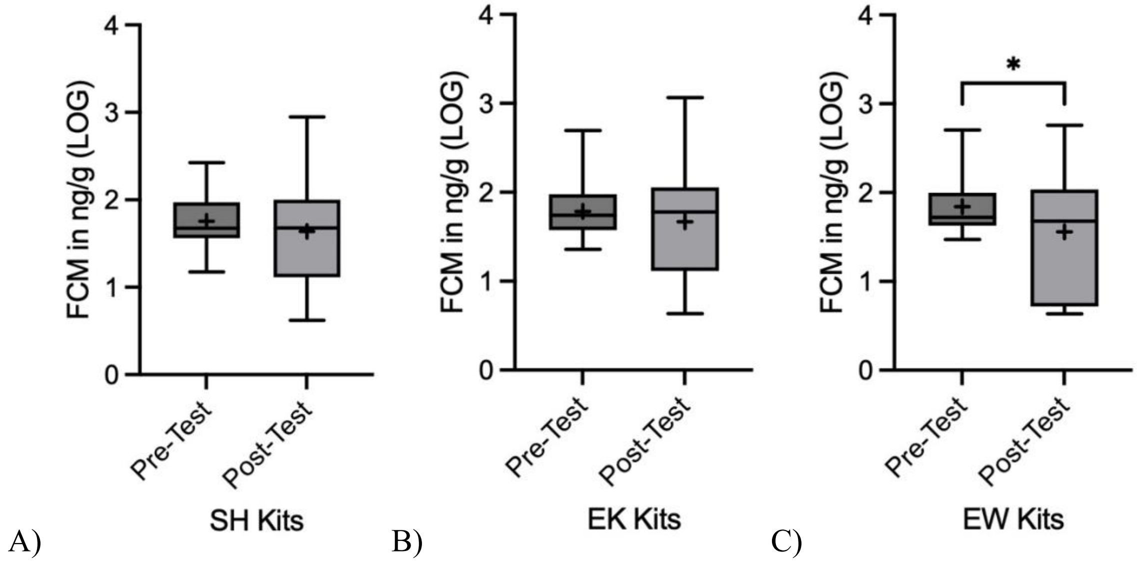


Figure 4.6 Log-transformed pre- and post-test faecal cortisol metabolite (FCM) concentrations in ng/g in **A)** SH kits (n = 29 pairs), **B)** EK kits (n = 29 pairs), and **C)** EW kits n = 32 pairs). Black + signs show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value. Bars indicate significant relationships with * indicating p<0.05.

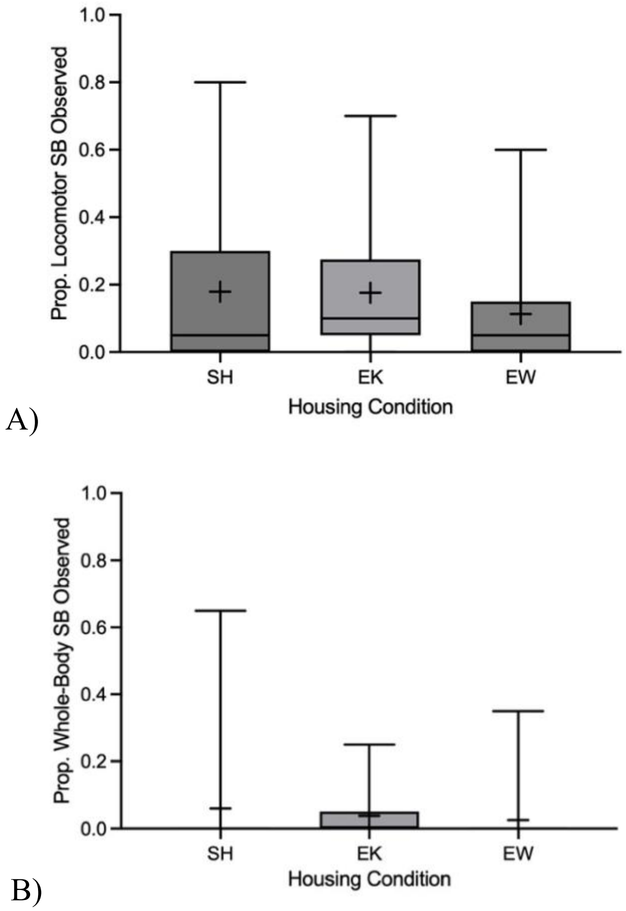


Figure 4.7 Average proportion of observations where **A)** locomotor SBs and **B)** whole-body SBs occurred in adult females of different rearing conditions. For each plot, n = 31, 37, and 36 sample points, respectively. Black squares show the means; center lines show the medians; box limits indicate the 25th and 75th percentiles as determined by GraphPad Prism software; whiskers extend from the minimum to the maximum value.

Table 4.9 Treatment effects on female kit behaviours as adults (SBs not included; represented in Figure 4.7). $n_{SH} = 31$; $n_{EK} = 37$; $n_{EW} = 36$.

Measure	SH	EK	EW	Statistic	P-value
	Mean ± SD	**Mean ± SD**	**Mean ± SD**		
Activity	0.182 ± 0.145	0.227 ± 0.142	0.204 ± 0.147	$X^2_2 = 1.856$	0.395
Resting	0.485 ± 0.325	0.446 ± 0.280	0.556 ± 0.289	$X^2_2 = 2.616$	0.270
Lying awake	0.050 ± 0.061	0.043 ± 0.049	0.040 ± 0.058	$X^2_2 = 0.819$	0.664
Lying awake in cage[a]	0.040 ± 0.060	0.034 ± 0.054	0.042 ± 0.068	$X^2_2 = 0.171$	0.918
Lying awake in NB[a]	0.008 ± 0.033	0.007 ± 0.023	0.002 ± 0.014	$X^2_2 = 0.978$	0.613
Inactivity	0.042 ± 0.061	0.066 ± 0.075	0.061 ± 0.079	$X^2_2 = 2.781$	0.249
Inactivity in NB[a]	0.005 ± 0.021	0.000 ± 0.000	0.005 ± 0.019	$X^2_2 = 2.311$	0.315

[a] Locations were only recorded on three out of five days of behaviour scans.

4.5 Discussion

Overall, several of the predicted benefits of EW and EK housing on behavioural measures of welfare were supported, with EW housing seemingly having the strongest effects. The predicted impacts of these enriched housing strategies on physiological measures of welfare (e.g., reduced basal faecal cortisol in EW dams, reduced post-stress faecal cortisol responses in EW kits, or reduced spleen weights in EW males as adults) were not found, though potential explanations for these results are further discussed below.

In support of our hypotheses for the EW intervention, there was a reduction in stereotypic behaviour observed in EW dams in the period leading up to whelping, along with an increase in resting behaviour. Dam SB may have been reduced through multiple avenues; the time available for SB performance may have been directly limited due to time spent interacting with the rope enrichment and/or extra nest building materials, or the internal drive to perform SB (if frustration-induced; Mason et al., 2007) may have been reduced by greater opportunity to perform motivated nest building behaviours. Other hypotheses for underlying causes of SB, such as boredom (Mason & Latham, 2004, Wemelsfelder, 2005), may also have been addressed by the greater variety of stimuli to interact with in EW housing. However, lying awake, a behaviour that has been hypothesized to be associated with boredom (in mink; Meagher & Mason, 2012 but see Polanco et al. 2021; in dogs; Harvey et al., 2019) was not affected. From rodent research, lying awake is also proposed to be associated with depression-like states as behavioural passivity in response to adverse situations (in mice; MacLellan et al., 2022), and EW housing had no effect on basal cortisol in dams in the peri-whelping period; thus, it is unlikely that depression-like states or other conditions associated with HPA axis activation were mitigated by EW housing. Resting, meanwhile, is associated with positive welfare (distinct from lying awake in that the subject's eyes are closed; reviewed in Fureix & Meagher, 2015). These results collectively indicate that additional nest building materials and a hanging rope enrichment conferred benefits to dam welfare as measured through behaviour in the peri-whelping period, if not evidenced physiologically.

Nest construction scores of EW dams were also improved compared to dams of other conditions. This difference reflects the achievement of walled nests (higher on all sides than the dam when lying down) with a partial overhang in EW pens, whereas SH and EK dams were not able to achieve these overhangs. EW dams often made use of all the materials provided (including the sisal rope), incorporating the entire amount into their nests. It is therefore possible that the premium materials provided to EW dams facilitated this improvement in nest construction, while standard chopped straw and wood shavings alone may be more limiting to nest shape. These results also demonstrate that dams will readily make use of the additional bedding materials provided in this study. We did not collect direct measures of nest temperature, but it is reasonable to believe that incorporation of these materials and improved nest construction would have benefited nest temperatures based on previous studies demonstrating the co-occurrence of high walled, roofed nests and higher nest temperatures (Malmkvist & Palme, 2008, Schou et al., 2018).

It was also predicted that increased behavioural opportunities for EW dams would positively impact kit-directed maternal care behaviour, including behaviours like nursing, grooming, and retrieving kits. However, the only measure of maternal care behaviour impacted by EW housing was time spent out of the nest box by dams (i.e., time where kit-directed behaviours were lacking). This result may reflect increased time spent interacting with the rope in the pen area; EW dams were occasionally recorded re-entering the nest box with unwound rope fibres or tugging on their rope enrichments from the nest box entrance, though it is uncertain whether this was always the case since the perspective of video footage was limited to the nest box. This could be viewed as an interruption to maternal care caused by maternal enrichment, however, other maternal care behaviours were not affected as they were in another study that claimed a negative impact of EE on maternal care (Li et al., 2016). It should also be noted that spending time away from kits has been shown to be beneficial for dams, particularly as kits age (e.g., reductions in dam SB; Buob et al., 2013; Dawson et al., 2013; Hansen, 1990; Jeppesen, 2004; increases in time spent resting; Dawson et al., 2013; decreases in symptoms of mastitis; Buob et al., 2013), and therefore increases in time spent away from kits may indirectly improve the maternal care behaviours dams perform by improving their welfare.

It could in fact be inferred from EW dams' increased time out of the nest box that they were more efficient in their maternal care, spending similar amounts of time performing kit-directed behaviours while in the nest box relative to SH and EK dams. This is further supported by the distinction made between 'quantity' and 'quality' of maternal care in other mammalian maternal care studies (defined as time physically spent with offspring and frequency of affiliative interactions with offspring, respectively; Aspillaga-Cid et al., 2021). While quantity, or time spent with offspring, was reduced in EW, time spent performing active maternal care behaviours was not significantly impacted. Thus, SH and

EK dams may have spent greater portions of their time in the nest box performing non-maternal behaviours. It could also be interpreted that EW dams were exhibiting increased effort to reinforce/maintain the nest structure based on anecdotal evidence of them re-entering the nest with rope from the pen area, thus also spending their out-of-nest box time performing maternal behaviour to some extent. Although measures of nursing sickness and/or mastitis were not collected in this study, it is also possible that greater time spent away from kits could have benefitted dams through the prevention of excessive suckling of kits (Buob et al., 2013, Dawson et al., 2013). Moreover, the trend towards decreased kit mortality observed from first weight to three weeks in EW litters supports that the EW intervention may have benefitted reproductive success, despite no observed changes in maternal care; this effect may be revealed as significant if further, farm-wide studies using larger sample sizes are conducted using this intervention.

It should also be noted that our sample size for these measures of maternal care was reduced due to technical difficulties encountered with the video equipment, which limited statistical power to detect differences. Recording of time spent nursing may also have been confounded by the quality and perspective of nest box camera footage, as kits were often not visible due to the dams' nursing postures (active bouts of nursing were only recorded when at least one kit could be seen attached to the nipple, or when kits were presumed to be suckling based on body position if the head was out of view). Similarly, bouts of nursing behaviour may have appeared shorter in dams who adjusted their posture more frequently, as kits would briefly appear in view before being concealed again.

Despite this limitation, the data collected are interesting in that it seems mink dams nurse almost continuously throughout the day (occupying approximately 70% of their observed time budget, and this may be an under-estimation). Patterns and circadian rhythm effects on maternal care are not well understood in mink, unlike other species whose maternal care is thoroughly documented (e.g., rabbits are known to nurse offspring roughly twice per day in bouts of less than 10 minutes each, while mice perform 25-35 nursing bouts of roughly 20-30 mins per day, and distinct sequences of maternal care have been identified for each species; Champagne et al., 2007, González-Mariscal et al., 2016; Jilge & Hudson, 2001). Such documentation of maternal care behaviours is useful in identifying whether dams are providing high- or low-quality maternal care, based on what is standard for the species. For example, assessment of 'fragmented' patterns of maternal care, which are known to have negative consequences for offspring HPA axis development in rodents (Ivy et al., 2008, Molet et al., 2016, Couto-Pereira et al., 2016), is only possible when species-specific sequences of maternal care have been determined. It could also be investigated in future studies whether less frequent nursing or nursing in shorter bouts is indicative of higher-quality milk production (i.e., milk of higher caloric content). Previous studies in mink have successfully quantified protein and fat content of maternal milk across postpartum weeks (Fink et al., 2001, Tauson et al., 2004), and similar methods could be applied to determine if enrichment of the dam increases milk quality, thus increasing kit weights despite reduced frequencies of nursing bouts or nursing bout durations, as has been found in rodents (DeRosa et al., 2022).

In video recordings with limited visibility such as those used in the present study, more information about quality of nursing could potentially be derived by categorizing the nursing postures of dams. There has been extensive research on nursing postures which are beneficial for offspring development in rodents, such as arched-back nursing (Myers et al., 1989) which is also known to be highly correlated with licking and grooming of pups (Meaney, 2001), and its proposed analogue in dogs, vertical nursing (Bray et al., 2017). Meanwhile, maintaining a flat or inert position while pups are attempting to nurse is considered lower quality maternal care (described in Champagne et al., 2003; Peña and Champagne, 2013). Nursing postures were not defined or categorized as such in the present study and have not previously been defined in mink, aside from postures that block access to teats entirely (Dawson et al., 2013). Investigation of the impact of nursing postures on kits' ease of access to milk and correlation with licking and grooming behaviour may therefore be informative to assess in future studies of mink maternal care. However, it should also be noted that most existing research on the role of maternal nursing, licking, and grooming on long-term offspring fear behaviour and/or HPA axis reactivity has featured species that are social. There is limited evidence that this particular mechanism applies in solitary carnivores like mink, and though there has been recent work demonstrating that early separation from the mother with or without sibling presence in cats (a relatively solitary carnivorous species) affects later emotional reactivity of kits (Martínez-Byer et al., 2023), this study did not directly measure outcomes of early maternal separation or lessened maternal care on kit HPA axis responsivity.

Relatedly, our prediction that pre- and post-stress faecal cortisol in EW kits would not significantly differ due to improvements in HPA axis regulation was not supported. EW kits' post-stress faecal cortisol metabolite concentrations were in fact significantly decreased compared to that of the pre-stress period, though this result also does not directly oppose our prediction since post-stress cortisol levels would be expected to increase if feedback sensitivity of the HPA axis was reduced. Moreover, there was no change in SH or EK kits' faecal cortisol across these periods. These results are highly unexpected given that a previous study using these methods of cortisol sampling in mink did find increases in faecal cortisol following both a 15-minute period of immobilization in a carrying cage and a 2-hour period of 'handling' in which mink were trapped, immobilized in a carrying cage, and repeatedly sampled for blood (Malmkvist et al., 2011).

However, in the present study pelt grading was implemented as an alternative to prolonged immobilization stress, which may be the source of this discrepancy. Pelt grading is a novel experience for kits and involves capture, restraint on a pelt grading table under a bright light, and manipulation of the pelt to assess hair nap, thus it was presumed to be sufficient to evoke a stress response (moreover, there is evidence from a previous study that fearful temperaments in mink increase following pelt grading; Bak & Malmkvist, 2020). However, we cannot exclude the possibility that the experience was too brief to induce measurable changes in faecal cortisol as kits were typically returned to their pens after only one or two minutes of handling. A recent study was also unable to detect differences in minks' faecal cortisol before and after 15-min immobilization in a carrying cage and relocation to a new pen due to high individual variation in cortisol levels at each sampling point, and relatively high baseline means which the authors propose may have blurred cortisol responding due to a ceiling effect (Malmkvist et al., under review). Thus, there may be high levels of variation in individual responses to handling or immobilization stress of certain durations and intensities. Responses to these stressors may also vary between farm populations, as fear of humans has been known to do (Meagher et al., 2011), and fear traits are known to be highly heritable and subject to selection in mink (Berg et al., 2002; Hansen, 1996; Malmkvist & Hansen, 2001, 2002; Thirstrup et al., 2019). Relatively low levels of fear behaviours were exhibited by our subjects during pelt grading; approximately twenty percent of kits assessed for stress responsiveness demonstrated struggling, biting, or urination during handling, and fear vocalizations occurred in very low numbers on average (mean of 1.05 vocalizations across all treatments combined).

The pelt grading event may also have been perceived by kits as similar to past stressors (i.e., previous handlings for immunizations, weight recordings, or pen moves); rodent studies have demonstrated the potential for a high degree of adaptation of corticosterone responses after repeated exposure to acute stressors, particularly if these stressors occur in adolescence – perhaps as a result of the greater ability of juveniles to adapt their behaviour as a stress-coping strategy (Sadler & Bailey, 2016; Papilloud et al., 2018). It could also be postulated that kits were demonstrating blunted HPA axis responses to stress (hypo-responsiveness or non-responsiveness to stress can occur in cases of extreme chronic stress; Herman et al., 2016), though there was limited evidence of chronic stress markers in our subjects. For example, the spleens of males across all groups were relatively heavy, which is hypothesized to be indicative of greater ability to invest in lymphocyte production or storage and thus good health and low-stress conditions (Nunn, 2002). However, we were presently unable to assess hormonal activity at other stages of the HPA axis response (e.g., ACTH or CRH levels), nor other tissue-related measures of stress (e.g., adrenal weights), so speculation on this point is limited.

As summarized in Chapter 3, EK kits showed more overall and more sustained enrichment use across the juvenile period compared to kits in standard housing. However, as shown here, the predicted effects of EK housing on other kit behaviours were largely unsupported. During the group-housing phase, neither positive welfare-related behaviours (social play or resting) nor behaviours potentially associated with boredom and/or stress (forms of waking inactivity and aggression) differed between SH, EK, and EW. During pair-housing, EK kits did demonstrate reduced inactivity in the nest box compared to kits of other conditions and increased lying awake in the pen compared to SH kits (10-13 weeks post-whelping). However, given that each of these forms of inactivity are suggested to be associated with negative states in mink (fear and boredom, respectively; Fureix & Meagher, 2015; Meagher & Mason, 2012; Meagher et al., 2013, 2017), it is difficult to determine whether EK enrichments were able to improve kit affective states in this period. Moreover, social play occurred more often in EW kits than in EK kits during pair housing. This may be indicative of reduced stress in EW kits compared to EK kits, as social play is known occur primarily in healthy, unstressed animals (Burghardt, 2005). However, cortisol concentrations were not assessed during juvenile behavioural observations, and prior to pelt grading at six months of age there was no difference in pre-test (basal) faecal cortisol metabolite concentrations between kits of different conditions.

The main shortcoming of the EK intervention was that it was not successful at reducing SB development in the kits as adults. In fact, EK kits showed signs of developing more whole-body SB than SH or EW kits. Although there was no statistical difference in overall performance of whole-body SB, the interquartile range for this subtype in EK mink was larger than that of mink in other treatments. Types of SB were distinguished in the present study due to previous evidence that sub-types of SB are heterogeneous in mink, although thus far only scrabbling and head-based forms have been suggested to be distinct from locomotor forms (both stationary and those involving translocation) in their causation and treatments (Dallaire et al., 2011; Díez-León et al., 2013, 2019; Polanco et al., 2017, 2018). Given that locomotor forms and whole-body, stationary forms are known to co-occur in mink (Polanco et al., 2017; Malmkvist et al., under review), the greater tendency of EK mink to display both is interesting. Overall activity also did not differ between groups, so this is an unlikely explanation for any differences in SB expression. Increases in enclosure complexity have been suggested to impede the space available to perform SB, or make them more complex in appearance (e.g., an established route-tracing stereotypy in a cape hunting dog was impeded by introduction of a hanging chain and the route was then adjusted to avoid the chain; Fentress, 1976; also c.f. Bergeron et al., in press on oral behaviours in hungry animals), so it is possible that the extra mobile and hanging EEs for EK kits acted as obstacles that caused alternative, stationary forms of SB to develop.

Moreover, our finding that performance of locomotor SB or any forms in general were not reduced in EK mink contradicts the findings of previous studies where access to a multitude of physical enrichments in early life or adulthood positively impacted SB (Campbell et al., 2013; Dallaire, 2012; Díez-León et al., 2016; Hansen et al., 2007; Meagher et al., 2013; Meagher & Mason, 2012). However, these impacts were often delivered through EE being present in the subjects’ pens at the time of SB assessment; or, in the case of Díez-León et al. (2016), the earliest SB assessment was conducted after only five weeks of removal from EE. The present study is the first to investigate whether providing EE to mink exclusively as juveniles can attenuate performance of SB up to one year following removal. In bank voles (Ödberg, 1987) and deer mice (Hadley et al., 2006; Powell et al., 2000), rearing in EE is shown to have protective effects against the development of SB even after long-term placement in standard pens. In other species, meanwhile, removal of temporary EE can exacerbate stereotypy (e.g., in primates: Bayne et al., 1992; in pigs: Day et al., 2002; in CD-1 mice: Latham & Mason, 2010). The latter scenario is thought to result from frustration due to placement in environments with fewer behavioural opportunities relative to their more stimulating, rewarding environments (a so-called 'negative contrast' effect), whereas animals who have not experienced these enriched conditions do not have the same frustrations (Crespi, 1942; Pecoraro et al., 1999; Wiegmann et al., 2003). It is therefore plausible that EK mink became accustomed to greater, more diverse enrichment and experienced frustration after subsequent placement in standard EE with only one, permanently present enrichment, resulting in development of frustration-induced SB. However, behavioural frustration in negative contrast scenarios typically also correlates with increased glucocorticoid output (Latham & Mason, 2010), which was not observed in EK kits relative to kits of other conditions during faecal cortisol sampling two months after EE removal (i.e., at pelt grading).

Moreover, in Díez-León et al. (2016), mink reared in EE and then placed in standard housing did not demonstrate exacerbated SB following EE removal and in fact showed reductions in locomotor SB compared to mink that had never experienced enrichment. This may be due to the timing of EE removal: enriched mink were placed in standard pens for five weeks in mid-November, when mink were six or seven months of age, and their SB was assessed in these standard pens before being returned to enriched pens. SB does not fully develop in mink until seven months of age (Jeppesen et al., 2000), at which point extra EEs for EK mink in the present study had already been removed. Thus, EE may only have protective effects against SB development in mink when supplied from rearing until approximately seven months of age, a potentially critical age of SB development. Interestingly, Axelsson et al. (2009) introduced enrichments to mink at seven months of age when subjects had already begun exhibiting SB, and one of the two farms housing trial mink did show an enrichment-related decrease in SB while the other did not. Thus, it is possible that seven months is a critical age for the provision of EE in mink, but provision leading up to this age is more effective than introduction once SBs have already begun to develop. In the aforementioned studies with deer mice, EE was also provided early in life (from 4-14 or 14-21 weeks of age: Hadley et al., 2006; detailed timing of provision is unknown in Powell et al., 2000), and SB in deer mice is thought to become stable by 6 weeks of age (Tanimura et al., 2010). Thus, the mice also had access to enrichments prior to and during the period in which SB becomes fully developed, and this EE strategy was also protective against future development of SB.

Of course, the lack of SB reduction in EK mink, or lack of statistical difference in whole-body SB of mink across housing conditions, may also have resulted from a false negative. Our sample of mink followed for SB development as adults was limited due to pelting of select individuals in their first year of life (according to standard farm practices) and other factors such as mortality, thus affecting our statistical power to detect differences. In future research it would be beneficial to follow a larger sample of females (and potentially males, as well) through to adulthood to observe their SB development after differential housing as juveniles. Alternatively, the amount of behavioural data collected from the available mink could be increased (i.e., observations could be conducted across a longer period than five days, or the number of scans per day could be increased). Regarding our speculations about timing of removal for EK enrichments, we recommend that future studies attempt to house mink with the nature of EE provided in this study until seven months of age to determine if this duration of enrichment is sufficient to deliver lasting preventative effects on SB, even after long-term placement in standard housing.

4.6 Conclusion

Overall, the EW intervention had a greater relative number of benefits than the EK intervention: the nest building materials and hanging rope provided in EW positively impacted dam behaviours, including stereotypic behaviour and resting prior to whelping, nest shapes were improved, and there was a trend towards decreased kit mortality up to three weeks post-whelping. Maternal stress and maternal care delivered to kits did not appear to be impacted by this intervention, though EW dams may have been more efficient in their kit-directed maternal care behaviour. Since nursing and grooming of kits was not performed at higher levels in EW dams, it follows that EW kit stress responsiveness did not appear to be affected; though, the stress event used for this test may have been inadequate to observe an HPA axis response due to insufficient restraint durations or habituation of kits' cortisol responses to repeated handling. The EK intervention was successful at reducing a potential behavioural indicator of fear in the late juvenile period, but a potential indicator of boredom was simultaneously increased.

Stereotypic behaviour development in the kits as adults was also not affected by EK housing; this demonstrates that additional enrichment as juveniles may not attenuate stereotypic behaviour performance in adult mink after one year of placement in standard housing. It would be valuable to determine in future studies if juvenile enrichment of this nature has long-term benefits when maintained until seven months of age, when stereotypic behaviours are known to become established in mink. In terms of potential on-farm applications of these enrichment strategies, this study demonstrates that dams will utilize additional nest building materials (particularly crumpled paper tissue) and will unwind hanging rope enrichments to weave into their nests. Further, access to these materials may improve reproductive success; however, benefits of these materials to maternal care and offspring stress physiology should be further investigated.

5. General Discussion

American mink have been the main furbearing species farmed under commercial conditions for the past 60 years and are the primary contributor to profits in the fur industry worldwide (Oaten, 2016). However, the fur industry, and animal-based industries generally, have been facing increasing pressure to adapt housing and husbandry practices to promote better animal welfare. For these reasons, among other concerns regarding environmental and anthropo-zoological health impacts of the industry (e.g., Fenollar et al., 2021), several countries have begun to phase out the practice of fur farming, but in many countries the industry is still profitable and demand for furs remains high (*Strong Demand and Rising Prices on Mink Skins*, 2021). In fact, a country with traditionally high production of mink whose fur industry was particularly impacted by recent viral outbreaks (i.e., the SARS-CoV-2 virus) has lifted a temporary ban on fur farming, and plans to resume operations (*Denmark*, 2022). In Canada and the United States, mink farming was continued through the viral pandemic (see Table 6.1 for a summary of annual production in recent years), and there appear to be no plans to cease production (see Doucet, 2021 for exception regarding a ban on fur farming in the province of British Columbia).

It is imperative that husbandry methods and cage environments for farmed mink be continually adapted based on the recommendations of animal welfare research to ensure the continued success and social sustainability of the fur industry. As discussed in previous chapters, there are several outstanding issues with mink farming practices which should be a priority of research. Two such issues, which were the focus of this thesis, include the widespread prevalence of stereotypic behaviour in farmed mink populations

Table 5.1 *North American mink pelt production statistics in recent years (millions)*

Year	2016	2017	2018	2019	2020	2021	2022
Canada	2.14[a]	2.22[a]	1.76[a]	1.21[a]	1.01[a]	No data	No data
United States	3.45[b]	3.40[b]	3.17[b]	2.74[b]	1.44[b]	1.57[b]	1.33[b]

[a](Government of Canada, 2021)
[b](National Agricultural Statistics Service (NASS), Agricultural Statistics Board, United States Department of & Agriculture (USDA), 2023)

and the inadequacy of nest building materials and other aspects of the whelping environment, which lead to poor dam welfare and moderate levels of kit mortality (European Commission, 2001). In this thesis, these issues were targeted by implementing various enrichment strategies in the juvenile period and the peri-whelping period, respectively, with the goal of identifying effective physical enrichment strategies in mink, preventing the development of stereotypic behaviour, reducing kit mortality, and promoting good welfare states and long-term fitness in dams and their kits. This discussion will review the primary outcomes of the research undertaken in this thesis in relation to each of these objectives, as well as practical implications for farming practices and recommendations for future research.

5.1 Summary of thesis outcomes

The objectives of this thesis were, in part, to determine a) how early-life provision of diverse and novel physical enrichments affect kit behaviour in the juvenile phase; b) how enrichment provided exclusively from 1-4 months of age mediates stereotypic behaviour development in kits; and c) which enrichment type(s) are used most by kits. For the latter purpose, use of both ‘mobile’ and ‘fixed’ enrichment types that were regularly exchanged to renew novelty was investigated. It was determined in Chapter 4 that measures of kit behaviour in the juvenile period were not positively affected by this physical enrichment strategy, aside from performance of inactivity in the nest box which was significantly decreased in the EK group during pair-housing (a behavioural sign hypothesized to be associated with fear; Fureix & Meagher, 2015; Meagher et al., 2013) and increased enrichment use in the EK group compared to standard-housed kits across the entire observation period (demonstrated in Chapter 3).

Although the measure of increased EE use itself is not validated as a measure of good welfare, increased and/or sustained levels of enrichment use are thought to be necessary to confer the welfare impacts of EE. Use of enrichments is also related to concepts such as exploration of the environment and performance of goal-directed behaviours presumed to be rewarding; novel objects and prey-like toys have been demonstrated to be valuable to mink, and the specific enrichments provided here have previously been found to confer welfare benefits (aside from hockey balls, though similar play balls have been used in past studies; Cooper & Mason, 1997, 2000; Díez-León et al., 2013; Díez-León & Mason, 2016; Meagher et al., 2014). The present finding of increased EE use in the EK group is therefore interesting since it demonstrates that providing mink with multiple, diverse, and novel enrichments can increase enrichment use above providing one, constant enrichment. Moreover, there was evidence of less habituation to enrichment in EK housing than in standard housing. Mobile items were used more than hanging items overall, which conflicts with the preferences of mink previously indicated by other studies and supports our speculation that these studies were confounded by either lack of equivalent novelty between mobile and fixed items or a conflation of uses of structural

EE, which can be used for resting, and other types of fixed and mobile EE, which are actively used.

Despite the increased EE use observed in EK kits as juveniles, the timing of EE provision or removal in this study was ineffective at preventing stereotypic behaviour development; performance of locomotor and whole-body forms of stereotypic behaviour did not statistically differ between mink of different groups one year following placement of EK mink in standard housing (interestingly, head-based forms and scrabbling were not observed in this cohort). However, the age of EE introduction implemented in this study appeared to be effective in that kits made use of enrichments beginning at six weeks of age (i.e., when observations of EE use began), and EE use was maintained, if not increased, until 13 weeks of age (i.e., when observations of EE use concluded). It is likely that the timing of EK enrichment removal was the source of this ineffectiveness, as interrupting access to EK enrichments prior to seven months of age when stereotypic behaviour becomes established in mink (Jeppesen et al., 2000) may have limited their ability to modulate stereotypic behaviour development.

In this thesis we also aimed to determine how greater nest building opportunities for dams in the peri-whelping period affect a) dam welfare, b) maternal behaviour and offspring survival, and c) stress responsiveness of kits. Provision of more numerous, high-quality nest building materials to dams in EW conferred benefits to behaviours associated with good welfare (e.g., increased resting) and reduced dams' performance of stereotypic behaviour, though their basal cortisol levels were not affected by this intervention. Measures of maternal care directly provided to kits were relatively unaffected by EW housing, but nests built by EW dams were better constructed than nests built by dams with standard nesting materials and there was a trend towards decreased kit mortality in EW litters. This effect may have been due to improvements in internal temperatures in EW nests; as demonstrated by Malmkvist & Palme (2008), nests with higher walls and full or partial roofs have improved nest temperatures, and these features were often achieved by EW dams. It is also discussed in Chapter 4 that maternal care in EW dams may have been more efficient than maternal care in standard-housed dams, since EW dams spent greater percentages of time out of the nest box.

However, there is limited evidence that enrichment of the whelping environment was able to confer long-term benefits to EW kit stress responsiveness. Kits in SH and EK housing conditions showed negligible changes in faecal cortisol before and after experiencing an experimental stressor, while EW kits had significantly reduced faecal cortisol following the stressor. This result is unexpected and does not reliably demonstrate more efficient HPA axis responsivity in EW kits compared to kits of other conditions. However, there was evidence of increased social play in EW kits during pair-housing compared to kits in the EK condition, and social play is thought to occur in healthy, unstressed animals (Burghardt, 2005). Although there were no measures of HPA axis activity collected during pair-housing, this behavioural measure may indicate reduced basal stress in EW kits compared to EK kits.

5.2 Practical implications for commercial mink farming

Of the items provided to EK mink, the pig's ear, the plastic ring, and the wiffle ball were the most-used mobile enrichments, and the sisal rope was the most-used hanging enrichment. These items were identified as having more numerous uses, greater malleability, or greater controllability than the other object types provided (Abou-Ismail & Mendl, 2016; Axelsson et al., 2009; Pisula et al., 2021; Sambrook & Buchanan-Smith, 1997); thus, these properties may be preferable in enrichments for mink. Although the plastic ring, an enrichment that is standard to provide on farms in Canada, was commonly used by kits in EK, access to an array of EE significantly increased use of enrichments compared to only a standard ring. This indicates that the current practice of providing only one mobile enrichment in pens is insufficient to promote optimal EE use.

Although increases in item use corresponding with renewal of novelty were not always statistically significant, there were qualitative increases in item use in weeks where familiar items were exchanged for novel ones. Moreover, these hypothesized novelty-induced increases in use appeared to be more sustained in weeks where pig's ears were introduced. Thus, exchanging EE for mink may be more effective at maintaining kits' interest in the items if their properties remain mentally stimulating for longer periods. If provided in farm settings, items that are less stimulating or have fewer uses (e.g., golf balls or hockey balls) may need to be exchanged as often as on a weekly basis to prevent habituation effects and deliver greater impacts on mink behaviour. Thus, less stimulating items may be more labour-intensive for farms to provide given that they require more frequent exchange to maintain their welfare benefits. More stimulating items, meanwhile, may incur greater costs to maintain or replace (the most-used items in the present thesis were destructible; both the pig's ears and sisal rope were anecdotally observed to be depleted within a minimum of two days and a maximum of 14 days), but may attract more use over time and would require less frequent exchange. An alternative option may be to provide items that were presently found to attract relatively high, sustained use (e.g., wiffle balls and plastic rings), while also presenting lower replacement costs and requiring less effort from farmers in terms of frequent item exchange.

This thesis also informs the age at which EE can effectively be introduced in commercially farmed mink; kits of all conditions used enrichments to some extent as soon as six weeks of age (i.e., from the first week after weaning; approximately 4.94% of active time in EK kits and 2.71% of active time in kits of other conditions), indicating that enrichments of both the mobile and hanging variety are useful at this time. Use of mobile enrichments was higher at this age compared to use of hanging enrichments (approximately 37.0% and 14.6% of EE use observations in EK kits, respectively), suggesting that mobile EE may be more appropriate to provide at or before six weeks of age than hanging EE; though, this difference may be due to the greater availability of mobile enrichments in the cage compared to number of kits, or the fact that one new mobile enrichment was introduced to the cage at this time. Use of the ring enrichment did increase significantly at seven weeks of age in both EK and standard housed kits, which aligns with findings stated by Jonasen (1987); however, this author suggested that object play does not emerge until seven weeks of age in mink kits, whereas here, play object use was observed earlier.

In terms of improving nest building material provision for dams, this thesis demonstrated that dams will readily make use of crumpled paper tissue, curled excelsior aspen shavings, and unwound sisal rope, and that these materials will additionally improve the structure of the nest. Dams most often used the entirety of these materials (including unwinding rope from the hanging rope enrichment and incorporating it into the nest), followed by a combination of crumpled paper and aspen shavings. When only one material was used in isolation, the crumpled paper tissue appeared most popular; therefore, crumpled paper tissue may be a highly valued bedding material for mink, but preferences for bedding may differ by individual. Furthermore, provision of these materials is beneficial for dams in that it can increase their resting behaviour and decrease their performance of stereotypic behaviour. There has been concern surrounding the provision of enrichment for dams in the peri-whelping period as a potential source of interruption to maternal care, but maternal care provided to kits did not appear negatively affected by the additional nest building materials or hanging rope provided; although dams may have spent more time in the cage as a result of interaction with the rope, this did not significantly reduce their performance of other kit-oriented behaviours or their reproductive success (i.e., kit mortality).

5.3 Recommendations for future research

As discussed in Chapter 3, it cannot be concluded from the results of this thesis whether variations in enrichment use across weeks of observation, particularly between post-weaning Week 1 and Week 2, were a result of developmental or environmental factors. For example, low enrichment use at six weeks of age compared to subsequent increases at seven weeks of age were speculated to reflect impacts of weaning, which is stressful for kits (Houbak and Jeppesen, 1987 as reported in Nimon & Broom, 1999). However, as no measures of HPA axis activity or behavioural indicators of stress (e.g., vocalizations; Jeppesen et al., 2000) were collected from kits at this time, it cannot be determined whether kits' stress levels differed between these two weeks of observation, or whether sufficient levels of stress can reduce enrichment use in mink. Conversely, some studies demonstrate that intervention with EE can mediate stress effects and promote explorative behaviour, particularly in early life (Bak & Malmkvist, 2020; Mkwanazi et al., 2018). This is an area that would be beneficial to explore in future research, particularly if it could be determined whether placement in EE at weaning can reduce the negative impacts of weaning, with or without inducing measurable changes in exploratory behaviours oriented towards enrichments.

The findings discussed in Chapter 3 also led to the proposal of different categorization systems for types of EE. The most common categorizations of physical EE currently used in the literature are ‘fixed’ (or ‘hanging’) and ‘mobile’ enrichments, though it was highlighted in this thesis that EEs classified as fixed or hanging may not share the same functions or satisfy the same behavioural needs in mink. The hanging lengths of rope or chain used in this study, although typically defined as fixed, more closely resembled mobile enrichments based on their use for manipulation, tugging, and chewing, yet they were not mobile to the same extent as loose items on the cage floor. Thus, we proposed that it may be appropriate in future studies to subdivide the category of ‘fixed’ or ‘hanging’ enrichments into fully fixed structural enrichments, which provide an additional resting space, versus fixed but manipulable objects which can only be climbed, pulled, chewed, etc., when investigating the effects of enrichment properties on their use.

It may also be interesting in future research to quantify the proportion of active or inactive time mink spend using different enrichment types, and the relative effectiveness of these forms of use in improving welfare parameters. Certain fixed EEs have demonstrated benefits when used passively (Buob et al., 2013; Dawson et al., 2013; Hansen et al., 2007; Hansen et al., 1994; Hansen et al., 2011; Jeppesen, 2004), but these studies did not specify whether EEs were only used during periods of inactivity, or for what proportion of the total time spent inactive. It is plausible, for instance, that these fixtures may also provide active climbing opportunities (e.g., rapidly jumping up and down from structures), and it has not been determined whether one of these forms of fixed EE use is more beneficial than another. Likewise, analyzing use of mobile EE or fixed but manipulable EE with respect to time spent active may reveal further information regarding their value for mink or the specific mechanisms of their welfare benefits. Mobile EE is typically recorded in use while being chased or chewed, but can also be used passively (e.g., resting in the ring enrichment was anecdotally observed in the present thesis). It is therefore possible that these enrichments may fulfill behavioural needs unrelated to active use, and there is an opportunity to determine if mink who more frequently rest in or around mobile EEs during periods of inactivity demonstrate greater welfare benefits.

Similarly, it has recently been suggested by Decker et al. (2023) that EE need not be ‘used’, as defined in most studies, to confer welfare benefits. The behavioural motivations addressed by enrichment may be quickly satisfied, thus reducing their use; or, use of enrichment in some contexts may be a means to an end rather than rewarding in itself (e.g., stereotypic burrow digging was reduced in gerbils provided with artificial burrows, indicating that digging itself might not be rewarding and the end result, the burrow, is what is desired; Waiblinger & König, 2004; Wiedenmayer, 1997). Decker et al. (2023) also posit that the presence of EE can benefit welfare through offering opportunities for control and choice (i.e., having the option to interact with EE is better than not having EE), or that the presence of certain EE is beneficial since ancestrally it would have signalled good environments for the species (e.g., opportunities to hide or substrates to burrow or nest in). Thus, in future research it should be considered that unconventional forms or levels of enrichment use, as well as the mere presence of EE, may confer welfare benefits.

Regarding further research on the whelping environments of mink, it was suggested in Chapter 4 that maternal care behaviours and consequent effects on kit stress response development be further investigated in mink. Commercially farmed mink offer a valuable opportunity to study maternal care behaviour in a model non-social carnivore; most research regarding quality of maternal care has been conducted in rodents, pigs, non-human primates, humans, and dogs (summarized in Lezama-García et al., 2019). Moreover, large sample sizes of mothers and offspring are available on mink farms and their behaviours in the nest box can be observed relatively easily. Although we encountered difficulty with video recordings of maternal care in this thesis, future studies could improve upon these methods to allow detailed quantification of maternal care behaviours or derive further information about quality of nursing behaviour through the identification of nursing postures (e.g., 'arched-back' nursing and 'vertical' nursing have been identified as beneficial nursing postures in rodents and dogs, respectively; Bray et al., 2017a, 2017b). Nursing postures have not previously been defined in mink, aside from postures that block access to teats entirely (Dawson et al., 2013).

Moreover, documentation of patterns and circadian rhythm effects on maternal care has been beneficial in assessing 'fragmented' patterns of maternal care in rabbits and mice compared to what is standard for the species (e.g., Jilge & Hudson 2001, Champagne et al. 2007, González-Mariscal et al. 2016). Fragmented maternal care is known to have negative consequences for offspring HPA axis development in rodents (Couto-Pereira et al., 2016; Ivy et al., 2008; Molet et al., 2016) and this would be beneficial to investigate in mink. Further research in general regarding the effects of high- or low-quality maternal care on stress response development in mink kits would be a valuable contribution both to the fields of neurobiology and animal welfare; determining to what extent maternal care mediates later susceptibility to chronic stress and stress-related disorders in mink would inform whether promotion of better maternal care through amelioration of whelping conditions for dams can contribute to better offspring welfare. As discussed in Chapter 4, the stressor used to evaluate HPA axis responsivity in the present thesis was likely insufficient to elicit measurable changes in faecal cortisol, and thus we could not determine whether EW housing improved offspring stress physiology; this test should ideally be replicated in future studies with a standard stress event such as a 15-minute immobilisation session (Malmkvist et al., 2011).

5.4 Conclusion

The research undertaken in this thesis revealed novel insights about the relative effectiveness of different means of enrichment and timings of intervention. Enrichment of the perinatal/peri-whelping environment was relatively more beneficial for mink (including dams and their kits) as assessed through behaviours related to good welfare compared to enrichment of the post-weaning juvenile environment, though neither intervention had measurable effects on basal stress, stress responsivity, spleen weights, or development of stereotypic behaviour in kits. In terms of potential on-farm applications of these enrichment strategies, this thesis demonstrates that dams will utilize nest building materials such as crumpled paper tissue and curled excelsior shavings and will unwind hanging rope enrichments to weave into their nests. Further, access to these materials may improve reproductive success; however, benefits of these materials to maternal care and offspring stress physiology should be further investigated. It was also demonstrated that enrichments with more numerous uses, greater malleability, or greater controllability were most effective at promoting EE interaction, though future studies should determine whether physical enrichment of this nature has long-term protective effects on stereotypic behaviour if maintained until seven months of age rather than removed at four months of age. This research contributes to our knowledge of the effects of housing and husbandry on the health and welfare of farmed mink, and further research on the topics proposed in this thesis may be instrumental in continuing to adapt farming practices to promote good welfare in farmed animals.

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Appendix

A1. Post-hoc comparisons of enrichment use by week of observation

Table A1.1 Post-hoc comparisons by week of Hanging EE use in EK pens (sqrt-transformed)

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
W1	-	W2	-0.03831	0.0153	-2.503	256	0.362
W1	-	W3	-0.03217	0.0153	-2.103	256	1.000
W1	-	W4	-0.02129	0.0153	-1.391	256	1.000
W1	-	W5	-0.11503	0.0146	-7.882	263	< .001
W1	-	W6	-0.06775	0.0146	-4.642	263	< .001
W1	-	W7	-0.00162	0.0146	-0.111	263	1.000
W1	-	W8	-0.00604	0.0146	-0.414	263	1.000
W2	-	W3	0.00613	0.0153	0.401	256	1.000
W2	-	W4	0.01702	0.0153	1.112	256	1.000
W2	-	W5	-0.07673	0.0146	-5.257	263	< .001
W2	-	W6	-0.02944	0.0146	-2.017	263	1.000
W2	-	W7	0.03669	0.0146	2.514	263	0.351
W2	-	W8	0.03227	0.0146	2.211	263	0.781
W3	-	W4	0.01089	0.0153	0.711	256	1.000
W3	-	W5	-0.08286	0.0146	-5.678	263	< .001
W3	-	W6	-0.03558	0.0146	-2.438	263	0.432
W3	-	W7	0.03055	0.0146	2.093	263	1.000
W3	-	W8	0.02613	0.0146	1.791	263	1.000
W4	-	W5	-0.09375	0.0146	-6.424	263	< .001
W4	-	W6	-0.04646	0.0146	-3.184	263	0.046
W4	-	W7	0.01967	0.0146	1.348	263	1.000
W4	-	W8	0.01525	0.0146	1.045	263	1.000
W5	-	W6	0.04728	0.0138	3.435	256	0.019
W5	-	W7	0.11341	0.0138	8.238	256	< .001
W5	-	W8	0.10899	0.0138	7.917	256	< .001
W6	-	W7	0.06613	0.0138	4.803	256	< .001
W6	-	W8	0.06171	0.0138	4.482	256	< .001
W7	-	W8	-0.00442	0.0138	-0.321	256	1.000

Table A1.2 Post-hoc comparisons by week of Ring EE use in EK pens (sqrt-transformed)

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
W1	-	W2	-0.04746	0.0141	-3.3748	257	0.024
W1	-	W3	-0.04786	0.0141	-3.4032	257	0.022
W1	-	W4	-0.03716	0.0141	-2.6426	257	0.244
W1	-	W5	-0.01971	0.0134	-1.4725	264	1.000
W1	-	W6	-0.01428	0.0134	-1.0667	264	1.000
W1	-	W7	-0.00607	0.0134	-0.4531	264	1.000
W1	-	W8	-0.03300	0.0134	-2.4651	264	0.401
W2	-	W3	-4.00e−4	0.0141	-0.0285	257	1.000
W2	-	W4	0.01030	0.0141	0.7322	257	1.000
W2	-	W5	0.02774	0.0134	2.0725	264	1.000
W2	-	W6	0.03318	0.0134	2.4783	264	0.387
W2	-	W7	0.04139	0.0134	3.0919	264	0.062
W2	-	W8	0.01446	0.0134	1.0799	264	1.000
W3	-	W4	0.01070	0.0141	0.7606	257	1.000
W3	-	W5	0.02814	0.0134	2.1024	264	1.000
W3	-	W6	0.03358	0.0134	2.5082	264	0.357
W3	-	W7	0.04179	0.0134	3.1218	264	0.056
W3	-	W8	0.01486	0.0134	1.1098	264	1.000
W4	-	W5	0.01745	0.0134	1.3034	264	1.000
W4	-	W6	0.02288	0.0134	1.7092	264	1.000
W4	-	W7	0.03110	0.0134	2.3229	264	0.587
W4	-	W8	0.00416	0.0134	0.3108	264	1.000
W5	-	W6	0.00543	0.0127	0.4294	257	1.000
W5	-	W7	0.01365	0.0127	1.0787	257	1.000
W5	-	W8	-0.01329	0.0127	-1.0502	257	1.000
W6	-	W7	0.00822	0.0127	0.6493	257	1.000
W6	-	W8	-0.01872	0.0127	-1.4796	257	1.000
W7	-	W8	-0.02694	0.0127	-2.1289	257	0.958

Table A1.3 Post-hoc comparisons by week of Mobile EE use without ring in EK pens (sqrt-transformed)

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
W1	-	W2	-0.01867	0.0231	-0.808	257	1.000
W1	-	W3	-0.04673	0.0231	-2.022	257	1.000
W1	-	W4	-0.00409	0.0231	-0.177	257	1.000
W1	-	W5	-0.06627	0.0220	-3.013	264	0.079
W1	-	W6	-0.07683	0.0220	-3.493	264	0.016
W1	-	W7	-0.10067	0.0220	-4.577	264	< .001
W1	-	W8	-0.09252	0.0220	-4.207	264	< .001
W2	-	W3	-0.02806	0.0231	-1.214	257	1.000
W2	-	W4	0.01459	0.0231	0.631	257	1.000
W2	-	W5	-0.04759	0.0220	-2.164	264	0.878
W2	-	W6	-0.05816	0.0220	-2.644	264	0.243
W2	-	W7	-0.08200	0.0220	-3.728	264	0.007
W2	-	W8	-0.07385	0.0220	-3.358	264	0.025
W3	-	W4	0.04265	0.0231	1.845	257	1.000
W3	-	W5	-0.01954	0.0220	-0.888	264	1.000
W3	-	W6	-0.03010	0.0220	-1.369	264	1.000
W3	-	W7	-0.05394	0.0220	-2.453	264	0.415
W3	-	W8	-0.04579	0.0220	-2.082	264	1.000
W4	-	W5	-0.06218	0.0220	-2.827	264	0.142
W4	-	W6	-0.07275	0.0220	-3.308	264	0.030
W4	-	W7	-0.09659	0.0220	-4.392	264	< .001
W4	-	W8	-0.08844	0.0220	-4.021	264	0.002
W5	-	W6	-0.01056	0.0208	-0.508	257	1.000
W5	-	W7	-0.03441	0.0208	-1.655	257	1.000
W5	-	W8	-0.02626	0.0208	-1.263	257	1.000
W6	-	W7	-0.02384	0.0208	-1.147	257	1.000
W6	-	W8	-0.01569	0.0208	-0.755	257	1.000
W7	-	W8	0.00815	0.0208	0.392	257	1.000

Table A1.4 Post-hoc comparisons by week of Mobile EE use with ring in EK pens (sqrt-transformed)

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
W1	-	W2	-0.02939	0.0236	-1.2434	257	1.000
W1	-	W3	-0.04835	0.0236	-2.0456	257	1.000
W1	-	W4	-0.01316	0.0236	-0.5568	257	1.000
W1	-	W5	-0.05058	0.0225	-2.2487	264	0.710
W1	-	W6	-0.05789	0.0225	-2.5738	264	0.297
W1	-	W7	-0.07623	0.0225	-3.3892	264	0.023
W1	-	W8	-0.08530	0.0225	-3.7926	264	0.005
W2	-	W3	-0.01896	0.0236	-0.8022	257	1.000
W2	-	W4	0.01623	0.0236	0.6866	257	1.000
W2	-	W5	-0.02119	0.0225	-0.9421	264	1.000
W2	-	W6	-0.02850	0.0225	-1.2671	264	1.000
W2	-	W7	-0.04684	0.0225	-2.0826	264	1.000
W2	-	W8	-0.05591	0.0225	-2.4859	264	0.379
W3	-	W4	0.03519	0.0236	1.4888	257	1.000
W3	-	W5	-0.00223	0.0225	-0.0991	264	1.000
W3	-	W6	-0.00954	0.0225	-0.4242	264	1.000
W3	-	W7	-0.02788	0.0225	-1.2396	264	1.000
W3	-	W8	-0.03695	0.0225	-1.6430	264	1.000
W4	-	W5	-0.03742	0.0225	-1.6636	264	1.000
W4	-	W6	-0.04473	0.0225	-1.9887	264	1.000
W4	-	W7	-0.06307	0.0225	-2.8041	264	0.152
W4	-	W8	-0.07214	0.0225	-3.2075	264	0.042
W5	-	W6	-0.00731	0.0213	-0.3438	257	1.000
W5	-	W7	-0.02565	0.0213	-1.2063	257	1.000
W5	-	W8	-0.03472	0.0213	-1.6329	257	1.000
W6	-	W7	-0.01834	0.0213	-0.8625	257	1.000
W6	-	W8	-0.02741	0.0213	-1.2891	257	1.000
W7	-	W8	-0.00907	0.0213	-0.4266	257	1.000

Table A1.5 Post-hoc comparisons by week of Total EE use in EK pens (sqrt-transformed)

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
W1	-	W2	-0.06585	0.0312	-2.109	257	1.000
W1	-	W3	-0.08374	0.0312	-2.682	257	0.218
W1	-	W4	-0.03575	0.0312	-1.145	257	1.000
W1	-	W5	-0.15653	0.0297	-5.264	264	< .001
W1	-	W6	-0.12554	0.0297	-4.222	264	< .001
W1	-	W7	-0.12113	0.0297	-4.074	264	0.002
W1	-	W8	-0.13436	0.0297	-4.518	264	< .001
W2	-	W3	-0.01789	0.0312	-0.573	257	1.000
W2	-	W4	0.03010	0.0312	0.964	257	1.000
W2	-	W5	-0.09068	0.0297	-3.049	264	0.071
W2	-	W6	-0.05969	0.0297	-2.007	264	1.000
W2	-	W7	-0.05528	0.0297	-1.859	264	1.000
W2	-	W8	-0.06851	0.0297	-2.304	264	0.616
W3	-	W4	0.04799	0.0312	1.537	257	1.000
W3	-	W5	-0.07278	0.0297	-2.448	264	0.421
W3	-	W6	-0.04180	0.0297	-1.406	264	1.000
W3	-	W7	-0.03738	0.0297	-1.257	264	1.000
W3	-	W8	-0.05061	0.0297	-1.702	264	1.000
W4	-	W5	-0.12078	0.0297	-4.062	264	0.002
W4	-	W6	-0.08979	0.0297	-3.020	264	0.078
W4	-	W7	-0.08538	0.0297	-2.871	264	0.124
W4	-	W8	-0.09861	0.0297	-3.316	264	0.029
W5	-	W6	0.03099	0.0281	1.103	257	1.000
W5	-	W7	0.03540	0.0281	1.260	257	1.000
W5	-	W8	0.02217	0.0281	0.789	257	1.000
W6	-	W7	0.00441	0.0281	0.157	257	1.000
W6	-	W8	-0.00882	0.0281	-0.314	257	1.000
W7	-	W8	-0.01323	0.0281	-0.471	257	1.000

Table A1.6 Post-hoc comparisons by week of Ring EE use in SH and EW pens (sqrt-transformed)

Comparison							
Week		Week	Difference	SE	t	df	$p_{bonferroni}$
W1	-	W2	-0.04560	0.00871	-5.234	552	< .001
W1	-	W3	-0.05479	0.00871	-6.289	552	< .001
W1	-	W4	-0.03867	0.00871	-4.439	552	< .001
W1	-	W5	-0.02169	0.00869	-2.496	568	0.359
W1	-	W6	-0.01295	0.00869	-1.491	568	1.000
W1	-	W7	-0.02047	0.00869	-2.355	568	0.528
W1	-	W8	-0.00301	0.00869	-0.347	568	1.000
W2	-	W3	-0.00920	0.00871	-1.055	552	1.000
W2	-	W4	0.00693	0.00871	0.795	552	1.000
W2	-	W5	0.02391	0.00869	2.751	568	0.172
W2	-	W6	0.03265	0.00869	3.757	568	0.005
W2	-	W7	0.02513	0.00869	2.892	568	0.111
W2	-	W8	0.04259	0.00869	4.901	568	< .001
W3	-	W4	0.01612	0.00871	1.850	552	1.000
W3	-	W5	0.03310	0.00869	3.809	568	0.004
W3	-	W6	0.04184	0.00869	4.815	568	< .001
W3	-	W7	0.03433	0.00869	3.951	568	0.002
W3	-	W8	0.05178	0.00869	5.959	568	< .001
W4	-	W5	0.01698	0.00869	1.954	568	1.000
W4	-	W6	0.02572	0.00869	2.960	568	0.090
W4	-	W7	0.01821	0.00869	2.095	568	1.000
W4	-	W8	0.03566	0.00869	4.104	568	0.001
W5	-	W6	0.00874	0.00864	1.012	552	1.000
W5	-	W7	0.00123	0.00864	0.142	552	1.000
W5	-	W8	0.01868	0.00864	2.162	552	0.868
W6	-	W7	-0.00751	0.00864	-0.870	552	1.000
W6	-	W8	0.00994	0.00864	1.151	552	1.000
W7	-	W8	0.01745	0.00864	2.020	552	1.000