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Elucidating the causes and consequences of individual behaviour: personality & plasticity in the marine gastropod, Littoraria irrorata.

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Elucidating the causes and consequences of individual behaviour: personality and plasticity in the marine gastropod, *Littoraria irrorata.*

A thesis presented for the degree of Doctor of Philosophy

By

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SUMMARY

During the last four decades, advancing perspectives in behavioural ecology have generated a surge of research focusing on individual behavioural variation. The resulting literature has highlighted the substantial effects of this variation on ecological and evolutionary processes, whilst also leading to the widely accepted view that individual variation may represent the 'end product' of natural selection. Despite receiving considerable recent attention, many questions remain unanswered, and thus there is still keen interest in unravelling the proximate and ultimate causes of behavioural diversity, and in improving on our current understanding of its maintenance within natural populations. This has led to a number of theoretical developments centring on links between behavioural, physiological, and life-history traits (the Pace-of-Life Syndrome hypothesis - POLS), which are predicted — along with factors affecting individual state — to underpin behavioural expression among- and within-individuals. Also central to these theoretical explanations are trade-offs between current reproduction and future survival, where more risk-prone individuals are expected to benefit from increased resource gains and faster growth, at the expense of higher mortality by predation. In order to examine these predictions, this thesis aimed to build on existing knowledge by responding to recent calls for more stringent empirical testing of key hypotheses, and by addressing important outstanding questions relating to the causes, constraints and consequences of individual behaviour. Specifically, the work examined individual risk-taking behaviour in the saltmarsh periwinkle (*Littoraria irrorata*). An important consumer on the intertidal marshes of the Eastern USA, *L. irrorata* ecology — including their characteristic circumtidal migrations on saltmarsh vegetation — has been studied extensively, but as yet, not at the individual level. Therefore, the work comprises four empirical studies, which aimed to examine (a) the consistency of risk-related behaviours, (b) the influence of local environmental conditions on behavioural consistency and flexibility, (c) associations between individual behaviour and factors affecting individual state, and (d) the possible trade-offs associated with behaviour and individual fitness.

Findings revealed, for the first time in this species, that risk-taking behaviour (boldness), activity, and latency to climb plant stems with the incoming tide are consistent individual attributes (personalities), and that these traits associate to form a risk-related behavioural syndrome. In addition, findings revealed considerable behavioural flexibility (plasticity) across environmental contexts, including diel and tidal cycles, and in response to temperature gradients — highlighting the importance of local environmental conditions in shaping individual behaviour. The work also provides a rare example of domain general plasticity, where behavioural responses carry over across contextual gradients, suggesting underlying physiology as a common mechanism, and raising the

possibility of correlational selection on plasticity. Further, evidence presented for covariation between boldness, resting metabolic rate (RMR) and somatic growth, indicated clear among- and within-individual correlations, providing compelling support for the POLS hypothesis. This, along with findings indicating that behaviour appears to be influenced by intrinsic and extrinsic factors associated with risk perception, provides evidence that individual behavioural differences may be driven by underlying physiology, as well as with factors affecting individual information state. However, contrary to theoretical predictions, findings also revealed that bolder individuals were more likely to survive exposure to a key marsh predator, thus raising questions relating to the maintenance of behavioural diversity in *L. irrorata*. In particular, where higher risk-taking propensity does not appear to be involved in fitness trade-offs under the conditions studied. Taken together, these findings illustrate the importance of behavioural variation in determining individual performance under varying conditions, whilst also contributing to the growing body of literature examining the causes and consequences of individual behaviour. Finally, the work also provides a solid foundation for future studies examining the role of individual behaviour in the ecology of this species.

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CONTRIBUTIONS TO EMPIRICAL WORK

This thesis is the product of my own research, undertaken in collaboration with my supervisor, Dr Ian McCarthy, and Dr Peter Biro. I conceived and designed all experiments, carried out data collection, data analysis and am the primary author on all chapters. The following is a breakdown of all other significant contributions:

CHAPTER TWO (in prep): Cornwell, T.O, McCarthy, I. D., Beaumont, K. L., Biro, P. A. Individual behaviour and circadian rhythms: the influence of diel cycle on risk-taking in the saltmarsh periwinkle, *Littoraria irrorata*.

I.D.M supported the conception of the study. K.L.B supported data collection in the field. P.A.B supported and gave advice on statistical analysis. Both I.D.M and P.A.B have read through early drafts and have supported manuscript revisions. All authors have supported preparations for manuscript submission.

CHAPTER THREE (published): Cornwell, T.O, McCarthy, I. D., Snyder, R. A., Biro, P. A. (2019) The influence of environmental gradients on individual behaviour: Individual plasticity is consistent across risk and temperature gradients. *Journal of Animal Ecology, 88, 511-520.*

I.D.M supported the conception of the study and the experimental design. P.A.B led on the statistical analysis, using SAS (SAS Institute, 2012). I followed and repeated the process in R (R Core Team, 2017). This process allowed me to develop my understanding of linear mixed-effect modelling and provided valuable experience of fitting these models as part of future research and to independently analyse data in all other chapters. R.A.S supported experimental set up during fieldwork. All authors contributed conceptually to the study, supported manuscript drafts and revisions, and gave final approval for publication.

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I.D.M supported the conception of the study and the experimental design. P.A.B supported and gave advice on statistical analysis. All authors contributed conceptually to the study, supported manuscript draft revisions, and gave final approval for manuscript submission.

CHAPTER FIVE (in prep): <u>Cornwell, T.O, McCarthy, I. D., Beaumont, K. L., Biro, P. A. The fitness consequences of risk-taking behaviour: does boldness predict survival in the saltmarsh periwinkle, *Littoraria irrorata*?</u>

I.D.M supported the conception of the study and the experimental design. P.A.B supported and gave advice on statistical analysis. K.L.B supported data collection in the field. Both I.D.M and P.A.B have read through early drafts and have supported manuscript revisions. All authors have supported preparations for manuscript submission.

CONTENTS

CHAPTER ONE

General introduction

A seemingly endless source of challenging questions, variation is undoubtedly one of the most striking features of the natural world (Hallgrímsson & Hall, 2005).

Background

Historical perspectives on behavioural variation

Animal behaviour has long been incorporated into the study of ecological systems (Sutherland, 1996; Fryxell & Lundberg, 1998), with its importance emphasised by the fundamental role it plays in the study of biological systems (Hallgrímsson & Hall, 2005). As one of the most plastic phenotypic traits (Sol & Lefebvre, 2000; Nicolakakis et al., 2003; Bergmüller et al., 2010; Foster, 2013; Edgell et al., 2017), behaviour allows organisms to adjust to changing conditions, and thus, from an evolutionary perspective, is considered to be highly adaptive (Clark & Ehlinger, 1987; Hazlett, 1995; Wilson, 1998; Sih et al., 2004a; Dingemanse & Réale, 2005; Wolf & Weissing, 2010). It is also well understood that natural selection promotes a mix of behaviour phenotypes within a single population (Coleman & Wilson, 1998) and that this variation plays an important role in ecological and evolutionary processes (Dall et al., 2004; Sih et al., 2012). However, despite phenotypic variation being Darwin's fundamental observation, and the focus of the first two chapters of *On the origin of species* (Hallgrímsson & Hall, 2005), variation observed within single populations has, until recently, been somewhat neglected (Arnold & Bennett, 1984; Wilson et al., 1993; Réale et al., 2010a). This may have resulted from phenotypic variation being seen as ubiquitous in natural populations and as such, differences observed among individuals themselves, were not deemed to require any specific explanation (Wilson et al., 1993). However, with variation occurring at every level of biological organisation (Allegue et al., 2017), one could certainly be led to question why the study of individual behavioural variability remained peripheral within the fields of ecology and evolution for over a century after the publication of Darwin's seminal work (Hallgrímsson & Hall, 2005).

During this time, historical perspectives on phenotypic variation leaned towards the explanation of differences between taxa, and even the notion that different populations of a single species could adapt to varying conditions was not widely accepted until the 1980's (Dall et al., 2004). Furthermore, with the classical approaches looking for interpretation based on a population's average (Slater, 1981; Mather, 1998; Sih, 2017), ecological theory rested on the assumption that behavioural variation centred on a single optimum or on two or more coexisting evolutionary stable strategies (Wilson et al., 1993; Weiss & Adams, 2013). Therefore, behavioural differences exhibited among individuals were long considered to simply represent ecological 'noise', or non-adaptive residual variation (Cleasby & Nakagawa, 2011) occurring around the adaptive, population mean

(Bennett, 1987; Mather & Anderson, 1993; Wilson, 1998; Sih et al., 2004a; Careau et al., 2008; Wolf & Weissing, 2012). An approach that overlooked the importance of individual differences (Slater, 1981; Toms et al., 2010), and more generally, the full extent of observed variation (Bennett, 1987). Furthermore, with only sporadic attention being given to the idea of among-individual phenotypic variation in the early, and latter half of the 20th century (Crawford, 1937; Huntingford, 1976; Arnold & Bennett, 1984; Taigen & Wells, 1985), some have attributed its neglect to a focus on 'species-typical' behaviours or to the assumption that individual differences simply resulted from experimental error or uncontrollable environmental factors (Clark & Ehlinger, 1987; Mather & Anderson, 1993; Stamps et al., 2012). In fact, it was not until Bennett (1987) encouraged biologists to avoid "the tyranny of the golden [population] mean", and to focus on the valuable and under-studied variation occurring among individuals of the same species, that an important shift in research focus was instigated (Roche et al., 2016). Subsequently, and based on mounting evidence challenging the early conventions, the consideration of finer-scale biological variation placed further emphasis on exploring the adaptive individual strategies being employed within natural populations (Wilson, 1998; Dall et al., 2004). Thus, with more research exploring the fundamental Darwinian perspective, that evolution acts on the individual as the principal element of selection (Smith, 1982; Dall et al., 2004; Hill & Zhang, 2004), the importance of individual-level investigations began to gain recognition (Dall et al., 2004; Toms et al., 2010).

During the last four decades, this changing perspective has generated a dramatic increase in research focusing on behavioural variability among- and within-individuals (see reviews by Gosling, 2001; Stamps & Groothuis, 2010b; Roche et al., 2016; Stamps, 2016). This shift has been underpinned by two key findings. Firstly, that within a given population, consistent individual differences in behaviour are a common occurrence across the animal kingdom (Gosling, 2001; Réale et al., 2007; Sih, et al., 2004). Secondly, that these differences derive from highly-structured behaviour types, that is to say, that behaviours are stable over time and are correlated across contexts (Sih et al., 2004a; Dingemanse & Réale, 2005; Bell et al., 2009; Rodríguez-Prieto et al., 2010; Wolf & Weissing, 2012). By the early nineties, ecologists had begun to recognise the importance of individual behaviour in shaping ecological processes (Dall et al., 2004; Sih et al., 2012); where individuals adopt distinct, alternative strategies (Gross, 1996; Widemo, 1998). Subsequently, reports of consistent individual behaviour have been reported in a diverse range of species, across multiple taxa (Trillmich & Hudson, 2011; Wolf & Weissing, 2012), with cases not simply restricted to higher vertebrates (Bell, 2007). For example, consistent individual differences in behaviour have thus far been identified in mammals, birds, reptiles, amphibians, fish and invertebrates (e.g. Gosling & John, 1999; Gosling, 2001; Toms et al., 2010); including those without a centralised brain (e.g.

anemones; Briffa & Greenaway, 2011). Furthermore, it has also been stated that individual behavioural variation accounts for, on average, >30% of phenotypic variance within wild populations (Bell et al., 2009) and that individual behaviours can be heritable (Stirling et al., 2002; Bell et al., 2009; Dochtermann et al., 2015), further supporting their potential adaptive significance (Dingemanse et al., 2002; Van Oers et al., 2004; Réale et al., 2007; Sih & Bell, 2008; Smith & Blumstein, 2008; Stamps & Groothuis, 2010a). This major shift in our understanding has come with the suggestion that individual differences should be considered as the "end product" of natural selection and that individual behaviour, rather than the populations average, is the essence of explaining variation (Wilson et al., 1993). Therefore, the nature of individual behavioural variation has now become a focus of research in its own right, and it is widely accepted that individual variation provides the "raw materials" of evolution (Endler, 1986); a key foundation of modern biology (Budaev & Brown, 2011).

Evidence for the wide-ranging incidence of individual behavioural variation, along with its non-random distribution (Gosling & John, 1999; Gosling, 2001), suggests that the phenomenon could have significant consequences for ecology and evolution (e.g. Dall et al., 2004; Wolf & Weissing, 2012); particularly, where consistent individual behaviour may account for both optimal and sub-optimal behaviour occurring within natural populations (Carter & Feeney, 2012). This apparent ecological importance, as well as the consistency identified across taxa, has led to the proliferation of empirical and theoretical work on the subject (e.g. Dingemanse & Réale, 2005; Stamps & Groothuis, 2010a; Wolf & Weissing, 2012; Ballew et al., 2017; Strong et al., 2017). This, in turn, has led to rapid development within the field; from its conception, rooted in investigations of the repeatability (consistency) of individual behavioural characteristics (e.g. Wilson et al., 1993); to a more recent focus on its causes, constraints and consequences (e.g. Nussey et al., 2007; Smith & Blumstein, 2008; Wolf & Weissing, 2012; Dingemanse & Wolf, 2013; Snell-Rood, 2013; Sih et al., 2015; Dammhahn et al., 2018). Moreover, the study of individual behavioural variation has, over the last four decades, taken a firm hold in scientific literature with ecologists, physiologists and those from related fields turning to questions relating to the variation occurring among and within individuals to explain behavioural variation across the animal kingdom (Toms et al., 2010).

Terminology

Throughout the early literature, many terms were used to characterise the phenomenon of consistent among-individual behavioural variation (animal personality), and indeed critics of the field argue that it is "riddled" with terminological inconsistencies (Roche et al., 2016; Beekman & Jordan, 2017). This is unsurprising given that the term 'personality' was initially borrowed from

human psychology where, within a single text, some 49 definitions are given for the one expression (McAdams & Pals, 2006). In non-human animals, however, the term personality refers to individual behavioural tendencies that are consistent over time and that influence behaviour exhibited in different contexts (Stamps & Groothuis, 2010b). Here, the word context refers to the conditions; both environmental and otherwise, that an individual is exposed to at a given point in time or in a given situation (Wilson, 1998; Stamps & Groothuis, 2010b). However, other terms such as 'temperament' have also been used, similarly, to describe individual behaviour that differs consistently (or is repeatable) over time and in a given context (Réale et al., 2007). The term temperament, itself, had previously been used in applied ethology, when referring to the behaviour of agricultural animals (Kilgour & Dalton, 1983); however, where temperament commonly measures behavioural variation in one context, personality refers to behavioural variation with a more complex structure (see review by MacKay & Haskell, 2015).

Although terms such as temperament, personality and 'predisposition' are distinct from one another in human behaviour, they have all been used synonymously with regards to non-human animals (Toms et al., 2010). Likewise, definitions similar to or even interchangeable with that of personality have been used to describe an individual's temperament, predisposition and 'coping style' (Carere & Maestrpieri, 2013). Although borrowing terminology from other fields is likely to have complicated matters in the early literature, one particular concern with the term personality was the fear of anthropomorphism; with the idea that 'personality' should only be considered in the "human domain" (MacKay & Haskell, 2015). However, despite caution surrounding the term personality and the potential for anthropomorphism (Gosling & John, 1999; Toms et al., 2010; Budaev & Brown, 2011), it has been adopted by much of the recent literature. Nonetheless, some researchers have, relatively recently, preferred to negate potential anthropomorphism by referring to an individual's 'behavioural type' (Bell, 2007; Sih et al., 2012). Although, according to Bell (2007), this refers to the individual expression of a particular "configuration of behaviours" that, like personality, is repeatable but focuses on the individual rather than the difference between individuals. Despite these often arbitrary distinctions (Réale et al., 2007; Roche et al., 2016), more recent attempts to refine the associated terminology have supported the provision of comprehensive frameworks to guide future research (Réale et al., 2007; Stamps & Groothuis, 2010b; Budaev & Brown, 2011; Carter et al., 2013; Dingemanse & Araya-Ajoy, 2015; MacKay & Haskell, 2015; Roche et al., 2016). Therefore, based on the descriptions and justifications provided in the most recent literature, and in lieu of an in-depth discussion of the terminological inconsistencies, Table 1.1 provides a glossary of key terms that will be used going forward, within this thesis, to ensure that clarity and consistency are maintained hereafter.

Table 1.1 Glossary of terms used in the study of individual behaviour.

Animal personality	Consistent or repeatable among-individual (inter-individual) behavioural differences that are maintained over time and across contexts (Réale et al., 2007). Among-individual behavioural variation that is attributable to the combined effects of genetics and environment, which affect an individuals' phenotype (Dingemanse & Araya-Ajoy, 2015).
Behavioural plasticity	Behavioural variation occurring both among and within individuals (intra-individual variation), across contexts or environmental gradients. The extent to which individual behaviour varies, relative to other individuals within a given context or over time (Barbasch & Buston, 2018).
Behavioural syndrome	The property of a population or species. A collection of behavioural tendencies that correlate across situations and contexts — a suite of correlating personality traits — e.g. the correlation between aggression and boldness (Aggressiveness-boldness syndrome; Bell et al., 2005) or the correlation between behavioural, physiological and life-history traits (Pace-of-Life Syndrome; Réale et al., 2010).
Behavioural type	The property of the individual. A specific combination of behavioural tendencies forming part of an individual's behavioural syndrome - E.g. bold vs shy (Bell, 2007; Wolf & Weissing, 2012).
Context	A functional category of behaviour, e.g. mating, foraging, dispersal (Sih et al., 2004a).
Individual state	Refers to the 'state' of an individual as being a combination of factors that influence its behavioural actions (Houston & McNamara, 1999). These factors could include energy reserves, condition, physiology (metabolic rate, hormone level, immune state), morphology, age or size. Other factors, relating to an individual's information state, could include experience or social rank (Sih et al., 2015).
Reaction norm	A function describing the variation of a phenotype (e.g. behaviour, metabolism) across a given environmental gradient or through time (Roche et al., 2016). The set of behavioural phenotypes that an individual can produce across a range of environmental conditions and internal stimuli (Dingemanse et al., 2010b).
Repeatability	The proportion of phenotypic variance due to among-individual differences, denoted as R. Adjusted repeatability (R_i) — when calculated after controlling for confounding effects. Conditional repeatability — when estimated at a given value of a fixed factor (Nakagawa & Schielzeth, 2010).
Situation	A set of environmental conditions at a given point in time, e.g. season or risk of predation (Sih et al., 2004a).

Studying individual behavioural variation

Animal personality

With the study of personality in *Homo sapiens* dating back more than a hundred years (Galton, 1883), it is well established that humans exhibit consistent individual personality traits (Pervin & John, 1999). However, today some of the traits used to quantify variation in human behaviour have been shown to exist in other animals (Gosling, 2001; Sih et al., 2004b). These traits have long since been manipulated by humans working with a range of domesticated animals (Budaev & Brown, 2011), and it is unlikely that anyone working with live, non-human animals — in any context — would dispute the conspicuous behavioural differences exhibited among individuals of the same species (Wilson et al., 1993; Roche et al., 2016). This sentiment was expressed by Crawford (1937), whilst studying behaviour in captive chimpanzees (*Pan troglodytes*), in one of the first articles documenting the existence of animal personality. Although personality is not just a characteristic of domesticated or laboratory animals, its presence and indeed its importance in natural populations was, as aforementioned, not to be realised until much more recently. Despite taking over a century for scientists to appreciate the extent and influence of individual behavioural variation (Arnold & Bennett, 1984; Hallgrímsson & Hall, 2005), the ever-growing body of work focusing on this subject reports that some individuals may be consistently bolder/more prone to exhibit risk-taking behaviour (e.g. Wilson et al., 1993, 1994; White et al., 2013), more aggressive (e.g. Betini & Norris, 2012; Sanches et al., 2012), more sociable (e.g. Cote & Clobert, 2007; Cote et al., 2008, 2012) or show a higher propensity to explore (e.g. Verbeek et al., 1994; Fraser et al., 2001; Dingemanse et al., 2002; Garant et al., 2005) than other conspecifics within a given population.

Exhibition of personalities are often described along an axis, or behavioural continuum (Wilson et al., 1993, 1994; Coleman & Wilson, 1998; Gosling, 2001), which has been recognised for a range of personality traits, including aggression (e.g. Johnson & Sih, 2005; Mowles et al., 2012), exploratory behaviour (e.g. Wilson & Godin, 2009) and sociability (e.g. Cote et al., 2008, 2012). However, first described was the bold/shy continuum (Wilson et al., 1993); a fundamental and ecologically important axis of behavioural variation, in which bolder individuals are defined by a higher propensity for risk-taking than shyer conspecifics (Wilson et al., 2010; White et al., 2013). Personality traits can be exhibited to varying degrees along a given axis, and this in itself can influence the outcome of typical ecological challenges, such as those associated with learning (Dugatkin & Alfieri, 2003), predator-prey interactions (Ioannou et al., 2008), exploration (Wilson et al., 1993), and even interaction with man-made technologies (Darrow & Shivik, 2009).

An individual's position along a particular axis has been shown to depend on a variety of different factors, including but not limited to life history and overall body condition. For example,

Riesch et al. (2009) reported reduced boldness in extremophile fishes, *Poecilia mexicana,* and *Poecilia sulpurarua*, with lower than average body condition. Analogous findings were reported by López et al. (2005), stating that boldness increases with body condition in Iberian rock lizards (*Lacerta monticola*). López et al. (2005) also suggest that an individual's position on the shy/bold continuum may reflect its ideal anti-predator behaviour as a function of its health and "general quality". Furthermore, it is understood that an individual's position along this axis can determine the extent to which it is likely to trade-off potential risky behaviour for increased resource gains (Boissy, 1995; Ward et al., 2004; Stamps, 2007; Riesch et al., 2009). This suggests that an individual's position along a behavioural continuum is likely to have an influence on its ability to compete for resources (e.g. food and mates), its chances of survival and ultimately, its overall fitness (Smith & Blumstein, 2008). Indeed, the ecological and evolutionary consequences associated with the exhibition of personality (Dingemanse & Réale, 2005; Wolf & Weissing, 2012; Carere & Maestripieri, 2013; Snell-Rood, 2013) further highlight its importance and, at least in part, justify why animal personality is currently one of the fastest growing research areas within the fields of behavioural biology and ecology (Carere & Maestripieri, 2013).

Behavioural syndromes

In many cases, personality traits have been shown to correlate with one another across contexts; an occurrence referred to as a behavioural syndrome (e.g. Sih et al., 2004a, b; Garamszegi et al., 2012; Huang et al., 2018). Although sometimes described as analogous to (Bell, 2007), or as a direct replacement to animal personalities (Sih et al., 2012), behavioural syndromes are defined here as correlations occurring between 'suites' of personality traits (see Table 1.1), or as a functional assembly of personalities (Clark & Ehlinger, 1987; Sih et al., 2004a). Behavioural syndromes were initially reported by Huntingford (1976), where male aggression during the breeding season was positively correlated with bold tendencies outside of the breeding season, in three-spined sticklebacks (*Gasterosteus aculeatus*). This study gave rise to the 'boldness-aggression syndrome', which has subsequently been identified in many different taxonomic groups (Sih et al., 2004b). The boldness-aggression syndrome is just one example of trait correlations occurring across different contexts, suggesting that it is important to recognise the evolution of trait covariation (Bell, 2007). Particularly, because some behaviours may evolve together (under correlational selection) to form part of an individual's behavioural phenotype (e.g. Dochtermann & Jenkins, 2007), and thus focussing on single behavioural traits may provide misleading information (Bell, 2007). Behavioural syndromes provide an important avenue of research that has led to the development of hypotheses relating to the proximate causation of consistent individual behaviour (i.e. the pace-of-

life syndrome (POLS) hypothesis; Ricklefs & Wikelski, 2002; Réale et al., 2010b). The POLS hypothesis suggests that behavioural traits may have co-evolved with life-history and physiological traits, such as growth and metabolism, along the proactive-reactive behavioural continuum (Debecker et al., 2016) (see Figure 1.1). It is also suggested that consistent individual differences in behaviour could be maintained in natural populations as a result of fitness trade-offs, integrated into a POLS involving physiology and behaviour (Le Galliard et al., 2013).

PACE-OF-LIFE-CONTINUUM

SLOW ⟵————————⟶ **FAST**

Life-history traits

Slow growth	Fast growth
Delayed reproduction	Precocious reproduction
Long life	Short life

Behavioural traits

Shy/risk averse	Bold/risk prone
Low levels of aggression	High levels of aggression
High sociability	Low sociability
Less active	Highly active
Higher parental investment	Lower parental investment
Generally more plastic	Generally less plastic

Physiological traits

Low metabolism	High metabolism
Low sensitivity to oxidative stress	High sensitivity to oxidative stress
High immune response	Low immune response

Figure 1.1 Representation of the traits associated with the pace-of-life syndrome hypotheses (POLS), explaining predicted variation in behavioural, life history and physiological traits across a fast-slow continuum (adapted from Réale et al., 2010b).

Although behavioural syndromes are now considered commonplace, they are certainly not universal (Wolf, 2009), and even within a single species their structure may vary between populations. For example, the boldness-aggression syndrome has been found to be present in *G. aculeatus* populations under strong predation pressure, but not in populations under relatively low

predation pressure (Bell & Sih, 2007; Dingemanse et al., 2007). Others have also reported variation in behavioural syndrome structure across different life stages and suggest that the long term stability of these relationships should not be assumed (Wuerz & Krüger, 2015). Justification for these observations is currently centred on environmental changes (Killen et al., 2012, 2013; Wuerz & Krüger, 2015) or, in the case of variation occurring as a result of different life stages, changing hormone levels (Wuerz & Krüger, 2015). The reported instability of trait correlations has led to difficulties in explaining how behavioural syndromes develop and are maintained (Sih et al., 2004a; Dingemanse & Réale, 2005; Dingemanse & Wolf, 2013), In turn, this has led to a surge of interest in the causal mechanisms of the relationships associated with behavioural syndromes, which currently remain relatively poorly understood (Adriaenssens & Johnsson, 2013; Killen et al., 2013).

Behavioural plasticity

Although early animal personality research centres on estimates of behavioural repeatability (Réale et al., 2007; Bell et al., 2009), there is some debate as to the degree of repeatability (consistency) shown by individuals across different situations (see e.g. Wilson et al., 1994; Bell, 2005; Wilson & Stevens, 2005; Dingemanse et al., 2007). Certainly, where individuals are observed repeatedly, even in the same situation, their behaviour will not be identical across trials (Bell et al., 2009). This does not cast doubt over the existence of consistent behavioural variation itself (Budaev & Brown, 2011), but rather generates questions regarding the causes of these observed discrepancies. Behaviour is, after all, a plastic trait that is expected to vary depending on context (West-Eberhard, 1989). In addition to the differences in average behaviour observed among individuals, variance is also often observed in the consistency of behavioural expression within individuals (Nussey et al., 2007; Barbasch & Buston, 2018). However, early techniques used to estimate repeatability of behaviours do not account for variation occurring at the within-individual level (Dingemanse et al., 2010; Réale & Dingemanse, 2010). This variation in individual consistency indicates the presence of behavioural flexibility, or plasticity (Coppens et al., 2010; Dingemanse et al., 2010b; Réale & Dingemanse, 2010), where behaviour is said to be modified within individuals, depending on context (Briffa et al., 2013; Roche et al., 2016; Stamps, 2016; Abram et al., 2017). Thus, behavioural plasticity is measured as the change in an individual's predicted phenotype over time or as a function of a given environmental gradient (Fuller et al., 2005; Martin & Réale, 2008; Dingemanse et al., 2010b), and allows individuals to respond to changes in their immediate environment (Piersma & Drent, 2003) or to produce a range of behavioural phenotypes, fit for varying or changing situations (DeWitt et al., 1998).

Although behavioural plasticity has been extensively studied from a developmental perspective, recent research has focussed on the short-term plasticity of individuals with the aim of elucidating the causes and consequences of this within-individual variation (Dingemanse & Wolf, 2013; Snell-Rood, 2013; Stamps, 2016). In this sense, individual plasticity is important to study because an understanding of short-term, 'reversible' variation may facilitate the answering of many questions relating to individual behaviour (Alonzo, 2015). For example, plasticity indicates that the amount of variation that selection can act on is not always consistent across situations (Dingemanse et al., 2012), and that this variation is also likely to be heritable (Scheiner, 1993; Nussey et al., 2005). Thus, behavioural plasticity is essential for organisms to persist under ever-changing environmental conditions (Clark et al., 2018) and to adapt to the heritable variation, fuelling evolution (Brommer et al., 2008; Alonzo, 2015). There is, however, a growing awareness that behavioural variation within a given population cannot be attributed entirely to individual plasticity (Coleman & Wilson, 1998; Nussey et al., 2007), and although behavioural ecologists have often assumed that the existence of plasticity equates to a lack of behavioural consistency, this has been deemed a misconception (Sih et al., 2004a; Briffa et al., 2008; Dingemanse et al., 2010b; Toscano, 2017). Specifically, although personality and plasticity have often been considered separately, evidence points to a degree of both plasticity and relative consistency being likely in many situations (e.g. Briffa et al., 2008). Consequently, there have been suggestions that the two could be linked (Koolhaas et al., 1999; Schjolden & Winberg, 2007; Sih & Bell, 2008) or even interdependent (Westneat et al., 2011), and thus should be considered as complementary aspects of an individual's phenotype (Dingemanse et al., 2010b). In fact, behavioural plasticity may offer an explanation as to the varying degrees of among-individual consistency, often found across contexts (Wilson et al., 1994; Bell, 2005; Wilson & Stevens, 2005; Dingemanse et al., 2007). For example, shyer and less aggressive individuals have been shown to exhibit higher plasticity than bolder or highly aggressive conspecifics (e.g. Koolhaas et al., 1999; Sinn et al., 2008; Kontiainen et al., 2009). This suggests that in order to develop a better understanding of either personality or behavioural plasticity, it may be important to analyse both as part of a single experimental and statistical framework (Dingemanse et al., 2010b).

This approach has been facilitated by the study of individual reaction norms, which provides a tool to analyse and integrate both personality and plasticity (Fuller et al., 2005; Dingemanse et al., 2010b; Westneat et al., 2011; Roche et al., 2016). According to this statistical framework, the behavioural response of an individual to a contextual gradient is considered to be the 'trait of interest' (Dingemanse et al., 2010b). Therefore, the behavioural reaction norm of an individual describes its average behaviour as well as how its behaviour changes in response to environmental

variation (Dingemanse et al., 2010b). In this instance, both personality (elevation of the reaction norm) and the variety and extent of plasticity (slope of the reaction norm) can be presented simultaneously (see Figure 1.2) for a given population (Nussey et al., 2007; Mathot et al., 2012; Dingemanse & Wolf, 2013). The application of behavioural reaction norms has proven useful; however, it might be considered an over simplified view since the effective analysis of personality and plasticity is data dependent (Dingemanse & Dochtermann, 2013). For example, a limited number of data points may only provide estimates of plasticity, whereas estimates of consistency require repeated measures over time and/or across contexts (Réale et al., 2007; Beckmann & Biro, 2013; Biro & Stamps, 2015). Thus, it is important that experimental design incorporates large enough sample sizes (Dingemanse & Dochtermann, 2013) and sufficient repeat trials to allow for estimates of among- and within-individual consistency (Beckmann & Biro, 2013). Under these circumstances, the reaction norm approach provides a valuable tool with which to examine the different components of an individual's behavioural phenotype concurrently (Dingemanse et al., 2010b). As a result, the field has often turned to consider both personality and plasticity together in attempts to explain variation observed within a given population (Dingemanse et al., 2010b; Dingemanse & Wolf, 2013).

Another important aspect of behavioural plasticity gaining recent attention relates to the predictability of individuals (Stamps et al., 2012; Briffa, 2013; Briffa et al., 2013; Jolles et al., 2019). This within-individual (residual) variation (Figure 1.2f) provides estimates of how sensitive individuals are to a particular situation (Briffa, 2013), and has recently been demonstrated in invertebrates (e.g. Briffa et al., 2013; Chang et al., 2017b), fish (e.g. Stamps et al., 2012; Jolles et al., 2019), and birds (e.g. David et al., 2012). Importantly, individual predictability can provide insight to how consistently individuals express behaviours within a given context (or environment) (Urszán et al., 2018), in particular, where it has been shown to vary across environmental gradients, including temperature (Briffa et al., 2013) and in response to predation pressure (Briffa, 2013). Although the evolutionary significance of individual predictability has only recently begun to be addressed (see e.g. Bridger et al., 2015; Urszán et al., 2018), given its links with important processes often predicted to underpin variation in the expression of individual behaviour (e.g. varying levels of risk; Briffa, 2013, and metabolic scope; Biro et al., 2018), it should be an important consideration in future studies examining phenotypic variation in natural populations.
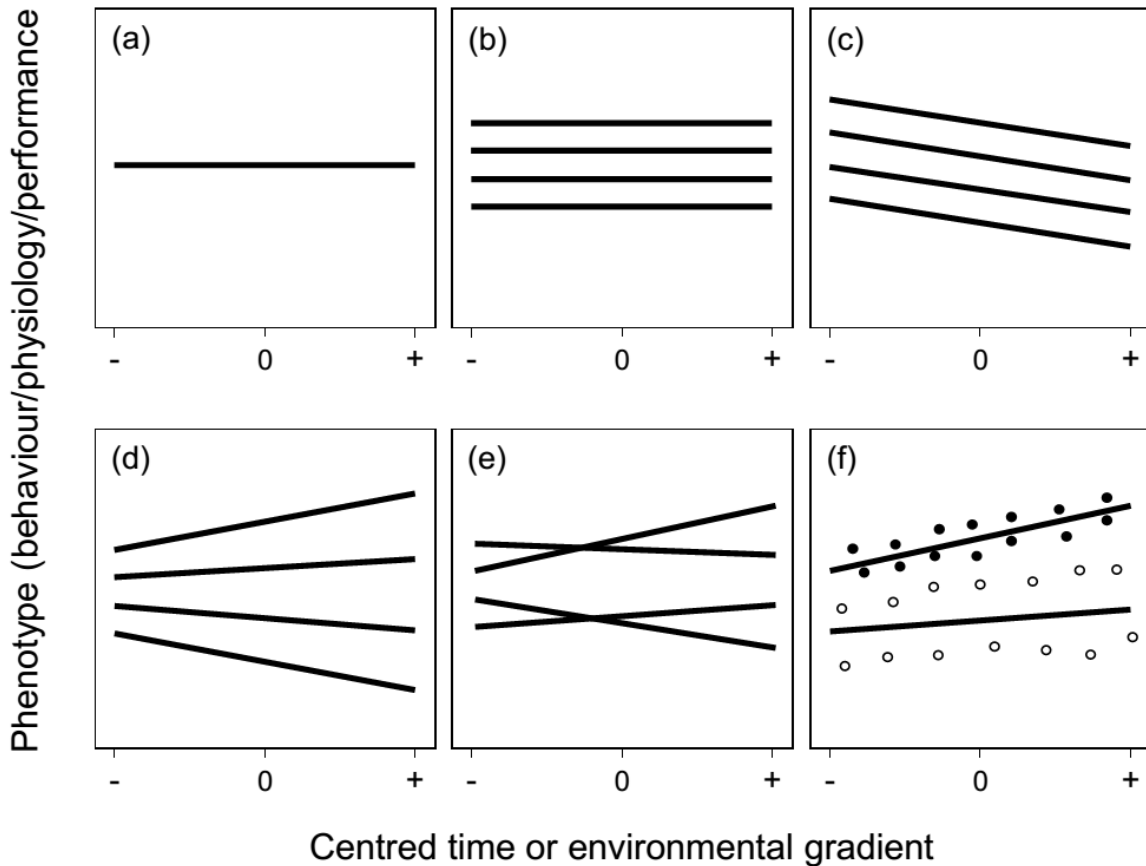
Figure 1.2 *Theoretical RN plots illustrating different types of variation within a population.* **(a)** *No among-individual differences (personality) and no plasticity exhibited — all individuals exhibit the same elevations (same intercepts) and do not respond to changes in time or context (slope = zero).* **(b)** *Individuals vary only in elevation (different intercepts = personality), no plasticity exhibited (slope = zero).* **(c)** *Personality and plasticity exhibited but individuals only vary in elevation — identical slopes (identical responses to change).* **(d)** *Personality and plasticity exhibited, individuals vary in elevation (=personality) and slope (different responses to changes over time or across environmental gradient — positive elevation-slope covariance.* **(e)** *Plasticity exhibited, individuals vary in elevation and slope, no elevation-slope covariance.* **(f)** *Includes residual (within-individual) variation, representing predictability. Closed circles = highly predictable, open circles = highly unpredictable* **(***Modified from Dingemanse et al. (2010b); Roche et al. (2016)).*

Experimental methods

As with the associated terminology, critics have also pointed out the inconsistencies relating to the methodologies employed within the field (Roche et al., 2016), a characteristic of many relatively young disciplines (Carter et al., 2013). Indeed, approaches used to measure and analyse individual behaviour in non-human animals are almost as diverse as the studies assessing the traits themselves (White et al., 2013), leading to confusion — at least in the early literature — as to how traits are defined and measured (Carter et al., 2013). In order to adopt a set of standardised tools with which to examine behavioural variation, the "open-field test" (Hall & Ballachey, 1936; Archer, 1973) provided a useful, general framework for exploring the ecology of individual variation (Réale et al., 2007). This approach involves measuring behavioural responses in an open, novel test arena, and became popular due to its simplicity, and due to the ease with which behaviours could be measured quickly and repeatedly (Perals et al., 2017). Thus, open-field tests were used frequently to assess activity and exploration, and were later modified to facilitate recording responses to novel objects (Sih et al., 2004a; Réale et al., 2010b).

Although the use of open-field tests, along with the addition of novel objects and startle response tests, proved important in demonstrating axes of exploration (Verbeek et al., 1994), boldness (Fraser et al., 2001) and activity (Kurvers et al., 2009), some have suggested that interpretation of the test outcomes was not always straightforward (Bell, 2007; Carter et al., 2013). For example, there are suggestions that these tests could be measuring a number of different traits at the same time, or simultaneously (Perals et al., 2017). Further, some suggest that recent studies employing open-field tests are entirely descriptive and that they lack the necessary experimental manipulations required to test *a priori* hypotheses relating to factors underpinning individual variation (Dall & Griffith, 2014). Therefore, it is important that researchers consider the ecology of their chosen study species and that experiments are designed to test theory-driven predictions, such as those associated with resources acquisition trade-offs (Houston, 2010) or social feedback (Wolf et al., 2011). This is particularly important when addressing questions relating to individual behavioural variation from an evolutionary or ecological perspective, since behavioural variation may be overlooked if experiments fail to account for behavioural or ecological variables intrinsic to the chosen study system (Dall & Griffith, 2014).

Another important consideration for research methodology relates to whether behavioural assays are conducted under natural or laboratory conditions (see Bell et al., 2009; Biro, 2012). Specifically, where laboratory studies often involve capturing and relocating animals from the field and placing them in potentially stressful, novel conditions. Under these circumstances, short term behavioural assays may lead to estimates of behavioural repeatability that do not reflect behaviour

under more familiar (or wild) conditions (Biro, 2012). Although some evidence supports the potential for studies undertaken using captive individuals, or under novel conditions, to predict behaviour in the wild (Réale et al., 2000; Boon et al., 2008; Herborn et al., 2010), caution should be taken by considering habituation and acclimation to novel (or laboratory) conditions (Biro, 2012). It has also been suggested that studies carried out under these conditions should include a high number of repeat observations (≥10 per individual) to provide robust characterisation of labile behavioural traits (Biro, 2012). In addition, others highlight the importance of even higher sample sizes being necessary to detect correlations between labile traits (Dingemanse & Dochtermann, 2013). Of course, this is not always feasible, where logistical constraints influence experimental design, and where an increased number of repeat observations may lead to confounds involving habituation (Edwards et al., 2013). However, in order to avoid mis-representing behavioural traits or underestimating repeatability, both acclimation and habituation, along with number of individuals, and the number of repeat behavioural measures, should be considered as part of any informed experimental design (Biro, 2012; Dingemanse & Dochtermann, 2013; Biro & Stamps, 2015).

Quantitative analysis

Since repeatable variation is said to provide raw materials on which selection can act (Endler, 1986), it is repeatability (*R*) that has often been used as the standardised method for quantifying consistency in among-individual traits (Bell et al., 2009). Studies focussing on behavioural repeatability have thus far provided insight to the role of consistent behaviours across different behavioural continua (Réale et al., 2007; Conrad et al., 2011). Furthermore, general findings suggest that some behavioural traits are more repeatable than others, and also that repeatability estimates are often higher in field-based studies compared to those observed under laboratory conditions (Bell et al., 2009). Additionally, repeatability estimates for many behaviours are higher when observation intervals are short (Bell et al., 2009), and increase with age in some species (Dall et al., 2004). Traditionally used in quantitative genetics to estimate the proportion of variation in different traits that is attributed to the difference between (among) individuals (McGraw & Wong, 1996; Hayes & Jenkins, 1997; Nakagawa & Schielzeth, 2010), behavioural repeatability can also be estimated in much the same way (Bell et al., 2009). In statistical terms, the propensity for individuals to exhibit consistent behaviours across contexts is quantified as a "behavioural correlation across situations" (Sih et al., 2004a). Thus, both product-moment and Spearman's rank correlation have also been widely used (Sinn & Moltschaniwskyj, 2005; Bell & Sih, 2007; David et al., 2012). However, until recently the most common statistic used to estimate broad-sense

repeatability, that is to say the extent to which traits scores are maintained over time (Biro & Stamps, 2015), was the intraclass correlation coefficient (ICC; Hayes & Jenkins, 1997). Here, repeatability can be estimated from variance components derived from a one-way ANOVA, and is calculated using the following formula, presented by Lessells and Boag (1987):

$$R = S^2_A / (S^2 + S^2_A)$$

Where S^2_A refers to the among-group variance and S^2 refers to the within-group variance.

More recently, it has been highlighted that these early methods for quantifying repeatability may be flawed since they do not account for the potential variation occurring over time (Biro & Stamps, 2015). Given that consistency over time is a fundamental characteristic of personality, traditional correlations and ICC may well result in inaccurate or biased repeatability estimates (McGraw & Wong, 1996; Hayes & Jenkins, 1997; Biro & Stamps, 2015), especially where investigators are working with small sample sizes, or where control of experimental conditions does not account for other identifiable variables. Examples of which include environmental factors, or those associated with individual state (e.g. size, sex, appetite or maturity; Biro & Stamps, 2015). In a recent attempt to improve the validity of repeatability estimates, Biro and Stamps (2015) reinforce suggestions made by McGraw and Wong (1996), and later by Hayes and Jenkins (1997), that the majority of studies testing for repeatability in animal behaviour — where inferences were made about consistency over time — neglect to consider shared effects of time on behaviour. For example, where confounding effects need to be controlled for statistically, it is possible to calculate estimates of repeatability that are conditioned on the fixed effects of a given model (Nakagawa & Schielzeth, 2010; Dingemanse & Dochtermann, 2013; Biro & Stamps, 2015; Houslay & Wilson, 2017). This has been termed adjusted repeatability (R_j) and is of particular interest where behavioural changes over time differ among individuals (Nakagawa & Schielzeth, 2010). Further, this adjusted (or conditioned) repeatability is often estimated using linear mixed effects models (LMMs) and generalised linear mixed models (GLMMs; an extension of LMMs) for gaussian and non-gaussian data, respectively (see Dingemanse & Dochtermann, 2013; Stoffel et al., 2017).

Although measuring trait repeatability is informative, often yielding important results, it is only the first step in developing a better understanding of how variation occurs within complex labile traits (Roche et al., 2016). Furthermore, although behavioural consistency has been of interest to biologists for some time, more recent attention being given to the importance of both among- and within-individual plasticity, along with recent interest in studying individual variation from an adaptive perspective (Dingemanse & Dochtermann, 2013), has led to changes in the way data are analysed. For example, questions relating to the conditions favouring among-individual vs within-individual variance, or those focussed on the ecological and evolutionary processes influenced by

individual differences (see e.g. Bolnick et al., 2011; Fogarty et al., 2011; Sih et al., 2012; Wolf & Weissing, 2012), cannot be answered by using models that only account for among-individual variation (Cleasby et al., 2015). Therefore, these questions, as well as those relating to the covariation of labile traits, require the partitioning of variance components into their among- and within-individual levels (Dingemanse & Dochtermann, 2013; Houslay & Wilson, 2017; Niemelä & Dingemanse, 2018a; Royauté et al., 2018; Moirón et al., 2019). Additionally, it is also important to consider the need for longitudinal repeated measures data (Beckmann & Biro, 2013), and the need to accommodate the lack of independence between different observations (Biro, 2012; Niemelä & Dingemanse, 2018b). This new direction has been supported by the adoption of mixed-effects modelling techniques (Houslay & Wilson, 2017) the use of which, from both frequentist and Bayesian approaches, provides an overarching statistical framework for research focusing on both among- and within-individual behaviour (Dingemanse & Dochtermann, 2013; Cleasby et al., 2015; Houslay & Wilson, 2017).

Mixed effects models (LMMs & GLMMs) are used to analyse data collected in groups (in this case, of individuals), and can describe relationships between dependent variables and their fixed effects (independent variables), whilst also including random effects (grouping variables). This approach includes fitting individual or subject identity as a random intercept effect (Nakagawa & Schielzeth, 2010; Biro & Stamps, 2015), thus also providing a versatile framework for estimating different measures of repeatability (Stoffel et al., 2017). Further, mixed effects models also allow for the inclusion of time (e.g. date, trial number), and for other controlled variables to be specified as fixed effects, producing a separate 'fixed effects portion' intercept. In addition, they also allow for the inclusion of separate random slopes (random slope and intercept models), providing estimations of individual reaction norms by examining within-individual responsiveness across environmental gradients (Dingemanse et al., 2010b). By accounting for (co)variance at the among- and within-individual levels (Dingemanse & Dochtermann, 2013; Cleasby et al., 2015; Houslay & Wilson, 2017), mixed effects models also enable researchers to answer questions relating to the association between behaviour and other traits of interest, including those related to physiology, life history, and overall fitness (Houslay & Wilson, 2017).

However, one of the issues with implementing these more sophisticated analyses relates to their accessibility to ecologists, as although the tools used for partitioning variance are available, many examples focus on estimating genetic parameters and involve unfamiliar 'jargon' that make navigating the literature difficult (Dingemanse & Dochtermann, 2013). More recently, however, example data sets and online tutorials have made these resources more accessible and their effective use, more achievable. For example, Dingemanse and Dochtermann (2013) provide a comprehensive

'how to' guide to quantifying individual behavioural variation that outlines different approaches based on field-specific research questions. This along with many other excellent examples (Bolker et al., 2009; Martin et al., 2011; Cleasby et al., 2015; Mitchell et al., 2016; Houslay & Wilson, 2017; Stamps et al., 2018), should be considered as invaluable tools that can be used with a variety of software packages. Further, they provide the frameworks with which data are analysed as part of this work. Specifically, univariate mixed effects models (lme4; Bates et al., 2015) were used to examine among- and within-individual variation across environmental gradients, and multivariate mixed models (MCMCglmm; Hadfield, 2010) were used to examine covariation between multiple labile traits.

Causes, constraints and consequences

Maintenance, fitness and ecological trade-offs

The study of individual behavioural variation has led to an array of questions relating to how different behavioural phenotypes exist within single populations, for example whether they include heritable variation, and to what extent this variation has consequences for individual fitness (Bergmüller & Taborsky, 2010). Furthermore, although the ecological importance of individual behaviour is already somewhat evident from its wide-ranging taxonomic expression (Wilson et al., 2010), it may be possible to gain a more comprehensive understanding of how certain behaviours arise, and are maintained, by exploring specific ecological and evolutionary implications (Wilson et al., 2010). For example, it is well established that an individual's fixed traits (e.g. aspects of morphology such as heavy armour or overall size) can lead to ecological constraints and trade-offs (Sih et al., 2004a), however recent literature has highlighted the potential for less optimal behavioural plasticity to produce similar conflicts (Sih et al., 2012). Likewise, fitness trade-offs are also associated with individual traits due to time budget constraints (Sih et al., 2004a). For example, more active individuals may experience greater resource acquisition, however with more time spent foraging, they may also be under a higher risk of predation (Stamps, 2007; Smith & Blumstein, 2008). Similarly, where an environment is unpredictable, the perceived benefits of particular behavioural traits are less clear (Frost et al., 2007). One possible explanation for this was proposed by Smith and Blumstein (2008) who suggest that fitness associated with different personality traits depends on the context in which they are expressed. This gives weight to an earlier proposal suggesting that more aggressive individuals may benefit when competing for resources and for mates, but if similar levels of aggression are maintained under high predation pressure, survival should be negatively affected (Sih et al., 2004a). This may lead to correlations among demographic rates (births, deaths and movement between habitats; Sih et al., 2012) where, for example, bolder

individuals may have higher resource intake rates, which are likely to elicit higher birth rates. Conversely, under some circumstances bolder individuals taking greater risks will be subject to higher mortality (Sih et al., 2004a; Smith & Blumstein, 2008). Similar trade-offs are also predicted for life-history characteristics such as growth rates, which may also generate risk-mortality trade-offs, where faster growth is expected to generate a higher propensity for risk-taking, in foraging contexts (Stamps, 2007).

Furthermore, it has been hypothesised that personality traits may be adaptive where individuals at one end of a given axis have higher relative fitness than those at intermediate or opposing positions along the continuum (Wilson, 1998). In particular, where relationships exist between personality traits and measures of overall fitness, these traits are likely to be selected for under certain environmental conditions (Réale & Festa-Bianchet, 2003; Dingemanse et al., 2004). For example, as a particular behavioural trait is favourable in some environments e.g. less bold individuals may benefit from lower mortality where predators are in abundance, it is possible that being less bold (or shyer) may be a suboptimal strategy under other conditions (Ward et al., 2004; Sih et al., 2012). In this instance, behavioural variation may occur as a result of negative frequency-dependent selection, whereby exhibition of certain behaviours is likely to change the frequency of these behaviours in a given population. Specifically, the fitness benefits of a particular behaviour will decrease as the number of individuals expressing it increases (Wolf & Weissing, 2010). In this way, frequency-dependent selection can explain the adaptive coexistence of more than one behavioural trait within a given population (e.g. Wolf et al., 2007).

Another prediction is that the relative fitness benefits of certain behaviours may be lower where they are more susceptible to environmental change (Bell et al., 2009); an idea that has been supported by the varying fitness consequences of personality, under changing environments. These include varying predation pressure (Réale & Festa-Bianchet, 2003), changes in resource availability (Dingemanse et al., 2004), and different social circumstances (Both et al., 2005). Similar adaptive explanations centre on the intensity of species interactions, where bolder, and possibly more aggressive individuals are said to have a stronger impact on competitors and prey. Support for this idea was presented as part of a meta-analysis by Smith and Blumstein (2008), which reported greater reproductive success in bolder individuals (more so in males than in females) but, again, that boldness may incur survival costs. This further reinforces the idea that personality traits are maintained as a result of fitness trade-offs across contexts (the trade-off hypothesis; Sih et al., 2004a), and that trade-offs can lead to single populations existing with dramatically varying levels of a particular trait. This theme is also consistent with models explaining the evolution and maintenance of personality in wild populations proposed by Stamps (2007) and Wolf et al. (2007).

For example, Stamps (2007) suggests that individuals with faster growth rates, relatively early maturation and early reproduction will also be bolder, more aggressive and more active. If valid then life-history characteristics may also contribute to adaptive explanations for the evolution of animal personality, and further explain the coexistence, consistency and the structure of behavioural syndromes (Wolf et al., 2007).

Where evidence supports the theory that trade-offs can act as a fundamental mechanism maintaining behavioural diversity (Sih et al., 2012), it may be possible to identify causation. For example, Niemelä et al. (2015) report that bolder nymphs of the field cricket (*Gryllus campestris*) suffer from higher mortality than shyer conspecifics as a consequence of longer flight initiation distances. Similarly, North American ground squirrels (*Tamiasciurus hudsonicus*) that were relatively bold (active) were also found to be less likely to survive overwinter than shyer (less active) conspecifics (Boon et al., 2008). Additionally, some findings also suggest that fitness can be influenced by the combined effect of prey and predator personality traits (e.g. Pruitt et al., 2012; Belgrad & Griffen, 2016a). However, some empirical literature focusing on the influence of personality on fitness report mixed findings, for example Patrick and Weimerskirch (2014) found that personality correlated with reproductive success in black browed albatross (*Thalassarche melanophris*). Although, these results showed a high level of sex-dependence, in that shyer males and bolder females have higher relative fitness, and the strength of these relationships was shown to depend on the quality of the year from a foraging perspective (Patrick & Weimerskirch, 2014). On the contrary, some have reported evidence that contradicts predictions made by the trade-off hypothesis. Examples of which include smaller and bolder black tegula snails (*Chlorostoma funebralis*) experiencing higher survival than shyer conspecifics in staged predator encounters (Foster et al., 2017), and faster growth in more fearful (risk-averse) wild cavies (*Cavia aperea*) (Guenther, 2018). It is, however, worth noting the suggestion that single behavioural traits may be limited in their ability to predict fitness consequences (e.g. survival) as it is likely that other factors, behavioural or otherwise, would also be selected upon, depending on the context (e.g. overall size; White et al., 2013). Thus, although there are strong theoretical arguments for the maintenance of personality as a result of fitness trade-offs, it is also likely that a number of other factors also contribute to determining overall fitness, and that explanations of these variables require a solid understanding of the ecology of each study species (Dall & Griffith, 2014).

Proximate and ultimate causation

Despite much work aiming to elucidate the maintenance of behavioural variation within natural populations, the proximate and ultimate mechanisms causing behavioural traits to vary non-

independently from each other are still poorly understood (Dingemanse et al., 2004; Sih et al., 2004a, 2015; Stamps & Groothuis, 2010b; Herczeg & Garamszegi, 2012). Indeed, one of the fundamental, outstanding questions is that of the evolutionary basis of individual variation (Réale et al., 2007). Specifically, since behaviour is assumed to be one of the most labile aspects of an organisms phenotype (Sol & Lefebvre, 2000; Nicolakakis et al., 2003; Bergmüller et al., 2010; Foster, 2013; Edgell et al., 2017), and given that natural selection is predicted to produce optimal behaviour (Houston et al., 2007), a reduction in phenotypic variation might be expected to occur over time. This seems to suggest that animal personality and behavioural syndromes are all the more implausible, and thus determining the driving forces underpinning these aspects of individual phenotype has emerged as a major focus of behavioural research (Sih et al., 2015; Belgrad et al., 2017).

Possible causal explanations have been suggested based on four behavioural approaches (Tinbergen, 1963). For example, one proximate explanation for the correlation of behaviours (behavioural syndromes) may involve a common, mechanistic link between traits (Stamps, 2016; Mitchell & Biro, 2017). This might involve either the same genes (pleiotropy) or the same hormones acting on several target traits (Ketterson & Nolan Jr, 2017). In this case, limited plasticity and the incidence of behavioural correlations (as well as correlations between behavioural, life-history and physiological traits) may be constrained by evolution, resulting in changes in one trait producing a correlative response in another (Bell, 2005). A functional explanation may involve combinations of traits being favoured by correlational selection (Bell, 2007; Bell & Sih, 2007), whereby multiple behaviours emerge as adaptive responses to different environmental pressures (Carere et al., 2010; Dingemanse & Wolf, 2010). However, it has been predicted that behaviours under morphological or physiological constraints are more stable when compared to those influenced by an individual's immediate environment (Castellano et al., 2002; Smith & Hunter, 2005). From a developmental perspective, early differences in environmental conditions may potentially set individuals on different courses, exposing them to circumstances (environmental or biological) that shape suites of correlating behaviours (Stamps, 2003). Alternatively from an evolutionary perspective, since personality has been found to be heritable (Dingemanse et al., 2002; Van Oers et al., 2004; Schuett et al., 2010), offspring may depend on acquiring successful traits from their ancestors (Bell, 2007).

It is, of course, fundamentally important to determine why individual behaviour differs (Beekman & Jordan, 2017), however, despite the explanations discussed, a comprehensive understanding of the underlying processes is still lacking (Bell, 2017). Furthermore, it is important to note that in some cases these explanations may be linked, in particular where ultimate (evolution & ontogeny) explanations may be underpinned by proximate (mechanism & function) causes. That

is to say that ultimate and proximate explanations are not necessarily mutually exclusive (Tinbergen, 1963). While current explanations relating to adaptive personalities and evolutionary constraint centre on the possible ultimate causation, and although the maintenance of behavioural variation is likely to be a result of ecological and life-history trade-offs, current understanding of the potential proximate drivers of personality and plasticity rests on a few prevailing theories. These include the Pace-of-Life Syndrome hypothesis (Ricklefs & Wikelski, 2002; Réale et al., 2010b), and the state-behaviour feedback model (Houston & McNamara, 1999; Dingemanse & Wolf, 2010; Sih et al., 2015) — two frameworks that provide the theoretical underpinning of much of the research currently aiming to elucidate the existence of individual behavioural diversity.

State-dependent behaviour

According to the state-dependent feedback model, among-individual behavioural differences could be explained if they result from differences in slowly changing, or even fixed individual state variables (Belgrad et al., 2017). In this context, factors that govern individual state include aspects of physiological condition, morphology and environment (Sih et al., 2015; Belgrad et al., 2017). However, the state of an individual can include any variable known to influence the cost and benefits of its behavioural expression (Houston & McNamara, 1999), and thus behaviour could be adjusted in response to these state-governing factors, in order to optimise trade-offs between energy intake and mortality (Näslund & Johnsson, 2016). Under this framework, small differences in individual state can, through positive feedback loops, result in behavioural differences among individuals (Houston & McNamara, 1999; Wolf & Weissing, 2010; Sih et al., 2015). For example, individuals that tend to be bolder may gain more resources — if their higher propensity for risk-taking is correlated with greater resource intake — leading to increased state-related differences (i.e. greater resource disparity) between bold and shy individuals (Houston & McNamara, 1999; Sih et al., 2015). However, there is also the possibility that negative feedbacks could erode behavioural differences over time (Bergmüller & Taborsky, 2010; Luttbeg & Sih, 2010; Sih et al., 2015). This has been suggested as a potential outcome of exposure to anthropogenic contaminants known to affect individual state (e.g. contaminants that reduce activity; Egea-Serrano et al., 2011; Miaud et al., 2011). For example, more active individuals may experience increased exposure relative to less active conspecifics, leading to lower activity levels and to attenuation of among-individuals variance (Montiglio & Royauté, 2014).

Research examining state-dependent behaviour often focusses on correlations between intrinsic state variables such as metabolism (Toscano & Monaco, 2015; Careau et al., 2019), hormones (e.g. Calcagnoli et al., 2014), hunger levels (Stocker & Huber, 2001; Belgrad & Griffen,

2016b), or the assessment of risk (e.g. Briffa, 2013). However, evidence for these relationships is mixed, with a recent meta-analysis reporting that only a very small proportion of personality variation is explained by intrinsic state (3-8%; Niemelä & Dingemanse, 2018a). Therefore, it is also important to consider behavioural variation across temporal and environmental scales that are known to influence individual state. For example, behavioural changes have been shown to occur in response to early experience (Bell & Sih, 2007; Urszán et al., 2015, 2018), predation pressure (Briffa et al., 2008), parasitism (Barber & Dingemanse, 2010), food availability (Kontiainen et al., 2009), temperature (Biro et al., 2010), breeding season (Belgrad et al., 2017), and time of day (Dingemanse et al., 2002). Importantly, it is also quite likely that both extrinsic and intrinsic factors are linked under natural conditions, for example hormones are known to mediate the effect of parasites on behavioural expression (e.g. Lafferty & Shaw, 2013). Therefore, despite some mixed support (Niemelä & Dingemanse, 2018a), examinations of state-dependent behaviour represent an important avenue of future research, particularly when addressing questions relating the relative importance of local ecological conditions and the potential interactions between intrinsic and extrinsic state variables (Sih et al., 2015).

The Pace-of-Life Syndrome hypothesis

In addition to work on state-dependent behaviour, recent attempts to elucidate the proximate causes of among-individual behavioural variation have highlighted the potential importance of links between behaviour, life-history (e.g. growth, maturation) and physiology (e.g. hormones, metabolism and immunity) (Biro & Stamps, 2008; Careau et al., 2008; Réale et al., 2010b). The observed associations between these traits has led to the development of the POLS hypothesis (Ricklefs & Wikelski, 2002), where co-correlating traits are expected to be expressed as part of a fast-slow continuum (see Figure 1.1). Furthermore, it is suggested that POLS form as a result of trade-offs between present and future reproduction (Réale et al., 2010b; Le Galliard et al., 2013; Royauté et al., 2015; Salzman et al., 2018). Indeed, there has been an increase in accounts of associations between behaviour and other POLS attributes (Wolf et al., 2007; Biro & Stamps, 2008; Careau et al., 2010; Réale et al., 2010b; Sih & Del Giudice, 2012; Le Galliard et al., 2013; Niemelä et al., 2013), leading to the suggestion that trade-offs between survival and reproduction may be associated with the evolution of individual behavioural differences (Hall et al., 2015). For example, investment in current reproduction will likely favour 'fast-paced' individuals that are expected to be bolder (more risk-prone), with relatively high metabolism, higher growth rates and ultimately, due to their comparatively 'risky' lifestyle, a shorter life expectancy (Stamps, 2007; Biro & Stamps, 2008, 2010; Réale et al., 2010b; Royauté et al., 2018). Equally, investment in future reproduction

should favour shyer (risk-averse) individuals with 'slow' pace-of-life characteristics, such as slower metabolism, lower growth rates, slower maturation, high offspring investment and increased longevity (Stamps, 2007; Biro & Stamps, 2008; Réale et al., 2010b; Royauté et al., 2018).

Conceptually, the POLS hypothesis is simply an extension of the r-and k-selection theory (MacArthur & Wilson, 1967; Pianka, 1970), with the addition of physiology (Gaillard et al., 2006; Jones et al., 2008), and more recently, behavioural traits (Wolf et al., 2007; Réale et al., 2010b). Early empirical testing of the assumptions of the POLS showed promise, with findings including interspecific level associations between exploratory behaviour and age at first reproduction in muroid rodents (Careau et al., 2008); relationships between personality traits, life-history characteristics and metabolic rate in domestic dog breeds (Careau et al., 2010); and correlations between risk-taking behaviours, residual reproductive rate, and survival in superb fairy wrens (*Malurus cyaneus*) (Hall et al., 2015). However, despite evidence in support of POLS, some studies report mixed findings (e.g. Le Galliard et al., 2013), while others provide evidence that counters proposals presented as part of the POLS hypothesis (Niemelä et al., 2013; Bridger et al., 2015; Závorka et al., 2015; Velasque & Briffa, 2016). For example, Niemelä et al. (2013) identified a link between immune response and boldness in crickets (*Gryllus integer*); however, counter to the prediction that bolder individuals should show lower immune system investment, they found these variables to be positively correlated. In addition, Bridger et al. (2015) present evidence suggesting that bolder hermit crabs (*Pagurus bernhardus*) are less fecund than shyer individuals; once again countering predictions made by the POLS hypothesis. A similar outcome was also reported by Wilson et al. (2010), where bolder eastern mosquito fish (*Gambusia holbrooki*) were also found to be less fecund. Further, despite much attention being given to testing the predictions made by the POLS hypothesis over recent years, a recent meta-analysis reported a general lack of support for its predicted associations (Royauté et al., 2018).

Where empirical findings are mixed or contrary to POLS predictions, some alternative explanations have been offered. Bijleveld et al. (2014), for example, conclude that personality may drive physiological adjustments, rather than the other way around, and suggest that these adjustments mitigate the survival costs of exploratory behaviour. Other suggestions include the idea that "sensitivity" to resource abundance may be higher in fast-paced individuals (Závorka et al., 2015), or that environmental conditions may determine associations between life-history and behavioural traits (Niemelä et al., 2013). In addition, it is suggested that some traits (e.g. aggression and activity) may not automatically result in increased resource gains (Montiglio et al., 2018), and that the conditions under which studies are constrained may lead to varying degrees of association (Dammhahn et al., 2018). For example, detection of POLS may be determined by a variety of

environmental and ecological variables such as variation in predation risk, and individual state (Montiglio et al., 2018). Although frequent correlations have been observed between life-history, physiological and behavioural traits, there is clearly a highly variable nature to these associations (Killen et al., 2013). This variability may also hint at highly complex relationships between individual behavioural and physiological traits (McKenzie et al., 2015). Indeed, it is also assumed, as with the expression of personality, that these associations may be influenced by different ecological selection pressures (Biro & Stamps, 2008; Réale et al., 2010b; Binder et al., 2016). Consequently, it may be necessary to give further consideration to environmental effects (Killen et al., 2013), as well as to variation occurring across developmental stages of a given organism (Careau et al., 2015). Furthermore, McKenzie et al. (2015) highlight the important complexities of designing experiments aiming to evaluate causal relationships between physiology and personality; an idea initially put forward by Killen et al. (2013). Another potential issue here echoes previous discussions concerning the consistent measurements of behavioural traits in a comparable manner across species (e.g. Bell, 2007; Réale et al., 2007; Budaev & Brown, 2011; Carter et al., 2013), which has led to calls for 'more stringent' empirical research testing the predictions made by the POLS hypothesis (Dammhahn et al., 2018).

Energy metabolism

Integral to relationships predicted by both the POLS and state-dependent behaviour frameworks, energy metabolism has provided significant insight to behavioural and life-history evolution in a variety of taxa (e.g. Seebacher et al., 2006; Wiersma et al., 2007; Williams et al., 2010). Indeed, the fundamental processes of energy acquisition and allocation are integrative with an animal's physiology and behaviour (Horodysky et al., 2011), and are central to all biological processes (Biro et al., 2018). Furthermore, it has been reported that individual behaviour, specifically an animal's ability to compete and its overall dominance, is significantly affected by its metabolic rate (McCarthy, 2001). This, along with its relative among-individual stability over time (Metcalfe et al., 2016), has led to particular interest individual metabolic rate (MR) as part of explanations for the existence of individual behavioural variation (Careau et al., 2008; Biro & Stamps, 2010; Réale et al., 2010b; Metcalfe et al., 2016). Proximate explanations for the relationship between measures of MR and behaviour focus on the relatively 'expensive' metabolic processes necessary to maintain a fast or slow pace-of-life (e.g. Careau et al., 2008; McGhee et al., 2013; Binder et al., 2016; Krams et al., 2017). Specifically, that relatively high MR may necessitate increased risk-taking and greater investment in foraging as a result of increased energy demands (Biro & Stamps, 2010; Killen et al., 2011; Careau & Garland, 2012; Metcalfe et al., 2016; Monceau

et al., 2017), influencing an individual's capacity to make energy, and in turn, its rate of growth (e.g. Biro & Stamps, 2010). As a consequence, associations between behaviour and metabolism have been investigated as the primary focus of POLS studies in a variety of species (e.g. Biro & Stamps, 2010; Killen et al., 2012; Krams et al., 2013a, b; Careau et al., 2015, 2019; Mathot et al., 2015; McKenzie et al., 2015; Myles-Gonzalez et al., 2015; Biro et al., 2018).

The number of studies linking between individual differences in measures of MR and behaviour has rapidly increased over recent years (see reviews by Mathot & Dingemanse, 2015; Metcalfe et al., 2016), with many observations providing evidence for the co-correlation of fast POLS traits (higher metabolic rate and boldness, aggression, higher activity levels). These patterns have been identified in mammals (e.g. Gębczyński & Konarzewski, 2009; Lantová et al., 2011; Biro et al., 2018), birds (e.g. Bryant & Newton, 1994), fish (e.g. McCarthy, 2001; Huntingford et al., 2010; McKenzie et al., 2015; Myles-Gonzalez et al., 2015) and invertebrates (e.g. Krams et al., 2013a, 2017). This mounting evidence for positive relationships between personality and metabolic rate provides support for the association between behaviour and measures of MR, across taxa and continues to be a productive area of research. However, some findings report mixed (Careau et al., 2015, 2019; Mathot et al., 2015), or context-dependent (Killen et al., 2011, 2012; Mathot et al., 2015; McKenzie et al., 2015) relationships, while others report negative (Hammond et al., 2000; Mathot et al., 2015; Velasque & Briffa, 2016) or non-significant (Le Galliard et al., 2013; Royauté et al., 2015) correlations. Many of these studies, however, centre on minimal measures of MR (standard or resting rates), whereas recent developments suggest that aerobic scope (= maximum MR - resting MR) might be a more appropriate predictor of behavioural plasticity (Biro et al., 2018). In addition there are also recent suggestions that among-individual variation in mitochondrial efficiency (as it relates to the production of adenosine triphosphate; ATP) may also be important in explaining variation in performance (Salin et al., 2019).

Analogous with the recurrent situation in many avenues of research encompassed by, or connected with, individual behavioural variation, investigations observing patterns of association employ mixed approaches that lead to conflicting views on how future research should proceed (Careau & Garland, 2015; Mathot & Dingemanse, 2015). For example, one of the major assumptions of these investigations, which often goes untested, is that MR can be used as a proxy for energetic constraints. Further, it has been argued that without thoroughly testing this assumption, it is not possible to interpret patterns identified between metabolism and personality (Mathot & Dingemanse, 2015). Indeed, some suggest that there may not be a causal link between behaviour and MR (Krams et al., 2017), and that observed correlations may be driven by a shared proximate mechanism (Biro & Stamps, 2010). Thus, some studies call for further investigation in order to

elucidate the potential mechanisms generating these relationships; especially those that are context-specific (e.g. Mathot et al., 2015). Furthermore, as mentioned previously, there have been suggestions that environmental conditions may influence the observed relationships (Niemelä et al., 2013), and that this should be considered prior to any conclusions being presented. There is, in fact, mounting evidence suggesting that correlational selection pressure is likely the main driver acting on the evolution of favourable trait combinations (Sinervo & Svensson, 2002; Lancaster et al., 2014), and it has been argued that environmental stressors are a major influence on the reported inconsistencies with associations between MR and behaviour (Killen et al., 2013; Kandler & Bleidorn, 2015). This is a logical argument, given that metabolic rate is influenced by a variety of biological and environmental conditions (Horodysky et al., 2011), and so it is plausible that the relationships between MR and behaviour may also be influenced by different or changing conditions. Especially since it appears that environmental stressors are able to mask, reveal and modulate the covariation of physiological and behavioural traits (Killen et al., 2013).

The effects of environmental variables on behaviour, physiology and genetic correlations have been investigated previously (Sgrò & Hoffmann, 2004; Dingemanse et al., 2010a; Cameron et al., 2013); however, few specifically with regards to their impact on the relationships between physiology and behaviour (see review by Killen et al., 2013). Empirical evidence supporting the idea that environmental variables significantly affect associations between behaviour and physiology has thus far been reported in three distinct taxa; mammals (Chappell et al., 2004; Lantová et al., 2011), fish (Finstad et al., 2007; Killen et al., 2012), and invertebrates (Belgrad et al., 2017). For example, Finstad et al. (2007) found that the link between metabolic rate and performance (measured by activity) in juvenile Atlantic salmon (*Salmo salar*) depends on habitat complexity and the availability of protective cover. Similar results were observed in the deer mouse (*Peromyscus maniculatus*), where activity levels and resting metabolic rate were found to correlate but that the strength of this correlation varied significantly with temperature change (Chappell et al. 2004). Although these examples provide compelling evidence, it is yet to be established whether similar observations can be identified in different species across a wider range of taxonomic groups. Furthermore, the notion that environmental stressors impact on the relationships between behaviour and physiology is still relatively novel; however, it is certainly worthy of further investigation. This will support a greater understanding of how individuals and species respond to environmental change and other variables such as anthropogenic activity (Killen et al., 2013; Delarue et al., 2015), and may further explain behavioural variation in wild populations. Despite ongoing debate as to the most suitable approaches, the link between physiology and individual behaviour is certainly evident

from the available literature. Consequently, this will surely stimulate future research aiming to provide a comprehensive explanation of the origins of personality and behavioural plasticity.

Ecological and evolutionary consequences

It is generally accepted that individual behaviour can influence individual fitness (Piersma & Drent, 2003; Dingemanse & Réale, 2005; Réale et al., 2007; Biro & Stamps, 2008; Smith & Blumstein, 2008; Stamps & Groothuis, 2010a), affecting both reproduction (Both et al., 2005; Sinn et al., 2006; Wilson et al., 2010; Rangassamy et al., 2015) and survival (Boon et al., 2008; Smith & Blumstein, 2010; White et al., 2013; Foster et al., 2017; Moirón et al., 2019). Furthermore, some have reported greater within-individual variance under higher predation risk (Maye et al., 2007; Stamps et al., 2012), suggesting that predictability of behaviour, in particular, could be directly related to survival. Additionally, individual behaviour has also been reported as being associated with individual stress levels (Archard et al., 2012; Oswald et al., 2012), and to influence sexual selection (Schuett et al., 2010), as well as learning and cognition (Carere & Locurto, 2011; Cole & Quinn, 2012; Sih & Del Giudice, 2012; Griffin et al., 2015). Some studies, for example, have shown that bolder or more aggressive individuals make faster decisions than shyer/less aggressive conspecifics (Mamuneas et al., 2015; Chang et al., 2017a), without being less accurate (but see Wang et al., 2015). This has been suggested as a functional explanation for why, in many species, bolder individuals lead and shyer conspecifics follow (e.g. Dyer et al., 2009), as having bold 'leaders' within a population may lead to faster group decisions without compromising accuracy in decision making (Mamuneas et al., 2015).

More recently, an even broader picture has been developing, with suggestions that individual behaviour can influence a variety of factors that may impact on the general structure of ecological networks (Bolnick et al., 2011; Sih et al., 2012). These factors include, but are not limited to foraging (Kurvers et al., 2009; Bergvall et al., 2011), competition (Cole & Quinn, 2012), dispersal (Cote & Clobert, 2007; Cote et al., 2013; Thorlacius et al., 2016), predator/prey interactions (e.g. Pruitt et al., 2012; Foster et al., 2017; Blake et al., 2018), and social dominance (Colléter & Brown, 2011; David et al., 2011; Favati et al., 2014). Further to these local effects, evidence also suggests that individual behavioural variation may also have significant implications for some of the major applied issues within the study of ecology (Réale et al., 2007). The emerging picture strongly indicates that ecological and evolutionary processes (including their interactions) are influenced by individual behavioural variation, and that this should be considered as a key area of ecological and evolutionary biological research (Wolf & Weissing, 2012). This should be unsurprising, given that the patterns of both genetic and phenotypic variation are able to determine the direction and the

result of natural selection (Barrett & Schluter, 2008; McNamara & Leimar, 2010). However, until recently, research focussing on within-population behavioural variation has tended to focus on a limited number of issues (Wolf & Weissing, 2012). Indeed, the importance of individual behavioural variation did not become fully apparent until it was studied explicitly in relation to issues such as biological invasions (Sol & Lefebvre, 2000; Fogarty et al., 2011; Chapple et al., 2012; Juette et al., 2014; Thorlacius et al., 2016), epidemiology (Lloyd-Smith et al., 2005), population stability, and extinction risk (Pruitt, 2013; Pruitt & Modlmeier, 2015).

The subject is now receiving significant attention, with a number of review articles compiling both theoretical and empirical work. For example, a review produced by Sih et al. (2012), suggested that species abundance and distribution can be significantly affected by an individual's ability to cope with different situations, such as foraging and predation — as part of the fitness trade-offs associated with different behavioural traits. Furthermore, this work supports the notion that spatial ecology can be personality-dependant and that this can have significant influence on the ecology of invasive species (Sih et al., 2012). Other cases for the importance of individual variation for ecological and evolutionary process have been posited by Wolf and Weissing (2012), and by Mittelbach et al. (2014), providing varied empirical and theoretical support for the influence of personality on ecological important issues, including population productivity, the resilience of populations, social evolution, and community structure. With these ecological issues in mind, and as our understanding of the influence of among- and within-individual variation develops, it is to be expected that some aspects of the field should be applied to conservation and wildlife management strategies (Berger-Tal et al., 2016; Merrick & Koprowski, 2017; Bremner-Harrison et al., 2018). In particular, phenotypic management relating to species re-introductions (Bremner-Harrison et al., 2004; Watters & Meehan, 2007; Kelleher et al., 2018), translocations (Baker et al., 2016), selective harvesting (Biro & Post, 2008), and habitat restoration (Watters et al., 2003). This is already being considered in detail for some taxa that have been studied extensively. For example, Conrad et al. (2011) provide an overview of the implications of individual behaviour for ecology and the management of fisheries; highlighting the importance of individual behaviour in developing effective species management strategies. Similarly, despite less work centring on this group, Kelleher et al. (2018) provide a framework for the application of individual behaviour to amphibian conservation; focussing on breeding and post-reintroduction survival. These examples highlight a growing awareness of the applications of among- and within-individual behavioural research, based on its ecological and evolutionary implications, and provide a solid foundation and direction for future studies.

Project overview

Intertidal environments and the saltmarsh periwinkle (<u>Littoraria irrorata</u>)

Intertidal organisms are subjected to daily fluxes of various environmental parameters that can influence labile traits (Dahlhoff et al., 2002), as well as species interactions (Menge & Olson, 1990), and overall distribution (Harley & Helmuth, 2003). These environments are often characterised by steep environmental gradients, such as those associated with temperature, tidal flux, and food availability (Dahlhoff et al., 2002). For example, *Spartina alterniflora*-dominated saltmarshes are highly structured, heterogeneous environments (Cantero et al., 1998), providing a variety of environmental gradients that can influence the behavioural responses of their resident species (Komers, 2008). With physical conditions regularly shifting between the marine and the terrestrial (Manica et al., 2000), saltmarsh inhabitants experience daily tidal inundation, extreme changes in salinity, as well as varying thermal and desiccation stress between both the high and low marsh (Baxter, 1983), and within the *S. alterniflora* canopy (Gömez-Cornejo, 1993). Under these conditions, organisms often rely on behavioural and physiological mechanisms to avoid exposure to unfavourable conditions (Manica et al., 2000). Furthermore, where previous suggestions link fluctuating environments to increased variability in behavioural phenotypes (Luttbeg & Sih, 2010), intertidal habitats provide ideal opportunities to study behavioural variation — particularly in relation to environmental gradients.

Figure 1.3 *The saltmarsh periwinkle (<u>Littoraria irrorata</u>).*

The saltmarsh periwinkle (*Littoraria irrorata*; Figure 1.3) is an abundant herbivore, and an important consumer on the intertidal marshes of the Southern Atlantic and Gulf coasts of the United States (Vaughn & Fisher, 1992; Iacarella & Helmuth, 2011; Stagg & Mendelssohn, 2012). It plays

an important role in ecosystem productivity by affecting nutrient cycling, microbial communities, and other invertebrates (Kemp et al., 1990; Silliman & Bertness, 2002; Atkins et al., 2015). It also acts as a vital link between saltmarsh primary and secondary production, and has been proposed as a possible contributor to marsh die-back events (Silliman & Zieman, 2001; Silliman et al., 2005). *L. irrorata* is often closely associated with the emergent vegetation, commonly, smooth cordgrass (*Spartina alterniflora*) (Stiven & Hunter, 1976; Stiven & Kuenzler, 1979; Rader, 1984), which provides refuge from predators (Vaughn & Fisher, 1988), as well as a key source of nutrition (Hamilton, 1976; Warren, 1985). It is also well known for its habitual, vertical migrations on cordgrass stems (Stiven & Kuenzler, 1979), occurring in synchrony with the incoming tide (Hovel et al., 2001). While attached to the stems, *L. irrorata* contribute to a "facultative, proto-farming mutualism" (Morton, 2018) whereby, snails use their radula to create longitudinal 'scars' on the plants leaves. These are subsequently invaded by fungal pathogens that are, in turn, consumed by *L. irrorata*; a procedure that contributes to top-down suppression of marsh productivity (Silliman & Newell, 2003). This shredding and decomposition of *S. alterniflora* stems influences the cycling of organic matter and may also regulate plant productivity. As well as feeding on fungal pathogens, *L. irrorata* is also known to feed on standing, dead stems (Bärlocher & Newell, 1994a), with previous research reporting considerably higher growth rates in snails fed exclusively on standing stems, both alive (with fungal growth) and dead, compared to those fed on marsh detritus alone (Bärlocher & Newell, 1994a).

In addition to foraging, climbing behaviour in *L. irrorata* has been linked to increased predation risk from fish (*Fundulus* spp), turtles (e.g. *Malaclemys terrapin*), and crabs (particularly the blue crab, *Calinectes sapidus*) at high tide (West & Williams, 1986; Vaughn & Fisher, 1988, 1992), as well as to higher substrate temperatures (increased risk of desiccation and osmotic stress; McBride et al., 1989; Iacarella & Helmuth, 2011), and extremes in salinity (McBride et al., 1989; Henry et al., 1993). Consequently, it is generally accepted that behavioural flexibility, in relation to vertical migrations, allows *L. irrorata* to alter their movements to avoid dangerous situations where predation risk, high winds, high temperatures or desiccation can be to the detriment of the animal (Hovel et al., 2001). Although climbing behaviour in this species has been well-documented, there is little known in regard to within-population variation (Vaughn & Fisher, 1992) and as yet, there has been no exploration of whether *L. irrorata* climbing behaviour varies at the among-individual level. Furthermore, studies are yet to consider how this climbing behaviour might be influenced by personality traits or whether these snails exhibit consistent individual climbing behaviour in general. Exploring this idea is likely to provide further insight to the behavioural ecology of *L. irrorata* and may help to elucidate factors influencing individual fitness, where for example, climbing behaviours

make up part of an animals' behavioural phenotype. Although research is yet to focus on behavioural variation occurring among- and within-individuals in *L. irrorata*, previous work has provided significant insights into the ecology of the species (Stiven & Hunter, 1976; Baxter, 1983; Vaughn & Fisher, 1988; McBride et al., 1989; Graça et al., 2000; Silliman & Newell, 2003; Iacarella & Helmuth, 2011). This is of particular importance, where such explanations are necessary in order to account for the observed structure of behavioural variation (Dall & Griffith, 2014; Montiglio et al., 2018).

Furthermore, despite efforts to highlight the potential importance of studying individual behaviour of invertebrates in general (Mather & Logue, 2013; Kralj-Fišer & Schuett, 2014), and despite invertebrates representing over 95% of all animal species (Scheffers et al., 2012), until recently the majority of research within the field has focussed on vertebrate animals (see Gosling, 2001). The now increasing interest in invertebrates as model organisms can be attributed, in part, to the relative ease with which they can be bred, reared, and maintained under laboratory conditions, as well as their relatively short life cycles (Kralj-Fišer & Schuett, 2014; Labaude et al., 2018). These factors facilitate many avenues of behavioural research that may be more challenging when using vertebrate species, and in particular, they support the incorporation of larger relative sample sizes, careful control, and allow for the use of many repeat measures during longitudinal studies. All of which have recently been highlighted as important challenges associated with studying and analysing individual behaviour (Niemelä & Dingemanse, 2018a; Royauté et al., 2018). Furthermore, since most invertebrate taxa are still under represented within the field of individual behaviour (Labaude et al., 2018), this work provides a rare opportunity to explore patterns of behavioural variation in a novel species, and to further elucidate areas of behavioural variation in invertebrates.

General methodology: study location

All studies undertaken as part of this thesis were carried out in collaboration with the Virginia Institute of Marine Science (VIMS), College of William & Mary, and all data collection was undertaken at the VIMS Eastern Shore Laboratory (ESL), Wachapreague, VA, USA (37°36'28.6"N 75°41'11.8"W) (Figure 1.4). Experimental materials were obtained from the high salinity marshes located behind the U.S. Atlantic barrier island system along the Virginia coastline, consisting of expansive monotypic *S. alterniflora* salt marsh. The region has limited barrier island development and limited public access, resulting in low anthropogenic stressors that might confound behavioural responses. A mean high water of 1.28m and mean low water of 0.05m result in regular inundation of *S. alterniflora* marsh in the upper intertidal range to an approximate 0.3m height (NOAA, 2018).
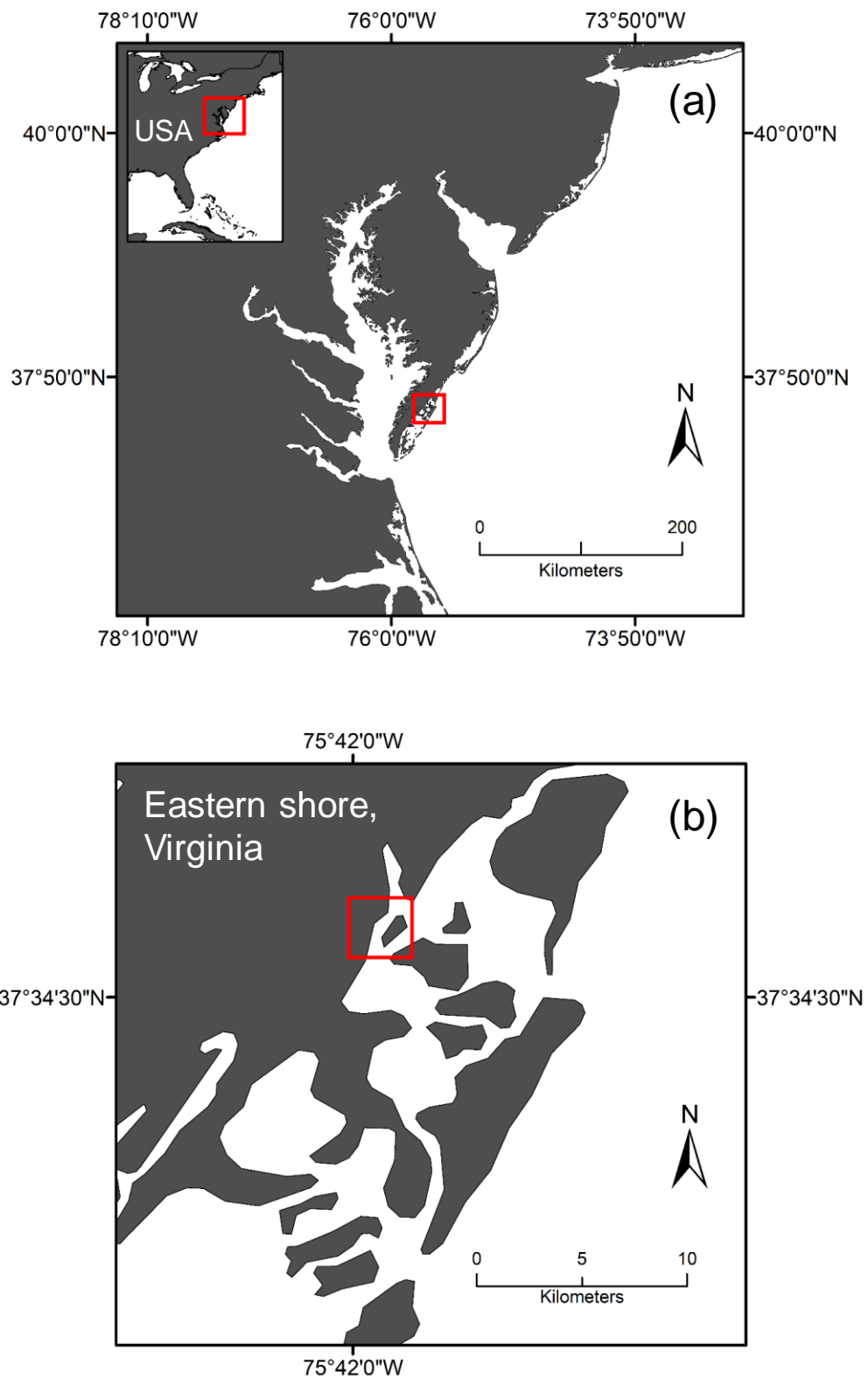
Figure 1.4 The location of the Chesapeake Bay and Eastern Shore, along the East coast of the United States of America **(a)***, and the location of the Virginia Institute of Marine Sciences (VIMS), Eastern Shore Laboratory (ESL), Wachapreague, VA, USA* **(b)***.*

General methodology: experimental design

Experiments were conducted either in the laboratories themselves or in outdoor plots, under semi-natural conditions (exposed to ambient temperatures, light cycles, water quality and weather patterns). Due to the importance of obtaining accurate repeat measurements of behaviour (Niemelä & Dingemanse, 2018b) across relatively large numbers of individuals, 'replica' marsh plots were used to house study specimens as part of each of the four experimental studies. Experimental design differed between studies and thus detailed descriptions of each setup are provided within the appropriate chapters. As an overview, marsh plots (Figure 1.5) were created such that experimental animals experienced ambient conditions similar to those on the natural marsh (e.g. changing temperature, weather, and tidal flux), whilst also facilitating careful and accurate recording of behaviour throughout the day and overnight. In each of the four experimental studies, estimates of plant biomass were made for the natural marsh and for plant biomass in each marsh plot using a power regression between stem height and stem dry weight (g) ($y = 0.0002x^{2.21}$, $r^2 = 0.887$) presented by Thursby et al. (2002). Since plant biomass is not a key focus of this work, the aim was to use estimates to ensure consistency between natural plant biomass and that of the experimental plots. Thus, it was possible to rapidly estimate dry plant biomass (g dry weight m^{-2}) by calculating the average stem height from all stems over 10cm (smaller stems contribute little to the overall biomass; Thursby et al., 2002), within a 0.5m^2 quadrat, and by using the regression equation to calculate the average individual dry stem weight. This number was then multiplied by the total number of stems to provide an estimate of total dry weight, scaled to grams m^{-2}. The procedure was repeated five times within the natural marsh and for each replica marsh plot.
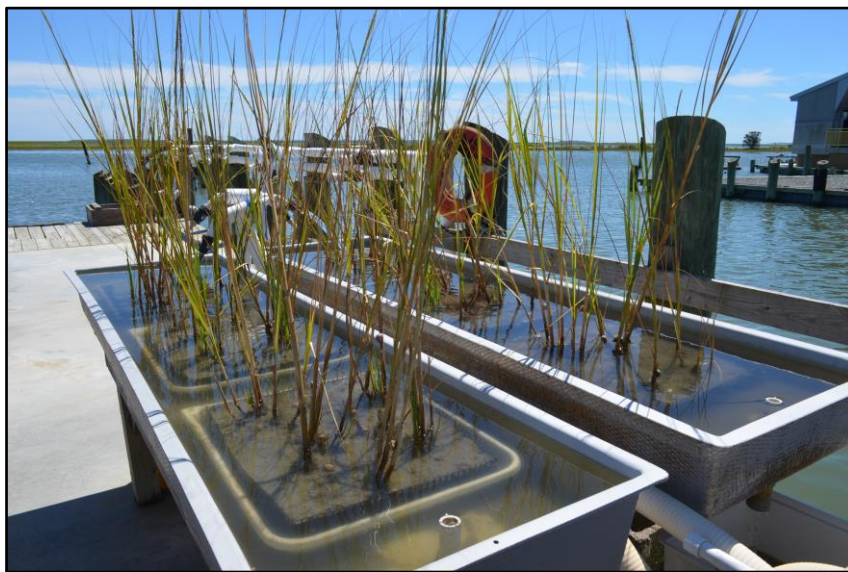
Figure 1.5 *Representative example of marsh plots created to house study* <u>*L. irrorata*</u> *specimens.*

Thesis outline

As our understanding of individual behavioural variation has developed, new concepts have altered perceptions of behavioural adaptation to include a view that individual behaviour is, at least in part, constrained by characteristics of the individual (e.g. Réale et al., 2010a). Looking for explanations relating to the causes, constraints and consequences represents the current focus for individual behaviour research and has generated a number of theoretical frameworks, as well as a calls for further empirical work. Therefore, by empirically testing predictions made by the prevailing theories, and by building on recognised associations at the species level (whilst also considering the influence of environmental stressors), it may be possible to contribute to a deeper understanding of the mechanisms underpinning among- and within-individual behaviour. In addition, although many avenues are likely to continue to provide valuable insights to this complex field, the continued investigation of the presence of individual phenotypic variation in lesser studied taxa, whilst adhering to standardised methodology (e.g. Dingemanse & Dochtermann, 2013; Biro & Stamps, 2015), will support the development of group-specific frameworks, whilst identifying more generalised patterns occurring across test species (Foster et al., 2017). Further, this work considers ongoing developments and discussions surrounding the most appropriate methods for future studies (e.g. Biro & Stamps, 2015; David & Dall, 2016; Dammhahn et al., 2018; Mathot & Frankenhuis, 2018; Montiglio et al., 2018), which provide valuable guidance for the following empirical research.

In order to support this developing field and to make a significant and novel contribution to an improved understanding of how and why individual behavioural traits evolve, it is important to consider the associations between behavioural differences and fundamental aspects of physiology and evolutionary biology (Roche et al., 2016). Consequently, the overall aim of this work was to elucidate aspects of individual behavioural variation in a novel study species, *Littoraria irrorata*. Although this species has been the subject of considerable past research, much of this work is centred on broad ecological concepts (e.g. Vaughn & Fisher, 1992; Iacarella & Helmuth, 2011), as well as their characteristic climbing behaviours (McBride et al., 1989; Hovel et al., 2001). Therefore, the potential finer-scale variation in behaviour exhibited by *L. irrorata* has, as yet, been overlooked. Thus, through a series of experiments designed to examine personality and plasticity under varying conditions on the saltmarshes of Virginia's Eastern Shore, this work is expected to provide the first account of individual behavioural patterns in this species. Initially, the work aimed to identify consistent individual behaviour in *L. irrorata* and to examine variation across 24-hour diel cycles (**CHAPTER 2**). In response to recent calls to consider the implications of local ecological conditions, short- and long-term environmental changes, and species specific natural history (Dammhahn et al., 2018; Montiglio et al., 2018), the work also aimed to examine behavioural

consistency and flexibility in response to two important environmental variables known to influence individual state; tide and temperature (**CHAPTER 3**). Further, in order to test important theoretical predictions made by the POLS hypothesis, the associations between behaviour, life history and physiology were examined using a multivariate mixed modelling approach (**CHAPTER 4**). Subsequently, the work aimed to examine the fitness consequences associated with behavioural variation in this species by examining the among-individual associations between boldness and survival during staged predator-prey encounters (**CHAPTER 5**). Finally, each chapter addresses important (and/or outstanding) questions relating to the possible causes and constraints acting on individual behaviour, with a focus on two of the prevailing theories underpinning its proximate and ultimate causation; The pace-of-life hypothesis (Ricklefs & Wikelski, 2002; Wikelski et al., 2003; Wiersma et al., 2007; Réale et al., 2010b) and that of state-dependent behaviour (Wolf & Weissing, 2010; Sih et al., 2015). It is also expected that this work would contribute to developing a broader understanding of the factors influencing among- and within-individual behaviour — with a view to generating predictions relating to their causes, constraints and consequences.

The main objectives of the work were to (1) repeatedly measure risk-taking behaviour, through a standardised assay, (2) examine individual responses across contexts, (3) quantify the among- and within-individual associations between behavioural, physiological, life-history traits, as well as between behavioural traits and measures of fitness using current statistical approaches (i.e. mixed effects models), and (4) to experimentally test predictions made by the prevailing theories in order to examine the validity of each. The structure of the final thesis was intended to provide original research articles, suitable for publication, and as such, to provide a substantial, novel contribution to the growing body of research currently being conducted within this field.

CHAPTER TWO

Individual behaviour and circadian rhythms: the influence of diel cycle on individual risk-taking in the saltmarsh periwinkle, *Littoraria irrorata*.

The results of this chapter are currently being prepared for journal submission.

Abstract

The ability of individuals to detect and respond to changing environmental conditions requires a balance between consistent and flexible behaviour. Both behavioural consistency (animal personality) and the expression of behaviour as a function of environmental variation (behavioural plasticity) have been extensively studied in recent years. However, despite a variety of environmental contexts being considered, and despite recent evidence for among-individual consistency in circadian behaviours (sleep or sleep-like behaviours), thus far little attention has been given to how individual behavioural variation changes across diel cycles (24h light – dark cycle) in wild populations. In order to elucidate the possible influence of diel cycles on the expression of individual behaviour, three behavioural traits related to risk-taking; boldness, activity, and individual climb latency during incoming tide, were examined using a multivariate mixed modelling approach, in the saltmarsh periwinkle (*Littoraria irrorata*), as part of a controlled laboratory experiment. Results revealed clear variation among individuals in all three behavioural traits across diel contexts, indicating behavioural sensitivity to day-night cycle. Boldness, activity and climb latency were all found to be repeatable (R_j = 0.29 - 0.48), and varied consistency depending on context. Among-individual covariation provided detectable correlations between boldness and activity, boldness and climb latency, and between activity and climb latency, indicating the presence of a behavioural syndrome linking all three traits. These results provide the first account of personality in *L. irrorata*, as well as substantial evidence for the influence of diel context on individual behavioural variation related to risk. Thus, also providing support for state-dependent behaviour in this species, whilst also highlighting the importance of local ecological conditions in assessing the stability and adaptive value of individual behaviour.

Introduction

Circadian rhythms influence many aspects of physiology and behaviour in a wide range of organisms (Ito & Tomioka, 2016), providing predictable cues relating to changing environmental factors such as temperature and light (Závorka et al., 2016). However, despite their importance in relation to behavioural processes, the implications of circadian rhythms have been somewhat neglected by studies centring on individual behavioural variation (Randler, 2014) — the subject of much attention in recent years (e.g. Sih et al., 2004a; Stamps & Groothuis, 2010b; Carere & Maestripieri, 2013; Roche et al., 2016; Stamps, 2016). This is somewhat surprising given recent research highlighting the importance of local ecological conditions, short- and long-term environmental changes, and species-specific natural history in the study of individual behavioural differences (Dammhahn et al., 2018; Montiglio et al., 2018). In particular, where these factors may mask patterns of behavioural variation or correlations between phenotypic traits (Dammhahn et al., 2018), and where they are known to influence individual state (Sih et al., 2015). Yet, despite some recent attention being given to repeatable variation of sleep, or sleep-like behaviours, in vertebrate animals at the among- and within-individual levels (Steinmeyer et al., 2010; Stuber et al., 2015; Alós et al., 2017), studies examining the influence of diel cycles on consistent individual behavioural differences (animal personalities), behavioural flexibility (plasticity), and trait correlations (behavioural syndromes) are currently scarce (but see Biro et al., 2014; Watts et al., 2015; Závorka et al., 2016; Alós et al., 2017), particularly in invertebrate animals.

The entrainment of circadian rhythms is regulated by the pineal hormone melatonin, typically released during periods of darkness, which has been found to control patterns of activity, behaviour, and sleep in vertebrates (Redman et al., 1983; Jiang et al., 1995; Sugden et al., 2004). In invertebrates, melatonin can also influence locomotor activity (Yamano et al., 2001; Bentkowski et al., 2010), as well as reproduction (Balzer & Hardeland, 1991; Gao & Hardie, 1997) and limb regeneration processes (Yoshizawa et al., 1991; Tilden et al., 1997). In many cases, the adaptive significance of circadian rhythms has often been assumed due to their ubiquity in nature, and due to the expected fitness advantages that they provide (Sharma, 2003). Specifically, circadian rhythms offer a selective advantage where organisms are able to anticipate and respond to daily changes in light through endogenous cellular mechanisms, by modifying behaviour and physiology to suit current environmental conditions (Green et al., 2002; Panda et al., 2002; Sharma, 2003). For example, many species are more active during the night, when foraging activities may be less risky (Nelson & Vance, 1979; Johnson & Covich, 2002; Keitt et al., 2004). In these cases, it might be expected that individuals would be more risk-averse during the day, particularly where key predators

are visually orientated and tend to avoid hunting when periods of darkness are likely to compromise the efficiency of successful prey capture (Regular et al., 2011).

Currently, however, little is known about how individual animals mitigate risk across diel cycles in the wild since repeatable traits related to risk, such as boldness and activity are almost invariably only quantified on a diurnal basis (but see Biro et al., 2014; Yli-Renko et al., 2015; Závorka et al., 2016) — resulting in a lack of information regarding the exhibition of nocturnal behaviours, and any potential variation occurring across day/night contexts (Závorka et al., 2016). Furthermore, since natural daily light cycles are an important environmental variable, previously shown to influence circadian variation in activity (Zann, 1973; Keitt et al., 2004; Monterroso et al., 2013) and metabolism (Laposky et al., 2008), it has been suggested that behavioural studies conducted only under diurnal conditions may bias our understanding of among-individual behavioural variation in wild populations (Závorka et al., 2016). However, since animals are able to manage the risk of predation by modulating their circadian activity patterns (Reebs, 2002; Kronfeld-Schor & Dayan, 2003; Hut et al., 2013), it is important to consider how diel cycles influence risk-taking behaviour, especially in cathemeral species, known to be active throughout light and dark periods.

To that end, individual behavioural traits related to risk-taking; boldness, activity, and with individual climbing behaviour, during incoming tide, were quantified over time and in relation to diel cycle in the saltmarsh periwinkle (*Littoraria irrorata*), as part of a controlled laboratory experiment. By measuring behavioural traits repeatedly over time and across day and night contexts, this study aimed to identify (a) whether these behavioural traits show context-specific consistency at the among-individual level, (b) whether there is among-individual variation in plasticity across these contexts, and (c) whether diel cycle influences among- and within-individual associations between risk-related behaviours in this species. Previous work examining physiological changes in *L. irrorata* has described daily rhythms in oxygen consumption, with a significant increase in O_2 consumption at night (Shirley & Findley, 1978; Shirley et al., 2007). Further, it is suggested that these findings may reflect changes in activity, such as foraging and general movement to more optimal conditions (Shirley et al., 2007); possibly prompted by reduced predation pressure at night (Shirley & Findley, 1978). This seems plausible given that blue crabs (*Callinectes sapidus*), a major predator of *L. irrorata*, are visually-oriented (Hamilton, 1976; Baldwin & Johnsen, 2011), and although known to forage across the diel cycle (Clark et al., 1999), feed mainly during dawn and dusk (Wolcott & Hines, 1989). In addition, since temperature has previously been shown to influence behaviour in *L. irrorata*, with activity increasing proportionally with an increase in

temperature within a range of 18-38°C (Bingham, 1972b), to avoid systematic confounds between temperature and light, temperature was controlled as part of this study.

Previous work on *L. irrorata* has attributed their circumtidal vertical migrations on the stems of the cordgrass (*Spartina alterniflora*), to a species-specific predator avoidance strategy (West & Williams, 1986; Vaughn & Fisher, 1988, 1992; Carroll et al., 2018), and thus climb latency should be considered as a risk-related behavioural trait. Further, although increased activity at night has also been reported previously in littoral gastropods (Zann, 1973), this has thus far not been examined explicitly in *L. irrorata* (Shirley & Findley, 1978). However, since activity, boldness and oxygen consumption (as a proxy for metabolic rate) are hypothesised to covary among individuals (Careau et al., 2008; Biro & Stamps, 2010; Careau & Garland, 2012), and given that activity is often expected to form a behavioural syndrome with measures of boldness (Sih et al., 2004a; Dingemanse et al., 2007), it was predicted that, on average, individuals would be bolder and more active (move further) during night-time observations. Further, it was also predicted that climb latency during the incoming tide would covary with boldness and activity across day and night contexts. It was also predicted that bolder/more active individuals would be less likely to form aggregations (group together) as a possible strategy for reducing the risk of predation. Finally, individual behavioural traits were expected to vary temporally across the many repeat measures, reflecting habituation to experimental protocols and/or uncontrolled external factors that vary over time (Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016).

Materials and methods

Experimental design

The experimental set up consisted of a single water table (approx. 220 x 67 x 31cm) connected to a flow-through system drawing water from and returning it to the adjacent Wachapreague channel. Within the water table, three 'marsh plots' (0.20m^2) were created by filling open top white photo trays (53 x 38 x 12cm) with 12cm deep marsh cores, complete with cordgrass roots and intact stems, taken from the adjacent *S. alterniflora*-dominated saltmarsh (37°36'30.0"N 75°41'07.5"W). Marsh plots were constructed such that estimated biomass density (biomass m^{-2} ± SD, 222 ± 6.2g) and average *S. alterniflora* stem height (mean ± SD, 139 ± 13.2cm) were consistent across each plot, and comparable with that observed in the surrounding marsh (biomass m^{-2} ± SD, 264 ± 82g; stem height ± SD, 163 ± 35.6cm). The experiment was conducted in a temperature-controlled laboratory with conditions maintained at 28–28.5°C, and under a 14L:10D diel light cycle, approximating average local conditions during the summer.

Collection and husbandry

Adult snails ($N = 81$; 21.03 – 28.77mm shell length, 2.31 – 4.62g total wet weight) were collected haphazardly, at low tide, from an 8m^2 patch of mid-marsh adjacent to the Eastern Shore Laboratory (37°36'30.0"N 75°41'07.5"W), in July 2018. All snails were transported to the laboratory and were held in seawater for 30 minutes to allow them to fully hydrate (Iacarella & Helmuth, 2011). Individuals were then stimulated to retract into their shells and to close their opercula by applying gentle pressure to the foot of each animal using a blunt-ended seeker (dissection probe), to minimize variation of mantle cavity fluid across specimens (Henry et al., 1993; Iacarella & Helmuth, 2011). Excess water was then removed from each of the shells prior to being weighed (g), measured (shell length, mm), and individually number-marked, by using permanent marker covered with non-toxic aquarium glue (Stagg & Mendelssohn, 2012). Sex was then determined, by visual inspection for the presence or absence of the male reproductive organ (43 females and 38 males). Only animals with fully intact shells, and without any obvious parasites, were included in the study.

Subsequently, snails were randomly allocated to one of three groups and each group was then assigned to one of the three experimental marsh plots (27 individuals per plot), where they were able to feed *ad libitum* on living and standing dead cordgrass stems, as well as benthic detritus. Snail density in each plot (≈ 135 snails m^{-2}) was comparable to the densities found in the marsh area from which they were collected (102 ± 25 snails m^{-2}). Individuals were allowed 48 hours to acclimate to the experimental setup prior to the commencement of the experiment. During this time, individuals were counted twice daily to ensure that they remained within their assigned plot. Any snails found to have escaped were relocated to the centre of their assigned plot. During the acclimation period, two snails went missing and one was found dead, thus these individuals were omitted from the experiment, reducing the overall sample size to $N = 78$ individuals (42 females and 36 males), and the number of snails in each plot to 26 (≈ 130 snails m^{-2}).

Procedural overview

All measurements were recorded over ten days between 14th – 24th August 2018. Behavioural trials were conducted either between 10:00 – 13:00 (daytime) or between 22:00 – 01:00 (night-time) on alternating days, beginning on the 14th August with the first daytime trials. This resulted in 10 observations of each trait per individual (78 individuals x 10 repeats = 780 observations per snail). At the end of each day, all individuals were randomly reassigned to one of the three groups, and each group was then randomly assigned to one of the three marsh plots to nullify any plot effect on behavioural responses across repeated trials. During night-time

observations, visibility was facilitated by using red LED head lamps (Vansky®) to minimise disturbance to the study subjects. On commencement of behavioural trials, individuals were given aggregation scores based on where they were found at the beginning of the observation period, either (0) <5mm from the nearest conspecific or (1) >5mm from the nearest conspecific. Tidal inundation was then simulated within the water table during each observation period by approximating the tidal patterns seen in the surrounding, natural marsh. Maximum water level within the water table, at high tide (250mm), and water flow rates were approximated by referring to a guide measure placed in the natural marsh (at the point of specimen collection), and by adjusting the flow rate to the water table accordingly. After each high tide — on completion of behavioural trials (detailed below) — conditions were maintained for 1 hour before water was slowly drained (over ~ 3hrs) to low tide conditions. A period of at least 9 hours was allowed between successive observation periods.

On completion of the experiment, identification marks were removed from all individuals and snails were returned to the marsh in the area of initial collection.

Climbing latency

Prior to tidal inundation, snails were individually positioned such that they were 50mm away from the nearest cordgrass stem. During tidal inundation, a timer was started as soon as the water reached the level of the marsh plot substrate, and individual climbing latencies were recorded, via repetitive scan sampling of all animals, as the time taken (in seconds) for each individual to initiate it's climb up its nearest stem. Once a climb had been initiated, that individual's climbing latency was recorded, and it was removed from the experimental plot and placed into a polyethylene container (26cm dia.), containing aerated seawater, to ensure accurate recording of all individuals, and to provide time to recover prior to the subsequent boldness tests). Climb latencies were recorded in seconds and ranged from 73.8 – 1390.2s (mean ± SD, 514 ± 263.77s).

Boldness

Subsequently, and after approximately 45 minutes under high tide conditions, boldness was measured using latency to emerge from a hiding response (emergence test; Seaman & Briffa, 2015). Each animal was lifted from its container and held over a white photo tray containing sufficient water to cover the snails (representing high-tide conditions). Pressure was applied to the foot of the snail using a blunt-ended seeker (dissection probe), causing the foot to be retracted into the shell and full closure of the operculum over the shell aperture. The snail was then placed on the photo tray, with the aperture facing upwards. Re-emergence latencies were recorded as the time taken (in

seconds) for the operculum to reopen and for the first antennae to become visible to the observer and ranged from 5.0 – 153.9s (mean ± SD, 42.95 ± 25.95). All observations of boldness were conducted by a single observer (T.O.C); ensuring consistency across trials.

Activity

Once response latencies had been recorded, individuals (in groups of 26) were haphazardly positioned 150mm apart on a large worktop (≈ 2 x 4m), with apertures facing down. The starting position of each snail was marked in pencil and a timer was set for 15 minutes. After 15 minutes, the final positions were marked, and the snails were returned to their respective marsh plot. Straight line distances between start and end points were measured to gain an estimate of activity (straight line distance travelled) for each individual and ranged from 6 – 390mm (mean ± SD, 111.8 ± 75.2mm). After each group observation, the table was wiped clean, and once responses for all three groups had been recorded, snails were returned to their respective experimental plots. Groups were tested in a random order for each observation period.

Statistical analysis

A multivariate 'character state' mixed model (MMM) was used to estimate adjusted repeatability (R_j) of — and correlations among — boldness, activity (distance travelled), and climb latency, (1) during the day, (2) at the night, and (3) across diel (day/night) contexts at both the among- and within-individual levels. The character state approach suggests that observations in discrete environments (or contexts, e.g. day vs night) are best modelled as distinct environment-specific sub-traits (Via et al., 1995; Houslay & Wilson, 2017), and thus responses for all three traits were divided into discrete day or night 'sub-traits' (i.e. six 'character states').

Boldness was modelled as the inverse (1/latency) of individual re-emergence latencies and was ln-transformed, while activity and climb latency were sqrt transformed, to meet the assumptions of normality. All response variables were then centred and standardised to a mean of 0 and a variance of 1 (z-transformed) to facilitate convergence (Schielzeth, 2010). To each of the six character states, trial number, sex, total wet weight, and aggregation score were fitted as fixed effects. To facilitate the interpretation of the main effects, sex was treated as a continuous variable (0 = female, 1 = male), as was aggregation score (0 = no group, 1 = group). Individual trial number (effect of time), and wet weight (g) were all centred and standardised to a mean of 0 and a variance of 1 (Houslay & Wilson, 2017). To test for individual differences in mean-level boldness, activity and climb latency, as well as individual differences in behavioural patterns over time (e.g. Stamps et al., 2012), individual identity (ID) and trial number were additionally specified as random effects. The random slope effect

of trial number quantifies individual differences in responsiveness (changes in behavioural traits) across trials, and tests for possible habituation to experimental protocols.

Parameters were estimated using the Markov chain Monte Carlo (MCMC) method with the 'MCMCglmm' package (Hadfield, 2010), in R (R Core Team, 2017). The posterior distributions and autocorrelation plots of five independent chains were compared to ensure convergence and adequate chain mixing, each with 950,000 iterations, 60,000 burn-ins, and thinning every 275 iterations. An 'uninformative', parameter-expanded, model prior was used for among-individual (co)variances, and two 'unstructured' variance-covariance matrices were specified. The first (I-matrix) accounts for the random effects of individual 'ID' and 'trial number', estimating the among-individual variance of each of the six character states (VAR_{ind}), and the covariances between them (COV_{ind}). The second (R-matrix) accounts for the residual variation (within-individual variance; VAR_e) and provides estimates of individual unpredictability, as well as estimates of covariance between the repeat measures of boldness, activity and climb latency, in each of the diel contexts (COV_e).

Model (co)variances were used to estimate among-individual (r_{ind}) and within-individual (r_e) correlations between traits by dividing the corresponding covariance between two variables by the product of the square root of their variances (Dingemanse & Dochtermann, 2013):

$$(\boldsymbol{r_{ind}} = COV_{ind} / (sqrt(VAR_{ind})*sqrt(VAR_{ind})))$$

$$(\boldsymbol{r_e} = COV_e / (sqrt(VAR_e)*sqrt(VAR_e)))$$

Since these correlations can be either positive or negative, it is possible to use the 95% credible intervals (CI) to assess statistical significance (Houslay & Wilson, 2017). In this case, only correlations with CIs excluding zero were considered significant. Among-individual (co)variances were also used to detect behavioural plasticity across day/night contexts, by calculating the among-individual correlations (as above) between environment-specific character states (e.g. boldness in the day and boldness at night), which, in the absence of cross-context plasticity, will be $r = +1$. In this case, where CIs for correlations between environment-specific character states excluded +1, it was inferred that significant among-individual plasticity was present across contexts. Within-individual (co)variance for each character state was also calculated (as above) to estimate within-individual (residual) plasticity across contexts.

Finally, context-specific adjusted repeatabilities (R_j) for boldness, activity and climb latency (i.e. conditioned on the fixed effects) were estimated by dividing their respective among-individual variance estimates by the sum of their among-individual and residual variances, following Nakagawa and Schielzeth (2010):

$$(R_j = \text{VAR}_{ind} / (\text{VAR}_{ind} + \text{VAR}_r))$$

The posterior modes of the random effects were then used to plot individual reaction norms (RNs) for each trait across diel contexts (Figure 2.1), and to create graphical representations of r_{ind} to illustrate among-individual correlations between each of the character states in each diel context (Figure 2.2).

Results

Mean-level results

On average, there was a tendency for reduced boldness at night (Est = -0.164, CI [-0.408 ; 0.065]) compared to the day (Est = 0.312, CI [0.015 ; 0.607]), where there is only a small overlap between 95% CIs. By contrast, average activity tended to increase at night (Est = 0.272, CI [-0.007 ; 0.530]) compared to the day (Est = -0.021, CI [-0.288 ; 0.264]), and there was a weak tendency for increased climb latencies at night (Est = -0.178, CI [-0.434 ; 0.103]) compared to the day (Est = -0.269, CI [-0.515 ; -0.015]), however, the overlap between 95% CIs indicate that these trends were not significant. The mean-level effects of diel context are represented by the red trend lines in Figure 2.1. Snails also showed a weak tendency for longer climb latencies to result in a higher aggregation scores at night (Est = 0.186, CI [0.013 ; 0.374], pMCMC = 0.049), but this trend was not detected during the day. On average, sex had an effect on climbing behaviour at night, with males tending towards higher climb latencies than females during the day (Est = 0.479, CI [0.203 ; 0.724], pMCMC = 0.001) but not at night. By contrast, average boldness and average activity were not significantly affected by trial number, weight or sex during the day or at night, nor were they related to aggregation scores (for full fixed effects output see Table 2.1).

Table 2.1 *Fixed effects fitted to the multivariate mixed model for response variables boldness, activity, and climb latency across day and night contexts in <u>L. irrorata</u>. Shown are posterior means, lower and upper 95% confidence intervals and pMCMC (statistically significant pMCMC values (p < 0.01) are in bold).*

Trait	post.mean	l-95% CI	u-95% CI	pMCMC
Boldness (D)				
Intercept	0.3125	0.0155	0.6071	
weight	0.0505	-0.1184	0.2333	0.5653
sex	0.0776	-0.2897	0.4156	0.6729
trial	-0.0169	-0.0720	0.0420	0.5647
group	-0.1349	-0.3116	0.0416	0.1464
Boldness (N)				
Intercept	-0.1643	-0.4077	0.0645	
weight	0.0978	-0.0245	0.2345	0.1322
sex	0.1943	-0.0741	0.4413	0.1452
trial	-0.0164	-0.0736	0.0350	0.5666
group	-0.0486	-0.2150	0.1143	0.5653
Activity (D)				
Intercept	-0.0206	-0.2884	0.2636	
weight	0.0565	-0.1090	0.2069	0.4863
sex	-0.0714	-0.3928	0.2272	0.6253
trial	0.0027	-0.0522	0.0536	0.9113
group	0.0272	-0.1379	0.1929	0.7383
Activity (N)				
Intercept	0.2720	-0.0066	0.5298	
weight	-0.1187	-0.2588	0.0204	0.0921
sex	-0.1135	-0.4114	0.1428	0.4066
trial	-0.0193	-0.0914	0.0437	0.5814
group	-0.1179	-0.2988	0.0492	0.1847
Climb latency (D)				
Intercept	-0.2692	-0.5145	-0.0148	
weight	-0.0782	-0.2103	0.0566	0.2583
sex	0.4790	0.2033	0.7242	**0.0012**
trial	-0.0295	-0.0807	0.0293	0.3089
group	-0.0184	-0.1907	0.1534	0.8452
Climb latency (N)				
Intercept	-0.1783	-0.4343	0.1028	
weight	0.0109	-0.1441	0.1471	0.8724
sex	0.1977	-0.0852	0.4910	0.1866
trial	-0.0519	-0.1099	0.0059	0.0809
group	0.1861	0.0125	0.3736	0.0488

Individual-level results

Among-individual correlations between environment-specific character states for boldness (r_{ind} = 0.81, [0.604 ; 0.913]), activity (r_{ind} = 0.60, [0.325 ; 0.763]) and climb latency (r_{ind} = 0.767, [0.535 ; 0.884) reveal significant among-individual plasticity across diel contexts, where 95% credible intervals exclude +1 for all traits. Individuals also differed in their responses to changes in diel cycle, with a trend for higher among-individual variation in boldness during the day (Est = 0.577, [0.371 ; 0.828) than at night (Est = 0.273, [0.161 ; 0.410]). Although, note that since 95% CIs overlap slightly, this trend was not considered significant. Individuals with higher than average boldness during the day tended towards shyer behaviour at night, however, individuals that were shyer than average during the day tended to become bolder at night, indicating that individual reaction norms (RNs) are converging at night (Figure 2.1a).

Individuals also differed in their sensitivity to diel context with regards to activity, with some individuals exhibiting substantially higher activity at night compared to the day, whereas for others activity was substantially lower at night. Overall, despite no significant difference between among-individual variation in activity across day (Est = 0.51, CI [0.316 ; 0.728]) and night (Est = 0.360, CI [0.215 ; 0.529]) observations, a trend for higher variation during the day (Figure 2.1b) suggests that individual RNs for activity are also converging at night. Individual sensitivity to diel context was also unclear for climb latency, with no significant difference between among-individual variation during the day (Est = 0.297, CI [0.167 ; 0.448]) compared to night (Est = 0.381, CI [0.217 ; 0.565]) (Figure 2.1c).

Individual unpredictability (within-individual variation; VAR_e) did not differ significantly across diel context for boldness (Day; 0.60, CI [0.516 ; 0.699], Night; 0.53, CI [0.448 ; 0.613), activity (Day; 0.55, CI [0.464 ; 0.639], Night; 0.57, CI [0.477 ; 0.664]) or climb latency (Day; 0.64, CI [0.544 ; 0.735], Night; 0.65, CI [0.547 ; 0.760]), since 95% CIs overlap for all traits, across diel context.
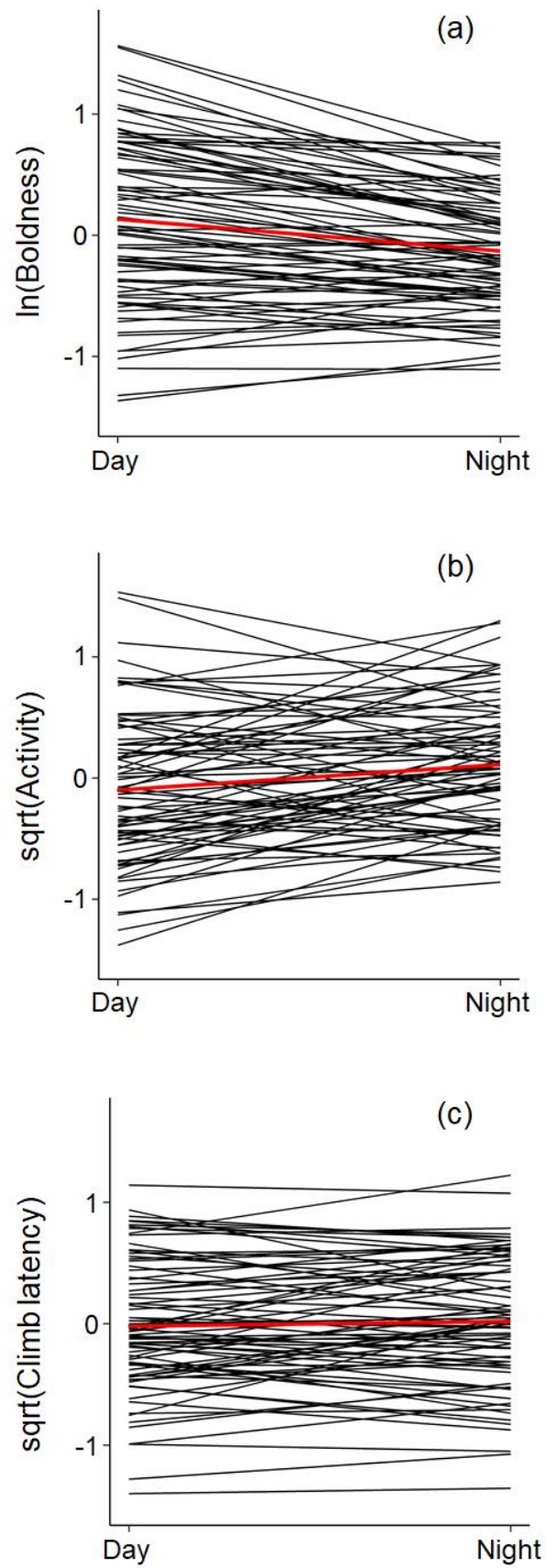
Figure 2.1 *Individual reaction norms representing variation across diel context (day & night) for boldness* **(a)***, activity* **(b)***, and climb latency* **(c)** *in* <u>*L. irrorata*</u>*. Black lines represent individual reaction norms, and red lines represent the mean-level trends for the population (N = 78). Trait values are expressed in units of standard deviation (see methods).*

Individuals did not differ in their trends of boldness or climb latency over time (trial number), across repeat trials during either day or night observations. The random effect of trial, which captures any behavioural changes over time, including possible habituation was estimated to be zero for boldness and climb latency, where individual predicted mean values were maintained relative to one another, and where the posterior distributions for variance components relating to trial number included zero. However, individuals did differ in their trends of activity over time, but this effect was only significant during night-time observations (Est = 0.03, CI [0.007 ; 0.062]). Individuals exhibiting higher activity in earlier trials tended to reduce activity in later trials, whereas individuals with relatively low activity in earlier trials tended to become more active in later trials (intercept-slope correlation, r = -0.67, [-0.858 ; -0.294]). The presence of a negative intercept-slope correlation indicates that individual RN's for activity are converging in later trials (i.e. lower variation among individuals in later trials compared to early trials) (Figure 2.2).
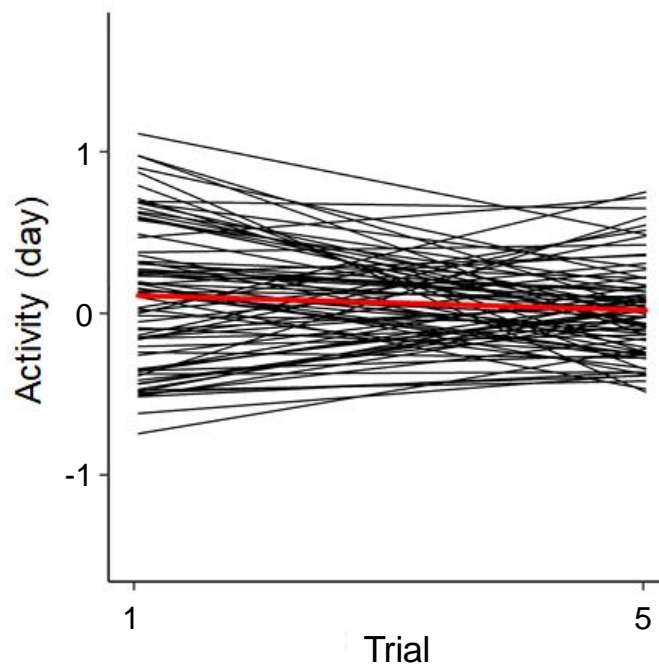
Figure 2.2 *Individual reaction norms representing variation in night-time activity (over time) in L. irrorata. Black lines represent individual reaction norms, and red lines represent the mean-level trends for the population (N = 78). Trait values are expressed in units of standard deviation (see methods).*

After accounting for the fixed effects of weight, sex, trial and aggregation score, both boldness and activity were moderately repeatable, with trends for reduced consistency at night (Table 2.2). Adjusted repeatability (R_j) was also moderately repeatable for climb latency, however,

in this case consistency increased from day to night (Table 2.3). The consistency of individual behavioural traits means that among- and within-individual covariance was possible between all three traits (boldness, activity and climb latency), during the day and at night.

Table 2.2 *Among-individual variance (VAR$_{ind}$), residual (within-individual) variance (VAR$_e$), and adjusted repeatability (R$_j$) boldness, activity and climb latency during the day (D) and at night (N) in* L. irrorata *(N = 78). All estimates were extracted from a single multivariate mixed model.*

Trait	VAR$_{ind}$	95% CI lower	upper	VAR$_e$	95% CI lower	upper	R$_j$	95%CI var-L	var-U
Boldness (D)	0.577	0.371	0.828	0.603	0.516	0.699	0.48	0.379	0.594
Boldness (N)	0.273	0.161	0.410	0.530	0.448	0.613	0.31	0.227	0.445
Activity (D)	0.511	0.316	0.728	0.549	0.464	0.639	0.47	0.362	0.586
Activity (N)	0.360	0.215	0.530	0.566	0.477	0.664	0.38	0.274	0.506
Climb latency (D)	0.297	0.167	0.448	0.635	0.544	0.735	0.29	0.212	0.431
Climb latency (N)	0.381	0.217	0.565	0.653	0.547	0.760	0.38	0.253	0.484

The results also revealed significant negative covariances between boldness and activity at both the among-individual (COV$_{ind}$ = -0.188, CI [-0.345 – -0.026]), and at the within-individual levels (COV$_{e}$ = -0.124, CI [-0.188 – -0.056]) during the day, but only at the within-individual level at night (COV$_e$ = -0.158, CI [-0.224 – -0.092]). These estimates provided moderate correlations between boldness and activity at the among-individual (r_{ind} = -0.38, [-0.584 – -0.01]) (Figure 2.3a) and within-individual levels (r_{cov} = -0.21, [-0.316 – -0.101]) during the day, and at the within-individual level at night (r_{cov} = -0.29, [-0.394 – -0.181]). There were also significant positive covariances between boldness and climb latency at the among-individual level during the day (COV$_{ind}$ = 0.173, CI [0.047 – 0.311]) and at night (COV$_{ind}$ = 0.135, CI [0.029 – 0.244]), with estimates providing moderate positive among-individual correlations for day time (r_{ind} = 0.34, [0.150 – 0.658]; Figure 2.3b) and during night-time observations (r_{ind} = 0.41, [0.131 – 0.658]) (Figure 2.3b). Finally, there were significant negative among-individual covariances between activity and climb latency during the day (COV$_{ind}$ = -0.257, CI [-0.391 – -0.13]) and at night (COV$_{ind}$ = -0.203, CI [-0.324 – -0.081]) and significant negative within-individual covariance between during the day (COV$_e$ = -0.113, CI [-0.181 – -0.046]). These estimates provided strong negative among-individual correlations between climb latency and activity during the day (r_{ind} = -0.68, [-0.831 – -0.457]) and at night (r_{ind} = -0.58, [-0.769 – -0.319]) (Figure 2.3c), and a weak within-individual correlation during the day (r_{cov} = -0.19, [-0.298 – -0.081]).
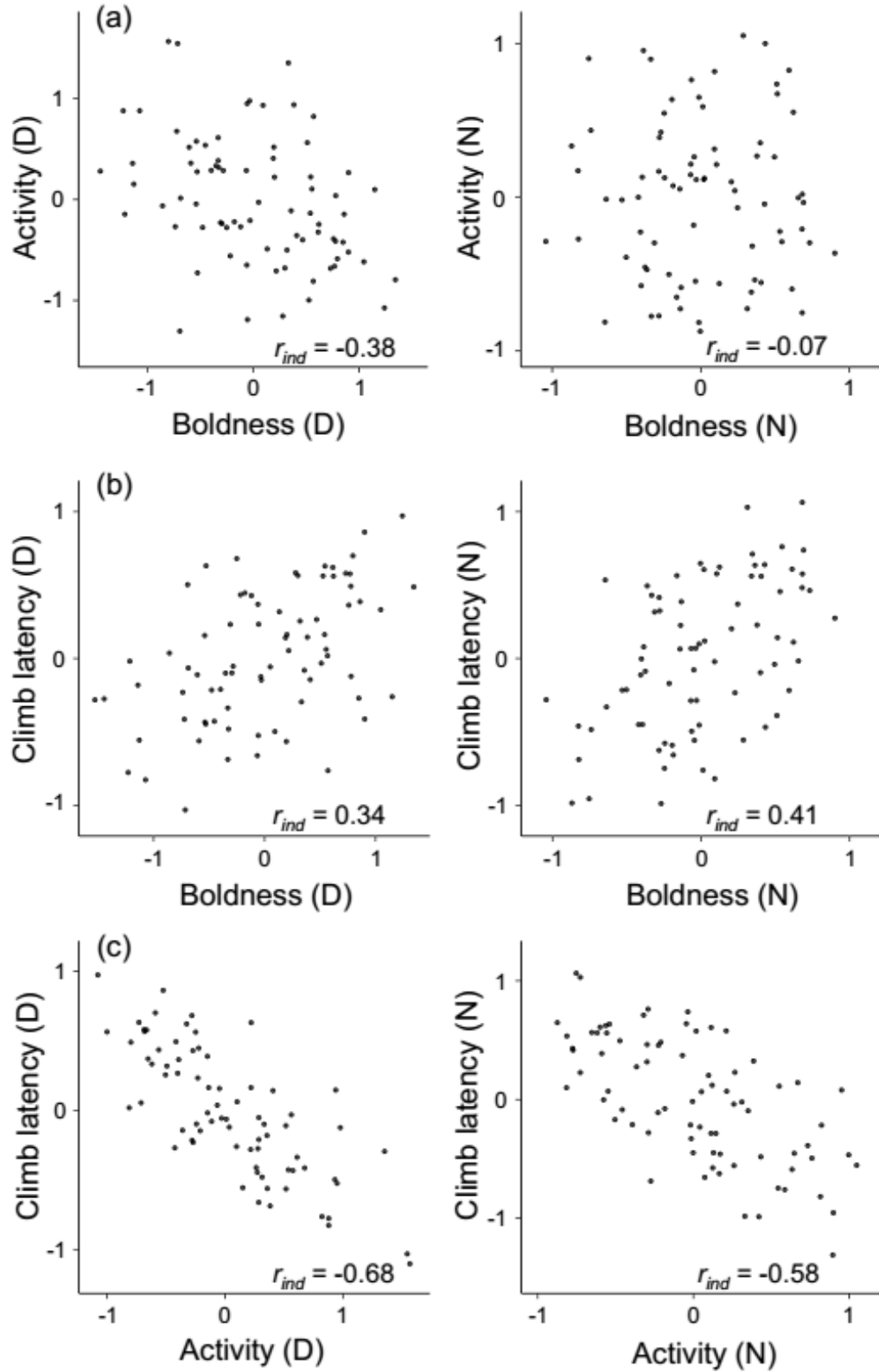
Figure 2.3 *Representation of the among-individual correlations (r_{ind}) during the day (left panels) and at the night (right panels) between* **(a)** *boldness and activity,* **(b)** *boldness and climb latency, and* **(c)** *activity and climb latency in* <u>*L. irrorata*</u>. *Data are based on the posterior modes of the random effects from the multivariate linear mixed model.*

Discussion

The influence of local environmental conditions, short- and long-term environmental changes, and species-specific natural history are often discussed as part of explanations for the proximate causation of individual behavioural variation (Sih et al., 2015; Dammhahn et al., 2018; Montiglio et al., 2018); in particular, where these variables are expected to affect individual state (Dingemanse & Wolf, 2010; Luttbeg & Sih, 2010; Wolf & Weissing, 2010; Sih et al., 2015). However, very few studies have thus far examined the influence of diel cycles on individual behaviour, and even fewer focus on invertebrate behaviour (but see Biro et al., 2014; Watts et al., 2015). Thus, this study aimed to examine the influence of diel cycle on the consistency of risk-related behavioural traits, and the covariance between them, under controlled conditions in a novel study species, *Littoraria irrorata*. As predicted, after accounting for the fixed effects, the results indicated moderate among-individual repeatability across diel contexts for boldness (Day R_j = 0.48; Night R_j = 0.31) and activity (Day R_j = 0.47; Night R_j = 0.38), corresponding with previous findings for invertebrate organisms under similarly controlled conditions (Nespolo & Franco, 2007; Bell et al., 2009). Furthermore, climb latency was also found to be moderately repeatable (Day R_j = 0.29; Night R_j = 0.38), indicating, for the first time, that climbing behaviour in *L. irrorata* may be considered a consistent individual (personality) trait.

Also, in accordance with *a priori* predictions, mean-level activity tended to increase from day to night; however, somewhat unexpectedly, average boldness trended in the opposite direction, and coincided with increased climb latency (snails were slower to climb). This finding does not support the expectation that boldness should increase from day to night as a result of decreased predation pressure (Shirley & Findley, 1978), and is contrary to findings reported previously in crayfish (*Cherax destructor*) (Biro et al., 2014), in which boldness was found to increase at night. However, while it has been reported that *C. sapidus* (blue crabs) feed mainly at dawn and dusk (Wolcott & Hines, 1989), they are also — along with other snail predators — including diamond back terrapins (*Malaclemys terrapin*) and killifish (*Fundulus* spp), known to also feed at night (Clarke & Johnston, 1999; Clark et al., 2003; Lipcius, 2013). In addition, *L. irrorata* has been shown to rely on visual cues for movement and orientation (Hamilton, 1977, 1978a), and are known to have better vision than most gastropods (Hamilton & Winter, 1982; Hamilton et al., 1983). Therefore, it is possible that periods of darkness may actually pose a greater risk of predation than periods of daylight, where the snails' vision may be less effective, thus affecting an individual's information state (e.g. their perception of risk: Rodríguez-Prieto et al., 2010; Briffa, 2013; Dorset et al., 2017). However, this idea is only supported at the mean-level, while within-individual variance

components (residual variation) indicated that individuals were more predictable in their expression of boldness at night (lower within-individual variance), and that there was no clear differences in predictability for activity or climb latency between contexts. So, although the propensity for risk-taking was generally lower at night, as would be expected in response to higher risk (Dammhahn & Almeling, 2012; Thomson et al., 2012), individual expression of boldness was in fact, more predictable. The opposite to what would be expected in high risk situations (Maye et al., 2007; Brembs, 2011), and to that reported previously in hermit crabs (*Pagurus bernhardus*) (Briffa, 2013), and in amphibian (*Rana dalmatina*) tadpoles (Urszán et al., 2018).

Despite the lack of a clear pattern indicating whether diel cycles likely represent different levels of risk, these results certainly suggest complex patterns of behavioural variation in response to changes in diel context. This is further supported by the finding of significant among-individual plasticity across contexts, for all three traits, where 95% credible intervals for among-individual correlations between environment-specific character states did not include +1. This is consistent with a previous report of significant diel flexibility in behavioural traits (e.g. Watts et al., 2015), and again, supports suggestions that individual behaviour is influenced by changes in environmental conditions that affect individual state (Dingemanse & Wolf, 2010; Luttbeg & Sih, 2010; Wolf & Weissing, 2010; Sih et al., 2015). Further, the finding that some individuals were more plastic than others in their expression of boldness and activity across diel contexts (see reaction norm plots; Figure 2.1) could indicate that the expression of these traits is controlled by one or more underlying variables (Stamps, 2016; Mitchell & Biro, 2017). For example, individual metabolism (Van Dijk et al., 2002; Careau et al., 2008; Biro & Stamps, 2010; Metcalfe et al., 2016; Biro et al., 2018), which is directly influenced by temperature (generally lower at night) in ectothermic animals (Clarke & Johnston, 1999). However, since temperature was controlled in this study, such that it was consistent (28-28.5°C) across diel context, the effect of time of day was isolated from that of temperature. Thus, the results seem to suggest that diel cycle itself may represent an important environmental variable, influencing both mean- and individual-level expression of different behavioural traits in this species.

Importantly, the clear among-individual correlations between boldness and activity, boldness and climb latency, and between activity and climb latency, support the prediction that these traits would form a risk-related behavioural syndrome, under the conditions of this study. However, the association between boldness and activity appears to be context-dependent, with no clear among-individual correlation between these traits at night (Figure 2.3). The robust partitioning of paired traits also revealed residual (within-individual) correlations between boldness and activity in both day and night observations, and a weak but detectable residual correlation between activity and

climb latency during the day. Contrary to *a priori* expectations, the associations between activity and boldness, and between activity and climb latency were negative. One possible explanation for these negative covariances may relate to the measure of activity used in this study. Specifically, although *L. irrorata* have been reported to show a propensity to ascend the closest stem on the commencement of incoming tide (Hamilton, 1977), it is possible that bolder individual may be more active (Sih et al., 2004a; Dingemanse et al., 2007), undertaking more localised movement in search of foraging opportunities, whereas shyer individuals may be moving further (in a straight line) in search of the closest stem (Hamilton, 1977). This conforms to anecdotal observations of movements during foraging bouts and, if true, could explain the negative correlations seen here. This is even more plausible given the negative association between activity and climb latencies, where shyer individuals appear to move further and commence climbing more quickly.

An alternative hypothesis might centre on previous reports of increased O_2 consumption in *L. irrorata* at night (Shirley & Findley, 1978; Shirley et al., 2007), and theorised links between individual metabolism and energetically 'expensive' behaviours (Van Dijk et al., 2002; Careau et al., 2008; Biro & Stamps, 2010; Biro et al., 2018), as well as the understanding that *S. alterniflora* stems not only provide protection from predators but also a source of food (Bärlocher & Newell, 1994b; Stagg & Mendelssohn, 2012). In this sense, individuals that would normally be characterised as shy (based on emergence tests), may actually be expending more energy travelling as far as necessary in search of both safe refuge as well as future foraging opportunities. Further, food was available *ad libitum*, and individuals were observed to be feeding on both the marsh substrate as well as on the S. *alterniflora* stems throughout the study period. Thus it is possible that boldness was not required for high feeding rates — an idea put forward previously when discussing behaviour in crayfish, *Cherax destructor* (Biro et al., 2014). In any case, the mean-level effect of reduced boldness at night remains somewhat perplexing but could indicate a species-specific alternative strategy to cope with increased risk. Specifically, where distance travelled increases in the absence of climbing opportunities but where boldness and climb latencies increase within high-risk situations. In order to test the validity of this prediction, future studies should examine both, measures of individual metabolism along with risk-related behavioural traits concurrently.

Notably, this study found no evidence for among-individual variation in temporal patterns of boldness or climb latency, and only limited evidence for this variation (temporal plasticity) in activity during night-time observations (Figure 2.2). However, it is important to note that despite data centring (see methods) providing conservative variance component estimates, it is possible that this result is influenced by a regression to the mean, and thus inferences should be made with caution here. However, studies often find evidence for individual changes in behaviour over time, which are

indicative of habituation to experimental protocols (Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016), but the lack of such variation here indicates that predicted mean values were highly consistent over time, in a given diel context. Thus, in addition to evidence for among-individual plasticity across diel context, this study presents strong evidence for consistency of behavioural traits over time, a key characteristic of personality.

In summary, this study provides evidence that *L. irrorata* exhibits significant diel plasticity in behaviour across discrete environmental contexts (day vs night), whilst also maintaining highly significant among-individual correlations across contexts. Given the lack of temporal plasticity (with the exception of activity during night-time observations), and the repeatability of each behavioural trait, the study also provides strong evidence for consistency in behavioural traits related to risk (boldness, activity and climb latency). Furthermore, the study also revealed significant multivariate correlations between boldness and activity, boldness and climb latency, and between activity and climb latency (to varying degrees) across diel contexts, indicating the presence of a behavioural syndrome, involving these three risk-related behaviours. Therefore, this study provides rare evidence for the influence of diel cycles on individual behaviour, and provides support for the idea that individual behavioural differences are, at least in part, driven by differences in individual state (Dingemanse & Wolf, 2010; Luttbeg & Sih, 2010; Wolf & Weissing, 2010; Sih et al., 2015); in this case, resulting from changing environmental context. Finally, the study highlights the potential importance of diel cycle as a short-term environmental variable that may provide further insight to the exhibition of individual behaviour in cathemeral species.

CHAPTER THREE

The influence of environmental gradients on individual behaviour: Individual plasticity is consistent across risk and temperature gradients.

The results of this chapter have been published:

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Abstract

The expression of individual behaviour as a function of environmental variation (behavioural plasticity) is recognised as a means for animals to modify their phenotypes in response to changing conditions. Plasticity has been studied extensively in recent years, leading to an accumulation of evidence for behavioural plasticity within natural populations. However, despite the recent attention given to studying individual variation in behavioural plasticity, there is still a lack of consensus regarding its causes and constraints. One pressing question related to this is whether individual plasticity carries over across temporal and environmental gradients. That is, are some individuals more plastic (responsive) than others in general? To address this question, the influence of temporal and environmental gradients on individual behavioural responses were examined in the marine gastropod, *Littoraria irrorata*. Individual boldness was measured repeatedly over time and in response to tidal cycle (high vs low, an index of risk) and daily temperature fluctuations (known to affect metabolism), in a controlled field experiment. On average, boldness increased from high to low tide and with increasing temperature but decreased marginally over time. Individuals also differed in their responses to variation in tide and temperature, but not over time. Those that were relatively bold at high tide (when predation risk is greater) were similarly bold at low tide, whereas shy individuals became much more 'bold' at low tide. Most notably, individuals that were more responsive to tide (and thus risk) were also more responsive to temperature changes, indicating that plasticity was correlated across contexts ($r = 0.57$) and that bolder individuals were least plastic overall. This study provides a rare and possibly first example of consistency of individual behavioural plasticity across contexts, suggesting underlying physiology as a common mechanism, and raises the possibility of correlational selection on plasticity.

Introduction

Plasticity has long been recognised as a means for animals to modify their phenotypes in response to varying conditions and has been extensively studied, but primarily from a developmental perspective. More recently, the study of plasticity has shifted to examining short-term reversible physiological and behavioural plasticity, from an individual perspective, with a focus on understanding the causes and consequences of individual behavioural variation (see Dingemanse & Wolf 2013; Snell-Rood 2013; Stamps 2016). Individual variation in plasticity is important to study because it indicates that the amount of variation that selection can act upon is not constant across environments (Dingemanse et al., 2012), and indicates that plasticity may be heritable (e.g. Scheiner, 1993; Nussey et al., 2005). Furthermore, plasticity can have important ecological implications (Toscano, 2017) such as those associated with population expansion (Schou et al., 2017), stability and persistence (Dingemanse & Wolf, 2013).

Evidence for individual variation in behavioural plasticity is rapidly accumulating, showing that responses to environmental variation often differ significantly among individuals, for a wide range of environmental and ecological gradients (e.g. Briffa et al., 2008; Westneat et al., 2011; Hewes et al., 2017; Mitchell & Biro, 2017; Toscano, 2017, see also reviews by Dingemanse & Wolf, 2013; Stamps, 2016). Most recently, two studies have shown that individual differences in plasticity are consistent over time and can be heritable (Araya-Ajoy & Dingemanse, 2017; Mitchell & Biro, 2017), indicating that individual behavioural plasticity is a consistent individual attribute, at least for the gradient(s) considered. However, despite the recent evidence for behavioural plasticity and the attempts to elucidate its mechanisms (Mathot et al., 2012; Mitchell & Biro, 2017), there is still a lack of an overall understanding of its origins (Snell-Rood, 2013) and the factors that constrain it (Stamps, 2016). One recent suggestion centres on the assumption that individual plasticity may correlate across environmental gradients (or is domain-general), as a result of common proximate mechanisms (Stamps, 2016), such as those related to individual physiology, e.g. metabolic rate (Mitchell & Biro, 2017) and metabolic scope (Biro et al., 2018). However, despite previous investigations examining covariance among reaction norm (RN) slopes (i.e. correlation of plasticity across gradients), support for this assumption is currently absent (Westneat et al., 2011; Biro et al., 2014; Mitchell & Biro, 2017) and is very rarely studied. Consequently, domain generality of behavioural RNs remains an outstanding question and, if valid, could help reveal the factors underpinning behavioural phenotypic variation (Stamps, 2016; Mitchell & Biro, 2017).

Recent proposals also suggest that among-individual variation in behaviour, including plasticity, may arise from differences in individual state. In this context, an individuals' state could

be related to relatively labile internal characteristics such as energy metabolism and energy reserves (Careau et al., 2008; Biro & Stamps, 2010; Metcalfe et al., 2016) as well as to individual information state variables such as social rank (Bergmüller & Taborsky, 2010; Colléter & Brown, 2011), risk perception (e.g. Dorset et al., 2017) and prior experience with external stimuli (Frost et al., 2007; Urszán et al., 2015, 2018). Individual state can also be influenced by environmental factors such as temperature (Biro et al., 2010; Briffa et al., 2013; Nakayama et al., 2016; Schou et al., 2017), time of day (Dingemanse et al., 2002; Biro et al., 2014), predator-prey interactions (Urszán et al., 2015, 2018; Foster et al., 2017) and habitat quality (Belgrad & Griffen, 2017; Belgrad et al., 2017). Yet, empirical studies that evaluate the relationships between individual state and individual behavioural differences, particularly relating to plasticity under different contextual gradients, are still lacking (but see Nakayama *et al.* 2016; Mitchell & Biro 2017).

Therefore, this study quantified how boldness (risk-taking propensity) in the saltmarsh periwinkle (*Littoraria* irrorata) was simultaneously influenced by repeated handling over time (trial number), along with two environmental gradients that can affect changes in internal physiological or information state: tidal inundation and daily temperature fluctuations, under semi-natural conditions. The study aimed to identify (a) whether there is evidence of among-individual variation in plasticity across contextual gradients and over time, and (b) whether plasticity is correlated across these different gradients. *L. irrorata* is a widespread herbivore on saltmarshes of the Eastern USA and is typically found on or amongst the stems of the habitat-forming cordgrass, *Spartina alterniflora*, across high, mid and low marsh areas (Hamilton, 1978b; Stiven & Kuenzler, 1979). An important prey species for numerous saltmarsh consumers, *L. irrorata* is known to undertake daily vertical migrations on *S. alterniflora* stems (Stiven & Kuenzler, 1979; Henry et al., 1993), to avoid predation at high tide (West & Williams, 1986; Vaughn & Fisher, 1988, 1992; Carroll et al., 2018). Thus, tide was used as an index of predation risk in this study.

An increase in mean boldness with increased temperatures was predicted due to increased metabolism (Clarke & Johnston, 1999) and the hypothesised links between metabolism and behaviour (Careau et al., 2008; Biro & Stamps, 2010; Metcalfe et al., 2016). Further, individual variation in metabolic responsiveness to temperature (Careau et al., 2014) should lead to individual variation in behavioural responsiveness to temperature (Biro et al., 2010; Briffa et al., 2013). With respect to risk, it was predicted that boldness would increase on average at low tide when risk of predation is low (West & Williams, 1986; Vaughn & Fisher, 1988, 1992). It was also expected that individuals would differ in their responses to risk (e.g. Dammhahn & Almeling, 2012; Thomson et al., 2012; Briffa, 2013) due to differences in perception of risk and stress responsiveness (e.g. Carere et al., 2010; Dorset et al., 2017). Finally, individuals were also expected to differ in temporal

changes in boldness across the many repeated behavioural assays, as these differences may reflect habituation to protocols and/or some other internal or external factor varying over time (e.g. Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016). In addition, since unpredictable behaviour under threat of predation is thought to increase the chances of evading capture (Brembs, 2011; Jones et al., 2011), it was expected that predictability (residual intra-individual variation) of behaviour would increase in response to risk perception at high tide (Maye et al., 2007; Briffa, 2013).

Methods

Experimental design

The experimental set up consisted of two water tables (approx. 248cm x 61cm x 20cm) connected to a flow-through system, drawing seawater from, and returning to the adjacent Wachapreague channel. In each table, two 'replica marsh plots' (0.30m^2) ($N = 4$) were created by filling open top mesh cages (60 x 50 x 5cm) with 5cm deep marsh cores, complete with cordgrass roots and intact stems, taken from the nearby marsh system in Wachapreague, VA (37°36'31.2"N 75°41'09.3"W).

Incoming and outgoing tides were simulated daily, within each flow-through table, by replicating the tidal patterns seen in the surrounding, natural marsh. Daily maximum water level within the marsh plots, at high tide (mean ± SD, 11.8 ± 1.7cm), along with incoming tide duration were approximated by recording the rate of incoming water on the natural marsh, referring to a guide measure placed in the natural marsh at the point of core collection, and by adjusting the flow to the water tables accordingly. At low tide, substrate within the marsh plots, like that of the natural marsh, were not submerged. Estimated biomass-density (biomass m^{-2} ± SD, 215.3 ± 2.1g) and average stem height (mean ± SD, 143 ± 12.8cm) of *S. alterniflora* were consistent in each marsh plot and were comparable to that observed in the surrounding, natural marsh from which the cores and the study subjects were collected (biomass m^{-2} ± SD, 255.3 ± 103.1g; stem height ± SD, 178 ± 32.8cm).

Collection and husbandry

Adult snails (as defined by Hamilton, 1978b), $N = 80$; 17-28mm shell length, were collected haphazardly from a patch of high-marsh (approx. 5m^2), at low tide, from nearby *S. alterniflora*-dominated saltmarsh (37°36'31.2"N 75°41'09.3"W) in July 2016. Individuals were transported to the VIMS Eastern Shore Laboratory where they were placed in seawater for 30 minutes to allow all individuals time to fully hydrate (Iacarella & Helmuth, 2011). To minimize variation in the mantle

cavity fluid (Henry et al., 1993; Iacarella & Helmuth, 2011) and before being weighed (total wet weight, g), each specimen was stimulated to retract into its shell and to close the operculum by applying gentle pressure to the exposed foot with a blunt-ended seeker (dissection probe). Excess water was then removed using tissue paper. Shell length (mm) was measured using Vernier callipers and all individuals were weighed (mean ± SD, 2.98 ± 0.85g). Sex was then determined, for each individual, by visual inspection for the presence or absence of male reproductive organs (46 females and 34 males). In this species, the male sex organ is visible on gently lifting the shell while the foot of the snail is attached to a flat surface.

The snails were number-marked using acrylic paint covered with clear, non-toxic aquarium glue (Stagg & Mendelssohn, 2012) and transported to the marsh plots. 20 snails were randomly assigned to each of the four experimental plots. In each plot, snail densities (≈ 66 snails m^{-2}) were comparable to the densities found in the surrounding marsh areas (mean ± SD, 85 ± 15 snails m^{-2}). All individuals were free to feed, *ad libitum*, on living and dead cordgrass and benthic material within the replica marsh plots.

Individual snails were checked twice daily to ensure they remained within their assigned marsh plot. Any escapes were recorded and wandering snails placed back into the centre of their respective marsh plot. During the experiment, seven individuals were found to be missing and were omitted from the analysis, reducing the overall sample size to $N = 73$ individuals (43 females and 30 males). Finally, on completion of behavioural trials, all snails were returned to the marsh after having their identification marks removed.

Data collection

A bioassay approach was used to determine the effects of tidal gradient extremes (high vs low tide) and daily temperature changes (range: 23°C – 34°C) on individual boldness. Here, individual boldness was determined by the latency to emerge from a hiding response (Seaman & Briffa, 2015). Each snail was lifted from its resident marsh plot and held over a white photo tray. Pressure was applied to the foot of the snail with a blunt-ended seeker, causing the foot to be retracted into the shell and for the operculum to be fully closed over the shell aperture. The snail was then placed on the photo tray, with the aperture facing upwards. Response latencies, recorded as the time taken (in seconds) for the operculum to reopen and for the first antennae to become visible to the observer (Seaman & Briffa, 2015), ranged from 2 – 183s (mean ± SD, 30.79 ± 22.70s). All observations were conducted by a single observer (T.O.C).

Individual latencies were recorded in a random order, twice daily for twelve consecutive days, August 1 – 12, 2016; during which time the magnitude of tidal range remained relatively consistent in the natural marsh (mean ± SD, 1.13 ± 0.22m). All recordings took place between 07:00 – 18:00 and were dictated by the tidal cycle. For the first six days, latencies were recorded first at high tide (am) and then at low tide (pm); for the last six days, this pattern was reversed so that low tide (am) observations were recorded before high tide (pm) observations. This design, together with tide times changing by one hour each day, ensured no systematic confound between tide, temperature and time of day. Specifically, although temperatures were influenced by incoming water, high tide temperatures were not always higher or lower than low tide temperatures. At high tide, snails could be found both above and below the water line, although the majority climbed just above the water and were found feeding, 'attached' or 'glued' to the cordgrass stems (Iacarella & Helmuth, 2011). Others remained below the water line, at the base of stems, or attached to stems at the water line. At low tide, all snails were above the water line, fewer snails could be found climbing and the majority were found moving around and or feeding on the exposed marsh surface substrate.

Substrate surface temperature (mean ± SD, 30.2 ± 3.35°C) was recorded continuously, in each marsh plot, over the period of the study using waterproofed iBUTTON (DS1920) digital temperature loggers (Maxim integrated^{TM}, CA, USA). With a single temperature logger in each plot, a subset of individual snail temperatures was also taken using a non-contact infrared digital thermometer (MD-H6-UKA, Hyology). Simple, one-way ANOVAs were performed to ensure snail temperatures were consistent at the mean level with water temperature during high tide (ANOVA: $F_{1,50} = 2.449$, $p = 0.124$) and with substrate temperature at low tide (ANOVA: $F_{1,50} = 2.772$, $p = 0.102$). Substrate surface temperature was found to be consistent across all marsh plots and between the plots and the surrounding, natural marsh (ANOVA: $F_{4,115} = 0.0137$, $p = 0.999$).

Statistical analyses

Boldness was modelled as the inverse (1/latency) of re-emergence latencies following a hiding response, using a linear mixed effects model. Boldness data were ln-transformed to ensure normality and then standardised to a mean of zero and variance of one (z-transformed). Linear mixed model analysis was undertaken to examine the influence of tide, temperature and time (trial number) on boldness using a fully saturated model containing intercept, tide (high vs low), temperature, trial number, time of day, weight, plot and sex, as fixed effects.

The mean level model:

ln (1/latency) = intercept + tide + temp + trial + time + sex + plot + weight

To test for individual differences in mean level boldness and responsiveness to tide, temperature and trial number, the intercept, tide, temperature and trial number effects were additionally specified as random (see below for more details).

The effect of tide was defined such that high tide = 0 and low tide = 1, and temperature data was left-centred, meaning that the intercept variance represented among-individual variation in boldness at high tide and at 23°C. The random slope effect of tide quantifies among-individual variation in responsiveness (changes in boldness) from high tide to low tide. Individual differences in behaviour patterns across days (e.g. Stamps et al., 2012), and individual differences in behavioural responses to temperature (Biro et al., 2010), were quantified by the random slope effects of trial number and temperature, respectively. Specifying each of these effects as 'random' fits a parameter describing the population mean and a variance parameter describing variation across individuals for each parameter (see Singer & Willett, 2003; West et al., 2011).

An 'unstructured' variance-covariance matrix was specified, meaning that covariance parameters were fit to describe correlations between individual intercept and slope values (cov_{is}) and between individual slope values (cov_{ss}), in addition to the intercept (var_i) and slope (var_s) variance parameters. We also fitted a separate residual variance parameter ($var_{residual}$) for high and low tide situations to test the prediction that high tide situations represent higher risk and thus, at high tide, individuals might exhibit less predictable behaviour (Briffa, 2013). This was achieved by creating a new categorical predictor variable for tide. Analysis was implemented using SAS Proc Mixed, using ReML. The Kenward-Roger method was used to calculate denominator degrees of freedom for the fixed effects (generating non-integer df values), and a type III sums of squares approach for fixed effects (Littell et al., 2006). Covariances were re-expressed as a correlation coefficient, using the standard formula:

$$r = COV / \text{sqrt(var*var)}.$$

The analysis revealed significant and substantial random slope effects and consequently, repeatability should not be calculated in the 'standard' way due to the rank order of individuals changing across contexts (with tide and temperature, but not trial number: Biro & Stamps 2015). With repeatability varying between contexts, the model of summing variance, presented by Singer and Willett (2003) was followed. The model included additional terms to accommodate the additional variance and covariances for the two significant random slope (*s*) effects that were found to be significantly different from zero (e.g. Biro et al., 2014). The following equation was subsequently generated to express situation-specific repeatability for a given tide (X_1) and temperature (X_2) context:

$$R = \frac{(var_{int} + 2*cov_{is}*X_1 + var_s*X_1^2 + 2*cov_{is}*X_2 + 2*cov_{ss}*X_1*X_2 + var_s*X_2^2)}{(var_{int} + 2*cov_{is}*X_1 + var_s*X_1^2 + 2*cov_{is}*X_2 + 2*cov_{ss}*X_1*X_2 + var_s*X_2^2 + var_{residual})}$$

Where var_{int} is the random intercept variance, var_s is the random slope variance, cov_{is} is the covariance between slopes and intercepts and $var_{residual}$ is the residual (within-individual) variance.

Results

Mean level results

On average, snails showed a very weak tendency for reduced boldness across trials (Est = -0.0004, SE = 0.001, $F_{1,1521}$ = 7.8, $p < 0.005$), however there was a strong trend for increased boldness with increases in temperature from 23°C to 34°C (Est = 0.15, SE = 0.006, $F_{1,109}$ = 634, $p < 0.0001$). There was also a strong tendency for increased boldness at low tide (Est = 0.74, SE = 0.05, $F_{1,72.1}$ = 218, $p < 0.0001$) compared to high tide (Est = -0.65). Both time of day ($F_{1,947}$ = 0.94, $p = 0.33$) and sex ($F_{1,67}$ = 0.45, $p = 0.51$) had no effect on mean level boldness. However, weight did have a modest effect ($F_{1,67}$ = 5.1, $p = 0.027$) with smaller snails being, on average, bolder than larger individuals. Finally, marsh plot may have had a small effect ($F_{3,67}$ = 2.3, $p = 0.09$). The mean-level effects of tidal inundation, temperature and trial number are represented by the red trend-lines in Figure 3.1.
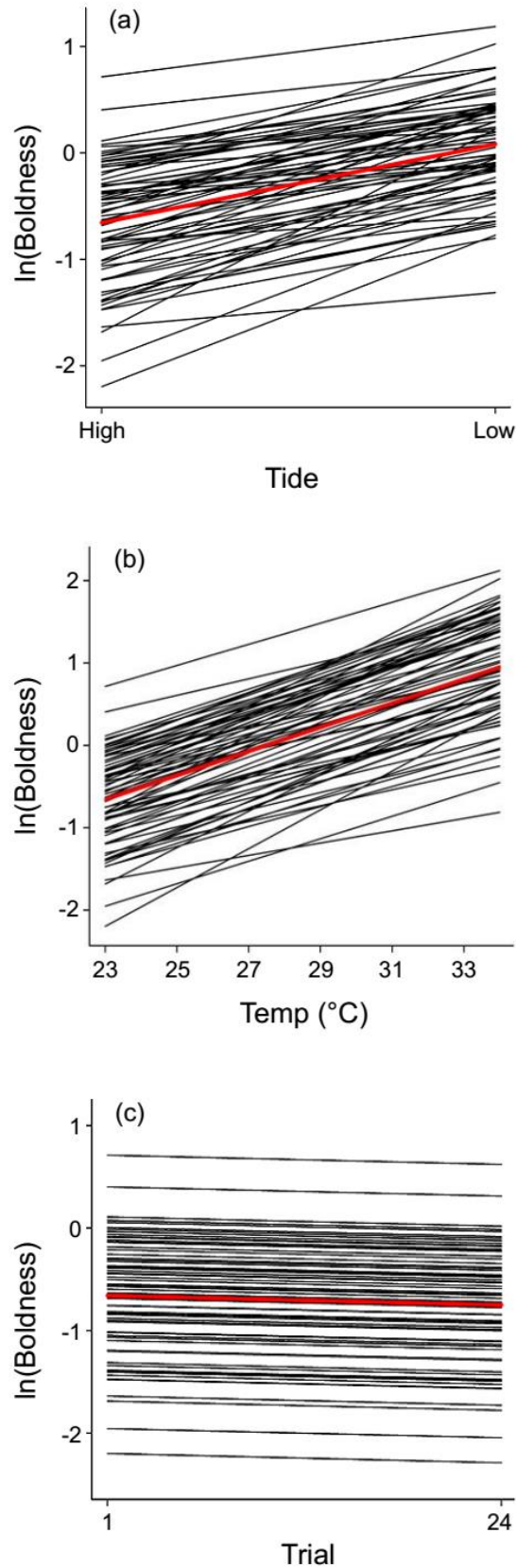
Figure 3.1 *Individual reaction norms representing variation across tides* **(a)***, temperatures* **(b)***, and trials* **(c)** *for* <u>*L. irrorata*</u>*. Black lines represent individual reaction norms and red lines represent the mean-level trend for the population (N = 73). Boldness values are expressed in units of standard deviation (see Methods).*

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Individual-level results

There was substantial individual variation in boldness at high tide (random intercept effect; variance$_{\text{high tide}}$ = 0.38, SE = 0.076, $p < 0.0001$), and individuals also differed substantially in responses to changes in tidal cycle, from high to low tide (slope variance = 0.15, SE = 0.030, $p < 0.0001$; see Figure 3.1a). Individuals exhibiting higher than average boldness at high tide tended to also exhibit higher boldness at low tide, whereas individuals that were relatively shy at high tide tended to become bolder at low tide (correlation between intercepts and slopes, $r = -0.57$, covariance = -0.136, SE = 0.04, $p = 0.0007$; Figure 3.2a). The presence of a significant negative intercept-slope covariance indicates that individual RNs are converging at low tide (i.e. lower variation among individuals at low tide compared to high tide; Figure 3.1a).

Individuals also differed in their sensitivity to temperature, with some exhibiting substantially increased boldness with increasing temperatures and others less so (slope variance = 0.0015, SE = 0.00033, $p < 0.0001$; see Figure 3.1b). There was some indication that individuals exhibiting higher than average boldness at 23°C were less responsive to increases in temperature, but this effect was small and uncertain (correlation between intercepts and slopes, $r = -0.25$, covariance = -0.0060, SE = 0.0037, $p = 0.107$; Figure 3.2b).
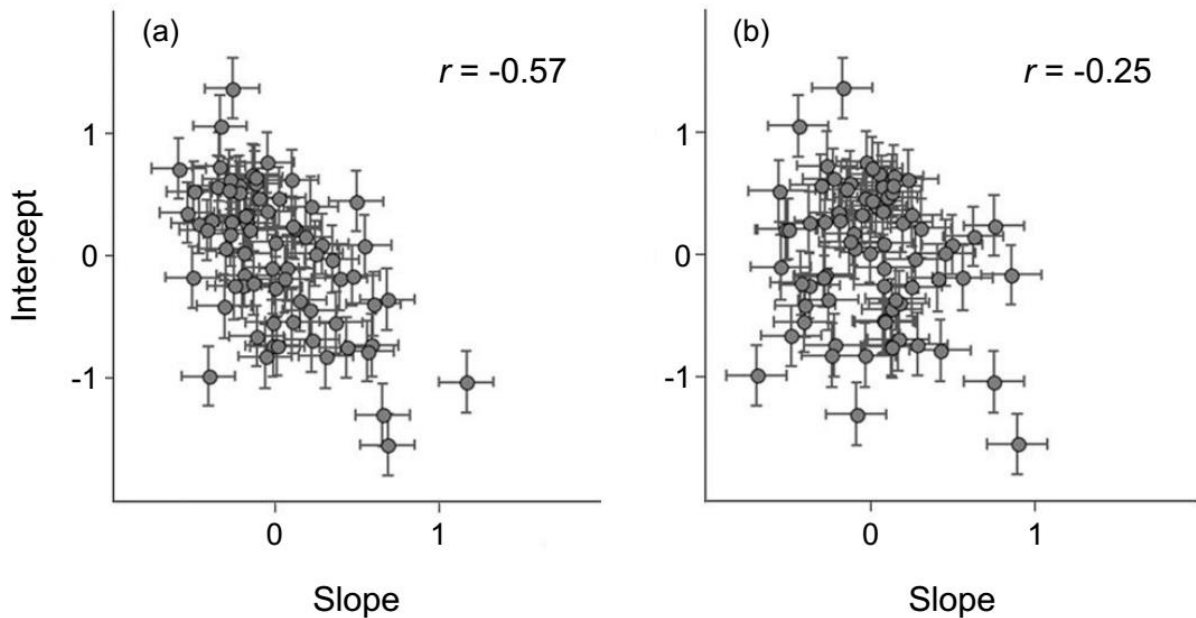
Figure 3.2 *Estimates of individual intercepts and slopes with regard to tide* **(a),** *and temperatures* **(b).** *Error bars denote standard error of the predicted values for each individual. Correlation values were estimated from the mixed model, using model variances and covariances.*

After accounting for tide and temperature effects, individuals did not differ in their trends of boldness over time, across the many repeated trials. The random effect of trial number, which captures any time-varying factor, including possible habituation, was estimated to be zero - thus, there were no individual differences in temporal RN slopes (Figure 3.1c) meaning that individual predicted mean values were maintained relative to one another; this term was thus removed from the model and all estimates of tide and temperature effects (above) are from this reduced model.

Individual plasticity, the responsiveness of individuals to tide and temperature, was correlated across situations such that individuals with stronger positive responses to tide also exhibited stronger positive responses to temperature (covariance between slopes, $r = 0.57$, $cov_{ss} = 0.0086$, SE $= 0.0025$, $p = 0.0005$; Figure 3.3).
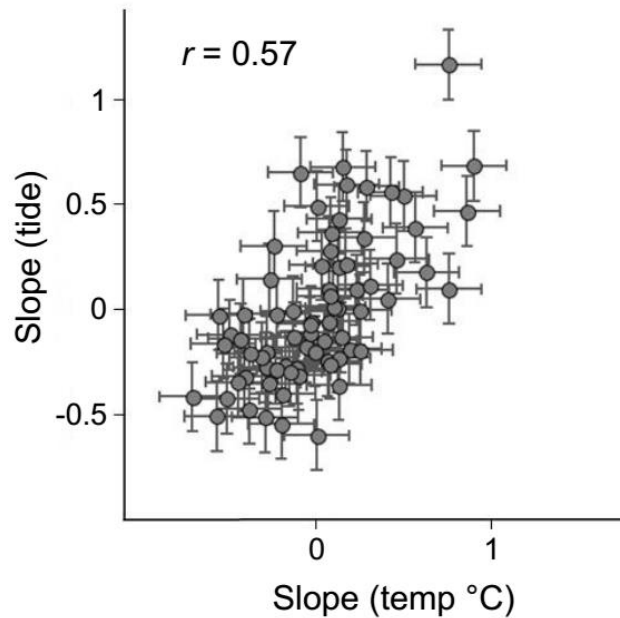
Figure 3.3 *Individual predicted slopes for reaction norms with respect to tide and to temperature for <u>L. irrorata</u>; standard errors of these values are shown. The significant correlation indicates more responsive (plastic) individuals with regard to tidal response were also more responsive to increases in temperature. Correlation value was estimated from the mixed model, using slope variances and the covariance.*

The inclusion of a separate residual variance for high and low tide situations provided an improved fit for the data and proved highly significant (Likelihood ratio test: $X_1^2 = 216$, $p < 0.0001$). This separate residual variance provided estimates of unpredictability, another form of plasticity, which was three times higher at high tide (Est $= 0.29$, SE $= 0.015$) than at low tide (Est $= 0.092$, SE $= 0.0049$). Based on the (co-)variances and residual variance at high tide, repeatability of boldness

at high tide and 23ºC was $R = 0.57$, and at high tide and 34ºC was $R = 0.61$. At low tide and 23ºC it was $R = 0.74$, and at low tide and 34ºC was $R = 0.85$.

Discussion

Discussions on the causes and consequences of behavioural plasticity often include domain generality as a common assumption, meaning that some individuals are expected to be more plastic, in general, than others (DeWitt et al., 1998; Sih & Del Giudice, 2012). However, thus far there is a lack of empirical evidence to support this in labile behavioural traits (Stamps, 2016; Mitchell & Biro, 2017). Here, results reveal that there was indeed a correlation between individual reaction norm (RN) slopes for tide and temperature, such that individuals that increased boldness more in response to increased temperature also increased boldness more in response to low tide conditions. This study therefore seemingly provides the first support for consistent behavioural plasticity across environmental gradients. The results also revealed that individual predicted mean values of boldness were highly consistent over time in a given tide and temperature context, given the lack of any temporal plasticity (Figure 3.1c). Boldness was also highly predictable in a given tide and temperature context, given the rather high situation-specific repeatability (R) estimates (0.57 to 0.85), indicating that individual reaction norms were quite distinct and predictable.

The finding, that some individuals were more plastic than others in their expression of boldness across tide and temperature gradients, could support the idea that phenotypic behavioural variation is underpinned by one or more common underlying variables (Stamps, 2016; Mitchell & Biro, 2017), such as those associated with individual metabolism (Van Dijk et al., 2002; Careau et al., 2008; Biro & Stamps, 2010; Metcalfe et al., 2016; Biro et al., 2018) or individual information states (e.g. Rodríguez-Prieto et al., 2010; Briffa, 2013; Dorset et al., 2017). In the context of this study, the propensity for boldness (risk-taking) should be higher under less risky situations (Dammhahn & Almeling, 2012; Thomson et al., 2012). Indeed, this was observed for the mean-level effect at low tide, a finding that supports previous work on *L. irrorata* showing that the incoming tide represents a considerably higher risk of predation (Warren, 1985; West & Williams, 1986; Vaughn & Fisher, 1988, 1992). The observed individual differences in responsiveness to tide (=risk) could be linked to differences in metabolism (Killen et al., 2011, 2012; Robison et al., 2018), providing a mechanistic 'bridge' for why responsiveness to risk was linked to temperature. Indeed, ectotherm metabolism is directly affected by changes in external temperature (Clarke & Johnston, 1999), and the expected mean-level increase in boldness with temperature was observed, in addition

to among-individual responses to temperature. This seems to indicate individual variation in sensitivity to metabolic state (Biro et al., 2010; Pruitt et al., 2011; Careau et al., 2014; Mitchell & Biro, 2017), which in turn might explain the covarying behavioural patterns observed. If valid, similar patterns of correlated plasticity in other traits previously linked to risk-taking, such as activity and aggression, would be expected where behavioural syndromes are present (Sih & Bell, 2008). Similar patterns might also be expected for individual growth; where bolder individuals are expected to experience higher growth rates as a result of increased food intake (Stamps, 2007; Biro & Stamps, 2008; Biro et al., 2014).

In this study, individuals that were less responsive to changes in tide-related risk and to temperature also tended to be bolder overall. Evidence for this comes from the strong negative covariance between predicted boldness at high tide compared to low tide. Simply put, bolder individuals had relatively high and flat reaction norms with respect to tide and temperature contexts. This seems to suggest that bold individuals have reduced scope to express variation in behaviour and/or low sensitivity to external and internal cues that influence information gathering relating to risk and affect metabolism, respectively. Thus, supporting the suggestion that bolder (or more pro-active) individuals should be generally less flexible than shyer (re-active) individuals (see e.g. Benus, Koolhaas & Van Oortmerssen 1987; Benus *et al.* 1990; Koolhaas *et al.* 1999). Low responsiveness to risk cues and temperature could be due to low-stress responsiveness (Groothuis & Carere, 2005) or potentially due to a reduced aerobic scope that in turn reduces the latitude for behavioural flexibility (Biro et al., 2018).

An individual's assessment of risk has previously been found to affect plasticity of a variety of traits including boldness (Briffa, 2013) and exploration (Quinn et al., 2012) as well as behaviour relating to foraging (Quinn & Cresswell, 2005), parental care (Ghalambor, 2002) and nest site placement (Peluc et al., 2008). Consequently, assessment of risk should be considered an important factor in modifying behaviour in variable environments. Indeed, individual boldness changed significantly across high and low tide situations, which carry different levels of risk. Individual response to risk was also evident where individuals were significantly less predictable in a high risk (high tide) situation. This finding, although contrary to those reported for amphibian (*Rana dalmatina*) tadpoles (Urszán et al., 2018), is consistent with similar observations in intertidal invertebrates (Briffa, 2013), and with previous suggestions that behaviour should be less predictable under high-risk situations (Maye et al., 2007; Brembs, 2011). This provides further insight into state-behaviour relationships; where extrinsic information regarding risk (perception of risk), associated with different situations, may well be considered as an important factor affecting individual state.

The highly labile nature of behavioural traits makes studying individual variation in plasticity challenging, from both experimental and statistical (sample size) perspectives. That this study was able to detect covariance between individual plasticity on two environmental gradients is likely due to a combination of substantial among-individual variation, substantial samples sizes (N = 73 IDs x 24 reps = 1752) that enhance power and precision of model parameters, and the semi-controlled conditions leading to individual RNs that were quite predictable and distinct from one another. This was reflected by the rather high situation-specific repeatability of boldness across all situations (range: $R = 0.57 – 0.85$). Repeatability was lower at high tide and 23°C and highest at low tide and 34°C, in significant part due to residual (intra-individual) variation being much lower at low tide than high tide.

Notably, the study found no evidence of among-individual variation in temporal patterns of boldness (i.e. no temporal plasticity). Often, studies find evidence for individual changes in behaviour over time indicative of acclimation or habituation to protocols (Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016). Lack of temporal plasticity indicates that individual predicted mean values were highly consistent over time in a given tide and temperature situation. Thus, in addition to showing that individuals differed in plasticity in response to two environmental gradients, this study also provides strong evidence of consistent individual differences in boldness over time, a hallmark of personality.

To summarise, the most novel and interesting observation is that of covariance between individual temperature and tide RN slopes indicating some individuals are more plastic than others in their expression of boldness across tide and temperature gradients. Both tide along with its risk-related information state, and temperature with its internal metabolic state, had significant influences on the behaviour of *L. irrorata* and therefore, this study provides further support for the influence of state variables as possible drivers of individual behaviour. Although this variation in plasticity might represent a strategy for coping with risk, future work should incorporate predation into the experimental design in order to examine the fitness consequences of the variation observed here (Briffa, 2013). The findings of this study also support previous suggestions that fluctuating environments may increase the variability of behavioural phenotypes (Luttbeg & Sih, 2010), and lead to the question of whether intertidal environments, themselves, select for greater flexibility in behavioural response to risk. In this regard, *L. irrorata* represents a potential model organism for future studies in which to compare labile behavioural traits of individuals exposed to the highly variable saltmarsh environment.

CHAPTER FOUR

Integration of physiology, behaviour and life-history traits: personality and pace-of-life in *Littoraria irrorata*.

Abstract

Attempts to unravel the proximate and ultimate causes of individual behavioural and life-history variation have often pointed to predicted correlations between behavioural, physiological and life-history traits, forming pace-of-life syndromes (POLS). The POLS hypothesis predicts that high levels of production (e.g. growth, fecundity) require high levels of foraging effort and risk-taking, supported by high metabolism. Despite tremendous interest in this topic, the POLS hypothesis still has limited empirical support, which has led to calls for more stringent empirical tests of the hypothesis and its assumptions. To that end, this study examined the associations between risk-taking behaviour (boldness), resting metabolic rate (RMR) and somatic growth rate in $N = 72$ marine gastropods, *Littoraria irrorata*, under controlled laboratory conditions using a longitudinal repeated measures design. After accounting for the effects of sex, size, and day of study, a multivariate mixed model revealed that bolder individuals had higher RMR ($r_{ind} = 0.32$), and grew faster ($r_{ind} = 0.59$), whereas RMR and growth were not significantly correlated ($r_{ind} = 0.13$). Further, if individuals were bolder than their average level on a given day, then their RMR was also higher (within-individual covariance: $r_e = 0.22$). This study represents rare and compelling support for the POLS hypothesis, studying its three key components (behaviour, energetics and life history), likely resulting from careful control, concurrent sampling of each trait, and rigorous analysis of the among- and within-individual patterns of variation and covariance.

Introduction

Recent attempts to explain the evolution of life history and behavioural diversification have highlighted the importance of links between behaviour, physiology and life-history characteristics, which are predicted to form pace-of-life syndromes (POLS; Ricklefs & Wikelski 2002). The POLS hypothesis highlights that individuals within single populations align along a gradient of slow to fast life history (LH) — in particular, it emphasises physiological constraints as being key to constraining life-history variation along this continuum, and points to the integration and co-evolution of behavioural, physiological and life-history characteristics (Ricklefs & Wikelski, 2002). Keen interest by behavioural ecologists to understand the development and maintenance of consistent behavioural phenotypes (*aka* animal personalities) subsequently led to similar hypotheses being proposed, and refined. For example, Stamps (2007) suggested that trade-offs between growth and mortality could explain the existence of personality trait variation for behaviours related to resource acquisition, such as activity, boldness or territoriality. Later theoretical developments included how resting metabolic rate (RMR) and LH productivity (growth and reproduction) could act as proximate constraints on behaviour to explain animal personality, where behavioural variation is maintained as a result of trade-offs between productivity and mortality, or present and future reproduction (Biro & Stamps, 2008, 2010; Careau et al., 2008; Wolf & Weissing, 2010). Further theoretical development then brought together an even greater suite of physiological, behavioural and life-history traits (Réale et al., 2010b).

In essence, these POLS hypotheses predict that individuals with faster LH should also have higher metabolic rates, fast growth, more active and bolder behaviour, and lower stress responsiveness. While early literature reviews provided substantial evidence for positive correlations between metabolism and behaviours that provide and/or consume significant energy (Careau et al., 2008; Biro & Stamps, 2010), and between behaviours that provide energy for the production of new biomass (Biro & Stamps, 2008), a large number of subsequent studies have not (Dammhahn et al., 2018). Indeed, a recent meta-analysis found little to no evidence for positive correlations among these traits as predicted by the POLS hypothesis (Royauté et al., 2018). However, it did find significant but weak correlations between behaviour and hormones ($r = 0.18$) and between behaviour and growth rate ($r = 0.23$); the work concluded that weak or no correlations may have resulted from methodological challenges associated with studying and analysing individual behaviour (Niemelä & Dingemanse, 2018a; see also Royauté et al., 2018).

A challenge when studying this topic is the fact that behaviour and physiological traits are highly labile, meaning they are sensitive to study protocols and methods, and to environmental

variation (Beckmann & Biro, 2013; Carter et al., 2013; Biro et al., 2014). This is why it is important to examine this topic in controlled studies, using many individuals and repeated measures, where physiology, behaviour and life history are concurrently sampled (Biro & Stamps, 2008, 2010; Careau et al., 2008; Biro et al., 2014). Unfortunately, about a third of recently reviewed POLS studies had no repeated measures for the labile traits considered (Royauté et al., 2018), which tends to downwardly bias any correlation that may be present (Adolph & Hardin, 2007). Conversely, there is also the possibility of upward bias where studies use analyses that fail to account for the error around individual-level predictions, leading to anticonservative hypothesis tests and small confidence intervals, and to correlation estimates which do not capture the full uncertainty of trait relationships (Houslay & Wilson, 2017). In addition, another recent meta-analysis also found little support for correlations between behaviours and metabolism or hormones, and painted an even bleaker picture of the quality of data and analyses used in most studies (Niemelä & Dingemanse, 2018a). This has led to calls for more stringent empirical research testing the predictions made by the POLS hypothesis (Biro et al., 2014; Dammhahn et al., 2018).

Of particular interest within the POLS hypothesis is the expected association between behavioural traits and metabolic rate (Careau et al., 2008; Biro & Stamps, 2010; Réale et al., 2010b; Metcalfe et al., 2016). Proximate explanations for this relationship centre on relatively expensive metabolic 'machinery' being necessary to support a comparatively active, fast paced, productive and risky life style (Careau et al., 2008; Biro & Stamps, 2010). Therefore, rapid growth, high fecundity and/or frequent reproduction should require high levels of foraging activity and boldness, with high RMR also expected as it likely reflects the underlying machinery needed to support this (Biro & Stamps, 2010; Killen et al., 2011; Careau & Garland, 2012; Auer et al., 2016; Monceau et al., 2017).

Until recently, the majority of research has focussed on testing associations between one behavioural trait and one life-history trait (e.g. Ward et al., 2004; Réale et al., 2009; Mas-Muñoz et al., 2011; Ariyomo & Watt, 2012; Kluen et al., 2013; Biro et al., 2014; Monceau et al., 2017; Niemelä & Dingemanse, 2018a) or between a behavioural trait and metabolism (e.g. Réale et al., 2010b; Killen et al., 2011, 2012; Krams et al., 2013a; McKenzie et al., 2015; Auer et al., 2016; Binder et al., 2016; Velasque & Briffa, 2016; White et al., 2016; Krams et al., 2017). However, given the multidimensional nature of individual variation (Réale et al., 2010b), interpreting pairwise correlations of individual traits can be problematic (Dingemanse et al., 2010a; Mathot & Frankenhuis, 2018). Therefore, it is important to consider the associations between multiple traits in order to provide reliable assessments of the relationships between them.

Here, the multidimensional associations between risk-taking propensity, metabolism and growth are examined together in a controlled laboratory experiment, using the saltmarsh periwinkle

(*Littoraria irrorata*). The study aimed to examine (a) the repeatability of these behavioural and physiological traits, and (b) the among-individual associations between boldness, RMR, and overall growth, as well as the within-individual association between boldness and RMR using a multivariate mixed model approach. Due to the hypothesised functional role played by risk-taking behaviour in mediating life-history trade-offs (Stamps, 2007; Wolf et al., 2007; Biro & Stamps, 2008), it was expected that faster overall growth would be observed in those individuals most prone to risk-taking behaviour. Further, where risk-taking behaviour may be necessary during foraging to satisfy the higher energy demands of individuals with higher metabolism (Biro & Stamps, 2010; Killen et al., 2011; Careau & Garland, 2012; Monceau et al., 2017), it was expected that individual propensity for risk-taking (boldness) would covary with RMR in a positive direction. Based on these predictions, and the idea that higher relative growth should associate with higher RMR (e.g. Biro & Stamps, 2010), it was also expected that individuals with relatively high RMR would exhibit faster overall growth.

Materials and methods

Collection and husbandry

This study used the remaining snails from Chapter 3 ($N = 72$, 17-28mm shell length), collected at low tide from a 5m^2 area of high-marsh, adjacent to the Eastern Shore Laboratory (37°36'31.2"N 75°41'09.3"W) in July 2016. All snails were transported to the laboratory and excess water was removed from shells prior to being individually marked, by number, using acrylic paint covered with non-toxic aquarium glue (Stagg & Mendelssohn, 2012). Sex was then determined by visual inspection for the presence or absence of the male reproductive organ (42 females and 30 males). Only animals with fully intact shells and without any obvious parasites were included in the study.

Snails were initially allocated (randomly) to one of four outdoor 'replica marsh plots' (0.30m^2) ($N = 4$), where conditions were comparable with that of the surrounding natural marsh. Marsh plots were created by filling open top mesh cages (60 x 50 x 5cm) with 5cm deep marsh cores, complete with cordgrass roots and intact stems, taken from the adjacent *Spartina alterniflora*-dominated saltmarsh (37°36'31.2"N 75°41'09.3"W). Marsh plots were placed in flow-through tables (248cm x 61cm x 20cm) connected to a flow through-system, drawing water from and returning it to the adjacent Wachapreague channel. Tidal inundations were simulated daily, within each flow-through table, by replicating the tidal patterns seen in the surrounding, natural marsh. Daily maximum water level, at high tide (mean ± SD, 11.83 ± 1.67cm), and water flow rates were

replicated, approximately, by referring to a guide measure placed in the natural marsh, visible from the marsh plots.

Within each plot, estimated plant biomass density (biomass m^{-2} ± SD., 215.3 ± 2.1g) and plant height (mean ± SD 143cm ± 12.83) were comparable to that observed in the surrounding, natural marsh (biomass m^{-2} 255.3 ± 103.1g; stem height 178cm ± 32.83, mean ± SD). Snail density in each plot (≈ 60 snails m^{-2}) was also comparable to the densities found in the surrounding marsh areas (85 ± 15 snails m^{-2}). Individuals were housed outdoors in these plots for 30 days to allow them to feed, *ad-libitum*, on living and dead *Spartina alterniflora* stems, and on marsh detritus. Animals were monitored daily to ensure all individuals were present and any escaped individuals were returned to the centre of their designated plots. This interval, plus the additional 12d during which time behaviour and metabolism were observed, permitted quantification of growth variation among individuals under semi-natural conditions, over a total duration of 42d; on average, snails increased in length by 0.6% over this interval, representing an estimated increase in mass of 1.62%.

After the initial 30-day period, marsh plots were transported into the laboratory where they were maintained, in ambient conditions (comparable to those experienced outdoors) for a further twelve days, where feeding could continue *ad-libitum*. Previous work on this species has highlighted the significant influence of both temperature and tidal cycle on individual behaviour (see CHAPTER 3), and thus, both tide and temperature were controlled for during the subsequent behavioural trials and metabolic rate measurements. Consequently, snails were maintained at 28-28.5 °C, and under high tide conditions, which represent conditions indicative of high predation risk (West & Williams, 1986; Vaughn & Fisher, 1988, 1992; see also CHAPTER 3).

After completion of the experiment, all snails were returned to the marsh after having their identification marks removed.

Growth rates

Shell length was measured after 24 hours of acclimation to the marsh plots, and again before release on the final day of the experiment 42d later. Measurement time was approximately equal across individuals and took no longer than 1 minute. Digital photographs were taken of all individuals prior to being returned to their designated marsh plots. Images were later used to re-measure shell length with ImageJ (Image processing and Analysis in Java - Rasband 2011) to provide more accurate measures of individual length (±0.001mm). Shell length was then used to estimate wet mass (g), to account for size effects on growth, by applying a length-mass regression described by Crisp (1984), based on length and wet mass measurements taken from a subset of snails collected, from the same area, prior to the experiment ($N = 62$) (Regression: y = 0.00001x$^{2.783}$, $R^2 =$

0.595). However, given the strength of this relationship and the variability these estimates would introduce, it was decided that shell length would be used in the subsequent data analysis.

Risk-taking propensity

Once in the laboratory, and after 24 hours of acclimation to controlled conditions, individual boldness was scored as the inverse of latency to emerge from a hiding response as done previously (CHAPTERS 2&3). Each snail was lifted from its resident marsh plot and held over a white photo tray. Pressure was applied to the foot of the snail with a blunt ended seeker (dissection probe), causing the foot to be retracted into the shell and for the operculum to be fully closed over the shell aperture. The snail was then placed on a photo tray, with the aperture facing upwards. Response latencies were recorded as the time taken (in seconds) for the operculum to reopen and for the first antennae to become visible to the observer. All observations were conducted by a single observer (T.O.C) ensuring consistency across trials. Individual response latencies were recorded in a random order, once daily (beginning at 9am and ending at ≈ 2pm), for nine consecutive days, yielding a total behavioural sample size of 648 (= 72 individuals x 9 repeats).

Resting metabolic rate

Resting metabolic rate (RMR, $\mu molO_2/h^1$) was calculated using oxygen uptake measurements obtained for aquatic respiration, using stop-flow respirometry, at least five hours after the behavioural trials undertaken on days 3, 6 and 9 (between 2-8pm). Feeding was prevented after behavioural observations, allowing for clearance of any food consumed prior to behavioural trials (see Killen et al., 2011). Furthermore, snails generally remained stationary during respirometry trials, and thus the minimum MR measurement achieved during a trial was defined as its resting MR, hereafter its RMR.

Each respirometer (volume = 140ml) contained a stirring bar, to prevent the formation of oxygen partial pressure (pO_2) gradients during trials and was fitted with an oxygen sensitive optode (Fibox 4, PreSens Gmbh. Regenburg, Germany). Respirometers were supplied from a central reservoir with fully aerated, filtered seawater; maintained at a constant 28-28.5°C and a salinity of 34ppt. The respirometers were covered with a dark, opaque plastic sheet to create a darkened environment and to reduce disturbance during acclimation and during trials. A preliminary set of experiments determined 1-2h as the acclimation time required for stress-related metabolic elevation to subside and for subsequent stable measures of O_2 uptake in the study animals (see Figure 4.1). Using this information, each snail was placed in an individual respirometer and was conservatively given 2h to acclimate and settle into the experimental environment prior to any measurements being

taken. During these preliminary trials, no faecal matter was present in the respirometry chambers
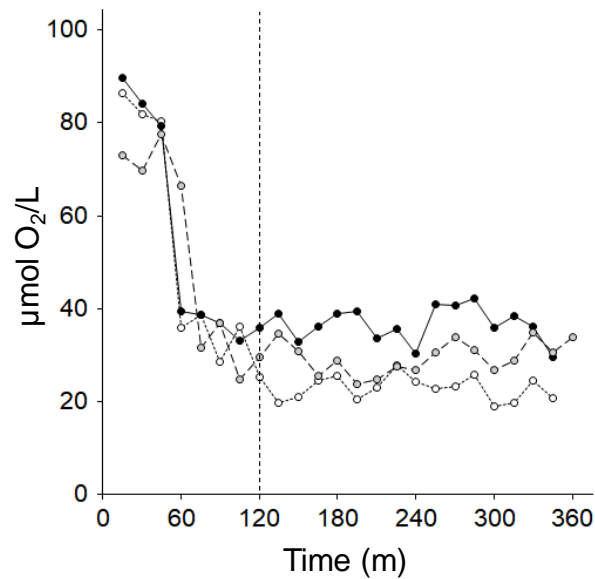
Figure 4.1 *Metabolic rate (μmol O₂/L) traces of three representative* <u>L. irrorata</u> *during preliminary respirometry trials. Each dot represents O₂ consumption over a 15-minute period. Vertical dashed line indicates minimum acclimation time (2h) used in subsequent trails as the pre-trial acclimation period.*

indicating that animals were indeed in a post-absorptive state after feeding ceased at least 5h prior.

After each initial acclimation period, water flow to respirometers (12 + 1 control) was stopped and the decline in μmol O₂/L was measure every 15 minutes, over a 60-minute period, for each animal. Respirometry chambers were not flushed between measures, however O₂ did not fall below 70% during trials. O₂ decline was measured using the Fibox 4 fiber optic oxygen meter (PreSens Gmbh. Regenburg, Germany), which automatically standardises barometric pressure, temperature and salinity during trials. RMR was recorded as the lowest measure for each individual over the 60-minute period.

To control for any background O₂ fluctuations during the experiment, oxygen consumption was also measured in a control respirometer (sans snail) during each trial. Residual declines in O₂ from the control respirometers (due to microbial, algal activity) were accounted for during the final calculations. As a further control, individual snail shells were scrubbed to remove debris and algae prior to respirometry trials.

Statistical analysis

A single multivariate mixed model (MMM) was used to simultaneously estimate the following: adjusted repeatability of boldness and RMR, correlations among boldness, RMR and growth at the among-individual level, and the correlation between boldness and RMR at the within-

individual (residual) level. Residual correlation between growth and other traits is not possible because it is a single value representing growth over 42d. Boldness was modelled as the inverse (1/latency) of individual re-emergence latencies and was ln-transformed to meet the assumptions of normality. RMR was also ln-transformed to meet the assumption of normality and to ensure a linear relationship with size (shell length). The response variables boldness and RMR, were then centred and standardised to a mean of 0 and a variance of 1 (z-transformed) to facilitate convergence (Schielzeth, 2010).

Fixed effects of trial, sex and shell length (mm) were fitted for all three traits. To facilitate the interpretation of these fixed effects, sex was treated as a continuous variable (female = 0, male = 1) and centred on the mean, trial number (effect of time) was mean-centred, and shell length was ln-transformed, then centred and standardised to a mean of 0 and a variance of 1 (Houslay & Wilson, 2017). Finally, individual 'ID' was included as a random (intercept) effect, along with a random slope effect of trial number. However, this effect was estimated to be zero and thus the results presented are from the reduced model.

Two unstructured variance-covariance matrices were specified. The first (the 'I-matrix') accounts for among individual variation in each trait (random intercept effect of individual 'ID'), estimating the among-individual variance of boldness, RMR and growth (VAR_{ind}) and the covariances between them (COV_{ind}). The second ('R-matrix') accounts for the residual variation (within-individual variance; VAR_r) and provides an estimate of covariance between the residual repeated measures of boldness and RMR (COV_e).

Parameters were estimated using the Markov chain Monte Carlo (MCMC) method. To ensure convergence and adequate chain mixing, the posterior distributions and autocorrelation plots of five independent chains were compared, each with a total of 750,000 iterations, 60,000 burn-ins and a thinning interval of 275 iterations (R package 'MCMCglmm' 2.21, Hadfield 2010). Before running the MMM, an 'uninformative', parameter-expanded, model prior was specified. For the within-individual variance, it must be noted that since only a single measure of overall growth was recorded for each individual, growth has no residual (within-individual) variance and as such, within-individual correlations involving this trait must be 0. However, specified variances must be positive, so the within-individual variance was fixed for growth at a small positive number (0.0001); fixing the residual variance in this way thus makes all variance in growth expressed only at the among-individual level in the I-matrix.

Model (co)variances were used to estimate among-individual (r_{ind}) and within-individual (r_e) correlations between response variables by dividing the corresponding covariance between two variables by the product of the square root of their variances (Dingemanse & Dochtermann, 2013):

$$(\textbf{\textit{r}}_{\textbf{ind}} = \text{COV}_{ind} / (\text{sqrt}(\text{VAR}_{ind})*\text{sqrt}(\text{VAR}_{ind})))$$

$$(\textbf{\textit{r}}_{\textbf{e}} = \text{COV}_{e} / (\text{sqrt}(\text{VAR}_{e})*\text{sqrt}(\text{VAR}_{e})))$$

Since these correlations can be either positive or negative, the 95% credible intervals (CI) were used to assess statistical significance, and only correlations with CIs excluding zero were considered significant.

Adjusted repeatability (R_j) for boldness and RMR (i.e. conditioned on the fixed effects) were estimated by dividing their respective among-individual variance estimates by the sum of their among-individual and residual variances following Nakagawa and Schielzeth (2010):

$$(\textbf{\textit{R}}_{\textbf{j}} = \text{VAR}_{ind} / (\text{VAR}_{ind} + \text{VAR}_{r}))$$

The posterior modes of the random effects were then used, following a very informative tutorial by Houslay and Wilson (2017), to create graphical representations of r_{ind} to illustrate associations between each of the variables of interest (see Figure 4.2).

Results

Mean-level results

On average, snails showed a weak tendency for increased boldness (0.025, [0.0036 – 0.045]) and increased RMR (0.032, CI [0.008 – 0.054]) across trials. Shell length had a significant effect on boldness, RMR and overall growth, with larger individuals tending towards higher RMR (0.839, CI [0.742 – 0.942]), longer response times (-0.249, CI [-0.411 – -0.071]) and slower growth (-0.097, CI [-0.147 – -0.042]). Sex had no significant effect of for any of the three traits (see Table 4.1).

Table 4.1 *Fixed effects fitted to the multivariate mixed model for response variables RMR, boldness and overall growth in* <u>L. irrorata</u>. *Shown are posterior means, lower and upper 95% confidence intervals and pMCMC (statistically significant pMCMC values (p < 0.01) are in bold).*

Trait	Post.mean	L - 95% CI	U - 95% CI	pMCMC
RMR				
Intercept	-0.0392	-0.1435	0.0694	
Shell length	0.8396	0.7418	0.9423	**<0.0001**
Sex	0.0414	-0.1655	0.2421	0.6943
Trial	0.0318	0.0084	0.0539	**0.0091**
Boldness				
Intercept	0.0020	-0.1657	0.1723	
Shell length	-0.2495	-0.4112	-0.0714	**0.0011**
Sex	0.2492	-0.1078	0.5880	0.1583
Trial	0.0248	0.0037	0.0452	0.0251
Growth				
Intercept	1.0014	0.9489	1.0536	
Shell length	-0.0967	-0.1475	-0.0416	**0.0006**
Sex	0.0495	-0.0608	0.1583	0.3457

Individual-level results

After accounting for the fixed effects of trial, sex and shell length, both boldness (R_j = 0.49, CI [0.391 – 0.588]) and RMR (R_j = 0.44 CI [0.279 – 0.578]) were moderately repeatable, meaning that covariance among the traits was possible. Indeed, the multivariate mixed effects model revealed significant, positive covariance between boldness and RMR at both the among-individual (COV_{ind} = 0.081, CI [0.006 – 0.163]), and at the within-individual (COV_e = 0.064, CI [0.009 – 0.114]) levels. These estimates provided moderate correlations at both the among-individual (r_{ind} = 0.32, CI [0.07 – 0.59]) (Figure 4.2) and within-individual levels (r_e = 0.22, CI [0.04 – 0.38]). Finally, there was also a significant positive covariance between boldness and growth (COV_{ind} = 0.093, CI [0.049 – 0.138]), but no significant covariance between RMR and growth (COV_{ind} = 0.011, CI [-0.012 – 0.0364]) at the among-individual level. These estimates indicated a strong positive correlation between boldness and growth (r_{ind} = 0.58, CI [0.415 – 0.750]) but not between RMR and growth (r_{ind} = 0.13, CI [-0.141 – 0.397]) (Figure 4.2).
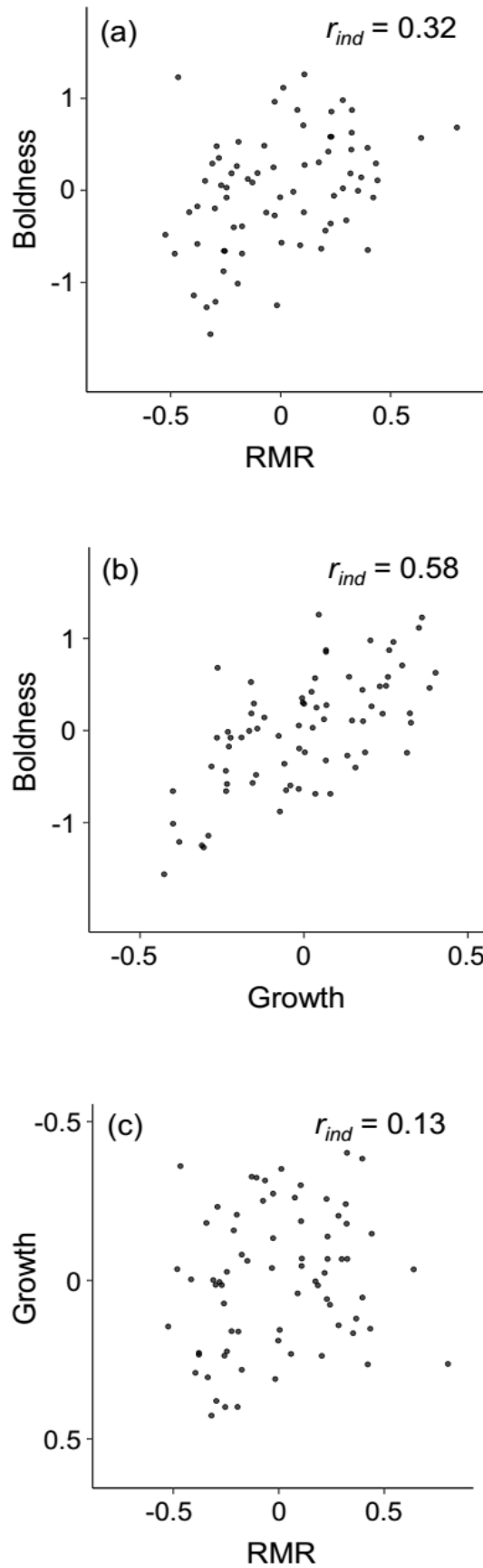
Figure 4.2 *Representation of the among-individual correlations between boldness and RMR* **(a)***, boldness and growth* **(b)***, and growth and RMR* **(c)** *in* <u>*L. irrorata*</u>*. Data are based on the posterior modes of the random effects from the multivariate linear mixed model.*

Discussion

The aim of this study was to examine correlations between boldness, RMR and somatic growth in *L. irrorata*, in order to test predictions made by the POLS hypothesis. The results revealed among-individual correlations between boldness and RMR ($r_{ind} = 0.32$), and between boldness and growth ($r_{ind} = 0.58$). Simply put, those with relatively high growth rates had shorter anti-predator response times and thus were considered bolder; bolder individuals also had relatively higher RMR. Thus, these results provide rare and compelling evidence for among-individual covariation between boldness and RMR, and between boldness and growth, commensurate with the predictions made by the POLS hypothesis (Ricklefs & Wikelski, 2002; Stamps, 2007; Careau et al., 2008; Biro & Stamps, 2010; Réale et al., 2010b; Wolf & Weissing, 2010).

The significant, positive association between behaviour and metabolism (RMR), at both the among- and within-individual levels reinforces previous suggestions that individual behavioural variation may be underpinned by energetic constraints (Van Dijk et al., 2002; Careau et al., 2008; Biro & Stamps, 2010; Biro et al., 2018). In particular, where RMR may reflect differences in the idling cost of the 'metabolic machinery' required for periods of maximal energy expenditure (Biro & Stamps, 2010; Auer et al., 2017), such as those experienced during predator avoidance. In this case, relatively high RMR animals may be generating higher energy output whilst also requiring more energy to sustain themselves. Therefore, boldness affecting foraging and food intake rates would be expected to covary with RMR to pay the cost of higher overall RMR. Further, given our *ad libitum* food conditions and standardised conditions, observed links between metabolism and behaviour may be genetically correlated (e.g. Gębczyński & Konarzewski, 2009; Careau et al., 2011; Brzek et al., 2016), an underlying premise of the POLS hypothesis (Réale et al., 2010b).

The high among-individual correlation between boldness and overall growth ($r_{ind} = 0.59$), along with the observation that study animals spent much of their time foraging, indicates that boldness plays a functional role in resource acquisition in *L. irrorata*, as predicted by the POLS hypothesis (Stamps, 2007; Biro & Stamps, 2008). This also supports the idea that intrinsically productive individuals should exhibit consistently high levels of behaviour that affect food intake rates (Stamps, 2007; Biro & Stamps, 2008), and adds to limited existing evidence for covariation between boldness and growth rate at the among individual level (e.g. Biro et al., 2014). It is also possible (as previously suggested, Biro et al., 2014) that motivation to feed is, for those with higher than average growth rates, a proximate driver for higher levels of boldness, which would be consistent with previous work examining growth enhancement through artificial selection or gene manipulation which resulted in higher feeding intake and risk-taking (Johnsson & Abrahams, 1991;

Johnsson et al., 1996; Sundström & Devlin, 2011). Further, it is important to note that since food was available *ad libitum* throughout the study, the possibility of a genetic correlation between boldness and growth cannot be discounted, especially given the high r_{ind} observed here (Dochtermann, 2011), or that correlations may reflect developmental plasticity in a common direction.

Despite a positive trend for higher overall growth in individuals with higher RMR, these findings indicate that RMR and overall growth were not strongly correlated ($r_{ind} = 0.13$). This might be contrary to the idea that increased growth rates (and thus higher food intake) should be associated with morphological and physiological systems geared towards processing more food per unit time (Biro & Stamps, 2008). However, inference here is likely limited by statistical power, where the number of individuals ($N = 72$) is far fewer (even with the 9 repeat samples per individual) than would be necessary to detect a true correlation of ~ 0.10 (see Dingemanse & Dochtermann 2013). This is, therefore, an important consideration for future studies. Nevertheless, increased growth has been shown to associate with morphological and physiological traits that improve digestive efficiency in different taxa (Hemsworth et al., 1994; Selman et al., 2001; Geverink et al., 2004; Biro et al., 2006; Walsh et al., 2006), and some studies suggest that higher productivity might require higher RMR (Metcalfe et al., 1995; McCarthy, 2000; Ksiazek et al., 2004), likely related to larger morphological structures (e.g. digestive organs, liver, heart etc), which have higher intrinsic mass-specific rates (Ksiazek et al., 2004). It is also possible, given the relatively low growth rates reported here ($\approx$ 0.6% average length gain), that these results could reflect changes in growth rate with individual size in *L. irrorata*, where growth declines with size (and age) (Bingham, 1972a). Since the majority of our specimens (shell length; 17 – 28.3mm) were adult (as defined by Hamilton, 1978b), it is possible that further growth would not increase fecundity to the degree that it might in younger individuals. Thus, energy is likely being channelled to activity, rather than reproductive output. In order to test the assumptions relating to the weak correlation between growth and RMR, future studies should consider quantifying associations between growth and RMR in different size classes to determine whether younger individuals, yet to reach maturity exhibit higher growth with higher RMR, as predicted by the POLS hypothesis.

The results also indicate moderate among-individual repeatability in boldness ($R_j = 0.49$) and RMR ($R_j = 0.44$), corresponding with previous findings of invertebrate organisms tested under controlled laboratory conditions (Nespolo & Franco, 2007; Bell et al., 2009), adding to existing evidence of consistent boldness (personality) in *L. irrorata* (CHAPTERS 2 & 3). Further, the observed (co)variance structure suggests that the model of a risk-prone, risk-averse (bold-shy) continuum appears to be valid for this population, under stable temperature and at high tide

situations. Both of which have previously been shown to influence among- and within-individual repeatability in this species (CHAPTER 3). Although individual differences in behavioural changes over time are a common observation, indicating varying levels of habituation to experimental protocols (Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016), no evidence for individual behavioural changes in boldness or RMR over time (i.e. temporal plasticity) were observed here. This finding is similar to that reported previously for this species (CHAPTERS 2 & 3) and suggests that individual predicted mean values were consistent over time. Thus this study also provides further evidence of consistent individual differences in boldness over time, a key characteristic of animal personality (Biro & Stamps, 2015).

In summary, this study identified significant positive correlations between behaviour (boldness) and metabolism (RMR), and between boldness and growth, with a trend (albeit insignificant) towards increased overall growth in relatively high MR animals. Importantly, the association between RMR and boldness was found to be significant at both the among- and within-individual levels. Overall, the study provides rare support of a pace-of-life syndrome between boldness, metabolism and somatic growth, which are particularly important given the recent limited support for among-individual correlations between behaviour, physiology, and life-history traits presented recently (Niemelä & Dingemanse, 2018a; Royauté et al., 2018). Further, these results may also highlight the importance of a combination of careful control, longitudinal repeated measures, and rigorous analysis accounting for the among- and within-individual patterns of variation and covariance in unravelling these associations. Finally, based on assumptions made in previous work on individual behaviour in *L. irrorata* (CHAPTERS 2 & 3), these results further reinforce the idea that behavioural variation in this species is influenced by factors associated with individual state, in this case, individual metabolism (Van Dijk et al., 2002; Careau et al., 2008; Biro & Stamps, 2010; Biro et al., 2018).

CHAPTER FIVE

The fitness consequences of risk-taking behaviour: does boldness predict survival in the saltmarsh periwinkle, *Littoraria irrorata*?

The results of this chapter are currently being prepared for journal submission.

Abstract

Recent research centring on consistent individual behavioural traits (personalities) has led to a growing appreciation of their potential to influence individual fitness traits, such as growth, reproduction, and survival. Theoretical explanations for the link between behaviour and fitness centre on trade-offs between current reproduction and future survival, where more risk-prone individuals are expected to benefit from increased resource gains at the expense of higher mortality by predation. However, despite attempts to examine this prediction, current evidence shows that the associations between risk-taking and survival vary considerably between study systems. Further, despite models predicting that positive associations between risk-taking and mortality occur at the among-individual level, few published studies have included among-individual covariance estimates, from repeated measures of behaviour, using analyses which allow for the partitioning of (co)variance to its among- and within-individual levels. Therefore, this study examined the among-individual associations between risk-taking behaviour (boldness) and survival rates in a marine gastropod (*Littoraria irrorata*), during exposure to a crustacean predator (*Callinectes sapidus*), using a multivariate mixed model approach. After accounting for the fixed effects of size, sex, and time of day, the study revealed that bolder individuals experienced greater survivability ($r_{ind} = 0.29$) and survived for longer ($r_{ind} = 0.37$) than shyer conspecifics. Thus, indicating that bolder snails are more likely to survive in general, and for longer, during repeated interactions with predatory crabs. Therefore, this study adds to the currently limited evidence for covariation between risk-taking and survival, and also adds to the growing body of literature showing that individual personalities influence individual fitness, and that predation may select positively for boldness in some species.

Introduction

Elucidating the adaptive nature of among-individual behavioural diversity has represented a major focus within the fields of behavioural ecology and evolutionary biology in recent years, with suggestions that consistent individual differences (personalities) can significantly influence components of individual fitness, such as growth, reproduction and survival (Dingemanse et al., 2004; Dingemanse & Réale, 2005; Réale et al., 2007; Stamps, 2007; Bergmüller et al., 2010; Smith & Blumstein, 2010; Sih et al., 2012; Wolf & Weissing, 2012). Further, the link between individual behaviour and individual fitness traits is a common assumption, underlying evolutionary hypotheses proposed to explain the maintenance of individual behavioural diversity (Dingemanse & Réale, 2005; Réale et al., 2007, 2010b; Stamps, 2007; Biro & Stamps, 2008; Smith & Blumstein, 2008; Dammhahn et al., 2018). For example, life-history theory posits that trade-offs between current and future reproduction depend on distinct alternative strategies (Stearns, 1989). These strategies, most often associated with risk-taking behaviours (e.g. boldness) at the individual level (Réale et al., 2010b; Dammhahn et al., 2018), are explained by adaptive personality theory as having co-adapted with life-history characteristics (Stamps, 2007; Wolf et al., 2007). In this sense, individual risk-related behavioural traits are expected to mediate trade-offs between the risk and reward associated with different behavioural phenotypes (e.g. faster growth, early maturation, current reproduction and future survival) (Stamps, 2007; Réale et al., 2010b; Dammhahn et al., 2018). In particular, as predicted by the Pace-of-Life Syndrome hypothesis (POLS; Ricklefs & Wikelski, 2002), individuals with a propensity for risk-taking should benefit from an increase in resource gains, at the expense of future survival (Wolf et al., 2007; Dingemanse & Wolf, 2010; Réale et al., 2010b).

Increased resource gains are expected to lead to faster growth (Stamps, 2007; Wolf et al., 2007; Biro & Stamps, 2008) and ultimately, larger body sizes, both of which are widely accepted to have a significant influence on survival, fecundity, as well as reproductive and competitive ability (see e.g. Sedinger et al., 1995; Biro et al., 2006). However, evidence suggests that despite the advantages offered by maximising growth rates, faster growth can incur fitness costs offsetting some of the potential benefits of obtaining larger body sizes more quickly (Biro et al., 2006; Chiba et al., 2007). For example, growth rates across a range of taxa are often reported to lie below their maximum physiological potential (e.g. Nicieza & Metcalfe, 1997; Wu & Dong, 2002; Lindström et al., 2005). Additionally, maintenance of behavioural diversity is expected to occur in natural populations where phenotypes associated with increased resource acquisition are also associated with higher predation risk, often leading to mortality prior to gaining the benefits of greater body size (Stamps, 2007; Smith & Blumstein, 2008). For example, increased foraging effort is often

reported to lead to higher mortality by predation (Anholt & Werner, 1995; Gotthard, 2000; Biro et al., 2003b, a; Stoks et al., 2005). Thus, it is predicted that the propensity for risk-taking will be selected against in some populations — particularly, where predation pressure is high (Bell & Sih, 2007; Kortet et al., 2010; Réale et al., 2010b).

The notion that risky behaviour should result in higher mortality was supported by an earlier meta-analysis reporting empirical evidence for negative associations between boldness and survival (Smith & Blumstein, 2008). However, there is also evidence that associations between behaviour and fitness can be context dependent, for example selection may act differently during different life stages (e.g. Biro et al., 2006; McCormick & Meekan, 2010; Mayrand et al., 2019), as a result of environmental variation (e.g. Kain & McCoy, 2016; MacPherson et al., 2017; Lapiedra et al., 2018; Santicchia et al., 2018), or with different levels of competition (Boulton et al., 2018). Similarly, despite much attention being given to this topic, empirical research has shown that associations between behaviour and fitness (particularly survival) differ markedly between study systems. For example, in some systems, risk-related behaviours such as activity and boldness have been shown to associate negatively with survival (Riechert & Hedrick, 1990; Storfer & Sih, 1998; Niemelä et al., 2015; Hulthén et al., 2017), but positively in others (Réale & Festa-Bianchet, 2003; Magnhagen & Staffan, 2005; Blake & Gabor, 2014; Foster et al., 2017; Piquet et al., 2018; Richardson et al., 2019). These differences could be explained by ecological variation, including different predator foraging strategies (Belgrad & Griffen, 2016a), different focal species ecologies (e.g. Riechert & Hedrick, 1990; Biro et al., 2004; Richardson et al., 2019), or by different results generated from lab and field studies (Biro et al., 2004; Pruitt et al., 2012; Foster et al., 2017). However, the relative importance of these and other factors is still poorly understood, and thus further studies examining the associations between individual behaviour and individual fitness are required to identify patterns across study systems (Foster et al., 2017).

Since predation risk could represent an ecological mechanism for balancing the costs and benefits of different behavioural strategies in natural populations (Stamps, 2007; Smith & Blumstein, 2008; Kortet et al., 2010), it is also likely to represent a mechanism for selection against higher growth rates in some populations (see e.g. Biro et al., 2006). Thus, examining potential covariation between risk-taking behaviour and mortality by predation may be critical in providing further insights into behaviourally mediated trade-offs between risk and reward in natural populations. However, despite previous work examining associations between risk-taking behaviour and survival, very few published studies have thus far included among-individual covariance estimates, from repeated measures of behaviour, using analyses which allow for the partitioning of (co)variance to its among- and within-individual levels (Niemelä & Dingemanse,

2018a, b; Royauté et al., 2018; Moirón et al., 2019). Therefore, it is important to consider associations between individual risk-related behavioural traits and measures of fitness, at the partitioned level, in different study systems in order to test theoretical predictions, and to further elucidate the costs and benefits of different behavioural strategies.

To that end, this study examined the among-individual associations between individual risk-taking propensity (boldness) and survival — during exposure to staged interactions with a predator, the blue crab (*Callinectes sapidus*) — as part of a controlled laboratory experiment, using the marine gastropod (*Littoraria irrorata*). In addition, the study also aimed to examine whether this association was influenced by environmental context (i.e. boldness measured under control and predator cue treatments), using a multivariate mixed model approach. An abundant consumer on the saltmarshes of the Eastern United States, *L. irrorata* is an important prey species for a number of saltmarsh predators (Hamilton, 1976; Crist & Banta, 1983; Tucker et al., 1995; Silliman & Bertness, 2002; Dietl & Alexander, 2009). In particular, the blue crab, which presents a significant threat of predation in the high marsh during tidal inundation, is thought to, at least in part, drive snail circumtidal ascension of cordgrass stems at high tide (e.g. Hamilton, 1976; Warren, 1985). Furthermore, *C. sapidus* has previously been shown to exhibit a preference for, and to readily consume live snails during controlled experiments (e.g. Hamilton et al., 1983; West & Williams, 1986), and *L. irrorata* have been shown to respond to crab predator cues in the form of predator effluent (Duval et al., 1994; Carroll et al., 2018). Thus, blue crabs were used as part of the staged predator-prey interactions in this study.

Previously, *L. irrorata* have been observed to exhibit temporally consistent individual differences in risk-taking propensity, and on average boldness has been shown to increase from high to low risk situations (high to low tide), indicating that behavioural responses in this species may be driven, at least in part, by varying predation risk (see CHAPTER 3). Therefore, individuals were expected to be bolder under controlled conditions (the control treatment) compared to those in which predator effluent was present (the predator treatment). It was also predicted that boldness would vary among individuals due to differences in risk perception and stress responsiveness (Carere et al., 2010; Dorset et al., 2017). With respect to the associations between boldness and survival, it was predicted that bolder individuals would suffer from higher mortality (Wolf et al., 2007; Dingemanse & Wolf, 2010; Réale et al., 2010b) — both in terms of overall survival and the number of days survived — during staged predator encounters. Finally, individual boldness was expected to vary across the many repeat trials, indicative of habituation to experimental protocols, such as to repeated exposure to predator effluent (Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016).

Methods

Experimental design

The predation trial system consisted of a large flow-through water table (approx. 366 x 91 x 40cm), connected to a recirculation system, containing filtered seawater. Seawater was maintained within a constant temperature range (28 – 28.5°C) and salinity of 32ppt throughout the study. Cylindrical polyethylene containers (26cm dia., $N = 8$), with bases removed, were evenly distributed along the water table and directly connected to the inflow to allow for consistent water movement within each container. Containers were held in place using dive weights and the water level in all containers was maintained at a depth of 10cm to replicate high tide conditions in the nearby upper intertidal marsh from where the study organisms were collected. Cordgrass stems, collected from nearby saltmarsh, were cut to a height of 19cm (allowing snails to climb higher than *C. sapidus* are able to reach; Hamilton, 1976) and fixed around the inner wall of each container as a source of food for the snails. These stems were replaced every two days during predation trials to ensure food was available *ad libitum* throughout the experiment.

Prior to, and between predation trials, snails were housed outside in specially constructed 'replica marsh plots' (0.20m^2) ($N = 6$) (37°36'28.0"N 75°41'10.5"W), where snails were exposed to ambient temperatures and weather conditions. Tidal cycle was simulated daily by replicating tidal patterns experienced in the natural marsh, at the point of specimen collection. Estimated daily maximum water level at high tide (mean ± SD 10.4 ± 1.9cm) was approximated within each plot by referring to a guide placed in the marsh, at the point of material collection. Marsh plots were created by filling white photo trays (53 x 38 x 12cm) with 10cm deep marsh cores, complete with cordgrass roots and intact stems, taken from the adjacent *Spartina alterniflora*-dominated saltmarsh (37°36'30.2"N 75°41'08.1"W). Estimated plant biomass density (biomass m^{-2} ± SD, 216 ± 12.9g) and average *S. alterniflora* height (mean ± SD, 98 ± 16.2cm) were comparable with those of the surrounding marsh (biomass m^{-2} ± SD, 231 ± 36.5g; stem height ± SD, 116 ± 43.6cm). Marsh plots were positioned within flow-through water tables (approx. 248cm x 61cm x 20cm), connected to a flow-through system, drawing water from and returning it to the adjacent Wachapreague channel. There, individuals were free to feed, *ad libitum*, on living and dead *S. alterniflora* and benthic material. Water tables were checked daily to ensure all individuals were accounted for and any escapees were returned to their marsh plots.

Collection and husbandry

Sub-adult and adult *L. irrorata* (as defined by Hamilton, 1978), 7.24 – 22.74mm shell length ($N = 120$), were haphazardly collected from a high-marsh patch (approx. 5m^2) of nearby saltmarsh

(37°36'30.2"N 75°41'08.1"W) at low tide, in August 2018. Snails were transported to the laboratory and placed in seawater for 30 minutes to ensure all individuals were fully hydrated (Iacarella & Helmuth, 2011). With a blunt ended seeker (dissection probe), gentle pressure was applied to the exposed foot of each specimen to stimulate retraction into the shell and to close the operculum, thus minimizing variation in the mantle cavity fluid (Henry et al., 1993; Iacarella & Helmuth, 2011). Excess water was removed using tissue paper before initial weight (total wet weight, g) was recorded for each individual, and shell length (mm) was measured using Vernier callipers. Visual inspection, for the presence of male reproductive organs determined individual sex (74 females and 46 males). Snails were then number-marked using a fine point permanent marker covered with clear, nontoxic aquarium glue before being transferred, randomly, to one of the 6 marsh plots.

Male *C. sapidus*, 140-150mm carapace length (*N* = 10), were collected using crab traps, set (for approximately 12 hours) off the VIMS dock, adjacent to the Eastern Shore Laboratory (37°36'27.6"N 75°41'09.6"W). All individuals were transported to the laboratory, where they were measured (carapace length, mm) and number-marked using fine point permanent marker covered with clear, non-toxic aquarium glue. Crabs were housed individually (to avoid cannibalism; Guerin & Stickle, 1992) in aerated, 18.93L cylindrical containers, within a large fibreglass holding tank. Crab containers were provided with fresh sea water daily and were fed *ad libitum* on crushed snails for two weeks prior to predation experiments to chemically "label" the predators with snail alarm substances (Jacobsen & Stabell, 1999; Wollerman et al., 2003). All crabs were starved for 18 hours prior to the commencement of the staged predation trials to standardise hunger levels and to allow clearance of the digestive tract (McGaw & Reiber, 2000).

Predation effluent preparation

Two of the crabs were each transferred to separate 18.93 L cylindrical containers, each with 2 litres of filtered, aerated sea water and were fed *ad libitum* on crushed *L. irrorata* for 2 weeks prior to predation trials. During boldness testing, on the morning of each trial, one crab was removed from its container and the remaining water (containing crab effluent) was used for the predator treatment. The crab was then provided a new container with filtered, aerated seawater and was fed in preparation for subsequent trials. This process was repeated such that each crab was used once every two days throughout the duration of the boldness trials.

Boldness trials

To characterise individual boldness, snails were randomly assigned to one of 8 (18.93L) cylindrical enclosures (15 per enclosure) containing 1 litre of either filtered sea water (control

treatment) or sea water containing crab effluent (predator treatment). Each container was covered to ensure all snails were submerged until their trial commenced. Snail responses were recorded in groups of 5, each of which was individually removed from their assigned enclosures, in a random order, and held over a white photo tray containing water with the same treatment as the previous container. Retraction into the shell and closure of the operculum was provoked by applying pressure to the foot of each snail with a blunt-ended seeker (dissection probe). Once fully retracted, the snails were placed into the photo tray with shell apertures facing upward. Response latencies were recorded as the time taken for the operculum to reopen and for the first antennae to become visible to the observer, and ranged from 3.9 – 164.3s (mean ± SD, 43.3 ± 26.5) under the control treatment, and from 4.3 – 378.5s (mean ± SD, 92.6 ± 60.7) under the predator treatment. Between trials, enclosures were washed with clean filtered sea water, and snails were allowed to recover in empty 18.93L containers (approx. 1hr), prior to the experiment being repeated in the alternative treatment. Trials were repeated every two days (20 days total), and treatment order was reversed for each trial to avoid order bias. After each set of behavioural trials, snails were returned to their marsh plots, where they remained until subsequent trials commenced.

Predation trials

In order to examine whether survival was related to individual behaviour, snails were exposed to predators during staged predator encounters, resulting in two measures; the number of days survived (survival time), and overall survival (survived or died). Snails were randomly allocated to one of the 8 cylindrical containers (15 snails in each), each filled with filtered aerated sea water (to a depth of 10 cm, matching the mean high tide level in the marsh plots), and were allowed 15 minutes to acclimate before a randomly selected *C. sapidus* was placed in the centre of each container. The containers were then left undisturbed for 3 hours (approximately the time period of tidal inundation at the point of material collection). At the end of this period, crabs were removed and returned to their holding containers. The number of consumed snails and their individual identification numbers were recorded, and all surviving snails were returned to their marsh plots to await subsequent trials. On each day, remaining snails were randomly allocated to a container to ensure each comprised an approximately even number of snails (≈15) for each trial. This led to some containers being unused as the trials progressed. Crabs were also rotated across the containers daily to avoid sampling bias, resulting from size and personality differences among crabs (Pruitt et al., 2012). Predation trials were conducted for 18 days, after which all crabs and surviving snails were returned to the marsh after having their identification marks removed.

Statistical analysis

A character-state, multivariate mixed model (MMM) was used to estimate adjusted repeatability of boldness measured under both control (boldness (C)) and predator (boldness (P)) treatments, and to examine among-individual correlations between measures of survival and boldness. Residual (within-individual) correlations between boldness and survival were not possible because measures of survival are single measures of the length of time (days) and overall survival (survived or died). Measures of boldness were modelled as the inverse (1/latency) of individual re-emergence latencies and were ln-transformed to meet the assumptions of normality. Ln-transformed boldness was then centred and standardised to a mean of 0 and a variance of 1 (z-transformed) to facilitate convergence (Schielzeth, 2010). The further response variables, survival time and overall survival were each divided by their mean values to allow investigation of the among-individual covariance between boldness and measures of relative fitness (Houslay & Wilson, 2017). Overall survival was modelled as a binary response (0 = died, 1 = survived), using family "threshold" (link = probit) in the multivariate model.

The fixed effects of sex and shell length (mm), were fitted for all five traits, and time (trial number) was fitted as a fixed effect for both measures of boldness. To facilitate the interpretation of the main effects, sex was treated as a continuous variable (0 = female, 1 = male), while trial number and shell length were both centred and standardised to a mean of 0 and a variance of 1. To test for individual differences in mean-level boldness, individual identity (ID) was additionally specified as a random effect. Trial number was also fitted as a random slope effect in an initial model; however, the estimated effect was zero and thus the following results are from the reduced model.

Parameters were estimated using the Markov chain Monte Carlo (MCMC) method, with the 'MCMCglmm' package (Hadfield, 2010) in R (R Core Team, 2017). The posterior distributions and autocorrelation plots of five independent chains were compared to ensure convergence and adequate chain mixing. Each with chain length, thinning interval and burn-in period such that 2000 samples were collected from the posterior. An 'uninformative', parameter-expanded model prior was used for among-individual (co)variances, and two 'unstructured' variance-covariance matrices were specified. The first (I-matrix) accounts for the random effects of individual 'ID', estimating the among-individual variance of each of the five dependent variables (VAR_{ind}) and the covariances between them (COV_{ind}). The second (R-matrix) accounts for the residual variation (within-individual variance; VAR_r) and provides an estimate of covariance between the repeat measures of boldness in each of the predator treatments (COV_e).

Model (co)variances were used to estimate among-individual (r_{ind}) correlations between traits by dividing the corresponding covariance between two variables by the product of the square root of their variances (Dingemanse & Dochtermann, 2013):

$$(r_{ind} = COV_{ind} / (sqrt(VAR_{ind})*sqrt(VAR_{ind})))$$

The 95% credible intervals (CI) were used to assess statistical significance. In this case, only correlations with a CIs excluding zero were considered significant.

Finally, adjusted repeatabilities (R_j) for each measure of boldness (i.e. conditioned on the fixed effects) were estimated by dividing their respective among-individual variance estimates by the sum of their among-individual and residual variances, following Nakagawa and Schielzeth (2010):

$$(R_j = VAR_{ind} / (VAR_{ind} + VAR_r))$$

The posterior modes of the random effects were then used to create graphical representations of r_{ind} to illustrate associations between each measure of boldness and each of the fitness traits (Figure 5.2).

Results

Mean-level results

On average, there was a weak tendency for reduced boldness under the predator treatment (boldness (P): -0.0006, CI [-0.122 – 0.126]) compared to the control treatment (boldness (C): 0.0002, CI [-0.135 – 0.144]); however, the overlap between 95% CIs suggest that this trend was not significant, thus there was no clear effect of treatment on boldness. This mean-level trend is represented by the red trend line in Figure 5.1. Trial number (time) had no effect on either measure of boldness (Table 5.1), however shell length did have a significant effect on boldness, with larger individuals tending towards longer response times, under the control (-0.201, CI [-0.339 – -0.068]) and predator treatments (-0.190, CI [-0.305 – -0.063]). Shell length also had a significant effect on survival time, with larger snails tending towards shorter survival times (-0.190, CI [-0.306 – -0.080]), but not on overall survival (-0.062, CI [-0.131 – 0.049]). There was no effect of sex on any of the four traits (see Table 5.1).

Table 5.1 *Fixed effects fitted to the multivariate mixed model for response variables boldness (C) (control), boldness (P) (predator), survival time, and overall survival in* <u>L. irrorata</u>. *Shown are Posterior means, lower and upper 95% confidence intervals and pMCMC (statistically significant pMCMC values (P<0.01) are in bold).*

Trait	Post.mean	l-95% CI	u-95% CI	pMCMC
Boldness (C)				
Intercept	0.00027	-0.13480	0.14389	
Shell length	-0.20113	-0.33935	-0.06764	**0.004**
Sex	-0.19865	-0.48386	0.09662	0.18
Trial	-0.00437	-0.01614	0.00916	0.509
Boldness (P)				
Intercept	-0.00061	-0.12161	0.12619	
Shell length	-0.19022	-0.30464	-0.06340	**0.003**
Sex	-0.10895	-0.38114	0.14404	0.409
Trial	-0.01156	-0.02608	0.00207	0.104
Days survived				
Intercept	0.99966	0.88277	1.12207	
Shell length	-0.18978	-0.30601	-0.07980	**0.001**
Sex	-0.29697	-0.54957	-0.07663	0.022
Overall Survival				
Intercept	0.07739	0.00081	0.15099	
Shell length	-0.06156	-0.13094	0.00233	0.072
Sex	-0.14801	-0.27955	-0.00558	0.033

Individual-level results

After accounting for the fixed effects of time, shell length and sex, boldness was moderately, and significantly repeatable under both control (R_j = 0.60, CI [0.536 ; 0.671]) and predator treatments (R_j = 0.49, CI [0.411 ; 0.561]). Among-individual covariation between treatment specific character states for boldness (COV_{ind} = 0.499, CI [0.369 ; 0.646]), provided a clear correlation (r_{ind} = 0.93, CI [0.895 ; 0.968]), but also revealed among-individual plasticity across treatments, since 95% credible intervals excluded +1. There was no clear difference between individual sensitivity to the different treatments, with 95% CI's for among-individual variation overlapping between the control treatment (Est = 0.594, [0.441 ; 0.756) and the predator treatment (Est = 0.482, [0.356 ; 0.632]). Individual snails with higher than average boldness tended to become less bold in under the predator treatment, whereas those with lower than average boldness tended to become bolder, suggesting that individual reaction norms (RNs) are converging under the predator treatment (Figure 5.1).

The inclusion of a separate residual variance for predator treatments provided estimates of predictability (another form of plasticity), which was higher under the predator effluent treatment (Est = 0.503, CI [0.461 ; 0.546]) than under the control treatment (Est = 0.384, CI [0.352 ; 0.417]).
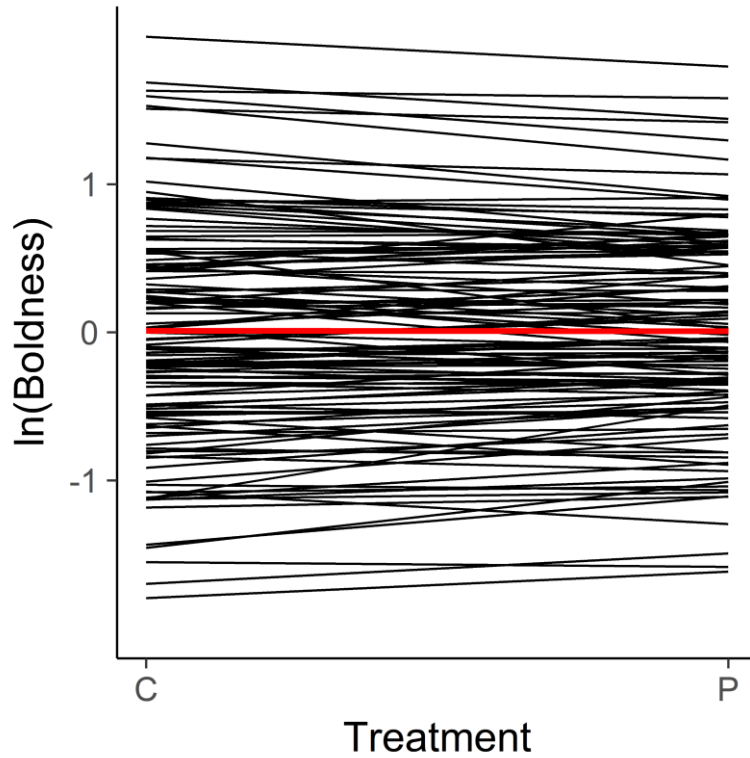
Figure 5.1 *Individual reaction norms representing variation across treatments (control & predator effluent) for boldness in L. irrorata. Black lines represent individual reaction norms, and red lines represent the mean-level trends for the population (N = 120). Trait values are expressed in units of standard deviation (see methods).*

There were significant positive covariances between overall survival and boldness under both treatments (Table 5.2), providing moderate positive among-individual correlations (boldness (C): r_{ind} = 0.29, CI [0.114 ; 0.455] ; boldness (P): r_{ind} = 0.30, CI [0.131 ; 0.470]). However, the mean boldness values in the surviving snails (30% = 36 individuals), compared to those which were predated (70% = 84 individuals) were not found to be significant under either treatment (boldness (C): t = -1.64$_{118\,DF}$, p = 0.104 ; boldness (P): t = 0.30, p = , t = -1.21$_{118\,DF}$, p = 0.229, Figure 5.2).

There were also significant positive covariances between boldness, under both control and predator treatments, and the number of days survived (Table 5.2). These estimates provided moderate among-individual correlations (boldness (C): r_{ind} = 0.37, CI [0.197 ; 0.527] ; boldness (P): r_{ind} = 0.39, CI [0.222 ; 0.543], Figure 5.3).

Finally, estimated covariance between survival time and overall survival (Table 5.2), indicated a strong positive among-individual correlation between the two measures of survival (r_{ind} = 0.62, CI [0.512 ; 0.726]).

Table 5.2 *Among-individual covariance estimates (COV$_{ind}$) for associations between measures of boldness and survival in <u>L. irrorata</u> extracted from a single multivariate mixed model. All are shown, along with 95% confidence intervals (95% CI).*

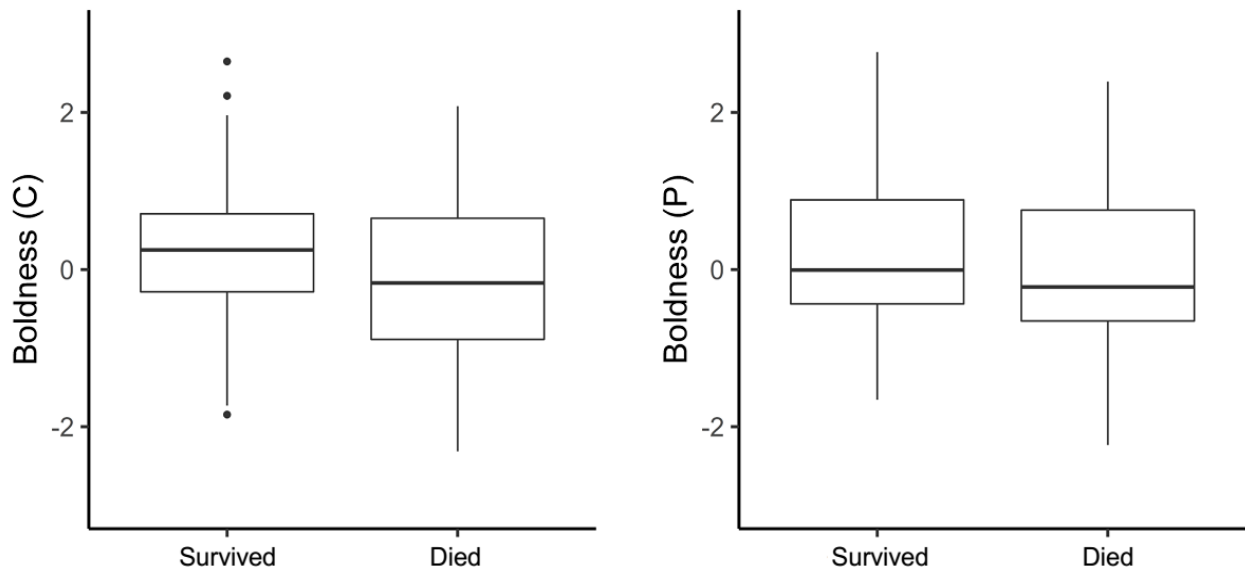| Trait 1	Trait 2	COV$_{ind}$	L - 95% CI	U - 95% CI
Boldness (C)	**Overall survival**	0.08380	0.02530	0.14130
	Days survived	0.18697	0.09392	0.15448
Boldness (P)	**Overall survival**	0.07732	0.01632	0.13724
	Days survived	0.17645	0.09514	0.27882
Overall survival	**Days survived**	0.42567	0.32170	0.53812

Figure 5.2 *Mean boldness for surviving and dead snails under control (Boldness (C)) and predator (Boldness (P)) treatments. Shown are the mean (z-transformed) boldness scores ± standard error of 120 snails (survived = 36, died = 84).*
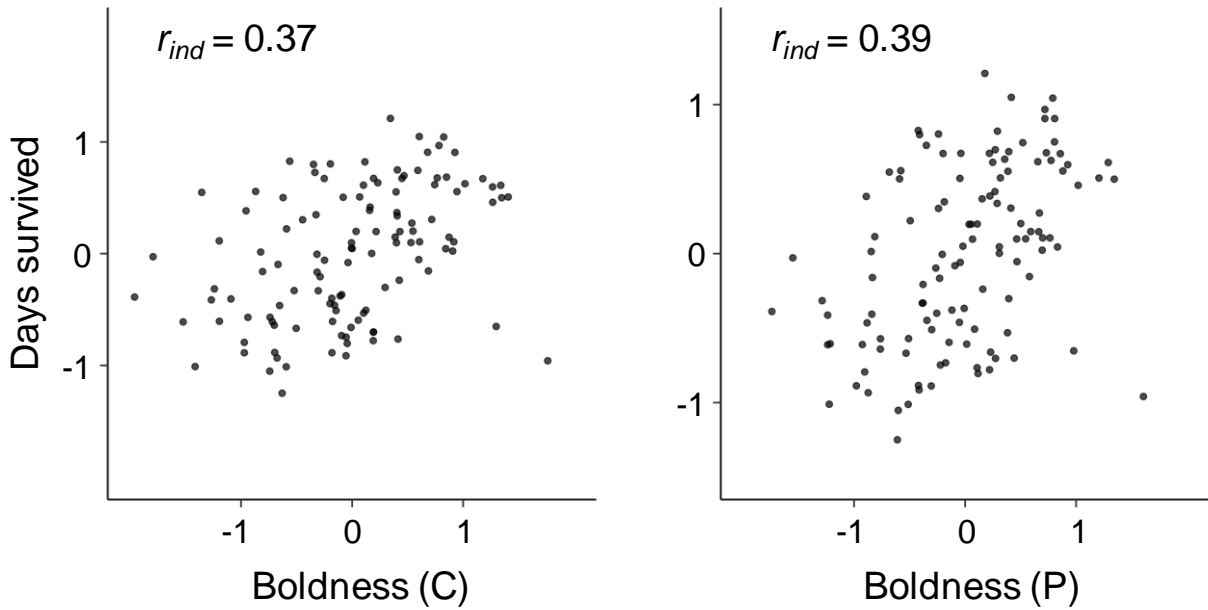
Figure 5.3 Representation of the among-individual correlations between days survived and boldness, under control (Boldness (C)) and predator treatments (Boldness (P)). Data are based on the posterior modes of the random effects from the multivariate linear mixed model.

Discussion

This study aimed to examine among-individual associations between boldness (measured within high and low risk situations) and survival, in *L. irrorata*, in order to test the theoretical prediction that riskier behaviour should lead to increased resource gains (higher growth; CHAPTER 4), at the expense of survival (e.g. Stamps, 2007; Wolf et al., 2007; Dingemanse & Wolf, 2010; Réale et al., 2010). Results revealed clear positive among-individual correlations between boldness, and both the number of days survived ($r_{ind} = 0.37 - 0.39$), and overall survival probability ($r_{ind} = 0.29 - 0.30$). Simply put, bolder individuals were more likely to survive overall, and for longer, than shyer conspecifics. Therefore, these results suggest that predator-mediated selection tends to favour more risk-prone snails, under the conditions of this study. In addition, the results do not provide support for positive covariation between boldness and increased mortality, as predicted by the POLS hypothesis (Ricklefs & Wikelski, 2002; Stamps, 2007; Careau et al., 2008; Biro & Stamps, 2010; Réale et al., 2010b; Wolf & Weissing, 2010). Further, there was no clear indication that associations

between measures of fitness and risk-taking behaviour were dependent on experimental context, and thus these results do not provide evidence for context specific (high vs low risk) associations between behaviour and survival in *L. irrorata*.

Despite previous work reporting the predicted positive associations between risk-taking, foraging success, and increased mortality (Riechert & Bishop, 1990; Biro et al., 2004, 2006), others examining this link have reported higher survivorship in more risk-prone individuals across a range of taxa, including mammals (Réale & Festa-Bianchet, 2003), fish (Smith & Blumstein, 2010; Blake & Gabor, 2014; Boulton et al., 2018) (but see Hulthén et al., 2017), and invertebrates (Foster et al., 2017). Thus, despite being contrary to expectations, the positive among-individual correlations between boldness and survival found here are not entirely novel. Further, although an earlier meta-analysis identified significant negative associations between boldness and survival (Smith & Blumstein, 2008), this analysis was based on the synthesis of studies that did not partition behavioural (co)variance to the among- and within-individual levels — still a rarity in the current empirical literature (Dammhahn et al., 2018; Niemelä & Dingemanse, 2018a, b; Moirón et al., 2019). On the contrary, a more recent meta-analysis exploring these associations at the among-individual level, found no evidence of a negative association between boldness and survival (Moirón et al., 2019), but reported a significant positive correlation between increased boldness and survival for studies conducted on wild populations. Thus, although the behavioural tests and the predation trials in this study were conducted under controlled laboratory conditions, the resulting trait correlations further highlight the importance of examining the associations between behaviour and fitness at the among-individual level (Dammhahn et al., 2018; Niemelä & Dingemanse, 2018a,b; Moirón et al., 2019).

This recent evidence for positive correlations between boldness and survival in some systems has led to predictions that more risk-prone individuals may compensate for increased predation risk with improved performance, such as increased speed, better physical defences, superior energy stores, or otherwise be in better condition (Luttbeg & Sih, 2010). An intriguing possibility, given a previous report linking defensive shell architecture and risk-related behaviours in aquatic snails (Ahlgren et al., 2015), as well as previous evidence of more defensive shell architecture in snails from relatively high predation areas (Moody & Aronson, 2012). Further, although no failed predation attempts were observed after capture, previous research has noted that many snails do carry shell scars from failed predation attempts by *C. sapidus* (Warren, 1985; Greenfield et al., 2002; Dietl & Alexander, 2009), and that the presence of these scars has been shown to correlate with increased resistance to predation — possibly resulting from scarred shells having significantly thicker lips than non-scarred conspecifics (Greenfield et al., 2002). This

suggests that defensive shell architecture may have developed in this species, and that failed predation attempts may reflect the increased energy expenditure required to access snails with more defensive shells (Ahlgren et al., 2015). Furthermore, since individual variation in risk-taking behaviour is heritable (Dochtermann et al., 2015), it might be expected that without trade-offs between growth and survival, there would be minimal population variability in behavioural traits relating to risk. Thus, it is possible that individual variation in boldness in *L. irrorata* is determined by variation in individual experience, or physiological or morphological traits related to overall condition (Foster et al., 2017). However, it is also possible, given that *L. irrorata* are preyed upon by a range of different predators, that maintenance of behavioural diversity is dependent on different predators interacting differently with prey behaviour (Pruitt et al., 2012; McGhee et al., 2013; Blake & Gabor, 2014; Belgrad & Griffen, 2016b; Blake et al., 2018), and thus higher than average boldness may lead to increased mortality when faced with different predators, under natural conditions.

In addition to the positive correlations between boldness and measures of survival, the results also indicate that smaller (also bolder) snails were more likely to survive, not overall, but for longer than larger conspecifics. This finding corresponds to previous work on the influence of boldness on predation mortality in another gastropod species, *Chlorostoma funebralis* (Foster et al., 2017), where it was suggested that predators may be showing a preference for larger individuals, or that larger snails may be more easily detected and handled. Indeed, previous work on *L. irrorata* predation by *C. sapidus* suggests that crabs demonstrate size selection for intermediate sized snails of between 14-18mm (West & Williams, 1986), a range that made up 50% of the total sample, with only 20% being >18mm (18-22mm). However, more recent evidence suggests that *C. sapidus* are more effective when feeding on smaller crabs (higher crab width:shell length ratios) than on larger individuals (Schindler et al., 1994; Moody & Aronson, 2012). Despite this, West & Williams (1986) also found that crabs could predate snails no further than 7cm above the water surface. This is particularly interesting, given that smaller snails were generally observed to be out of the water more often, and generally higher up the *S. alterniflora* stems than larger individuals during predation trials. A similar observation was made by Stanhope et al. (1982), where smaller snails were observed to be more abundant above the water line. This may provide an alternative explanation for higher survival among smaller and bolder individuals, as it is possible that shyer (larger) individuals were more likely to be attacked by *C. sapidus* than bolder (smaller) individuals if smaller individuals spend more time out of the water. However, this anecdotal observation was not tested explicitly here, and therefore it should be a consideration for future studies examining associations between behaviour and survival in this species.

Finally, the results only revealed a very weak average trend for reduced boldness in the predator treatment compared to the control treatment. This was particularly unexpected given previous work reporting reduced boldness in *L. irrorata* under simulated high risk conditions (high tide, CHAPTER 3), and where individual assessment of risk has been shown to influence the plasticity of risk-related behaviours (e.g. Briffa, 2013). However, given that both measures of boldness were recorded under high tide conditions, it is possible that snail responses to incoming tide, and its associated risk, are a stronger driver of state-dependent behaviour than those associated with specific predator cues. Indeed, recent work reported significant variation in behavioural responses to waterborne predator cues, depending on home-marsh geography, and suggested that *L. irrorata* behaviour is also influenced by airborne cues (Carroll et al., 2018). However, the potential influence of external cues was not controlled for here, and thus the proximity of predators within the experimental area may have generated airborne scent cues, influencing behavioural responses in the study specimens. Further, this study design did not allow for the among-individual variation in cue sensitivity to be quantified, nor did it control for variation in cue concentration. Therefore, it is also possible that the lack of a clear trend for boldness across treatments may reflect among-individual canalization (developmental stability) between the control and the predator treatments (Kain & McCoy, 2016).

In summary, this study identified significant positive among-individual correlations between risk-taking behaviour (boldness) and survival. Importantly, these associations between boldness and survival are contrary to predictions made by one of the central hypotheses explaining the maintenance of among-individual behavioural variation (Sih et al., 2004a; Stamps, 2007; Biro & Stamps, 2008; Réale et al., 2010a; Dammhahn et al., 2018), and supports a recent suggestion that theory may need revising (Moirón et al., 2019). Further, the work raises the question of whether *L. irrorata* are exhibiting phenotypic compensation, where trade-offs between risk and reward may be mediated, at least in part, by individual differences in defensive shell morphology (Ahlgren et al., 2015). Despite the unexpected finding that bolder individuals did not incur a higher survival cost than shyer individuals during exposure to a predator, the influence of individual behaviour on individual performance is still evident here. Thus, this study adds to the growing body of work examining the effects of individual behaviour on predator-prey interactions and provides possible avenues for future work examining variation in defensive morphology, as a potential mechanism for maintaining behavioural diversity in natural populations.

CHAPTER SIX

General discussion

Individual behaviour in *Littoraria irrorata*

General context

Recent discussions on the causes, constraints and consequences of individual behavioural variation include the assertion that empirical studies should include more rigorous empirical testing of key theoretical predictions (Biro et al., 2014; Dammhahn et al., 2018; Montiglio et al., 2018). These discussions have also placed emphasis on the inclusion of longitudinal repeat measures of individual labile traits (Niemelä & Dingemanse, 2018b), the consideration of temporal plasticity (Biro & Stamps, 2015), and the implementation of robust statistical analysis, allowing for the partitioning of variation occurring at the within- and among-individual levels (Dingemanse & Dochtermann, 2013; Biro & Stamps, 2015; Cleasby et al., 2015; Houslay & Wilson, 2017). Therefore, this thesis aimed to provide empirical examinations of key theories underpinning the causes and consequences of individual variation, with each study centring on individual risk-taking propensity and the bold-shy continuum — a fundamental axis of behavioural variation (Wilson et al., 1994). More specifically, the work examined the influence of factors affecting individual state, the fitness consequences of behavioural variation, and links between behaviour, physiology and life history. Further, given that invertebrates remain under-represented in research centring on individual behaviour (Mather & Logue, 2013; Kralj-Fišer & Schuett, 2014; Labaude et al., 2018), these examinations were undertaken using a novel invertebrate species, the saltmarsh periwinkle (*Littoraria irrorata*). In addition, based on the most current statistical approaches (Nakagawa & Schielzeth, 2010; Bell & Peeke, 2012; Dingemanse & Dochtermann, 2013; Biro & Stamps, 2015; Cleasby et al., 2015; Houslay & Wilson, 2017), robust partitioning of among- and within-individual variance was applied to quantify individual responses to changing environmental conditions, whilst also exploring behavioural consistency, and examining associations between important labile traits.

Behavioural consistency: personality and behavioural syndromes

Given the lack of previous research examining individual variation in *L. irrorata*, it was important to determine the degree to which behavioural consistency is exhibited in this species. This was explored as part of all empirical work and involved repeated measures of risk-taking behaviour (boldness), along with measures of activity, and the saltmarsh periwinkle's characteristic circumtidal climbing behaviour (CHAPTER 2). Behavioural consistency (repeatability) was estimated across a number of different environmental contexts, beginning with an exploration of behaviour across 24h day/night cycles. Boldness, activity and latency to climb with the incoming tide were all found to be repeatable traits ($R_j = 0.29 - 0.48$), providing the first evidence for consistent individual behaviour (personality) in this species, and importantly, suggesting that climb

latency should be considered a consistent individual behavioural attribute. That boldness (propensity for risk-taking) was found to be repeatable was further supported by subsequent results, with estimates ranging from $R_j = 0.31 - 0.85$, across the four empirical studies. Boldness was most consistent at high temperatures and under low tide conditions ($R_j = 0.85$; CHAPTER 3), and was least consistent during night-time observations, under high-tide conditions ($R_j = 0.31$; CHAPTER 2). Further, although there was limited evidence for temporal plasticity in activity (Figure 2.2) — likely a result of habituation to experimental protocols (Bell & Peeke, 2012; Dingemanse et al., 2012; Stamps et al., 2012; Briffa et al., 2016) — there was no evidence for among-individual variation in temporal patterns of boldness (temporal plasticity). This lack of temporal plasticity indicates that individual mean values for boldness were highly consistent across repeat trials, providing strong evidence for consistent individual differences over time, a hallmark of personality (Biro & Stamps, 2015). In addition, although boldness did not appear to be influenced by sex differences, there was evidence suggesting that smaller individuals tended to be bolder, with size having a modest influence on risk-taking behaviour in *L. irrorata*. A finding that corresponds with previous reports in other gastropod species (Foster et al., 2017), and that suggests size may be important aspect of individual state, giving rise to state-dependent behavioural differences (Wolf & Weissing, 2010).

Further to the repeatability of individual behavioural traits, there was also evidence for among-individual correlations between boldness, activity and climb latency (Figure 2.3), suggesting the formation of a risk-related behavioural syndrome during diurnal periods, and indicating that boldness, activity and climb latency may be under correlational selection (Bell, 2007). This is particularly likely where these traits showed a lack of independence from one another, suggesting that they may represent different aspects of a given individual's behavioural phenotype (Dochtermann & Jenkins, 2007). That a clear correlation between activity and boldness was not detected during night-time observations (CHAPTER 2) might simply reflect limited statistical power, where the number of individuals sampled ($N = 78$) was fewer (10 repeats per individual, per context = 780 samples) than would be necessary to detect a true correlation of ~ -0.10 Dingemanse & Dochtermann, 2013). Alternatively, this finding might suggest that the stability of this syndrome is influenced by changes associated with different diel contexts. For example, it has been suggested that the formation of behavioural syndromes may be adaptive, depending on local selection pressure (e.g. Dingemanse et al., 2007). This has been shown previously in three-spined sticklebacks (*Gasterosteus aculeatus*), where correlations between activity and exploration were only found to be significant under predation pressure (Dingemanse et al., 2007). Thus, the finding that behavioural syndrome structure appears to change with diel cycle may indicate that day/night contexts represent

different levels of predation pressure (risk) — perhaps offering insight into how some species might mitigate risk across day and night contexts. At the very least, these findings indicate that diel cycle (rarely studied with regards to personality and plasticity) can influence behavioural consistency, as well as the strength of associations between labile behavioural traits in this species.

However, given the uncertainty around possible sample size limitations, this should be a consideration in future examinations of co-correlating behavioural traits in *L. irrorata*, and indeed in any other animal model known to be active across a given diel cycle. Furthermore, there are a number of other factors that require attention; specifically, future studies should examine to what extent risk varies across diel cycles, including variation occurring over periods of dawn and dusk. This is particularly important since this species' most abundant predator (*C. sapidus*) is thought to forage more intensively at these times (Wolcott & Hines, 1989). Assessment of relative risk might be achieved by following the procedures used to examine mortality by predation (CHAPTER 5), and by extending these to include variation over 24 hour periods.

Behavioural flexibility: plasticity, predictability & domain generality

In addition to evidence for consistent among-individual behavioural differences (personality), this work also provides evidence for plasticity across contexts at both the population and individual levels. Specifically, among-individual plasticity was clearly evident across the gradients and contexts studied, consistent with previous work examining behavioural variation across day/night contexts (Watts et al., 2015), temperature gradients (e.g. Pruitt et al., 2011; Briffa et al., 2013; Abram et al., 2017), and in response to varying predation pressure (e.g. Urszán et al., 2018). In addition, the work also appears to provide the first evidence of among-individual plasticity across tidal cycles (CHAPTER 3), thus emphasizing the importance of short- and long-term environmental change, and local ecological conditions as factors influencing individual behavioural expression (Dammhahn et al., 2018; Montiglio et al., 2018).

With regard to mean-level plasticity, boldness tended to decrease with lower temperatures, as shown previously in ectothermic animals (Biro et al., 2010; Briffa et al., 2013), and there were also mean-level trends for reduced boldness at low tide, during night-time observations, and when measured in the presence of predator effluent. Although the strength of these tendencies varied substantially depending on context, it is possible that shyer behaviour occurring under these conditions was a result of higher relative risk (Dammhahn & Almeling, 2012; Thomson et al., 2012). Furthermore, a mean-level decrease in boldness under high-tide conditions supports previous work on *L. irrorata*, and the long standing assumption that incoming tide represents a considerably higher risk of predation (Warren, 1985; West & Williams, 1986; Vaughn & Fisher, 1988, 1992; Hovel et

al., 2001). However, somewhat surprisingly, there was also a reduction in mean-level boldness during night-time observations; a finding opposing the assumption that *L. irrorata* should experience reduced predation risk during periods of darkness (Shirley & Findley, 1978), and which was contrary to previous findings reported in crayfish (*Cherax destructor*) (Biro et al., 2014). Although unexpected, it is quite conceivable that the risk of predation is actually higher at night, since many predators of *L. irrorata* are active across the diel cycle (Clarke & Johnston, 1999; Clark et al., 2003; Lipcius, 2013). This is made more plausible considering *L. irrorata* rely heavily on visual cues (Hamilton, 1977, 1978a) and have better developed vision than most gastropods (Hamilton & Winter, 1982; Hamilton et al., 1983); possibly leading to more risk-averse behaviour during periods of darkness, when important senses are not functioning optimally.

However, this idea was only supported at the mean-level, since individual unpredictability (within-individual variance) tended to be lower at night (0.53, CI [0.448 – 0.613]) than during the day (0.60, CI [0.516 – 0.699]). This is the opposite to what should be expected given previous suggestions that an individual's behaviour should be less predictable under relatively high-risk situations (Maye et al., 2007; Brembs, 2011). Further, although this result confers with similar findings in agile frog (*Rana dalmatina*) tadpoles (Urszán et al., 2015, 2018), it is contrary to those described for hermit crabs (*Pagurus bernhardus*) (Briffa, 2013), and to those reported here for boldness in *L. irrorata* under high-tide conditions, and in the presence of predator effluent. These findings, therefore, may indicate a more complex pattern of variation occurring across diel contexts, and one that may involve interactions between environment and other factors, such as physiological condition (e.g. Belgrad et al., 2017), or between diel cycle and environmental variables, including tidal cycle and temperature; both of which fluctuate within the saltmarsh across a given 24 hour period. Although it is only possible to speculate as to the mechanisms underpinning this variation, it is important to note that this experiment controlled for natural variation in tidal cycle and ambient temperature to avoid systematic confounds between these variables (temperature maintained at 28 – 28.5°C across day/night contexts). However, since previous work has shown that boldness in ectotherms can become more unpredictable at higher temperatures (Briffa et al., 2013), it is unlikely that the higher than normal temperatures experienced during night-time observations would have led to anything other than even more unpredictable behaviour. Thus, future studies aiming to examine the effect of diel cycle should consider the inclusion of different temperature regimes, including possible interactions between temperature and light.

Despite the uncertainty regarding predictability across diel contexts, this work provides evidence that exposure to high-risk situations can lead to shyer behaviour, and increased unpredictability in *L. irrorata*. In particular, where individual boldness was significantly more

predictable at low tide and under benign condition (absence of predator treatment), thus supporting the assumption that *L. irrorata* are responding to the increased risk of predation at high tide (Warren, 1985; West & Williams, 1986; Vaughn & Fisher, 1988, 1992; Hovel et al., 2001), rather than just responding to being submerged during tidal inundation. Interestingly, the evidence presented for variation occurring in response to risk, or more generally, that plasticity across different environmental gradients may be underpinned by one or more common underlying variables (Stamps, 2016; Mitchell & Biro, 2017), is further supported by the evidence for domain general plasticity in this species. Specifically, the finding that individual reaction norm (RN) slopes for tide and temperature were strongly correlated ($r = 0.57$; Figure 3.3), providing what appears to be the first empirical evidence of consistent behavioural plasticity across environmental gradients. Put simply, individuals that were more responsive to changes in tidal cycle, were also more responsive to increased temperature. Domain-general plasticity is a common assumption (DeWitt, 1998; Sih & Del Giudice, 2012), however examinations are rare and support, thus far, has been absent (Westneat et al., 2011; Biro et al., 2014; Mitchell & Biro, 2017). Therefore, this result could help to elucidate important factors underpinning individual behavioural variation (Stamps, 2016). Particularly, given the suggestion that correlation of plasticity across environmental gradients is likely driven by common proximate mechanisms (Stamps, 2016), such as those related to differences in individual information state or experience (Wolf & Weissing, 2010; Sih et al., 2015) or to individual physiology e.g. aspects of metabolic phenotype (e.g. Auer et al., 2015; Metcalfe et al., 2016; Biro et al., 2018; Salin et al., 2019).

Causes and consequences

State-dependent behaviour

In context of this work, it is possible that responsiveness to changes in risk could also be linked to differences in metabolism (Killen et al., 2011, 2012; Robison et al., 2018), providing a potential mechanistic explanation for why responsiveness to risk was also linked to temperature (CHAPTER 3). This is particularly plausible given that temperature is known to directly affect metabolism in ectothermic animals (Clarke & Johnston, 1999). Further, given the observed mean-level increase in boldness with increasing temperature, in addition to the among-individual variation in response to temperature, these findings appear to indicate variation in individual sensitivity to metabolic state (Biro et al., 2010; Pruitt et al., 2011; Careau et al., 2014; Metcalfe et al., 2016; Mitchell & Biro, 2017), which may explain the observed pattern of behavioural covariation. If valid, it would be feasible to expect similar patterns of correlated plasticity in other behavioural traits related to risk,

such as those examined here (e.g. activity & climb latency), where behavioural syndromes are present (Sih & Bell, 2008). Further, given the link between responsiveness to tide and temperature, these results may support the earlier suggestion that individual responses to diel context may interact with changes in tide, and temperature. This is particularly viable where changes in diel context are expected to represent different levels of risk, and where patterns of O_2 consumption in *L. irrorata* have previously been shown to vary across day/night contexts (Shirley & Findley, 1978; Shirley et al., 2007). However, one important assumption here is that individual behavioural traits related to risk should be linked to individual differences in metabolism. Indeed, attempts to explain the proximate and ultimate causes of behavioural variation often point to this predicted relationship (Careau et al., 2008; Biro & Stamps, 2010; Careau & Garland, 2015; Metcalfe et al., 2016). Further, links between behaviour and physiology frequently include associations between these traits and life-history characteristics, specifically, as part of the Pace-of Life Syndrome (POLS) hypothesis (Ricklefs & Wikelski, 2002; Réale et al., 2010b).

Pace-of-Life

On examining the associations between boldness, resting metabolic rate (RMR) and somatic growth in *L. irrorata*, results revealed clear among-individual correlations between boldness and RMR (r_{ind} = 0.32; Figure 4.2a), and between boldness and growth (r_{ind} = 0.58; Figure 4.2b), indicating that bolder individuals had relatively high growth rates and relatively high RMR compared to shyer individuals. Thus, providing strong evidence for among-individual covariation between boldness and metabolism, and between boldness and growth, under the conditions of this study — as predicted by the POLS hypothesis (Ricklefs & Wikelski, 2002; Stamps, 2007; Careau et al., 2008; Biro & Stamps, 2010; Réale et al., 2010b; Wolf & Weissing, 2010). These findings also provide support for the suggestion that behavioural variation is underpinned by energetic constraints (Van Dijk et al., 2002; Careau et al., 2008; Biro & Stamps, 2010; Metcalfe et al., 2016; Biro et al., 2018), particularly where RMR might reflect differences in the idling costs of metabolic machinery required for periods of maximum energy expenditure (e.g. Biro & Stamps, 2010; Auer et al., 2017). These might include periods of increased risk, where predator avoidance behaviour is necessary, and where relatively high RMR individuals might generate higher energy output. In this case, these individuals should also require more energy to sustain themselves and thus, bolder individuals should have higher foraging and food intake rates than shyer individuals. Furthermore, the high among-individual correlation between growth and boldness reinforces the idea that boldness may play a functional role in resource acquisition in this species, another prediction made by the POLS hypothesis (Stamps, 2007; Biro & Stamps, 2008).

Given the evidence presented for among-individual correlations between POLS traits, it was expected that risk-taking behaviour in *L. irrorata* would play a role in mediating trade-offs between risk and reward. For example, it is often predicted that propensity for risk-taking will be selected against in populations under high predation pressure (Bell & Sih, 2007; Kortet et al., 2010; Réale et al., 2010b). Specifically, the faster growth observed in bolder individuals should come at the expense of future survival, where risk-prone individuals are expected to experience higher mortality by predation (Stamps, 2007; Wolf et al., 2007; Dingemanse & Wolf, 2010; Réale et al., 2010b). However, on testing this prediction (CHAPTER 5), results provided strong evidence to the contrary, with bolder individuals experiencing higher survivorship and surviving for longer than shyer conspecifics during stage predator prey encounters. Despite being contrary to predictions, the finding supports previous work on risk-taking and survival in another marine gastropod, the black tegula (*Chlorostoma funebralis*) (Pruitt et al., 2012; Foster et al., 2017); indicating that predator-mediated selection may favour risky behaviour in this species. Therefore, propensity for boldness appears to confer fitness benefits both in terms of increased growth and higher survivorship under predation from blue crabs in *L. irrorata*.

If this is the case, then why, given that individual behavioural variation is heritable (Dochtermann et al., 2015), has genetic variability in boldness not eroded away in *L. irrorata* populations? This would certainly be expected if bolder individuals are also in better general condition (Luttbeg & Sih, 2010), perhaps compensating for increased risk with better physical defences such as more defensive shell morphology (e.g. Moody & Aronson, 2012; Ahlgren et al., 2015). A likely explanation is that boldness in *L. irrorata* has negative effects on other measures of individual performance (Foster et al., 2017), or that boldness is selected against by other snail predators, such as diamond back terrapins (*Malaclemys terrapin*), or killifish (*Fundulus* spp) (Clark et al., 2003; Lipcius, 2013). This seems particularly plausible given evidence that associations between behaviour and survival can be influenced by different predator hunting strategies (Belgrad & Griffen, 2016a), and given the range of different species preying on *L. irrorata*, within their natural habitat. In this case, higher than average boldness may lead to increased mortality when encountered by different predators, under natural conditions. Another possible explanation, although somewhat speculative, might be that bolder snails are indeed more likely to be captured by *C. sapidus*, but that failed predation attempts lead to a higher proportion of bolder individuals developing shell scarring that has been shown to increase predation resistance (Warren, 1985; Greenfield et al., 2002; Dietl & Alexander, 2009). In turn, leading to selection against scarred (possibly bolder) snails due to the increased energy costs of accessing a food resource contained in a more robust shell. This explanation would also be in line with assumptions of the state-behaviour-

feedback models (Wolf & Weissing, 2010; Sih et al., 2015), suggesting that bolder behaviour may be reinforced by experience (i.e. increased resource gains and failed predation attempts) (Sih et al., 2015). Although shell scarring was not included as a factor in this experiment, the idea may provide the foundation of future work on the influence of among-individual morphological variation on behaviour and survival in this species, going forward.

Conclusions & future directions

Perspectives for future research

Although the expression of individual risk-taking behaviour in *L. irrorata* was often observed to be in line with theoretical predictions, some of the findings presented within this thesis hint at more complex relationships between behaviour and the highly variable nature of saltmarsh environments (Cantero et al., 1998). In particular, the work generates questions relating to the relative importance of short-term environmental variation (e.g. day/night contexts), trade-offs between risk and reward, and possible mechanisms maintaining behavioural diversity, all of which undoubtedly require further attention. Although, recommendations for future work have been touched on throughout, of particular interest to the continuation of this work would be the testing of predictions relating to the apparent lack of fitness costs associated with bolder behaviour in *L. irrorata*. Specifically, the possibility that bolder individuals compensate for increased risk of predation with better physical defences such as more protective shell morphology (i.e. phenotypic compensation; Ahlgren et al., 2015). Indeed, variation in shell morphology has been studied previously in this species (Greenfield et al., 2002; Dietl & Alexander, 2009; Moody & Aronson, 2012), with evidence for shells having developed more extensively calcified apertural lips and narrower apertural openings in areas of high predation, compared to those from low-predation areas (Moody & Aronson, 2012). In addition, previous work also emphasizes the possibility that shell scarring resulting from failed predation attempt may improve physical defences in subsequent predator encounters (Greenfield et al., 2002; Dietl & Alexander, 2009). Examining phenotypic compensation, as a possible explanation for higher survival rates in bolder *L. irrorata*, could be achieved by expanding on methodology presented here (CHAPTER 5) by incorporating the use of geometric morphometrics software (e.g. SHAPE; Iwata & Ukai, 2002) to examine key shell characteristics (Ahlgren et al., 2015). In addition, the use of longitudinal mark-recapture experiments might also aid in determining the relative effectiveness of shell features, and their links with behaviour, under field conditions, while examining the effects of predation more generally, rather than focussing on one key predator. Importantly, testing the phenotypic compensation hypothesis may help to further elucidate the mechanisms underpinning the maintenance of behavioural diversity in *L. irrorata*.

Another possible extension of this work might include examinations of variation in behavioural syndrome structure in different populations of *L. irrorata*, where known differences in selection pressure can be accounted for (see e.g. Dingemanse et al., 2007). In particular, given suggestions that *L. irrorata* might be a contributor to marsh die-back events (Silliman & Zieman, 2001; Silliman et al., 2005), one interesting avenue of future research would be to consider the effect of these events on selection pressure, and to examine behavioural variation in both degraded (after die-back events) and healthy saltmarsh. Especially since *S. alterniflora* density is known to be much lower in degraded areas (Stagg & Mendelssohn, 2012), perhaps leading to increased predation pressure and behavioural change over time.

Conclusions

This work provides the first evidence of consistent among-individual behaviour (personality), and of individual behavioural variation across environmental gradients (plasticity) in the saltmarsh periwinkle (*Littoraria irrorata*). It also provides strong support of two key theories underpinning the potential causes of among- and within-individual phenotypic variation. Firstly, the work identified clear correlations between boldness, resting metabolic rate, and somatic growth, in accordance with predictions made by the Pace-of-Life Syndrome hypothesis (Ricklefs & Wikelski, 2002; Stamps, 2007; Careau et al., 2008; Biro & Stamps, 2010; Réale et al., 2010b; Wolf & Weissing, 2010). In addition, the finding that plasticity carries over across tide and temperature gradients, where both tide and temperature had significant influences on the behaviour of *L. irrorata*, provides strong support for the influence of state variables as possible drivers of individual behaviour (Dingemanse & Wolf, 2010; Luttbeg & Sih, 2010; Wolf & Weissing, 2010; Sih et al., 2015). In this case, taken together these findings support the idea that variation in individual behaviour under changing conditions may be underpinned by one or more underlying variables, such as those influencing individual state, including risk perception (Rodríguez-Prieto et al., 2010; Briffa, 2013; Dorset et al., 2017) and/or individual metabolism (e.g. Auer et al., 2015; Metcalfe et al., 2016; Biro et al., 2018; Salin et al., 2019)

In addition, the work may also highlight the importance of a combination of careful control, longitudinal repeated measures, and effective partitioning of variance components when examining questions relating to the causes and consequences of individual behavioural variation in natural populations. More generally, this thesis provides a solid foundation for future studies examining variation of labile traits, where fluctuating environments may increase the variability of behavioural phenotypes (Luttbeg & Sih, 2010). In this sense, *L. irrorata* represents an appealing model organism

for future studies in which to compare the behaviour of individuals exposed to the highly variable saltmarsh environment.

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