How does inter-individual variation affect group level behaviour?

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Abstract

No two animals are the same. Individual differences in personality, memory or internal state may lead two different animals to make different choices or show different behavioural phenotypes. These inter-individual differences are key to understanding the life history strategies animals have adopted to adapt to their environments. However, despite the importance of inter-individual differences to our understanding of animal behaviour, there are still significant gaps in our knowledge of how inter-individual differences may affect group level behaviours. In this thesis I therefore aimed to determine how inter-individual differences in personality or memory may affect group level behaviour in social and subsocial invertebrates. In this thesis I describe lab-based behavioural trials on social and subsocial model systems (*Temnothorax albipennis* and *Oniscus asellus* respectively) to empirically test whether differences in inter-individual memories or personality affects group level decision-making and stability. I then further investigated the possible mechanisms behind our findings using agent-based modelling. The thesis shows that differences in both personality and memory played an important role in the emergence of group level behaviours and suggested that greater integration of the fields of animal personality and collective behaviour could greatly benefit our understanding of animal behaviour. I also explored the ethics and implications of carrying out animal behaviour studies. I suggested that research into invertebrate personality has many possible benefits both through tangible conservation interventions, as well as benefitting our theoretical understanding of animal interactions. However, I also highlighted the importance of continued re-evaluation of the ethics of the methods used in invertebrate research in light of shifting research into invertebrate cognition and public perception. I hope this work will spark further work into the role which inter-individual differences may play in group level behaviours as well as further interest in exploring the ethics and implications of this types of work.

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Declaration

I declare that this thesis is a presentation of original work and my contribution is detailed below. This material has not previously been presented for an award at this, or any other, university. All sources are acknowledged as references.

Chapter I: Introduction

Written by Eleanor Drinkwater (ED) under the supervision of Elva Robinson (EJHR) and Jamie Wood (AJW)

Chapter II: How do inter-individual differences in behaviour affect aggregation stability?

The experiment was designed by ED under the supervision of EJHR with input from AJW. The blinded observations of woodlice behaviour were performed by Alexander Dodds, Herbie Garland, Marina Martin Maroto, Kathryn Moyes, Fay Bennet, Peter Danks, Daniel Russel, Katie Richie and Joseph Vaughan. Jenny Salloux scored the blinded video trial. ED carried out the unblinded video trials. Simon N Chapman provided advice on the use of AFT models.

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Chapter III: In a changeable environment, can individual memory help house-hunting ant colonies to make decisions about nest choice?

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Chapter IV: Consensus decision making: can individual personality affect group level personality: an agent based modelling approach

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Chapter V: New directions for research in invertebrate personality: applications to vertebrate translocation studies?

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Chapter I: Introduction

"I have called this principle, by which each slight variation, if useful, is preserved, by the term of Natural Selection." (Darwin, 1860)

I.I Introduction

By the principle of Natural Selection, selection can lead to the evolution of an optimum phenotype, allowing animals to thrive in an immense range of challenging and unexpected environments. From fish like the magadi talapia (*Alcolapia graham*) which are adapted to survive in high pH lakes which reach temperatures of up to 40˚C (Kavembe *et al.,* 2015), to birds like the ground tit (*Parus humilis*) which have adapted to the hypoxic conditions of the Tibetan plateau (Qu *et al.,* 2013), natural selection has allowed animals to adapt in and thrive in remarkable and diverse habitats (Kavembe *et al.,* 2015; Lan *et al.,* 2017; Yang *et al.,* 2016).

In addition to selection resulting in complex and varied physical phenotypes, selection has also resulted in a vast array of behavioural phenotypes. Complex behaviours including cultivation of fungus by leaf cutter ants (*Acromyrmex ambiguus*) (Saverschek & Roces, 2011), or optimum thermoregulation through tunnel construction by the Namibian desert spider (*Ariadna* spp.) (Mulder *et al.,* 2019), have allowed species to adapt to new niches and thrive in challenging environments.

With the advantages of behavioural adaptation, we might expect to see selection towards a single behavioural optimum for any given environmental niche, i.e. species exhibiting behavioural niche specialisation. However in reality a wide range of behaviours can be expressed within the same species in the same environment (Cole & Quinn, 2014; Cote *et al.,* 2011; Jandt *et al.,* 2014). Within one group of a single species, some individuals may show bolder behaviour with traits such as less fear of novel objects and more explorative behaviours than other conspecifics (Herborn *et al.,* 2010; Richardson *et al.,* 2017). Other individuals may show shyer behaviours with traits like taking longer to recover from disturbance or showing less explorative tendencies (Briffa & Greenaway, 2011; Hui & Pinter-Wollman, 2014; Magnhagen & Bunnefeld, 2009).

Inter-individual behavioural differences can arise from a variety of factors (Boogert *et al.,* 2014; McComb *et al.,* 2001; Ortigosa & Rowe, 2002). Variation in behaviour which is consistent over time and context is referred to as “personality” (reviewed by Wolf & Weissing, 2012). Personality can arise from a range of factors including genetics, long term memory and developmental conditions. In addition to long-term variation in personality there are other key factors which can lead to short term variation in behaviour including differences in internal state which could include hunger or disease (Kekäläinen *et al.,* 2014; Ortigosa & Rowe, 2002) or differences in short-term memories (Burns *et al.,* 2016; Stroeymeyt *et al.,* 2011). Behavioural variation resulting from these or other factors have implications for the short and long term behavioural strategies adopted by individuals (Aplin *et al.,* 2013; Ortigosa & Rowe, 2002).

In social animals, variation in memories also plays a crucial role in group-level behaviour and survival (Brown & Irving, 2014; Modlmeier *et al.,* 2012). Matriarchs in killer whale (*Orcinus orca*) pods, for example, have a key role in retaining memories of fishing grounds (Brent *et al.,* 2015) and can lead the pods to prime foraging opportunities. In guppies (*Poecilia reticulata*) on the other hand, individuals with a consistently lower tendency to explore can reduce the exploration of the group overall (Brown & Irving, 2014), which in turn may affect the foraging opportunities or predation risk for individuals within the group. These examples highlight how both variation in memory retention as well as variation in consistent inter-individual behaviour can have important implications for group level behaviours.

Despite the importance of understanding inter-individual variation to both individual (Kralj-Fišer & Schuett, 2014) and group survival (Jandt *et al.,* 2014), there are still many gaps in our knowledge in how inter-individual variation affects group dynamics. In this thesis I will focus on exploring how individuals affect group level behaviours. Specifically, I will focus on how variation in individual memories, and consistent inter-individual variation in behaviour affects group decision making. I will also explore the implications and ethics of this type of work. The reasons for focussing on each of these sections will be explored in more detail below.

I.II Personality and decision making

Individual animals within one species show consistent predispositions to different behavioural patterns, for example one individual in a group may be consistently bolder, and another individual shyer than other group members (Kralj-Fišer & Schuett, 2014; McDougall *et al.,* 2006). Behavioural bias can be adaptive or maladaptive depending on the context (Arnqvist & Henriksson, 1997; Quinn & Cresswell, 2005). Examples of behavioural traits being adaptive or maladaptive include aggression in fishing spiders (Sih *et al.,* 2004) and boldness in fish (Hulthén *et al.,* 2017). In fishing spiders high levels of aggression are beneficial during the juvenile stages of development as aggressive feeding strategies (including cannibalism of conspecifics) allow the juvenile to utilize more feeding opportunities, but in the adult stages highly aggressive females are likely to cannibalise males, thereby reducing mating opportunities (Arnqvist & Henriksson, 1997). In other animals bold behaviour may provide individuals with more opportunities to mate and access new resources; however, these bold behaviours may also lead to more risky behaviour and higher risks of predation (Hulthén *et al.,* 2017). In the common roach (*Rutilus rutilus*), for example, boldness in captivity directly predicts probability of predation in the wild, with bolder individuals being more likely to be predated (Hulthén *et al.,* 2017). In these cases, a behavioural predisposition may have an advantage in one context but be maladaptive in another.

In addition to the importance of behavioural predisposition being beneficial or maladaptive in different environments, it may be the case that there are differing strategies which are effective in the same environment (Both *et al.,* 2005). In great tits (*Parus major*) both very slow exploring (shy) and very fast exploring (bold) pairs of birds are successful at raising chicks (Both *et al.,* 2005). Bold birds are more successful at securing better territories so have an advantage in provisioning the nest over shy birds with worse territories, while shy birds are more effective at adapting to changing foraging opportunities, so are better able to provision their offspring in a changing environment (Both *et al.,* 2005). Given the variability of many environments, a variety of different strategies may be equally successful as shown by the success of bold and shy pairs of *P.major.*

1.2.1 Drivers and modulators of inter-individual personality

According to Tinbergen's framework of "the four whys" (Tinbergen, 1963), one of the four fundamental problems which ethologist must address is, how has a behaviour developed through the course of an animal's life? For understanding how inter-individual variation developed through the course of an animals life, we must explore a range of elements from innate factors like genetics (Dingemanse, 2002) to external factors like developmental conditions (Aspaas *et al.,* 2016; Boogert *et al.,* 2014)

The genetics of an individual is likely to play an important role in determining variation in inter-individual behaviour (Dingemanse, 2002; Van Oers *et al.,* 2004). The genetic component of behavioural variation in animals, has been shown through studies in birds in which the explorative tendencies of offspring can be predicted by the exploration or risk taking behaviours of the parents (Dingemanse, 2002; Van Oers *et al.,* 2004). Work in big horn sheep has gone even further than hereditary studies, and has mapped two quantitative trait loci to two different areas on the big horn sheep genome which are likely to be linked to bold or shy behaviours (Poissant *et al.,* 2013). Both of these studies demonstrate that while genetics are not the only element which determines inter-individual variation, some aspects of behavioural variation are likely to have a genetic basis.

Developmental conditions have also been shown to be important to the development of behavioural variation (Boogert *et al.,* 2014). For example, zebra finches (*Taeniopygia guttata*) show consistent behavioural differences in social behaviours between chicks fed the stress hormone corticosterone during development, compared to control chicks (Boogert *et al.,* 2014). In European lobsters (*Homarus gammarus*) individuals reared in an enriched environment or an empty container later showed differences in a range of behaviours from shelter use to aggression (Aspaas *et al.,* 2016). Difference in behavioural variation in response to developmental conditions could be a form of behavioural plasticity, allowing long term adaptation to different environments at the level of an individual. Selection could therefore be acting on the responsiveness of individuals to cues during development, but this needs to be investigated further.

Immediate social environment can also modulate the behaviours of a focal individual. In mosquitofish (*Gambusia holbrooki*), individuals in larger groups explore more than smaller groups (Ward, 2012), while ravens (*Corvus corax*) change their approaches to novel objects depending on the social environment (Stöwe *et al.,* 2006). Ravens show consistent variation to in approach time to a novel object when tested alone, but in a social environment the relative speed of approach is be modulated by the immediate social context of the trial (Stöwe *et al.,* 2006). Dominant male birds for example would approach the novel item first when in a pair with a female, but not a male (Stöwe *et al.,* 2006). Examples from both mosquitofish and ravens show how a social environment may have a modulating influence on expression of behaviours irrespective of underlying behavioural variation.

There is still much to be learned about the mechanisms behind variation in animal behaviour, but it is likely that the observed differences are due to a combination of innate factors like genetics and set factors like developmental conditions (Aspaas *et al.,* 2016; Boogert *et al.,* 2014), then modulated by factors like parasite load (Kekäläinen *et al.,* 2014), recent experience (Franks *et al.,* 2007; Stroeymeyt, 2011a) and immediate social environment (Stöwe *et al.,* 2006).

I.III Terminology and controversy of animal behavioural variation

Consistent behavioural variation between individuals can be explored in different ways. The number of different ways in which behavioural variation can be studied, coupled with a lack of universally adopted definitions for the field has led to inconsistencies and controversy about terminology in this field (Beekman & Jordan, 2017).

One of the more controversial terms used to describe consistent variation in behaviour is 'animal personality' (Beekman & Jordan, 2017). The term personality is widely used (Magnhagen & Bunnefeld, 2009; Barber & Dingemanse, 2010; Modlmeier *et al.,* 2012; Udino *et al.,* 2016; Wexler *et al.,* 2016), but has been criticized as an unneeded buzz-word due to terminology already being used to describe consistent variation in separate fields (Beekman & Jordan, 2017). The terminology of personality can be justified however, as an accessible term which can be used across many different taxa facilitating an exciting cross-pollination of ideas about variation across fields which previously may have used different terminology.

In this thesis, for consistency, behavioural variation which shows consistency across time and multiple contexts, for example an animal being consistent in the way they explore and response to a novel object over time, will be referred to as personality (Biro & Stamps, 2008). Consistency over a single context however, like the exploration tendencies of an individual in a certain environment, will be referred to as consistent inter-individual variation.

I.IV Frameworks for understanding the evolution of variation in animal behaviour

Another problem identified in Tinbergen's framework of "the four whys"(Tinbergen, 1963), is the question; how did a behaviour evolve over the history of a species? For those studying personality, this question is still a central question within the field.

There are several hypotheses to explain why personality within a population may have evolved (Bergmüller & Taborsky, 2010; Cole & Quinn, 2014).

One key hypothesis is the pace of life hypothesis (Réale *et al.,* 2010). In the pace of life framework it has been suggested that both shy and bold behavioural strategies are selected for in a single population (Cole & Quinn, 2014). Bold individuals may be more likely to take risks which could have benefits, for example in utilizing novel food sources, or foraging in places where more shyer individuals may avoid; this risky life-history strategy could be highly productive in the short term but could lead to increased mortality in bolder individuals (Cole & Quinn, 2014; Blight *et al.,* 2016). Shyer individuals, on the other hand, may show more reluctance to forage in potentially risky environments or try novel food sources, which may mean they miss out on valuable opportunities, but in the longer term may be less likely to be predated or poisoned, and therefore have a higher long-term productivity (Cole & Quinn, 2014). Both strategies have benefits in different environments, therefore in a changeable environment both strategies could be simultaneously selected for.

The pace of life hypothesis is a key hypothesis to explain the role of behavioural variation; however, there are additional drivers which could also have a role in the evolution of variation in animal behaviour. One hypothesis which could provide additional insights into the role of behavioural variation is the social niche hypothesis where inter-individual conflict could be reduced by different individuals in a group adopting different social strategies (Bergmüller & Taborsky, 2010), and that consistent behavioural variation could be an effective way of achieving this. Overall, selection for more extreme social niche adaptation, driven by inter-individual conflict, could result in the evolution of consistent differences in behaviour in a population; however this is likely to occur in a system-specific manner, reflecting the social structure of the species.

I.V Behavioural variation in social groups

As well as its importance in affecting individual survival and reproductive success, individual behavioural variation has an increasingly recognized role in group level behaviour and group level decision-making (Aplin *et al.,* 2014; Blight *et al.,* 2016; Delgado *et al.,* 2018; Réale *et al.,* 2007; Scharf *et al.,* 2012). In great tits (*Parus major*) and three-spined sticklebacks (*Gasterosteus aculeatus*), individual personality has been linked to social network position (Pike *et al.,* 2008; Aplin *et al.,* 2013), with bolder individuals occupying more central network positions (Pike *et al.,* 2008), and playing a key role in connecting more social groups that are separate (Aplin *et al.,* 2013). Shyer individuals are found to have fewer but often stronger social bonds. These studies highlight how individuals with different personalities may occupy different positions in a group, this diversity of personality in a group may have implications for group level behaviour.

In most types of social structure, selection happens at the level of the individual. However, while selection occurs at the level of the individual, the social environment can lead to selection for certain phenotypes to fit particular social niches within that social structure (Bergmüller & Taborsky, 2010). The exception to this are eusocial societies, in these societies since the colony is the reproductive unit, which means selection occurs at the level of the group. In eusocial societies more behavioural variation at the individual level has been linked to the group being more successful and productive (Blight *et al.,* 2016; Modlmeier *et al.,* 2012).

Variation of personality in a group can lead to beneficial emergent processes in allowing the group to behave flexibly in different environments (Michelena *et al.,* 2010). For example, in herd animals bold individuals may explore new food patches when grazed patches become crowded, while shy individuals are more likely to forage with other group members, this combination of shy and bold individuals therefore prevents over-crowding of feeding sites, while still ensuring group cohesion (Michelena *et al.,* 2010). If emergent processes lead to better foraging opportunities, there could be selection on individuals to be more likely to choose groups with higher diversity. Work on three-spined sticklebacks*,* (*Gasterosteus aculeatus*) has shown individuals do make association decisions based on personality (Harcourt *et al.,* 2009), however, currently little is known about how diversity of personalities may affect the decision of animals to join or leave a group. More work is needed to understand how the emergent processes observed in groups with diverse personalities may affect group choice. However, while the relationship between group choice and personality is unclear, personality variation can lead to emergent group level behaviours which could benefit the group.

The role of behavioural variation has been explored in species with complex social networks and social bonds like great tits (*Parus major*) and blue tits (*Cyanistes caeruleus*) (Aplin *et al.,* 2013; Cole & Quinn, 2014), however, there are many other species and environments where simpler forms of social groupings which may lack complex social networks and individual recognition (Jeanson *et al.,* 2005). One of the most basic of such groupings are animal aggregations. Aggregations occur when individuals of one or more species are distributed in a habitat in a non-uniform manner (Broly *et al.,* 2013), this spatial grouping of animals is fundamental to the social behaviours and interactions which emerge from these groups.

I.VI Animal aggregation

Aggregations fall into two categories. Firstly, there are aggregations which depend on a shared attractant, like moths that are drawn to a light. Secondly there are aggregations in which inter-individual attraction is also at play (Broly *et al.,* 2013). In aggregations with inter-individual attraction in some species there may be complex social networks at play (Kendal *et al.,* 2015); however, in other species there may be inter-individual attraction, but limited evidence for individual recognition beyond broad dividers like species, group or nest-mate recognition (Lihoreau & Rivault, 2008).

Within animal aggregations, decisions by the group to behave in a particular way can be made in multiple ways. Cases where the whole group needs to decide on one decision or choice the decision are known as consensus decision making (Conradt *et al.,* 2009), examples of these include ants colonies having to decide which new nest site to relocate to (Pratt, 2005). Another type of group decision making is known as combined decision making (Conradt *et al.,* 2009), in this type of decision-making individuals can make decisions independently without having to come to consensus, like woodlice choosing to leave an aggregation (*pers. obs*). In both types of decision making, in groups which are too large for all the individuals to sense each other directly, individuals within the group will make decisions based on local information, leading to the overall group behaviour emerging in a self-organised way (Conradt & List, 2009).

I.VI.I Current models for aggregation/group level decision-making

While animal aggregations are important and widespread in nature, mathematically modelling aggregation behaviours can be challenging. Early studies on fish shoaling behaviour relied on an analytical modelling approach which treated fish shoals like a chemical lattice, where interaction between fish could be understood in the same way that the repulsion (Breder, 1954) and attraction between atoms in a lattice may be understood (Schellinck & White, 2011). A more recent example of an analytical model which models collective decision-making is the Group-Behaviour model developed by Conradt & Roper (2005) which also takes an analytical modelling approach to understanding modelling collective decision making. However, in this case it is used to determine the costs for group members if decisions are made one “leader” in the group, or by the group collectively. Their work suggests benefits for group decision making over decisions made by one leader alone (Conradt & Roper, 2005). In both of these models the analytical approach has the benefit of being clear and communicable and generalizable across systems (Grimm & Railsback, 2005), however despite these benefits there are also benefits of using other modelling approaches to understand collective behaviour (Grimm & Railsback, 2005).

An additional approach to investigating collective behaviours is agent-based modelling (Conradt & Roper, 2009; Pogson, 2016; Rands *et al.,* 2003). One example of an important agent based model which has provided key insights into collective behaviour is the Leader-Follower model (Rands *et al.,* 2003), this model suggests that during foraging individuals with more resources should act as followers, while individuals with less resources are likely to be more motivated to forage and therefore would lead foraging (Rands *et al.,* 2003). These findings are similar to those identified in another agent based model, (the Lead According to Need model), in which decisions are led by individuals with a greater “need” as well as those which place less importance in group cohesion (Conradt *et al.,* 2009). These models highlight the importance of asymmetry in “voting power” in aggregations. Within one aggregation individuals may (for a range of reasons) have a greater impact on their neighbouring individuals than others (Conradt, 2012).

Agent based models can be useful tools in understanding the link between individual behaviours and emergent group level behaviour (Oosten *et al.,* 2010; Pogson, 2016). One of the features of group living animals is that each of the animals will react to the surrounding environment (including the other animals in the group), and the other animals in the group in turn react to that animal as part of their own environment. This circular system of causation leads to the complex emergent behaviours seen in many systems (Grimm & Railsback, 2005). Agent based modelling allows this changeable interdependence of individuals in a group to be modelled (Grimm & Railsback, 2005) in order to investigate rules from which complex behaviours may emerge. By using the simple environment of an agent based model and simplified interacting agents, theoretical principles can be tested and compared to observations of living systems (Pratt *et al.,* 2005). Agent-based models do have limitations however, as while an agent-based model can determine whether an emergent behaviour can emerge from individual rules, there is the possibility that the same collective behaviour could emerge from a range of different starting rules, and the natural system may actually be acting by a different set of rules with the same eventual outcome. It is therefore challenging both to determine the starting conditions of a given model, as well as the level of detail needed by a model (Grimm *et al.,* 2005). Too simple a model could give too simplistic an outcome, however too complex a model could also be unhelpful given high computing requirements, and the possibility of losing sight of the larger questions though too many details (Grimm *et al.,* 2005). In modelling complexity a model should fall within the "Medawar zone" (Grimm *et al.,* 2005), an optimum point between overly complex models and overly simplistic models where the greatest amount of information can be learned, with the minimum computing power. However, identifying this optimum zone in modelling can be challenging (Grimm *et al.,* 2005).

One approach for finding the right resolution for modelling is pattern orientated modelling (Grimm *et al.,* 2005), where instead of modelling all the complexities of a system, particular patterns in behaviour are observed, and the model is built around these real life observations (Grimm *et al.,* 2005). The need for agent-based models to have detailed parameters based on observations is a second limitation of agent based models, as the significant amounts of data which are needed to accurately parametrise the model (Pratt *et al.,* 2005) can be challenging and time-consuming to collect, even when focussing on particular patterns in a system. Without accurate parameters the model is unlikely to act in a way which reflects a natural system. Given the need of agent-based models for high amounts of data for parametrization it is important to use a tractable study species or system for work involving agent-based models.

A suitable study system to collect sufficient data to develop of a model to investigate the role of behavioural variation in group level aggregation behaviour would need simple social structure, aggregation behaviours and be experimentally manipulatable. Woodlice fit all of these criteria due to their physiology and social behaviours, which are described in detail below.

I.VII Woodlouse natural history

Woodlice belong to the isopod sub-order Oniscidae. Oniscidae is an unusual clade as it is one of the only branches of crustaceans which has adapted fully to a terrestrial environment (Oliver & Meechan, 1993). This adaptation has required a range of both physiological (Oliver & Meechan, 1993) and behavioural adaptations to allow the animals to live on land and, importantly, avoid desiccation.

Species of woodlice display a range of social behaviours allowing individuals to survive in many different terrestrial environments. At one extreme are species like the desert dwelling *Hemilepistus reaumuri* woodlice which dig tunnels, form monogamous pair bonds, and live in family groups (Linsenmair, 1974). On the other side are species like the rough (*Porcellio scaber*), and shiny woodlice (*Oniscus asellus*) which form large mixed-species aggregations. While they do show attraction to other woodlice, these highly aggregative species do not appear to form social bonds and are not thought to display inter-individual recognition, though to our knowledge this has not been tested. There are multiple reasons why the shiny and rough woodlice form these large aggregations. Some of the key reasons are explored below.

I.VII.I Increased reproductive opportunities

In woodlice, like many other species, the increased mating opportunities could be an important factor behind aggregation (Parrish & Edelstein-Keshet, 1999). In addition to finding a mate, aggregation also has an impact on the reproductive state on the females in the aggregation. If aggregated in female only groups, females will have a higher rate of parturial moulting (which provides the female with pouch for her future offspring), than females kept singly. If co-housed with a male, the rate of parturial moulting is higher still (Broly *et al.,* 2013).

I.VII.II Coprophagy

Another benefit of group living is opportunities for coprophagy (consumption of faeces). Coprophagy is an important secondary food source for woodlice, without which woodlice show slowed growth patterns (Hassall & Rushton, 1982). Rapid growth is beneficial to woodlice as a majority of woodlouse mortality occurs while the individuals are juveniles (Broly *et al.,* 2013).

I.VII.III Predator defence

Aggregations could provide some protection against predators. Woodlice are known to be predated by a range of species including spiders, shrews and centipedes (Oliver & Meechan, 1993). While some species of spider are thought to be repelled by the tegumental glands of the woodlouse, spiders of the genus *Dysderia* (otherwise known as woodlouse spiders), are specialized in predating woodlice: they have specifically adapted jaws allowing efficient predation of this group (Oliver & Meechan, 1993). While there are no studies directly linking aggregation behaviours to reduced predation in woodlice, other study systems have shown an important role of aggregation in reducing predation (Brighton *et al.,* 2020; Van der Marel *et al.,* 2019) through the effects of dilution (Brighton *et al.,* 2020), confusion (Hogan *et al.,* 2017; Olson *et al.,* 2013) as well as better vigilance (Van der Marel *et al.,* 2019;

Ward *et al.,* 2011). In woodlice juveniles are at the greatest risk from predation (Broly *et al.,* 2013). Females have been hypothesized to synchronize their reproductive patterns via a mechanism of ecdysteroid ingestion through coprophagy (Broly *et al.,* 2013). This would result in a mass release of juveniles, potentially conferring anti-predator benefits (Broly *et al.,* 2013) to woodlice reproducing in aggregations.

I.VII.IIII Water retention

The final and most important driver of aggregation is water retention. Woodlice lack epicuticular lipids, making them very vulnerable to water loss (Broly *et al.,* 2013). By aggregating in a small space, like under a piece of bark, the shared humidity from the water loss from all individuals in the aggregation can rapidly increase the humidity of the microenvironment. This increased humidity in the immediate environment surrounding the aggregation can reduce further water loss from individuals within the aggregation, protecting them from desiccation.

The importance of hygroreception (ability to detect moisture or humidity) to woodlouse survival is shown by the redundancy of woodlice having hygroreceptors on both their large second antennae, as well as through the very diminished first antennae (Schumalfuss, 1998). The second antennae perform a range of crucial functions, including water regulation and olfactory sensing (Schumalfuss, 1998). The large second antennae however may be vulnerable to loss during predator attack, while the reduced first antennae are protected under the body of the woodlouse and are therefore less likely to be lost during predator attack. If the larger second antenna are lost the woodlouse can therefore rely on the first antenna to avoid desiccation until the second antennae can be regrown at the next moult (Schumalfuss, 1998). The two separate hygroreceptor organs likely reflects the importance of hygroreceptor and avoiding dehydration to woodlouse survival.

I.VII.V Justification for the study species

While worldwide there are around 900 species of woodlouse, in the UK the number is limited to 38 species (Oliver & Meechan, 1993). From these UK species we have chosen for this project the shiny woodlouse (*Oniscus asellus*). As well as being a large species (reaching up to 18mm) (Oliver & Meechan, 1993) and very common in the UK, this species is also a good model to study aggregation for multiple physiological reasons. In the wild this species is observed in both single and mixed species aggregations of up to hundreds of individuals (*pers obs*). A key driver of aggregation behaviours seen in *O. asellus* and other woodlouse species is the need to conserve water. In comparison to other UK species of woodlouse, *O. asellus* is particularly susceptible to desiccation. One of the unusual physiological features of *O. asellus* is the lack of pleopodal lungs (Wright & Ting, 2006). Species of woodlice with pleopodal lungs are typically less permeable than species without lungs (Wright & Ting, 2006), leading to the suggestion that pleopodal lungs reduced the need for permeability, therefore reducing water loss (Wright & Ting, 2006). In addition to a lack of pleopodal lungs, *O. asellus* is unable to draw water sequestered in tissues into the haemolymph (Holdich & Mayes, 1976), which could make them even more sensitive to water loss in comparison to other UK species which are able to draw water from their tissues. The propensity of *O. asellus* to lose water could in turn, make them highly motivated to aggregate in dry conditions in order to prevent desiccation. This high motivation to aggregate we expect will make them a good candidate to understand behavioural variation in the context of aggregation behaviour.

Overall, we argue that woodlice (particularly *O. asellus*) are a good model for understanding the link between inter-individual variation and group level decision-making. It is important that we continue to explore the role of inter-individual variation as despite the important implications this area of behaviour has to individual and group decision making, there are still many areas gaps in our knowledge about how inter-individual variation may affect the behaviour of a group. We will explore this in more detail in chapter II and III.

I.VIII Memory and collective behaviour

In addition to the role which consistent innate behavioural differences may have on individual decision making, memory is also likely to play an important role in determining behaviour at the level of the individual, and by extension the level of the group. Memory can be described as the acquisition, processing, retention and retrieval of information (Fagan *et al.,* 2013). Individuals may initially gain memory genetically, as seen in newly hatched Atlantic salmon (*Salmo salar L.*) which instantly recognise pike as a predator despite having no previous experience of pike and having never experienced predation attempts (Hawkins *et al.,* 2004). After birth, memories can also be gained through social learning, which can be seen in Blue Tits that can learn to avoid certain foraging opportunities from seeing conspecifics reacting to a distasteful foraging experience (Hämäläinen *et al.,* 2017). Additionally, memories can also be gained through the physical experiences of an individual, like the northern quoll *Dasyurus hallucatus,* which can be trained to avoid cane toads through contact with cane toad baits which have been laced with aversive chemicals (Fagan *et al.,* 2013; Indigo *et al.,* 2018).

Different types of information can be held in memories, including spatial information (information about location), and attribute information (information about attributes encountered which could be features like types of food available in a food patch or shelter quality) (Fagan *et al.,* 2013). Memory has many benefits including improving foraging accuracy and allowing individuals to re-find key areas; however there are also costs associated with memories, like the metabolic costs of laying down memories, the risks of gaining inaccurate memories through social learning or the risks of retaining outdated memories (Fagan *et al.,* 2013).

Individuals within a group may possess different memories which are likely to affect how they make decisions, and in turn will affect the decision making of the group (Brent *et al.,* 2015; McComb *et al.,* 2001). It is therefore important to consider variation in memories between individuals when considering group level behaviours (Czaczkes *et al.,* 2015; McComb *et al.,* 2001).

Different individuals within a group may have a disproportionate effect on group decision-making based on their knowledge and prior experiences (Brent *et al.,* 2015; McComb *et al.,* 2001). In social animals like hooded crows (*Corvus corone cornix*) and chimpanzees (*Pan troglodytes*), inexperienced animals may copy the behaviours of more experienced individuals (Kendal *et al.,* 2015; Sonerud *et al.,* 2001), which allow the inexperienced animals to solve foraging problems or find new foraging patches. In some cases like matriarch elephants (*Loxodonta africana*) (McComb *et al.,* 2001) and killer whales (*Orcinus orca*) (Brent *et al.,* 2015), a single long-lived individual could have a significantly greater knowledge base than other individuals, and therefore have a disproportionately strong role in the decision making and survival of the group.

Previously, cognitive capacity was thought to be linked to brain size with larger brains being capable of more complex cognition including learning and memory tasks; however work on invertebrate systems has called assumptions about brain size into question (Chittka & Niven, 2009; Mendl *et al.,* 2011; Mery, 2013). Model systems like *Caenorhabditis elegans* have demonstrated that even the nematode worms have the capacity to lay down memories linking a spatial area with attributes like food, while *Drosophila melanogaster* has been a model to study a range of learning and memory tasks including social learning (Thornquist & Crickmore, 2019) and associative learning (Iliadi *et al.,* 2017; Mery, 2013). In addition to these well-characterised model systems, other invertebrates have shown extraordinary abilities to learn and lay down memories: bees for example can remember how to carry out a socially learned task, then develop a better solution to the task than the taught solution (Loukola *et al.,* 2017).

Interestingly, the ability to lay down memories does differ between even closely related species (Mery, 2013). The closely related species parasitic wasps *Cotesia glomerata* and *Cotesia rubecula* for example differ greatly in their spatial memory, reflecting their prey distribution and likelihood of finding new prey at already visited sites (Mery, 2013; Smid *et al.,* 2007). The interspecies variation in how memory is used reflects the ecological niches and challenges faced by different species (Mery, 2013).

In eusocial invertebrates (which have the challenge of organising rapid collective decision making), memories are retained both internally at the level of the individual as well as in the form of an external signal (Czaczkes *et al.,* 2015; Dussutour *et al.,* 2009; Jones *et al.,* 2019). In ants, external memories can be laid down in the form of pheromone trails (Czaczkes *et al.,* 2015; Franks *et al.,* 2007), which are composed of chemical signal with different properties, allowing complex signals to be laid down (Czaczkes *et al.,* 2015; Dussutour *et al.,* 2009). The pheromone signals can act as attractants, which attract ants along certain routes or repellents where they can repel ants from following unprofitable routes (Robinson *et al.,* 2008). Also, both long- and short-lasting pheromones are laid down on foraging trails; the short-lasting pheromone recruits other ants strongly (Dussutour *et al.,* 2009) but are quickly degraded if the foraging source is depleted and the pheromone is no longer re-laid, which in turn reduces recruitment. Long-lasting pheromone, on the other, hand recruits other ants only weakly and acts as a long term external memory of the route as a previous site of feeding, which will then be occasionally checked by workers (Czaczkes *et al.,* 2015; Dussutour *et al.,* 2009; Jackson *et al.,* 2006).

Even with pheromone trails however, internal memories are still important to ants (Czaczkes *et al.,* 2015). Pheromone trails appear to help memory formation in ants, because on trails with pheromones ants make fewer errors and learn the route more quickly (Czaczkes *et al.,* 2015). In addition to facilitating learning, pheromone trails also complement individual memory (Czaczkes *et al.,* 2011). When internal memory is combined with trails, ants are more confident in the route and therefore appear to invest less time in route checking, leading to an increase in speed of up to 30% (Czaczkes *et al.,* 2011). Internal memories however, are often more accurate than the pheromone trails, so when there is a discrepancy between internal memory and pheromone signals ants will use their internal memories over the pheromone trails (Czaczkes *et al.,* 2011) unless environmental conditions prevent internal memories from being used (Jones *et al.,* 2019). When light levels are too low to use visual cues, for example, ants revert back to putting more importance on the trails than on internal memories (Jones *et al.,* 2019). The ability of ants to use external and internal memories is crucial to their ability to make consensus decisions (Czaczkes *et al.,* 2015; Jones *et al.,* 2019).

I.IX Temnothorax as a model organism

Temnothorax albipennis is a species of ant which is used as a model system to disentangle the link between individual behaviour and collective decision making (Dornhaus & Franks, 2006; Pratt *et al.,* 2005). *Temnothorax albipennis* lives in rock cavities, which are often lost or degraded due to weathering or disturbance, which means that *T. albipennis* has to be able to rapidly make consensus decisions about alternative shelters (Pratt, 2005), this propensity for decision making, coupled with the detailed research on inter-individual communication (Franks & Richardson, 2006) and individual decision making (Stroeymeyt *et al.,* 2011a) in this species , makes them a good model for collective decision making.

During collective decision making, *T. albipennis* show positive and negative bias in shelter choice when they are forced to emigrate to a new shelter (Franks *et al.,* 2007; Stroeymeyt *et al.,* 2011a; Stroeymeyt *et al.,* 2011b). These ants will show negative bias against shelters which they previously experienced to be poor quality: when forced to migrate they will preferentially move into poor shelters which are novel over equally poor shelters of which they had prior experience (Franks *et al.,* 2007). On the other hand, ants will also show positive bias towards familiar nearby shelters which are of good quality over equally good quality novel shelters (Stroeymeyt *et al.,* 2010; Stroeymeyt *et al.,* 2011a).

There are still significant gaps however in our understanding of the extent to which ants show bias when confronted with a changing environment. Previous studies have looked at the bias towards or against shelters which ants have previously visited (Burns *et al.,* 2016), however in a changeable environment the conditions of the previously visited shelters can change. It is unknown whether ants can update their memories if the conditions of nearby shelters change, or the nearby shelters are lost altogether. Understanding if ants can distinguish between these different types of degradation and show bias accordingly would provide valuable insights into the role of individual memory and collective behaviour. In this thesis we therefore aim to investigate how individually held memories (rather than shared pheromone memories) can lead to bias in collective decision making in a changing environment. We will explore this more in chapter IV.

I.X Understanding the ethical implications for behavioural studies on invertebrates

In any animal study it is important to consider the ethical implications for the work that is being done. In research on vertebrates there are clearly defined guidelines to help researchers ensure that work is carried out in line with accepted ethical standards (Schuppli *et al.,* 2004; Lindsjö *et al.,* 2016).

In research on invertebrates however, ethical standards and guidelines are far less developed. Unlike vertebrates, (with a few notable exceptions (Smith *et al.,* 2013; Fiorito *et al.,* 2015)) invertebrates are not covered under the animal protection legislation for research. This lack of regulation surrounding invertebrate use in research is largely due to the perception that invertebrates do not perceive pain.

Pain perception is challenging to determine, as it can be difficult to determine if the reaction of an animal is due or nociception or pain perception (Barr *et al.,* 2008). Nociception is the perception of tissue damage or noxious stimuli (Barr *et al.,* 2008) and may result in responses like a withdrawal reflex. The perception of pain, however, requires the perception of damage to be processed and responded to with a negative association (Sneddon, 2006). Markers may be used to try and detect pain perception, which include long term behavioural changes or learned avoidance behaviours (Sneddon, 2006). Despite historical assumptions that invertebrates lack the capacity to perceive pain, recent work has called these assumptions into question (Elwood, 2012).

Recent developments in our understanding of invertebrate cognition (Mendl *et al.,* 2011; Elwood, 2012; Loukola & Chittka, 2017) and increasing appreciation that these animals may have the capacity to experience suffering (Barr *et al.,* 2008) raise important ethical questions for work on invertebrates. In this thesis we therefore discuss these ethical questions in more detail in chapter VI, where we review the current state of ethical guidelines for the use of invertebrates and argue that there needs to be further discussion surrounding our development of ethics in invertebrates.

1.11 Untapped opportunities for the understanding of invertebrate behavioural variation

While it is important to carefully consider the ethics of work done on any animal, it is also important to consider the opportunities and benefits which could be gained by researching a system. The study of animal behavioural variation has not been immune to criticism, with some critics questioning how worthwhile the field of animal behavioural variation is to our understanding of animal behaviour (Beekman & Jordan, 2017; Jungwirth *et al.,* 2017). While there are some concerns surrounding repeatability, inconsistency of definitions and the design of certain experiments, which are valid concerns. However, overall there are many exciting opportunities presented by the study of animal behavioural variation, and more specifically invertebrate behavioural variation (Kralj-Fišer & Schuett, 2014).

The study of invertebrate behavioural variation can greatly contribute to our theoretical understanding of the role of behavioural variation in animal systems (Kralj-Fišer & Schuett, 2014). As well as providing tractable systems for testing theories about animal behavioural variation in a laboratory setting, many groups of invertebrates, while closely related, show different life history strategies (Powers & Avilés, 2007), for example different types of sociality (Powers & Avilés, 2007) or different stages of adaptation to aquatic and terrestrial environments (Warburg, 1968). This combination of extant diversity of extant species and strategies, and the tractability of many of these invertebrate system to their study in a laboratory system makes many ideal study species, which could contribute key insights into our theoretical understanding of the role of behavioural variation in animal behaviour.

In addition to the potential benefits of improving our theoretical understanding of animal behaviour, the study of invertebrate behavioural variation could also have applied benefits to animal conservation (McDougall *et al.,* 2006). In the past few years, conservation practitioners have been exploring ways in which understanding animal behavioural variation could improve conservation outcomes (Bremner-Harrison *et al.,* 2004; Martin-Wintle *et al.,* 2017; Merrick & Koprowski, 2017). However, this so far has been restricted mainly to studies of vertebrate study systems. There could be important untapped potential in the study of invertebrate behavioural variation to help improve conservation outcomes. We explore this in more detail in chapter V.

I.XII Summary of aims

Overall, in this thesis I set out to explore the role of behavioural variation and individual memory in invertebrates with different degrees of sociality. I will then consider the ethics and applications of this work and argue for greater discussion surrounding invertebrate ethics, and wider exploration into the practical implications and applications of understanding invertebrate behavioural variation.

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Chapter II: How do inter-individual differences in behaviour affect aggregation stability?

II.I Abstract

Understanding individual variation in behaviour is crucial to understanding emergence of group level behaviours. One important aspect of group behaviour is the stability of the group. Group stability can benefit animals living in aggregations; however, too much stability can be maladaptive and lead to problems like overgrazing or overcrowding. Group stability is often studied in the context of social ties, but animal aggregations do not require social ties to form or function. By understanding group stability in the absence of social ties we can determine the extent of the role of other elements of behaviour including individual behavioural variation. In this study we used the woodlouse Oniscus asellus *to experimentally test if the presence of more explorative or less explorative individuals in a group affected aggregation stability. To do this we assembled groups of eight woodlice which contained either explorative individuals, non-explorative individuals or a mixture of explorative and non-explorative individuals. We found that the groups which included explorative individuals took significantly less time to leave an aggregation site than groups of just non-explorative individuals, but found that there was no difference in the time taken to leave an aggregation site between groups which had a mixture of explorative and non-explorative individuals, and groups of just explorative individuals. Our results show that individual variation in behaviour has an important role in determining group stability and suggests that even a small number of explorative individuals can catalyze the break-up of an aggregation. This link between individual variation in behaviour and group stability highlights how small numbers of animals with a particular behavioural type can have disproportionate effects on the group even in the absence of social ties. We hope this work will encourage further work into understanding the link between individual and group level behaviours in the absence of strong social ties.*

II.II Introduction

Inter-individual variation, i.e. behavioural differences between individuals within a group, is an important component of group level behaviour (Aplin *et al.,* 2013; Michelena *et al*., 2009; Walton & Toth, 2016; Wray *et al*., 2011). Individual variation influences a wide range of behaviours including foraging (Kurvers *et al.,* 2010) and dissemination of information (Carter *et al*., 2014); however, while inter-individual behavioural variation is known to be important to group level behaviours, there are still significant gaps in our understanding of the role and mechanisms this variation may play in many group level behaviours (Bode *et al.*, 2011).

The ability of individual animals to form stable groups over an appropriate time frame can have implications for survival at the level of both the individual and the group (Hass & Valenzuela, 2002; Yagi & Hasegawa, 2011). Living in an aggregation can provide multiple benefits (Krause & Ruxton, 2002), affecting the probability of predation (Matsuda *et al.,* 2010; Marshall *et al.,* 2016), as well providing a protective microhabitat for individuals within the aggregation (Briffa & Greenaway, 2011; Lutermann *et al*., 2010; Stahlschmidt *et al*., 2011). Microhabitats which emerge from animal aggregations can enhance temperature regulation in the case of animals like emperor penguins or rabbit pups which huddle to conserve heat (Bautista *et al*., 2013; Gilbert *et al*., 2006), or the regulation of humidity in the case of animals like woodlice (Stahlschmidt *et al.,* 2011).

Despite benefits of aggregation, too much stability in a group may be maladaptive. In a changing environment, some flexibility to leave the aggregation and move to a better location can be beneficial (Michelena *et al*., 2010). In cases where patches of food are limited for example, too much stability in a group could lead to over-exploitation and competition (Sibbald *et al*., 2009). If groups contain some individuals which show less than average cohesive behaviour, these less cohesive individuals may be more likely to explore other food patches, which in turn could encourage other members of the group to also adopt these newly found food patches (Michelena *et al.,* 2010).

In terms of aggregation stability, the effects of consistent inter-individual variation can be considered in light of two collective behaviours, firstly the fragmentation of an aggregation as some individuals leave the original aggregation site (Michelena *et al.,* 2010), and then reaggregation of the group in a new site as the aggregation reforms in another location. In large groups this fragmentation and subsequent aggregation is likely to happen by a process of self-organization. Self-organization occurs when it is not possible for an individual to have a global overview of the group, and therefore individual decisions are made based on local physical and social conditions (Conradt & Roper, 2009).

One important social condition to which aggregation behaviours of many social animals can be linked is the existence of social ties, where individuals stay physically close to other individuals with whom they share strong social affiliation (Ling *et al.,* 2019). Bolder individuals may exhibit more social ties than shyer individuals (Aplin *et al.,* 2013); the different strengths of these ties associated with bolder or shyer individuals mean that some individuals have a bigger impact on group stability than others (Aplin *et al*., 2014; Hauschildt & Gerken, 2015). This inequality in impact that different individuals can have on a group (Conradt, 2012) is likely to affect group level behaviours.

Not all animals which display aggregative behaviour are known to form social ties or have social networks which impact behaviour (Jeanson *et al.,* 2005; Pogson, 2016; Wada-Katsumata *et al.,* 2015). In many species of woodlice, individuals aggregate (Broly *et al*., 2014; Devigne *et al*., 2011; Pogson, 2016) and show social attraction (Devigne *et al.,* 2011), but unlike many other social species, there is no evidence of social networks or preference for social partners in species of woodlice which form large aggregations.

Woodlice are a good study system to understand aggregation as many species are highly motivated to aggregate (Barnes *et al.*, 2015; Broly *et al*., 2013; Broly & Deneubourg, 2015; Broly *et al.,* 2014; Dias *et al.*, 2012). Aggregations are important for woodlice because they provide a microclimate with an artificially raised humidity, protecting the individuals within it from desiccation (Dias *et al.,* 2012). This aggregation behaviour is likely to have emerged as a key factor which allowed the aquatic ancestors of modern woodlice (Oniscidae) to adapt to living on land (Broly *et al.,* 2013). Among woodlice, the shiny woodlouse (*Oniscus asellus*) is particularly prone to desiccation (Dias *et al.,* 2012), and therefore may be expected to form aggregations even more readily than species less susceptible to desiccation, making it a particularly good system to study aggregation behaviours.

Woodlice exhibit consistent inter-individual variation in behaviour (Tuf *et al.,* 2015). In particular individual woodlice show consistent variation in their immobility responses to different threats including touching, pinch and water stimuli: the individual variation in tonic immobility responses is consistent over time and context (Tuf *et al.,* 2015), which are key hallmarks of consistent inter-individual variation in animals. We now have the opportunity to explore how consistent variation in inter-individual behaviour may impact group behaviour in the absence of stable social networks.

In this study we use the woodlouse *O. asellus* to investigate the influence that consistent inter-individual variation composition has on group level behaviours. Specifically, we hypothesize that the presence of exploratory individuals in a group will decrease the stability of an aggregation, and contribute to it breaking apart, while the presence of non-exploratory individuals will increase aggregation stability and contribute to it persisting. We also hypothesize that if the effects of explorative and non-explorative individuals are equal, then we would expect these effects to mitigate each other, resulting in mixed groups of explorative and non-explorative woodlice being behaviourally distinct from groups made of only explorative or non-explorative woodlice, and intermediate in aggregation stability.

II.III Materials and methods

Study species collection and maintenance

Over a thousand woodlice (*O. asellus*) were collected from the University of York campus (lat:53.9456, long:1.0579) and nearby surrounding woodland in the Autumn of 2019 and kept in the laboratory for at least 7 days prior to trials, allowing acclimatization to the laboratory 12-12 hour light-dark cycle.

Sets of 60-300 woodlice were collected throughout the experiment. Woodlice collected on the same day were housed together in 170mmx120mmx45mm holding boxes containing 100ml of set plaster of Paris which was initially dampened with 15ml of water. Each box also contained a sheet of absorbent paper (replaced when broken down), a tube of water stopped at one end with cotton wool, and a 25mmx25mmx8mm shelter. The woodlice were also provided with potato *ad lib.*, supplemented weekly. Water was added to each box as required to maintain humidity. Each housing box required a different volume of water to maintain high humidity because the housing boxes contained different numbers and sizes of woodlice due to different numbers and sizes of woodlice being collected on different days, as well as woodlice being removed from the housing boxes after their use in experiments. To control for the effects of using woodlice from different housing boxes, for each batch only woodlice from one housing box were used, therefore the treatments within a batch were from the same housing box. A batch is a group of 60 woodlice used in the first stage of the experiment.

Selecting woodlice for each trial

Random number generation was used when selecting experimental subjects to prevent bias towards selecting woodlice from particular areas of their housing box which could have different microclimatic conditions (for example areas nearer to the water tube would be damper). The woodlouse housing box was divided into 6 numbered segments. The R sample function (R Core Team, 2015) was used to generate random numbers between 1 and 6 (with replacement). These numbers dictated from which segment each woodlouse would be selected for a batch. Each batch comprised 60 woodlice placed into three 90mm diameter petri dishes of 20 woodlice each (sub-batches, see Figure 1). All woodlice in one batch were sourced from the same location, collected on the same day and had been previously housed in the same box.

Individual marking

To allow for individual identification, each woodlouse within each sub-batch of 20 woodlice (see Figure 1) was painted with a unique colour code using oil paints, applied with a cocktail stick.

Consistent inter-individual variation trials

Behavioural observations were carried out to assign woodlice to a bold or shy group. As shown in Figure 1, after painting, each sub-batch of 20 woodlice was placed in a fresh petri-dish (90mm x 15mm) (the test arena) with a 25mm by 25mm by 7mm shelter for 20 minutes to acclimatize to the test arena. Following acclimatization, woodlice were observed for 15 minutes. During the acclimatization woodlice could settle inside or outside the shelter. Woodlice were scored as “explorative” if they were seen to leave (the whole body emerged from shelter) or to enter (any part of the woodlouse excluding antennae enters the shelter) at least once during the observation period. This assay is a proxy for the woodlouse propensity to explore their environment. If they did not enter or leave the shelter during this time they were scored as “non-explorative”.

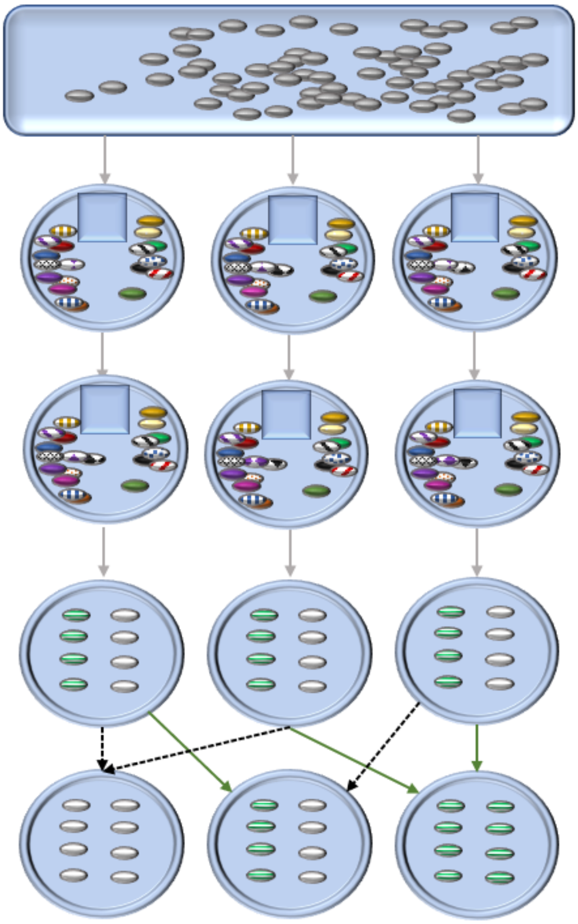
After the observation period, the sub-batch of woodlice were placed in a fresh petri-dish for 20 minutes. The petri dish also contained half a paper towel (115mm x 310mm) dampened with 2ml of water to prevent desiccation between trials. After this rest period, the same sub-batch of woodlice were placed back into the test petri-dish with the shelter to acclimatize for 20 minutes (the dish and shelter had been cleaned with 70% ethanol during the rest period). After acclimatization, the woodlice were observed a second time for 15 minutes and scored as explorative or non-explorative as previously described.

Choosing explorative or non-explorative woodlice

The results of the consistent inter-individual variation trials were used to identify individual woodlice which were consistently more or less explorative (“explorative” or “non-explorative”) (shown in Figure 1). These individuals were then assembled into treatment groups of 8 that either comprised all explorative, all non-explorative or a mixture of explorative and non-explorative individuals, in the following way:

For each sub-batch, if there were four or fewer individuals in a sub-batch which were active (explorative) in both observation periods, those were selected as “explorative”. If more than four individuals were active in both observation periods, then a random number generator was used to select four as explorative. If fewer than four individuals were active in both observation periods, after designating the individuals which were active in both as “explorative”, a random number generator was used to select which of the individuals that were active only in one trial would be used as explorative individuals. This allowed the most active individuals to be chosen.

Similarly, if there were four individuals that were inactive (non-explorative) during the consistent inter-individual variation trials, these were designated as “non-explorative”. If there were more than four individuals that were inactive for both trials, a random number generator was to select which four individuals would be designated as “non-explorative” individuals. There were never fewer than four woodlice non-explorative in a trial.



(1) Woodlice collected on one day are kept in one home box for at least 7 days to acclimatize.

(2) 60 woodlice (one batch) are taken from the housing box and split into three sub-batches of 20 woodlice. These are individually painted to allow identification then scored for activity.

(3) After a break woodlice are scored for activity a second time.

(4) From the consistent inter-individual variation trials, the 4 most explorative woodlice (green) and the 4 least explorative woodlice (white) are selected.

(5) The 4 most explorative/non-explorative woodlice from each sub-batch are recombined to form 3 treatment groups: all explorative (green), all non-explorative (white) or mixed (green and white).

Figure 1: *Description of how explorative and non-explorative groups were made up. The grey ovals represent woodlice in the housing box prior to consistent inter-individual variation trials. The multicoloured groups of ovals represent woodlice which have been individually painted to allow identification during consistent inter-individual variation trials. Green striped ovals represent explorative woodlice while white ovals represent non-explorative woodlice.*

In most cases, three treatment groups (explorative, non-explorative and mixed) were then made up from the three sub-batches used in the consistent inter-individual variation trials, in such a way that each treatment group comprised two sets of four woodlice from different sub-batches as shown in Figure 1.

In some cases (10/22) it was not possible to make three treatment groups from one batch of 60 woodlice, as there were not always enough woodlice classified as explorative to conduct all three treatments concurrently (this required 12 explorative woodlice; 8 for the explorative treatment group and 4 for the mixed treatment group). In these cases, if at least eight woodlice were classed as explorative then only explorative and non-explorative treatment groups were formed; if four woodlice were classified as explorative then only non-explorative and a mixed treatment groups were formed. This resulted in more explorative than mixed trials being conducted.

As woodlice were painted within their original sub-batches of 20, there were sometimes replications in colour patterns within the newly assembled treatment groups; these woodlice were repainted to have unique colour combinations. As a sham treatment, the woodlice which did not need to be repainted were stroked with a cocktail stick which was wetted with distilled water.

Testing differences between the treatment groups in relocation dynamics

Test arena set up

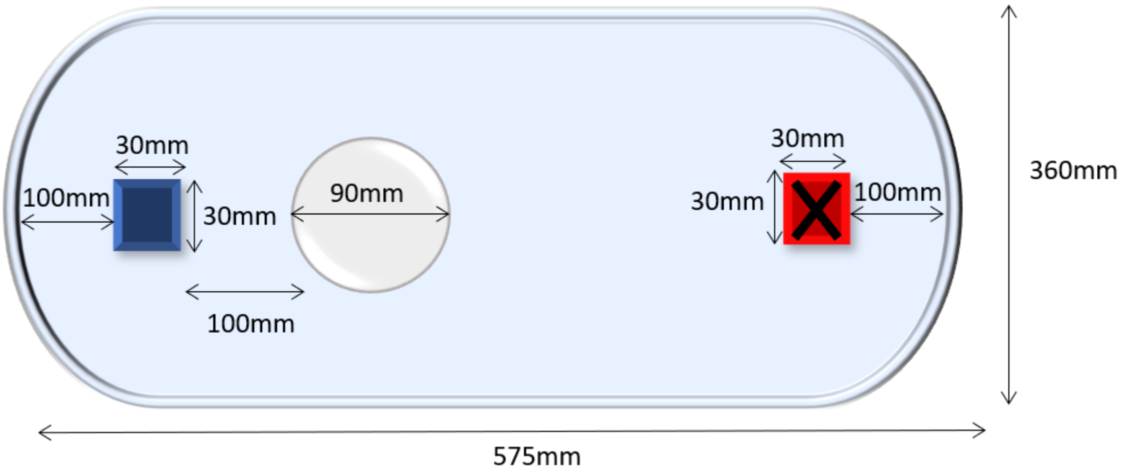


Figure 2: *Diagram of the test arena (not to scale). At one end is the good quality shelter (blue), in front of this is a petri-dish (shown as a grey circle) and at the other side of the test arena is the poor-quality shelter (red with a black cross). Each shelter is 100mm away from the edge of the test arena. Woodlice start the experiment in the poor shelter.*

The test arena had a floor area of 1.1m^2. At one end there was a poor shelter, at the other was a good shelter. Both shelters had a volume of 7000mm^3. The poor shelters had a lid with holes (3: 2x 55mm^2, 1x 120mm^2); good shelters had intact lids and contained a 50mm x 50mm piece of absorbent paper with 0.5ml of water added to it, making the ‘good’ shelters darker and more humid. In front of the good shelter was a petri dish: this prevented the woodlice walking in a straight line from one shelter to the other, making the exploration task more challenging for the woodlice. Before each trial, the test arena and shelters were cleaned with 70% ethanol. Woodlice were placed in the poor shelter at the start of the experiment.

Relocation trials

In total, 22 trials were carried out containing all non-explorative individuals, 19 trials were carried out containing all explorative individuals and 15 trials were carried out with a mixture of explorative and non-explorative individuals. Three trials (one explorative, one non-explorative and one mixed) were excluded from the analysis as the trials were stopped early for technical reasons. Each group of woodlice was used for only one treatment. These woodlice were then removed from the experiment and returned to the areas in the wild from which they were collected.

At the start of each trial, the eight woodlice were placed in the poor shelter (Figure 2) and prevented from leaving by a cover blocking the entrance and gaps in the lid. The woodlice were then allowed to acclimatize for 10 minutes. After the 10-minute acclimatization the cover was removed and the woodlice were allowed to leave and re-enter either of the shelters freely. From this point, one or two observers blinded to the identity of the treatment watched the woodlice for 110 minutes. The observers recorded the time and woodlouse colour when (1) any woodlouse left the poor shelter (completely out of shelter) (2) entered the good shelter (counted as when the woodlouse is completely under the shelter). All trials except three were carried out blind; of the three trials which were carried out not blinded due to logistical constraints, one of the unblinded trials was video recorded to check for observer bias. The video was scored by a blinded observer, these scores were then used to check for observer bias. A one-way consistency intra-class correlation model using the package "irr" (Gamer *et al.*, 2019) was used to check for observer bias. The correlation model compared the time for woodlice to enter the new shelter for the first time recorded by the non-blinded scorer, in comparison to the blind scorer, this comparison had a ICC score of 0.999, which suggests excellent (Koo & Li, 2016) levels of correlation between the blind and non-blind observers, and therefore suggest the scores recorded by the unblinded observer were not biased.

Analysis

The data represent ‘time to event’ data, so survival analysis was used with the R packages “survival” (Therneau & Lumley, 2014), and “dplyr” (Wickham *et al.*, 2015). To plot these data “ggplot2” (Wickham, 2016) was used. Some survival analysis methods are based on the assumptions of proportional hazards, therefore initially the package “survival” was used to test the assumption of proportionality in the data. The proportional hazards assumptions were not met with these data, so accelerated failure time (AFT) models with a Weibull distribution (using the survreg function of the “survival” package) were used. The package “SurvRegCensCov” (Hubeaux & Rufibach, 2014) was used to extract the Hazard Ratio.

To test whether treatment affected the time at which an individual left the poor shelter for the first time, or if treatment affected the time it took for an individual to find a new shelter after leaving the poor shelter, AFT models with a Weibull distribution were constructed with treatment as the predictor and Gaussian frailty term added as a random factor to take batch into account. The dependent variables were either time to emerge or time from emergence to finding the new shelter. Data were right censored for woodlice which did not emerge in the time of the observation.

II.IV Results

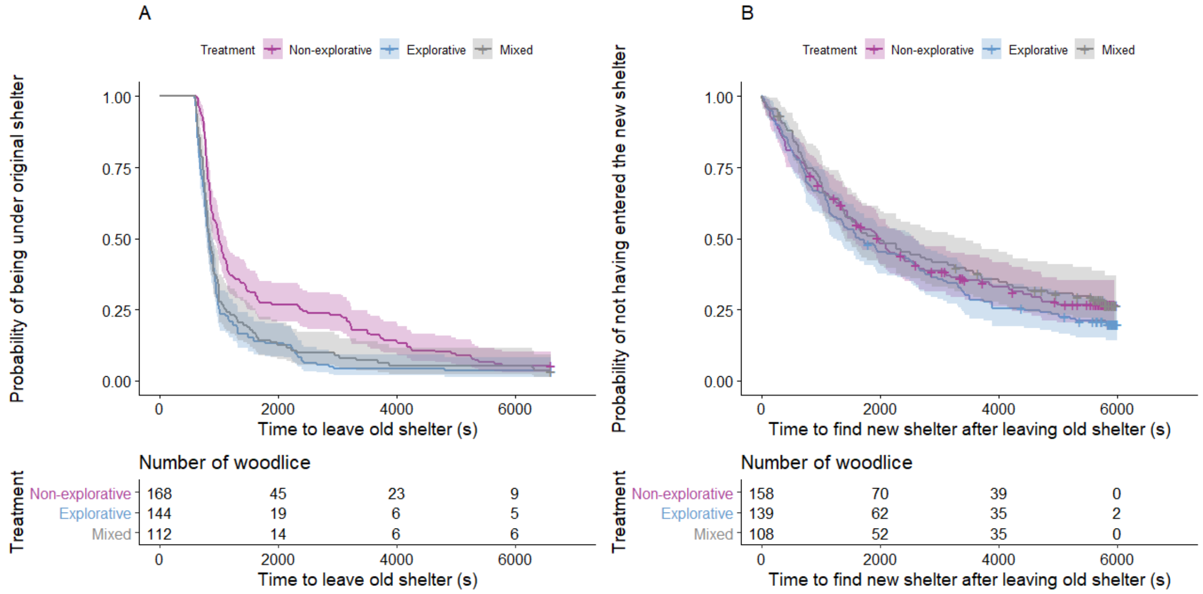


Figure 3: ***A:*** *Survival curves showing (**A**) the model fit of the probability of woodlice staying in the shelter over time (s) from start of the experiment; (**B**) the model fit of the probability of woodlice finding a new shelter over time(s), from the time they left the original shelter. For both parts: 95% confidence interval for point estimates of survival curves are shown in a paler colour around the survival curve. + shows data which has been right censored. Tables show empirical event data.*

Aggregation stability

Treatment group significantly affected the time taken for woodlice to leave the old shelter (Figure 3A, Table 1). Explorative groups (made of only explorative individuals) and mixed groups (made of both explorative and non-explorative individuals) took significantly less time to leave the shelter than groups made up of only non-explorative woodlice (Table 1). The mixed groups behaved like explorative groups, i.e. there was no significant difference between the time taken for woodlice from the mixed groups to leave the shelter and the time for woodlice from explorative groups to leave the shelter (Table 1). Suggesting that the presence of explorative individuals play an important role in affecting the time for the group to leave the shelter. Treatment condition did not significantly affect the time taken for woodlice to find the new shelter after leaving the original shelter (Figure 3B, Table 1).

Table 1: Comparison of time different treatment groups of woodlice took to leave the old shelter or join the new shelter

Time taken for woodlice to leave old shelter	**Hazard Ratio (HR)**	**z**	**Df**	***p***
Treatment: explorative vs mixed	0.777	1.78	20.3	0.075
Treatment: explorative vs non-explorative	0.541	4.99	20.3	<0.005*
Treatment: non-explorative vs mixed	1.438	-2.62	20.3	<0.005*

Time for woodlice to find new shelter after leaving the old shelter	**Hazard Ratio (HR)**	**z**	**Df**	***p***
Treatment explorative vs mixed	0.841	10.8	1.11	0.267
Treatment explorative vs non-explorative	0.889	10.8	0.85	0. 394
Treatment non-explorative vs mixed	0.947	10.8	0.36	0.722

* Denotes significance ($p<0.05$)

II.V Discussion

Our results show that consistent inter-individual variation has an important role in determining aggregation stability in *O. asellus* and provides evidence of emergent group level behaviour based on individual variation. We found that aggregation stability differed between treatment groups with different combinations of inter-individual variation. We also found that this aggregation stability was determined by differences in the time woodlice took to leave a shelter. There was no difference in the time different woodlice in different treatments took to discover the new shelter. Woodlice which left the old shelter later would arrive at the new shelter later, conversely woodlice which left the old shelter earlier would arrive at the new shelter earlier irrespective of group. Therefore, the time woodlice took to leave the aggregation ultimately determined group dynamics.

Emergence from old shelter

If woodlice with different behavioural types acted independently then it would be expected that non-explorative groups would emerge most slowly, the explorative groups would emerge most quickly, and the mixed groups would emerge, on average, between the two. While our non-explorative groups did indeed form the most stable aggregations, the lack of difference between the mixed and explorative groups, demonstrates that even a small number of explorative individuals leads to the emergence of explorative-type group level behaviours and unstable aggregations. This challenges previous modelling work which suggested that distribution of personalities in aggregative animals like woodlice do not affect collective behaviours (Pogson, 2016), and highlights how understanding the distribution of consistent inter-individual variation in behaviour within an aggregation is important to understanding group level aggregation dynamics.

The link between group composition and aggregation stability could have important implications for individual survival in both facultatively social isopods like woodlice, and also in other aggregative species. Less stable or smaller aggregations could reduce the benefits of aggregating (Broly *et al.,* 2014), which could in turn affect the survival probability of individuals in the aggregations. However, while aggregation stability is important, it is also important that a group is able to adapt to a changing environment, and can move to a better location if needed (Dornhaus *et al.,* 2004; Burns *et al.*, 2016).

In other social species, explorative individuals are more likely to break from an original aggregation location and move to a better location (Aplin *et al.,* 2014; Michelena *et al.,* 2009), which in turn could reduce the stability of the aggregation overall. A lack of explorative individuals in an aggregation could lead to a scenario where a group will continue to stay in as substandard location rather than moving to a better location - as can be seen in this experiment, where non-explorative groups spent longer in a substandard environment than groups with explorative individuals. These results highlight the importance of even a small number of explorative individuals in an aggregation to initiate the breakup of an aggregation and ultimately facilitate movement to a better environment. This finding shows similarities to findings in social species, like guppies (Brown & Irving, 2014) where a proportion of the group with particular traits had a non-linear effect on the group behaviour; however unlike our findings with woodlice here, in guppies it was the shyest individuals rather than the boldest individuals which exerted the greatest influence over the group, by disproportionately reducing exploration (Brown & Irving, 2014).

The influence of explorative individual woodlice on the aggregation fragmentation behaviour could give weight to several different hypotheses for how emergent collective behaviour in woodlice may occur. Broly *et al.* (2015) suggested that tactile cues in woodlice could facilitate woodlice to change between “calm” or “excited” behavioural states, and that woodlice in “calm” behavioural states may settle others in the aggregation, while “excited” woodlice could stimulate other individuals to become more active (Broly & Deneubourg, 2015). It could be the case that different behavioural types have different probabilities of shifting between calm and excited states, with explorative woodlice more likely to be in or shift to the excited state. If explorative woodlice were more likely to be excited, they would be more likely to move around the aggregation and to affect more woodlice than woodlice in a non-excited state. A second hypothesis is that in aggregative species like woodlice, an aggregation becomes less attractive as more individuals leave; therefore when even a small number of explorative individuals leave the aggregation, the site of the aggregation becomes less attractive (Broly *et al.,* 2014), and the probability of even non-explorative woodlice leaving is higher. It is likely that the emergent behaviours observed could be linked to a combination of the effects of these two hypotheses, i.e. behavioural contagion (Broly & Deneubourg, 2015) and the degradation of aggregation site quality associated with explorative individuals leaving (Broly *et al.,* 2014).

Discovery of new location

In this study we observed no effect of group composition in the time it took for individuals to find a new shelter after leaving the poor shelter. The lack of difference in time to find and enter the new shelter is surprising, as the explorative individuals may be expected to cover more ground than non-explorative individuals (Blight *et al.*, 2016; Carere *et al.*, 2005; Sneddon, 2003). It could be the case that being outside a shelter for an extended period is stressful for both non-explorative and explorative individuals, and this increased stress could override an individual's disposition to explore. Something similar has been noted in mammals where explorative tendencies may not be expressed when there is a threat (Carter *et al.*, 2012). It may be that due to the danger involved in moving during the daytime, all woodlice search at the maximum possible speed.

Overall, the link between behavioural composition and group level aggregation stability in woodlice presents many questions for future work. There are several key questions which would be important to investigate further: Firstly, are these observed emergent behaviours consistent in other contexts like foraging as well as aggregation? And secondly, how much of an impact do these emergent behaviours have on the survival of the individual or the group in the wild? Both questions could provide future insight in the role consistent inter-individual variation plays in aggregation behaviours, and we encourage further research in this area.

Limitations of the study

It must be noted however that there are several methodological limitations of this work. Firstly, individual woodlice were tested for consistency in behaviour only across a very short period of time within the same day, this was done to reduce the risks of desiccation and stress responses associated with repeated testing as well as challenges of marking woodlice. There is a risk that observed patterns were driven by potentially short-term differences in individual states, there is therefore a need for longer term studies to determine the long-term repeatability of this type of behaviour. Secondly, individuals were tested for their exploratory behaviour in group rather than individually. This approach of taking the individuals which were boldest or shyest in a group would have led to groups which were relatively bolder or shyer than each other, but would have varied in the extent of boldness or shyness in absolute terms. This approach was used as this species of woodlouse naturally aggregates and therefore the approach of measuring the relative behaviours in the context of an aggregation was thought to provide an assessment of more natural behaviour and avoid eliciting behaviour which could be interpreted as a stress response to being placed along in an arena without an aggregation or shelter. We would encourage future work to be done potentially on nocturnal foraging patterns of individual woodlice as they leave an aggregation, as this may give individual specific data without removing the individual from their social environment. Finally, it was not assessed whether the behaviour was repeatable across the individuals tested, and woodlice that did not behave consistently across trials were classified as unexploratory. It could be the case that flexibility in behavioural states is a particular dimension of woodlouse personality which has not yet been explored. There is still very little understood about the axes which woodlouse personality falls along. Here we have assumed only a bold-shy continuum; however, there may be other dimensions like flexibility of behaviour or sensitivity to other woodlice which may play key roles to the emergence of woodlouse behaviour at the individual and group level. It is important therefore that future studies thoroughly explore the potential dimensions and distributions of woodlouse personality in other to fully understand how personality affects group level behaviours.

II.VI Conclusion

In conclusion, in order to understand the how group level behaviours are modulated by inter-individual variation, it is important that we study this phenomenon in a variety of social systems. Here we highlight the potential role of woodlice as a study system to study the role of consistent inter-individual variation in aggregations (Barnes *et al.,* 2015; Devigne *et al.,* 2011). We have shown that group-level behaviours are modulated by inter-individual variation, and that explorative woodlice have a bigger impact on aggregation fragmentation than non-explorative ones, providing evidence of emergent behaviours in a species which aggregates in the absence of a social network. The link between consistent inter-individual variation composition and aggregation stability highlights the importance of furthering our understanding of how distributions of personalities type in an aggregation may affect group behaviours (Aplin *et al.,* 2014; Brown & Irving, 2014; Michelena *et al.,* 2010)

II.VII Acknowledgements

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Chapter III: Consensus decision making. Can individual consistent inter-individual variation affect group level personality: an agent-based modelling approach

III.I Abstract

Understanding how individuals make decisions, and how these decisions influence a group is a crucial to understanding collective behaviour. Many factors affect how an individual acts in a group, and how the group collectively reacts to the individual. This interplay between actions and reactions of different individuals in a group leads to complex emergent behaviours. A range of factors affect the actions and interactions of individuals in a group including social hierarchy and individual nutritional state. One factor which has been explored less in the context of emergent behaviours is consistent inter-individual variation. In this paper we use experimental data to parameterize an agent-based model to explore the link between consistent inter-individual behavioural variation of woodlice (Oniscidae) and emergent aggregation behaviours. We find that consistent inter-individual variation can play a significant role in the emergence of aggregative behaviours in woodlice. Additionally, we find that groups with just small numbers of explorative individuals show aggregation patterns which are similar to groups made up completely of explorative individuals. We suggest that in this system explorative individuals amplify activity in the aggregation through behavioural contagion, increasing the probability of other woodlice in the aggregation becoming active. This work highlights the importance of considering the integration of animal variation when modelling collective behaviour, as well as the need for further work investigating the mechanisms by which consistent inter-individual differences in behaviour may shape group level personality.

III.II Introduction

Understanding how individuals make decisions is crucial to understanding group stability (Conradt & Roper, 2005). Individual animals within a group continually have to decide whether to stay with the group or to leave. The probability of each individual making the decision to stay or leave will affect the stability of a group. There are multiple benefits to group living including greater access to information about foraging opportunities (Aplin *et al.,* 2012; Boogert *et al.,* 2008), enhanced predator defence through shared vigilance and the dilution effect (Cresswell & Quinn, 2011; Hass & Valenzuela, 2002), and the benefits of a local microclimate. For example, animals like new-born rabbits (*Oryctolagus cuniculus*) benefit from the microclimate caused by living in a group through huddling for warmth (Bautista *et al.,* 2013), while woodlice (Oniscidea) benefit from increased humidity in an aggregation (Bautista *et al.,* 2013; Broly *et al.,* 2013). However, there also could be benefits to leaving a group, including reduced competition for food (Beauchamp & Fernández-Juricic, 2005). The importance of food availability in the decision to leave a group is shown by social spiders (*Holocnemus pluchei*) which are more likely to adopt a solitary strategy if undernourished (Jakob, 2004). Another reason an animal may leave a group is the possibility of finding a social group with more opportunities for mating or less social conflict (Huang *et al.,* 2017), as observed in female cichlid fish (*Neolamprologus pulcher*) for which the chance of having a high social rank ranking is an important factor in group choice (Reddon *et al.,* 2011). How individuals make decisions can be affected by a variety of short term factors, including the reproductive or nutritional state of an individual (Fischhoff *et al.,* 2007; Hansen *et al.,* 2015).

In addition to decision making being affected by short term factors like hunger, individuals can also show longer term innate consistent variation in behaviour and decision making (Cole & Quinn, 2014; Kralj-Fišer & Schuett, 2014). Consistent inter-individual variation in behaviour emerges from a combination of factors, including genetics (Dochtermann *et al.,* 2015; Poissant *et al.,* 2013; Van Oers *et al.,* 2004; Zablocki-Thomas *et al.,* 2019) and developmental conditions (Boogert *et al.,* 2014), and has been increasingly recognised as an important part of group decision making and behaviour (Kolay *et al.,* 2020; Planas-Sitjà, 2020; Spiegel *et al.,* 2017; Tang & Fu, 2019).

Despite the importance of consistent inter-individual variation in behaviour to group behaviours across a range of study systems (Magnhagen & Bunnefeld, 2009; Michelena *et al.,* 2010; Sibbald *et al.,* 2009), it is unclear to what degree consistent inter-individual variation in behaviour is relevant to all taxa and contexts. One example of this is the suggestion that variation in personality may not play a role in aggregation behaviours in woodlice (Pogson, 2016). This model by Pogson raises interesting questions about the role of consistent inter-individual variation in behaviour in different social systems. Many of the studies which have looked at consistent inter-individual variation in the context of sociality have used study systems with well-defined social structures such as great tits (*Parus major*). In flocks of *P. major* individuals occupy different positions in a social network (Aplin *et al.,* 2013) as well as different spatial positions in the flock (Aplin *et al.,* 2014), and these in turn are affected by consistent inter-individual variation in behaviour (Aplin *et al.,* 2013). Other systems like schooling fish have less defined social structures but have also shown an important role of individual variation in behaviour in group level behaviour (Magnhagen & Bunnefeld, 2009). However, while the social structure may be far more transient to those observed in certain bird flocks, fish do show some spatial structure within a school. Fish like mullet (*Liza aurata)* for example show some spatial structure in schools as fish with higher aerobic capacity occupy a position at the front of a school, while fish with a lower aerobic capacity make up the rear of the school where there is less drag (Killen *et al.,* 2012). It is less well understood how consistent inter-individual variation in behaviour can affect the behaviour of aggregations of animals without social ties or consistent structure. Invertebrate systems like woodlice can be used as models to understand aggregation dynamics (Broly *et al.,* 2013; Broly & Deneubourg, 2015; Pogson, 2016). Aggregations seen in many species of woodlouse have no known strong social ties. It could therefore be hypothesised that consistent inter-individual variation in behaviour is less important to collective decision-making in these less structured aggregations than in more structured animal social groups; alternatively, it could be that existing models do not capture the role of consistent inter-individual variation in behaviour in facultative aggregations. In either case, this highlights a knowledge gap in our understanding of the role of consistent inter-individual variation in behaviour in group level behaviours across different social contexts.

In chapter two of this thesis we demonstrated empirically that individual woodlice (*Oniscus asellus*) show consistent variation in activity level, and that these inter-individual differences in woodlouse behaviour affect the way a group of woodlice leaves a shelter; these empirical results contrast with the predictions of an existing model (Pogson, 2016). While these empirical findings are important to further our understanding about the link between individual and group level behaviour, there are still many unanswered questions about the underlying mechanism behind the impact which individual variation may have on group level behaviour.

One of the most surprising findings of chapter two was that groups of *O. asellus* composed of a mix of active and inactive individuals left the shelter as fast as groups made of only active individuals, and that groups made of only inactive individuals were significantly slower to leave the shelter than mixed groups or groups made of only active individuals. The inequality between the impact of active and inactive individuals raises interesting questions about how behavioural variation could affect group level behaviour in aggregations, and how the Pogson (2016) model could be adapted to bring it more in line with these empirical observations.

The Pogson model is an agent-based model. Agent based modelling encodes individual agents with a set of rules before allowing them to interact. This approach has the benefit of allowing flexibility in defining individual agents, as well as allowing emergent behaviour to be modelled from the bottom up (Bazghandi, 2012). In addition to the benefits of agent-based modelling there are also drawbacks including challenges like high computing requirements, as well as the challenges of setting appropriate parameters (Bazghandi, 2012). The sensitivity of agent-based models to the encoded parameters make it essential to base parameters on real world values wherever possible.

Re-parameterising this existing model with empirical data could lead simulations in which individual consistent inter-individual variation in behaviour does have an important role in woodlouse aggregation behaviour. In chapter two we show the importance of individual variation to shelter leaving behaviour; however, it is unclear what the mechanism behind these emergent behaviours may be. In the experimental study we suggest that the inter-individual differences which lead to emergent behaviours are from differences in the likelihood of woodlice to be active or inactive. In this extended and re-parameterised model, we can test whether group level differences in aggregation stability could emerge from consistent inter-individual differences in the likelihood of woodlice to be active or inactive, combined with interactions with environmental conditions including the presence of other active and inactive woodlice in the local area. This modelling approach could provide key insights into the possible mechanisms which could lead to the group level behaviours observed in woodlouse aggregations.

Overall, there is much that could be learned from taking a modelling approach to investigate the link between individual and group-level behaviour in woodlice. Additionally, access to empirical data on one aspect of individual and group level behaviour will allow us to robustly re-parameterise and adapt the existing model, before going on to ask more mechanistic questions. We therefore aim to parametrize an adapted model with empirical data, then use this adapted model to test the following hypothesis:

1. **If individual woodlice (Oniscidae) have consistent differences in their probability of being active (referred to here as differences in consistent inter-individual variation in behaviour), then composition of a group will affect group stability**

III.III Methods

This modelling approach is presented following the Overview, Design concepts, and Details (ODD) framework for modelling description (Grimm *et al.,* 2006). Initially the published model was re-coded from scratch, and the figures in the (Pogson,2016) paper recreated using Python to check similarity between the models (see Appendix one). We then built upon the published model to integrate the findings of chapter two (that woodlice do show consistent variation over time in behaviour), to test our hypotheses detailed above.

III.IV Overview

Purpose

The purpose of this model is to test the hypothesis stated in the introduction, specifically to investigate if consistent inter-individual variation in behaviour in woodlice can affect different aspects of sheltering behaviour in woodlouse groups, as well as to investigate how active and inactive individuals may affect other woodlice in the group.

State Variables and Scales

This model has two levels, individual and environment. Individuals are characterised by the state variables of personality and individual identity.

The spatial structure of the model is as follows; the replicates take place in a circular arena with a radius of 100mm (see Figure 1). On opposite sides of the arena are two circular shelters with a radius of 17.5mm. Within this arena a group of 8 woodlice can move anywhere in the arena including over the top of each other; woodlice regularly move over each other in natural settings (*pers. obs*). This spatial structure is based on the Pogson model.

The model has one key environmental parameter, this is the quality of the environment: if a woodlouse is under a shelter the woodlouse would be less likely to move than if it was outside the shelter (not taking the impact of sociality into account), because we assume that the shelter is perceived as higher quality. These environmentally dependent movement probabilities were controlled by the values of p_i shown in Table 1.

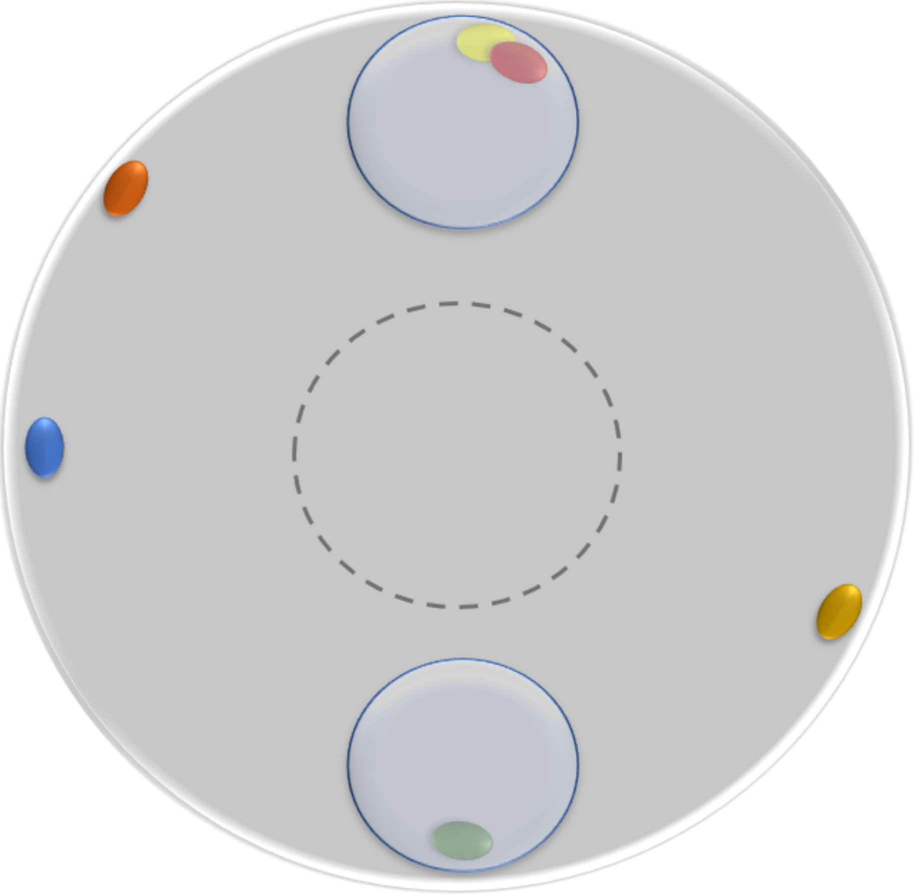


Figure 1: *Spatial arrangement of the model based on the model by Pogson* (2016). *The large grey circle represents the arena, the small circles represent shelters in the arena, the small colourful ovals represent the woodlice.*

III.V Process overview and scheduling

This model proceeds in time steps of one second. In each time step each woodlouse will either move forward a specified distance or remain non-moving. If moving, the distance will be randomly generated from a normal distribution within one standard deviation on either side of a mean of 7.34 mm s^{-1} mm. The average distance of 7.34 mm s^{-1} per second is used because female woodlice move at a velocity of 6.10mm s^{-1} while male woodlice move at a velocity of 8.59mm s^{-1}, therefore a mean velocity of 7.34 mm s^{-1} will be used in these simulation experiments. (The action of one woodlouse moving a step forward or being non-moving will be referred to as taking an action). The order in which each woodlouse takes an action at each time step is randomised at every time step.

Once a woodlouse starts moving the woodlouse will continue moving until the minimum number of steps is reached, at every step the minimum number of steps is randomly drawn from a normal distribution with a mean of 19 and a standard deviation of one, which means the woodlouse will move an average of 19 steps forward once it starts moving. The average movement of 19 steps has been chosen because under empirical experimental conditions female woodlice of *Porcellio scaber* move an average of 70970mm over an average of 658 moves in eight hours (Bayley, 1995). Male *P. scaber* woodlice move on average 155400mm over 668 moves in the same timeframe (Bayley, 1995). From these the mean distance of one move can be calculated as 142 mm. When taking into account the average velocity of a woodlouse (7.34mm s^{-1}), this means that to cover 142mm, 19.3 steps (rounded to 19 steps) would then be taken in order to mirror the tendency of woodlice to move a distance after starting to move (Bayley, 1995). As the individual step length varies (see above) but is on average 7.34mm, the length of the total distance moved while moving will vary but will on average fall close to 142mm. After an average of 19 steps the moving or non-moving status of a woodlouse is recalculated. The 19 steps taken by the woodlouse after it starts moving deviates from the Pogson model in which woodlice only move five steps before the activity of the woodlouse is recalculated.

Whether or not a woodlouse moves in one time step depends on whether the probability of moving is greater than a random number generated at each time step as well as whether a woodlouse has exceeded their average of nineteen steps of movement following activation. This structure is based on the model developed by Pogson (2016).

Table 1 – Overview of processes, parameters and default values of parameters of the original Pogson woodlouse model

Parameter or variable	Abbreviation	Value
Sociality when in contact with non-moving woodlice	f_g	0.5
Probability of movement if under shelter, or in the open	p_i	0.1, 0.8
Personality	f_r	± 0.1
Number of non-moving neighbouring agents within a detectable radius	n	0-39

Pogson's original model of an individual's movement probability (P_M) is calculated using the values in Table one in the following way:

$$P_M = p_i f_g^{n} + f_r$$

To adapt the model to include the influence of moving woodlice, the probability of movement is calculated using the values in Table 2 in the following way:

Equation 1:

$$P_M = p_i f_a^{mA} f_g^{mG} + f_r$$

Table 2 – Overview of processes, parameters and values of parameters of the adapted woodlouse model

Parameter or variable	Abbreviation	Value
Sociality when in contact with non-moving woodlice	f_g	>1*
Sociality when in contact with moving woodlice*	f_a	<1*
Probability of movement (if under shelter or not under shelter)	p_i	0.1, 0.8
Consistent inter-individual variation in behaviour	f_r	± 0.1
Number of moving woodlice within detection radius of the focal woodlouse	mA	0-7*
Number of non-moving agents within detection radius of the focal woodlouse	mG	0-7*

**** These values will be explored later in this chapter***

Some of parameters used in this model were largely based on the values used by the Pogson (2016) paper. Probability of movement (if under shelter or not under shelter), sensing distance, and the range of parameter values were based on the Pogson (2016) model. More research is needed to definitively test whether these values are accurate; however in the absence of empirical data, the values used in the Pogson (2016) model are used.

Several of the parameters differed from the Pogson (2016) paper. The number of woodlice used in each trial (eight) differed from the number used in the Pogson (2016) paper, eight woodlice were used as this reflected the number used in the empirical trials used to parameterise this model. The velocity of woodlice was based on findings by Bayley (1995), discussed in more detail in section III.V above (process overview and scheduling).

We use a Metropolis step and if the probability is greater than 1 it is automatically accepted. In this adapted equation, f_a is greater than one and f_g is smaller than one, meaning that detecting moving woodlice increases the probability of movement, and detecting non-moving woodlice decreases the probability of movement. The ratio between f_a and f_g affects the overall probability of movement as shown in Figure 2**.**

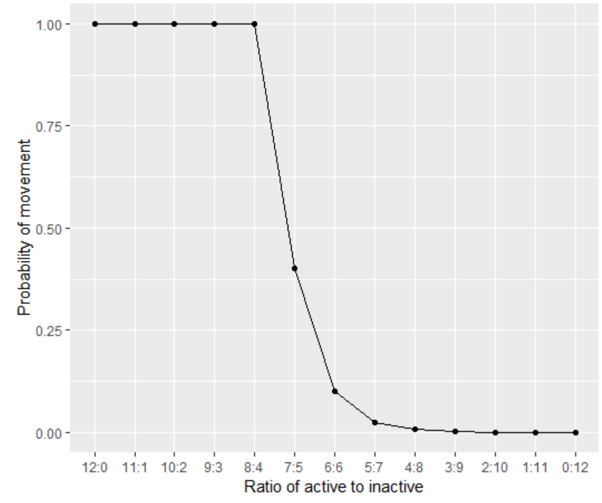
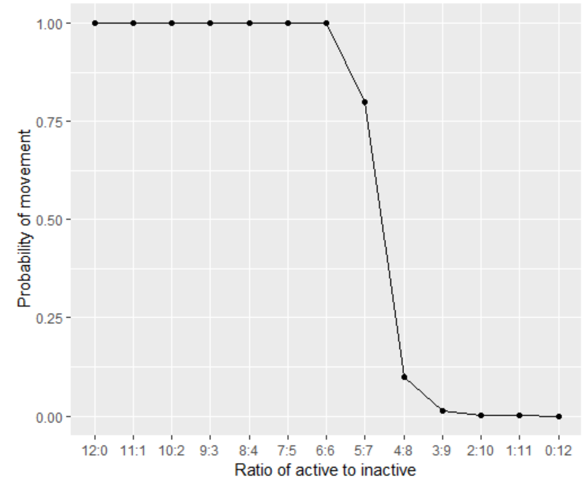
	
$f_a = 2$ $f_g = ½$	$f_a = 4$ $f_g = ½$
(p_i is kept at 0.1, and f_r is kept at 0)	(p_i is kept at 0.1, and f_r is kept at 0)

Figure 2: *Example of how changing the ratio of* f_a *and* f_g *can alter the probability of movement assuming woodlouse is in the shelter, and excluding any consistent inter-individual variation in behaviour effects*

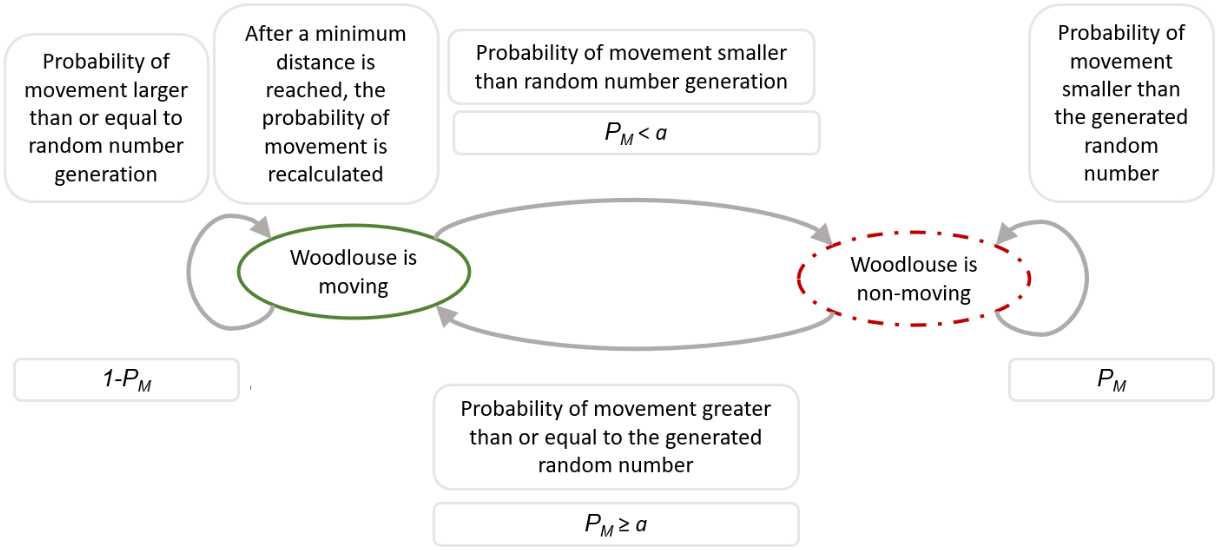


Figure 3: *When a woodlouse is in a non-moving state the probability of movement is calculated at each step by comparing* P_M *to a number drawn from a uniform random distribution. Once a woodlouse becomes active the woodlouse will move until the minimum movement distance is reached. After the minimum movement distance is reached then* P_M *will be compared to a number drawn from a uniform random distribution, depending on this number the woodlouse will either changed to a non-moving state or will continue moving. If the woodlouse continues moving for a greater distance than the minimum movement distance, then probability of movement is calculated at each step by comparing* P_M *to a number drawn from a uniform random distribution.*

III.VI Design concepts

Sensing: Woodlice can sense their immediate surroundings in this model; specifically, they can sense the presence of other woodlice within a 5mm diameter (based on estimations from the Pogson (2016) model). They are also able to sense if the neighbouring woodlice are moving or non-moving. As well as other agents, woodlice can also sense the shelters: if they are within 25mm of the shelter they will act as if they are under the shelter.

Interaction: If the focal woodlouse senses other woodlice nearby, these neighbouring woodlice will affect the probability of the focal woodlouse moving. If the neighbouring woodlice are moving then the probability of the focal woodlouse moving will be increased, if the neighbouring woodlice are non-moving then the probability of the focal woodlouse moving will be decreased (shown in Equation 1)

Stochasticity: consistent inter-individual variation in behaviour of individual woodlice is selected from a uniform distribution of values to represent a wide variation of personalities.

III.VII Details

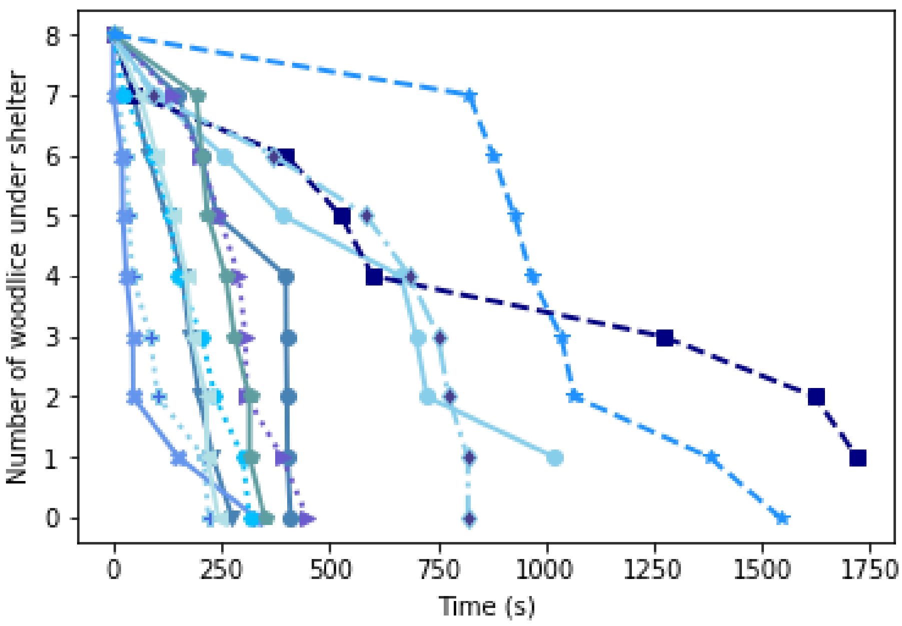
Initialization

Each replicate starts with 8 woodlice under one of the shelters, mimicking the experimental setup in chapter two. Initial starting coordinates were selected at random within one shelter. Woodlice are randomly assigned an initial orientation, and none of them are in motion at the start of the simulation experiment (mimicking a stable woodlouse aggregation); woodlice then walk in a straight line until encountering the edge of the arena. This may mean that a woodlouse may encounter the edge of the arena which borders the shelter, or that a woodlouse may leave the shelter and walk across the arena until encountering the edge of the arena on the other side. When a woodlouse encounters the edge of the arena the woodlouse turns either right or left at random and continues walking around the arena in that direction. Once a woodlouse has started moving along the edge of the arena in one direction, the woodlouse then continues following arena edge in that direction until the end of the simulation experiment. As the shelters were placed at the edge of the arena, woodlice would move through them as they follow the edge of the arena. In this arena the woodlice could enter the shelter from one side, continue through the shelter and leave through the other side of the shelter.

III.VIII Finding the model parameters

In order to parameterise this model with empirical experimental data, we first went back to the empirical woodlouse experiment in chapter two and took the data from the thirteen groups each of which was made up of a mixture of 4 non-explorative and 4 explorative individuals (for more details of empirical experimental methods see chapter two). We calculated the time until each individual leaves the shelter for the first time. For each of these thirteen groups we calculated the mean rate of leaving, as well as the mean within-group variation in the time to leave the shelter, which was done using the total sum of squares. Sum of squares is calculated by subtracting the mean time to leave the shelter for each group from each data point in the group, then summing the squared outputs: the mean of the sum of squares was then taken across the 13 groups.

A



B

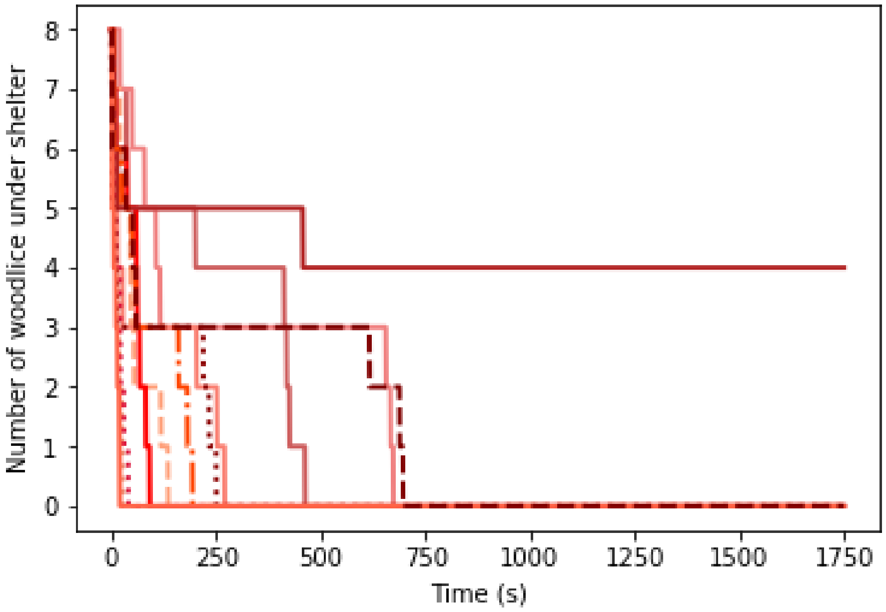


***Figure 4**: Empirical data and simulation data of woodlice leaving shelter. In both graphs time to leave the shelter is plotted against number of woodlice remaining under the shelter at the point the decision to leave the shelter was taken. **A**: Empirical data: Empirical data showing the time for woodlice to leave a shelter. These empirical data are from the leaving trials conducted on mixed groups in chapter 2; each line depicts a different group. **B**: Simulation data: Simulation data showing shelter-leaving behaviour of woodlouse agents where f_a =2, p_i =(inside shelter = 0.1, outside shelter=0.8), f_g =1/16 and f_r=0. The different lines represent different groups of 8 woodlice.*

Table 3: *Mean variance of time for woodlice to leave the shelter and average leaving rate calculated from the empirical and simulation data shown in Figure 4.*

Data type	**Mean variance in time for woodlice to leave the shelter (sum of squares)**	**Average rate of leaving (woodlice leaving per second)**
Empirical	**89068.709**	**0.0220**
Simulation	**58642.75**	**0.089**

In order to then parameterise our model, we started with the parameters used in the Pogson model (see Table 1) and changed each of the inputs of f_a sequentially to nearest power of 2 while keeping the other inputs constant in line with Table 1 (the model input combinations are shown in Table 4 in Appendix 2). Each model was initially run for 80 time steps and replicated 13 times to match the 13 trials of our empirical data. To determine which combination of parameters would generate results which were closest to empirical data, the rate of leaving and the variation in time for woodlice to leave the shelter from the model output were compared to the rate and variation from the empirical experimental data (the model outputs from each input combinations are shown in Table 5 in Appendix 2). Using this method, the model which ranked most similar to the empirical experimental data output was selected and a second round of parameter testing was carried out sequentially changing f_g while using the parameter values used in the Pogson model, except f_a which was kept constant as the value selected in round one (model input combinations are shown in Table 6 in Appendix 2).

Following this investigation of parameter space, the following parameters were chosen for the simulation experiments: f_a =2, p_i =(inside shelter = 0.1, outside shelter=0.8), f_g =1/16. The outputs of the model with these parameters is shown in Figure 4 and Table 3, where f_r=0, i.e. the model we used to look at the parameter space does not allow for individual woodlouse behavioural variation.

III.VIII Simulation experiment testing

Experiment 1: Is sheltering behaviour in woodlice affected by consistent inter-individual variation in behaviour?

Having parameterised the model using a set of data on woodlice leaving a shelter, we then used our first simulation experiment to ask whether consistent inter-individual variation in behaviour affects sheltering behaviour of a group of woodlice. We ran simulations of three different treatments: in the ‘explorative’ treatment the woodlice have consistent inter-individual variation in behaviour randomly generated from one of the distributions with values between 0 and 0.1, in the ‘non-explorative’ treatment the woodlice have consistent inter-individual variation in behaviour randomly generated from one of the distributions with values between 0 and -0.1, in the ‘mixed’ treatment four woodlice will be have their consistent inter-individual variation in behaviour generated between the values of 0 and 0.1, and four woodlice between the values of 0 and -0.1. Each treatment was replicated 13 times and run for 1750 one second steps, matching empirical experimental levels of replication. We then compared two different measures of group cohesion between treatments. Group cohesion was measured in two ways. Firstly, the time individuals spend stationary was calculated. Secondly, the number of woodlice that finished the replicate in the shelter which contained the highest number of woodlice (winning shelter) was also measured.

III.IX Statistical analysis

Statistical tests were carried out using the software R version 3.5.3. We note that performing statistical tests on simulated data becomes meaningless when very high numbers of replicates are generated; however, here we use just 13 replicates, to match our empirical data, and so we apply a similar statistical approach to the simulation results as we would to empirical data, but also report effect sized for more information.

Individual time non-active

The mean time each individual woodlouse spent in a non-active state was calculated for each replicate (8 woodlice). As there were 13 replicates of each treatment, this led to 13 mean values per treatment. Pair-wise comparisons were carried out using a two-samples unpaired Wilcoxon test (using R package rstatix (Kassambara, 2020)) to compare the means of each replicate between each pair of treatments, and Wilcoxon effect sizes (r) were calculated using R package rstatix (Kassambara, 2020).

Number of woodlice choosing the winning shelter

The shelter with most woodlice under it at the end of each simulation was designated as the winning shelter. Next to determine if there was a difference in the number of woodlice under the winning shelter between treatments at the end of the simulation experiment, the mean number of woodlice for each replicated for each of the 13 replicates in each treatment was calculated. The means numbers under the winning shelter at the end of the simulation were then compared between treatments using a Two-samples unpaired Wilcoxon test, and Wilcoxon effect sizes (r) (using R package rstatix (Kassambara, 2020)).

III.X Results

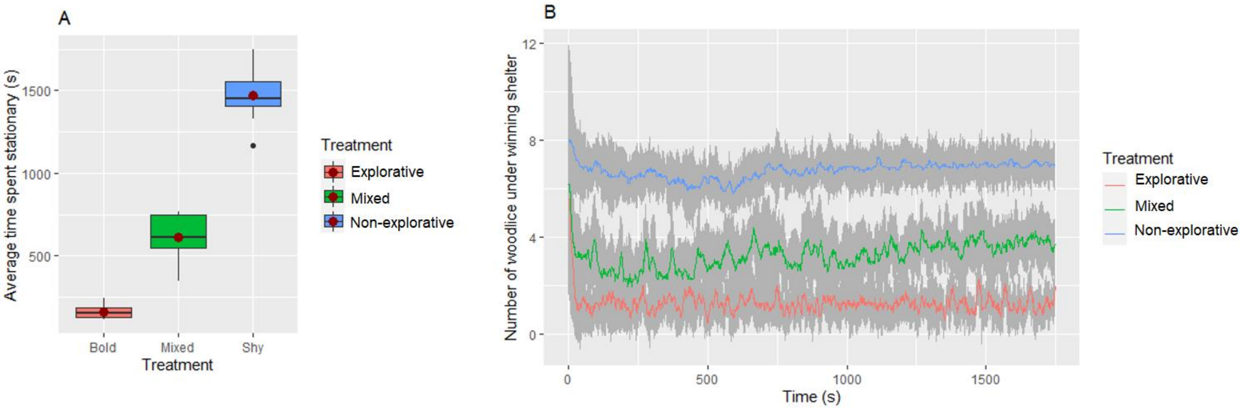


Figure 5: Results from simulation experiment 1: *Using the parameters derived from the empirical data f_a and f_g (2 and 1/16 respectively), the role of consistent inter-individual variation in behaviour in group sheltering behaviour was tested in two ways.* ***(A)*** *The average time which individual woodlice spent in a non-moving state.* ***(B)*** *The average number of woodlice under the winning experiment at each time point. Standard deviation around the mean is shown in grey in B.*

Experiment 1:

Using the parameters derived from the empirical data f_a and f_g (2 and 1/16 respectively), the role of consistent inter-individual variation in behaviour in group sheltering was tested. Individual woodlice in explorative treatment groups spent significantly less time in a non-moving state than woodlice in non-explorative treatment groups (test: Wilcoxon, test-statistic= 0, **N**=13, ***p***<0.005), and the effect size of this was high (Wilcoxon effect size **r** (13)= 0.841). Individual woodlice in explorative treatment groups also spent significantly less time in a non-moving state than woodlice in mixed treatment groups (test: Wilcoxon, test-statistic= 0, **N**=13, ***p***<0.005), and the effect size of this was high (Wilcoxon effect size **r** (13)= 0.841). Woodlice in mixed treatment groups also spent significantly more time non-moving than woodlice in non-explorative groups (test: Wilcoxon, test-statistic=169, **N**=13, ***p***<0.005) and the effect size of this was high (Wilcoxon effect size **r** (13)= 0.841) (this test statistic uses a rank based metric therefore as there is no overlap between the data in any of the groups the effect size is the same between groups).

The number of woodlice under the shelter at the end of the simulation experiment was also compared between treatments. There were on average significantly more woodlice under the shelter at the end of the simulation experiment in non-explorative treatment groups than explorative treatment groups (test: Wilcoxon, test-statistic= 2, **N**=26, ***p***<0.005) and the effect size of this was high (Wilcoxon effect size **r** (13) = 0.841). There was no significant difference between the number of woodlice under the shelter between the explorative and mixed groups (test: Wilcoxon, test-statistic= 57, **N**=26, ***p***=0.15), and the effect size of treatment on the average number of woodlice under the shelter was low (Wilcoxon effect size **r** (13) = 0.288).There were also on average fewer woodlice under the shelter at the end of the simulation experiment in mixed treatment groups than in non-explorative treatment groups (test: Wilcoxon, test-statistic= 2.5, **N**=26, ***p***=<0.005), and the effect size of this was high (Wilcoxon effect size **r** (13) = 0.837).

III.XI Discussion

This model highlights that inter-individual differences in consistent inter-individual variation in behaviour can influence the emergence of collective behaviours, with different personalities leading to different group level behaviours in a nonlinear manner even in simple social structures like animal aggregations. Asymmetry in the influence different individuals have over a group has been documented in collective behaviours from voting patterns to complex animal behaviours (Conradt & List, 2009). This asymmetry can be linked to a variety of factors, including social hierarchy (King *et al.,* 2008; Sueur & Petit, 2008) or differences in knowledge (Dyer *et al.,* 2009; Flack *et al.,* 2012; Stroeymeyt *et al.,* 2011). This model suggests that activity level (and by extension consistent inter-individual variation in behaviour) may also be a factor which leads to asymmetry in the influence of different individuals in a group.

In this model we found that the model parameters which were similar to the empirical experimental data placed the influence of non-moving individuals far higher than moving individuals (approx. eight times higher), (i.e. a non-moving woodlouse was significantly more likely to make neighbouring woodlice non-moving than a moving woodlouse was likely to make neighbouring woodlice moving). This could be because the empirical data are from the woodlouse *O. asellus* which readily forms aggregations (Broly *et al.,* 2013). These stable aggregations have benefits including microhabitat formation and antipredator benefits (Broly *et al.,* 2013). In forming stable aggregations woodlice show a strong non-specific attraction to other aggregated woodlice (Devigne *et al.,* 2011). It may be that the effects of a stationary individual on neighbouring woodlice need to be higher than the effects of a moving woodlice, otherwise the arrival of a woodlice to an aggregation would cause the nearby woodlice in the aggregation to become active, which could lead to the fission of the aggregation and the loss of the benefits of group living. Therefore, the greater influence of stationary individuals on neighbouring woodlice in comparison to active individuals may be an important factor which allows woodlice aggregations to persist.

Despite finding that inactive individuals had a greater influence over their neighbours than active individuals, we also found that mixed groups of non-explorative and explorative individuals appeared to have acted more like groups made of just explorative individuals (both in this model and in our experimental findings in chapter two) than like groups of just non-explorative individuals. Looking at experiment 1A, for example, while there was a significant difference between the mean time individuals spent in a non-moving state between all treatments, the difference between the means of the mixed and explorative treatments was approximately half the difference than between the means of the mixed and non-explorative groups (a difference of 451s and 863s respectively). Similarly, in experiment B there was a greater difference between the mean number of woodlice settled under the winning shelter at the end of the trial when the mixed treatment was compared to the non-explorative treatment, than when the mixed treatment was compared to the explorative treatment (a mean difference of 3 woodlice or 2 woodlice respectively). There was also a large effect size of treatment group on the number of woodlice settled at the end the experiment when the mixed treatment was compared to the non-explorative treatment, but only a small effect size when the mixed treatment was compared the explorative group. These findings suggest similarities in the behaviour of groups of woodlice which are in the explorative groups and mixed groups.

In this model explorative individuals were more likely to become active at any given time than non-explorative individuals, before any external factors were taken into account. It could be the case that that explorative woodlice (which are already at a high probability of becoming active) are more susceptible to becoming active due to the activity of a nearby individual than non-explorative woodlice (which are at a lower innate probability of becoming active). In groups of explorative or explorative and non-explorative woodlice, the effect of an active individual could be amplified, as more explorative woodlice become active. As more woodlice become active the effect of active individuals is increased, and there are less inactive individuals which would reduce the influence of the non-active individuals. Therefore, one explanation for our findings is that explorative individuals may play an important role in amplifying the movement within an aggregation.

This suggestion of the amplification of behaviour in woodlice is similar to the “behavioural contagion” hypothesis for woodlouse behaviour put forward by Broly and Deneubourg (2015). The behavioural contagion hypothesis for woodlice suggested that the switch between active or inactive states could be driven by a “contagion” of the state of nearby woodlice on a focal individual (Broly & Deneubourg, 2015). Similar ideas have been put forward in other systems for example in fish individuals will align themselves with the direction of the fish in front of them (Katz *et al.,* 2011), or in broods of chicks (*Gallus domesticus*) which show contagion of behavioural alertness following one of their group experiencing stress (Edgar & Nicol, 2018). In these examples this alignment of behaviour with other animals in the group has strong adaptive advantages. In the case of fish, alignment of individuals allows group cohesion and movement, in the case of chicks a heightened alertness could prepare the group for threats. In woodlice further work would need to be done to understand definitively what adaptive advantage this behavioural contagion may have. It could be the case that (as in chapter two) an aggregation was in a non-favourable location, and therefore individuals would have to move to avoid desiccation. In cases like this behavioural contagion could allow even members of the group move from the original aggregation site and seek out a better site. An alternative reason could be that an adaptation to avoid predators. Woodlice are the prey for a variety of creatures (Oliver & Meechan, 1993), and a large aggregation of woodlice could be a good source of nutrition. Behavioural contagion of activity throughout the aggregation would lead to rapid fission of the aggregation, which would allow more of the woodlice to escape the predator than would have escaped if their only cue for a predator was the predator itself. It could be the case that behaviour like behavioural contagion was selected for one of these threats (e.g. predation) but is also effective against other threats (e.g. desiccation). However further studies of woodlice species with a different balance of costs between desiccation and predation would have to be carried out to understand this.

While behavioural contagion in animal behaviour has been well characterised in the literature (Boogert *et al.,* 2008; Broly & Deneubourg, 2015; Edgar & Nicol, 2018), less work has been done to integrate behavioural contagion or alignment into the framework of animal personality. However, while there is limited work on behavioural contagion and consistent inter-individual variation in behaviour in animals, work done on other types of emergent behaviour does suggest that consistent inter-individual variation in behaviour plays an important role in modulating group behaviour (Brown & Irving, 2014; Sasaki *et al.,* 2018). In guppies (*Poecilia reticulata*) exploration behaviour of a shoal is correlated with the personality of the shyest individual and the sociality of the most social fish in the group (Brown & Irving, 2014). Similarly in homing pigeons (*Columba livia*) individuals with different personalities are likely to affect group behaviours to different extents; however unlike guppies, in homing pigeons bolder individuals are more likely to be higher in the leadership hierarchy than shyer individuals, and therefore have a greater role in deciding the direction of collective motion (Sasaki *et al.,* 2018). These examples highlight how inter-individual variation in behaviour can affect group behaviours, it is therefore important that we extend our understanding of the role of personality to other aspects of collective behaviour like behavioural contagion.

Given the current gaps in our understanding of the interplay between consistent inter-individual variation in behaviour and emergent behaviours, it must be noted that models like the one described in this paper have limitations, and while these models are parameterised to our data, the observed behaviours could have emerged from different processes. It is important to consider the context in which consistent inter-individual variation in behaviour is being displayed: it could be the case that we are looking at these behaviours in too broad a manner. It may be the case for example that there may be a more nuanced form of communication of behavioural contagion, with woodlice responding more to woodlice which are more agitated and active than woodlice which are moving slowly into the aggregation. This differentiation between "agitated" and merely "active" individuals could be important in allowing the woodlice in the aggregation to differentiate between movement and a real predator threat. However, these suggestions were outside of the scope of the empirical study put forward in chapter two, as well as being outside the scope of this model. These suggestions however do highlight how much there is still to learn in order to fully understand the role of consistent inter-individual variation in behaviour in emergent group level behaviours.

Despite the limitations of this study, this model does suggest one way in which the observed emergent behaviours described in chapter two may have emerged. We would suggest that this model could be a useful starting point for future work linking consistent inter-individual variation in behaviour to emergent group level behaviours.

III.XII Conclusion

In conclusion, in this chapter we put forward a model which integrates inter-individual variation in behaviour with emergent group level behaviour. We suggest that consistent inter-individual variation in behaviour can have an important role in modulating group level behaviours. We suggest that the emergent behaviours could be due to the amplification of behaviour by individuals with personalities which are more susceptible to stimuli. However, this study also highlights how much there is still to learn in terms of how consistent inter-individual variation in behaviour and emergent behaviour may interact in social animals.

III.XIII References

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III.XIV Appendix I

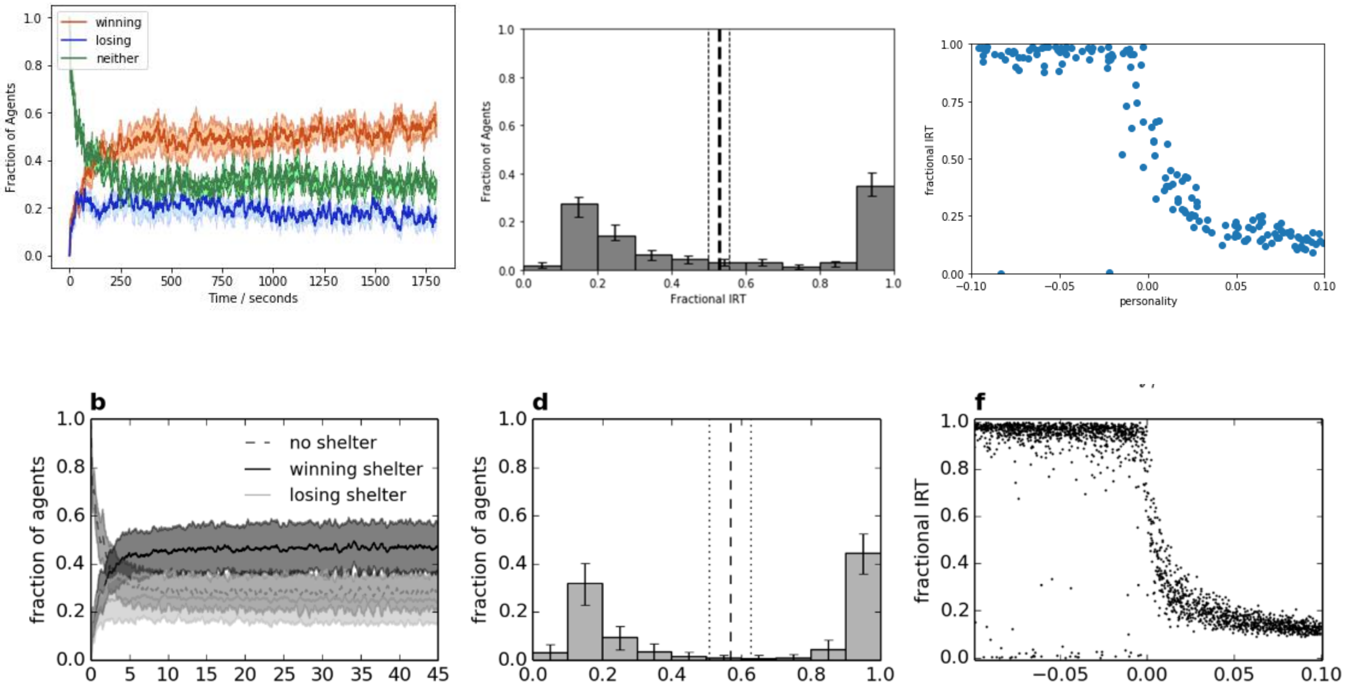


__Figure 6:__ Our replication of the figures from the Pogson model, the figures on the top row were taken from the original Pogson paper, the figures on the bottom row were replicated using our model, the differences are likely due to the Pogson model running 50 times the simulations than were run in our model, and the number of agents being 40 whereas our model was run with 12 agents.

III.XV Appendix II: Iterative exploration of parameter space

Table 4: Iteration 1: Proposed values for initial model testing

Iteration 1: Proposed values for initial model testing		
p_i	f_a	f_g
Changing f_a while p_i and f_g are stable		
0.1 / 0.8	2	0.5
0.1 /0.8	4	0.5
0.1 / 0.8	8	0.5
0.1 / 0.8	16	0.5

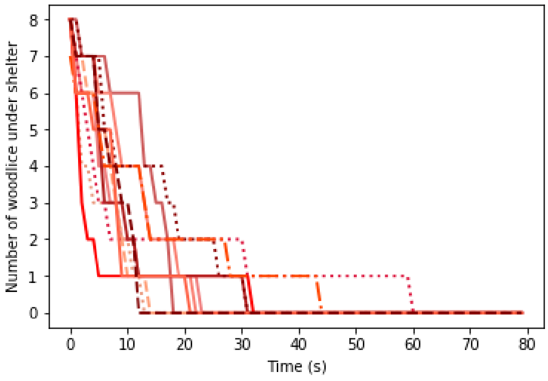
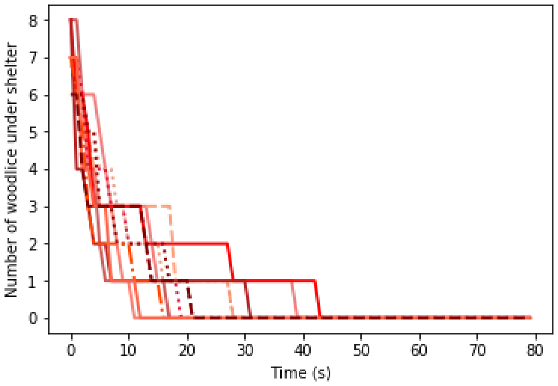
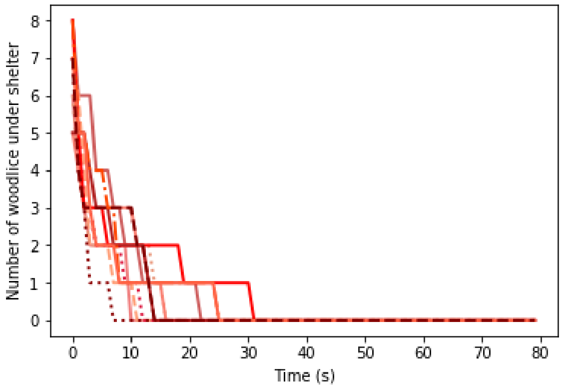
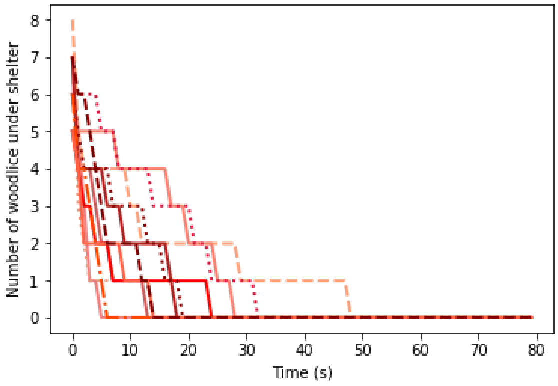
	
Testing relationship between f_g and f_a	Testing p_i while f_g and f_a are stable
p_i =(outside: 0.8, under shelter: 0.1) f_a =2, f_g =0.5, f_r =0	p_i = (under shelter: 0.1, outside: 0.8), f_a =4, f_g =0.5
	
Changing f_a while p_i and f_g are stable	Changing f_a while p_i and f_g are stable
p_i = (under shelter: 0.1, outside: 0.8), f_a =8, f_g =0.5	p_i = (under shelter: 0.1, outside: 0.8), f_a =16, f_g=0.5

***Figure 7**: Parameter space exploration through changing f_a while keeping all other inputs stable*

Table 5: Changing f_a by consecutive powers of 2 while p_i and f_g are stable

Proposed combinations of values			**Results**		
Changing f_a by consecutive powers of 2 while p_i and f_g are stable					
p_i	f_a	f_g	***Average variation (1sf)***	***Average rate (1sf)***	***Rank relative to empirical experimental data***
inside: 0.1, outside: 0.8	2	0.5	200	0.40	Variation:1 Rate: 2
inside: 0.1, outside: 0.8	4	0.5	100	0.40	Variation: 2 Rate: 2
inside: 0.1, outside: 0.8	8	0.5	80	0.50	Variation: 3 Rate: 1
inside: 0.1, outside: 0.8	16	0.5	80	0.40	Variation:3 Rate: 2

Based on the ranking of the model outputs above, the following models will be tested for 1750 steps.

Table 6: Iteration 2 - proposed values for initial model testing

Iteration 2: Proposed values for initial model testing		
p_i	f_a	f_g
Reducing f_a by consecutive powers of negative 2 while keeping the other inputs stable		
inside: 0.1, outside: 0.8	2	0.5
inside: 0.1, outside: 0.8	2	0.25
inside: 0.1, outside: 0.8	2	0.125
inside: 0.1, outside: 0.8	2	0.0625

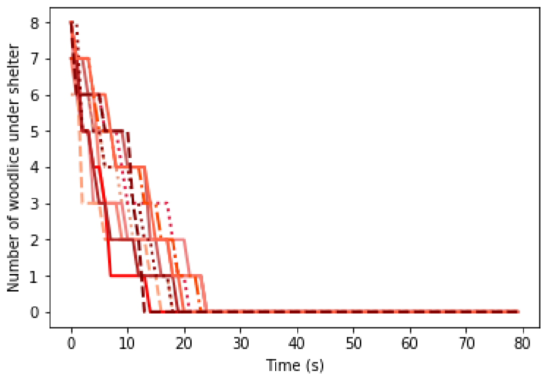
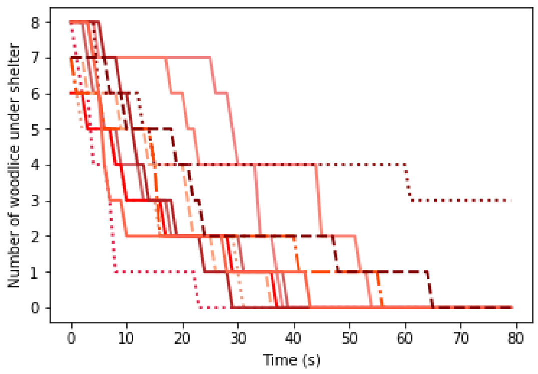
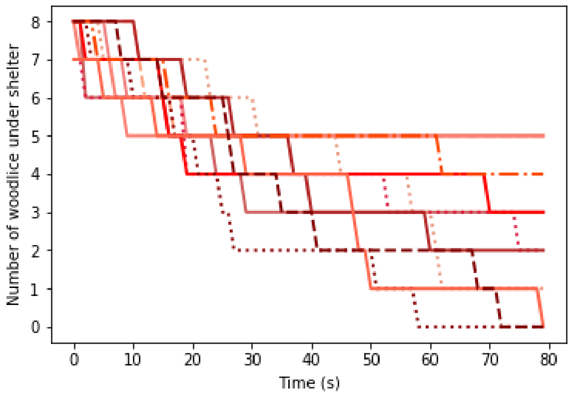
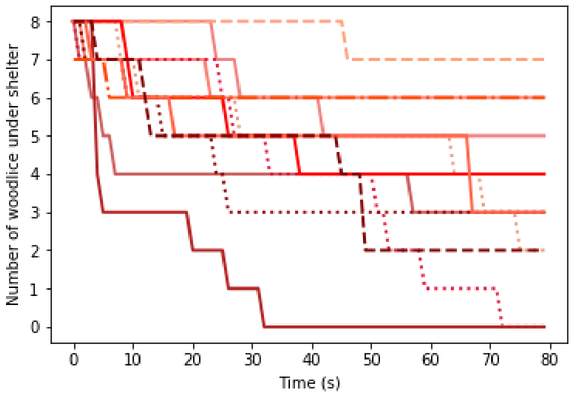
	
Reducing f_a while keeping the other inputs stable	Testing relationship between f_g and f_a
p_i = (under shelter: 0.1, outside: 0.8), f_a =2, f_g =0.5	p_i = (under shelter: 0.1, outside: 0.8), f_a =2 f_g=0.25
	
Changing p_i while f_g and f_a are stable	Changing p_i while f_g and f_a are stable
p_i = (under shelter: 0.05, outside: 0.8), f_a =2, f_g =0.125	p_i = (under shelter: 0.1, outside: 0.8), f_a =2, f_g=0.0625

***Figure 8**: Parameter space exploration through changing f_g while keeping all other inputs stable*

Table 7: Reducing f_a while keeping the other inputs stable

Proposed combinations of values			**Results**		
p_i	f_a	f_g	***Average variation (1sf)***	***Average rate (1sf)***	***Rank relative to empirical experimental data***
Reducing f_a while keeping the other inputs stable					
inside: 0.1, outside: 0.8	2	0.5	40	0.4	Variation: 4 Rate: 3
inside: 0.1, outside: 0.8	2	0.25	300	0.2	Variation: 3 Rate: 2
inside: 0.1, outside: 0.8	2	0.125	400	0.1	Variation: 1 Rate: 1
inside: 0.1, outside: 0.8	2	0.0625	500	0.1	Variation: 2 Rate: 4

Chapter IV: In a changeable environment, can individual memory help house-hunting ant colonies to make decisions about nest choice?

IV.I Abstract

Memory is crucial to the ability of animals to navigate and make choices about their environment. In social animals, memories held by or shared by individuals in a social group can play an important role in group-level decision making. The ant Temnothorax albipennis *uses both individual memory and shared memory in the form of pheromones to make decisions. Based on prior experiences,* T. albipennis *shows bias towards high quality nests and bias against poor quality nests. However, it is unclear whether ants like* T. albipennis *can use changes in quality to inform decision-making. In this study we allowed* T. albipennis *colonies to explore boxes containing nests which were either consistently good, consistently poor, degraded through the course of the exploration period or removed during the course of the exploration period. The home nest of the colony was then destroyed and bias towards or against the explored nests was assessed during their emigration. We found that colonies took significantly longer to enter a box in which they had previously experienced a nest having disappeared than they did to enter a box in which they had previously experienced either a consistently good quality nest, or a nest that had been degraded from good to poor quality. These findings suggest ants have an ability to update their memories about neighbouring nest sites, both by remembering useable nest sites and forgetting nest sites which are no longer usable.*

IV.II Introduction

Memory is crucial to the way animals navigate and interact with their environment (Arenas & Roces, 2016; Smid *et al.,* 2007; Stroeymeyt *et al.,* 2010). Having accurate memories can benefit animals in a range of areas including foraging (Aplin *et al.,* 2013; Noser & Byrne, 2015), finding a mate (Anderson *et al*., 2013; Thornquist & Crickmore, 2019) and finding a nest (Stroeymeyt *et al.,* 2011a; Stroeymeyt *et al.,* 2010). However, just as important as the ability to lay down memories, is the ability to update or forget information in an appropriate way. Carrying incorrect or outdated memories can have maladaptive consequences which could put the individual in danger or lead to energy and time being wasted in a search for a resource which is no longer present (Dunlap & Stephens, 2012; Gordon *et al.,* 1992; Merkle *et al.,* 2014).

Individual memories can be split into short-term and long-term memories (Smid *et al.,* 2007). Short-term memories persist for only a short period and can occur after only a single exposure to a stimulus. Long-term memories, on the other hand, are more energetically costly, requiring the production of protein to facilitate long-term storage of the memory, and tend to only be laid down after multiple exposures to a stimulus (Smid *et al.,* 2007). In both cases the ability to lay down, but also forget or update a memory is important to the individual being able to display behavioural plasticity in a changing environment.

Similar to the importance of memories to an individual animal, in social animals accurate memories can be important to the survival of other group members. Memories are retained in social groups of animals in different ways. In certain groups, individuals may retain different memories with some individuals playing a greater role than others in guiding the groups based on past experiences, like matriarchs in a herd of elephants (McComb *et al.,* 2001) or a pod of killer whales (Brent *et al.,* 2015). In other social systems, like some ant colonies, memories are shared collectively through pheromone trails (Czaczkes *et al.,* 2015), as well as being retained at an individual level (Czaczkes *et al.,* 2015; Schwarz & Cheng, 2011). Both individual memories and shared pheromone memories are important to collective decision making in the ant genus *Temnothorax* (Bowens, *et al.,* 2013; Cao & Dornhaus, 2012).

Temnothorax albipennis is a valuable model system to understand collective decision-making (Dornhaus & Franks, 2006; Franks *et al.,* 2007; Pratt *et al.,* 2005). Surviving in rock cavities which are prone to degradation through erosion or weathering, *T. albipennis* colonies have to be able to make rapid collective decisions to emigrate quickly to more suitable environments (Visscher, 2007). The different stages of the emigration are well characterised: initially there is an exploration stage where scouts investigate nearby sites, then there is an initial stage of recruitment where scouts will lead other workers to the potential nest site through a series of tandem runs until a quorum of around 5-20 ants is reached at the new nest site (Pratt *et al.,* 2002). Following quorum being reached, the next stage of emigration is active transport during which adult ants, pupae, larvae and eggs are carried to the nest (Pratt *et al.,* 2002).

Emigration decisions are informed by the prior experience of scouting workers. During *T. albipennis* emigrations, colonies show bias for or against new nests based on their prior experience of that location (Franks *et al.,* 2007; Stroeymeyt *et al.,* 2011a; Burns *et al.,* 2016). If a nest is destroyed, the colony will favour emigrating to a novel site over a site which was previously familiar to them as low quality (Franks *et al.,* 2007). Conversely, if a colony is given the choice between a novel site or a site familiar to the colony as high quality, the colony will preferentially emigrate to the familiar site (Stroeymeyt *et al.,* 2010; Stroeymeyt *et al.,* 2011a). This demonstrates that collective decisions can be influenced by both positive and negative information about available options.

While knowledge of available nest site quality clearly influences colony decisions in *T. albipennis*, it is unclear if this species is able to update memories in cases where a nearby good nest is degraded or destroyed. In their natural environment this could easily occur if erosion causes a natural cavity to change shape or even break open entirely. It is also unclear whether these ants react differently to nests which have been degraded (but could still provide some shelter) compared to those that have been lost entirely (and therefore could no longer provide any shelter).

While both pheromone trails and individual memories are important to decision making in *T. albipennis* (Franks *et al.,* 2007; Stroeymeyt *et al.,* 2011a), in this study we will focus on individual memory over pheromone memory. Focussing on individual memory will allow us to manipulate the presence and absence of nests without the confounding element of some nests being marked with pheromones and other being unmarked. Therefore, this study sets out to investigate how memories held at the level of the individual within an ant colony may be updated in response to nearby nests either being degraded or removed, and how these memories in turn affect nest site choice when ants are given the choice of a poor quality nest in a novel location, or a poor quality nest in the location of the nest they remember the nest being degraded or removed. We set out to test the following three hypotheses:

(1) **Ants can 'forget' sites when they disappear**. Specifically, given the choice between a novel nest site and either i) a nest at the site of a nest that has previously been good then removed, or ii) a nest site which has been consistently good, ants will show preference for and move most quickly to the location of the consistently good nest site. (A vs C in Figure 1)

(2) **Ants can distinguish between a downgrade and a disappearance.** Specifically, given the choice between a novel nest site and either i) a nest site which has previously been good then degraded or ii) a nest at the site of a nest that has previously been good then removed, ants will show preference for and move most quickly to the location of the nest which has been degraded. (C vs D in Figure 1)

(3) **Ants avoid sites that have undergone a downgrade, all else being equal.** Specifically, given the choice between a novel nest site and either i) a nest site which has been good then degraded to poor or ii) a nest site which has previously been consistently poor, ants will show preference for and move most quickly to the location of the nest which has been consistently poor. (B vs D in Figure 1)

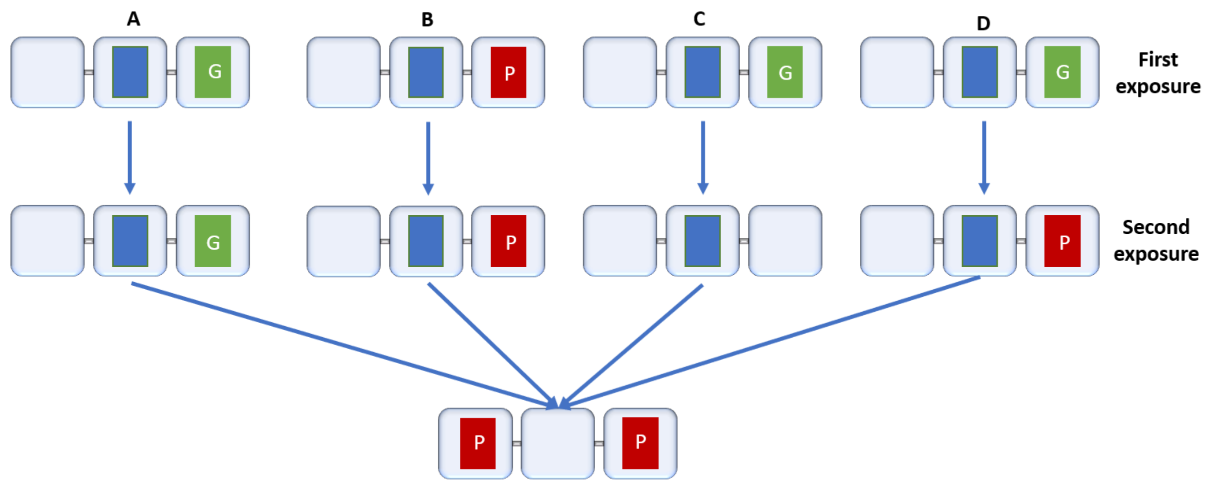


Figure 1: *This diagram shows the four different treatments of the experiment. Blue shows the original home nest, green shows good quality nests (G) and red shows poor quality nests (P). Control treatments are: consistently good (**A**), consistently poor (**B**). Change treatments are: good then removed (**C**), good then degraded (**D**). After exposure to the treatments, the home nest was destroyed, and the ants chose between two new poor-quality nests. For simplicity here treatment nests are depicted on the right; in the experiments their position was systematically varied.*

Hypothesis 1 represents negative contrast behaviour (Pellegrini *et al.,* 2004) whereby we would predict that the experience of a decrease in site quality would lead to a negative bias against the location. Hypothesis 1 predicts that ants will choose a nest site which they have consistently experienced as high quality over a nest site which was high quality but then the nest was removed, even though when the actual choice is made, both nests offered are poor quality. This involves comparing treatment A and treatment C on Figure 1.

Hypothesis 2 tests the constant update hypothesis (Clayton *et al.,* 2001) as it tests the ability of the ants to fully update their memories about what is available, and discriminate between a site which could still provide some shelter (even if it is not as good a nest than it was previously), and a site which would no longer provide any shelter. Hypothesis 2 predicts that the absence of any nest would be more aversive than a low-quality nest, and therefore ants should show a preference for the location of the degraded nest site over that of the removed nest site. This involves comparing treatment C to treatment D in Figure 1.

Hypothesis 3 tests a second form of negative contrast behaviour. It predicts that ants will show avoidance of the location of a nest which has been good, but then downgraded to poor, relative to their preference for a nest site which has been consistently poor, because if ants do show negative contrast behaviour, they should display negative bias against the degraded nest even though when the actual choice is made, both nests offered are poor quality. This involves comparing treatment B to treatment D in Figure 1.

IV.III Materials and methods

Power analysis

The power analysis for the experiment (Appendix I) was calculated with the programme G*Power (Buchner *et al.,* 2020) using results from Burns *et al.,* (2016) to provide effect sizes. The Burns *et al*. (2016) study was used to calculate the power analysis as there were similarities between their experimental design and the experimental design of this study. The Burns *et al*. (2016) study compared the house hunting behaviours of *Temnothorax albipenis* ants which were exposed to a nest site which constantly fluctuated in quality to the house hunting behaviours ants which were exposed to a nest site of consistently mediocre quality. While in this study the nest quality changed only once during the course of the experiment rather than consistently fluctuating, it was expected that the differences in house hunting behaviours would be likely to have a similar effect size as those observed in the Burns *et al.,* (2016) study. This analysis indicated a sample size of 32 would be sufficient for this study.

Collection

Sixty-seven colonies of *Temnothorax albipennis* were collected from the Isle of Portland in February 2018. These were maintained in the laboratory at 23-24°C and provided with water, 20% sucrose solution and mealworm pieces until the time of the experiment in October 2018. The light cycle was 12:12 during most of this period. Due to a fault in the temperature-controlled room, the light cycle was variable during parts of the experiment; however all ant colonies experienced the same light cycles.

Colony selection

For the experiment, 48 colonies were selected, on the basis that they had both brood and at least one queen. Colony sizes were estimated from photographs using ImageJ. In cases where brood was too densely stacked to accurately count individual items, the area of brood was calculated and divided by the average area of a prepupa (estimated from measurement of six prepupae). All colonies used in the experiment were queenright with the exception of one colony which lost its queen within three weeks of starting the experiment. Each colony was used for 2 trials: one change treatment (C or D in Figure 1) and one control (A or B in Figure 1).

Assignment of colonies to first trial

Experimental colonies were size matched between the four treatments (A-D in Figure 1). Colonies were sorted by size first by number of adult ants rounded to the nearest 10, then by number of brood items. The sorted list of colonies was then split into sections comprising four colonies, and within each section the four colonies were randomly assigned to one of the four treatments for the initial trial. The order of the use of each colony within a treatment was then randomised.

Assignment of colonies to second trial

Inter-trial timings were kept constant between colonies, i.e. colonies which had been used on the morning of day one of trial one, were also used in the morning day one of trial two; this was done to ensure intervals between each treatment were constant.

Colonies were assigned such that each colony experienced a change treatment trial and control treatment trial. Half of the colonies experienced change treatment trials first, and half experienced control treatment trials first. During trial one, two colonies escaped; these were replaced in trial two with size-matched colonies.

The arena

The experiment was conducted in a lidded arena made up of three 12cm x 12cm square boxes attached together using 5cm lengths of tube with a 0.5cm internal diameter (Figure 3A). Boxes were lined with Fluon and, along with the tubes, were cleaned with 70% ethanol prior to each trial.

The nests

Nests were made of 7.6cm by 5cm by 0.1cm plastic rectangles with a 3cm by 6cm cavity cut inside the plastic and a nest entrance 0.1cm wide. The nest piece was placed between two 7.6cm by 5cm glass slides to allow observation (Figure 3). In “good” nests an opaque piece of plastic was placed over the top of the nest and slides to make the interior of the nest dark; in “poor” nests the nests were left without an additional cover. In this experiment the home nest was always of “good” quality.

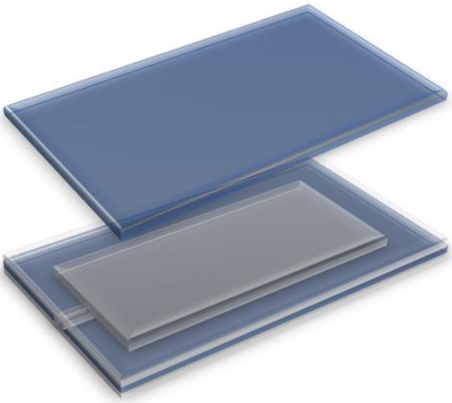


Figure 2: *Nest configuration made up of a plastic piece between two glass slides, the nest lid is made either from opaque plastic or an additional glass slide. In this diagram the opaque lid is lifted to show the internal structure of the nest.*

Treatments

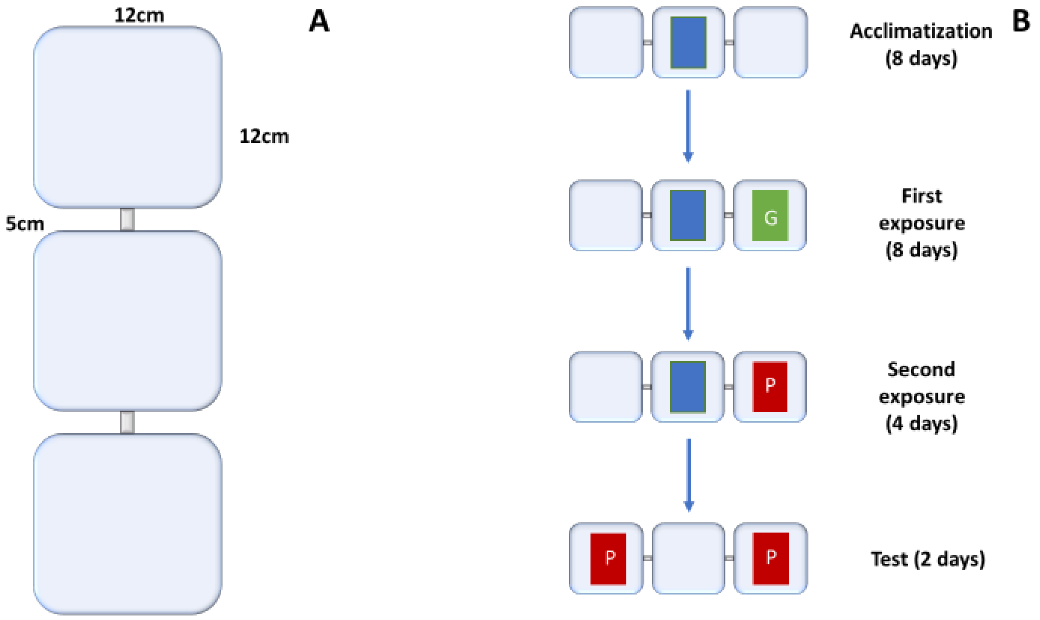


***Figure 3A:** Arena configuration made up of three 12cm by 12cm adjoining boxes connected by 5cm of tubing. **3B:** Timeline of the experiment with the acclimatization period, a first and second exposure and then the emigration test. The example illustrated here is treatment D (see **Figure 1**) but the same timeline was used for all treatments.*

Each trial began with an acclimatization period of eight days, at the start of which the ants were added to the arena and occupied the only nest available (this nest was always a "good" quality nest and will be referred to as the home nest). Following acclimatization, the ant colonies were exposed to either a change treatment or control treatment as shown in Figure 1.

In this experiment the nest site where the change treatment or control treatment nest was during the first and second exposure will be referred to as the familiar nest site. The box containing the familiar nest site will be referred to as the familiar box. The left/right position was of the familiar box was systematically varied between trials.

Emigration tests

On the test day of the experiment (day 20) a poor-quality nest, cleaned with 70% ethanol, was added to each of the two adjoining boxes (Figure 3A). Then the home nest was destroyed by removing the nest lid and nest-surround (see Fig 2) leaving the ants exposed on the base of the old nest. Any ants on the removed nest parts were gently brushed back into the central box using a soft paint brush.

The experiments were observed visually by an observer and the time that certain stages of emigration were reached was recorded as described in Table 1. The observations of emigration were continued for five hours after the home nest was destroyed. 48 hours after the destruction of the home nest the nests were visually re-checked to determine what the final nest choice of the colonies, in cases where the choices were not unanimous photos were taken of the nests to allow the number of ants in each nest to be counted.

Table 1: Stages of emigration in ants

Term	**Definition**
First entry into each adjoining box	*The time at which the ant is completely inside one of the adjoining boxes with no part of the ant still touching the joining tube*
First entry into each nest	*The time at which the ant is completely inside the nest*
First tandem run	*The time at which a tandem run occurs either in the adjoining tubes or the adjoining box containing the tandem run's destination nest following discovery of newly added nest.*
First active transport	*The time at which an ant carries an adult ant or brood item into the nest and the carrier places the carried ant or brood item down.*
Completion of emigration	*Defined as time at which last brood item is carried out of the central box*
Final state	*Defined as which nest site the colony emigrated to after 48 hours.*

IV.IV Analysis

Time data were analysed using survival analysis using the R package "Survival"(Therneau, 2015). The hazards structure of the data was tested and for our data the proportional hazards assumption was not met. The data were therefore analysed using Accelerated Failure Time models with a Weibull distribution.

To assess if there were differences in the proportions of colonies that chose the nest in a familiar location between treatments, two-tailed z-tests were used.

To test if colony size predicted the time for a colony to reach each of the following three stages of emigration - time to enter the familiar box, time to enter the familiar shelter, time to start performing tandem runs - three separate GLMs with gaussian error structures were carried out. For each of the GLMs, time to event was used as the response variable, while colony size was included as the predictor variable (as a fixed effect).

Figures were produced using “ggplot2”(Wickham, 2016). Transport events and time to complete emigration were available in only in a small number of cases (43 of 93 cases or 9 out of 93 cases respectively), so the decision was made not to analyse these further. In cases where colonies were split (eight additional cases) adult ants were counted from photographs using ImageJ and the nest with the highest number of ants was assigned as the chosen nest. For one trial, no data except first entry into each adjoining box was recorded, due to interruption by a fire alarm evacuation.

IV.V Results

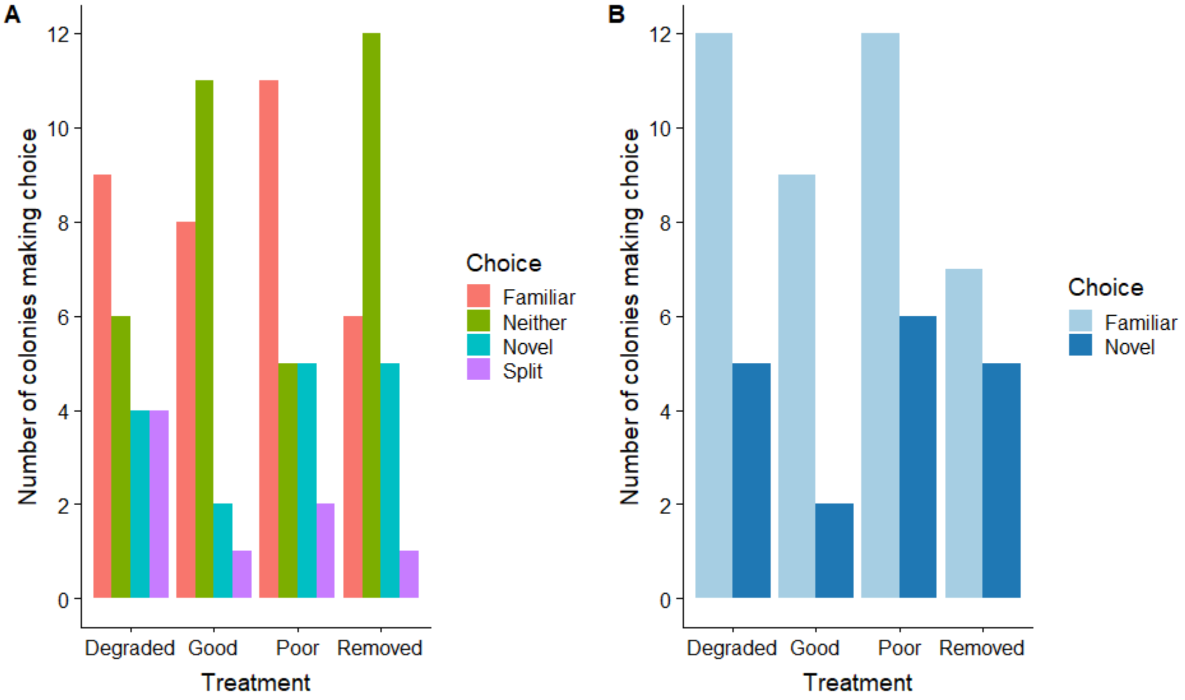


***Figure 4**: Colony choices after 48h. **A**: Number of colonies which chose either the familiar or novel nest, chose neither nest, or split the colony between the familiar and novel nests. **B**: Number of colonies which chose familiar or novel nests, including split colonies based on which contained more adult ants*

In some of the trials (34), colonies did not choose either nest, but rather stayed at the location of the destroyed home nest or moved into one of the corners of the home nest box or to the tubes which connected the home box to adjoining boxes, but in 51 trials the colonies did make a unanimous choice. In cases where colonies were split (eight additional cases) adult ants were counted after 48 hours, and the nest site with the highest number of ants was included in the analysis as the chosen nest site.

Hypothesis 1:

Ants can 'forget' sites when they disappear. Specifically, given the choice between a novel nest site and either i) a nest at the site of a nest that has previously been good then removed, or ii) a nest site which has been consistently good, ants will show preference for and move most quickly to the location of the consistently good nest site. (A vs C in Figure 1)

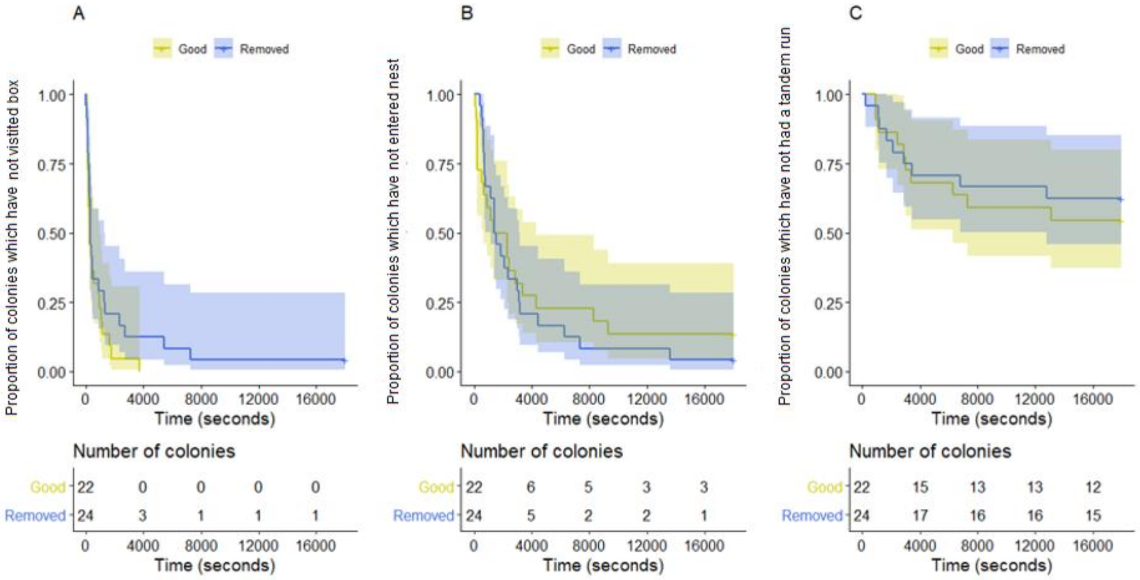


Figure 5: *Time for the colonies to perform the following behaviours: (**A**) visiting the box containing the familiar nest site, (B), entering the shelter at the familiar location, (**C**), performing the first tandem runs to the familiar nest site when the treatments are consistently good (yellow) or good then removed (blue). Confidence intervals (95%) are shown. + indicates censored data, i.e. event had not yet occurred at time 18,000s. Table shows event data.*

Following the destruction of the home nest, colonies took significantly longer to visit the box containing the removed nest site than the box containing the good nest site (Fig. 5A, AFT **z**= 2.04, **N**=46, ***p***= 0.0417), but there was no significant difference between the time taken to enter the nest site which was previously good or removed (Fig. 5B, AFT **z**=-0.85, **N**=46, ***p***= 0.396), nor to perform tandem runs to the nest site which was previously good or removed (Fig. 5C, AFT **z**= 0.52, **N**=46, ***p***= 0.606).

Forty-eight hours after the destruction of the home nest there was no significant difference between the proportions of colonies choosing the familiar or novel nest sites between the good then removed treatment and the consistently good treatments (Pearson's chi-squared test χ-squared=0.276, **N**=46, ***p***= 0.599) (shown in bar 1 and 2 of **Figure 4B**).In the treatment with the treatment where the nest was good then removed, 12/24 colonies chose the familiar (removed) nest site and the rest of the colonies chose the novel site. In the consistently good treatment, 9/22 colonies chose the familiar (good) nest site the remaining colonies chose the novel site.

Hypothesis 2:

Ants can distinguish between a downgrade and a disappearance. Specifically, given the choice between a novel nest site and either i) a nest site which has previously been good then degraded or ii) a nest at the site of a nest that has previously been good then removed, ants will show preference for and move most quickly to the location of the nest which has been degraded. (C vs D in Figure 1)

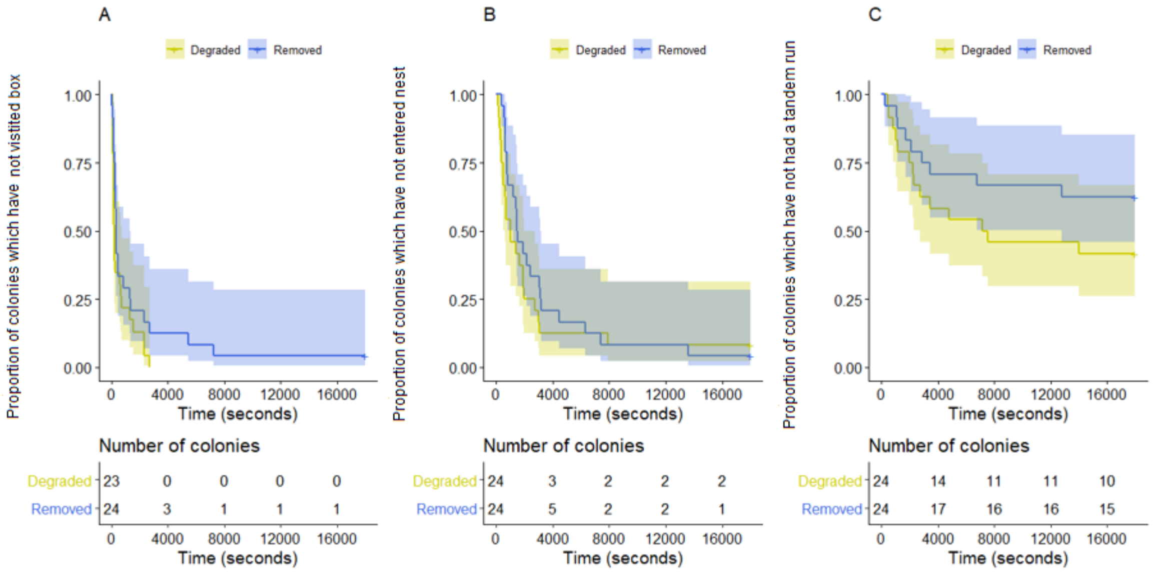


***Figure 6**: Time until colonies performed the following behaviours: (**A**) visiting the box containing the familiar nest site, (**B**) entering the shelter at the familiar location, (**C**) performing the first tandem runs to the familiar nest site when the treatments are good then degraded (yellow) or good then removed (blue). Confidence intervals (95%) are shown. + indicates censored data, i.e. event had not yet occurred at time 18,000s. Table shows event data.*

Following the destruction of the home nest, colonies took significantly longer to enter the box previously containing the removed nest site than the box containing the degraded nest site (Fig. 6A, AFT **z**= 2.22, **N**=47, ***p***= 0.0266) (one colony in the degraded treatment had an entry into the familiar box at time 0, this was not included in analyses as Accelerated Failure Time models require non-zero values – note that this removal is conservative, as including it would have made the observed effect stronger), but there was no significant difference in time to enter the nest at the site which was previously degraded or removed (Fig. 6B, AFT **z**= 0.39, **N**=48, ***p***= 0.7), or to perform tandem runs to the nest at the site which was previously degraded or removed (Fig. 6C, AFT **z**=1.46, **N**=48, ***p***= 0.144).

Forty-eight hours after the destruction of the home nest there was no significant difference between the proportions of colonies choosing the familiar or novel nest sites between the good then removed treatment and the consistently good treatments (Pearson's chi-squared test X-squared = 1.394, **N**=48, ***p***= 0.24) (shown in bar 2 and 4 of Figure 4B). In the good then removed treatment 7/24 colonies chose the familiar (removed) nest site and the rest of the colonies chose the novel site. On the other hand, in the consistently good treatment, 12/24 colonies chose the familiar (good) nest site and 11 colonies chose the novel site.

Hypothesis 3:

Ants avoid sites that have undergone a downgrade, all else being equal. Specifically, given the choice between a novel nest site and either i) a nest site which has been good then degraded to poor or ii) a nest site which has previously been consistently poor, ants will show preference for and move most quickly to the location of the nest which has been consistently poor. (B vs D in Figure 1)

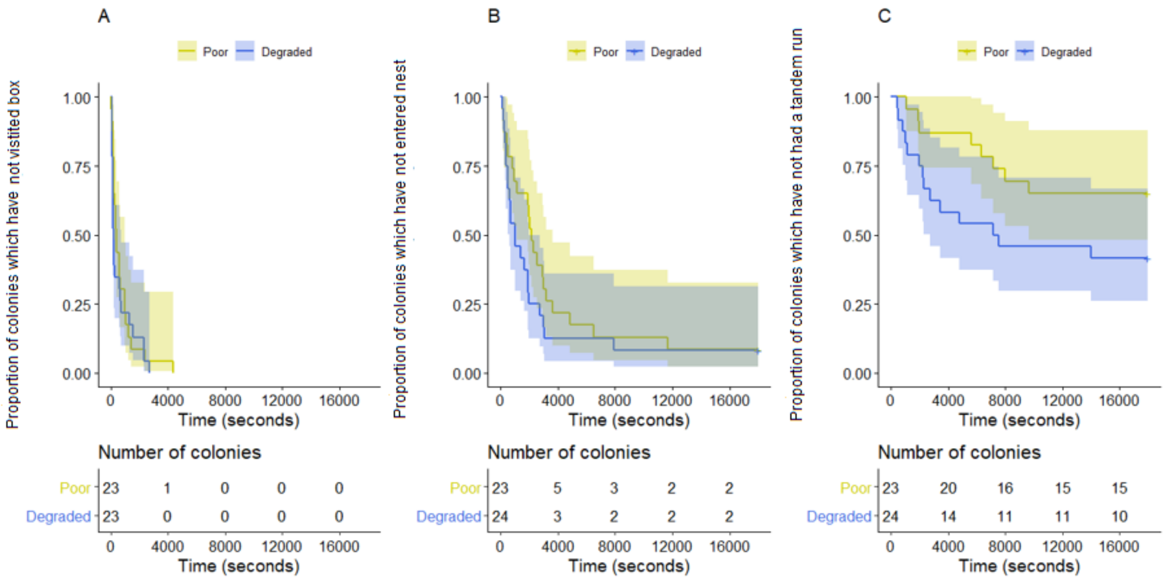


Figure 7*: Time until the colonies performed the following behaviours: (**A**) visiting the box containing the familiar nest site, (**B**) entering the shelter at the familiar location, (**C**) performing the first tandem runs to the familiar nest site when the treatments are consistently poor (yellow) or good then degraded (blue). Confidence intervals (95%) are shown. + indicates censored data, i.e. event had not yet occurred at time 18,000s. Table shows event data.*

Following the destruction of the home nest, there was no significant difference between the time taken for ants to enter the box previously containing the poor or degraded nest (Fig. 7A, AFT **z**= -0.54, **N**=46, ***p***= 0.59), to enter the nest at the site which was previously poor or degraded (Fig. 7B, AFT **z**= -0.91, **N**=47, ***p***= 0.3644), or to perform tandem runs to the nest at the site which was previously poor or degraded (Fig. 7C, AFT **z**= -1.75, **N**=47, ***p***=0.08). (One colony in the degraded treatment had an entry into the familiar box at time 0, this was not included in analyses as Accelerated Failure Time models require non-zero values).

Forty-eight hours after the destruction of the home nest there was no significant difference between the proportions of colonies choosing the familiar or novel nest sites between the consistently poor treatment and the degraded treatment (Pearson's chi-squared test X-squared = <0.0001, **N**=47, ***p***= 1) (shown in bar 2 and 4 of Figure 4B). In the treatment where the familiar nest was poor consistently, 12/23 colonies chose the familiar (removed) nest site and the rest chose the novel site. On the other hand, in the treatment where the nest was degraded, 12/24 colonies chose the familiar (degraded) nest site and the rest of the colonies chose the novel site.

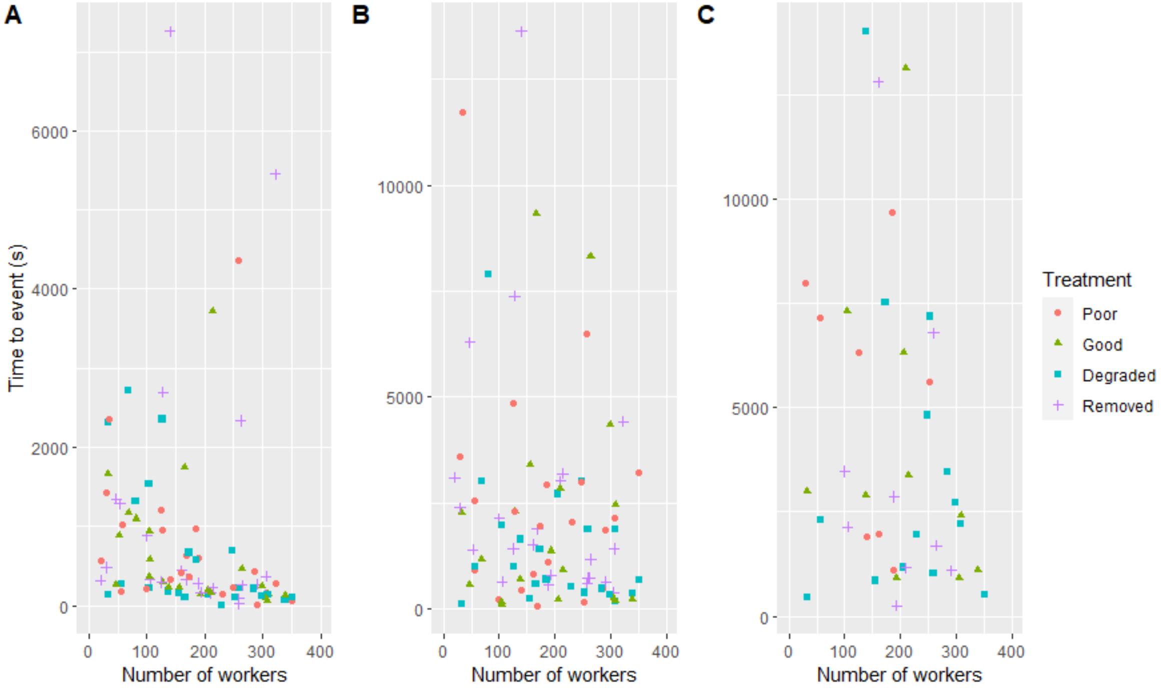


***Figure 8**: Relationship between colony size and time to (A) enter the box containing the familiar nest site, (B) enter the familiar nest site or (C) perform the first tandem runs to the familiar nest site.*

Colony size does not predict the first entry to the box containing the familiar nest site (GLM t=-1.761, df=91, ***p***= 0.0861). There is a borderline negative relationship between colony size and the first entry to the familiar nest site (GLM t=-1.977, df=79, ***p***=0.0516) which means smaller colonies took slightly longer to enter the familiar nest site. Colony size does not predict time to the first tandem run to the familiar nest site (GLM t=-1.508, df=40, ***p***=0.140 respectively).

IV.VI Discussion

In this study we found that ants have the ability to update outdated memories about house moving options over a period as short as a few days. We found some support for both our first and second hypothesis; namely that ants can “forget” nest sites when they disappear, and that ants can distinguish between a downgrade and a disappearance. However, we observed these effects only in the initial scouting behaviour of these colonies and not in recruitment patterns nor in final nest choice. We also found no evidence for our third hypothesis; namely that ants avoid sites that have undergone a downgrade. This result could instead indicate that ants update their memories after the downgrade and “forget” that the nest was ever of a good quality. These findings are interesting as they show that ants do have the ability to update their memories, but that in this experimental set-up these updated memories did not lead to an impact on their final nest choice.

Our first hypothesis (ants can “forget” sites when they disappear) is supported because ants take significantly less time to enter a box that had contained a good nest during their previous exploration than a box from which the nest had previously been removed. In both cases the other option was a novel nest. Interestingly this bias between formerly good and formerly removed nest sites was observed only in the initial exploration of the nest boxes and did not continue into later stages of recruitment or lead to a significant bias in final nest choice. This could be because both the actual nests present during the emigration were poor quality nests, and the ants rapidly updated their assessment during the emigration. Therefore, the initial head-start that the high-quality nest received from early discovery may not have been consolidated because scouts re-assessed the nest and found it to be poor.

Being able to remember then re-find nearby good quality nest sites, while forgetting sites which had been removed or destroyed, would benefit *T. albipennis* colonies as these memories would reduce search time for a new nest site and prevent the colony spending time searching for a nest site which no longer exists (Stroeymeyt *et al.,* 2011a). This ability to overwrite previously positive memories should be important in the ecological context of *T. albipennis* because this species lives in a changing environment where nearby rock cavities may be rapidly lost due to weathering. The ability to update positive memories has been documented in other taxa, for example bumblebees can update their memories and communication of foraging locations if they are moved (even if it takes several foraging trips to do so) (Chatterjee *et al.,* 2019), similarly Blue Jays (*Cyanocitta cristata*) overwrite their memories about cues associated with foraging and show more rapid learning when foraging conditions are changeable (Dunlap & Stephens, 2012). However, since memories about nearby nests would have adaptive advantages, it is unclear why initial bias observed in scouting did not lead to differences in final nest choice. It is possible that the lack of bias shown in site selection *T. albipennis* could be due to limitations of this study which are discussed later.

We also found some evidence for our second hypothesis: ants can distinguish between a downgrade and a disappearance. Specifically, we found that ants entered the box which had contained a degraded nest site faster than the box from which the nest had previously been removed. These data could be explained in several different ways. It could be the case that ants show bias against the removed nest, given the large drop in quality from good to removed (negative contrast effects like this has been observed in ant foraging (Wendt *et al.,* 2019)). Alternatively, ants could show a preference for a poor nest over no nest. Preference for a poor-quality nest over a removed nest would be adaptive as a poor-quality nest would still provide some protection for a colony while a destroyed nest would not. This finding is interesting, however, as previous studies have shown that ants will show bias against sites which were previously of worse quality than the home nest (Stroeymeyt *et al.,* 2011b) (which in this case the poor nest was) and this bias is not seen in this experiment. Similar to the first hypothesis, we found no evidence that this initial bias persisted into later recruitment or final nest choice.

We found no evidence for our third hypothesis: ants avoid sites that have undergone a downgrade, all else being equal. For this hypothesis we could predict either a hang-over effect with ants showing bias towards the shelter which was good before it was degraded, or we could predict a negative-contrast effect where the ants expecting a good quality nest may then show negative bias against the nest which was now degraded to a poor quality in comparison to a consistently poor nest. Ants do display contrast behaviour where the quality of a resource is judged relative to the expected quality of the resource (Wendt *et al.,* 2019). From this negative contrast behaviour at the individual level we could also expect bias at the colony level as, through a collective emergent effect, colonies show bias against neighbouring nests which are of a poorer quality than the home nest (Stroeymeyt *et al.,* 2011b). Contrast behaviour in house hunting can lead to maladaptive decisions with ants choosing novel nests which are of a worse quality than the familiar neighbouring nests the colony has become biased against (Stroeymeyt *et al.,* 2011b). While other studies have shown contrast behaviours in ants, in these results we see that ants behave the same way whether the nest has been degraded to a poor state or if it has been consistently poor. These findings are in line with the idea that *T. albipennis* update their memories about surrounding nest site conditions and overwrite inaccurate information over the course of a few days. This updating would have an advantage, because both the hangover effect and negative contrast behaviour could lead to maladaptive decision making.

The mechanism by which ant colonies remember surrounding nests relies on both individual memories and group memories shared through pheromone trails (Franks *et al.,* 2007; Stroeymeyt, *et al.,* 2011a). Individual memories are important because informed individuals with previous experience of surrounding sites recruit more quickly to good quality nearby sites than naive individuals, and have a disproportionate effect on recruitment, leading to a bias in recruitment towards the good familiar nest sites (Stroeymeyt *et al.,* 2011a). Furthermore, this biased recruitment to a good quality familiar nest still occurs (though at a slower speed) when pheromone cues are rotated, suggesting a key role for individually retained memory over pheromone trails in house hunting behaviour (Stroeymeyt *et* *al.,* 2011a). However, other work highlights the importance of pheromone cues over individual memories during house hunting (Franks *et al.,* 2007). As well as being used for recruitment, during house hunting pheromone cues may also act as negative markers against substandard nest site choices (Franks *et al.,* 2007), a phenomenon observed in ant foraging trails (Robinson *et al.,* 2005). This is shown by the way in which removal of pheromones or re-orientation of visual cues results in negative bias against familiar mediocre nests sites being lost, and ants showing random choice during house hunting (Franks *et al.,* 2007). These examples demonstrate how in different contexts individual memory and pheromone trails may play different roles in house hunting, and that both of these need to be considered when interpreting how house hunting behaviours may have emerged from the decisions of individual workers in a colony.

In this study we found that, while ant colonies did show experience effects resulting in an initial bias in which areas were explored first, the bias did not translate to recruitment or final nest choice. Certain limitations of our experimental set up could play a role here. To make more equal our comparison between treatments in which the nests were removed (and therefore the pheromones in that nest would have also been removed) or not removed (and therefore the pheromones on that shelter would have been left intact), all the familiar nests were replaced with new nests prior to the ants making their choice, and the boxes containing both the familiar nest site and novel nest site were cleaned with ethanol. However, the pheromone trails were left intact in the home nest box. In the experiment, the ants reached the box containing the consistently good familiar nest site (which could have been located through following the intact pheromone trails in the home nest box) more quickly than the unfamiliar box. In contrast, they did not reach the actual nest (where pheromone cues were absent) more quickly. It could therefore be the case that in this case pheromone trails play an important role in remembering the location of a good nearby nest site (Franks *et al.,* 2007), and in forgetting nearby nest sites which are good then removed. Further work both removing the pheromones in the box containing the home nest and leaving all the pheromone trails intact would have to be done to determine what is underlies the behaviour observed.

The phenomenon of making judgements about the value of a resource based on memories has been observed in other taxa. For example negative contrast effects have been shown in multiple vertebrate species from fallow deer (*Dama dama*) (Bergvall *et al.,* 2007) to rats (*Rattus norvergicus*) (Pellegrini & Mustaca, 2000). In many of these experiments animals which are presented a lower quality food after being presented with a higher quality food will consume less of the poorer quality food, than if they had not previously been presented with the higher quality food (Bergvall *et al*., 2007; Flaherty *et al*., 1994; Pellegrini & Mustaca, 2000). In these examples it is likely that animals are using memories to predict future outcomes (McNamara *et al.*, 2013), for example if previous food options had been good then it is likely that other future food options will be good, therefore a poorer option can be avoided, conversely if previous foraging opportunities have been poor the future opportunities are likely to be poor therefore a poor option should not be avoided (McNamara *et al*., 2013). It has been suggested that this ability to use memories to determine the relative value of resources, and therefore show contrast behaviours is be beneficial in changeable environments where the quality of resources may vary over time, or when there is local choice of different food resources to choose from (Bergvall *et al*., 2007; McNamara *et al*., 2013). Furthermore, that the extent to which contrast behaviour is displayed is hereditary and is likely to be under selection (Flaherty *et al*., 1994). It could be the case contrast behaviour observed in this experiment could be linked to the changeability of the environments inhabited by *Temnothorax albipennis*, and their ability to adapt to them; however, this also raises interesting questions about the heritability and selection for contrast behaviours at the level of both the individual and the colony in eusocial animals.

IV.VII Conclusion

In conclusion, in this experiment we address questions about how ants use memory to influence group-level decision making and ask whether ants can use individual memory to show bias against options that have decreased in quality. Our findings show that ant colonies are able to update their memories about the presence or absence of nearby good and degraded nest sites, but interestingly there was limited evidence that these memories influenced recruitment and decision making. These findings highlight how there are still many important questions to be addressed in understanding the link between individual and group level memories, both in eusocial animals like ants, as well as in animals with other social structures. For example, how do animals prioritize using individual or group level information in different environments, is there variation between individuals about how efficiently individuals forget or update memories, or are there personality syndromes which link inter-individual variation in learning ability and memory retention? Overall, this is still a developing area of research which has much to contribute in terms of our theoretical understanding of the role of individual variation in social animals.

IV.VII References

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IV.VIII APPENDIX I:

We used G*Power (Buchner *et al.,* 2020) to estimate the sample size needed to carry out this experiment. We carried out an a priori analysis using a Wilcoxon Mann-Whitney test. We carried out a 2-tailed analysis with an error probability of 0.05, a power of 0.8 and a normal distribution. We used effect size data from Burns *et al.* (2016), specifically, we used the differences in the number of tandems runs before quorum between a fluctuating environment (median=5, interquartile range=6) and a constant environment (median=0, interquartile range=0). Based on these figures the G*Power program showed that for a paired test a total sample size of 16 was needed, 8 per treatment. As we were carrying out three paired tests, we estimated that we would need at least 32 trials (8 for each change treatment or control treatment).

Chapter V: New directions for research in invertebrate personality: applications to vertebrate translocation studies?

V.I Abstract

Personality plays a key role in vertebrate translocation success in both wild-to-wild and captive-to-wild translocations. Greater understanding of the role personality plays in translocation has important implications for conservation as well as for our theoretical understanding of personality in different ecological settings. Vertebrate translocation studies are often constrained by small sample sizes, slow generation times and other practical considerations. It would therefore be beneficial to develop complementary systems to study the role of personality in translocation. One possibility is to explore how non-vertebrate systems can provide insights into the way in which personality affect translocation as well as adaptation of behaviour to captivity. Similarities and differences in the personality may play in invertebrate and vertebrate systems during captive breeding and translocation could guide the development of appropriate invertebrate model systems, to better understand the potential importance of personality in these contexts. We argue that invertebrate research and translocations are often not as constrained by the limitations of vertebrate systems and could therefore provide a powerful tool for developing a theoretical framework to understand the role of personality in translocation success. We propose that applying the study of personality in invertebrates to the field of reintroduction biology has the potential to provide new insights not only into the study of reintroduction and translocation, but also a greater understanding of the role of personality in different ecological contexts.

V.II Introduction

One factor increasingly recognised as potentially important to translocation is behavioural variation (Richardson *et al.,* 2017; Sinn *et al.,* 2014). Behavioural variation which shows consistency across time and multiple contexts, for example an animal being consistent in the way they explore and response to a novel object over time, can be referred to as personality (Biro & Stamps, 2008). Personalities in both vertebrate (Bergmüller & Taborsky, 2010; Zablocki-Thomas *et al.,* 2019) and invertebrate systems (Kralj-Fišer & Schuett, 2014; Parthasarathy *et al.,* 2019), vary between individuals even within one population. A few illustrative examples of personality traits in invertebrates include the bold-shy axis of the beadlet anemone (*Actinia equina*) (Briffa & Greenaway, 2011), and the colony level personality in the ant *Aphaenogaster senilis* (Blight *et al.,* 2016). Personality appears to be derived in part from a heritable component (Carere *et al.,* 2005; Zablocki-Thomas *et al.,* 2019), but also can be modulated by conditions like stress and environmental enrichment during development (Aspaas *et al.,* 2016; Boogert *et al.,* 2014; Jimeno *et al.,* 2019). There is still debate however, over the benefit of being constrained to a particular behavioural phenotype over different environments rather than reacting to each stimuli independently (Wolf & Weissing, 2012). Multiple hypotheses have been put forward to explain this phenomenon (Bergmüller & Taborsky, 2010; Biro & Stamps, 2008, 2010). In both vertebrates and invertebrates, it is thought that personality can be either beneficial or detrimental (Cole & Quinn, 2014; Sinn *et al.,* 2014) in different conditions; an effect that makes personality potentially an important consideration when assembling a founder group for translocation.

Translocations, including captive-to-wild and wild-to-wild translocations can provide key insights into a range of ecological questions (Bremner-Harrison *et al.,* 2004; Haage *et al.,* 2017; Hare *et al.,* 2020), as well as being crucial tools in the conservation of vertebrates (Griffith *et al.,* 1989; Hare *et al.,* 2020), invertebrates (Amaral *et al.,* 1997; King & Balfour, 2020; Shepherd & Debinski, 2005; Wynhoff, 1998) and plants (Abeli & Dixon, 2016; Zimmer *et al.,* 2019). While many of these translocations may be primarily for conservation purposes (Johnson *et al.,* 2010; Larter *et al.,* 2000), studying these translocations can also provide theoretical insights into how aspects of behaviour like personality noted in captivity may have implications for life history in the wild (Bremner-Harrison *et al.,* 2004; Haage *et al.,* 2017; Hare *et al.,* 2020).

Work on translocation and captive care has already provided insights into animal personality during captive care through work demonstrating a link between behaviour and post-release behaviours in vertebrates (Richardson *et al.,* 2017; Sinn *et al.,* 2014), as well as changes in behaviour associated with captivity (Hare *et al.,* 2020; McDougall *et al.,* 2006). However, studies of vertebrate captive breeding and translocation, while highly valuable, can face some key limitations. Understanding how personality may change with captivity may be restricted by both slow generation times and the permissible interventions for both ethical and practical reasons. Furthermore, understanding the role of personality on translocation success may be hampered by small release numbers. All these constraints often mean that results have limited power and can be challenging to interpret.

Invertebrate translocation studies have the potential to contribute to our understanding of the link between personality through both wild-to-wild and captive-to-wild translocations. With appropriate systems selected to study particular processes, invertebrate systems offer tremendous potential to understand multiple considerations of translocation and captivity, including personality.

This is not to say that invertebrate studies could replace those done on vertebrates and there are many cases where invertebrates studies would not improve our understanding of vertebrate translocations. For example, studies on the physical and economic practicalities of translocation, like descriptions of translocations of the methods for Bolivian river dolphins (*Inia boliviensis*) (Aliaga-Rossel & Escobar-Ww, 2020), or analyses of the financial costs of carnivore translocations (Weise *et al*., 2014) are specific to a species or group of species which would mean broad inferences from invertebrates would not be useful. Similarly studies on taxon specific physiology or behaviours associated with translocations can be carried out only on the taxon of interest. For example, work on brushtail possums (*Trichosurus*) has highlighted how possums are physiologically adapted to different geographic areas, which may affect decisions about translocation (Cooper *et al*., 2018). These examples highlight how taxon specific translocations studies can provide key information with important conservation implications.

While there are many elements of vertebrate translocations which could not be inferred from other systems, it would be beneficial to explore the areas which could benefit from extrapolating findings from more tractable study systems, including invertebrates. Examples of areas where our understanding of translocation of vertebrates could benefit from studies on invertebrates include our understanding of broad theoretical questions about translocation, like the link between captive breeding and changes to behaviour and physiology and what this would mean to translocation (Dojnov *et al*., 2012; Gilligan & Frankham, 2003; Lewis & Thomas, 2001;Frankham & Loebel, 1992), or how individual personality affects the behaviour of a group (Planas-Sitjà *et al.,* 2018) and whether that could affect translocation outcomes, or post-translocation behaviour.

We therefore aim to suggest how behavioural studies of invertebrates associated with the translocation of individuals could provide benefits to the field of reintroduction biology, while also providing greater insights into the role of personality in different contexts. Through this, we hope to spark discussion about the potential benefits that the study of invertebrate behaviour could bring to both invertebrate and vertebrate conservation. We will first discuss the benefits of studying personality in invertebrate systems which lend themselves to large sample sizes, we will then go on to discuss the feasibility of recognising and recapturing individual invertebrates in the laboratory and the field.

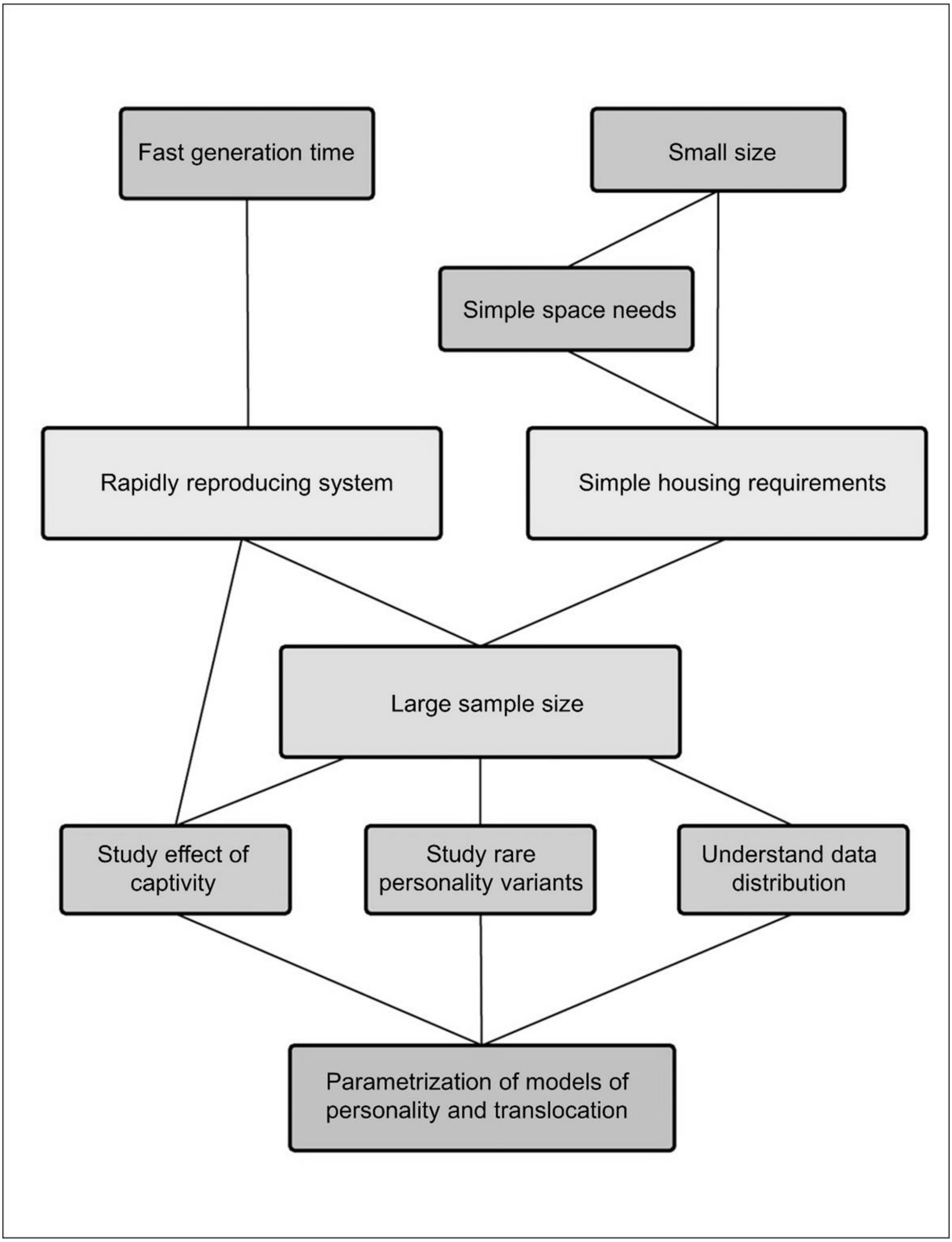

Figure 1: *Framework of the benefits of using invertebrates for studies on the impact of personality on translocation outcome. The small sizes and modest housing requirements of many species of invertebrate could make them useful models to study translocation*

The benefits of using invertebrate translocations to study animal behaviour

To understand how wild-to-wild or captive-to-wild translocation may be affected by personality, it is important to have a sample which captures personality variation across natural or captive populations of the study species (see Box 1). Personality are multivariate measures typically calculated from two or more behavioural assays (Aplin *et al.,* 2013; Aplin *et al.,* 2014). The multivariate nature of these data result in the potential for a huge range of complex personality profiles. Hypothetically, if there were strong selection for one behaviour type, there would be little variation in behavioural profiles and small sample sizes might be appropriate. In reality, in most cases, vertebrates and invertebrates do show a range of profiles, likely due to a variety of different biological factors (Wolf & Weissing, 2012). Small samples can therefore lead to errors in accurately determining the distribution of data, as well as resulting in missing rarer, but ecologically important, variants.

It is important to establish that the critical details contained within personality distributions may not be accessible using meta-analysis. For multiple studies on the same species, with personality trait data collected in a similar way, meaningful meta-analysis may be possible, however, it is unusual for data of this type to be available (Stewart, 2010). Meta-analysis of personality in multiple species on the other hand would be highly challenging if not impossible. Given that species-specific differences in distributions of personality traits are not fully understood, this could lead to the importance of particular variants in different species being overlooked. This is particularly important in the study of captive animals being prepared for translocation, as there is even greater variance in the behaviours of captive than wild animals (McPhee & Silverman, 2004), given relaxed selection pressures associated with captive management allowing perpetuation of less beneficial behavioural variation.

Small available sample sizes can be a significant drawback to understanding personality variation in translocation studies on vertebrates. In the two recent IUCN Global Reintroduction Perspectives papers (Soorae, 2013, 2016), 98 cases of animal translocations were collected (in this analysis of these papers, multiple species or subspecies within one case study were treated as separate cases, and analysis is done on final release numbers rather than number initially transported). Of the 98 cases, 80 reported the numbers of individuals released. Within cases which reported release number, the best represented groups were mammals and birds (38 and 21 studies respectively), while other groups were less well represented, (reptiles (9), fish (6), amphibians (3), invertebrates (2)). Given that among mammals, birds and reptiles, a few cases have a sample size of hundreds of individuals, while a large proportion of the studies had release numbers under 50 individuals (63%, 47% and 44%) respectively, the median is reported rather than the mean. The median numbers for release were as follows: mammals (34), birds (50), reptiles (60), fish (786), amphibians (4110) and invertebrates (881). The very high numbers of fish and amphibians is largely accounted for by the release of eggs, tadpoles and young juveniles. The use of eggs and young juveniles of fish and amphibians could present challenges to using these systems to study personality, as pre-release behavioural testing and long-term tagging may be difficult, particularly in cases where young metamorphose. In the invertebrate studies on the other hand, to our knowledge, most of the individuals released were in their adult stage before release, it would therefore be possible to permanently mark individuals prior to release in one of a variety of ways (Davy-Bowker, 2002; Sendova-Franks & Franks, 1995; Weslien & Lindelow, 1990) Invertebrate translocation projects have been encouraged to have large release sizes, as this has been identified as a crucial factor in the success of the translocation project (Bellis *et al.,* 2019) invertebrates could therefore be beneficial in facilitating studies on translocation with large sample sizes which could allow a fuller understanding what role personality may play in translocation.

The practical benefits of using invertebrate systems does not mean that invertebrate studies should replace vertebrate studies, rather they could be seen as an additional opportunity to understand personality and a useful tool to inform and support vertebrate studies. For example, the feasibility of invertebrate studies could allow investigation of models of different types of personality axis (Watanabe *et al.,* 2012) or even frameworks linking particular personalities with particular conditions

(Aspaas *et al.,* 2016; Segev & Foitzik, 2019). Models like these could be developed and parameterised in invertebrate systems providing key insights into animal personality.

Box 1: Why is a large sample size important for understanding personality?

Case study:

Great tit (*Parus major*) personalities are seen as multi-dimensional. The bold-shy axis is determined through a range of assays that can include: explorative behaviour, reaction to a novel object, reaction to conspecifics. These are continuous values, resulting in a high number of potential personality profiles. In great tits there is broad variation in behaviour, which is thought to be due to different personality profiles representing different successful behavioural strategies (Cole & Quinn, 2014).

If there is little variation in personality profiles, then small samples may capture the variance. However, many studies on personality have shown a wide spectrum of personality types, reflecting that there are multiple potential successful behavioural strategies in one population. With multiple personality types, distribution will be more spread as shown in the hypothetical example shown in Figure 2, and poor sampling may not provide capture enough information to be representative of the true profile. This is particularly true in complex, multidimensional animal personality data.

Application:

If a model were developed to predict post-release survival of animals accounting for a personality type, the model would be seeded with a starting distribution of behavioural phenotypes. Parameterising such a model from data that under-samples behavioural variants could miss key individual profiles that may be particularly ecologically important, and thus lead to inaccurate model predictions.

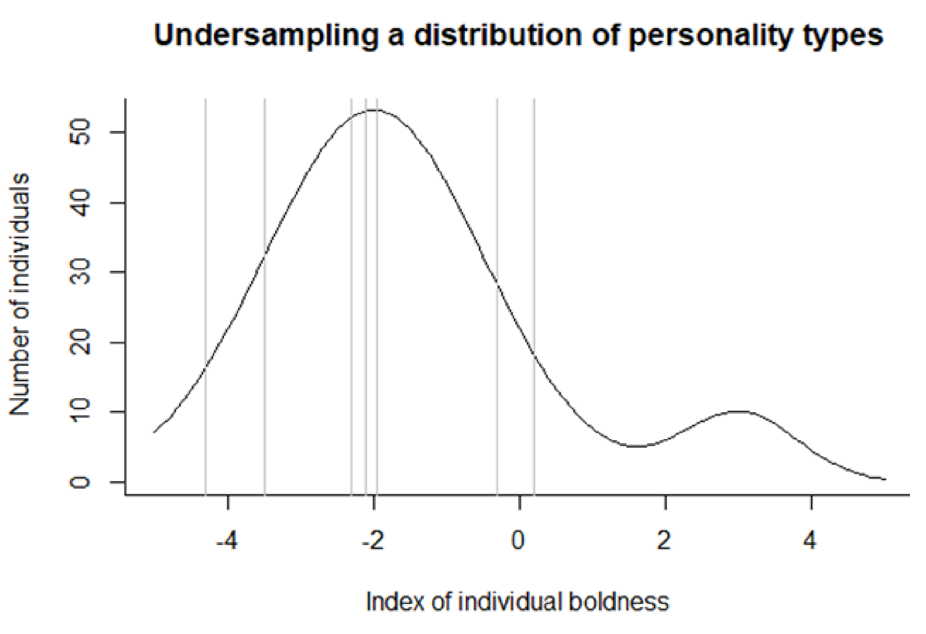

Figure 2: *The risks of under sampling data with an unknown distribution. The distribution represents variance of personality types in a population, the grey lines represent sampling of seven individuals from the population. This representation demonstrates how under sampling can result in rarer variants being missed, leading to an inaccurate understanding of the distribution of personality types. In real studies there are often far more dimensions to the personality making appropriate sample sizes highly important.*

V.II.I Benefits of studying invertebrates to understand adaptation of personality during captivity

<u>Space requirements</u>

Many groups of invertebrates have very modest requirements for ethical captive care (see Figure 1). Modest requirements for care can be clearly illustrated with the model organism the fruit fly (*Drosophila melanogaster*). In the Gilligan and Frankham 2003 study which investigated genetic changes associated with captivity, 1000 fruit flies were split to groups of 50, and each group kept in a 270ml bottle (Gilligan & Frankham, 2003). Similarly, work on burying beetles looking at adaptation to changed rearing conditions have maintained beetles after eclosion in 12 cm x 8 cm x 2 cm boxes (Schrader *et al.,* 2015). These small space requirements allow studies to be unconstrained by the problems with fixed enclosures that are often a caveat of captive vertebrate zoo studies, and also make the possibility of larger sample sizes far more feasible to possible in a captive setting than may be feasible with vertebrate studies. It cannot be overstated how important sample size is in meaningfully sampling the tails of the distribution (Box 1).

Despite increasing awareness of the importance of ethical approaches to working with invertebrates, there are no widely-adopted ethical guidelines for minimum space requirements for most invertebrate species (Drinkwater *et al.,* 2019; Horvath *et al.,* 2013). The lack of clear guidelines means that there needs to be careful consideration of appropriate rearing conditions during the translocation process (Drinkwater *et al.,* 2019; Horvath *et al.,* 2013). In certain species like the sweet potato weevil (*Cylas formicarius*) a lack of difference in behaviour have been found between mass reared and wild weevils (Kuriwada *et al.,* 2010, 2014), suggesting that in this species limited space during rearing does not have negative implications for development. *Drosophila melanogaster* (Sokolowski *et al.,* 1997) on the other hand evolves different foraging behaviours when raised with different fly densities, showing that differences in individual space availability may impact behaviour, which would need to be considered when planning the space requirements for invertebrate behaviour studies. These considerations, while important, should not reduce the potential of many species of invertebrate as model systems. Similar considerations of rearing conditions and density would have to be made with vertebrates, and in many cases the space requirements of many model invertebrate systems would still be significantly smaller, more feasible and less costly than those required by vertebrate systems, allowing greater sample sizes (Box 1).

The limited space requirements of many invertebrate systems contrast with many cases in studies on captivity on vertebrates, particularly larger animals, where the experimental design is dependent on the practical and ethical constraints of the study system, a key consideration of which is space. For example, maned wolf (*Chrysocyon brachyurus*) personality assays have been carried out in enclosures of different sizes containing different habitats and enrichment due to the fixed enclosure structure of the zoo (Silva & Azevedo, 2013). Despite efforts to carry out the work to the highest possible standard, lack of standardisation of enclosure size could present limitations to experiments investigating personality. The need for standardized enclosures, animal housing facilities and licencing may also mean that carrying out studies on adaptation to captivity in vertebrates may be practically or financially inhibitive to many research groups. Invertebrate studies, on the other hand, could provide an additional and feasible tool to gain more insights into the adaptation of personality traits to captivity.

Fecundity

In addition to the benefits of limited space requirements, some species of invertebrate, particularly established study species like *Drosophila melanogaster*, are known for their short generation times, which is far shorter than many vertebrate systems. The generation times of *Drosophila melanogaster* has already led to multiple advances in our knowledge of genetic adaptation to captivity (Frankham & Loebel, 1992; Gilligan & Frankham, 2003). However, there are still important gaps in our knowledge as to how captivity may alter the personality of an animal over time. Given the precedent for invertebrate work to better understand adaptation to captivity (Archard & Braithwaite, 2010; Dojnov *et al.,* 2012; Frankham & Loebel, 1992; Lewis & Thomas, 2001; Olzer *et al.,* 2019), there could also be scope for the use of invertebrate studies to better facilitate our understanding of how animal personality may adapt to captive conditions.

V.II.II Feasibility of studying invertebrate personality in the field

Recapture

Despite the discussed benefits of studying personality of invertebrates during translocation there may be concerns over the feasibility of conducting behavioural studies on invertebrates, particularly concerning the recapture and identification of individuals. While translocation studies may be challenging in certain invertebrate groups, there are a wide range of invertebrates with traits which would make them highly suitable to be feasible models for invertebrate translocation (see Table 1).

Traits which would be beneficial for studies of personality in invertebrates in the field studies include: (1) Conspicuousness: invertebrates like certain species of butterfly (Fred & Brommer, 2015; Harris, 2008) or dragonfly (Bried & Ervin, 2006) can be identified at a distance. (2) Fixed location: species like social spiders (Burgess, 1976), bumblebees (Svensson *et al.,* 2000) and social wasps (Borges *et al.,* 2017) build fixed webs or nests respectively which can be monitored while the site is maintained. (3) Attraction to traps: moths (Baker & Yvonne, 1978; Beck & Linsenmair, 2006), beetles (Shore & McLean, 1988) and butterfly groups like *Nymphalidae* (Mas & Dietsch, 2003) are attracted to light traps, pheromone traps, or baited traps respectively. (4) Limited dispersal: species limited to fixed habitat boundaries like rock-pools (Briffa & Greenaway, 2011), ponds (Davy-Bowker, 2002) or specific host plants (Chapman *et al.,* 2007). (5) Slow dispersal: beetles like the milkweed beetle (*Tetraopes tetraophthalmus*) show very slow dispersal (McCauley *et al.,* 1980), which would mean monitoring effort would be confined to a limited area.

These examples demonstrate just a few of the ways in which many species of invertebrate have traits which would make them feasible models to understand translocation. Therefore, in addition to the discussed benefits of invertebrate groups allowing high sample sizes, ease of housing (in captive-wild translocations), and in some cases high fecundity, many groups of invertebrates have traits which would facilitate post-release monitoring.

Table 1: Examples of invertebrate studies investigating translocation and adaptation to captivity, show the feasibility of these types of study:

Authors	*Species*	*Area*
(Vaughan-Higgins *et al.,* 2016)	Short-haired bumblebee (*Bombus subterraneus*)	Disease risk analysis for the reintroduction of the short-haired bumblebee
(Hannon & Hafernik, 2007)	Damselfly (*Ischnura gemina*)	Reintroduction of the rare damselfly *Ischnura gemina* (Odonata: Coenagrionidae) into an urban California park.
(Watts *et al.,* 2008)	Weta (Orthoptera: Anostostomatidae)	History of weta (Orthoptera: Anostostomatidae) translocation in New Zealand: Lessons learned, islands as sanctuaries and the future
(Green, 2005)	Auckland tree weta (*Hemideina thoracica*)	Using artificial refuges to translocate and establish Auckland tree weta *Hemideina thoracica* on Korapuki Island, New Zealand
(Amaral *et al.,* 1997)	The American burying beetle (*Nicrophorus americanus*)	Conservation Status and Reintroduction of the Endangered American Burying Beetle
(Phillips *et al.,* 2009)	Crayfish (*Orconectes virilis*)	Experimental reintroduction of the crayfish species *Orconectes virilis* into formerly acidified Lake 302S (Experimental Lakes Area, Canada)
Examples of papers using invertebrates to investigate impacts of captive care on adaptation		
(Dojnov *et al.,* 2012)	Longhorn beetle *Morimus funereus* (Coleoptera: Cerambycidae)	Adaptations to captive breeding of the longhorn beetle *Morimus funereus* (Coleoptera: Cerambycidae); application on amylase study
(Gilligan & Frankham, 2003)	Fruit fly (*Drosophila melanogaster*)	Dynamics of genetic adaptation to captivity

(Lewis & Thomas, 2001)	Large white butterfly (*Pieris brassicae*) (L.)	Adaptations to captivity in the butterfly *Pieris brassicae* (L.) and the implications for ex situ conservation
(Frankham & Loebel, 1992)	Fruit-fly (*Drosophila melanogaster*)	Modelling problems in conservation genetics using captive *Drosophila* populations: Rapid genetic adaptation to captivity
(Hammer *et al.,* 2014)	Butterfly (*Heliconius erato*)	Metamorphosis of a Butterfly-Associated Bacterial Community

Individual identification

Similar to concerns over recapture, there may also be concerns over the feasibility of re-identification of the same individual invertebrate. However, there are a plethora of methods which make re-identification of individual invertebrates simple and achievable.

Classical methods of differentiating between individual invertebrates include paint marking (Sendova-Franks & Franks, 1995), gluing visual markers or numbers (Davy-Bowker, 2002), and clipping or hole-punching the invertebrate (Boiteau, 2005). These methods have proved effective in both the field and the laboratory and have provided important insights into population dynamics (Davy-Bowker, 2002), spatial movement (Auckland *et al.,* 2004), and behavioural ecology (Baguette *et al.,* 1998). More technologically advanced options include harmonic radar (Makinson *et al.,* 2019) and radio-telemetry (Vinatier *et al.,* 2010), the tags of which can either work passively or actively. Passive radio-tags can be as light as 89µg including the adhesive (Robinson *et al.,* 2009). These tags appear identical, but each encodes a unique ID. These tags have the benefit of allowing double blind studies on invertebrates that are indistinguishable from each other to the eye, a process that may be more difficult in vertebrate studies in which individuals often have distinctive markings or characteristics. Active tags, while heavier than passive tags, transmit positional data as well as individual identification, allowing researchers to accurately follow long-term movements, for example in bee foraging behaviour (Hagen *et al.,* 2011).

Overall, there are multiple well established methods to identify individuals within one species (Boiteau, 2005; He *et al.,* 2019; Robinson *et al.,* 2009; Sendova-Franks & Franks, 1995), which make both studies on personality in captivity and monitoring post-release translocation feasible in invertebrates. In certain cases, appearance similarities within a species can even be beneficial by helping researchers to avoid unconscious bias in recording, particularly if a tagging method like radio telemetry is used.

V.II.III Is it feasible to use invertebrates to understand adaptation of personality to captivity?

For captive-to-wild translocations it is important to consider the effects which captivity may have on the biology and behaviour of the animal during captivity. Invertebrate studies have already provided key insights into adaptation to captivity, particularly into the genetic adaptations to captivity (Frankham & Loebel, 1992; Gilligan & Frankham, 2003), allowing development of models to better understand adaptation to captivity (Frankham & Loebel, 1992; Olzer *et al.,* 2019), as well as insights into how environmental enrichment during development may change behaviour of an individual (Aspaas *et al.,* 2016). There are still gaps in our knowledge however as to how personality may be modulated by captivity.

Both individual (Briffa & Greenaway, 2011; Muller *et al.,* 2010; Pamminger *et al.,* 2014; Parthasarathy *et al.,* 2019), and group level behaviour (Jandt *et al.,* 2014; Pinter-Wollman *et al.,* 2012; Segev & Foitzik, 2019) have become widely researched areas in invertebrate behaviour studies. These studies have been effective in developing both standardized methods of measuring personality in a range of different species and developing our understanding of personality in invertebrates.

Despite developments in understanding of the genetic aspects of adaptation to captivity (Frankham & Loebel, 1992; Gilligan & Frankham, 2003), as well as invertebrate behaviour (Kralj-Fišer & Schuett, 2014; Olzer *et al.,* 2019), there is little literature focussed on the adaptations of invertebrate personality to captivity with a view towards conservation. We argue that given our increasing knowledge of personality in invertebrates and increasing awareness of the importance of personality to translocation in vertebrates, it would be an opportune time to start to utilise invertebrate model systems to better understand how personality adapt to captivity.

V.III Conclusion

The study of personality in invertebrates has the potential to provide new insights into the field of reintroduction biology, as well as a greater understanding of the role of personality in different ecological contexts. Fast generation time and simple housing requirements of many invertebrate taxa make personality studies with large sample sizes and standardised conditions more feasible than the same studies in vertebrates. Large sample size is imperative in the study of animal personality, given the wide variation in behavioural phenotypes, and the risk of missing rare, ecologically important variants.

Understanding the distribution of personality profiles of invertebrate study systems, and how these profiles relate to post-translocation activity, could allow informed parameterisation of translocation models, for which dense personality sampling of other systems may be challenging. This does not mean that studies on invertebrates could replace vertebrate studies, as there will ultimately be many taxon-specific differences between different study systems. However, for developing our understanding of the underlying principles of the role individual personality can play in post-translocation success, and with it a deeper understanding of how personality may affect survival in wild and captive settings, studies on invertebrate translocations could be crucial.

There are many ways in which studying invertebrate personality could provide key insights into personality and the role which personality may play in translocation and captive care. For understanding the role which personality may play in translocation future directions include: (1) the development of robust models of personality and translocation success parameterized with invertebrate studies, (2) evolutionary studies on personality changes associated with captivity, (3) investigation into how developmental conditions affect personality and probability of translocation success. It is possible that the use of invertebrate systems would greatly benefit the study of each of these areas; the potential benefits of studying invertebrate personality in the context of translocation should not be overlooked.

We hope that this paper will spark discussion about the potential benefits of applying studies on personality to translocation of invertebrates to both invertebrate conservation as well as the understanding of animal translocation as a whole.

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Chapter VI: Keeping invertebrate research ethical in a landscape of shifting public opinion

VI.I Abstract

Invertebrate study systems are cornerstones of biological and biomedical research, providing key insights into fields from genetics to behavioural ecology. Despite the widespread use of invertebrates in research there are very few ethical guidelines surrounding their use. Focussing on two ethical considerations faced during invertebrate studies – collecting methods and euthanasia - we make recommendations for integrating principles of vertebrate research into invertebrate research practice. We argue, given emerging research on invertebrate cognition and shifting public perception on the use of invertebrates in research, it is vital that the scientific community revisits the ethics of invertebrate use in research. Without careful consideration and development of the ethics surrounding the use of invertebrates by the scientific community, there is a danger of losing public support. It is imperative that the public understand the significance of research that uses invertebrates and that scientists demonstrate their ethical treatment of their experimental subjects.

VI.II Introduction

Ethics in research shift constantly, and ethical standards are neither universal or immutable (Ferdowsian & Beck, 2011). Dramatic shifts in perception and attitudes towards ethics in vertebrate research in just the last century demonstrate just how far and how fast ethical standards can move. When, in 1982, Rollin presented a review to the US Congress of the available literature on providing analgesics for laboratory animals, the Library of Congress had only two papers (Rollin, 2006) on this subject. In 2011 there were over 11,000 relevant papers in the same library (Rollin, 2011). As well as an increased appreciation for the importance of controlling pain in animals in research, there have been shifts in scientific protocol with the development of the three R's principles (reduction, refinement and replacement), as set out in the book "The Principles of Humane Experimental

Technique" (Russell & Burch, 1959). Despite the initially slow reception of the book (Balls, 2009), these principles are now key to modern research practices, having been adopted and promoted across the international research community (Farnaud, 2009; Lindsjö *et al.,* 2016). Examples of bodies which now oversee the implementation the three Rs, as well as other aspects of animal welfare, include the Australian and New Zealand Council for the Care of Animals in Research and Teaching (established in 1987)(University of Adelaide, 2018), the Canadian Council on Animal Care (established 1968) (CCAC, 2019), and the National Centre for the Replacement, Refinement and Reduction of Animals in Research in the UK (established 2004) (N3Rs, 2019).

Historical shifts in ethical stances towards vertebrate experimentation highlight how rapidly ethical norms have moved to stay in line with scientific understanding of animal suffering. Keeping ethical frameworks current with our understanding of the systems that we are working on is critical to ensuring that our work is carried out with the highest levels ethical and moral integrity.

VI.III Moral obligations of researchers and effects of previous shifts in ethical frameworks

Shifting views of the public and scientific community, and the legislation that have followed these shifts in the past have provided hugely important improvements in animal welfare by today's standards. A key example of this is the British Act of 1876 (Cruelty to Animals Act), in no little part sparked by the public reaction (and similarly outraged reaction from a section of the scientific community (Dewsbury, 1990)) to the highly publicised rise in anatomical studies being carried out in France at the time (Rollin, 2006). Infamous examples of these studies included cases like the public dissection of a dog carried out in the UK lasting two days without anaesthetic, leaving the animal without pain relief on the dissecting table overnight (Franco, 2013). Cases like this highlight how important shifts in ethical views from the public and scientific community are to push through legislation preventing studies which by today's standards are inexcusably cruel.

Changes in attitudes to ethics, particularly within the use of animals in research, have also provoked concerns over the costs to the development of science that restricting practices may cause. Even the British Act of 1876 (Cruelty to Animals Act) was subject to concerns and criticisms surrounding its possible impact on science (Dewsbury, 1990). Similar fears are voiced today over contemporary ethical issues. One recent case study includes concerns that unease over the use of human cells being included in chimeras could halt the progress of chimera research, and the potential loss of medical advances that could be gained from their study (Hyun, 2016; Inoue *et al.,* 2016).

VI.IV Potential concerns from the scientific community about calls to consider invertebrate ethics

We expect that, similarly to times of change in vertebrate ethics (Cohen, 1986; Dewsbury, 1990), suggestions of change within the ethics of invertebrate research will be met with concern from some branches of science about potential limits to research progress. We would like to make clear that we are not arguing against using invertebrates in research, nor against euthanising invertebrates during research. Rather, we are arguing for careful consideration and discussion surrounding which methods are most appropriate for use on any given system, particularly in terms of ensuring ethical euthanasia of study organisms, and during collection of wild invertebrates.

For vertebrates, there is already a well-established field investigating the appropriateness of different methods for procedures that have welfare implications, such as euthanasia (Shine *et al.,* 2015; Valentim *et al.,* 2016; van Rijn *et al.,* 2011). These studies allow researchers to make informed decisions on the appropriateness of different methods. However, in invertebrates, this research is lacking in many systems, with gaps in research into even simple metrics like comparing the time different euthanasia methods take to work. These types of study would be highly valuable, allowing researchers to make informed decisions on how appropriate a method may be for their study species. Many researchers already aim to do this (Cooper, 2011; Lewbart & Mosley, 2012), and we hope that this article will encourage further discussion, research and debate around this topic.

VI.V Risks of mismatched ethical expectations between the scientific community and the public

Continual reassessment and consideration of ethical frameworks has the secondary function of not only ensuring the highest level of care for study subjects, but also of protecting scientists and the research they do from unexpected backlash from the public. While the motivations behind developing ethical frameworks to protect scientists, and developing frameworks to protect their study subjects may come from different places, they converge towards the same results and both should be considered in the debate surrounding invertebrate ethics.

When considering the role of ethical frameworks in protecting researchers from public backlash, the historical literature is littered with examples showing how mismatched expectations in ethics can have severe negative consequences for researchers and the research they conduct (Knaiz, 1995; Pettite, 2017). In recent history, examples can be taken from the 1970s and 1980s with the rise of the animal liberation movement, where polarised opinions surrounding animal ethics resulted in some factions turning to violent acts like arson, letter bombs and harassment, as well as protest (Knaiz, 1995; Wilson, 2004).

One case from study the animal liberation movement described in detail by Pettite (2017), is the public protests against the “great cat mutilation” in the 1970s, the aftermath of which involved the retirement of the scientist, Lester Aronson, and the dissolution of the American Museum of Natural History’s Department of Animal Behaviour (AMNH). It was claimed that Aronson’s work at the AMNH on cat sexuality complied with existing regulation and was accepted within the scientific community (Pettite, 2017); however, in 1970s New York perceptions towards cats were shifting from pests to pets with the ability to feel. Protests broke out outside the museum, arguing against the ethics of the research and attacking Aronson’s morals personally (Pettite, 2017). We do not believe that currently shifting perceptions in invertebrates would result in a repeat of the ethical struggles of the 1970, but use this as an extreme example to demonstrate how important preserving public trust in the ethical frameworks used in laboratories is to maintaining links and open discourse with the public.

Today, given the prevalence of social media, and ease of organising online campaigns, researchers are more vulnerable than ever to rapid public outrage to perceived ethical transgressions. Recent examples of the campaigns against Christine Lattin and Christopher Filardi demonstrate how both established and junior researchers can been targeted in online animal rights campaigns despite their work being carried out within ethical guidelines set by the scientific community as well as government legislation. In the case of Lattin, a viral video about her work on birds was circulated by PETA and helped to fuel a campaign of harassment at her place of work and home (Grimm, 2017). In the case of Filiardi, petitions circulated demanding him to be fired and jailed reached thousands of signatures, after he took a single specimen of rare bird for a museum collection (Filardi, 2015; Johnson, 2018). In both cases the ethical guidelines from the scientific community and government legislation did not match with the public perception of what ethical standards within science were expected to be. These mismatches in ethical perception, and the negative consequences resulting from them, highlight how important both up-to-date ethical frameworks are, as well as public education about current ethical norms are to protecting researchers from public backlash.

In these cases, there was an ethical gap in viewpoints despite the ethical frameworks centred on vertebrates, which have already been considered and developed in detail. So far, the ethics surrounding invertebrate experimentation has received far less attention. Recent developments in our understanding of invertebrate consciousness (Klein & Barron, 2016; Mendl *et al.,* 2011) and recent concern from the charity sector about the ethics of experiments on invertebrates (Barkham, 2017; Knapton, 2017), point to a need to revisit the ethics of invertebrates in science, to prevent the development of an ethical gap between researchers and the public.

VI.VI Current state of ethics for invertebrates

Invertebrates are key experimental models in a diverse range of research fields from medical biology (Rittschof & Schirmeier, 2018; Sanz *et al.,* 2017) to behavioural ecology (Barron & Klein, 2016; Hollis & Guillette, 2015; Kralj-Fišer & Schuett, 2014). However, despite the importance and widespread use of invertebrates in research there are few ethical guidelines governing their use in science. Legal protection of invertebrates in research is inconsistent between countries: for example, regulation of crustaceans euthanasia in New Zealand (Ministry for Primary Industry, 2017), but not in the UK. Currently, what ethical guidance there is comes from guidelines on invertebrate use recommended by scientific societies like the Association for the Society for Animal Behaviour (ASAB, 2018). These society guidelines are used as a reference by editors considering papers for publication in journals associated with the society, however outside decisions on society journal publications and small society research grants, these guidelines are not widely enforced. While existing legislation and journal-led guidelines are clearly important, we would argue that more can be done to standardise and encourage consideration of invertebrate ethics in research.

VI.VII Ethical exceptions among invertebrates

Among invertebrates, crustaceans and cephalopods are granted some ethical protection which aims to reduce suffering. For crustaceans the protection does not extend to research but covers transport and euthanasia in certain countries. These include New Zealand where crabs, rock lobsters and crayfish have to be insensible before death (Ministry for Primary Industry, 2017), as well as Switzerland which requires crustaceans to be stunned before death, and where crustaceans cannot be transported in ice or ice water. The regulations in banning transport of crustaceans in ice has also been recently adopted by Italy (Anti-Vivisection League vs the People, 2017).

Cephalopods on the other hand, have greater legislative protection. Recently the EU introduced extensive regulation, with legislation covering an estimated 700 species of cephalopods (Fiorito *et al.,*

2014) during research under Directive 2010/63/EU (Berry *et al.,* 2015). This was a milestone decision based on the recommendations of a scientific panel who concluded there was evidence for pain perception in cephalopods; this decision was not uncontroversial, however, with concerns voiced over the impact this new status may have on science (Fiorito *et al.,* 2014). Following the changes to EU legislation, the UK then changed its own legislation bringing it more in line with the EU with the regulation of all living cephalopods (except cephalopod embryos) in research (*Animals (Scientific Procedures) Act 1986, Act Amendment Regulations*, 2012). Outside Europe, the status of ethical regulation of the use of cephalopods is less clear. In Canada the legality of animal research is outside federal control due to the Constitution Act 1867, but instead is controlled at a provincial level. However, to gain federal funding institutional certification is needed from the Canadian Council on Animal Care (CCAC, 1993) (CCAC). The CCAC suggests that "cephalopods and some other higher invertebrates", have complex nervous systems and may be eligible for inclusion under certain ethical frameworks (CCAC, 1993).

The consideration of cephalopods, and more recently the limited inclusion of crustaceans, in legislative frameworks (see Table 1) to reduce suffering sets a precedent for including invertebrates in the conversation surrounding standards of care for animals used in research. In cases where these invertebrates have been included under ethical legislation, inclusion has been largely due to the perception these animals show advanced cognition and the ability to experience pain or suffering (Fiorito *et al.,* 2015; Rowe, 2018). It could be the case that these are "exceptional" invertebrates, different to all other invertebrates in their cognitive abilities and ability to experience pain, or it may be the case that future research demonstrates similar capabilities in other species, and that these are the first of many which will be afforded regulation as further understanding of invertebrate cognition is gained.

VI.VIII Recent advances in understanding invertebrate cognition

Understanding cognition in invertebrates is crucial to invertebrate ethics, as perception that a species or group has the cognitive capacity to experience pain or suffering has been key to the development of existing legislation protecting first vertebrates, and now certain invertebrates (Fiorito *et al.,* 2015; Rowe, 2018). The capacity and complexity of invertebrate brains and their resultant cognitive abilities is an area of considerable contemporary study and debate (Barron & Klein, 2016; Chittka & Niven, 2009; Klein & Barron, 2016; Perry *et al.,* 2017). While it was once assumed that large brains were needed for cognitive complexity, it is now appreciated that that brain size has less of a role in determining cognitive capacity than once supposed (Chittka & Niven, 2009; Perry *et al.,* 2017). Instead, structural features of brain architecture like modularity and interconnectivity have a greater role (Chittka & Niven, 2009). Findings that the structure of the brain is more important than brain size challenges previous assumptions that because many invertebrates have small brains they have little cognitive complexity, and raises the possibility of more cognitive complexity in invertebrates than previously assumed (Chittka & Niven, 2009). Further evidence for the role of brain architecture in dictating cognitive capacity comes from the study of complex behaviours now known to occur in invertebrate systems. Invertebrates display many behaviours once thought to be exclusive to larger-brained organisms, including ability to complete complex social learning tasks, recognise multiple individuals of the same species and even use tools (Perry *et al.,* 2017). However, it is still not understood whether invertebrate cognition extends to pain, defined as “a subjective experience of discomfort, despair and other negative affective states” (Adamo, 2016) and consciousness, defined as “marked by the presence of subjective experience” (Barron & Klein, 2016).

Recent behavioural and physiological work has gone so far as to suggest that there is some evidence for consciousness in invertebrates. Behaviourally, bees which were subject to a simulated dangerous environment went on to show “pessimistic” cognitive bias, suggesting capacity for subjective experiences (Mendl *et al.,* 2011), while bees which have been injured will self-administer analgesic (Groening *et al.,* 2017). With regard to physiology, analogous structures found in the invertebrate and vertebrate brain have been used to suggest that similarities in capacity for consciousness may exist (Barron & Klein, 2016; Klein & Barron, 2016).

VI.IX Changing attitudes to invertebrates

Given the long-term appreciation of cephalopod cognition, it is perhaps unsurprising that dialogue surrounding ethical concerns about improving invertebrate ethics often hinges on cephalopods. Current concerns about their care can be seen in recent petitions on banning live consumption of octopus in US restaurants, one of which gained over 47,000 signatures (Wolverton, 2019).

However, in light of research on lobster pain perception (Barr *et al.,* 2008; Elwood, 2012), there has also been a flurry of petitions in multiple countries, demanding a range of tighter ethical controls over treatment of crustaceans. In the UK, a recent petition demanding the British Government include lobsters and crabs under the Animal Welfare Act, exceeded 41,000 signatures (Crustacean Compassion, 2018). In the USA, PETA has started campaigns against the current practices used for killing lobsters for supermarket consumption (Toliver, 2018). Other countries who have already taken steps to improve crustacean welfare are summarised in table 1.

Table 1: Summary of important changes to invertebrate ethical legislation

Date	Summary of action	Country	Legislation
2010	Regulation on the treatment of an estimated 700 species of cephalopods in research	EU wide	Directive 2010/63/EU (Berry *et al.,* 2015)
2012	Use of all living cephalopods (except cephalopod embryos) in research is regulated.	UK	(The Animals (Scientific Procedures) Act 1986, Act Amendment regulations 2012.
2017	Crabs, rock lobsters and crayfish must be insensible before death.	New Zealand	(Ministry for Primary Industry, 2017)
2017	Transport of crustaceans in ice banned.	Italy	(Anti-Vivisection League vs the People, 2017)
2018	Crustaceans to be stunned before death, and where crustaceans cannot be transported in ice or ice water.	Switzerland	(Schweizerische Eidgenossenschaft, 2018)

Addressing invertebrates more broadly, animal rights organisations (PETA, 2017; Peta2, 2018), and individuals on social activism websites (Geer, 2015) have voiced concerns about the ethical treatment of invertebrates. While there has been less uptake from the wider public on these issues from a purely ethical angle; there is increasing real public concern about the plight and decline of pollinators, with over 99,000 people signing a petitioning against neonicotinoids to the UK government (Petitions, 2015) after concerns were raised about the impact of these pesticides on pollinators (Rundlöf *et al.,* 2015; Van der Sluijs *et al.,* 2013; Whitehorn *et al.,* 2012).

The current interest and concern about declining pollinators may appear to be outside the scope of considering invertebrate ethics in research, but in fact it highlights the importance of strong public education about the practices involved in studying invertebrates in the field. In many cases the critical research to investigate invertebrate declines, including pollinators, requires the killing of thousands of invertebrate specimens. An example of public concerns about the ethics of conducting research that involves invertebrate mortality, given the decline in pollinators, is the 2017 Great Wasp Survey (Knapton, 2017). The Great Wasp Survey was designed as a public science project with public recorders building and setting up wasp traps, collecting the trapped wasps, and sending them to scientists to be identified. Although the project was intended to understand wasp species distribution across the country, and to provide data to support conservation, the project was aggressively criticized for killing pollinators (Barkham, 2017). In fact, the project captured no queens, had a very limited by-catch and just two weeks of citizen engagement resulted in data comparable to four decades of expert sampling (Sumner *et al.,* 2019).

Public perception of invertebrate studies is important to multiple aspects of carrying out work on invertebrates. Large scale citizen science projects, publicly funded projects, or work which relies on volunteer recorders, all depend on a positive public response to the work being done, and the view that the work is ethically justified. It is therefore important that projects with ecological sampling, and public participation be ethically transparent and that steps are taken to mitigate potential ethical concerns.

VI.X Conservation concerns

Most of the public concerns about studies which take specimens from the wild (both vertebrate and invertebrate), centre on the conservation issues this may cause (Barkham, 2017; Johnson, 2018; Knapton, 2017). These types of concern should be taken seriously when considering invertebrate ethics. While the impact of long-term sampling on invertebrates has not been well studied, among the studies which have been done, conservation concerns have been raised over a few very specific forms of sampling. These include examples like destructive sampling of bromeliads to investigate invertebrate communities which live within them (Jocque *et al.,* 2010), the off-target effects of formalin use for earthworm sampling on environmental microbial communities (Čoja *et al.,* 2008) and lethal sampling being used to monitor rare or translocated invertebrates (Bowie *et al.,* 2006; Bowle & Frampton, 1998). In each of these examples, less destructive alternatives to these sampling methods have been investigated (Bowle & Frampton, 1998; Čoja *et al.,* 2008; Jocque *et al.,* 2010). Outside these very specific examples, there is little evidence to suggest that the most collecting carried out as part of scientific studies poses any serious conservation threat to invertebrates. However, this is an area which would benefit from more systematic and data-driven assessment of sampling impacts.

Despite the lack of evidence for scientific collection impacting invertebrate communities, many research centres and individual studies already apply a principle of reducing possible impacts as far as possible. One example of a research centre applying these principles is the Nouragues Research Centre in French Guiana which prohibits the use of non-selective sampling methods like light traps or fogging (Centre national de la recherche scientifique, 2019) in order to reduce the impact of studies on bycatch species. Another example, this time from an individual study, is the previously discussed Big Wasp Survey, which aimed to reduce the impact that wasp collecting may have by ensuring collection only took place late in the summer, so most collected wasps would be nearing the end of their reproductive lives (Big Wasp Survey, 2017).

Overall, there is already some progress within the scientific community to mitigate impact that studies involving invertebrate collection may have, particularly in cases where the species are rare (Bowle & Frampton, 1998), or where sampling methods are damaging to the local environment (Čoja *et al.,* 2008; Jocque *et al.,* 2010). We argue that ethically, and in line with public opinion, this should be encouraged. However, there also needs to be allowances for well justified studies which use non-selective trapping methods, as in some cases long term data collected in a non-specific manner can be the only way to collect data with important conservation outcomes (Hallmann *et al.,* 2017; Lister & Garcia, 2018). In the cases of large scale non-selective trapping however, public engagement and education may also be important to communicate the justifications for the work, and to ensure a gap in ethical perspectives between the public and scientific communities does not emerge.

VI.XI Suggestions for improving ethical practices around invertebrates

Mounting evidence for increased public awareness of and concern for invertebrates in research, particularly those collected from the wild, plus a developing understanding of the potential capacity for at least some invertebrate species to experience pain or to suffer, suggests a need for invertebrate ethics to be revisited by the research community, and discussion opened with the public. Addressing these concerns will be important, not only to ensuring an appropriate standard of the welfare the invertebrate study systems, but also to maintaining public support for invertebrate-based research.

Here we present a set of five suggestions to improve invertebrate research ethics. In this paper we focus on case studies of euthanasia and wild collecting methods. These areas have been chosen as there are cases of each of these being the recent focus of public concern (Knapton, 2017), or legislative change (Rowe, 2018). We hope that exploring these areas will spark discussions about the other ethical questions surrounding invertebrate use in research.

VI.XI.I (I) Power analysis

Power analysis is a useful tool to determine the smallest number of individuals that can be used in an experiment while still providing appropriate statistical power, a practice long encouraged in work on vertebrates (Festing *et al.,* 1998; Shaw *et al.,* 2002), and used in many invertebrate studies already (Arnqvist & Henriksson, 1997; Brereton *et al.,* 2011; Evans *et al.,* 2003). Adoption of pre-study power analysis as standard practice among those who research invertebrates, and acceptance by journals of lower samples sizes (given appropriate justification of power), could be an effective way of reducing the numbers of invertebrates used in trials.

VI.XI.II (II) Selection of specific trapping methods to reduce bycatch

During sampling work, in addition to lethal sampling of focal species, with many trapping methods bycatch of non-target species is inevitable. The limited evidence available on target species suggests sampling for research has little effect on study populations (Gezon *et al.,* 2015), but very little work has been done on the impacts of trapping on non-target species. Even without population-level impacts of bycatch, if we were to apply similar ethical principles to invertebrate systems as are applied to vertebrate systems with the importance of reduction, refinement and replacement, reducing the amount of off-target mortality should be encouraged (Russell & Burch, 1959). In many cases these principles are already in place, driven by practical benefits of reduced specimen processing and sorting times (Cha *et al.,* 2015).

VI.XI.III (III) Alteration of trapping protocol to minimize bycatch

Certain adaptations of trapping methods are employed to reduce non-target bycatch and can have an important role in changing which species are likely to be caught, hence reducing the impact of trapping on non-target species. Examples include altering the funnel structure of pheromone traps (Martín *et al.,* 2013), changing the size of pitfall traps (Brennan *et al.,* 1999) or even changing the colour of traps (Clare *et al.,* 2000). Many important studies on this area have already been carried out (Brennan *et al.,* 1999; Cha *et al.,* 2015; Pendola & New, 2007). Further research into methods of reducing off-target species capture could be effective in maintaining public support, particularly in large field studies, or studies with public involvement.

VI.XI.IIII (IIII) Make bycatch available for future use

In many cases reducing bycatch entirely may not be possible. In these cases, there may be real benefits to making bycatch available, accessible and advertised for study by other researchers (Buchholz *et al.,* 2011), and making the associated data open access. This would not be feasible for all bycatch, but high-quality or well-preserved bycatch, particularly if carried out as part of a large or long-term trial could contain a plethora of important information about a system that was not the focus of the study (Skvarla & Holland, 2011). In some cases, bycatch is already being used in other studies: one example is a project monitoring cerambycid diversity being conducted using the bycatch of a project specifically monitoring Asian Longhorn beetles (*Anoplophora glabripennis*)(DiGirolomo & Dodds, 2014). Making more bycatch available for study could provide important insights into the sampled systems and, in some cases, reduce the need for sampling similar areas a second time, reducing invertebrate mortality, as well as reducing the costs of these studies. Methods developed to enable collaboration among ecologists (Buchholz *et al.,* 2011) could be beneficially adopted more widely.

VI.XI.V (V) Where possible minimizing invertebrate suffering

Minimising animal suffering is key to the development of ethical guidelines for vertebrate studies, as well as for the small number of invertebrates which currently have ethical protection. It is likely to also be an important area of focus of invertebrate ethics. The main challenge for developing protocols to minimise invertebrate suffering stems from difficulties in determining whether or not an invertebrate is suffering, particularly when the perception of pain and suffering in invertebrates is not fully understood (Adamo, 2016). While more research is undoubtedly needed to investigate pain perception in invertebrates, in the short term it may be possible to look to the vertebrate for proxies of suffering.

A variety of proxies has been adopted tackle the challenge of assessing pain in vertebrates (Flecknell & Roughan, 2004), these include changes in movement, changes in food consumption, change in behaviour in response to a noxious stimuli (Flecknell & Roughan, 2004), or even reduction in response to noxious stimuli when analgesic is applied (Sneddon, 2003). Similar proxies, like retraction from a noxious stimuli have been used in invertebrates to assess potential suffering during procedures like euthanasia (Gilbertson & Wyatt, 2016). These authors argue that while a behaviour like retraction in response to a stimuli could be a reflex, if there is a choice of methods with no significant disadvantages, it could be ethical to choose the method with in which the animal shows a less marked behavioural reaction to the stimuli, until it has been shown definitively that the response is a reflex rather than an indication of suffering (Gilbertson & Wyatt, 2016).

VI.XII Conclusion

The current state of invertebrate ethics, and communication of these ethical standards need to be re-explored in light of our developing understanding of invertebrate cognition and pain perception and public perception of invertebrate studies. While invertebrate research ethics develops, the literature surrounding the already more developed vertebrate research ethics are rich in guidelines and philosophy which could be adapted to invertebrate use. As well as revisiting the ethics of using invertebrates in research, it is also highly important as a field to engage the public to highlight the need for often lethal invertebrate studies, as well as the ethical measures employed to reduce negative impacts. To ignore the changing public perceptions of invertebrate studies could mean losing public support for invertebrate studies.

Author's contribution statement

ED and EJHR conceived the presented idea, ED, EJHR and AGH advanced the presented idea and developed the theoretical framework. ED wrote the manuscript with input from EJHR and AGH. All authors discussed and contributed to the final manuscript.

Data Accessibility

Not applicable as no original data presented.

VI.XIII References

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Chapter VII: Thesis Discussion

VII.I Thesis overview

In this thesis I have presented a series of studies investigating the link between inter-individual variation and group level behaviours in social invertebrates. I have also explored the ethics and implications of carrying out research on invertebrates. In **chapter II** we explored the link between inter-individual variation and group stability in the facultatively social isopod *Oniscus asellus*. We showed that inter-individual variation in behaviour has a significant effect on group level behaviour. We also showed that not all behavioural phenotypes in *O. asellus* have an equal effect on group level behaviour, which could mean that group composition has important implications for group stability. In **chapter III** we developed an agent-based model which takes into account inter-individual variation in behaviour, as well as the unequal effects that different behavioural phenotypes have on surrounding individuals. Using outputs from the model we suggested that less active (or shy) individuals may have a greater effect on surrounding individuals than more active (or bold) individuals. In **chapter IV** we moved from the facultatively social isopods to the eusocial ant *Temnothorax albipennis.* In chapter IV we explored the role of inter-individual variation in memories on group level behaviour in *T. albipennis*. We found some effect of memory on group level house hunting behaviour and suggest that similar to inter-individual variation in behaviour, inter-individual variation in memory could have an important role in group level behaviours. In **chapter V** we explored how research into inter-individual variation in behaviour in invertebrates can benefit both our understanding of animal behaviour and also have potential benefits for wider conservation. In **chapter VI** we explored the ethics of using invertebrates in research. We highlighted how invertebrate ethical standards are far behind the standards required for other taxa and suggested the some of the ethical considerations applied to vertebrates should be considered for invertebrates.

VII.II Individual variation and group level behaviour in social and invertebrates

In this thesis we have shown that group level behaviours of social invertebrates are affected by both consistent individual variation in behaviour (chapter II and III) as well as memories of prior experiences (chapter IV). We have also shown that differences in individual behaviour have important implications for group level behaviour not just in highly organised colonies (chapter IV), but also to animals living in loose aggregations (chapter II and III).

For the facultatively social woodlouse *O. asellus* we showed that individuals had consistently different activity levels, and these differences in activity affected aggregation stability in a non-linear way (chapter II). We found that groups made of only inactive woodlice were slower to leave a sub-standard shelter than either groups made of active woodlice or groups made of active and inactive woodlice. We also found that there was no difference in emergence behaviour between groups made of active individuals and mixed groups of active and inactive individuals. The behaviour we observed could have emerged from behavioural contagion (Broly & Deneubourg, 2015), in which the activity of individual woodlice affects the likelihood of neighbouring woodlice showing active or inactive behaviour. In addition to the effects of behavioural contagion, our modelling work in chapter III could suggest that while the behaviour of all woodlice affect the behaviour of the woodlice around them, stationary woodlice have a stronger effect on stabilizing the aggregation than active woodlice have on destabilising the aggregation. While more work would be needed to definitively prove the mechanism behind this behaviour, it is clear that consistent differences in behaviour have important implications for group level behaviours in *O. asellus.*

In the ant *T. albipennis*, we also found that variation in individuals was likely to affect group level behaviour. However, unlike the work in *O. asellus*, in *T. albipennis* we explored how variation in memories could affect group level decision making. We found that in colonies where workers had memories of neighbouring nest-sites, colonies showed bias in certain house-hunting behaviours. Specifically, we found colonies took significantly longer to enter a box containing a nest site which had been removed than either a nest site which had been good or had been degraded. These findings suggest ants have an ability to update their memories about neighbouring nest sites, both by remembering useable nest sites and forgetting nest sites which are no longer usable. However, unlike previous studies (Stroeymeyt *et al.,* 2011a; Stroeymeyt, *et al.,* 2011b), these differences in scouting behaviour did not translate to differences in final choice of nest, which could be due to the lack of pheromones in our experimental set up, although more work would be needed to confirm this. It is clear however, that at least some stages of colony house hunting behaviour (scouting behaviour) are affected by the experiences which the individual ants in the colony have had in the past.

VII.III Ethics and invertebrates

While carrying out any work on live animals it is also important to consider the ethics and implications for the work being done. Research on invertebrates has the crucial applications to human medicine (Iijima-Ando & Iijima, 2010; Sanz *et al.,* 2017; Wilson-Sanders, 2011) as well as conservation (Brown *et al.,* 2017; Frankham & Loebel, 1992; Lewis & Thomas, 2001) and our understanding of animal behaviour (Alem *et al.,* 2016; Chittka & Niven, 2009; Kralj-Fišer & Schuett, 2014; Modlmeier, *et al.*, 2012; Perry *et al.*, 2017). However, despite the importance of invertebrate research, there are few guidelines on the ethical use of invertebrates. We argue that the current standards of ethical treatment of invertebrates needs to be reviewed in light of shifting public opinion (Crustacean Compassion, 2018; O’Connor, 2018) as well as current research demonstrating higher levels of cognition in invertebrates than previously assumed (reviewed in Perry, *et al.,* 2017). It is crucial the entomology community begins to develop best practices for working with invertebrates ethically to ensure the important work on invertebrates develops unhindered in an ethical way.

These ethical perspectives and recommendations put forward in chapter VI and published in 2019 (Drinkwater *et al.,* 2019) have sparked discussion in the scientific community (Creedy *et al.,* 2020; Padget, 2020; Salman *et al.,* 2020; Soulsbury *et al.,* 2020). In particular there has been greater consideration and discussion about the impacts which broad targeting sampling methods may have (Creedy *et al.,* 2020; Salman *et al.,* 2020), as well as calls for the uptake non-lethal collecting methods like trunk refugia which can be used to collect target species without causing mortality to off-target species (Salman *et al.,* 2020). There has also been discussion about the ethics and potential impacts of tagging and marking of invertebrates (Batsleer *et al.,* 2020; Padget, 2020). Finally, there has been further discussion about how the 3Rs can be better integrated invertebrate studies (Soulsbury *et al.,* 2020). The positive reception of our work on invertebrate ethics is encouraging as it suggests that there is an interest in improving invertebrate ethics, and hopefully these ongoing discussions will spark further developments in this field.

VII.IV Limitations and future directions

One clear limitation of this work is that we only explored individual variation across one behavioural axis in each study; in doing so we were unable to explore directly whether the behaviours observed in either *O. asellus* or *T. albipennis* were modulated by personality across multiple axes. In other species certain behavioural phenotypes show non-independence. In great tits (*Parus major*) for example, individuals show behavioural correlations across multiple behavioural axes (Aplin *et al.,* 2013; Carere *et al.,* 2014; Snijders *et al.,* 2014). For example bold great tits show lower neophobia and show faster exploration tendencies (Cole & Quinn, 2014). Shy great tits on the other hand show higher neophobia and slower exploration tendencies (Cole & Quinn, 2014). These correlated personality traits or behavioural traits are likely to be linked to adaptation of different great tits in a group having adapted to a high risk but higher reward or a lower risk lower reward strategy (Carere *et al.,* 2005; Cole & Quinn, 2014). While personality across multiple axes are important to the systems they have been studied in, the role of personality across multiple axes is far less clear in either of our study systems (*O.asellus* or *T. albipennis*).

Personality across multiple axes may play an important role in the systems we have studied. In *O.asellus* for example we observed that within the length of our experimental trials, certain individuals were more active than other individuals (chapter II and IIII). It could have been the case that these behaviours were modulated by other behavioural axes. For example, it may be the case that some individuals are more sensitive to reduced humidity and also less attracted to other individuals (Devigne *et al.,* 2011), and this increased the probability of them becoming more active and leaving. If this were the case woodlice may be adopting different life history strategies (Cole & Quinn, 2014), with certain individuals (active) risking predation for more humid conditions, while others (less active) avoiding predation by staying with a group but risking desiccation. However, in systems other than great tits personality across multiple axes has emerged in different ways. For example, in some species, certain personality phenotypes have been linked to sexual selection (Schuett *et al.,*2010), in rainbow kribs (*Pelvicachromis pulcher*) for example, females showed preferences of males with dissimilar levels of boldness to themselves (Scherer, *et al.,* 2017). Similarly, in birds there is evidence for sexual selection for personality across multiple axes (Garamszegi *et al.,* 2008; Ophir *et al.,* 2005). Male collared flycatchers, (*Ficedula albicollis*) advertise their boldness when they sing (Garamszegi *et al.,* 2008), while female Japanese quail (*Coturnix japonica*) on the other hand will preferentially choose less aggressive males over more aggressive males (Ophir *et al.,* 2005). It could be the case in these examples that personality traits like boldness or aggression also correlate with parental care behaviours or mating habits which could be damaging to the female, leading to sexual selection for correlated personality traits (Garamszegi *et al.,* 2008; Ophir *et al.,* 2005). In other animals correlated personality traits may have evolved in response to different hunting strategies, in the jumping spider *Portia labiate* for example, aggression is linked to decision making style, with docile spiders showing better problem solving of challenging problems than more aggressive spiders (Chang *et al.,* 2018). Other work looking at the jumping spiders *Portia labiate* and *Cosmophasis umbratica* showed a link between hunting success and the respective personalities of the predator and prey (Chang *et al.,* 2017), with aggressive spiders being more successful in hunting prey which showed unexpected behaviours, and docile spiders being more successful in hunting predictable prey (Chang *et al.,* 2017), suggesting the possibility of selection for different correlated personality traits to facilitate different hunting strategies. Given the wide range of evolutionary reasons which may have led to the selection for personality across different behavioural axes it is challenging to determine what personality axes may be at play in either of the systems explored in this thesis.

In this thesis the length of time we could spend scoring individual woodlouse personality was limited by their propensity to desiccate which resulted in short windows of observation. These time constraints would have made extending the study to multiple personality axes challenging. However, there are other approaches which could be employed to collect observations more efficiently. One approach to scoring behaviour could be to use tracking software such as idTracker (Pérez-Escudero *et al.,* 2014). Tracking software like idTracker would allow other aspects of individual behaviour like average turning speed and distance more to be collected rapidly. This software requires small differences in individual appearance to track individuals, therefore *O. asellus* may be able to be tracked; however there are other species of woodlice which are kept in captivity which have a great deal more variation in markings and may be simpler to track automatically. The spotty morphs of *Porcellio laevis* (known colloquially as dairy cow woodlice) for example are widely kept in captivity and show variation which could allow tracking software like idTracker to easily distinguish between individuals. Additionally, spotted woodlouse morphs would remove the need for researchers to paint mark individuals which has challenges of paint mark loss and the risks of affecting individual behaviour (Naranjo, 1990; Packer, 2005). Overall, the use of a species like *P. laevis* combined with a tracking software could allow a multimodal approach to be taken to explore multiple axes of individual variation simultaneously.

Widening the range of species used would allow the role of individual behavioural variation and group level decision making be explored over a wider range of social structures. In this thesis we have shown that woodlice can be a model for linking individual behavioural variation and group level behaviours.

The chapters of this thesis which use woodlice (Oniscidae) focus on *O. asellus*, a temperate species which lives in polygamous aggregations of up to hundreds of individuals (*pers. obs*). While aggregative behaviour has been suggested to be an ancestral adaptation for land-dwelling woodlice (Broly, Deneubourg *et al.,* 2013), some species have since adapted more complex social behaviours. For example, *Hemilepistus elongatus* woodlice are polygamous (Röder & Linsenmair, 1999), but unlike *O. asellus*, females show parental care after their emergence, bringing food back to the burrow to her young for some days (Röder and Linsenmair, 1999). In *Hemilepistus reaumuriform* on the other hand, woodlice form monogamous pair bonds and recognise family members by olfactory cues (Röder & Linsenmair, 1999). Since woodlice show a range of social structures which have diverged from their original aggregative social structure, looking at individual variation in the context of these divergent social structures could provide key insights in selection on individual behavioural variation in different social contexts.

An alternative direction to take future work would be to investigate drivers of inter-individual behaviour which are additional to memory and personality: one possible driver which could be investigated in more detail is parasitism. Parasitism has been linked to changes in behavioural variation traits (Barber & Dingemanse, 2010). There are many examples of behavioural manipulations caused by parasite infections, including extreme examples, like the infection of a cricket with a gordian worm (*Paragordius tricuspidatus*): infection with this worm eventually causes the host to jump into water, allowing the worm to emerge and continue its lifecycle aquatically (Lefèvre & Thomas, 2008). Despite the dramatic examples of host behavioural manipulation by parasite, there are still gaps in our understanding of the link between parasitism and animal behavioural variation. Parasitism and stress caused by the parasitic infection may contribute to developmental conditions, but there has been limited work on parasitism within the framework of animal personality or consistent inter-individual behavioural variation. One study on Eurasian minnows (*Phoxinus phoxinus*) showed small changes in behavioural repeatability after infection with a trematode parasite, but no overall changes in boldness repeatability or boldness after infection (Kekäläinen *et al.,* 2014); however, more experimental work needs to be done to confirm this link, and more theoretical discussion is needed to determine if parasitism is a driver of behavioural variation. It could be that parasitism may be a modulator rather than a driver of behavioural variation. Unlike genetics or behavioural variation changes driven by developmental conditions, the effects of parasitism may depend on presence of a parasite (Kekäläinen *et al.,* 2014). Outside immediate infection, parasitism may have a role as a driver of evolutionary selection as different behavioural variation traits may expose individuals to different risks of parasitism, which in turn could result in selection pressures for or against different behavioural variation traits (Barber & Dingemanse, 2010). The link between behavioural variation traits and selection due to parasitism is still to be explored, and to our knowledge has not yet been shown directly.

One good model system for understanding the link between parasitism and inter-individual variation could be the infection of woodlice with *Wolbachia* bacteria. *Wolbachia* is a maternally transmitted intracytoplasmic endosymbiont found widely in wild woodlouse populations (Moreau & Rigaud, 2001). This is passed down from a female woodlouse to her offspring and causes genotypic males to become functional females, facilitating future female mediated bacterial transmission (Moreau & Rigaud, 2001). While it has been found that these feminised males perform behaviours like mating (Moreau & Rigaud, 2001), it is unclear the extent to which this bacteria may alter the behaviour of the population. Male and female woodlice which are not affected by *Wolbachia* have different patterns of activity with males showing more active behaviour and travelling greater distances than females (Bayley, 1995). It has also been suggested that females may require higher humidity than males particularly when carrying young (Howard, 1980). It could be the case that if *Wolbachia* causes a shift in consistent behavioural patterns in a population to less active female movement behaviours, this could lead to highly stable aggregation behaviours. This idea could be tested by either collecting groups of woodlice from the wild which are already infected by *Wolbachia* and comparing these to groups which are uninfected, or infecting a population in a laboratory and testing for changes in behaviour of that population over time. Individual activity level, and group level activity could be calculated for infected and uninfected woodlice as described in chapter II. This work could provide interesting insights into whether infection with a parasite could affect long term individual and group level behaviour. If *Wolbachia* infection is found to have a significant effect on behaviour in the long term, this would raise interesting questions about how personality should be defined as well as how we should integrate infections into long term studies of inter-individual variation.

VII.V General discussion

VII.V.I Inter-individual variation in behaviour

Variation in individual behaviour affecting group level decision making is not restricted to social invertebrates (Aplin *et al.,* 2014; Bode *et al.,* 2011; Brown & Irving, 2014; Michelena *et al.,* 2010; Ward, 2012). In this thesis we have looked at how individual variation affects group level decision making in two species of social invertebrate; however the central question of how emergent group behaviours arise from the actions of the individuals within that group, is key to any species which forms social groups or aggregations (Aplin *et al.,* 2014; Brown & Irving, 2014; Hauschildt & Gerken, 2015; Michelena *et al.,* 2010; Michelena *et al.,* 2009).

Understanding the action of individuals on group behaviours in animal aggregations has wide implications for both non-human animals (Hui & Pinter-Wollman, 2014; Modlmeier *et al.,* 2012; Wray *et al.,* 2011) (chapter IV) as well as for humans (*Homo sapiens*) (Cimellaro *et al.,* 2019; Cimellaro *et al.,* 2017). In humans, researching the link between individual behaviour and group level behaviour provides insights into crucial behaviours, like evacuation behaviour in the event of a natural disaster in the presence or absence of social connections (Madireddy *et al.,* 2015; Sadri *et al.,* 2017). In non-human animals, understanding the role of individuals in group behaviours has also provided insights into important behavioural questions, like how do groups efficiently balance predator avoidance and foraging (Festa-Bianchet, 1988; Hebblewhite & Merrill, 2009; Pays *et al.,* 2013), or how do collective foraging behaviours emerge from individual decisions (Festa-Bianchet, 1988; Hauschildt & Gerken, 2015; Michelena *et al.,* 2010). In both human and non-human animal systems a range of factors need to be considered to understand collective behaviour. Two important factors to consider are social networks (Aplin *et al.,* 2012; Bode *et al.,* 2011; Croft *et al.,* 2005; Sadri *et al.,* 2017) as well as personality (Krause, *et al.,* 2010; Sasaki *et al.,* 2018, Scharf *et al.,* 2012; Wilson *et al.,* 2013). However, personality and position in a social network are often interconnected (Aplin *et al.,* 2013; Krause *et al.,* 2010; Wilson *et al.,* 2013), and therefore hard to disentangle.

Model systems like woodlice (Oniscidea) can provide an unusual perspective into animal aggregation behaviours (Broly *et al.,* 2013; Broly *et al.,* 2014). Given the loose aggregative form of sociality observed in many temperate species of woodlouse (Broly *et al.,* 2014), work on woodlice provides the opportunity to begin disentangling the effects of personality from the effects of social network position, as this group does not appear to show strong social ties or social fidelity.

Previous work looking at personality in social systems has highlighted the link between personality and social network position, with bolder (or fast-exploring) individuals holding a more central network position than shyer (or slow exploring) individuals (Aplin *et al.,* 2013). This link between personality and network position provides support for the social niche hypothesis (Aplin *et al.,* 2013; Bergmüller & Taborsky, 2010), which argues that competition for position in a group selects for certain behavioural phenotypes, which is one explanation for behavioural variation in social species.

In this thesis we have shown that inter-individual variation in behaviour has an important role in the emergent group level behaviours in woodlouse *O. asellus* (chapter II). Since *O. asellus* lacks structured social organisation the species is unlikely to be under strong selection pressure under the social niche framework (Bergmüller & Taborsky, 2010). It is likely that in social animals which have complex social structures, social niche specialization is an important in driving the evolution of inter-individual variation (Bergmüller & Taborsky, 2010). However, in species with less structured social organisation, other hypotheses for the evolution of inter-individual variation like the pace of life hypothesis (which suggests the evolution of variation in response to more or less risky life history strategies (reviewed in

Réale *et al.,* 2010)) may better explain the evolution of personality. Species which aggregate in the absence of complex social organisation could provide important insights into the evolution of personality, however, more needs to be done to investigate the evolution of personality in these simple social structures.

While heritable differences are one important driver of consistent inter-individual behavioural variation in animals (Dochtermann *et al.,* 2015), other factors like different experiences of individual animals also play an important role in shaping inter-individual behavioural variation over the long (Boogert *et al.,* 2014) or short term (Stroeymeyt *et al.,* 2011b). In this thesis we show that in the ant *T. albipennis* prior experience of individuals in a group is an important aspect of group level behaviour, and that memories of these experiences can be updated (chapter IV). This thesis therefore highlights the importance of considering how memories of recent events may shape individual behaviour.

There is also a link between how much knowledge an individual has and the impact which they have on conspecifics. In homing pigeons (*Columba liviahas*), the most informed individual will lead less informed individuals along a previously flown route (Flack *et al.,* 2012). Similarly, in *T. albipennis*, ants that have previously experienced a nearby nest will have a disproportionate impact on recruitment to that known nest site in the case of a colony emigration events (Stroeymeyt *et al.,* 2011a). It is therefore important to consider the effects of experiences on the individual, and how that will affect the group over the long (McComb *et al.,* 2001) or short term (Stroeymeyt *et al.,* 2011a).This is particularly true in personality studies on the of wild or wild collected animals which will differ in their short or long term behaviours due to prior experience rather than heritable behavioural phenotype.

Heritable inter-individual differences and the influence they have on a group need to be considered in terms of the level at which the selection may be happening. In the case of eusocial animals selection occurs at the level of the colony (Bergmüller & Taborsky, 2010; Hall & Goodisman, 2012; Nowak, Tarnita, & Wilson, 2010; Pinter-Wollman, 2012), with selection for different personality profiles happening at the level of the colony as the single reproductive unit (Jandt & Gordon, 2016; Jennifer

M. Jandt *et al.,* 2014; Pinter-Wollman, 2012). With other social animals however, selection for different personalities happens at the level of the individual rather than at the level of the colony (Dingemanse & Réale, 2005; Ingley & Johnson, 2014; Oers & Mueller, 2010), as in many cases the animal can choose to leave a particular or join a different group (Harcourt *et al.,* 2009; Reddon *et al.,* 2011). A link between individual decision making about group choice and personality is shown in three spined sticklebacks (*Gasterosteus aculeatus*), where given a choice, fish will choose to join a group with a higher boldness (Harcourt *et al.,* 2009), even if they are shy and therefore reduce the boldness of the group. Overall, more research is needed to understand how inter-individual differences in experience and personality affect group level personality, whether through group level selection (as is the case with eusocial animals) or through selection at the level of the individual and subsequent decisions of those individuals to leave or join different groups.

VII.V.II Ethics and applications of using invertebrates in research

Recent years have provided insights into individual and group behaviour which have challenged many assumptions about invertebrate behaviour (reviewed Perry *et al.,* 2017); however while our understanding of invertebrate behaviour has rapidly evolved our ethical standards of the treatments for these animals has remained largely unchanged. With the exception of a few exceptional groups like cephalopods (Fiorito *et al.,* 2015), there is little in the way even of guidance for the ethical care of invertebrates in a laboratory setting.

While the evidence for suffering in invertebrates is still an area of debate, I would argue that the precautionary principle could be applied to many areas of entomology research without serious negative implications for research. Simple steps like determining which method of euthanasia will euthanise the study species the most swiftly and with the least apparent disturbance to behaviour (Gilbertson & Wyatt, 2016) could be one step towards applying the precautionary principle to invertebrates without having a detrimental impact on invertebrate research. Some work has already been done in molluscs and crustaceans to determine best practice for euthanasia (Gilbertson & Wyatt, 2016), the protocols developed in these systems could perhaps be adapted to other systems.

It is important that the entomological community comes together to address the issue of invertebrate ethics is particularly important given the crucial work done on invertebrate systems every year (Iijima-Ando & Iijima, 2010; Sanz *et al.,* 2017). Invertebrate studies provide us with not just answers to key questions of invertebrate behaviour, but they also provide us with tools to understand some of the fundamental questions of biology like why do individuals differ (Kralj-Fišer & Schuett, 2014), and how emergent behaviours evolve (Modlmeier *et al.,* 2012), as well as applicable insights into conservation strategies (Brown *et al.,* 2017; Frankham & Loebel, 1992; Gilligan & Frankham, 2003; Lewis & Thomas, 2001) or mechanisms for human diseases (Iijima-Ando & Iijima, 2010; Sanz *et al.,* 2017; Wilson-Sanders, 2011). Given the importance of the work on invertebrates, it is important that researchers lead the change in invertebrate ethics, in order to ensure that invertebrates are cared for in an ethical way while research can also be carried out effectively.

VII.V.III Conclusion

In conclusion, inter-individual variation, whether caused by personality, differences in memories or other factors play a key role in determining the animal behaviour at the level of the individual as well as at the level of the group. However, in many cases the social context of the focal individual may have an important role in determining how these differences may be displayed at the individual or group level. In this thesis we have highlighted both the importance of considering different aspects of inter-individual behaviour when considering group level behaviour, as well as the value of considering the use of different types of social structure to address similar questions. We hope this body of work will spark future debate about how group level behaviours may emerge from different sources of inter-individual variation across different social systems, as well as further discussion about how the ethical frameworks for these types of study can be developed further.

VII.VI References

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