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Personality, life-history traits and pace of life in the hermit crab Pagurus bernhardus

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Personality, life-history traits and pace of life in the hermit crab *Pagurus bernhardus*

by

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By Mariana Velasque Borges

Abstract. Consistent between-individual differences in behaviour (termed "animal personality") may be driven by adaptive differences in behavioural and physiological life-history traits. The Pace of Life Syndrome (POLS) hypothesis predicts a suit of correlations between those life-history traits along a fast-slow continuum. Therefore, according to the POLS, individuals that are fast-paced would be bolder, more explorative, show high growth-rates, lower immunity and a higher metabolic rate. A mechanistic link between such traits could also explain variation in cognitive traits, where bold individuals are faster at a given task but pay less attention to external cues and therefore make decisions less accurately. Here, I tested the POLS hypothesis focusing on between and within-individual variance in boldness, metabolic rate (MR), cognitive performance (as decision-making performance) and exploration in the hermit crab *Pagurus bernhardus*. In addition, I also investigated the potential role of anthropogenic disturbances (constant light exposure) as a driver of between and within-individual variation in boldness. Hermit crabs demonstrated consistent between-individual differences in boldness and exploration, providing evidence for the presence of animal personality. However, variation between individuals in boldness, exploration and cognitive performance were not underpinned by variation in MR. Although there were no between-individual correlations among MR and behaviour, MR did co-vary with within-individual variance in boldness. My results indicate that less predictable hermit crabs, on average, have a higher MR during startle responses compared with those that are relatively consistent in their behaviour. Boldness was positively

correlated with exploration rate, indicating that more explorative were also bolder, as well as cognitive performance, as bold individuals had a better performance than shy. Finally, constant light exposure is likely to modify hermit crab personality and physiology. Hermit crabs kept under a constant light regime were less bold and had a higher metabolic rate, than when kept under standard light and dark regime, indicating possible effects light pollution in this species. These results only partially support the POLS hypothesis.

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At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Sub-Committee.

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Thesis format, contribution of co-author and contributions to knowledge

Thesis format and contribution of co-authors

This thesis, with the exception for the General Introduction (Chapter 1) and the Conclusion (Chapter 6), is a collection of chapters in manuscript style. Chapter 1 introduces the thesis and its objectives, providing a literature review of the subject and Chapter 6 summarise all finds providing guidelines for future research. Chapter 2 has been publicised in a special edition of a peer-reviewed journal ('*Behaviour*') and Chapters 3 to 5 will be shortly submitted.

Contributions to Knowledge

Chapter 2: The opposite effects of routine metabolic rate and metabolic rate during startle responses on variation in the predictability of behaviour in hermit crabs.

This study was the first to investigate the potential for co-variation between: between (V_{BI}) and within-individual (V_{WI}) variation in behaviour and energy expenditure in the hermit crab *Pagurus bernhardus*. In hermit crabs, between individual variation (i.e. animal personality) in boldness (i.e. startle response duration) is not underpinned by variation in energy expenditure, measured as metabolic rate (MR) under two conditions, routine MR and MR during the startle response (startled MR). However, there was an V_{WI} increase with startled MR and decreased with routine MR. These results provide evidence that crabs with lower routine MR behave more predictably and that there is an increase in MR during the induction of startle response duration.

Chapter 3: Cognition, personality and energetics in hermit crabs

This study was the first to investigate the relationship between the consistency of a behavioural trait involved in risk-taking (boldness), cognitive performance and energy expenditure, in order to test key predictions of the Pace of Life Syndrome (POLS) hypothesis. This chapter provides evidence that cognitive performance, in terms of both decision-making time and accuracy in decision-making (shell assessment), is not dependent on energy expenditure in hermit crabs. Furthermore, in hermit crabs, accuracy in decision-making seems to be associated with boldness as bold individuals were (surprisingly) more accurate on average than shy ones, and this association was independent of decision-making time, contrary to the POLS.

Chapter 4: Behavioural syndrome and pace of life: the effect of metabolic rate in boldness and exploration

This study provides the first evidence of a positive association between two correlated behavioural traits (i.e. a behavioural syndrome) and energy expenditure (measured as routine metabolic rate) in hermit crabs. Exploration, measured as spontaneous alternation, and boldness was consistently different between hermit crabs indicating that both are animal personality traits, and they were positively correlated. More explorative animals were bolder than non-explorative animals, they also had higher routine metabolic rate. This was the first study to investigate individual consistency in exploration as alternation performance (investigated in a plus-maze) in invertebrates.

Chapter 5: Under the influence of light: how constant artificial light affects the expression of personality and energetic consumption in hermit crabs

This is the first evidence that exposure to constant light alters not only the average behaviour of hermit crabs, but also changes their oxygen consumption, indicating changes to metabolic rate. My results indicate that hermit crabs kept under constant light condition were consistently less bold and had a higher metabolic rate, than when kept under a standard light and dark regime (12:12h light/dark). I also demonstrated that hermit crabs have a different behavioural pattern during day and night and that such behavioural differences increase under constant light conditions. These results could reflect the effect of light pollution on behaviour and energy consumption in hermit crabs in natural environments.

Chapter 1

General introduction

Introduction

Animals, including humans, possess several behavioural traits in common. For instance, animals can learn (Abrahamson, 1981; Spearet al., 1990; Galef, 1992; Schneuwly, 1993; Mackintosh, 1994; Castro & Wasserman, 2010; Dickinson, 2012), communicate (Wilson, 1972; Bradbury & Vehrencamp, 2000; Laidre, 2012; Naguib, 2006), make tools (Eisenberg, 1981; Seed & Byrne, 2010) and recently it was found that they also possess 'personality traits' (Dingemanse & Réale, 2005; Locurto, 2006; Biro & Stamps, 2008; Careau et al., 2008; Adriaenssens & Johnsson, 2009; Schuett et al., 2010; vanOers & Mueller, 2010).

Personality is originally a term from psychology, and it refers to the behavioural tendencies that differ between individuals and where these differences between individuals are (relatively) consistent over time or situations (see Glossary) (Caspi, et al., 2005; Réale *et al.,* 2007; alternatively, behavioural type: Sih et al., 2012). Over the last decade the idea of personality has been increasing applied more broadly to non-human animals, since most (and perhaps all) animal species show consistent between-individual differences in patterns of behaviour (Gosling, 2001; Dingemanse & Réale, 2005; Locurto, 2006; Bell, 2007, 2012; Biro & Stamps, 2008; Careau et al., 2008; Adriaenssens & Johnsson, 2009; Schuett et al., 2010; Stamps & Groothuis, 2010; vanOers & Mueller, 2010; Weiss & Adams, 2010; Briffa, Bridger, & Biro, 2013; Carter et al., 2013). In fact, consistent differences in behavioural tendencies have been demonstrated in animals that do not possess a centralised brain, such as anemones (Briffa & Greenaway, 2011). When discussing non-human animals, the term 'animal personality' is usually used to refer to behavioural differences between individuals that are consistent over time and are maintained (to a greater or lesser extent) across situations.

So far, ethologists have discovered that some individuals can be consistently more aggressive, bolder or more exploratory than others. Moreover, these consistent differences in behaviour can be heritable (Brodie, 1996; Stirlinget al., 2002; Kölliker, 2005; Bell et al., 2009; vanOers & Mueller, 2010; Dochtermann et al., 2015) and therefore potentially adaptive (Sih et al., 2004a, b; Dingemanse & Réale, 2005; Réale et al, 2007; Sih & Bell, 2008; Smith & Blumstein, 2008). In addition, personality traits are often correlated. For instance, individuals that exhibit high levels of boldness (see Glossary) might also show high levels of activity (Mazué et al., 2015), aggressiveness (Verbeek et al., 1996, Koolhaas et al., 2001), a tendency to be more competitive and to be more exploratory (see Glossary) (Sih et al., 2004). Such suites of correlated behaviour have been termed as 'behavioural syndromes' (see Glossary) (Réale et al., 2007; Sih & Bell, 2008), coping styles (Archard & Braithwaite, 2010; Koolhaas et al., 1999) or 'temperament' (Réale et al., 2000). Furthermore, if behavioural syndromes are stable over time, they could also vary consistently between individuals, and therefore represent an aspect of personality (Biro & Adriaenssens, 2013; Westneat et al., 2015). Hence, behavioural differences might not only exist between individual behaviours, but also in suites of correlated behaviours that are (relatively) consistent different between individuals.

Although the presence of animal personality refers to the presence of consistent differences in behaviour between individuals, it does not necessarily imply that individuals would be behave similar (Sih et al., 2004). For instance, when facing a change in environmental conditions, individuals are likely to adapt their responses, exhibiting considerable plasticity in their behaviour (see Glossary) (behavioural plasticity; Briffa et al., 2008; Schuett et al., 2009; Montiglio et al., 2013). In addition, when individuals are observed multiple times in the same situation, their responses will not be identical across observations (Bell et al., 2009). Initially, such unexplained

variation was seen as random noise, resulting from measurement errors or uncontrolled variables (Mather & Anderson, 1993; Stamps et al., 2012) and therefore ignored. However, there is an increasing in evidence that such variability is not necessarily a result of some non-controlled variation (Fiske & Rice, 1955), but might represent an intrinsic form of variation that corresponds to an individual's 'predictability' or 'consistency' in behaviour. In this case, this source of variation is called within-individual variability (V_{WI}; Dingemanse et al., 2010; Dingemanse & Dochtermann, 2013), intra individual variation in behaviour (Nesselroade, 1991; Siegler, 1994; Salthouse, 2007; Ram & Gerstorf 2009; Stamps et al., 2012) or residual (i.e. unexplained) behavioural variance (Westneat et al., 2014).

In animals, the level of the within-individual variation in behaviour appears to show a direct relationship with fitness (Piersma & Drent, 2003), affecting sexual selection (e.g. Schuett et al., 2010), foraging and survival (e.g. Okuyama, 2015; for review see Westneat et al., 2015). For instance, it has been proposed that males that possess a lower within-individual variation in behaviour (are more consistent) when competing to assess females, will spend an unnecessary amount of energy during a competition with a weaker male (DeKort et al., 2009), which could result in fitness consequences. Similarly, under a higher predation risk, some individuals tend to exhibit higher within-individual variance in behaviour (Maye et al., 2007; Stamps et al., 2012), suggesting that V_{WI} could have evolved as a way to escape predation, given that, presumably, less predictable individuals are potentially more difficult to be preyed when compared to more predictable ones (Bednekoff & Lima, 1998, 2002; Maye et al., 2007; Brembs, 2011).

Here, I will use the term 'animal personality' to refer to consistent between individual differences in behaviour (single behaviour) within a population. Note that

'repeatability' (R) is an effect size estimate for this pattern, which effectively quantifies the amount of variation in behaviour that is due to differences in (mean) behaviour among individuals; formally repeatability expresses the proportion of total variation in behaviour that is due to variation between individuals (see below for details). And 'behavioural syndrome' is used to refer to the correlation between two or more behavioural traits, that are consistently different between individuals. Thus, both animal personality and behavioural syndrome, are features of a population. To refer to an individual's mean expression of a specific behaviour (e.g. 'explorative', 'aggressive', 'shy' or 'bold'), I will use the term 'behavioural type' (see Glossary) (Bell, 2007). Thus, it is consistently different behavioural types that lead to the presence of animal personality at the population level. Finally, I will use the term 'within-individual variation in behaviour' to refer to the presence residual variation in behaviour within-individuals (Dingemanse et al., 2010; Dingemanse & Dochtermann, 2013) that is not explained by differences among individuals. Note, however, that this within-individual variance can differ between individuals (i.e. some individuals are more 'predictable' than others) and (on average) it can vary between situations (i.e. in some situations all individuals are, on average, more predictable compared to other situations), such that there may be plasticity in V_{WI} as well as in individual mean level responses (e.g. Briffa, 2013).

Why do individuals behave differently? Current ideas about the emergence and maintenance of animal personality

As defined above, animal personality is the presence of individual differences in behaviour that are consistent across time and/or situations. As a result, the study of animal personality demands repeated measurements of the same individuals at different

times (Briffa & Greenaway, 2011; Bell, 2012; Kralj-Fišer & Schuett, 2014) and/or across different situations (Hayes & Jenkins, 1997; Dall & Griffith, 2014), requiring longitudinal data. Such behavioural consistency could result in major effects on survival (Møller & Garamszegib, 2012) and fitness (Krebs & Davies ,2009), as they are likely to mediate the interaction between an individual and its environment (Dingemanse & Réale, 2005). Furthermore, there is sufficient evidence that such consistent between individual variation in behavioural traits and suites of correlated behaviours (behavioural syndromes) co-vary with other physiological and morphological traits. This suggests that differences in behavioural types could be linked to life-history (see Glossary) differences (Stamps 2007; Wolf et al., 2007), especially if behavioural types are expressed on a fast–slow life-history continuum (e.g. Gaillard et al., 1989; Bielby et al., 2007; Jones et al., 2008). For instance, individuals that are fast-lived are predicted to have a higher mortality rate, a fast development, produce a large number of offspring in relatively short time increments, and also have a higher metabolic rate (Wikelski et al., 2003; Wiersma et al., 2007).

Although personality traits and suites of correlated behaviours have been intensively studied over the last 20 years, the mechanisms behind their emergence and maintenance in a population or species are still not well understood (Dingemanse et al., 2004; Dall et al., 2004; Sih et al., 2004a; Stamps & Groothuis, 2010; Sih et al., 2015). The key problem is that if Natural Selection should produce optimal ways of behaving, this process is expected to erode phenotypic differences between individuals. This could be said of any aspect of phenotype, but consistent behavioural differences seem especially surprising given that behaviour is typically assumed to be the (potentially) most labile (rapidly changing and highly reversible) aspect of phenotype. To explain the presence of animal personalities, four types of explanation have been proposed: evolutionary constraints (genetic, hormones, morphology), adaptive personality

differences, state dependent feed-back and life-history trade-offs (Sih et al, 2007). Each of these potential drivers of animal personality are discussed below.

Evolutionary constraints

Behaviour is considered to be one of the most plastic phenotypic attributes (Sol & Lefebvre, 2000; Nicolakakis et al., 2003; Lefebvre & Sol, 2008; Schuett et al., 2010) changing in expression across situations situation (i.e. behavioural plasticity) (Schuett et al., 2009). However, the presence of consistent differences between individuals across time and situations seems, to an extent, to be at variance with this assumption; since any one individual is unlikely to express the full range of behavioural responses seen in the population as a whole, it appears that at least there must be some constraints on behavioural plasticity. Such limited plasticity, as well as the presence of correlations between behavioural and physiological traits, can be explained by the presence of evolutionary constraints. In this model, behavioural and physiological traits are assumed to have a shared proximate link, such as common hormone regulating both traits (Ketterson & Nolan, 1999) or genetic correlations (vanOers et al., 2005). As a result, the change in one trait can produce an indirect but correlated response in the other (Bell, 2005), constraining the evolution of both traits. Thus, animal personalities might represent nothing more than variation around an adaptive population-level mean.

Adaptive personality

Alternatively, the presence of personality and suites of correlated behaviours can be domain-specific, whereby consistent between individual variation in behaviour is present but only in specific situations (Bell, 2005). For instance, individuals could behave consistently shyer and also less explorative only in the presence of predators, limiting the expression of a given behavioural type. In this model, behaviour and

physiological traits emerge as adaptive responses to a selective pressure (e.g. Carere et al., 2010, Dingemanse & Wolf, 2010), instead of resulting from a constraint on evolution (constraint hypothesis). Therefore, different selective pressures may generate or erode behavioural variation according to the particular situation (Wilson, 1998). For instance, great tits (*Parus major*) show differences in exploration according to the abundance of resources (Dingemanse et al., 2002, 2003, 2004, Dingemanse & de Goede, 2004).

Another adaptive explanation is that differences in behavioural types could arise from negative frequency dependent selection. Here, the utility of behaving in a particular way changes with the frequency of that behaviour in the population, specifically the fitness benefits decrease in proportion to the number of individuals expressing the trait (Would et al., 2008). For instance, the frequency of producers (individuals that actively search for new food sources) and scroungers (individuals that exploit food sources discovered by other's individuals) is dependent on frequency of that behavioural strategy in the population. Therefore, the higher number of producers in a population, less beneficial this type of behaviour would become, favouring the selection of scroungers (e.g. Barnard & Sibly, 1981). Negative frequency dependent selection can lead to the evolution of populations that reach stable equilibria containing a mix of behavioural types (i.e. there are a mixed Evolutionarily Stable Strategies, ESS).

State dependent feed-back

While both adaptive and constraint hypotheses provide ultimate explanations for behavioural types, in order to fully understand behavioural variation, we should also consider potential proximate mechanisms. In some cases, these proximate explanations may underpin the ultimate explanations described above. Moreover, it seems clear that

ultimate and proximate explanation need not be mutually exclusive (Tinbergen, 1963). The state dependent feed-back model suggests that individual differences in behaviour are the result of dynamic feedbacks between environmental characteristics and their outcome. In this way, small initial differences in state (e.g. age, sex, size, energy balance) can, through positive feedback, generate differences in behaviour, which can modify the following state and the subsequent behaviour (Houston & McNamara, 1999; Dingemanse & Wolf, 2010; Sih et al., 2015).

For instance, bolder individuals may gain more rewards (e.g. mating, territory, food) which then exacerbates initial differences in state between bolder and shyer individuals (e.g. size, energy balance) (Biro & Stamps, 2010; Houston, 2010; for review see Sih et al., 2015). Therefore, by positive feedback, the change in the initial state leads to an increasing and/or the maintenance of the behavioural tendency (e.g. being bolder). Alternatively, if differences in state have negative or little effect on behavioural types, they can erode or limit differences in behaviour, by negative feedbacks. For instance, a behavioural trait can be inhibited if leads to an increase to exposure to contaminants (e.g. heavy metals) (Montiglio & Royauté, 2014).

Life-history trade-offs

Individual differences in life-history strategies can also be attributed to the existence of life-history trade-offs (Roff & Fairbairn, 2007; Wolf et al., 2007; Réale et al., 2010). Accordingly, individual variation in personality traits are maintained by a trade-off between survival and reproduction. Thus, within a population some individuals would have a higher investment in current reproduction (but a lower investment in survival), whereas others would invest more in future reproduction (Wolf et al., 2007). As a result, individuals with a higher investment in future reproduction, would behave accordingly, exhibiting less risk prone behaviour, foraging with less intensity but also

gathering fewer rewards, and thus, having a lower short-term reproductive performance (Wolf et al., 2007; Réale et al., 2010). Therefore, behaviours are expressed in a fast-slow continuum in conjunction with correlated non-behavioural life-history traits (e.g. physiological; Sih et al., 2004). This theory integrates across behavioural and physiological traits, along a slow-fast continuum called pace-of-life syndrome hypothesis (see Glossary) (POLS, Réale et al., 2010).

The POLS is conceptually analogous to the r- and k-selection theory developed by MacArthur and Wilson (1967). Here, closely related species would exhibit differences in physiological (e.g. metabolic rate, size, hormonal, immunity) and behavioural traits, produced by differences in life-history traits between those species, favouring their coexistence (Ricklefs & Wikelski, 2002). The POLS concept explores the same principle, that differences in life-history traits promotes coexistence between individuals within a population or species, rather than between species. As a result, individuals supposedly behave consistently in a spectrum along a fast-slow continuum, characterising their 'pace of life' (Réale et al., 2010). Hence, such a continuum would reflect multiple aspects of the animal life-history, including survival, maturation, reproductive and metabolic rates, immunity and hormone levels as well as behaviours such as boldness and exploration (Réale et al., 2010).

Importance of animal personality and some current gaps in knowledge

The field of animal personality seen a substantial increase in studies over the last 20 years (Sih et al., 2004; Réale et al., 2007; Sih & Bell, 2008; Sih et al., 2012). This has led to the inclusion of between individual variation in others areas than behavioural ecology. As a result, there is increasing evidence that individual variation in behaviour can have an effect on invasion success (Cote et al., 2010; Fogarty et al., 2011), species

interactions (Biro et al., 2004; Toscano & Griffen, 2014), maintenance of biodiversity (Crutsinger et al., 2006; Crutsinger et al., 2009) and even in extinction risk (Pruitt, 2013). However, studies that integrate behavioural variation with the response to environmental changes are still rare (Briffa et al., 2013; Sih, 2013; Sol et al., 2013; Royauté et al., 2015; Royauté & Pruitt, 2015; White & Briffa, 2017), even though they could provide a better overview of the effects of environment in behaviour. These effects could be important because some personality types may perform differently in each situation (e.g. shyer individuals can be more reactive towards a change in contition) (Martin & Réale, 2008) or adapt faster to environmental changes (Sih, 2013), such as anthropogenic disturbances (e.g. light pollution, noise, pollutants) (Royauté et al., 2015). In addition, investigating the role of between individual differences in response to a change in conditions, could increase the reliability of the results, as individuals would be assessed multiple times.

It is clear that correlations between life-history traits (physiological and behavioural) are associated with differences in fitness. Here, I divided mechanisms behind their emergence and maintenance into proximate (state dependent feed-back) and ultimate causes (evolutionary constraints, life-history trade-offs and adaptive personality differences). However, it is important to note that although such schemes can be useful for organising our thinking about animal behaviour (or any other traits in biology) (Tinbergen, 1963), different 'causes' should not be thought of as occurring in isolation from one another. For instance, between individual differences in behaviour may arise as an adaptive response to a selective pressure (adaptive explanation) and are likely to generate life-history trade-offs, which, via positive feedback, can be reinforced over an animal's life, for example. Since proximate and ultimate mechanisms are

difficult to untangle without a selective study (Sinervo & Svensson, 1998), I am going to explore here consistent among-individual differences in both behaviour and key life-history traits (body size; metabolic rate) from the POLS perspective. More specifically, I investigate whether between and within-individual variation in behaviour (boldness, cognitive performance and exploration) co-vary with metabolic rate. In Chapter 2 I explore the association between boldness and metabolic rate, at routine and during recovery form a startle response, as well as the relationship between metabolic rate (routine and during recovery form a startle response) and V_{WI}. In Chapter 3 I explored the association between boldness, cognitive performance, in terms of both decision-making time and accuracy in decision-making (shell assessment), and metabolic rate during routine and decision-making. In Chapter 4 I investigated the association between boldness, exploration and routine metabolic rate, using alternation performance in a plus-maze as index of exploration. Furthermore, behavioural variations can also indicate the relationship between individual and its environment, indicating of how an organism response to environmental pressures (e.g. Hedrick, 2000). Therefore, I also investigated the role of anthropogenic disturbances driving variations in behavioural types (Chapter 5).

Behavioural types and metabolic rate

Traditionally, animal personality is investigated along five behavioural axes: shy-boldness, activity, aggressiveness, sociality and exploration–avoidance (Gosling et al., 2003). Subsequently it was suggested by Réale et al. (2007) that these 'Big 5' personality traits could be generalised to adequately describe the major axes of consistent among-individual variation in behaviour expected in most animal species, not just humans. Among them, exploration (see Glossary) and boldness (see Glossary) have been receiving particular attention (Réale et al., 2007; Houston, 2010). This occurs

because the former influences processes related to foraging, and dispersion and therefore, its expression is correlated with the risk of predation (Anholt & Werner, 1998; Mangel & Stamps, 2001; Biro et al., 2004). While the latter is the individual propensity of taking risks (Réale et al., 2007; Conrad et al., 2011). Therefore, variations in explorative behaviour, could reflect variation in boldness and vice versa (Sih et al., 2012; Wolf & Weissing, 2012). In addition, as both traits are directly involved with energetic gain and mortality (e.g. bolder and more explorative individuals can gather more food but also have a higher mortality), variation in both traits could also reflect underlying physiological traits (Stamps, 2007; e.g. Biro & Stamps, 2008). Thus, these two axes of variation in behaviour seem to be of special appeal to animal behaviour researchers, particularly those whose outlook is grounded in evolutionary and ecological theory (i.e. behavioural ecologists).

One explanation for the relationship between exploration and boldness, is that a higher exploration and boldness may increase foraging level and therefore increase the chance of encounter with food, but also but increases the chances of encounters with predators, creating trade-off between survival and reproduction (Biro & Stamps, 2008). Alternatively, variation in behavioural types can be the result of a constraints imposed by physiological traits, such as energetic demands (see for review Mathot & Dingemanse, 2015). Here, a higher energetic demand can affect individual decision-making, driving individuals to forage with a higher frequency (modifying exploration) or in places with a higher predation risk (see for review Mathot & Dingemanse, 2015). Although the causal direction of such associations (whether differences in behavioural drives variations in energetic use or if energy use drives variation in behavioural types) is sometimes difficult to discern, they are likely to generate a similar effect: a correlation between behavioural types and energetic consumption. Regardless of the direction of causation, there remains a paucity of empirical evidence for these

associations in the first place (see exceptions below). While the theory surrounding animal personality research has advanced rapidly, empirical research has, it could be argued, failed to keep up. Partly this may be due to the inherent complexity of the statistical approaches (see below) required to deal with longitudinal data that may be significantly heteroskedastic, and empiricists need to learn to 'run before they can walk'. However, what seems to still be required, as a first step in uncovering the casual relations between variation in repeatable behaviour and life-history, are studies describing how (or 'if') these traits might co-vary. These studies should look variation in a suite of behavioural contexts (see Glossary) and across situation (see Glossary), both between and within-individuals.

The measure of the metabolic rate can provide a reliable proxy for the individual's energetic consumption (Mathot & Dingemanse, 2015). Metabolic rate (MR) is an approximate estimation of the individual's rate of energy expenditure and it can be assessed in different ways. For instance, the basal MR (BMR) (see Glossary) is defined as the minimum energetic requirement for self-maintenance and therefore, it is estimated under conditions that will eliminate such effects (Speakman, et al., 2004), usually involving in a physical constraint of the individual's movement (minimising the waste of energy executing behaviour) and is mainly used in endothermic animals. In ectotherms, standard MR (see Glossary) (SMR) and routine MR (see Glossary) are used more generally. The former, similar to BMR, it is an estimation of the individual lowest MR, being measured in a resting animal (Speakman, et al., 2004). However, marine animals may impose a challenge to this measure, as their gas exchange is usually dependent on gill bailer movement (e.g. scaphognathites in crustaceans) (Nelson & Chabot, 2011). Therefore, in such cases the use of routine MR is more appropriate, as it is allows for some baseline levels of activity (Speakman, et al., 2004). Furthermore,

routine MR does not require restraint of the animal, allowing for further manipulations, such as measuring boldness.

Although the predicted relationship between behavioural types and energetic use seems logical, there is no consensus among the results from studies on a variety of species. For instance, exploration in the deer mouse, *Perumyscus maniculatus*, seems to be positively correlated to MR (Careau et al., 2011). In great tits, *Parus major*, exploratory behaviour is only weekly related to BMR (Bouwhuis et al., 2013), while in the brook charr (*Salvelinus fontinalis*) activity rate (estimated as the proportion of time spent moving in the field) had no relationship with RMR (Farwell & McLaughlin, 2009). This lack of agreement between studies, may indicate the presence of an overlooked element. In fact, in a recent meta-analysis Bell et al. (2009) found that two-thirds of the behavioural variation occurs within-individuals rather than between. This suggests that V_{WI} might represent a key trait that natural selection could act upon, its expression reflecting life-history trait variation underpinned by variation in MR (Velasque & Briffa, 2016; Chapter 2 in this thesis).

Alternatively, such conflicting results could be the result of measurement errors. For instance, individuals often exhibit behavioural responses to novel situations such as handling or being restrained (Mathot & Dingemanse, 2015), resulting in a personality-related bias in measurements (Roche et al., 2016). Therefore, shyer and more reactive individuals, associated with the slow POLS axis, would react more intensively to experimental manipulation (handling or restriction), generating a higher energetic consumption without a behavioural response (e.g. being bolder). Whereas, proactive individuals would acclimatise faster to the new situations, maintaining a normal level of energetic consumption (Careau et al., 2008). Such effects can be minimised with the reduction of the handling time and the increase of the resting time prior the

measurement (ensuring that the MR is not related to the response to novelty or to the handling).

Cognitive performance and animal personality

Cognition (see Glossary) is the process by which information is acquired, stored, processed and used (Shettleworth, 2010). Therefore, it could encompass a large variety of abilities such as categorization, learning (e.g. associative learning, habituation), behavioural inhibition, social learning, memory, resource assessment and decision-making (Shettleworth, 2010). Although its definitions are similar to those used for humans, cognitive performance (see Glossary) is not easy to quantify in non-human animals (Griffin et al., 2017). One way to estimate it is to quantify the change in behaviour, such as the time in which an animal takes to habituate to a new stimulus (e.g. Monteith, 1963), or to learn the association between a stimulus (e.g. light, noise) to a food reward or punishment (e.g. Faberet al., 1990; Menzel, 1993; Mallon et al., 2003). Alternatively, it is also possible to quantify an individual's performance during decision-making (Shettleworth, 2001), for instance by comparing the difference in the speed and accuracy of making relatively easy and difficult decisions.

Decision-making (see Glossary) is the cognitive process that results in the selection of a particular behaviour (or object) among a set of possible alternatives, based on given criteria or strategies (Wang & Ruhe, 2007). It involves acquiring, processing information from the environment (Wang & Ruhe, 2007) and it can be quantified with a single or multiples observation events (this is different from learning trials that require a certain number of trials until the animal achieve the level of performance required for the experiment). Therefore, when animals are faced with a choice, we would expect that individuals that spent more time gathering information would make a more accurate decision (less error prone) than animals that make a fast

assessment (e.g. Pachella, 1974), generating a trade-off between speed and accuracy (SATs) (Chittka & Osorio 2007; Chittka et al., 2009). For instance, depending on the urgency of the decision, ants (*Temnothorax curvispinosus*) may sacrifice accuracy for speed when deciding a new nest-site (Pratt & Sumpter, 2006).

Since making a fast, less accurate choice or a slow, more accurate choice is likely to generate fitness variation (e.g. a higher risk of predation while assessing the quality of a given resource), these decision-making processes are likely to be bound up with other life-history traits. Additionally, similar to other behavioural types, individuals may also fall onto a slow fast-continuum in respect of SATs (Trimmer et al, 2008). Therefore, it is possible that decision-making co-varies with personality types, where bold individuals would explore faster and make less accurate choices (Chapter 3).

Anthropogenic disturbances and behavioural variation

Human activities ranging from climate change (Walther et al., 2002) to noise pollution (Tidau & Briffa, 2016) have a great impact on natural environments. As a result, human activities have caused changes in populations dynamics, community composition (Vitousek et al., 1997) and in the behaviour of many species (e.g. Dowding et al., 2010), forcing individuals to adjust to the change in situation, if their behaviour is sufficiently plastic. For instance, in response to a human disturbance some species may change their foraging pattern (Legagneux & Ducatez, 2013) or even their behavioural type (Montiglio & Royauté, 2013). Therefore, such behavioural modifications can potentially improve the individual's probability of survival and reproduction, suggesting that less plastic individuals would be in disadvantaged in disturbed environments (e.g. Lowry et al., 2013).

Light pollution (see Glossary), caused by (electric) artificial lighting, is a fast - expanding current issue, increasing on average 6% per annum around coastlines (Hölker et al., 2010). The disruption of natural cycles of light and darkness caused by artificial lighting are linked with changes in spatial distribution (e.g. Vermeij & Bak, 2002), migration (e.g. Kiyofuji & Saitoh 2004; Ugolini et al., 2005) activity time (Titulaer et al., 2012), sexual maturation (e.g. Dominoni et al., 2013) and even in the predation risk (Rydell, 1992). Although it is also expected to cause changes in metabolic rate, the effect of light pollution in behavioural types is still unknown, and in Chapter 5 I describe an experiment designed to test this possibility.

Quantifying animal personality (and others statistical highlights)

Estimating animal personality

As initially stated, animal personality is the presence of between-individual behavioural differences that are consistent over time and/or situation (Gosling 2001, Sih et al., 2004, Réale et al., 2007, Biro & Stamps 2010). And, therefore, the presence of animal personality is dependent on two elements of variation: the variation between individuals (V_{BI}) and the variation that individual express over the multiple observations (within-individual variation - V_{WI}).Thus, animal personality is only said to be present when a significant amount of the total variance in the (longitudinal) data is explained by variance between individuals (V_{BI}) Expressed as such, the proportion of between individual variance can be estimated using repeatability (see Glossary) (R):

$$1 \qquad R = \frac{V_{BI}}{V_{BI} + V_{WI}}$$

The variance components to estimate repeatability can be obtained from models such as ANOVA (Wolak et al., 2012) or from linear mixed-effect models (LMM) (Nakagawa &

Schielzeth, 2010). Here, I estimate repeatability using the variance components extracted from hierarchical linear mixed models (see below for more information) with additional fixed effects (see Glossary) (Chapter 2) and from LMM (Chapter 2, 3, 4, 5) without including additional effects into their estimation. I will describe different types of LMM in the following section.

Linear mixed effect models

The study of animal personality requires longitudinal data, that is multiple observations of the same object (here the individual). Therefore, methods that accommodate this lack of independency between different observations (as required by t-test, ANOVA, ANCOVA for instance) are necessary (e.g. repeated measures ANOVA or LMMs).

LMMs models are extensions of the linear regression model, used for data collected in groups (individuals in this case). They describe the relationship between dependent and independent variables (called here fixed effects) in relation to one or more grouping variables (named random effects or components), following a normal distribution. The term random effects are used here to differentiate them from fixed effects, as the latter represents explanatory variables (e.g. sex, mass, body length) and the former the source of variability among the subjects. Therefore, in this context random effects represent the variation between individuals (Long, 2011).

A higher flexibility can be obtained using Generalized Linear Mixed Models (see Glossary) (GLMMs), which allows response variables from different distributions, such as categories, binary responses or distributions other than Gaussian, being preferred in personality studies, when compared to LMMs (Long, 2011). Nevertheless, they also assume a homoscedastic residual variance, that is when random effects have the same residual variance (as opposed to the heteroscedasticity, when residuals differ

across random effects). Therefore, GLMMs are not recommended when is necessary account for the within-individual variance in behaviour (especially in cases where this residual variance is known to differ between individuals) (Cleasby & Nakagawa, 2011). An alternative approach, that can cope with such data are, Hierarchical Generalized Linear Models (see Glossary) (HGLM) (Lee et al., 2006), and an extension of these called Double (or Doubly) Hierarchical Generalized Linear Models (DHGLM).

HGLMs allows the modelling of the residual variance for each individual using hyperparameters (Lee & Pawitan, 2006). These hyperparameters estimate between and within variance as a series of GLMs, simulating different regression models for each individual, and thus allows that different components possess independent and non-normal distribution. HGLMs are described to be robust and powerful with heavily tailed data (Lee & Nelder, 1996) and they can also be modified in order to include random effects for both the mean and dispersion parts (residual model variance) of the model, forming (Double)HGLMs (see Glossary) (Lee et al., 2006), as used here in Chapter 2.

Many models can be extended in order to include double hierarchical generalized linear models (DHGLM) (Verbeke & Molenberghs, 2000), such as generalized linear mixed models (Breslow & Clayton, 1993), random-coefficient models (Longford, 1993), mixed linear models (Verbeke & Molenberghs, 2000) and the Bayesian Markov Chain Monte Carlo (MCMC). However, for heavily skewed data models using the residual maximum likelihood (REML) are more recommended (Lee & Nelder, 1996). This is because REML models assume that there is significant variance attributed to the random effects that were not measured (unobserved random-effects; Lee & Nelder, 2006).

The choice of modelling approach depends on several factors such as number of fixed and random terms (see Figure 1.1), general trends in the data, the level of variance to be estimated and the hypotheses that will be explained by the model. In these studies, I used two types of modelling, DHGLM (Chapter 2) and HGLM (Chapters 3, 4 and 5) in two different approaches, REML (Chapters 2, 3, and 4) and Bayesian MCMC (Chapter 5). The choice of a different model (DHGLM or HGLM) was necessary where I investigated the presence of among individual differences in within-individual variation in behaviour (Chapter 2), requiring the estimation of the variance through DHGLM. In contrast the choice of a different statistical approach (REML or Bayesian MCMC) was due to the difficulty of estimating differences in repeatability, V_{BI} and V_{WI} (Chapter 5) using REML methods (executed in ASReml in R version). All models were fitted using R version 3.3.1 ('Bug in Your Hair').

In addition, models can also vary in function of the number of dependent variables. Models with a single dependent variable, are called 'univariate models' and they can include one or more independent variables measured over time (Long, 2011). The standard model for univariate regression for longitudinal data is given by:

2a $$y_{i,j} = (\beta_0 + ind_{0,j}) + e_{0,i,j}$$

2b $$\left[ind_{0,j}\right] \sim N(0, \Omega_{ind}): \quad \Omega_{ind} = [V_{BI}] \quad ,$$
$$\left[e_{0,i,j}\right] \sim N(0, \Omega_e): \quad \Omega_e = [V_{\mathrm{WI}}]$$

Where, $y_{i,j}$ is the (univariate) response of the individual i^{th} on the j^{th} occasion, β_0 is the mean value of average individual responses and each individual mean response. The inclusion of the random intercept allows the estimation of the individual contribution the population mean by modelling the differences in mean response between individuals ($ind_{0,j}$). Such random effects are assumed to be normally distributed (N) with a mean zero and a variance (Ω_{ind}) representing the between-individual variance (V_{BI}: variance across random intercepts of individuals). The residual error $e_{0,j,i}$ also

normally distributed, with a zero mean and a variance (Ω_e) representing the within-individual variance (V_{WI}). Therefore, such univariate models can be used to estimate repeatability (Equation 1).

The equation 2a can be expanded to include additional fixed effects (β_1 to β_{n+1}). Note that univariate models excluded covariance and other interactive effects (e.g. between or within-individual covariance) common in longitudinal data. Therefore, in situations where it is necessary to residual model variance (e.g. to decompose phenotypic correlations or when individuals are assessed at different times), a special model is required. Multivariate models can provide such level of flexibility, in addition, detailing how between and within-individual effects contribute to the mean effect. Furthermore, multivariate models also allow the use of more than one dependent variable, that are measured repeatedly over time. Multivariate models with two dependent variables are called bivariate models (e.g. exploration and boldness) and they can be estimated with the following model (as used on Chapter 4):

3a $$\begin{aligned} y_{i,j} &= (\beta_{0\mathrm{y}} + ind_{0,\mathrm{y},j}) + e_{0,\mathrm{y},i,j} \\ z_{i,j} &= (\beta_{0\mathrm{z}} + ind_{0,\mathrm{z},j}) + e_{0,\mathrm{z},i,j} \end{aligned} ,$$

Where, z and y represent the phenotypic attributes. Similar to the univariate model instance 'i' for individual 'j' is modelled by fitting random intercept for each level of the individual $ind_{0,j.}$ I also modelled $\beta_{0,y}$ and $\beta_{0,z}$ as being distinct (e.g. Matsuyama & Ohashi, 1997). The random effects are given by:

3b $$\begin{bmatrix} ind_{0,\mathrm{y},j} \\ ind_{0,\mathrm{z},j} \end{bmatrix} \sim \mathrm{MV}N(0, \Omega_{ind}): \quad \Omega_{ind} = \begin{bmatrix} V_{BI_y} & Cov_{BI_y,\,BI_z} \\ Cov_{BI_y,\,BI_z} & V_{BI_z} \end{bmatrix} ,$$
$$\begin{bmatrix} e_{0,\mathrm{y},j} \\ e_{0,\mathrm{z},j} \end{bmatrix} \sim \mathrm{MV}N(0, \Omega_e): \quad \Omega_e = \begin{bmatrix} V_{WI_y} & Cov_{BI_y,\,BI_z} \\ Cov_{WI_y,WI_Z} & V_{WI_Z} \end{bmatrix}$$

In this bivariate model, different from the univariate neither random intercept or residual nor the residual errors are independent. The random intercept is assumed to have a multivariate normal distribution with a variance-covariance structure (Ω_{ind}). The

variance-covariance structure, presented on equation 3b, is assumed to have between individual variance (V_{BIy} and V_{Biz};) and covariance ($COV_{BIy,BIz}$). The residual errors are equally assumed to be from a multivariate normal distribution, with means of zero, within-individual variance (V_{WIz} and V_{WIy}) and covariance ($COV_{Wiy,Wiz}$).

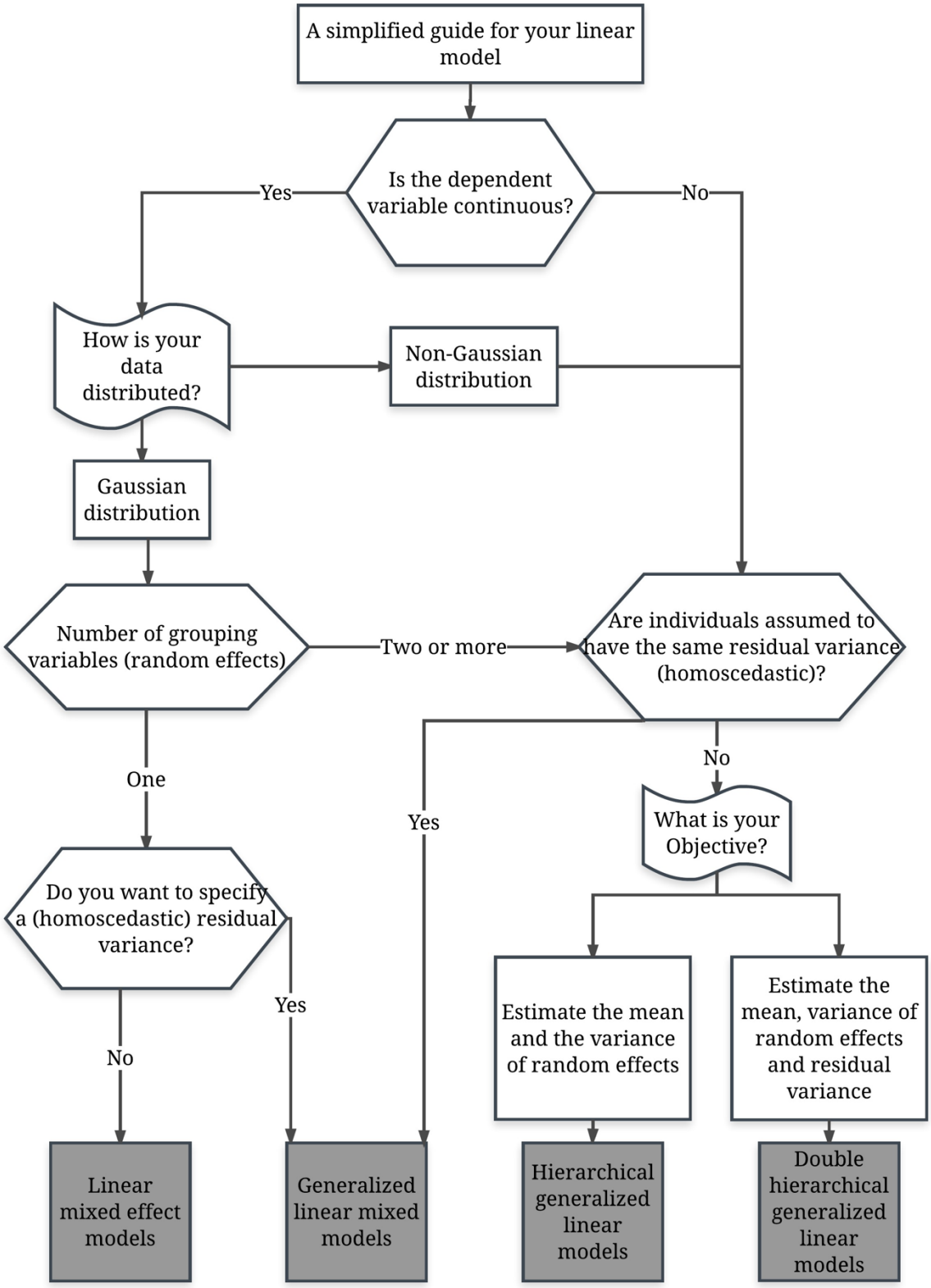

Figure 1.1: Decision tree aid the selection of the appropriate statistical techniques to analyse longitudinal data.

The hermit crab *Pagurus bernhardus*: a model system for the study of animal personalities

The hermit crab *Pagurus bernhardus* (Crustacea: Anomura) is one of the most common hermit crab species of Europe's coasts. They can be found in a great range of marine habitats including rock, seagrass and sand areas, with the depth varying according with their size (smaller individuals tend to live in shallower waters when compared to larger individuals; Lancaster, 1990). As with most hermit crabs*, P. bernhardus* has a strongly calcified carapace covering the cephalothorax but the soft abdomen is weakly calcified and therefore hermit crabs rely on occupying empty gastropod shells for protection (Taylor, 1981; Elwood et al.,1995). As a result, the type and size of gastropod shell can have an important effect on the fitness of hermit crabs. For instance, if the occupied shell is too small (compared with the hermit crab size), the protection against predators (e.g., Angel, 2000) and survival (e.g., Borjesson & Szelistowski, 1989) can be reduced. Whereas, occupying shells that are large (compared with the hermit crab size) imposes a higher energetic cost to carry it (Elwood & Neil, 1992). Consequently, hermit crabs may face a strong selective pressure to obtain a shell of the optimal size and species (Jackson &Elwood, 1989; Tricarico & Gherardi, 2007).

In addition, hermit crabs when threatened withdraw into their gastropod shells for protection. Previous studies have demonstrated that the time spent withdrawn into the shell before re-emerging (startle response duration) can be used as a repeatable index of boldness, being consistent different between individuals over time (Briffa, 2013; Briffa et al., 2013; Bridger et al., 2015) and across situations (Briffa et al., 2013; Courtene-Jones & Briffa, 2014). Furthermore, they show significant differences between individuals (Stamps et al., 2012) and situations (Briffa, 2013; White & Briffa,

2017) in the amount of variation within-individuals (V_{WI}). Therefore, *Pagurus bernhardus* is the model organism in this study.

Thesis objective

The overall objective of this thesis is to determine whether consistent between individual variation in behaviour (V_{BI}) and within-individual variation in behaviour (V_{WI}) can be explained by variation in metabolic rate and other life-history traits, across a range of behavioural contexts and environmental situations. The pace of life syndrome hypothesis (POLS) already posits that V_{BI} should be driven by variation in metabolic rate. Therefore, as well as testing this prediction I aim to determine whether there is any evidence that POLS could be extended to explain variation in V_{WI} as well. It seems logical that it could, since much of the theory underlying the POLS relates to how animals cope with risk and low predictability (i.e. high V_{WI}) could represent a strategy to reduce exposure to predation risk.

To answer these questions, I set four specific objectives that were answered by a series of experiments:

1. My first objective (Chapter 2) was to investigate whether consistent individual differences and within-individual variation in boldness are underpinned by variations in energy consumption as estimated by variation in metabolic rate) and hermit crab mass.

2. My second objective (Chapter 3) was to investigate whether consistent individual differences in boldness and energy expenditure could explain variation in cognitive performance shown during a shell assessment and decision-making task.

3. My third objective (Chapter 4) was to investigate the presence of a behavioural syndrome between exploration, quantified as spontaneous alternation performance, and boldness and whether variation in exploration and boldness are underpinned by variations in energetic consumption

4. My final objective (Chapter 5) was to investigate the effect of constant light on boldness (average behaviour and consistency) and energetic consumption.

Glossary

Aggressiveness: An individual's agonistic reaction toward conspecifics (Réale et al., 2007).

Animal personality: Defined as differences between individuals' average level of behaviour that are repeatable across time and/or contexts (Réale et al., 2007).

Basal metabolic rate (BMR): Lower metabolic rate of an adult endotherm, post-absorptive, non-productive and inactive while in his neutral thermal zone (McNab, 1997).

Behavioural plasticity: Is the individual change in behaviour as a function of a changing in condition (e.g. environmental conditions) (Dingemanse & Dochtermann, 2013).

Behavioural syndrome: Refers to the presence of correlation between two or more between or more personality traits that are consistent through time and/or across situations (Dingemanse et al., 2012).

Behavioural reaction norm: Describes the individual change in behaviour over an environmental gradient (Dingemanse et al., 2010).

Behavioural type: Is the individual mean expression of a specific behaviour (Bell, 2007).

Bivariate analysis: Binomial distribution: Used in statistics to describes data distributed in discrete probability where events are distributed in a sequence of n independent yes/no experiments (Crawley, 2007).

Boldness: Response to potentially risky situations (Réale et al., 2007) or propensity to take risks (Huntingford et al., 2010; Killen et al., 2011).

Cognition: Mechanisms that enable the acquisition, processing, storage and use of information. Including perception, learning, memory, and decision-making (Shettleworth, 2010).

Cognitive performance: Is the mechanism used to quantify individual in a cognitive trait (e.g. number of trials to an animal learn to responds to a noxious stimulus) (Shettleworth, 2010).

Context: Is the functional category in which the behaviour occurs (e.g. boldness, feeding courtship, aggression, exploration) (Briffa & Weiss, 2010).

Decision-making: Type of cognitive process that results in the selection of an option or course of action (Wang, 2007).

Double Hierarchical Generalized Linear Models (DHGLM): Extension to HGLM by allowing the estimation of an error component alongside the mean component (Lee & Nelder, 2006).

Exploration: The response of an individual to novel situations, including the behaviour towards a new habitat, new food or new object. Likely to be misleading once it could neophobia or boldness (Réale et al., 2007).

Generalized Linear Mixed Model (GLMs): Is an extension to the linear mixed models, on which the predictor contains both random and fixed effects. They can also accommodate non-normal linear mixed models (Long et al., 2011).

Hierarchical Generalized Linear Models (HGLM): Is an extension to the GLM by relaxing the assumption that error components are independent, allowing models with more than one error terms and also allows for dependencies between error terms (Lee & Nelder, 2006).

Individual variation: Differences among individuals within a population after accounting with variation (Réale et al., 2007).

Life-history traits: Traits associated with the individual life strategy, including growth rate, age and size at sexual maturity, the temporal pattern or schedule of reproduction, the number, size, and sex ratio of offspring, the distribution of intrinsic or extrinsic mortality rates (e.g., patterns of senescence) and patterns of dormancy and dispersal.

Light pollution: Presence light in areas where it is not needed, and thereby interferes with some visual act (Davies et al., 2014).

Multivariate statistics: Type of statistical analysis that allows the examination of multiple variables at the same time (Crawley, 2007).

Pace of life syndrome: Association between one or more life-history traits following a slow-fast continuum (Réale et al., 2010).

Personality type: Used here to refer to the various degrees of a personality trait (e.g., bold vs shy) (Réale et al., 2010).

Repeatability: Is the proportion of phenotypic variance explained by differences among individuals (Wray, 2013).

Routine metabolic rate (routine MR): Is metabolic rate of a post-absorptive, undisturbed ectotherm, allowing for some spontaneous activity (e.g., ventilation of gills) and maintenance of body posture (McNab, 1997).

Situation: Any external condition that can vary across a gradient, including social (e.g. group size or composition), habitat (e.g. temperature or perceived predation risk) and physiological (e.g. internal state) situations (Dingemanse et al., 2010).

Standard metabolic rate (SMR): Is the lowest metabolic rate of an ectotherm that is at rest during its normal period of inactivity, post-absorptive, and non-reproductive at a specified ambient temperature (McNab, 1997).

Univariate model: Type of regression on with a single dependent variable normally distributed (Crawley, 2007).

Chapter 2

The opposite effects of routine metabolic rate and metabolic rate during startle responses on variation in the predictability of behaviour in hermit crabs

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Abstract

Studies on animal behaviour have suggested a link between personality and energy expenditure. However, most models assume constant variation within-individuals, even though individuals vary between observations. Such variation is called within-individual variation in behaviour (V_{WI}). I investigate if V_{WI} in the duration of the startle response is associated with metabolic rates (MR) in the hermit crab *Pagurus bernhardus*. I repeatedly measured startle response durations and MR during each observation. I used double hierarchical generalized linear models to ask whether between and V_{WI} in behaviour was underpinned by MR. I found no association between the mean duration of the startle responses and either routine MR or MR during startle response. Nevertheless, I found that V_{WI} increased with MR during startle responses and decreased with routine MR. These results indicate that crabs with higher MR during startle responses behave less predictably, and that predictability is reduced during exposure to elevated temperatures.

Introduction

In many species individuals differ in their behaviour consistently over time and these differences are often maintained across situations (Gosling & John, 1999; Sih et al., 2004b; Réale et al., 2007; Briffa & Greenaway, 2011). Some individuals, for example, are consistently bolder (e.g. Brown & Braithwaite, 2004; Magnhagen & Borcherding, 2008; Bridger et al., 2015), more aggressive (Bell, 2007; Sih & Bell, 2008) or more cooperative (Bergmüller & Taborsky, 2007; Charmantier et al., 2007; Schürch & Heg, 2010) than other individuals from the same population. Such consistent between individual behavioural variation is termed 'animal personality' (Gosling, 2001; Drent et al., 2003; Dingemanse & Reale, 2005; Reale et al., 2007) and it seems to be ubiquitous in nature, ranging from humans to invertebrates (Gosling & John, 1999; Sih et al., 2004b; Reale et al., 2007; Briffa & Greenaway, 2011).

Several methods have been used to estimate such behavioural consistency, but the estimation of the 'broad sense' (as defined by Biro & Stamps, 2015) repeatability, which is the most widely used measure of repeatability (Hayes & Jenkins, 1997; Bell et al., 2009; Biro & Stamps, 2015). In general, repeatability values tend to be low to moderate (0.37-0.42; Bell et al., 2009). Thus, most variation in behaviour is unaccounted for as consistent inter-individual differences. One possibility for this is that unaccounted for environmental factors may contribute to variation in behavioural traits, so as to 'mask' the true amount of variation between individuals (e.g. Briffa & Greenaway, 2011). Another possibility is that significant amounts of variation occur within, rather than between individuals (Bell et al., 2009; Biro & Stamps, 2015). Since repeatability (R) is the proportion of total variance (variance between individuals [V_{BI}] + variance within-individuals [V_{WI}]) that is due to between individual differences (i.e. R = V_{BI} / (V_{BI} + V_{WI})), overall estimates of repeatability do not provide direct information

on the individual variance components. Thus, recent studies have focussed on understanding what factors may influence the within-individual or 'within-individual variance' component (V_{WI}) (Nesselroade, 1991; Siegler, 1994; Salthouse, 2007; Ram & Gerstorf, 2009; Stamps et al., 2012; Briffa et al., 2013). This V_{WI} is also sometimes referred to as predictability or individual consistency and as inter-individual variation in behaviour (IIV). In this case, individuals with a higher predictability will have lower V_{WI} scores. Such studies have demonstrated that V_{WI} is an important component of animal behaviour that seems to be related to learning (Maye et al., 2007; MacDonald et al., 2009; Bielak et al., 2010; Brembs, 2011), coping with risk (Briffa, 2013) and male dominance and sexual selection (Cowlishaw, 1996; Rogers & Cato, 2002; Delgado, 2006).

In general, studies on animal personality have been revealing links between behavioural types (mean behaviour) and the pace of life syndrome (POLS; e.g. Smith & Blumstein, 2008; Bell et al., 2009; Réale at al., 2010; Garamszegi et al., 2012; 2013; Urszán et al., 2015b). The POLS hypothesis aims to explain variation in life-history strategies and physiological traits, which are assumed to be expressed along a slow-fast continuum (Hille & Cooper, 2015). Individuals that follow a slow POLS are expected to be long-living, avoid risk, be less active and with a lower metabolic rate (MR). In contrast, those with a fast POLS are expected to be more active, take more risks, be more aggressive and have a higher MR (e.g. "live fast and die young"; Biro & Stamps, 2008; Smith & Blumstein, 2008; Réale et al., 2010). Thus, variation in the pace of life might represent an underlying mechanism for animal personality variation (Careau & Garland, 2012), both in terms of between individual differences in mean level behaviour and in terms of between individual differences in behavioural consistency or V_{WI}.

So far, studies investigating the link between behavioural traits and POLS have focussed on mean-level differences in behaviour, and taken together they indicate that the relationship between pace of life and animal personalities varies among study systems. In the deer mice (*Perumyscus maniculatus*) Careau et, al. (2011) found a positive relationship between exploration and MR. Similarly, more exploratory individuals of the superb fairy-wren (*Malurus cyaneus*) were less likely to be found 12 months later, indicating that these individuals are less long-lived. In contrast, the brown trout, *Salmo trutta*, seems to have a negative relationship between activity levels and mortality, indicating that longevity is not necessarily reduced in active individuals (Adriaenssens & Johnsson, 2013). This lack of consensus between studies, may indicate the presence of an overlooked element, such as within-individual variation in behaviour. In fact, in recent meta-analysis Réale et al. (2010) found that two-thirds of the behavioural variation occurs within-individuals rather than between individuals. Furthermore, recent studies focussing explicitly on the analysis of V_{WI}, showing that predictability can consistently vary between individuals (i.e. V_{WI} itself is repeatable; Biro & Adriaenssens, 2013), as well as at the mean level. Taken together, these results suggest that V_{WI} might play a key role in both natural and sexual selection and thus, be linked with POLS (Urszán et al., 2015b).

It seems logical, then, that variation in metabolic rate might drive between individual variation in behaviour both at the individual-mean level and at the level of between individual variation in V_{WI} (Careau et al., 2008; Houston, 2010). Nevertheless, studies investigating relationships between metabolism and V_{WI} are still rare (Mathot & Dingemanse, 2015). In the case of ectothermic marine invertebrates, metabolic rate is known to be driven by the temperature of the seawater. Thus, it is possible that individual consistency could be also dependent upon fluctuations in seawater temperature, if MR influences V_{WI}. In the European hermit crab, *Pagurus bernhardus*,

there is some evidence that startle response durations (where crabs hide in their empty gastropod shell upon disturbance, see below) increase at higher temperatures, although this effect was modified by the experimental order (Briffa et al., 2013). There was, however, a clearer effect at the level of V_{WI}, with crabs at a higher temperature treatment behaving less consistently (i.e. showing greater V_{WI}) than those subjected to lower temperature, across a 5°C temperature difference. Thus, individuals that presumably had higher metabolic rates (due to elevated temperature) showed greater within-individual variance in behaviour than those with lower metabolic rates.

One potential interpretation of this result is that individuals with high rates of metabolism have relatively high energy demands and therefore might be exposed to greater predation risk due to the need to service these energy demands through foraging (Briffa et al., 2013). Behaving less predictably might be a way to minimise these risks, and indeed *P. bernhardus* appear to behave less consistently in the presence of a predator (Briffa, 2013). Here, I directly investigate the links between metabolic rate and the duration of startle responses in the European hermit crab, while accounting for small fluctuations in temperature in the laboratory and for variation in crab mass. Hermit crabs occupy empty gastropod shells and a simple stimulus causes them to withdraw from their shells and slowly re-emerge. The latency to emerge from the shell ('startle response duration') is considered a measure of 'boldness' (Biro & Stamps, 2008; Briffa et al., 2013; Bridger et al., 2015) and its variation between and within-individuals is well studied in this species (Briffa, 2013; Briffa et al., 2013; Bridger et al., 2015). To avoid indirect associations between MR and the behavioural trait (Mathot & Dingemanse, 2015), I repeatedly measured the oxygen consumption in two situations: during routine behaviour (routine MR) and during the startle response period, where crabs are withdrawn into their shells (startled MR). I considered startled MR as the metabolic rate of a reactive individual during and after the application of a stimulus.

Routine MR is defined as the metabolic rate of an undisturbed, post-absorptive, resting individual, it also includes some level of random activity, maintenance of equilibrium and posture (Jobling, 1994; Killen et al., 2011; Mathot & Dingemanse, 2015).

Here I investigate the links between metabolic rate and boldness analysed across two levels, individual mean boldness and within-individual variation in boldness (V_{WI}). In addition to variation in metabolic rate I also analysed the effects of small (uncontrolled) fluctuations in temperature within the laboratory, since these could influence both metabolism and oxygen availability. I predicted that the MR would have a negative correlation with the duration of the startle response at the mean level of behaviour and in V_{WI} of behaviour. In other words, according to the POLS hypothesis, it is expected that individuals with a high metabolic rate would recover quickly from a perturbation and hence show startle responses of relatively short duration. Furthermore, if shorter startle responses equate to greater risk exposure, I would expect individuals with higher metabolic rate to show relatively low levels of behavioural consistency, and hence high levels of V_{WI}.

Materials and methods

Collection and maintenance of hermit crabs

I collected hermit crabs between November 2013 and January 2014 from the rocky intertidal at Hannafore Point, Cornwall, UK (50°20′N, -4°27′W), from where they were transported directly back to the laboratory at Plymouth. In the laboratory, I removed crabs from their shells by cracking in a bench vice. This stage is necessary because the behaviour of hermit crabs, including the duration of the startle response, could be affected by the shell mass (Briffa & Bibost, 2009). All crabs thus received a new *Littorina littorea* shell with 100% of its preferred mass (Briffa & Elwood, 2007). I only used male crabs (mean mass = 0.76g ± SE = 0.34g) free from obvious parasites, appendage damage or recent moult (N=40). Crabs were individually housed in white plastic dishes of 16cm of diameter, filled to 4cm depth with seawater, with continuous aeration and feed daily *ad libitum* with cubes of white fish at the end of each observation (i.e. there was always excess food available in the housing dishes, outside of the observation periods). The seawater was from the laboratory supply, which is regularly obtained from the seaward side of Mount Batten pier (50°36′N, -4°13′W) in Plymouth Sound, at spring tides. They were acclimated for ten days in a temperature controlled room (mean temperature= 12.21º± SE =1.05ºC), followed by ten days of daily observation of startle response durations (see below).

Determination of routine and metabolic rate

I determined the routine and startled metabolic rate for each crab using the oxygen uptake as a proxy (Dupont-Prinet et al., 2010). This was measured daily using a sealed 'Kilner' jar (polyethylene terephthalate, PET), blackened-out and a non-invasive optical oxygen sensor with a temperature probe (OxySense GEN III 5000 series, OxySense,

Dallas, TX). Each jar had a sensor spot attached on the inner wall with silicone glue. The sensor spot reacts with the oxygen present inside the jar and, when read by the sensor, shows the remaining concentration of O_2, allowing a non-invasive measurement (a closed respirometer). I closed the jar underwater using a rubber washer to ensure the absence of air bubbles. To prevent oxygen stratification and ensure moderate mixing of water, I placed the jar onto a multi-channel magnetic stirrer (MIX 15 eco; 2mag AG, Munich, Germany) with a magnetic flea inside. A mesh was placed between the hermit crab and the magnetic flea to ensure no contact. In order to control for possible bias from algal or bacterial activity in oxygen measurements, I only used filtered seawater. Additionally, oxygen measures can also be influenced by the jar material, as some types of plastic can absorb or release oxygen (Stevens, 1992) (although significant O_2 exchange is less likely with the high-quality PET material that I used here, compared with other plastics). Therefore, to account for both microbial activity and potential oxygen exchange with the jar material, I measured the oxygen consumption in two extra jars containing the same seawater used in the experiments and one empty *L. littorea* shell ("blank") similar to those occupied by the crabs. I then accounted for any microbial activity and any the effect of the jar's material in the final calculation of metabolic rate (Calosi, et al., 2013).

I allowed hermit crabs rest for 15 minutes before starting routine MR measures of oxygen consumption. To allow an accurate estimation of oxygen concentration inside the jar I obtained readings every 5 minutes, during 25 minutes (i.e. 5 measures of O_2 concentration in total per estimate). These measures were then used to estimate the rate of O_2 uptake by the hermit crab (see below for the calculation). Previous observations indicate a stabilization of oxygen consumption after 15 minutes of resting, and therefore, a minimization of stress imposed by the manipulation.

I then recorded the startled MR immediately after the routine MR. I obtained the startled MR measurements immediately after inducing a startle response (Briffa et al., 2008; Stamps et al., 2012). As previously stated, I considered startled MR as the metabolic rate of an individual during and after a stimulus, thus I measured startled MR and startle responses simultaneously. In the current study, where crabs are housed in sealed jars for the measurement of metabolic rate, it was not possible to induce startle responses in the same way as previous studies by manually handling the crabs. This is because the manual handling procedure would entail opening the respirometry jar and then resealing it after the response had been induced. These procedures would lead to (a) a time delay in the onset of measurements of oxygen concentration and (b) the potential for additional disturbance to the crabs (e.g. unintentional movements of the jar during resealing) that would be difficult to quantify. Therefore, I used an alternative technique where the startle response was induced by carefully lifting the jar (avoiding early disturbance to the crab) by 30cm and then releasing it so that it fell back onto the bench. This induced hermit crabs to withdraw into their gastropod shells in a similar way to the manual handling technique used in previous studies, with the vast majority of crabs falling into an aperture facing upwards position following the drop. Furthermore, the use of a physical impact on the jar is similar to an approach used in other studies of hermit crabs where an object has been dropped onto the top of an unsealed crystallising dish to induce startle responses (see Elwood & Briffa, 2001; Briffa & Elwood, 2001). Initial observations indicated that when aligned in a horizontal position and dropped from 30cm, the jar will rarely spin or bounce when hits the bench, independent of the hermit crab position inside the jar. When it occurs, routine MR measures and the startle response induction were made after two hours of rest.

I timed the latency of recovery from the point at which the jar is released to the point at which the perieopods first make contact with base of the jar. As the startle

response varies between and within-individual hermit crabs it was not possible to measure oxygen concentrations over a set time period as in the case for the estimation of routine MR. Instead I measured the oxygen concentration inside the jar at a higher time resolution of every five seconds. These measurements commenced at the point at which the hermit crab withdrew into its shell and continued until five minutes after its recovery. These repeated measures of oxygen concentration were then used to estimate the rate of oxygen uptake during and following the startle response (see below for calculation).

I obtained 10 startle response durations along with 10 measures of Routine MR and startled MR for each of 40 individuals yielding 400 observations in total. Measures made using closed respirometers are never constant due to the continuous use of oxygen by the organisms inside the jar. Thus, I used the decrease in oxygen concentration to calculate the O_2 consumption per individual. In humid environments, the oxygen consumption is dependent on the oxygen solubility, which in turn is dependent on both temperature and salinity. Although I conducted measures in temperature controlled rooms, small fluctuations still occurred, which could affect both behaviour and oxygen consumption rates. I calculate the O_2 consumption rate using the slope from a linear regression of oxygen consumption over time minus the blank variations. The slope was then multiplied by the oxygen solubility coefficient (adjusted for salinity and temperature). Thus, rate of O_2 consumption was calculated using:

$$Rate\ of\ O_2 uptake\ (\mu\text{moles}\ O_2 h^{-1}) = C(t) \times (V_r) \times ({}^{60}/_{t_1 - t_2})$$

Where, C(t) is the O_2 consumption rate (from the linear regression of oxygen consumption over time), V_r is the total volume of water inside the jar (jar volume minus the hermit crab volume) and t_0, t_1, is the measurement period (in minutes; Widdows & Staff, 2006; Calosi, et al.,2013). To create a standardized measure and allow

comparisons between individuals, I divided the rate of O_2 uptake by the individual body mass (without the shell; Porter & Brand, 1995).

Data analysis

Previously, mean level behaviour and V_{WI} have been calculated using general linear-mixed effects model (GLMM) in a two-step analysis. First, a random regression model is fitted (Stamps et al., *2012*; Briffa et al., 2013; Cleasby et al., 2015). With this model, it is possible to obtain the expected values, followed by the residual individual standard deviation (riSD). Although widely used in human personality, and recently animal personality, research this method has some limitations (Cleasby et al., 2015; Bridger et al., 2015). GLMMs assume homoscedastic residual variation in behaviour (the same for all individuals), which is violated if individuals do indeed differ in V_{WI} (Cleasby et al., 2015; Bridger et al., 2015). Secondly, a two-step analysis might inflate type 1 errors, by not carrying forward the uncertainty estimates from step 1.

More recently (e.g. Bridger et al., 2015) an alternative approach has been used, where the mean and the variance for each individual are modelled iteratively using hyperparameters. These models are extensions of GLMM called hierarchical GLMs (HGLM). HGLMs models sets of interlinked GLMs, allowing parameters non-normally distributed (Lee & Nelder, 1996). Additionally, such models allow the specification of separate models for the mean (relative to V_{BI}) and standard deviation model (SD model, relative to V_{WI}), both incorporating fixed and random effects. Such models are termed Double HGLM (DHGLM; Lee & Nelder, 2006). I used the software ASReml-R (Butler et al., 2009) in R version 3.2.1 (R Development Core Team, 2012) to fit a DHGLM model, as follows.

In the mean model, I included observation number, mass, routine and startled metabolic rate as fixed effects. As I had small temperature variations, I also include temperature as a fixed effect (covariate) to account for this. I modelled a random intercept and a random slope effect to allow for between individual variation in responses to repeated observations (see supplementary material S1). I tested for correlation between all fixed effects and none were significant (see supplementary material S1). In the SD model, I included mass, temperature, routine and startled metabolic rate as fixed effects, and random intercepts for each individual (random slopes are not possible in the SD model because I only obtained one set of repeated measures within per individual, allowing a single estimate of residual variance; see supplementary material S1). As the model is expected to be robust against outliers (Lee & Nelder, 2006), I opted to use non-transformed data. I used Wald-F test to test the significance of fixed effects and z-ratio for random.

Ethical note

No animals were harmed during the experiment and at the end of the experiment all individuals appeared healthy and were supplied with excess food (as above) and a new gastropod shell before being returned to the sea (same collection point).

Results

The mean startle response duration did not vary with either routine ($\chi^2_1 = 0.41$, $p = 0.5227$) or startled ($\chi^2 = 0.06$, $p = 0.8140$) metabolic rate, and there was no significant effect of either mass ($\chi^2 = 0.24$, $p = 0.6227$) or temperature ($\chi^2 = 1.20$, $p = 0.27$; Table 2.1) on the startle response duration. There was, however, a clear pattern of reduction in startle response duration across successive observations ($\chi^2 = 18.89$, $p = 0.02617$, Table 2.1), indicating habituation. A significant random intercept indicated that there was between individual variation in the mean startle response duration ($\chi^2 = 11.37$, $p = 0.0006$, Table 2.1).

In the standard deviation model, the analysis of the fixed effects indicates that V_{WI} in startle response duration increases with temperature ($\chi^2 = 28.9$, $p < 0.001$) and with startled metabolic rate ($\chi^2 = 10.4$, $p = 0.00062$) but decreases with crab mass ($\chi^2 = 25.1$, $p < 0.001$) and with routine metabolic rate ($\chi^2 = 55.4$, $p < 0.001$; Table 2.1; Figure 2.1). In summary, crabs behave more consistently when exposed to higher average temperatures and if they have a high metabolic rate during the startle response. In contrast, crabs that were large or had a higher routine MR showed an increasing of consistency in startle response duration.

The random intercepts of the mean and SD model indicate the presence of significant within-individual variation, but not significant between individual variation (Table 2.1). To provide a standardised measure of the proportion of variance due to between individual variation in behaviour I also calculated the repeatability (R_c; Biro & Stamps, 2015) of startle responses and both routine and startled MR. For each variable, I fitted a model on only a constant (intercept) and the observation as a fixed effect and the individual's ID as random effect (Wilson et al., 2010). The significance of repeatability was obtained using a likelihood ratio test (LRT) method, which compares

the likelihoods of the linear mixed model described above (with the individual's ID as a random effect) and a general linear model without random effect (Nakagawa & Schielzeth, 2010), distributed as Chi square with one degree of freedom. The repeatability of the startle response was 0.013 (χ^2=28.33, p<0.001). Since it is not possible to directly generate confidence intervals around this estimate of repeatability obtained from the variance components of the model, and to allow comparison with other studies where data on traits such as MR are absent, I also calculated an unadjusted (in relation to the model fixed effects) repeatability, based on a linear mixed model. Here the repeatability was R_{LMM} = 0.111 ± SE = 0.042 (95% CI = [0.034, 0.193], P < 0.0001). The repeatability of the routine MR was 0.00133 (χ^2=61.26, p<0.001) and startle MR was 0.00006 (χ^2=1.91, p=0.166).

Table 2.1: The fixed effects and their statistical significance of the mean model and for standard deviation model of the duration of the startle response.

Sub-model	Parameter name	Effect	SE	df	Wald χ^2	p-value
Mean	**Intercept**	**96.9998**	**113.269**	**1**	**11.3699**	**0.00055**
	Observation	**-8.5687**	**3.363**	**9**	**18.8876**	**0.02617**
	Mass	-22.1446	135.238	1	0.2421	0.62271
	Temperature	12.1099	42.623	1	1.1993	0.27346
	Routine MR	-0.0155	0.475	1	0.4085	0.52272
	Startled MR	-0.5088	0.435	1	0.0553	0.81403
SD	**Intercept**	**7.142901**	**0.9231**	**1**	**19041.8**	**<0.0001**
	Mass	**-1.19803**	**0.2335**	**1**	**25.1**	**<0.0001**
	Temperature	**0.39201**	**0.0729**	**1**	**28.9**	**<0.0001**
	Routine MR	**-0.00511**	**0.0007**	**1**	**55.4**	**<0.0001**
	Startled MR	**0.00229**	**0.0007**	**1**	**10.4**	**0.00062**

(Mean effect sizes of factors and covariates with their effects, standard error, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 2.2: Estimated variance components of the mean model for behavioural traits. σ^2 is the variance of each component. Statistical significance is assessed by comparing variance to the Z-Ratio; effects are considered to be statistically significant if Z> 2 (Wilson et al., 2010). Significant variables are printed in bold.

Component	$\boldsymbol{\sigma^2}$	SE	Z-Ratio
Between individual	2122.309	3939.88	0.53868
Within-individual	43583.42	5653.985	**7.7084441**

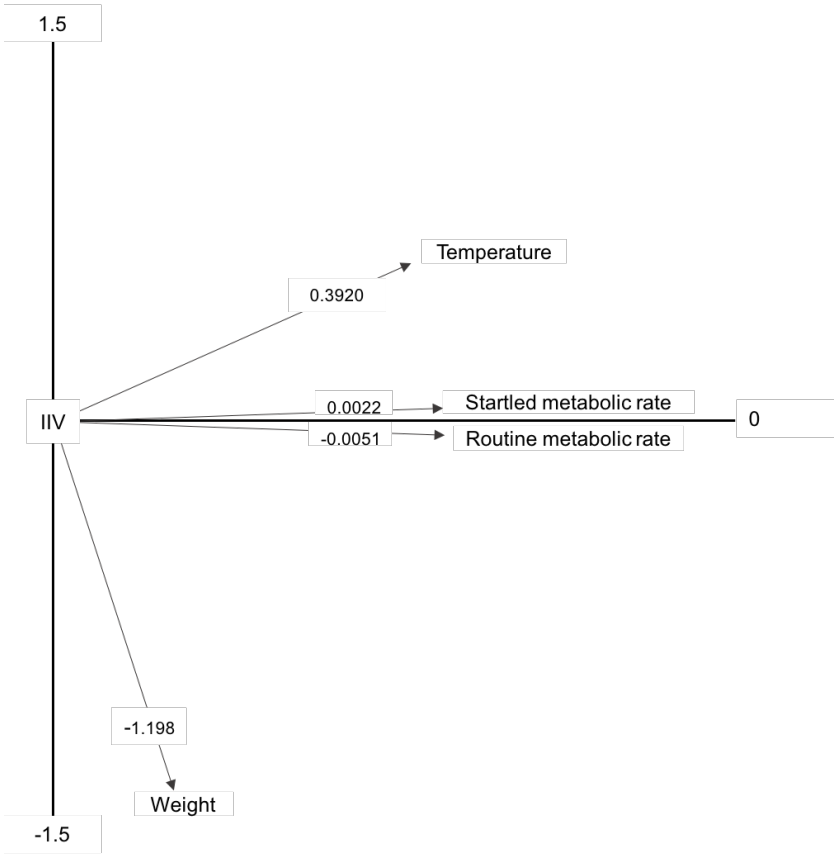

Figure 2.1: Representation of the effects of four parameters on V_{WI}. Values were extracted from the standard deviation model on the DHGLM. The angle of the arrows represents the effect size of each parameter on V_{WI}.

Discussion

Previous studies highlighted the importance of investigating within-individual variance in behaviour in addition to the mean level (Briffa, 2013; Brommer, 2013; Dingemanse & Dochtermann, 2013; Bridger et al., 2015). In the hermit crab *Pagurus bernhardus,* I found that body mass, temperature, routine and startled metabolic rate had no relationship in the mean duration of the startle response, and thus, contrary to the prediction, boldness in *P. bernhardus* does not appear to be related with POLS. However, I found a significant relationship between V_{WI} in startle response and these life-history traits (body mass, temperature, routine MR and startled MR). Additionally, *P. bernhardus* also exhibited a higher within-individual variation in behaviour than between individual variation, consistent with a previous meta-analysis (Bell et al., 2009). Behavioural traits, in general, have low to moderate repeatability (0.37-0.42; Bell et al., 2009), which has been shown in previous studies in *P. bernhardus* (e.g. Stamps et al., 2012; Briffa, 2013; Briffa et al., 2013). In this study, however, although there was significant repeatability (i.e. 95% CIs did not cross zero) my estimates were much lower than for those previous studies on the same species. One possible explanation for the lower repeatability is the method used to stimulate the startle response. As the startle response is associated with defence, being manually handled (e.g. Stamps et al., 2012; Briffa, 2013; Briffa et al., 2013) could be a less intense stimulus for hermit crabs and thus, result in a more predictable response than a free fall of 30cm, as handly could be associated with predation risk. Furthermore, a recent study on tadpoles of the frog *Rana dalmatina* has shown that novel stimuli might yield lower repeatability compared to stimuli that the animal is likely to be more familiar with (Urszán et al., 2015a). Hermit crabs are frequently perturbed in their habitat (due to wave action, encounters with predators or indeed with other hermit crabs), which is

simulated by the manual handling protocols used in previous studies. In contrast, they are unlikely to experience frequent 30 cm falls. Nevertheless, this approach was necessary in order to obtain respiration rates during recovery form disturbance and this technique still resulted in significant repeatability, allowing analysis of the variance components of interest.

Several studies have documented the repeatability of metabolic rate across several taxa (McCarthy, 2000; Broggi et al., 2007; Nespolo & Franco, 2007). So far, metabolic rate appears to be a repeatable trait (Nespolo & Franco, 2007), with a potentially stable state (Konarzewski et al., 2005) and linked to the activity level (Nespolo & Franco, 2007; Sundt-Hansen et al., 2009). Nevertheless, such studies are mainly focussed on endotherms (for review see Nespolo & Franco, 2007), and by having a higher self-maintenance cost, tend to have a higher and more constant metabolic rate (basal metabolic rate; Stearns, 1992). While invertebrates, as ectotherms, are strongly influenced by ambient temperature and, in general, show lower metabolic rates, even in activity (Alexander, 1999; Nespolo & Franco, 2007). Those differences could explain the lower repeatability estimate in routine MR, as it was measured with minor temperature variation and with some random levels of hermit crab activity. And, in hermit crabs, such uncontrolled environmental variance combined with an unnatural method to stimulate the startle response (by dropping), may generate lack of repeatability in startled MR.

Indirect associations between behaviour and POLS have been largely criticized (e.g. growth-related traits) with the suggestion that direct associations between behaviour and metabolic rate are required for adequate testing of the POLS hypothesis (Adriaenssens & Johnsson, 2009). Although logical, such correlations have a mixed support (e.g Bryant & Newton, 1994; Ketola & Kotiaho, 2012; Krams et al., 2013).

Mathot and Dingemanse (2015) suggested that studies attempting to link metabolic rate and behaviour may be biased if metabolic rate is measured in only one (or a few) occasions. This is because a single measure of metabolic rate may be insufficient to infer results regarding the energy management of individuals. In this study, however, I measured both types of metabolic rate (routine MR and startled MR) repeatedly with measures of MR being taken immediately prior to and coinciding with each behavioural observation. With this large amount of data, I nevertheless failed to observe any association between mean startle response duration and either routine MR or startled MR. In contrast, I found a significant association with WI-level variation in startle responses. Contrary to the prediction, mine results indicate a negative correlation between V_{WI} and routine MR, indicating that individuals showing low levels of consistency are those with the lower routine metabolic rates. In contrast, individuals with low levels of consistency had higher metabolic rates during startle response.

Despite the POLS predicting a positive relationship between activity, superficial exploration and energetic expenditure, there is no consensus on its prediction regarding within-individual variation in behaviour (Coppens et al., 2010; Careau et al., 2012; Niemelä et al., 2012b). Coppens et al., (2010) and Niemelä et al., (2012b) for example, suggested that a fixed behavioural strategy (lower V_{WI}) should be less energetically demanding (due to lower costs for cognitive activities) and thus more common in slow-paced strategy individuals. Urszán et al., (2015a) found similar results, and a positive relationship between gain in mass and low V_{WI} in exploratory behaviour in tadpoles (*Rana dalmatina*). The results, however, indicate that in *P. bernhardus* the POLS strategy may be conditional on behavioural consistency.

Individuals with low routine MR were those that subsequently revealed high V_{WI} in startle response durations. Conversely, startled MR increased with V_{WI}. Thus, in the

context of linking metabolic rate to ideas about the pace of life, both the situation in which metabolic rate is estimated and the level of behavioural variation under analysis appear critical. Indeed, Mathot & Dingemanse (2015) point out that both behaviour and physiological traits are highly flexible, and thus, any slow-fast classification of individuals must be carefully assessed in more than one situation. In *P. bernhardus* the variability in hiding time seems to be a strategy to cope with risk (Briffa et al., 2013; Briffa, 2013). The results reinforce such findings, since the startled MR was only correlated in the V_{WI} level, and not in the mean-level. Therefore, it appears that, at least in potentially stressful situations, within-individual variation in behaviour, rather than individual mean levels of behaviour, might be linked with underlying variation in metabolic rate (Dall et al., 2004).

Habituation is a type of learning in which an individual after repetitive stimulation exhibits a waning of its response (Thorpe, 1956). It occurs when a continued response to a nonthreatening stimulus is considered to be energetically costly (Raderschall et al., 2011). Previous investigations in *P. bernhardus*, however, did not detect any habituation (Briffa et al., 2008; Briffa et al., 2013; Bridger et al., 2015). This study demonstrates a mean level reduction in startle response duration with observation, and thus habituation with the startle response stimulation. Another possible explanation is the way in which I induced the startle response. As habituation is described to occur faster with weaker stimuli (Rankin et al., 2009), it is possible that the dropping stimulus is less intense than manually handling, generating habituation and thus a lower (but still significant) repeatability in startle response.

Previous studies in *P. bernhardus* have shown that the mean-level boldness is sensitive to ambient temperature. Hermit crabs when transferred to a different temperature treatment (10ºC to 15ºC and 15ºC to 10ºC) had a significant increase in

mean-level boldness (Briffa et al., 2013). In contrast to that study where temperature was manipulated, in the current experiment I attempted to make observations across stable temperature conditions. Hence, the temperature variation during the current experiment was much lower, and maybe insufficient to generate a general trend in mean level behaviour. Nevertheless, at the V_{WI} level these relatively small and routine temperature fluctuations appear to have been sufficient to generate a relatively strong positive effect. Individuals seemed to exhibit a higher variation in behaviour when exposed to higher temperatures. This result is consistent with the hypothesis that an increase in temperature, in poikilotherms, could result in increasing in the unpredictability in behaviour (Dingemanse et al., 2010; Briffa, 2013). However, I did not find any clear correlation between routine MR or startled MR and the temperature of the seawater, indicating that the small temperature fluctuations experienced during the experiment were not sufficient to drive changes in metabolic rate (see supplementary material S1). Therefore, it is interesting that temperature appears to have a greater effect on V_{WI} compared to either measure of MR. A possible explanation is that at greater temperatures poikilothermic predators may be more active (Petersen & Kitchell, 2001). However, I also note that a direct interpretation of the effects of temperature in this study may be less easy to interpret in comparison where temperature variation was specifically manipulated across a wider temperature range (e.g. Briffa et al., 2013). Since the small fluctuations in temperature occurred within and between observations it is unlikely that the animals were in a steady temperature state.

I also found that the mean duration of the startle response in *P. bernhardus* have had no relationship with the hermit crab body mass, similar to other studies (Briffa et al., 2008; Bridger et al., 2015). But, it appears to have a strong negative effect on the V_{WI} level. Heavier individuals, were the ones with lower V_{WI}. Growth in crustaceans is a step-wise process, resulting from sequential moulting (Liberto & Mesquita-Joanes,

2014), and in the case of hermit crabs it is further constrained by access to suitable gastropod shells. Although it could be influenced by several factors (e.g. sex and environmental conditions during ontogeny), size, in general, varies with age (size-age relationship; Liberto & Mesquita-Joanes, 2014) so here it may be the case that V_{WI} decreases with age.

Although there is not much consensus on why animals vary in the predictability of their behaviour, a few studies have shown that V_{WI} reduces with age and previous experience (Maye et al., 2007; Brembs, 2011; Urszán et al., 2015b). If so, V_{WI} could represent adaptive stochastic variation in behaviour, facilitating trial and error learning (Brembs, 2011). That is possible because individuals tend to adjust their behaviour according to environmental conditions and internal state (behavioural plasticity; Briffa, 2013). However, such behavioural flexibility is likely to be costly (as I demonstrated; Dall et al.,2004; Careau et al., 2008), and thus, individuals tend to develop a behavioural strategy that becomes less flexible with age and previous experience (Dall & Cuthill, 1997; Dall et al., 2004). In contrast, Bridger et al. (2015) found that V_{WI} in hermit crabs increases with mass. Although both studies investigate the variation in startle response in *P. bernhardus,* Bridger et al. (2015) induced the startle response by lifting and replacing the hermit crabs at the base of the tank, while here I dropped the jar causing the animal to withdraw inside the shell. Nevertheless, I obtained a similar relationship between the mean duration of the startle response and crab weight (Briffa et al., 2008; Bridger et al., 2015), indicating that the method on which I induced startle response may not be the responsible by such conflicting results. An alternative explanation is that Bridger et al. (2015) controlled for mass, using lighter crabs across a smaller size range, which could hide effects of ontogenetic variations in behavioural trends (Bridger et al., 2015). In studies of V_{WI} in hermit crabs to date, I have used hermit crabs from a single size class, as defined by the species of gastropod shell that

they occupy. Further studies, using hermit crabs form different size classes, which are distinct due to the different species of gastropod shell that crabs of markedly different sizes occupy, would enable us to gain further insights into how V_{WI} varies with age.

Studies on animal personality have increased over the last decade. They have investigated the maintenance of a given behavioural trait over time, situation, across environmental conditions (Sih et al., 2004; Bell & Sih, 2007; Réale et al., 2007; Cote et al., 2008). However, the knowledge behind physiological and behavioural correlations in both mean level behaviour and V_{WI} still is in early stages (Fresneau et al., 2014), mainly due to the lack of statistical tools to divide the variance into mean and V_{WI} levels (Cleasby et al., 2015). My findings demonstrate that relationships between behavioural traits and underpinning physiology can be variable within-individuals, dependent on current activity rates.

Chapter 3

Cognition, personality and energetics in hermit crabs

Abstract

Cognition is the process by which animals acquire, process, store and manipulate information about their environment. It comprises of perception, memory, learning and decision-making and, in consequence, is considered to be an energetically demanding component of life-history variation. Thus, cognitive performance, quantified as a trade-off between speed and accuracy, is expected co-vary with other life-history traits such as boldness and metabolic rate. Using the common hermit crab *Pagurus bernhardus*, I determine whether there is a trade-off between speed (i.e. speed in which hermit crabs assess new shell) and accuracy (i.e. correct choice of a better quality shell or rejection of a poorer quality shell) and whether it is correlated with metabolic rate (MR) and among individual differences in mean boldness. To estimate cognition, I evaluate the assessment of a new gastropod shell with a higher or lower quality as the shell currently occupied by the hermit crab. Surprisingly, I did not find support for the presence of a trade-off between speed and accuracy; rather, fast decisions were more accurate than slow ones. Furthermore, there was also a positive correlation between boldness and accuracy, indicating that bolder individuals were more accurate than shy individuals, independent of decision time. None of these patterns (speed, accuracy or boldness) co-varied with routine metabolic rate. Moreover, although metabolic rate was elevated during shell assessment there was no difference in metabolic rate between an easy and a difficult task. This indicates that the lack of a speed-accuracy trade-off here cannot be explained by a facultative increase in energy allocation during difficult tasks.

Introduction

Over the last decade studies in animal behaviour found that animals often exhibit consistent between individual differences in boldness, activity, aggressiveness, exploration and sociability (Sih et al., 2004a, b; Réale et al, 2007; Sih & Bell, 2008; Jennings et al., 2013), usually termed animal personality. It was also found that such personality traits are often correlated, forming behavioural syndromes (Réale et al, 2007; Sih & Bell, 2008). For instance, individuals that exhibit high levels of boldness also show high levels of activity (Mazué et al, 2015), a tendency to be more competitive and to be more exploratory (Sih, et al., 2004). One explanation for such limited behavioural plasticity is that it is the result of behavioural trade-offs (Sih et al, 2004), for example if there is a trade-off between risk and reward. This trade-off favours specialisation (that arises either developmentally as in canalisation or is determined genetically through frequency dependent selection), so that some individuals follow a risk-prone bold strategy while others follow a shyer risk-averse strategy. These different strategies both attempt to maximise fitness but in different ways. Fast or bold individuals might rapidly acquire resources (e.g. food, territory, mates) but at the cost of greater predation risk and reduced survival. Slow or shy individuals gain resources less quickly but this is balanced by less predation risk and greater longevity in comparison with bolder individuals. This variation in how individuals respond to risk results in a slow-fast continuum across the population as a whole, often referred to as the bold-shy axis (Chittka et al., 2003; Chittka et al., 2009). While animal personality research traditionally focusses on behaviour, behaviour is but one labile (rapidly changing and highly reversible) aspect of phenotype, which may correlate with other labile traits. It is, therefore, expected that other labile life-history traits (e.g. physiological) might co-vary along a this fast-slow continuum, in a model called the pace-of-life syndrome (POLS)

(Biro & Stamps, 2008; Smith & Blumstein, 2008; Réale et al., 2010). For example, 'risk prone' individuals may have higher activity, growth, foraging, earlier maturation, all of which demand higher metabolic rates (Biro & Stamps, 2010; Réale et al., 2010).

Decision-making is the cognitive process that results in the selection of optimal choices or courses of action among a set of possible alternatives based on certain criteria (Wang et al., 2004; Wilson & Keil, 2001). Hence, when faced with choices, individuals need to be able to discriminate and choose accurately, gathering information in a process called resource-assessment. One well-known factor that may constrain accuracy is decision-making speed (Wickelgren, 1977; Chittka & Osorio, 2007; Chittka et al., 2009), whereby greater accuracy (fewer errors) may be at the cost of a slower assessment (Chittka & Osorio, 2007; Chittka et al., 2009). Assessing resources more thoroughly but slowly might be costly for a number of reasons. First, there are incidental costs such as the elevated risk of predation while distracted by the resource, delay in access to the resource and there is always the chance that the resource could be taken by another individual while the assessment process is underway. Second, there are intrinsic costs, that is, the time and energy devoted to a lengthy assessment that will be lost to other activities. Such a trade-off might be relatively stable over time. For example, fast exploring bumblebees, *Bombus terrestris*, tend to be less thorough when foraging in artificial flowers, and consequently are more error-prone than slow individuals (Chittka et al., 2003). In other examples, similar to other personality traits (Briffa et al., 2008), the trade-off might be subject to plasticity across situations (see Glossary). For instance, depending on the urgency of the decision, ants (*Temnothorax curvispinosus*) may sacrifice accuracy for speed when deciding on a new nest-site (Pratt & Sumpter, 2006).

This correlation between time spent assessing a new resource and accuracy in decision-making is called the speed-accuracy trade-off (SATs) (Wickelgren, 1977; Chittka et al., 2009; Sih & Del Giudice, 2012). Similar to other personality traits, SATs also fall across a slow fast-continuum (Trimmer et al, 2008), with some individuals being consistently slow and with more accurate decisions, whereas others are consistently fast and less accurate (Moiron et al., 2016). Thus, there is the possibility that SATs contribute to wider behavioural syndromes such that bolder individuals explore faster and make less accurate choices. In other words, resource assessment could co-vary with other personality traits that are associated with how individuals manage risk (Sih & Del Giudice, 2012; Griffin et al., 2015, Mamuneas et al., 2014). Nevertheless, there is mixed support for the prevalence of SATS, let alone a correlation between SATS and other forms of consistent variation in behaviour. Some studies failed to find support for the presence of a speed-accuracy trade-off at all (Mamuneas et al., 2014; Proulx et al., 2014; Mamuteas et al., 2014; Moiron et al., 2015), while it was demonstrated in others (Chittka et al., 2003; Shadlen & Kiani, 2013; Ducatez et al., 2015). One explanation for this discrepancy is based on the possibility that accuracy in decision-making demands both time and energy to gather information and to evaluate choices (Sih & Del Giudice, 2012). If so, facultatively increasing energy usage during assessment tasks could allow animals to sample information from the environment at a faster rate while lowering the frequency of errors (e.g. allocating more energy to sensory systems) (Froment et al., 2014), effectively removing (or reducing) the constraints that drive any trade-off. The possibility that some animals might be able to facultatively allocate energy to information gathering and decision-making might explain the range of differing results from studies of SATs (Chittka et al., 2003; Ducatez et al., 2015; Mamuneas et al., 2014; Proulx et al., 2014; Moiron et al., 2016), and why the most able individuals can be both fast and accurate (Mamuteas et al.,

2014). While the ability to titrate energy expenditure against the complexity of a resource assessment task might vary among species (leading to divergent results across different study systems) it might also vary between individuals within the same species and even within the same population. Such a situation would be analogous to the individual * environment (I x E) interaction effects known as 'behavioural reaction norms' (Dingemanse et al., 2010) that are already analysed within the animal personality framework.

Thus, the decision-making process, and any possibility of allocating elevated energy expenditure to cope with it, is likely to vary with resource quality, as this will drive the complexity of the assessment task. Optimality models predict that when presented with a clear option between resources that are very different in quality, animals would be able to readily discriminate between high and low quality resources, favouring high-quality options over low quality ones (Jackson & Elwood, 1989; Sih et al., 2004; Shettleworth, 2010; Frankenhuis & Del Giudice, 2012). Conversely, when resource units are of similar quality, animals may need more time to discriminate between them and make a choice (Jackson &Elwood, 1989). This could lead to relationships between the difficulty of the task, speed and the accuracy of the decision, where the greater the difference between available resource units, the faster an animal can gather information prior to making an accurate decision (Jackson & Elwood, 1989).

Hermit crabs are ideal models for investigating resource assessment and the decision-making process. Their growth, reproduction and survival are dependent on the occupation of gastropod shells of appropriate size and species (Vance, 1972; Bertness, 1981; Taylor, 1981; Elwood et al., 1995, Tricarico & Gherardi, 2007). For example, if hermit crabs occupy shells that are too small, protection against predators (e.g., Angel, 2000) and survival (e.g., Borjesson & Szelistowski, 1989) are likely to be reduced and

its growth limited (e.g., Angel, 2000). Conversely, occupying shells that are too large imposes a higher energetic cost to carry it (Elwood & Neil, 1992). Therefore, hermit crabs are under strong selective pressure to make decisions that allow them to obtain a shell of the appropriate size (Jackson &Elwood, 1989; Tricarico & Gherardi, 2007). When threatened, hermit crabs quickly withdraw into their shells for protection and the duration of this startle response (i.e. time spent withdrawn into the shell before re-emerging) has been used as an index of boldness. Studies using the hermit crab *Pagurus bernhardus* as model organism have demonstrated consistent between individual differences in boldness over time (Briffa, 2013; Briffa et al., 2013; Bridger et al., 2015) and across situations (Briffa et al., 2013; Courtene-Jones & Briffa, 2014) but have also shown that behavioural plasticity across situations is nested within this wider pattern of consistent between individual differences. Hermit crabs also show well defined activities during shell assessment and selection (Reese 1963; Elwood & Stewart 1985; Scully, 1986; Jackson & Elwood, 1989). Therefore, they are an ideal study species for investigating trade-offs in accuracy and speed during resource assessment and whether this trade-off is linked to wider personality traits and underlying variation in metabolic rate.

In this study, I investigate the possibility that lower accuracy in decision-making is correlated with faster decision-making, and thus I will determine-whether there is a trade-off between speed and accuracy during shell assessment by hermit crabs. If such trade-off is present, I would expect to see that when crabs are presented with a choice between highly optimal and less optimal shells, there should be a positive correlation between the time spent assessing the shells and the probability of choosing the best shell. I also ask whether SATs co-vary with boldness, where bolder animals are expected to make faster but less accurate decisions. Furthermore, I ask whether decision-making performance is underpinned by variation in metabolic rate, that is,

whether there are positive correlations between metabolic rate and assessment duration and assessment accuracy. Finally, I also investigate whether investigation time and energy allocation might show plasticity (at the sample mean level) with the difficulty of the assessment task; that is will crabs adjust assessment times, taking longer to make their decision when shells are of similar quality and making faster decisions when they are more distinct. Moreover, if shell choice is energetically demanding, I would expect a higher O_2 consumption when hermit crabs are assessing shells compared to routine MR. Crucially, if SATS can be undermined by a facultative increase in energy allocation, then MR when choosing between two shells of similar quality should be greater in comparison to a situation where the difference in shell quality is more marked.

Methods

From November 2014 to March 2015 I collected hermit crabs from the intertidal at Hannafore Point, Cornwall, U.K and transported them directly to the laboratory at Plymouth University. There, using a bench vice, I cracked the gastropod shell open to remove the hermit crab without causing any damage. I also only used male crabs with similar size (mean mass = 0.75g ± SE = 0.2g), free from obvious parasites, damage to appendages or recent moult (N=100).

I randomly allocated individuals into four groups (N=25 in each group; A, B, C and D) and supplied each crab with a new *Littorina littorea* shell, in which the new shell mass varied across the groups. In each group, hermit crabs received shells with different weights in the two phases of the experiment (described below). Hermit crabs received a new *L. littorea* shell, with suboptimal mass, as judged by the mass of the crab (Briffa & Elwood, 2007). Crabs in the groups A and B received shells that were 85% of the predicted preferred shell weight (Briffa & Elwood, 2007). Groups C and D were assigned with shells of 75% of the predicted preferred shell weight.

Startle response duration

Once supplied with their new shells, hermit crabs were housed in individual containers, of 16cm diameter and 4cm depth of aerated seawater at 15°C and 12:12h light:dark cycle and left for ten days of acclimation period. On the 11th day, I induced the startle responses by a handling protocol, where crabs were lifted out of their tank for 5 seconds and replaced in an inverted position on the base of the tank. This causes them to withdraw into their gastropod shell. I then timed the latency of recovery from the point at which its walking legs first made re-contact with base of the tank (Briffa et al., 2008). I induced startle responses once a day, for a period of 5 days.

Decision-making task

At the end of the startle response phase (i.e. on the 16th day), I assessed decision-making time and accuracy, routine metabolic rate (MR) and MR during decision-making. In a respirometry chamber filled with sea water (see Determination of the metabolic rate), I introduced a second empty shell, with weight varying according with the hermit crab group. In groups A and C, I added a shell that was 60% of predicted preferred shell weight, while crabs in groups and B and D received shells 100% of their preferred weight. (Figure 3.1). Thus, there were four treatment combinations, determined by (i) whether the new shell should be rejected (groups A and C) or accepted (groups B and D) and (ii) whether the decision to reject or accept should be relatively easy (larger differential in shell size between current and new shell; groups A and D) or relatively difficult (smaller differential in shell size; groups B and C) (Table 3.1).

To enable accurate measurement of O_2 consumption, respirometry chambers must be sealed underwater to exclude air from them. During this process, it was important to prevent the hermit crabs form interacting with the new shell, allowing for separate metabolic rate measures to be obtained (a) during routine activity inside the chamber and (b) during shell investigation. Therefore, the new shell was kept near the chamber lid, held in place by a piece of fabric (sterilized before each observation). One end of the fabric was firmly attached to the inside of the lid using Super Glue®, while the other end was attached to a magnet (also sterilized before each observation) and firmly held in place by a magnet located outside of the chamber (Figure 3.2a). Following routine O_2 consumption measurement, the magnet was removed (Figure 3.2b), releasing the shell onto the base of the chamber and allowing the crab to freely

access it (Figure 3.2c). To prevent disturbance, I only released the new shell when the hermit crab was located in a safe distance from the fall.

There is evidence that *Pagurus bernhardus* is able to visually assess shell quality prior to physical contact (Elwood & Neil, 1992), thus, I considered the beginning of the shell assessment to be the point at which the shell was released from its net and fell to the bottom of the jar. At this point, I initiated a stopwatch and started the collection of oxygen consumption data during shell assessment and eventual decision-making ("MR during decision-making"). I considered the end of the assessment and decision-making process to be the point at which the crab rejected either the new shell or its original shell. I deemed the original shell to have been rejected if the crab chose to move out of it and into the new shell. I deemed the new shell to have been rejected if the crab ceased investigating it, and had no physical contact with it, for at least 300s. I continuously monitored oxygen consumption until either the original shell was rejected (i.e. the crab moved out of it and into the new shell) or until the new shell was rejected; in the latter case, I back-calculated the correct durations for O_2 measures by removing the final 300s of measurement for those crabs that rejected the new shell. Crabs were deemed to have made an accurate decision if they chose the shell of higher quality, either because the hermit crab moved to the new shell if a better shell was offered (groups B and D) or remained on the initial shell if a poorer shell was offered (groups A and C). Thus, I recoded two aspects of shell-assessment and decision-making, speed and accuracy.

Table 3.1: The experimental design showing the four treatment groups (A-D) defined by the percentage of preferred shell weight for initial shells and the new shell that they could choose to change into.

Group	**Initial shell**	**New assessed shell**	**Difference**	**Expected outcome**
A	80%	60%	-25%	Reject new shell
B	80%	100%	20%	Accept new shell
C	75%	60%	-20%	Reject new shell
D	75%	100%	33%	Accept new shell

During the shell assessment, and the decision-making process, individuals were offered new shells with the shell size varying according with the allocated group, followed by the expected outcome of the different shells and the difference in percentage of the difference in weight from the occupied shell to accessed shell.

Determination of metabolic rate

To investigate whether individuals with a better ability in assessing resource quality consume more energy (both average energy consumption and during the decision-making) I measured the metabolic rate (MR) during routine activity (routine MR) and during decision-making. I measured MR during decision-making immediately after the estimation of routine MR (see below for more information).

I conducted all MR measurements in closed chambers (Figure 3.2), using oxygen uptake as a proxy for metabolic rate. In a closed system, oxygen availability reduces with the consumption and this can be monitored with an oxygen sensitive sensor spot (PreSens Precision Sensing GmbH, Regensburg, Germany) attached to the inner wall. The sensitive spot reacts with the O_2 available, allowing a non-invasive measure, as well as more precise measures (when compared to open chambers), as prevented gas exchange during the readings. The sensor spot is them read by a Fibrox 4 trace machine (PreSens Precision Sensing GmbH, Regensburg, Germany), attached to a temperature sensor (Pt100, Bioengineering AG, Wald, Switzerland). To prevent oxygen stratification, and to ensure adequate mixing of water, I placed the chamber onto a multi-channel magnetic stirrer (MIX 15 eco; 2mag AG, Munich, Germany) with a magnetic flea inside. To prevent contact between the magnetic flea and the hermit crab, I placed a mesh inside between them.

I sealed the chambers underwater. To minimize bacterial and algal activity, I used filtered sea water. I also measured the oxygen consumption in three extra chambers (‘blanks’), containing a single *Littorina littorea* shell, with a similar size as used by the crabs, and filtered sea water (as above). The difference in oxygen

concentration over time in the blank indicates microbial activity, and was accounted at the final estimation of the individual metabolic rates (see below).

I obtained the O_2 consumption rate using the slope of a linear regression of the oxygen consumption over time minus the blank O_2 consumption rate (Calosi, et al., 2013). Although I conducted the metabolic rate measurements in a temperature controlled room, there was small fluctuations in temperature, which can affect oxygen solubility values (Widdows & Staff, 2006). I accounted for such small fluctuations in temperature in the estimation of the oxygen solubility coefficient. Then, I multiplied the slope by the oxygen solubility coefficient and adjusted for salinity and temperature. I calculated the rate of O_2 consumption using:

$$Rate\ of\ O_2 uptake\ \left(\mu\text{moles}\ O_2 h^{-1}\right) = C(t) \times (V_r) \times \left({}^{60}/_{t_1 - t_2}\right)$$

Where, C(t) is the O_2 consumption rate (from the linear regression of oxygen consumption over time), V_r is the total volume of water inside the jar (jar volume minus the hermit crab volume) and t_0, t_1, is the measurement period (in minutes; Widdows & Staff, 2006; Calosi, et al., 2013). To standardise metabolic rate measures against variation in body mass I divided the rate of O_2 uptake by individual body mass (Porter & Brand, 1995).

I individually sealed hermit crabs under water to prevent the formation of air bubbles and allowed hermit crabs resting for minimum 30 minutes before starting routine MR measures of oxygen consumption. The routine MR is defined as the metabolism of an undisturbed, post-absorptive, resting individual, it also includes some level of random activity, maintenance of equilibrium and posture (Jobling, 1994; Killen et al., 2011; Mathot & Dingemanse, 2015). Therefore, I monitor the oxygen consumption during the resting period (using the Fibrox 4 graphic output), initiating the

routine MR measurements with the stabilization of the O_2 consumption (as demonstrated in Figure 3.3). This is important because individuals can differ on their response to a stressful situation (e.g. handling).

Statistical analysis

Prior to analysis, I log-transformed startle response duration ($\log_{10}(x + 3)$), time assessing new shell ($\log_{10}(x +1.5)$), routine MR ($\log_{10}(x +1.5)$) and MR during decision-making ($\log_{10}(x +1.5)$) to improve normality.

Repeatability of startle responses

I estimated the repeatability of the startle response duration based on a linear mixed model, using the REML method for Gaussian data (R package rptR) (Nakagawa & Schielzeth, 2010). I also estimated the repeatability of the startle response separately for the crabs in each of the categories of original shell weight (75% or 80% of its preferred shell weight), as the shell weight can modify startle response duration. To determine what influences the startle response duration I fitted a saturated model containing the startle response as dependent variable and crab mass, sampling occasion (1-5), the weight of the occupied shell (if 75% or 80%) and routine MR as fixed effects. I allowed a random intercept for each crab.

The accuracy and speed of decision-making

To investigate the presence of a trade-off between speed and accuracy I fitted a univariate model with a binomial distribution (see Glossary) using accuracy (accurate response = rejecting a smaller shell or accepting a larger shell; inaccurate response = rejecting a larger shell or accepting a smaller shell) in decision-making as the dependent variable and mass, potential change in shell quality (according to the difference in size

between the occupied and the assessed shell, Figure 3.1), decision time, the individual average startle response duration and the decision time x potential change in shell quality interaction effect as independent variables. The model also allowed us to investigate whether the startle responses co-vary with accuracy in decision-making. In order to determine whether decision time co-varies with startle response I fitted a similar univariate model using decision time as dependent variable.

The probability of changing shells

To determine whether there is a relationship between the startle response duration and the probability of changing shell (irrespective of accurate shell choice or shell quality), I fitted a univariate model with a binomial distribution using the shell change as the dependent variable (whether hermit crabs changed shells or not) and mass, decision time. It would also have been interesting to include accuracy of the choice as a predictor of whether shells were exchanged, however, it was not possible to reliably analyse the data in this way because the model that did include accuracy could not converge, most likely as a result of low sample sizes in some combinations of shell exchange and accuracy.

Metabolic rate and decision-making

To investigate whether shell choice is energetically demanding I fitted a univariate model (see Glossary) using MR during decision-making as the dependent variable and potential change in shell quality, decision time and accuracy and the interactions between decision time * accuracy in decision-making, accuracy * potential change in shell quality, decision time * potential change in shell quality, decision time * accuracy * potential change in shell quality as independent variables. I conducted a similar analysis (i.e. univariate model) to determine if individuals with higher metabolic rate

perform better (faster decisions and higher accuracy) in decision-making using the routine MR as dependent variable. I also compared the difference between routine MR and MR during decision-making using a paired t-test. Additionally, I perform a third univariate model, using the change in metabolic rate (MR during decision-making - routine MR) to investigate whether the increase in metabolic rate during decision-making explains the hermit crab performance. I used the difference in metabolic rate as dependent variable and potential change in shell quality, decision time and accuracy and the interactions between decision time * accuracy in decision-making, accuracy * potential change in shell quality decision time * potential change in shell quality, decision time * accuracy * potential change in shell quality as independent variables.

I used the software ASReml-R (Butler et al., 2009), fitted using REML (residual maximum likelihood), in R version (3.3.1), to fit the analysis described above (see supplementary material S2). The effects of the fixed components were evaluated using Wald's chi-square test and p-values and random effects using Z-test.

Ethical note

No animals were harmed during the experiment and at the end of the experiment all individuals appeared healthy and were supplied with excess food (as above) and a new gastropod shell before being returned to the sea.

Day	1-10	11	12	13	14	15	16
Behaviour	Acclimation	Startle responses (SR)					Shell choice
		SR1	SR2	SR3	SR4	SR5	

Figure 3.1: Time-line of the experiment. Individuals were acclimatized for 10 days prior to the beginning of the experiment, followed by five days of startle response induction (SR1- SR5). On the 16th day, hermit crabs would receive a new shell with the optimum weight varying according with the allocated group (Table 3.1)

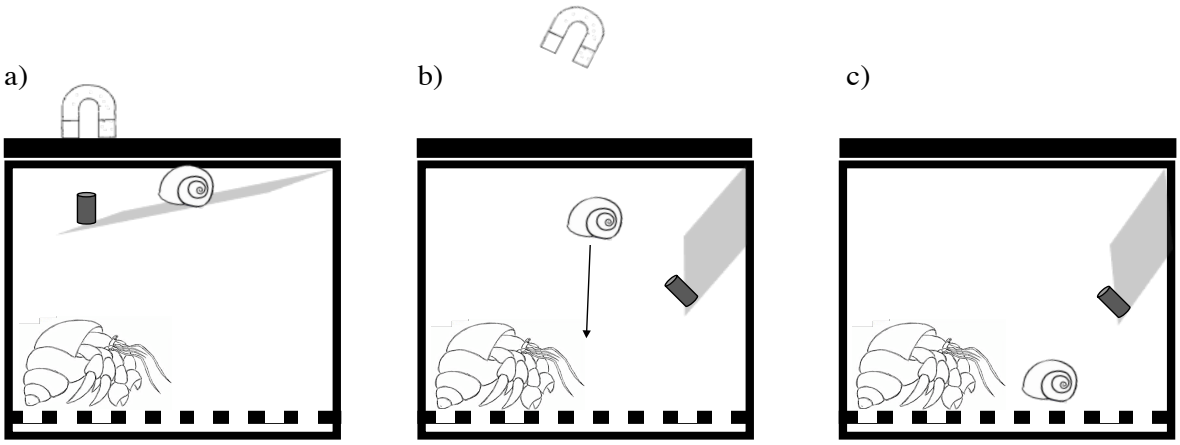

Figure 3.2: The respirometer chamber that allows the study of metabolic rate and decision-making in hermit crabs. a) chamber setup during routine MR. A fabric sling held in place by magnets (both inside and outside the chamber) prevents the contact between the hermit crab and the new shell. b) with the removal of the external magnet, the fabric releases the new shell. c) once released, the new shell can be investigated by the hermit crab.

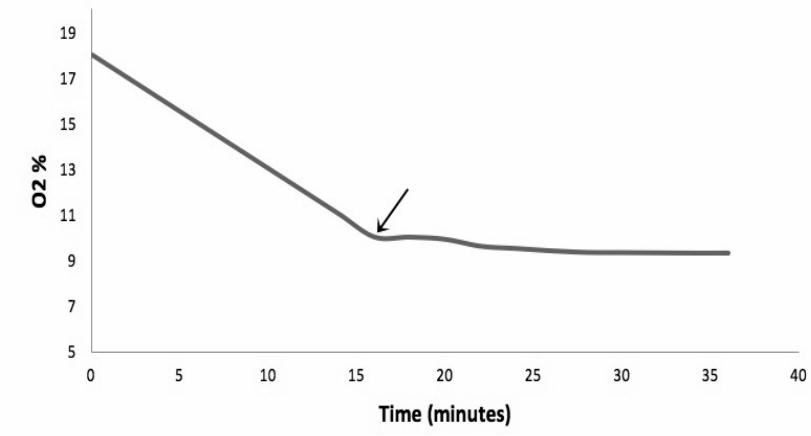

Figure 3.3: Representation of Fibrox 4 graphic output. The arrow represents the point when the oxygen consumption starts to stabilize and mark the beginning of the routine MR measurements. The period anterior of the arrow is correspondent to the period following handling

Results

Repeatability of startle responses

There was significant repeatability in startle-response duration for all groups of crabs (i.e. those initially occupying shells of both 75% (R_A = 0.472 ± S.E. = 0.07; CI = 0.0.324, 0.593) or 80% (R_A= 0.503 ± S.E. = 0.067; CI = 0.366,0.627) of the shell preferred weight), and with both groups combined (R_A= 0.472 ± S.E. = 0.048; CI = 0.375, 0.56). The variance within-individual was significant (Z-Ratio = 15.72) but not between-individuals (Z-Ratio = 0.67).

Factors affecting startle response duration

The parameter estimates for fixed and random effects from these models are given in Tables 3.2 and Table 3.3 respectively. I found a significant random intercept (Table 3.2), confirming the presence of the significant between individual variation in behaviour estimated using repeatability above. There was no temporal trend across the 5 observations (Table 3.3) and mass also had no effect in startle response duration (Table 3.3). Startle response duration did not vary with initial shell size (Table 3.3) or with routine MR (Table 3.3).

The speed and accuracy of decision-making

The parameter estimates of the following model are given in Table 3.4. There was a positive relation between accuracy and decision time (Table 3.4), such that crabs that chose to reject or accept the new shell more quickly were more likely to make the correct decision. There was no effect of crab mass (Table 3.4) or potential change in shell quality (Table 3.5) on the accuracy of the decision. However, there was a significant correlation between accuracy and startle response duration (Table 3.5) where

the likelihood of making the right decision declined with increasing startle response durations. The parameter estimates of the decision time model are given in Table 3.6. There was also a significant interaction effect between decision time and the potential change in shell quality, on the probability of a correct decision (Table 3.6). When crabs stood to gain in shell quality or to experience a 25% loss in quality by exchanging shells, the chance of making the right decision increased with decision time. However, for crabs that would experience a larger loss (-25%) by exchanging shells the chance of making the right decision decreased with decision time.

The probability of changing shells

There was a significant positive association between decision time and the probability of changing shells (Table 3.7) and mass (Table 3.7). There was no correlation between the decision time and the startle response duration (Table 3.7).

Metabolic rate and decision-making

There was no effect of potential change in shell quality (Table 3.8), decision time (Table 3.8) and accuracy (Table 3.8) on routine metabolic rate, and there were no two or three-way interaction effects (Table 3.8).

Similarly, there was no effect of potential change in shell quality (Table 3.9) or accuracy in decision-making (Table 3.9) on the metabolic rate during decision-making. However, metabolic rate decreased with the time taken to make a decision (Table 3.9). There were no interactions between decision time and accuracy (Table 3.9), accuracy and potential change in shell quality (Table 3.9), decision time and potential change in shell quality (Table 3.9) and there was no three-way interaction (Table 3.9).

Metabolic rate during decision-making was elevated compared to routine activity (paired t-test: $t_{99} = 17.76$, $p < 0.0001$). However, analysis of the change in metabolic rate (MR during shell investigation – MR routine) indicates that the amount by which metabolic rate increased was not influenced by the potential change in shell quality (Table 3.10), decision time (Table 3.10) or accuracy in decision-making (Table 3.10). Similarly, the change in metabolic rate was not driven by any interaction effects (Table 3.10).

Table 3.2: The fixed effects and their statistical significance of the startle response duration.

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	747.11	1	648.14	<0.001
Observation	1.8	1	1.56	0.212
Mass	0.23	1	0.2	0.652
Initial shell size	1.78	1	1.54	0.214
Routine MR	0.2	1	0.18	0.674

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 3.3: Estimated variance components for startle response duration. σ^2 is the variance of each component. Statistical significance is assessed by comparing variance to the Z-Ratio; effects are considered to be statistically significant if Z> 2 (Wilson et al., 2010). Significant variables are printed in bold.

Component	Effect	σ^2	SE	Z-Ratio
Between individual	0.00006	0.00005	0.00009	0.67440
Within-individual	**1.15270**	**1.00000**	**0.07334**	**15.71623**

Table 3.4: The fixed effects and their statistical significance of the accuracy in decision-making.

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	**413.89**	**2**	**413.89**	**<0.001**
Mass	3.39	2	3.39	0.183
Potential change in shell quality	3.33	6	3.33	0.766
Decision-making time	**374.85**	**2**	**374.85**	**<0.001**
Startle response duration	**8.12**	**2**	**8.12**	**0.017**
Decision-making time * Potential change in shell quality	**46.18**	**4**	**46.18**	**<0.001**

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 3.5: Number of hermit crabs with accurate (selection of a shell with a higher quality) and inaccurate decisions (selection of a shell with a lower quality) per treatment group. Treatment group represents the potential change in shell quality (in parenthesis).

Group	Accurate decisions	Inaccurate decisions
A (-25%)	24	1
B (20%)	8	17
C (-20%)	24	1
D (33%)	7	18

Table 3.6: The fixed effects and their statistical significance of the decision-making time.

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	**2551.88**	**1**	**2090.66**	**<0.001**
Mass	**9.25**	**1**	**7.58**	**0.0059**
Potential change in shell quality	**16.19**	**3**	**13.27**	**0.004**
Accuracy in decision-making	**11.65**	**1**	**9.54**	**0.002**
Startle response duration	0.21	1	0.17	0.677
Accuracy in decision-making* Potential change in shell quality	1.28	3	1.05	0.789

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 3.7: The fixed effects and their statistical significance of the probability of changing shells.

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	**398.68**	**2**	**398.68**	**<0.001**
Mass	**7.58**	**2**	**7.58**	**0.0059**
Decision-making time	**359.24**	**2**	**359.24**	**<0.001**
Startle response duration	3.44	2	3.44	0.178

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 3.8: The fixed effects and their statistical significance of routine metabolic rate

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	**1519.71**	**1**	**465.6**	**<0.001**
Potential change in shell quality	4.17	3	1.28	0.734
Decision-making time	0	1	0	0.997
Accuracy in decision-making	0.2	1	0.06	0.805
Decision-making time * accuracy in decision-making	3.82	1	1.17	0.279
Accuracy in decision-making* Potential change in shell quality	3.25	3	1	0.802
Decision-making time * Potential change in shell quality	9.63	3	2.95	0.399
Decision-making time * accuracy in decision-making* Potential change in shell quality	0.05	1	0.01	0.904

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 3.9: The fixed effects and their statistical significance of metabolic rate during decision-making.

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	6032.6	1	3069.28	**<0.001**
Potential change in shell quality	6.4	3	3.26	0.353
Decision-making time	**8.1**	**1**	**4.13**	**0.042**
Accuracy in decision-making	1.1	1	0.57	0.451
Decision-making time * accuracy in decision-making	0.3	1	0.15	0.696
Accuracy in decision-making* Potential change in shell quality	2	3	1.03	0.794
Decision-making time * Potential change in shell quality	3.2	3	1.65	0.647
Decision-making time * accuracy in decision-making* Potential change in shell quality	2.3	1	1.2	0.274

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 3.10: The fixed effects and their statistical significance of the change in metabolic rate (routine MR – MR during decision-making).

Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Intercept	1496.66	1	305.665	<0.001
Potential change in shell quality	14.64	3	2.989	0.393
Decision-making time	5.78	1	1.18	0.277
Accuracy in decision-making	1.1	1	0.225	0.635
Decision-making time * accuracy in decision-making	6.26	1	1.279	0.258
Accuracy in decision-making* Potential change in shell quality	0.56	3	0.115	0.990
Decision-making time * Potential change in shell quality	13.55	3	2.768	0.429
Decision-making time * accuracy in decision-making* Potential change in shell quality	1.73	1	0.353	0.552

(Mean effect sizes of factors and covariates with the sum of squares, degrees of freedom, Wald's chi-square test and p-values; significant variables are printed in bold).

Discussion

In a recent review, Sih and Del Giudice (2012) suggested that cognitive speed-accuracy trade-offs are often expressed in a slow fast continuum, suggesting a connection between fast–slow behavioural types (i.e. the boldness-shyness axis) and SATs. In this study, I investigated whether there is a trade-off between speed and accuracy in decision-making and whether shell investigation varies with metabolic rate and repeatable between individual variation in startle response duration, an index of boldness. Contrary to my expectations, I found that in most situations where a new shell was offered to hermit crabs faster decisions were more accurate than slower decisions. Thus, in situations where an exchange of shells would be beneficial (20% and 33% increase in shell weight) those that decided to change made their decision more quickly than those that decided to reject the new shell. Similarly, in the situation where changing shells would result in a 25% loss of shell quality, crabs that rejected the new shell made their decision more quickly than those that accepted it. It was only in the group where an exchange would have resulted in a less marked loss of shell quality (-20%) that I found evidence for a trade-off between accuracy and speed. Here, crabs that (correctly) rejected the new shell took longer to make their decision than those that accepted it. Over most of the experiment it therefore appears that crabs that made correct decisions also made them quickly. Thus, individual hermit crabs may differ in their cognitive abilities, such that those performing better in terms of speed also performed better in terms of accuracy. Such variation in the cognitive task may be part of a wider behavioural syndrome of variation between individuals. Indeed, although the cognitive task was only performed once by each crab, I found that the accuracy of decision-making co-varied with (repeatable) startle response durations. Although I

expected a correlation between startle responses and accurate shell choices, the direction of the result was again contrary to the initial prediction. Bolder individuals did not make less accurate choices as expected (Sih & Del Giudice, 2012). Rather, the probability of making the correct decision declined as startle response duration increased, indicating that the boldest individuals had assessed the choice of shells more accurately. This effect of boldness on accuracy, however, cannot be explained by variation in the time taken to make the decision since decision time did not vary with boldness. Whereas fast decisions were more accurate than longer ones, the results also show that hermit crabs were more likely to change shell when it was investigated for longer periods of time. This indicates that the increase in assessment time could lead to an inaccurate changing of shells, where hermit crabs would choose a shell with a lower quality than the occupied ones. This is a surprising result and the reasons for it are unclear at present. One possibility is that there is a threshold of effort in shell investigation, which once committed to investigating the new shell increases the likelihood of an exchange. Another potential explanation is that crabs already occupying poor quality shells are primed for exchanging shells in a way that makes them 'inappropriately optimistic' about the quality of any new shells that they encounter (Houston et al., 2012). However, both explanations for this particular result, although intriguing, remain speculative at this point.

Thus, similar to previous studies, I have found that repeatable startle responses in hermit crabs are also linked to other behaviours including aggression (Mowles et al., 2012; Courtene-Jones & Briffa, 2014), shell investigation (Mowles et al., 2012) and now the speed and accuracy of decision-making about shells. Mowles et al. (2012) investigated the latency of shell investigation but did not investigate the accuracy of this behaviour. I now show that that these traits also have the potential to contribute to a behavioural syndrome in hermit crabs, where the boldest individuals are not only the

most aggressive and inquisitive but also appear to make decisions more effectively than shyer individuals. the original expectation was that such potential behavioural syndromes (although in this experiment I was unable to test the stability of any such syndrome for logistical limitations involved in the repeated re-cracking of hermit crabs out of their gastropod shells) could be underpinned by variation in metabolic rate as predicted by the POLS hypothesis. Similar to a previous study, however, I found no evidence of a correlation between boldness and routine metabolic rate (Velasque & Briffa, 2016). Thus, it is perhaps not surprising that although shell investigation is revealed to be metabolically demanding (since metabolic rate is elevated during this activity) the accuracy of the decisions made are not influenced by either routine metabolic rate, metabolic rate during shell investigation or by the amount that metabolic rate is increased (compared to routine) during shell investigation. Combined with the lack of trade-off between speed and accuracy and the unexpected direction of the correlation between boldness and decision speed, the data on metabolic rate suggest that any syndrome of boldness and cognitive ability in hermit crabs is not underpinned by variation in metabolic rate.

I found no temporal trend across the 5 observations, evidencing absence of habituation to the startle response induction (Briffa et al., 2008; Stamps et al., 2012; Briffa et al., 2013; Bridger et al., 2015). In addition, the presence of consistent differences between individuals in startle response was independent of the occupied shell mass, contrary to previous findings here hermit crabs occupying poor quality shells had longer startle response (Briffa & Bibost, 2009). One possible explanation is that hermit crabs occupied shells with similar mass (80% and 75% of the hermit crab preferred shell weight), while Briffa & Bibost (2009) use shells with a greater difference in quality (75% and 100%). And as consequence, it is possible that the

difference in shell mass between groups was insufficient to detect influence startle response duration.

As in previous studies, the startle response duration is not affected by the hermit crab mass (Briffa et al., 2008; Bridger et al., 2015). As mass is an indicative of age in crustaceans (Lancaster, 1988; Liberto & Mesquita-Joanes, 2014), the results reinforce the evidence that boldness is unlikely to be related with ontogenetic variation in hermit crabs. Similarly, neither accuracy, nor probability of changing shells are related with mass. This is important as shell assessment is a complex process previously assumed to be dependent not only on the perceived value of the offered shell (Elwood & Neil, 1992) but also on previous experiences (Hazlett, 1995, 1996). Although mass had no relationship with the decision-making accuracy, heavier, and thus older, individuals spent more time investigating a new shell. As experience is correlated with age (Elwood & Neil, 1992; Krause & Ruxton, 2002) I would expect ontogenetic variation to be related with faster assessment and a higher accuracy (Chittka et al., 2009), contrary to the findings. Note, however that in this study I have used a relatively narrow range of crab sizes, given the overall size range of this species. This was because I focussed on intertidal crabs in the size range that occupy *L. litorea* shells, so that differences in the effects of preferred shell species (which change as the crabs undergo significant growth) on behaviour could be excluded from the experiments. However, in order to fully assess the effects of crab mass (and hence age) it may be necessary to extend future studies to encompass smaller individuals (that prefer *Littorina obtusata* shells) and the largest subtidal individuals (that prefer *Buccinum undatum* shells).

Neuronal cells are energetically expensive, requiring more energy than other cells even during rest (Mink et al., 1981). As cognitive performance is conditioned on the mass of neuronal tissue relative to body mass (Smith, 1990) increasing cognitive

performance could demand an increased energy expenditure both during and between episodes of cognitive work. As a consequence, in natural environments where energy is limited, individual performance can be constrained. This could occur not only due to the number of neuronal cells (Mink et al., 1981) but also as a result of the energetic demand to acquire and process information (Laughlin & Sejnowski, 2003). Therefore, I expected that animals with a higher energetic expenditure to have higher decision-making performance and that during tasks that demand certain levels cognitive ability, a higher usage of energy (compared with the regular usage). The effect should be particularly expected in individuals that are both fast and accurate. In relation with energetic usage during decision-making, the results partially support this prediction. During routine behaviour, the metabolic rate was significantly lower than during shell investigation, reinforcing the idea that resource assessment and decision-making are energetically demanding. Nevertheless, such increasing energetic usage was unrelated to decision-making performance because there was no correlation between speed or accuracy and metabolic rate. Individuals however, spent more energy when assessing shells for longer periods of time, and that such increase was independent of accuracy. I also found similar results when using the change in metabolic rate (MR during decision-making - routine MR). Therefore, the results in several respects are at variance with the predictions (grounded in the POLs hypothesis) for links between boldness, SATs and metabolic rate. It is worth noting that these predictions have been made in the context of vertebrate biology, but the brains of vertebrates and the decision-making centres of invertebrates have some important differences that could affect these predictions. In particular, it is possible that in contrast to vertebrates, invertebrate 'brains' (i.e. the decision-making centres in the vast majority of animal species) divide information across parallels pathways (McNab, 2002). This would reduce several processes, when compared to a vertebrate brain. Such 'parallel processing' could allow

for lower energy consumption (Laughlin & Sejnowski, 2003), explaining why decision-making was not as energetically demanding as expected in this study. Alternatively, any increase in metabolic rate (between decision-making and routine MR) could be the result of an increased physical activity, rather than neuronal activity, during the shell assessment, as shells often turned or moved during this process (Elwood & Neil, 1992). In this case, I would expect no large differences in increased energy expenditure as the physical cost of investigating shells would be similar regardless of the difficulty of the task.

Another explanation is that the manipulation of shell weight to investigate performance in decision-making is a task with relative simplicity and, therefore, does not require a significant increase in MR. This could also explain why most individuals were both fast and accurate. In fact, the trade-off between speed and accuracy seems to be context-dependent, being found only in individuals assessing shell with less marked loss of quality (-20%). Hence, future work manipulating other shells features (e.g. Jackson & Elwood, 1989) would be useful to further investigate the trade-off between speed-accuracy in decision-making performance and its energetic cost. For instance, the manipulation of the shell exterior increasing weight (e.g. Jackson & Elwood, 1989) or using different shell colour increasing conspicuousness (e.g. Briffa & Twyman, 2011) could be used to increase shell complexity turning decision-making into a more (physically) laborious task.

A recent hypothesis suggests a link between variation in cognitive performance and wider behavioural syndromes (Sih & Del Giudice, 2012). The data only partially support this idea as bolder crabs appeared to assess shells more accurately but I found no link between boldness and the speed of decision-making. Due to logistical constraints (i.e. to avoid repeatedly removing crabs from their shells) I only assayed

shell assessment and decision-making once in each crab, and therefore I cannot determine whether these cognitive tasks are repeatable. Nevertheless, the overall conclusion from the data is that cognitive performance in hermit crabs is related to variation in repeatable startle response durations (an index of boldness) such that both behaviours may be linked in a wider behavioural syndrome. However, although decision-making is energetically demanding neither this nor boldness appear to vary with metabolic rate. Therefore, the presence of such a behavioural syndrome in hermit crabs cannot be explained by variation in the pace of life.

Chapter 4

Behavioural syndrome and the pace of life syndrome: the effect of metabolic rate on boldness and exploration

Abstract

Animal personality is defined as the presence of consistent between-individual differences in behaviour. Among repeatable personality traits, boldness and exploration have been a particular focus of interest, one reason being that they are often correlated ('behavioural syndromes'). Such correlation between behavioural traits is predicted by the Pace of Life Syndrome (POLS) hypothesis, which suggests that differences in behavioural types will be correlated with others life-history traits (e.g. size, metabolic rate, immunity) along a fast-slow continuum. I tested predictions of this hypothesis in the hermit crab *Pagurus bernhardus*, by investigating if differences in a behavioural syndrome (i.e. exploration and boldness) are driven by variation in metabolic rate (routine metabolic rate). Exploration (estimated as spontaneous alternation in a plus-maze) and boldness (estimated as the startle response duration) were consistently different between individuals and positively correlated at both between and within-individual levels of variation, indicating the presence of behavioural syndrome in this species. My results indicate that the average change in boldness is correlated with average change in exploration (between individual correlation) and that the individual change in boldness between two observations correlated with its changes in exploration over the same period (within-individual correlation). Furthermore, changes in the syndrome (a combination of exploration and boldness) were positively correlated with changes in metabolic rate, as more explorative and bolder hermit crabs had a higher routine metabolic rate than less bold and explorative individuals. the findings provide evidence that there is a behavioural syndrome between boldness and exploratory behaviour and that variations in these traits could be promoted by variations in energetic expenditure, as predicted by the POLS hypothesis.

Introduction

The presence of consistent between individual differences in behaviour, termed animal personality, has been shown in several species (Sih et al., 2004a, b; Réale et al., 2007; Sih & Bell, 2008; Jennings et al., 2013). Indeed, there has been increasing evidence that for most animal species we should expect consistent variation in boldness, aggressiveness, activity, sociality or exploration between individuals from the same population (Réale et al., 2007). It was also discovered that such personality traits are often correlated across different behavioural contexts, forming stable behavioural syndromes (Wilson, 1998; Gosling, 2001; Dingemanse et al., 2003; Sih et al., 2003; Bell & Stamps, 2004; Bell, 2007; Stamps, 2007; Wolf et al., 2007). Thus, individuals that are more active also tend to be relatively more aggressive (Mazué et al., 2015), bolder and more explorative compared to less active individuals (Réale et al., 2007). In addition, the strength and structure of the syndrome can also vary across ecological situations, such as the predation regime (e.g., Huntingford, 1976; Dingemanse et al., 2007; Archard & Braithwaite, 2011). For instance, correlations between boldness and aggressiveness in the three-spines stickleback, *Gasterosteus aculeatus*, are stronger in populations where predators are present (Bell, 2005; Bell & Sih, 2007; Dingemanse et al., 2007).

Personality traits (i.e. behaviours that vary consistently between individuals) have been intensively studied over the last 20 years, however, the mechanisms behind their emergence and maintenance are still not well understood (Stamps & Groothuis, 2010; Sih et al., 2015). It has been suggested that such behavioural differences between individuals may be the result of life-history trade-offs, in particular trade-offs between reproduction and survival (Wolf et al., 2007; Biro & Stamps, 2008; Réale et al., 2010). Here, individuals with higher survival rates would take few risks and, as a consequence,

would gather low rewards (e.g. food, mating, territories) in the short term. In contrast, individuals with a higher reproductive rate would be more risk prone (with a higher mortality rates), but also maximise their short-term reward. As life-history trade-offs can impact different life-history traits in opposing ways, it is likely that others life-history (physiological and/or behavioural) traits would be correlated, following a slow-fast continuum (Réale et al., 2000; Boon et al., 2007; Dammhahn 2012; Korsten et al., 2013; Montiglio & Royatué, 2014). For example, increasing investment in reproduction (e.g. increasing territory, increasing the body size, amount of sperm produced), could lead to an increase in energetic demand (e.g. metabolic rate), requiring a higher foraging rate. Such correlation between life-history traits according to a slow-fast continuum is predicted by the pace of life syndrome (POLS) hypothesis (Réale et al., 2010). Indeed, a key trait that might underpin variation in pace of life is variation in metabolic rate.

Exploration is often associated with foraging, dispersal, defence and mate searching (Réale et al., 2007; Biro & Stamps 2008; Cote et al., 2010). It is an important component of life-history and one of the major behavioural dimensions, influencing process such as dispersal and foraging (Réale et al., 2007). Its study usually involves the measure of an animal's movement pattern (e.g. activity, time spent in a sheltered place) in a given environment (familiar or unfamiliar) (Carter et al., 2012), as in a standard open field test. However, information on how individuals move through the environment (e.g. movement randomness, speed) often excluded from such studies (e.g. Fox et al., 2009; Boyer et al., 2010) and they can provide information on the intrinsic (without cues, bias or reinforcement) pattern of movement. For instance, randomness in movement might increase the likelihood of discovering an unexploited resource (Ramey et al., 2009). Therefore, analysing the consistency of such intrinsic patterns of movement could be especially important for understanding the extent to which animals

can adjust their behaviour to cope with highly fluctuating and unpredictable environments (Inglis, et al., 2001). For instance, the foraging strategy in honey bees, *Apis mellifera,* differs between subspecies (Winston & Katz, 1982). Europeans honey bees, have evolved in a more stable environment, and thus, during foraging they tend to rely more on learning and social cues leading to relatively consistent foraging patterns (Leadbeater & Chittka, 2007). In contrast, African honey bees tend to be opportunistic foragers, and engage more in random foraging, as they are more adapted to more unstable environments (Mistro et al., 2005). Therefore, a more random exploratory behaviour may increase an individual's likelihood of discovering new resources, in unpredictable environments (Chiussi et al., 2001; Weissburg & Dusenbery, 2002). In other animals, the consistency of exploration is little understood. As well as the question of whether behavioural differences varies between individuals and populations. However, it is likely that similarly to boldness, exploration is linked to risk prone behaviours and ultimately to variation in the pace of life (Wolf et al., 2007; Réale et al., 2010).

Variation in individual exploration can be investigated by measuring 'spontaneous alternation' (e.g., Lalonde 2002). This is the behavioural pattern that results from an individual's tendency to alternate their successive choices, such that they are more likely to visit spaces that have been least recently visited in the absence of reinforcement (Richman et al., 1986; Lalonde, 2002; Hughes, 2012). Such exploration pattern appears to be innate (Ramey et al., 2009), and have been demonstrated in several organisms, from *Paramecium* sp. (Aderman & Dawson, 1970; Harvey & Bovell, 2006) to rodents (Dember & Fowler 1959; Still, 1966) and humans (Schultz, 1964). Because alternation performance has a potential fitness effect (e.g. higher chance of encountering mating partners or food), individuals with a higher expression of a given exploratory tendency may gather more rewards. On the other

hand, constantly entering new areas may also increase the chance of encountering predators and other dangers. Thus, the potential trade-offs inherent in spontaneous alternation seem similar to those already proposed to underpin other personality traits related to risk, such as consistent between individual variation in boldness. Therefore, it is possible that animals would exhibit repeatable differences in spontaneous alternation between individuals, such that this behaviour also represents a personality trait. In addition, spontaneous alternation could also be associated with other life-history traits (e.g. metabolic rate, immunity, body size), exhibiting a positive correlation between behavioural tendencies and physiological traits as predicted by the POLS hypothesis.

Most hermit crab species occupy empty gastropod shells to protect their weekly calcified abdomen. As a result, their growth and survival is dependent on the occupation of an appropriate sized shell (Elwood et al.,1995, Tricarico & Gherardi, 2007). For instance, if the occupied shell is considered large, it may impose a higher energetic cost to carry it (Elwood & Neil, 1992), while smaller shells may not provide optimum protection against predators (e.g., Angel, 2000). When threatened, hermit crabs withdraw into their shells for protection. The latency to re-emerge after the withdraw is referred to as the "startle response duration" (Briffa et al., 2008) and it has been used as an index of boldness. Thus, hermit crabs may have a strong selective pressure to occupy optimum shell sizes (Jackson &Elwood, 1989) in natural environments. If they occupy a suboptimal shell they are expected to show increased motivation to investigate new shells that they encounter (Neil & Elwood, 1986). In contrast, the effect of shell size on exploratory behaviour in hermit crabs is unknown. On the one hand, they might increase exploration (e.g. greater spontaneous alternation) to increase the chance of encountering a new shell. On the other hand, if a small shell equates to greater risk because it offers less protection, they might reduce their exploration, showing less spontaneous alternation.

Previous studies in *P. bernhardus* have shown that the startle response duration is repeatable over time (Briffa, 2013; Briffa et al., 2013; Bridger et al., 2015) and across situations (Briffa et al., 2013; Courtene-Jones & Briffa, 2014). However, this pattern of consistent between individual variation in startle responses is also subject to behavioural plasticity across situations and there are significant among-individual differences in behavioural reaction norms (i.e. the amount of plasticity varies between individuals). Here, I tested the hypothesis that *P. bernhardus* consistently differ in their spontaneous alternation and startle response duration, indicating that both behaviours represent personality traits. Furthermore, if both are repeatable I predict that they co-vary in a stable behavioural syndrome, since both latency to emerge and exploration influence the amount of risk that an individual is exposed to. If the behavioural syndrome is present, I also attempt to investigate whether is underpinned by variation in metabolic rate, as suggested by the pace of life syndrome hypothesis. Finally, by manipulating the shell weight relative to optimum shell weight, I will determine the extent to which these patterns are plastic across situations, and whether individuals show different amounts of plasticity across situations (i.e. differences in behavioural reaction norm) (Dingemanse et al., 2010; Stamps & Groothuis, 2010).

Methods

From November 2015 to April 2016 I collected hermit crabs from the intertidal at Hannafore Point, Cornwall, U.K and transport them to the laboratory at Plymouth University. I removed each crab from its gastropod shell by cracking the shell in a bench vice without causing any damage to the crab. I only use male crabs with similar mass (mean mass = 0.91 ± SE 0.011g), free from obvious parasites, damage to appendages or recent moult (N=100). I randomly allocated individuals into two groups (N=50 in each group), and supplied each crab with a new *Littorina littorea* shell, in which the new shell mass varied across the groups. In each group, hermit crabs received a new shell with 50% or 100% of the predicted preferred shell weight (Briffa & Elwood, 2007). I housed hermit crabs in individual containers, of 16cm diameter and 4cm depth of aerated seawater at 15°C and 12:12h light:dark cycle and left for ten days of acclimation period.

Experimental design

I assessed each hermit crab for spontaneous alternation (see below for details) once a day over five consecutive days in two periods (10 times in total) (Figure 4.1), with a different optimal shell weight (50 or 100% of its optimal shell weight) in each moment (Figure 4.1). I also stimulated the startle response duration at the end of each individual observation.

After the first five days of observations, I removed the shell initially supplied and then supplied with a new L. littorea shell. Hermit crabs that initially received 100% of the predicted preferred shell weight received a new shell with 50% of the predicted preferred shell weight. Hermit crabs occupying 50% shells received 100% of the predicted preferred shell weight. Thus, all crabs experienced both shell sizes during the

experiment, but not in the same instant (i.e. in different experimental periods). After 10 days of resting, I restarted the investigation of both exploration and startle response stimulus for another five consecutive days.

At the start of each observation, I placed hermit crabs in the centre of a plus-maze (see above). To avoid interference, I recorded its movements for 65 min, ensuring 60 minutes of observation (without an observer in the room) with a video camera (Sony Handycam HDR-CX190). I removed the seawater at the end of each session, in preparation for the next subject to remove possible chemical cues or trails left by the previous hermit crab.

Spontaneous alternation scoring

To quantify exploration, I placed a white plastic plus-maze (arm length: 14cm, arm width: 4cm, arm high: 5cm) filled with seawater. In each extremity of the maze I placed one of four images attached onto the rear wall (Figure 4.2) of different shape (a star, a triangle, a circle and a new moon), with the same surface area ($3cm^2$, due to use of object with different shapes) and shade (black) to potentially aid hermit crabs in navigation. At the end of each arm of the maze I placed an identical piece of elliptic glass marbles (1cm x 0.2cm) and a black line mark in the last 1/3 of each arm (4.5cm from the end). The black mark indicated the "threshold" that hermit crabs had to cross in order to score the entry of each arm as an arm choice, while the glass marbles provided structure (Ramey et al., 2009).

Group	Predicted preferred shell weight					Predicted preferred shell weight				
A	50%					100%				
B	100%					50%				
Period	A					B				
Observation	1	2	3	4	5	6	7	8	9	10
Day	11	12	13	14	16	26	27	28	29	30

Figure 4.1: Experimental design. Percentage represents the shell sizes (based on the hermit weight) provided to hermit crabs within each block of the experiment. Observations days (1-5 and 6-10) were preceded with 10 rest days where the crabs could acclimate to their new shell. On each observation day crabs were observed once in a plus-maze to estimate spontaneous alternation for 65 minutes, followed by the induction of the startle response (after the completion of the plus-maze observation).

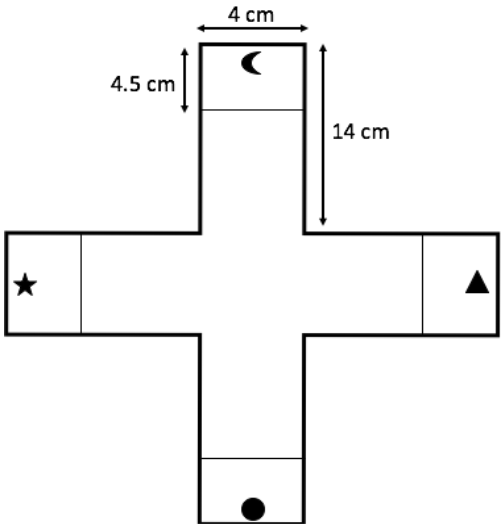

Figure 4.2: Experimental setup and plus-maze dimensions. Grey lines represent the threshold and the symbols are the landmarks provided to aid exploration.

To consider an entry as an arm choice, hermit crabs had to cross the threshold (at least half of its body), located at 1/3 of the arm length. I measure spontaneous alternation in a similar way as other studies of continuous spontaneous alternation. I marked the time at which hermit crab crossed this mark, excluding from the analysis repeated sequential entries in the same arm (McNay & Gold, 2001; Lennartz, 2008). I considered as successful alternation (1) when hermit crabs chose four different arms within a rolling window of five consecutive choices. Any other pattern of movement would be scored as unsuccessful alternation (0).

I estimated the individual performance in each observation as the ratio of successful alternation to the number of possible alternations (the number of all arm choices - 4). For example, considering that a given hermit crabs made the following arm choices: A-D-C-B-A-D-A-D-C-B then, the number of successful alternation would be four out of six possible alternations (A-D-C-B-A-D-A-D-C-B; A-D-C-B-A-D-A-D-C-B; A-D-C-B-A-D-A-D-C-B; A-D-C-B-A-D-A-D-C-B), and the score would be 4 / 6 = 0.67.

I also investigated whether their pattern of movement inside the maze occurs as a function of their dominant side. In this case, as *P. bernhardus* is dextral, I investigate if there was a dominant direction of movement, as turning right, left or forward in the centre of the maze. Using the same video recordings from the spontaneous alternation experiment, I marked each turn inside the arm. I considered a turn when the hermit crab had all his body inside an arm, differently from the spontaneous alternation experiment (which used the threshold to consider an arm choice). Therefore, in each different arm entrance, I recorded the direction of the movement, whether it turned right, left or moved forward. I used the BORIS software (Friard & Gamba, 2016) to record body turn.

Metabolic rate measurements

To investigate whether more exploratory individuals have a higher routine metabolic rate (routine MR), I measured the routine MR of all individuals on four occasions, immediately following the first and last (fifth) observations of spontaneous alternation within each experimental block. Therefore, all individuals would have two routine MR estimations while occupying shells of both 50% and 100% of the preferred shell weight. I estimated metabolic rate using oxygen uptake as a proxy in a closed chamber respirometer. I used an oxygen sensitive sensor spot (PreSens Precision Sensing GmbH, Regensburg, Germany) attached into the inner wall of the chamber with a silicone rubber compound (as specified by the manufacturer). The use of the sensitive spot allows us to have non-invasive measures with a higher precision, preventing any gas exchange during the readings.

I sealed all chambers underwater, preventing the presence of air bubbles that may affect the reading. I only used filtered sea water, minimising bacterial and algae activity. I also measured the oxygen consumption in three extra chambers (blanks), containing a single *L. littorea* shell, with a similar size as used by the crab (and the same sea water as above). If different (different oxygen concentration in the blank compared with the hermit crab chamber), I would account for microbial activity during routine MR estimation. To prevent stress and possible error measurements, I allowed hermit crabs to rest for 30 minutes before starting routine MR measures of oxygen consumption, followed by 40 minutes of measures.

Due to the continuous oxygen consumption by the hermit crab, measures in this closed chamber are never constant. Therefore, I used the difference in oxygen concentration over time to estimate the oxygen consumption inside the chamber, which was latter read by the sensor spot and recorded by a Fibox 4 trace machine (PreSens

Precision Sensing GmbH, Regensburg, Germany), attached to a temperature sensor (Pt100, Bioengineering AG, Wald, Switzerland). To prevent oxygen stratification, and ensure enough mixing of water, I placed the chamber onto a multi-channel magnetic stirrer (MIX 15 eco; 2mag AG, Munich, Germany) with a magnetic flea inside. I placed a mesh between the hermit crab and the magnetic flea to prevent contact between them.

I obtained the O_2 consumption rate using the slope of a linear regression of the oxygen consumption over time, minus the blank O_2 consumption values (Calosi, et al., 2013). Then, I multiplied the slope by the oxygen solubility coefficient and adjusted for salinity and temperature. Although I conducted the metabolic rate measurements in a temperature controlled room, there was small fluctuations in temperature, which can affect oxygen solubility values (Widdows & Staff, 2006). I accounted for these small fluctuations in temperature in the estimation of the oxygen solubility coefficient (as described above). I calculated the rate of O_2 consumption using:

$$Rate\ of\ O_2 uptake\ \left(\mu\text{moles}\ O_2 h^{-1}\right) = C(t) \times (V_r) \times \left({}^{60}/_{t_1 - t_2}\right)$$

Where, C(t) is the O_2 consumption rate (from the linear regression of oxygen consumption over time), V_r is the total volume of water inside the jar (jar volume minus the hermit crab volume) and t_0, t_1, is the measurement period (in minutes; Widdows & Staff, 2006; Calosi, et al., 2013). In order to estimate the metabolic rate and create a standardized measure, allowing the comparisons between individuals, I divided the rate of O_2 uptake by individual body mass (Porter & Brand, 1995).

Data analysis

Prior to the data analysis, I log-transformed startle response duration ($\log_{10}(x + 2)$), spontaneous alternation scores ($\log_{10}(x +1.5)$) and routine MR ($\log_{10}(x +1.5)$) to improve normality. Behavioural syndromes are between individual covariance between

traits (Dingemanse & Dochtermann, 2013). Therefore, only repeatable behaviours can form a behavioural syndrome. Thus, prior to any analysis, I first estimated the repeatability of both startle response duration and spontaneous alternation based on a linear mixed model, using the REML method for Gaussian data (R package rptR) (Nakagawa & Schielzeth, 2010). I calculated repeatability across all observations and also estimated separately for the data collected in each shell size (50% or 100% of its preferred shell weight), as the shell weight can modify the hermit crab's behaviour.

The primary aim was to determine which fixed effects significantly influenced each trait. Therefore, I fitted two univariate models, one for each behaviour (i.e. the response variable was either startle response duration or the spontaneous alternation index) including as fixed predictors shell weight (50% or 100%), hermit crab mass, the day (day 1-5) and the individual's average metabolic rate (average MR of the period). To investigate whether individuals differed in how they reacted to the change in shell size, for both startle response duration and spontaneous alternation, I initially specified random intercepts per individual and a random slope effect across the two and the shell sizes (described as an 'individual x environment interaction' by Dingemanse et al., 2010). However, this model did not achieve convergence (I found a similar pattern in the multivariate analysis), so I restricted out final analysis to an intercept only model in each case.

Behavioural syndromes are referred as suites of correlated behaviours across observations or situations. Traditionally, correlations between two behavioural traits are investigated pairwise correlations between behavioural traits (i.e. Pearson's or Spearman's correlations). However, when behaviour is measured multiple times on multiple traits, it is possible that the correlation between the traits may be divided into between and within-individual components. Thus, an overall correlation between traits

could be resulted from (a) a relationship between the two behaviours within-individual's change in each behaviour (i.e. within-individual correlation, where if individual *i* increases the expression of behaviour X at instance *j*, behaviour Y also increases) , (b) from a relationship between the average responses of individuals for the two behaviours (i.e. between-individual correlation, individual i_1 expresses X and Y at a high rate on average whereas individual i_2 expresses X and Y at a low rate on average), or (c) some combination of within and between individual correlations. Therefore, it is necessary to implement models that partition the variance and covariance structure for all behaviours, using multivariate statistical techniques (see Glossary). Here, I implemented multivariate mixed modelling approaches to estimate between and within-individual correlations between exploration and startle response, while taking in account the effects of the predictor variables (fixed effects). I included as fixed effect shell weight (50% or 100%), hermit crab mass, the experimental block and the occasion (day 1-5) and the average metabolic rate (average MR of the experimental block).

To test whether there was a behavioural syndrome between spontaneous alternation behaviour and startle response duration, I built three models with similar fixed effects structure (as described above), but with different error structures (or residual component, correspondent to the within-individual variance-V_{WI}) and random effects (correspondent to the between individual variance-V_{BI}) (see supplementary material S4 for more information). The first model (M1), was an unconstrained model, allowing the startle response and exploration to co-vary (indicating that these traits are correlated in the individual average response and also that these attributes have correlated changes within-individuals). In the second model (M2), I constrained the between individual co-variances to zero (indicating that these traits are correlated in the individual average response). In the third model (M3), I constrained the within-

individual co-variances (that these attributes have correlated changes within-individuals) to zero (Dingemanse & Dochtermann, 2012).

To test the significance of between and within-individual correlation I compared the covariance structure between these three models using log-likelihood ratio tests (LRT) (Wilson et al., 2009). If M1 was significantly different from M2 and M3 and with a lower log-likelihood (logLik) value, I would have considered both between and within-individual correlation to be significant. Using the unconstrained model (M1) I also estimated both between and within-individual covariance between startle response duration and exploration (see supplementary material S4 for calculation). A significant between individual covariance indicates that the individual mean values of startle response correlated with the mean values of exploration. Whereas a significant within-individual correlation indicates that a change in startle response duration between two observations is correlated with its changes in exploration over the same observations. While I used LRT tests to infer significance, it is also worth noting that in previous studies the estimate of correlation have been grouped into three broad categories that describe how strong the effect is ($|r| \sim 0.1$: weak effect, $|r| \sim 0.3$: medium effect, $|r| \sim 0.5$: strong effect) (Royauté et al., 2013). For mixed effects models, I used the software ASReml-R (Butler et al., 2009), fitted analyses described above using REML (residual maximum likelihood), in R version. I evaluated the effects of the fixed components using Wald's chi-square test and p-values and random effects using Z-test.

Finally, I determined whether hermit crabs, when confronted with a choice (in the middle of the maze), would have a preferred direction (whether it turned right, left or moved strait in the centre of the maze). To determine whether there was a preferred exploratory movement, I used one-way repeated measures ANOVA, using the numbers of turns in each side. I conducted this analysis in R (version 3.3.1). If their movement

inside the maze was related with a preferred direction of turning, I would expect hermit crabs turning right more frequent than left or moving forward. I log transformed (log_{10} +1.5) the count data to better fit the model assumptions.

Ethical note

No animals were harmed during the experiment and at the end of the experiment all individuals appeared healthy and were supplied with excess food (as above) and a new gastropod shell before being returned to the sea.

Results

There was significant repeatability in spontaneous alternation (R_A = 0.09 ± S.E. = 0.25; CI = 0.044, 0.0142) and startle-response duration (R_A = 0.102 ± S.E. = 0.26; CI = 0.054, 0.15) for all individuals (occupying both 50% or 100% of the shell preferred weight) combined. The repeatability of spontaneous alternation was also significant for individuals occupying 50% (R_A = 0.113 ± S.E. = 0.04; CI = 0.035, 0.189) and 100% (R_A= 0.037 ± S.E. = 0.29; CI =0.001, 0.102) of the shell preferred weight. Similarly, the repeatability of the startle response was significant for individuals occupying 50% (R_A = 0.066 ± S.E. = 0.034; CI = 0.006, 0.137) and 100% (R_A= 0.115 ± S.E. = 0.04; CI = 0.076, 0.243) of the shell preferred weight. As assessed by overlap of 95% CIs, there were no differences in repeatability between the two shell sizes for either behaviour.

The parameter estimates for random and fixed effects of the univariate models are given in Tables 4.1 and Table 4.2 respectively. For startle response durations, I found no temporal trend across the 5 observations within each block (χ^2_1 = 1.30, p = 0.25) and mass also had no effect on startle response duration (χ^2_1 = 0.5, p = 0.49). Startle response duration did not vary with initial shell size (χ^2_1 = 0.8, p =0.38) nor with routine MR (χ^2_1 = 1.20, p =0.27). Similarly, I found no temporal trend (χ^2_1 = 2.20, p = 0.14) or effect of mass (χ^2_1 = 0.70, p = 0.42) on the spontaneous alternation behaviour. Additionally, the spontaneous alternation behaviour did not vary with the routine metabolic rate (χ^2_1 = 2.60, p = 0.11) or with the shell weight (χ^2_1 = 0.30, p = 0.58)

The LRT test revealed significant differences between the covariance model and the zero-covariance model, indicating a negative correlation between startle response duration and exploration ($LRT_{M1\text{-}M2}$: χ^2 = 5.84, df = 1; P = 0.016; $LRT_{M1\text{-}M3}$: χ^2 = 5.57, df = 1; P = 0.018; logLik M1 = 1796.09; logLik M2= 2190.38; logLik M3 = 2305.36). I found a significant between individual (r_{ind} = -0.23) and significant within-individual

covariance (r_e = -0.53) correlation between startle response duration and spontaneous alternation. This indicates that the individual mean values of the startle response duration had a negative correlation with the individual mean values of exploration. Furthermore, within-individual change in startle response duration between observations is negatively correlated with changes in exploration over the same observations.

In the univariate model, I found no effect of mass, shell weight, routine MR, or observation on the startle response duration (Table 4.2). Similarly, exploration was not related with mass, shell weight, routine MR , or observation (Table 4.2).

For the multivariate model (Table 4.3), there was no significant change in the combined startle response-spontaneous alternation variable across observations but had a significant increase across experimental blocks. there was also a significant effect of mass and routine metabolic rate, where there was a negative effect of startle response duration and a positive effect of exploration with the increase in mass and routine MR, different from the univariate model. Shell weight had a significant effect whereby both exploration and startle response duration increased with the increase of the shell weight.

Finally, when confronted with an arm choice (in the middle of the maze), hermit crabs tend to have a directional exploration movement ($F_{3, 2685} =$ 1897; $p<0.001$), tuning left more often ($t = 49.55$; $p<0.001$; Figure 4.3) than right ($t = 45.71$; $p<0.001$) or moving forward ($t = 33.88$; $p<0.001$).

Table 4.1: Estimated variance components in univariate linear mixed models for startle response duration and spontaneous alternation behaviour. σ^2 is the variance of each component.

Univariate model	Component	Effect	σ^2	SE	Z-Ratio
Startle response duration	Between experimental perid variance	0.008	0.019	0.005	0.640
	Between individual variance	**$2.63e^{-08}$**	**$1.01e^{-07}$**	**$1.18e^{-09}$**	**22.305**
	Within-individual variance	**0.2595**	**1.0000**	**0.0116**	**22.305**
Spontaneous alternation behaviour	**Between experimental block variance**	**$6.75e^{-10}$**	**$1.47e^{-07}$**	**$3.03E^{-11}$**	**22.305**
	Between individual variance	**$2.95e^{-11}$**	**$6.40e^{-09}$**	**$1.32e^{-12}$**	**22.30**
	Within-individual variance	**$4.60e^{-03}$**	**1**	**$2.06e^{-04}$**	**22.30**

Statistical significance is assessed by comparing variance to the Z-Ratio; effects are considered to be statistically significant if Z> 2 (Wilson et al., 2010). Significant variables are printed in bold.

Table 4.2: The fixed effects and their statistical significance in univariate linear mixed models for startle response duration and spontaneous alternation behaviour.

Univariate model	Parameter name	Sum of Sq.	df	Wald χ^2	p-value
Startle response duration	**Intercept**	**2519.40**	**1**	**9707.90**	**<0.001**
	Mass	0.12	1	0.50	0.49
	Shell weight	0.20	1	0.80	0.38
	Routine metabolic rate	0.32	1	1.20	0.27
	Observation	0.34	1	1.30	0.25
Spontaneous alternation behaviour	**Intercept**	**116.306**	**1**	**25267.80**	**<0.001**
	Mass	0.003	1	0.70	0.42
	Shell weight	0.001	1	0.30	0.58
	Routine metabolic rate	0.012	1	2.60	0.11
	Observation	0.010	1	2.20	0.14

(Mean effect sizes of factors and covariates with their effects, standard error, Wald's chi-square test and p-values; significant variables are printed in bold).

Table 4.3: The fixed effects and their statistical significance from the multivariate model in spontaneous alternation behaviour (SA) and startle response duration. Contrasts are provided for effect size and standard error.

Parameter		Effect	SE	DF	Wald χ^2	p-value
Mass	**SA**	**0.1102**	**0.0075**	**2**	**33287**	**<0.001**
	SR	**-0.5947**	**0.0467**			
Shell weight	**SA**	**0.0019**	**0.0001**	**2**	**5203**	**<0.001**
	SR	**0.0098**	**0.0006**			
Routine MR	**SA**	**3.99e $^{-06}$**	**1.81e-^{06}**	**2**	**1541**	**<0.001**
	SR	**-2.04e $^{-05}$**	**1.11e^{-06}**			
Observation	SA	0.0266	0.0020	2	3	0.1913
	SR	0.1326	0.0124			
Experimental block	**SA**	**0.3400**	**0.0100**	**2**	**12**	**0.0027**
	SR	**1.7053**	**0.0749**			

(Mean effect sizes of factors and covariates with their effects, standard error, Wald's chi-square test and p-values; significant variables are printed in bold).

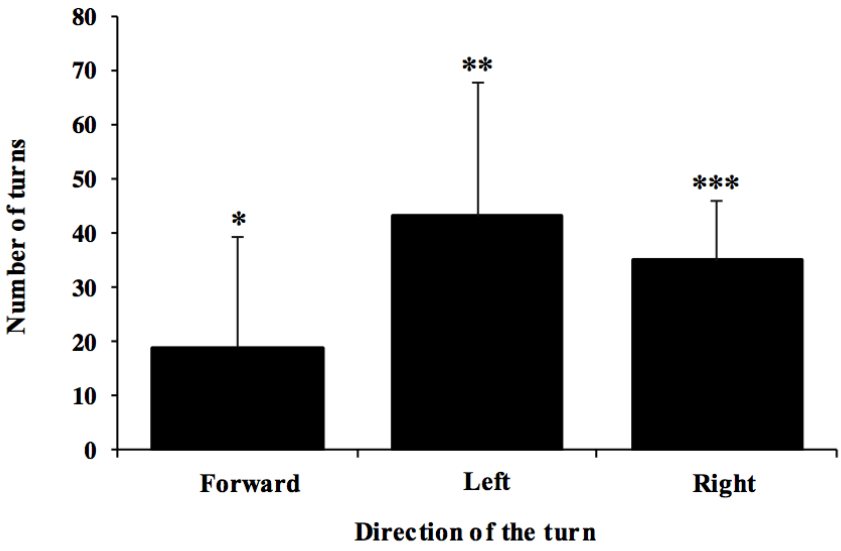

Figure 4.3: The average number of turns in each direction (right, left and forward) made by hermit crabs when in the center of the maze ± SE. Asterisk denotes a significance difference between

Discussion

Here I investigated the presence of correlation between boldness and exploratory behaviour, measured as spontaneous alternation performance. I found a negative correlation between these two repeatable traits, evidencing the presence of behavioural syndrome. Hermit crabs with shorter startle response duration, and thus bolder, had a higher alternation performance than shy ones. In addition, bold hermit crabs with a higher spontaneous alternation performance also had a higher routine metabolic rate, as predicted by the pace of life syndrome hypothesis. the results indicated that exploration tendencies were repeatable within sessions and across situations, 50% and 100% of the optimum shell weight. I found similar results in the startle response duration, with individuals being shyer with the increase in shell weight, in accordance with previous studies (Briffa et al., 2008; Bridger et al., 2015).

The correlation between startle response duration and alternation performance was independent of observation, indicating absence of habituation, similar to both univariate models (Briffa et al., 2008; Stamps et al., 2012; Briffa et al., 2013; Bridger et al., 2015). I have also shown that there is a positive correlation between metabolic rate and exploration in the bivariate model, demonstrating that individuals with a higher metabolic rate are bolder and more explorative. More explorative and bolder individuals (with a higher alternation performance) are likely to be fast explorers, exploring a new environment fast, but less thoroughly, covering more distance, and thus spending more energy (Sih & Bell, 2008). Such high consumption of energy can be maintained by a positive feedback loop, once individuals with a higher routine MR may take more risks, explore a bigger area, but they are more likely to gather more rewards (Biro & Stamps, 2010; Houston, 2010). Furthermore, such a correlation between life-history traits are likely to be heritable (Dochtermann et al., 2015), and therefore, they can be the result of

genetic covariance (Cheverud, 1996; Sinervo & Svensson, 2002). In this study, routine MR did vary with startle response duration (Velasque & Briffa, 2016) and with exploration as proposed by Careau et al. (2008). Although I did not find a significant relationship between both personality traits and metabolic rate in the univariate models, bivariate model the bivariate model results are consistent with the pace-of-life syndrome hypothesis (Réale et al., 2010). One explanation for this conflicting result is that the changes in routine metabolic rate is better explained by startle response duration and exploration combined. Thus, it is possible that it is the syndrome, rather the individual behaviours (boldness and exploration) that is driven by variation in metabolic rate. Furthermore, it possible that the syndrome is under selection pressure, rather than a single behavioural trait (Réale et al., 2010). Alternatively, correlations between behavioural traits can also originate from a shared and fixed mechanism that underpins both (e.g. same hormone regulation for both behaviours or a pleiotropic effect). Therefore, suites of correlated behaviours should be viewed as coupled traits, rather than independent ones (Price & Langen, 1992; Wilson et al., 2010) and thus, changes in metabolic rate, for instance, should lead to a shift in both the correlation between the linked behaviours (i.e. in the bivariate model) but not in either behaviour on its own.

In crustaceans, mass is a loose indicator of age, such that heavier individuals also tend to be older than lighter ones (Lancaster, 1988; Liberto & Mesquita-Joanes, 2014). Therefore, the results could have indicated an ontogenic change in both alternation performance and startle response behaviour, with an increase in both exploration and boldness with age. This result could be explained by positive feed-back like process. For instance, if more explorative and bold individuals have a higher gain, it possible that, by positive feedback (i.e. state-dependent feedbacks), there is an increase in such behavioural tendencies with the increasing in age (Sih & Bell, 2008).

I also found that spontaneous alternation performance and startle response duration differ according with the shell weight. Individuals occupying 100% of their optimum shell weight were more bold and explorative than individuals on 50% shells. Previous studies in *P. bernhardus* indicate that hermit crabs occupying poor quality shells had longer startle responses than those occupying optimum shell weight (Briffa & Bibost, 2009). Therefore, hermit crabs occupying sub-optimal shell could be more vulnerable (e.g. sub protected) to predators, and therefore, exhibiting a decrease in exploration and increasing in startle response duration. The spontaneous alternation pattern can be mediated by several factors, including spatial, odour or body turn cues (see for a review Richman et al., 1986). Animals may have a higher tendency of movement caused by encounter with predators, food, even the disposition of the habitat. The movement pattern in *Artemia* sp., for example, seems to be based on their previous moment. When animals were forced to turn left in an earlier moment (forced by a multiple T-maze), they tended to turn right at the next choice (when both choices, right or left, were provided) (Çarkoğlu et al., 2015). Alternatively, animals can use their own body as a cue to move, exhibiting bias towards one side. I shown exploration in *P. bernhardus* may also be biased (i.e. exhibiting preference when turning), where individuals would turn left more often than right or move forward, without reinforcement.

Exploration and boldness are important aspects of life-history traits (Réale et al., 2010), being extensively investigated in personality studies (Carter et al., 2012). For example, exploration tends to be correlated with aggressiveness, dispersal, sociability (Fraser et al., 2001; Dingemanse et al., 2003; Krackow, 2003; Cote & Clobert, 2007; Cote et al., 2010). However, their definition and study are often conflated (see Carter et al., 2012 for review). For instance, boldness can be defined as propensity to take risks (usually investigated under novel situations) (Coleman & Wilson, 1998; Toms et al.,

2010) or the individual's response to a risky situation (e.g. presence of predator) (Réale et al., 2007). Therefore, its study can include the behavioural response to a novel environment, situation or the response to predation risk (Toms et al., 2010). While the study of exploration usually involves the measure of the exploration of a novel object or environment (Carter et al., 2012). In both cases, the presence of a new compound could induce anxiety in the animal and it could be misinterpreted as variation in shyness/boldness (Sih & Bell, 2008; Carter et al., 2012; Perals et al., 2017). Here, I attempt to avoid such confounds by taking independent measures of startle response duration and spontaneous alternations, providing separate indexes of boldness and exploration.

Spontaneous alternation is the behavioural pattern that is assumed to represent an innate (i.e. does not require reinforcement) tendency to explore novel ambient from those recently visited, it can increase the likelihood of discovery cues to a new resource or new unexploited resources, reducing the effect of competition and potentially increasing fitness (e.g. patch, mate, food, shelter) (Chiussi et al., 2001; Weissburg & Dusenbery, 2002). Therefore, such a behavioural pattern may be beneficial to the individual during exploration, but it could also increase the risk of predation (e.g. encounter with predator) in a similar way to boldness.

Here I have shown that spontaneous alternation is consistently different between individuals. I also have shown that variation in spontaneous alternation and startle response underpinned by variation in routine metabolic rate. Therefore, a higher exploration and boldness may indeed reflect a fast pace of life where acquisition of new resources is prioritized over longevity. However, I should also note that this result is correlative and I cannot rule out the possibility that higher rates of MR could not have

been driven by a recent high activity (during exploration) prior to the respirometry part of the experiment.

Chapter 5

Under the influence of light: how constant artificial light affects the expression of personality and energetic consumption in hermit crabs

Abstract

Variation in behaviour caused by change in environmental conditions are an important part of behavioural ecology. However, more studies are necessary for a better understanding of behavioural modifications caused by anthropogenic disturbances, such as light pollution. Here, I investigate the effect of permanent light driving variations in a personality trait and metabolic rate in European hermit crab *Pagurus bernhardus*. I used Bayesian mixed models to estimate average behavioural change (i.e. sample mean level behavioural plasticity), consistency and between and within-individual variation in boldness in response to permanent light in laboratory. Hermit crabs kept under constant light were consistently less bold and had a higher metabolic rate, than when kept under a standard light and dark regime (12:12h light/dark), however there was no effect of light in consistency in behaviour. As boldness is associated with response to risk, the results could reflect the effect of light pollution in behaviour, where hermit crabs may experience an increase in the predation risk and energetic consumption in natural areas with artificial light at night (i.e. light pollution).

Introduction

Most species have evolved under natural and predictable regimes of moonlight, sunlight and starlight (e.g. nocturnal, crepuscular, diurnal). For those species, light offers navigational aid, helps to regulate and coordinate maturation and reproductive events, regulates physiology (Davies et al., 2014) and visually informs guided behaviours such as predation and communication (Gaston & Spicer, 2013). In natural environments light follows a predictable and cyclic pattern of change, providing environmental 'Zeitgebers' (timing cues; Aschoff et al., 1974). This 'clock' is synchronized not only by the Earth's rotation (which creates day and night cycle) but also by the tilting of the Earth's axis relative to the Sun (Panda et al., 2002), allowing organisms to anticipate seasonal changes and adjust their behaviour and physiology accordingly (Aschoff, 1960; Pittendrigh, 1981; Saper et al., 2005). On a daily time-scale, these patterns of activity are called circadian rhythms and they have been observed in plants, animals, fungi, and bacteria (Roenneberg & Merrow, 2005; Edgar et al., 2012).

Formally, circadian rhythms refer to endogenous free-running periods, that an organism can maintain even under constant conditions (e.g. total darkness) for at least 24 hours. Thus, circadian rhythms can be distinguished from simple responses to external cues (including light; Aschoff, 1981). For instance, mimosa plants are able to fold leaflets during night and unfold them in the daytime, maintaining this pattern even in constant darkness (De Mairan, 1729). The endogeneity of such rhythms also implies that they could be reset once the organism is exposed to a different external stimulus, or Zeitgeberg, in a process called entrainment (Pardini & Kaeffer, 2006). Therefore, rhythmicity appears to be an important force, allowing organisms to anticipate and react to environmental change, regulate metabolic processes, their behaviour and physiology, and thus potentially offering a selective advantage (Enright, 1970; Green et al., 2002).

In the majority of animals, the entrainment of circadian rhythm is regulated by the hormone melatonin, which is typically released under dark conditions (Collins et al., 1994; Jiang et al., 1995; Ochoa-Sanchez et al., 2011; Zhdanova et al., 2001). Although melatonin production is nearly ubiquitous in nature, occurring in plants and all animal taxa, except sponges (Feuda et al., 2012), the way in which it operates varies with life-history. For example, in vertebrates, melatonin production controls sleep patterns, behaviour, activity and blood pressure (Jiang et al., 1995; Ochoa-Sanchez et al., 2011; Zhdanova et al., 2001). In invertebrates, melatonin production appears to inhibit movement rather than induce sleep (Anctil et al., 1991; Bentkowski et al., 2010). Constant light in vertebrates, can induce melatonin suppression causing major physiological and behavioural disruptive effects. These include disorientation, inappropriate attraction (Rydell, 1992), or repulsion from light (Stone et al., 2012), distortion of signals, disruption to periods of rest, reproductive failures (Eisenbeis et al., 2006), disruption of memory formation (Rawashdeh et al., 2007) and causing metabolic alterations (Knutson et al., 2007; Dolgin, 2013). These individual level affects can, in turn, lead to changes in patterns of intra- and inter-species competition and predation (Longcore & Rich, 2004). However, the effects of constant light in groups other than vertebrates is not well understood (Balzer & Hardeland, 1991; Vivien-Roels & Pévet, 1993; Hardeland & Poeggeler, 2003; Feuda et al., 2012; Roopin & Levy, 2012).

For marine animals, light availability can also provide environmental cues for predicting current velocity and tidal height by the peak of lunar brightness every 29 days using the so-called “Lunar clock” (Naylor, 2010). This lunar clock could be masked by artificial lighting at night, reducing the ability of marine animals to predict these regimes (Hölker et al., 2011). Such disruption may also interfere with the synchronization of spawning events, decreasing cross fertilization (Davies et al., 2013) and even disrupt the diel migration of zooplankton (Ashjian et al., 1998). The

magnitude of any behavioural effects, however, is not well understood in marine invertebrates (Davies et al., 2014).

Artificial light regimes also have the potential to influence antipredator behaviour (Troscianko et al., 2009, Yorzinski et al, 2015). Although many marine animals use cryptic colouration to reduce the chance of detection, the effectiveness of crypsis tends to be enhanced under dark conditions and reduced during daylight (Feltmate & Williams, 1989; Halle, 2000). Hence, many animals are typically more active at night, undertaking activities such as foraging during this period of reduced detectability (Speakman, 1999; Halle, 2000; Monterroso et al., 2013; Maeno et al., 2014). Thus, if light conditions are artificially prolonged (i.e. if there is light pollution) there may be a reduction in night time activity rates (Starr et al., 2012). Furthermore, under such conditions animals may become more risk averse (i.e. shyer) under elevated predation risk (Davies et al., 2014; Maeno et al., 2014). On the other hand, the extent to which such behavioural plasticity (i.e. a reduction in night time activity under extended light conditions) is seen will be dependent on the influence of circadian rhythms, and the strength of their entrainment (Dominoni et al., 2013; Longcore et al., 2013; Davies et al., 2014). Thus, if consistent patterns of daily variation in activity rates are strongly entrained, the effects of artificially extended daily periods of light may become apparent gradually (over multiple days) rather than immediately (Aschoff et al., 1960; Aschoff, 1980).

Behaviour is considered to be plastic when it can be rapidly adjusted according to a changing environmental condition or during interactions with other individuals (Sih et al., 2004; Briffa et al., 2008; Lange & Del-Claro, 2014). However, individuals also tend to show some degree of behavioural consistency across time, even across changing situations (e.g. Dingemanse & Wolf, 2010), indicating limits to behavioural plasticity

(Briffa et al., 2008). Consistent behavioural differences between individuals from the same population is characterized as 'animal personalities' (Dall et al., 2004; Sih et al., 2012). The presence of animal personality has been demonstrated in many species, including marine invertebrates (Briffa et al., 2008; Briffa & Bibost 2009; Briffa & Twyman, 2011; Mowles et al., 2012; Watanabe et al., 2012).

'Boldness' is a measure of the propensity to take risks. Several studies have demonstrated that boldness can vary consistently between individuals of the same species, providing evidence that it as a personality trait, and it can also differ between situations, evidencing behavioural plasticity (as seen in Chapter 4). For example, hermit crabs, *Pagurus bernhardus*, occupy empty gastropod shells to protect their weakly calcified abdomen. When perturbed they show a characteristic startle response of withdrawing into the shell and the latency to re-emerge gives a measure of boldness (bolder individuals re-emerging more quickly). Despite it being demonstrated that startle response duration is consistently different between individuals (Briffa et al., 2008; Briffa, 2013), it can also be plastic, changing according to situations, such as the level of predation risk (Briffa et al., 2008, Briffa 2013) and differences in shell quality (Briffa & Bibost, 2009). Therefore, while I might see a degree of behavioural consistency in night-time behaviour (potentially varying across individuals) due to the entrainment of circadian rhythms, I might also see behavioural plasticity in response to a change in the normal daily light regime. Artificial light at night has being linked with numerous behavioural changes, disorienting animals (Salmon et al., 2005), causing repulsion (Beier, 1995; Beier, 2006) or attraction (Jaeger & Hailman, 1973; Frank 1988; Wiese et al., 2001) to light, increasing predation risk or influencing the ability of predators to detect and prey capture (Buchanan, 1993; Lima, 1998; Ringelberg ,1999), for instance. In addition to changes in behaviour, a change to the light regime might also influence key physiological mechanisms that are expected to underpin variation in

activity rates. For example, activity rates are expected to be driven by underlying variation in metabolic rate (Friesen et al., 1989). In addition, activity rates in crustaceans might be influenced by haemocyanin concentration (Spicer & Baden, 2000), the oxygen transport molecule that determines the scope for aerobic activity. In addition, activity rates in crustaceans might be influenced by haemocyanin concentration, the oxygen transport molecule that determines the scope for aerobic activity, and which can be rapidly adjusted in response to stress (Spicer & Baden, 2000).

Although modifications in light regime by artificial lighting is a global phenomenon, coastal areas are one of the most affected due to extensive development in these areas (Cinzano et al., 2001; Longcore & Rich, 2004). However, its effects on marine and terrestrial species are not well known (Longcore & Rich, 2004). Here, I investigated the effect of constant light on the personality and physiology of the hermit crab *Pagurus bernhardus*. More specifically, I investigate the effect of artificial lighting at night on the: (i) maintenance of personality (i.e. the repeatability of behaviour); (ii) behavioural plasticity (iii) changes to the normal diel pattern of activity, (iv) metabolic rate and (v) haemocyanin concentration. I predict that animals kept under permanent light would exhibit longer duration of the startle response (being shyer) than animals under a standard light and dark regime. I also predict that intra-daily variation in activity pattern would be reduced due to a more homogenous light regime. Thus, under constant light, hermit crabs should exhibit similar startle response duration in day and night measurements, while individuals under a standard light and dark regime exhibit more marked differences in startle response durations between these time periods. If differences between individuals (between individual variance, 'V_{BI}') are reduced this would tend to reduce repeatability, but if differences within-individuals (within-individual variance, 'V_{WI}') are reduced this would tend to increase repeatability.

Therefore, to fully understand the effect of a change in light regime on animal personality, an approach is needed where both variance components are investigated.

Methods

I collected hermit crabs during May 2014 from the rocky intertidal at Hannafore Point, Cornwall, UK. Since the mass of the gastropod shell can affect hermit crab behaviour (Briffa & Bibost, 2009), all crabs were removed from their shells by cracking the shell with a bench vice. I then assigned each crab a new *Littorina littorea* 100% of its preferred weight. I only used male hermit crabs free form parasites and appendage damage (mean mass = 0.51g ± SE = 0.27g, N = 40).

Hermit crabs were housed in individual containers, of 16cm diameter and 4cm depth of aerated seawater at 15°C. Under these conditions the crabs were allocated to one of two light regimes, either a 12:12h light:dark cycle (group LD; N = 20) or continuous illumination (group LL; N = 20). They were left for a ten day acclimation period, followed by 10 days of behavioural observation.

Behavioural assays

I induced the startle response (LL, N = 15; LD, N =15) using a handling protocol, where crabs were lifted out of their tank and replaced in an inverted position on the base of the tank. This causes them to withdraw into their gastropod shell. I timed the latency of recovery from the point at which the crab is replaced in the tank to the point at which the walking legs re-contact with base of the tank (Briffa et al., 2008). Although many marine animals are assumed to be more active at night, *P. bernhardus* under standard light conditions (12:12h light: dark) is more active during the day than at night (peak of activity at 9:00 and lower activity at 22:30h) (Michell, 1973). Therefore, I induced startle responses twice every 24h at 9:00 (day time observations) and 21:00 (night time

observations). I made night-time observations under the 12:12h light dark cycle with low levels of red light in order to avoid influencing crab behaviour (Hazlett, 1966; Sinn & Moltschaniwskyj, 2005).

After the collection of the set of observations, the light/dark regime conditions were reversed. Crabs which initially experienced 12:12h light: dark treatment (LD) were transferred to permanent light treatment (LL) and those initially experienced LL were transferred to the LD treatment (Figure 5.1). The usage of the crossover design allowed us to identify whether the period of the experiment (A or B) would be a confounding factor, masking the effect of the light regime (Briffa et al., 2013). Observations at these new light conditions restarted after a further ten days of acclimation, as described above. Thus, all crabs experienced a 10 day acclimation period, a 10 day period of twice daily observations, followed by a second 10 day acclimation and 10 day observation period.

	Period					
	A			B		
Days	1-10	11-20		21-30	31-40	
Treatment order	*10 day acclimation*	Day SR (x10)	Night SR (x10)	10 day acclimation	Day SR (x10)	Night SR (x10)
LL-LD	Light-Light	Light	Light	Light-Dark	Light	Dark
LD-LL	Light-Dark	Light	Dark	Light-Light	Light	Light

Figure 5.1: Schematic representation of the time-line of the experiment, showing how the treatments (Light – Light, Light – Dark) were applied to the two treatment orders (LL-LD, LD-LL) across the two periods (A,B) of the experiment.

Metabolic rate measurements

To investigate if the metabolic varies in response to the light conditions, I measured the routine metabolic rate (routine MR) of 10 individuals (LL-LD, N= 5; LD-LL, N = 5) (i.e. different individuals to those used in the main behavioural experiment), exposed to the same conditions as described above. I used a similar crossover design, in which hermit crabs first experiencing LL experiment were then transferred to LD and vice versa. Thus, I measure each hermit crab's metabolic rate in two light regimes, LL and LD. To minimize measurement errors, I measured routine MR in the same room in which the animal was maintained. I restarted routine MR measures after 10 days of acclimation in the new treatment.

I measured routine MR throughout 24 hours using the oxygen uptake as a proxy in a closed chamber respirometer. I used an oxygen sensitive sensor spot (PreSens Precision Sensing GmbH, Regensburg, Germany) attached into the inner wall of the chamber with a silicone rubber compound, as specified by the manufacturer. The usage of the sensitive spot, allowed a non-invasive measure, as well as more precise measures, as prevented gas exchange during the readings.

Measures conducted in closed chambers are never constant due to the continuous oxygen consumption by the animal. Therefore, I used the difference in oxygen concentration over time to estimate the oxygen consumption inside the chamber, which can read by the sensor spot and recorded by a Fibox 4 trace machine (PreSens Precision Sensing GmbH, Regensburg, Germany), attached to a temperature sensor (Pt100, Bioengineering AG, Wald, Switzerland). To prevent oxygen stratification, and ensure enough mixing of water, I placed the chamber onto a multi-channel magnetic stirrer (MIX 15 eco; 2mag AG, Munich, Germany) with a magnetic

flea inside. I placed a mesh between the hermit crab and the magnetic flea to prevent contact between them.

I sealed the chambers underwater to prevent the presence of air bubbles affecting the measure. To minimize bacterial and algal activity, I only used filtered sea water. I also measured the oxygen consumption in three extra chambers ('blank'), containing a single *L. littorea* shell, with a similar size as used by the crab, and sea water as described above. The microbial activity was accounted for during routine MR estimation. I obtained the O_2 consumption rate using the slope of a linear regression of the oxygen consumption over time minus the blank O_2 consumption rate (Calosi, et al., 2013). Then, I multiplied the slope by the oxygen solubility coefficient and adjusted for salinity and temperature. Although I conducted the metabolic rate measurements in a temperature controlled room, there was small fluctuations in temperature, which can affect oxygen solubility values (Widdows & Staff, 2006). I accounted for such small fluctuations in temperature in the estimation of the oxygen solubility coefficient (as described above). I calculate the rate of O_2 consumption using:

$$Rate\ of\ O_2 uptake\ \ (\mu\text{moles}\ O_2 h^{-1}) = C(t) \times (V_r) \times \left({}^{60}/_{t_1 - t_2}\right)$$

Where, C(t) is the O_2 consumption rate (from the linear regression of oxygen consumption over time), V_r is the total volume of water inside the jar (jar volume minus the hermit crab volume) and t_0, t_1, is the measurement period (in minutes; Widdows & Staff, 2006; Calosi, et al., 2013). In order to estimate the metabolic rate and create a standardized measure, allowing the comparisons between individuals, I divided the rate of O_2 uptake by individual body mass (Porter & Brand, 1995). I allowed hermit crabs to rest for 30 minutes before starting routine MR measures of oxygen consumption. To prevent stress and possible disturbances in the animal, I kept the same individual during the 24 hours' measurements.

Haemocyanin concentration

After completing the behavioural observations, I extracted a haemolymph sample from all hermit crabs (LD; N = 20 and LL; N = 20), following the protocol described by Bridger et al. (2015) by inserting an insulin syringe into the infrabranchial sinus. Then, I transferred 10µl of the haemocyanin recently sampled into semi-micro cuvette containing 690 µl of double distilled water After mixing, I measured the haemocyanin absorbance at 337 nm in a spectrophotometer. I used the Nickerson & Van Holder (1971), extinction coefficient to determine the haemocyanin concentration. I euthanized all individuals used after the haemolymph collection, by placing into a saturated magnesium chloride solution.

Data analysis

I used three analyses to investigate the effect of light/dark regime on the startle response. In the first analysis, I quantified the effect of light and dark conditions on the duration of the startle response using a hierarchical generalised linear model (HGLM) implemented within a Bayesian framework (MCMC Bayesian approach implemented in the R package MCMCglmm; Hadfield, 2010). I then used a second HGLM to estimate treatment group and time specific repeatabilities (these could not be obtained from the primary model that was used to test for mean level effects; see details below). In the third analysis, I determined the effect of the light and dark condition on metabolic rate using a repeated measures ANOVA. Thus, I took the approach of using the simplest possible analysis that was adequate for the question of interest and the properties of the data.

In the first analysis, I fitted a model allowing a random intercept for each individual, allowing for between individual variation in startle responses ($V_{BI:}$ between

individual variance) and random slopes across the repeated observations ($V_{WI:}$ within-individual variance also called residual variance), which allowed the presence of individual variation across the observations. I assumed that the residual variance was normally distributed and uncorrelated across observations. I used the startle response duration as the predictor and included time at which I collected the startle response (diurnal or nocturnal), the treatment (LL or LD), the period (according to the crossover design), the occasion (day 1-20) on which the behaviour was observed, the hermit crab mass and the hermit crab mass, the haemocyanin concentration as fixed effects and the interactions between treatment * time and between treatment *period. I used two flat-non-informative priors to test the robustness of the model: (V = 1, n = 0.002) and (V= 1, n = 1.002). Both priors produced similar results, however, the first produced the lowest DIC (Deviance Information Criterion) (DIC: flat-non-informative prior 1 = 1572.22; flat-non-informative prior 2 = 1756.21), justifying its use in this analysis. I reported the posterior mode for fixed effects along with their 95% credible intervals (CIs).

To compare repeatability (and its's V_{BI} and V_{WI} components) across treatment groups I modelled another HGLM. In contrast to the model described above, this model has experimental block-specific random intercepts for individuals (LL day, LL night, LD day, LD night) (i.e. there is a block-specific G-structure, corresponding to V_{BI}). I used two non-informative priors to test the robustness of the model. The first prior was a flat-non informative prior (V = diag(4), nu = 1.002) and the second was an inverse-Wishart (V = diag(4), nu = 3.002), where n is the number of behavioural variables. Both priors produced similar results, however, the inverse-Wishart prior produced the lowest DIC (DIC: flat-non-informative prior = 3401.76; inverse-Wishart = 3386.84), being used in this analysis. Additionally, I modelled separate residual variances for each

experimental block (R-structure, corresponding to V_{WI}). I used a similar structure for fixed effects as in the model described above.

I estimated the posterior modes for repeatability in each experimental block (with 95% CIs). I also determined whether the repeatability estimates showed significant differences among the experimental blocks by calculating the posterior modal differences among blocks (ΔR; see supplementary material S5) and the 95% CI values of these differences (Royauté et al., 2015; White & Briffa, 2017; Osborn & Briffa 2017). I estimated the difference in repeatability, ΔR, between treatments within each time of day (R_{LL}-R_{LD}) and between each time of day within groups ($R_{LL\ during\ day}$-$R_{LL\ during\ night}$; $R_{LD\ during\ day}$-$R_{LL\ during\ night}$). I made similar calculations to assess the changes in the specific variance components of repeatability (ΔV_{BI} and ΔV_{WI}) between treatments and times of day.

Both of these models described above were implemented using Bayesian framework, and thus, delta and repeatability values were considered significant when 95% CIs of their posterior modes did not overlap zero. I specified a Markov Chain Monte Carlo (MCMC) for both models with 1.9×10^6 interaction, 9×10^5 interaction burn-in and a thinning interval of 1000. I fitted all models using Markov chain Monte Carlo methods (implemented with MCMCglmm in R3.0.2).

In the third analysis, I determine the effect of light on the metabolic rate using a two-way repeated measures ANOVA. As the metabolic data were not normally distributed, I apply Log_{10} +1 transformation prior to analysis. I also log transformed (Log_{10} +1) the startle response duration in all analyses to improve normality. I also checked whether the rooms (LD and LL) used on this experiment differed in light measures during day using T-test. Light levels did not differ between rooms used (unpaired t-test $t_{24} = 0.11$, $p = 0.92$) and were in average 385.54 lux (range = 301-446,

$n_{locations}$ = 25). Light levels at night in Hannafore Point averaged 0.127 lux (range = 0-1.4, $n_{locations}$ = 25).

Results

The effect of permanent light on boldness

The parameter estimates for both random and fixed effects of the HGML model and their 95% credible intervals (and estimated p values) are given in Table 5.1. Since significance is inferred via contrasts rather than via an overall p-value for categorical predictors in this type of model, each effect contains multiple p-values rather than just one, as in most statistical approaches. Therefore, for brevity, I do not reproduce the p-values reported in Table 5.1 in the text below.

The fixed effects components of the HGLM model provide strong evidence that the mean duration of the startle response had no temporal trend across the 20 observations and there was no correlation with the hermit crab mass. The model also provides strong support that the mean duration of startle response varied between individuals, that startle responses were greater in LL than in the LD group (Figure 5.2a), that startle responses were longer during the day than at night (Figure 5.2a) and they increased with haemocyanin concentration ($p<0.01$). There was strong evidence for the interaction between treatment and the time of day, indicating that the difference between day and night startle responses was more marked for the LD treatment compared to the LL treatment (Figure 5.2a). Although I found evidence that the duration of the startle response varied across periods, there was no effect of the interaction between the period and the treatment.

Comparing the repeatability and variance components of startle responses

I estimated the repeatability from the second HGLM (see supplementary material S5). The repeatability estimates (Table 5.2) provides strong evidence that the startle

response duration was repeatable in all treatments blocks and that there was no significant difference in repeatability between treatment groups within periods. The model also indicates the presence of significant among and within-individual variation in startle response duration between treatment groups within periods (Table 5.3) and that was no significant differences in V_{BI} periods between groups. The comparison of the V_{WI} between groups indicate a lower behavioural consistency in individuals on LD treatment was greater at night measures than in the day measures.

The effect of permanent light on metabolic rate

I found no difference in metabolic rate between diurnal and nocturnal measures between treatment groups ($F_{1,27} = 0.12$, $p = 0.73$, Figure 5.2b). Additionally, there is no difference between diurnal and nocturnal measures ($F_{1,27} = 1.28$, $p = 0.27$, Figure 5.2b), nevertheless, I found a significant support for an interaction between treatment and time on the metabolic rate, whereby oxygen consumption was significantly greater during the day compared to the night for the LL treatment, but there was no difference between day and night for the LD treatment ($F_{1,27} = 9.11$, $p = 0.006$, Figure 5.2b).

Table 5.1: Posterior summary statistics for the mean effect of startle response, showing posterior mean, lower and upper 95% CIs and P-values (for fixed effect only).

Parameter name	**Posterior mean**	**95% CI lower**	**95% CI Upper**	***p***
Fixed effects				
Observation	0.024	-0.016	0.062	0.22
Mass	-0.110	-0.354	0.166	0.40
Haemocyanin concentration	**0.007**	**0.004**	**0.011**	**<0.01**
Time (by contrast)				
Day	**1.838**	**1.217**	**2.422**	**<0.01**
Night	**1.827**	**1.241**	**2.448**	**<0.01**
Treatment (by contrast)				
LL	**1.730**	**1.048**	**2.489**	**<0.01**
LD	**2.142**	**1.601**	**2.792**	**<0.01**
Period (by contrast)				
A	**1.719**	**0.993**	**2.422**	**<0.01**
B	**1.833**	**1.219**	**2.371**	**<0.01**
Treatment x Time (by contrast)				
LL Day	**0.693**	**0.402**	**0.948**	**<0.01**
LL Night	**-0.696**	**-0.963**	**-0.434**	**<0.01**
LD Day	**0.692**	**0.384**	**0.929**	**<0.01**
LD Night	**-0.709**	**-0.967**	**-0.427**	**<0.01**
Treatment x Period (by contrast)				
LL – A	-0.289	-0.809	0.223	0.28
LL – B	0.285	-0.244	0.784	0.29
LD – A	0.270	-0.275	0.722	0.31
LD – B	-0.298	-0.782	0.203	0.26
Intercept	**2.150**	**1.503**	**2.720**	**<0.01**
Random intercepts (between individual variation, G-structure)				
Hermit Crab ID (intercept)	0.0009	0.0005	0.001	-
Hermit Crab ID (observation)	1.109	0.96	1.265	-

The significance of effects was tested with a a Wald's chi-square test. Significant variables are printed in bold. Contrast between categories are provided for the varibles: treatment, period, the interactions between treatment x time and between treatment x period.

Table 5.2: Posterior modes, upper and lower 95% CIs (in brackets) for MCMC repeatability estimates between treatment groups within periods and ΔR of differences between treatments (ΔR = LL-LD) and between the time on which the startle response was induced Δ*R* (Night-Day). Significant values are shown in bold

	Day	Night	Δ*R* (Night-Day)
LL	**0.38 [0.23,0.54]**	**0.21 [0.13, 0.39]**	-0.12 [-0.34, 0.07]
LD	**0.49 [0.35, 0.64]**	**0.35 [0.24, 0.50]**	-0.13 [-0.34, 0.04]
Δ*R*(LL-LD)	-0.16 [-0.32, 0.10]	-0.14 [-0.30, 0.09]	-

Table 5.3: Posterior modes, upper and lower 95% CIs (in brackets) for (a) among and (b) within-individual variation in startle response duration between treatment groups within periods and ΔV for the of differences between treatments (ΔV = LL-LD) and between the time on which the startle response was induced ΔV (Night-Day). Significant values are shown in bold

(a) Between individual variation, V_{BI}

	Day	Night	ΔV_{BI} (Night-Day)
LL	**0.52 [0.30, 1.05]**	**0.26 [0.13, 0.56]**	0.14 [-0.15, 0.79]
LD	**0.76 [0.38, 1.24]**	**0.52 [0.31, 0.98]**	-0.15 [-0.81, 0.33]
ΔV_{BI} (LL-LD)	-0.16 [-0.82, 0.54]	-0.27 [-0.67, 0.21]	-

(b) Within-individual variation, V_{WI}

	Day	Night	ΔV_{WI} (B-A)
LL	**0.94 [0.77, 1.05]**	**0.97 [0.79, 1.11]**	0.06 [-0.18, 0.24]
LD	**0.73 [0.63, 0.89]**	**1.03 [0.85, 1.21]**	**0.29 [0.04, 0.48]**
ΔV_{WI} (LL-LD)	-0.13 [-0.81, 0.51]	-0.11 [-0.31, 0.16]	-

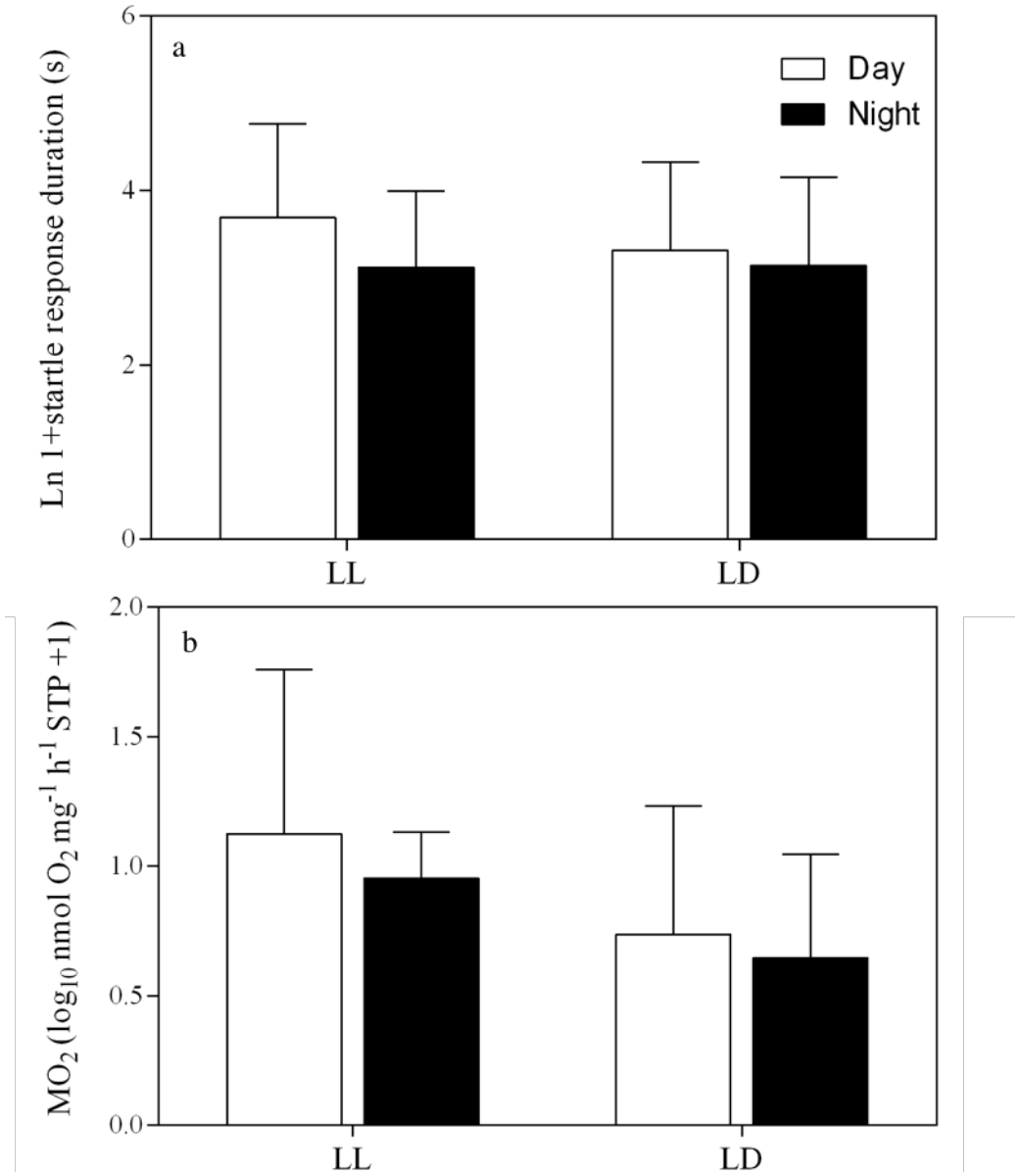

Figure 5.2: The interaction effects between treatment (LL and LD) and time (day or night) on: (a) the duration of the startle response and on the (b) the metabolic rate (MO_2). Metabolic rate is expressed as log_{10} nmol O_2 mg^{-1} h^{-1} STP (error bars represents standard deviation).

Discussion

I investigated the effect of constant light on personality traits and metabolic rate in the hermit crab *Pagurus bernhardus.* The data show that permanent light influences mean level startle response durations but has no effect on the repeatability. Individuals kept in permanent light (LL) were shyer (with longer startle response duration) than individuals experiencing standard light and dark conditions (LD). The usage of the crossover design allowed us to test the effect of the treatment avoiding confounding factors, such as time and habituation (Briffa et al., 2008; Briffa & Bibost, 2009; Briffa & Twyman ,2011; Mowles et al., 2012, Briffa et al., 2013). In fact, the startle response duration varied by period of the experiment, with longer startle responses during period B. Similarly, Briffa et al. (2013) found an influence of duration of the experiment on startle responses in *Pagurus bernhardus.* While many studies neglect to use a crossover design, it is only such a design that can reveal effects of the duration of the experiment, which otherwise might be mistaken for effects of the treatment. However, while Briffa et al. (2013) observed a decline in the startle response duration in the second half of the experiment, I observed an increase. Nevertheless, even with such effect, it was still possible to detect the differences in startle response duration that are due to the light treatment.

Previous studies in *P. bernhardus* have shown that the startle response duration is not affected by the hermit crab mass (Briffa et al., 2008; Bridger et al., 2015, Velasque & Briffa, 2016). This indicates that that ontogenetic variation in hermit crabs is unlikely to be related with boldness (at least within the restricted range of hermit crab masses in the size class of crabs used in this experiment), as mass is indicative of age in crustaceans (Lancaster, 1988; Liberto & Mesquita-Joanes, 2014). As in previous studies, I also found no temporal trend across the 20 observations (Briffa et al., 2008;

Stamps et al., 2012; Briffa et al., 2013; Bridger et al., 2015) signalling an absence of habituation to the startle response stimulus, within blocks of the experiment.

Artificial light at can make nocturnal animals more visible, facilitating predator's detection and increasing the predation risk for prey species (Troscianko et al., 2009; Prugh & Golden, 2014). Previous studies in *P. bernhardus* have shown that hermit crabs can be aware of the shell conspicuousness and the predation risk associated the conspicuousness of the shell, modifying the startle response duration according with the inherent situation (Briffa & Twyman, 2011). This experiment has similar results, in both treatments (with and without artificial light at night) individuals exhibited longer startle response during day and shorter at night. Nevertheless, hermit crabs in the permanent light treatment (LL) had shorter startle response duration at night time than individuals under standard dark and light regime (LD). It is possible that light at night has a similar effect in hermit crabs, increasing their conspicuousness and, as a consequence, increasing risk (Gaston & Spicer, 2013). Therefore, when such conspicuousness is reduced, animals may adjust their decision (e.g. exhibiting plasticity towards light regime), increasing startle response duration during the day. Michell (1973) has shown that *P. bernhardus* has a distinctive pattern of activity, being more active during day than at night, even in the presence of constant light. Thus, it is possible that startle response duration follows a similar pattern as activity, being longer during day, even when the predation risk is increased by the presence of light at night.

Low predictability, alongside with low boldness, is a potential strategy to cope with risk, as less predictable individuals might reduce the chance of being captured (Briffa et al., 2013; Briffa, 2013). Although I found that hermit crabs adapt their response with permanent light (increasing boldness), this study did not find any differences in predictability between light treatments. Permanent light did, however,

lead to an increase in energy consumption (when compared with standard light and dark regime). Therefore, it is possible that in a long-term exposure to constant light, hermit crabs need to increase the food consumption to support his high energetic demand (Speakman & McQueenie, 1996), which increases foraging and therefore the predation risk (Lima, 1988; Lima & Dill, 1990).

Animals in captivity tend to have a more homogeneous behaviour as result of reduction in environmental heterogeneity (Desy et al, 1990; Bell et al., 2009; Dammhahn & Almeling, 2012). However, captive animals still have some pattern of activity with phases of higher and lower activity, usually reinforced by light (e.g. circadian rhythms: Palmer, 1973). Thus, under constant light conditions, it is expected that such patterns would be minimized (Wyse et al., 2011; Rieswijk, 2015), producing a more homogeneous response (e.g. lower variance or higher repeatability). However, I found no evidence for this, with individuals experiencing the permanent light treatment had similar repeatability to individuals under the standard light and dark regime. The repeatability within groups (day versus night) was also similar. Similarly, individuals in both treatment groups and at both time periods exhibit significant among and between individual variance in boldness (V_{BI} and V_{WI} respectively). There was also no significant variation in V_{BI} between treatment groups and time. However, under the standard light and dark regime treatment (LD) there was a significant difference in the amount of within-individual variation (V_{WI}) between day and night; crabs had more within-individual variation in behaviour (less consistent) at night than during the day. One possible explanation is that *Pagurus bernhardus* is a diurnal species, maintaining its activity pattern even under the presence of constant light (Michell, 1973). Therefore, it is possible that the absence of light at night, other rhythms than circadian (tidal or lunar) are more pronounced, resulting in a higher variation in behaviour within-individuals. Alternatively, hermit crabs may be subjected to different pressures through

the day. For instance, it is possible that the predation risk increases during the night time and thus, decreasing in predictability (increases V_{WI}) may increase survival in natural conditions. That occur because low predictability (i.e. low behavioural consistency or high V_{WI}), alongside low boldness, is potentially a strategy to cope with risk, as less predictable individuals might reduce the chance of being captured (Briffa et al., 2013 Briffa, 2013). Although I found that hermit crabs decrease their startle response durations (increasing boldness) under permanent light conditions, this study did not find any differences in predictability between light treatments. Another effect of permanent light was the increase in energy consumption (when compared with standard light and dark regime). Therefore, it is possible that in a long-term exposure to constant light, hermit crabs need to increase food consumption to support higher energetic demands (Speakman & McQueenie, 1996), which increases the need to forage and therefore the predation risk (Lima, 1988; Lima & Dill, 1990).

The effect of light at night on the energetic demands has been investigated in several species, with mixed results. Artificial lighting, for example, seems to result in metabolic disruption leading to obesity in humans (Wyse et al., 2011), dogs and cats (Zoran, 2010). While in fishes, night time illumination increases general activity (Batty, 1987; Woodhead, 1957) and accelerates yolk consumption in larvae. This results in a premature hatch, indicating an elevated metabolic rate (Brüning et al., 2011). Similarly, the data show that hermit crabs experiencing constant light treatment had a higher metabolic rate than those under standard light dark regime, and this effect was independent of the crossover design or time (Figure 1). My finding also suggests that hermit crabs exposed to permanent light treatment also had a higher haemocyanin concentration than individuals at standard light and dark conditions. As haemocyanins are proteins responsible for oxygen transport in many arthropods and molluscs (Linzen

et al., 1985), such increase in its concentration can indicate a higher energetic demand of the crab, which can be supported by the increased in metabolic rate.

Artificial light is a modern, globally widespread (Cinzano et al., 2001) and fast expanding (Hölker et al., 2010), issue. And thus, over the last decade these concerns have been fuelling the need to understand the range of impacts that it may cause (Stone et al., 2012; Davies et al., 2014). Nevertheless, the impact on marine life is yet not well documented, especially in invertebrates. One reason is that coastlines tend to accumulate environmental stressors, such as chemical pollution, habitat fragmentation, artificial habitats, noise and eutrophication, potentially overriding any effects of light pollution or at least the assumptions about its importance (Longcore & Rich, 2004). In addition, the extent of the effect of artificial light on marine life also depends on several factors, including the intensity and spectrum of the artificial light, the organism perception (spectral sensitivity pigments), the timing and the local conditions (e.g. rocky shores provide more shade areas, minimizing the general impact of light pollution; Land & Nilsson, 2002; Longcore & Rich, 2004). Therefore, investigating the effect of artificial lightning in laboratory conditions could isolate its effect from others stressors. In this sense, this study shows possible outcomes of the effect of light pollution in *Pagurus bernhardus* under natural conditions. To my knowledge, there are no prior studies evaluating the effects of permanent light conditions as potential drivers (or disruptors) of variation in repeatable personality traits. Therefore, this study shows how light pollution may affect *Pagurus bernhardus* physiologically, increasing metabolic rate, and behaviourally by reducing within-individual variation in behaviour and decreasing boldness overall.

Changes in light and dark regimes are assumed to represent a significant source of behavioural and physiological changes for non-captive animal populations, and such

effects might even ramify to alter inter-specific interactions, and thus modify the ecosystem structure (e.g. there might be both top-down and bottom-up effects; Davies et al., 2012; Bennie et al., 2015). For instance, urban light can increase the activity during night in diurnal or crepuscular species, leading to an increasing in consumption and thus in a top-down effect (Longcore & Rich, 2004). Although my results (increased metabolic rate, and absence of differences in repeatability in permanent light) indicate that artificial light at night can cause hermit crabs to experiment an increasing in predation risk, which could reduce its population in affected areas. However, further experiments to explore these potential downstream effects of light pollution are clearly warranted. For instance, it has been shown that hermit crabs exposed to predator chemical cues (e.g. effluent form containers with a predator) or visual (e.g. predator model) cues, tend to adjust their behaviour (Briffa et al., 2008; Briffa, 2013) and could be used to simulate risk under constant light regime. Nevertheless, the current study shows a reduction in boldness and an increasing in metabolic rate.

Chapter 6

Discussion

Overview

Phenotypic variance is an important concept in natural selection. First because natural selection can promote phenotypic variance. For instance, competition for similar resources (e.g. food, optimum territory) can promote 'niche differentiation' (e.g. niche specialization) reducing within-species competition (Bergmüller & Taborsky, 2010). Alternatively, natural selection can also reduce phenotypic variance if it favours an average phenotype and selects against extreme variations (Orr, 2009). This potentially adaptive variation occurs across different levels of biological organisation. For instance, there is variation between species, between individuals within the same species and within each individual. Recently, this between and within-individual variation has received a high level of attention from behavioural ecologists due to the possibility that it could influence survival and reproduction (Dingemanse et al., 2010) and hence fitness. Here, I focus on two aspects of phenotypic variation: physiological and behavioural investigated both between and within-individuals. More specifically, I investigated whether variations in cognitive performance (as speed and accuracy during shell assessment), exploration (spontaneous alternation) and between and within variation in boldness (startle response duration) are underpinned by variations in metabolic rate, as predicted by the pace of life syndrome (POLS) hypothesis in the hermit crab *Pagurus bernhardus*. Finally, I also investigated how constant light affects boldness (mean level and consistency) and energetic consumption in *P. bernhardus*, in this way exploring how concepts such as POLS, and indeed animal personalities, might help us to better understand the effect of anthropogenic impacts on animal behaviour.

Research summary

Behaviour and energetic use

The POLS hypothesis attempts to explain the presence of consistency in behavioural and physiological traits as the result of life-history trade-offs (Roff & Fairbairn, 2007; Wolf et al., 2007; Réale et al., 2010). Where individuals would adopt a fixed strategy, investing in current or future reproduction (Wolf et al., 2007). As a result, individuals with a higher investment in future reproduction, would behave accordingly, exhibiting less risk prone behaviour, foraging with less intensity but also gathering fewer rewards, and thus, having a lower short-term reproductive performance (Wolf et al., 2007; Réale et al., 2010). Here, I explored one of the key predictions of the POLS concept: that bold and more exploratory individuals should have a higher energetic consumption than less exploratory and shy ones. My results partially support this prediction. First, I did not find evidence for a correlation between individual personality traits (i.e. boldness or exploration) and energetic usage (Chapters 2, 3 and 4). Nevertheless, when both exploration and boldness were combined forming a behavioural syndrome, they were positively associated with routine metabolic rate, as predicted by the POLS hypothesis. (Chapter 4). This difference in results is most likely due to the syndrome structure, where suites of correlated behaviours could be linked by proximate mechanisms (e.g. hormonal, genetic) and thus, changes in metabolic rate, for instance, should lead to a shift in the linked behaviours (i.e. exploration and boldness). Therefore, decoupled traits (isolated boldness or exploration) do not fully represent the structure of life-history trade-offs (Price & Langen 1992; Wilson et al., 2010). This explains why more explorative and bold individuals had a higher energetic consumption.

Recently it was suggested that POLS could be extended to include other life-history aspects such as decision-making (Sih & Del Giudice, 2012). In Chapter 3, using

shell assessment, I investigated whether decision speed (time to accept or reject a new shell) and decision accuracy (choice of a shell with a potential higher quality) co-vary with between individual differences in boldness and metabolic rate. I demonstrated that not only there isn't a trade-off between speed and accuracy, with faster assessments leading to more accurate decisions, but also that bold individuals were more accurate than shy ones.

In hermit crabs, the decision decision-making seems to be energetically demanding (Chapter 3), with the metabolic rate being significantly higher during decision-making when compared to routine. Nevertheless, the increasing in energy use during decision-making was not related with the cognitive performance (both decision time and accuracy). Decision-making MR, however, covaried with decision speed, where individuals that assessed shells for longer period of time also had a higher energetic consumption. This result, combined with the fact that faster assessments led to a higher accuracy in decision-making suggests that shell assessment is not as complex as indicated by previous studies (e.g. Elwood et al., 1979; Dowds & Elwood, 1985; Elwood & Stewart, 1985; Elwood & Niel, 1992). Alternatively, it is also possible that accuracy (i.e. shell choice) is not an energetically demanding process (when compared with shell investigation), but might represent an outcome of the shell comparison, and thus, may not impose additional energetics costs to the individual.

This possible outcome was addressed by previous authors, where it was suggested that the use of a single estimation of the individual oxygen consumption at rest may not fully capture the energetic demands of the animal behaviour (Speakman, 1999; Mathot & Dingemanse, 2015). Therefore, I attempted to collect repeated measures of behaviour (Chapter 2) and metabolic rate per individuals (Chapter 2 and 4). Furthermore, I also combined the estimation of basal energetic expenditure (i.e. routine

MR) with the individual energetic consumption while performing the behaviour (i.e. MR during the startle response induction in Chapter 2 and MR during decision-making in Chapter 3). However, between individual differences in behaviour were not related to energetic consumption (Chapters 2, 3 and 4). Furthermore, the simultaneous measure of metabolic rate during startle response (i.e. 30cm free fall) also seemed to be potentially problematic. Once by being unfamiliar to the stimulus, the behaviour exhibited by hermit crab could be wrongly interpreted as boldness. In fact, they seem to habituate to the startle response stimulus only when it was caused by the 30cm free fall (in opposition to manual handling), suggesting that the recovery time could be associated with a rudimental type of learning (i.e. habituation) (Speakman, 1999; Amdam et al., 2010). Nonetheless, I did not find any relationship between metabolic rate and the startle response induced by manual handling in further experiments (Chapters 3 and 4), increasing the reliability of the findings in Chapter 2.

Identifying the behaviour structure (i.e. whether behavioural traits are independent or correlated forming syndromes) has been described as crucial in behavioural ecology (Sih & Bell, 2008). Particularly because, correlations between behavioural traits could explain the maintenance and selection of different behavioural types within a population (Wolf & Weissing, 2010). For instance, correlated behaviours and physiological traits can be adaptive, depending on local selective pressures (Dingemanse et al., 2004; Dochtermann & Jenkins 2007), and thus, might be population specific (Bell, 2007). Correlation between activity, exploration and aggression of the three-spined stickleback, for example, *Gasterosteus aculeatus*, was only significant in populations where the predator was present (in opposition to the predator naïve) (Dingemanse et al., 2007), indicating that the presence of a syndrome was adaptive. Syndromes can also be caused by a constraintmechanism, in which, behavioural traits shared a fixed mechanism (e.g. same hormone regulation both behaviour or a

pleiotropic effect of genes), thus, changes in one trait lead to a shift in a second behavioural or physiological trait. Future studies will be necessary to determine whether the correlation between the behavioural syndrome (i.e. exploration and boldness) and energetic expenditure are caused by a constraint mechanism or if they are adaptive. For example, if different populations (with different selective pressures) of *P. bernhardus* vary on the expression of these traits (e.g. stronger, weaker or absence of correlations between behavioural and physiological traits) it could indicate that boldness, exploration and metabolic rate are adaptive, instead of constrained.

Repeatability in life-history traits

Here, I used repeatability to investigate the consistency of three life-history traits: boldness (Chapter 2, 3, 4 and 5), exploration (Chapter 4) and metabolic rate (Chapter 2). Although hermit crabs were collected in the same location, and tested with the same standard test for startle response duration (except for Chapter 2), repeatability, between and within-individual variation changed across this study. Thus, I obtained the highest repeatability estimate in boldness in Chapter 3 (R_A= 0.472) and the least when assessing boldness alongside exploration (Chapter 4, R_A = 0.102). While these studies indicate that different behaviours (and some times the same behaviour assessed using different methods, e.g. startle response duratiosn) have different repeatabilities there is also the possibility that some of the differences in repeatability estimates could be driven by temporal differences (e.g. seasonal or across years) in which the experiments took place. Nevertheless, there are still some overall patterns for repatable behaviour in hermit crabs, which are discussed below.

Hermit crabs exhibited consistency in boldness throughout all experiments (Chapter 2, 3, 4 and 5). However, the degree of consistency varied according with the method employed to induce startle response (i.e. by the 30cm free fall in Chapter 2),

according with the occupied shell size (Chapter 3 and 4), light condition (Chapter 5) and with other unaccounted factors (e.g. temperature). For instance, the induction of the startle response by the 30cm free fall (Chapter 2) seemed to produce lower repeatability estimates than the proposed by Bell et al. (2009). In comparison, manual handling often, except in Chapter 4, resulted in moderate repeatability (Chapters 3 and 5), in accordance with previous studies in *P. bernhardus* (e.g. Stamps et al., 2012; Briffa, 2013; Briffa et al., 2013). Light regime (Chapter 5) and shell size (Chapters 3 and 4) also seem to produce, non-significant differences in repeatability.

Exploration and boldness are important aspects of life-history traits (Réale et al., 2010), being key traits in personality studies (Carter et al., 2012). However, their definitions are often conflated, which might increase the apparent co-dependence between those traits, leading to misleading results (see Carter et al., 2012 for review). For instance, boldness is usually defined as propensity to take risks (usually investigated under novel situations) (Coleman & Wilson, 1998; Toms et al., 2010), whereas exploration the individual response to a novel situation or level of superficial exploration (Réale et al., 2007). Therefore, its study can include the behavioural response to a novel environment, situation or the response to predation risk (Toms et al., 2010). Here, I demonstrate that spontaneous alternation (Chapter 4) in hermit crabs is consistent (i.e. repeatable), but also correlated with other life-history aspects, and it could be used as a new independent index of exploration.

I also found a lower, but significant repeatability estimate of routine and startled MR (Chapter 2), contradicting previous studies (e.g. McCarthy, 2000; Broggi et al., 2007; Nespolo & Franco, 2007). One explanation is that such studies are mainly focussed on endotherms (for review see Nespolo & Franco, 2007). Which by having a

higher maintenance cost, may maintain a more constant metabolic rate (basal metabolic rate; Stearns, 1992).

Beyond consistency in behaviour

Over the last 20 years there was an increasing in information regarding the presence of personality. However recent findings have been suggesting another important component of behavioural variation where individuals might (consistently) vary in behaviour responding to an environmental gradient (behavioural plasticity) or even exhibit non-explained behavioural variation, termed within-individual variation in behaviour (V_{WI}). Here, I investigate the link between energetic consumption (routine MR and startled MR) and within-individual variation in boldness.

The POLS predicts a positive relationship between life-history traits, but, unfortunately there is no consensus on its prediction regarding within-individual variation in behaviour (Coppens et al., 2010; Careau et al., 2012; Niemelä et al., 2012). Some authors for instance, suggests that fixed behavioural strategy (lower V_{WI}) should be less energetically demanding (due to lower costs for cognitive activities) and thus more common in slow-paced strategy individuals (Coppens et al., 2010; Niemelä et al., 2012). In hermit crabs, however, the relationship between behavioural consistency (V_{WI}) and energetic use seem to be plastic (Chapter 2). As behavioural consistency decreased (higher V_{WI}), with the increase in metabolic rate during the startle response induction (startled MR) and increase (lower V_{WI}), with routine MR.

Studies in residual behavioural variation are still in earlier stages, mainly due to the lack of statistical knowledge to estimate variation, but there is evidence that V_{WI} is an important component of individual's life-history traits (Piersma & Drent, 2003). In hermit crabs for instance, V_{WI} is associated with increased predation risk, where

individuals behaved less predictably in the presence of a predator (Briffa, 2013).My results reinforce such findings, since the startled MR was only correlated with startle responses at the V_{WI} level, and not at the mean-level. Therefore, it appears that, at least in potentially stressful situations, within-individual variation in behaviour, rather than individual mean levels of behaviour, might be linked with underlying variation in metabolic rate.

Others physiological traits

In crustaceans, mass is a loose indicator of age and therefore heavier individuals also tend to be older than lighter ones (Lancaster, 1990; Liberto et al., 2014). Although I attempt to control for mass, opting for individuals with similar weight thought the experiments, small variations in mass between individuals were inevitable. Overall, mass had no effect on boldness (Chapter 3, 4 and 5) and exploration (Chapter 4), indicating that differences in behavioural types are unlikely to be the result of ontogenic changes. However, when boldness and exploration are combined (i.e. using multivariate analysis) they are positively associated with the individual mass. This indicates an ontogenic change in both exploration and startle response behaviour, with an increase in both exploration and boldness with age. Such differences can be the result of state-dependent feedback, on which more explorative and bold individuals gain more resources (e.g. food, territories), thus, by positive feedback, there is an increase in such behavioural tendencies with increasing age (Sih & Bell, 2008). This possibility reinforces the idea that the study of isolated behavioural traits (e.g. boldness or exploration alone) may not fully represent an individual's strategy.

Is there a fast-slow behavioural type in hermit crabs?

The idea that animals must trade-off future to current reproduction is central to many areas of behavioural ecology, explaining such apparently diverse observations such as patterns of growth (e.g. Robinson & Doyle, 1985; Houston et al.,1993; Blomquist, 2009), number of offspring (e.g. Jensen, 1996), mating behaviour (e.g. Abrahams, 1993), differences between sexes (e.g. Robinson & Doyle, 1985) and foraging behaviour (e.g. Lima & Dill, 1990). Recently, the idea of trade-offs was also used to explain the presence of between individual differences in behaviour (i.e. animal personality) (Wolf et al., 2007; Réale et al., 2010), through a mechanism of correlation between multiple life-history traits.

In the personality literature, behaviour is traditionally investigated along five behavioural axes: shy-boldness, activity, aggressiveness, sociality and exploration–avoidance (Gosling et al., 2003). They can be investigated using specific tests that might not fully represent each behavioural axis (see Carter et al., 2012 for review). For example, activity and exploration or aggressive and sociality might not be decoupled (see Carter et al., 2012 for review). Furthermore, the distinction of behaviour in five isolated behavioural axes may not have biological meaning, explaining why I only found support for the POLS when two behaviours were examined in combination (Chapter 4) or when the I examined the variance in behaviour (V_{WI} Chapter 2). However, most studies still focus in sets of isolated behavioural traits, potentially explaining why studies in POLS have mixed support (e.g., Bryant & Newton, 1994; Ketola & Kotiaho, 2012; Krams et al., 2013). Moreover, the POLS explains suites of correlated behaviour assuming that they are the result of natural selection (e.g. adaptive explanation and life-history trade-off for animal personality), thus, it is expected that life-history correlations would vary between populations (e.g. Dingemanse et al., 2007)

and species (Sih & Bell, 2008). Thus, although the POLS provides reasonable explanations for life-history traits, generalisations towards its assumptions should be avoided.

Another important point, is that POLS is based on the presence of life-history trade-offs and they are only used to explain traits that are selected in opposing directions (Schluter et al., 1991). However, this theory considers that individuals are subjected to similar pressures (e.g. uniform presence of predators, parasites for food), when it is likely to vary in patches (Bell, 2012). Therefore, some individuals might perform better than others, without trade current for future reproduction. In fact, I did not observe such trade-off in hermit crabs, where faster decisions lead to higher accuracy in shell choice (Chapter 3). Furthermore, bolder individuals were also more accurate than shy ones, indicating that shy crabs may be underperforming in natural environments. Therefore, in hermit crab differences in boldness could be caused by differences in individual quality (e.g. shy individuals might be hungry or with parasites), rather than representing the life-history strategy.

Shifts in personality traits in response to permanent light

Artificial lighting is a modern, and fast expanding (Hölker et al., 2010) issue. However, its consequence in animal behaviour is not well known (Stone et al., 2012; Davies et al., 2014), especially in marine in invertebrates (Longcore & Rich, 2004; Davies et al., 2014). In Chapter 5, I investigated possible outcomes of the effect of light pollution in *Pagurus bernhardus* under natural conditions. In the laboratory, I tested the effects of constant light on boldness and metabolic rate (routine MR). To answer whether constant light affects behaviour, I compared the average behaviour, behavioural repeatability, between and within-individual variation in behaviour between groups

exposed to permanent light (LL treatment) and to normal light conditions (12h:12h light and dark - LD).

Permanent light seems to affect only the mean level behaviour (i.e. there was no differences in repeatability nor between individual differences in behaviour) and energetic consumption. When individuals were kept in permanent light (LL), they were shyer (with longer startle response duration) and had a higher metabolic rate compared with individuals experiencing standard light and dark conditions (LD). Artificial light at night can increase the individual visibility, facilitating the predator's detection, and thus, it is possible that this change in behavioural types corresponds to a plastic response to increased conspicuousness (e.g. Briffa & Twyman, 2011).

In Chapter 4, I show boldness and exploration are correlated in both between and within-individual components. Consequently, the average boldness is correlated with the average exploration (between individual level) and that the individual change in boldness is correlated with the individual change in exploration (within-individual level). This suggests that permanent light is also likely to reduce exploration in hermit crabs. Such effect combined with my results (increased metabolic rate, and absence of differences in repeatability in permanent light) indicate that hermit crabs can experience an increase in the predation risk in areas with artificial lighting, which could lead to a reduction of this species abundance further experiments would be necessary the effect of light pollution.

Future prospects

The study of animal personality has provided a great contribution to the study of animal behaviour, by demonstrating the importance of investigating individual variation in behaviour alongside mean level variation, generating directions and further questions.

First, the POLS suggests that differences in life-history traits between individuals of the same specie could provide a mechanism of coexistence between them. Thus, studying how personality traits differ between males and females, may provide more information on the mechanisms that underlie behavioural variation (Pruitt & Riechert, 2009; Schuett & Dall, 2009; Chapman et al., 2013; Fresneau et al., 2014). Once, males and females diverge in response to different selection pressures and thus, exhibit different life-history traits.

As shown in Chapter 4, bolder and more exploratory individuals also have a higher routine MR, potentially maintained by positive feedback (e.g. more explorative and bold individuals may gather more resources, which increases their energetic demands, leading to an increasing or maintenance of the bold and exploratory behaviour). It would be interesting to investigate whether changes in reward may lead to changes in the behavioural syndrome (i.e. positive feedback). This is possible by manipulating food intake and comparing changes in behaviour between groups with high and low food regimes.

Lastly, light pollution represents could represent a threat to biodiversity, however, its effects on behaviour are not well known (Davies et al., 2014). Chapter 5, was the first study to investigate the effect of permanent light as a potential driver (or disruptor) of variation in repeatable personality traits. I have shown that hermit crabs under permanent light conditions are bolder and have a higher metabolic rate than those under normal light conditions. However, it is necessary in future investigations to assess the extent of the effect of light pollution in other life-history aspects. For instance, Chapter 4 indicates exploration is correlated with boldness, and therefore changes in boldness (caused by permanent light) are likely to result in changes in exploration. However, the effect of light pollution driving changes in exploration and survival of

hermit crabs still needs to be addressed. Another aspect that needs to be addressed is the combined effect of permanent light with others pollutants. For example, coastlines tend to accumulate environmental problems (e.g. eutrophication, heavy metals, noise pollution). Therefore, to better draw conclusions of the effects of these impacts on animal behaviour in natural environments, it is necessary to address the combined (and potentially interactive) effect of multiple stressors.

Synthesis

Here, I have shown that, contrary to the POLS hypothesis, neither boldness or exploration individually are underpinned by the individual energetic expenditure (routine or startled MR). I also have shown that boldness and exploration have a positive between and within-individual correlation forming a behavioural syndrome. And that variations in the syndrome are underpinned by variations in routine MR. Indicating that bolder individuals were also more explorative and with a higher energetic consumption, highlighting the importance of investigating sets of behavioural traits. Metabolic rate (both routine and startled MR) and behavioural traits (boldness and exploration) were repeatable though this work, providing evidence for the presence of animal personality. The estimation of exploration via spontaneous alternation was also repeatable and correlated with boldness. Thus, I recommend spontaneous alternation as a reliable estimation of exploration, which, alongside activity, could be used to gague behavioural responses to a novel environment or situation or the response to predation risk (Toms et al., 2010).

Using double hierarchical generalized linear models, I asked whether variances in between and within-individual variance in behaviour are underpinned by variations in metabolic rate during routine and startled MR. There was an increasing in within-

individual variance in behaviour (lower predictability) with the increase with startle MR and a decreased with routine MR.

In relation to cognitive performance, I did not find support for the presence of a trade-off between speed in decision-making and accuracy in shell choice. As faster assessments lead to a higher accuracy in decision-making (i.e. choice of a shell with higher quality). Furthermore, accuracy was significantly correlated with boldness, with bolder individual being often more accurate than shy ones, and this variance was not underpinned by decision-making time nor differences in energetic consumption. Finally, constant light exposure is likely to modify hermit crab personality and physiology. Hermit crabs kept under a constant light regime were less bold and had a higher metabolic rate, than when kept under standard light and dark regime, indicating possible effects light pollution in this species.

The POLS hypothesis attempts to explain consistent between individual behavioural differences and suites of correlated life-history traits as a result of conflicting life-history choices (i.e. life-history trade-offs). Thus, it has the potential to give insights of the selective pressure that a given population (or species) is exposed to. Because of the inherent facility behind its study (correlations are theoretically simpler to be studied than a selective experiment to determine causes of behavioural changes), it has been an attractive framework for behavioural ecologists to use. However, studies on POLS are often inconclusive (mixed evidence supporting POLS) and the lack of evidence to support this hypothesis is often attributed to the method used (e.g. Le Galliard et al., 2012; Velasque & Briffa, 2016). However, aside form methodological issues, the mixed array of result could indicate that mechanisms other than life-history trade-offs are generating differences in behavioural types (e.g. constraint, state-dependent feedback). Alternatively, it also could indicate the presence of an overlooked

element of life-history traits, as encountered here (i.e within-individual variation in behaviour or suites of correlated behaviours), and thus, changes in a personality trait (i.e. boldness) will not reflect the changes associated with physiological trait (i.e. metabolic rate). Therefore, such decoupled traits do not represent the life-history structure, explaining why more explorative and bold individuals had a higher energetic consumption. It also important to note that the idea of a syndrome linking boldness, exploration and metabolic rate, should also be seen with caution, given that they might be part of part of more complex syndromes ultimatley dirven by other traits (e.g. immunity, activity, number or quality of offspring) that have yet to be assessed.

Appendix

Supplemental material - S1

Description of the mean and SD models

If Y_{ij} denotes the startle response of the i^{th} hermit crab on the j^{th} occasion then I assume that (Y_{ij}) is normally distributed with mean μ_{ij} and standard deviation σ_i, the model can be expressed as:

1 $(Y_{ij}) = (\beta_1 + \delta_{0i}) + \beta_2 \text{Temperature}_i + \beta_3 \text{Mass}_i + \beta_4 \text{Routine metabolic rate}_i + \beta_5 \text{Activity metabolic rate}_i + (\beta_6 + \delta_{1i}) \text{Occasion}_{ij}$

-β_1 is the expected value when all of the covariates are equal to zero (i.e. the intercept)

-β_2 to β_5 represent fixed effects for the covariates.

-δ_{0i} represents the random intercept effect

-δ_{1i} represents the random slope associated with occasion.

I assumed that the random effects were normally distributed with means of zero and unknown variances. The SD model can be expressed as:

2 $(\sigma_i) = (\gamma_1 + \phi_{0i}) + \gamma_2 \text{Temperature}_i + \gamma_3 \text{Mass}_i + \gamma_4 \text{Routine metabolic rate}_i + \gamma_5 \text{Activity metabolic rate}_i$

-γ_1 represents the sample mean

-γ_2 to γ_5 represent fixed effects for the covariates.

-φ_{0i} represents the random intercept, assumed to have a mean of zero and standard deviation of $\tau\sigma,0$.

Table 1- Wald-F test for autocorrelation estimation between fixed effects

Parameter name	Wald X^2	p-value
Mass x Routine MR	0.6648	0.4149
Temperature x Routine MR	0.5983	0.4392
Mass x AMR	0.6497	0.4202
Temperature x AMR	0.8106	0.3680
Routine MR x AMR	1.2142	0.2705

R codes for the double hierarchical genitalized linear model

```
library(asreml)
library(asremlPlus)
library("dae")
library("car")
library(latticeExtra)

setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Paper")
hcrab=read.csv("Maridata.csv", header=TRUE)


sr <- as.numeric(hcrab$SR)     #   Startle response duration
id <- as.factor(hcrab$ind)     #   Individual number
obs <- as.factor(hcrab$Obs)    #   Observation number
o2r <- as.numeric(hcrab$O2R)   #   Routine metabolic rate
o2a <-as.numeric(hcrab$O2A)    #   Startled metabolic rate
wt <- as.numeric(hcrab$Wt)     #   Mass
temp <- as.numeric(hcrab$Temp) #   Room temperature (during the measurements)


set.seed(123)
N= 400                              # No. of observations
k=40                                # No. of individuals with repeated measurements
ID <- factor( rep(1:k, each=N/k) ) # Define individuals as a factor. For later use
Z <- diag(k)%x%rep(1,N/k)
u <- rnorm(k,0,2)                   # Random effects in the mean. Simulated
 variance=4
u.d <- rnorm(k,0,1)                # Random effects in the dispersion. Simulated
 variance=1
sigma2e <- exp(Z%*%u.d)
e <- rnorm(N, 0, sqrt(sigma2e))
y <- Z%*%u + e


##Model 1
```

```
mean.w <- c(rep(1,N))
res.var = 0
conv.crit = 0.00001
max.iter = 20
i.iter = 0
while (i.iter<max.iter & abs(res.var-1)>conv.crit)
{stop
  mean.model <- asreml(sr~ o2a + o2r + wt+ obs + temp
                       ,random = ~ id
                       , weights=mean.w
                       , calc.like=TRUE
                       , control = asreml.control(maxiter=100))
  asreml.hv <- mean.model$hat
  res.var <- mean.model$sigma2   #Residual variance
  res<-resid(mean.model, type = "response")
  hv.true <- asreml.hv*mean.w/res.var

# These are checks for extreme cases

 tol.val=0.0001
  for (i in 1:N) {
    if (abs(res[i])<tol.val) res[i]=tol.val
    if (hv.true[i]>(1-tol.val)) hv.true[i]=(1-tol.val)}
}
y_d<-(res^2)/(1-hv.true)                        #Response for variance model
var.w <- (1-hv.true)/2                          #Weights for variance model
var.model <- asreml(y_d~o2a+o2r+wt+temp,
                    weights=var.w, family=asreml.Gamma(link=log), control =
 asreml.control(maxiter=100))
mean.w <- 1/fitted(var.model)


###Model 2

mean.w2 <- c(rep(1,N))
res.var2 = 0
conv.crit = 0.00001
max.iter = 20
i.iter = 0
while (i.iter<max.iter & abs(res.var-1)>conv.crit)
{stop
  mean.model2 <- asreml(sr~ o2a + o2r + wt+ obs + temp
                       ,random = ~ id
                       ,rcov = ~ id:exp(obs)
                       , weights=mean.w2
                       , calc.like=TRUE
                       , control = asreml.control(maxiter=100))
  asreml.hv2 <- mean.model2$hat
  res.var2 <- mean.model2$sigma2   #Residual variance
  res2<-resid(mean.model2, type = "response")
  hv.true2 <- asreml.hv2*mean.w2/res.var2

# These are checks for extreme cases

  tol.val=0.0001
  for (i in 1:N) {
    if (abs(res2[i])<tol.val) res2[i]=tol.val
    if (hv.true2[i]>(1-tol.val)) hv.true2[i]=(1-tol.val)}
}
y_d2<-(res2^2)/(1-hv.true2)                      #Response for variance model
var.w2 <- (1-hv.true2)/2                        #Weights for variance model
var.model2 <- asreml(y_d2~o2a+o2r+wt+temp,
```

```
                    weights=var.w2, family=asreml.Gamma(link=log), control =
 asreml.control(maxiter=100))
mean.w2 <- 1/fitted(var.model2)


##Model 3

mean.w3 <- c(rep(1,N))
res.var3 = 0
conv.crit = 0.00001
max.iter = 20
i.iter = 0
while (i.iter<max.iter & abs(res.var3-1)>conv.crit)
{stop
  mean.model3 <- asreml(sr~ o2a + o2r + wt+ obs + temp + o2a:obs + o2r:obs + wt:obs
                        ,random = ~ id
                        ,rcov = ~ id:exp(obs)
                        , weights=mean.w3
                        , calc.like=TRUE
                        , control = asreml.control(maxiter=100))
  asreml.hv3 <- mean.model3$hat
  res.var3 <- mean.model3$sigma2   #Residual variance
  res3<-resid(mean.model3, type = "response")
  hv.true3 <- asreml.hv3*mean.w3/res.var3

 # These are checks for extreme cases

  tol.val=0.0001
  for (i in 1:N) {
    if (abs(res3[i])<tol.val) res3[i]=tol.val
    if (hv.true3[i]>(1-tol.val)) hv.true3[i]=(1-tol.val)}
}
y_d3<-(res3^2)/(1-hv.true3)                      #Response for variance model
var.w3 <- (1-hv.true3)/2                        #Weights for variance model
var.model3 <- asreml(y_d3~o2a+o2r+wt+temp,
                     weights=var.w3, family=asreml.Gamma(link=log), control =
 asreml.control(maxiter=100))
mean.w3 <- 1/fitted(var.model3)

ap<- asreml(y_d3~o2a+o2r+wt+temp+sr,
            weights=var.w3, family=asreml.Gamma(link=log), control =
 asreml.control(maxiter=100))


##Check for convergence: yes
mean.model$conv
mean.model2$conv
mean.model3$conv
var.model$conv
var.model2$conv
var.model3$conv

##COMPARE MODELS
info.crit.asreml(mod1)
info.crit.asreml(mean.model)
info.crit.asreml(mean.model2)
info.crit.asreml(mean.model3) ##Smaller AIC, better convergence, and smaller
 LogLik, Use model 3

## Summary and Wald-F

anova(mean.model3)
anova(var.model3)
summary(var.model3)$varcomp
```

```
coef(mean.model3)$fixed
summary(mean.model3, nice= TRUE)

wald(mean.model3,all=T, denDF = "numeric", ssType= "conditional")
wald(var.model3,all=T, denDF = "numeric", ssType= "conditional")

##Find the z-ratio (solution is the effect of the variable)
print(summary(mean.model3, all=T)$coef.fi)
print(summary(var.model3, all=T)$coef.fi)

## wald-f
print(wald(mean.model3, print.ranef = TRUE))
print(wald(var.model3, print.ranef = TRUE))

## Check for effect

coef(mean.model3)$fixed ##for fixed effects
coef(var.model3)$fixed  ##for fixed effects

##Done!
```

Supplemental material – S2

R codes for the repeatability in startle response duration

```
setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 3")

cog=read.csv("accuracy_total.csv", header=TRUE)
names(cog)

library(rptR)

id<- as.factor(cog$ID)                          # Individual number
shell.1<- as.factor(cog$Shell.1)                # Percentage shell weight
 (occupied)
sr<- as.numeric(cog$SR)                         # Startle response duration


##Total repeatability

rpt(sr, ID, datatype = "Gaussian", method = "REML")

#Repeatability by shell weight

cog$seventyfive <- ifelse(cog$sr=="seventyfive",cog$shell.1,NA )
cog$eighty <-ifelse(cog$sr=="eighty", cog$shell.1,NA )

rpt(seventyfive, ID, datatype = "Gaussian", method = "REML")
rpt(eighty, ID, datatype = "Gaussian", method = "REML")
```

R codes for the hierarchical generalized linear model of startle response duration

```
rm(list=ls(all=TRUE))
setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 3")
```

```
cog=read.csv("accuracy_total.csv", header=TRUE)
names(cog)

library(asreml)
library(asremlPlus)
library("dae")
library("car")
library(latticeExtra)



id<- as.factor(cog$ID)                          # Individual number
wt<- as.numeric(cog$wt)                         # Mass
shell.1<- as.factor(cog$Shell.1)                # Percentage shell weight
 (occupied)
obs<- as.factor(cog$Occasion)                   # Observation number
sr<- as.numeric(cog$SR)                         # Startle response duration
shell.2<- as.factor(cog$Shell.2)                # Percentage shell weight
 (assessed)
bs<-as.factor(cog$bs)                           # If the shell assessed has a
 better quality
chg<-as.factor(cog$chg)                         # If the shell was changed
speed<-as.numeric(cog$speed)                    # Decision-making time
MRcog<-as.numeric(cog$MRcog)                    # Metabolic rate during shell
 assessment
RMR<-as.numeric(cog$Quality)                    # Routine metabolic rate
Quality<-as.numeric(cog$RMR)                    # Potential change in shell
 quality
accuracy<-as.numeric(cog$accuracy)              # Accuracy in decision making

# Startle response duration

Startle<- asreml(sr ~  obs + wt + shell.1 + RMR
                     , random= ~ id
                     , rcov = ~ units
                     , var=T,init=1
                     , data= cog
                     , na.method.X="include"
                     , na.method.Y="include")

 ## Check for convergence: yes
 Startle$conv


###Summary and Wald-F
anova(Startle)
summary(Startle)
print(summary(Startle, all=T)$coef.fi)
wald.asreml(Startle, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of accuracy in decision-making

```
setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 3")

cog=read.csv("accuracy_total.csv", header=TRUE)
names(cog)

library(asreml)
library(asremlPlus)
```

```
library("dae")
library("car")
library(latticeExtra)



id<- as.factor(cog$ID)                          # Individual number
wt<- as.numeric(cog$wt)                         # Mass
shell.1<- as.factor(cog$Shell.1)                # Percentage shell weight
 (occupied)
obs<- as.factor(cog$Occasion)                   # Observation number
sr<- as.numeric(cog$SR)                         # Startle response duration
shell.2<- as.factor(cog$Shell.2)                # Percentage shell weight
 (assessed)
bs<-as.factor(cog$bs)                           # If the shell assessed has a
 better quality
chg<-as.factor(cog$chg)                         # If the shell was changed
speed<-as.numeric(cog$speed)                    # Decision-making time
MRcog<-as.numeric(cog$MRcog)                    # Metabolic rate during shell
 assessment
RMR<-as.numeric(cog$RMR)                        # Routine metabolic rate
accuracy<-as.numeric(cog$accuracy)              # Accuracy in decision making
Quality<-as.numeric(cog$RMR)                    # Potential change in shell
 quality

# ASREml does not accept binomial distribution, so I need to trick it. It can be
 done by transforming the binome into two categories and specifying the family

cog$Yes <- ifelse(cog$Final.with.a.better.shell =="Yes",cog$accuracy,NA )
cog$No <-ifelse(cog$Final.with.a.better.shell =="No",cog$accuracy,NA )

Acy<- asreml(cbind(yes,no) ~ wt + sr + Quality*speed + Quality + speed
                 , random= ~ id
                 , rcov = ~ units
                 , family = asreml.binomial(link = "logit")
                 , var=T,init=1
                 , data= cog
                 , na.method.X="include"
                 , na.method.Y="include")

 ## Check for convergence: yes
 Acy$conv

###Summary and Wald-F
anova(Acy)
summary(Acy)
print(summary(Acy, all=T)$coef.fi)
wald.asreml(Acy, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of decision-making time

```
rm(list = ls(all = TRUE)) ###Need to erase the configuration of the past model

setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 3")

cog=read.csv("accuracy_total.csv", header=TRUE)
names(cog)

library(asreml)
```

```
library(asremlPlus)
library("dae")
library("car")
library(latticeExtra)



id<- as.factor(cog$ID)                          # Individual number
wt<- as.numeric(cog$wt)                         # Mass
shell.1<- as.factor(cog$Shell.1)                # Percentage shell weight
 (occupied)
obs<- as.factor(cog$Occasion)                   # Observation number
sr<- as.numeric(cog$SR)                         # Startle response duration
shell.2<- as.factor(cog$Shell.2)                # Percentage shell weight
 (assessed)
bs<-as.factor(cog$bs)                           # If the shell assessed has a
 better quality
chg<-as.factor(cog$chg)                         # If the shell was changed
speed<-as.numeric(cog$speed)                    # Decision-making time
MRcog<-as.numeric(cog$MRcog)                    # Metabolic rate during shell
 assessment
RMR<-as.numeric(cog$RMR)                        # Routine metabolic rate
accuracy<-as.numeric(cog$accuracy)              # Accuracy in decision making
Quality<-as.numeric(cog$RMR)                    # Potential change in shell
 quality


SP<- asreml(speed ~ wt + sr + Quality*accuracy + Quality + accuracy
                  , random= ~ id
                  , rcov = ~ units
                  , var=T,init=1
                  , data= cog
                  , na.method.X="include"
                  , na.method.Y="include")

 ## Check for convergence: yes
SP$conv

###Summary and Wald-F
anova(SP)
summary(SP)
print(summary(SP, all=T)$coef.fi)
wald.asreml(SP, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of the probability of changing shells

```
# ASREml does not accept binomial distribution, so I need to trick it. It can be
 done by transforming the binome into two categories and specifying the family

cog$Yes <- ifelse(cog$Final.with.a.better.shell =="Yes",cog$chg,NA )
cog$No <-ifelse(cog$Final.with.a.better.shell =="No",cog$chg,NA )

changing<- asreml(cbind(yes,no) ~ wt + sr + speed + accuracy
                  , random= ~ id
                  , rcov = ~ units
                  , family = asreml.binomial(link = "logit")
                  , var=T,init=1
```

```
                  , data= cog
                  , na.method.X="include"
                  , na.method.Y="include")

 ## Check for convergence: yes
changing$conv

###Summary and Wald-F
anova(changing)
summary(changing)
print(summary(changing, all=T)$coef.fi)
wald.asreml(changing, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of routine metabolic rate

```
rm(list = ls(all = TRUE)) ###Need to erase the configuration of the past model

setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 3")

cog=read.csv("accuracy_total.csv", header=TRUE)
names(cog)

library(asreml)
library(asremlPlus)
library("dae")
library("car")
library(latticeExtra)


id<- as.factor(cog$ID)                          # Individual number
wt<- as.numeric(cog$wt)                         # Mass
shell.1<- as.factor(cog$Shell.1)                # Percentage shell weight
 (occupied)
obs<- as.factor(cog$Occasion)                   # Observation number
sr<- as.numeric(cog$SR)                         # Startle response duration
shell.2<- as.factor(cog$Shell.2)                # Percentage shell weight
 (assessed)
bs<-as.factor(cog$bs)                           # If the shell assessed has a
 better quality
chg<-as.factor(cog$chg)                         # If the shell was changed
speed<-as.numeric(cog$speed)                    # Decision-making time
MRcog<-as.numeric(cog$MRcog)                    # Metabolic rate during shell
 assessment
RMR<-as.numeric(cog$RMR)                        # Routine metabolic rate
accuracy<-as.numeric(cog$accuracy)              # Accuracy in decision making
Quality<-as.numeric(cog$RMR)                    # Potential change in shell
 quality


Routine <- asreml(RMR ~ Quality + accuracy + speed + Quality*accuracy +
 speed*accuracy + Quality*speed + Quality*accuracy* speed
                  , random= ~ id
                  , rcov = ~ units
                  , var=T,init=1
                  , data= cog
                  , na.method.X="include"
                  , na.method.Y="include")
```

```
## Check for convergence: yes
Routine$conv

###Summary and Wald-F
anova(Routine)
summary(Routine)
print(summary(Routine, all=T)$coef.fi)
wald.asreml(Routine, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of metabolic rate during decision-making

```
cogmr <- asreml(MRcog ~ Quality + accuracy + speed + Quality*accuracy +
 speed*accuracy + Quality*speed + Quality*accuracy* speed
                 , random= ~ id
                 , rcov = ~ units
                 , var=T,init=1
                 , data= cog
                 , na.method.X="include"
                 , na.method.Y="include")


## Check for convergence: yes
cogmr$conv

###Summary and Wald-F
anova(cogmr)
summary(cogmr)
print(summary(cogmr, all=T)$coef.fi)
wald.asreml(cogmr, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of change in metabolic rate (routine MR – MR during decision-making)

```
dif= (MRcog - RMR) ## Creating the difference in metabolic rate

DifMR <- asreml(dif ~ Quality + accuracy + speed + Quality*accuracy +
 speed*accuracy + Quality*speed + Quality*accuracy* speed
                 , random= ~ id
                 , rcov = ~ units
                 , var=T,init=1
                 , data= cog
                 , na.method.X="include"
                 , na.method.Y="include")


## Check for convergence: yes
DifMR$conv

###Summary and Wald-F
anova(DifMR)
summary(DifMR)
```

```
print(summary(DifMR, all=T)$coef.fi)
wald.asreml(DifMR, ssType="conditional", denDF="numeric")
```

Supplemental material – S3

R codes for the repeatability in startle response duration and spontaneous alternation

```
library(rptR)
setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 4")

Spalt=read.csv("Spontaneous alternation.csv", header=TRUE)


ID<-as.numeric(Spalt$ID)                    # Individual number
SR<-as.numeric(Spalt$SR)                    # Startle response duration
S.A<-as.numeric(Spalt$S.A)                  # Number of total alternations


## Transforming the data
LogSR=log10(SR+2)                  # Log startle response
Spalt$LogSR=LogSR                  # Incorporating Log of startle response into
 the sata set
LogS.A= log10(S.A + 1.5)           # Log of spontaneous total alternation
Spalt$LogS.A=LogS.A                # Incorporating Log of total alternation


#Total repeatability

rpt(LogSR, ID, datatype = "Gaussian", method = "REML")
rpt(LogS.A, ID, datatype = "Gaussian", method = "REML")


#Startle response duration repeatability by shell weight

SH=paste(Spalt$SH,sep="_")
Spalt$SH<- c(SH)
SH
hundred <- ifelse(Spalt$SH =="hundred",Spalt$LogSR,NA )
Spalt$hundred = hundred

fifty = ifelse(Spalt$SH =="fifty",Spalt$LogSR,NA )
Spalt$fifty = fifty

rpt(hundred, ID, datatype = "Gaussian", method = "REML")
rpt(fifty, ID, datatype = "Gaussian", method = "REML")

#Spontaneous alternation repeatability by shell weight

rm(list = ls(all = TRUE)) ###Need to erase the configuration of the past model

library(rptR)
setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 4")

Spalt=read.csv("Spontaneous alternation.csv", header=TRUE)
```

```
ID<-as.numeric(Spalt$ID)                    # Individual number
SR<-as.numeric(Spalt$SR)                    # Startle response duration
S.A<-as.numeric(Spalt$S.A)                  # Number of total alternations


## Transforming the data
LogSR=log10(SR+2)                  # Log startle response
Spalt$LogSR=LogSR                  # Incorporating Log of startle response into
 the sata set
LogS.A= log10(S.A + 1.5)           # Log of spontaneous total alternation
Spalt$LogS.A=LogS.A                # Incorporating Log of total alternation


SH=paste(Spalt$SH,sep="_")
Spalt$SH<- c(SH)

hundred <- ifelse(Spalt$SH =="hundred",Spalt$LogS.A,NA )
Spalt$hundred = hundred

fifty = ifelse(Spalt$SH =="fifty",Spalt$LogS.A,NA )
Spalt$fifty = fifty

rpt(hundred, ID, datatype = "Gaussian", method = "REML")
rpt(fifty, ID, datatype = "Gaussian", method = "REML")
```

R codes for the hierarchical generalized linear model of startle response duration

```
rm(list=ls(all=TRUE))

library(asreml)
library(asremlPlus)
library("dae")
library("car")
library(nadiv)

setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 4")

Spalt=read.csv("Spontaneous alternation.csv", header=TRUE)


ID<-as.numeric(Spalt$ID)                    # Individual number
SR<-as.numeric(Spalt$SR)                    # Startle response duration
Obs <- as.numeric(Spalt$Obs)                # Observation number
block = as.factor (Spalt$block)             # Experimental block
S.A<-as.numeric(Spalt$S.A)                  # Number of total alternations
WT<-as.numeric(Spalt$WT)                    # Mass
SH = as.numeric(Spalt$Shell.W)              # Shell weight
RMR = as.numeric (Spalt$RMR)                # Routine metabolic rate

LogSR=log10(SR+2)                  # Log startle response
Spalt$LogSR=LogSR                  # Incorporating Log of startle response
LogS.A= log10(S.A + 1.5)           # Log of spontaneous total alternation
Spalt$LogS.A=LogS.A                # Incorporating Log of total alternation
LogRMR= log10(S.A + 1.5)           # Log of routine metabolic rate
Spalt$LogRMR=LogRMR                # Incorporating Log of routine metabolic rate
```

```
startle<- asreml(LogSR) ~ MR + WT + SH + Obs + LogRMR
            , random=~ units:us(trait):ID + block
            , rcov=~units:us(trait)
            , na.method.X="include"
            , na.method.Y="include"
            , data = Spalt
            , maxiter=20000000)

## Check for convergence: yes
startle$conv

###Summary and Wald-F
anova(startle)
summary(startle)
print(summary(startle, all=T)$coef.fi)
wald.asreml(startle, ssType="conditional", denDF="numeric")
```

R codes for the hierarchical generalized linear model of spontaneous alternation

```
spont<- asreml(LogS.A) ~ MR + WT + SH + Obs + LogRMR
            , random=~ units:us(trait):ID + block
            , rcov=~units:us(trait)
            , na.method.X="include"
            , na.method.Y="include"
            , data = Spalt
            , maxiter=20000000)

## Check for convergence: yes
spont$conv

###Summary and Wald-F
anova(spont)
summary(spont)
print(summary(spont, all=T)$coef.fi)
wald.asreml(spont, ssType="conditional", denDF="numeric")
```

R codes for the multivariate model

```
###
m1<- asreml(cbind(LogS.A,LogSR) ~ (MR + WT + SH + Obs + block):trait
            , random=~ units:us(trait):ID
            , rcov=~units:us(trait)
            , na.method.X="include"
            , na.method.Y="include"
            , data = Spalt
            , maxiter=20000000)

summary(m1)
wald(m1)
print(summary(m1, all=T)$coef.fi)

m2<- asreml(cbind(LogS.A,LogSR) ~ (MR + WT + SH + Obs + block):trait
             ,random=~diag(trait):ID
```

```
                ,rcov=~units:diag(trait):units
                , na.method.X="include"
                , na.method.Y="include"
                , data = Spalt
                , maxiter=2000000)

summary(m2)

###Constrained model: zero correlation between logSR and Log S.A
##In ASReml-R, you can do this by specifying the covariance matrix as a diagonal
 matrix (i.e. diag instead of us)

m3<- asreml(cbind(LogS.A,LogSR) ~ (MR + WT + SH + Obs + block):trait
            ,random=~diag(trait):ID + Part
            ,rcov=~units:diag(trait):units
            , na.method.X="include"
            , na.method.Y="include"
            , data = Spalt
            , maxiter=2000000)

## Check for convergence: yes
m1$conv
m2$conv
m2$conv


## Check for LogLik

summary(m2)
summary(m3)
summary(m1) --> better model!

#### LRT test

2*(m1$loglik-m2$loglik)

#calculate the associated significance
1-pchisq(2*(m1$loglik-m2$loglik),1)

2*(m1$loglik-m3$loglik)

#calculate the associated significance
1-pchisq(2*(m1$loglik-m3$loglik),1)



###Summary and Wald-F
anova(m1)
summary(m1)
print(summary(m1, all=T)$coef.fi)
wald.asreml(m1, ssType="conditional", denDF="numeric")
```

Supplemental material - S4

R codes for the average effect of light on startle response

```
rm(list=ls(all=TRUE))

library(MCMCglmm)

setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 2")

lightdark=read.csv("Experiment_2_1_treatment and light.csv", header=TRUE)


ID = as.numeric(lightdark$ID)                                  # Individual number
Srtime = as.factor(lightdark$Srtime)                           # Time on which the
 startle response was estimated (e.g. AM or PM)
Treatment = (lightdark$Period)                                 # Whether individuals
 were in LL or LD treatment
Period= (lightdark$Torder)                                     # Period the experiment
treatment_group = (lightdark$treatment_group)                  # Combination of treatment
 and time of the startle response
Obs = (lightdark$Occasion)                                     # Observation number
wt = (lightdark$wt)                                            # Mass
SH = (lightdark$shell.weight)                                  # Shell weight
hacyn = (lightdark$hacyn)                                      # Haemocyanin
 concentration
SR= (lightdark$sr)                                             # Startle response
 duration
Logsr=log10(SR+1)                                              # Log startle response


##Flats non-informative prior

prior.1<-list(R=list(V=1, n=0.002),G=list(G1=list(V=1, n=0.002)))
prior.2 list(R=list(V=1, n=1.002),G=list(G1=list(V=1, n=1.002)))

M1 <- MCMCglmm (logsr ~ 1 + Period + hacyn + Obs + Srtime + Treatment +
 Treatment*Srtime Treatment*Period
                , random=~ idh(ID):units
                , rcov=~idh(Occasion):units
                , prior= prior.1
                , family="gaussian"
                , pl=TRUE
                , pr= TRUE
                , data=lightdark
                , DIC= T
                , singular.ok = TRUE
                , nitt=1900000, thin=1000, burnin=90000,verbose=T)

M2 <- MCMCglmm (logsr ~ 1 + Period + hacyn + Obs + Srtime + Treatment +
 Treatment*Srtime Treatment*Period
                , random=~ idh(ID):units
                , rcov=~idh(Occasion):units
                , prior= prior.2
                , family="gaussian"
                , pl=TRUE
                , pr= TRUE
```

```
                , data=lightdark
                , DIC= T
                , singular.ok = TRUE
                , nitt=1900000, thin=1000, burnin=90000, verbose=T)

##Check DIC

HPDinterval(as.mcmc(M1))
HPDinterval(as.mcmc(M2)) ## Use this model


# autocorrelation diagnostics, should be < 0.1 for proper convergence
diag(autocorr(M1$Sol)[2, ]);diag(autocorr(M1$VCV)[2, ])
```

R codes for the repeatability, between and within-individual variation in behaviour in response to permanent light

```
rm(list=ls(all=TRUE))

library(MCMCglmm)

setwd("~/Documents/Pesquisa/Doutorado/PhD Analysis/Experiment 2")

lightdark=read.csv("Experiment_2_1_treatment and light.csv", header=TRUE)


ID = as.numeric(lightdark$ID)                                  # Individual number
Srtime = as.factor(lightdark$Srtime)                           # Time on which the
 startle response was estimated (e.g. AM or PM)
Treatment = (lightdark$Period)                                 # Whether individuals
 were in LL or LD treatment
Period= (lightdark$Torder)                                     # Period the experiment
treatment_group = (lightdark$treatment_group)                  # Combination of treatment
 and time of the startle response
Obs = (lightdark$Occasion)                                     # Observation number
wt = (lightdark$wt)                                            # Mass
SH = (lightdark$shell.weight)                                  # Shell weight
hacyn = (lightdark$hacyn)                                      # Haemocyanin
 concentration
SR= (lightdark$sr)                                             # Startle response
 duration
Logsr=log10(SR+1)                                              # Log startle response

# Specifying that the variance will be divided in four groups (the interaction
 between time on which the startle response was estimate and the treatment)

Group_Period=paste(lightdark$treatment_group,sep="_")
lightdark$Group_Period<- c(Group_Period)

## Inverse-Wishart prior

prior.IW<- list(R=list(V=diag(4), nu= 3.002),
                      G=list(G1=list(V=diag(4), nu=3.002)))

## Flat-non informative prior

prior.1<- list(R=list(V=diag(4), nu=1.002),
               G=list(G1=list(V=diag(4), nu=1.002)))##fits better the data set
```

```
# This prior estimates a 4x4 covariance matrix for the random effects
# with the variances of each subgroup on the diagonal
# Below is the matrix specified for the among-individual variance
matrix(c("VID_AM_LD",0,0,0,
         0,"VID_PM_LD",0,0,
         0,0,"VID_AM_LL",0,
         0,0,0,"VID_PM_LL"),nrow=4,ncol=4,byrow=T)

# Model with Inverse-Wishart

m1.IW <- MCMCglmm(logsr ~ 1 + Period + hacyn + Obs + Srtime + Treatment +
 Treatment*Srtime Treatment*Period
                                  , random=~ idh(Group_Period):ID
                                  , rcov=~idh(Group_Period):units
                                  , prior= prior.IW
                                  , family="gaussian"
                                  , data=lightdark
                                  , singular.ok=TRUE
                                  , nitt=1900000, thin=1000,
 burnin=90000,verbose=T)


# Flat-non informative prior

m1.p1 <- MCMCglmm(logsr ~ 1 + Period + hacyn + Obs + Srtime + Treatment +
 Treatment*Srtime Treatment*Period
                                  , random=~ idh(Group_Period):ID
                                  , rcov=~idh(Group_Period):units
                                  , prior= prior.1
                                  , family="gaussian"
                                  , data=lightdark
                                  , singular.ok=TRUE
                                  , nitt=1900000, thin=1000,
 burnin=90000,verbose=T)
##Check DIC

HPDinterval(as.mcmc(m1.p1))
HPDinterval(as.mcmc(m1.IW)) ## Use this model



plot(m1.IW$Sol)
plot(m1.IW$VCV)

summary(m1.IW)

# autocorrelation diagnostics, should be < 0.1 for proper convergence
diag(autocorr(m1.IW$Sol)[2, ]);diag(autocorr(m1.IW$VCV)[2, ])



# extract variance components
posterior.mode(m1.IW$VCV);HPDinterval(m1.IW$VCV)



# double check that these correspond to the proper variance components for your
 model!
VID_AM_LD=m1.IW$VCV[,1];VR_AM_LD=m1.IW$VCV[,5]
VID_PM_LD=m1.IW$VCV[,2];VR_PM_LD=m1.IW$VCV[,6]
VID_AM_LL=m1.IW$VCV[,3];VR_AM_LL=m1.IW$VCV[,7]
VID_PM_LL=m1.IW$VCV[,4];VR_PM_LL=m1.IW$VCV[,8]
```

```
#in tis data AM_LD woudl mean field-lab treatment group in period A
# your equivalent woudl be something like LL treatment group in the am (e.g. AM_LL,
 LL_PM, LD_AM, LD_PM)

# Calculate Repeatability per group and phase
R_AM_LD=m1.IW$VCV[,1]/(m1.IW$VCV[,1]+m1.IW$VCV[,5])
R_PM_LD=m1.IW$VCV[,2]/(m1.IW$VCV[,2]+m1.IW$VCV[,6])
R_AM_LL=m1.IW$VCV[,3]/(m1.IW$VCV[,3]+m1.IW$VCV[,7])
R_PM_LL=m1.IW$VCV[,4]/(m1.IW$VCV[,4]+m1.IW$VCV[,8])
posterior.mode(R_AM_LD);HPDinterval(R_AM_LD) #these are the posterior mode
 repeatability estimates with 95% CIs
posterior.mode(R_AM_LL);HPDinterval(R_AM_LL) #specific for each block of the
 experiment
posterior.mode(R_PM_LD);HPDinterval(R_PM_LD)
posterior.mode(R_PM_LL);HPDinterval(R_PM_LL)

# compare repeatability across blocks
#deltaR with 95% CIs for the delta. If these don't cross zero the diff in R is
 'significant'

#Lab to lab group (phase 2 - phase 1) this is the equivalent of LL morning versus
 LL night

deltaR_LL = (R_PM_LL-R_AM_LL)
posterior.mode(deltaR_LL);HPDinterval(deltaR_LL)

#Lab to field group (phase 2-phase 1) this is the equivalent of LD morning versus
 LD night

deltaR_LD = (R_PM_LD-R_AM_LD)
posterior.mode(deltaR_LD);HPDinterval(deltaR_LD)

# Phase 1 (lab to field - lab to lab) this is the equivalent of LL versus LDduring
 day measures
deltaR_AM = (R_AM_LL-R_AM_LD)
posterior.mode(deltaR_AM);HPDinterval(deltaR_AM)

# Phase 2 (lab to field - lab to lab) this is the equivalent of LL versus
 LD  during night
deltaR_PM = (R_PM_LL-R_PM_LD)
posterior.mode(deltaR_PM);HPDinterval(deltaR_PM)


#Compare among individual variation - basically the same for the above but doing it
 for G structure (V-BI)
#deltaVID

posterior.mode(VID_AM_LD);HPDinterval(VID_AM_LD) #these are the posterior mode
 repeatability estimates with 95% CIs
posterior.mode(VID_AM_LL);HPDinterval(VID_AM_LL) #specific for each block of the
 experiment
posterior.mode(VID_PM_LD);HPDinterval(VID_PM_LD)
posterior.mode(VID_PM_LL);HPDinterval(VID_PM_LL)


deltaVID_LL = VID_AM_LL-VID_PM_LL          ####Among individual variation during LL
 treatment morning versus night
posterior.mode(deltaVID_LL);HPDinterval(deltaVID_LL)

deltaVID_LD = VID_PM_LD-VID_AM_LD         ####Among individual variation during LD
 treatment morning versus night
posterior.mode(deltaVID_LD);HPDinterval(deltaVID_LD)
```

```
deltaVID_AM = VID_AM_LL-VID_AM_LD       ####Among individual variation morning LL
 treatment versus LD treatment
posterior.mode(deltaVID_AM);HPDinterval(deltaVID_AM)

deltaVID_PM = VID_PM_LL-VID_PM_LD        ####Among individual variation night LL
 treatment versus LD treatment
posterior.mode(deltaVID_PM);HPDinterval(deltaVID_PM)

#Compare within-individual variation - basically the same for the above but doing
 it for R structure (V-WI)
#deltaVR

posterior.mode(VR_AM_LD);HPDinterval(VR_AM_LD)
posterior.mode(VR_AM_LL);HPDinterval(VR_AM_LL)
posterior.mode(VR_PM_LD);HPDinterval(VR_PM_LD)
posterior.mode(VR_PM_LL);HPDinterval(VR_PM_LL)



deltaVR_LL = VR_PM_LL-VR_AM_LL        #### Within-individual variation during LL
 treatment morning versus night
posterior.mode(deltaVR_LL);HPDinterval(deltaVR_LL)

deltaVR_FL = VR_PM_LD-VR_AM_LD        #### Within-individual variation during LD
 treatment morning versus night
posterior.mode(deltaVR_FL);HPDinterval(deltaVR_FL)

deltaVR_P1 = VID_AM_LL-VID_AM_LD        #### Within-individual variation morning LL
 treatment versus LD treatment
posterior.mode(deltaVR_P1);HPDinterval(deltaVR_P1)

deltaVR_P2 = VR_PM_LL-VR_PM_LD        #### Within-individual variation night LL
 treatment versus LD treatment
posterior.mode(deltaVR_P2);HPDinterval(deltaVR_P2)
```

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The opposite effects of routine metabolic rate and metabolic rate during startle responses on variation in the predictability of behaviour in hermit crabs

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Abstract
Studies on animal behaviour have suggested a link between personality and energy expenditure. However, most models assume constant variation within individuals, even though individuals vary between observations. Such variation is called intraindividual variation in behaviour (IIV). We investigate if IIV in the duration of the startle response is associated with metabolic rates (MR) in the hermit crab *Pagurus bernhardus*. We repeatedly measured startle response durations and MR during each observation. We used double hierarchical generalized linear models to ask whether among and IIV in behaviour was underpinned by MR. We found no association between the mean duration of the startle responses and either routine MR or MR during startle response. Nevertheless, we found that IIV increased with MR during startle responses and decreased with routine MR. These results indicate that crabs with higher MR during startle responses behave less predictably, and that predictability is reduced during exposure to elevated temperatures.

Keywords
pace-of-life syndrome, intraindividual variation, personality, predictability, life-history strategies.

1. Introduction

In many species individuals differ in their behaviour consistently over time and these differences are often maintained across situations (Gosling & John, 1999; Sih et al., 2004; Réale et al., 2007; Briffa & Greenaway, 2011). Some individuals, for example, are consistently bolder (e.g., Brown & Braithwaite, 2004; Magnhagen & Borcherding, 2008; Bridger et al., 2015), more aggressive (Bell, 2007; Sih & Bell, 2008) or more cooperative (Bergmüller &

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Taborsky, 2007; Charmantier et al., 2007; Schürch & Heg, 2010) than other individuals from the same population. Such consistent among-individual behavioural variation is termed 'animal personality' (Gosling, 2001; Drent et al., 2003; Dingemanse & Reale, 2005; Reale et al., 2007) and it seems to be ubiquitous in nature, ranging from humans to invertebrates (Gosling & John, 1999; Sih et al., 2004; Reale et al., 2007; Briffa & Greenaway, 2011).

Several methods have been used to estimate such behavioural consistency, but the estimation of the 'broad sense' (as defined by Biro & Stamps, 2015) repeatability is the most widely used measure of repeatability (Hayes & Jenkins, 1997; Bell et al., 2009; Biro & Stamps, 2015). In general, repeatability values tend to be low to moderate (0.37–0.42; Bell et al., 2009). Thus, most variation in behaviour is unaccounted for as consistent inter-individual differences. One possibility for this is that unaccounted for environmental factors may contribute to variation in behavioural traits, so as to 'mask' the true amount of variation among individuals (e.g., Briffa & Greenaway, 2011). Another possibility is that significant amounts of variation occur within, rather than between individuals (Bell et al., 2009; Biro & Stamps, 2015). Since repeatability (R) is the proportion of total variance (variance between individuals (V_{BI}) + variance within individuals (V_{WI})) that is due to between individual differences (i.e., $R = V_{\mathrm{BI}}/(V_{\mathrm{BI}} + V_{\mathrm{WI}})$), overall estimates of repeatability do not provide direct information on the individual variance components. Thus, recent studies have focussed on understanding what factors may influence the within individual or 'intra-individual variance' component (IIV) (Nesselroade, 1991; Siegler, 1994; Salthouse, 2007; Ram & Gerstorf, 2009; Stamps et al., 2012; Briffa et al., 2013). This IIV is also sometimes referred to as predictability or individual consistency. In this case, individuals with a higher predictability will have lower IIV scores. Such studies have demonstrated that IIV is an important component of animal behaviour that seems to be related to learning (Maye et al., 2007; MacDonald et al., 2009; Bielak et al., 2010; Brembs, 2011), coping with risk (Briffa, 2013), male dominance and sexual selection (Cowlishaw, 1996; Rogers & Cato, 2002; Delgado, 2006).

In general, studies on animal personality have been revealing links between behavioural types (mean behaviour) and the pace of life syndrome (POLS; e.g., Smith & Blumstein, 2008; Bell et al., 2009; Reale at al., 2010; Garamszegi et al., 2012, 2013; Urszán et al., 2015b). The POLS hypothesis aims to explain variation in life-history strategies and physiological traits,

which are assumed to be expressed along a slow-fast continuum (Hille & Cooper, 2015). Individuals that follow a slow POLS are expected to be long-living, avoid risk, be less active and with a lower metabolic rate (MR). In contrast, those with a fast POLS are expected to be more active, take more risks, be more aggressive and have a higher MR (e.g., 'live fast and die young'; Biro & Stamps, 2008; Smith & Blumstein, 2008; Réale et al., 2010). Thus, variation in the pace of life might represent an underlying mechanism for animal personality variation (Careau & Garland, 2012), both in terms of among individual differences in mean level behaviour and in terms of among individual differences in behavioural consistency or IIV.

So far, studies investigating the link between behavioural traits and POLS have focussed on mean-level differences in behaviour, and taken together they indicate that the relationship between pace of life and animal personalities varies among study systems. In deer mice (*Perumyscus maniculatus*) Careau et, al. (2011) found a positive relationship between exploration and MR. Similarly, more exploratory individuals of the superb fairy-wren (*Malurus cyaneus*) were less likely to be found 12 months later, indicating that these individuals are less long-lived. In contrast, the brown trout, *Salmo trutta*, seems to have a negative relationship between activity levels and mortality, indicating that longevity is not necessarily reduced in active individuals (Adriaenssens & Johnsson, 2013). This lack of consensus between studies, may indicate the presence of an overlooked element. In fact, in recent meta-analysis Réale et al. (2010) found that two-thirds of the behavioural variation occurs within individuals rather than between individuals. Furthermore, recent studies focussing explicitly on the analysis of IIV, showing that predictability can consistently vary among individuals (i.e., IIV itself is repeatable; Biro & Adriaenssens, 2013), as well as at the mean level. Taken together, these results suggest that IIV might play a key role in both natural and sexual selection and thus, be linked with POLS (Urszán et al., 2015b).

It seems logical, then, that variation in metabolic rate might drive among-individual variation in behaviour both at the individual-mean level and at the level of among individual variation in IIV (Careau et al., 2008; Houston, 2010). Nevertheless, studies investigating relationships between metabolism and IIV are still rare (Mathot & Dingemanse, 2015). In the case of ectothermic marine invertebrates, metabolic rate is known to be driven by the temperature of the seawater. Thus, it is possible that individual consistency could be also dependent upon fluctuations in seawater temperature, if

MR influences IIV. In the European hermit crab, *Pagurus bernhardus*, there is some evidence that startle response durations (where crabs hide in their empty gastropod shell upon disturbance, see below) increase at higher temperatures, although this effect was modified by treatment order (Briffa et al., 2013). There was, however, a clearer effect at the level of IIV, with crabs at a higher temperature treatment behaving less consistently (i.e., showing greater IIV) than those subjected to lower temperature, across a 5°C temperature difference. Thus, individuals that presumably had higher metabolic rates (due to elevated temperature) showed greater within-individual variance in behaviour than those with lower metabolic rates.

One potential interpretation of this result is that individuals with high rates of metabolism have relatively high energy demands and therefore might be exposed to greater predation risk due to the need to service these energy demands through foraging (Briffa et al., 2013). Behaving less predictably might be a way to minimise these risks, and indeed *P. bernhardus* appear to behave less consistently in the presence of a predator (Briffa, 2013). Here, we directly investigate the links between metabolic rate and the duration of startle responses in the European hermit crab, while accounting for small fluctuations in temperature in the laboratory and for variation in crab mass. Hermit crabs occupy empty gastropod shells and a simple stimulus causes them to withdraw from their shells and slowly re-emerge. The latency to emerge from the shell ('startle response duration') is considered a measure of 'boldness' (Biro & Stamps, 2008; Briffa et al., 2013; Bridger et al., 2015) and its variation between and within-individuals is well studied in this species (Briffa, 2013; Briffa et al., 2013; Bridger et al., 2015). To avoid indirect associations between MR and the behavioural trait (Mathot & Dingemanse, 2015), we repeatedly measured the oxygen consumption in two situations: during routine behaviour (routine MR) and during the startle response period, where crabs are withdrawn into their shells (startled MR). We considered startled MR as the metabolic rate of a reactive individual during and after the application of a stimulus. Routine MR is defined as the metabolic rate of an undisturbed, post-absorptive, resting individual, it also includes some level of random activity, maintenance of equilibrium and posture (Jobling, 1994; Killen et al., 2011; Mathot & Dingemanse, 2015).

Here we investigate the links between metabolic rate and boldness analysed across two levels, individual mean boldness and within-individual variation in boldness (IIV). In addition to variation in metabolic rate we also

analyse the effects of small (uncontrolled) fluctuations in temperature within the laboratory, since these could influence both metabolism and oxygen availability. We predicted that the MR would have a negative correlation with the duration of the startle response at the mean level of behaviour and in IIV of behaviour. In other words, according to the POLS hypothesis, we should expect individuals with a high metabolic rate to recover quickly from a perturbation and hence show startle responses of relatively short duration. Furthermore, if shorter startle responses equate to greater risk exposure, we would expect individuals with higher metabolic rate to show relatively low levels of behavioural consistency, and hence high levels of IIV.

2. Materials and methods

2.1. Collection and maintenance of hermit crabs

We collected hermit crabs between November 2013 and January 2014 from the rocky intertidal at Hannafore Point, Cornwall, UK (50°20′N, 4°27′W), from where they were transported directly back to the laboratory at Plymouth. In the laboratory we removed crabs from their shells by cracking in a bench vice. This stage is necessary because the behaviour of hermit crabs, including the duration of the startle response, could be affected by the shell mass (Briffa & Bibost, 2009). All crabs thus received a new *Littorina littorea* shell with 100% of its preferred mass (Briffa & Elwood, 2007). We only used male crabs (mean mass $\pm$ SE $= 0.76 \pm 0.34$ g) free from obvious parasites, appendage damage or recent moult ($N = 40$). Crabs were individually housed in white plastic dishes of 16 cm of diameter, filled to 4 cm depth with seawater, with continuous aeration and feed daily ad libitum with cubes of white fish at the end of each observation (i.e., there was always excess food available in the housing dishes, outside of the observation periods). The seawater was from our laboratory supply, which is regularly obtained from the seaward side of Mount Batten pier (50°36′N, 4°13′W) in Plymouth Sound, at spring tides. They were acclimated for ten days in a temperature controlled room (mean temperature $\pm$ SE $= 12.21 \pm 1.05$°C), followed by a ten days of daily observation of startle response durations (see below).

2.2. Determination of routine and active metabolic rate

We determined the routine and startled metabolic rate for each crab using oxygen uptake as a proxy (Dupont-Prinet et al., 2010). This was measured daily using a sealed ‘Kilner’ jar (polyethylene terephthalate, PET),

blackened-out and a non-invasive optical oxygen sensor with a temperature probe (OxySense GEN III 5000 series, OxySense, Dallas, TX, USA). Each jar had a sensor spot attached on the inner wall with silicone glue. The sensor spot reacts with the oxygen present inside the jar and, when read by the sensor, shows the remaining concentration of O_2, allowing a non-invasive measurement (a closed respirometer). We closed the jar underwater using a rubber washer to ensure the absence of air bubbles. To prevent oxygen stratification and ensure moderate mixing of water, we placed the jar onto a multi-channel magnetic stirrer (MIX 15 eco; 2mag, Munich, Germany) with a magnetic flea inside. A mesh was placed between the hermit crab and the magnetic flea to ensure no contact. In order to control for possible bias from algal or bacterial activity in oxygen measurements, we only used filtered seawater. Additionally, oxygen measures can also be influenced by the jar material, as some types of plastic can absorb or release oxygen (Stevens, 1992) (although significant O_2 exchange is less likely with the high quality PET material that we used here, compared with other plastics). Therefore, to account for both microbial activity and potential oxygen exchange with the jar material, we measured the oxygen consumption in two extra jars containing the same seawater used in the experiments and one empty *L. littorea* shell ('blank') similar to those occupied by the crabs. We then accounted for any microbial activity and any the effect of the jar's material in the final calculation of metabolic rate (Calosi et al., 2013).

We allowed hermit crabs rest for 15 min before starting routine MR measures of oxygen consumption. To allow an accurate estimation of oxygen concentration inside the jar we obtained readings every 5 min, during 25 min (i.e., 5 measures of O_2 concentration in total per estimate). These measures were then used to estimate the rate of O_2 uptake by the hermit crab (see below for the calculation). Previous observations indicate a stabilization of oxygen consumption after 15 min of resting, and therefore, a minimization of stress imposed by the manipulation.

We then recorded the startled MR immediately after the routine MR. We obtained the startled MR measurements immediately after inducing a startle response (Briffa et al., 2008; Stamps et al., 2012). As previously stated, we considered startled MR as the metabolic rate of an individual during and after a stimulus, thus we measured startled MR and startle responses simultaneously. In the current study, where crabs are housed in sealed jars for the measurement of metabolic rate, it was not possible to induce startle responses

in the same way as previous studies by manually handling the crabs. This is because the manual handling procedure would entail opening the respirometry jar and then resealing it after the response had been induced. These procedures would lead to (a) a time delay in the onset of measurements of oxygen concentration and (b) the potential for additional disturbance to the crabs (e.g., unintentional movements of the jar during resealing) that would be difficult to quantify. Therefore, we used an alternative technique where the startle response was induced by carefully lifting the jar (avoiding early disturbance to the crab) by 30 cm and then releasing it so that it fell back onto the bench. This induced hermit crabs to withdraw into their gastropod shells in a similar way to the manual handling technique used in previous studies, with the vast majority of crabs falling into an aperture facing upwards position following the drop. Furthermore, the use of a physical impact on the jar is similar to an approach used in other studies of hermit crabs where an object has been dropped onto the top of an unsealed crystallising dish to induce startle responses (see Briffa & Elwood, 2001; Elwood & Briffa, 2001). Initial observations indicated that when aligned in a horizontal position and dropped from 30 cm, the jar will rarely spin or bounce when hits the bench, independent of the hermit crab position inside the jar. When it occurs, routine MR measures and the startle response induction were made after 2 h of rest.

We timed the latency of recovery from the point at which the jar is released to the point at which the perieopods first make contact with base of the jar. As the startle response varies among and within individual hermit crabs it was not possible to measure oxygen concentrations over a set time period as in the case for our estimate of routine MR. Instead we measured the oxygen concentration inside the jar at a higher time resolution of every 5 s. These measurements commenced at the point at which the hermit crab withdrew into its shell and continued until 5 min after its recovery. These repeated measures of oxygen concentration were then used to estimate the rate of oxygen uptake during and following the startle response (see below for calculation).

We obtained 10 startle response durations along with 10 measures of Routine MR and startled MR for each of 40 individuals yielding 400 observations in total. Measures made using closed respirometers are never constant due to the continuous use of oxygen by the organisms inside the jar. Thus, we used the decrease in oxygen concentration to calculate the O_2 consumption per

individual. In humid environments the oxygen consumption is dependent on the oxygen solubility, which in turn is dependent on both temperature and salinity. Although we conducted measures in temperature controlled rooms, small fluctuations still occurred, which could affect both behaviour and oxygen consumption rates. We calculate the O_2 consumption rate using the slope from a linear regression of oxygen consumption over time minus the blank variations. The slope was then multiplied by the oxygen solubility coefficient (adjusted for salinity and temperature). Thus, rate of O_2 consumption was calculated using

$$\text{Rate of } O_2 \text{ uptake } (\mu\text{mol } O_2/\text{h}) = C(t) \times (V_r) \times (60/t_1 - t_2),$$

where $C(t)$ is the O_2 consumption rate (from the linear regression of oxygen consumption over time), V_r is the total volume of water inside the jar (jar volume minus the hermit crab volume) and t_0, t_1, is the measurement period (in min; Widdows & Staff, 2006; Calosi, et al., 2013). To create a standardized measures and allow comparisons between individuals, we divided the rate of O_2 uptake by the individual body mass (without the shell; Porter & Brand, 1995).

2.3. Data analysis

Previously, mean level behaviour and IIV have been calculated using general linear-mixed effects model (GLMM) in a two-step analysis. First, a random regression model is fitted (Stamps et al., 2012; Briffa et al., 2013; Cleasby et al., 2015). With this model it is possible to obtain the expected values, followed by the residual individual standard deviation (riSD). Although widely used in human personality, and recently animal personality, research this method has some limitations (Bridger et al., 2015; Cleasby et al., 2015). GLMMs assume homoscedastic residual variation in behaviour (the same for all individuals), which is violated if individuals do indeed differ in IIV (Bridger et al., 2015; Cleasby et al., 2015). Secondly, a two-step analysis might inflate type 1 errors, by not carrying forward the uncertainty estimates from step 1.

More recently (e.g., Bridger et al., 2015) an alternative approach has been used, where the mean and the variance for each individual are modelled iteratively using hyperparameters. These models are extensions of GLMM called hierarchical GLMs (HGLM). HGLMs model sets of interlinked GLMs, allowing non-normally distributed parameters (Lee & Nelder, 1996). Additionally, such models allow the specification of separate models for the mean and

standard deviation model (SD model), both incorporating fixed and random effects. Such models are termed Double HGLM (DHGLM; Lee & Nelder, 2006). We used the software ASReml-R (Butler et al., 2009) in R version 3.2.1 (R Development Core Team, 2012) to fit a DHGLM model, as follows.

In the mean model we included observation number, mass, routine and startled metabolic rate as fixed effects. As we had small temperature variations, we also include temperature as a fixed effect (covariate) to account for this. We modelled a random intercept and a random slope effect to allow for among individual variation in responses to repeated observations (see the Appendix for detailed descriptions). We tested for correlation between all fixed effects and none were significant (see the Appendix for detailed descriptions). In the SD model we included mass, temperature, routine and startled metabolic rate as fixed effects, and random intercepts for each individual (random slopes are not possible in the SD model because we only obtained one set of repeated measures within each individual, allowing a single estimate of residual variance; see the Appendix for detailed descriptions). As the model is expected to be robust against outliers (Lee & Nelder, 2006), we opted to use non-transformed data. We used the Wald-F test to test the significance of fixed effects and Z-ratio for random.

2.4. Ethical note

No animals were harmed during the experiment and at the end of the experiment all individuals appeared healthy and were supplied with excess food (as above) and a new gastropod shell before being returned to the sea.

3. Results

The mean startle response duration did not vary with either routine ($\chi^2_1 = 0.41$, $p = 0.5227$) or startled ($\chi^2 = 0.06$, $p = 0.8140$) metabolic rate, and there was no significant effect of either mass ($\chi^2 = 0.24$, $p = 0.6227$) or temperature ($\chi^2 = 1.20$, $p = 0.27$; Table 1) on the startle response duration. There was, however, a clear pattern of reduction in startle response duration across successive observations ($\chi^2 = 18.89$, $p = 0.02617$, Table 1), indicating habituation.

In the standard deviation model, the analysis of the fixed effects indicates that IIV in startle response duration increases with temperature ($\chi^2 = 28.9$, $p < 0.001$) and with startled metabolic rate ($\chi^2 = 10.4$, $p = 0.00062$) but

Table 1.
The fixed effects and their statistical significance of the mean model and for standard deviation model of the duration of the startle response.

Sub-model	Parameter name	Effect	SE	df	Wald χ^2	p
Mean	Intercept	96.9998	113.269	1	11.3699	0.00055*
	Observation	−8.5687	3.363	9	18.8876	0.02617*
	Mass	−22.1446	135.238	1	0.2421	0.62271
	Temperature	12.1099	42.623	1	1.1993	0.27346
	Routine MR	−0.0155	0.475	1	0.4085	0.52272
	Startled MR	−0.5088	0.435	1	0.0553	0.81403
Variance	Intercept	7.142901	0.9231	1	19041.8	<0.0001*
	Mass	−1.19803	0.2335	1	25.1	<0.0001*
	Temperature	0.39201	0.0729	1	28.9	<0.00018
	Routine MR	−0.00511	0.0007	1	55.4	<0.0001*
	Startled MR	0.00229	0.0007	1	10.4	0.00062*

Mean effect sizes of factors and covariates with their effects, standard error, Wald's chi-square test and p-values. *Significant variable.

decreases with crab mass ($\chi^2 = 25.1$, $p < 0.001$) and with routine metabolic rate ($\chi^2 = 55.4$, $p < 0.001$; Table 1; Figure 1). In summary, crabs behave more consistently when exposed to higher average temperatures and if they have a high metabolic rate during the startle response. In contrast, crabs that were large or had a higher routine MR showed an increasing of consistency in startle response duration.

The random intercepts of the mean and SD model indicate the presence of significant within-individual variation, but not significant between-individual variation (Table 2). To provide a standardised measure of the proportion of variance due to between individual variation in behaviour we also calculated the repeatability (R_c; Biro & Stamps, 2015) of startle responses and both routine and startled MR. For each variable, we fitted a model on only a constant (intercept) and the observation as a fixed effect and the individual's ID as random effect (Wilson et al., 2010). The significance of repeatability was obtained using a likelihood ratio test (LRT) method, which compares the likelihoods of the linear mixed model described above (with the individual's ID as a random effect) and a general linear model without random effect (Nakagawa & Schielzeth, 2010), distributed as Chi square with one degree of freedom. The repeatability of the startle response was 0.013 ($\chi^2 = 28.33$, $p < 0.001$). Since it is not possible to directly generate confidence intervals

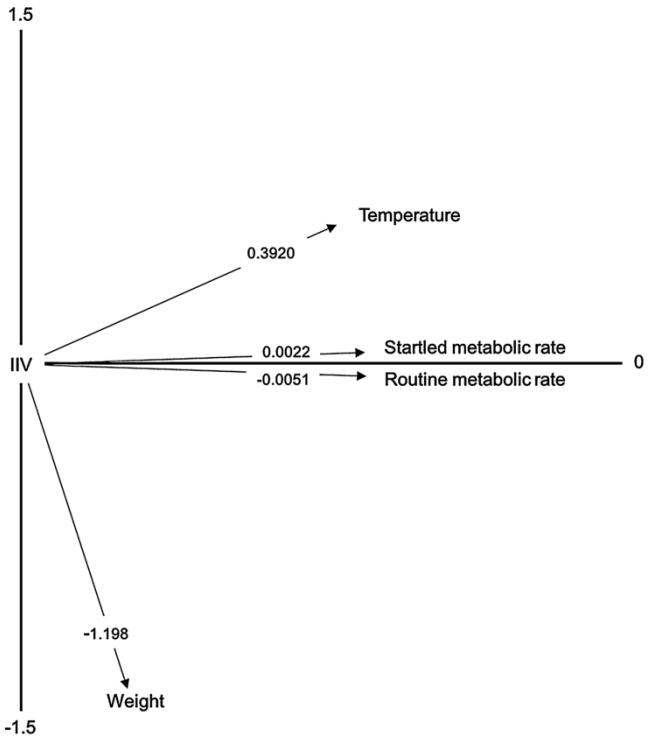

Figure 1. Representation of the effects of four parameters on IIV. Values were extracted from the standard deviation model on the DHGLM. The angle of the arrows represents the effect size of each parameter on IIV.

around this estimate of repeatability obtained from the variance components of our model, and to allow comparison with other studies where data on traits such as MR are absent, we also calculated an unadjusted (in relation to the model fixed effects) repeatability, based on a linear mixed model. Here the

Table 2.
Estimated variance components for behavioural traits. σ^2 is the variance of each component.

Component	σ^2	SE	Z-ratio
Between-individual	2122.309	3939.88	0.53868
Within-individual	43 583.42	5653.985	7.7084441*

Statistical significance is assessed by comparing variance to the Z-ratio; effects are considered to be statistically significant if $Z > 2$ (Wilson et al., 2010). *Significant variable.

repeatability was $R_{LMM} = 0.111 \pm SE = 0.042$ (95% CI = [0.034, 0.193], $p < 0.0001$). The repeatability of the routine MR was 0.00133 ($\chi^2 = 61.26$, $p < 0.001$) and startle MR was 0.00006 ($\chi^2 = 1.91$, $p = 0.166$).

4. Discussion

Previous studies highlighted the importance of investigating intra-individual variance in behaviour in addition to the mean level (Briffa, 2013; Brommer, 2013; Dingemanse & Dochtermann, 2013; Bridger et al., 2015). In the hermit crab *Pagurus bernhardus*, we found that body mass, temperature, routine and startled metabolic rate had no relationship in the mean duration of the startle response, and thus, contrary to our prediction, boldness in *P. bernhardus* does not appear to be related with POLS. However, we found a significant relationship between IIV in startle response and these life history traits (body mass, temperature, routine MR and startled MR). Additionally, *P. bernhardus* also exhibited a higher intra-individual variation in behaviour than between-individual variation, consistent with a previous meta-analysis (Bell et al., 2009). Behavioural traits, in general, have low to moderate repeatability (0.37-0.42; Bell et al., 2009), which has been shown in previous studies in *P. bernhardus* (e.g., Stamps et al., 2012; Briffa, 2013; Briffa et al., 2013). In this study, however, although there was significant repeatability (i.e., 95% CIs did not cross zero) our estimates were much lower than for those previous studies on the same species. One possible explanation for the lower repeatability is the method used to stimulate the startle response. As the startle response is associated with defence, being manually handled (e.g., Stamps et al., 2012; Briffa, 2013; Briffa et al., 2013) could be a less intense stimulus for hermit crabs and thus, result in a more predictable response than a free fall of 30 cm. Furthermore, a recent study on tadpoles of the frog *Rana dalmatina* has shown that novel stimuli might yield lower repeatability compared to stimuli that the animal is likely to be more familiar with (Urszán et al., 2015a). Hermit crabs are frequently perturbed in their habitat (due to wave action, encounters with predators or indeed with other hermit crabs), which is simulated by the manual handling protocols used in previous studies. In contrast, they are unlikely to experience frequent 30 cm falls. Nevertheless, this approach was necessary in order to obtain respiration rates during recovery form disturbance and this technique still resulted in significant repeatability, allowing analysis of the variance components of interest.

Several studies have documented the repeatability of metabolic rate across several taxa (McCarthy, 2000; Broggi et al., 2007; Nespolo & Franco, 2007). So far, metabolic rate appears to be a repeatable trait (Nespolo & Franco, 2007), with a potentially stable state (Konarzewski et al., 2005) and linked to the activity level (Nespolo & Franco, 2007; Sundt-Hansen et al., 2009). Nevertheless, such studies are mainly focussed on endotherms (for review see Nespolo & Franco, 2007), and by having a higher self-maintenance cost, tend to have a higher and more constant metabolic rate (basal metabolic rate; Stearns, 1992). While invertebrates, by being ectothermic, are strongly influenced by ambient temperature and, in general, show lower metabolic rates, even in activity (Alexander, 1999; Nespolo & Franco, 2007). Those differences could explain the lower repeatability estimate in routine MR, as it was measured with minor temperature variation and with some random levels of hermit crab activity. And, in hermit crabs, such uncontrolled environmental variance combined with an unnatural method to stimulate the startle response (by dropping), may generate lack of repeatability in startled MR.

Indirect associations between behaviour and POLS have been largely criticized (e.g., growth-related traits) with the suggestion that direct associations between behaviour and metabolic rate are required for adequate testing of the POLS hypothesis (Adriaenssens & Johnsson, 2009). Although logical, such correlations have a mixed support (e.g., Bryant & Newton, 1994; Ketola & Kotiaho, 2012; Krams et al., 2013). Mathot & Dingemanse (2015) suggested that studies attempting to link metabolic rate and behaviour may be biased if metabolic rate is measured in only on one (or a few) occasions. This is because a single measure of metabolic rate may be insufficient to infer results regarding the energy management of individuals. In our study, however, we measured both types of metabolic rate (routine MR and startled MR) repeatedly with measures of MR being taken immediately prior to and coinciding with each behavioural observation. With this large amount of data, we nevertheless failed to observe any association between mean startle response duration and either routine MR or startled MR. In contrast, we found a significant association with IIV-level variation in startle responses. Contrary to our prediction, our results indicate a negative correlation between IIV and routine MR, indicating that individuals showing low levels of consistency are those with the lower routine metabolic rates. In contrast, individuals with low levels of consistency had higher metabolic rates during startle response.

Despite the POLS predicting a positive relationship between activity, superficial exploration and energetic expenditure, there is no consensus on its prediction regarding within individual variation in behaviour (Coppens et al., 2010; Careau et al., 2012; Niemelä et al., 2012b). Coppens et al. (2010) and Niemelä et al. (2012b) for example, suggested that a fixed behavioural strategy (lower IIV) should be less energetically demanding (due to lower costs for cognitive activities) and thus more common in slow-paced strategy individuals. Urszán et al. (2015a) found similar results, and a positive relationship between gain in mass and low IIV in exploratory behaviour in tadpoles (*Rana dalmatina*). Our results, however, indicate that in *P. bernhardus* the POLS strategy may be conditional on behavioural consistency.

Individuals with low routine MR were those that subsequently revealed high IIV variability in startle response durations. Conversely, startled MR increased with IIV. Thus in the context of linking metabolic rate to ideas about the pace of life, both the situation in which metabolic rate is estimated and the level of behavioural variation under analysis appear critical. Indeed, Mathot & Dingemanse (2015) point out that both behaviour and physiological traits are highly flexible, and thus, any slow-fast classification of individuals must be carefully assessed in more than one situation. In *P. bernhardus* the variability in hiding time seems to be a strategy to cope with risk (Briffa, 2013; Briffa et al., 2013). Our results reinforce such findings, since the startled MR was only correlated in the IIV level, and not in the mean-level. Therefore, it appears that, at least in potentially stressful situations, within-individual variation in behaviour, rather than individual mean levels of behaviour, might be linked with underlying variation in metabolic rate (Dall et al., 2004).

Habituation is a type of learning in which an individual after repetitive stimulation exhibits a waning of its response (Thorpe, 1956). It occurs when a continued response to a nonthreatening stimulus is considered to be energetically costly (Raderschall et al., 2011). Previous investigations in *P. bernhardus*, however, have did not detect any habituation (Briffa et al., 2008, 2013; Bridger et al., 2015). Our study demonstrates a mean level reduction in startle response duration with observation, and thus habituation with the startle response stimulation. One possible explanation is the way in which we induced the startle response. As habituation is described to occur faster with weaker stimuli (Rankin et al., 2009), it is possible that the dropping stimulus is less intense than manually handling, generating habituation and thus a lower (but still significant) repeatability in startle response.

Previous studies in *P. bernhardus* have shown that the mean-level boldness is sensitive to ambient temperature. Hermit crabs when transferred to a different temperature treatment (10 to 15°C and 15 to 10°C) had a significant increase in mean-level boldness (Briffa et al., 2013). In contrast to that study where temperature was manipulated, in the current experiment we attempted to make observations across stable temperature conditions. Hence, the temperature variation during the current experiment was much lower, and maybe insufficient to generate a general trend in mean level behaviour. Nevertheless, at the IIV level these relatively small and routine temperature fluctuations appear to have been sufficient to generate a relatively strong positive effect. Individuals seemed to exhibit a higher variation in behaviour when exposed to higher temperatures. This result is consistent with the hypothesis that an increase in temperature, in poikilotherms, could result in increasing in the unpredictability in behaviour (Dingemanse et al., 2010; Briffa, 2013). However, we did not find any clear correlation between routine MR or startled MR and the temperature of the seawater, indicating that the small temperature fluctuations experienced during the experiment were not sufficient to drive changes in metabolic rate (see the Appendix for detailed descriptions). Therefore, it is interesting that temperature appears to have a greater effect on IIV compared to either measure of MR. A possible explanation is that at greater temperatures poikilothermic predators may be more active (Petersen & Kitchell, 2001). However, we also note that a direct interpretation of the effects of temperature in this study may be less easy to interpret in comparison where temperature variation was specifically manipulated across a wider temperature range (e.g., Briffa et al., 2013). Since the small fluctuations in temperature occurred within and between observations it is unlikely that the animals were in a steady temperature state.

We also found that the mean duration of the startle response in *P. bernhardus* have had no relationship with the hermit crab body mass, similar to other studies (Briffa et al., 2008; Bridger et al., 2015). But, it appears to have a strong negative effect on the IIV level. Heavier individuals, were the ones with lower IIV. Growth in crustaceans is a step-wise process, resulting from sequential moulting (Liberto & Mesquita-Joanes, 2014), and in the case of hermit crabs it is further constrained by access to suitable gastropod shells. Although it could be influenced by several factors (e.g., sex and environmental conditions during ontogeny), size, in general, varies with age (size-age

relationship; Liberto & Mesquita-Joanes, 2014) so here it may be the case that IIV decreases with age.

Although there is not much consensus on why animals vary in the predictability of their behaviour, a few studies have shown that IIV reduces with age and previous experience (Maye et al., 2007; Brembs, 2011; Urszán et al., 2015b). If so, IIV could represent adaptive stochastic variation in behaviour, facilitating trial and error learning (Brembs, 2011). That is possible because individuals tend to adjust their behaviour according to environmental conditions and internal state (behavioural plasticity; Briffa, 2013). However, such behavioural flexibility is likely to be costly (as we demonstrated; Dall et al., 2004; Careau et al., 2008), and thus, individuals tend to develop a behavioural strategy that becomes less flexible with age and previous experience (Dall & Cuthill, 1997; Dall et al., 2004). In contrast, Bridger et al. (2015) found that IIV in hermit crabs increases with mass. Although both studies investigate the variation in startle response in *P. bernhardus*, Bridger et al. (2015) induced the startle response by lifting and replacing the hermit crabs at the base of the tank, while here we dropped the jar causing the animal to withdraw inside the shell. Nevertheless, we obtained a similar relationship between the mean duration of the startle response and crab weight (Briffa et al., 2008; Bridger et al., 2015), indicating that the method variation may not be the responsible for such conflicting results. An alternative explanation is that Bridger et al. (2015) controlled for mass, using lighter crabs across a smaller size range, which could hide effects of ontogenetic variations in behavioural trends (Bridger et al., 2015). In studies of IIV in hermit crabs to date, we have used hermit crabs from a single size class, as defined by the species of gastropod shell that they occupy. Further studies, using hermit crabs form different size classes, which are distinct due to the different species of gastropod shell that crabs of markedly different sizes occupy, would enable us to gain further insights into how IIV varies with age.

Studies on animal personality have increased over the last decade. They have investigated the maintenance of a given behavioural trait over time, situation, across environmental conditions (Sih et al., 2004; Bell & Sih, 2007; Réale et al., 2007; Cote et al., 2008). However, the knowledge behind physiological and behavioural correlations in both mean level behaviour and IIV still is in early stages (Fresneau et al., 2014), mainly due to the lack of statistical tools to divide the variance into mean and IIV levels (Cleasby et al., 2015). Our findings demonstrate that relationships between behavioural

traits and underpinning physiology can be variable within individuals, dependent on current activity rates.

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Appendix

A.1. Description of the mean and SD models

If Y_{ij} denotes the startle response of the i-th hermit crab on the j-th occasion then we assume that (Y_{ij}) is normally distributed with mean μ_{ij} and standard deviation σ_i, the model can be expressed as:

$$\begin{aligned}(Y_{ij}) = {} & (\beta_1 + \delta_{0i}) + \beta_2 \text{Temperature}_i + \beta_3 \text{Mass}_i \\ & + \beta_4 \text{Routine metabolic rate}_i + \beta_5 \text{Activity metabolic rate}_i \\ & + (\beta_6 + \delta_{1i}) \text{Occasion}_{ij} \end{aligned} \quad (1)$$

where β_1 is the expected value when all of the covariates are equal to zero (i.e., the intercept), β_2 to β_5 represent fixed effects for the covariates, δ_{0i} represents the random intercept effect and δ_{1i} represents the random slope associated with occasion.

We assumed that the random effects were normally distributed with means of zero and unknown variances. The SD model can be expressed as:

$$\begin{aligned}(\sigma_i) = {} & (\gamma_1 + \phi_{0i}) + \gamma_2 \text{Temperature}_i + \gamma_3 \text{Mass}_i \\ & + \gamma_4 \text{Routine metabolic rate}_i + \gamma_5 \text{Activity metabolic rate}_i \end{aligned} \quad (2)$$

where γ_1 represents the sample mean, γ_2 to γ_5 represent fixed effects for the covariates and ϕ_{0i} represents the random intercept, assumed to have a mean of zero and standard deviation of $\tau_{\sigma,0}$.

Table A.1.
Wald-F test for autocorrelation estimation between fixed effects.

Parameter name	Wald χ^2	p-value
Mass × Routine MR	0.6648	0.4149
Temperature × Routine MR	0.5983	0.4392
Mass × AMR	0.6497	0.4202
Temperature × AMR	0.8106	0.3680
Routine MR × AMR	1.2142	0.2705