# Implementing On-Farm Enrichment: Effects on the Motivation of Lambs and Dairy Cows


BY

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# Abstract

Farming practices have evolved with a focus on promoting positive welfare through allowing livestock more control of their free time. Targeted enrichment, designed to meet specific motivations, can foster positive emotion by allowing animals to act on their motivations, leading to satisfaction of needs. Effective enrichment should be actively used by the animal and fulfill a motivation to interact or have access to it (suggesting the enrichment is positively valued by the animals). Multi-modal enrichment which combines multiple types of enrichment (i.e. physical, cognitive, sensory, social, nutritional) could address this concern through encouraging an array of motivated behaviours. Future standards for farm animal care may also recommend including these strongly motivated enrichments into daily farm practice to avoid causing negative emotions, such as frustration, linked to the anticipation of a delayed or non-existent event or reward, as revealed by a literature review. However, the implementation of multi-modal enrichment and its interactions with animal motivation needed further examination, both on a theoretical level and for practicality of use on-farm. A study on lambs provided with or without multi-modal enrichment found that although both groups learned how to navigate a test maze and were just as flexible in finding the reward, the enriched lambs were calmer while the control lambs appeared more agitated, suggesting an impact of enrichment on reactivity. This study did not directly investigate animals' motivation towards the enrichment, but towards a reward in another context, and was focused on young sheep, so the next study aimed to understand how frequency of access to enrichment directly impacted motivation of adult dairy cattle. Movement-restricted dairy cows were given three days (3x) or one day (1x) of outdoor access and their behaviour, along with handlers', was recorded during the outdoor period. Both 3x and 1x had stronger motivation to go outside than to return to the barn, although handling interventions and weather may have impacted motivation. Though this study was useful in understanding if dairy

cows place value on going outside, regardless of how often they are given the opportunity, the next step was to implement going outside in a realistic setting to determine how group size and handler interventions affect a producers' ability to transition their herd to daily outdoor exercise. Seventy-six lactating cows were split into seven groups, with a new group being added each week until all groups were going outside into one of two exercise paddocks. It was found that increasing the number of cows did not require additional staff or time per outing, and that the number of interventions by handlers remained the same (go interventions) or decreased (stop interventions) after one week. It was also found that behaviour did not differ between paddocks, even during or after the introduction of a new group. This suggests that both cows and handlers are quick to adapt to outdoor access and that producers can begin to transition their herd in increments. All of these specified works were integral in identifying how multi-modal enrichment impacts animal motivation, both towards the enrichment and in other contexts, and how multi-modal enrichment can be used practically by producers in modern production practices.

## Résumé

Les pratiques agricoles ont évolué pour offrir aux animaux plus de contrôle sur leur temps, en mettant l'accent sur leur bien-être. L'enrichissement ciblé, conçu pour répondre à des besoins spécifiques, favorise des émotions positives en permettant aux animaux d'agir selon leurs motivations, satisfaisant ainsi leurs besoins. Un enrichissement efficace doit être activement utilisé par l'animal et doit répondre à une motivation d'interaction ou d'accès, ce qui suggère qu'il est perçu positivement. L'enrichissement multimodal, combinant plusieurs types d'enrichissement (ex. physique, cognitif, sensoriel, social, nutritionnel), pourrait répondre à cette problématique en encourageant une gamme de comportements motivés. Les normes futures concernant les soins des animaux d'élevage pourraient également recommander aux producteurs l'inclusion de ces enrichissements fortement motivés dans leurs pratiques quotidiennes afin d'éviter la provocation d'émotions négatives, comme la frustration, liées à l'anticipation d'un événement ou d'une récompense retardée ou inexistante, comme révélé dans une revue de littérature. Cependant, la mise en œuvre de l'enrichissement multimodal et ses interactions avec la motivation animale nécessite un examen plus approfondi, tant au niveau théorique qu'au niveau pratique. Une étude menée sur des agneaux, exposés ou non à un enrichissement multimodal, a démontré que bien que les deux groupes aient appris à naviguer un labyrinthe et aient démontré la même flexibilité pour obtenir la récompense, les agneaux enrichis étaient plus calmes, tandis que ceux du groupe témoin étaient plus agités, ce qui suggère que l'enrichissement peut avoir un impact sur la réactivité. Cette étude n'a pas directement étudié la motivation des animaux vers l'enrichissement, mais vers une récompense dans un autre contexte, et elle s'est concentrée sur les jeunes. Alors, la prochaine étude a cherché à comprendre comment la fréquence d'accès à l'enrichissement influence la motivation des vaches laitières adultes. Des vaches ayant un accès limité à l'exercice ont été réparties en deux groupes : un avec un accès

extérieur trois jours par semaine et l'autre un jour par semaine. Leur comportement et celui des membres du personnel ont été observés pendant ces périodes. Les résultats ont démontré que les vaches des deux groupes étaient plus motivées pour sortir que pour retourner à l'étable, bien que les interventions du personnel et les conditions climatiques aient pu influencer cette motivation. Bien que cette étude ait été bénéfique pour comprendre si les vaches laitières accordent de l'importance à l'accès extérieur, peu importe la fréquence d'accès fournie, l'étape suivante consistait à tester la provision d'accès extérieur dans un cadre réaliste pour déterminer comment la taille des groupes et les interventions du personnel influencent la capacité des producteurs à faire la transition vers la provision d'exercice extérieur quotidien pour leur troupeau. 76 vaches en lactation ont été réparties en sept groupes, avec un nouveau groupe étant ajouté à chaque semaine, jusqu'à ce que tous les groupes aient accès à un des deux enclos d'exercice. Il a été constaté qu'augmenter le nombre de vaches n'entraînait pas de besoin supplémentaire en personnel ni de plus de temps par sortie. De plus, le nombre d'interventions du personnel est resté stable (interventions go) ou a diminué (interventions stop) après une semaine. De plus, le comportement des vaches n'a pas différé de manière significative entre les enclos, même après l'introduction d'un nouveau groupe. Cela suggère que les vaches et le personnel s'adaptent rapidement à l'accès extérieur, et que les producteurs peuvent commencer à introduire progressivement des sorties extérieures quotidiennes pour leur troupeau. Toutes les études décrites ont été essentielles pour comprendre comment l'enrichissement multimodal influence la motivation des animaux, tant en matière d'enrichissement que dans d'autres contextes, et comment ces pratiques peuvent être utilisées de manière pratique par les producteurs dans les systèmes de production modernes.

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The most predominant part of my life has been spent in academia, yet the most valuable lessons I have learned have been during the last four years I spent earning my PhD. Though only my name appears on the title page of this thesis, the growth and hard work necessary to finish this document would not have been possible without the support I have received during my time as part of the CowLife McGill laboratory.

Firstly, I want to thank my supervisor, Dr. Elsa Vasseur, who has shown me how to be a strong leader - one who sometimes needs to give their students the much needed kick-in-the-pants necessary to keep the train chugging down the track. Elsa has encouraged, guided, and pushed me to be a better student and a better person. She was involved in all aspects of my work including supervision of project design and implementation, assistance with theoretical concepts, decisions related to data analysis and results interpretation, editing and feedback on all publications, presentations, and vulgarisations, and above all else, being the leading hand I needed to finish this degree. Without her advice and input, this thesis would not have been possible. Thank you Elsa, for seeing promise in me.

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# Contribution to Knowledge

**Chapter 2**

Current animal research is shifting its focus towards improving the welfare of animals through promoting positive emotions. Although studies on motivation of animals are becoming more prevalent, there has yet to be an in-depth analysis into how motivation is connected to animal emotion. This warranted a comprehensive review of the literature to better understand what work has been done on motivation and other concepts that may impact it. This chapter contributed to knowledge by thoroughly covering the methodologies used to measure animal motivation, anticipation, and frustration, and the research gaps associated with each method. The review also identified the interrelationship between the three concepts using a newly developed relationship chain. As all of the concepts were interconnected, it was suggested that future work develop a theoretical framework on how to utilize these concepts in tandem when trying to understand the full story on animal emotion.

**Chapter 3**

Public concern related to farm animal welfare has promoted the addition of environmental enrichment. However, the impacts of enrichment at an early age, and on motivation in a context outside of where the enrichment is provided, is understudied. This chapter evaluated how enrichment in the home pen of lambs from birth to fattening affected the animals' learning, behavioural flexibility, and motivation, towards finding a reward in a test maze. Control and enriched lambs learned just as quickly, were equally flexible, and were similarly motivated, to find the reward across different types of tests (i.e., training, reversal, and disappearance). Though the enrichment did not impact how quickly lambs found the reward, the behaviours exhibited suggested that enriched lambs were better able to adapt to isolation in the maze, and may therefore have seen an improvement in reactivity compared to control. These findings confirm that non-cognitive enrichment may not be enough to impact cognitive functioning (such as learning and flexibility), but that enrichment provided in early life could positively impact animal reactivity, even in situations where enrichment is not present.

**Chapter 4**

Original contributions of this chapter aimed to understand if and how animals are motivated for enrichment in a new context with adult animals, further developing the knowledge gathered from the previous chapter. Research has shown that adult dairy cows prefer to go outdoors vs. staying inside, but this study answered a new question related to how frequency of going outside impacted motivation. This study further confirmed that cows are strongly motivated to go outside, but also demonstrated principally that regardless of how many times cows went outside (3x/week vs. 1x/week), that motivation for the enrichment did not change, further prompting its use in agricultural systems.

**Chapter 5**

The National Farm Animal Care Council's Canadian Dairy Code of Practice recently updated its requirements, so that by 2027, producers cannot keep their animals tethered for the entire production cycle. As most dairy cattle in Canada are kept in tie-stall systems, the new requirement will elicit a substantial change in the dairy industry. Although the code does not specifically state that cows must go outside, current welfare trends suggest that producers would benefit from transitioning their herd to having outdoor access both from a profit standpoint (e.g. consumers refusing to buy milk from animals kept inside 24/7 in the future), and from a welfare standpoint (e.g. previous research demonstrating both physical and mental benefits to dairy cows). However, it had yet to be investigated how a producer could practically implement outdoor access coming from a movement-restricted system. This is why a living lab experiment was conducted where the research team consulted on and then observed how McGill’s Macdonald Campus Farm implemented this transition. It was found that both cows and staff members quickly adapted to the outing procedure after a few outings, when implemented slowly over time. This contributed to both researchers’ and producers’ knowledge of what best methodology to use when making this change.

## Contribution of Authors

Four co-authored manuscripts are presented in this thesis.

Authors of manuscripts 1, 2, 3, and 4 (i.e. Chapters 2, 3, 4, and 5, respectively) are as follows: Jasmine Muszik (primary author, manuscripts 1-4), Elsa Vasseur (manuscripts 1-4), Nadège Aigueperse (manuscripts 1-4), Raphaëlle Botreau (manuscript 2), Marjorie Cellier (manuscripts 1, 3, and 4), and Abdoulaye Baniré Diallo (manuscript 5).

Jasmine Muszik co-conceptualized manuscripts 1-4, designed, conducted, analyzed and prepared data for research studies resulting in the findings presented in manuscripts 1-4. Jasmine was the primary author on all four manuscripts.

Elsa Vasseur supervised the primary author, co-conceptualized manuscripts 1-4, and oversaw, reviewed, and assisted in all aspects of theoretical conceptualization, study design, and presentation of findings. Elsa also reviewed and co-authored manuscripts 1-4.

Nadège Aigueperse co-supervised the primary author, as well as co-conceptualized manuscripts 1 and 2, and oversaw and reviewed theoretical concepts of manuscripts 1-4 and statistical analysis of data on manuscripts 1-3. Nadège also co-authored manuscripts 1-4.

Marjorie Cellier co-conceptualized manuscripts 1, 3, and 4. She oversaw study design, and data analysis and collection, for manuscripts 1, 3, and 4. She reviewed and co-authored manuscripts 1, 3, and 4.

Raphaëlle Botreau oversaw study design, data collection, and reviewed and co-authored manuscript 2.

Abdoulaye Baniré Diallo contributed to the broader research program, under which manuscript 5 was conducted, by helping to shape the program's scientific direction and implementation, and as well as securing funding.

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# Chapter 1 – General Introduction and Thesis Objectives

## 1.1 Introduction

Farm animals worldwide are often kept in intensive environments with little control over their free time. This can lead to negative emotions which consequently impact the welfare of the animal (Boissy et al., 2007; Mellor, 2012; Paul and Mendl, 2018). As the general public is becoming increasingly aware and vocal about farming practices, it benefits producers to find ways to allow animals to act on their motivations. A previous collection of research highlights the importance of understanding animal motivation — the internal drive of an animal to perform behaviour as a result of their perceived physiological or psychological state, whether that be to interact or avoid — and improving systems to allow animals to act on these motivations (Jensen and Toates, 1993; Manteuffel et al., 2009).

One way to meet the specific needs or desires of animals is through enrichment. Enrichment is a change or addition to the environment that aims to improve welfare through stimulating and enabling animals to act on their physical, behavioural, and/or cognitive motivational needs (Botreau et al., 2023a, 2023b). Enrichment allows animals to have agency, on top of promoting experiences which have the potential to increase the positive emotions felt by the animal. Effective enrichment is characterized by its active use by animals (Boissy et al., 2007). However, to maximize its impact, enrichment should go beyond single interventions. Multi-modal enrichment which encompasses a combination of physical, cognitive, sensory, social, and/or nutritional elements, has been proposed as a comprehensive strategy to encourage a wide range of motivated behaviours (Botreau et al., 2023).

However, while the benefits of enrichment are widely acknowledged, and although animal motivation is becoming a more prevalent topic in ethology research, there remains significant gaps in our understanding of the complex relationship between motivation, emotion, and enrichment. Little is known about the mechanisms that connect motivation to emotion in farm animals, including how motivation can influence the anticipation of a specific outcome resulting in a positive or negative emotion when the anticipated outcome is met or not. Anticipation has been found to affect sensory input, where it has been suggested that emotional response may depend more on how the animal regards the experience than on the situation itself (Lecorps & Weary, 2024). So, not only should motivated be investigated, but also the animals' anticipation related to the motivation.

Additionally, while enrichment can provide opportunities to act on motivation, its impact on the underlying processes of motivation, and therefore emotion, remains underexplored. The question of how the methodology used to apply the enrichment (such as group size or frequency of application) affects motivation also remains to be answered. Gaining insights into how animals react to enrichment will address knowledge gaps that limit our ability to optimize enrichment implementation.

Furthermore, while theoretical insights into motivation and enrichment are valuable, their practical application in commercial farming systems presents unique challenges. Producers face constraints such as operation costs, labour requirements, and demanding schedules (Smid et al., 2021), which can hinder the widespread adoption of enrichment practices. Understanding how to integrate enrichment into daily farm routines without compromising efficiency or animal motivation is critical for making welfare improvements accessible and sustainable.

This thesis will contribute to the literary evidence supporting positive welfare approaches through identifying the interactions between enrichment and animal motivation, and will highlight the importance of aligning theoretical advancements with practical applications in agriculture.

## 1.2 General and Specific Objectives

The general objective of this thesis (as explored in the following chapters) was to identify, both in theory and in an application, how environmental enrichment impacts animal motivation. More specifically: 1) how motivated behaviour has and can be used to understand animal emotion; 2) how enrichment affected motivation for a reward outside of the enriched environment; 3) if and how animals were motivated for the enrichment itself; and 4) how implementing enrichment in a real-world setting impacted motivation. The literature review in Chapter 2 aimed to evaluate the interrelationships between motivation, anticipation, and frustration, to better understand how motivation affects emotion (and therefore animal welfare) and vice versa. The ideas described in this chapter were used in practice in the subsequent chapters, firstly for evaluating how enrichment could be measured in a context where the enrichment was not present (i.e. in a test maze). Chapter 3 aimed to connect motivation for a reward with early life enrichment to see if indirect effects could be identified: particularly, how lambs housed with or without enrichment from birth to fattening would respond to finding a reward in a test maze away from the housing environment. The results of this chapter which demonstrated that motivated behaviour could be influenced by enrichment, led to the purpose of Chapter 4 which was to understand motivation towards the enrichment itself. Chapter 4's objective was to understand how the frequency of enrichment impacts motivation for the enrichment. Specifically, this chapter aimed to investigate if allowing cows outside once a week vs. three times a week would affect the motivation to go outside vs. returning to the barn. After having identified motivation for the enrichment, Chapter 5 expanded on these results by studying how enrichment application could impact motivation and more precisely, how larger group sizes and handler interventions affects motivation and the ability of cows and handlers to adapt to the outing procedure when transitioning from tie-stall to regular outdoor access.

# Chapter 2 – The Relationship Between Motivation, Anticipation, and Frustration in Animals: A Scoping Review

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## 2.1 Abstract

Motivation, anticipation, and frustration, are important concepts for understanding animal emotion. Denying or delaying an outcome that an animal is motivated for, expects, and performs specific behaviour in preparation of, can cause frustration, impacting the emotional welfare of the animal. However, diversity in the definitions and methodology related to these concepts can lead to confusing or conflicting results, and may complicate replication of previous work. A scoping review (conducted according to PRIMSA guidelines) was completed where a multi-step screening process identified 112 final primary research papers. The objectives of the review were to determine the ways in which the three concepts have been studied in previous literature, the relationship between the main concepts, and the gaps in knowledge regarding these concepts. Advantages and drawbacks of tests and variables used in the selected papers were explored, along with the repeatability, the applicability within and between individuals or species, and the interpretation of results. There were no universally accepted definitions of motivation, anticipation, or frustration, and the methods used to find or show evidence of the concepts varied, even within a species using the same test. However, a clear connection between concepts was confirmed, where it was suggested that future researchers develop a framework to measure the concepts together to understand the full scope of how different decisions or practices may impact animal emotion.

**Keywords – positive emotion, behaviour, arousal, valence, welfare, satisfaction**

## 2.2 Introduction

Captive animals often do not have agency over their environment or situation as they act as commodities for humans, relying on our care for maintenance and survival. However, the inability to perform their behavioural repertoire in a suitable setting can lead to negative emotional states as the animal's perceived needs aren't satisfied (Bracke & Hopster, 2006; Cromwell et al., 2018). Despite the absence of a universal definition, emotions are generally regarded as short-term, but intense, internal states caused by a specific stimulus that triggers an automatic and unconscious response (such as fight, flight, or freeze) (Anderson & Adolphs, 2014; Boissy et al., 2007). This process includes a behavioural reaction that cannot be hidden by the animal, thereby making emotions an observable measure of welfare.

Emotions can be both positive or negative, (i.e. valence), and high or low intensity (i.e. arousal) (Figure 2.1 (Liu et al., 2018)). Several "emotion wheels" have been developed to depict the relationship between arousal and valence, however they originate from studies on humans and there is a wide range of emotions that have been added to these wheels, with authors at times including non-emotion terms such as "tense" or "tired" (see Figure 2.1). These wheels are useful, nonetheless, in visually representing the two necessary components for evaluating an emotion: the valence and level of intensity. They also allow for developing methods to categorize and understand emotions, although one has yet to be developed for animals. Emotions are internal states individually experienced by the animal that cannot be communicated to us linguistically and may be expressed differently due to physiological differences. It is therefore important to try to understand animal emotion by exploring what mechanisms control it.

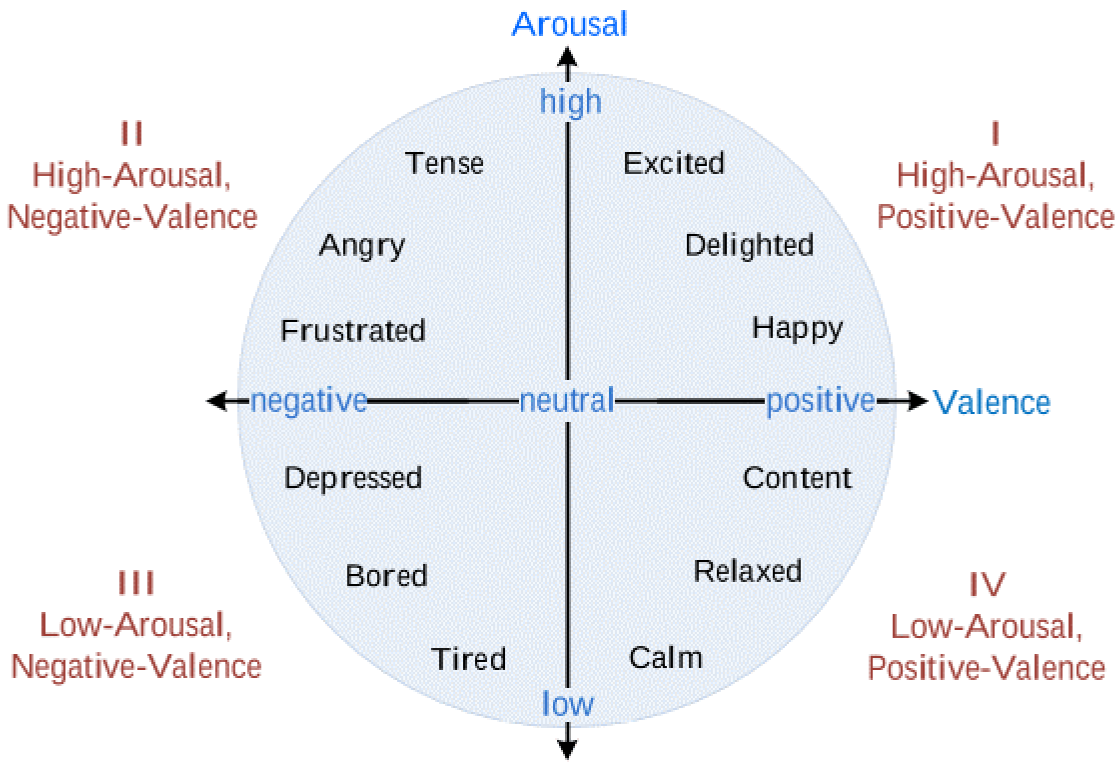


*Figure 2.1 An emotion wheel developed for human emotion by Liu et al. 2018 which illustrates the range in which emotions can be categorized (both by arousal and valence).*

For the purpose of this article, the working definition used for motivation is the internal drive to perform behaviour related to an animal's perceived physiological and/or psychological state (Jensen & Toates, 1993; Manteuffel et al., 2009). Motivation has two components; arousal (positive or negative) and goal (approach or avoidance)(Antle & Silver, 2016). Motivation can be induced and modulated by a psychological or physiological need or desire. The strength of the motivation, also depends on the context and the animal's previous experience, preferences, expectations, etc. (Kirkden & Pajor, 2006). It can arise spontaneously or be induced by a stimulus. If the animal first becomes aroused via a stimulus (ex. sound of food dispensing in feeder) this can lead to goal-oriented behaviour (ex. approaching the feeder to eat) in an attempt to satisfy the perceived needs (Antle & Silver, 2016). Although stimuli are not always necessary to incite motivation, they are often used in research to train the animal to associate a cue with an outcome. In fact, if an animal has motivation and has associated a stimuli, cue, or event with a specific outcome, this learning can lead to anticipation of an outcome.

In the context of this review, we define anticipation in animals as a high arousal state that results in an increase in activity and/or the performance of preparatory behaviour in expectation of a particular event or outcome (Clegg et al., 2018; McGrath et al., 2016; Peters et al., 2012).If a positively expected outcome does not happen (due to decisions by a caretaker or external factors), this could lead to frustration and thus, negative emotion (inversely, a negatively expected outcome that does not happen can result in relief) (Anderson et al., 2020).

For clarity in this review, the term frustration will be defined as a highly negative and extremely intense emotion whereby activity is increased and/or frustrated behaviour (and at times abnormal behaviour) is performed due to the expectation of an event or outcome not being met (Bokkers & Koene, 2002; Bremhorst et al., 2019; Delgado & Jacobs, 2016). These three concepts, motivation, anticipation, and frustration, can be studied together to get a better idea of the psychological experience of the animal (particularly in cases where the animal is offered something recurrently and predictably). This can be useful to understand how to meet biological and/or behavioural needs. For example, these concepts could be used to investigate the effectiveness of enrichments aimed at fulfilling motivation and promoting positive experiences (Botreau et al., 2023).

Past research has utilized a variety of tests to understand motivation, anticipation, and frustration, but there is often a lack of standardization for these tests as they cannot be applied to all species or even within the same species due to the differences in the objectives of the test, the measures taken, species differences, and more (Kirkden & Pajor, 2006). Additionally, there is a need for better understanding of how motivation is related to anticipation and frustration, so that we can employ management strategies that take into account the relationship between concepts to better consider and manage emotional states. If we can understand what an animal anticipates, is motivated for, and would be frustrated without, we can tailor our decisions related to housing, feed, etc., to try to reduce negative emotions and increase positive ones. So, a scoping review is necessary to understand how motivation, anticipation, and frustration are commonly defined and studied in animals, and how these concepts interact.

The objectives of this scoping review are to; 1) explore the different methods used in the field of ethology to define and measure motivation, anticipation, and frustration in animals to better understand their relationship, and 2) determine the current gaps in knowledge about these concepts.

## 2.3 Methods

The protocol used was the Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) (Pages, 2021).

### *2.3.1 Eligibility Criteria*

Full text publications in English from any location, were included. As the first objective of this review was to understand the methodology used to study the main concepts of motivation, anticipation, and frustration, only primary research was incorporated in the final selected papers. Review papers on the individual topics were used supplementarily for background information, but were not included in the final selection of papers and were not used for analysis. Articles detailing research on animals kept in captivity were included (ex. research, zoo, farmed animals).

Studies that covered more than one of the concepts and their interactions were also included to better understand the relationship between concepts.

Papers using quantitative measures were included as they directly pertained to the main concepts of motivation, anticipation, and frustration, in animals themselves. Mixed-method studies were also included to ensure that papers using a variety of methods (ex. qualitative behaviour assessment along with quantitative biological measures) to investigate the main concepts were not missed. Studies that focused on animal behaviour and welfare were included as one of the concentrations of the review was how each concept relates to one another in the field of ethology.

Further, studies on animal psychology were included as they help to understand the underlying mechanisms that control motivation, frustration, and anticipation, along with interpretation of why these occur and how they are related. Papers with outcome measures for any of the main concepts were included as they were often used in conjunction with studying animal behaviour, and thus would help in uncovering behavioural or psychological studies of the concepts in animals. Finally, only studies published in the last 20 years (i.e. from 2002 onwards) were included, as they would have the most relevant information and would use the most current methods.

*2.3.2 Search Strategy*

The literature search used to identify references was conducted on two electronic databases: Web of Science and Scopus. First, a comprehensive search strategy was established to identify relevant material. Five main domains were created including: motivation, anticipation, frustration, animal, and measures. The following domain combination was applied to the search algorithm: (motivation) AND ((anticipation) OR (frustration)) AND (animal) AND (measures). After inputting the five domains and several possible keywords for each into the two databases, the article abstracts, titles, and keywords from the results were evaluated.

Sixty-three string searches were done using a combination of keywords to identify the most relevant search terms and combinations to use, resulting in the final string. As motivation, anticipation, and frustration were the three main concepts, they were used as the theme for the first three strings, where synonyms or related terms were used (ex. the animal can either be relieved or frustrated at an outcome, so both were searched in case the paper mentioned one or the other in hopes of identifying papers that discuss frustration or anticipation). As well, types of tests that might be used to identify or prove one of the main concepts were incorporated into one string such as preference tests (which can help to identify motivation or anticipation) or human approach tests (which could test all three concepts), etc.

The search string combinations are available in Table 2.1 (using Web of Science as an example). The same search strategy was used in Scopus, for a final search date of April 22, 2022. Finally, a secondary search was performed using hand-searching techniques to find articles missed by the databases, along with screening of the reference lists of articles meeting the eligibility criteria to distinguish any additional documents. After the additional search, no pertinent secondary articles were identified (see Figure 2.2).

**Table 2.1** Web of Science search string history with the individual strings and combination of strings (searching for topic (TS) which includes title, abstract, and keywords) and records (number of articles) found for each string.

| Number | String | Records Found |
|---|---|---|
| #1 | TS=((motiv*)) | 582,103 |
| #2 | TS=((anticipat* OR “expected state*”)) | 184,538 |
| #3 | TS=((frustrat* OR dissatisf* OR “behav* restriction” OR relief OR disappointment)) | 239,815 |
| #4 | TS=((animal*)) | 1,333,764 |
| #5 | TS=((“human approach test*” OR “choice test*” OR “preference test*” OR “operant test*” OR defin* OR behav* OR operational* OR welfare)) | 7,735,080 |
| #6 | #1 AND (#2 OR #3) AND #4 AND #5 | 8,132 |

*2.3.3 Study Selection*

All references were exported from the two databases to Endnote X9 reference management software to remove all duplicate articles (Clarivate, 2013). Additional records were not added as the total number of references found (n=8,901) was deemed relevant and substantial enough to not warrant any further searching. Thus, the next step was to de-duplicate the references by removing articles that were identified twice. Once duplicates were removed, the remaining articles were uploaded to Rayyan, an online systematic review tool, to filter out (in a two-step procedure) irrelevant or unsuitable studies (Ouzzani et al. 2016). Firstly, articles were excluded based on title, abstract, and keywords, if considered ineligible or irrelevant based on the previously described criteria. Secondly, articles were assessed for eligibility by full-text and any not fitting the criteria were removed. Figure 2.2 depicts the full screening process.

**PRISMA 2009 Flow Diagram**

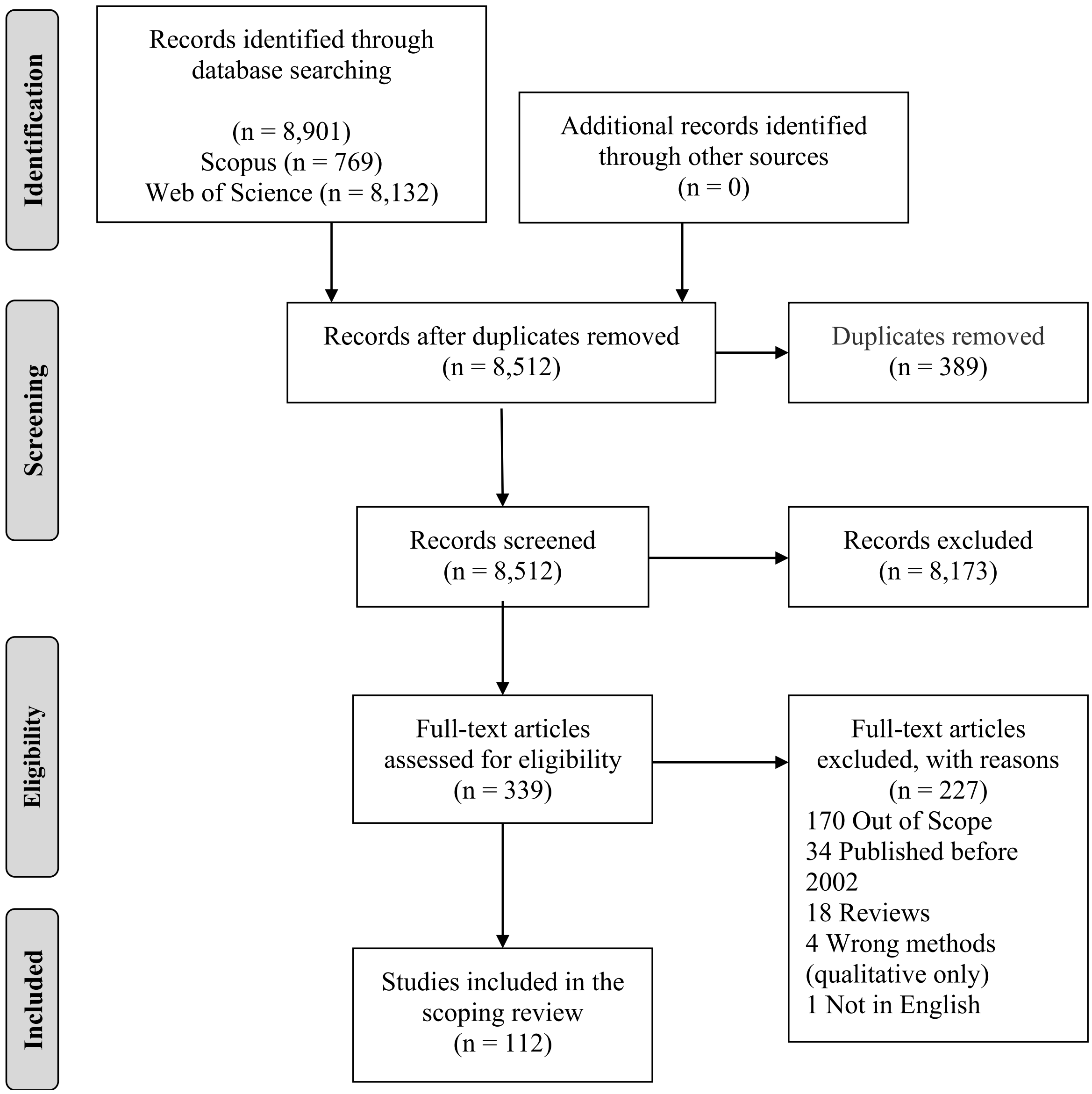

*Figure 2.2 Prisma diagram displaying the search and screening process to find eligible articles, where the initial search process identified 8,901 articles which were then screened and removed or included based on eligibility criteria until a final 112 selected articles remained.*

### *2.3.4 Data Charting*

Data extraction was done using a sheet created by the authors that included the following headings: Author(s); Publication year; Country of origin; Objective(s)/purpose; Concept(s) covered (ex. motivation & anticipation); Concept defined? If so, how?; Sample size; Treatment(s); Species used; Animal characteristics (ex. age, sex, etc.); Type of setting (research, farm, zoo, etc.); Housed alone or pairs/groups; Methods (test used or not); Measures taken (behavioural); Ethogram included (yes or no); Measures taken (physiological/environmental); Key findings relevant to the review. A single reviewer performed all of the screening and extraction procedures. The review team had weekly discussions throughout this process to mitigate the possibility of human error.

## 2.4 Results

Figure 2.2 uses the PRISMA flow diagram to depict the selection of articles pertinent to this review. A total of 8,901 studies (8,132 from Web of Science and 769 from Scopus) were found through the selection process. The de-duplication method removed 389 articles, leaving 8,512 to be screened. After excluding based on title, abstract, and keywords, 339 articles were left to be assessed using full text. Of these, 112 matched eligibility criteria and were thus included in this scoping literature review.

### *2.4.1 Study Characteristics*

The selected studies originated from 23 different countries, with the majority of the work coming from the United States of America, 17% (n=19), England, 13% (n=15), and

Australia, 9% (n=10) (Figure 2.3). Over half of the studies, 61% (n=68), were published between 2012 and 2022, while the rest were published between 2002 and 2011 (39%, n=44).

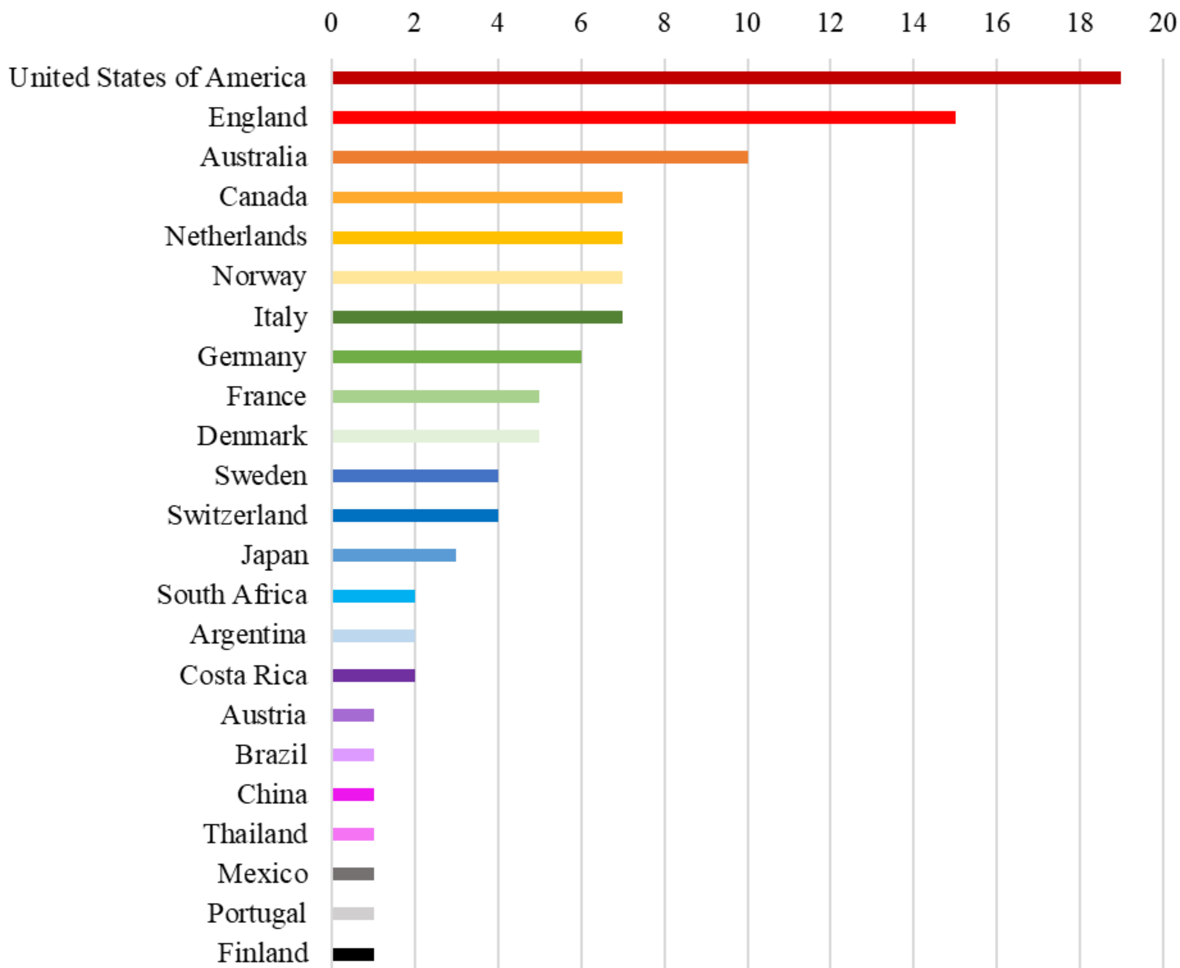


*Figure 2.3 Graphical depiction of countries where selected articles were published, including the number of studies originating from each country.*

Sample sizes of the studies ranged from n=1 to n=525, with 53% (n=59) of studies using experimental treatments and 47% (n=53) not including treatments (ex. often observing individual responses to the same application or test). Half of the articles, 50% (n=56) used farm animal species, with the next largest category being wildlife kept in zoos, rehabilitation centers, or research facilities (20%, n=22). Rodents (i.e. mice and rats) were the next most used subject (15%, n=17), followed by marine species (8%, n=9) and companion animals (7%, n=8) (Figure 2.4). However, in terms of location, 46% (n=52) of studies took place in a farm setting, 45% (n=50) were done in research labs, 8% (n=9) in zoos, and less than 1% (n=1) in shelters.

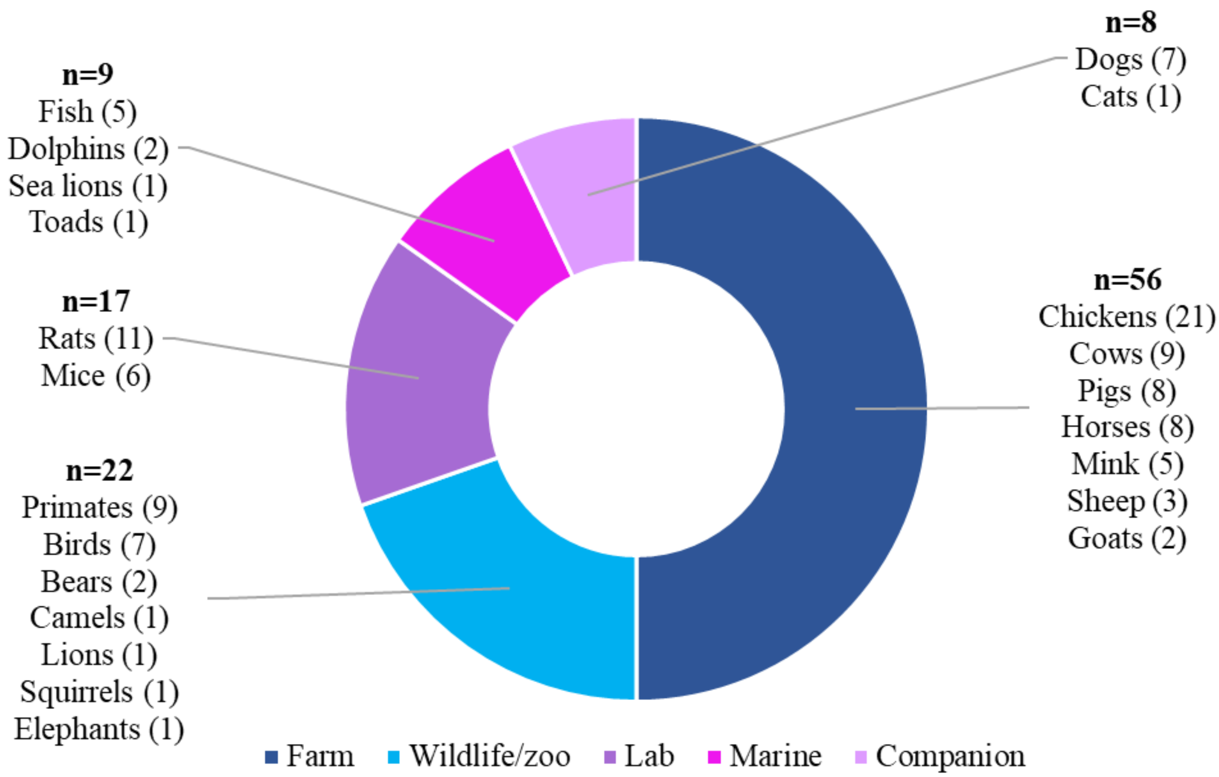


*Figure 2.4 Circle chart representation of number of articles using each category of species, with farm animals being the largest (n=56 studies) and companion animals being the smallest (n=8 studies).*

Additionally, a predominant number of papers included age and/or maturity (ex. youth, adult, etc.) (76%, n=85) and whether the animals were male (18%, n=20), female (32%, n=36), or mixed sexes (26%, n=29). Of those that stated the animals were young, only a few specified whether the animals were weaned (3%, n=3). For those that specified if the animals were female, only 2% (n=2) mentioned whether they were pregnant, or lactating (2%, n=2). Starting weights of the animals were occasionally reported (11%, n=12) (typically included for feed related trials). 92% (n=103) of the included studies reported how the subjects were housed; individually (27%, n=28), in pairs (10%, n=10), groups (62%, n=64), or with their mother (1%, n=1).

For measures taken during the studies, there was a range of behavioural measures such as general behaviour (walking, eating, vocalizations, etc.), the posture/position or facial expression of the animal (ex. position within test arena or ear position), abnormal or agonistic behaviour (ex. head shaking, bar biting, etc.), and behaviour directed towards humans (ex. making contact with the experimenter). Several studies also included sexual measures (like copulation time) or personality traits (like dominance, reactivity) with respect to their effect on the animals' motivation, frustration, or anticipation.

There were also observations on the duration or frequency of the behaviour itself (ex. duration of lying time), or the latency to perform the behaviour (such as latency to press a lever during an operant test). These could also come in the form of time budgets or whether the behaviour was present or absent. Lastly, choice during preference testing was noted, along with the number of correct responses/errors or number of responses/rewards consumed during learning or motivation tasks. Only 38% (n=43) of the selected articles incorporated an ethogram or a list of behaviours with some definitions, including two papers whose ethograms did not define the full range of behaviours analyzed. There were five papers that did not have an ethogram but did include descriptions of the behaviour(s) in the body of text (at times lacking in clarity of when the behaviour starts and stops or having simple or unclear definitions (ex: Mun et al., 2015, defined walking as "animal is actively moving", which could also be applied to other locomotory activities), while the other 65 articles (58%) did not include an ethogram, nor specify how the behaviours were defined.

Along with behavioural observations in living conditions, the primary way to assess the main concepts was to use tests of preference or aversion (for motivation), operant or learning tasks (for motivation and/or anticipation), theory of mind or predictive future tests (for anticipation), induced frustration or impossible tasks (for frustration), and inequity or delayed gratification tests (for motivation and/or frustration) (Figure 2.5).

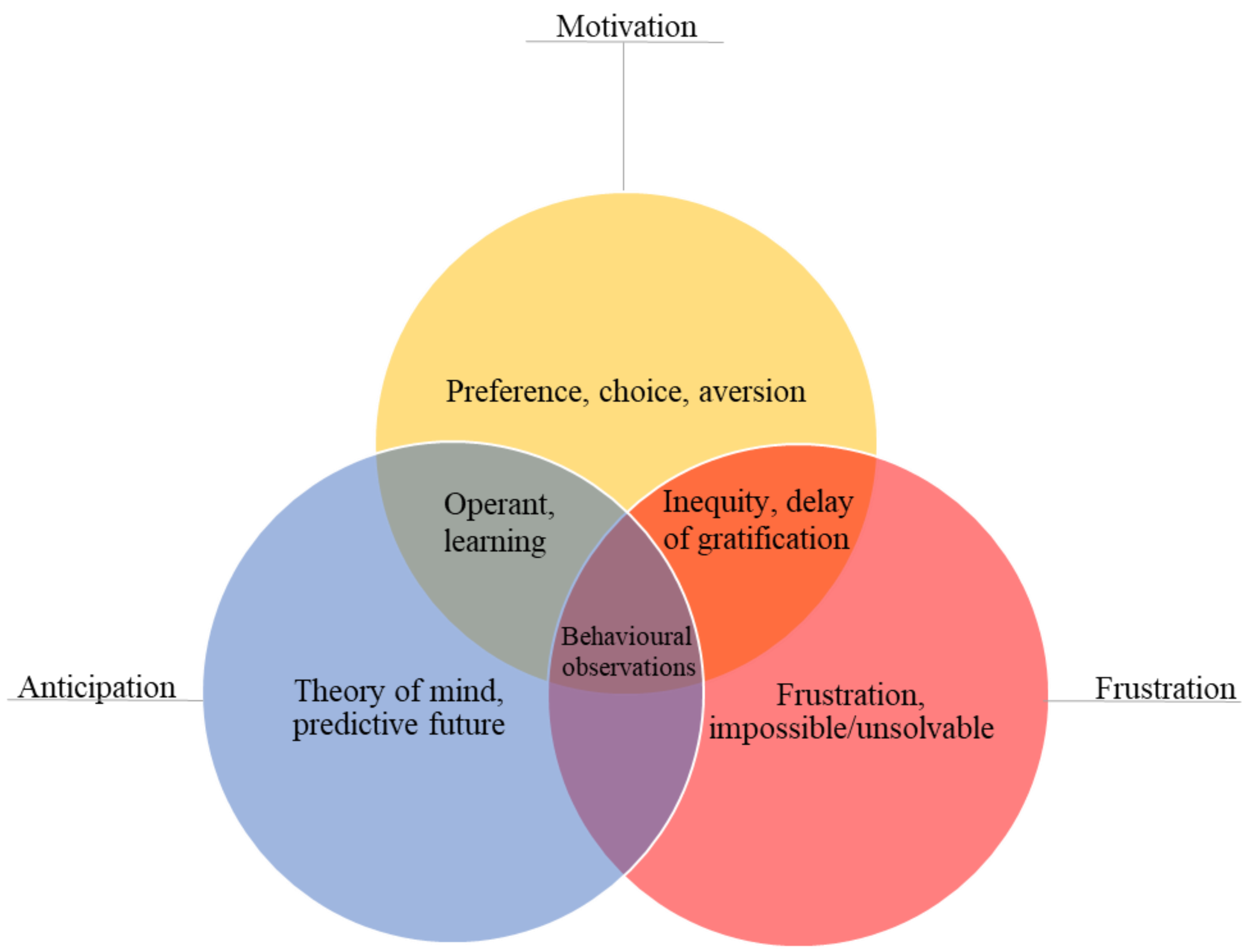

*Figure 2.5 Venn diagram illustrating the types of tests used to understand each concept, including the overlap between all papers that used behaviour as an indicator of the concept (which was selected for in the eligibility criteria).*

There were 40 articles (36%) that included physiological data such as: blood samples (ex. cortisol) (35%, n=14), heart rate or heart rate variability (28%, n=11), weights (28%, n=11), brain scans/images (7.5%, n=3), milk samples (7.5%, n=3), respiration rates (5%, n=2), saliva samples (5%, n=2), fecal samples (5%, n=2), or temperature of body, limbs, etc. (5%, n=2). Out of the 40 articles, 45% (n=18) used physiological measures (with some articles using more than one) to further explain or give reason for their results in relation with our concepts (ex. author reports that a peak in heart rate, along with preparatory behaviour after seeing a learned cue, suggests that the animal was anticipating).

### *2.4.2 Article Themes*

The main themes of the objectives of the articles were as follows: observing stereotypic behaviour or behaviour during/after frustration inducing tasks (25%, n=28, with four of these studies also investigating anticipation); understanding decision-making, learning, or anticipation (35%, n=39); animal motivation and preference (20%, n=22); implications of restricted feeding/drinking or changes in feeding routine on the concepts (11%, n=12); response to inequity or delay of gratification (7%, n=8); using facial expressions/ear positions/eye whites to assess emotion (4%, n=5); and predicting the future/theory of mind in animals (4%, n=4). Four studies used these themes (i.e. anticipation, facial expressions/eye whites, and motivation) as a means to validate a test or method. There were seven studies that did not clearly state an objective.

With regards to the three main concepts, 82 studies investigated only one of the main concepts: motivation (17%, n=19), anticipation (24%, n=27), or frustration (32%, n=36). The other 30 covered two of the main concepts: motivation and anticipation (12%, n=13), motivation and frustration (9%, n=10), or anticipation and frustration (6%, n=7). There were no studies that covered all three of the main concepts.

A majority (74%, n=83) of studies did not include a definition of any of the main concepts. Twenty-nine studies (26%), out of the original 112, included one or multiple definitions of the concepts, where 17 (59%) of these were quoted or paraphrased definitions from previous literature. Out of the 29 papers with definitions, five were about motivation, nine defined anticipation, and 15 were about frustration. When comparing the different definitions of a single concept to each other (ex. only looking at definitions of motivation) the most common terms were: for motivation: animal (only used in two definitions); for anticipation: animals (found in five of the definitions), anticipatory behaviour (found in four of the definitions), and reward (found in two of the definitions); and for frustration: animal (common between two of the definitions), reward (found in two definitions), aversive (used in two definitions) and psychological state (found in three of the definitions).

## 2.5 Discussion

### *2.5.1 How are Motivation, Anticipation, and Frustration Defined?*

When trying to understand a concept, it is important to first be familiar with its definition(s) to discern the components that make up or explain what the concept is and how it works. However, when it comes to motivation, anticipation, and frustration, this is difficult to do as there is no consensus in the field of ethology on what exactly each concept is and how we should try to understand it in animals. Though four studies did include previously established definitions, 13 authors attempted to create their own definitions, using original and portions of past definitions (ex. quoting or paraphrasing parts of other definitions). There was some commonality in the terms used however. For example (aside from the names of the concepts themselves), behaviour, reward/non-reward, state(s), emotion(s), response, expecting/expectation(s), and goal/goal-directed/goal-oriented were of the most frequently found words in the definitions across all concepts. However, when looking at the common terms used in definitions within a single concept, there were fewer (one, three, or four) words that were used in multiple definitions. These terms often only appeared in two or three definitions and were more universal terms like animal or reward.

So, although there was overlap in the terms used across the concepts, there lacked cohesion within a concept as the common terms were only found in two-five of the definitions. This might be reflective of the relationship between concepts as they have a close connection; but it also highlights the divide in the field about how we define and measure these concepts in animals. In this review, 83 papers (74%) chose not to define the concept which they were attempting to measure (whether what they were measuring was clearly stated or implied). Studies that did not provide an explicit definition of one of the concept were nonetheless included if they claimed to have demonstrated evidence for, or measured, the concept in their results. In such cases, categorization was based on the study's reference to the concept or the use of terminology associated with it. There were 29 studies that did include a definition (26%), but no two papers used the same one, demonstrating a need for universal definitions to ensure that what is being measured between researchers is the same concept.

### *2.5.2 How Do Motivation, Anticipation, and Frustration Relate?*

As found through the overlap in tests/methods and results in the selected articles, there is a clear relationship between the concepts of motivation, anticipation, and frustration. The link between the three main concepts is also shown by the 27% (n=30) of selected studies investigating multiple concepts within one paper (ex. motivation and anticipation) and by the overlap in use of tests for the different concepts (Figure 2.5). As stated by Balci et al. (2010):

“Motivational states mainly affect the decision to initiate anticipatory responding”, already suggesting a link between motivation and anticipation. While motivation and anticipation themselves are not emotions (rather mechanisms that inform how the animal interprets and behaves towards an outcome), they can influence or be influenced by one another and by the emotion of the animal (such as frustration) (Anderson et al., 2020; Anselme, 2010).

Motivation starts the relationship chain where an animal has a learned or innate need or desire that can lead to an emotion. In order to have an expectation, an animal must first have learned an associated cue or have a learned experience (such as habituating to a test and its potential outcomes) before knowing what preparatory behaviour should be performed (Spruijt et al., 2001). The expectation of the outcome (i.e. predicting what will happen) is one necessary aspect of anticipation but the animal also needs to have increased arousal and/or perform preparatory behaviour as a response to the expectation in order for researchers to understand and measure the anticipation (i.e. you can have expectations without anticipation, but you cannot have anticipation without expectation) (Clegg et al., 2018; McGrath et al., 2016; Spruijt et al., 2001). Moreover, if they have no or low levels of motivation, the animal may not exhibit a strong anticipation as a result (Anselme, 2010; Clegg et al., 2018).

As shown in Figure 2.6, when an animal is provided with a positively associated cue, they have motivation to interact or have access to the perceived reward, so it is likely that they will expect, anticipate, and prepare for it (Anderson et al., 2020). If the expectation they have is not met (ex. not being given a feed reward), the animal may become frustrated as a result (Anderson et al., 2020). This can have a feedback effect on the motivation, and thus the anticipation, through either increasing the animal’s motivation for the reward (potentially leading to increased levels of frustration the next time they are not rewarded) or by decreasing their motivation and/or expectations to lessen the impact of negative emotion (Anselme & Robinson, 2019).

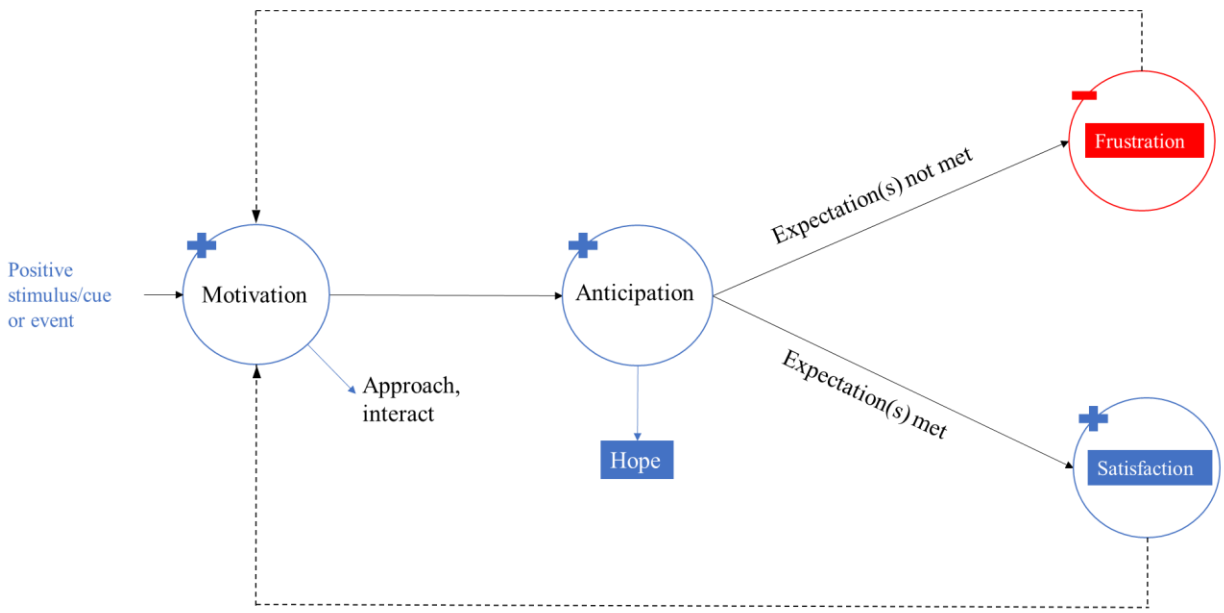


*Figure 2.6 Visual chain representation of potential effect of an associated positive cue or event on the motivation of the animal, along with the anticipation (ex. positive emotion could be hope for the event to occur or reward to be received) and the emotional response if the expectation is met or not with the result of a negative (frustration) or positive (satisfaction) emotion.*

With a negatively associated cue, the motivation is to get away from or avoid the outcome, where the anticipation may lead to fear in expectation of being physical or mentally affected and the result (i.e. the event happens or not) can lead to anger (negative emotion) or relief (positive emotion) (Figure 2.7). Realistically, animals cannot be completely free from negative emotions, but by mitigating them where possible while also increasing the opportunity for positive emotion, we have the potential to improve the welfare of future generations.

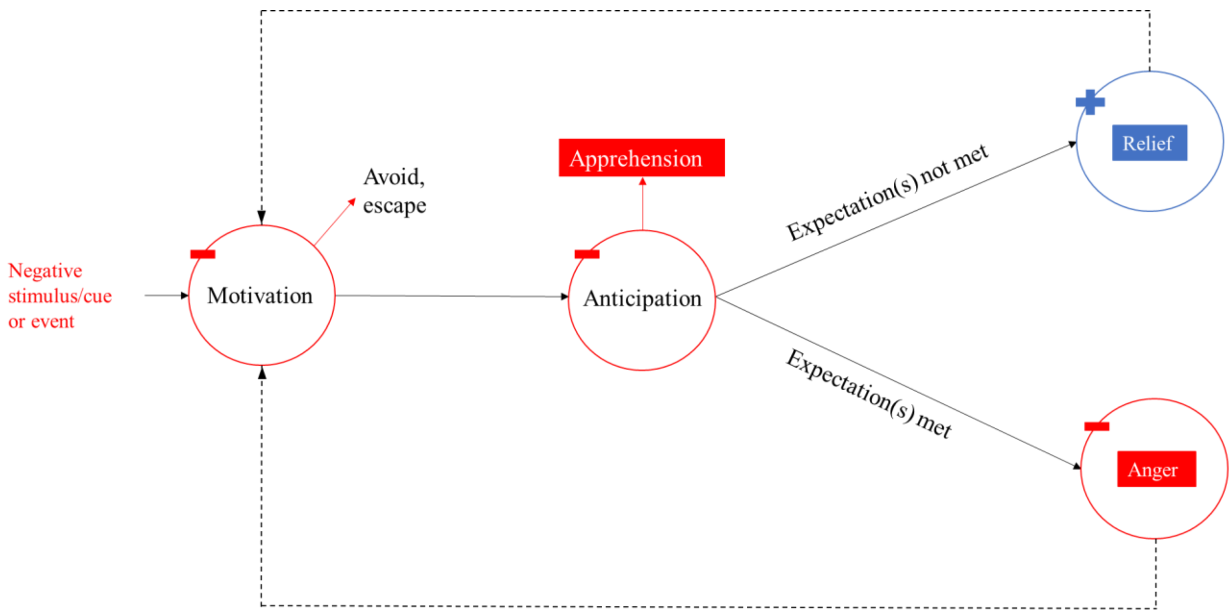


*Figure 2.7 The impact of a negative stimuli or experience on the chain of motivation and anticipation and the resulting emotion if the expected aversive event occurs (anger) or does not (relief).*

### *2.5.3 Methodology Used to Investigate the Main Concepts*

Regarding the methods of the selected studies, there was diversity in how the authors attempted to study one or more of the main concepts. A little over half of the papers used treatment groups, often to demonstrate or understand which criteria, linked to the treatment, could control or influence motivation, anticipation, or frustration, in animals. The papers not using treatments typically wanted to understand the individual responses of the animal to a test, a signal or reward, etc., to determine if the animal has or can exhibit motivation, anticipation, or frustration, and if they do, in what ways or using which behaviour. So, the two main purposes were to identify if the animal could exhibit one or more of the concepts or to find animal indicators of the concept(s).

There was variability in the number of animals used and how they were studied, ranging from one to 525. When the sample sizes were as small as one, the individual was treated as a representative of the species or of all animals for the anticipation concept (Krebs et al. 2017; Podturkin et al. 2022). While it is important to understand if an individual can possess motivation, anticipation, or frustration, when only one individual is used it is also important for authors to avoid generalization of results to an entire species, or other animals, as individual differences and previous experience may affect the results. Particularly in these cases where the aim was to create methodology for invoking and studying anticipation as a measure of well-being, these methods may not ultimately be suitable for other individuals.

On the other hand, studies with larger sample sizes over 100 animals (such as Buck et al., 2014; Hansen & Moller, 2008; Starling, Spurrett, et al., 2020; Starling, Wilson, et al., 2020) can magnify errors in the data, such as finding statistical significance despite trivial differences. Further, the large the sample size, the greater the variation in behavioural response, making results more difficult to interpret. Thus, the results of the included articles with substantial sample sizes were analyzed scrupulously. A disparity in sample size further complicates the ability to create or determine standard methodology for studying the main concepts as the aim is to apply consistent and repeatable approaches across the same or different groups. When the included studies range from one to 525 animals, variation in sample sizes can lead to distortion or misinterpretation of the quality of the methods/measures and add another layer of complexity when seeking clear and replicable tests or techniques for studying motivation, anticipation, and frustration.

For all the concepts, the reward used during testing was often feed related (ex. Barbano & Cador, 2003; Bokkers & Koene, 2002; Bremhorst et al., 2019; Ludvig et al., 2011; Olsson et al., 2002; Vasquez et al., 2021, etc.). This can be explained due to the incentive value feed has which is necessary to study the main concepts. The more palatable the food, the more motivation/anticipation/frustration it induces. We can therefore use the type of feed and its palatability as a factor to vary the animal's motivation and see the effect induced. The use of feed, as a vital homeostatic need with a strong incentive effect, makes it possible to highlight the existence of the concepts and to see how they are expressed in different species. But above all, this value must be controlled.

The use of different feed (for the same species) makes any comparison of methodology or results complicated by a possible gradient in incentive value, depending on the feed. Moreover, the behaviours expressed are not necessarily the same, depending on the nature of the motivation. Thus, by focusing on feed rewards, studies have potentially missed behaviours expressed in cases of motivation/anticipation/frustration linked to other needs or desires necessary for animal wellbeing, such as response to other aspects of their environment like the enrichment provided, choice of bedding, social companions, etc.

Using varied rewards may be more useful in understanding the complex needs of animals. For example, Clegg et al. in 2018 measured anticipation of dolphins using a variety of floating toys to determine if level of anticipatory behaviour could predict whether the dolphins would later participate in the expected event or interact with the reward. They found that more anticipatory behaviour before an event related to increased levels of participation after, which they suggested relates to the dolphins having a positive perception of the floating toys and placing intrinsic value on them. So, there was both anticipation and motivation for a non-feed reward. They also identified that one toy was highly preferred over another, demonstrating that one reward had higher intrinsic value and was potentially more important to the animal's welfare than the second option. This toy might have even been preferred over a feed reward, although this would need to be tested.

This point further solidifies the fact that authors should not assume or generalize results to all possible types of reward (as with generalizing results to other animals or species as previously mentioned), as different contexts, motivations, and even individual preference can influence the response to a chosen reward. This also has an impact on the behaviours and ethograms to be used, as different rewards, or even the same reward in a new context, could result in different behaviours, even within the same species or individual (Krebs et al., 2022).

As there were several different species used in the selected studies, the type of behaviour(s) recorded varied greatly from more generalized behaviour common across multiple animals (such as feeding, locomotory activities, etc.), to species-specific behaviour (ex. vacuum-chewing in horses, mouth fighting in fish, etc.). This appears fitting given the assortment of species and of the reward (when they are given one), due to the variation in physiological and behavioural needs between species. However, there lacked a uniformity of variables used when the same test was applied on a single species. For example, two studies employed a novel object test to investigate neophobia (that can be likened to motivation to explore) in chickens, both of them using group tests. The first study recorded the number of animals that approached the object within one body length, and the second measured both individual frequency and duration of pecks at the object, and the duration of head fixation and duration from fixation to contact with object (Dixon et al., 2008; Lindholm et al., 2018). This is one example of lack of cohesive methodology between authors. This concern is even more general than the main concepts: the non-harmonization of variables and associated interpretations for the same test, which is supposed to measure the same concept, makes interpretation difficult and impossible to compare as the tests and associated interpretations are not repeatable from one experiment to another, even for the same species.

An equally important but opposite issue is the use of the same variable(s) in multiple contexts, with different tests, to study different concepts. One example is jumping which was recorded in four studies, including operant and learning tests (for anticipation), a frustration test (for frustration), and an appetitive/consummatory test (for motivation). Authors used this measure to make conclusions about all three concepts, where the same behaviour was suggested to represent play (motivation), anticipation, and frustration. These are just two examples of the lack of cohesion between methods and variables used by different authors, which makes trying to differentiate and measure the concepts of review difficult (although the evolution of the tests throughout its years of use, the specific objectives within each article, and the country of origin may play a larger factor in these discrepancies).

To combat this, the pattern or sequence of behaviour, along with magnitude, should also be considered to reduce confusion and to ensure that the interpretation related to one or more concepts is as accurate as possible. It should be noted that 65 articles (58%) did not include the ethogram used to define their behaviours of interest. It cannot be known whether different authors really used different or identical variables/behaviours for the same test/species, and which behaviour could be used for several species or is specific to only one. This lack of transparency may make study replication less feasible, and again, lead to further differentiation among methods.

Repeatability and validity of tests or variables used is one aspect that needs further discussion when studying these concepts. Sandem et al., in 2002, aimed to evaluate the sensitivity and specificity of eye whites as indicators of emotion in cattle. This was one of four studies with an objective to validate either a test or a behaviour (Bremhorst et al., 2021; Olsson et al., 2002; Sandem et al., 2002; Taylor et al., 2020 ). Discussion of the test's validity itself was only included in these four specified papers, although mentions of replication could be found throughout the selected papers, where authors suggested study replicates or future research for finding suitable methods to test the three main concepts. It is recommended that authors comment on the validity and repeatability of the tests they are using, both for transparency of results and for succeeding researchers who will use the same/similar tests or variables to make appropriate decisions regarding methodology.

Several of the selected articles were able to demonstrate that these concepts were present in animals (ex. Beran et al., 2012; Daneri et al., 2007; De Petrillo et al., 2017; Krebs et al., 2017; Kuhne, 2016; Raby et al., 2007; Villain et al., 2020; Vindas et al., 2012). However, some studies found that animals could not demonstrate one of the concepts. For example, Paxton and Hampton (2009) found that rhesus monkeys were unable to anticipate future thirst events, even in as little time as 15 minutes into the future. However, this may be a limitation of the study, as Beran et al. (2012) found that both rhesus and capuchin monkeys could anticipate future events, pointing out that motivation may be the limiting factor when trying to study anticipation. The first study used a predictive choice test where the animals could select a large feed reward with a large delay in receiving water, or a small reward with a short delay in receiving water, while the other study used an operant test involving operating a joystick to correctly select future matches on a screen to receive a feed reward. So, it is important to note that it is possible to study motivation, anticipation, and frustration, but more cohesion is needed on the best method(s) to use for each species.

It can be difficult to equate these types of results as they may test different types of anticipation or motivation (ex. anticipation of one’s thirst vs. anticipation of visual stimuli, and motivation for feed vs. motivation for water). While it is useful to gather information on the different aspects of the three main concepts, having such a large variety in methods and measures used (even within a species) can lead to confusion regarding the concepts (i.e. whether they are present or not, how best to test them, etc.) Although, these concepts are relatively new within animal research, so the diversity in methodology could also be explained by the consideration to try to find 'best' measures for these concepts which have yet to be found.

The other missing component in the selected studies was the acknowledgment of possible misinterpretation of valence related to the concept. Only a few types of tests (ex. operant tests like weighted door) could themselves identify both the strength and the valence, where it can be inferred from the motivation to have access to the reward, that the animal relates its consumption to a positive emotion (i.e. the harder the animal works to access the reward, the stronger the motivation, the more likely the reward is viewed positively). Although this may be inherent in other tests as the aim is often to see the animals' response to a positive or negative stimuli/event, several authors did not comment on the valence, or rather the potential for misunderstanding the valence, when discussing their results. This is a necessary piece of information as whether the animal views the reward/experience as positive or negative, and the result of the expected outcome, can impact the emotion of the animal. Thus, failing to comment on how the valence may have impacted results (ex. you expect a reward to be viewed positively, but the animal does not show strong motivation for it, therefore it might not be as positively perceived as predicted), could hinder future studies attempting to use the same method/reward or further complicate the understanding of the concepts due to lack of clarity on the impact of valence.

The inconsistency in methodology may be attributed to several factors, including the relatively recent emergence of these concepts within the field of ethology, the absence of widely accepted protocols which may require different approaches between species or even individuals, and/or the inherently subjective nature of behavioural and psychological research which can lack consensus even in human research. Regardless, the development and adoption of a common test or standardized method for identifying these concepts would be beneficial, as it would facilitate clearer interpretation, improve comparability across studies, and contribute to a more coherent understanding of how best to measure and evaluate these concepts in animals.

*2.5.4 Measuring Motivation*

Motivation requires arousal and goal, where valence can be inferred from the reaction of the animal (positive stimuli = approach, negative stimuli = avoid). Several studies misinterpreted the valence in their methodology and interpretation of results. For example, motivation was mainly investigated using preference (also called choice) or aversion tests (Barbano & Cador, 2003; Bigiani & Pilenga, n.d.; Bremhorst et al., 2021; Brosnan et al., 2015; Daggett et al., 2019; de Jonge et al., 2008; De Petrillo et al., 2017; Gaultier et al., 2011; Hernandez-Gonzalez et al., 2007). Preference/choice tests provide the animal a choice between two or more options, where the selection is recorded. The goal is to understand which outcome the animal has a preference for. These tests are helpful in understanding the choice of the animal, but the downfall is that they do not tell us how strongly the animal is motivated for that choice, outside of the context of this specific choice, or how the animal feels about the choice (Kirkden & Pajor, 2006). For example, if we give the animal the choice between feed A or feed B, they may dislike both but choose feed B as it is viewed slightly more positively than feed A. Thus, they are slightly more motivated to eat B over A. This may mislead us to believe that the animal enjoys feed B when they do not.

The emotion of the animal towards their selected option is not known and can only be interpreted through behaviour exhibited towards the choice such as the speed at which the feed is consumed (using the previous example) if the study has opted to include these details. Barbano and Cador in 2005 used a preference test in rats, where the choice and latency to reach the food was recorded. Having the additional information on how quickly the animal ran to the reward can help indicate the strength of preference and valence towards the outcome. There is also the possibility to combine this test with other motivation tests to give further context to the animal's motivation. In 1997, Manser et al. combined a preference test with an operant test, where rats needed to push a weighted door to access either a room with an empty cage, a room with a nest box, or a room with a nest box and nesting material. The goal was to see the valence and strength of motivation through room choice and how much weight the rats were willing to push to access the room. This type of study reduces the likelihood of imprecise or inaccurate interpretation by giving additional information related to the choice and could be suggested for use in current research.

Aversion tests, also used to study motivation, are used to see strength of motivation to avoid an outcome through employing a negatively perceived stimulus (ex. the sound of a predator). The avoidance behaviour (such as latency to escape) can tell us how strongly the animal wants to get away from or avoid a negative stimulus (Kirkden & Pajor, 2006). This is important for welfare as understanding what causes distress for an animal can help us to avoid putting them in situations that will negatively impact them. There are multiple limitations with aversion tests, such as if animals are chronically stressed by outside factors, like frequent rough handling or poor home environment conditions, which may lead to a pessimistic view of any new stimuli or experiences, potentially causing non-aversive stimuli to elicit avoidance behaviour. Secondly, these tests focus on the negative emotional response of the animal, where current research is moving away from studies involving imposition of negative states like fear and moving towards exploring positive states like joy (due to ethical concerns and general interest).

So, although aversion tests are useful for investigating strength of motivation to avoid a negative stimuli, it is recommended to use other means of testing motivation to avoid the drawbacks associated with this test.

A test that was appropriate for looking at the strength of the motivation was the operant test (to be discussed further in *2.5.5 Assessing Anticipation*), where the animal was taught to perform a specific behaviour or task in order to receive a reward or avoid a negative stimuli (i.e. operant aversive test).The animal would be required to do a certain number of tasks or work (such as lever pressing or pushing a weighted door open), which indicates how much effort they will expend to get what they want, where the goal is to identify how strongly they are motivated for the outcome (Balci et al., 2010; Beran et al., 2012; Bokkers et al., 2004; Buck et al., 2014; Hayes et al., 2021; Kuhne, 2016; Wood et al., 2006). If an animal is quick to perform the task, they may view the outcome as extremely positive or extremely negative, depending on if the result is a reward or punishment (Anselme, 2010; Kirkden & Pajor, 2006). Similar limitations previously mentioned for aversive tests apply here when the outcome is viewed aversively.

When the result is viewed positively, the main concern for operant tests is the ceiling effect in which the animal is no longer physically able to perform the task, despite the motivation being there. Olsson et al. in 2002, found that feed-deprived hens were willing to push a weighted door to the maximum weight physically possible to have access to a feeder. However, once the door was too heavy to open, the hens often still made an attempt to try again. As the weight stops the hens from reaching the maximum point of motivation (where they can't physically push anymore, but still want to), it is difficult to determine how strong the motivation is past the point of the maximum weight the animal can handle. Although, this is still a useful test in knowing how motivated an animal is until the ceiling effect is reached, and seeing the animal attempt to continue to work past the point of their physical limit does tell us that the reward or outcome is further valued, past the point of the ceiling effect.

Delayed gratification tests were used in studies where the researcher wanted to know if and how motivation changed (increased or thwarted) when there is a postponement in receiving or having access to a reward that is expected and desired (Normando et al., 2013; Reefmann et al., 2009; P. Taylor, 2020). These tests involve training the animal to expect a reward, then delaying the reward in subsequent tests to evaluate how behaviour changes. For example, Taylor et al. in 2020 found that feed-deprived hens were more likely to peck the feed container during the delay period than non-deprived hens, which they concluded indicates a stronger felt motivation for the reward. Studies like these are also useful in understanding the strength of the motivation and since the reward is expected to be viewed as positive, a denial in reward might lead to negative emotion. However, the behaviour suggested to represent motivation in this case is actually indicative of frustration, where the animal has been denied that which they are motivated for. So, although it does tell us information relating to motivation, it was actually investigating the aspect of frustration without acknowledgement.

There were other instances where authors stated the results were suggestive of one concept, but were either mistaken (i.e. confusing one concept with another) or neglected to comment on the other concept(s) despite the fact that they were also being measured. This is why delay of gratification tests were also used to understand frustration (discussed further in *2.5.6 Finding Frustration*), where the reaction to the reward delay is reported as frustration behaviour or used to further explain the motivation for the reward (Brucks et al., 2017). Although, at what point the behaviour changes from being indicative of motivation to signs of a negative emotion like frustration is difficult to determine and may complicate analysis of results.

Inequity tests were used to measure valence of motivation towards equity as the goal is to see if the subject will share, withhold, or forgo a reward. In this test, the animal is often given the option to choose to give equal amounts of feed to their conspecifics, to eat more than their conspecific(s), or to not eat at all. Typically, these studies allowed the subject animal to dole out the rewards (often in the form of treats) to be either equal between subjects or in favour of themselves or their partner(s) (i.e. one receives more than the other) (Brosnan et al., 2015; Brucks et al., 2017; Hopper et al., 2014). Most animals were motivated to give equal reward instead of allowing themselves to have more than their counterpart(s) (Fletcher, 2008; Hopper et al., 2014). These studies demonstrated a motivation for equity through evaluating the choice in the test as a measure of valence towards having the same or less reward than a counterpart (where it is proposed that animals see equity as positive and inequity as negative).

This may be important for understanding social relationships and animal motivation to work together or to appease other animals. Oberliessen and Kalenscher (2019) postulated that animals prefer equity due to motivational mechanisms that maintain sharing as a cooperative function, where sustentation between individuals led to long term pay off. This could suggest that animals will make decisions in the inequity test based on the need or desire to fulfill this motivation. However, more investigation is needed on how inequity impacts emotional status (discussed in *2.5.6 Finding Frustration*). These tests often do not include the strength of motivation, as with preference tests, the choice (to share or not) is the primary indicator of the motivation. Adding an operant task where the animal needs to work to share or work to keep the feed to themselves could be recommended to assess the motivational strength for equity (in addition to the valence of the choice).

*2.5.5 Assessing Anticipation*

Anticipation happens after an animal has learned to expect an outcome, but before the outcome happens (with or without a stimulus signalling the event), where the number of behavioural transitions and the behaviour itself can be observed to distinguish the strength and valence of arousal towards the outcome. The number of transitions in behaviour relates to the arousal level, where a highly anticipated event (whether negative or positive) should elicit a greater response by the animal, thus leading to increased activity. A neutral outcome, or one that is not particularly desired or feared for example, would result in lower activity level (Anderson et al., 2020).

The behaviour itself can identify the valence, where an animal that positively associates the outcome might behave differently when faced with a negatively perceived outcome. For instance, De Petrillo et al. in 2017 observed more scratching and hiccupping in capuchins before choosing a risky option (they either receive one reward or seven) than before choosing a safe reward (four reward items guaranteed). They posited that these behaviours related to a general state of anxiety in selecting the risky option before the outcome was revealed. Although, it may be difficult at times to correctly identify the valence, where Cless and Lukas (2017) found that pacing in captive polar bears could indicate positive affect in anticipation of an event or could be a method to help the animal cope with stressors when in negative affect. Both anticipatory characteristics of arousal should thus be used collectively for appropriate identification of anticipation.

As with motivation, operant tests were also used for understanding anticipation, where more work to reach or avoid an outcome signified strong anticipation (positively and negatively, respectively). However, for studies using this test, the results were interpreted in terms of strength of anticipation for reward, rather than (or in addition to) the motivation for reward.

These tests can include a cue to signal the beginning of the test or not, depending on the set-up and task involved. Two authors, Hayes et al. (2021) and Wood et al. (2006), chose to look at how many times the animal would perform the task before receiving the reward as an example of preparatory behaviour. One study by Beran et al. (2012) trained rhesus and capuchin monkeys to make correct matches using a joystick that connected to a screen and evaluated their ability to predict future matches. It was found they were able to change their behaviour to make educated guesses about future events, suggesting that animals not only have anticipation, but can adapt their behaviour to maximize the possibility for reward (Beran et al., 2012). As motivation and anticipation are connected, there may be issues with concluding that the behaviour in operant testing is indictive of only one of these concepts, when in fact it is often both (ex. Beran et al., 2012; Paxton & Hampton, 2009; Raby et al., 2007). In an operant study on mice in 2010, Balci et al. aptly remarked that "motivational states mainly affect the decision to initiate anticipatory responding, with limited effects on both the perceived interval and the decision to terminate anticipatory responding". Accordingly, authors should consider reflection on this in their discussion (in any anticipatory test), to avoid missing a key factor that influences anticipation: the motivation.

Another assessment that attempts to address if animals can predict their future is the 'theory of mind' test. In this test, the author's aimed to determine if and how the subject can understand (without being cued) that their current sense of reality can be different than that of their future reality (ex. Beran et al., 2012; Paxton & Hampton, 2009; Raby et al., 2007). Although these tests are typically used for judging cognitive ability, it was used by Correia et al. (2007) and Raby et al. (2007) to see if the animal could give up their current motivation to eat to instead store the feed (also called 'caching') in expectation of future hunger. Both studies used western scrub-jays as test subjects where the birds could either eat in the present but receive no feed at a later point in time, or could forgo eating to cache the feed in trays for consumption later. Both authors found that birds would forego their current motivational state for feed by caching in preparation of future needs (Correia et al., 2007; Raby et al., 2007).

Both of these predictive tests were able to demonstrate animals' ability to anticipate and adapt as necessary. This type of test measures the strength of the arousal, as caching indicates that the animal deems the preparatory behaviour important for the outcome and increased caching actions denote stronger anticipation. One drawback is that the behaviour in this test may not be interpretable for the expectation and valence of the outcome, as whether caching is due to hope (that caching will prevent future hunger) or apprehension (that others will steal its feed if not cached) or both, is unclear.

Additionally, this test differs from operant tests in that it still requires some learning to be able to make an accurate prediction (ex. association between caching and hunger), but it doesn't require a specific stimulus to trigger anticipatory behaviour. So, when preparatory behaviour actually begins/ends might be challenging to ascertain. Moreso, being able to predict an outcome without a signal is not the same as being trained to associate a stimulus to an event. Predicting what will happen next is easier for an animal when it has been taught to expect something specific. However, predicting an unknown future requires a higher level of thinking. Therefore, the results presented in both scenarios are not equivalent and cannot be compared. This adds further challenge to studying anticipation uniformly, when dependent on the test, one author may be researching a different type of anticipation than another (one with cues and one without), where the study without cues may also struggle to correctly identify when anticipatory behaviour begins and ends.

Other studies used only the number of behavioural changes (i.e. changes in activity level) to look at the strength of arousal of anticipation (Cless & Lukas, 2017; McGrath et al., 2016; Peters et al., 2012; Spangenberg & Wichman, 2018). Although, it again misses one aspect of emotion, where we know the level of arousal, but not the valence. Without this knowledge, it is difficult to understand the animal's perception and potential response, along with what management steps should be taken. Therefore, both behaviour and activity level should be considered when trying to evaluate these concepts and their relationship to animal emotion.

*2.5.6 Finding Frustration*

Unlike the previous concepts, the valence of frustration is already known. Frustration arises due to denial or delay of an expected outcome, resulting in a negative emotion. So, as a negative state needs to be imposed (i.e. the outcome is expected to be negatively received), the important factor to consider is how strongly felt the emotion is and how it is displayed. As stated earlier, research is moving away from imposing negative states, but ample work has already been done on frustration, which justifies the examination of this concept.

As with motivation, inequity and delayed gratification tests were also used to evaluate frustration. In this case, however, the behaviour (or transitions in behaviour) demonstrated during the inequity or delay were considered indicative of frustration. Inequity tests were used to see animals' (ex. primates, dogs) responses to not being given an equal reward as their counterparts, and two inequity studies even purposely gave some individuals less and recorded their reactions (Brosnan et al., 2015; Brucks et al., 2017; Fletcher, 2008; Hopper et al., 2014). For delay of gratification tests, an animal was trained to expect a reward and then that reward was delayed or denied completely and the animals' response was recorded. In the case of both inequity or delay tests, not being given the same amount/value of reward as a partner or an expected reward being denied could lead to frustration. For example, Normando et al. (2013)

found that cows increased their agnostic behaviour when feed was delayed, regardless of the frequency of the delay during the week.

As the inequality or delay/denial time can vary depending on the study, when the animal has understood the reward discrepancy and switches from preparatory behaviour to frustration behaviour could be indistinct. A limitation already discussed is the inability to determine when one concept ends and another begins, in this case the difference between anticipation and frustration. Especially if the behaviour of anticipation and frustration are the same/similar - using again the example of Cless and Lukas' 2017 study in which pacing in polar bears was speculated to be both a sign of anticipatory behaviour and a representation of frustration. The authors do suggest future research should compare anticipatory pacing with general pacing to reveal when pacing in anticipation becomes 'centrally controlled pacing' (Cless & Lukas, 2017). Both anticipation and frustration can result in high arousal which would elicit more behavioural transitions due to increased activity level, so transitions alone are not enough to distinguish between the two. One option could be to combine the measure of transitions with behaviour itself that is clearly related to frustration.

Stereotypic behaviour, abnormal repetitive actions that serve no goal or function, and agonistic behaviour, were often considered in studies investigating frustration as they have been universally recognized as behaviour caused by frustration (as a mechanism to cope with an unfulfilled need) (Ahola et al., 2011; Campbell et al., 2013; Fangmeier et al., 2020; Freymond et al., 2020; Hansen & Moller, 2008; Jones et al., 2011; Kohari et al., 2017; Latham & Mason, 2010; Tatemoto et al., 2019; Vickery & Mason, 2004). As stereotypies are well described, in studies where the author aims to see the presence or absence of stereotypies in relation to a frustrating context, it is much simpler to identify if and when the animal feels frustration.

Comparably, studies where the only differentiator between anticipation and frustration is time (ex. Bremhorst et al., 2019, 2021; Briefer et al., 2015, p. 2; Gygax et al., 2013) are less straightforward when determining the transition point.

Stereotypic behaviours were also used in frustration tests or impossible/unsolvable tasks which purposely induced frustration in the animal and the resulting behaviour was observed (Alterisio et al., 2018; Bremhorst et al., 2019, 2021; Delgado & Jacobs, 2016; Freymond et al., 2020; Gygax et al., 2013; Kuhne, 2016; Piotti et al., 2021; Vasquez et al., 2021; Webster & Brosnan, 2021). Impossible/unsolvable tests train the animal to do a specific action or behaviour in order to receive a reward (ex. feed is placed on a table in front of a horse where they can reach to grab it), then in subsequent rounds the task is made "impossible" (ex. feed is placed too far away for the horse to reach it) (Alterisio et al., 2018). Results from this test were also used to conclude whether the target animal could experience frustration and to understand how they respond to it once it is felt. These tests represent progress towards finding less ambiguous outcomes, as the point at which the animal is unable to solve the problem/task is likely the starting point of frustration. Along with the observation of stereotypic behaviour, the results from impossible/unsolvable tests could be more straightforward in pinpointing frustration. Finding contexts in which frustration is apparent can help caretakers and researchers alike to find solutions that avoid or reduce the amount of frustration being experienced by captive animals.

### *2.5.7 Future Directions for Motivation, Anticipation, and Frustration Research*

The first concept, motivation, is important to study as it can help us to understand the needs and/or desires of an individual animal or a species-specific motivation, which can be used to make informed decisions regarding welfare. Previous research has spent substantial time understanding what animals want and are motivated for, but the remaining question is: how strongly is that motivation felt, and how does the outcome impact the emotion of the animal? If an event is recurring, where the animal has the opportunity to learn and expect a future outcome, the preparatory stage of anticipation could be evaluated to understand what events are viewed as immensely negative or positive for the animal (as anticipation entails a high arousal state, but the valence will depend on the individual's perception of the signal and associated outcome). However, as previously mentioned, valence is an important aspect to consider for knowing whether the animal interprets the stimuli/outcome as positive or negative. Frustration is also connected to the animals' needs or desires, but directly tells us the perceived valence of the outcome and if we see an absence of frustrated behaviour and low activity level when a positive stimuli is applied, the animal could be satisfied with the outcome (or relieved if the stimuli is perceived to be negative).

Although two out of three concepts do not directly tell us about the emotion of the animal, we can combine the results from each to infer (through the context given and the exhibited behaviour) how animals feel about the environment and enrichment we provide, and whether they are perceived positively by the animal or not. Moreover, understanding and measuring one concept can tell us about one aspect of the chain, but having information about all three (motivation, anticipation, and frustration) is a step towards better understanding the full picture of the animal's needs and/or desires. For example, if we see the motivation to interact and preparatory behaviour in anticipation of an event, we could assume that denying that opportunity will result in frustration, but how strongly that emotion will be felt, and in what quantity or frequency the opportunity is sufficient to avoid frustration, is not known unless we measure the frustration as well.

While the variety in methods makes it difficult to compare what previous researchers have done, it also allows for a wide selection of options for trying to study these concepts. It appears that the main connection between all of the selected studies is the use of behaviour, which is also a direct measure of animal welfare (Dawkins, 2004; Whay et al., 2003). There is potential for using methods that combine all concepts, such as using an operant and preference test together (motivation) (as identified in Manser et al., 1998) where behaviour before the test is measured for anticipation and behaviour after the reward is denied is measured for frustration. For example, using multiple weighted doors that lead to chambers/rooms with the choices being evaluated, where after learning the options, anticipation and motivation are measured, and then once the preferred choice is determined, it could be denied/removed to measure frustration. This could, theoretically, be used within and between species. However, as different species have unique physiology to one another, the behaviour being considered might not be applicable to other animals.

Determining universal parameters, or rather a framework, for measuring motivation, anticipation, and frustration across species, could be useful in creating uniformity within the field, to further improve the understanding of the main concepts. So, future study should be done with a focus on creating standardized methods to include in a framework that allows for consistency when studying these concepts in animals.

## 2.6 Conclusion

The purpose of this scoping review was to determine how the three main concepts: motivation, anticipation, and frustration, were defined and related, and to identify any gaps in knowledge when researching these concepts. It was confirmed that there was not a universal definition used for each concept as many authors chose to omit a definition or if included, borrowed parts or whole quotes from previous works, where there lacked uniformity in the terms used to describe each concept. Despite this, the selected articles were useful in understanding the connection between motivation, anticipation, and frustration (Figures 2.6 and 2.7). However, the methods and variables chosen to measure these concepts in animals varied greatly, even within a species and/or using the same tests. As well, most authors focused on studying one or two concepts, but using each concept in conjunction would help to get a full picture of the emotion of the animal.

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## Connecting Statement 1

The comprehensive review highlighted the missing gaps related to understanding animal motivation. Definitions of motivation, anticipation, and frustration were not universally agreed upon, and the approaches to identify or demonstrate these concepts were not consistent, even in studies involving the same species and experimental conditions. One of the other takeaways of the review was how motivation, and specifically behavioural measures of motivation, can be used to understand and make conclusions about animal emotion as it relates to animal welfare. This review also found that the included studies tended to test response to feed reward, but the ramifications of other management decisions on motivation (particularly, enrichment and its applications) were not as well investigated. As enrichment use is becoming more prevalent in commercial farming, its impacts on animal motivation would be useful for tailoring welfare practices towards the needs and/or desires of the animal.

In the following chapters, numerous types of enrichment will be applied in a variety of contexts, using both young and adult animals from multiple species, to study the effects on motivation. Specifically, Chapter 3 will build on behavioural measures, presenting on how enrichment generally impacts motivation in other contexts. Enrichment will be applied in-pen for lambs and then, outside of where the enrichment was available, motivation in a test maze will be observed. Knowing if enrichment affects motivation outside of where the enrichment is provided, could influence how and when producers use enrichment.

# Chapter 3) Early Life Multi-Modal Enrichment Impacts on Learning, Flexibility, and Motivation of Lambs in a Test Maze


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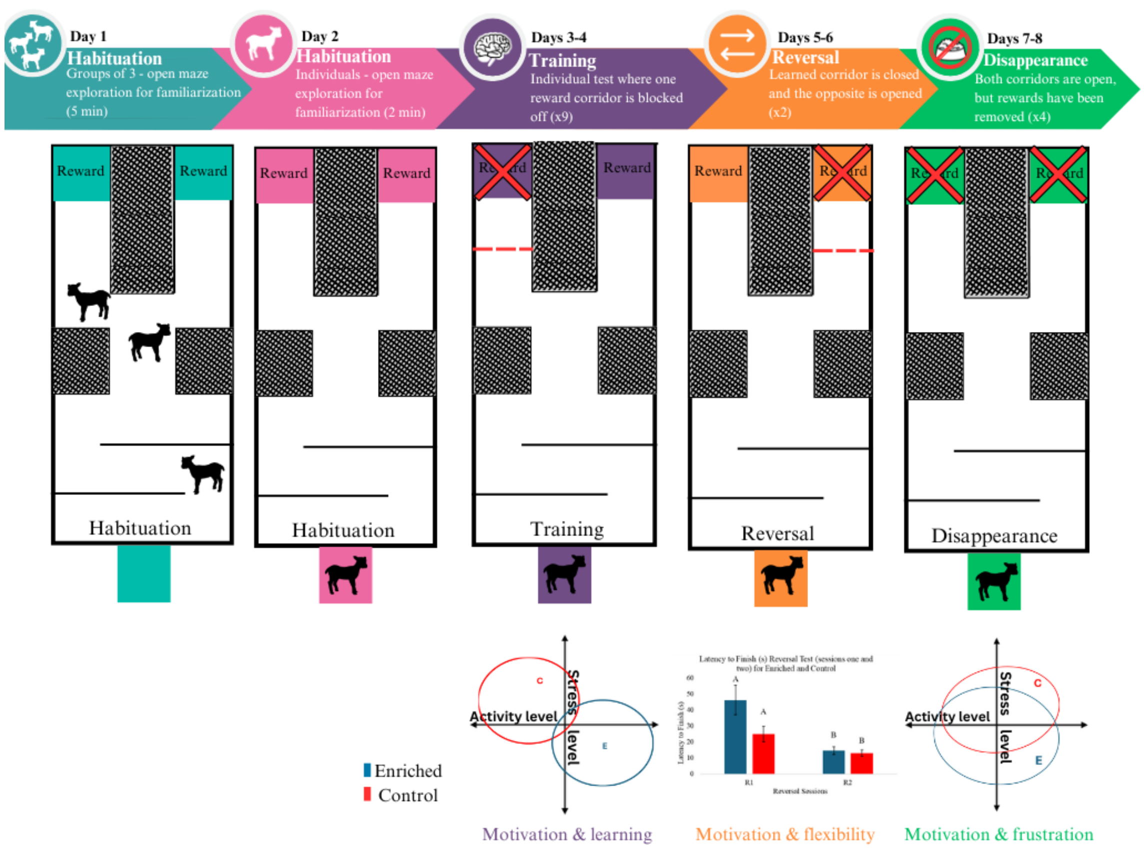
## 3.1 Graphical Abstract



## 3.2 Abstract

Early life enrichment has shown benefits on the cognitive ability of livestock and animals have shown motivation to have access to positive stimulation. However, how environmental enrichment impacts lambs from birth to fattening and in a context outside of where the enrichment was provided needs further investigation. This study used a longitudinal randomized controlled design to explore how multi-modal enrichment in the home pen impacted lambs' learning, flexibility, and motivation for a reward whilst in a test maze. Lambs were split into two groups: control which received no in-pen enrichment and enriched which received multi-modal in-pen enrichment. The animals were individually tested in a maze outside of the pen environment using multiple types of tests. Training tests were used to evaluate the lambs' ability to learn and motivation to go to one end of the maze for a reward while the other end had no reward. A reversal test was used to determine the lambs' behavioural flexibility and motivation to adapt learned behaviour when the end where the reward was previously found was reversed to the other side. Lastly, a disappearance test was used to understand motivation and resulting emotion when the reward was removed from both ends of the test maze. No treatment differences for learning or flexibility ($p > 0.05$) were found, but there was a difference in motivation to search the maze, including discrepancies in types of behaviour performed, revealing that enriched lambs were more comfortable with isolation when in the maze. Further, a session effect was found for training, reversal, and disappearance tests ($p < 0.05$), where animals were faster and more flexible over time. Lambs from both groups were more motivated to find the reward across training and reversal tests, but less motivated over time in the disappearance test. Enriched lambs persisted in searching for a solution longer than control, suggesting animals who do not receive enrichment in the home pen may be less hopeful for a reward in other contexts. Although further research on the impacts of early life enrichment on learning,

flexibility, and motivation is needed, enrichments employed from a young age are recommended to improve the welfare of sheep.

**Keywords: reactivity, frustration, behavioural robustness, affective state**

## 3.3 Introduction

In early life, a young animal's brain is more plastic, referring to its ability to mature and mold adaptively based on their environment and their experiences (Hedges and Woon, 2011). Early life stress can have long-term cognitive effects, causing deficits in learning and memory, for example, lambs who were faced with daily aversive experiences were found to make fewer correct choices in a learning test than control lambs (Destrez et al., in 2013). The early lambing (1-2 months after birth) and weaning (2-3 months after birth) stages are especially sensitive for sheep, as they learn in what contexts they should interact or avoid (Ligout et al., 2011). Thus, early life experience is also important for learning behaviour that gives feedback to the motivation of the animal.

Motivation is a goal-directed drive that urges the performance of behaviour necessary to meet the animals' perceived physiological and/or psychological state (Jensen and Toates, 1993; Manteuffel et al., 2009). How motivated an animal is for a reward, for example, can determine how hard they are willing to work to learn how to access that reward, and how hard they will persevere in performing behaviour that they have learned results in reward access. One way to evaluate the effects of early life enrichment on motivation and learning are to study flexibility, which is the ability of the animal to adapt their behaviour in response to changes in the environment (Uddin, 2021; Raoult et al., 2021). This can be evaluated using reversal learning, where the animal is taught to do a task one way, then the 'rules' of the task are reversed or changed to see if the animal can modify their previously learned behaviour (Uddin, 2021; Raoult et al., 2021). If the animal is able to adapt to the new rule, they are considered to be more flexible than animals that fail to switch.

A common test of reversal learning is using spatial reversal in a test maze which involves training the animal to navigate to one end of the maze for a reward while the other end has nothing, then the subsequent end with the reward is swapped. Calves who were provided diversity in the diet were found to be faster at learning a T-maze reversal than control calves, where they suggested that control calves had reduced flexibility due to lack of feeding enrichment (Horvath et al., 2017). However, how in-pen enrichment impacts reversal learning in lambs is not as well understood.

Enrichment, that is a manipulation or change to the environment that improves welfare through allowing animals to act on their motivated behaviour, is becoming more commonplace in livestock housing (Botreau et al., 2023a,b). Previous studies found that providing enrichment can improve reactivity, such as reducing fear behaviour during an isolation test (Vandenheede and Bouissou, 1998) or cognitive bias , such as increasing optimism towards an ambiguous outcome in a judgement bias task (Stephenson and Haskell, 2022). This can help to improve overall welfare through reducing stress in situations that may arise during early life such as being separated from their flock or being exposed to unfamiliar stimuli (Vandenheede and Bouissou, 1998; Stephenson and Haskell, 2022). However, little research exists on effects of early life enrichment on lambs' motivation and flexibility, specifically whether enrichment provided in the home environment has impacts on motivation for a reward in another context (i.e. outside of the home pen) and if it affects flexibility to change a previously learned behaviour. Further, the denial of an expected reward or the ability to perform motivated behaviour could lead to frustration (Anderson et al., 2020), but the removal of a reward in relation to whether an animal is housed in an enriched environment or not, needs further investigation. To test whether enrichment can help to improve reaction to being denied a reward, a disappearance test can be employed where the expected reward is removed completely to evaluate the animals' response (Seehuus et al., 2013; Latham and Mason, 2010).

The aim of this study is to determine how multi-modal enrichment in the home pen from birth to fattening impacts lamb's motivation and flexibility relating to a feed reward in a test maze (during training, reversal, and disappearance tests). Specifically our hypotheses are; 1) enriched lambs will be faster to find a feed reward than control lambs during training and reversal tests, 2) enriched lambs will be more motivated than control lambs to search for the missing reward during disappearance tests, and 3) enriched and control lambs will display different behavioural motivations after learning to navigate the maze.

## 3.4 Material and Methods

*3.4.1 Ethics Statement*

The animals and research procedures used in this study (project #33551-2021102211369923) were approved by and followed the standards set forth by the regional ethics committee of the French Ministry of Education and Research.

*3.4.2 Animals and Housing Conditions*

This study used 40 lambs from 20 pregnant Romane bred ewes (60-day gestation) (i.e. 2 lambs per ewe (n=40)), from birth until the fattening period from the UMR Herbivores unit of the Université Clermont Auvergne (INRAE, VetAgro Sup), F-63122 Saint Genès-Champanelle, France. The lambs were raised with their mother until weaning. The animals were housed in two perimortem rooms (2 batches per room) in breeding pens (46.5m²). In each room, there was an ewe pen (26.57m²) and a lamb pen (19.93m²) connected by gates (each 1.5cm long) with rubber flooring covered by straw that was replaced weekly.

The lambs were split into two groups: a control group (n=20) where lambs were reared without enrichment (with the exception of social enrichment, due to the nature of the pens) and an enriched treatment group (n=20) where lambs were reared from birth to fattening with multi-modal enrichment that covered several categories including: physical, sensory, and nutritional.

There was a box with diffused olfactory scents that changed each day, twice a day in a repeating pattern over seven weeks. Alcohol was used a baseline in week one, then in the subsequent weeks, two scents were dispensed each day (one from 10:30am-12:30pm, and another from 2:30pm-4:30pm) in the order of; thyme, then chamomile (day one), bitter orange, then lavender (day two), chamomile, then bitter orange (day three), and lavender then thyme (day four). This schedule was determined to be the least aversive order based on a preliminary study. There was also a white Kerbl L-shaped nylon brush (43x10cm), yellow fixed rectangular nylon brush (43x24cm), white cotton hanging rope (12mm in diameter) and blue polypropylene hanging rope (14mm in diameter), suspended orange plastic chewing disc (17.8x14.7cm), and red Hippo-Tonic suspended ball (17.5cm in diameter), a feed additive, and multi-level plywood climbing platform with anti-slip coating (60x120x120cm) in the enriched pens (see Figure 3.1).

*Figure 3.1 Images (©Raphaëlle Botreau) from lamb pens illustrating the control group (top) and enriched group (bottom) which features multi-modal enrichment including; white and yellow brushes (top and bottom left), olfactory scents (bottom middle), hanging ropes and toy (top center) and red ball (middle left), feed additive (top right), and climbing platforms (middle right).*

### *3.4.3 Test Maze Setup*

The test maze was used as a way to measure motivation for reward and frustration when denied reward of control and enriched lambs, including latency to reach the reward and behaviour whilst in the maze. Over the course of the trial, only 14 lambs from each group were tested due to time constraint and as housing was too small to fit all lambs once they reached the finishing stage. The test maze (12m x 4.5m) consisted of a start box (0.75m x 0.5m) where the lambs were originally placed before the start of any tests (see Figure 3.2). Near the beginning of the maze was an experimenter zone where the test experimenter sat during testing. The maze consisted of 3m long alternating opaque walls that forced the animal to follow a specific pathway until reaching a narrowed lane (1.5m width) that split into two corridors, one to the left and one to the right, that each led to a reward zone after passing through the exit zone which included exit gates that were only opened when the test was completed to let the animal out of the maze. There were also 3 cameras placed around the maze to capture the behaviour of the animal during testing (see Figure 3.2). Noldus MediaRecorder software was used to synchronize the videos.

### *3.4.4 Habituation*

During the habituation process, the animals were given time to become familiar with the setup of a testing maze over a 2-day period, the first day in groups of three for five minutes (where rewards were available in both reward zones) and the second day individually for two minutes (where the animals alternated between having the left and the right corridors open). This process was repeated three times per group or per individual, where the animals were observed to ensure they have explored all areas of the maze, including the reward corridors, and had consumed the concentrate in both reward zones (for groups) or in at least one of the reward zones (for individuals). Animals that did not reach one of the reward zones within two minutes of the individual habituation, were gently encouraged forward by a handler to ensure experience traversing the entire maze.

### *3.4.5 Training Tests*

Training was carried out over two days, where individuals were made to pass three times each day (i.e., sessions T1-T6) in the maze with a target corridor open while the other was blocked off to force the lamb to learn to go one direction for the reward. The lambs were first placed in the start box for ten seconds before the door to the maze was opened and the animal was given 30 seconds to exit the box (after this time, they were gently encouraged by a handler to enter the maze). Once inside the maze, the animal's behaviour was recorded for 120 seconds, live (latency to vocalize and number of vocalizations) and on video (latency to finish test, percentage of walking, immobile, exploring, or looking at the experimenter, frequency in exit or reward zone, and frequency of agitated behaviour (i.e. running, jumping, trotting)). Once the animal reached the reward, they were given 10 seconds to eat the reward before the handler led them towards the closest exit gate. If the animal had not reached the reward within 120 seconds, the animal was gently pushed towards the reward and left for 10 seconds where they could consume (or not) the reward (to ensure they understood where and what the reward was). Training was done to understand the motivation of lambs for reward.

### *3.4.6 Reversal Tests*

The reversal tests were carried out over two days, where the individuals made two passes each day. The first passes were simply a reminder of the trained corridor using methods described in 3.4.5 (i.e. sessions T7 and T8). The second pass involved closing the opposite corridor to the one that the animal was trained on, to "reverse" the location of the reward, where the animal was left in the maze until they found the reward in the opposite corridor or a time of 120 seconds was reached (i.e. sessions R1 and R2). This test was done to understand flexibility and whether the lambs were able to adapt their behaviour to find the new reward location.

*3.4.7 Disappearance Tests*

After all previous tests were completed, a disappearance test was run over a two-day period. The first passage was a repeat of training in which the reward was present in the trained corridor (i.e. session T9). The following sessions involved the complete removal of the reward from both corridors, where the lamb had four opportunities (two per day) to search the entirety of the maze for the (missing) reward for the duration of 120 seconds (i.e. sessions D1-D4). The purpose of this test was to understand the motivation of the animal for the reward and frustration when the reward was denied. For the disappearance test, variables observed on video that were not recorded for the other tests included latency to reach a reward zone, frequency in the beginning zone, frequency of frustrated behaviour (foot scraping, running, trotting, jumping), and number of zone changes.

*3.4.8 Behavioural Measures*

In all tests, the main behaviour of interest was the duration (in seconds) it took for the animal to reach the reward (or a finish zone in the case of the disappearance test). The duration, frequency, and/or percentage of behaviour during the tests was also recorded, where the behaviour of interest was dependent on the test itself. These behaviours (latency to finish the test, vocalizing, looking at the experimenter or open corridor, immobile, zone frequency (i.e. beginning, reward, exit, or obstacle zone) and number of zone changes, exploring, walking, nervous behaviour (i.e. running, trotting, jumping), food searching, and frustration behaviour (i.e. running, trotting, jumping, foot scraping) were previously annotated by a trained observer (at INRAE) on the software program Noldus Observer XT 16 (Wageningen, Netherlands).

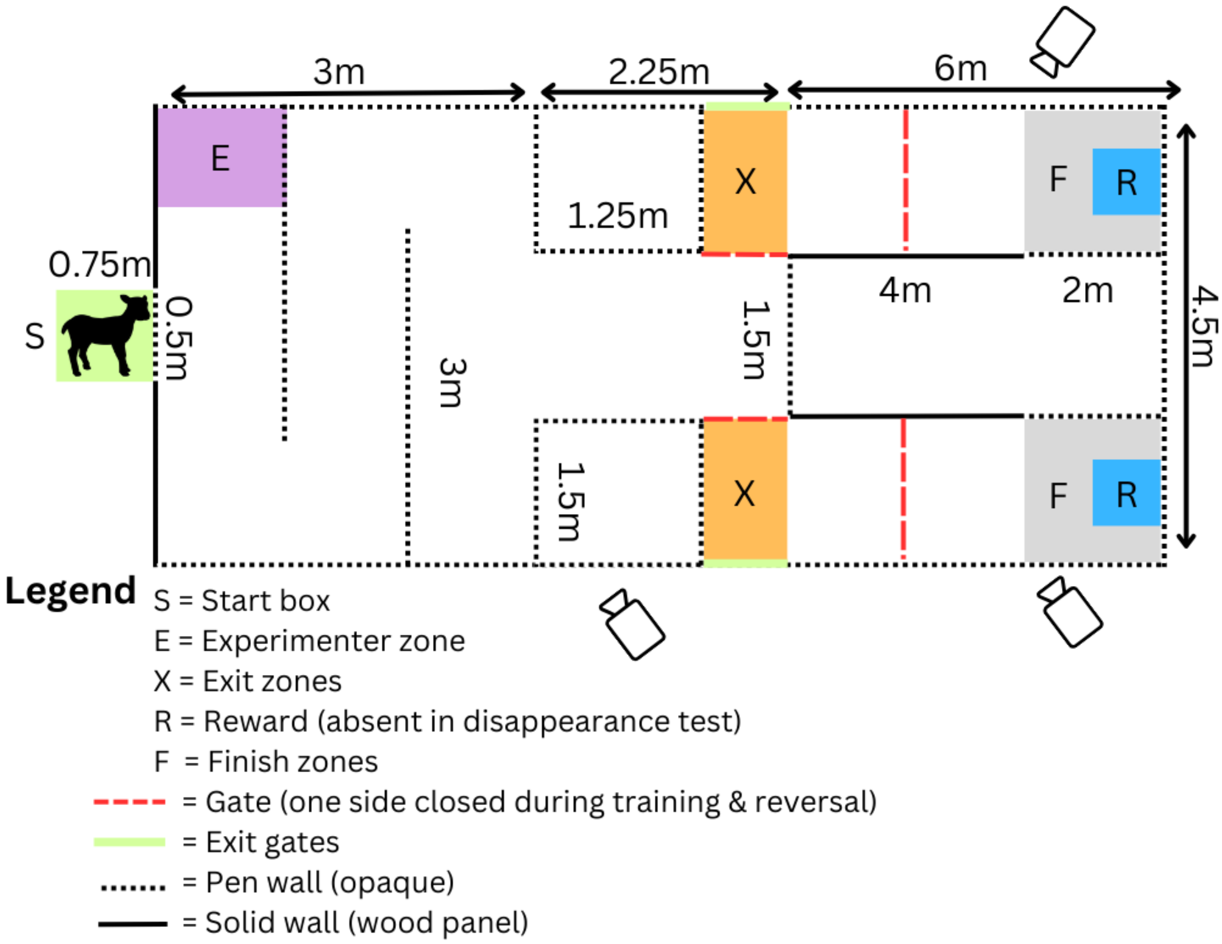


*Figure 3.2 Diagram of the maze used for testing the lambs in which the individual was placed in the start box and was trained to navigate the maze to find a feed reward (in blue). This placement was counterbalanced between groups, where animals were trained to expect one side of the maze to be open and the other side to be closed off. The side that was learned was subsequently closed off and the opposite side was opened during the reversal test. During the disappearance test, the reward was removed entirely and both corridors of the maze were available to explore.*

### *3.4.9 Statistical Analysis*

All statistical analysis was done on R® version 2021.09.2+382.pro1 Ghost Orchid (250 Northern Ave, Boston, MA 02210) with the Factoshiny package for Principal Component Analysis (PCA); lme4, lmerTest and emmeans, for model analysis. Two linear mixed models (LMr) were employed, where treatment, session, and interaction of treatment and session were considered fixed effects, and lamb nested in mother (or just lamb when mother did not have an effect which was tested by model comparison) was considered a random effect. The models are as follows:

$$\gamma_{ijk} = \mu + \text{treatment}_i + \text{session}_j + \text{treatment*session}_k + \text{mother(lamb)}_l + e_{ijkl}$$

$$\gamma_{ijk} = \mu + \text{treatment}_i + \text{session}_j + \text{treatment*session}_k + \text{lamb}_l + e_{ijkl}$$

Q-Q plots were used to assess normality of the data graphically and Levene tests for homogeneity. The Gamma law was used to transform the data when conditions for normality were not met. Post-hoc pairwise comparisons via Estimated Marginal Means (emmeans) with a Tukey adjustment were performed when necessary. Significance was declared at $P \leq 0.05$, and tendencies between 0.1 and 0.05.

Fisher tests were done to compare how many animals went to a reward zone or not, how many animals visited a reward zone two times or fewer vs. more than two times, and how many animals visited one or fewer vs. two or more zones (i.e. any zones of the maze).

A Principal Component Analysis (PCA) was done on the behaviour exhibited during the tests (e.g. latency to finish, running, trotting, etc.). Variables were removed or combined based on contribution and biological significance. The coordinates of the final PCAs were extracted and also assessed using a linear mixed model with Gamma law and post-hoc comparisons as previously defined.

## 3.5 Results

### *3.5.1 Training Test Results*

For latency to finish the training test, the interaction of treatment and session were found to be significant, where both groups took longer to finish the test in the first two sessions (T1 and T2) compared to the other sessions (T3 and following; Figure 3.3). For enriched sheep, the first two sessions were different from the others, but not from each other ($p < 0.05$). For control sheep, the first session was different from sessions two, three, four, seven, eight, and nine ($p < 0.05$). Control session five was different from sessions four, seven, eight, and nine. Session six was also different from sessions seven to nine. Between groups and when comparing the same session (i.e. enriched T1 vs. control T1), there was no difference ($p > 0.05$).

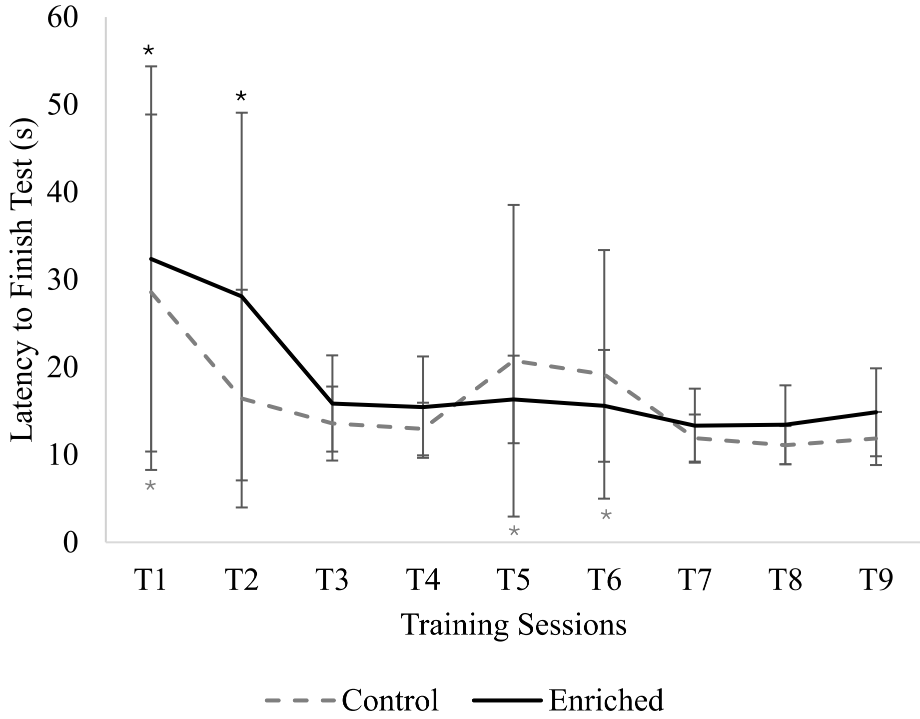



*Figure 3.3 Average latency to finish in seconds for control and enriched groups for the nine training sessions (sessions T7-9 are specific training tests for reversal (T7, T8) and disappearance (T9)). Sessions with * are significantly different from those without (comparing only between sessions of the same group (e.g. enriched T1 vs. enriched T3) ($p < 0.05$). Session comparisons between groups in the same session (e.g. enriched T3 vs. control T3) were not significant ($p > 0.05$).*

The PCA for training tests revealed two dimensions related to sheep behaviour with eigenvalues greater than one. These two dimensions explained 71.82% of the variance, where the first dimension was named “Reward Access” (which included latency to finish, latency to vocalize, percentage of exploring and immobile, opposite frequency of exit zone and reward zone) and the second dimension was named “Agitation Level” (which included frequency of agitated behaviour and looking at experimenter opposite walking percentage) (Figure 3.4).

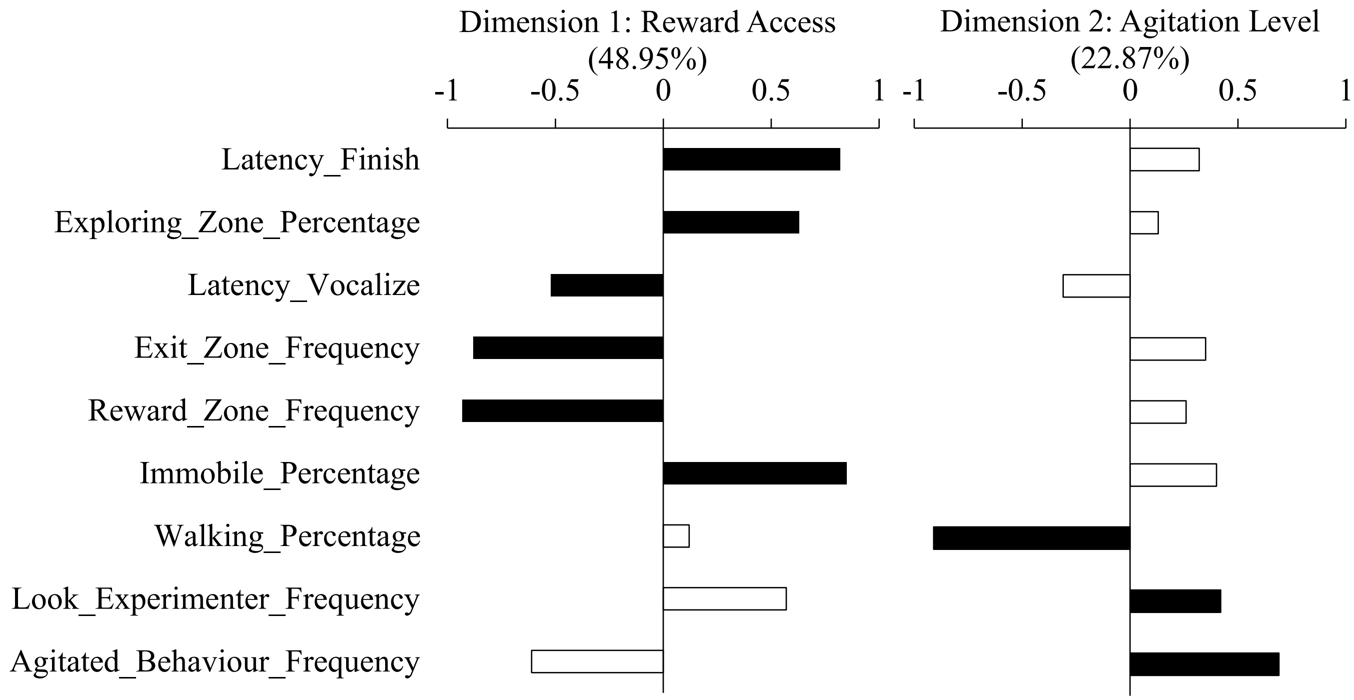



*Figure 3.4 Coordinates of variables from training tests PCA including which dimension each variable had a higher contribution to (i.e. solid black bars).*

The comparisons of treatment, session, and interaction between treatment and session from the PCA coordinates showed difference of session for Dimension 1 ($p < 0.05$), and a tendency for session in Dimension 2, although post-hoc comparisons did not identify any pairwise differences for Dimension 2 ($p > 0.05$). For Dimension 1, there was an increase in "Reward Access" between session one (T1) and all other sessions (T2 and following; $p = 0.03$ between session one and two, $p < 0.0001$ between session one and all other sessions). There was also an increase of "Reward Access" between session two and the last three sessions of training ($p < 0.001$). "Reward Access" during training sessions five and six were also lower than sessions seven and eight (T5-T7 and T5-T8: $p = 0.03$, T6-T7: $p = 0.05$, T6-T8: $p = 0.005$). All other sessions of the training were not different ($p > 0.05$).

*3.5.2 Reversal Test Results*

In evaluating behavioural flexibility, no difference in how many lambs correctly started the reversal test (i.e. went to the learned side first) ($p > 0.05$) or how many passed the test (i.e. went to the learned side then reversed and went to the other reward zone) were found ($p > 0.05$). However, there was a decrease in latency to finish the reversal test between the first and second session of the test (R1 and R2, $p < 0.05$).

The PCA for reversal tests revealed two dimensions with eigenvalues greater than one that explained 65.29% of the variance. The first dimension was named "Search Strategy" (which included latency to finish test, frequency of looking at the open corridor, and frequency of exploring opposite number of zone changes frequency) and the second dimension was named "Contact Seeking" (which included latency to vocalize opposite vocalizing frequency) (Figure 3.5).

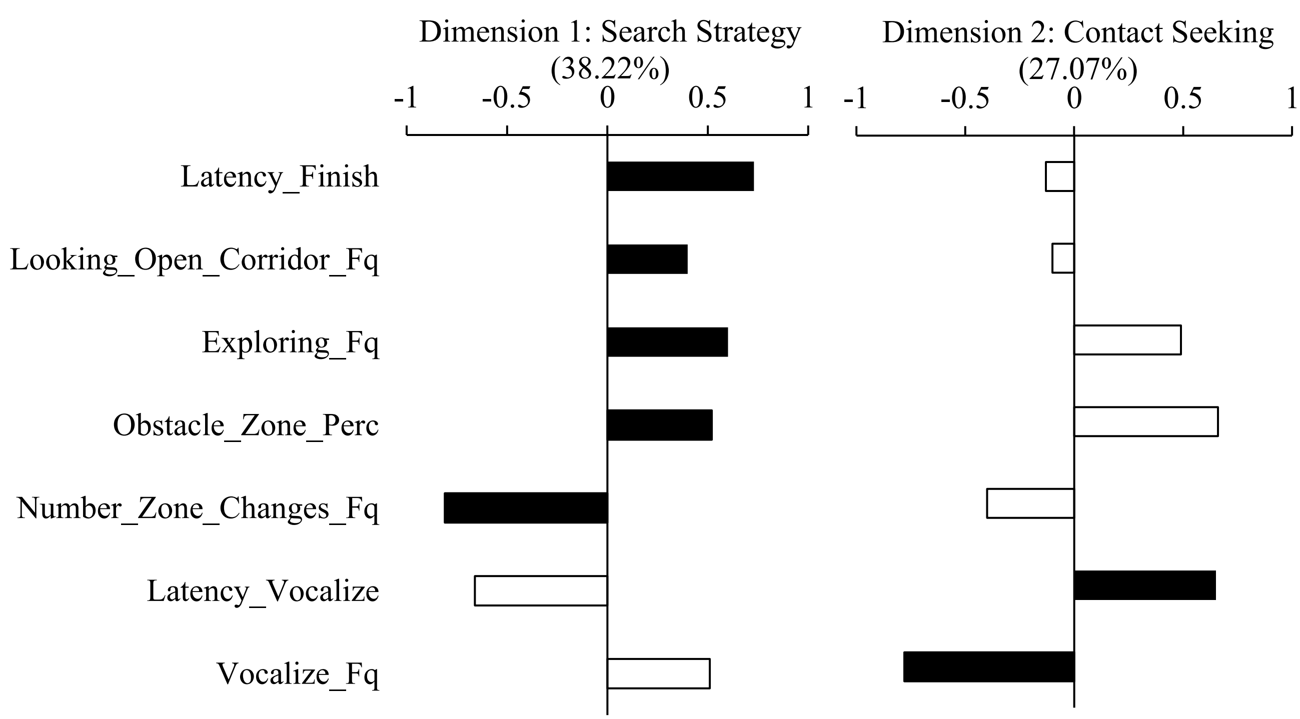
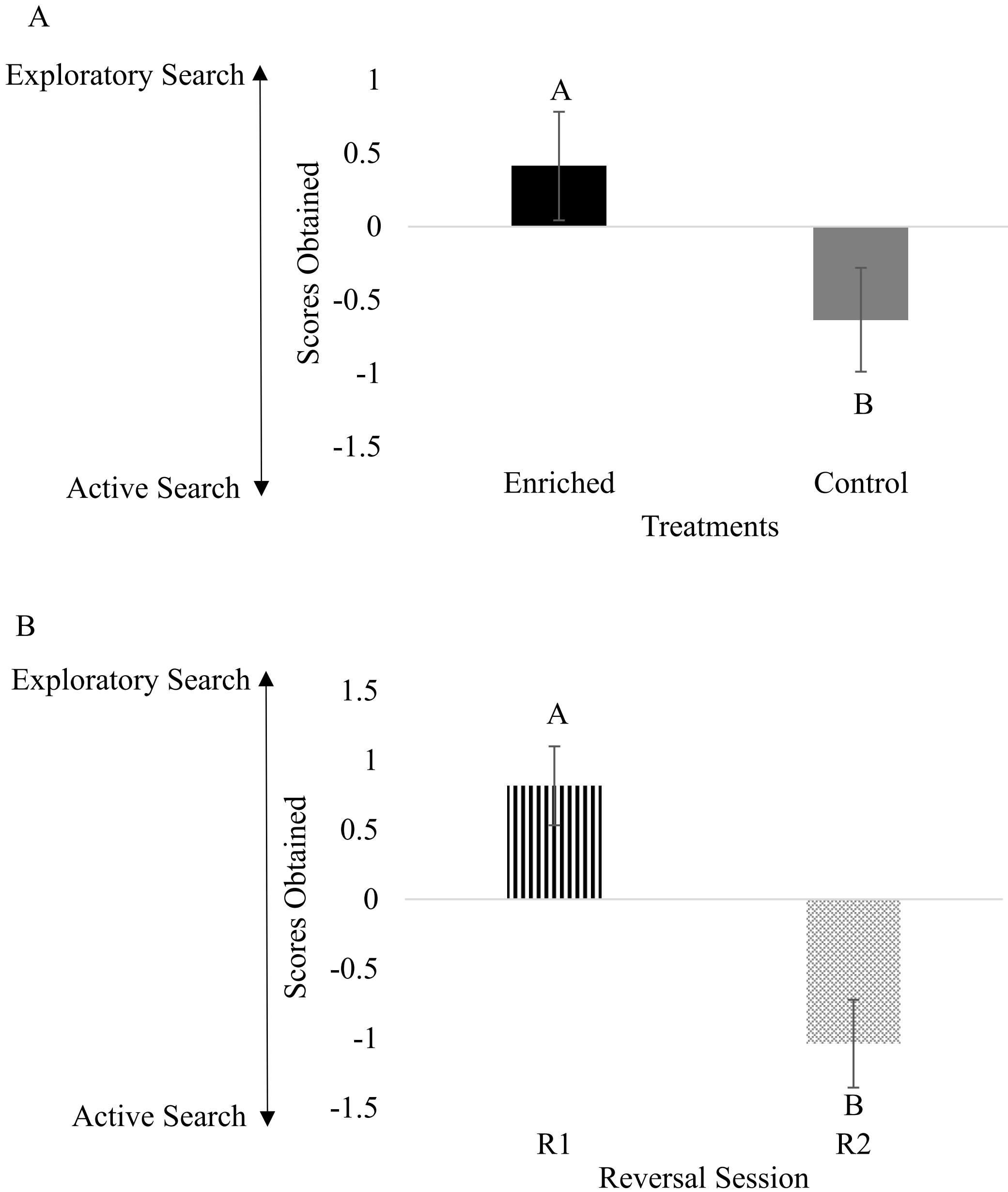


*Figure 3.5 Coordinates of variables from PCA of reversal tests including the dimension that each variable had a higher contribution to (i.e. solid black bars).*

The PCA coordinates analyzed for effects of treatment, session, and interaction between treatment and session, found difference of treatment and session for Dimension 1 ($p = 0.03$, $p < 0.0001$), and difference for session in Dimension 2 ($p = 0.002$). For Dimension 1, there was a difference in treatment where enriched lambs had a higher score, and therefore used an exploratory "Search Strategy" (exploring zones, staying in obstacle zone longer, and looking at open corridor), whereas control lambs had a lower score, implying a more active "Search Strategy" (changing zones more frequently) (Figure 3.6A). Both treatments, however, were found to use a more active "Search Strategy" in the second session (Figure 3.6B). Dimension 2 showed a session difference where both groups had higher score in session one, meaning higher "Contact Seeking" behaviour vs. a lower score in session two, and therefore lower "Contact Seeking" behaviour (Figure 3.6C).


A
Exploratory Search
Active Search
Scores Obtained
1
0.5
0
-0.5
-1
-1.5
A
B
Enriched
Control
Treatments
B
Exploratory Search
Active Search
Scores Obtained
1.5
1
0.5
0
-0.5
-1
-1.5
A
B
R1
R2
Reversal Session

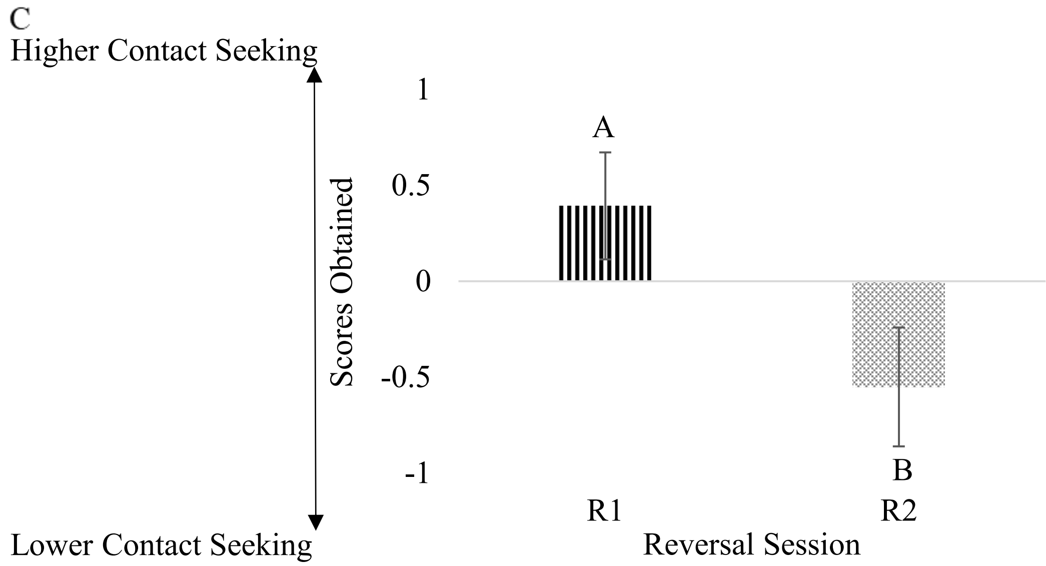



*Figure 3.6 Estimated Marginal Means (Emmeans) and Standard Errors (S.E.s) for Dimension 1 (Search Strategy) for treatment enriched and control (A) and session R1 and R2 (B), and Dimension 2 (Contact Seeking) for session R1 and R2 (C) of the reversal tests. Letters denote significant differences ($p < 0.05$).*

### *3.5.3 Disappearance Test Results*

Latency to reach the finish zone of the disappearance test showed an increase from sessions one (D1) and two (D2) to sessions three (D3) and four (D4) of the test ($p < 0.05$) (Figure 3.7). There was also an increase in latency to reach a finish zone between sessions three and four. There was no difference between treatments or interaction of treatment and session ($p > 0.5$ for both).

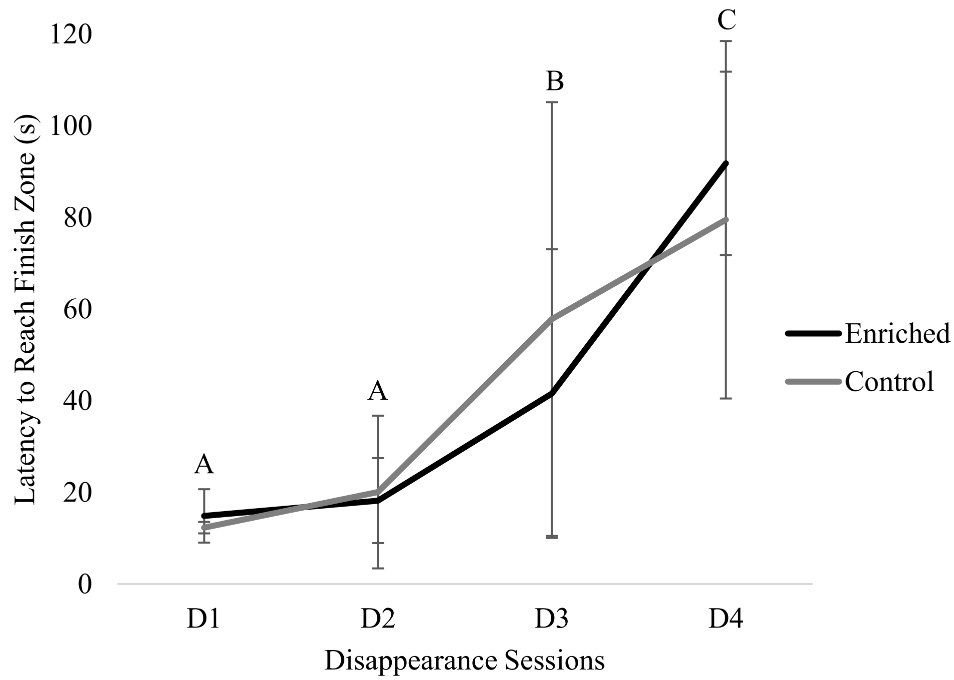



*Figure 3.7 Average latency and Standard Errors (S.E.s) of enriched and control to reach a finish zone (seconds) in each session of the disappearance test (D1 to D4). Letters denote significant differences.*

No differences between treatments were found for how many individuals went to a reward zone or not ($p = 1$), how many animals made two or more reward zone visits ($p = 0.09$), or how many times the lambs changed to a different zone of the maze ($p = 1$). There was also no treatment difference for entering more than one zone within a session ($p = 0.2$, $p = 0.2$, $p = 1$, $p = 0.5$, for disappearance sessions D1-D4, respectively) (Figure 3.8). However, the total number of zone changes in the exit zone showed a decrease in how many individuals entered more than one zone across sessions ($p = 0.05$), particularly a tendency between session one and three in a post-hoc comparison ($p = 0.06$).

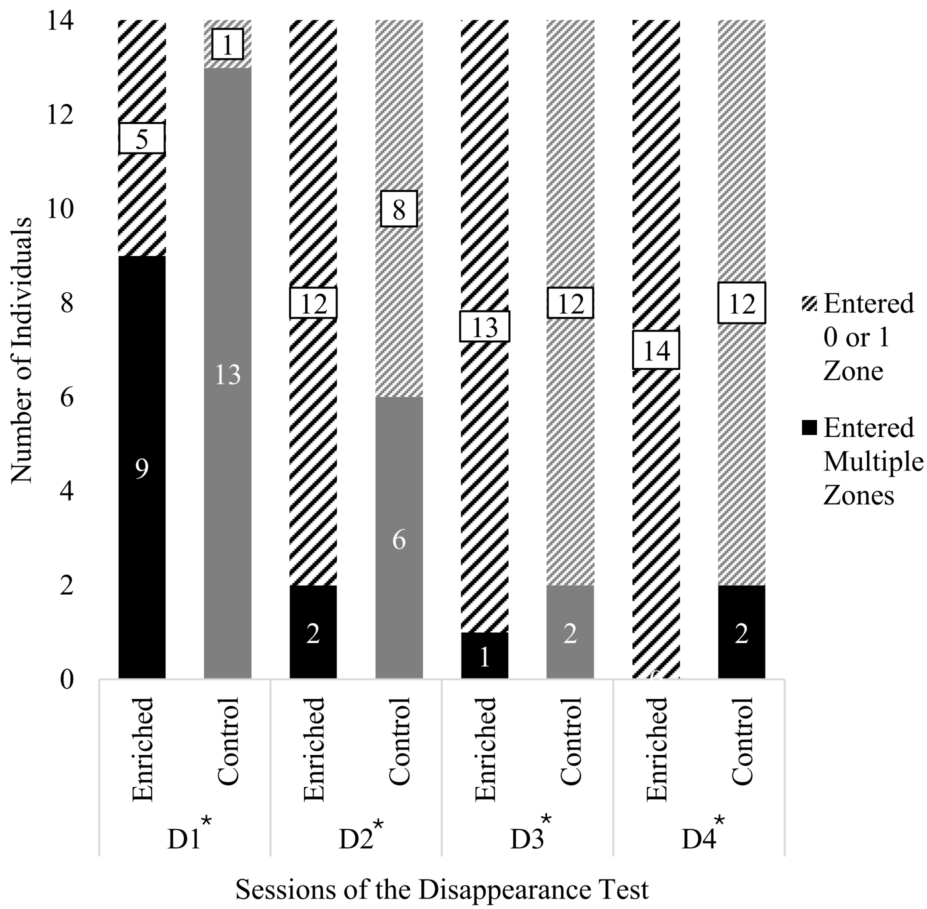



*Figure 3.8 Number of individuals from enriched and control who entered zero or one reward zone or multiple zones of the maze during the four sessions (D1-D4) of disappearance test. * Denotes significant differences across sessions ($p < 0.05$).*

Finally, the PCA for the disappearance test revealed two distinct dimensions with an eigenvalue greater than one. The dimensions were noted as "Reward Access" (Dimension 1) and "Agitation Level" (Dimension 2) and explained 56.04% of the variance (Figure 3.9). Dimension 1 included latency to reach a finish zone, percentage spent in the beginning zone of the maze, vocalizing frequency, and looking at experimenter frequency opposing reward zone percentage and food searching frequency. Dimension 2 included frequency of frustration behaviour and exploring frequency opposing immobile percentage (Figure 3.9).

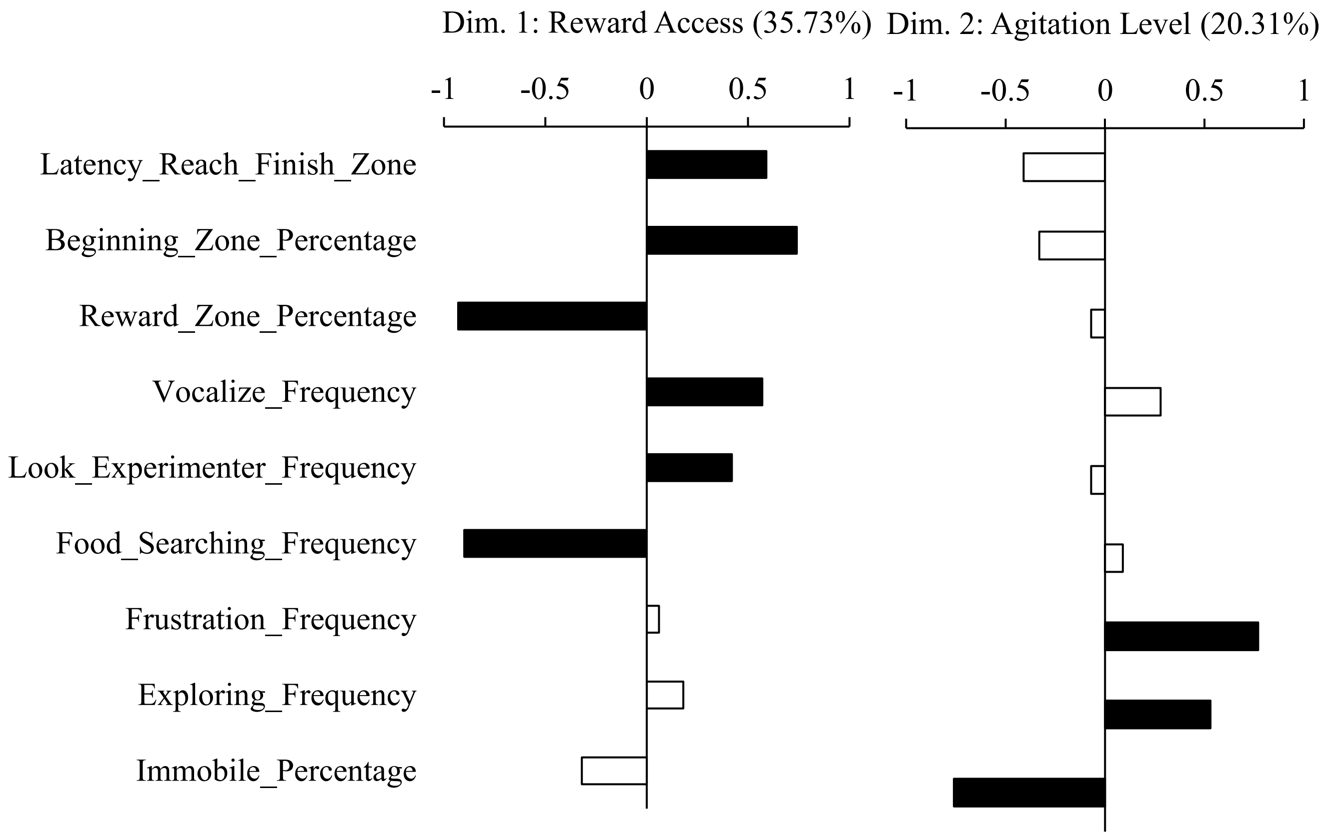



*Figure 3.9 Coordinates of variables from the disappearance test PCA, including black bars to denote which dimension each variable had a higher contribution in.*

Comparisons on the PCA coordinates revealed an effect of treatment and session interaction for Dimension 1 ($p = 0.04$) (Figure 3.10) and an effect of session for Dimension 2 (p < 0.001) (Figure 3.11). For Dimension 1, between groups, there was no difference in “Reward Access” between session one (D1, $p = 0.99$), but there was between session one (D1) of control lambs compared to sessions two-four (D2-D4) of enriched lambs ($p < 0.0001$). Control session two was only different from enriched session four ($p = 0.02$), where control spent more time accessing the reward in session two than enriched in session four. No difference was found between control session two and enriched session three or control session three and enriched session two ($p > 0.05$). Finally, control session three was not different from enriched session four ($p > 0.05$).

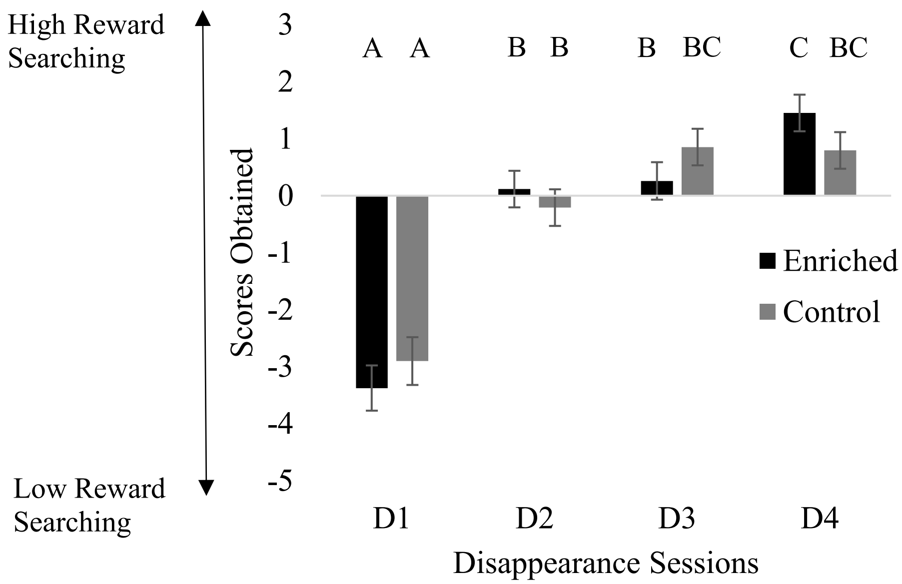



Figure 3.10 *Estimated Marginal Means (Emmeans) and Standard Errors (S.E.s) for the treatment (enriched and control) and session (D1 to D4) interaction of Dimension 1: Reward Access. Letters denote significance between sessions.*

For Dimension 2, there were no treatment effects, but session effects. There was a tendency towards a decrease in "Agitation Level" (i.e. less frustration and exploring, but more immobile behaviour) between sessions one and four ($p = 0.097$). Similarly, there was a tendency towards a decrease in agitation between session two and three ($p = 0.061$) and a decrease in agitation between session two and four ($p = 0.0001$). There was no difference between session three and four ($p > 0.05$).

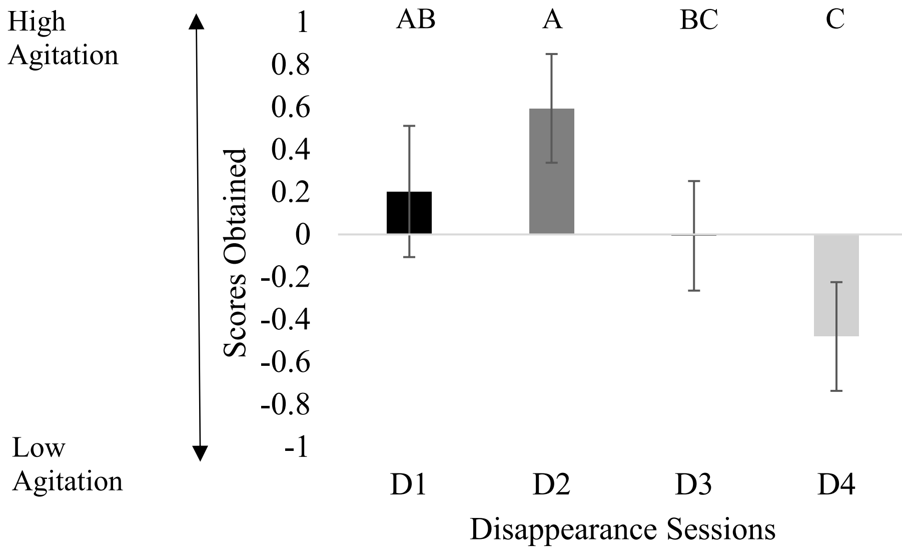



*Figure 3.11 Estimated Marginal Means (Emmeans) and Standard Errors (S.E.s) for sessions of the disappearance test in Dimension 2: Agitation Level of the Principal Component Analysis. Letters denote significance between sessions.*

## 3.6 Discussion

### *3.6.1 Training Tests*

During training, enriched lambs initially took longer to finish the test in session one, but were faster with repeated exposure while control lambs got faster after session one, but had no significant learning progression beyond session two. Regardless, this suggests that both treatments only needed one to two sessions to learn how to navigate the maze (similar to results found in other sheep maze studies such as Hernandez et al., 2009 and Lee et al., 2006). Both treatment groups also appeared to understand the training even when the 'rules' were changed for the reversal and disappearance training tests (in T7-T8, and T9, respectively). Contrary to our first hypothesis, there was no difference between treatment groups for latency to finish and thus, no difference in learning ability, which could be explained by the lack of cognitive enrichment employed to the enrichment group (Botreau et al., 2023a). Although other types of enrichment were used (physical, sensory, and nutritional), none that specifically targeted the learning abilities of the lambs were employed (e.g. no puzzles were used), which may account for the lack of discrepancy between enriched and control's ability to solve the test.

Spatial learning could be affected moreso by the drive of the animal rather than an effect of enrichment, where it could be difficult to separate motivational differences from learning differences (Zentall, 2021). That is to say, that the lack of discrepancy in learning may be masked by both enriched and control lambs' motivation to complete the maze and be reunited with conspecifics in the home pen after the test. Additionally, the associative processes (such as how animals connect an action with a response) may not be related to cognition in that they do not follow the principles of logical, rational thinking and instead, may happen more automatically, without deeper reasoning (Dickinson, 2012). The learning that lambs must do to finish the maze test may not relate to their cognitive ability (in regard to learning), and the lack of cognitive enrichment may not have been the reason for a lack of difference in latency to finish the test. Cognition could be better tested using behavioural flexibility (Erhard et al., 2004), as it requires a change in thought processes which was further explored in the reversal test (see *3.6.2*).

There was no difference between treatments for the "Reward Access" or "Agitation Level", but there was between session. The lack of treatment effect could be because the test may not have been cognitively challenging enough to cause a difference in groups or the amount of training repetitions may have been too many, which could also account for the increase in latency to finish over the sessions. Multi-modal enrichment in the pen might also not have been enough to impact the cognitive abilities of lambs. Similarly, it was found that pigs housed in a barren or enriched environment did not differ in their ability to solve a T-maze memory test (Jansen et al., 2009), but that both sets of pigs got better at solving the maze with secondary testing, which could account for why lambs in our trial were faster to solve the test with more training, even when the parameters of the test were slightly altered (i.e. the reversal and disappearance training tests).

There was an increase of “Reward Access” over sessions where lambs in the first session were more distracted with other kinds of behaviour (like exploration, observation, etc.). However, as the number of sessions increased, the lambs spent less time exploring or observing and went to the reward and exit zones more directly as they learnt with repetition. Repeated exposure to test conditions has been found to improve animal learning (Jansen et al., 2009; Destrez et al., 2013), as repetition can build pathways for memories to be more easily retained and for longer periods of time (Zhan et al., 2018).

Lambs in both treatment groups had a tendency to have a higher “Agitation Level” in the first two sessions of the training tests. One explanation could be that the feed reward may not have been satisfying enough. Lambs showed stronger preference for being reunited with their conspecifics, and thus, went to the exit zone instead of the reward zone (causing the test to continue longer) which could further agitate them. Another explanation could be that the lambs may not be as experienced with change and find it more stressful, resulting in agitation (Veissier et al., 2009).

*3.6.2 Reversal Tests*

For the reversal test, there was no difference between control or enriched lambs for how many animals correctly started (i.e. went to the learned side first) or succeeded (i.e. switched to the new side after seeing the obstacle) the test, but similar to what was found during training, the lambs in both groups were faster to learn the new rule upon repeated testing. The increased speed with repetition was similar to reversal test findings in lambs (Hunter et al., 2015; Johnson et al., 2012; Erhard et al., 2009) and in male sheep (Erhard et al., 2004). In both reversal tests, lambs were able to switch to the new reward side, demonstrating behavioural flexibility as they had to adapt their previously successful technique of going to the trained side, to change to the new correct reward side (Uddin, 2021). Both treatment groups going faster during the second session also demonstrated motivation to find the new answer to the test after learning the ‘new’technique of switching once encountering the obstacle.

Interestingly, “Search Strategy” differed where enriched lambs were more exploratory while control lambs were more actively looking to find the solution of the reversal. The control lambs entered more zones than the enriched ones, and they showed more signs of distress in not finding the reward such as vocalizing sooner and changing zones more than enriched. Vocalizations can be used to measure agitation of sheep in socially isolating situations (Atkinson et al., 2015; Viérin and Bouissou, 2003; Cockram et al., 1994; Romeyer and Bouissou, 1992), and have been previously linked to active locomotion due to fear in isolation testing (Barnard et al., 2015). So, it is likely that the control were less comfortable with being isolated in the maze. Enriched lambs were observed more frequently looking at the open corridor and exploring the arena and obstacle zone. Enriched lambs may therefore have been more proactive in their approach (that is, deliberate investigation for a solution and spending more time searching the obstacle area for the ‘goal’ of the test) while control lambs may have been more reactive, such as running around frantically in hopes of escaping in any way possible (Atkinson et al., 2015; Luo et al., 2020; Viérin and Bouissou, 2003). Additionally, lambs from both treatments were faster to vocalize and vocalized more often in session one than in subsequent sessions of the reversal test, indicating higher “Contact Seeking” on the first round. Again, this may relate to higher discomfort the first time the lambs were not able to find the solution.

Contrary to our second hypothesis, home pen enrichment did not impact motivation to search for the new reward but did support our third hypothesis regarding the treatments using different motivated behaviour. Thus, enrichment might be useful in reducing the reactivity of lambs to stressful contexts outside the home pen. Enrichment has been found to increase exploration and decrease fearfulness, so it could be the case that the home pen enrichment was enough to decrease the enriched lambs fear of isolation within the maze (Zebunke et al., 2013). However, the reduction in "Contact Seeking" could also indicate that the lambs learned how to navigate the reversal test and were therefore more comfortable with the maze environment rather than due to the in-pen enrichment directly.

*3.6.3 Disappearance Tests*

Motivation, in this case the perseverance to find the reward, may have decreased for both treatment groups. Between sessions of the disappearance tests, there was an increase in latency to reach the finish zone where both control and enriched lambs were slower to go to the finish zone after the first two sessions, and even slower after the third. This could also be because of similarities in motivation between groups to reach the finish, as both treatments may have had similar desire to consume the reward or to return to the home pen (i.e. motivation to be reunited with conspecifics) or it could be due to the animals 'giving up' on searching because of its pointlessness as there was no longer a solution to end the test sooner.

No treatment or interaction effects were found for how quickly both treatment groups went to a finish zone. The groups also did not differ in the number of times that they continued to go to a reward zone, but this number decreased over time for both enriched and control lambs. This may suggest that perseverance to search for the reward during disappearance tests decreased when the animals were faced with an 'impossible' task because of the lack of solution to voluntarily end the test before the allotted time (as was the case in previous training and reversal tests) (Latham and Mason, 2010). Impossible/unsolvable tests are often used to induce frustration, which in turn may negatively impact future motivation towards the same task (Alterisio et al., 2018; Piotti et al., 2021). Similarly, an increase in frustration behaviour in horses were observed when a previously solvable task became unsolvable (Alterisio et al., 2018). Thus, one interpretation could be that the tested lambs may have felt frustrated upon finding no solution to the disappearance test, resulting in a negative feedback on motivation to search in subsequent session (i.e. longer latency to reach reward and less zone searching). This decrease in motivation could therefore cause the lambs to simply wait for the test to be over instead of continuing to search. This is further demonstrated when exploring the “Reward Access” and “Agitation Level” during the disappearance test.

There was a trend for less agitation after the first two sessions (with an increase in session two) where lambs did not explore or show as much frustrated behaviour in the third or fourth sessions but spent more of their time immobile by the last session, which could also illustrate less distress with repeated sessions. It is possible that the lambs did more exploring in the first session as they expected to find a solution like in the other tests (i.e. training and reversal). Then, after again failing to find a way out of the maze the second time, there was an increase in frustration behaviour in session two as the lambs still could not find the answer to the disappearance tests. After being forced to wait twice until the session was over, by the third session the lambs likely realized there was no escape to the maze and became more resolute, instead choosing to stay immobile near the front of the maze until the door to go out was opened and the test ended. Animals develop learned detachment in unpredictable and uncontrollable situations (Dwyer, 2004), and although being placed in the test maze was likely predictable to the lambs after multiple sessions (based on experimenters following the same protocol each time), it could still indicate that the lambs in the disappearance test learned to stop searching due it its futility.

Interestingly though, the enriched group was found to persist searching for the reward even after a lack of reward in the second session, and in fact increased their efforts by the last tests, even moreso than the control group. Enriched lambs might therefore be more hopeful to find a solution, which could imply that having enrichment in the home pen might influence how optimistic the lambs were towards finding a reward or to returning to their social group (which is highly valued in prey species like sheep) (Stephenson and Haskell, 2022; Dwyer, 2004). This could also show that enriched lambs were more comfortable even when not receiving a reward due to having satisfying home pen enrichments to return to after the end of the test.

Though the multi-modal enrichment used was not sufficient for impacting early life learning abilities or expression of frustration, it did have an effect on “Search Strategy”, reactivity, and perseverance for reward expressed through different motivated behaviours, which supports our final hypothesis. It was shown that lambs were faster and more flexible with repeated attempts and enriched animals may have been more comfortable with the isolation and more hopeful of solving the maze tests. Our results may lead to the recommendation of incorporating enrichments in lamb home environment where possible as they could provide benefits on reactivity and motivation, although future research on these aspects, specifically the learning and flexibility, need to build upon this approach, where the incorporation of cognitive enrichment in the multi-modal enrichment provided is suggested.

## 3.7 Conclusion

Contrary to our hypotheses, enriched and control lambs were similarly motivated and able to learn how to find a reward in the test maze during training. Both treatment groups were flexible in adapting their behaviour during the reversal test, although enriched lambs seemed more comfortable than control with being isolated during the test. This could suggest that the enrichment provided in their home environment positively affected lambs' reactivity towards a stress-inducing situation. Finally, during the disappearance test, "Reward Access" decreased for both groups (with enriched lambs persisting to search for the reward longer than control), and so did the "Agitation Level". The unsolvability of the disappearance test may have initially caused some confusion, followed by frustration when a solution was no longer available, with both enriched and control lambs becoming more resolute and immobile after repeated sessions of the test. However, as enriched lambs continued to search for the reward longer, this could indicate that enriched animals were more hopeful for a resolution, hence why they continued to explore. Our hypothesis that the treatment groups would display different motivated behaviours during testing was supported by our findings. This increased persistence in enriched lambs may reflect a greater sense of hope or an enhanced ability to cope with frustration when faced with unsolvable tasks. These traits suggest that multi-modal in-pen enrichment may foster problem-solving resilience and reduce the negative emotional impacts of stress-inducing situations outside of the home environment. By promoting these adaptive behaviours, early life enrichment can have meaningful and lasting benefits for farm animals. So, the use of early life environmental enrichment is encouraged to improve the welfare of sheep and other livestock.

**Declaration of Competing Interest**

Authors declare that there are no competing interests.

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## Connecting Statement 2

Having established in Chapter 3 that enriched and control lambs showed similar learning, flexibility, and motivation to search for a reward, the search strategies reflected that enriched lambs exhibited greater persistence and reduced agitation during tests compared to control. This suggests that early-life enrichment enhances coping strategies and adaptability, highlighting its value in improving welfare. While Chapter 3 focused on how enrichment impacts motivation, the following chapter will present how motivated animals are for enrichment itself. To diversify the scope of this thesis, there will be a transition from the use of young animals in Chapter 3 to adult animals in Chapter 4, as well as species transition from sheep to dairy cattle. The motivation of movement-restricted dairy cattle for outdoor access have been already established in past literature, so Chapter 4 will add a dimension by investigating if motivation is impacted when the enrichment is given at different frequencies. This chapter will continue to use behavioural measures, as suggested from the comprehensive review presented in Chapter 2, to make conclusions related to the motivation and more specifically, animal emotion, when given or denied the ability to perform motivated behaviour during the outing.

# Chapter 4 - Frequency of Outdoor Access on Motivation of Movement-Restricted Dairy Cattle for an Outdoor Area

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## 4.1 Abstract

Farm animal motivation is being given greater consideration in the development of improved standards for animal care as allowing animals the ability to act on their own needs and/or desires can impact the mental well-being of the animal. This can be achieved by employing opportunities for enrichment that allow the animal a greater sense of control, particularly when housed in restrictive environments. In this study, 36 lactating Holstein dairy cows housed in movement-restricted environment (in this case tie-stall housing) were split into two treatments provided outdoor access: 3x, which went outside three times a week (n=18), and 1x, which went outside once a week (n=18). The cows were randomly blocked in groups of three into six outdoor paddocks and provided 1h of outing time, where the trip duration and frequency of behaviour going out to the paddocks and going back inside to the barn were recorded. Behaviour once inside the paddocks was also recorded. Finally, the number of behavioural transitions whilst outside was analyzed. LMr comparisons and Principal Component Analysis

(PCA) were used to assess motivation for the outdoor area. T-tests revealed that cows were quicker to go outside than to return to the barn, regardless of outing frequency, suggesting a stronger motivation for the outdoor area than the standard environment. Trip Speed and Stop Quality dimensions were identified from the PCA; both also revealed a stronger motivation to go out than to return to the barn. Time budgets of outdoor behaviour during outing were found to be similar to other studies of cattle behaviour during outdoor access, suggesting that the change in environment from tie-stall to outdoors did not affect behaviour. Further, cattle from both treatments had low behavioural transitions, thus low arousal, suggesting that cows were comfortable being outdoors. Cows were strongly motivated to go out even at lower frequencies and were quick to adapt to the outdoor area. Providing outdoor access can act as an enrichment that improves animal welfare through allowing dairy cattle to act on their motivation to go out, while promoting positive emotions associated to being in a stimulating environment where a range of behaviours is possible.

**Keywords: exercise, enrichment, desire, frustration, handling intervention**

## 4.2 Introduction

The past decade has brought about a change in the way animal welfare is studied, particularly in response to public perception of livestock farming. In recent years, research has shifted from physiological measures and a focus on negative welfare (e.g. how to reduce animals' pain and suffering) to an approach including positive welfare (e.g. incorporation of enjoyable experiences) and understanding of the animal's behaviour as a measure of psychological welfare, more specifically of the relationship between behaviour and the underlying motivations that guide it (Rault et al. 2022).

Motivation is the internal drive of the animal which informs goal-directed behaviour based on the animals physiological or psychological perceived needs (Jensen and Toates 1993; Manteuffel et al. 2009). Motivation is either innate or learned, and can be positive (e.g. the need/desire to approach, interact, or have access to a reward/event) or negative (e.g., the need/desire to escape or avoid an event) (Antle and Silver 2016). If the animal deems an experience or stimuli as positive, they will be motivated for it, even willing to work in order to access or experience it (McConnachie et al. 2018; Tucker et al. 2018; Von Keyserlingk et al. 2017). Thus, motivation could be helpful in further understanding how the animal perceives their environment and makes decisions in response to environmental changes.

This likely explains why enrichment is being integrated at an increasing rate to the housing of livestock. Defined as a change or addition to the environment that stimulates and enables the animal to act on their physical, behavioural, and/or cognitive motivational needs in a manner that improves their welfare, enrichment allows animals to have agency over how they spend their free time, on top of promoting positive experiences which have the potential to increase the positive emotions felt by the animal (Botreau et al. 2023a, 2023b). Additionally, multi-modal enrichment (i.e. incorporating two or more categories of enrichment, which comprise physical, cognitive, sensory, nutritional and/or social enrichments) represents another, possibly more effective, method to improve welfare as it stimulates the animal in a variety of ways (Strappini et al. 2021). For example, multi-modal enrichment (including physical enrichment: larger pens, straw bedding, logs; and cognitive and sensory enrichment: chains, sticks, and cardboard boxes) was found to induced positive emotion in pigs (Douglas et al. 2012). However, for an addition to be considered enrichment, it should enhance the life of the animal, and the animal should be motivated to interact with or utilize it (Botreau et al. 2023b).

In Canada, dairy cows are often kept inside, year-round, in individual stalls with little to no enrichment. Compared to animals that have access to an exercise or outdoor area, cows in tie-stalls do not have as much opportunity for mental stimulation, exercise, or the ability to express their behavioural repertoire. Being kept in a non-enriched environment can lead to boredom, frustration, or even distress in confined animals (e.g., in mink: Meagher and Mason 2012). Outdoor access could be used to combat these issues as it can be considered a multi-modal enrichment and can include all enrichment categories: physical (more space to move and perform locomotory activities compared to being in the stall), sensory and cognitive (new sights, sounds, smells, etc. and challenges that would not be experienced inside the barn), nutritional (ability to graze on fresh grass if weather permits), and social (mixing with more animals from the herd resulting in increased social interactions). It has been shown that dairy cows prefer to go outside rather than to stay in the barn at all times (Shepley et al. 2016, 2017, 2020). However, showing a preference to go out does not answer to how strong the motivation for outdoors is compared to being indoors and whether it is viewed as a positive reward. Further, the resulting emotion from being able to act on one’s motivation for  outdoor access, , has not been yet investigated.

Moreover, allowing cows access to the outdoors has been shown to bring both physical and mental benefits (Nejati et al. 2024; Palacio et al. 2023; Aigueperse et al. 2023; Aigueperse and Vasseur 2021). However, the way that animals are handled was found to influence future responses and may impact the perception of the enrichment (Boissy and Bouissou 1995, Aigueperse et al. 2023). Aigueperse and Vasseur (2021) found that cows imposed with more movement restrictions during the trip to go outside did not exhibit an improvement in their reactivity to humans. They hypothesized this may be because the handling-related restrictions being viewed as aversive by the cows, who, conversely, did not associate going outside with a positive event. However, it is still unclear how human-animal interventions, with respect to going outside, might play a role in influencing the animal's motivation to go out, and although physical benefits to going out have been found, different frequencies of access and their impact on motivation have yet to be determined (Nejati et al. 2024).

Thus, the objective of this study was to assess the motivation of movement-restricted dairy cattle for outdoor access, a model of multi-modal enrichment, when going out three days/week vs. one day/week.

## 4.3 Material and Methods

### *4.3.1 Ethics Statement*

The animals and research procedures used in this study were approved by the Animal Care Committee of McGill University and affiliated hospitals and research institutes (#2016-7794). Standard Operating Procedures (SOPs) regarding the handling and outing were followed by staff members throughout the process of taking the cattle outside. The handler intervention steps (i.e., the order and type of actions handlers were instructed to follow) to encourage cows forward when they stopped during the outing and to slow the cows down when they moved too quickly were adapted from Aigueperse et al. 2023.

*4.3.2 Housing and Care*

The animals were fed TMR 4x/day (around 6am, 10am, 4pm, and 8pm) and had *ad libitum* access to water. They were milked twice a day at 5am and 5pm. The cows were housed in individual tie-stalls measuring 186.4cm x 141.5cm, with tie rails at a height of 122cm and 36cm from the manger wall. Wood chips (2cm depth) were used as bedding and were replaced as necessary.

*4.3.3 Outing Setup and Procedure*

The alleyway was examined daily for risk factors such as large rocks or bad traction and the appropriate corrective measure(s) (e.g., rock removal, addition of sand to the alleyway) was applied to reduce the risk of injury to the animals. Metadata was also collected daily right before the outing for the temperature (°C), traction of alleyway and paddocks (Table 4.1), wind speed (km/h), wind chill factor, if it was raining or snowing, snow accumulation (cm), and dryness of ground. Lastly, if a cow was not taken out, it was recorded whether they were dry, lame/injured, or in heat. Feed, water, and shelter were not provided during outdoor access. Before going outside, halters were tied to the animals, tails were untied, and grit was spread on the floor in the barn to add traction.

**Table 4.1** Average temperatures (˚C) and average traction condition recorded right before outings across five weeks.

| Week | Average Temperature | Average Traction Condition |
|---:|---:|---:|
| 1 | 5.0 | Good |
| 2 | 8.8 | Good |
| 3 | 3.5 | Slippery |
| 4 | -2.4 | Slippery |
| 5 | -2.2 | Slippery |

During the trip, an observer (standing approximately 23m from the alleyway) operated two tripod cameras, rotating the tripods to allow the cameras to capture the movement and behaviour of the group of animals and handlers as they traversed the alleyway. After starting the recordings, the observer used a stopwatch to note the length of time it took each group of animals to travel from the starting area of the alleyway to the end of it (see Figure 4.1).

Thirty-six lactating Holstein dairy cattle (mean DIM 121.1±57.29, mean parity 2.1±1.02) housed in a tie-stall operation were selected from the Macdonald Campus Dairy Cattle Complex located in Sainte-Anne-de-Bellevue, Quebec. The animals were split into two treatment groups; a 3-exits (n=18) that went outside 3 days/week (on Mondays, Wednesdays, and Fridays), and a 1-exit (n=18) that went outside 1 day/week (on Thursdays). The treatment groups were allowed access to an outdoor exercise area that was divided into 6 paddocks (each $117m^2$ (9m x 13m)), with 3 cows allocated to each paddock during outings (blocked by DIM and parity; see Figure 4.1). Leading to the outdoor area was an alleyway (approx. 40m long with a 12m long waiting area) connected to the barn. Paddock groups were rotated weekly, over a total duration of five weeks (from November to December 2021), to allow the groups to experience all areas of the exercise area and to decrease bias related to the specific aspects of each paddock (e.g., one paddock had more mud than others).

A staff member from the barn individually collected three cows from adjacent stalls in the barn (in the same treatment and paddock group) and led them by the halter to the waiting area in the alleyway. Once all three cows were collected, the waiting area gate was opened and two handlers walked in front of the group (trying to maintain a distance of approx. 1m), leading the cows to the paddocks using flags and physical or non-physical contacts to slow down the animals when they started to run, jump, caracol, or force their passage (Figure 4.1). A third person was positioned behind the animals to encourage them to move forward when they resisted moving or were waiting/inactive for more than 30 seconds. The cows were directed into their assigned paddock and were left out for a minimum of 60 minutes before being brought back in.

The return to the barn followed the same procedure as going out (without a waiting area), where cows were let out one paddock at a time (the first group returning first, and so on), with two handlers in front and one behind following the group until the final cow from each group had entered the barn. This was repeated until all paddock groups were inside. For the last two weeks of the trial, the methods from Aigueperse et al. 2023 were adapted, where a fourth person was added in an attempt to stop the animals from jumping to decrease the risk of injury. This person stood further ahead of the animals (approx. 10m) and also used a flag.

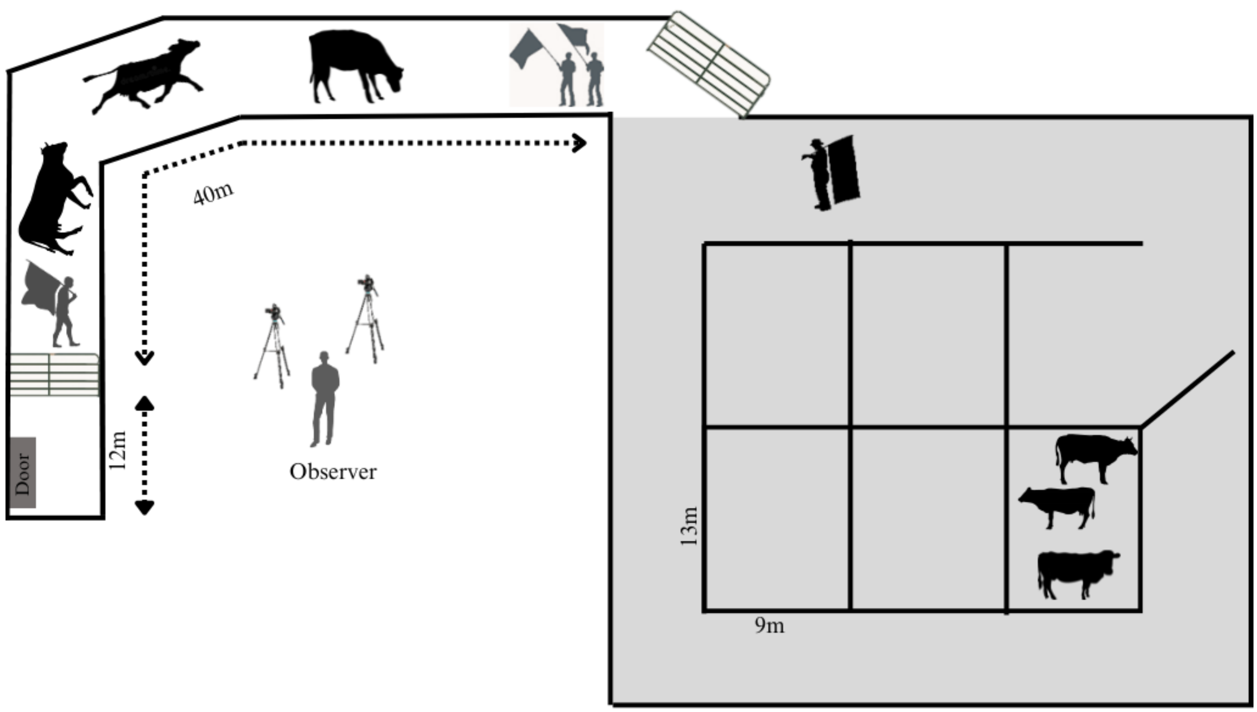


*Figure 4.1 Depicts set-up of waiting area (12m long) (the area between the door and the first fence) as well as the alleyway (approx. 40m long) with handlers (carrying flags) positioned two at the front and one at the back. In the fourth week, an additional handler with a flag (approx. 10m from the outing group) was added to further slow down the cows. Also shows the outdoor area divided into six smaller paddocks (each 117m2 (9m x 13m)), each containing one block of cows (i.e. three per paddock). The observer recorded trip durations live using a stopwatch, and cow behaviour and handler interventions using two tripod cameras from a point approximately 23m away from the alleyway (which were later annotated on Noldus Observer XT 14).*

*4.3.4 Motivation Measures*

Duration (in seconds) of the trip going outside and back into the barn was recorded live by the camera operator using a stopwatch (see Figure 4.1). For trips going outside, the start of the trip was defined as the time when the waiting area was opened and cows were free to move, and the end of the trip was when all four legs of the last cow had crossed the final gate of the alleyway leading to the exercise yard. For trips returning to the barn, the trip started when any cow from the group being taken in crossed the line from the outdoor area into the alleyway with at least one hoof (i.e. past where the open gate that connected the paddocks to the alleyway was), and the trip was finished once all three animals had successfully crossed the barrier of the door going into the barn with all four legs (Figure 4.1).

Both duration and frequency of cow behaviour and handler interventions during the trips (to and from the outdoor area) were recorded using two video cameras on tripods which were set-up outside, at a distance of approximately 23m from the alleyway (see Figure 4.1). Cows' behaviour and handler interventions during outings were annotated by two blind observers on the software program Noldus Observer XT 14 (behaviours defined in Tables 4.2 and 4.3). The observers were first trained by an expert using multiple practice videos and a ‘gold standard’ video which included clips of all behaviours present in the ethogram. Inter and intra-observer reliability was checked within the software program every 10 videos, using the gold standard video, to ensure 85% reliability with the expert and with themselves.

**Table 4.2** Ethogram of motivation measures including category, behaviour, and definitions used for cow behaviour. Adapted from 1) Aigueperse et al. 2023, 2) Lindahl et al. 2016, 3) Somers et al., 2012.

| **Behaviour** | **Definition** |
|---|---|
| Move (itself) | Cow starts to move without intervention by handler[1,2] |
| Move (handler) | Cow starts to move directly after contact or non-contact by handler[1,2] |
| Move (cow) | Cow starts to move because of another cow[1,2] |
| Doesn't Move/Resists | Does not start to move despite contact or non-contact by handler: further encouragement needed OR cow attempts to escape by going against the direction of the handler (e.g. handler is pulling cow to halt, but cow runs forward anyways)[1,2] |
| Freezing | Cow stops moving forward, with ears facing forward, full attention toward item or person in focus, body is tense[1,2] |
| Balking | Cow stops forward movement, lunging its head or body backwards, voluntarily or because of other cow(s) or handler[2] |
| Running | Cow moves at a quick and steady pace, trotting or galloping forward |
| Caracoling | Cow alternates between running at a quick steady pace and bucking (lowering its head and raising its hindquarters into the air while kicking out with the hind legs)[1,3] |
| Jump | Cow launches itself forward onto the front two legs, placing its weight forward, then landing on its hind legs, alternating between the front and hindlegs[3] |
| Kicking | Cow kicks with hind leg(s) toward the handler[1,2] |
| Head-butting | Cow thrusts its head into or toward the handler[1,2] |
| Forcing | Cow uses its entire body to push through or climb over fencing, other structures, or push past humans, excluding when the handler is in contact with the cow (see "doesn't move/resists")[1,2] |

**Table 4.3** Ethogram of motivation measures of handler interventions including category, behaviour, and definitions used for supplementary variables. Adapted from 1) Aigueperse et al. 2023, 2) Lindahl et al. 2016.

| **Behaviour** | **Definition** |
|---|---|
| Arm raising | The handler stretches arms wide outwards from the body, to appear larger, to slow down or stop the cow or to encourage it to move[1] |
| Arm waving | The handler, while standing behind or in front of the animal, stretches arms wide outwards from the body and waves arms either up and down or side to side, to slow down or stop the animal(s) or to encourage it to move |
| Flag waving | The handler swings a flag (a stick with a piece of rectangular material attached) back and forth or holds it out in front of the cow to move, slow down, or stop the cow |
| Patting | The handler taps any part of the cow quickly and gently using a flat hand, fist, or object (e.g. flagpole) to encourage the cow to move[2] |
| Poking | The handler quickly thrusts their finger(s) onto any part of the animal(s) to encourage it to move |
| Pulling | The handler, standing beside or in front of the animal, exerts forward force on the halter to move the animal in the direction of the force |
| Pushing | The handler exerts force onto the front or rump of the cow to physically displace the animal OR applies pressure, using the foot, to an animal's declaws to encourage the cow to move or stop[1,2] |
| Rubbing | The handler exerts a firm pressure on any part of the animal(s) in a repetitive motion using the hand(s) or an object to encourage the animal(s) to move |
| Yelling | The handler speaks or makes noise from the mouth using a strong and loud voice to encourage the cow to move or slow down/stop[2] |
| Whistling | The handler forces or sucks air between a small hole created by the lips to emit a high-pitched sound to encourage the animal(s) to move or slow down/stop |
| Speaking | The handler communicates in a natural speaking pitch using spoken language to encourage the animal(s) to move or slow down/stop |
| Tongue snapping/clicking | The handler places the tongue on the roof of the mouth flicking it downwards to hit the bottom of the mouth produce a clicking or popping noise to encourage the animal(s) to move or slow down/stop |
| Clapping | The handler strikes the palms of their hands together to emit noise to encourage the animal(s) to move or slow down/stop |

For each outing (going out or in), a new cow was randomly selected from each trio to be observed and its behaviour annotated, acting as a behavioural representative for her group. For all cow behaviour, it was noted when there was a change in behaviour, involving a stop of movement (e.g., walking stops and animal starts waiting) or the beginning of movement (e.g., from freezing to running), as well as for what reason (by themselves or because of another cow or human) to better understand the reasons underlying the observed behaviour changes. Handler interventions, as described in the Table 4.3, include duration and frequencies of: physical or non-physical contacts, behaviours involving noises, or if there was no intervention or the intervention was not visible to the observer. It was also observed which person initiated the intervention (a person at the front or back of the group or if multiple people were involved). This allowed us to understand, along with the cow's own behaviour, whether the animal needed encouragement to move or to stop, and the level of intensity of the intervention (e.g., multiple people pushing the cow to go towards the outdoor area likely indicating a lower motivation to go out from the cow).

Whilst cows were in the paddocks, live scan samplings were done by a trained observer who stood outside of the outdoor area (approximately 10m away), out of sight of the cows, to avoid influencing their behaviour. The scans were done on all cows (identified by collar colours; n=18), with one scan being completed every 10 seconds for a total of 30 minutes. Observed behaviours were categorized into: idle/ruminating, vigilance, exploration, locomotion, eating, social interaction, and maintenance behaviours. Scans were conducted to develop time budgets for the treatment groups, to identify how cows responded to the outing. These samples were also used to calculate average number of behavioural transitions per week as an indicator of arousal. Both time budgets and transitions were used to determine if cows were comfortable being outside (through evaluating percentage of behaviours performed in comparison to 'typical' percentages of cow behaviour whilst outside as well as average number of transitions to determine arousal level), to understand whether the trip speeds going out were related to motivation to go out or aversion (to the procedure, other cows, or handler interventions).

## 4.4 Statistical Analysis

Statistical analysis was done on R Studio® version 2021.09.2+382.pro1 Ghost Orchid (250 Northern Ave, Boston, MA 02210) with the Factoshiny package for Principal Component Analysis (PCA); lme4, lmerTest. and emmeans for model analysis. To analyze the data, a statistical approach inspired by Aigueperse et al. (2023) was used. The analysis investigated the difference in duration of going out and returning to the barn for each day of outing and for each treatment group (3-exits and 1-exit), to see if there was a difference between motivation to access the outdoors vs. to return to the barn. This difference was compared to 0 (the value if the two durations were equal) where multiple comparisons were taken into consideration using a t-test with a Bonferroni adjustment. A Wilcoxon signed rank test was used when conditions for normality were not met.

The duration to go out was also analyzed between treatments and across weeks (1-5) using linear mixed-effects models (LMr) to evaluate any treatment differences in motivation and evolution of motivation over time. In the model, the fixed effects were: treatment (3-exits and 1-exit), week (1-5), treatment*week interactions, and the random effect was: block (groups of 3 cows formed by parity and stage of lactation).The model is as follows:

$$\gamma_{ijk} = \mu + \text{treatment}_i + \text{week}_j + \text{treatment}_i * \text{week}_j + \text{block}_k + e_{ijk}$$

Normality of the data was graphically assessed using Q-Q plot. When necessary, post-hoc comparisons were performed by means of Tukey tests. Significance was declared at $P \leq 0.05$, and tendencies between 0.1 and 0.05.

Behavioural frequencies accounted for trips of different durations having different frequencies of behaviours for cow and handler interventions, where frequency by second was calculated by dividing the number of behaviours that occurred by the duration of the trip. A Principal Component Analysis (PCA) was done on the behavioural frequencies (by second) for the randomly selected cow that was observed for each trip, where certain variables were grouped together including: FreeStop (i.e. moving by itself, moving because of another cow), Caracoling (i.e. caracoling and jumping), StopRbalk (i.e. move because of handler, balking because of itself or another cow, balking because of handler), and Resist (i.e. resisting, kicking, head-butting, and forcing). These were first grouped based on the aforementioned paper by Aigueperse et al. (2023) to validate the methods used, with the handler interventions added as supplementary variables to understand their effects.

The variables of handling interventions were grouped as such: Noise to Move (i.e. yelling, speaking, whistling, clapping, and tongue snapping or clicking performed from the handler at the back of the group to encourage movement forward), Noise to Stop (i.e. speaking, yelling, or clapping from a handler in front of the group to encourage cows to slow down or stop), Noise Mixed (i.e. yelling, clapping, speaking, or tongue snapping/clicking performed by multiple handlers, where the intent to move or stop is not determinable), Contact to Move (i.e. patting, poking, pushing, rubbing, and pulling to encourage movement forward), and Movement Mixed (i.e. arm raising or arm waving, where the intent to move or stop is not determinable). Pushing to stop and flag waving were also graphed with handler interventions.

Numerical comparisons were conducted to summarize the average time budgets of 3x and 1x across all observational scans during the trial. Time allocation to different activities was expressed as percentages of scans per day. These descriptive analyses were performed using Microsoft Excel. For behavioural transitions whilst in the paddocks (used to evaluate cow arousal), a repeated measures ANOVA was conducted to analyze the effects of treatment, week, and the interaction of treatment and week. The model included treatment, week, and the interaction as fixed effects and cow as a random effect:

$$\gamma_{ijk} = \mu + treatment_i + week_j + treatment*week_k + cow_l + e_{ijkl}$$

## 4.5 Results

*4.5.1 Differences Between Go-out and Go-in Trip Durations*

The difference between going out and going in was larger than 0 for three out of five weeks for both treatments ($p<0.05$), meaning that the cows went out faster than they returned to the barn (Figure 4.2). For the 3x treatment, that included weeks one (Wilcoxon; W = 78; $p = 0.001$), two (Wilcoxon; W = 64: $p = 0.003$), and three (Wilcoxon; W = 66; $p = 0.02$). For the 1x treatment, weeks one (t-test; t = 2.49; $p = 0.03$), three (t-test; t = 2.48; $p = 0.03$), and four (t-test; t = 2.03; $p = 0.05$) were different from 0, and there was a tendency for the cows to be faster to go out in week two (t-test; t = 1.68; $p = 0.08$).

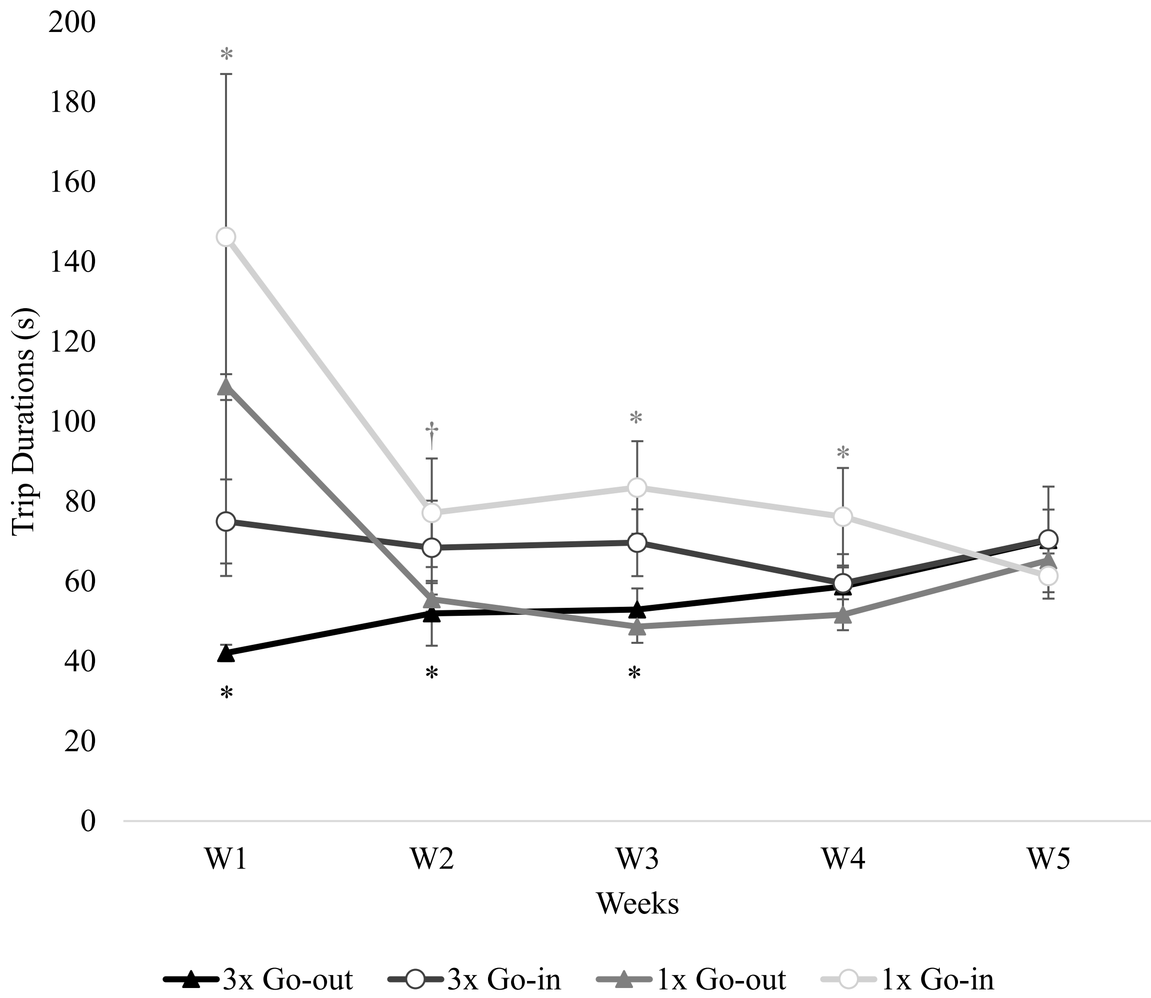



*Figure 4.2 Graphical depiction of differences in trip durations to go-out (Δ) and go-in (o), comparing across the five weeks of the trial for the 3x (black) and 1x treatment (grey). * Denotes statistically significant differences between duration of go-out and go-in within a treatment (p<0.05), † represents a tendency between the two trips of a same treatment (0.05<p<0.1).*

*4.5.2 Cows' Behaviour During Trips*

The PCA revealed two dimensions related to cow behaviour with eigenvalues greater than one. These dimensions explained 56.6% of the variance, where the first dimension was named "Trip Speed" ranging from slow to fast (which included running and speed, opposing trip duration) and the second was named "Stop Quality" ranging from excited to frustrated (which encompassed free stop behaviours, and caracoling/jumping behaviours opposing resisting and balking behaviours) (Figure 4.3). Variables of the handler interventions during the trip were also added to the PCA, where frequencies of noise to stop and move, contact to move, and flag waving were included on Dimension 1 (ranging from behaviours to move the cows to behaviours to stop the cows), while frequencies of pushing to stop, mixed noise interventions and mixed movement interventions (to stop and to move) were included on Dimension 2.

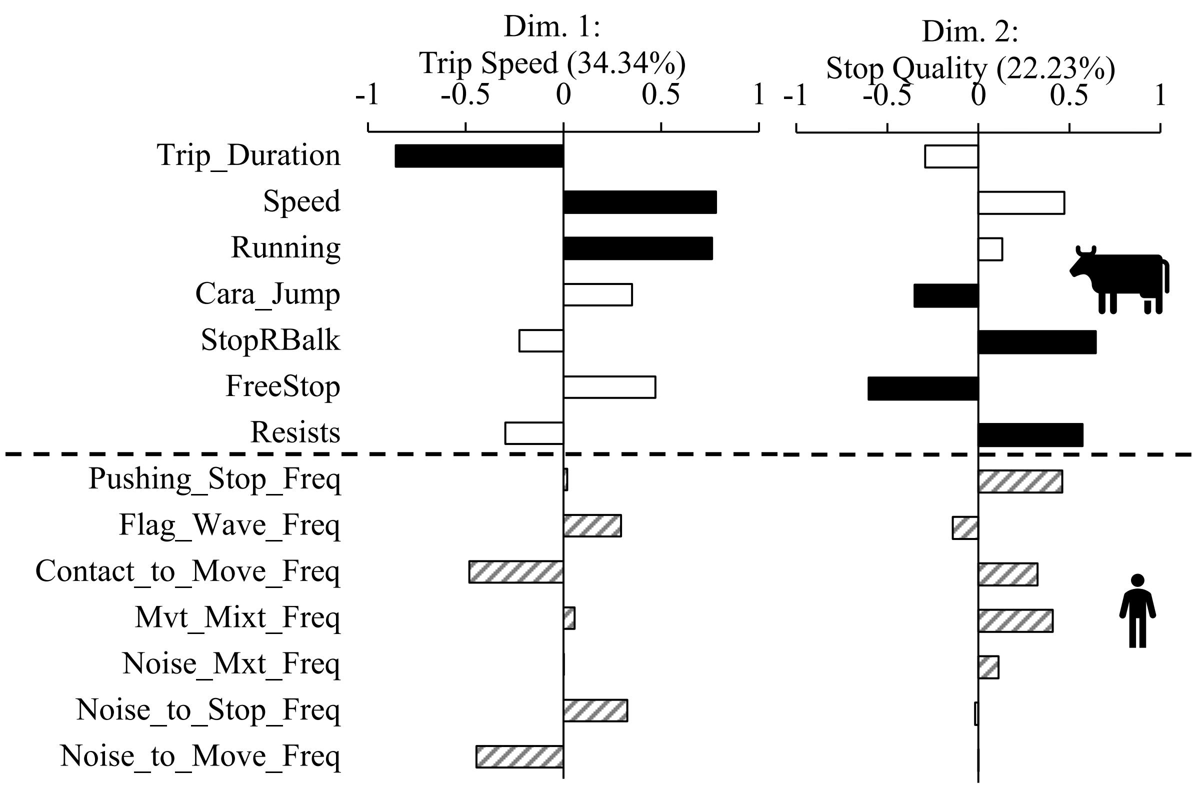
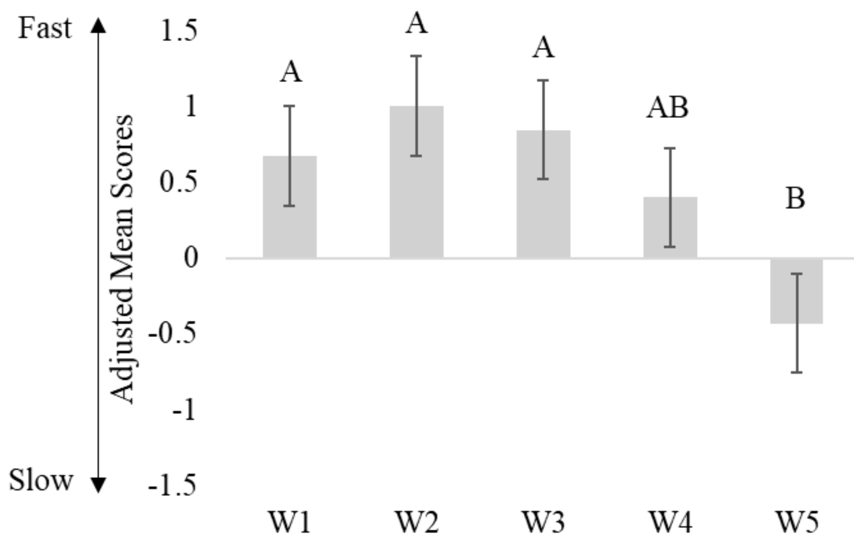


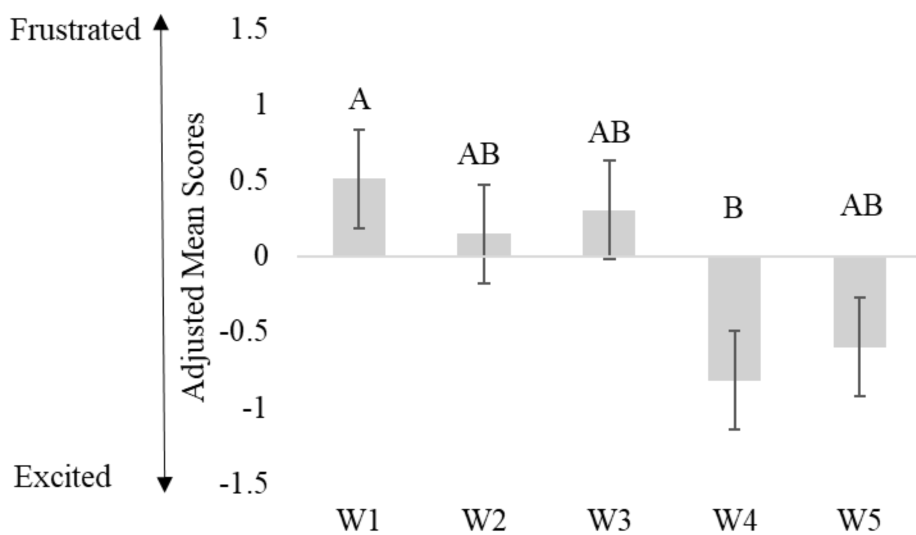
*Figure 4.3 Coordinates of variables from PCA including which dimension (either Dimension 1: Trip Speed (left side) or Dimension 2: Stop Quality (right side)) each variable had a higher contribution to (i.e. solid black bars for cows). The top eight variables are cow behaviour (including trip duration, speed, frequencies of running, free stopping behaviour, stopRbalk behaviour, resisting behaviour, caracoling/jumping behaviour, and percentage of vocalizing) whilst the bottom five variables are handler intervention behaviours (that is frequency of pushing to stop, flag waving, contact to move, movement mixed, noise mixed, noise to stop, and noise to move).*

For go-out trips, the LMr comparisons of treatment, week, and the interaction of treatment and week, from the PCA coordinates revealed, that the scores were different between the weeks for Dimension 1 ("Trip Speed"; $p = 0.002$). Cows slowed down over time, specifically in the final week of the trial, compared with weeks one, two, and three (Figure 4.4A). For Dimension 2 ("Stop Quality"), there were differences between weeks ($p = 0.02$), with cows from both treatments scoring lower (i.e., being more excited) in week four compared to week one (Figure 4.4B), as well as for treatments, with 1x cows scoring lower on excitement behaviours and 3x cows scoring higher on frustration behaviours ($p = 0.004$)(Figure 4.4C). For the go-in trips, no significance was found for treatment, week, or the interaction for Dimension 1. For go-in Dimension 2, the treatment was significant ($p < 0.001$). Subsequent pairwise comparisons revealed that 1x had a lower score (i.e., cows more excited) than 3x who had a higher score (i.e. more frustrated). (Figure 4.4D).

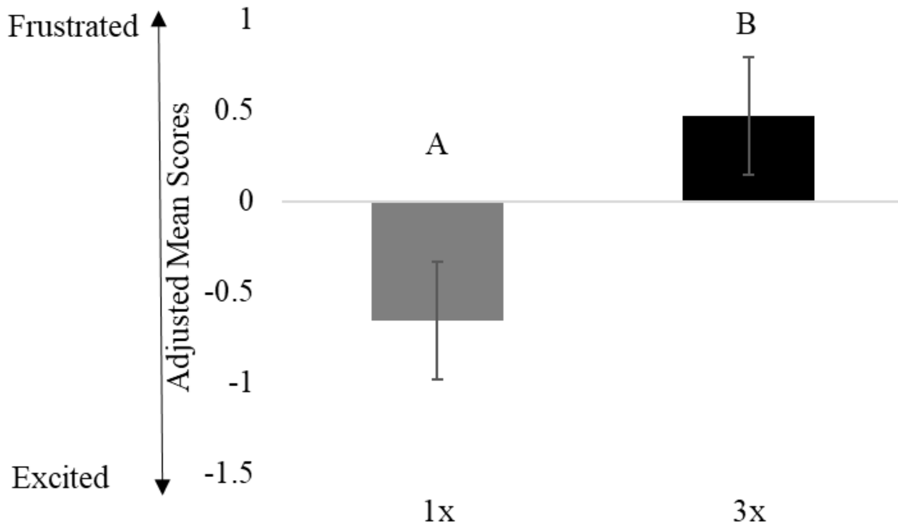
A


Fast
Slow
Adjusted Mean Scores
1.5
1
0.5
0
-0.5
-1
-1.5
A
A
A
AB
B
W1
W2
W3
W4
W5

B


Frustrated
Excited
Adjusted Mean Scores
1.5
1
0.5
0
-0.5
-1
-1.5
A
AB
AB
B
AB
W1
W2
W3
W4
W5

C


Frustrated
Excited
Adjusted Mean Scores
1
0.5
0
-0.5
-1
-1.5
A
B
1x
3x

D

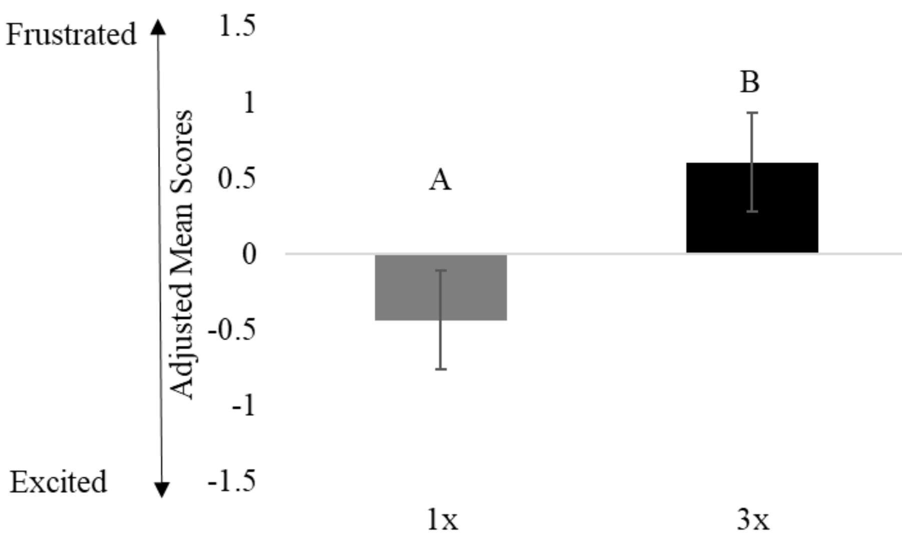

Frustrated
Excited
Adjusted Mean Scores
1.5
1
0.5
0
-0.5
-1
-1.5
A
B
1x
3x

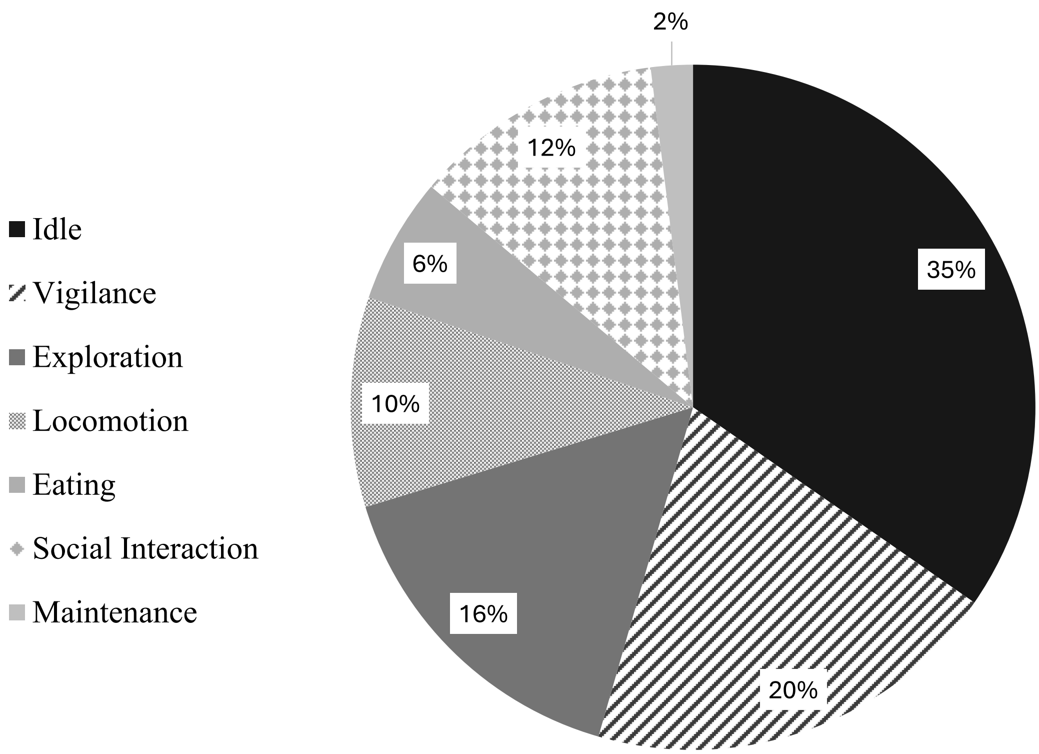
*Figure 4.4 Graphical depiction of the detailed results from the PCA including Estimated Marginal Means (Emmeans) and Standard Errors (SEs) of the LMr of the cows' scores for Trip Speed (A for go-out by weeks of both treatments) and Stop Quality (B for go-out by weeks of both treatments, C for go-out by treatment, and D for go-in by treatment). Different letters denote significant difference between weeks (for A and B) or treatments (for C).*

#### *4.5.3 Cows' Behaviour Whilst Outside*

When doing numerical comparison of time budgets (Figure 4.5), no major differences were found between treatments. 3x cows spent slightly more time idle and ruminating than 1x cows (35% vs. 28%), slightly more time engaging in social interactions (12% vs. 7%), and similarly little time engaging in maintenance behaviour than 1x cows (2% vs. 1%). Inversely, 1x cows spent slightly more time performing vigilance behaviours (25% vs. 20%), but similar time exploring (18% vs. 16%), eating (9% vs. 6%), and engaging in locomotion (12% vs. 10%) compared to 3x cows.

For behavioural transitions in the outdoor exercise yard, there were no differences in treatment, week, or the interaction ($p < 0.05$).

A



Idle
Vigilance
Exploration
Locomotion
Eating
Social Interaction
Maintenance
2%
12%
6%
35%
10%
16%
20%

B

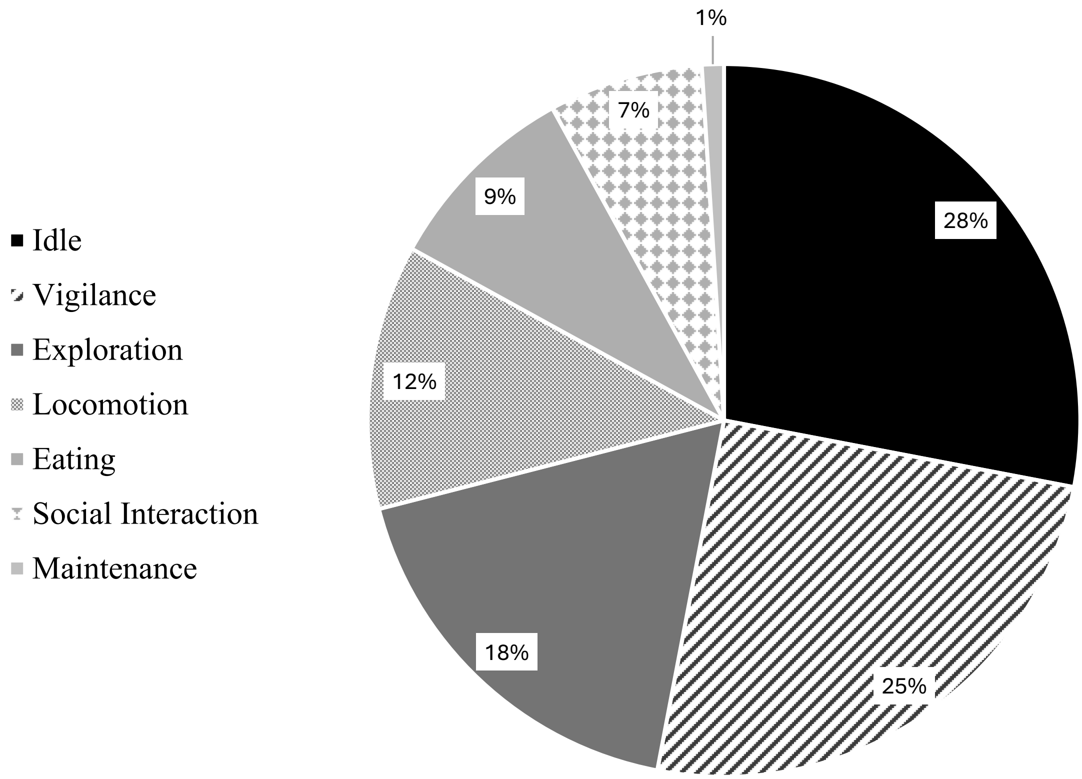



*Figure 4.5 Time budgets of 3x (A) and 1x (B) cows during outing, including overall percentage of time spent performing idle, vigilance, exploration, locomotion, eating, social interaction, and doing maintenance behaviours.*

## 4.6 Discussion

## 4.6.1 Animal Motivation to Go-Out vs. Go-In

*4.6.1.1 Differences in Trip Duration*

When examining trip differences, both treatments were quicker to go out than to return in week one (habituation week). This difference remained until week three for 3x treatment, and until week four for 1x treatment group, indicating that cows had greater motivation to reach the outdoor area than to return to their stalls, even over time (Aigueperse et al. 2023). Previous studies had found that cows were motivated for pasture, even over varying distances, temperatures, and humidities (Charlton et al. 2013), and that dairy cows are willing to work harder to access an outdoor area than to access fresh feed (Von Keyserlingk et al. 2017). In both cases, the authors attributed their results to a high motivation, likely due to going outdoors being important to cows; these results suggest that going out is inherently valued by cows under multiple contexts. In our study, the outdoor experience was perceived by the cows as positive, which would explain their persistent motivation (i.e. faster speeds going out vs. returning).

In the final two weeks of the trial, colder weather and poorer traction in the alleyway led to more corrective actions being implemented to make the ground conditions suitable for the cows to be allowed out (Table 4.1). Despite the successful corrective actions, these conditions may have slowed the cows' speed when entering the alley upon their exit from the barn, resulting in slower trip speeds that matched return trips in the fourth week (3x only) and fifth week (both treatments). It has been discussed in previous work that winter surface conditions may make cows more cautious, slowing their speed despite their motivation to move quickly (Aigueperse et al. 2023). After an hour outside and having already traversed the alleyway to go out, cows likely adapted to the ground conditions and were quicker upon return trips.

Between the first three weeks and the last two weeks, there was also a noticeable decrease in temperature compared to being in the barn, along with snow occurring in the last two weeks. The temperature change and sight of snow, a first for these cows, may have affected their motivation to leave the barn in the final two weeks. It was found that cows with prior outdoor exposure, especially experience being out during winter conditions, had a higher preference for staying outside (Shepley et al. 2016). Although our cows were not given a choice to stay inside, lack of experience with changing outdoor environments could have reduced their motivation to go out and increased motivation to go in. However, it is unclear whether it is truly a change in motivation or rather an impact of caution towards changing ground conditions on the way out and more confidence upon the return after acclimatization, as previously discussed. Corrective measures aimed to ensure good ground conditions regardless of weather, but cold weather remains a concern by handlers to limit slip and falls and therefore risk of injuries, when deciding if cows should be allowed outside.

The slower pace in the last two weeks might also signal decreased reactivity, where cows habituated to the outing procedure and were better adapted to handle the environmental changes related to going out. As the cows in the 3x had more experience going out, this could explain why they their speeds to go out vs. go in stabilized after three weeks, whereas 1x took an additional week to slow down when going out. It could be also that the cows are less sensitive to being brought outdoors which reduces their emotional response, though the behaviour itself speaks more to the emotional state in these weeks (see *4.6.1.2*). It could also be suggested to incorporate other measures such as observing the behaviour of cows in the barn after returning from the outing to see if they are satisfied and thus, have adjusted well to outdoor access.

In the fourth week of the trial, there was also an additional human handler with a flag introduced to slow the cows. While this addition potentially affected the 3x group's momentum, as they seemingly adjusted to this change by exhibiting slower speeds upon the go-out trips, the fact that the 1x group only experienced it once in week four could explain why no difference in speed was observed until the following week (upon their second exposure). It is possible for the extra flag and handler to have caused a change in the cows' behaviour due to the change in procedure and possibly additional human interventions during the outing, resulting in the observed variations in trip difference.

In past studies, non-forceful interventions were found to result in slightly elevated heart rate, without being correlated with dangerous incidents such as cows pushing or kicking human handlers (Lindahl et al. 2016); the addition of an additional flag-bearing human may thus have represented the safest method of intervention to attain the objective of slowing down the cows' speed. Yet, while safety is crucial, even minor protocol changes can impact the cow's ability to act on one's motivation, and too many interventions can ultimately lead to frustration in cows due to repeated thwarting of their motivation, with potential negative effects on their motivation to access the outdoor yard and on the interventions with human handlers involved in the process, as was observed in a previous research project (Aigueperse et al. 2023). Thus, balancing safety and the cows' motivation during outings should both be taken into consideration.

Regardless of outing frequency, cows showed a preference for going out vs. returning to the barn across weeks, indicating a consistent motivation to go out. Thus, outdoor access could positively influence emotion through allowing cattle to act on their motivated behaviour as well as improving adaptability to changing environments. Using multi-modal enrichment, such as outdoor access, is useful for improving welfare through promoting targeted expression of behaviour such as running, as well as providing a mentally and physically stimulating environment.

*4.6.1.2 Animal Trip Behaviour*

**4.6.1.2.1 Dimension 1: Trip Speed Behaviour**

The two PCA dimensions were similar to those found in 2023 by Aigueperse et al. whose methodology was adapted for this trial. There were no differences between treatments for "Trip Speed" on go-out animal behaviours, signifying that cow motivation to go to the outdoor area was the same regardless of frequency of outing. This highlights that having only one outing a week or having three, does not change how motivated cows are to go outside. This could be interpreted, along with our previous results, that even when only allowed to go out once a week, cows prefer the outdoors compared to the barn.

Although the motivation did not differ between treatments, a week effect was found in the Dimension 1 scores during go-out trips: the “Trip Speed” scores decreased with time (after three weeks) denoting a decrease in speed and number of running behaviours. This complements our previous results where there was an increase in trip duration in the last few weeks. This could be due to a learning process (Rørvang and Nawroth 2021). In the first three weeks of outings, cows may be unsure of how to react which could have resulted in some cows running to try to escape the handlers/situation. Once they realize what the procedure entails and start to habituate to outings, this may reduce agitation or stress, leading to lower speeds in the last week (Marçal-Pedroza et al. 2021). This could also show motivation for outdoors where in the beginning cows are more excited and try harder to run and push past the handlers to go out until they learn they will be slowed down and need to adjust behaviour accordingly (Von Keyserlingk et al. 2017). In the final two weeks, the change in ground conditions and handling may have also changed their reaction (as described in *4.6.1.1*). Both treatments seemed to have learned what to do and reacted according to previous experience and/or changing environment in these weeks (Rørvang and Nawroth 2021).

There was no difference of treatment, week, or interaction of treatment and week, for go-in “Trip Speed” behaviours, possibly meaning the motivation to return to the barn was not impacted by frequency of outing. However, the persistence in slower speeds going in again highlights the preference for the outdoors vs. the barn (Arnott et al. 2017). This corresponds to the “outdoor enthusiasts” profile found by Aigueperse et al. in 2023 in their Fall trial which also found that cows were faster to go-out than to go-in. This also supports previous work that found that outdoor access is valued by cows shown through strong motivation to be outside (Von Keyserlingk et al. 2017). Confirming that outdoor access is a positively associated experience, making cows more inclined to stay outside and thus, take longer to go back inside.

As previously mentioned, other works have shown a preference for the outdoors in comparison to indoors (including: Shepley et al. 2016, 2017, 2020), but the impact of frequency of outing on cow motivation for the outdoors has yet to be explored. This result is important for understanding and demonstrating that even when implementing outdoor access at a lower frequency, it does not decrease how strongly cows want to go outside. Thus, outdoor access is a valued enrichment that cows have strong motivation for, regardless of how often it is implemented. Since the goal of enrichment is to improve the welfare of livestock, it could be suggested that outdoor access be applied, even incrementally, so as to promote positive experiences for dairy cattle.

**4.6.1.2.2 Dimension 2: Stop Quality Behaviour**

There was a difference between treatment groups in "Stop Quality" behaviours, for both go-out and go-in. When going out, 1x cows showed locomotory behaviour like jumping, caracoling, and free stopping behaviours such as moving by itself or because of another cow after stopping, for all weeks but the last. Boissy et al. (2007) and Somers (2012) associated locomotor play (such as jumping and rapid movements) with positive states and the expression of playful excitement in young animals. Boissy et al. also saw that locomotor play was one of the most common forms of play in calves (2007). Here, however, the behaviours were observed in adult cows, which may slightly alter the interpretation. To identify these behaviours as "play", we might need to link them to other behaviours such as vocalizing or sudden movements, which may also be responses to the frustration of being held back, or anticipation linked to regained free movement access. Play in animals is often characterized by repetitive actions with no apparent purpose. If jumping and caracoling behaviours are observed sporadically, it could be an expression of acute excitement rather than play. This implies that 1x cows were excited as they performed what appeared to be locomotor play.

On go-out trips, it was found that 3x cows performed locomotion behaviour like jumping and caracoling as well as free stop like 1x cows, but also some resistant behaviours such as balking, freezing, and resisting. Balking and freezing may have resulted from the animal's fight or flight response to handlers' intervention trying to move or slow down/stop the cows. Using more forceful handling techniques such as shouting have been shown to be aversive to cows, resulting in higher heart rates and fear behaviour like balking, freezing, and resisting (Lindahl et al. 2016). Refusing to move after an interaction could be due to frustration from being forced to move or stop or due to unwillingness to go out (Veissier et al. 2008).

The supplementary handler interventions variables that were mapped with these behaviours were pushing the cow to stop, mixed noise and mixed movement interventions. However, as the behavioural sequence is not known, these behaviours could come before or after the cows' response, making these interactions more difficult to interpret. However, it is likely that the 3x cows performed free stopping and resistant behaviours due to expecting to go out and being stopped more often than 1x or wanting to go out slowly and being forced to move, causing frustration as their motivated behaviours were being thwarted. The 3x cows may have also been more sensitive to the handlers' interventions as lower arousal level and repeated exposure to handling could have led to more attentive and receptive response to interventions, making it easier for handlers to attempt to slow or move the cows. When the 3x cows were performing the behaviours they learned were less likely to result in an intervention and were still faced with adversity from the humans, this could've caused frustration and resulted in more bold or resistant behaviours as a consequence.

In 2011, Mason and Burn stated that restricting motivated behaviour harms welfare through causing frustration (i.e. a negative emotion). It has been found that thwarting cow behaviour can result in frustration and even an increase in active behaviours after thwarting (Veissier et al. 2008). In our study, there was potential for thwarting of behaviour both due to cows being confined in the barn for the majority of their lifetime and due to the influence of handlers during trips. Further, Aigueperse et al. in 2023, whose methodology was reproduced here, found multiple motivational profiles including 'thwarted motivation' where trip duration to go-out increased after experiencing higher number of handler interventions, and cows performed more negative behaviours towards handlers such as forcing their way through. 3x may have experienced this frustration when being continually stopped mid-behaviour, which resulted in a rebound of bold behaviours like resisting.

The "Stop Quality" behaviours shown by the treatment groups also differed when it came to going inside to the barn. The 3x cows exhibited more negative interactions when going in, which included resistant behaviours like forcing their way through or past the handlers, balking/freezing, and refusing the move, possibly as a result of trying to run (shown in Dimension 1) and being stopped by a handler, typically through a physical contact.

On the other hand, the 1x cows continued performing excited behaviours during return trips, possibly using this secondary opportunity to perform locomotor play. The movement restrictions related to tie-stall housing could also contribute to the types of behaviours cows showed when being allowed outdoors, as being able to use the full range of motion and experiencing a new/changing environment could facilitate the expression of positive emotions

(Mee and Boyle 2020; Botreau et al. 2023a,b). The number of outings cows need to experience to start developing expectations is not precisely known, yet what appears clear is that their cumulative experience does result in cows learning how the outing process works, and could thus lead them to become more confident, and perhaps, bolder as a result (in the case of 3x cows). This effect could be particularly pronounced in instances where cows learned which behaviours result in handler interventions, only to see the procedures modified, resulting in frustration and/or new adaptation to a changing procedure. Consistency when applying outdoor access could enhance enrichment aspects through minimizing stress or confusion, supporting learning and habituation, and promoting behavioural adaptation, so that the enrichment implementation itself does not negatively affect emotional state.

The difference in "Stop Quality" between treatments suggests that the frequency of outings influences the animals' motivated behaviour when returning indoors. The 3x group appeared more forceful and stubborn, while the 1x group showed more excitement, spending time jumping and caracoling. The 1x group, having fewer outings, encountered fewer physical interventions overall, compared to the more resistant 3x group due to the nature of the frequency of outings. Over time though, both treatments adapted their behaviour to match that of the handler interventions. This may be due to the cows needing a few outings to learn the expectations during the procedure (Young 1959).

As one of the aims of giving cows the opportunity to go outside is to enrich their lives and allow them to act on motivated behaviour in the hope of encouraging positive welfare, impeding their ability to do so may be counter to the goal of outdoor access. Our results present a potential tipping point at which trying to control motivated behaviour too much can cause frustration, feeding back into bolder and more resistant behaviours which may negate the reason for the handler intervention in the first place (e.g., slow down the cow to reduce the risk of injury). However, further validation is needed to identify at what point this occurs during the outing process and if reducing interventions after seeing signs of frustration could cause a positive rebound in motivation. Observing that cows are motivated to go outside, including performing what could be play behaviours (jumping, caracoling, etc.), demonstrates further the invaluable worth that animals place on having access to enriching experiences. Rewarding enrichments like having outdoor access should be considered vital to promoting positive welfare.

## 4.6.2 Animal Behaviour in Paddocks

### *4.6.2.1 Time Budgets*

Visual inspection of time budgets revealed no major variations between treatments in the percentage of time spent performing each category of behaviour. The only slight differences were for idle and ruminating (3x spending 7% more time idle and ruminating than 1x), social interactions (3x spending 5% more time socializing than 1x), and vigilance behaviours (3x spending 5% more time vigilant than 1x). A multiple trial study on the effects of exercise to fulfil cattle behavioural needs comparing indoor vs. outdoor exercise, outing duration, and size of exercise area, found on average that cows spent 25-70% of their time idle and ruminating (Cellier et al. 2024). Both treatments in our study fell within this range (3x at 35%, 1x at 28%), suggesting that the time spent idle and ruminating falls within a similar baseline pattern for cows, and that differences in the exercise frequency does not appear to affect the natural behaviour of cattle. Multiple studies have found a link between rumination and relaxation, where longer bouts or higher percentage of rumination are indicative of a relaxed emotional state (Marçal-Pedroza et al. 2021; Mattiello et al. 2024). Thus, this would suggest that both treatments were relaxed, and perhaps comfortable, during the outing.

Similar to idle and ruminating, time spent socially interacting was not greatly different to the aforementioned study (Cellier et al. 2024) at 5%, where our cows spent 12% and 7% of their time socializing (for 3x and 1x, respectively). This could again suggest that our animals behaved in a typical manner to other cows given exercise as enrichment, even when provided access at lower frequencies. However, as the social interactions included in our study combined both positive and negative interactions in a single behavioural category, it is difficult to know whether the animals engaged in these interactions in a relaxed or aggressive state. This information would have provided a better insight on the emotional state of cows once in the exercise yard, thus we suggest that future studies split social interactions into positive or negative interactions to better understand the modality of these behaviours.

The only deviation was for vigilance behaviour in which our treatments performed a higher percentage of vigilance (25% and 20% vs. 0.3-1.3%) than the compared study (Cellier et al. 2024). This difference could be due to many reasons such as familiarity with going out (a novel experience for our cows, but not necessarily for all trials in Cellier's study), type of exercise area (our always being outside, while theirs had a mixture of environments), or even number of outings per week (3x and 1x in our study vs. 2x and 5x in theirs). Being outdoors could increase vigilance due to new sights (changes in lighting, snow, etc.), new sounds (e.g., traffic nearby), smells, etc. that could change from one day to the next (Welp et al. 2004). So, the differences seen in behaviour could also easily be linked to these external effects, where the emotional state could vary (vigilant due to fear, curiosity, or other) and is not clearly determined based on our results.

Regardless of frequency of outing, it appears that both treatments were equally able to adjust to the outing procedure and that even at lower frequencies, both treatments performed in a normal range of behaviours, with the exception of vigilance. Behaviour did not seem to be negatively affected, however future studies would be suggested to further differentiate between positive and negative behaviours such as the different types of social interactions, for clearer indication of how outdoor access affects emotional state.

*4.6.2.2 Behavioural Transitions*

There were no differences in behavioural transitions between the 3x treatment or the 1x treatment, or between weeks. As mentioned before, cows spent most of the time idle and ruminating, which are relaxed behaviours (Marçal-Pedroza et al. 2021; Mattiello et al. 2024). As well, cows did not appear to be in a heightened state of arousal as the average number of behavioural transitions per week (i.e. less than three) were low (Crump et al. 2019). Thus, cows appeared to be exhibiting comfort with the outdoor experience, further demonstrating how adaptable they were to the new procedure regardless of outing frequency.

### 4.6.3 Implication of the Impact of Frequency of Outdoor Access on Motivation

The frequency at which dairy cows were allowed outdoor access did influence motivation, both during the trips to go out and come in, and inside the paddocks during the outing itself. There was an inherent connection between “Trip Speed” and “Stop Quality”, where more forceful behaviours were associated with a higher speed (3x), while excited behaviours were associated with a slower speed (1x). However, both treatments were motivated to go out, regardless of frequency, so outdoor access was likely a valued enrichment by cattle. Acting on an animals’ needs/desire could positively impact the welfare of the animal, and in this trial, both treatments benefitted from having the opportunity to go out (Kirkden and Pajor 2006).

The type of handler interventions seemed to influence cow responses, where more invasive interactions involving physical contact may have impacted the behavioural sequence and thus, the response of the 3x animals (Breuer et al. 2003; de Passillé et al. 1996; Lindahl et al. 2016). In our specific case, being prevented from fulfilling behavioural motivations seemed to incite aggression to push past the handlers doing the thwarting or in resistance to move after being thwarted. The 1x treatment group was faced with fewer physical contacts than 3x, possibly because the handlers felt more comfortable addressing excited behaviours like jumping with motion and noise (e.g., flag waving and/or clapping) rather than intervening physically. This might be because handlers perceive it as more difficult or dangerous to try to physically restrain or stop a jumping/caracoling cow, due to the vertical height and greater potential for trampling. This may be why 1x cows continued to act excited, both due to frequency of outing and because the preferred intervention was viewed as less aversive, suggesting they maintained a positive association with going outside (Tirloni et al. 2013).

The behaviour performed by the two treatment groups whilst outside was similar, and the time budgets were comparable to another study on outdoor access for dairy cows (Cellier et al. 2024). Further, behavioural transitions showed low arousal level for both treatments, where there were few behavioural transitions on average per week. These results suggest that the animals were comfortable and adjusted well to having outdoor access.

In all, trip durations and trip behaviour confirm the importance of allowing cows to act upon their motivation towards enrichment, in particularly to go outside, regardless of outing frequency. Dairy cattle were found to have strong motivation to go out and their behaviour was not negatively affected whilst outside and in fact, the cows seemed comfortable during the outing. Outdoor access could thus be an enrichment that benefits the mental well-being of movement-restricted animals.

## 4.7 Conclusion

Dairy cows who are allowed outside, even just once a week, are strongly motivated for outdoor access and show speediness such as running. Depending on whether their motivation is thwarted by handlers, they exhibit either excited or resistant behaviour when going outside. Regardless of the frequency of outing, cows were comfortable with the outing experience, shown through low arousal and relaxed behaviours such as idle and ruminating. Handler interventions could also impact motivation and should therefore be carefully considered as to how often and in what measure interventions should be applied, to ensure a positive experience to both the cows and the handlers. Allowing dairy cattle more freedom to act on their motivational needs and/or desires, particularly in situations they deem valuable, could positively improve animal welfare.

**Declaration of Competing Interest**

Authors declare that there are no competing interests.

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## Connection Statement 3

Chapters 3 and 4 looked at the implementation of enrichment across species in similar contexts (a.k.a research settings) while Chapter 5 will investigate the implementation of the same enrichment within same species in different contexts, one in a research setting and one that mimics a commercial setting, to allow further scaling-up of our research results. Chapter 4 verified that even with lower frequencies of outdoor access, that dairy cattle were strongly motivated to go outside vs. return to the barn. However, it was also found that external factors such as handling could impact motivation for the outdoors. Further, Chapter 4 evaluated smaller groups in a strict environment controlled by the researchers, where producers may not have the ability to implement such methodology. Chapter 5 will take the knowledge gained from the previous chapters and apply it in a living lab experiment with larger group sizes, akin to commercial farms, and adapted handling practices to see how cattle motivation to go outdoors is affected.

Chapter 5 is the accumulation of all the theoretical knowledge on enrichment impacts on motivation from previous chapters applied to a real-world setting, where the takeaways will be integral for producers wishing to transition their herd from movement-restricted systems to having regular outdoor access. The comprehensive review revealed how behavioural measures of motivation could be used to make conclusions regarding how animal perceive and react to enrichment. While Chapters 3 and 4 applied this in a controlled setting, Chapter 5 will investigate which behavioural measures can be practically collected on-farm to evaluate the success of the transition to outdoor access, but also which handling procedures should be adopted to best make this transition efficient and readily adoptable.

# Chapter 5 - On-Farm Implementation of Regular Outdoor Access on Motivation of Movement-Restricted Dairy Cattle: A Living Lab Experiment

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## 5.1 Abstract

Outdoor access can physically and mentally benefit dairy cows and presents a way for producers to satisfy the upcoming requirements set by the National Farm Animal Care Council (NFACC) Canadian Code of Practice for the Care and Handling of Dairy Cattle. To understand how producers could transition their herd from a movement-restricted system to having regular outdoor access in a practical way without negatively impacting animal motivation, a living lab experiment was conducted. A farm with 76 lactating dairy cattle, split into seven groups based on row configuration in the barn, was introduced to 1h of outdoor access in weekly increments with a new group being added each week. Handlers were stationed at strategic points throughout an alleyway leading to one of two outdoor paddocks, to assist in moving or slowing cows as

necessary. Groups were brought out individually, but entire paddocks were brought in together to mimic realistic commercial farm methods. The duration of the trip going out and returning to the barn, the number of staff and cows present during each trip, the number of handler interventions to move or slow the cows, and the behaviour of cows whilst outside (time budgets and behavioural transitions), was recorded. It was found that even with more cows being added (i.e. an additional group of 10 cows on average each week, $p < 0.001$), the length of time taken for cows to go out did not change (average 4-16sec per cow, $p > 0.05$). Further, the behaviour outside was not impacted by the addition of new groups ($p > 0.05$). This suggested consistency of cattle motivation and ease of management even at larger group sizes (i.e. starting at only 10 cows in week one and ending with the entire herd of 76 cows by week five). Average handler interventions decreased after week one from 15 to less than five interventions a week for stop interventions and only increased by 5-10 interventions in week five for go interventions due to cows turning around to go back to the barn during the trips ($p < 0.05$). Additionally, the number of handlers present did not change throughout the weeks (i.e. one of two handlers average, $p > 0.05$). It is likely that handlers were not as confident in the first week of the trial, using more interventions to compensate, but became more comfortable with experience and thus, did not require more handlers or more interventions even as the number of cows increased. Both cows and handlers adapted quickly to the transition and producers can similarly implement outdoor access in gradual steps to allow time for an adjustment period.

**Keywords: outdoor transition, enrichment, time budget, handling**

## 5.2 Introduction

In Canada, dairy cows are often kept inside, year-round, in individual stalls that might not provide as much opportunity for mental stimulation, exercise, or the ability to express their behavioural repertoire as other systems (Popescu et al. 2013; Palacio et al. 2023; Cellier et al. 2024). Although this practice is used for a number of reasons (ex. weather conditions, ease of management and monitoring, etc.), being kept in a less enriched environment can lead to boredom, frustration, or even distress for confined animals (Meagher and Mason 2012; Russell et al. 2024). Recently, the National Farm Animal Care Council (NFACC) Dairy Cattle Code of Practice updated its requirements to necessitate that by 2027 Canadian dairy producers using tie-stall barns must allow their cattle regular time outside of the stall (NFACC 2023). To assist producers in this transition, best methods for implementing the change while optimising welfare need to be identified.

Outdoor access could be a solution as it is a multi-modal enrichment that can include all enrichment categories: physical (more space to move and perform locomotory activities compared to being in the stall), cognitive and sensory (new sights, sounds, smells, etc. and challenges that would not be experienced inside the barn), nutritional (ability to graze on fresh grass if weather permits), and social (mixing with more animals from the herd resulting in increased social interactions) (Botreau et al. 2023b). Further, allowing cows access to the outdoors has been shown to have both physical and mental benefits (Nejati et al. 2024; Palacio et al. 2023; Aigueperse and Vasseur 2021) and cows have shown preference for being outdoors (Charlton et al. 2013; Smid et al. 2018; Shepley et al. 2016, 2017, 2020). Being able to act on their motivation to go out could lead to positive emotion (Muszik et al. 2024, Manuscript in review). Additionally, a survey of Canadian dairy cattle handlers reported that 70% of respondents agreed that prioritizing conditions that improved emotional states was very or extremely important (Lutevele et al. 2025). Producers themselves also recognize the relationship between emotion and animal welfare is important to consider (Hötzel et al. 2025), and should therefore make management decisions that optimize positive experiences, such as having outdoor access.

It has been found that cattle are motivated to access an outdoor area (Aigueperse et al. 2023; Muszik et al. 2025, Manuscript in preparation), but the way that animals were handled when being brought outside influenced future responses to going out (Aigueperse et al. 2023), and thus handling may impact the perception of the enrichment (Boissy and Bouissou 1995). Aigueperse and Vasseur (2021) demonstrated that cows imposed with more movement restrictions to limit cows from running and reduce risk of injuries during the trip to go outside, did not exhibit an improvement in reactivity to humans, unlike other sub-trials where less handling was used to limit cows from running and reduce the risk of injuries. They hypothesized that handling restrictions may have been viewed as aversive, preventing cattle from associating going outside with a positive event. Further, it was found that handling interventions may have thwarted cattle motivation, resulting in resistance behaviour such as pushing past the handler (Muszik et al. 2025, Manuscript in preparation). So, reducing handler presence and behavioural interventions could positively affect motivation, although this remains to be studied.

As commercial farms will likely implement outdoor access with larger, frequently mixed groups (Smid et al. 2023), it is important to understand whether larger groups enhance the novelty and dynamic nature of going outside, while also positively influencing motivation through increased social opportunities and improved reactivity to a constantly changing environment (Boissy and Le Neindre 1990). Social relationships have been found to influence dairy cows' use of an outdoor area when in pairs (Nogues et al. 2024). So, outdoor behaviour should be evaluated to determine if and how larger groups impacts animal motivation for outdoor access. Larger groups of fearful or bold animals may increase the amount of time or staff need to take animals out or bring them back to the barn, which could deter producers from attempting to continue outdoor access implementation. Smid et al. in 2023 found that Canadian dairy farms with larger lactating herds had a lower likelihood of providing outdoor access. It should be determined if being in a larger group size and continuous mixing between individuals whilst outside has an impact on motivation for subsequent outings, and thus the achievability and ease of transition from tie-stalls to regular opportunity for outdoor access. If it can be demonstrated that larger group size and less handler interventions can positively impact motivation for enrichment, this may improve producers' willingness and ease to make the transition.

Therefore, the objective of this study was to identify practical methods for producers wanting to implement outdoor access for movement-restricted dairy cattle when the entire herd are being transitioned to go outside. Further, one aim was to identify how group size and handling interventions impact cow motivation for the outdoors using a living lab approach. It is hypothesized that even with larger group sizes and continuous remixing of animals, cows and handlers will adapt to the outing procedure after a few outings, and that adjustment of the handling methods to less interventions as the handlers and the cows get adapted to the procedure, will not negatively impact the transition or motivation of the herd to going outside when using less handling restrictions.

## 5.3 Methods

### *5.3.1 Ethics Statement*

The animals and research procedures used in this study were approved by the Animal Care Committee of McGill University and affiliated hospitals and research institutes (#2016-7794).

*5.3.2 Outdoor Access Implementation*

This study used the entire lactating herd (76 cows consisting of: 68 Holsteins, 5 Jerseys, 2 Brown Swiss and 1 Ayrshire) approximately 166 DIM ± 97.78, average parity 2.47 ± 1.55, located at the Macdonald Campus Dairy Cattle Complex (Sainte-Anne-de-Bellevue, Quebec). The animals were split into seven groups for going-out (n=13, n=15, n=11, n=11, n=10, n=8, n=8, respectively). The groups were chosen based on the stall design of the barn in which one group consisted of an entire row within the barn. The groups were given access to one of two outdoor paddocks (42m x 34m for groups 1-3 and 65m x 21m for groups 4-8, respectively) for 1 hour/day per outing, with a target of 5 outings/week (not including weekends). The number of outings per week was dependent on environmental conditions and handlers availability.

The trial took place from May 2022 to July 2022. If outing conditions were met (i.e. weather and ground conditions deemed acceptable by the staff based on a previously established Standard Operating Procedure (Macdonald Campus Farm Cattle Complex, 2023), that is: not raining heavily, visibility not reduced, and/or alleyways and paddocks not too muddy and/or slippery as to hinder cows' movement or cause injury) the animals were brought outside in their respective groups with a new group being introduced each week (with the exception of group 6 who was added in week seven instead of week six) to allow for at least three outings for the original group(s) to habituate to the procedure. Group 1 was let out on week one, then group 2 was added in week two, and so forth until the eighth week when the final group was added and all seven groups were being brought out (see Figure 5.1). The first paddock had 13 cows in week one, 28 in week two, and 39 cows in weeks three to eight. The second paddock had 11 cows in week four, 21 in week five and six, and 37 in weeks seven and eight. All new groups were brought out separately. A waiting area (12m) was added for the first one to two outings of each group to easily manage and help habituate cows to the alleyway and outing procedure.

Following a living lab approach (Veeckman et al. 2013), this schedule and procedures were created as a collaborative effort between research and farm staff to ensure the procedure was as manageable and applicable as possible to the farm environment and management, while data could be collected by the research team to document the implementation of the new routine. After the habituation period, the following outings included untying and directing the cows from their stalls to the farm door where they could enter the alleyway (approx. 40m in length or 52m when the waiting area was not used) leading to the outdoor areas. Handlers were positioned at three points along the alleyway to encourage the cows to move forward or to stop when needed (Figure 5.2).

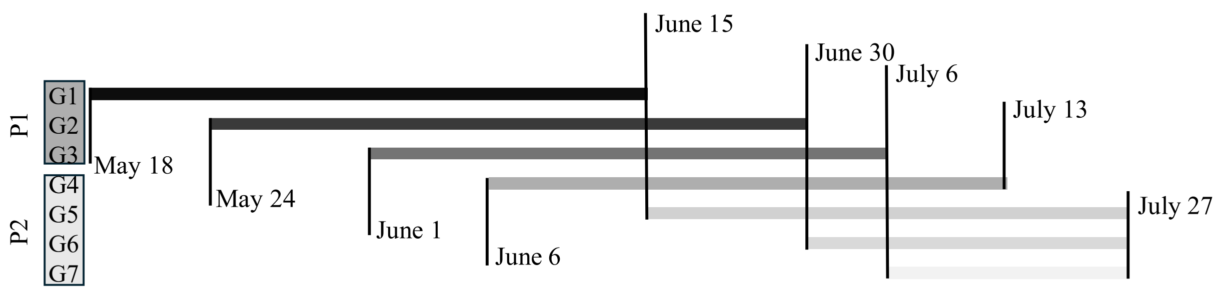



*Figure 5.1 Observational timeline depicting the weeks when each group (G1-G7) was introduced into paddock 1 or 2 (P1 and P2, respectively) and when observations for that group finished, including the habituation period where each group was given a minimum of three outings to learn the procedure before a new group was introduced. The new group that was added each week was brought out separately from the previous groups in which the animals were first brought to a waiting area (a 12m long area in the alleyway starting at the barn door) before the gate was opened.*

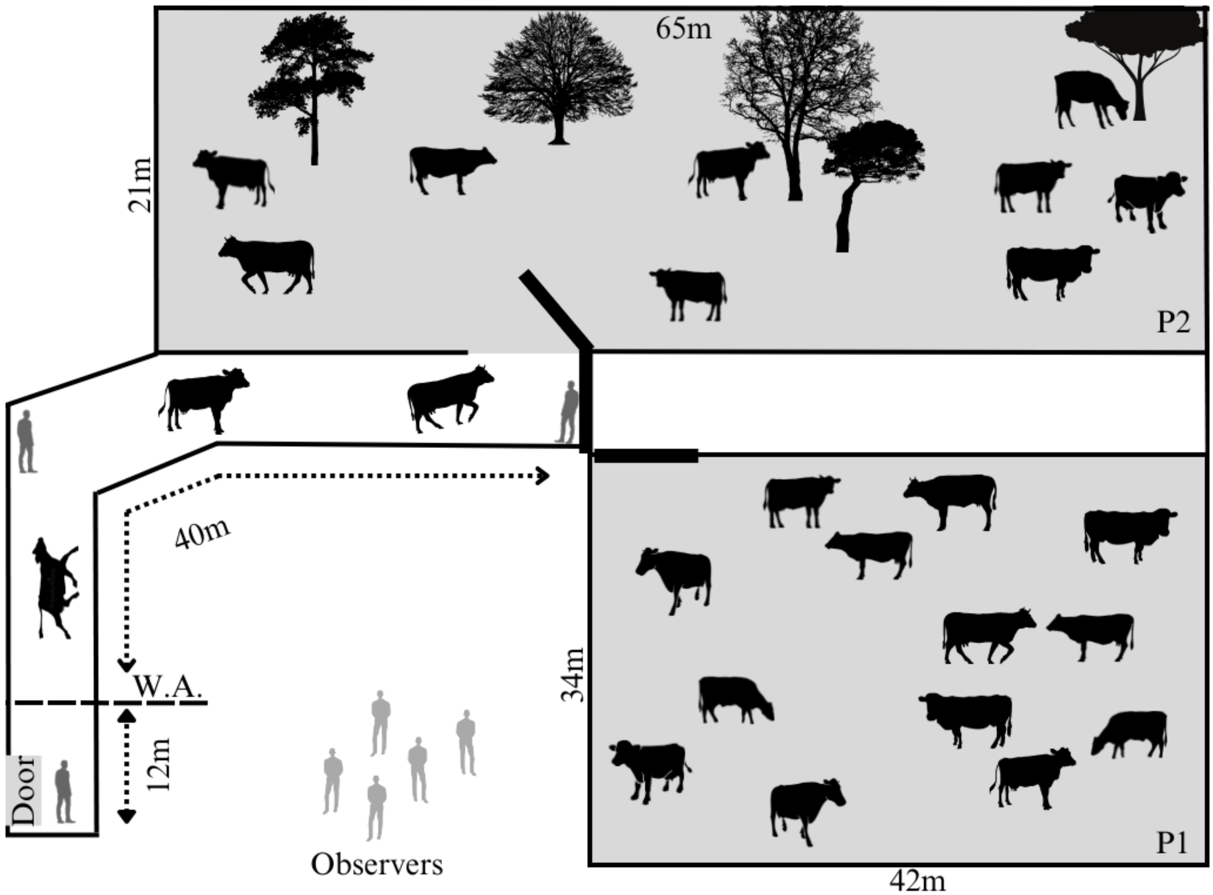



*Figure 5.2 Graphic of the outing procedure and paddocks (42m x 34m for groups 1-3 and 65m x 21m for groups 4-7, respectively). The barn door leading to the outdoor area was propped open to allow cows to exit at their leisure once untied from stalls, with handlers positioned strategically around the alleyway (approx. 40m in length with the 12m waiting area (W.A.) or 52m without W.A.) to encourage cows to slow down or speed up if necessary. The observers (in light grey) were positioned approximately 10m from the alleyway. Handlers (in dark grey) were strategically stationed at three points in the alleyway to encourage cows to stop or move as necessary.*

*5.3.3 Measures*

To measure motivation for the outdoor exercise area, duration (in seconds) of the trip going outside and back into the barn was recorded by group (going out) or by paddock (going in) using two stopwatches by one of the observers (following procedures developed by Aigueperse et al. 2023, and adapted from Muszik et al. 2025, Manuscript in preparation). One stopwatch was to record the duration from the start of the trip (defined as when the first cow enters the alleyway through the opened farm door) to when the first cow reaches the outdoor area (for the trip going out) or end of alleyway (for the trip returning to the barn), and the second to record from the start of trip to when the last cow reaches the exercise yard or end of alleyway. This was done to find an average trip duration per cow per week of the trial. Five observers were trained by an expert in the first two weeks of outings, and were shadowed for one outing in the fourth (i.e. middle) and eighth (i.e. end) week to ensure 90% inter and intra reliability was reached. The trained observers did live observations of the handler interventions, focusing on how many times a physical or non-physical contact was used to move or stop the cow(s) (Table 5.1).

**Table 5.1** Motivation measures (recorded live) including handlers interventions, modifier, and definition adapted from Muszik et al. 2025,  Frequency of Outdoor Access on Motivation of Movement-Restricted Dairy Cattle for an Outdoor Area [Manuscript in preparation].

| Behaviour | Modifier | Definition |
|---|---|---|
| **Go** | Physical contact | The handler encourages the cow to move forward by physically manipulating the cow using hand(s), body, or object(s) |
| | Non-physical contact | The handler encourages the cows to move forward by making a noise such as clapping, whistling, tongue clicking, speaking, etc. or waving of the hand(s), arm(s), or flag(s) |
| **Stop** | Physical contact | The handler tries to make the cow stop moving by physically manipulating the cow using hand(s), body, or object(s) |
| | Non-physical contact | The handler tries to make the cow stop moving by making a noise such as clapping, whistling, tongue clicking, speaking, etc. or waving of the hand(s), arm(s), or flag(s) |

To identify how larger group size affects cows' motivation for outdoor access, behaviour during the outing was also recorded live using scan samplings by the trained observers who stood outside of the outdoor area (approximately 10m away), out of sight of the cows to avoid influencing cow behaviour. This procedure was done following methodology adapted from Cellier et al. 2024.The scans were done on five randomly selected cows per group (identified by collar colours). One scan was recorded every 10 seconds for a total of 30 minutes. These behaviours were categorized into: idle (including ruminating), vigilance, exploration, locomotion, eating, positive and negative social interaction, maintenance behaviour, and vocalizing (Table 5.2).

Scans were conducted to develop time budgets to identify if there were behavioural differences between the two paddocks by averaging the scans of all groups in each paddock for comparison using descriptive statistics. Scans were also used to compare between outings that happened immediately before, during, and after the introduction of a new group. This was done by averaging the scans of the old group(s), (i.e. group(s) that had already been introduced to outings), on the day prior to the addition of a new group for the 'immediately before', and averaging the scans of all groups together (including both old and new) on the day of and the day after the addition of the new group for the 'during' and 'after'.

**Table 5.2** Time budget behaviours recorded whilst cows were outside (adapted from Cellier et al. 2024).

| Category | Behaviour | Description |
|---|---|---|
| Idle | | Body supported by four legs, immobile, body relaxed, head down or extended. Cow can be ruminating (i.e. repetitiously chewing partly digested feed). |
| Vigilance | | Body supported by four legs, immobile, body tensed, head raised, and ears facing forward, tensed |
| Exploring | | Sniffing or licking grass, fencing, trees or other structures/objects in the environment (cow can be ruminating) |
| Locomotion | Walking | Movement forward with the feet, one or two feet lifted at a time, as cow moves at a slow pace |

| | | |
|---|---|---|
| | Trot/Running | Fast movement forward with front or hind legs in parallel, touching the ground and then lifting simultaneously with a brief period of suspension in the air at a quick or rapid pace |
| | Caracole | Alternating trot/run and buck |
| | Leap (jumping) | Movement upwards and/or forward, the two forelegs are lifted from the ground as animal launches its weight up and/forth, the hind legs can also be lifted |
| Eating | | Grabbing, chewing, and/or consumption of grass |
| Positive social interaction | | Allogrooming, sniffing other cows, head and/or body contact without force |
| Negative social interaction | | Bucking, kicking, head butting with force, and/or fighting |
| Maintenance | Grooming | Tongue in contact with own skin/fur or scratches with the foot or the head or object (not including the ground) |
| | Urination | Cow lifts tail to eliminate liquid matter |
| | Defecation | Cow lifts tail to eliminate fecal matter |
| Vocalizing | | Cow omits noise from mouth |

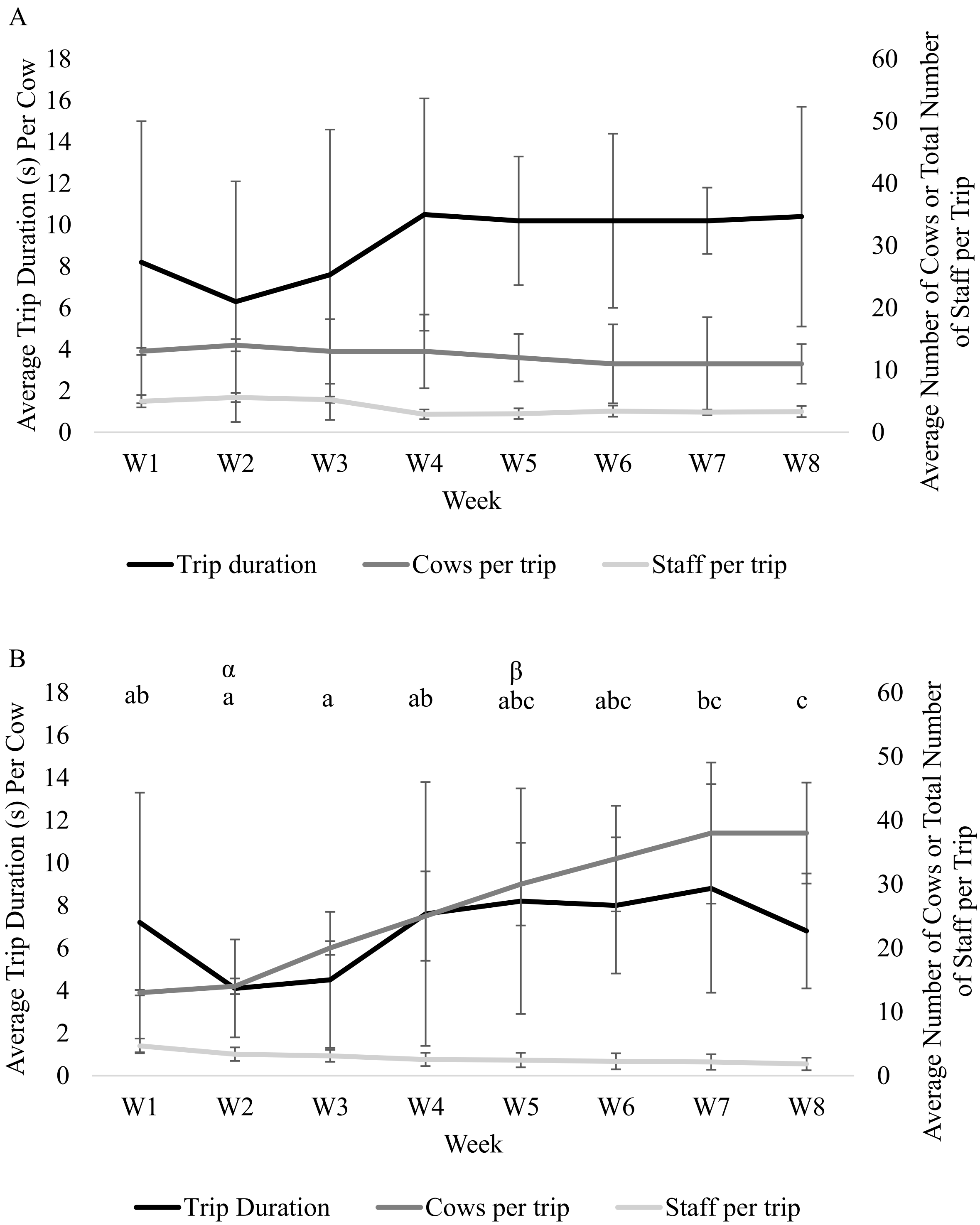
*5.3.4 Statistical Analysis*

R Studio® version 2021.09.2+382.pro1 Ghost Orchid (250 Northern Ave, Boston, MA 02210) was used for statistical analysis. Tests for normality were done using Shapiro-Wilk and the Bartlett test (for unpaired data) for homoscedasticity, and data transformed when conditions were not met. Q-Q plots were also used to assess normality of the data graphically. Trip durations were also tested using a one-way ANOVA. Average number of cows and total number of handlers per trip was tested using the Kruskal-Wallis and Dunn test with a correction of Bonferroni Holm for the go-out trip, while the trip go-in was tested using Welch's t-tests. The handler interventions were tested using Welch's ANOVA with Games-Howell test for post-hoc comparison. Significance was declared at $p \leq 0.05$, and tendencies between 0.1 and 0.05.

Descriptive statistics were conducted to summarize the average time budgets of the two paddocks, as well as average time budgets for the group(s) a day before, the day of, or the day after a new group was introduced. Time allocation to different activities was expressed as percentages of scans per time period (all scans across the five weeks for paddock time budgets and all scans per day of observation for before, during, and after, the introduction of a new group). Descriptive analyses were performed using Microsoft Excel.

## 5.4 Results

The trip durations were not different between weeks for the go-out (figure 5.3A), but there was an increase of trip duration (4s to 8s) between weeks two and five for go-in ($p = 0.03$) (Figure 5.3B). There was no difference between weeks for number of cows during the go-out trip ($p = 0.2$), but there was an increase in number of cows for the go-in trips over the weeks ($p < 0.001$). These increases occurred between weeks one and eight ($p = 0.02$), two and seven ($p = 0.01$) and two and eight ($p < 0.001$), weeks three and seven ($p = 0.03$) and three and eight ($p = 0.003$), and finally between weeks four and eight ($p = 0.006$). The number of handlers present during the trip did not change during the go-out or the go-in trip ($p > 0.05$).


A
Average Trip Duration (s) Per Cow
Average Number of Cows or Total Number of Staff per Trip
0
2
4
6
8
10
12
14
16
18
10
20
30
40
50
60
W1
W2
W3
W4
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W6
W7
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Week
Trip duration
Cows per trip
Staff per trip
B
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Average Trip Duration (s) Per Cow
Average Number of Cows or Total Number of Staff per Trip
W1
W2
W3
W4
W5
W6
W7
W8
Week
Trip Duration
Cows per trip
Staff per trip

*Figure 5.3 Average trip durations, average number of cows per trip, and total number of handlers per trip (with standard deviations) for trip go-out (A) and trip go-in (B). Greek letters denote significant difference between average trip durations while latin letters denote significant differences between average number of cows per trip. Significance declared at p ≤ 0.05.*

For handler interventions, an increase in go behaviours was found for the go-out and go-in trip in week five compared to all other weeks ($p < 0.05$) (Figure 5.4). Stop behaviours during week one were higher than all other weeks for both trip go-out and go-in ($p < 0.05$).

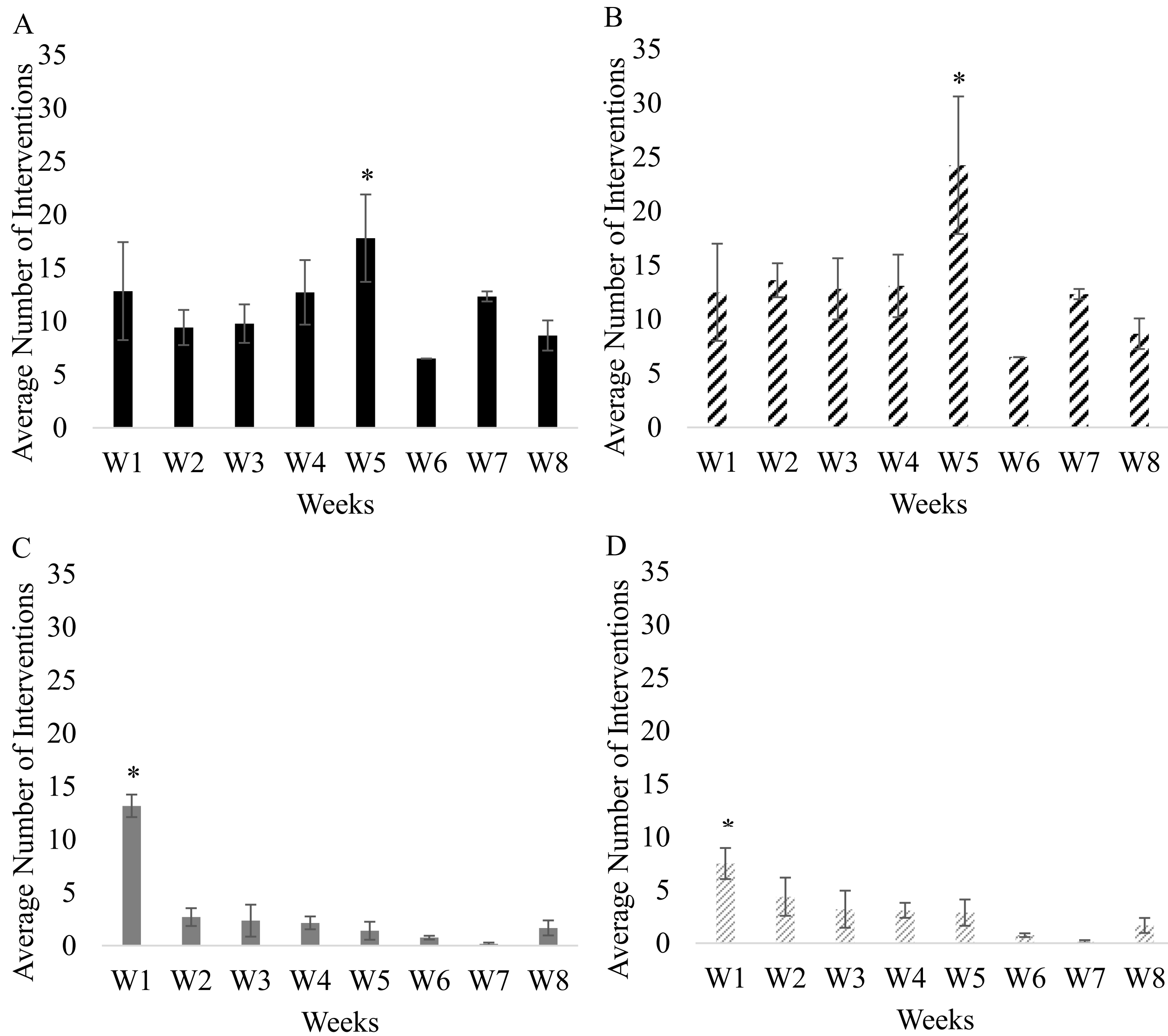
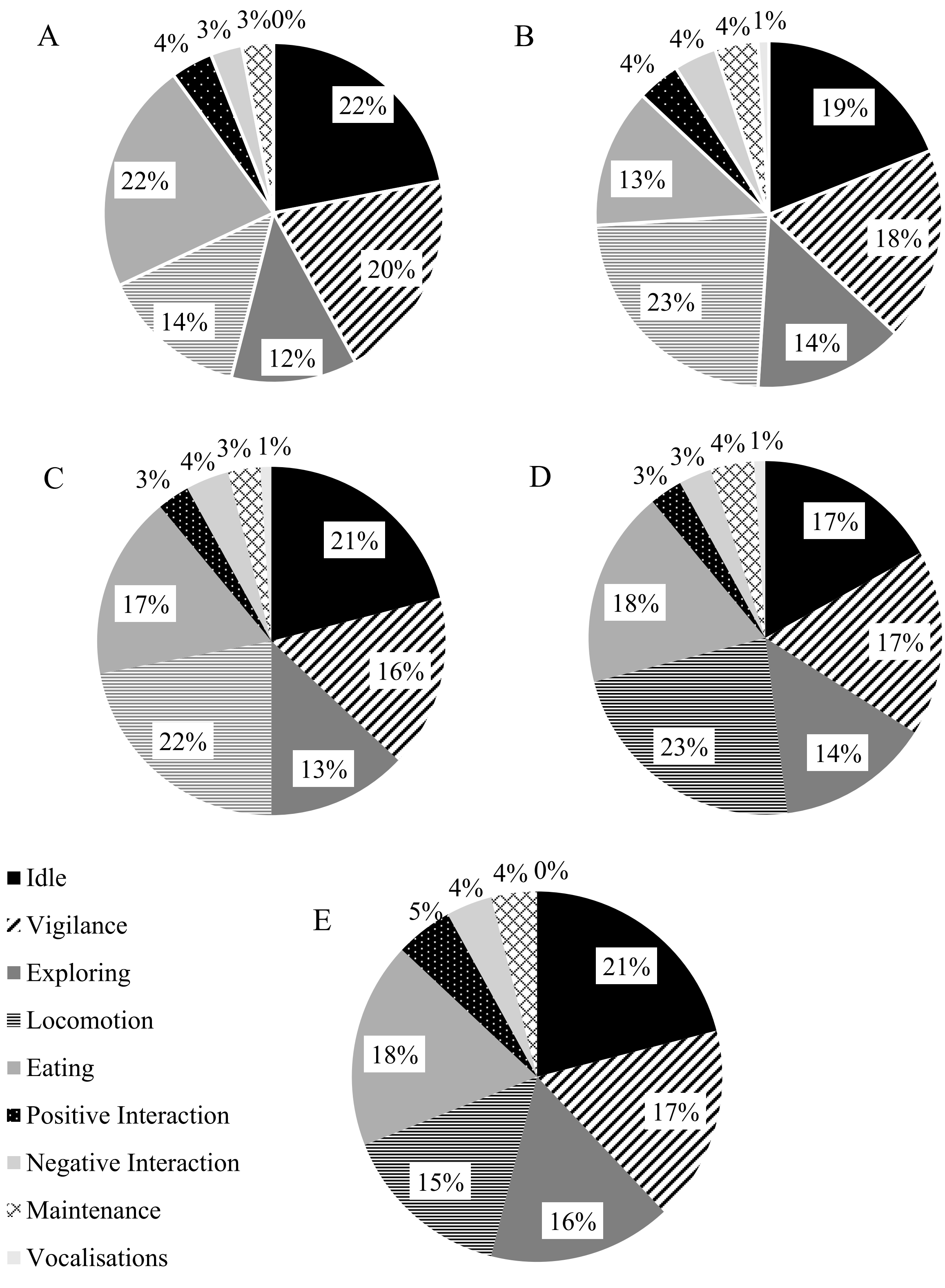


*Figure 5.4 Average number of go interventions for go-out (A) and go-in (B) per week with standard deviations, along with average number of stop interventions and standard deviations per week for go-out (C) and go-in (D). * Denotes significance, which was declared at $p < 0.05$.*

Time budgets were examined using descriptive statistics where no major observable differences in behaviour whilst outside between paddocks 1 or 2. There was also no observed differences between paddock behaviour on the day immediately before, the day of, or the day after outings when a new group of cows was introduced (Figure 5.5). Cows in both paddocks spent about 17-22% of the time idle, 16-20% vigilant, 14-16% of the time exploring, 14-23% in locomotion, 13-22% eating, 3-5% in positive social interaction and 3-4% in negative social interaction, 3-4% of the time doing maintenance behaviour, and 0-1% of the time vocalizing.


A
22%
20%
12%
14%
22%
4%
3%
3%
0%
B
19%
18%
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23%
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3%
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D
17%
17%
14%
23%
18%
3%
3%
4%
1%
E
21%
17%
16%
15%
18%
5%
4%
4%
0%
Idle
Vigilance
Exploring
Locomotion
Eating
Positive Interaction
Negative Interaction
Maintenance
Vocalisations

*Figure 5.5 Time budgets (i.e. percentage of scans) of cattle behaviour (including idle, vigilance, exploring, locomotion, eating, positive and negative social interactions, maintenance behaviour, and vocalizations) per paddock 1 (A) and paddock 2 (B) during all outings of the trial, and for outings immediately before (C), during (D), and after (E), the introduction of a new group of cows (includes both paddocks).*

## 5.5 Discussion

We predicted that go-out trip durations would not differ throughout the weeks, as groups were taken out individually and group size was relatively consistent. The cows' go-out trip durations were averaged using multiple outings within each week, so the addition of a new group could have been masked within these averages as both old and new groups durations were averaged together, resulting in fairly consistent outing times. If there was an effect of the new group, the average trip duration did not increase regardless (meaning the cows did not slow down over time) which suggest cows' motivation to go-out remained similar even with repeated exposure to the outdoors (Aigueperse et al. 2023). Additionally, the short trip durations (four to sixteen seconds per cow) suggest a quickened pace to go out, even for new groups. However, the trips going out and in could not be compared due to individual groups going out, but multiple groups from each paddock coming in, so motivation to go out vs. return to the home environment could not be conclusively extrapolated. This methodology mimics commercial settings though, so is more applicable to scaling-up for any herd size.

The same prediction of no weekly differences in go-out trip was applied to number of cows and handlers present, so it is rational to see no changes between trips. However, where we expected and did see differences was on the go-in trip. Due to the nature of the outings and for logistical reasons, the farm protocol established for the trip going back into the barn was to take cows in by paddock instead of by group. All groups in paddock 1 (groups one to three) or paddock 2 (groups four to seven), were therefore taken in together. As a new group was added each week, the number of cows in the paddocks increased, such that the first four weeks included significantly less cows on average than the last two weeks (i.e. the weeks when the entire herd was being taken out). What was unexpected was that the trip durations only had an increase between weeks two and five for trip go-in, where it was originally predicted that trip durations would increase as more cows per paddock were being taken inside. Week five had a higher number of animals turning around to go back to the paddock during the go-in trip (based on personal observation), which either required further intervention from the handlers (causing increase in interventions) or was due to increased interventions from handlers causing cows to want to turn around, which may have caused this trip duration increase. This could suggest that the motivation of the cows was affected by handler intervention, specifically in week five (Aigueperse et al. 2023).

The number of handlers did not change for either trip, suggesting that staff were just as capable of implementing outdoor access for a larger group size as a smaller group size, with no additional hands required. As well, even with a growing group size for go-in trips, number of handlers required for the procedure remained constant. For scaling-up proposed to commercial settings, this determined that whether the group size is ten (like in our first week) or forty (like in our last week), that if implemented incrementally, the number of handlers necessary to take out either group size does not increase even if number of cows does. In a survey done by Smid et al. in 2021, ease of management was one of the reasons why Canadian dairy producers did not provide outdoor access. However, our study demonstrated that a small number of staff could adapt to an increasing group size without needing additional help or time. This result may help to mitigate worries related to labour intensiveness of outdoor access implementation.

Having fewer handlers and low interventions can also act as low-stress handling, which has been recommended for ease of moving cows (Tirloni et al. 2013; Breuer et al. 2003; Lindahl et al. 2016). There was no increase in go or stop interventions over time, and in fact the amount of stop behaviours decreased after the first week. The go behaviours did not change (with the exception of week five for go behaviour, which as previously stated, may have caused staff members to increase interventions to get cows to turn around), which may suggest that cows' motivation remained consistent over time as the staff did not need to further encourage the cows to move (Lange et al. 2021). As staff may feel more uncertain in the first week of implementation, it may explain why the number of stop interventions was higher. As staff became more confident in what to expect, these additional interventions stabilized. It was previously identified that repeated hands-on experience with cattle improved handling ease (Adams et al. 2019), so it is likely the same case here. The consistency in trip durations, staff members needed, and handlers interventions, across time illustrates that despite handling more animals during go-in trips, the amount of time and labour did not increase. Moreso, the decrease in stop interventions also shows the adaptation of cows and handler to going outside despite more animals being added to the process. This further supports the on-farm implementation recommendation that producers gradually introduce outings, as slowly introducing more animals over time minimizes the need for additional staff or extended outing durations.

Regarding cow behaviours exhibited whilst outside, the paddock or addition of a new group did not appear to affect the time budgets which were also similar to those of previous research, suggesting that even when the dynamics of the outdoor area were different or changing, cow behaviour was not impacted (Cellier et al. 2024). Cows have been found to adapt quickly to new procedures such as using automated milking machines or being regrouped with unfamiliar animals (Jacobs and Siegford 2012; Jensen 2018). Despite having new animals from the herd being added to the outdoor paddocks, this did not seem to disrupt the previous groups (e.g. no increase of fighting). It was predicted that adding new animals to the paddocks would increase social behaviours as cows become more familiar with the new animals or even fight to establish a dominance hierarchy (Jensen 2018). This lack of change, despite the new group, may suggests that cows' motivations to perform specific behaviours were not influenced by the addition of new animals and did not negatively impact the social dynamics.

Noticeably, the cows showed a proclivity for certain behaviours over others even in different paddocks or with new animals added. For example, they consistently spent more time eating compared to doing maintenance behaviour. This showed a relatively similar scope to time budgets of other studies that recorded behaviour during outdoor access in a variety of seasons and durations (Cellier et al. 2024, Shepley et al. 2020). The observation of cow behaviour in the paddock further corroborates previous findings for implementing routine outdoor access in increments as it did not appear to impact the cows' ability to adjust to new grouping whilst outside.

This study has demonstrated that slowly introducing outdoor access, whether that be over weeks, months, etc. is feasible, even for farms with fewer staff available, as cows and handlers adapt well and quickly after just a few outings. The whole process should be accompanied by a good strategy tailored to the on-farm environment and management, to ensure a safe and low-stress handling methods. Previous work has found that individual animals may display different motivational profiles when going outdoors, which can be influenced by handling (Aigueperse et al. 2023), so the feedback that cow behaviour and handler interventions may have on cow motivation as well as the ease of the outing procedure, should be adjusted to fit the needs of each farm. Although this study was conducted over eight weeks, producers can adapt the timeline to align with their specific daily routines, staffing number, and herd size, while still optimizing the benefits of incremental, low-stress handling techniques for outdoor access.

## 5.6 Conclusion

This study aimed to find practical methods for introducing outdoor access, where a living lab experiment successfully identified how larger groups and less handling interventions impacted motivation of cows and adaptability or animals and staff to going outside. Increasing group size during go-in trips did not affect trip duration or staffing requirements, indicating consistent cow motivation and manageable workload even with larger groups. Handler interventions to stop cows decreased after the first week. This suggests that handlers and cows were more confident with subsequent outings. The introduction of new cows did not affect the behaviour or motivation of existing groups, further demonstrating the adaptability of the animals to outdoor access. Thus, this living lab experiment has shown a real-world application that can be used as a guide for producers towards taking steps to implement outdoor access. This incremental methodology can be implemented by producers to introduce their herd to outdoor access without necessarily requiring additional staff members or time. As outdoor access becomes more readily adopted as an enrichment strategy for farm animals, the suggested outing procedures highlighted in our study can act as a first step in furthering Canadian dairy producers' mission towards best practices for animal welfare.

**Declaration of Competing Interest**

There are no competing interests to declare.

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# Chapter 6 - General Discussion and Conclusion

We, as caretakers and protectors of our non-human counterparts, have a responsibility to do our best by animals. Though our continued efforts have gradually shifted longstanding philosophies from considering animals as no more than mere machines (Hatfield, 2015), to accepting that animals feel pain and can suffer (Broom, 2011), it is still difficult to evaluate how our decisions impact animals when viewing their reactions from a human-centric perspective.

It is clear – from a human-based viewpoint – that not being able to act on our motivations can lead to negative consequences that affect our overall health. We are capable of complex speech which allows us to verbalize the emotions that come with this type of denial or disappointment. However, our awareness and understanding of what animals expect and desire is not as well developed because, in short, we do not speak the same language. Thus, we rely on behavioural cues to have even a glimpse into what animals might feel, as behaviour resulting from an emotional response cannot be concealed (Anderson and Adolphs, 2014). However, the link between behaviour and therefore, emotion, in response to current agricultural practices needed further exploration, which was evaluated using theoretical and practical applications on-farm.

The themes and studies presented in this thesis worked to address this research gap by first identifying the interrelationships between and methodology used to study animal motivation and anticipation (i.e. mechanisms that influence emotion), and frustration (i.e. a negative emotion that could adversely impact welfare). Chapter 2's scoping review found that previous literature not only lacked a universal definition of these three main concepts, of which this thesis has attempted to standardize definitions, but failed to fully investigate how these concepts relate to the use of an emerging practice in agriculture today – environmental enrichment. To promote good welfare standards, producers must continuously adapt to new rules and requirements. A

2020 survey found that older producers (20+ years of experience) were not as likely to use enrichment as younger producers (less than 20 years of experience) (Pierozan et al., 2020). This might be because more experienced producers do not see the benefit in changing their established practices, or share the common concern of having unexpected negative consequences to changing their daily routine. This is worrisome, as a 2021 census found that over 60% of Canadian producers were over the age of 55 ("Canada's 2021 Census of Agriculture", 2022). So, strong evidence of animal motivation for enrichment was needed, as producers may be more inclined to consider enrichment if it was shown that it does not negatively impact, and more importantly can positively impact, their animals or their management. Further, producers may spend time and money implementing enrichment, so it was vital to demonstrate its value to continue promoting its use.

Additionally, in the comprehensive review in Chapter 2, the effectiveness of multi-modal enrichment related to these concepts was not an objective of any of the included articles. This was of interest as enrichment that does not enhance farm animal welfare through fulfilling motivational needs does not serve its purpose, where using multiple types of enrichment may be more appropriate for addressing the behavioural needs of the animal (Botreau et al., 2023b). So, the scoping review revealed multiple missing connections that served as a basis for our overarching research question which was: "How does multi-modal enrichment impact motivation of farm animals?".

What was evident in the included articles of the comprehensive review, was that past researchers all utilized behavioural measures to investigate emotion related to one of the three concepts. This result prompted the studies completed in Chapters 3, 4, and 5, to use behavioural responses to make conclusions on how animals react to and show motivation for enrichment. A variety of behaviour was used as multiple ages, species, and contexts, were explored. This was done with the intention of understanding the impact on motivation within and across species and tests, which was missing from the included articles of the review. Widening our scope also allowed for elaborating on the results, and therefore benefits, of enrichment for different farm animals and in multiple contexts.

Though the scoping review was ideal for providing answers about gaps in the literature, there were some limitations. First, one limitation of the review was that it was expanded to cover all animals as preliminary searches returned limited number of studies on these concepts in sheep or cattle, our target species. This was useful for gaining general knowledge on animal motivation, anticipation, and frustration, but also meant that the results and specifically, methodology used, varied to a greater extent. Further, where overlaps did occur, such as in the tests used to study the main concepts, the interpretability of these tests could have been clearer by just using behaviour itself. Behaviour is the most direct way of measuring emotion as the fight, flight, or freeze, response cannot be concealed by the animal (Anderson and Adolphs, 2014). Though interpretation of behaviour can vary between observers, a well-defined ethogram can help to reduce intra/inter-observer variability, and behavioural measures can be less invasive, faster, and easier to record than physiological measures. Behavioural measures were identified as being the standard variable(s) of interest in the selected articles, but the mixture of species and contexts could not inform what specific behaviours were best to use for each animal or situation. Hence, why our following studies used a diversity of behavioural measures, as explained previously, since the comprehensive review did not include similar studies that could be used as a basis for replication and rather acted as foundation for which our research questions were generated.

Another limitation, not in completing the review, but of the applicability of its findings to our other Chapters, was that a key result of the review could not be directly accomplished in this thesis. In the review, it was suggested that future work develop a framework for using all three concepts of motivation, anticipation, and frustration, in conjunction. Though motivation was used to make interpretations about expectations and emotion, and frustration behaviour was found in Chapter 4 for example, measures of anticipation were not included. One possibility, which would be suggested for future work, would have been to record the sheep before the beginning of the maze tests in Chapter 3 or record the cows before going outside in Chapters 4 and 5, to evaluate anticipation and its relation to the other two concepts. Further, in Chapter 4, the animals being left inside whilst the other group went out, could've been recorded for frustration. It is proposed that future researchers either create a procedure to employ measures of all three concepts in one test or design a new test that inherently measures all three. This may also act to reduce previously discussed issues related to having an overabundance of measures to choose from for the same test (particularly when being used for the same species).

In Chapter 3, it was demonstrated that multi-modal enrichment may not specifically impact the learning or behavioural flexibility of lambs. Although cognitive enrichment such as puzzles were purposely excluded to understand if other types of enrichment were enough to influence cognitive abilities, it would be suggested to target these abilities using cognitive enrichment for future work looking to investigate cognitive functioning (Lee et al., 2006). Despite this, the multi-modal enrichment was still enough to positively improve reactivity of lambs to being isolated in the maze. Sheep are prey species who prefer to flock together, and it has been found that lambs are more likely than older sheep to find social isolation stressful due to being at higher risk of predation (Dwyer, 2004). If producers can positively influence lambs ability to cope with stressful situations such as being separated from their mother or conspecifics, this is useful for practices such as weaning or routine vaccination which animals will inevitably experience (Luo et al., 2020). Improving reactivity could make these practices easier for producers and could alleviate discomfort of the animals involved (Atkinson et al., 2022; Viérin and Bouissou, 2003). So, the use of multiple types of enrichment at an early age is recommended as it could positively improve animal welfare (Abou-Ismail, 2011; Strappini et al., 2021). One possible limitation to this interpretation is that the artificial nature of the maze may limit ecological validity. Lambs' behaviour in a test maze may not fully represent their reactions in real world application. Further, motivation for the reward could not be fully distinguished from motivation to end the test and be reunited with conspecifics in the home-pen. However, as this chapter aimed to investigate the effects of enrichment on motivation outside the home environment, rather than to assess reactivity to isolation or the specific goals of lambs within the test maze, interpretations were proposed cautiously with the hope of highlighting the potential benefits of enrichment on motivation and to encourage its adoption in production systems. The following two chapters took this a step further to show that animals were strongly motivated for enrichment and quickly adapted to having access to it.

In Chapter 4, it was found that cows were more motivated to go outside than to return to the barn regardless of frequency of outing, but that external factors such as handling may impact motivation for enrichment. This study acted as a first step for investigating how frequency of enrichment impacts the outing process as producers may be more inclined to start with fewer outings due to factors such as weather, infrastructure, staff availability, or other (Smid et al., 2021). It was important to demonstrate that even with only one outing a week, cows were excited to go outside (shown through behaviours such as fast trip speeds, jumping, etc.), which verified their motivation for having the opportunity to be outside of their individual barn stalls. It is well understood that cows place value on having outdoor access (Aigueperse et al., 2023; Loberg et al., 2004; Shepley et al., 2016; Shepley et al., 2017; Von Keyserlingk et al., 2017), but Chapter 4 confirmed that this desire to go outside is present even when rarely afforded the opportunity to go out. As the comprehensive review (Chapter 2) revealed that denying experiences that animals are motivated for could lead to negative emotion and thus poor mental welfare, this study illustrates to producers the significance of allowing cows to go out, even if they start at lower frequencies.

Additionally, putting greater restrictions, not just on where animals can go, but on what they do, impacted their behavioural response. Aigueperse et al. in 2021 found that greater movement restrictions may have been negatively perceived by the cows, which was similarly found in Chapter 4. Cows performed frustrated behaviour when more intrusive interventions were placed on them, which producers need to consider if implementing outdoor access as this not only impacts their herd, but could affect their staff's safety or their human-animal relationship with the cows (depending on how aggressively the animals react). Low-stress handling (such as using a flag) reduces stress and could prevent aggressive reactions towards handlers (Tirloni et al., in 2013). Therefore, handling techniques and frequency of intervention should be carefully considered before implementing outdoor access.

Limitations of Chapter 4 included paddock set-up as there was less space for the cows to perform natural behaviours, less chance to socialize with other animals, and less resources available as some paddocks had less grass than others (although the rotation of groups in paddocks attempted to compensate for this) and no feed or water was provided whilst outside. So, the outing area could not mimic as closely a realistic paddock set-up to that of a pasture

(Beaver et al. 2021). Although this did not appear to affect the cows' behaviours or motivation for the outdoor area, we attempted to rectify these issues in Chapter 5 where the full extent of the paddock areas were provided to larger groups.

Another possible factor that could limit the interpretations of Chapter 4's results were the humans themselves. As each handler  has a different level of experience and thus, comfortability with handling large animals like cows, this may have impacted certain individuals likeliness to react with more restrictive interventions. The handler behaviours were considered as a group, so this effect would be masked, but future work could look at individual handler differences in reaction and personality during outing, as previous work has found that attitudes of handlers could also impact animals' response to that person (Napolitano et al. 2020; des Roches et al. 2016; Hemsworth 2003). So, it is possible that one handler is more likely to affect a cows' motivation to go out than another.

Another restriction related to personality was the interpretation of cattle group behaviour towards outdoor access. As animals themselves have unique personalities, there may be hidden factors that influence an individual cows' motivation for going outside such as reaction to handling, ability to cope with environmental change, or group dynamics (Finkemeier et al., 2018; Kaiser and Müller 2021; Marino and Allen 2017; Neave et al., 2020). A single cow was randomly selected from each group for each trip and considered representative of the behaviour of the entire group. However, this approach may limit the ability to draw valid conclusions about group-level behaviors, as individual differences could have influenced the data, potentially masking broader trends. Future research and producers themselves are suggested to consider how personality may affect motivation for outdoor access. Individual motivation could be taken into consideration when deciding which groups to bring out together or even which paddocks to house certain animals in, based on their personality (Foris et al. 2018).

To elaborate on Chapter 4's findings, Chapter 5 utilized the innovative approach of a living lab experiment to test the application of outdoor access in a real-world setting. Where the previous chapters focused on the theoretical and contextual effects and responses to enrichment, this chapter was essential for bridging the gap between theory and practice by allowing examination of how principles established in earlier research could be adapted to real farm environments. This approach mimicked the dynamic conditions producers face, particularly when considering the integration of outdoor access into their operations. It is not often that researchers are able to directly co-create and observe their theories applied in a real-world setting in the same way that this study was conducted. This type of experiment gave an insight into the potential obstacles that a producer might face as well as the potential solutions to overcome them. Collaborating with the staff at Macdonald campus, a robust and scalable methodology was developed. This adaptability ensured that the practices tested could be applied to farms of varying sizes and resource levels, making the findings broadly applicable.

A significant outcome was the observation that the increase in number of cows had no major effect on the number of handlers needed or the trip duration. This result is noteworthy as it directly addresses producer concerns by highlighting that even farms with less handlers available and/or larger herds can still employ these techniques without concern about ease of management or herd size (i.e. reasons not to provide outdoor access as indicated by producers in a 2021 survey by Smid et al.). As well, the consistency in go interventions and reduction in stop interventions corroborates the idea that cows do not require intensive handling to be able to understand the outing process. Chapter 4 had already demonstrated the impact of frequent and/or invasive restrictions, so with that in mind, this chapter utilized low-stress handling techniques to avoid the same consequences.

Despite its strength in application-based techniques, Chapter 5 also faced limitations. One drawback was not being able to directly identify motivation, as the trips going in and out were not uniform (due to the nature of individual groups going out, but all groups in a paddock going in together), so the trip durations could not be compared. Consequently, the study could not definitively extrapolate motivation for outdoor access versus returning to the barn, as was done in Chapter 4. However, as this study was not designed to clearly differentiate between motivation for outdoor access versus returning to the barn, and as the methodology was meant to mimic commercial procedures, alternative factors were considered to interpret the motivation such as the number of go interventions needed to move encourage cows outside, which did not change over time. So, cows motivation seemingly remained the same throughout the study. Even the time budgets revealed that adding new groups and having different features within the paddocks did not change the behaviour performed by the cows, again emphasizing that motivated behaviour was not so easily altered by details such as being mixed with new cows.

Additionally, a main concern for producers considering outdoor access is the implication on production aspects. Studies have investigated outdoor access on lying time (ex. Hernandez-Mendo et al. 2007; Cai et al. 2024; O'Driscoll et al. 2015, etc.), on milk production (ex. Loberg et al. 2004; Huricha et al. 2024; Dechow et al. 2011, etc.), on lameness and other health measures (ex. Palacio et al. 2023; Nejati et al. 2024; Mee & Boyle 2020, etc.). However, these studies are not often conducted in commercial settings using similar parameters as the living lab study in Chapter 5 (i.e. entire herd with less handling), which could make their interpretations difficult to extract for real world comparison. Additionally, Chapters 4 and 5 did not record behaviour while cows were in their stalls in the barn, missing the opportunity to evaluate how outdoor access might influence daily behavioural metrics relevant to production. Data such as calving interval, milk quantity (L), milk fat (kg), milk protein (kg), somatic cell count (SCC) and somatic cell score (SCS), were available for use in Chapter 5, however due to the scope of the thesis, this data was not analyzed. So, although Chapter 5 was limited in being able to address physiological parameters, it would be suggested that future work attempt to combine these measures with the results of the behavioural data. This could answer to the impact of outdoor access implementation on production and also potentially corroborate the benefits of outdoor access on dairy cattle, further encouraging producers to adopt this practice.

By tackling real-world challenges, Chapter 5 validated key findings from previous chapters whilst also providing a practical roadmap for producers seeking to implement outdoor access. Producers in Smid et al.'s 2021 survey stated that they were not aware of the motivation studies conducted on outdoor access, despite multiple studies being carried out over the last several years (Aigueperse et al. 2023; Charlton et al. 2013; Legrand et al. 2009; Shepley et al. 2016; Shepley et al. 2017; Von Keyserlingk et al. 2017). As previous studies were still theoretical in nature, this chapter may aid in disseminating working methodologies for producers through true to life applications that meet the upcoming requirements of the Canadian Dairy Code of Practice (NFACC 2023). This research highlights the potential for scalable, low-stress, and efficient enrichment implementation practices that align with both animal welfare priorities and operational feasibility.

Overall, the chapters presented in this thesis worked together to produce results that were important for providing answers related to animal motivation, as well as for producers wishing to enrich and enhance the lives of their livestock through the incorporation of multi-modal enrichment. This research has provided a comprehensive exploration of enrichment practices and animal emotion by ways of a scoping literature review in Chapter 2, detailed effects and responses to enrichment in controlled testing environments in Chapters 3 and 4, and demonstrating the applicability of these insights through a living lab experiment in Chapter 5. By addressing gaps related to how motivation is defined and measured, to how enrichment impacts motivation, and consequently how motivated animals are to have access to said enrichment, the work outlined in this thesis will become a stepping stone for future research into these overarching concepts.

In conclusion, the central aim of this thesis was to identify ways to improve the emotional welfare of farm animals through applying enrichments that positively impact animal motivation. The comprehensive review, along with three on-farm studies, presented new perspectives on how enrichment impacts animal's behavioural responses, as well as how enrichment can be applied to maximize emotional welfare. It was found that enrichment can improve reactivity to isolation and that animals are motivated to have access to enrichment, even when it is provided at lower frequencies, demonstrating its inherent value. Additionally, animals and handlers adapt quickly to enrichment implementation, particularly when applied gradually over time. Though future study is needed on how to take individual motivational profiles into consideration, and of the relationship of enrichment on emotion, these findings positively contribute to our knowledge of animal motivation and offers guidance for producers wishing to implement enrichment into their daily practice to reap the benefits it can provide their farm animals.

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