The behavioural, metabolic and proteomic effects of temperature stress on bold and shy beadlet anemones (*Actinia equina*)

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Philosophy by

Daniel Kit Maskrey

September 2021

[Page intentionally left blank]

ABSTRACT

Thesis author: Daniel Kit Maskrey

Thesis title: The behavioural, metabolic and proteomic effects of temperature stress on bold and shy beadlet anemones (*Actinia equina*)

Environmental change caused by human activity is an ever-increasing threat to global biodiversity. A key determinant of the survival of animal populations under climate change is intraspecific phenotypic variation. This variation occurs at multiple levels, including between-individual differences in behavioural phenotypes (personalities), varied metabolic rates, and contrasting patterns of proteomic expression. Furthermore, an animal's behavioural phenotype is split into two levels of its own, such that individuals vary consistently both in their mean level behaviours, and in the residual variation, or unpredictability, that they show around those means. Studies indicate that these different phenotypic levels could often be associated with one another: unpredictability may be constrained, or act as a constraint on, mean level personality; metabolic rate could be positively or negatively correlated with an animal's propensity to take risks, termed their boldness; and proteomic expression under stress may differ between personality-types. All of these phenotypic levels provide important mechanisms for animals to mitigate against or cope with climate change, but few investigations have yet explored the relationships between them in the context of phenotypic flexibility during environmental shifts, termed plasticity. In this thesis, I explore how acute thermal perturbation, which is predicted to increase in frequency under continued climate change, affects the relationships between behavioural, metabolic, and proteomic phenotypes. Using the beadlet anemone (*Actinia equina*), a common intertidal species living in a thermally heterogeneous environment, I examine plasticity in two boldness-related behaviours: startle response-time (SRT), defined as the time it takes an anemone to re-extend its tentacles after a threatening stimulus; and immersion response-time (IRT), defined as the time to re-extend tentacles after simulated tidal immersion. In my first and second data chapters, I show that both behaviours exhibit between-individual variation in temperature-driven plasticity at the mean level, and that this extends to the level of unpredictability for IRT. I also show behaviour-dependent associations between temperature-driven behavioural plasticity, genotypic differences, and environmental history. In my third and fourth data chapters, I expand my investigations of each behaviour to encompass temperature-driven plasticity at other phenotypic levels. I find directionally unexpected associations between IRT

and metabolic rate, and clear relationships between proteomic expression under thermal stress and SRT. The data presented here provide strong evidence for multi-level associations in temperature-driven phenotypic plasticity, and indicate that several strategies for dealing with thermal perturbation exist in *A. equina*. They advocate taking account of physiological and molecular correlates when investigating behavioural plasticity in a changing climate, and suggest that some individuals living in intertidal populations are likely to be more at risk from climate change-induced mortality than others. Overall, this thesis reveals the remarkable complexity of intraspecific variation in phenotypic plasticity, and drives home the need for ecologists and conservationists to consider this variation when assessing organismal vulnerability to climate change.

TABLE OF CONTENTS

[Page intentionally left blank]

LIST OF FIGURES

LIST OF TABLES

[Page intentionally left blank]

GLOSSARY OF TERMS

Term	Definition
Personality	Consistent differences between individuals in their behaviour across both time and context (Sih et al., 2004).
Behavioural Plasticity	The alteration of an animal's behavioural phenotype in response to environmental change (Snell-Rood, 2013).
Behavioural Unpredictability	Unexplained behavioural variation within individuals (Stamps et al., 2012).
Boldness	An animal's propensity to take risks. Bold animals show riskier behaviour than shy animals (Beckmann & Biro, 2013).
Startle Response-Time (SRT)	A boldness-related behaviour in *Actinia equina*, measuring the re-emergence of feeding tentacles after a disturbance (Briffa & Greenaway, 2011).
Immersion Response-Time (IRT)	A boldness-related behaviour in *Actinia equina*, measuring the re-emergence of feeding tentacles after a simulated tidal emersion-immersion cycle (Maskrey et al., 2020)
Pace of Life Syndrome Hypothesis (POLS)	Posits that "fast" behavioural traits, such as being bolder or more exploratory, should be correlated with "fast" physiological and life-history traits, such as increased metabolic rate and faster growth (Réale et al., 2010)
Routine Metabolic Rate (RMR)	The metabolic rate of an animal during normal activity (Metcalfe et al., 2016).
Intermittent-Flow Respirometry	A method of measuring the oxygen consumption of animals by flushing respirometers to replenish oxygen concentration after a set time-period. Intermittent-flow respirometers allow researchers to easily take multiple repeated measures of metabolic rate (Svendsen et al., 2016).
Proteome	All of the proteins an animal is expressing at a given time (Tomanek, 2011).
Proteomic Profile	The degree of expression of different proteins across an animal's proteome (Nieto-Barajas et al., 2012).
Protein Functional Grouping	Proteins that play roles in the same, or similar, molecular pathways, cellular functions, or complex cellular structures (Nieto-Barajas et al., 2012).
Ribosome	Organelle, made of multiple protein components which themselves form subunits, whose primary function is to facilitate the translation of mRNA to protein (Moore & Steitz, 2002).
Spliceosome	A dynamic protein complex, found in the cellular nucleus, whose primary role is to alter transcribed mRNA molecules before they reach the ribosome (Matera & Wang, 2014).
Proteasome	A multi-functional protein complex, whose primary function is in degrading damaged or unnecessary proteins (Sorokin et al., 2009).
Heat Shock Protein (HSP)	A family of molecular chaperone proteins known for their role in facilitating cellular stress resistance (Bowler, 2005).

[Page intentionally left blank]

ACKNOWLEDGEMENTS

My name may adorn the front of this thesis, but it would certainly not exist without the invaluable support of my supervisors, collaborators, colleagues, family, and friends. Whether it be designing studies, spending late nights and weekends in the lab, overcoming seemingly insurmountable analytical knots, writing up chapters, or simply summoning the will to keep at it, everything I have achieved over the past four years has been made incalculably easier by the people surrounding me.

First and foremost, thank you to Jack Thomson, my primary supervisor. I could not have asked for a more supportive, enthusiastic, and knowledgeable researcher to work with, and his tolerance of my random knocks at his office door during countless workdays is undoubtedly deserving of an award of some sort. I feel immensely grateful to have been able to learn from Jack, and it has been great to have a supervisor not only concerned with my current work and wellbeing, but also with expanding my skillset into other areas for the future. His advice permeates every chapter of this thesis.

Thank you too to my two other main supervisors, Lynne Sneddon and Kate Arnold. Lynne's calm knowledge of behavioural, metabolic and molecular techniques, alongside her vast experience of aquaculture, has made this PhD far less daunting. I am especially grateful to her for always vehemently defending my corner, for the lunch and dinner catch-ups, and for connecting me with other researchers of her own extremely high calibre. Kate's forensic examination of my experimental designs, analyses, and write-ups, meanwhile, has improved this thesis exponentially. Her ability to dig me out of experimental rabbit-holes and to nudge me towards vastly improved study designs, even over the course of single meetings, has been absolutely invaluable.

Alongside my three main supervisors, thanks must also go to other co-supervisors, advisors, collaborators, and colleagues. Thank you to my co-supervisor Rob Beynon, for sharing his encyclopedic knowledge of proteomics, even after his retirement; to my co-supervisor and industry sponsor David Wolfenden, for always being an email away whenever I had any questions about aquaculture; and to Shaun Killen, for showing me how to make respirometers, for helping with my metabolic analyses and write-up, and for his hospitality in Glasgow. Thanks to Deborah Simpson for purifying and quantifying all the proteomic profiles contained here, and for carrying out preliminary proteomic analyses; to Philip Brownridge for his bioinformatic assistance; to Cat Franco for laying the building blocks of

my proteomic work; and to Rosemary Maher for helping me analyse and visualise my proteomic data. I am also grateful to Leslie Connor, Stephen Crowley, Rees Sweeney, and Barry Cotterill for technical assistance, and to my advisory panel, Sam Patrick and Nova Mieszkowska, for their thoughtful advice and helpful support.

I have had a fantastic time in Liverpool over the past four years, and such a positive experience simply would not have happened without the friends I made along the way. Thank you to Ruth, for your endless positivity and support, for always being there for a chat and a cup of tea, and for your very yellow living room. Thank you to Holly, for disagreeing with me *just* enough on every topic under the sun, and for all the amazing days and nights out. Thank you to Steph, for the kubb (or is it kewb?), and for taking me under your wing as soon as I arrived. Thank you to George, without whom I don't know how I would have coped in lockdown, and to Christian, for always asking about my thesis even after long-since leaving Liverpool. Thank you too to all my other close Liverpool friends, who have all helped me to grow immensely, both personally and professionally, over the past four years, including (but not limited to) Padraig, Tom, Jamie, Val, Callum, Emily, Lucia, Joel, Tommy, Rhi, Curtis, Alice, Jack, Emma, and James.

I am also hugely grateful to everyone else, family and friends, who has been there for me over the past four years. Thanks to Rhodri, Ben, Adam, Luke, Cam, and Jake for keeping me sane during lockdown and for the endless fun we've had over the years. Thanks to Jacob and to Esme for their constancy, and to Max, for being the closest thing to a cousin without actually being one. Thank you to my Gran, for always being my number one fan, and to my Grandad, for his pride in me even before I'd started. Thank you to Nicola, Nick, and Blue, for being such brilliant characters who I know I can always turn to. Thank you to my Mum, who has been an absolute rock over the past few years and long before. None of what I've achieved would have been possible without her.

Finally, Guille. It is not overstating things to say that without your help on countless rainy days in Llandudno, this thesis would be completely different. I am so grateful to have started my PhD at the same time as you and am in awe of your overriding concern for others. Good luck in the final stretch of your own PhD, and I'm looking forward to heading over to Madrid soon!

A NOTE ON THE TEXT

Chapters 2-5 were written as manuscripts for publication in scientific journals and share a common theme of exploring behavioural and physiological stress responses in *A. equina*. They have been minorly adapted for this text but remain largely as they were originally written. As such, some repetition exists between these chapters, particularly in introductory and methodological sections.

[Page intentionally left blank]

Chapter 1:

General Introduction

[Page intentionally left blank]

1.1 BETWEEN-INDIVIDUAL BEHAVIOURAL DIFFERENCES

The world is host to a vast array of biodiversity, exhibiting a diverse spectrum of phenotypes across an assortment of animal taxa. Natural selection is a key driver of phenotypic variation and is thus crucial to both maintaining and controlling phenotypic diversity (Murray, 1972). The maintenance of diversity by selection occurs at both interspecific and intraspecific levels, leading to variation within species in a range of ecologically relevant phenotypic traits (Bolnick et al., 2003). In the last 20 years, animal behaviour has come to the forefront of ecological research as one such trait.

1.1.1 Overview

Animals within the same species do not behave uniformly. This lack of uniformity is not random, and individuals show consistency in how they behave across both time and context (Sih et al., 2004). In turn, intraspecific variation in a given behaviour shows stability in both direction and magnitude, such that different individuals display distinct behavioural types (Bell, 2007). The reasons for this have long puzzled ecologists, as the rigid behavioural types exhibited across the spectrum of a population may not all be adaptively optimal (Wolf & McNamara, 2012; Wolf & Weissing, 2012). In an evolutionarily costless system, the best strategy for a population should be to show complete behavioural flexibility in the face of environmental challenge (Dall et al., 2004). In this way, behaviours could remain at selective peaks and mitigate against the detrimental effects of environmental perturbation on less flexible areas of an animal's phenotype (West-Eberhard, 1989). However, work on other traits has long indicated that phenotypic flexibility comes with its own set of selective costs (Dewitt et al., 1998; Scheiner, 1993). As such, animals must trade off the reduced short-term demands of a more rigid phenotype with the ability to rapidly adapt to environmental shifts (Dall et al., 2012; Dingemanse & Wolf, 2013). Complex patterns thus arise, whereby individuals are consistent in their behaviours (personality; Bell et al., 2009), vary in the scope and direction of their behavioural flexibility (plasticity; Dingemanse et al., 2010; Martin et al., 2011), and vary on an individual basis in the degree to which their behaviours are consistent (unpredictability; Biro & Adriaenssens, 2013; Stamps et al., 2012).

1.1.2. Personality

Animal personality is defined as consistent differences between individuals in their behaviour across both time and context (Dall et al., 2004; Sih et al., 2004). To elucidate the strength of personality variation in a given population, repeated behavioural measurements are taken from multiple individuals and the proportion of the variance that is explained by individual differences is determined (Dohm, 2003; Wilson, 2018). Personality is likely to be present across much of the animal kingdom (Bell et al., 2009), and significant levels of between-individual behavioural variation have now been documented in many taxa including in mammals (Debeffe et al., 2015; Ferrari et al., 2013; Seyfarth et al., 2012), birds (Harris et al., 2020; Nicolaus et al., 2012; Williams et al., 2012) and fish (Killen et al., 2012; Kniel & Godin, 2019; Mitchell et al., 2016). Evidence has more recently also extended to invertebrates (reviewed in: Kralj-Fišer & Schuett, 2014) such as crustaceans (Belgrad et al., 2017; Bridger et al., 2015), arachnids (Chang et al., 2017; Kralj-Fišer & Schneider, 2012), and cnidarians (Briffa & Greenaway, 2011; Collins et al., 2017).

In measuring between-individual behavioural variation, researchers have described specific types of behavioural response which can be observed across taxa (Carter et al., 2013). These include aggression (Kilgour et al., 2018), exploration (Le Galliard et al., 2013), activity and foraging (Patrick & Weimerskirch, 2014), and, most widely studied, boldness (Ballew et al., 2017; He et al., 2017; Lane & Briffa, 2017). Boldness is most commonly defined as an individual's propensity to take risks (Carter et al., 2013), but the most effective method of measuring this trait, and whether single behaviours can really encompass "risk-taking", is not always clear (Beckmann & Biro, 2013). As such, researchers often seek to place animals on a "boldness-shyness" axis, where boldness can be defined by multiple correlated behavioural traits (e.g. Carter & Feeney, 2012; Maskrey et al., 2018; White et al., 2016). Boldness is a relevant trait on which to focus when investigating the selective implications of personality as it should represent an obvious trade-off whereby bolder animals forage, explore and reproduce more efficiently than shy animals, but are also at increased risk of mortality via predation, competitive interactions, and pathogens or parasites (Réale et al., 2010). Not all evidence supports this hypothesis. For example, in crustaceans, shy rockpool prawns (*Palaemon elegans*) forage more effectively (Maskrey et al., 2018) and shy shore crabs (*Carcinus maenas*) exhibit higher body condition (Fürtbauer, 2015) than bold individuals. Meanwhile, in guppies (*Poecilia reticulata*), bold individuals may experience higher survival under the threat of predation than shy individuals (Smith & Blumstein, 2010). Nonetheless,

meta-analyses suggest that, in general, animals do face a trade-off between short-term life-history benefits and long-term survival that is related to their boldness (Royauté et al., 2018; Smith & Blumstein, 2008). This trade-off becomes particularly significant in the face of environmental change, as greater boldness is often (but not always, see: Biro et al., 2013; Thomson et al., 2012) associated with more limited behavioural plasticity (Coppens et al., 2010; Geffroy et al., 2020; Mathot et al., 2012; e.g. Jolles et al., 2019; Kareklas et al., 2016; Ólafsdóttir & Magellan, 2016), which could have important implications for survival (Killen et al., 2016).

1.1.3. Plasticity

Behavioural plasticity, where an individual alters its behaviour either temporarily or permanently in response to environmental change (Snell-Rood, 2013), was initially a particular stumbling block in developing evolutionary theories of personality (Adriaenssens & Johnsson, 2013). This was because behaviour was long-viewed as being almost completely plastic and thus unlikely to be under any strong directional selection (West-Eberhard, 1989). However, data has increasingly pointed to variation in both the scope and direction of plastic responses between individuals (Ghalambor et al., 2010; Stamps & Biro, 2016). As evidence has also mounted that the heritability of personality is largely analogous to that of other labile phenotypic traits (Dochtermann et al., 2014, 2019; Prentice et al., 2020; Van Oers et al., 2005), it has become clear that the plasticity of behaviour is likely to be selectively constrained (Snell-Rood, 2013). As such, between-individual variation in behavioural plasticity may be driven by a trade-off. In spatially and temporally heterogeneous environments, a wider range of behavioural phenotypes (Mouchet et al., 2021) displaying greater behavioural plasticity (Dingemanse & Wolf, 2013) is likely to be selectively beneficial (Scheiner, 2013). In homogeneous environments, meanwhile, the costs of maintaining behavioural plasticity, such as displaying adaptively detrimental variation around a steady phenotypic optimum (Auld et al., 2010; Nakagawa et al., 2007), could make it selectively damaging (Dall et al., 2004). Variation between individuals in their plastic behavioural responses has thus come to the fore as a potential driver of fitness outcomes.

Appropriate behavioural responses to environmental shifts can be of great adaptive value to an individual (Abram et al., 2017; Dingemanse & Wolf, 2013; Mery & Burns, 2010); in American pikas (*Ochotona princeps*), individuals that are more plastic under thermal perturbation experience less stress and collect more food (Hall & Chalfoun, 2019).

Furthermore, in a range of birds, species that exhibit higher levels of behavioural plasticity in the face of environmental shifts are at lower risk of extinction than those that are more rigid (Ducatez et al., 2020). The scope for this adaptively beneficial "activational" behavioural plasticity, defined as a short-term, reversible behavioural response driven by acute environmental perturbation (Snell-Rood, 2013), is often intertwined with an individual's personality. For example, in black finger crabs (*Ozius truncates*), individuals that are bolder at high temperatures show more behavioural change when exposed to lower temperatures than individuals that are shyer (Biro et al., 2013). Conversely, in blue tits (*Cyanistes caeruleus*), shyer birds show greater reductions in feeding rates when exposed to high temperatures than bolder birds (Herborn et al., 2014). Behavioural consistency within a given environment and plastic responses to environmental perturbation are thus associated in what is commonly referred to as a behavioural reaction-norm (Dingemanse et al., 2010). Reaction-norms lead animals to exhibit a range of behavioural strategies for dealing with environmental challenge which can vary in their selective value (Abram et al., 2017; Adriaenssens & Johnsson, 2013; Hall & Chalfoun, 2019; Mery & Burns, 2010). Figure 1.1 visualises how different reaction-norm scenarios can constrain different aspects of behaviour.

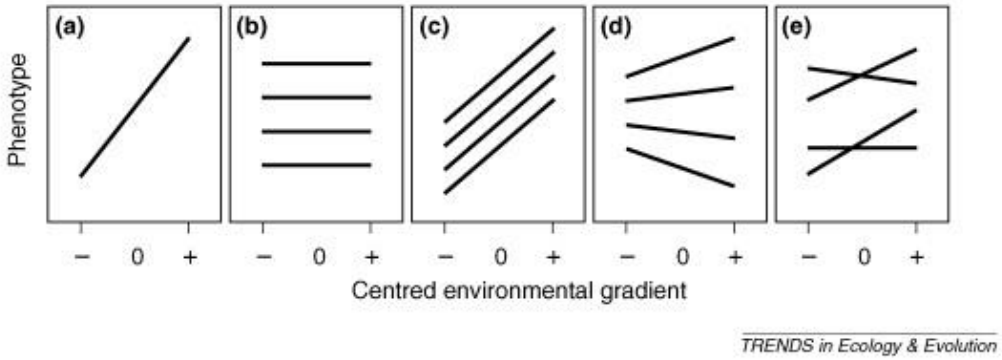


Figure 1.1. Five reaction-norm scenarios, where an animal's reaction-norm is represented by the intercepts (personality) and slopes (plasticity) of their behaviour across two environmental scenarios. In panel (a) Plasticity is present, but all individuals show the same personality within environments and the same plastic response across environments. In panel (b) individuals show personality variation, but no plasticity is present, so intercepts in both environments remain the same. In panel (c) individuals show personality variation and plasticity, but the plastic response is identical in all individuals. In panel (d) individuals show variation in both their personality and their plasticity, and the slope of their behavioural response is positively correlated with their behaviour in the first environment. In panel (e) individuals show variation in both their personality and their plasticity, but there is no correlation between their behaviour in the first environment and the slope of their behavioural response. From Dingemanse et al., 2010, published with permission of the copyright-holder.

1.1.4. Unpredictability

Reaction-norms are further complicated by residual (unexplained) within-individual behavioural variation, defined in this thesis as unpredictability (as described in: Stamps et al., 2012) but also often termed predictability (as described in: Biro & Adriaenssens, 2013). Recent advances in modelling methodologies (Cleasby et al., 2015; Lee & Nelder, 2006; Westneat et al., 2015) have determined that individuals not only differ in mean level behaviour, but also in the amount of variance they show around behavioural means (their unpredictability; Figure 1.2). Between-individual variation in unpredictability is increasingly well-documented across a range of behavioural traits in species including rescue dogs (*Canis lupus familiaris*; Goold & Newberry, 2017), guppies (*P. reticulata*; Mitchell et al., 2016; Prentice et al., 2020), and jumping spiders (*Cosmophasis umbratica*; Chang et al., 2017). Individual differences in unpredictability, alongside the question of why they might persist, are thus important to address when investigating intra-species behavioural variation.

One mechanism by which unpredictability might be maintained could be through direct selection, with individuals using unpredictable behaviour as a predator mitigation strategy to reduce their risk of mortality (Briffa, 2013; Martin et al., 2017). Alternatively, unpredictability could be under indirect selection via associations with other behavioural traits. Unpredictable behaviour might, for example, be associated with mean level activational plasticity (Westneat et al., 2015) and thus affect an individual's ability to rapidly adapt to environmental change. Associations between mean level behavioural traits and unpredictably are increasingly evident in the literature. For example, more docile yellow-bellied marmots (*Marmota flaviventris*) show lower unpredictability in their docility than less docile animals (Martin et al., 2017), and bolder sticklebacks (*Gasterosteus aculeatus*) are not as unpredictable in their risk-taking as their shyer counterparts (Jolles et al., 2019). If unpredictability is in fact under both direct and indirect selection, it is possible that it could be constrained by, and act as a constraint on, other personality traits (Biro & Adriaenssens, 2013; Chang et al., 2017; Okuyama, 2015). As such, unpredictability itself could often be described as a component of an animal's behavioural syndrome. The traditional view has been that behavioural syndromes are comprised of suites of correlated mean level traits, for example, aggression, activity and exploration in sticklebacks (*G. aculeatus*; Dingemanse et al., 2007), or activity, risk-taking and sociability in anemonefish (*Amphiprion ocellaris*; Wong et al., 2013), which could adaptively constrain or complement one-another (Sih et al., 2012). However, recent research supports the view that behavioural syndromes also often

encompass the complex associations between personality, plasticity, and unpredictability (Mitchell et al., 2016; e.g. Jolles et al., 2019; Martin et al., 2017).

As part of an adaptively constrained behavioural syndrome, unpredictability should show plastic responses to changing environmental conditions (Stamps, 2016) that covary with other behavioural traits (Mitchell & Houslay, 2021). These responses could influence an animal's fitness (Jennings et al., 2013) but have thus far seen limited investigation, primarily in vertebrate taxa (Highcock & Carter, 2014; Horváth et al., 2017; Mitchell & Biro, 2017; Nakayama et al., 2016), because of the highly complex and data demanding modelling approaches required to explore them (Mitchell et al., 2016; Mitchell & Houslay, 2021). Despite research in hermit crabs (*Pagurus bernhardus*) finding that unpredictability can change in response to predation risk (Briffa, 2013), environmental temperature (Briffa et al., 2013), and environmental contaminants (Nanninga et al., 2020), this lack of study is particularly apparent in invertebrates. Further work, particularly in the face of climate change-induced selection for specific plastic behavioural strategies (Sih et al., 2011; van Baaren & Candolin, 2018), is therefore required. Any comprehensive study investigating the behavioural effects of environmental change should, in turn, encompass mean and residual-level behavioural variation within and between environments.

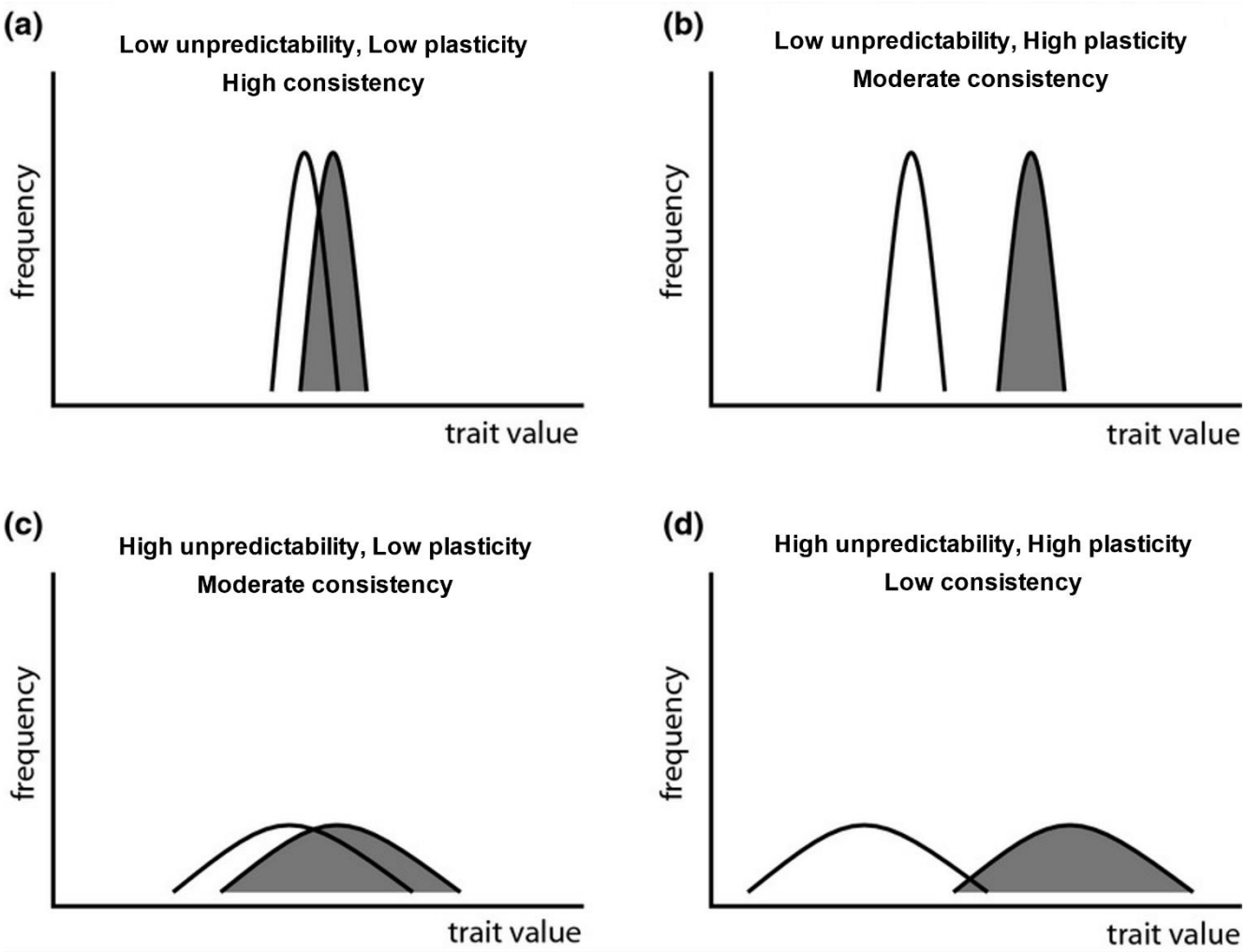


Figure 1.2. The different distributions in each panel represent within-individual variation (unpredictability) in observations of the same behaviour in two different environments. Different distributions of behavioural responses affect both the consistency of behaviour across environments (personality) and the magnitude of cross-environmental behavioural change (plasticity). Unpredictable behaviours have wider distributions. Adapted from Cleasby et al., 2015, published under the paper's open access agreement.

1.2. CLIMATE CHANGE AND INDIVIDUAL BEHAVIOUR

1.2.1. Climate change driving behavioural change

Anthropogenic climate change is likely to be the key driver of environmental challenges facing animal populations in the near future (Doney et al., 2012; Helmuth et al., 2006; Parmesan, 2006). Changes to abiotic factors such as temperature (IPCC, 2018) and environmental chemistry (Dodd et al., 2015), and biotic factors including species composition (Dijkstra et al., 2011), are occurring across the globe. These changes present animal populations with novel selective pressures against which they must either mitigate or risk perishing (Beever et al., 2017; Buckley & Kingsolver, 2019). Behavioural change is

particularly crucial to alleviating these novel pressures as behaviour is more labile than most other areas of an animal's phenotype (Brommer, 2013). As such, both developmental (i.e. gradual, less easily reversible behavioural changes brought about by longer-term neurological and epigenetic alterations; Snell-Rood, 2013) and activational behavioural plasticity are key mechanisms by which animals can respond to climatic shifts (Kelly, 2019; Sih, 2013; Sih et al., 2011; Tuomainen & Candolin, 2011). Examples of activational behavioural responses to improve selective outcomes when faced with environmental challenges include changing activity levels (Rodriguez-Dominguez et al., 2019), dispersing to new locations (Wong & Candolin, 2015), and altering interactions with conspecifics (Rudin & Briffa, 2012) or predators (Bell & Sih, 2007). Those animals that exhibit the most effective plastic behavioural strategies are, in turn, expected to be at a selective advantage as environmental shifts continue (Fox et al., 2019; Kelly, 2019). As such, studies investigating between-individual variation in these strategies can shed light on the most vulnerable individuals within a given population or habitat, and thus be of great value to conservationists (Beever et al., 2017; Caro, 2016)

1.2.2. Temperature

One of the most important variables being affected by human-induced environmental change is temperature (IPCC, 2018). In the thirty years up to 2006, global air temperatures increased by an average of 0.2^{o}C per decade (Hansen, 2006), and this trend is predicted to continue and even steepen (Arnell et al., 2019). Alongside this, temperature fluctuations across the globe are predicted to increase in frequency, leading to more heatwaves and cold snaps (IPCC, 2013; Mbokodo et al., 2020; Perkins-Kirkpatrick & Gibson, 2017). More regular acute temperature fluctuations will have important selective consequences (Hemraj et al., 2020; Seuront et al., 2019; Vajedsamiei et al., 2021; Weitzman et al., 2021), so how individuals vary in their mean and residual behavioural responses to these fluctuations (e.g. Biro et al., 2013; Briffa et al., 2013) is likely to impact their fitness (Abram et al., 2017).

Variation in these responses often depends on an animal's environment of origin. For example, at the population level, houseflies (*Musa domestica*) from warmer climates are more active than those from cold climates, and retain activity levels for longer when exposed to near-lethal temperatures in the laboratory (Kjærsgaard et al., 2015). Laboratory tests in a range of alpine reptiles, meanwhile, found that populations from cold, highland habitats, bask more (and thus expose themselves to increased risk) than those from lowland habitats

(Caldwell et al., 2017). The nature of an individual's plastic behavioural response to temperature is not only dictated by population-level environmental conditions, but also by intra-population variation. As previously described, populations living in heterogeneous environments are predicted to exhibit high levels of between-individual behavioural variation (Mouchet et al., 2021) and wider ranges of behavioural strategies for dealing with environmental change (Dingemanse & Wolf, 2013; Laskowski & Bell, 2013). The selective value of these strategies is likely to be altered by climate change-induced thermal perturbation (Chapperon et al., 2016; Vajedsamiei et al., 2021; Weitzman et al., 2021). As such, as heatwaves and cold snaps become more common, populations living in heterogeneous environments could risk a loss of diversity if certain behavioural strategies cease to be of selective value (van Baaren & Candolin, 2018). Studies of different animals from the same population, taken from multiple locations across a heterogeneous habitat, could test this hypothesis and thus shed light on which individuals, living in which locations, might be at most risk from anthropogenic heatwaves and cold snaps.

1.3. BEHAVIOUR AND THERMAL PHYSIOLOGY

1.3.1. Pace-of-life syndrome

Physiological variation is another important driver of individual susceptibility to thermal stressors (Schulte et al., 2011), and is likely to be an important proximate influence on between-individual behavioural differences (Biro et al., 2018; Coppens et al., 2010; Killen et al., 2016; Koolhaas et al., 2010). A physiological characteristic of particular relevance to thermal variation is metabolic rate, as temperature increases are associated with higher metabolic demand in many species (Clarke & Fraser, 2004; Clarke & Johnston, 1999). Although it seems intuitive that metabolic requirements should be closely correlated with risky behaviours such as foraging (Careau et al., 2008; Houston, 2010), empirical evidence for this theoretical association, called the pace-of-life syndrome hypothesis (POLS; Réale et al., 2010), is mixed. POLS suggests that bold, "fast-lived", animals should trade-off long-term survival chances against short term fitness, and thus be metabolically primed to be more active, forage more efficiently, and grow faster (Montiglio et al., 2018), while also exposing themselves to higher risks of mortality from predation (Stamps, 2007) or energetic deficits (Careau et al., 2008). Shyer animals, meanwhile, should show "slower" life-histories and lower short-term fecundity, but similar fitness in the long-term due to higher survival rates (Hall et al., 2015; Santicchia et al., 2018). POLS further proposes that behavioural,

physiological and life-history traits should evolve concurrently (Montiglio et al., 2018; Réale et al., 2010), with variable mechanistic associations leading particular phenotypic levels to drive coevolution in others (Dammhahn et al., 2018; Eckerström-Liedholm et al., 2019). In silverside fish (*Menidia menidia*), for example, a "faster" life-history may drive individuals to exhibit bolder behaviours, as animals selected for faster growth rates also exhibit concurrent increases in their foraging activity (Chiba et al., 2007). Contrastingly, in field crickets (*Gryllus integer*), individuals that are risk-averse early in their ontogeny invest heavily in later-life immune responses (Niemelä et al., 2012), suggesting that, in this case, early-life personality may influence physiological investment in later-life survival. While some studies have found evidence for POLS (Cornwell et al., 2020; Debecker & Stoks, 2019; Polverino et al., 2018), overall support for the hypothesis is decidedly mixed, leading a meta-analysis from Royauté et al in 2018 to conclude that it may not be a valid generalised ecological framework. Figure 1.3 shows a complete lack of overall directional association between metabolic physiology and boldness across the literature.

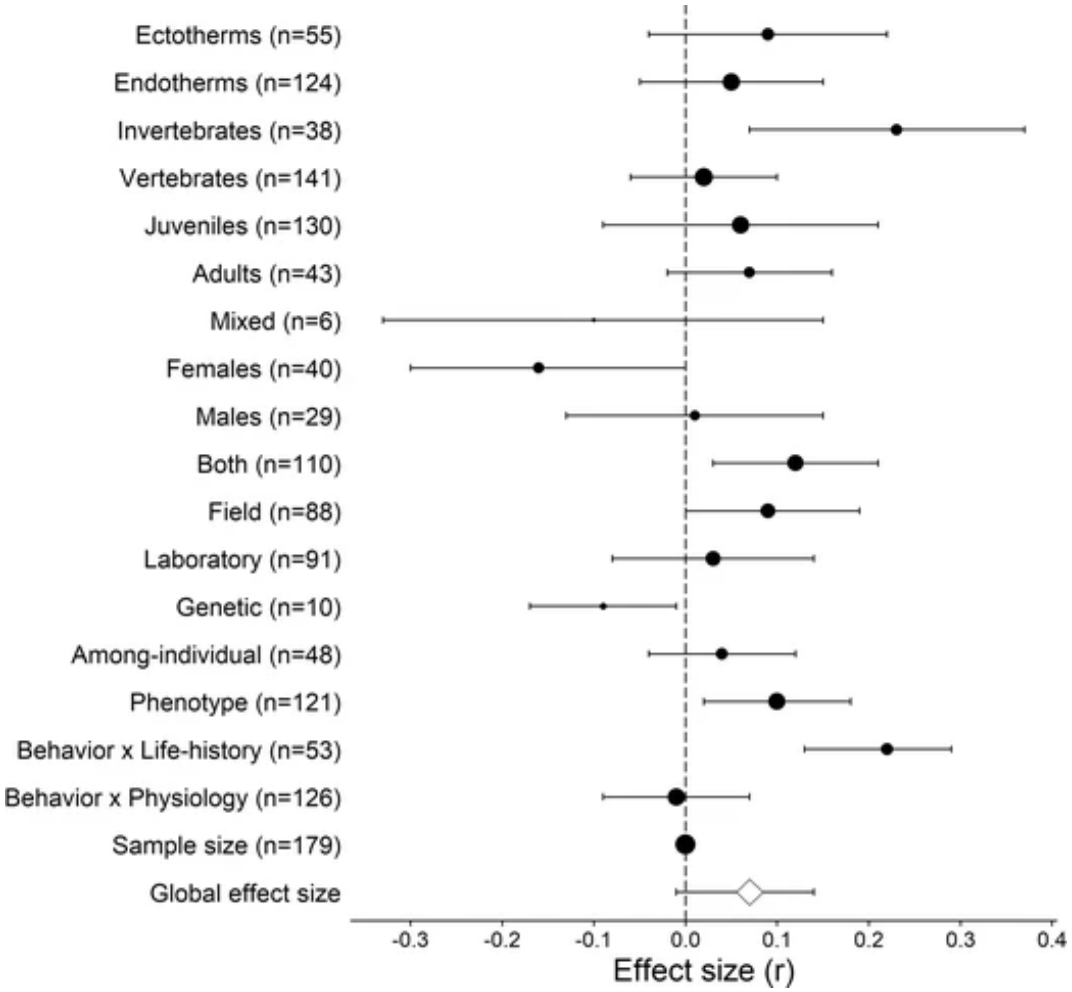


Figure 1.3. Mixed meta-analytical evidence for the pace of life syndrome hypothesis (POLS) across biological categories, where positive values with 95% confidence intervals not crossing zero indicate support for POLS. The overall support for POLS is contained within the "Global effect size" category. From Royauté et al., 2018, published with permission from the copyright-holder.

A possible reason for currently mixed evidence for POLS could be that many studies fail to account for important environmental factors affecting the relationships between boldness and metabolism (Dammhahn et al., 2018; Montiglio et al., 2018), particularly environmental temperature (Goulet et al., 2017; Michelangeli et al., 2018). Physiology and behaviour are both highly (although not completely) labile, and thermal environment is a driver of between-individual variability in both boldness (Angiulli et al., 2020; Cornwell et al., 2019; Forsatkar et al., 2016) and metabolic rate (Killen et al., 2016; Schulte, 2015; Seebacher et al., 2015). Further, the trade-offs hypothesised by POLS should be particularly stark at high temperatures, where animals are likely to find themselves under both increased energetic demand (Schulte, 2015) and increased risk of predation (Miller et al., 2014; Twardochleb et al., 2020).

Trade-offs between energetics and predation risk should be clearer still in ectothermic animals, which depend on their environment to maintain thermal homeostasis (Angilletta et al., 2002; Deutsch et al., 2015) and whose metabolic physiology is intrinsically linked to environmental temperature (Clarke & Johnston, 1999; Seebacher et al., 2015). Given this close association, ectotherms rely particularly heavily on behavioural strategies to mitigate against the metabolic effects of thermal heterogeneity (Abram et al., 2017; Kearney et al., 2009; Sears et al., 2016). For example, grain aphids (*Sitobion avenae*) disperse away from warm environments to cooler microhabitats under high temperatures, and can face energetic deficits and perish if they disperse poorly (Ma et al., 2018). Littornid snails (*Littorinidae sp.*), meanwhile, can directly alter the metabolic effects of their immediate thermal environment by reorientating themselves or retracting into their shell (Ng et al., 2017). Despite these close associations between behaviour and metabolism, ectotherms are disproportionately underrepresented in studies investigating POLS (Royauté et al., 2018), and have received very little attention in relation to different thermal conditions (Goulet et al., 2018). Further, although environmental variation could change associations or lead to dissociation of POLS (Killen et al., 2013; Montiglio et al., 2018), no studies in any species have yet tested the relationship between metabolic rate and boldness under changing thermal conditions. How individual differences might shape these patterns has also gone unexplored, so investigations of boldness and metabolic rate in the same ectothermic individuals across multiple temperatures could help clarify several understudied aspects of POLS.

1.3.2. The proteome

While changing metabolic rate is a measurable outcome of an animal's physiological thermal stress response, it is not the underlying driver of that response. Rather, metabolic rates are both limited (Lesser & Kruse, 2004; Storey & Storey, 2004) and driven (Bennett, 1988; Wang et al., 2018) in large part by changes to the active molecular components of an animal, namely, the proteins it expresses (Tomanek, 2011). Proteomic expression, governed in part by the transcriptome of an animal at a given time (Feder & Walser, 2005), is a major proximate driver of all physiological processes in that animal (Torson et al., 2020), and particularly important when mitigating against thermal stress (Black et al., 2007; Choresh et al., 2007; Gleason & Burton, 2015; Madeira et al., 2013). Behaviour is also reliant on the expression of proteins. Neuronal transmission and neuroendocrine responses, for example, can drive behavioural variation (Gosling, 2008; Mondal et al., 2006; Staes et al., 2016, 2021; Trainor & Hofmann, 2006), while stress resistance proteins, such as heat-shock proteins

(HSPs), are associated with behavioural stress responses (Busby et al., 2012; Larios-Soriano et al., 2020). Intraspecific variation in these proteomic responses may, in turn, be associated with between-individual differences in behaviour (Nonnis et al., 2021; Pusch et al., 2018; Toni et al., 2019).

One such proteomic stress response that differs between individuals is the response to thermal stress. In starlet sea anemones (*Nematostella vectensis*), for example, the enrichment of different molecular pathways under heat stress depends on an individual's developmental conditions (Rivera et al., 2021); larvae whose parents had recently been exposed to heat stress showed increased HSP expression at high temperatures when compared with larvae whose parents had not. Similarly, in topshells (*Trochus histrio*), high temperatures elicit sex-specific differences in proteomic profiles such that, during heat stress, males express significantly higher levels of HSPs than females (Grilo et al., 2018). Given that intraspecific differences in proteomic and behavioural stress responses are likely to be associated with one-another (Nonnis et al., 2021; Pusch et al., 2018; Toni et al., 2019), the question arises whether proteomic change might drive varied behavioural mitigation strategies under anthropogenic heatwaves. Few studies have yet begun to explore this (but see: Alfonso et al., 2020; Pusch et al., 2018), and fewer still have addressed it at a whole-proteome or whole-transcriptome level (but see: Nonnis et al., 2021; Toni et al., 2019). Of these two approaches, whole-proteome methods offer a truer picture of an animal's molecular stress response (Diz et al., 2012) as up to 50% of proteomic expression is not explained by transcriptomic analyses (Feder & Walser, 2005).

Whole-proteome approaches could also elucidate the relationships between behavioural and molecular stress responses in a way that previous studies have not. As of yet, studies investigating hypotheses such as behavioural coping styles (Koolhaas et al., 1999), which attempt to explain associations between stress responses at different phenotypic levels, have almost exclusively focused on specific neuroendocrine correlates of behaviour (Coppens et al., 2010; Koolhaas et al., 2010; e.g. Baugh et al., 2012; De Bruijn & Romero, 2011; Houslay et al., 2018; Mell et al., 2016; Wong et al., 2019). However, since stress-responsive molecules and pathways do not provide the whole picture of thermal mitigation (Gormally & Romero, 2020), broader proteomic approaches could provide a more comprehensive picture of molecular changes than pathway-specific studies. The expression of biosynthetic proteins, for instance, involved in many essential cellular processes, shows varied responses under heat stress. On one hand, in species such as brown anemones (*Aiptasia pallida*) and marine snails

(*Tegula sp.*), biosynthetic processes are down-regulated, possibly as a strategy to mitigate against their metabolic costs (Oakley et al., 2017; Tomanek & Somero, 1999). On the other, species including sclerantian coral (*Anomastraea irregularis*) and ark shells (*Scapharca subcrenata*) up-regulate biosynthetic pathways to help stabilise cellular structure and function under thermal pressure (Ning et al., 2021; Onyango et al., 2021). Apoptotic pathways, too, show changes under heat-stress, either being up-regulated to ensure efficient cellular turnover (Richier et al., 2006; Yang et al., 2017), or down-regulated in order to maximise cellular survival (Kvitt et al., 2016; Pernice et al., 2011). Widespread focus on the relationships between specific neuroendocrine pathways and behaviour (Coppens et al., 2010; Koolhaas et al., 1999, 2010), rather than more generalised responses, has also led to a particular dearth in the literature where invertebrate taxa are concerned. There has as yet been no investigation of how intraspecific variability in invertebrate proteomic stress responses might be related to the growing, but still under-represented, field of invertebrate personality (Kralj-Fišer & Schuett, 2014).

1.4. STUDY SYSTEM

1.4.1. Marine invertebrates

Sea temperatures over the past 50 years have been rising at over 50% of the rate of air temperatures (IPCC, 2013) and the regularity of marine heatwaves is increasing (Darmaraki et al., 2019; Fordyce et al., 2019; Leggat et al., 2019). Whilst thermal perturbation will be particularly problematic for all ectotherms (Kearney et al., 2009), marine ectotherms, which live especially close to their thermal maxima (Pinsky et al., 2019), are likely to be placed under particularly high pressure. The temperature responses of one group of marine ectotherms, fish, have received a great deal of attention in both the behavioural (e.g. Biro et al., 2010; Cerqueira et al., 2016; Forsatkar et al., 2016; Lienart et al., 2014) and physiological literature (e.g. Birnie-Gauvin et al., 2017; Killen, 2014; Madeira et al., 2013; Norin et al., 2016). The behavioural responses of marine invertebrates and how these relate to thermal physiology, meanwhile, have received much less focus, despite the fact that they are also at risk of temperature-induced mortality (Chapperon et al., 2016; Hemraj et al., 2020; Ragazzola et al., 2021).

The thermal stress responses of marine invertebrates are well studied at the whole-proteome level (Grilo et al., 2018; Ning et al., 2021; Oakley et al., 2017; Onyango et al., 2021; Richier et al., 2006; Tomanek & Somero, 1999) and these responses are well-established to

incorporate changes in stress-responsive protein expression. For example, in snakelock anemones (*Anemonia viridis*), one family of HSPs is upregulated in the mitochondria when animals are exposed to heat shock (Choresh et al., 2007). In Pacific oysters (*Crassostrea gigas*), meanwhile, thermal stress elicits similar increases in HSP expression, while also driving down proteins related to protein metabolism and biosynthesis (Lim et al., 2016). Alongside proteomic expression, marine invertebrate behaviour also varies under different thermal regimes (Cornwell et al., 2019; Ng et al., 2017) and behavioural differences in these organisms could be associated with fitness via factors such as predation risk (Briffa et al., 2013) and body condition (Fürtbauer, 2015). Nonetheless, no studies to my knowledge have yet related personality with proteomic stress responses in any marine invertebrate.

Marine invertebrates are also an ideal taxon with which to study POLS and temperature. While Royauté et al (2018) shows weak evidence for POLS overall, it also suggests that the framework may be more applicable to invertebrates than vertebrates (Figure 1.3.). Vertebrate and invertebrate organisms may be facing subtly different selective pressures (Kralj-Fišer & Schuett, 2014) which could be driving this discrepancy, and these could be exacerbated or changed by thermal stress (Killen et al., 2013). Further, investigations of POLS in invertebrates are disproportionately under-represented in the literature (Royauté et al., 2018) and this is particularly true in marine environments (but see: Cornwell et al., 2020). As increasingly frequent marine heatwaves exacerbate thermal fluctuations in shallow (Hemraj et al., 2020) and intertidal (Weitzman et al., 2021) regions, addressing the lack of knowledge of the physiological correlates of behaviour in marine invertebrate taxa may provide important information as to the most vulnerable individuals in populations.

1.4.2. The Beadlet Anemone, *Actinia equina*

The intertidal zone is highly heterogeneous (Bockelmann et al., 2002; Menge, 1976). Animals living on the seashore live very close to their thermal limits and experience very different thermal conditions depending on the height on the shore at which they live (Brahim et al., 2019; Cornwell et al., 2019). This leads populations of intertidal organisms to exhibit a range of thermal preferences (Allcock et al., 1998; Chapperon et al., 2016; Dias et al., 2018) and to be particularly susceptible to anthropogenic temperature changes even compared with other marine taxa (e.g. Weitzman et al., 2021).

The beadlet anemone, *Actinia equina*, is one such organism. *A. equina* is a small to medium-sized anemone (averaging 2-3 cm diameter and 4-6g wet weight in UK populations; Monteiro

et al., 1997) which uses its underside, called a pedal disc, to adhere to rocky substrates on the shore (Quicke et al., 1983). As a largely sessile, opportunistic predator with a wide-ranging diet that includes crustaceans, molluscs, and orgainic detritus (Chintiroglou & Koukouras, 1992), *A. equina* uses specialised tentacles containing stinging cells, called nematocysts (Allcock et al., 1998), to capture prey as it passes in the water. Individuals display intraspecific aggression when competing for space on the shore (Brace et al., 1979) but they can forms groups, or aggregations, which are often genetically identical due to the species' ability to propagate both sexually and asexually (Turner et al., 2003).

Unlike many intertidal animals (Harley et al., 2006; Helmuth et al., 2006), *A. equina* has very wide thermal ranges (Griffiths, 1977a, 1977b), allowing it to be exposed to extreme temperature variation without risk of mortality. *A. equina* individuals further display variable thermal physiology depending on the height on the shore from which they were collected (Navarro et al., 1981; Ortega et al., 1984) and exhibit several genetically distinct morphotypes, which themselves prefer specific shore heights (Quicke et al., 1983) and show consistent differences in their behaviour (Collins et al., 2017). The more aggressive red morphotype prefers the more stochastic, thermally extreme high-shore, while the less aggressive green morphotype prefers the more thermally homogeneous low-shore (Brace & Quicke, 1986; Collins et al., 2017; Quicke et al., 1983, 1985). Table 1.1 provides information on known differences between the two morphotypes. Alongside population-level variation, *A. equina* also shows behavioural variation at the level of the individual, varying in two boldness-related behaviours. The first, startle response-time (SRT), is well described in the literature (Briffa & Greenaway, 2011; Lane & Briffa, 2017; Osborn & Briffa, 2017) and involves simulating a predation threat, leading an anemone to retract its tentacles, before recording the time to tentacle re-emergence. The second, immersion response-time (IRT), is measured for the first time, to my knowledge, in this thesis (Maskrey et al., 2020). IRT again measures time to tentacle re-emergence, but rather than simulating a predation threat, it simulates a tide, causing an anemone to retract its tentacles upon emersion and re-extend them upon re-immersion. Tentacle extension is risky for *A. equina* because it exposes individuals to greater risk of predation (Edmunds et al., 1976). As such, both SRT and IRT represent an ecologically relevant trade-off between foraging and predation risk (Bell et al., 2006) that is likely to be exacerbated at high temperatures due to increased energetic demand (Schulte, 2015). Figure 1.4 shows the different states of *A. equina* individuals near the start of an IRT assay. The multiple levels of behavioural and physiological variation exhibited by *A.*

equina could influence different individuals' ability to deal with extreme temperature events (Abram et al., 2017; Vajedsamiei et al., 2021), and make it an ideal intertidal invertebrate model with which to study relationships between physiology and behaviour.

Table 1.1. Differences between the two morphotypes as they were described in the literature prior to the research contained in this thesis. Arrows up or down indicate which morphotype displays higher levels of a given trait.

Trait	Red Morphotype	Green Morphotype
Aggression (Brace & Reynolds, 1989)	↑	↓
Size of Weaponry (Allcock et al., 1998)	↑	↓
Competitive ability (Brace et al., 1979)	↑	↓
Strength of adhesion to substrate (Quicke et al., 1983)	↑	↓
Preference for high-shore (Quicke et al., 1983, 1985)	↑	↓
Boldness (Collins et al., 2017)	↑	↓

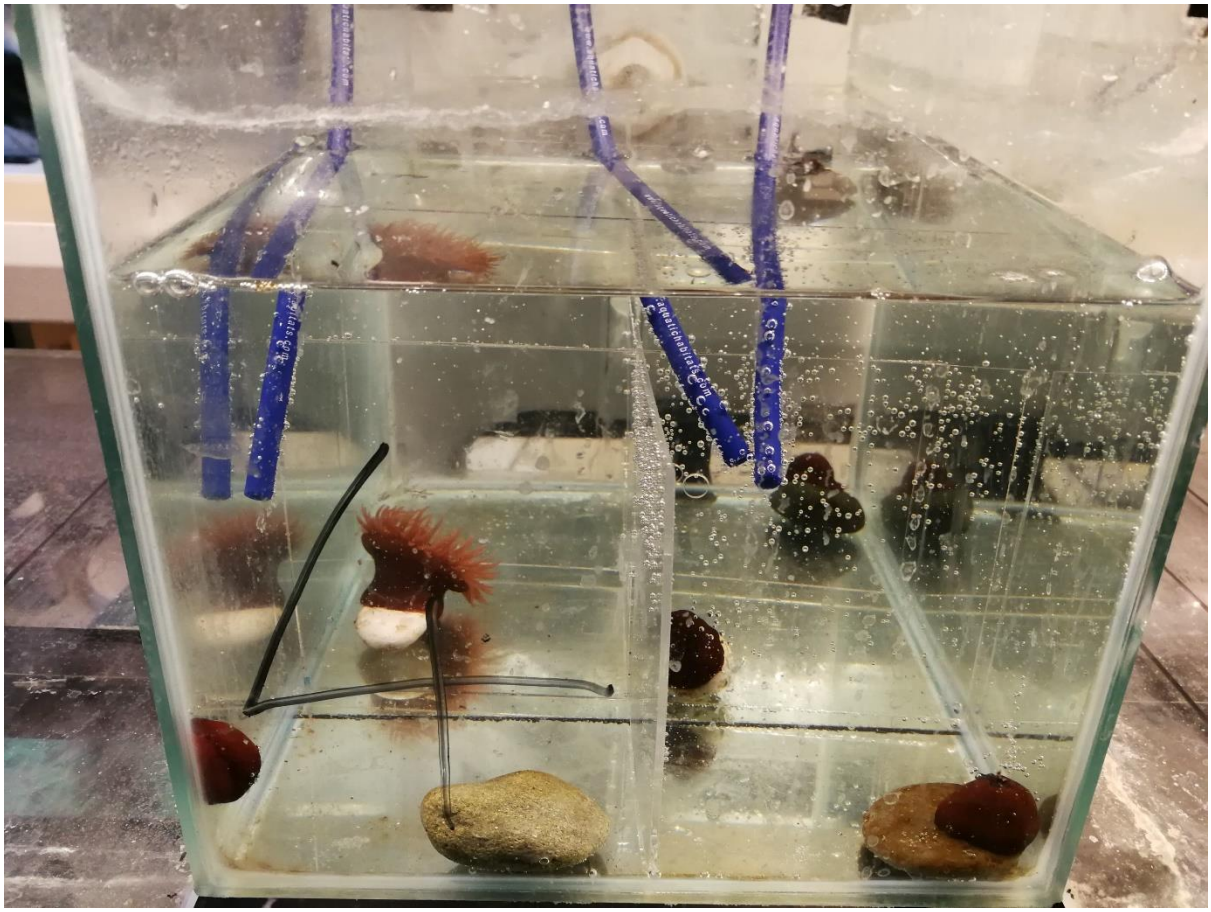


Figure 1.4. Anemones in the laboratory having recently been re-immersed during an IRT assay. Four individuals (front left, front right, middle right, back right) have their tentacles fully retracted, as they would be immediately after eliciting an IRT or SRT, and one individual (middle left) has its tentacles extended, as they would be at the conclusion of an IRT or SRT.

1.5. OVERVIEW OF AIMS AND METHODS

This thesis aims to quantify behavioural variation in *A. equina* across different thermal contexts and to determine how this covaries with metabolic and proteomic responses to temperature. Using animals from Llandudno North Beach in North Wales (lat: 53.330359, long: -3.828975) and from New Brighton in North-West England (lat: 53.4400, long: -3.0565), I address this aim over the course of four data chapters investigating three hypotheses.

1.5.1 Hypothesis 1

"Different *A. equina* individuals will show consistent differences in their startle response-times (SRTs) and immersion response-times (IRTs), and individuals will vary in how those behaviours change across temperatures. The morphotype and environmental history of an individual will also affect these patterns."

I expected that some animals at each grouping-level would display more adaptively advantageous behavioural changes at extreme temperatures than others. I predicted that animals with a preference for the more thermally extreme high-shore would show more adaptively advantageous behavioural responses to extreme temperatures than those with a preference for the low-shore. I further expected that boldness would be related to plasticity, and that bolder individuals might show less plasticity to changes in temperature and consistently lower levels of unpredictability across temperatures, in line with previous work indicating that bolder animals often have reduced scope for behavioural variability.

In **Chapter 2** I investigate this at the mean level. I took repeated assays of SRT and IRT at three different temperatures in experimental treatments: 11^{o}C (baseline), 18^{o}C (high), and 23^{o}C (near-lethal). I also ran control treatments where individuals were retained at 11^{o}C throughout. I used several modelling approaches to explore the influence of morphotype, shore height and individual level differences on behavioural responses to temperature, alongside investigating the existence of a boldness-shyness continuum in *A. equina*.

> Published: "Maskrey, D. K., Sneddon, L. U., Arnold, K. E., Wolfenden, D. C. C., & Thomson, J. S. (2021). The impact of personality, morphotype and shore height on temperature-mediated behavioural responses in the beadlet anemone *Actinia equina*. *Journal of Animal Ecology*, *89*(10), 2311-2324."
>
> Author Contributions: DKM, JST, LUS and KEA designed and formulated the study. DKM, DCCW, JST and LUS all contributed to the set-up of laboratory apparatus. DKM carried out all laboratory experiments with assistance from DCCW. All authors contributed extensively during the formulation of the manuscript.

I then consider the unpredictability of IRT and SRT at different temperatures in **Chapter 3**. I used a crossed-over design and a temporal control to investigate behavioural unpredictability at low and high temperature extremes. I employed double-hierarchical mixed modelling to investigate between-individual variation in unpredictability and how different individuals, of different morphotypes and from different shore heights, plastically altered their unpredictability at different temperatures.

> Published: "Maskrey, D. K., Sneddon, L. U., Arnold, K. E., Wolfenden, D. C. C., & Thomson, J. S. (2021). Temperature-driven changes in behavioural unpredictability and personality in the beadlet sea anemone, *Actinia equina*. *Animal Behaviour*, *181*, 13-27"

Author Contributions: The study was formulated by D.K.M., J.S.T. and K.E.A. D.K.M. and D.C.C.W. jointly set up the laboratory, with assistance from J.S.T. and L.U.S. D.K.M. carried out experimental work assisted by D.C.C.W. All authors provided extensive contributions to the manuscript.

1.5.2. Hypothesis 2

"Anemones will show consistent between-individual differences in their metabolic rates both within and across temperatures. These patterns will be influenced by morphotypic differences and will covary with an individual's boldness."

In conjunction with generally higher energetic demand on ectotherms at high temperatures, I expected all animals to increase their metabolic rates as temperatures rose, but to varying degrees based on individual and morphotypic variation. On the basis of results from Chapter 2, I expected individual IRTs to be correlated with individual metabolic rates at all temperatures. This correlation would be in line with POLS and would become clearer at high temperatures due to greater energetic demand driving trade-offs between foraging and risk-aversion.

I address this hypothesis in **Chapter 4**. I used another crossed-over design and intermittent-flow respirometry to investigate the relationship between routine metabolic rate (RMR) and IRT across temperatures. Making use of Bayesian random regression and multivariate approaches, I examined the influence of morphotype and individual differences on RMR, how that was affected by thermal variation, and whether an individual's RMR was related to their IRT within and across temperatures.

In review at *Functional Ecology* (September 2021): "Maskrey, D. K., Killen, S.S., Sneddon, L. U., Arnold, K. E., Wolfenden, D. C. C., & Thomson, J. S. Differential metabolic responses in bold and shy sea anemones during a simulated heatwave"

Author Contributions: DKM, SSK, LUS, KEA, and JST formulated the study. Laboratory set-up and animal husbandry was carried out by DKM and DCCW. DKM carried out experimental work and analyses, with assistance from all authors. All authors contributed extensively to the manuscript.

1.5.3. Hypothesis 3

"Anemones will show a strong proteomic thermal stress response which will be associated with their boldness."

Here, I expected to see clear up-regulation of stress response proteins such as HSPs, alongside changes in the expression of energetically costly biosynthetic processes and of

apoptotic pathways. I further predicted that this stress response would be least apparent in bold individuals, in this case measured as SRT, given the low thermal plasticity they exhibited in Chapter 2.

Chapter 5 explores this hypothesis. I took tentacle samples from bold and shy animals, some under temperature stress and others at a neutral temperature, and examined discrepancies in their proteomic expression. Using a variety of proteomic and genomic statistical methodologies, alongside simple linear models, I investigated *A. equina's* proteomic thermal stress response and how that response was related to an individual's mean SRT, measured at a baseline temperature.

> "Maskrey, D. K., Beynon, R.J., Sneddon, L. U., Arnold, K. E., Franco, C., Simpson, D.M., Brownridge, P.J., Wolfenden, D. C. C., & Thomson, J. S. Personality and the proteome: proteomic effects of temperature stress on the beadlet sea anemone (*A. equina*)". In preparation.
>
> Author Contributions: DKM, JST, LUS, KEA, and RJB formulated the study. DKM and DCCW set up the animal laboratory with assistance from LUS and JST. Husbandry was carried out by DKM and DCCW. DKM ran behavioural assays and took tentacle samples. CF and DMS purified, quantified, and ran Progenesis analyses on proteomic profiles. PJB provided bioinformatic support throughout and provided initial BLAST IDs. DKM ran all other analyses. All authors contributed extensively to the manuscript.

[Page intentionally left blank]

Chapter 2:

The impact of personality, morphotype and shore height on temperature-mediated behavioural responses in the beadlet anemone *Actinia equina*

[Page intentionally left blank]

2.1. KEYWORDS

Behavioural plasticity, Boldness, Climate change, Environmental history, Marine invertebrate, Morphotype, Personality, Temperature fluctuation

2.2. ABSTRACT

1. Between-individual variation in behavioural phenotype, termed personality, is an important determinant of how populations cope with acute environmental fluctuation related to climate change.
2. Personality in the beadlet sea anemone (*Actinia equina*) is linked to genetically distinct morphotypes, which are associated with different heights on the shore. In the intertidal zone, high-shore environments experience more environmental fluctuation due to longer periods of exposure, and animals adapted to live in these environments are predicted to deal more effectively with environmental perturbation than their low-shore counterparts.
3. We collected beadlet anemones of two different morphotypes from three different shore heights. We investigated variation in two behaviours at three different temperatures and in a temporal control treatment where the temperature was not changed: startle response-time, the time it took an anemone to re-extend its tentacles after a threatening stimulus, and immersion response-time, the time to re-extend tentacles after simulated tidal immersion. These behaviours reflect risk taking and allow individuals to be categorised as bold, shy or intermediate based upon response-times.
4. Both behaviours showed significant changes as the temperature increased. For immersion response, the morphotype associated with the low-shore lengthened response-times at high temperatures. For startle response, all animals lengthened their response-times at high temperatures but animals collected from the low-shore lengthened theirs to the greatest degree. At the individual level, although control individuals exhibited temporal changes in their response-times, a clear effect of temperature was present in both behaviours. Shy and bold individuals became more intermediate at higher temperatures in immersion response (this effect was present to a lesser degree in control individuals), while intermediate individuals raised their response-times at higher temperatures for startle response.

5. Given that prolonged tentacle retraction reduces foraging opportunities and can negatively impact respiratory efficiency, our data suggest that some individuals within a single population of A.equina, particularly those associated with the lower-shore, may exhibit less effective behavioural responses to temperature-shifts than others. These findings demonstrate that acute temperature changes influence risk-taking, and could have profound short and long-term implications for survival in the face of climate change.

2.3. INTRODUCTION

Anthropogenic climate change is an ever-increasing threat to global biodiversity (IPCC, 2018; Parmesan, 2006). The effects of climate change can be seen particularly in the oceans; since 1971, global average ocean surface temperatures have increased at a rate of 0.11^{o}C per decade (IPCC, 2013), with 2018 having been the hottest year in the oceans since records began (Cheng et al., 2019). Within ocean habitats, the effects of climate change are especially apparent in intertidal zones, as they are already subject to extremely high levels of spatial and temporal heterogeneity (Brahim et al., 2019). Different heights on the shore vary substantially in their exposure to environmental fluctuation (Bockelmann et al., 2002). This leads to highly niche-specialised intertidal flora and fauna, adapted to live at different shore heights and so under differing levels of heterogeneity in their immediate environment (Allcock et al., 1998; Dias et al., 2018). Phenotypic differences across the gradient of the shore can also extend within species, and animals adapted to certain shore heights may be more susceptible to potential environmental changes than others (Brahim et al., 2019; Chapperon et al., 2016).

An important aspect of within-species phenotypic variation is consistent behavioural differences between individuals across times and contexts (context here defined as immediate environmental conditions), also termed personality (Dall et al., 2004; Sih et al., 2004). Personality is now widely documented across a variety of taxa (Bell et al., 2009), including many invertebrates (Kralj-Fišer & Schuett, 2014). Aggression, exploration and risk-taking (usually referred to as boldness) are all commonly measured personality traits (Carter et al., 2013). Personality variation is linked with how individuals respond to environmental stressors both behaviourally and physiologically (Koolhaas et al., 2010) and divergence in these responses to environmental challenge (e.g. Dong et al., 2008; Wong et al., 2019) could cause varied fitness levels in the face of environmental perturbation (Killen et al., 2016).

Investigating the effects of environmental change on personality variation can therefore contribute to our understanding of how different populations, and individuals within those populations, are likely to cope with anthropogenic climate change (Tuomainen & Candolin, 2011).

Variation in behavioural strategies to deal with different environmental conditions is becoming more widely demonstrated (Killen et al., 2013) and personality-types are often specialised to specific environments (Holtmann et al., 2017). In heterogeneous environments like the intertidal zone, biotic (e.g. food availability; Kolluru et al., 2007) and abiotic (e.g. temperature; Chapperon et al., 2016) selective pressures vary spatially and temporally (Araújo et al., 2011). This can lead to these environments having a diverse range of behavioural optima, which can drive the maintenance of personality variation (Dingemanse & Wolf, 2013). Environmentally-driven personality variation can extend to how different individuals plastically alter their behaviour in response to environmental fluctuations (Stamps, 2016). Where usual environmental conditions are less variable, more rigid personalities and a reduced scope for potentially energetically costly "activational" behavioural plasticity (i.e. a rapid phenotypic shift induced by an environmental stimulus; Snell-Rood, 2013) could provide an adaptive advantage (Dall et al., 2004). Meanwhile, in stochastic environments, a lack of behavioural plasticity can be detrimental to individual fitness (Abram et al., 2017) and to the overall health of a population (van Baaren & Candolin, 2018).

Thermal variation is one of the key environmental variables being affected by climate change (Hoegh-Guldberg & Bruno, 2010; IPCC, 2018), and environmental temperature shifts can have far-reaching physiological and behavioural effects across a range of species (Abram et al., 2017; Parmesan, 2006). As a species that inhabits the intertidal zone (Allcock et al., 1998), single populations of the beadlet anemone (*Actinia equina*) live across wide spatiotemporal thermal gradients (Harley et al., 2006). Personality variation in *A. equina* has a known genetic component: at least three distinct morphotypes (Allcock et al., 1998), two of which are readily determined by eye, show clear differences in their boldness and aggression (Collins et al., 2017). *A. equina* is highly sedentary, and morphotypes show significant ecological distinction in their distribution across shore heights (although there is some overlap; Allcock et al., 1998). This indicates that they are adapted to live at different heights on the shore (Quicke et al., 1983), which vary in their thermal exposure (Brahim et al., 2019). *A. equina* therefore provides the opportunity not only to investigate differences in

temperature-mediated behavioural shifts at the level of the individual, but to further uncover how these might be linked to population-level variation brought about by environmental heterogeneity (Monteiro et al., 1997). Those individuals of high-shore adapted morphotypes or simply collected from higher up the shore, that experience greater environmental fluctuation with changing tides, may have increased scope for behavioural plasticity in the face of environmental shifts (Dingemanse & Wolf, 2013). This could increase their robustness to climate change-induced temperature changes (Tuomainen & Candolin, 2011), as compared with lower-shore individuals, which deal with a much more homogeneous immediate environment and should therefore be more rigid in their behaviour (Dall et al., 2004; Snell-Rood, 2013).

In this study we measured startle response, a form of emergence test using the re-extension of feeding tentacles in recovery from a threatening stimulus (Collins et al., 2017; Lane & Briffa, 2017; Rudin & Briffa, 2012). In *A. equina*, startle response is commonly used as a proxy for boldness, since latency to recover from a disturbance can provide a measure of risk-taking (Beckmann & Biro, 2013). To investigate whether boldness in *A. equina* could be defined as an axis of behavioural variation (Carter & Feeney, 2012; Houslay & Wilson, 2017) and whether different behaviours falling on this axis might respond predictably to temperature shifts, we defined a second, potentially related behaviour: immersion response. This measurement used an anemone's latency to extend its foraging tentacles in response to simulated tidal fluctuations, which is inherently risky as it exposes tentacles to increased predation (Edmunds et al., 1974), as opposed to recovery from a threat.

We aimed to explore underlying differences between groups (i.e. different morphotypes and shore heights) and individuals in boldness, and how these related to variation in temperature-mediated behavioural shifts. To address this, we employed a graduated temperature increase, alongside a temporal control treatment, and took repeated behavioural measures from *A. equina* individuals at each temperature, or at each equivalent timepoint for the control. Our focus was specifically on acute temperature shifts of the type that might be brought about by extreme weather events, the frequency of which are expected to increase as the climate continues to warm (IPCC, 2013). As certain behavioural responses may be more adaptively advantageous in the face of increasing temperatures than others (Abram et al., 2017; van Baaren & Candolin, 2018), we hoped to gain an understanding of which groups and personality-types within a population of *A. equina* might exhibit less effective behavioural

changes, and thus be particularly susceptible in future to climate change-induced acute temperature changes.

2.4. METHODS

2.4.1. Collection and housing

Data collection took place between April and August 2018 across four, three-week blocks with each block randomly assigned to experimental or control treatments. Anemones were collected from the north shore of Llandudno, North Wales (lat: 53.330359, long: -3.828975). Within each block, anemones were removed from substrate using a flathead screwdriver, taking care not to cause tissue damage. A minimum of 1m was left between each anemone collected to avoid collecting clonal individuals (Foster & Briffa, 2014). Collected anemones were split between two morphotypes which are putatively associated with different heights on the seashore (Quicke et al., 1983). Although each morphotype was far more abundant at their associated shore height, overlap in their distributions allowed sample sizes of each morphotype to be split evenly across low, mid, and high shore heights (Appendix A2.A), which were defined by the stratification of the shore (e.g. Dias et al., 2018). The colour of the pedal disc was used to differentiate between the two morphotypes (Allcock et al., 1998). The high-shore-associated morphotype was defined as having a red or brown pedal disc (henceforth red) and the low-shore-associated morphotype was defined as having a green pedal disc (henceforth green). Where pedal disc colour was inconclusive, the presence or absence of a blue limbus around the disc was used to further differentiate the individual as green if present (Collins et al., 2017). Total sample size was 209, split between 101 (red= 65, green= 36) experimental individuals and 108 (red= 72, green= 36) controls (for a full explanation of sample sizes and associated ethical considerations, see Appendix A2.A.). Upon collection, each anemone was placed into a small, sealable plastic bag filled with seawater and air. Anemones were transported to the laboratory at the University of Liverpool within 4 hours of collection. None suffered mortality and all subsequently fed.

Upon arrival to the lab, each individual was transferred into a separate 9.5 cm x 9.5 cm compartment within a larger tank (30 cm x 20 cm x 20 cm), containing a single pebble onto which they could attach. Anemones were unable to physically interact but could potentially chemically interact via the flow-through system. Tanks were filled to a depth of ca.15 cm with seawater (RO water and Tropic Marin, Germany, Pro Reef Sea Salt) and were situated on a flow-through system, located in an 18^{o}C (±0.5^{o}C) temperature-controlled room. Overall,

10 tanks were used to house each block of anemones, with eight tanks housing six individuals and two tanks housing three individuals (see Appendix A2.A. for a diagram of this set-up). Tanks always housed the same number of individuals across data collection blocks. The system was kept at a salinity of 34ppt (±1), a pH of 8.1 (±0.3), and was regularly monitored for water quality. Anemones were all housed at a natural baseline temperature of 11^{o}C (±1^{o}C; www.seatemperature.org; see Appendix A2.B.). Water was chilled using an Aqua-Medic (Bissendorf, Germany) Titan 500 chiller, and heated using an Aqua-Medic (Bissendorf, Germany) 300W titanium heater. Animals were kept on a 12:12 hour (9am to 9pm) light:dark cycle and were allowed a minimum of two days to acclimate to their new environment. Anemones were fed ad libitum on the final day of acclimation with Tetra Marine Flakes (Tetra GMBH, Melle, Germany) and a 50% water change was carried out before commencing behavioural trials the following day (See Appendix A2.C. for a visualisation of within-block experimental schedules).

For ease of identification, morphotypes and shore heights of anemones were standardised in each tank. This was deemed an appropriate method of housing because (1) the flow-through system allowed us to keep all experimental tanks at a uniform temperature (±1^{o}C), and (2) the one-system set-up should have meant that individuals within each data collection block were being exposed to the same chemical cues regardless of the tank they were housed in.

2.4.2. Behavioural Trials

Timeline

Behavioural testing took place over the course of 13 days, subsequent to the initial acclimation period. In experimental treatments, anemones were initially subjected to three days of behavioural trials at 11^{o}C (±1^{o}C). Behavioural trials on each day consisted of first testing individual immersion response-times (IRTs) and testing their startle response-times (SRTs) 10 minutes after the conclusion of IRT observation. Half of the tanks were tested in the morning and half in the afternoon of each day. Tanks were randomly selected on days one and three, with tanks tested in the opposite order on day two to ensure that all individuals experienced testing at both times of day. Within time of day the order in which tanks were subjected to behavioural trials was randomised and, for SRT trials, so was the order in which individuals were subjected to trials within tanks.

At the end of the third day of behavioural testing at 11°C (±1°C), a 50% water change was carried out and during experimental blocks the water temperature was raised to 18°C (±1°C), which is at the upper limit of what this population might experience naturally (www.seatemperature.org), overnight at a rate of 1°C per hour. Anemones were given a further two days to acclimate to the new temperature and once again fed ad libitum on Tetra Marine Flakes the day before behavioural trials commenced. Behavioural testing was carried out using the same process as at 11°C (±1°C) before finally repeating the process again for the last temperature of 23°C (±1°C), which was deemed near-lethal as pilot work found that anemones from Llandudno began to denature at 24-25°C.

For control treatments, the timeline of behavioural testing remained the same but the temperature stayed at 11°C (±1°C) across the 15 days. Blocks of three repeats within experimental treatments are hereafter referred to as temperatures and blocks of three repeats in controls as timepoints.

Behaviour One: Immersion response-time (IRT)

In order to test IRT, tanks were drained by turning off the water inflow, temporarily removing the partitions separating anemones, and siphoning water directly into the system sump. This method caused minimal disturbance to animals other than the reduction in water level and took roughly five minutes per tank. Partitions were reinstated and each tank was left for 30 minutes before re-immersion, which was achieved by switching on the inflow. Response-time was captured using time-lapse photographs, taken every 30 s, using seven Crosstour 4k (Shenzhen Longtour Optics Co. Ltd, Shenzhen, China) action cameras and two GoPro Hero 4 (San Mateo, California, USA) action cameras. Each camera captured the response-times of three individuals per trial. Recording began before re-immersion commenced to ensure fast responses were not missed or incorrectly measured. 50 minutes of footage was captured for every camera, from which 45 minutes were used to measure IRT. We deemed 45 minutes an appropriate time-limit as pilot work found that most individuals re-extended their tentacles within 30 minutes of re-immersion.

IRT was defined as the length of time from when the water-line first touched the body of an individual to when an individual had fully re-extended its feeding tentacles such that the entire circumference of the collar (the upper rim of the anemone's column; Griffiths, 1977b) had been surpassed and there was no longer any visible extension occurring. How many pictures a response took was recorded, and this number was multiplied by the time-lapse

interval to convert values into seconds. Those individuals which did not re-extend their tentacles within 45 minutes were given a value of 2700 s. Photo analysis found that 5% of IRTs hit this upper bound. All behavioural values were extracted from photos by the same researcher (DKM), blinded, as far as possible given the colour differences inherent in the different morphotypes, to anemone type to avoid any inter-individual variation in results.

Behaviour Two: Startle response-time (SRT)

SRT was tested using a similar method to Rudin & Briffa (2012). Startle responses were elicited 10 minutes after the conclusion of the previous immersion trial by jetting each individual's oral disc with 50 ml of tank water from a 60 ml syringe located 1 cm above an anemone, causing them to retract their tentacles. Our method differed from Rudin & Briffa (2012) in that, on time lapse recordings, an individual's oral disc was not always visible and individuals rarely fully re-extended their tentacles. SRT was therefore defined as the time it took an anemone to re-extend its feeding tentacles such that 75% of the circumference of the collar had been surpassed. Pre-startle reference pictures were also taken, as some individuals did not have their tentacles fully extended beyond the collar at the start of trials. For these individuals, SRT was defined as the time it took for tentacles to return to their pre-startle degree of extension. Some individuals exhibited no or very limited tentacle extension at the beginning of a given trial and thus no response could be elicited. Of 1881 SRT data points, 156, spanning 89 individuals, were not quantifiable and thus excluded.

While the startle stimulus remained the same across individuals, different definitions were utilised for different datapoints as the visible behavioural response differed slightly depending on starting tentacle extension. Those individuals that began fully extended very rarely returned to their pre-startle degree of extension, even when exhibiting a large degree of recovery and returning to "normal" behaviour. Those individuals which did not start with their tentacles fully extended similarly rarely began with more than 75% of their tentacles extended beyond the collar, but regularly returned to their pre-startle degree of extension. The dual definition was thus the best method to maximise sample sizes and account for the slight difference in observable responses with as accurate a measure as was feasible. There was a relatively even split of individuals between the two definitions. Re-analysing a randomly selected subset of photos from the full dataset, it was found that 62% of individuals began a given trial with their tentacles fully extended, while 38% of individuals did not. See Appendix A2.D. for a full analytical justification of this.

Measurement and extraction of response-times was conducted in the same way as for IRTs. In this case, cameras recorded 100 minutes of time-lapse footage. Each individual's SRT was measured using the 90 minutes of footage immediately following the syringe stimulus and those that did not exhibit re-extension within this time were given a value of 5400 s. This was deemed an appropriate upper bound, beyond which re-extension of tentacles should no longer be defined as the recovery from a threat, based on a review of SRT ranges across the current literature (e.g. Collins et al., 2017; Rudin & Briffa, 2012). 22% of SRT measurements reached 5400 s in this study. These values were retained for analysis as our literature review indicated that these individuals were exhibiting maximally shy behavioural responses.

2.4.3. Statistical analysis

We carried out all analyses in R version 3.5.1 (R Core Team, 2020). Analysis of fixed effects on population means was carried out in lme4 (Bates et al., 2015), using extra features from lmerTest (Kuznetsova et al., 2017). All individual level behavioural analysis followed a Bayesian Markov Chain Monte Carlo framework, using the R package MCMCglmm (Hadfield, 2010). We ran models using both parameter-expanded and inverse-Wishart priors to ensure robustness to different specifications. DIC (Deviance Information Criterion, analogous to AIC values in REML analysis; Spiegelhalter et al., 2002) estimates did not differ meaningfully between the two prior specifications in any model and effect estimates remained similar. Only the inverse-Wishart results are reported. Response variables were always z-transformed in order to improve model convergence (Houslay & Wilson, 2017). All individuals were included in all analyses, regardless of missing values, as individuals with fewer observations can still markedly improve the power of behavioural models (Martin et al., 2011). All Bayesian models were run for 420000 iterations with a 20000 burn-in period and a thinning interval of 100. All models underwent full model checks; convergence and autocorrelation were checked by visual plot inspection and using both Heidelberger & Welch and Gelman-Rubin convergence tests. The significance of individual level estimates and differences between these estimates were both determined using 95% credible intervals (e.g. Debeffe et al., 2015; Highcock & Carter, 2014; Houslay & Wilson, 2017). Bayesian models are described in brief here, but for further details of these analyses see Appendix A2.E. Graphs were drawn with ggplot2 (Wickham, 2011).

Group level effects on population means

To investigate the impact of morphotype and shore height on IRT and SRT, and whether this was associated with temperature change, we ran separate univariate generalised linear mixed effects models on the full datasets (incorporating both control and experimental treatments) for each behaviour. We used stepwise model simplification to determine minimum adequate models. Behaviours were set as the response variable. IRT values were reciprocal root transformed to ensure model assumptions were met. Starting models contained morphotype, shore height, temperature (a three-level categorical effect in all models), time of day, data collection block and sampling occasion, which incorporated both time since feeding and time since collection, as fixed effects, and the first three were initially allowed to interact. Individual and tank were random effects. Given that tanks always housed the same number of individuals, this variable also incorporated the differing densities of anemones inherent in our housing design.

Repeatability variation across temperatures and timepoints

We assessed adjusted repeatability (hereafter, repeatability) across temperatures and timepoints (hereafter, when referred to together, called contexts) by splitting experimental and control treatments into separate datasets for IRT and SRT respectively. We ran univariate mixed effects models on each dataset and extracted across-context repeatability estimates from each (Appendix A2.E.). Morphotype, shore height and sampling occasion were fixed effects and individual was a random effect. Temperature was fitted as a further fixed effect in the experimental models and interaction terms were included in each model based on the minimum adequate models from our REML analysis.

A single axis of behavioural variation

To test whether IRT and SRT fell on the same axis of behavioural variation, we first ran a bivariate model on the whole dataset and extracted the between-individual correlation between the two behaviours (r_I; Appendix A2.E.). Both IRT and SRT were set as response variables with morphotype, shore height, temperature and sampling occasion as fixed effects and individual as a random effect. To investigate whether covariance remained stable within treatments (i.e. whether temperature change affected any correlation between the two behaviours), we ran two further bivariate models on control (with temperature no longer included as a fixed effect) and experimental datasets respectively.

Within-context repeatability, between-context correlations and between-individual variation in plasticity

To examine between-individual variation in plasticity (IxE) and the strength of correlations between behaviours across contexts we utilised a character-state approach, treating each context as a separate response variable (Dingemanse et al., 2010; Houslay & Wilson, 2017). Methods of controlling for temporal variation in the laboratory typically utilise variations of crossed-over designs (e.g. Briffa et al., 2013; Mitchell & Biro, 2017) or temporal controls similar to the one employed in this study (White & Briffa, 2017) and account for time statistically. However, a unique feature of the temporal control is that it allows the direct comparison of control and experimental patterns of change by partitioning treatments into separate datasets, and that is the method utilised here. Comparison with other methods found that the character-state approach fitted these data significantly better overall (Appendix A2.E.).

We specified a trivariate mixed effects model for each partitioned dataset, in which response variables were behavioural response-times in each context. Morphotype, shore height and time since last feeding were specified as fixed effects and individual was specified as a random effect. We extracted repeatabilities for each response variable (i.e. repeatability within-contexts). To investigate the presence of individual differences in plasticity (IxE) we calculated between-individual behavioural correlations between different combinations of contexts (Appendix A2.E.).

We extracted the posterior modes of each individual's response-times (analogous to BLUPs) for each context from each model to explore patterns of IxE between different contexts. Because of high levels of variance in the data, individuals were split into three starting personality-types for SRT and IRT respectively ("bold", "intermediate" and "shy") based on the mean of their predicted values in the first context. Equivalent mean values for the other contexts were extracted for each personality-type. The magnitude of changes in predicted response-times for each personality-type between contexts was calculated (Appendix A2.E.).

2.5. RESULTS

2.5.1. Temperature and group-level effects

Temperature interacted with morphotype ($F= 16.26_{1,1126}$, $P< 0.001$) but not shore height to impact the length of immersion response-times (IRTs). This effect was small in the context of the overall range of individual within-context means (130s-2110s; Appendix A2.F.) but was still clear and significant. The higher-shore-associated red morph shortened IRTs at 18ºC (becoming bolder). The lower-shore-associated green morph lengthened IRTs at 23ºC, such that red individuals exhibited shorter IRTs than their green counterparts at that temperature. Temperature interacted with shore height ($F= 10.68_{2,1703}$, $P< 0.001$) but not morphotype for startle response-time (SRT). Individuals from the low-shore lengthened SRTs at 18ºC (becoming shyer) while those from the mid and high-shore did not. At 23ºC mid and high-shore individuals also lengthened their SRTs, but low-shore individuals still exhibited the longest response-times. SRTs further differed between morphotypes ($F= 8.91_{1,198}$, $P= 0.003$), but this difference was independent of temperature. Green morphotypes exhibited consistently shorter SRTs than red morphotypes across all temperatures. Sampling occasion explained a significant amount of variance in both models (IRT: $F= 4.72_{8,1683}$, $P< 0.001$; SRT: $F= 10.18_{8,1528}$, $P< 0.001$).

These data show that those anemones living lower down the shore, or of the lower-shore-associated green morphotype, altered their behaviour differently at high temperatures than their higher-shore-associated counterparts. Figure 2.1 provides a visualisation of the effects of morphotype and shore height for each behaviour in control and experimental treatments.

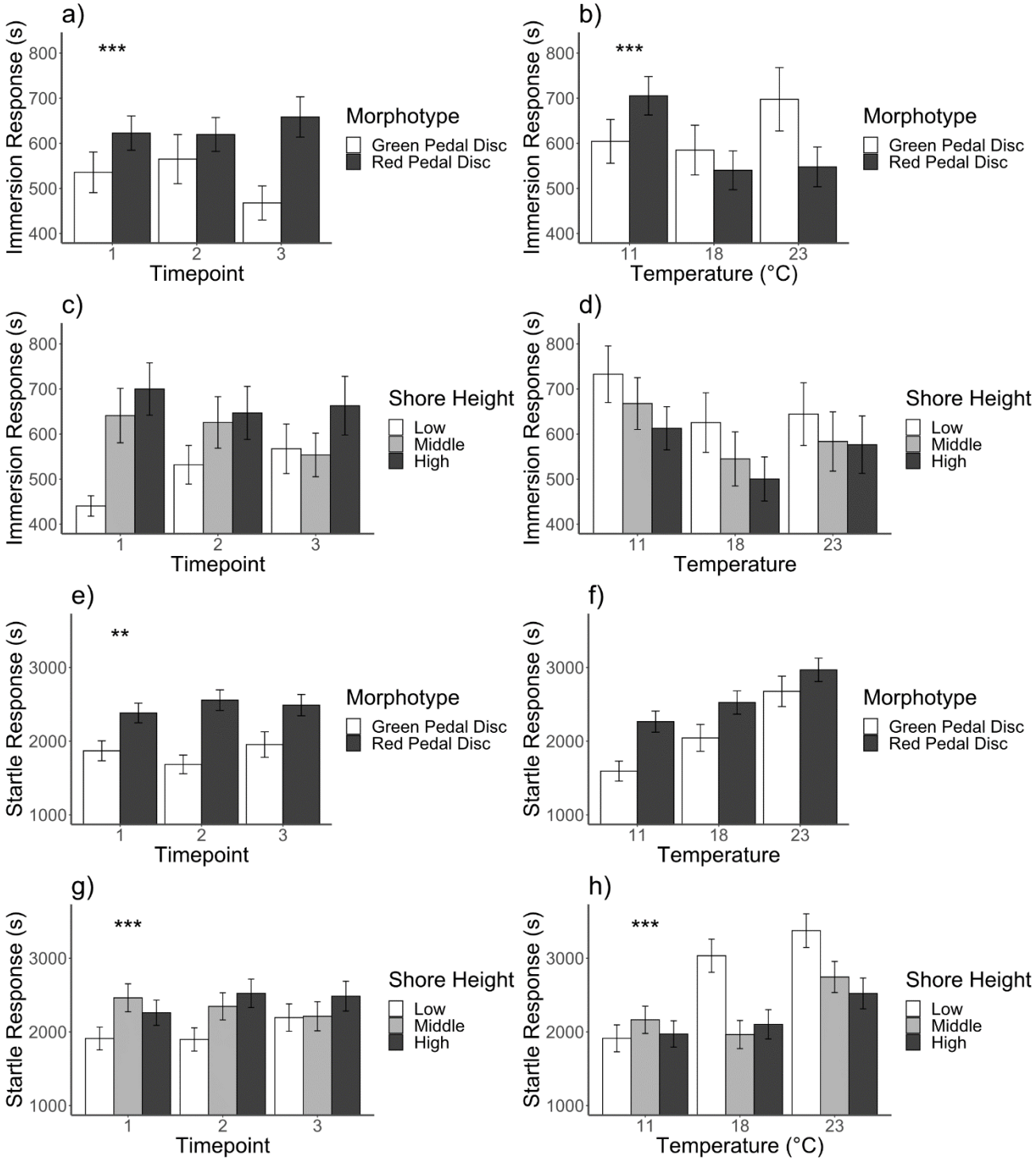


Figure 2.1. Variation in mean immersion and startle response-times, across timepoints for 108 control beadlet anemones (panels a, c, e, g) and temperatures for 101 experimental anemones (panels b, d, f, h), for different morphotypes/shore heights. Means are derived from 3 repeated measures per anemone within each timepoint/temperature. Significance of single morphotype/shore height terms, determined from generalised linear mixed effects models, is denoted by asterisks above control plots. Significant interactions between morphotype/shore height and temperature are denoted by asterisks above experimental plots. $P < 0.01$ is denoted by "**", $P < 0.001$ is denoted by "***".

2.5.2. Repeatability across contexts

Significant across-context repeatability was observed for SRT and IRT in both experimental (SRT: R= 0.45, 95% CI= 0.35, 0.55; IRT: R= 0.21, 95% CI= 0.13, 0.29) and control (SRT: R= 0.43, 95% CI= 0.33, 0.53; IRT: R= 0.19, 95% CI= 0.12, 0.27) treatments. Models containing an individual intercept term fit the data significantly better than models where this was removed for both SRT (Experimental With: DIC= 1956; Experimental Without: DIC= 2354; Control With: DIC= 2092; Control Without: DIC= 2454) and IRT (Experimental With: DIC= 2444; Experimental Without: DIC= 2567; Control With: DIC= 2626; Control Without: DIC= 2742), further indicating significant repeatability in both behaviours. Very similar estimates and strongly overlapping 95% credible intervals suggest that across-context repeatability in both behaviours was not significantly affected by temperature change. These results are indicative of personality and show that between-individual differences were maintained to the same degree in the presence and absence of a graduated temperature increase.

2.5.3. A single axis of behavioural variation

Bivariate models show significant between-individual correlations between IRT and SRT across the whole dataset (r_I= 0.55, 95% CI= 0.40, 0.69). Correlation coefficients remained stable for control data (r_I= 0.55, 95% CI= 0.37, 0.74) but were reduced in the experimental dataset (r_I= 0.40, 95% CI= 0.19, 0.64). These correlations show that a statistically significant portion of the between-individual differences in these behaviours fell on a single axis of variation. This axis could plausibly be defined as a "boldness-shyness" continuum, with faster responders in both behaviours being "bolder" (i.e. less risk-averse or more risk-prone). For the full and control datasets, correlation coefficients were moderate (r_i= 0.55), indicating that variation in one or both behaviours that was not explained by this axis was also present. The relationship decoupled to some extent when individuals were subjected to increasing temperatures (r_i= 0.40), indicating that patterns of temperature-related between-individual variation in plasticity (IxE) may have differed between these behaviours.

2.5.4. Repeatability within contexts

Repeatability estimates derived from character-state analyses remained significant within contexts for both experimental and control treatments (Table 2.1). IRT and SRT were more repeatable across short timeframes of three days, as within-context estimates were uniformly higher than across-context estimates derived from nine repeated measures taken over 13 days. Although some fluctuations in within-context estimates both between treatments and between contexts were present, estimates within each behaviour remained broadly similar. These similarities, coupled with highly overlapping 95% credible intervals (Table 2.1), indicate that no firm conclusions should be drawn concerning these differences (see: Bierbach, Laskowski, & Wolf, 2017).

Table 2.1. Adjusted repeatabilities (R), explaining the variation in behaviour attributable to between-individual differences, within timepoints for control (C) treatments and temperatures for experimental (E) treatments for immersion response-time (IRT) and startle response-time (SRT), alongside associated 95% credible intervals. Repeatabilities were extracted from trivariate Bayesian GLMMs utilising a character-state approach and are derived from 3 repeated measures per individual beadlet anemone within each timepoint/temperature.

Behaviour	Treatment	Timepoint	Temperature	Adjusted R	95% CI
IRT	C	1	11	0.34	0.24, 0.45
		2	11	0.26	0.17, 0.36
		3	11	0.28	0.18, 0.37
	E	1	11	0.38	0.27, 0.5
		2	18	0.32	0.23, 0.44
		3	23	0.32	0.21, 0.43
SRT	C	1	11	0.5	0.4, 0.61
		2	11	0.55	0.45, 0.65
		3	11	0.59	0.49, 0.69
	E	1	11	0.61	0.52, 0.72
		2	18	0.66	0.57, 0.75
		3	23	0.58	0.48, 0.68

2.5.5. Between-individual variation in plasticity and correlations between contexts

For IRT, IxE was present in both treatments. Correlations remained steady and significant between different timepoints in controls (Figure 2.2) and were relatively weak, consistent with relatively low across-context repeatability estimates. In experimental treatments, although there was clear overlap with the 95% CIs of control estimates, the patterns of across-context correlation were markedly different (Figure 2.3; for an alternate version of this figure, see Appendix A2.G.). Coefficients were reduced and non-significant, indicating no significant repeatability, between 11^{o}C and both higher temperatures (Figure 2.2) whilst increasing and becoming significant, indicating higher repeatability, between 18^{o}C and 23^{o}C (Figure 2.2). Looking at patterns of change, a temporal effect was present in control treatments (Figure 2.3). Both bold and shy individuals moved away from personality extremes, with bold individuals lengthening response-times and shy individuals shortening them between the first and second timepoints. Intermediate individuals did not alter their behaviour. This effect was also present in experimental treatments (Figure 2.3), but the magnitude of changes for both bold and shy individuals was greater (although again note some overlap in 95% CIs). These results indicate high temporal variability in IRTs, but also show differences between individuals in how they altered their IRTs in response to temperature change. Bold and shy anemones became more intermediate in their IRTs when the temperature was increased than they did over time alone.

Correlations between contexts also reveal evidence for IxE in SRT for both control and experimental treatments (Figure 2.2). Coefficients in the two treatments were similar, but investigating patterns of change shows that temperature, as well as time, had an effect at the individual level. Predicted response-times in control treatments show shy individuals shortening their response-times and thus becoming bolder over time (Figure 2.3). Changes in experimental treatments differed from those in controls and, unlike the equivalent control timepoints, the majority of these changes occurred between 11^{o}C and 18^{o}C. Shy individuals shortened their SRTs to a greater degree than in control treatments, and intermediate individuals lengthened theirs (Figure 2.3). Intermediate individuals only showed significant levels of plasticity in experimental treatments, while shy individuals showed significantly shorter SRTs over time in both treatments (Figure 2.3). These data show that temporal variation was an important factor in determining individual level changes in SRT, but that

increasing temperatures affected how shy and intermediate individuals altered their SRTs across contexts. The SRTs of the boldest individuals remained stable in both treatments.

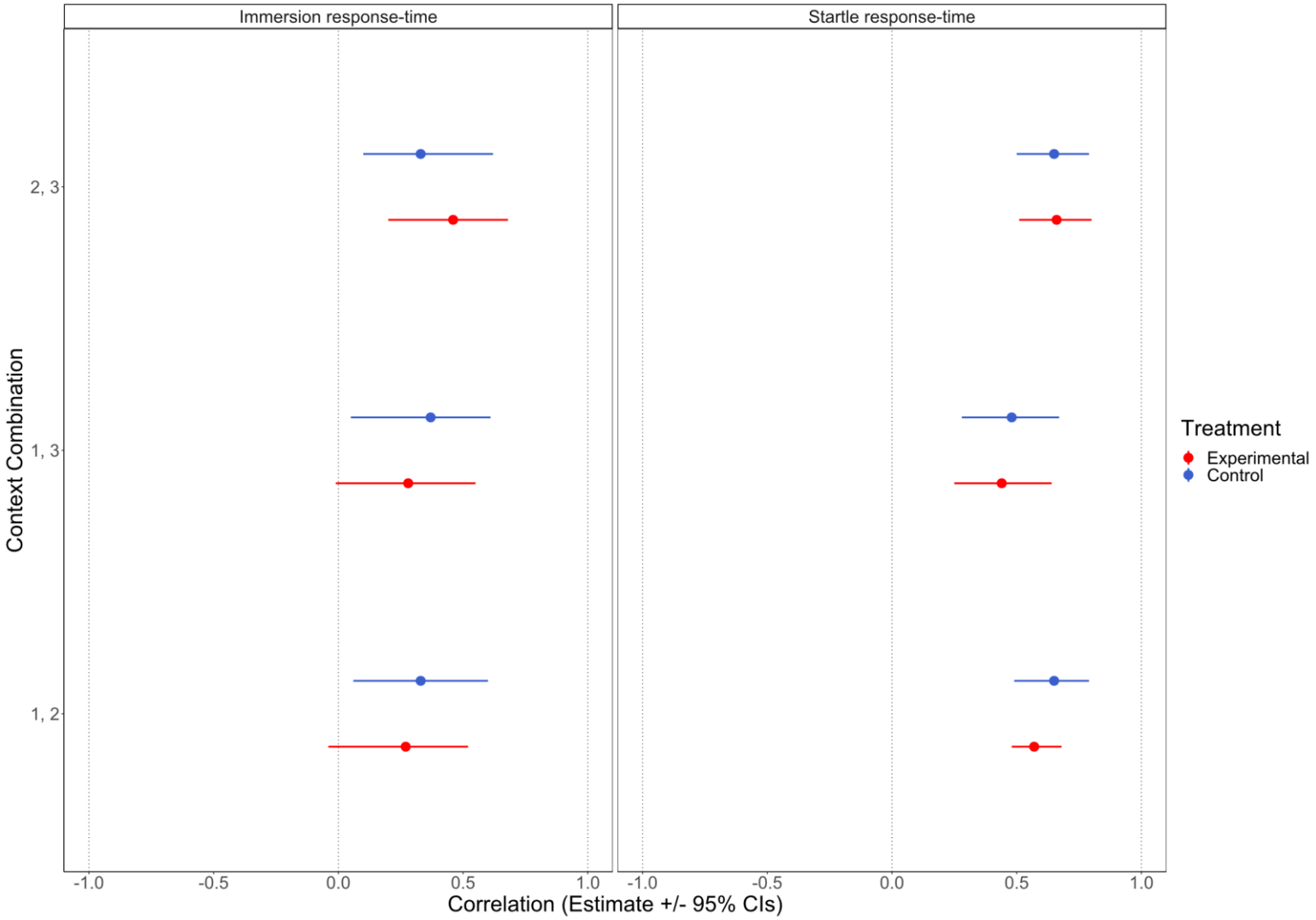


Figure 2.2. Correlation coefficients and associated 95% credible intervals between behaviours across different combinations of contexts, extracted from the posterior covariance structures of trivariate Bayesian GLMMs, for immersion response-time (IRT; left panel) and startle response-time (SRT; right panel). For experimental treatments contexts denote different temperatures (1= 11ºC, 2= 18ºC, 3= 23ºC) and for controls, contexts denote the same numbered timepoint.

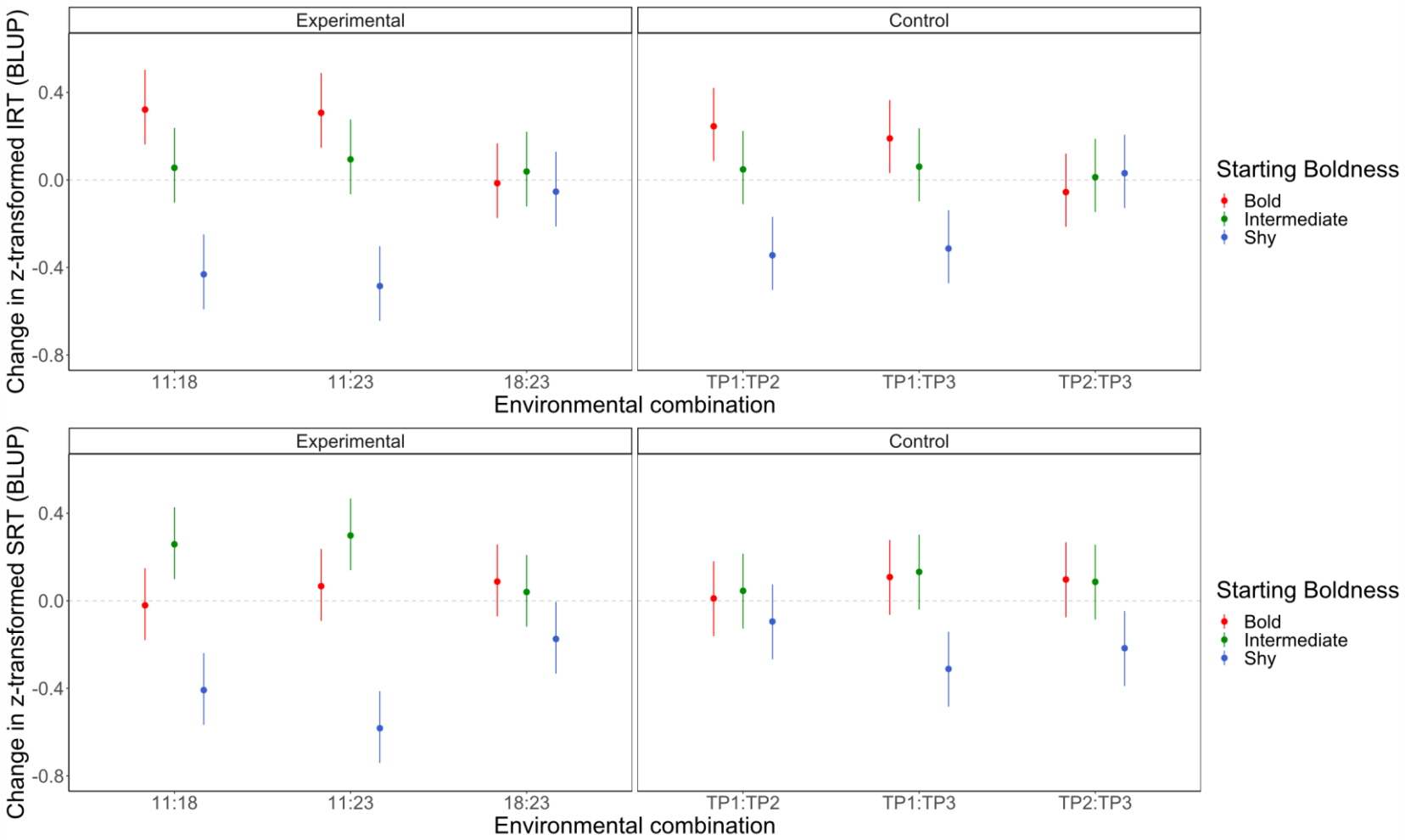


Figure 2.3. The magnitude of change in the mean of the Bayesian BLUPs extracted from trivariate GLMMs for immersion response-times (IRTs; top panels) and startle response-times (SRTs; bottom panels) and their associated 95% credible intervals across different combinations of temperatures for experimental treatments (left panels) or timepoints for control treatments (right panels). Different colours denote different personality-types of *A. equina* (based on response-times at 11ºC or timepoint one). Changes were deemed to be significant where 95% credible intervals did not cross zero, with negative changes indicating individuals becoming bolder and shortening their response-times, and positive changes indicating individuals becoming shyer and lengthening their response-times.

2.6. DISCUSSION

Temperature fluctuations due to climate change are predicted to increase the frequency of extreme weather events such as heat waves (IPCC, 2013). This could have detrimental and wide-ranging effects on organisms in their natural environment. By simulating this, our findings demonstrate that a potentially stressful temperature increase significantly affected risk-related behaviour in *Actinia equina*. We found consistent, mean level behavioural differences between individuals and between groups (i.e. morphotypes and shore heights). Overall repeatabilities in startle response-times (SRT) were consistent with estimates from recent studies of *A. equina* (e.g. Osborn & Briffa, 2017). These analyses also revealed a second repeatable behaviour in this species, in the form of immersion response-time (IRT). A moderate "boldness-shyness" continuum explaining variation in both behaviours did appear to be present and this decoupled to some degree in experimental treatments. We further quantified much of the variation inherent in the individual level behavioural effects of environmental change (IxE). By investigating across-context repeatabilities we found that individual consistency was not clearly affected by temperature. Across-context correlations and inspection of patterns, however, showed that at both the group and between-individual levels, SRT and IRT exhibited temperature-dependent change, the patterns of which differed between behaviours.

The different patterns observed for SRT and IRT suggest that temperature responses in these behaviours should be considered separately. While significant correlations between them indicate that a "boldness-shyness" continuum is present, moderate coefficients suggest that one or both behaviours may also fall on other behavioural axes. The explanatory importance of the relationship between these two behaviours may be further reduced as the temperature is increased, leading the axis to decouple and each behaviour to exhibit different patterns of temperature-related change. This suggests that the mechanistic underpinnings of these behaviours may differ and that the relevance of the relationship between them in terms of their temperature-related plasticity may be limited. These behaviours do reflect different demands made upon the animal. SRT indicates recovery from a threat, while IRT is indicative of the natural response to the cessation of air emersion, leading to the resumption of feeding and optimal respiration (Navarro et al., 1981).

In IRTs, temperature-related change at the group level might be attributable to increasing metabolic demand (Abram et al., 2017; van Baaren & Candolin, 2018). While we did not

measure metabolic rate in the present study, it has been shown to increase with ambient temperature in *Actinia equina*, with specific metabolic responses varying depending on an individual's environmental history (Navarro et al., 1981). In ectotherms, when metabolic rate increases individuals tend to exhibit faster behaviours to keep up with increased energetic costs (Abram et al., 2017). The expected pattern should have been for all individuals to re-extend their tentacles more quickly, shortening response-times, as the temperature rose, enhancing oxygen uptake and allowing foraging to meet metabolic demands. Although the green morphotype showed shorter (bolder) IRTs at 11^{o}C, red morphotypes shortened response-times to similar levels as their green counterparts at 18^{o}C, indicating enhanced metabolic demand. The green morphotype, associated with the low-shore and thus likely to be less well adapted to dealing with temperature fluctuation (Quicke et al., 1983), showed no similar reduction and exhibited possibly maladaptive longer IRTs at 23^{o}C. Differing behavioural thermal performance curves, whereby individuals quicken their behaviours up to a thermal maximum beyond which they can no longer effectively mediate them, could explain the discrepancy between morphotypes (Abram et al., 2017). It is possible that individuals of the green morphotype had surpassed their critical temperature at 23^{o}C. Red individuals, meanwhile, whose IRTs remained stable between 18^{o}C and 23^{o}C, may retain mechanisms to mediate their IRTs even when temperatures near potentially lethal levels.

At the between-individual level, high levels of within-individual behavioural variation (Stamps et al., 2012) may have been a factor in apparent time-related change in control IRTs, as between-individual correlations across contexts were low but remained uniform (see: Dingemanse et al., 2010). This would be consistent with the relatively low estimate of between-timepoint repeatability in this behaviour. Thermal stress, meanwhile, could have caused individuals to perform larger, more varied behavioural changes between 11^{o}C and 18^{o}C than the equivalent control timepoints, indicated by lower across-context correlations, and led to more consistency and thus a higher across-context correlation between 18^{o}C and 23^{o}C. When grouped based on personality-type, both shy and bold control individuals became less extreme in their average IRTs between the first and second timepoint. This could again indicate high levels of intra-individual variation, causing the IRTs of initially bold or shy individuals to appear more intermediate over longer timeframes. Patterns under temperature change retained this indication of high within-individual variation between 11^{o}C and 18^{o}C but, when coupled with higher across-context correlations between 18^{o}C and 23^{o}C, suggest that within-individual and population-level variance may have been reduced at higher

temperatures. Increased metabolic demand could feasibly reduce the adaptive scope for variability in behaviours directly related to metabolism (e.g. Velasque & Briffa, 2016) but this is in contrast to within-temperature repeatability estimates, which provide no indication that the consistency of IRTs increased with temperature. Although these estimates are based on fewer repeated measures than the across-context correlations and would thus be less likely to reveal specific patterns, future studies would be well-served to investigate this further. Understanding temperature-dependent changes in both metabolic rate and intra-individual variation will be crucial to determining whether patterns in IRT are indeed driven by metabolic changes, and whether this leads to increased stability of IRTs at higher temperatures.

In contrast to IRTs, all groups lengthened their SRTs as the temperature increased, indicating that temperature response in this behaviour was unlikely to have been solely driven by metabolic changes (Abram et al., 2017). Green individuals were bolder, showing shorter SRTs, at all temperatures but anemones from the low-shore lengthened their SRTs to a much greater degree at higher temperatures than their conspecifics from other shore heights. Low-shore individuals, which are immersed for longer periods of the day, may have more environmental scope to up-regulate this response. They may also be less acclimated to temperature fluctuations (Chapperon et al., 2016) and therefore be more stressed at high temperatures (Abram et al., 2017), leading them to be more risk-averse or physiologically unprepared in the face of further stressors (Koolhaas et al., 2010; Wong et al., 2019).

Varied temperature responses further extended to different personality-types for SRTs. Shy individuals followed a common pattern of habituation in control treatments where they became bolder upon repeated exposures to a stressor (e.g. Edwards et al., 2013; Houslay et al., 2019). When the temperature was changed, different personality-types exhibited different behavioural responses. Shy individuals became bolder by shortening their response-times to a greater degree than in control treatments, and the overall population-level trend to lengthen SRTs at higher temperatures may have been driven by intermediate individuals, which only exhibited significant plastic changes in experimental treatments. The stability of bold individuals in both treatments could indicate that they have less scope for plasticity in SRTs (e.g. Kareklas et al., 2016). Differences between personality-types in their degree and pattern of temperature-related SRT change could suggest varied molecular “coping styles”, where different personality-types exhibit different molecular and, in turn, behavioural responses to stress (Koolhaas et al., 2010; Wong et al., 2019). Although this phenomenon is well known in

vertebrates (e.g. Pusch et al., 2018; Thomson et al., 2011), the links between behavioural plasticity and molecular changes in invertebrates are poorly understood (Fürtbauer, 2015) and further work will be required to uncover the potential physiological basis of plasticity in SRTs. From an ecological perspective, these patterns of plasticity could come at a detriment to the survival of some groups, as they may be placed under increased metabolic stress whenever responding to threats at higher temperatures (Griffiths, 1977b; Sebens, 1981). Intermediate personality-types living lower down the shore may be particularly poorly equipped to mediate their SRTs under climate change-induced heat waves.

There has been increased call for conservation strategies to move beyond population-based approaches in the conservation of marine systems being exposed to environmental change (Brooker et al., 2016; Killen et al., 2016). These laboratory results indicate that more effective and targeted strategies might indeed be designed by considering finer-scale variation. Both of these behaviours are ecologically relevant and although SRT changes in relation to temperature may not be solely predicated on metabolic demand, elevated periods of tentacle retraction, especially at higher temperatures, still come at a metabolic cost (Griffiths, 1977b; Sebens, 1981). Both behaviours would thus be important when considering the survival of this species in the face of climate change-induced heat waves. When designing conservation strategies in heterogeneous environments, multi-faceted investigations of multiple behaviours (Carter & Feeney, 2012) are likely to provide indications of which individuals of a given species will be more vulnerable to acute, climate change-induced temperature shifts (See: Sih et al., 2012).

2.6. CONCLUSION

Acute, extreme temperature shifts associated with anthropogenic climate change could have significant effects on populations living in the intertidal zone. We show that morphotype, shore height, and individual level variation all affect behavioural responses to temperature change in *A. equina*. The highly complex relationship between behaviour and temperature in this species highlights the importance of incorporating multi-faceted behavioural approaches when designing strategies to predict the effects of anthropogenic climate change in these environments. A failure to target the most vulnerable groups or individuals in intertidal populations could lead to a loss of genetic diversity, leaving populations potentially more susceptible to future short-term and longer-term environmental perturbation.

2.7. ACKNOWLEDGEMENTS

Thank you to Leslie Connor for technical assistance. DKM was funded by a NERC ACCE PhD studentship (ref: 1950009) and Blue Planet Aquarium. I am grateful to Guillermo Garcia-Gomez, without whose help in the field, none of this work would have been possible. Further thanks to two reviewers and the associate editor for insightful comments on earlier versions of this chapter.

2.8. DATA AVAILABILITY STATEMENT

Data available from the Dryad digital repository: [*doi:10.5061/dryad.rn8pk0p6x] (Maskrey et al. 2020).*

APPENDIX TO CHAPTER 2: The impact of personality, morphotype and shore height on temperature-mediated behavioural responses in the beadlet anemone *Actinia equina*

A2.A. Sample sizes, housing and ethics

Actinia equina exhibits at least three morphotypes (Allcock et al., 1998), but only the low-shore morphotype is easily differentiated by eye from the two higher shore morphs (via pedal disc colour and limbus). The two higher shore morphotypes are only easily defined by genetic differentiation (Quicke et al., 1985) as they both display red pedal disc colouration (Quicke & Brace, 1984). In an attempt to mitigate for this, and to ensure as even a split as possible between the two red morphs, we collected 36 red individuals and 18 green individuals per block. We aimed to differentiate between these red morphs via holotrich nematocyst measurement (Collins et al., 2017), but variance in red nematocyst length meant this proved to be unfeasible. 7 *A. prasina* individuals were mis-identified as red morphs across both experimental blocks, leading total experimental red sample sizes to be 65. Figure A2.1 shows the housing set-up for anemones during each block and table A2.1 shows the sample sizes for each grouping factor during each data collection block.

This study did not require ethical approval since this species is not regulated under UK Home Office legislation. Across the four data collection blocks no animal mortality occurred and measured environmental parameters were consistently within normal tolerance ranges for this or similar species (Leal et al., 2012; Salt et al., 2013). All animals were returned to their collection site at Llandudno at the conclusion of testing, after tentacle samples had been collected under anaesthesia (2% $MgCl_2$) for a small subset of individuals. These animals are able to regenerate from high levels of damage, including self-inflicted damage to the epithelium (Palaoro et al., 2017) and lateral cutting (Turner et al., 2003), and multiple previous studies have taken samples of feeding and fighting (acrorhagi) tentacles with no adverse effects (e.g. Gashout & Ormond, 1979; Monteiro et al., 1997). Additionally, sample sizes were kept as low as possible without compromising the power of the study (see: Dingemanse & Dochtermann, 2013 for detailed power calculations with regard to individual level behavioural variation on which our sample sizes were based).

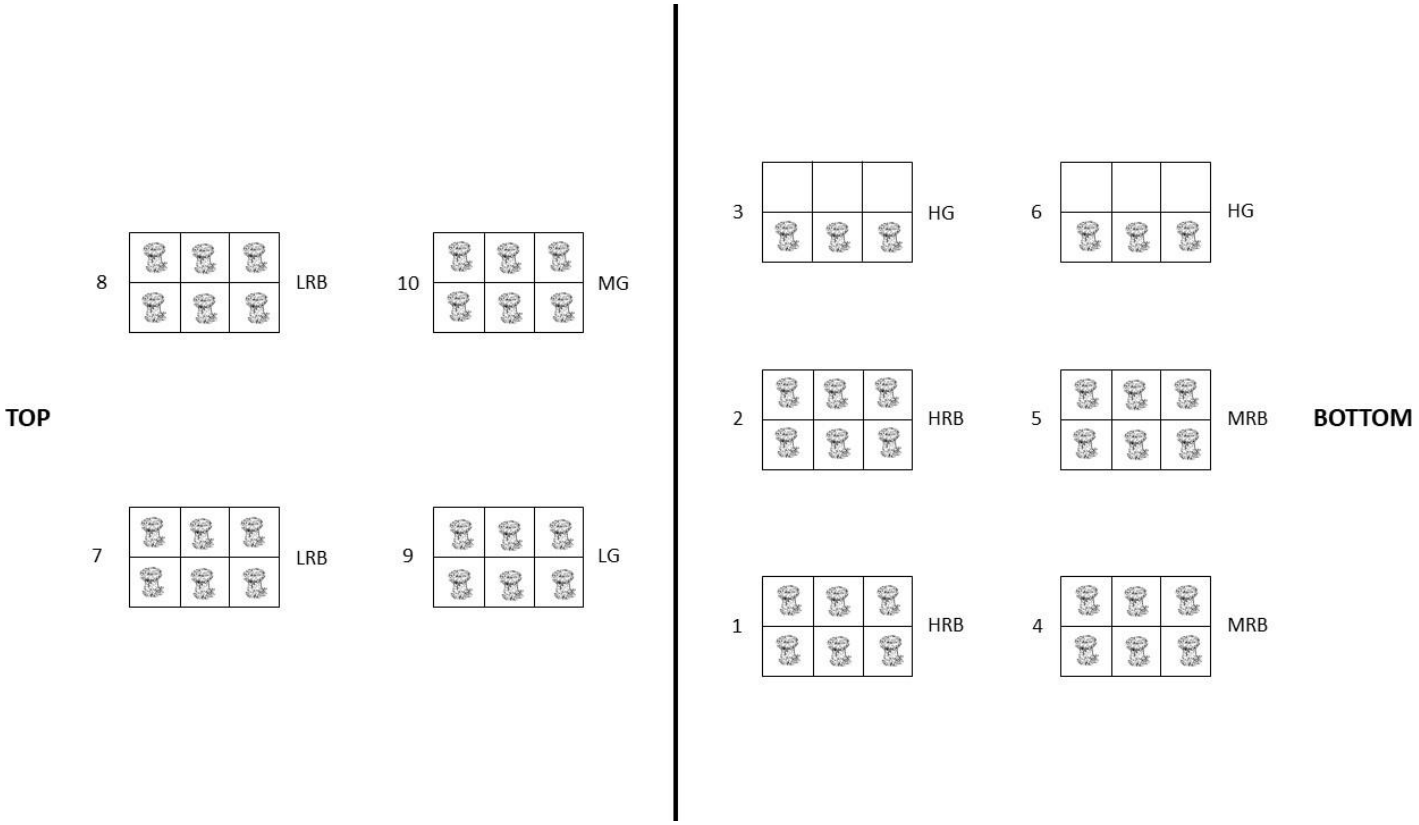


Figure A2.1. The within data collection block anemone housing set-up in the flow-through system. L, M and H denote low, mid and high-shore individuals respectively. G denotes the green morphotype, while RB denotes the red morphotype.

Table A2.1. The split of anemones between morphotypes and shore heights within a data collection block. L, M and H denote low, mid and high-shore individuals respectively. Green denotes the green morphotype, while red denotes the red morphotype.

	L	**M**	**H**
Red	12	12	12
Green	6	6	6

A2.B. Environmental control

Although average temperatures remained within ±1°C for all treatments (11°C ±0.67; 18°C ±0.29; 23°C ±0.64; Table A2.2), on two days during the 11°C portion of treatments temperatures exceeding 12°C were measured in the system. Similarly, on one occasion, the temperature reached 24.1°C during the first experimental treatment's 23°C period. Preliminary testing indicated that removal of these data did not significantly alter model fits, so they were retained for subsequent analysis.

Average ocean surface pH is currently 8.1-8.2 (±0.3 dependent on season and location; Raven et al., 2005), thus although our system experienced some small pH fluctuations, pH still fell within current normal levels.

Table A2.2. Full table of environmental measurements taken across the course of the study.

Date	**11-Apr**	**12-Apr**	**13-Apr**	**14-Apr**	**16-Apr**	**17-Apr**	**18-Apr**	**19-Apr**	**21-Apr**	**22-Apr**	**23-Apr**	**24-Apr**
Temp am		10.3	10.8	10.2		18.1	18	18.3		24	24	24
Temp pm	10.6	10.2	11	10.9	17.6	18.3	18.2	18	24	23.9	23.8	24.1
Salinity	33				33				33			
pH	8.1				8				8.1			
Nitrate	20				10				20			
Date	**27-May**	**28-May**	**29-May**	**30-May**	**01-Jun**	**02-Jun**	**03-Jun**	**04-Jun**	**06-Jun**	**07-Jun**	**08-Jun**	**09-Jun**
Temp am		11.5	11.8	11.8		12.2	11.8	11.8		11.7	11.8	11.9
Temp pm	11.4	11.9	12	12	11.7	12.7	12	11.9	11.8	12	11.9	12
Salinity	33				34.7				34.5			
pH	8				7.9				8.1			
Nitrate	5				10				10			
Date	**24-Jun**	**25-Jun**	**26-Jun**	**27-Jun**	**29-Jun**	**30-Jun**	**01-Jul**	**02-Jul**	**04-Jul**	**05-Jul**	**06-Jul**	**07-Jul**
Temp am		11.7	11.5	11.6		17.5	17.6	17.6		22.8	22.5	22.6
Temp pm	11.3	11.7	11.7	12.4	17.5	17.7	17.6	17.7	23	22.8	22.6	22.5
Salinity	33.24				34.6				34.5			
pH	8				8.2				8.2			
Nitrate	5				10				20			
Date	**24-Jul**	**25-Jul**	**26-Jul**	**27-Jul**	**29-Jul**	**30-Jul**	**31-Jul**	**01-Aug**	**03-Aug**	**04-Aug**	**05-Aug**	**06-Aug**
Temp am		11.2	11.3	11.5		11.4	11.2	11.3		11.7	11.6	11.6
Temp pm	11.5	11.3	11.4	11.9	11.6	11.7	11.4	11.5	11.3	12	11.7	11.8
Salinity	33.08				34.17				34.45			
pH	8.3				8.4				8.4			
Nitrate	5				10				10			

A2.C. Visualising the within-block schedule

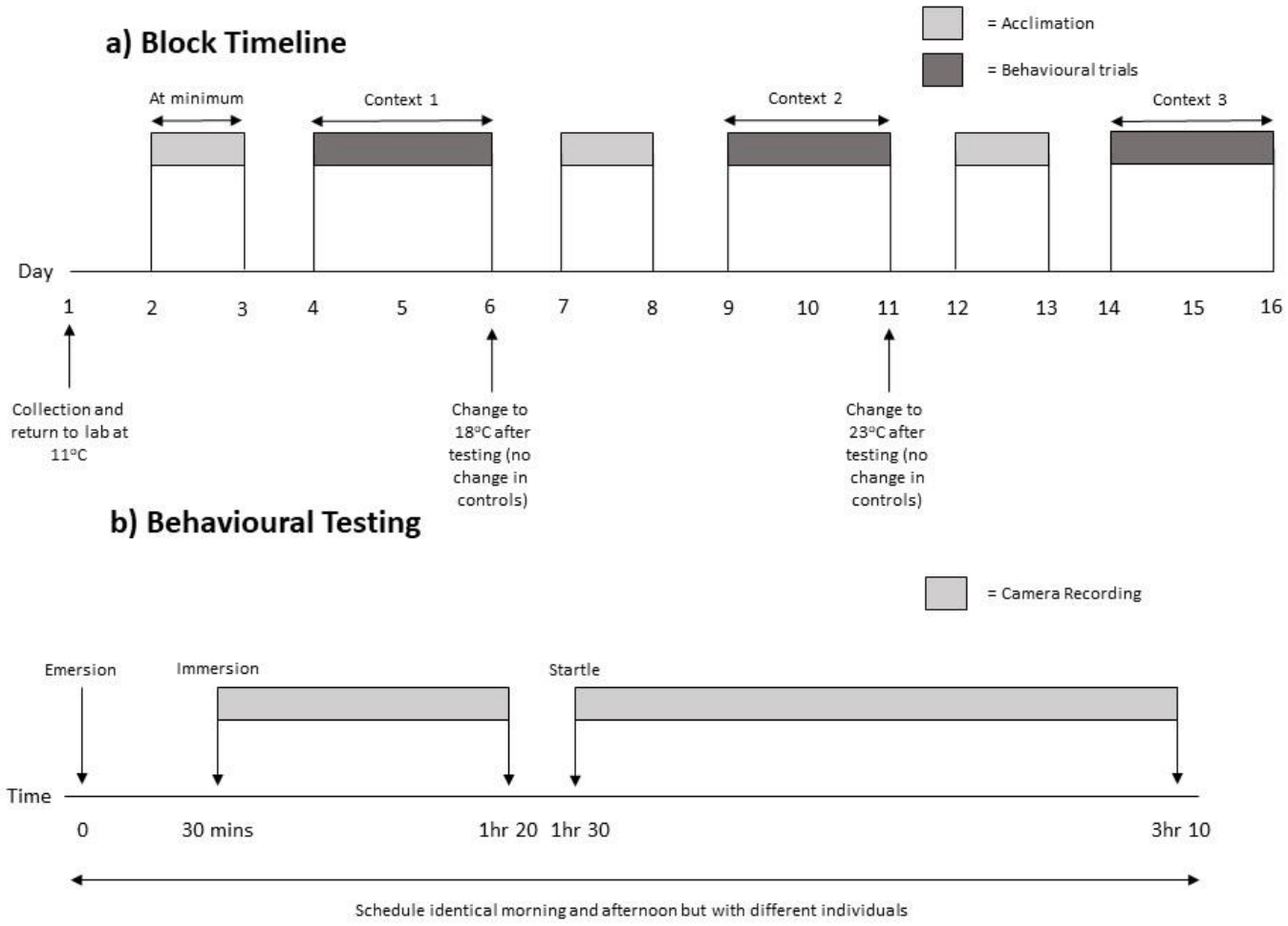


Figure A2.2. (a) A timeline of the experimental schedule for each experimental block. Below the timeline indicates where individuals were subject to environmental changes. Above the line indicates on which days individuals were acclimating to a new environment and on which days they were subject to behavioural testing; (b) The timeline of behavioural testing for a set of 27 individuals, showing when individuals were subject to immersion and startle response trials and timelapse video recordings. Emersion refers to the removal of water from tanks, and immersion refers to the re-introduction of water into tanks. Startle refers to eliciting fright responses by jetting 50 ml of tank water into anemones' oral discs using a syringe.

A2.D. The dual definition of SRT

To ensure that we were not inadvertently measuring two different responses by splitting our SRT definitions, we took a randomly selected experimental and a randomly selected control block (blocks 1 and 4) and re-analysed their raw photo data. We recorded whether an individual was fully extended or not before a startle response was elicited. Due to the relatively even split of 62% extended and 38% not fully extended, we did not deem it appropriate to analyse the two definitions separately. This would have led to large reductions in both between and within-individual sample sizes for the behaviours, which would have substantantially reduced the power of our main analyses to detect patterns in these data. We thus carried out two analyses to ensure that these definitions did not differ significantly from one another in the patterns they showed.

Firstly, we ran an identical model to the full SRT model presented in our paper but added initial extension as an additional fixed effect. This new variable was allowed to interact with temperature and also with treatment. Due to the smaller dataset, tank was fitted as a fixed, rather than random, effect so as not to overfit the model. No effect of initial extension was detected. To analyse a possible effect of data collection block, we then took the first 3 sampling occasions from each block and ran a further model with temperature removed, where starting extension was allowed to interact with block. Once again, no effect of initial extension was detected.

Secondly, we ran a short multivariate MCMC analysis on the same data (50000 iterations with a 5000 iteration burn-in and a thinning interval of 100) where response-times falling under each definition were both set as response variables. Morphotype, shore height, temperature and sampling occasion were specified as fixed effects, while individual was specified as a random effect. The residual variance-covariance matrix was constrained to 1. When model diagnostics had been checked, we extracted a correlation coefficient of 0.65 between the two definitions and associated credible intervals (0.46, 0.80). Although this coefficient is only moderately high, we deemed it acceptable for 3 reasons; a) the analyses in our paper show that time has significant effects on this behaviour and this analysis contains no time overlap within-individuals; b) a scatterplot of individual posterior modes (Figure A2.3) shows a clear positive relationship between the two measures; c) this correlation coefficient is higher than the across-context repeatabilities for SRT presented in our paper. We further calculated repeatabilities for both definitions from this model in the method of our

character-state analysis. While these were both much lower than those presented in our paper, due both to reductions in sample size at every level and to increased lengths of time between repeated measures for each definition, they were very similar for both definitions with highly overlapping credible intervals (Fully Extended: R= 0.27, 95% CI= 0.19, 0.36; Partially Extended: R= 0.26, 95% CI= 0.17, 0.36).

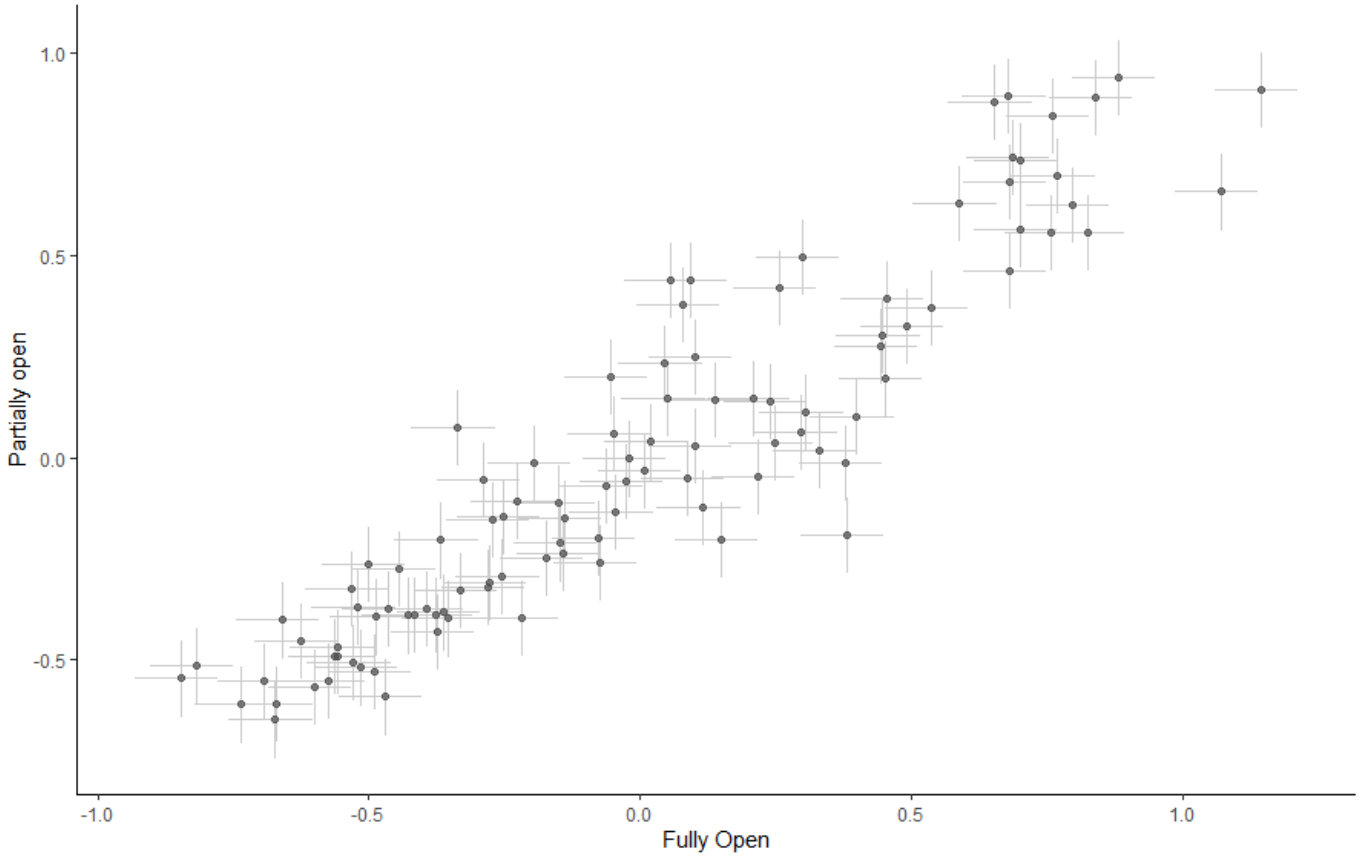


Figure A2.3. Associations between the posterior modes of each individual (Bayesian BLUPs) under each behavioural definition.

A2.E. Bayesian analytical detail

Univariate across-context repeatability estimation

Time of day, data collection block, and tank were all dropped as they had no effect on model DIC values and had been non-significant for both behaviours in the initial maximum-likelihood analysis. Once model assumptions had been checked we extracted adjusted repeatability estimates and associated 95% credible intervals (analogous to 95% confidence intervals from a REML analysis) for each behaviour from the G structure of each initial model's posterior distribution. As the variance of random effects is constrained to positive values in MCMCglmm (Letcher et al., 2011), repeatabilities were taken to be statistically significant where the distribution of posterior repeatability estimates was not pushed up against zero (determined from histograms of the variances) and 95% credible intervals did not come close to zero (e.g. Highcock & Carter, 2014).

Bivariate correlations between behaviours

In these models, because the two behaviours were each measured during different trials, residual (within-individual) covariation was not identifiable. Variance and covariance components of residual covariance matrices were thus fixed at 1. R_i and the associated 95% credible intervals were calculated from the between-individual covariance estimates from the posterior G structure of each model (Houslay & Wilson, 2017). Statistical significance was inferred where 95% credible intervals did not cross zero.

Comparison of character state models with random regression

Comparing partitioned random regressions with character state analyses found that DIC values were generally very similar between the two analyses, but that the character state approach fit experimental SRT data significantly better than the equivalent random regression (Character State DIC= 1807; Random regression DIC= 1866). Furthermore, inspection of individual level predictions from character state and equivalent random regression models found character state models to better fit the raw data (see: Houslay & Wilson, 2017).

Character-state calculations and significance of estimates

Within-context repeatabilities were estimated by calculating a mean from posterior repeatability estimates alongside associated 95% credible intervals. Significance of repeatability estimates were assessed by the same methods as above using 95% credible

intervals. To estimate across-context correlations, we extracted across-context covariance terms between the different response variables and converted these to correlation coefficients and their associated 95% credible intervals. As covariance terms are not constrained to positive values, correlation coefficients were deemed significant where 95% credible intervals did not cross zero (Hadfield, 2010). IxE was deemed to be present where 95% credible intervals of correlation coefficients did not include 1 (Houslay & Wilson, 2017).

Grouped analysis on the magnitude of behavioural change

In this analysis "bold" individuals were those whose mean predicted response-times fell below the first quartile of posterior estimates and "shy" individuals were those whose mean predicted response-times fell above the third quartile of posterior estimates. "Intermediate" individuals fell in between the two. 95% credible intervals for predicted values were extracted from each model alongside mean estimates, and changes were deemed to be significant where 95% credible intervals did not cross zero.

A2.F. Reaction norms with morphotype/shore height means

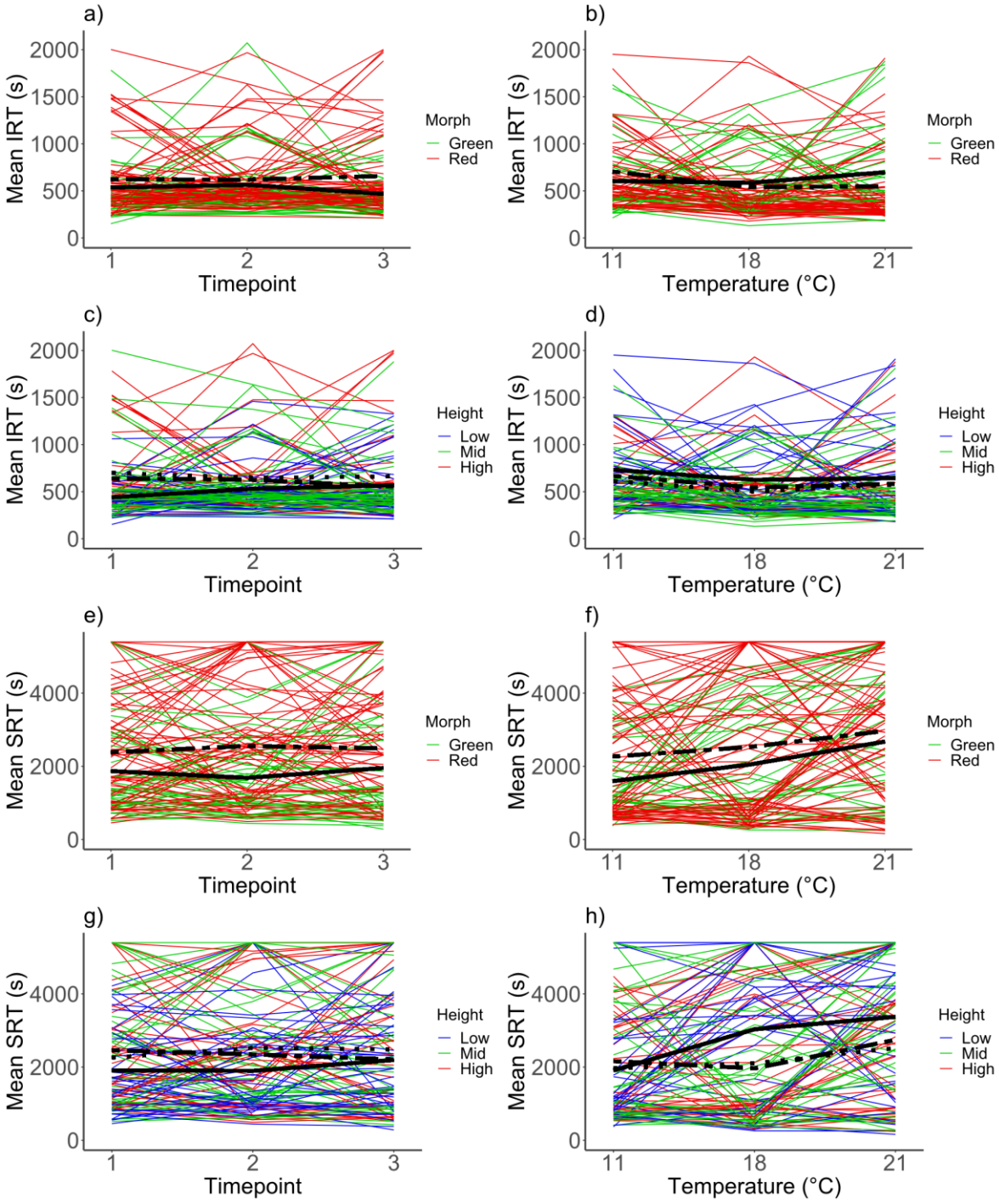


Figure A2.4. Plots of individual reaction norms using raw data means for each individual within each context (timepoint or temperature) for immersion response-time (IRT; panels a-d) and startle response-time (SRT; panels e-h). Panels a, b, e and f are coloured by morphotype while panels c, d, g and h are coloured by shore height. Panels a, c, e and g show control treatments, while panels b, d, f and h show experimental treatments. For panels a, b, e and f, the solid line indicates the mean for the green morphotype in each context, while the dashed line indicates the same for the red morphotype. For panels c, d, g and h the solid line indicates the mean for low-shore individuals in each context, the dashed line mid-shore individuals and the dotted line high-shore individuals.

A2.G. Alternative Visualisation of figure 2

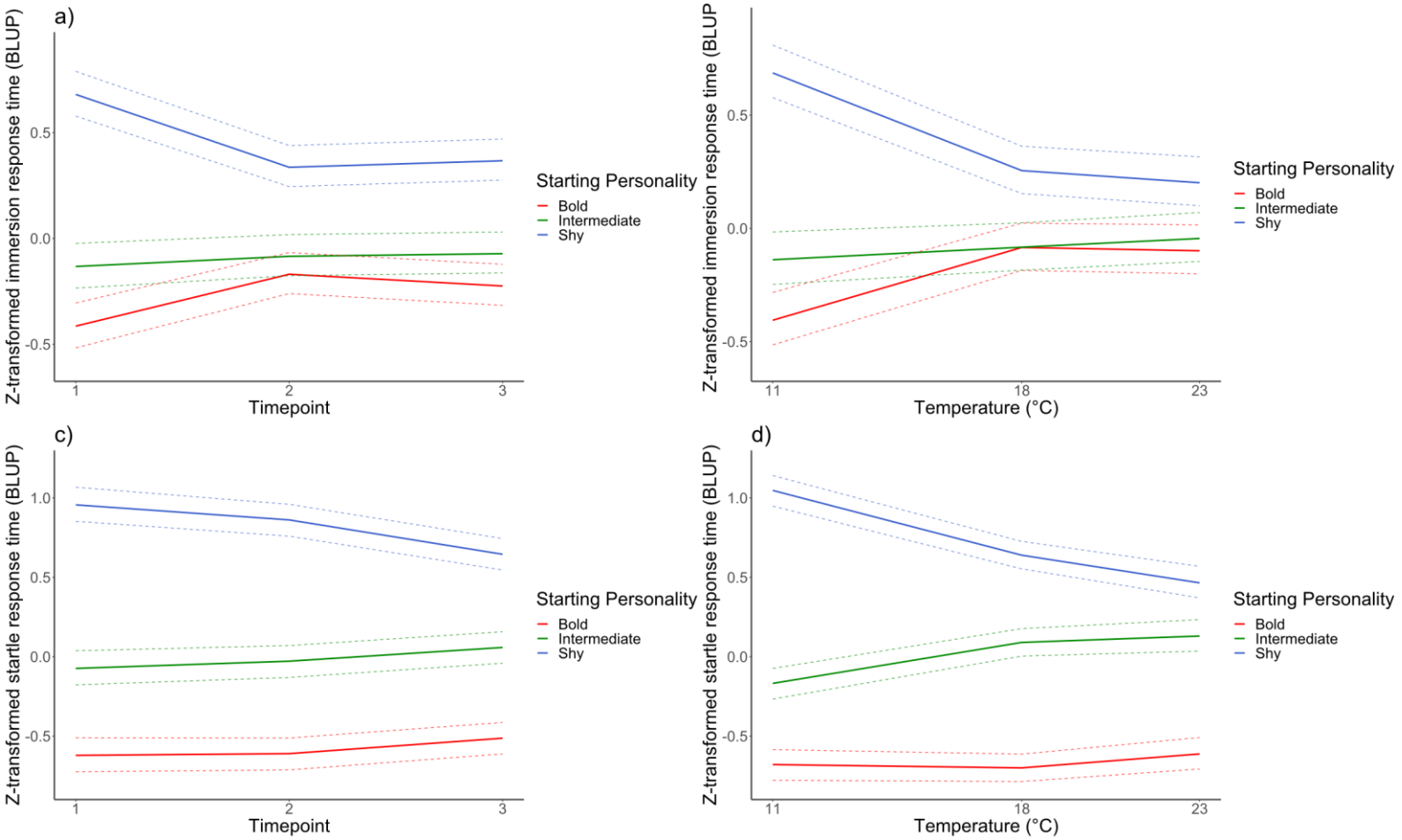


Figure A2.5. Visualising how the means of Bayesian BLUPs (the modes of predicted individual beadlet anemone response-times, extracted from the posterior distributions of Bayesian trivariate GLMMs), and their associated 95% credible intervals, changed across timepoints for 108 control individuals, or temperatures for 101 experimental individuals. Individuals with different personality-types at the first timepoint or temperature are indicated by separate colours. Showing a) Control startle response-times (SRTs); b) Experimental SRTs; c) Control immersion response-times (IRTs); d) Experimental IRTs.

Chapter 3:

Temperature-driven changes in behavioural unpredictability and personality in the beadlet sea anemone, *Actinia equina*

[Page intentionally left blank]

3.1. KEYWORDS

Behavioural plasticity, Boldness, Climate change, Marine invertebrate, Temperature fluctuation, Unpredictability

3.2. ABSTRACT

Unexplained behavioural inconsistency in individual animals, termed unpredictability, could account for more than 50% of variance in some behaviours. Unpredictability is likely to be selectively beneficial as a predator mitigation strategy and thus should be of adaptive value. Between-individual differences in behavioural unpredictability and how it changes across environmental contexts may thus have important consequences for selection, particularly in the face of extreme environmental changes. Associations between unpredictability and other risk-mitigating behavioural traits such as boldness could further influence individual fitness and population health. In this study, we investigated patterns of unpredictability in *Actinia equina* individuals at high and low temperature extremes. We investigated two boldness-related behaviours, immersion response (tentacle extension with submergence) and startle response (tentacle extension after a fright). We took bursts of six repeated measures of each behaviour, one at 6^{o}C and one at 21^{o}C in two crossed-over treatments, and two at 13^{o}C in a control treatment. Large sample sizes allowed us to use double-hierarchical linear mixed modelling to investigate between-individual variation in unpredictability and in the plasticity of unpredictability. Significant between-individual variation in unpredictability was present for both behaviours and was influenced by temperature. For the startle response, animals collected from less stochastic environments were more unpredictable at 21^{o}C than those from more stochastic environments. For the immersion response, animals were more unpredictable at 21^{o}C than at 6^{o}C; this difference was clearer in those individuals that started at the high, rather than the low, temperature. Unpredictability was further positively correlated with the mean level immersion response at both temperatures; intermediate and moderately shy individuals were more unpredictable than bold in both environments. Metabolic rate in *A. equina* increases as the temperature rises, so energetically taxing unpredictability, coupled with reductions in foraging associated with shyer behaviour, could prove selectively detrimental during heatwaves.

3.3. INTRODUCTION

It is now widely accepted that labile behavioural traits tend to differ consistently between individuals of the same species and these differences are often termed personality (Dall et al., 2004; Sih et al., 2004). When confronted with a challenge, such as a novel competitor or a change in their environment, personality traits can influence how different individuals alter their behaviour in a specific manner (Dingemanse et al., 2010; Stamps & Biro, 2016). Plastic behavioural change is in fact a key mechanism by which animals can mitigate the effects of stressors such as environmental fluctuation (Abram et al., 2017; Snell-Rood, 2013). Shifts in numerous environmental variables, including temperature (Briffa et al., 2013; Kjærsgaard et al., 2015), chemistry (White & Briffa, 2017) and oxygen levels (Norin et al., 2016), have been shown to have clear individual level behavioural effects. Differences between individuals in how their behaviour changes across environmental contexts could have important implications when addressing population and species level robustness to anthropogenic climate change (Beever et al., 2017; Sih et al., 2011).

Repeatable differences between individuals in the extent and nature of plastic changes in their behaviour across contexts and time are often termed differences in their reaction norms (Dingemanse et al., 2010). These differences can be linked to individual fitness under specific environmental scenarios (Tuomainen & Candolin, 2011; van Baaren & Candolin, 2018). Numerous studies across many taxa have now sought to investigate variation in environmentally driven reaction norms (reviewed in: Abram et al., 2017; Wong & Candolin, 2015). However, until recently, a key component of individual behavioural variation and, in turn, reaction norms, alongside mean level variation, has often been neglected. Residual intraindividual behavioural variation (rIIV; Cleasby et al., 2015; Westneat et al., 2015), hereafter termed unpredictability, is the short-term, reversible behavioural inconsistency shown within individuals across repeated behavioural measures (Stamps et al., 2012). Although the term ‘predictability’ is often used in the literature to describe rIIV (e.g. Briffa et al., 2013; Jolles et al., 2019), ‘unpredictability’ is a true representation of what rIIV measures. It requires no reference back to the acronym once it has been introduced, unlike ‘predictability’ which, being inverse to rIIV, tends to require regular clarification. The term ‘predictability’ is thus commonly used in unison with both the acronym and ‘unpredictability’ (e.g. Chang et al., 2017; Cleasby et al., 2015). Semantic inconsistency is an issue that regularly plagues behavioural ecology (Carter et al., 2013), and as such standardizing the term ‘unpredictability’ could be of value to the field to improve clarity. Given that the

average behavioural variance accounted for by differences in behavioural means, termed repeatability, is moderate (0.37–0.42; Bell et al., 2009), unpredictability may often account for a substantial proportion of between-individual behavioural differences (Stamps et al., 2012).

The maintenance of between-individual differences in unpredictability (Cleasby et al., 2015; Mitchell et al., 2016) is of adaptive interest (Westneat et al., 2015), and questions arise as to how it relates to an individual's overall behavioural strategy. One possibility is that maintaining the plastic scope of a behaviour necessitates reduced behavioural consistency (Dingemanse & Wolf, 2013), such that individuals with greater plastic scope exhibit greater unpredictability (e.g. Nakagawa et al., 2007). Another is that unpredictability itself is of adaptive significance (Prentice et al., 2020), possibly as part of a predator avoidance strategy (Briffa et al., 2013; Martin et al., 2017). This may prove adaptively beneficial for more risk-averse individuals, limiting the need for highly risk-averse mean level behaviours, which themselves can come at a detriment to other life history traits (Gasparini et al., 2019; Mathot et al., 2019).

If unpredictability is of adaptive importance, it should present as any other behavioural trait, showing its own patterns of consistency and plasticity, and potentially itself forming part of an adaptive behavioural syndrome (Briffa et al., 2013; Prentice et al., 2020). It is thus important in studies of plasticity to consider levels of unpredictability and how they might covary with mean level behaviour (Mitchell et al., 2016). Studies are increasingly addressing this by incorporating both mean and residual level behavioural variation, with recent examples including findings that anthropogenic contaminants can maladaptively influence unpredictability in hermit crabs, *Pagurus bernhardus* (Nanninga et al., 2020) and indications that differing degrees of unpredictability may help explain sex-specific differences in laterality in guppies, *Poecilia reticulata* (McLean & Morrell, 2020). Comparatively few studies, however, have investigated variation between individuals in how their unpredictability changes across environments (plasticity of unpredictability), and how this associates with possible covariation between unpredictability and mean level behaviour under environmental change (but see: e.g. Mitchell & Biro, 2017; Nakayama et al., 2016). Understanding how unpredictability plastically changes under environmental shifts will enable us to interpret how it could influence overall fitness and survival in a changing climate.

In ectotherms, temperature is directly related to metabolic demand (Abram et al., 2017; Killen et al., 2013). Risk-averse (shy) behaviours, such as increased latency to emerge after a fright, should become increasingly metabolically detrimental to an ectothermic animal as temperatures rise (Mathot et al., 2019), with animals trading off foraging time against risk aversion. Thus, if greater unpredictability in risky behaviour can be utilized as an alternative method of predator mitigation (e.g. Chang et al., 2017; Richardson et al., 2018), its adaptive value to an ectotherm should be inherently linked with environmental temperature. The frequency of weather events leading to drastic short-term temperature changes, such as heatwaves and cold snaps, is predicted to increase under anthropogenic climate change (IPCC, 2013). Between-individual differences in the unpredictability of ecologically relevant behaviours, how this unpredictability changes under extreme thermal contexts and how it relates to mean level effects could thus have important implications for the future survival of ectothermic organisms that are dependent upon environmental temperature, particularly if this is outside their tolerance range.

The beadlet anemone, *Actinia equina*, is an ideal study organism to investigate the relationship between individual behavioural unpredictability and temperature as it has particularly high thermal tolerances (although these are greater at high than low temperatures; Griffiths, 1977a) and can be exposed to extreme temperatures with minimal risk of mortality (Chapter 2). Further, temperature shifts in *A. equina* drive changes in metabolic rate (Chomsky et al., 2004b), proteomic expression (Choresh et al., 2001) and boldness-related behaviours (Chapter 2). Boldness in *A. equina* is measured via latency to return to normal tentacle extension after the presentation of different stimuli (e.g. Briffa & Greenaway, 2011; Chapter 2). Tentacle extension is inherently linked to both foraging efficiency and general metabolic processes (metabolic rate peaks when tentacles are at maximum extension; Griffiths, 1977b) in this species, so these behaviours are likely to represent a trade-off between risk mitigation and metabolic efficiency (Chapter 2). *Actinia equina* also lives across a gradient of intertidal shore heights and exhibits visually distinct morphotypes (Brace & Reynolds, 1989) which are known to differ in these behaviours (Collins et al., 2017). Morphotypes are themselves associated with different heights on the shore (Brace & Reynolds, 1989), which are characterized in part by the very different thermal patterns that they exhibit (Brahim et al., 2019). Single populations of *A. equina* can thus encompass several different thermal optima and a large degree of variation in plastic strategies to deal with thermal changes (Chomsky et al., 2004b; Navarro et al., 1981). These anemones further

show population level variation in unpredictability, with high levels of unpredictability potentially being associated with high-risk environments (Osborn & Briffa, 2017).

In the present study we aimed to understand how between-individual variation in the unpredictability of *A. equina* might influence responses to heatwaves or cold snaps. We investigated differences in unpredictability at morphotype, shore height and between-individual levels for two boldness-related behaviours, startle response-time (SRT) and immersion response-time (IRT). We sampled both between (2592 behavioural measures across 216 individuals) and within (12 repeated measures across two six-measure bursts) individuals at two temperature extremes, allowing us to use double-hierarchical linear mixed models to partition mean and residual level behavioural variation across and within those extremes. We were able to investigate between-individual differences in unpredictability and whether these differences changed in a nonuniform way across temperatures, indicating between-individual variation in the plasticity of unpredictability. We further determined whether unpredictability was plastically changing independently of mean level behaviour, and thus how it might fit into anemones' overall behavioural syndromes. To do this, we calculated correlations between unpredictability and mean level personality-type and quantified how these were affected by different temperature extremes.

3.4. METHODS

3.4.1. Collection and laboratory set-up

Data collection was carried out between December 2018 and February 2019 across four 10-day data collection blocks. During each block we collected 54 anemones from the rocky shore of Llandudno, North Wales (lat: 53.330359, long: -3.828975). Within each data collection block, each treatment combination (shore height*morphotype) had a sample size of nine. Collection of anemones, identification of red (high-shore-associated) and green (low-shore-associated) morphotypes and transfer to the University of Liverpool was carried out using well-established protocols for this population (Collins et al., 2017; Chapter 2). Total sample size was 216, split evenly across the four collection blocks.

For the duration of their time in the laboratory, anemones were housed in separate, 15 cm tall clear plastic cups, ca. 7 cm wide at their base, each containing >10 small drainage holes and a single pebble to which the anemone could bind. The pedal disc (underside) width of anemones used in this study ranged between 1.4 cm and 3.4 cm. All anemones had space for

movement in their cups and were able to fully extend their feeding tentacles during experiments. Cups were positioned within one of three tanks (80 cm x 45 cm x 40 cm), filled to a depth of ca. 12 cm with artificial sea water (RO water and Tropic Marin, Wartenberg, Germany, Pro Reef Salt) and situated in a 13ºC (±1ºC) temperature-controlled room. Each tank contained 18 anemones, split evenly such that they each contained three anemones of each morphotype from each shore height. Within tanks, anemones were housed in three clusters of six cups and could not physically interact with one another. These tanks were, in turn, situated in one of three larger flow-through systems being maintained at three different temperatures. Two experimental systems were maintained at 6ºC (±1ºC) and 21ºC (±1ºC), and this was switched halfway through behavioural testing in a crossed-over temperature change design accounting for treatment order (Briffa et al., 2013; Mitchell & Biro, 2017). To allow the direct comparison of temperature effects with temporal effects alone, a separate group of anemones remained at 13ºC (±1ºC) for each full 10-day block. The temperature of each system was randomized between blocks, with each system running each treatment at least once. To avoid any systematic effects, the tank used in a system was randomly selected for each block, with each tank used at least once.

Both experimental temperatures fall outside the average range of sea temperatures for Llandudno (7.5ºC–16.25ºC; www.seatemperature.org), so were deemed appropriate to simulate the temperature extremes that might be brought about by severe weather events. *Actinia equina* has been shown to be less physiologically tolerant to cold than hot temperature extremes (Griffiths, 1977a), so 6ºC, a temperature at the very lower bound of the normal range of extremes this population might experience (www.seatemperature.org), was chosen to reflect this. The other extreme, 21ºC, is nearing the thermal maximum for this population (Chapter 2). It is well above the normal high sea temperature extremes experienced by Lllandudno (17.3ºC), but it is possible that isolated tide pools could reach this temperature during a heatwave. We chose 13ºC as a 'baseline' control temperature as it is well within the normal range of temperatures for Llandudno and a rough midpoint between the two temperature extremes (www.seatemperature.org).

Once all anemones had been placed in their cups, they were given 60 h to acclimate prior to experimentation. Systems were maintained at a salinity of 33–35 ppt and a pH of 8.2 (±0.3) with regular water quality measurements of temperature, salinity, pH, ammonia (< 0.1 mg/litre), nitrite (< 0.25 mg/litre) and nitrate levels (< 20 mg/litre). We carried out 20% water changes once per week and full water changes at the conclusion of each data collection block.

Light was provided for individual tanks using Arcadia Classica T8 60 cm LED lights (Ely, U.K.) and the laboratory was kept on a 12:12 h (0830–2030) day:night cycle throughout the data collection period. Anemones were fed ad libitum on brine shrimp (Monkfield Nutrition, Ely, U.K.) 48 h into their 60 h acclimation period (as per Chapter 2).

3.4.2. Behavioural testing and morphological measurement

We carried out behavioural testing over the course of an 8-day period after the initial 60 h acclimation (for a full experimental timeline, a visualization of our crossed-over temperature schedule and a schematic of our set-up, see Appendix A3.A.). Every individual had its immersion response-time (IRT) and startle response-time (SRT) tested twice daily, once in the morning and once in the afternoon, for the first 3 days of this period. IRT was measured first on all occasions, always followed by a 10 min interval before we measured SRT. For IRT, the order of individual behavioural trials on each occasion was randomized between cup clusters. For SRT, the order was further randomized at the individual level within clusters. Both behaviours involved measuring individual latency to extend feeding tentacles after a stimulus. For IRT measurement each anemone was emersed by removing its cup from the tank and allowing water to drain via the drainage holes, leading to tentacle retraction, before being reimmersed after 30 min. IRT was defined as when an individual had fully re-extended its feeding tentacles (as per Chapter 2). SRT was measured after first discharging 50 ml of water at an anemone's oral disc (the mouth and the smooth tissue surrounding it; Appendix A3.B), causing it to retract its tentacles. SRT was defined as when an individual's feeding tentacles had re-extended such that they surpassed 75% of the anemone's collar (contractile tissue at the top of the body; Appendix A3.B) and only 25% of the collar was still visible, or as when an anemone returned to its pre-startle degree of extension if it began with less than 75% of its feeding tentacles extended beyond the collar. These measures are highly correlated and taken together strongly define the range of individual SRTs (Chapter 2).

Behavioural response-times were recorded using seven Crosstour 4k action cameras and two GoPro Hero 4 action cameras, which were mounted above tanks. Each camera was able to record the responses of six individuals simultaneously. Using timelapse photographs captured at 30 s intervals, we determined IRT from 45 min of footage, beyond which response-times were given a maximal value of 2700 s (Chapter 2). For logistical purposes, the upper bound of SRT was lowered from 5400 s (Chapter 2) to 4800 s. Preliminary analyses of data from Chapter 2 found that only 0.6% of SRT measurements fell between these values, so this

change was deemed appropriate. The temperature in tanks was recorded twice daily on days when behavioural testing took place, during both morning and afternoon testing in between the measurement of IRTs and the measurement of SRTs. Although anemone morphotypes differ in coloration, this was not clearly visible in photos, so the observer was fully blinded to anemone type when analysing them. D.K.M. carried out all behavioural testing and data extraction to avoid interobserver effects.

At the conclusion of 3 days of testing, thermal contexts were reversed within the two experimental systems overnight at a rate of 1^{o}C/h, to account for the influence of treatment order (as per Briffa et al., 2013). The temperature in the control system remained at 13^{o}C. Individuals were given a further 60 h to acclimate to their new thermal contexts and again fed ad libitum after 48 h. All three treatments were then subject to another 3 days of behavioural testing, providing up to 12 observations overall for each behaviour per individual.

For IRT anemones were occasionally not fully visible on recordings, and for SRT some individuals either did not have their tentacles extended or were detached from the substrate at the commencement of a given trial. Of 2592 possible measurements, 25, each from separate individuals and occurring no more than once per individual, were not quantifiable for IRT. For SRT, 447 of 2592 measurements, occurring across 164 individuals (and thus occurring more than once for some) were not quantifiable. Anemones were fed ad libitum on brine shrimp (Monkfield Nutrition, Ely, U.K.) each evening prior to behavioural testing, to maintain energy availability across all behavioural trials. This meant that anemones were fed on six occasions in total during each data collection block. After all testing had concluded, anemones were returned to their collection site.

3.4.3. Statistical analysis

Our aim was to quantify between-individual differences in the mean and residual variance components of each behaviour and how these covaried with one another. We wanted to investigate this at the level of both behavioural intercepts (repeatability) and behavioural slopes across timepoints and temperatures (plasticity). We utilized adapted versions of the methods detailed in full by Mitchell et al. (2016), fitted in a Bayesian Markov chain Monte Carlo framework with a double-hierarchical structure and a covariance matrix between mean and residual individual level effects.

All analyses were carried out using R version 3.6.2 (R Core Team, 2020), using the analytical software JAGS with the rjags overlay (Plummer, 2014). For modelling purposes data for both behaviours were split into control and experimental datasets, allowing for the direct comparison of model outputs.

3.4.4. Model set-up

All models were run for 400 000 iterations across three parallel chains after a 10 000 iteration burn-in period, with a thinning interval of 100. Fixed-effect parameters on the mean and the residuals were assigned inverse-Wishart priors, while random effect parameters were given uniform priors. Alongside visual inspection of posterior and autocorrelation plots, Heidelberger–Welch, Gelman–Rubin and autocorrelation diagnostic tests were carried out on all models to ensure successful convergence before any results were extracted. The posterior distributions of each sampling node were further inspected post hoc to ensure they met the assumptions of normality.

To partition the mean and residual variance components of SRT and IRT and investigate the covariance between these two levels of behavioural variation, we initially specified one double-hierarchical model per dataset. SRT or IRT (z-transformed) were set as the response variable in the mean level of the model. In control mean models morphotype, shore height, sampling occasion, data collection block and temperature (z-transformed) were fixed effects. Morphotype, shore height and sampling occasion were initially allowed to interact. Tank and individual ID were included as random effects as was a nested random effect of ID*timepoint, where response-times were partitioned into two bursts of six repeated measures. This was our random slope, which accounted for plasticity by addressing temperature or timepoint-driven individual*environment (IxE) effects on mean level behavioural variation. Hereafter, where mentioned together, temperatures and timepoints are referred to as contexts. Experimental mean models differed in that treatment order was also included as a fixed effect and that temperature (z-transformed), rather than sampling occasion, interacted with morphotype and shore height. The nested random effect in experimental models was ID*temperature, where response-times were partitioned into two bursts of six repeated measures based on temperature, rather than timepoint. All fixed effects were retained in mean models regardless of significance but clearly nonsignificant interaction effects, determined by whether the 95% credible intervals, CI, of their posterior estimates

substantially crossed zero, were removed to help avoid overparameterization (Spiegelhalter et al., 1998) in residual models.

At the residual level, for control models, morphotype, shore height and sampling occasion were specified as fixed effects, and these were initially allowed to interact in a series of two-way interaction terms. Experimental models differed in that temperature replaced sampling occasion and treatment order was also specified as a fixed effect, which was initially allowed to interact with temperature. All nonsignificant interaction terms were removed from final residual level models. Individual ID was specified as a random effect on the residuals in both models, and this was allowed to covary with the random effect of individual ID on the mean. Fitting both a random intercept and a random slope (i.e. ID*timepoint, the IxE effect) at the residual level caused models to overfit and led to failed convergence. However, our IRT dataset allowed for the specification of further models for our experimental and control datasets, respectively, where we specified covariance matrices between mean and residual ID effects at the within-context level, while retaining ID alone as a random effect on the mean (hereafter slopes models). This allowed for comparison of within- and across-context estimates of individual unpredictability via the examination of 95% CI. The fit of each of the model specifications was also compared through comparison of DIC values (deviance information criterion, analogous to Akaike information criterion in REML analysis: Spiegelhalter et al., 2002). Owing to the large computational load of retaining deviance estimates for each chain, DICs were calculated from further 200 000 iteration runs of the same models, where saved outputs contained only intercept and deviance samplers. There was no difference in the explanatory power of the two model specifications for the control IRT treatment, such that their DICs did not differ (intercept covariance DIC= 2059; slope covariance DIC= 2061). In contrast, DIC values were significantly higher in experimental models when intercepts covaried, as opposed to slopes (intercept DIC= 4091; slope DIC= 4019). This indicated that the experimental slopes model was more explanatory of our dataset. Estimates from this model are therefore presented here. To allow for reliable comparison, so too are estimates from the control IRT slopes model. For the purposes of comparing across-context and within-context unpredictability and the associated correlations with mean level IRTs, slopes model estimates are contrasted with equivalent estimates from intercept models.

Our SRT dataset did not allow for the same approach to investigating the plasticity of unpredictability due to high numbers of individuals exhibiting little to no variance at the

within-context level. We therefore only present across-context estimates of individual unpredictability for this behaviour and do not directly investigate patterns of individual behavioural plasticity in the unpredictability of SRTs across contexts. Eleven individuals exhibiting no SRT variance across all repeated measures were excluded from all SRT analyses. See Appendix A3.C. for a full discussion of the decision to retain maximal SRT values.

3.4.5. Extraction of effect estimates and individual level coefficients

To investigate how extreme temperatures affected each behaviour and whether these effects indicated temperature-driven IxE at both the mean and residual levels, we extracted effect estimates for fixed and random parameters from each model, alongside associated 95% CI.

At the mean level, we calculated across-context correlations between behavioural intercepts (R_{int}) and compared these between treatments. We further calculated the repeatability of behaviour across all 12 repeated measures, not accounting for context-related change (unconditional repeatability; R_u). This would be expected to be significantly reduced in experimental treatments as compared with controls in the presence of temperature-driven IxE. To investigate repeatability within different contexts and whether it was retained to a greater degree than across contexts, indicating between-individual differences in reaction norms, we calculated the conditional repeatability of each behaviour (i.e. accounting for context-related change; R_c). To investigate whether the magnitude of temperature-induced plasticity was greater than that elicited by temporal change alone, we compared all these estimates between treatments.

To investigate sample level unpredictability, and whether this differed between treatments, we extracted overall residual variance estimates from the residual level of each model. Then, to quantify between-individual variation in unpredictability, and whether its explanatory power was reduced across temperatures as compared with across timepoints, across-context CV_p (the coefficient of variation in predictability; Cleasby et al., 2015) values were calculated. For IRT these extended to within-context CV_p estimates from our slopes models, to similarly investigate between-individual differences in the slopes of unpredictability under extreme temperature change. To investigate whether mean level behaviour covaried with unpredictability and whether this covariance was impacted by extreme temperatures, correlations between individual mean level intercepts and estimates of individual unpredictability were extracted. To further investigate how mean and residual level

temperature-related IxE affected these correlations, estimates were extended for IRT by our slopes models. Full methods for extracting all of the above coefficients are available in Mitchell et al. (2016).

Significant correlations between mean level variation and unpredictability could have been relics of the bounded distribution of our data. To account for this, we calculated mean response-times in each treatment for both behaviours, and context-specific means for our further IRT analysis. The closer to the midpoint of possible distributions of each response-time these values were (i.e. 1350 s for IRT and 2400 s for SRT), the less likely that correlations between mean level individual variation and unpredictability were relics of our sampling distribution. A mean close to the midpoint would have allowed posterior estimates to have varied equally in both directions, and not biased unpredictability estimates in favour of bolder or shyer individuals.

3.4.6. Estimation of individual level unpredictability across and within contexts

To produce individual level point estimates of unpredictability allowing for informative plotting and comparisons, we ran simplified individual level linear mixed models (LMMs) to extract estimates of residual individual standard deviation (riSD; Jolles et al., 2019; Stamps et al., 2012). SRT or IRT were response variables and sampling occasion was the fixed effect. We estimated individual across-context unpredictability by extracting riSD across sampling occasions for both behaviours. For IRT we also extracted riSD within each context, to provide context-specific estimates of unpredictability for this behaviour, which could be related to our slopes models.

3.4.7. Ethical Note

Sea anemones are not covered by U.K. legislation, so no project licence was required for this work. The project was nevertheless approved by the ethical committee at Blue Planet Aquarium. The temperatures used in this study are within the low (www.seatemperature.org) and high (Chapter 2) tolerance limits of this species. No animals perished during experimentation, and upon the conclusion of each data collection block all animals were returned to the same rocky shore where they had been collected.

3.5. RESULTS

3.5.1. Mean level effects

At the mean level, IRT showed a strong temperature-driven IxE effect, as between-individual behavioural consistency was completely lost across, but retained within, temperatures. Across-context correlations between behavioural intercepts and unconditional repeatability estimates were both negligible and nonsignificant in experimental models (Figure 3.1a). This contrasted with controls, where both estimates were significant (Figure 3.1a). Conditional repeatability, meanwhile, was significant and similar in both treatments (Figure 3.1a).

For SRT, too, a clear mean level temperature-driven IxE effect was present, but coefficient estimates suggest that it may have been relatively weak. Estimates of across-context correlations in mean level individual behavioural intercepts were markedly lower in experimental than control treatments (Figure 3.1b), indicating significantly larger changes in individual SRTs across temperatures than were accounted for by time alone. Individuals showed moderate unconditional and conditional repeatability in both models (Figure 3.1b). These estimates did not differ significantly between treatments, in contrast to the clear IRT results. Estimates of the two types of repeatability did differ significantly within the experimental treatment, however, again indicating some effect of temperature.

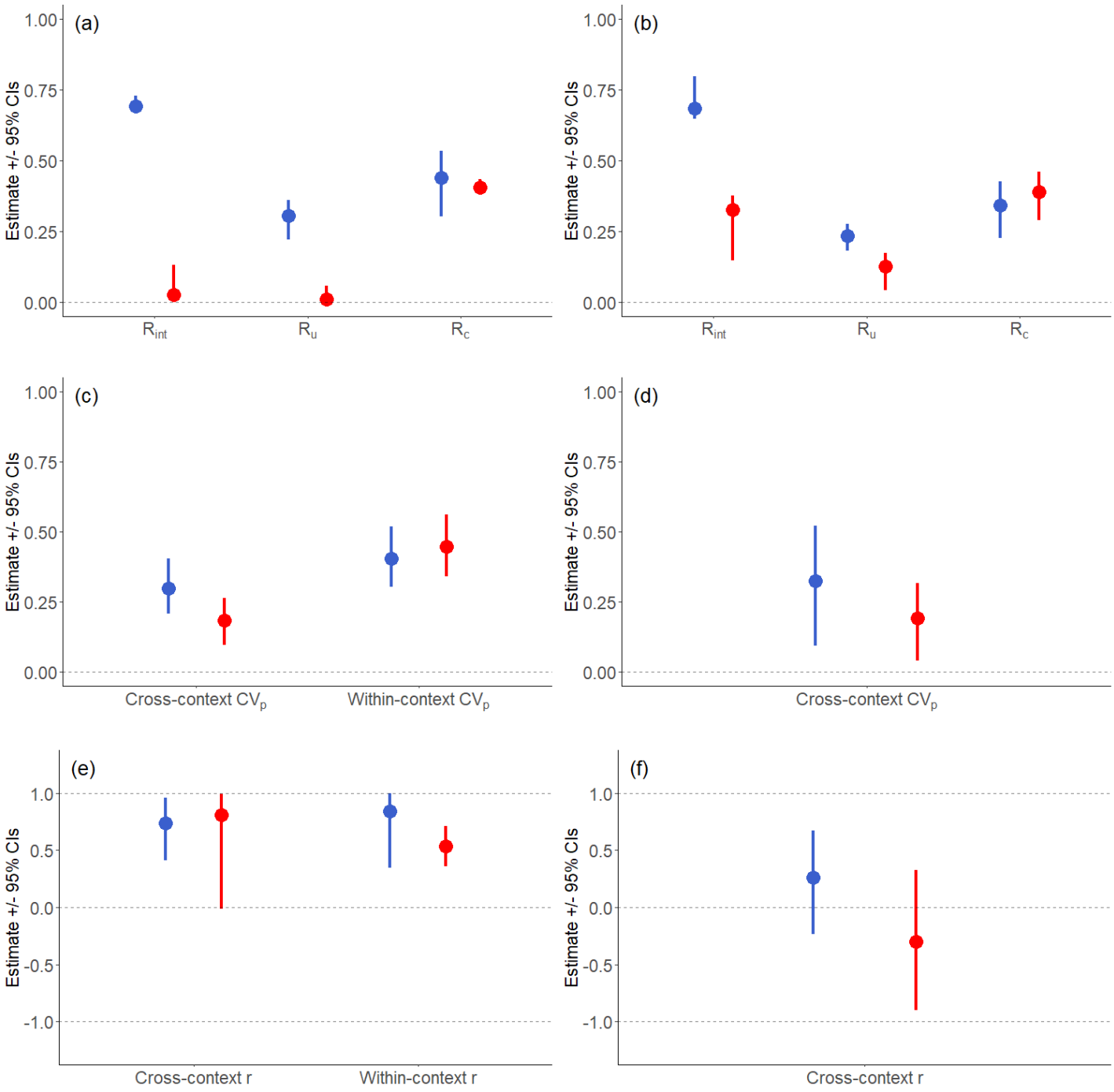


Figure 3.1. Between-individual behavioural estimates (±95% credible intervals, CI) from double-hierarchical GLMMs in the sea anemone, *Actinia equina*, showing: (a) the correlation between mean level individual behavioural intercepts across contexts (R_{int}), unconditional repeatability (R_u) and conditional repeatability (R_c) for immersion response-time (IRT); (b) R_{int}, R_u and R_c for startle response-time (SRT); (c) the coefficient of between-individual variation in unpredictability (CV_p) across and within contexts for IRT; (d) CV_p across contexts for SRT; (e) estimates of the correlations between individual mean response-time estimates and unpredictability estimates across and within contexts for IRT; (f) estimates of correlations between individual mean response-time estimates and unpredictability estimates across contexts for SRT. Dashed lines on panels (a)-(d) denote zero. Dashed lines on panels (e)-(f), from low to high, denote -1, 0, and 1.

3.5.2. Sample level unpredictability

Overall sample level unpredictability was moderate in both IRT models and was unaffected by temperature (control: residual variance= 0.35, 95% CI= 0.27, 0.45; experimental: residual variance= 0.38, 95% CI= 0.30, 0.47). In control models, the red morphotype showed more unpredictable IRTs than the green (estimate= 0.34, 95% CI= 0.17, 0.51) and individuals were slightly more unpredictable at later sampling occasions (estimate= 0.03, 95% CI= 0.01, 0.05). Shore height also fell on the bound of significance in the control model (estimate=0.11, 95% CI= 0.01, 0.22), indicating that individuals from lower down the shore may have been more unpredictable in their IRTs. None of these effects were significant in the experimental IRT model. Instead, temperature was the key explanatory variable; experimental individuals were more unpredictable in their IRTs at 21ºC (estimate= 0.13, 95% CI= 0.07, 0.20) than at 13ºC, and when they were first tested at 6ºC (cold–hot) rather than 21ºC (hot–cold; estimate= 0.21, 95% CI= 0.08, 0.34). Figure 3.2 shows this effect clearly, and further indicates that while population level unpredictability was significantly higher at 21ºC than at 6ºC under both treatment orders, the scale of this difference was much greater under the hot–cold treatment order than vice versa, driving the effect of treatment order shown in the model.

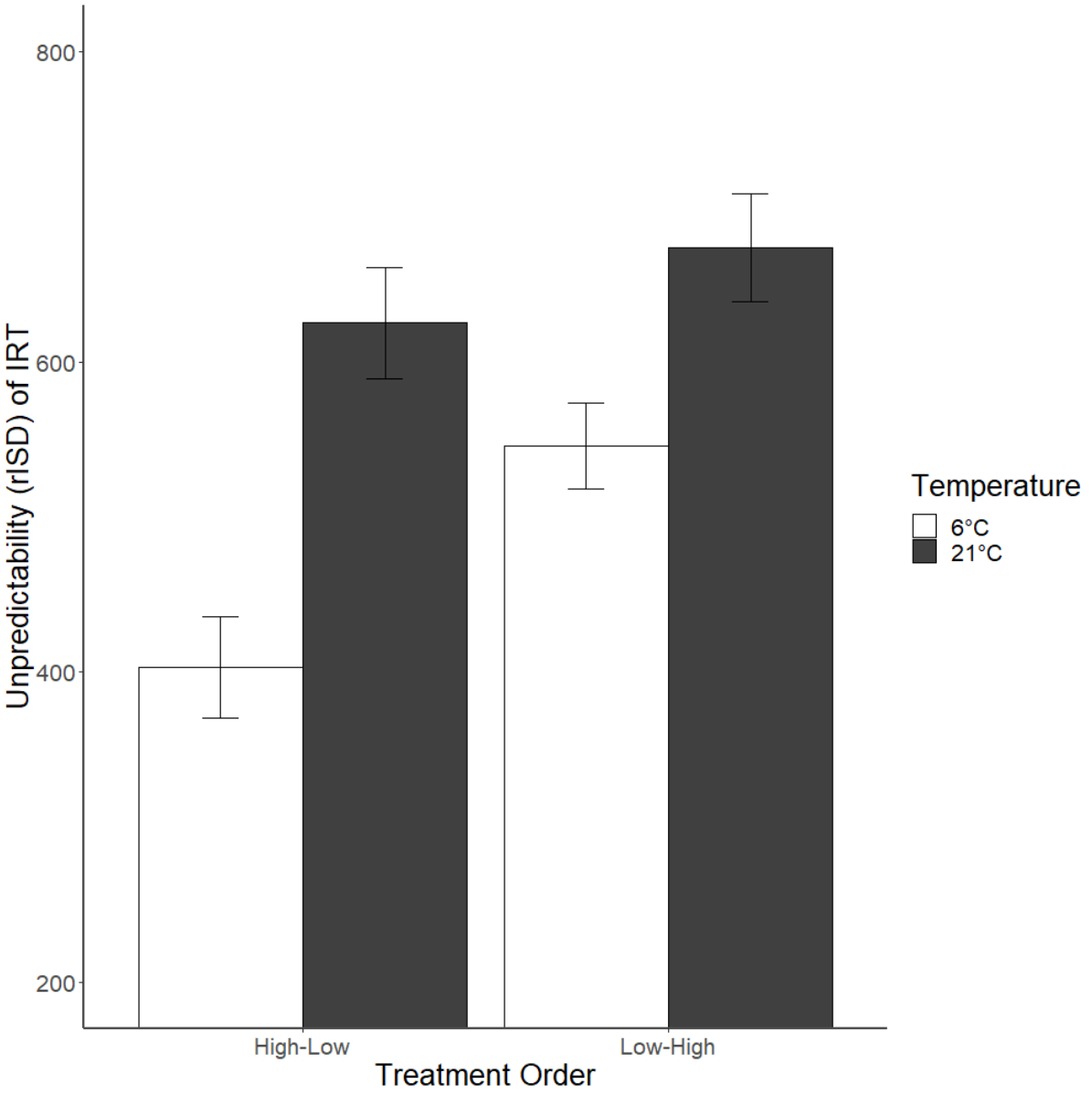


Figure 3.2. Population mean estimates (±SE) of the unpredictability of immersion response-time (IRT) under different treatment orders (high temperature then low temperature or vice versa) at different temperatures in the sea anemone, *Actinia equina*. Unpredictability is here measured as residual individual standard deviation (rISD), derived from simple individual level LMMs.

Sample level unpredictability in SRTs was also unaffected by extreme temperatures (control: residual variance= 0.60, 95% CI= 0.44, 0.79; experimental: residual variance= 0.57, 95% CI= 0.47, 0.69). In the experimental model, temperature interacted with shore height to affect residual variance, such that, at higher temperatures, individuals from lower down the shore were more unpredictable in their SRTs than those from higher up the shore (estimate= 0.07, 95% CI= 0.02, 0.13).

3.5.3. Between-individual variation in unpredictability

Between-individual variation was evident in the unpredictability of IRTs, and this was affected by temperature. The significantly better fit of the IRT slopes model (DIC= 4019) as compared with the intercepts model (DIC= 4091) indicates that individual rIIV estimates were changing in a nonuniform way across temperatures, consistent with between-individual variation in plasticity. This translated into a clear effect of temperature on between-individual differences in unpredictability estimates for IRT, which was not present under temporal change alone. Within-timepoint CV_p and across-timepoint CV_p (Figure 3.1c) did not differ for control individuals, indicating that between-individual differences remained similar across temperatures. In contrast, they did differ in experimental treatments, with within-temperature CV_p significantly higher than the across-temperature estimate (Figure 3.1c).

The reduced analysis of SRT did not indicate an effect of temperature on between-individual differences in unpredictability. Moderate but significant across-context CV_p estimates were extracted from both models (Figure 3.1d), indicating that between-individual variation in unpredictability was present for this behaviour. Across-context CV_p was lower in the experimental model than the control (Figure 3.1d), but not significantly so. As such, no temperature effect could be inferred.

3.5.4. Relationship between unpredictability and mean level behaviour

All IRT models indicated a strong positive correlation between mean response-times and individual unpredictability, where moderately shy individuals were most unpredictable (Figure 3.1e, Figure 3.3a-f). However, the nonsignificant, imprecise estimate of the correlation in the experimental IRT intercepts model indicates a lack of consistency in both individual means and unpredictability across temperatures (Figure 3.1e). The equivalent estimate from the experimental IRT slopes model, meanwhile, was significant and more precise, showing that this pattern was better preserved within temperatures. This again indicates that temperature-related IxE was playing a role in driving patterns of the unpredictability of IRTs, as well as of mean level IRTs, for experimental individuals. These correlation estimates are unlikely to have been relics of the bounded distribution of these data; population means of unpredictability consistently fell very close to the midpoint of the possible distribution of values, and where they did not (Figure 3.3e) the peak of unpredictability still fell at longer (shyer) individual mean response-times than both the midpoint and the population mean.

For SRT, individuals exhibiting intermediate mean level behaviour may have had more scope to behave unpredictably than those individuals falling closer to behavioural extremes. There was no correlation between individual mean level behaviour and unpredictability across timepoints or across temperatures (Figure 3.1f). Figure 3.3(g-h) shows this lack of a correlation clearly, with intermediate individuals exhibiting the greatest unpredictability in both treatments, and bold and shy individuals remaining more consistent. This could have been a relic of the bounded distribution of these data; in both treatments the population mean fell markedly above the midpoint of possible values, which could have biased posterior estimates in favour of bolder individuals being more unpredictable.

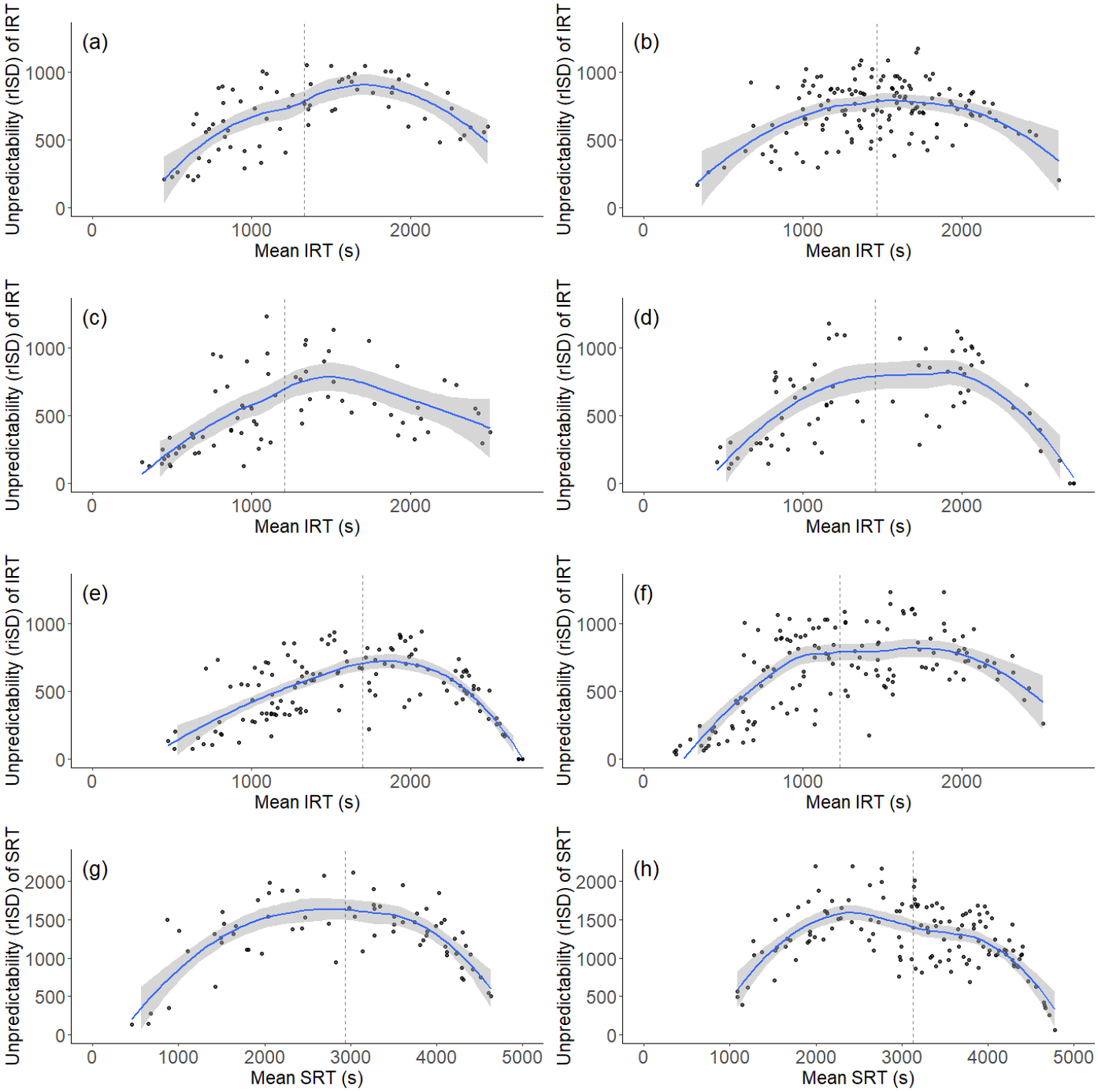


Figure 3.3. The relationship between unpredictability, here measured as point estimates of residual individual standard deviation (riSD) from simple individual level linear regressions, and raw individual mean behavioural response-times in the sea anemone, *Actinia equina*, showing: (a) across-context control immersion response-times (IRT); (b) across-context experimental IRTs; (c) control IRTs within timepoint 1; (d) control IRTs within timepoint 2; (e) experimental IRTs within 13ºC; (f) experimental IRTs within 21ºC; (g) across-context control startle response-times (SRT); (h) across-context experimental SRTs. Trendlines were fitted using local polynomial regression. Population behavioural means are denoted by vertical, dashed lines. Grey shading denotes 95% confidence intervals around the polynomial regression line.

3.5.5. Plasticity of unpredictability in IRT

Individual patterns of plasticity in unpredictability, and how this related to mean level behaviour, were affected differently by extreme temperatures than by time alone for IRT, translating to clear differences between control and experimental treatments. Figure 3.4a shows some consistency for control individuals in both residual and mean IRT estimates across timepoints, and unclear patterns for those individuals whose estimates did change. In particular, bolder control individuals showed strong consistency in their mean level behaviour, and more consistency in their unpredictability than their experimental counterparts. Figure 3.4b shows much clearer patterns in experimental treatments. Nearly all individuals showing high or moderate unpredictability at either temperature also showed longer (shyer) IRTs at that temperature than their less unpredictable counterparts, in line with within-context correlation estimates. Further, all the plotted individuals that were boldest at 6^{o}C and thus showed the lowest unpredictability at that temperature became more unpredictable and shyer at 21^{o}C. Similarly, many of the shyer, more unpredictable individuals at 6^{o}C became bolder and less unpredictable at 21^{o}C, although this second pattern was not uniform, instead being exhibited by a substantial subset of these individuals. Those individuals that did not conform to this pattern were shyer and more unpredictable across both temperatures.

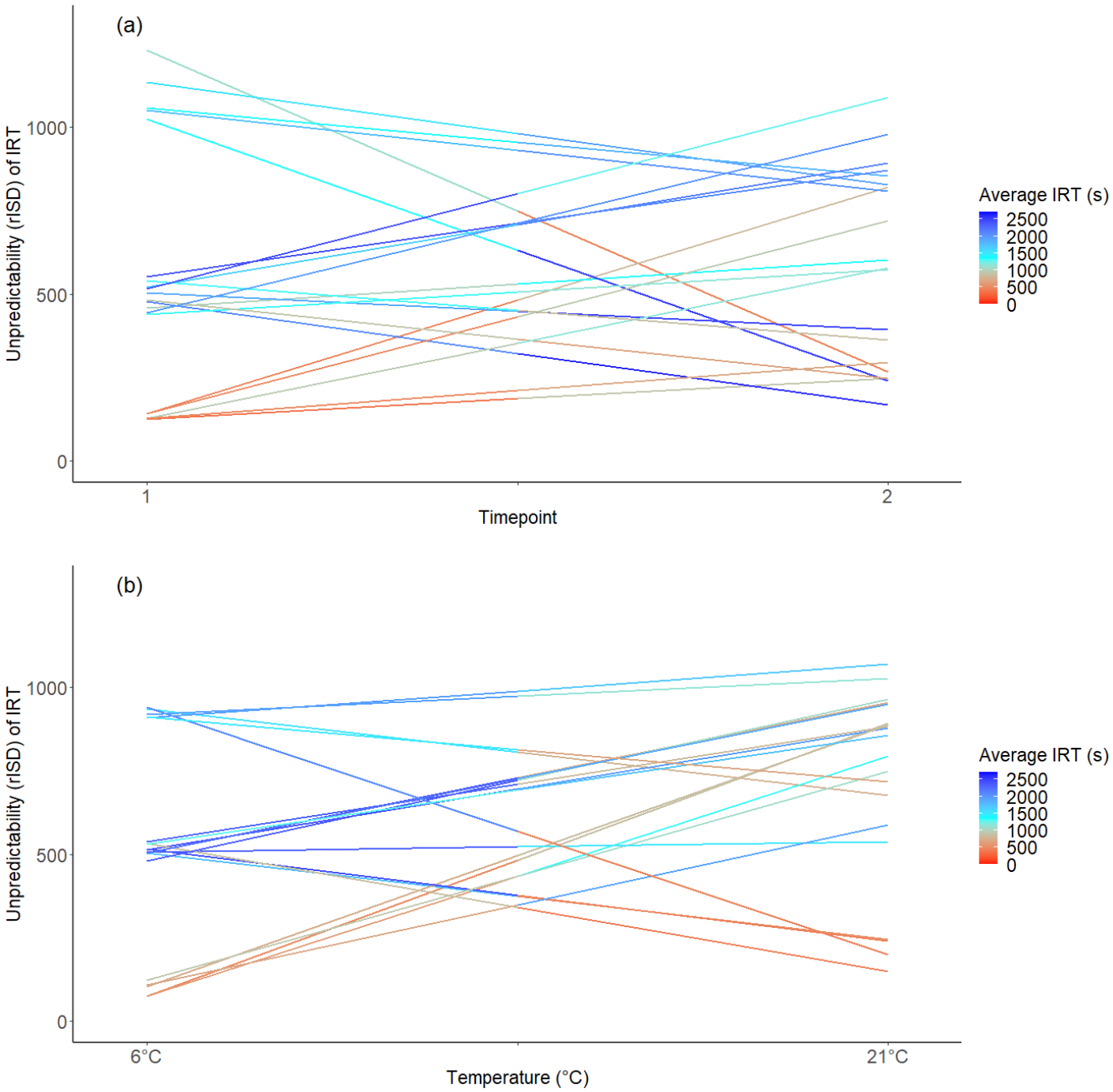


Figure 3.4. Plasticity of individual level unpredictability, here measured as within-environment point estimates of residual individual standard deviation (riSD) from simple individual level linear regressions, and its relationship with individual mean level responses for the immersion response-times (IRT) of 20 individuals at either (a) timepoint 1 and timepoint 2 in control treatments or (b) 6ºC and 21ºC in experimental treatments. The individuals with the five lowest estimates, five highest estimates and 10 estimates falling either side of the median in the first context are shown. Line colours denote raw individual mean response-times in each context following a colour gradient, with red denoting bold, cyan intermediate and blue shy individuals. For the purposes of plotting patterns, individuals exhibiting zero variance in either context are not shown.

3.5.6. Fixed effects on behavioural means

The impacts of the included fixed effects on mean level behavioural changes, under the influence of both temperature and time alone, are not the focus of this paper. The implications of the patterns exhibited by many of these effects are detailed elsewhere (Chapter 2) but for full results and discussion in relation to the models presented here, see Appendix A3.D.

3.6. DISCUSSION

Unpredictability is a key component of behaviour which could, in some cases, account for 50% or more of individual behavioural variation (Bell et al., 2009) and is likely to be of adaptive significance (Prentice et al., 2020). In the face of adaptive challenges such as climatic shifts, unpredictability should have important consequences for individual survival and, in turn, overall species robustness. In this study, we demonstrated significant between-individual differences in unpredictability for two repeatable behaviours in *A. equina*. We further revealed complex relationships therein between unpredictability, mean level behavioural variation and environmental temperature. We showed that individual unpredictability in *A. equina* was sensitive to temperature extremes, of the sort that might be brought about by heatwaves or cold snaps, as the residual variance of both behaviours was affected by temperature to differing degrees. Furthermore, IRT showed clear temperature-driven individual level changes in unpredictability. Individual mean IRTs also covaried with unpredictability, such that moderately shy individuals were the most unpredictable, and this relationship was significantly affected by changes in temperature.

Individual behavioural repeatability was not significantly affected by temporal variation alone for either behaviour in our anemones. This finding is in contrast to many studies of other species (e.g. Biro & Stamps, 2015; Mitchell et al., 2020) showing higher repeatability within short bursts of repeated measures than across measures with longer gaps between them, and may be due to the relatively short overall schedule of each of our data collection blocks. Unlike control treatments, estimates for experimental treatments indicate that individual consistency of SRT was reduced, and for IRT was completely lost, across the extreme temperature gradient, but was retained within contexts for both behaviours. This indicates that individuals were likely to be showing varied reaction norms in both behaviours under temperature change, such that different individuals might employ very different strategies to deal with extremes (e.g. Chapperon et al., 2016; Killen et al., 2013). Given that

inappropriate behavioural responses to environmental changes can be associated with fitness costs (Beever et al., 2017; Sih et al., 2011), individuals that exhibit less adaptively beneficial mean level behaviours at particular extreme temperatures might be at selective disadvantages during some weather events.

Temperature had clear effects on unpredictability within experimental treatments. For SRT, lower-shore individuals were more unpredictable than their high-shore counterparts at 21^{o}C. A temperature-driven increase in ectothermic predator activity (Twardochleb et al., 2020; Yamane & Gilman, 2009) could drive animals towards greater unpredictability as a risk mitigation strategy (Osborn & Briffa, 2017). The predominant predatory threat to *A. equina* are ectothermic aeolid nudibranchs (*Aeolidia papillosa;* Edmunds et al., 1974; Hall et al., 1982). Thus, predation risk to anemones is likely to be greater at higher water temperatures, and tentacle extension puts anemones at even greater risk (Edmunds et al., 1976). Individuals lower down the shore are likely to be under increased risk from nudibranch predators, as they are submerged for longer periods of the day (Bucklin, 1987), and thus might be disproportionately affected by any increased requirement for predator defence at high temperatures. Greater behavioural unpredictability could provide lower-shore individuals with another layer of predator defence besides startle responses themselves (Briffa et al., 2013; Martin et al., 2017). Another explanation could be that these individuals were less robust to high than low temperatures (e.g. Kjærsgaard et al., 2015), leading to increased physical stress and more residual variation. This pattern would also follow, as the low-shore is the least stochastic environment on the seashore and thus less likely to be severely impacted by heatwaves than less stable high-shore environments (Chapperon et al., 2016).

SRT analyses did not provide evidence that individual level unpredictability was significantly impacted by extreme temperature change. Note that had the calculation of within-environment unpredictability been feasible for SRT, it is possible it might have shown similar patterns to those shown by IRT. Nevertheless, individual level estimates indicate that temperature-related IxE may have had a stronger effect at the mean level, rather than at the level of unpredictability, for this behaviour. The lack of covariance between individual unpredictability and mean level behaviour could further suggest that individuals with intermediate mean level personality-types maintained their plastic scope (Dall et al., 2004) by remaining more unpredictable than those that were bolder or shyer. Although this could have been a relic of our sampling distribution, the upper bound of these data is likely to be of biological relevance (e.g. Briffa & Greenaway, 2011; Collins et al., 2017), and the peak of

unpredictability estimates did not fall at the lower (i.e. bolder) end of SRTs, instead falling roughly at the midpoint in both treatments. This is likely to indicate that bolder individuals were not showing artificially greater unpredictability and could further suggest that this analysis was picking up a true biological pattern. The pattern for intermediate individuals to be more variable would make biological sense, as individuals with more extreme mean level personality-types have previously been shown to be more consistent and less responsive to environmental change (e.g. Jolles et al., 2019; Thomson et al., 2011). This could indicate the coexistence of both variable and rigid startle response strategies in this population (Wolf & McNamara, 2012).

For IRT, the effect of temperature on unpredictability was clearer with patterns shown in experimental models being very different from controls. Anemones were significantly more unpredictable in their IRTs at 21^{o}C than 6^{o}C and this difference was more substantial when anemones were cooled than when they were heated (a similar pattern to that found in hermit crabs in Briffa et al., 2013). At 21^{o}C, greater unpredictability could have been driven by a trade-off between temperature-driven rises in both metabolic demand (Abram et al., 2017; Killen et al., 2013) and ectothermic predator activity (Twardochleb et al., 2020; Yamane & Gilman, 2009), which could be mitigated by increased unpredictability, allowing some individuals to lower their mean level IRTs at high temperatures (Chapter 2; Appendix A3.D.) Temperature compensation (Somero & Hochachka, 1969) might further explain why this difference between temperatures was more stark when animals were cooled rather than heated. At very high temperatures, ectotherms down-regulate the expression of metabolic enzymes and mitochondria, and decrease the affinity of those enzymes to their substrate to avoid unnecessary energy expenditure (Le Lann et al., 2011). When the temperature is then rapidly decreased, not only is an ectotherm's metabolic rate naturally lowered (Abram et al., 2017; van Baaren & Candolin, 2018), but there is also a delay in the up-regulation of metabolic molecules and organelles as the animal acclimates to the new temperature (Rohr et al., 2018). This drives a further temporary decrease in metabolic rate and could lead to a greater reduction in activity and foraging at 6^{o}C in the hot–cold treatment than vice versa. As foraging is inherently risky (Edmunds et al., 1976) this might feasibly further reduce the need to mitigate against predation by any means other than highly risk-averse IRTs at 6^{o}C in the hot–cold treatment, further decreasing unpredictability. While this pattern was not present in the opposite direction (i.e. greater unpredictability at 21^{o}C after heating than before cooling due to higher metabolic demands at 21^{o}C in the heating treatment), this could feasibly be

explained by *A. equina* acclimating more effectively, and thus more quickly, to high than to low temperatures (Griffiths, 1977a). Future studies should explore metabolic responses to temperature changes to determine whether metabolism correlates with behavioural traits.

Temperature further influenced between-individual unpredictability in IRTs, such that individuals changed not only their mean level behaviour in response to temperature but also their unpredictability. It is not apparent if these temperature-driven changes could be said to be plasticity of unpredictability. From these data, given the significant correlations between unpredictability and mean level IRTs at both temperatures in the experimental treatments, it appears that unpredictability was covarying with mean level plasticity, with the boldest individuals at both temperatures being less unpredictable, and intermediate and shyer individuals more so. Whether mean level behaviour or unpredictability was driving this relationship is unclear, but either way these data indicate that unpredictability may in fact form part of a multifaceted response to risk, in conjunction with shy behaviours, rather than being an alternative risk mitigation strategy that might alleviate the fitness costs of shyness.

Regardless of the mechanistic underpinnings, the observed changes in the unpredictability of IRTs could still be of adaptive significance (Westneat et al., 2015). Individual level unpredictability estimates in this study present a picture of several coexisting temperature response strategies. One group exhibited shyer, more unpredictable behaviour at low temperatures and bolder, less unpredictable behaviour at high temperatures, another group exhibited the opposite pattern, and yet another remained shy and more unpredictable at both temperatures. These strategies could plausibly be maintained by density-dependent selective processes (Wolf et al., 2007; Wolf & McNamara, 2012) and the stochasticity of rock pool temperatures meaning that neither high nor low temperature extremes are disproportionately represented across seasonal cycles (Dingemanse & Wolf, 2013). As the climate warms, more frequent climate change-induced heatwaves could alter this equilibrium, which might lead to one of these strategies becoming more adaptively advantageous than the others (see: Sih et al., 2011). Under increased frequency of heatwaves and generally higher temperatures, it could be expected that the large benefits of increased metabolic efficiency at high thermal extremes (Biro & Stamps, 2010; Dell et al., 2014), would be likely to outweigh the risks. As such, animals that might exhibit reduced foraging time in favour of predator mitigation at high temperatures under current global conditions, where heatwaves are still comparatively infrequent (IPCC, 2013), might be placed at a severe adaptive disadvantage as their frequency increases. This could be particularly plausible as highly unpredictable behaviour

itself comes at an energetic cost (Biro et al., 2018), which could compound the negative metabolic impacts of shyer mean level behaviour under more regularly occurring heatwaves.

The behavioural effects of extreme temperatures, and extreme temperature shifts, are far reaching. They encompass multiple facets of between-individual behavioural variation including behavioural unpredictability and could be of great adaptive significance as the climate changes. Under climatic shifts and the associated increasing frequency of extreme heatwaves, individuals whose behavioural temperature mitigation strategies are currently adaptively advantageous may find them to no longer be so. These individuals may thus become more susceptible to mortality than their conspecifics which could, in turn, drive a loss of population and community robustness to future challenges. Future studies should directly investigate associations between temperature shifts, behavioural traits and physiology, to further determine which behavioural strategies might be adaptively advantageous in the face of climate change.

3.7. ACKNOWLEDGEMENTS

Thank you to Leslie Connor for technical help and Guillermo Garcia-Gomez for invaluable assistance with animal collection. Thanks also to two anonymous referees and Dr Peter Schausberger for their insightful comments on the manuscript. D.K.M. was funded by a NERC ACCE PhD studentship (ref: 1950009) and Blue Planet Aquarium.

APPENDIX TO CHAPTER 3: Temperature-driven changes in behavioural unpredictability and personality in the beadlet sea anemone, *Actinia equina*

A3.A. Experimental schedule

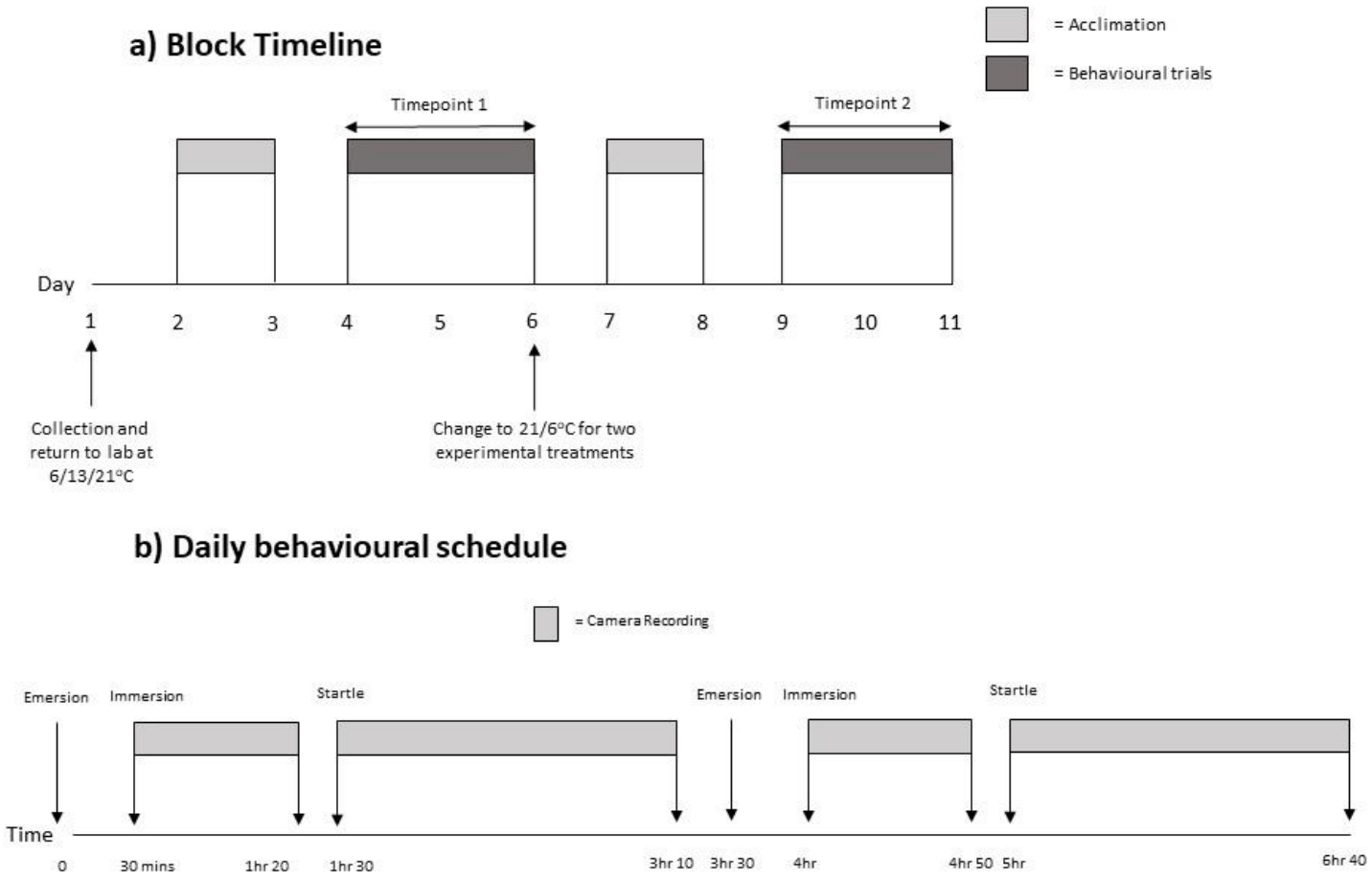


Figure A3.1. (a) The full experimental timeline for a single data collection block and (b) the daily behavioural schedule.

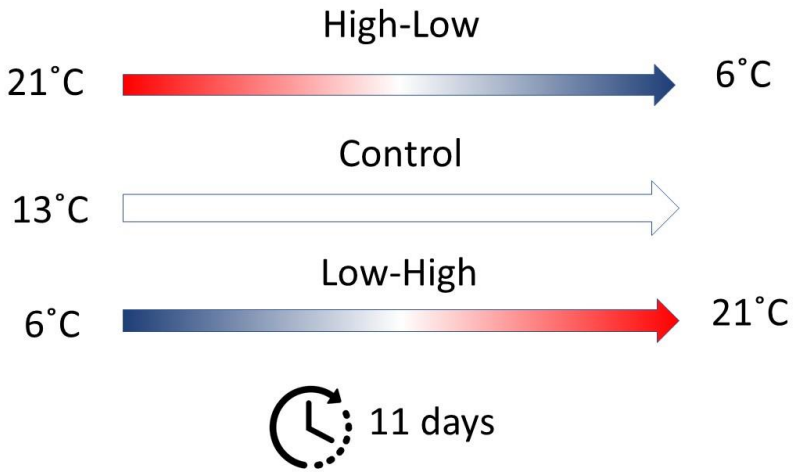


Figure A3.2. The crossed-over temperature schedule of a data collection block.

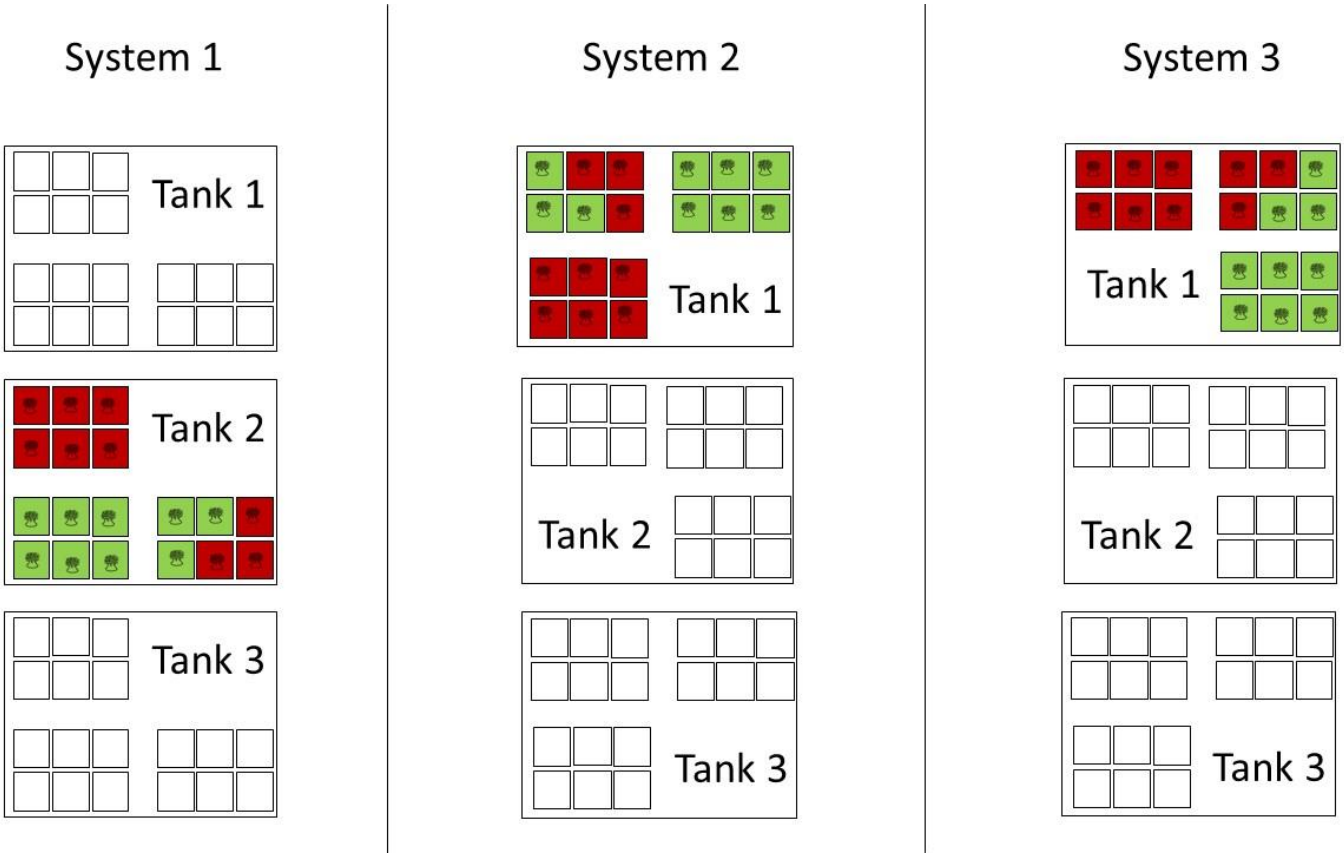


Figure A3.3. Schematic of the tank set-up for our first data collection block, showing the randomly selected tank within each larger flow-through system, each containing three tanks, with anemones clustered in groups of six cups within each tank. Cups are coloured by anemone morphotype.

A3.B. Anemone anatomy

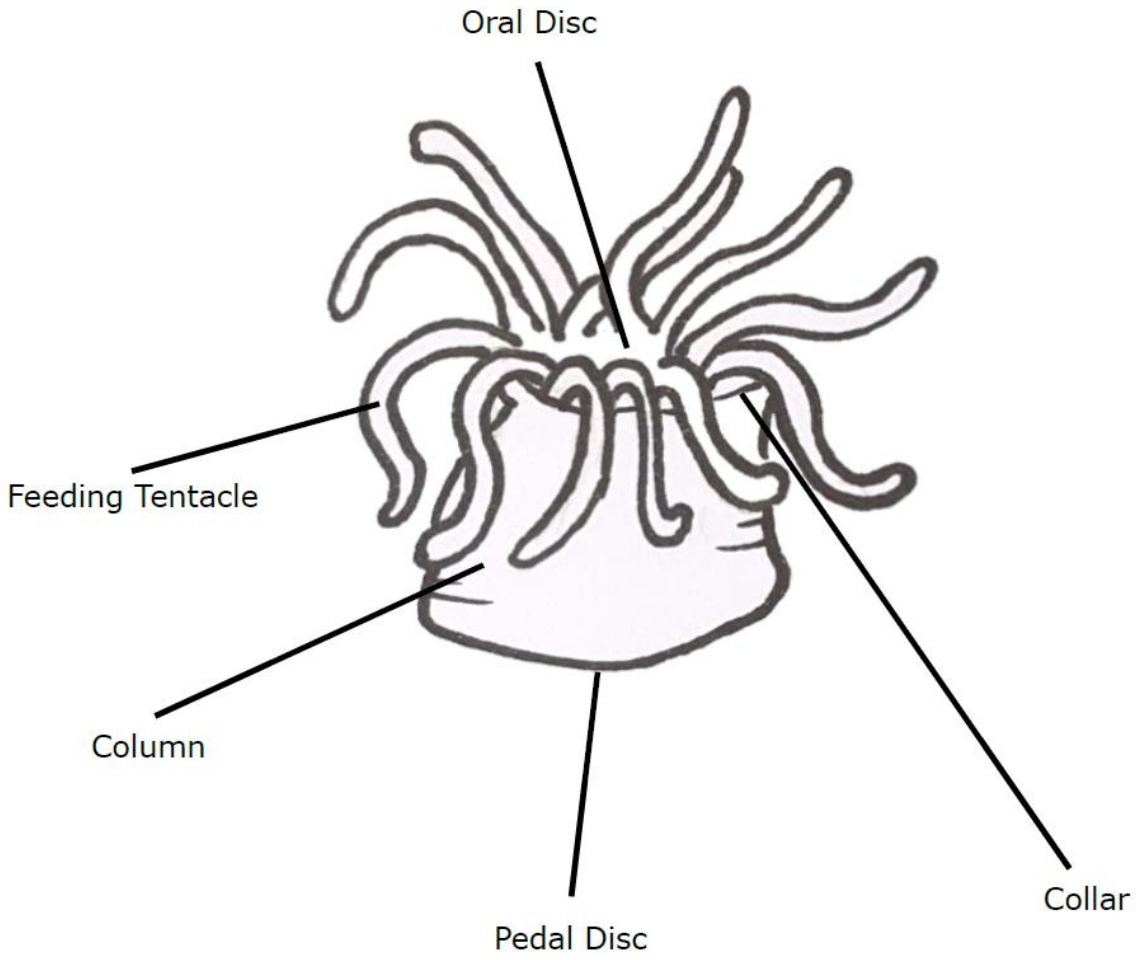


Figure A3.4. The basic anatomy of *A. equina*.

A3.C. Censoring response-times and retaining right-censored values

It was deemed inappropriate to remove those individuals exhibiting maximal values from either SRT dataset as (1) it cut the effective sample size of our control dataset by 27% and of our experimental dataset by 33%, (2) appending the data to this degree would have led to difficulties making any meaningful biological inferences as it removed the entire upper quartile of the data and thus all shy individuals within our sample, and (3) traditional methods for dealing with censored data were unsuitable as our data were not truncated (i.e. we did not remove individuals that exhibited response-times above a given value), and censored distributions invalidate individual behavioural estimates (Stamps et al., 2012). At the full dataset level for SRT, 11 individuals only exhibited maximum values and these individuals were removed from the analyses. The removal of these individuals was deemed less

problematic in a biological context, as it only appended the individual level data by 5%, meaning the majority of the shyest individuals were still retained for analysis.

Our data are right censored, as with many studies of latency (e.g. Beckmann & Biro, 2013; Sasaki et al., 2018; Thomson et al., 2012). Previous studies have suggested that this can bias estimates of intraindividual variability towards lower values than their true value (Stamps et al., 2012) and estimates of repeatability towards higher values than their true value (Urszán et al., 2015). It has been argued that, in these cases, the only way to unbias data is to remove individuals exhibiting a high proportion of maximal values (Stamps et al., 2012). This method biases latency datasets towards bold individuals and where the measurement of latency is related to the response to or recovery from a single stimulus, particularly where that response could be said to be time limited, it may be biologically unsound. In cases such as these, if a researcher had infinite time and resources to measure a response-time, shy individuals (i.e. long responders) might appear to exhibit very high levels of intraindividual variation and very low levels of repeatability. A question arises, however, as to when a given behaviour ceases to be a response to an acute stimulus. Further, in the case of this study, there is a natural time limit imposed on both responses by the tidal cycle, as an individual may be re-emersed when the tide goes out, at which point it will retract its tentacles (Chapter 2). Measurement of a supposed IRT or SRT is therefore likely to cease to be biologically relevant after a given time. As such, those individuals exhibiting responses that consistently fall outside a biologically relevant timeframe could be said to be exhibiting a genuinely maximally shy response-time, and therefore genuinely high repeatability and low intraindividual variation. For variables such as these it should therefore be biologically valid to set a maximal value and incorporate individuals exhibiting that value into between- and within-individual analyses. The timeframe a researcher chooses should, of course, not simply be arbitrary, and extensive pilot data collection or a thorough review of the literature should be carried out to determine what could be deemed a biologically relevant timeframe for a given response-time (carried out for both of our behaviours in Chapter 2).

A3.D. Fixed effects on mean behaviour

For a full table of mean level effects incorporated in different models, see table A3.1.

Startle response-time

In both experimental and control treatments, sampling occasion had a weak but significant effect on mean SRTs, with individuals exhibiting longer response-times at later repeats (control: estimate= 0.03, 95% CI= 0.01, 0.05; experimental: estimate= 0.06, 95% CI= 0.04, 0.08). Temperature was significant in the experimental model (estimate= 0.15, 95% CI= 0.05, 0.25), such that individuals exhibited shorter SRTs at 6^{o}C than at 21^{o}C. Shore height (estimate= -0.17, 95% CI= -0.34, -0.01) and the interaction between morphotype and temperature (estimate= -0.16, 95% CI= -0.30, -0.01) fell at the margin of significance in the experimental model. If significant, these estimates could indicate that individuals from lower down the shore were bolder than those from further up the shore, and that the red morphotype was bolder at higher temperatures. The interaction between morphotype and shore height was also retained in the experimental model as its 95% CI came very close to 0 (estimate= 0.22, 95% CI= -0.02, 0.45). The random effect of tank was also significant in the control model (estimate= 0.39, 95% CI= 0.17, 0.86). There was some small variation in tank temperature across control treatments, but this fell well within ±1^{o}C. Tanks were situated at different heights on their respective flowthrough systems, so it is feasible that these differences were driven by between-tank variation in flowrate.

Immersion response-time

Anemones showed longer response-times on later sampling occasions in both models (control: estimate= 0.06, 95% CI= 0.04, 0.08; experimental: estimate= 0.07, 95% CI= 0.06, 0.09). Morphotypes differed in their behaviour in both treatments. In the control model, the red morphotype showed significantly longer (shyer) response-times than their green counterparts (estimate= 0.37, 95% CI= 0.12, 0.63). In the experimental model, morphotype interacted with temperature (estimate= -0.28, 95% CI= -0.41, -0.15) such that red morphotypes showed shorter (bolder) responses at higher temperatures than their green counterparts. In the experimental treatment, IRTs further differed between treatment orders (estimate= -0.36, 95% CI= -0.5, -0.22), with anemones in the cold–hot temperature treatment exhibiting shorter responses than those in the hot–cold treatment.

Table A3.1. Table of fixed and random effect estimates taken from the mean level models of double-hierarchical GLMs**.** Models were run using the JAGs overlay rjags. Estimates are shown for startle response-time (SRT) and immersion response-time (IRT) in control and experimental beadlet anemones, *Actinia equina*, with 95% credible intervals in parentheses. Significance of effects, determined where 95% credible intervals did not cross or meet 0, or in the case of random effects were not pushed up against 0, is denoted by "**". N/a denotes a variable that was not included in a given model, either due to it being a control, or due to it being a non-significant interaction effect that was removed from the final run.

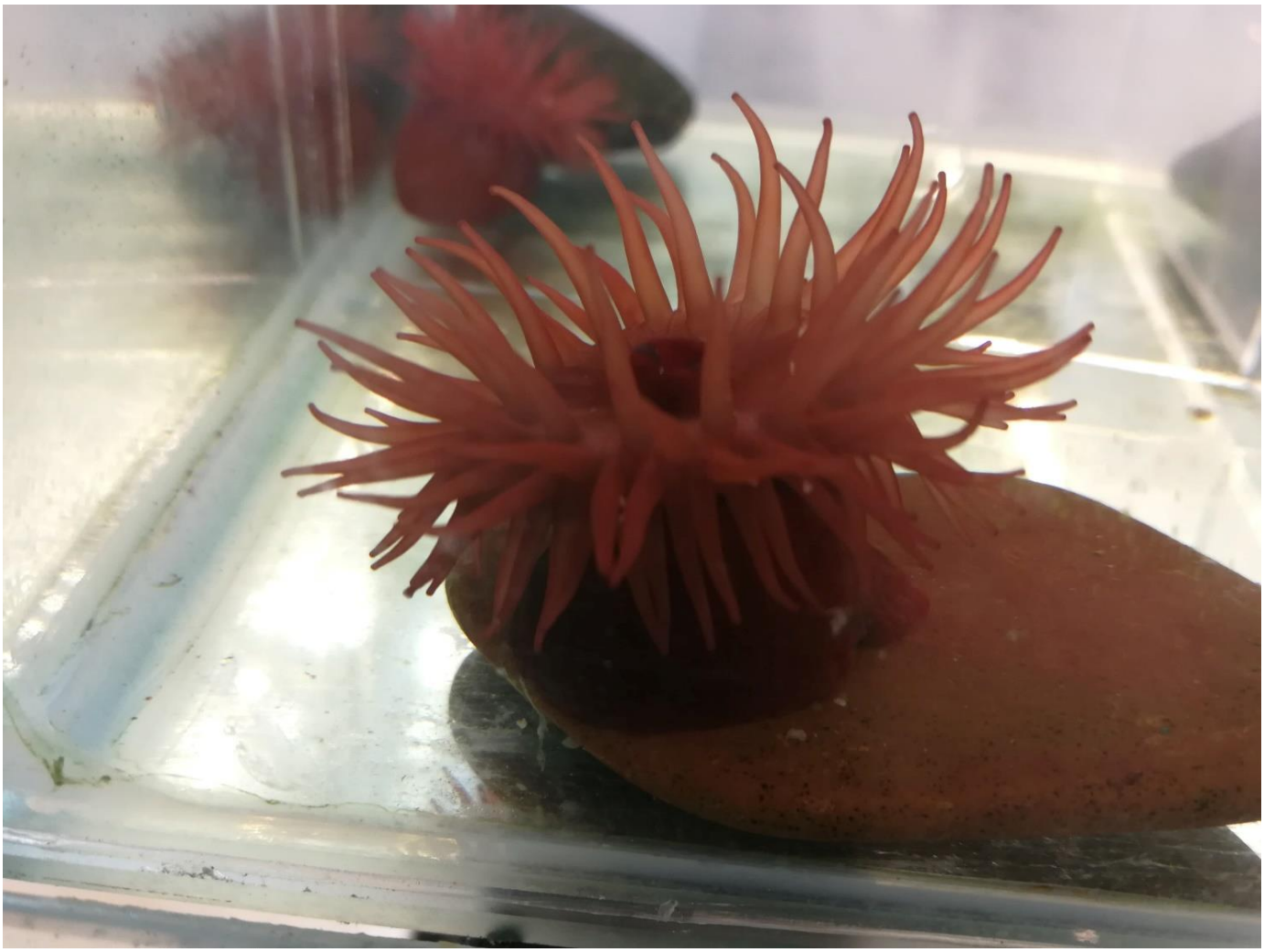
		SRT estimate		**IRT estimate**
Variable	**Control**	**Experimental**	**Control**	**Experimental**
Intercept	0.09 (-0.54,0.39)	0.02 (-0.20, 0.24)	-0.29 (-0.58, -0.02)	-0.03 (-0.21, 0.14)
Temperature	0.00 (-0.09, 0.08)	0.15 (0.04, 0.25)**	-0.03 (-0.10, 0.04)	-0.12 (-0.21, -0.02)**
Morphotype	0.13 (-0.16, 0.42)	0.05 (-0.15, 0.25)	0.38 (0.12, 0.63)**	0.21 (0.05, 0.37)**
Shore height	0.21 (0.00, 0.41)	-0.17 (-0.34, -0.01)**	0.09 (-0.07, 0.26)	0.05 (-0.04, 0.14)
Sampling occasion	0.03 (0.01, 0.05)**	0.06 (0.04, 0.08)**	0.06 (0.04, 0.08)**	0.07 (0.06, 0.09)**
Sampling block	0.05 (-0.20, 0.28)	0.03 (-0.06, 0.11)	0.07 (-0.07, 0.20)	0.07 (0.01, 0.13)**
Treatment order	N/a	-0.06 (-0.25, 0.12)	N/a	-0.36 (-0.50, -0.22)**
Temperature*Morphotype	N/a	-0.16 (-0.30, -0.01)**	N/a	-0.28 (-0.41, -0.15)**
Morphotype*Shore height	N/a	0.22 (-0.02, 0.45)	N/a	N/a
Individual	0.46 (0.32, 0.62)**	0.34 (0.17, 0.47)**	0.44 (0.30, 0.59)**	0.08 (0.00, 0.22)
Individual*Temperature	N/a	0.50 (0.40, 0.61)**	N/a	0.50 (0.44, 0.56)**
Individual*Timepoint	0.31 (0.16, 0.45)**	N/a	0.29 (0.18, 0.41)**	N/a
Tank	0.39 (0.17, 0.86)**	0.05 (0.00, 0.20)	0.11, (0.00, 0.38)	0.09 (0.00, 0.25)

Discussion

Our results add to a body of evidence that temperature is a significant predictor of behaviour for many ectotherms (e.g. Andrew et al., 2013; Mitchell & Biro, 2017). In both behaviours it appears that morphotype was the key fixed variable to interact with temperature and affect behaviour, and this corroborates previous findings that individuals of the green morphotype exhibit potentially detrimental behavioural responses to high temperatures as compared with their red counterparts (Chapter 2). These data also substantiate that shore height may be an important predictor of SRT but not of IRT at the mean level (Chapter 2). Further, time spent in the laboratory was a significant, if weak, predictor of both behaviours, as mean response-times across all models increased at later sampling occasions. Whether this was due to natural temporal changes in behaviour (e.g. Jolles et al., 2019), habituation to stimuli (e.g. Houslay et al., 2019) or a response to time spent in an artificial environment (e.g. Osborn & Briffa, 2017) remains unclear. Regardless of the reason, this finding indicates that caution should be exercised when keeping *A. equina* in the laboratory for long periods before behavioural testing. It is possible that the behaviour of this species may become less biologically relevant as it spends longer in an ex situ environment.

Chapter 4:

Differential metabolic responses in bold and shy sea anemones during a simulated heatwave



[Page intentionally left blank]

4.1. KEYWORDS

Climate Change, Boldness, Metabolism, Pace of life, Marine invertebrate

4.2. ABSTRACT

1. As climate change-induced heatwaves become more common, phenotypic plasticity at multiple levels is a key mitigation strategy by which organisms can optimise selective outcomes. In ectotherms, metabolic physiology is inherently related to temperature, and behavioural plasticity is crucial to alleviate the effects of thermal stress. Nonetheless, no study in any ectotherm has yet empirically investigated how changing temperatures affect between-individual differences in the associations between these two traits.
2. Using the beadlet anemone (*Actinia equina*), a common intertidal species living in a thermally heterogeneous environment, we investigated between-individual differences in the plasticity of metabolic rate and boldness to acute temperature shifts.
3. We used a crossed-over design and a temporal control to test the same individuals at a non-stressful temperature, 13^{o}C, and a high temperature extreme, 21^{o}C. At each temperature, we took repeated measurements of routine metabolic rate (RMR) and also measured a repeatable boldness-related behaviour, immersion response-time (IRT).
4. Individual differences were highly predictive of metabolic plasticity, and the plasticity of RMR was associated with IRT. At 13^{o}C, shy animals had the highest metabolic rates, while at 21^{o}C this relationship was reversed. Individuals that were bold at 13^{o}C also exhibited the highest metabolic rates at 21^{o}C.
5. Exposure to high metabolic demand and predation pressure during heatwaves could come at a short-term detriment to bold individuals. Equally, lower metabolic rates at baseline temperatures could be necessary for optimal survival as heatwaves become more common. These results provide novel insight into the relationship between metabolic and behavioural plasticity and help highlight the adaptive value of different personality-types under a changing climate.

4.3. INTRODUCTION

The pace-of-life syndrome hypothesis (POLS; Réale et al., 2010) posits that organisms must trade-off long-term survival against short term reproductive success, leading to predictable

correlations between “active” behaviours and energetic physiology. POLS predicts that individuals with more risk-prone, “bolder” personalities (consistent behavioural differences between individuals; Sih et al., 2004), should show “faster” physiological characteristics, such as a high metabolic rate, that are conducive to higher early fecundity and growth, but not to long-term survival (Montiglio et al., 2018; Réale et al., 2010). It is well established that life-history trade-offs can drive the maintenance of personality-types (Wolf & McNamara, 2012; Wolf & Weissing, 2010); bold individuals often place themselves at greater risk of mortality than shy conspecifics, but benefit from riskier lifestyles via increased foraging opportunities and more energetic scope for reproduction (Smith & Blumstein, 2008). POLS simply extends these trade-offs by predicting that the physiology of bolder individuals should be primed to maintain high levels of risky activity, exploration, and foraging, even under periods of heightened environmental stress (Montiglio et al., 2018; Réale et al., 2010). As such, according to POLS, bolder animals should exhibit reduced physiological stress reactivity, faster growth, and higher metabolic rates than shyer conspecifics (Biro & Stamps, 2010). The relationships proposed by POLS could be of great value to conservationists, indicating the life-history strategies present in a given population and informing whether some behavioural phenotypes might be more susceptible to novel selective pressures than others.

Evidence for POLS is mixed, with studies finding positive (Polverino et al., 2018), negative (Le Galliard et al., 2013), and inconclusive associations (Killen et al., 2012) between metabolic rate and boldness. As such, a recent meta-analysis concluded that empirical evidence for POLS is weak (Royauté et al., 2018). However, no studies have yet investigated how individual variation in the scope and nature of phenotypic plasticity to environmental fluctuation might affect relationships between bioenergetics and boldness (Killen et al., 2013; Montiglio et al., 2018). “Activational” phenotypic plasticity (Snell-Rood, 2013), an animal’s ability to rapidly alter aspects of its phenotype in response to acute environmental change (Seebacher et al., 2015; Stamps, 2016), could be crucial to understanding these relationships under a rapidly changing climate, where extreme weather events are becoming increasingly common (IPCC, 2013, 2018). Activational plasticity can have selective advantages, helping many species remain robust to fluctuating environments (Snell-Rood, 2013), and disadvantages, as maintaining scope for short-term plasticity is energetically costly (Dall et al., 2004). This leads to variation within populations in the abilities of different individuals to deal with acute environmental changes (Dingemanse & Wolf, 2013) and could

thus influence relationships between metabolism and boldness, and their selective implications under different environmental scenarios.

Although activational plasticity does not itself form part of POLS (Montiglio et al., 2018), the relationship between metabolism and behaviour is likely to be both species and context-dependent (Killen et al., 2012). In mosquitofish, for example, lineages from more stochastic environments grow faster, but also exhibit lower metabolic rates and are shyer than those from stable environments (Polverino et al., 2018). If environmental context is indeed a major driver of relationships between metabolism and behaviour, short-term environmental fluctuations and between-individual differences in plasticity to those fluctuations will inevitably impact any associations between a species' behaviour and its physiology (Killen et al., 2013; Montiglio et al., 2018). Between-individual variation in plasticity may be an especially important driver of relationships between behaviour and metabolism in heterogeneous environments, where the coexistence of different plastic strategies can be driven by spatial and temporal variation in both normal environmental conditions and environmental stochasticity (Dingemanse & Wolf, 2013; Wolf & McNamara, 2012). Despite the clear need to incorporate activational plasticity into investigations of POLS and its selective implications, we currently do not have any empirical information on how short-term environmental changes, and the overall stochasticity of an animal's environment, might affect the relationships between metabolism and behaviour.

When investigating how acute environmental changes affect these relationships, temperature is a particularly important environmental variable to consider. As climate change leads to higher average temperatures and increased frequency of heatwaves (IPCC, 2013, 2018), animal populations are being placed under substantial, novel selective pressures (Parmesan, 2006). Thermal physiology and thermal preferences are especially important drivers of associations between metabolism and behaviour in ectotherms (Abram et al., 2017), where many physiological processes, including metabolic rate, are intrinsically linked to environmental temperatures (Seebacher et al., 2015). In ants, for example, colonies from warmer environments are more likely to show the positive associations among "active" behaviours that are predicted by POLS (Segev et al., 2017). In parasitic wasps, meanwhile, adults with a thermal preference for low temperatures exhibit high metabolic rates, low foraging efficiency, and short lifespans when exposed to high temperatures (Le Lann et al., 2011). As an acute thermal stressor to which they are unable to immediately physiologically adjust, climate change-induced heatwaves present ectotherms with a different set of

ecological challenges than chronic temperature change (Vajedsamiei et al., 2021), and many species rely heavily on behavioural plasticity to address these challenges (Abram et al., 2017). Nonetheless, the effect of acute temperature rises on the relationship between ectothermic physiology and behaviour, and how this relates to thermal preferences, has yet to be investigated.

The beadlet anemone, *Actinia equina*, lives across a gradient of shore heights in the spatially and temporally heterogeneous intertidal zone (Allcock et al., 1998). Although *A. equina* is particularly robust to temperature stress (Griffiths, 1977b), making it an ideal organism with which to investigate the effects of thermal perturbation without risk of mortality, the heterogeneous nature of its environment leads it to show ranges of thermal preferences within single populations (Navarro et al., 1981). Anemones living further from the low-tide line (higher up the shore) experience increased exposure to stressful temperatures (Brahim et al., 2019) and greater thermal variation than those living lower down the shore (Chapperon et al., 2016). Multiple genetically distinct morphotypes have developed which favour different shore heights; the red morphotype is associated with the high-shore, and is thus predicted to deal more effectively with temperature extremes than the green morphotype, which is associated with the low-shore (Allcock et al., 1998). Morphotypes further display consistent differences in a relevant behaviour, immersion response-time (IRT), which could be closely associated with an anemone's metabolic rate (Chapter 2). IRT is a risk-related behaviour, and thus a gauge of an anemone's boldness, which measures how long after a simulated tide-cycle it takes an anemone to re-extend its feeding tentacles and resume foraging. Because *A. equina* is unable to feed when the tide is out, IRT should represent a trade-off, whereby if an individual is more cautious to predation risk after re-immersion, it will have less time available to forage and thus less energy available for metabolic processes. At high temperatures, where increased metabolic demand drives increased foraging (Abram et al., 2017), this trade-off should become particularly pronounced, as high temperatures are also likely to place anemones under greater risk of predation from other ectotherms (e.g. Twardochleb et al., 2020; Yamane & Gilman, 2009). Relationships between IRT and metabolic rate at different temperatures should thus provide clear indications of whether *A. equina* follows POLS and enable prediction of the metabolic responses of different individuals to thermal extremes.

In this study, we investigated for the first time how relationships between individual behavioural phenotypes and their metabolic rates were influenced by different temperatures.

We determined how individuals of two morphotypes of *A. equina* varied in their scope to respond behaviourally and physiologically to simulated heatwaves. We measured the relationships between an anemone's routine metabolic rate (RMR; Metcalfe et al., 2016; Velasque & Briffa, 2016), which is the metabolic rate of an animal undergoing normal activity, and its IRT. These were measured at two temperatures using a crossed-over temperature design, where one temperature was neutral and the other simulated a heatwave. Importantly, all experimental individuals had their IRTs and RMRs measured at both temperatures. We also incorporated a temporal control where the temperature remained neutral throughout. We expected that RMR would show general increases across all individuals at high temperatures and that because different animals would exhibit different degrees of plasticity, the relationship between temperature and RMR would vary between individuals. We also predicted that the degree of change in RMR between temperatures would differ between morphotypes, with discrepancies being influenced by the red morphotype's tolerance for higher, more variable temperatures than the green. In terms of the relationship between IRT and RMR, we anticipated that bolder animals, exhibiting shorter IRTs, would have higher RMRs at both temperatures, as predicted by POLS, and that this relationship would be stronger at high temperatures.

4.4. METHODS

4.4.1. Experimental schedule

Data collection was carried out between January and March 2020 over the course of four, nine-day data collection blocks. Each block began with three days of anemone collection from rocky sea defences at the top of the beach at New Brighton, UK (lat: 53.4400, long: -3.0565). Five animals were collected on each day, split between the red and green morphotypes, such that three of one morphotype and two of the other were collected. This uneven split, necessary due to 15 being the maximum number of individuals that could be investigated per block in our respirometry apparatus, was randomised and evened out over the course of the study. Overall, 30 anemones of each morphotype were used. Collection and identification of morphotypes was carried out using previously described methods (Chapter 2). Anemones were size-matched at the point of collection as far as was possible by measuring pedal disc diameters (PDD). Because the raw routine metabolic rate (RMR; Metcalfe et al., 2016; Velasque & Briffa, 2016) of larger individuals of all species is higher than that of smaller individuals (Glazier, 2005), and thus easier to accurately detect, only

anemones with a PDD of over 20mm, the largest individuals in the population, were selected. Further, because pilot work found the measurement of wet weight to be highly invasive and cause significant damage to anemones, and because the volume of *A. equina* is not static (Griffiths, 1977b) so cannot be reliably measured, this size-matching was also used to minimise any effect of anemone volume on metabolic results.

Each group of five anemones was returned to the laboratory, again using previously described methods (Chapter 2), and left to acclimate to their surroundings for 48 hours. Anemones were housed in a 13 ±1^{o}C temperature-controlled room on a 12h:12h day-night cycle. Each anemone was placed in a 7 cm x 15 cm plastic cup containing drainage holes and a rock to which the anemone could adhere. Cups were situated in one of two 80 cm x 45 cm x40 cm tanks filled to ca. 12 cm with artificial seawater, at a salinity of 34 ±1 ppt (RO water with Tropic Marin Pro Reef Salt, Germany). One of these tanks, which housed 10 individuals, was maintained at the ambient temperature of 13^{o}C (±1^{o}C), while the other, which housed 5 individuals, was maintained at 21^{o}C (±1^{o}C). Tanks were themselves situated in two flow-through systems, which each contained three tanks. Every tank contained anemones at least once and the order in which tanks were used was randomised across blocks. Across the four blocks, each system was maintained at each temperature twice, and the order of this was randomised. Water quality (salinity, pH, nitrate, nitrite, ammonia) and water temperature in holding tanks were regularly recorded and full water changes in each system were carried out before each block.

After 24 hours of the 48-hour acclimation period, anemones were fed *ad libitum* on defrosted *Artemia* (Monkfield Nutrition, Ely, UK). This gave anemones time to bind to substrate and limited variation between individuals in levels of satiation, whilst also allowing a period of 24 hours without food before metabolic experimentation commenced. After the full 48 hours, each group of five anemones was first subject to a ca. 5pm immersion response-time (IRT) measurement, using anemones' natural response to changes in tides to measure boldness. IRTs have previously been shown to be repeatable over time (Chapter 2) so only one measurement was taken before the commencement of metabolic testing. Cups containing anemones were removed from holding tanks and drained. Anemones were left emersed for half an hour, before being re-immersed in their holding tank. After re-immersion, IRT, defined as the length of time to re-extend feeding tentacles fully (Chapter 2), was recorded by two GoPro Hero 4 (GoPro Inc., San Mateo, CA) cameras mounted directly above the tank, taking time-lapse photographs every 30 s. 50 minutes of footage was recorded, of which the

45 minutes immediately after each individual's immersion was used to determine IRT. The number of photos an anemone took to re-extend its tentacles was recorded and converted into seconds. Anemones which showed no complete response within 45 minutes were given a maximum value of 2700 s (Chapter 2).

After their evening IRT measurement, anemones were gently separated from their pebbles and transferred to one of six freshly sterilised (with bleach-water and rinsed with fresh water) 14 cm x 6 cm 425 ml glass intermittent-flow respirometry chambers sealed with polypropylene lids (IKEA, *Älmhult*, Sweden) and each containing a 2.5 g magnetic stir bar with which anemones could not interact. One of these chambers was designated as the blank, and thus remained empty. For identification purposes, anemones of the same morphotype were grouped in adjacent chambers, but where the morphotypes were, and which chamber was designated as the blank, was randomised. Chambers were situated in a temperature-controlled respirometry tank (67 cm x 46 cm x 38 cm) filled to a depth of 20 cm with freshly made artificial seawater (salinity 34 ±1 ppt). The seawater was heated or chilled to the appropriate temperature (±0.3^{o}C) prior to anemone introduction. Chambers were also subject to blank measurements prior to the introduction of anemones to ensure that only negligible background respiration was present; where background was detected, chambers were re-sterilised to ensure minimal microbial activity. After their introduction, anemones were left for 12 hours overnight to acclimate and attach to their chamber. We used automated intermittent-flow respirometry (Svendsen et al., 2016) to measure aquatic RMR, so this 12-hour period also served to acclimate anemones to the intermittent-flow cycle. Measurements were unable to be conducted throughout the night due to difficulties with having the magnetic stir-bars move continuously for 12 hours. The intermittent-flow cycle consisted of a three-minute water flush period and a 38-minute closed period where oxygen consumption was measured. This measurement period was shorter than those used in previous *A. equina* respirometry studies (Navarro et al., 1981), but with this volume of chamber, pilot investigations found it produced sufficient data with which to measure oxygen consumption, while allowing more repeated metabolic measurements to be taken each day. After this 12-hour overnight acclimation period, experimentation was carried out for four hours in the morning and four hours in the afternoon, with a brief (ca.10 minutes) break period between the two where any small bubbles that had formed were removed from chambers. During trials, aquatic oxygen concentration within chambers never fell below 80% saturation. Four

metabolic slopes of oxygen consumption were produced for each anemone at each time of day (for full descriptions and pictures of metabolic apparatus, see Appendix A4.A.).

At the end of their first day of testing, groups were transferred back to one of the two holding tanks and the whole cycle was repeated, beginning with another 48h acclimation period. To account for temporal variation and treatment order effects we utilised a crossed-over design (Briffa et al., 2013) and a temporal control (White & Briffa, 2017). Within blocks, each group of five anemones was designated to one of three treatment groups, each with an overall sample size of 10 red and 10 green individuals:

- The temporal control (L-L) group was housed and tested at 13ºC throughout.
- The low temperature to high temperature group (L-H) was first housed and tested at 13ºC, then at 21ºC.
- The high temperature to low temperature group (H-L) was first housed and tested at 21ºC, then at 13ºC.

After the second round of metabolic testing, anemones were placed directly into a -20°C freezer in individually labelled plastic bags, to be stored for later drying and weighing. This method of euthanasia added an extra step compared with previous studies of *A. equina*, where anemones have been dried from live (e.g. Navarro et al., 1981; Rudin & Briffa, 2012). See Appendix A4.B. for a visualisation of this schedule.

4.4.2. Morphological measurement

To measure the dry weight of each individual, frozen anemones were placed in a Carbolite (Hope, UK) CWF100 muffle furnace, maintained at 110ºC for 90 hours. Dry anemones were then weighed using a Sartorius (Stonehouse, UK) R2000 balance scale, accurate to the nearest hundredth of a milligram.

4.4.3. Statistical analysis

Calculation of RMR

Metabolic data were analysed using the respR package (Harianto et al., 2019) in R version 3.6.2 (R Core Team, 2020). A minimum of two slopes were extracted for every chamber for both morning and afternoon sets of readings, providing up to six measurements of oxygen consumption per chamber, per day. The first 41 minute cycles in both the morning and afternoon were always excluded, as preliminary data indicated that RMRs settled during the

first cycle, such that they were different from subsequent slopes in a sampling repeat. After a wait period before recording of at least a minute to ensure only the linear portion of slopes were measured (Svendsen et al., 2016), the r^2 of recorded oxygen consumption slopes for all anemones fell above a threshold of 0.9. For each repeated measure, slopes from blank chambers were used to calculate background-adjusted whole-organism oxygen consumption (RMR) by subtracting blank oxygen consumption values from the values of each experimental chamber. Raw blank-adjusted measurements were converted to provide final whole-organism RMR estimates in milligrams of oxygen per hour (mgO_2/h; Appendix A4.C.).

Bayesian Analyses

All individual level models were run within a Bayesian Markov Chain Monte Carlo framework using the R package MCMCglmm (Hadfield, 2010). See Appendix A4.D. for details of general model specification and convergence checks, additional analytical details and discussion of a confirmatory analysis into the relationship between RMR and dry weight.

Repeatability and Plasticity of RMR

After comparative testing against a random intercepts model (Appendix A4.D.), effect estimates were extracted from a random slopes model to determine the degree of between-individual variation in RMR, and how that related to metabolic changes between different temperatures. RMR (z-transformed) was the response variable and morphotype, temperature, treatment, dry weight (z-transformed), sampling occasion, sampling day (i.e. whether it was an individual's first or second day of metabolic testing), and data collection block were all included as fixed effects. Temperature was allowed to interact, separately, with morphotype and treatment order. A random-slope effect, accounting for individual variation and the interaction between individual identity and metabolic plasticity to temperature (IxE), was specified as the random effect.

To investigate whether individual differences in metabolic rate were consistent across the two temperatures, adjusted across-context repeatability was calculated. To investigate individual variation in metabolic plasticity to temperature (IxE), the individual level slope effect, indicating the explanatory importance of individual differences in predicting plasticity between the two temperatures, was extracted. Finally, to explore how an individual's metabolic rate at 13^{o}C related to their metabolic plasticity to temperature, the correlation between individual intercepts at 13^{o}C and their slopes between 13^{o}C and 21^{o}C was

calculated. Statistical significance was inferred for fixed effects and covariance terms where 95% credible intervals did not cross zero. For repeatability and slope estimates, significance was inferred where 95% credible intervals were not close to zero and histograms of the term's posterior estimates were not pushed up against zero (Chapter 2).

The relationship between RMR and immersion response-time (IRT)

To investigate the relationship between RMR and IRT, how it might relate to POLS at different temperatures, and whether boldness at 13^{o}C or 21^{o}C could be used to predict RMR at 21^{o}C, two separate datasets were used (for justification see Appendix A4.D.). The '13^{o}C dataset' included all values measured at 13^{o}C, while the 'crossover dataset' included all values measured in the crossed-over temperature treatments. A bivariate mixed effects model was run on the 13^{o}C dataset to determine the covariance between the two traits at 13^{o}C, with RMR and IRT (both z-transformed) set as response variables. For the crossover dataset, a multivariate model was run where the four response variables were z-transformed RMR and IRT at each temperature. For both models, morphotype, dry weight (z-transformed), treatment, sampling occasion and data collection block were all fixed effects, with individual identity included as a random effect. Residual (within-individual) covariance was not identifiable and values within the residual variance-covariance matrices of each model were therefore fixed at 1. After the appropriate model checks had been carried out, between-individual correlations between IRT and RMR within each temperature and between temperatures, alongside associated 95% credible intervals, were extracted. Statistical significance was inferred where 95% credible intervals did not cross zero.

4.4.4. Ethical Note

No licenses or ethical approval were required to carry out this study as this species is not currently protected under UK legislation. Nonetheless, anemones were treated humanely, temperatures to which anemones were exposed fell within their tolerance ranges (Chapter 2), and no mortality occurred during the experimental periods.

4.5. RESULTS

4.5.1. Repeatability and Plasticity of RMR

There was a general trend across almost all animals for increased RMR at 21^{o}C such that sample mean RMR increased by over 50% from 0.12 mgO_2/h (SD= 0.04) at 13^{o}C to 0.19 mgO_2/h (SD= 0.06) at 21^{o}C. This translated into a significant modelled temperature effect on RMR, but the size of this effect was estimated with a large degree of uncertainty (estimate= 0.98, 95% CI= 0.01, 1.83). Both morphotype and dry weight were also significant predictors of RMR, and neither of these relationships were affected by temperature. The green morphotype exhibited significantly higher mean RMRs than the red morphotype at both temperatures (25% higher at 13^{o}C and 21% higher at 21^{o}C; estimate= -0.34, 95% CI= -0.59, -0.09), although figure 4.1 does show a wider range of RMR values for both morphotypes at 21^{o}C. Heavier individuals exhibited significantly higher RMRs than lighter individuals (estimate= 0.41, 95% CI= 0.28, 0.54). Data collection block also had a significant relationship with RMR (estimate= -0.12, 95% CI= -0.23, -0.01), such that animals in later blocks had lower RMRs than those in earlier blocks.

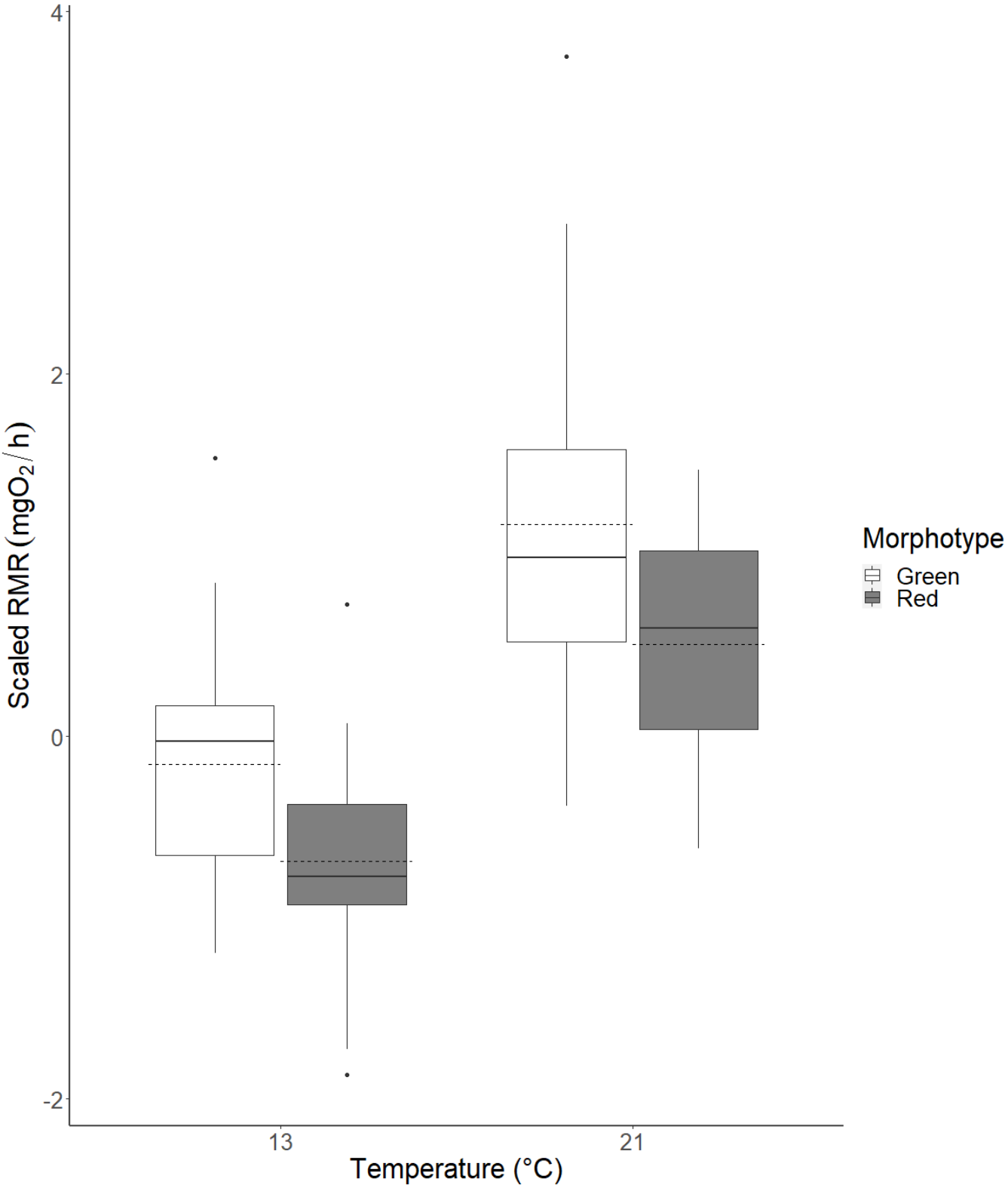


Figure 4.1. Variation between red and green morphotypes in z-transformed predicted mean routine metabolic rate (RMR) at 13ºC and 21ºC for all individuals (n= 60), derived from a Bayesian random slopes analysis. Boxes denote the median value, with the first and third quartiles forming the box limits, and dotted lines denoting the mean. Whiskers extend to encompass all data or 1.5 times the interquartile range. Any point falling outside the whiskers can be deemed to be an outlier.

At the individual level, repeatability estimates indicate that RMR varied consistently between individuals and that some of this variation was retained across temperatures, such that the rank-order of individual RMRs at 21^{o}C remained partially similar to the rank order at 13^{o}C. As such, adjusted across-context repeatability was low, but still significant (R_{adj}= 0.17, 95% CI= 0.11, 0.25). Most of the between-individual variation in RMR was explained by IxE, translating into a highly significant, strong slope effect (estimate= 0.70, 95% CI= 0.60, 0.80). Figure 4.2 shows that individuals differed greatly in the degree to which their RMR increased as the temperature was raised. 2/39 exhibited lower RMRs at 21^{o}C than at 13^{o}C and 2/39 showed minimal change between the two temperatures. How the RMR of different individuals changed between temperatures was associated with their RMR at 13^{o}C, with individuals that exhibited lower RMRs at 13^{o}C showing larger increases at 21^{o}C than those that exhibited higher RMRs at 13^{o}C. This translated into a significant negative correlation between individual RMRs at 13^{o}C and the gradient of individual slopes between the two temperatures (r= -0.47, 95% CI= -0.70, -0.19).

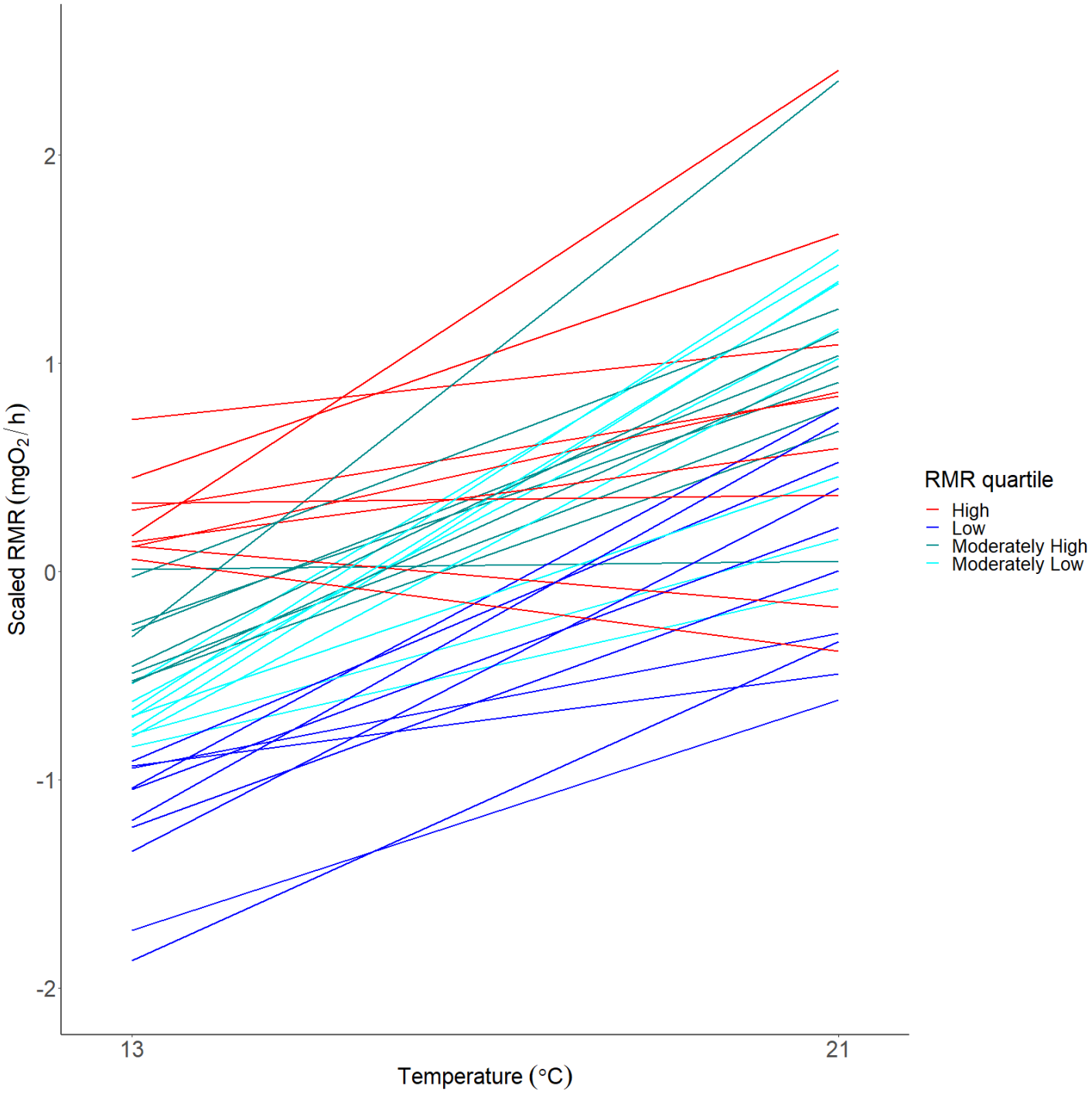


Figure 4.2. The change in individual level posterior mean predictions for experimental individuals (n= 39) for z-transformed routine metabolic rate (RMR) between 13ºC and 21ºC, with each line corresponding to a single individual's predicted RMR at each temperature. Individuals are coloured based on their RMR quartiles, compared with other experimental individuals, at 13ºC. Predictions are derived from a random regression model across 13ºC and 21ºC run using the full dataset incorporating control individuals (n= 60). For visualisation purposes, one individual that exhibited especially high scaled estimates at both temperatures (13ºC= 1.54, 21ºC= 3.76) is excluded from the plot.

4.5.2. The relationship between RMR and immersion response-time (IRT)

RMR was correlated with IRT, and the nature of this correlation was related to environmental temperature such that it did not always follow the assumptions of POLS. At 13ºC, shyer individuals (exhibiting longer IRTs) showed higher RMRs than bolder individuals, translating into a moderate, significant positive correlation between IRT and RMR at that temperature (r= 0.34, 95% CI= 0.03, 0.64; Figure 4.3a). At 21ºC, the pattern of this correlation was swapped, such that bolder individuals (with shorter IRTs) at either temperature exhibited higher RMRs at 21ºC than individuals that were shyer at either temperature (Figures 4.3.b, 4.3.d). This correlation, while still of interest, was not significant between IRT and RMR both measured at 21ºC (r= -0.29, 95% CI= -0.65, 0.06; Figure 4.3b). It was, however, at the bound of significance between IRT at 13ºC and RMR at 21ºC, as repeated runs of the model found the lower bound of the 95% credible interval to hover within 0.01 units either side of 0 (r= -0.34, 95% CI= -0.67, 0.00; Figure 4.3d). This indicates that individuals' IRTs at 13ºC were moderately predictive of their RMRs at 21ºC. It is worth noting that this significance may have been clearer but for the smaller effective sample size of the crossover dataset (n= 39). There was no relationship between RMR at 13ºC and IRT at 21ºC (r= 0.04, 95% CI= -0.34, 0.44; Figure 4.3c).

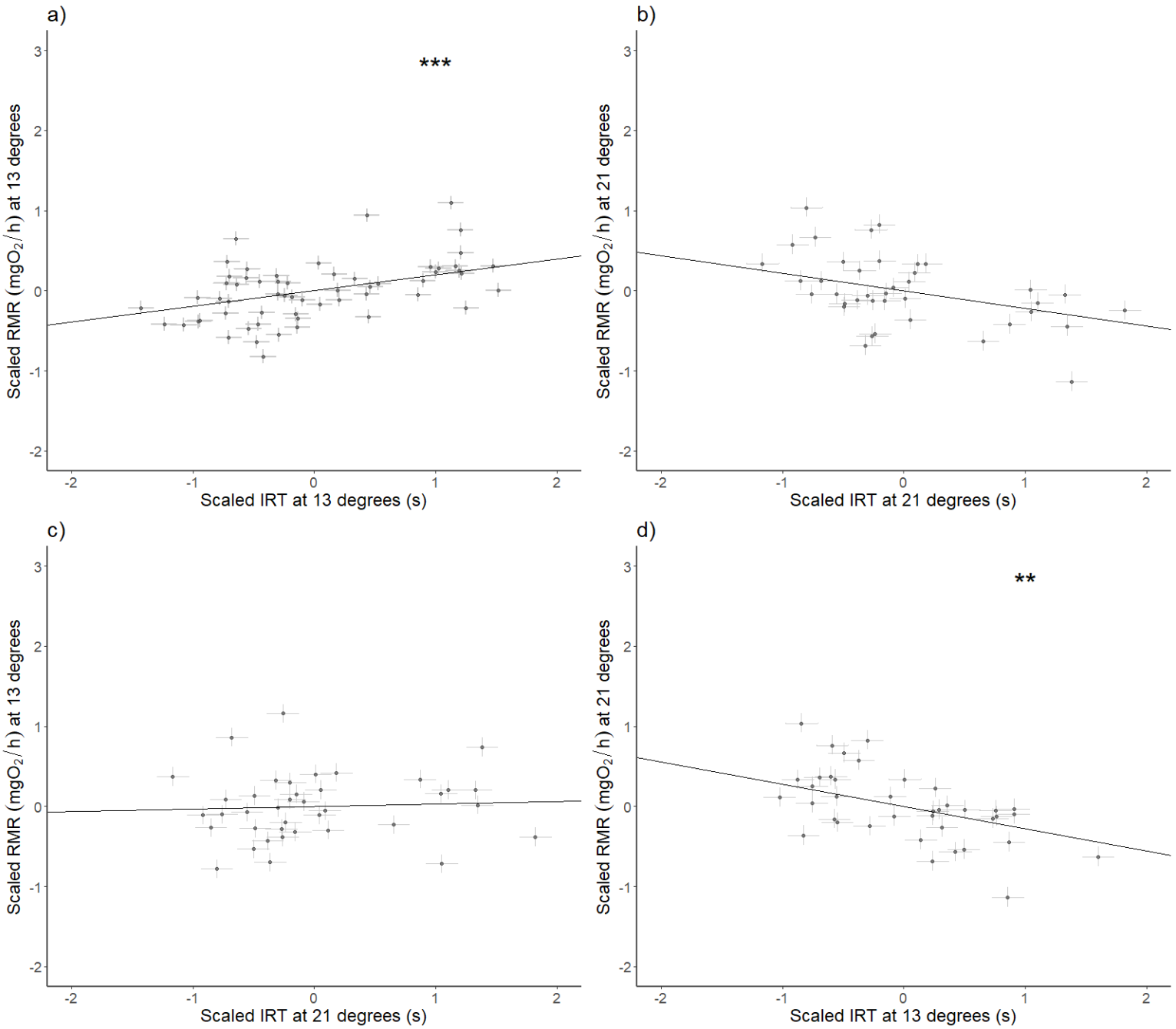


Figure 4.3. The relationship between individual level posterior mode estimates (Bayesian BLUPS) for z-transformed routine metabolic rate (RMR) and z-transformed immersion response-time (IRT) at a) 13ºC for both traits, b) 21ºC for both traits, c) 13ºC for RMR and 21ºC for IRT and d) 21ºC for RMR and 13ºC for IRT. "***" denotes a statistically significant relationship between the two traits. "**" denotes a relationship falling at the bound of statistical significance. Estimates are derived from bivariate (panel a) and multivariate (panels b,c and d) Bayesian mixed effects models. Error bars denote 95% credible intervals around each of the traits at each temperature.

4.6. DISCUSSION

As the climate warms and marine invertebrates experience extreme temperatures with increasing regularity (IPCC, 2013), the survival of individuals under the greatest metabolic demand at those temperatures may be placed in jeopardy (Montiglio et al., 2018). The pace-of-life syndrome hypothesis (POLS; Réale et al., 2010), which predicts positive correlations between boldness and metabolic rate, could be used to help indicate those individuals most vulnerable to warming, but how these correlations change when individuals are exposed to ecologically relevant heat stress has not been tested. In this study we found evidence in beadlet sea anemones for a complex association between routine metabolic rate (RMR) and immersion response-time (IRT) across temperatures, such that the correlations between the two did not follow the assumptions of POLS at 13ºC but did at 21ºC. We further showed that individual differences explained most of the variance in how RMR changed between temperatures. If individuals of certain personality-types are behaviourally or physiologically more sensitive to temperature stress, using more energy or experiencing reduced foraging opportunities or increased predation risk, then more regular heatwaves could put those individuals at a selective disadvantage.

Under baseline temperatures, shyer individuals exhibited higher RMRs, a result entirely at odds with POLS. One explanation for this could be that, at lower temperatures, shyer individuals were investing more than bolder individuals in metabolically costly processes such as growth. Although population-wide studies tend to find faster growth at warmer temperatures in marine invertebrates (reviewed in: Angilletta et al., 2004), this rule is not universal (Angilletta & Dunham, 2003). In *A. equina*, even very regular feeding regimens are not enough to counteract a loss of body-mass at high temperatures in laboratory environments (Chomsky et al., 2004b). At low temperatures, meanwhile, *A. equina* is able to grow even when fed only once or twice a week (Chomsky et al., 2004a). As such, while 24 hours of fasting should have eliminated the costs of digestion (Navarro et al., 1981), individuals likely still had flexibility in how they expended their energy at 13ºC. This flexibility could have provided the scope for different personality-types to employ different resource allocation strategies and for RMR to be positively correlated with growth (Killen, 2014). Shy individuals may thus have prioritised growth to a greater degree than bold individuals at low temperatures, both because of the greater flexibility in resource allocation that those temperatures afforded, and because low risks of predation from ectothermic predators at those temperatures (e.g. Twardochleb et al., 2020; Yamane & Gilman, 2009) should have

allowed them to forage more freely. Alternatively, the move from holding tanks to respirometry chambers, or the slight disturbance in the chambers caused by the intermittent-flow cycle itself, might have caused disproportionate stress responses in shyer animals as compared with their bolder conspecifics, driving their higher RMRs at 13ºC (e.g. Martins et al., 2011). Future studies could investigate the relationships between growth-rate, dry weight and IRT to see whether shy individuals do grow faster than bold individuals at baseline temperatures, and what implications this has for selection.

In contrast to baseline temperatures, individuals that were bolder at either temperature exhibited higher RMRs when under thermal stress than those that were shyer. One explanation for this could be shy individuals suppressing their RMRs to a greater degree than bold individuals at stressful high temperatures, in preparation for cooler "recovery periods" in the future (Schulte et al., 2011). Temperature-driven adaptive metabolic suppression (either limiting the increase or even decreasing metabolic rates rapidly by curbing processes with high energy requirements; Schulte et al., 2011) appears to be specific to intertidal and shallow-water marine invertebrates, having been so far documented in gastropods (McMahon et al., 1995) and molluscs (Vajedsamiei et al., 2021). Animals employing this strategy may be equipped during heatwaves to avoid their energetic demands exceeding their available energy supply but, as heatwaves become more frequent, suppression strategies could place individuals at a selective disadvantage by creating energetic deficits which they are unable to fulfil during progressively shorter recovery periods (Pörtner, 2012). Although they were of varying personality-types, those individuals suppressing their RMRs most in our sample could have been the four that failed to increase their RMRs at all at 21ºC (e.g. Vajedsamiei et al., 2021). It is also possible that these individuals had surpassed their metabolic thermal maximum (Schulte et al., 2011). Although the high RMRs of all four of these individuals at 13ºC might suggest this is plausible, it would seem unlikely that any of them had surpassed this threshold given that lethal temperatures for other UK populations of *A. equina* are at least two degrees higher than the 21ºC used in this experiment (Chapter 2).

The effects of individual and morphotypic variation on the plasticity of RMR could shed further light on its relationship with IRT. There was a general trend towards higher RMRs at 21ºC, which remained unexpectedly consistent between the two morphotypes. Importantly, however, as predicted, this response was not ubiquitous at the level of the individual, possibly indicating variation between individuals in strategies for dealing with extreme high temperatures. One potential explanation is that some individuals may compensate for

increased RMR induced by higher temperatures by reducing activity. This could have led to the observed negative correlation between IRT and RMR at 21^{o}C. After considering the available evidence, however, we believe that such a compensatory reduction in activity may be unlikely. Foraging tentacle expansion is the main energetically costly activity these anemones undertake (Griffiths, 1977b) and IRT is thus not only an excellent proxy for boldness but should also act as a proxy for activity. As such, if a change in activity was driving changing RMR, IRT at a baseline temperature should not have been more strongly correlated with RMR at 21^{o}C than IRT at 21^{o}C. Previous research also shows differences in how morphotypes change their IRTs, and thus their activity, across temperatures (Chapter 2). These discrepancies were not reflected in morphotypic patterns of RMR in these data, further indicating that changing activity was not driving changing RMR. Instead, it seems more likely that relationships between IRT and RMR were at least partly driven by individual physiology, either via varied growth strategies at baseline and RMR suppression at high temperatures as we speculate, or by other mechanisms.

Although these data indicate that individual variation was the key predictor of how RMR changed across temperatures and suggest that individual plasticity of RMR was associated with individual plasticity of IRT, it is also possible that these results could be indicative of important variables that we failed to measure. For example, the previous experience of these anemones, for which it was impossible to control, could have affected these results. Some animals might have been significantly more stressed than others before being transferred to the laboratory, and this could have led to unexpected patterns in both their RMRs and their IRTs. It is also worth noting here the significant effect of data collection block, which could have been indicative of early metabolic acclimation to environmental seasonality (Ortega et al., 1984), another effect that may not have been fully captured by these data.

This study shows that associations between RMR and boldness in *A. equina* are highly temperature-dependent. Individual differences were the key drivers of how RMR changed between temperatures, with some individuals likely to experience higher metabolic demand at high temperatures than others. Further, these results indicate that individual variation in trait plasticity could be part of the reason why evidence for POLS is so inconsistent. By measuring the boldness and metabolism of animals living in heterogeneous environments under different contexts, researchers could draw a clearer picture of POLS, facilitate a more comprehensive understanding of the adaptive value of different personality-types under climate change, and

gain insights into how different populations might respond to more regular climate change-induced heatwaves.

4.7. ACKNOWLEDGEMENTS

Thanks to Leslie Connor for technical support with the muffle furnace, to Guillermo Garcia-Gomez, Ruth Dunn, and Curtis Horne, who assisted with anemone collection, and to Dr Matthew Spencer for invaluable assistance setting up the 21^{o}C temperature control. DKM was funded by a NERC ACCE PhD studentship (ref: 1950009) and Blue Planet Aquarium.

APPENDIX TO CHAPTER 4: Differential metabolic responses in bold and shy sea anemones during a simulated heatwave

A4.A. Metabolic apparatus and measurement detail

Automated intermittent-flow cycles were precisely controlled by a National Instruments C Series Counter Input Module (National Instruments, Austin, USA). Water in the respirometry tank was fully aerated throughout testing by a Tetra Second Nature Whisper 800 air pump (Tetra GMBH, Melle, Germany). Each chamber contained a central 6 cm x 6 cm x 6 cm polypropylene mesh cage containing a magnetic stir bar, which was controlled by an IKAMAG multi-position magnetic stirrer plate (IKA England LTD, Oxford, UK) positioned under the respirometry tank. For the ambient 13^{o}C measurements, the temperature within the respirometry tank was maintained to a precision of ±0.2^{o}C by the laboratory's temperature control. At 21^{o}C, the temperature of the tank was maintained to a precision of ±0.3^{o}C; a waterproof DS1820B temperature sensor (Maxim Integrated Products, Sunnyvale, USA) was connected to an Arduino Uno (Arduino LLC, Boston, USA) and used to control a LightwaveRF wireless control plug socket (LightwaveRF, Birmingham, UK) which was itself connected to an Eheim thermocontrol 300W heater (Eheim, Deizisau, Germany) submerged in the tank. During experimentation, the temperature in the respirometry tank was recorded at half hour intervals, taking measurements directly from the temperature sensor using the serial monitor within the Arduino software. Oxygen concentration, and by extension, oxygen consumption, was measured at second intervals using two PyroScience Firesting O_2 dissolved oxygen sensors (PyroScience GmbH, Aachen, Germany) each connected to three PyroScience 3mm diameter robust oxygen probes fed directly into chambers. Probes were calibrated at the start of each day of metabolic testing. See figures A4.1, A4.2, and A4.3 for pictures of metabolic apparatus, and table A4.1 for a full checklist of information on intermittent-flow methodology.

Figure A4.1. Overhead view of apparatus, including 6 intermittent-flow pumps and a chiller pump in the top right corner.

Figure A4.2. Side view of 2 open respirometry chambers, showing external seals and piping.

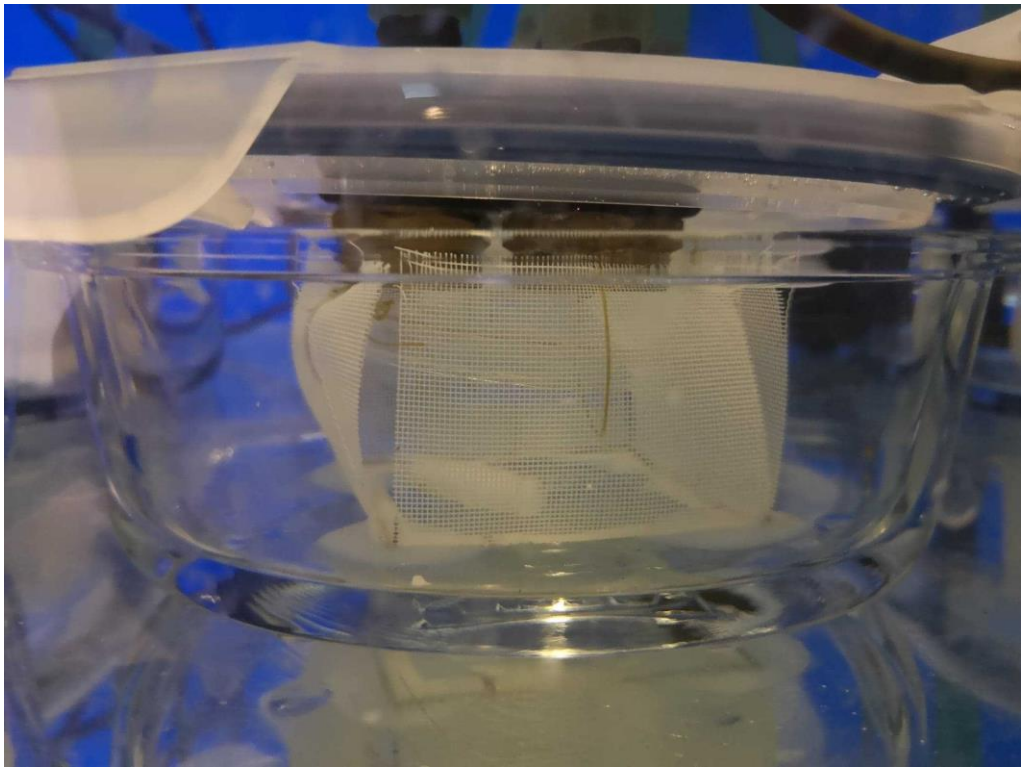


Figure A4.3. Close up of a single chamber, showing brass internal seals, mesh cage and magnetic stirrer.

Table A4.1. Full checklist of information on aquatic respirometry methods (from: Killen et al., 2021).

Number	Criterion and Category	Response	Value (where required)	Units
	EQUIPMENT, MATERIALS, AND SET-UP			
1	Body mass of animals at time of respirometry	Pedal disc diameter (PDD) threshold used as no reliable, non-invasive measure of anemone volume	20	mm
2	Volume of empty respirometers	422.5 ml		
3	How chamber mixing was achieved	Magnetic stirrer		
4	Ratio of net respirometer volume (plus any associated tubing in mixing circuit) to animal body mass	N/a, no reliable measure of live anemone volume so threshold PDD used		
5	Material of tubing used in any mixing circuit	N/a		
6	Volume of tubing in any mixing circuit	N/a		
7	Confirm volume of tubing in any mixing circuit was included in calculations of oxygen uptake	N/a		
8	Material of respirometer (e.g. glass, acrylic, etc.)	Glass, Polypropylene lid		
9	Type of oxygen probe and data recording	Pyroscience Firesting O_2		
10	Sampling frequency of water dissolved oxygen	1s		
11	Describe placement of oxygen probe (in mixing circuit or directly in chamber)	Directly		
12	Flow rate during flushing and recirculation, or confirm that chamber returned to normoxia during flushing	Chamber returned to normoxia (Appendix A4.C.)		
13	Timing of flush/closed cycles	3 minutes / 38 minutes		
14	Wait (delay) time excluded from closed measurement cycles	>60s		
15	Frequency and method of probe calibration (for both 0 and 100% calibrations)	Daily, calibrated to 100% dissolved oxygen saturation		

16	State whether software temperature compensation was used during recording of water oxygen concentration	Yes, standardised to temperature of treatment, not to real-time fluctuation		
	MEASUREMENT CONDITIONS			
17	Temperature during respirometry	Variable	13/21	^{o}C
18	How temperature was controlled	Arduino	±0.3	^{o}C
19	Photoperiod during respirometry	12:12 h		
20	If (and how) ambient water bath was cleaned and aerated during measurement of oxygen uptake (e.g. filtration, periodic or continuous water changes)	Periodic water changes and bleach sterilisation. Air stone for aeration.		
21	Total volume of ambient water bath and any associated reservoirs	61640 cm^3 (67 cm x 46 cm x 20 cm)		
22	Minimum water oxygen dissolved oxygen reached during closed phases	>80%		
23	State whether chambers were visually shielded from external disturbance	Yes, opaque shield around water bath		
24	How many animals were measured during a given respirometry trial (i.e. how many animals were in the same water bath)	Five		
25	If multiple animals were measured simultaneously, state whether they were able to see each other during measurements	N/a		
26	Duration of animal fasting before placement in respirometer	>24h		
27	Duration of all trials combined (number of days to measure all animals in the study)	24 days		
28	Acclimation time to the laboratory (or time since capture for field studies) before respirometry measurements	48 h x 2		
	BACKGROUND RESPIRATION			
29	Whether background microbial respiration was measured and accounted for, and if so, method used (e.g. parallel measures with empty respirometry chamber, measurements before and after for all chambers while empty, both)	Yes, parallel measures		

30	If background respiration was measured at beginning and/or end, state how many slopes and for what duration	N/a
31	How changes in background respiration were modelled over time (e.g. linear, exponential, parallel measures)	Parallel measures
32	Level of background respiration (e.g. as a percentage of SMR)	Temperature and trial-dependent
33	Method and frequency of system cleaning (e.g. system bleached between each trial, UV lamp)	System and chambers bleached between each trial
	STANDARD OR ROUTINE METABOLIC RATE	
34	Acclimation time after transfer to chamber, or alternatively, time to reach beginning of metabolic rate measurements after introduction to chamber	12 h
35	Duration over which metabolic rate was estimated	38 minutes x 6
36	Value taken as SMR/RMR (e.g. quantile, mean of lowest 10 percent, mean of all values)	N/a, individual slopes recorded for analyses
37	Total number of slopes measured and used to derive metabolic rate (e.g. how much data were used to calculate quantiles)	N/a, individual slopes recorded for analyses
38	Whether any time periods were removed from calculations of SMR/RMR (e.g. data during acclimation, periods of high activity [e.g. daytime])	No data were recorded during acclimation / attachment
39	r^2 threshold for slopes used for SMR/RMR (or mean)	0.9
40	Proportion of data removed due to being outliers below r-squared threshold	0%
	MAXIMUM METABOLIC RATE	
41	When MMR was measured in relation to SMR (i.e. before or after)	N/a

42	Method used (e.g. critical swimming speed respirometry, swim to exhaustion in swim tunnel, or chase to exhaustion)	N/a
43	Value taken as MMR (e.g. the highest rate of oxygen uptake value after transfer, average of highest values)	N/a
44	If MMR measured post-exhaustion, length of activity challenge or chase (e.g. 2 min, until exhaustion, etc.)	N/a
45	If MMR measured post-exhaustion, state whether further air-exposure was added after exercise	N/a
46	If MMR measured post-exhaustion, time until transfer to chamber after exhaustion or time to start of oxygen uptake recording	N/a
47	Duration of slopes used to calculate MMR (e.g. 1 min, 5 min, etc.)	N/a
48	Slope estimation method for MMR (e.g. rolling regression, sequential discrete time frames)	N/a
49	How absolute aerobic scope and/or factorial aerobic scope is calculated (i.e. using raw SMR and MMR, allometrically mass-adjusted SMR and MMR, or allometrically mass-adjusting aerobic scope itself)	N/a
	DATA HANDLING AND STATISTICS	
50	Sample size	60 overall, 20 control, 40 crossed-over
51	How oxygen uptake rates were calculated (software or script, equation, units, etc.)	respr R package followed by conversions to mgO_2/h
52	Confirm that volume (mass) of animal was subtracted from respirometer volume when calculating oxygen uptake rates	No, size was standardised to PDD as far as possible
53	State whether analyses accounted for variation in body mass and describe any allometric mass-corrections or adjustments	Yes, all analyses incorporated dry weight as a fixed effect

A4.B. Experimental schedule

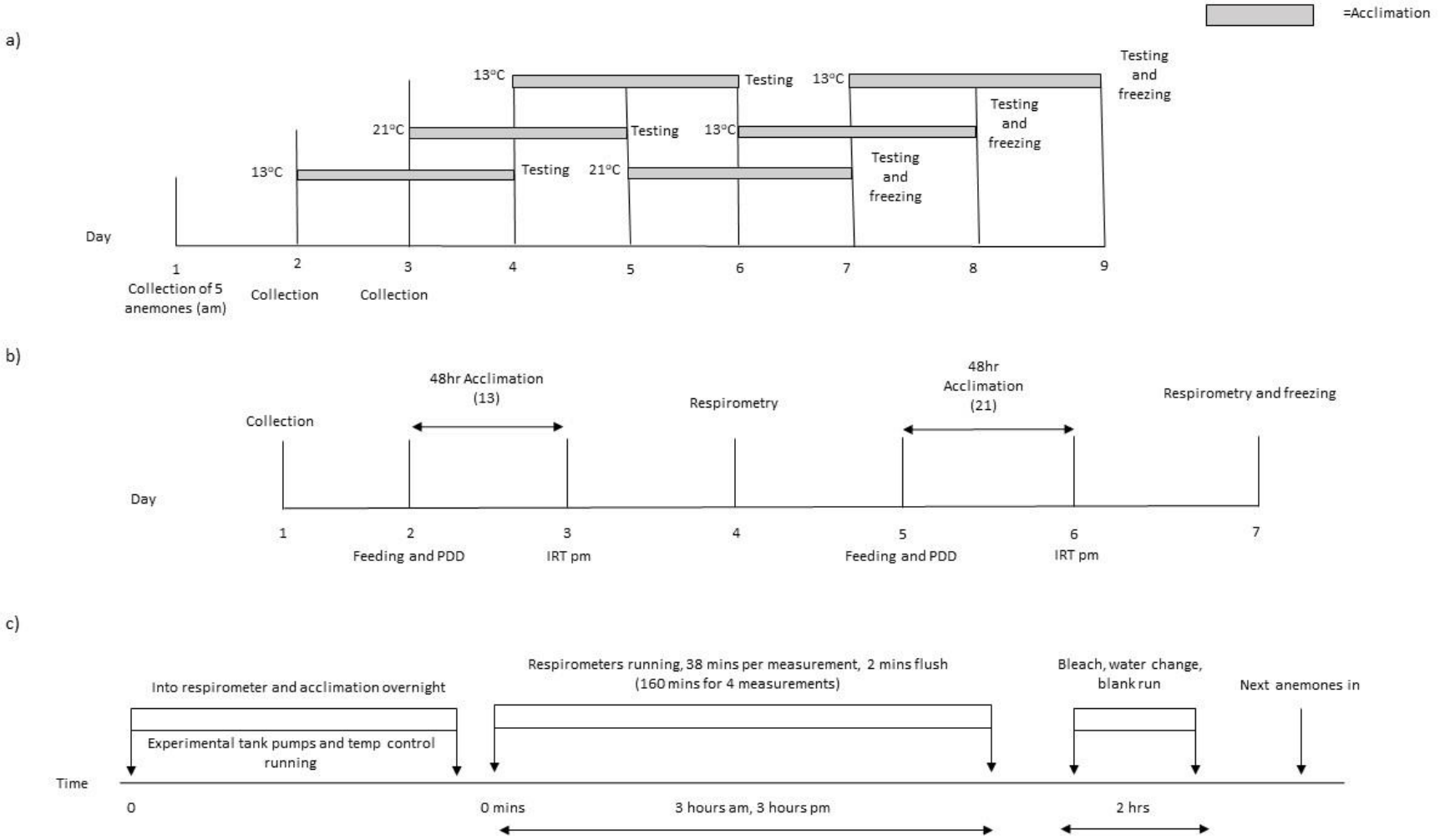


Figure A4.4. Showing a) A full example block schedule, b) the schedule for an L-H group (kept initially at 13ºC and then changed to 21ºC on day 4) and c) an example respirometry schedule.

A4.C. Example slopes and conversion

Raw oxygen concentration was measured in % O_2 concentration, and chamber volume less the volume of the magnetic stirrers was 422.5 ml. Because wet weight and anemone volume could not be reliably measured, the volume of anemones was not corrected for. Initial slopes were thus measured in % O_2/s/422.5 ml. These were first converted, using temperature-specific concentration conversion tables (Boyd & Pillai, 1985), to mgO_2/s/422.5 ml. These values were then multiplied by 3600 to give the slopes in mgO_2/h/422.5 ml, before being further multiplied by 0.4225 to give the values in mgO_2/h.

In the majority of measured slopes of oxygen consumption a steady, linear decline in oxygen concentration was observed after an initial wait period of at least one minute (Svendsen et al., 2016; Figure A4.5), but there were some instances where slopes showed more noise. The respR package provides mitigation for this by calculating rolling regressions (Harianto et al., 2019), allowing accurate slope estimation even with a substantial degree of noise in the data around the gradient of the slope (Figure A4.6). In some instances, there were periods of noise which influenced the results of the rolling regression (Figure A4.7). In these instances, where possible, the longest, most stable, periods of linear decline of oxygen concentration were used in slope calculations (White et al., 2016). Where this was not possible, the slope was discounted from the dataset. One individual, from the H-L treatment and of the green morphotype, was discounted from all analyses as it showed no measurable slopes at 21^{o}C. With this individual removed, of 826 oxygen consumption measurements (including blanks), 42 were measured using substantially truncated slopes. Of 708 anemone consumption measurements, nine were discounted from analyses.

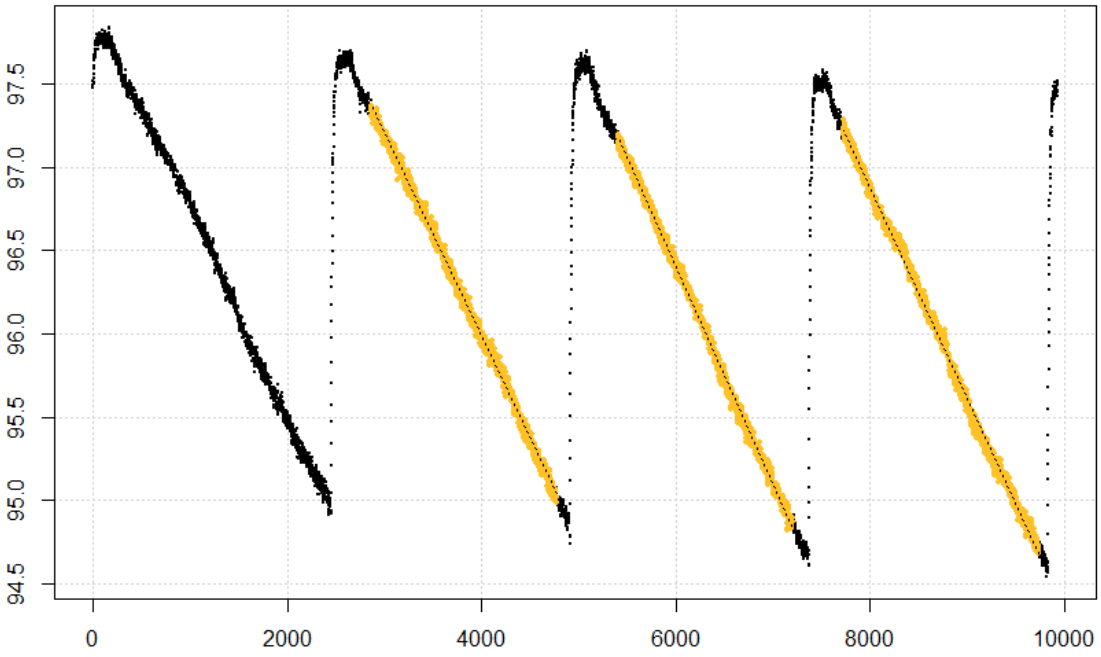


Figure A4.5. Example of metabolic slopes with no fluctuation or truncation.

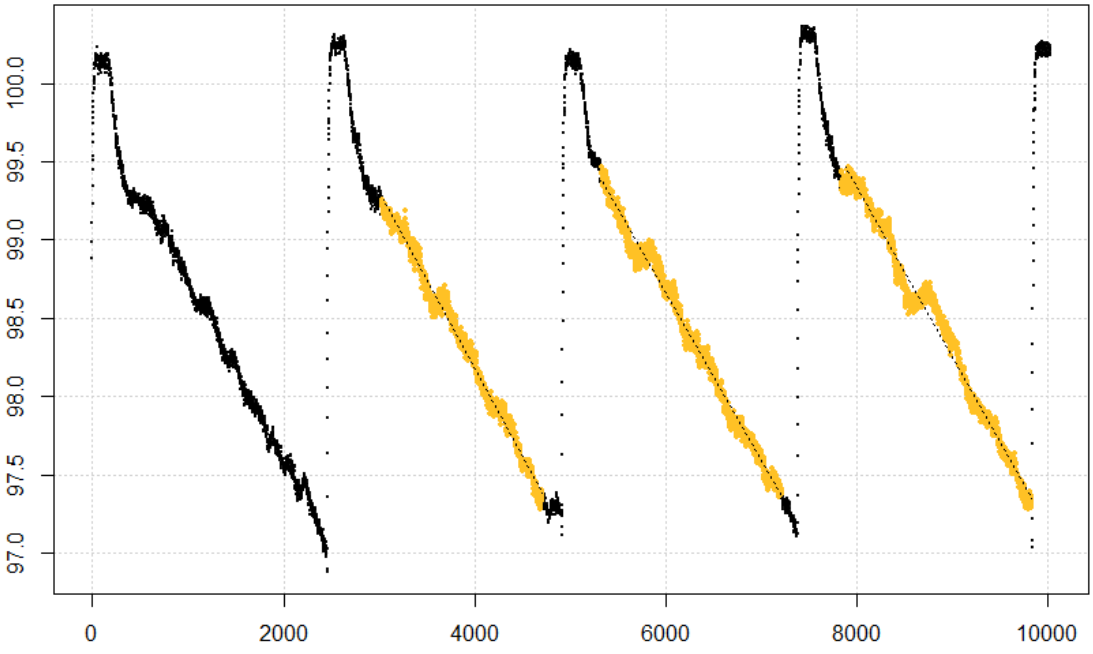


Figure A4.6. Example of metabolic slopes where fluctuations could be dealt with by rolling regression.

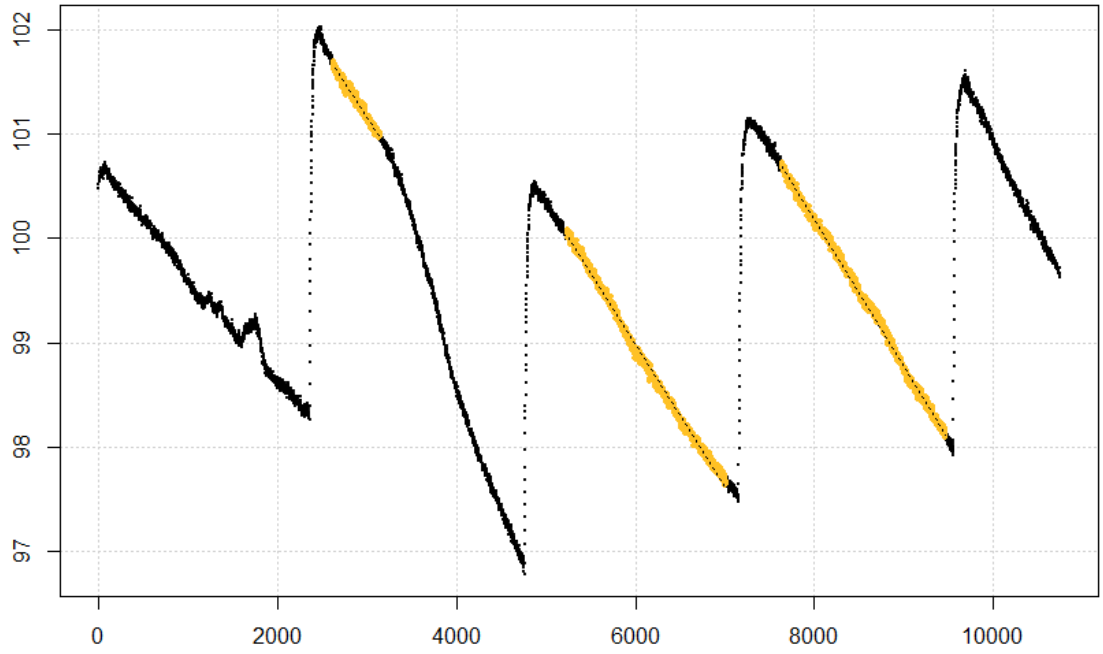


Figure A4.7. Example of a metabolic slope where a large degree of truncation was required.

A4.D. Statistical detail and extra analysis

Model Specification

Univariate models were fitted over uninformative Inverse-Wishart priors and run for 420000 iterations with a 20000-iteration initial burn-in period and a thinning interval of 100. Bivariate and multivariate models were fit similarly, but over 1000000 iterations with a 50000-iteration burn-in. To ensure successful convergence visual inspection of convergence and autocorrelation plots was carried out. To further confirm this, Heidelberger-Welch and Gelman-Rubin diagnostic tests were run on all models. Comparative models were also run over alternative, parameter-expanded priors, to ensure robustness to different prior specifications. Model estimates and deviance-information criteria (DIC; Spiegelhalter et al., 2002) did not differ meaningfully between the different specifications.

Metabolic plasticity model choice: random slopes

Two models were initially run on our full dataset. A random intercepts model, not taking into account individual level variation in metabolic plasticity to temperature, and a random slopes model. Once assumptions and convergence of both models had been checked, the two

models' DIC values (Spiegelhalter et al., 2002) were compared. The DIC of the random slopes model (DIC= 666) was dramatically lower than that of the random intercepts model (DIC= 1110), indicating very clearly that the former, incorporating a random slope effect, was a better fit to the data than the latter.

Covariance analysis split

The overall covariance analysis was split into a bivariate and a multivariate component to allow the incorporation of the control data into the 13ºC correlation estimate. The crossed-over nature of the multivariate dataset means that the multivariate model was still robust to both treatment order and temporal effects.

The relationship between RMR and dry weight

To explore the relationship between body size and RMR, a further bivariate model was run where RMR and dry weight were set as the response variables. Fixed and random effects were the same as the bivariate model above but with temperature set as an extra fixed effect. This confirmed a strong positive correlation between RMR and dry weight (r= 0.72, 95% CI= 0.57, 0.87; Figure A4.8). The magnitude of the correlation found here is in line with previous work in *A. equina* (Navarro et al., 1981) and also indicates that RMR in this species, when investigated in relation to dry weight, follows a standard scaling relationship (Brown et al., 2004).

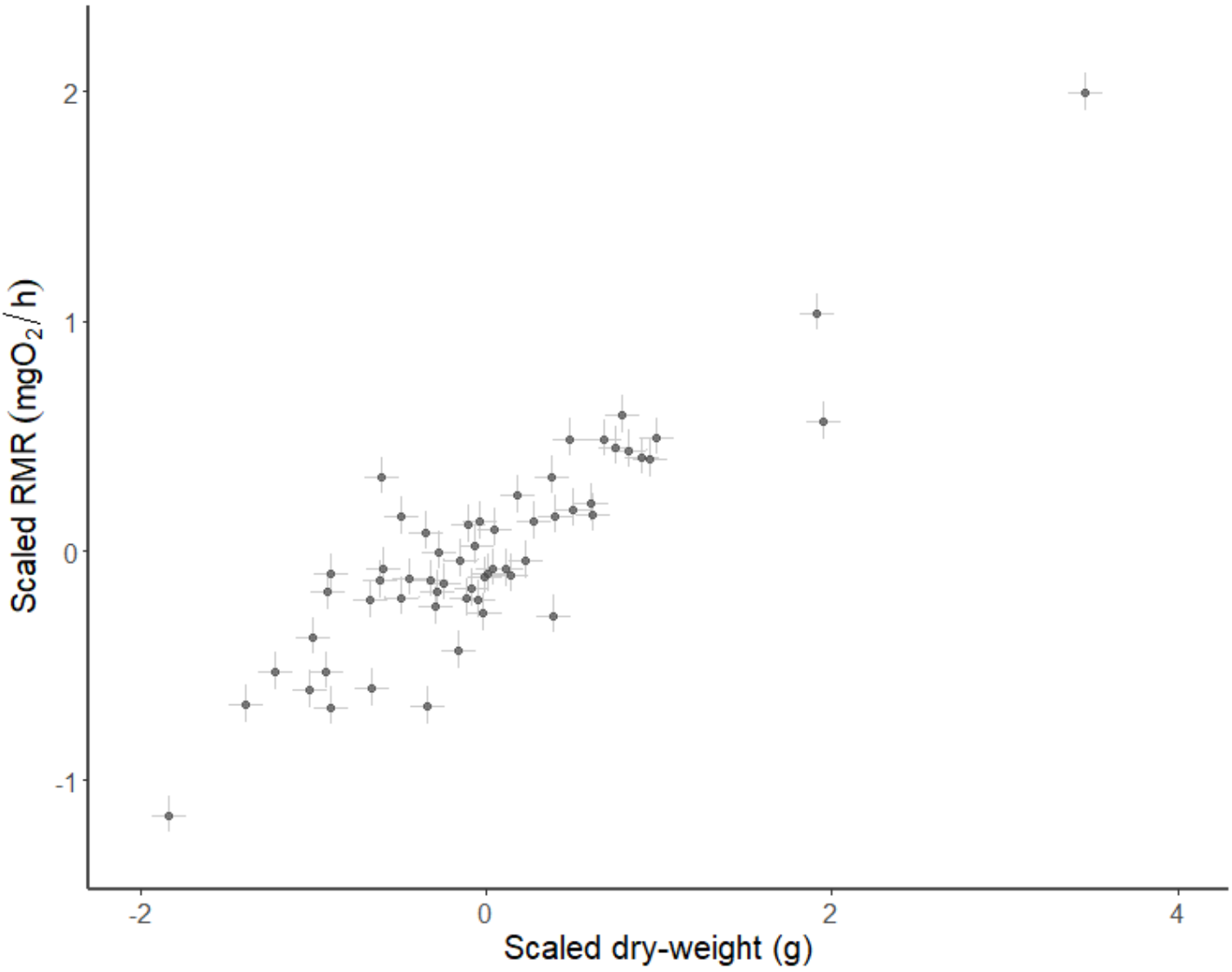


Figure A4.8. The relationship between the posterior mode estimates (Bayesian BLUPS) of individual RMR and individual dry weight, derived from a bivariate mixed effects model run on the full dataset (n= 60).

Chapter 5:

Different proteomic responses to temperature stress in bold and shy beadlet sea anemones

[Page intentionally left blank]

5.1. KEYWORDS

Climate change, Boldness, Proteomics, Coping Styles, Stress, Marine invertebrate

5.2. ABSTRACT

Behavioural, physiological, and molecular changes are all important mechanisms by which animals can mitigate against human-induced environmental perturbation. Proteomic changes, representing alterations to the active components of an animal's cells, are a particularly crucial mechanism with which to respond to environmental challenge. Between-individual variation in proteomic expression can be associated with individual behavioural consistency, termed personality, but investigations of the whole proteomes of animals of different personality-types in relation to their behavioural stress responses remain sparse. Here, in the first such study in an invertebrate, we assessed variation in the proteomes of bold and shy beadlet anemones (*Actinia equina*) under different thermal stress scenarios. We exposed some anemones to a graduated temperature increase, culminating at a near (sub)-lethal high temperature (23^{o}C), and maintained others at a baseline temperature (11^{o}C). We repeatedly measured anemones' startle response-times, their latency to re-extend their feeding tentacles after a disturbance, in both treatments at 11^{o}C, and took tentacle samples from the boldest and shyest animals at the conclusion of each treatment. We found a strong proteomic temperature response in *A. equina*, with individuals down-regulating biosynthetic proteins and apoptotic pathways, in favour of up-regulating proteins involved with stress resistance. We further found a whole-proteome effect of personality-type which was only apparent under heat stress: shy animals down-regulated biosynthetic pathways to a greater degree than bold animals and showed less up-regulation of proteins associated with cellular maintenance, proliferation, and stress resistance. As such, under extreme temperatures, shy individuals may suppress energetically costly cellular processes, thus saving resources to use under less stressful future environmental conditions. Contrastingly, bold individuals might maintain a greater degree of cellular function by up-regulating proteins involved in stress resistance. Both of these strategies could have their own adaptive value but, under increasingly regular exposure to high temperatures of the type which might be brought about by climate change, some personality-types may be placed under greater selective pressure than others.

5.3. INTRODUCTION

Animal personality, consistent variation between individuals in their behaviour within and across contexts, is a well-established, replicable, and measurable phenomenon (Dingemanse & Dochtermann, 2013; Sih et al., 2004). However, despite much interest in the adaptive underpinnings of personality (Duckworth, 2010; Moiron et al., 2020; Wolf & Weissing, 2010), the processes driving, or being driven by, consistent differences in behaviour, remain an important and poorly understood area of study. The active molecular components of an animal are essential factors in the induction and control of all its biological processes (Torson et al., 2020), including its behaviour (Gosling, 2008). As such, scientists cannot hope to understand the selective drivers underpinning personality variation without marrying the proximate cause of that variation, molecular differences, with behaviour. One hypothesis that attempts to address this is termed coping styles (Koolhaas et al., 1999). Coping styles place different personality-types, specifically risk-takers (bold animals) and risk-avoiders (shy animals), along a 'proactive-reactive' stress response axis (Coppens et al., 2010). Proactive individuals should show less pronounced molecular stress responses, bolder behaviours, and reduced behavioural flexibility, while reactive individuals should show the opposite (Geffroy et al., 2020; Wong et al., 2019). This intraspecific variation within groups should drive individuals within a population to respond differently to acute or chronic stressors such as those associated with climate change.

Although investigations of coping styles indicate associations between behaviour and molecular physiology across a range of species (Castanheira et al., 2017; Cockrem, 2007; Costantini et al., 2012; Ferrari et al., 2013), much of the work that has been carried out thus far has focused on specific neuroendocrine differences (e.g. Houslay et al., 2018; Wong et al., 2019). While these responses are useful to investigate, complex neuroendocrine pathways are not present in all taxa (Hartenstein, 2006; Malagoli & Ottaviani, 2017). As such, investigations of the relationships between behaviour and molecular physiology have largely been focused on vertebrates (Fürtbauer, 2015). Further, neuroendocrine responses alone may not offer a complete picture of an animal's stress response, often acting as one of many stress-induced molecular changes (Gormally & Romero, 2020). In chickens (*Gallus gallus domesticus*), for example, chronic stress drives increases in both corticosterone and heat shock protein (HSP) expression, and both molecular indicators are associated with personality (Pusch et al., 2018). In catfish (*Horabagrus brachysoma*), meanwhile, heat stress leads to the induction of multiple layers of molecular change, including changes to

gluconeogenesis (and, by extension, blood cortisol levels), increased expression of proteins associated with metabolic processes, and increased HSP expression (Dalvi et al., 2017). Whole-transcriptome or whole-proteome approaches could thus provide a more comprehensive picture of the associations between molecular stress responses and personality (Sneddon et al., 2005). Studies such as these have recently begun to receive increased interest in some taxa (Ferrari et al., 2020; Nonnis et al., 2021; Rey et al., 2013; Rey et al., 2021; Thomson et al., 2011; Toni et al., 2019), but have yet to be conducted in any invertebrate with a simpler body plan and divergent life-history. Considering proteomic changes in particular, given the direct effects these have on cellular processes and homeostasis (Feder & Walser, 2005; Tomanek, 2011), offers the potential to significantly broaden understanding of the interplay between molecular and behavioural stress responses. In turn, this could help predict whether some personality-types, and by extension some populations (Tomanek, 2010; Tomanek, 2008), might be more susceptible to novel stressors brought about by human-induced environmental change.

Intertidal invertebrates are at particular risk from novel climatic stressors as the highly stochastic environment (Brahim et al., 2019; Chapperon et al., 2016) which they inhabit is already regularly exposed to environmental extremes, particularly during tidal exposure (Harley et al., 2006; Helmuth et al., 2006; Scapini et al., 2019). One of the clearest changes being caused by climatic shifts is a significant rise in environmental temperatures (IPCC, 2018). This rise is especially apparent in marine environments (Doney et al., 2012) and, as it continues, not only are intertidal populations experiencing chronically higher environmental temperatures (Hoegh-Guldberg & Bruno, 2010), but also increasingly regular periods of acute thermal stress in the form of heatwaves (Weitzman et al., 2021). Heatwaves, such as those recently experienced in Canada (Ross et al., 2021), can place intertidal populations under severely heightened physiological stress (e.g. Hemraj et al., 2020; Vajedsamiei et al., 2021) and cause some individuals to perish (e.g. Garrabou et al., 2009; Seuront et al., 2019). Intertidal invertebrates can exhibit a host of proteomic responses to mitigate against these effects. Amongst other changes, they have been shown to up-regulate stress-responsive molecules such as heat-shock proteins (HSPs; Choresh et al., 2007; Dong et al., 2008; Ravaux et al., 2016), increase or regulate apoptotic activity (Gleason & Burton, 2015; Richier et al., 2006), or change the expression of proteins forming complex structures (Ning et al., 2021; Teranishi & Stillman, 2007). These structures include ribosomes and spliceosomes, which are involved in protein biosynthesis (Matera & Wang, 2014; Moore & Steitz, 2002),

and proteasomes, whose primary function lies in proteolytic pathways (Sorokin et al., 2009). Many intertidal taxa also show adaptive behavioural responses to temperature stress (Crickenberger et al., 2020) which vary between individuals (Biro et al., 2013; Briffa et al., 2013; Scapini et al., 2019), but no work has yet sought to marry behavioural and proteomic thermal stress responses in any intertidal invertebrate.

The beadlet anemone (*Actinia equina*) is an ideal organism with which to address this gap in the literature. This species is both particularly robust to temperature change, with thermal tolerances that can span ranges of 20 degrees or more (Griffiths, 1977b), and displays within-population variation in behavioural and physiological responses to heat stress (Navarro et al., 1981; Chapter 2; Chapter 4). Closely related anemone species also show varied proteomic responses at high temperatures (Choresh et al., 2007; Richier et al., 2006, 2008) and genetically determined thermal tolerances (Rivera et al., 2021), so associations between these responses and individual behaviour could have important selective implications in *A. equina*. For instance, bold anemones show little plasticity in their startle response-times (SRTs, a proxy for boldness measuring the time for an anemone to re-extend its feeding tentacles after a threatening stimulus; Briffa & Greenaway, 2011) when exposed to an extreme temperature (Chapter 2). This rigidity could be energetically beneficial as it provides more foraging opportunities (Griffiths, 1977b) under increased metabolic demand (Navarro et al., 1981), but could equally prove costly if higher temperatures lead to increased pressure from ectothermic predators (e.g. Twardochleb et al., 2020; Yamane & Gilman, 2009). If bold individuals show generally low phenotypic plasticity in response to temperature stress, they might show less pronounced up-regulation of stress-mitigating molecules such as HSPs (e.g. Pusch et al., 2018). In this scenario, pressures to maintain higher rates of biosynthetic and metabolic processes to repair damage caused by thermal stress (e.g. Ning et al., 2021; Richier et al., 2008; Vajedsamiei et al., 2021) could lead bold animals to maximise their time spent foraging out of energetic necessity, despite potential selective costs. Insights such as these into the interplay between molecular and behavioural stress responses in *A. equina* could, in turn, help inform investigators when examining climatic effects on intertidal invertebrates more generally.

In this study, we examined bold and shy (measured as SRT) *A. equina* individuals under two different temperature treatments. Half of the individuals of each personality-type were exposed to a graduated temperature-increase, culminating at a near-lethal temperature, while the other half remained at a baseline. We took tentacle samples for proteomic analysis from

each individual and examined how proteomic expression was related to both temperature treatment and personality-type. We expected that expression would differ significantly between hot and cold treatments. We predicted that all individuals in the hot treatment would exhibit changes in the expression of proteins related to biosynthetic processes and apoptotic pathways and would up-regulate stress resistance proteins, including HSPs. We further expected that these discrepancies would be influenced by personality-type, predicting that bold individuals would show less pronounced proteomic changes under temperature stress than shy individuals.

5.4. METHODS

5.4.1. Experimental design

Collection, housing and behaviour

Data collection took place between April and August 2018. Anemones were selected from a behavioural study detailed fully in Chapter 2. In brief, anemones were subject to either a 17-day graduated temperature increase from 11^{o}C to 23^{o}C (the temperature stress treatment) or spent 17 days at 11^{o}C (the control treatment). Behavioural trials were carried out for the first 15 days and proteomic sampling occurred on day 17. Anemones were selected for proteomic analysis based on the average of three startle response-times (SRTs), a measure of boldness using latency to extend feeding tentacles after a fright (Briffa & Greenaway, 2011; Collins et al., 2017), measured at 11^{o}C. Six of the boldest (shortest SRT) and six of the shyest (longest SRT) individuals were selected from separate pools of 101 in the temperature stress treatment and 108 in the control treatment, leading to an overall sample size of 24 animals. The day after proteomic sampling, anemones were returned to their collection site at Llandudno North Beach, North Wales (lat: 53.330359, long: -3.828975).

Tentacle Sampling

Before tentacle sampling for proteomic analysis commenced, individuals were first fully emersed from seawater. This took approximately 5 minutes and caused minimal disturbance to individuals. Anemones were then immediately (< 1 minute) immersed in 3 litres of 2% magnesium chloride ($MgCl_2$) solution and left to become fully anaesthetised for 30 minutes before sampling began.

For each anemone, 2 tentacle samples were taken using sterilised forceps and scissors. Upon collection, samples were placed in sterile, labelled Eppendorfs and flash-frozen immediately

in liquid nitrogen. Once sampling was complete, all tentacles were transferred to a -80ºC freezer, where they were stored for between three to five months prior to proteomic analysis. Sampling time was approximately 5 minutes per anemone and sampling order was randomised within bold and shy personality-types. For ease of identification, shy animals were sampled first and bold animals second. As all animals were fully anaesthetised, this standardisation should have had no impact on their stress levels at the time of sampling, or on their proteomic expression.

5.4.2. Protein Identification

Proteomics sample preparation

The 24 tentacle sections were removed from the -80ºC freezer and transferred to a 0.5 ml bead beating tube containing 1.4 mm ceramic beads and homogenised at 5,000 rpm for 30 s using a Minilys homogeniser (Bertin Technologies, Montigny-le-Brettoneux, France).

A protein assay was performed using a 75-fold dilution of sample (5 µl homogenate diluted to 375 µl). A volume of sample equivalent to 20 µg of protein was transferred to 0.65 ml low bind tubes and a balance volume of 25 mM ammonium bicarbonate buffer (ambic) added. 2.5 µl of freshly reconstituted 1%(w/v) Rapigest surfactant (Waters Ltd., Wilmslow, UK) was added and the samples incubated at 80°C for 10 minutes. Samples were reduced by the addition of 2.5 µl of 9.2 mg/ml dithiothreitol (DTT) in 25 mM ambic and incubated at 60°C for 10 minutes. Alkylation of cysteine residues was carried out by the addition of 2.5 µl of 33 mg/ml iodoacetamide followed by incubation in the dark at room temperature for 30 minutes. 2.5 µl of freshly reconstituted trypsin was added (0.5 µg) and samples incubated in a final digestion volume of 50 µl overnight at 37°C.

The following day the 24 digests were acidified by the addition of 0.5 µl of TFA and incubated at 37°C for 45 minutes to hydrolyse the Rapigest surfactant. Digests were centrifuged at 17,200 x g for 30 minutes and the clarified supernatants were transferred to fresh tubes. A 10-fold dilution of two digests in 0.1% TFA/3% ACN and analysis on a 30-minute LC-MS/MS gradient was carried out for sample ranging to determine final loading. Digests were transferred to total recovery vials (Waters, Wilmslow, UK) and 4 µl was injected on-column in a randomly assigned order (Appendix A5.A.).

LC-MS/MS analysis

Data-dependent LC-MSMS analyses were conducted on a Q Exactive quadrupole-Orbitrap mass spectrometer (Thermo Fisher, Waltham, MA, USA) coupled to a Dionex Ultimate 3000 RSLC nano-liquid chromatograph (Sunnyvale, CA, USA). Sample digest (4 μl) was loaded onto an Acclaim PepMap 100 C18 trapping column (Thermo Fisher, Waltham, MA, USA; 75 μm x 2 cm, 3 μm packing material, 100 Å) using a loading buffer of 0.1% (v/v) TFA, 2% (v/v) acetonitrile in water for 7 minutes at a flow rate of 9 μl/minute. The trapping column was then set in-line with an EASY-Spray PepMap RSLC C18 analytical column (Thermo Fisher, Waltham, MA, USA; 75 μm x 50 cm, 2 μm packing material, 100 Å) and the peptides eluted using a linear gradient of 96.2% A (0.1 % [v/v] formic acid):3.8% B (0.1% [v/v[formic acid in water:acetonitrile [80:20] [v/v]) to 50% A:50% B over 55 minutes at a flow rate of 300 nl/min, followed by washing at 1% A:99% B for 6 minutes and re-equilibration of the column to starting conditions. The column was maintained at 40^{o}C, and the effluent introduced directly into the integrated nano-electrospray ionisation source operating in positive ion mode. The mass spectrometer was operated in DDA mode with survey scans between *m*/*z* 300-2000 acquired at a mass resolution of 70,000 (FWHM) at *m*/*z* 200. The maximum injection time was 250 ms, and the automatic gain control was set to $1x10^6$. The 10 most intense precursor ions with charges states of between 2+ and 5+ were selected for MS/MS at a resolution of 17,500 with an isolation window of 2 *m*/*z* units. The maximum injection time was 50 ms, and the automatic gain control was set to $1x10^5$. Fragmentation of the peptides was by higher-energy collisional dissociation using a normalised collision energy of 30%. Dynamic exclusion of *m*/*z* values to prevent repeated fragmentation of the same peptide was used with an exclusion time of 20 s.

Progenesis Data Analysis

Label-free protein quantification was performed using Progenesis QI for Proteomics v.2.0 (Waters Ltd., Wilmslow, UK). Samples were automatically aligned according to retention time. All raw data files aligned well with high alignment scores, apart from a single shy individual from the first temperature treatment block, which had to be removed from the downstream analysis. Default peak picking parameters were applied and peptides with charges between 2^+ and 7^+ were retained. By assuming that a significant number of peptide ions were unaffected by the experimental conditions, normalisation was performed to adjust for variation in sample loading. The initial Progenesis experiment was between bold (n= 12)

and shy (n= 11) individuals to enable all data files to be represented in the aggregate reference file and database search.

A Mascot Generic File, created by Progenesis was searched in Peaks v10 (Bioinformatic Solutions Inc, Waterloo, ON, Canada) against the *Nematostellla vectensis* database downloaded from Uniprot (24,435 sequences), using a peptide mass tolerance of 10 ppm and a fragment ion tolerance of 0.01 Da. Carbamidomethyl cysteine and oxidation of methionine were selected as fixed and variable modifications respectively. A SPIDER search was then performed against more than 250 modifications and amino acid substitutions, and the results of this search adjusted to a psm false discovery rate of 1% (-10 lgP 38.5%, alc 50%). The 976 proteins identified were uploaded into Progenesis and peptides were assigned to proteins. Progenesis reports were generated and all proteins, including those identified via a single unique peptide, were included in subsequent analyses.

Protein Functional ID

Because many of the proteins in the *N. vectensis* database have yet to be fully functionally described, the FASTA sequences of identified proteins were input into the protein BLAST search engine (NCBI, USA) and run against the full database therein of non-redundant protein sequences. The named protein results displaying the lowest e values in these searches were used as protein identifiers. BLAST-identified results are referenced in all discussion of specific proteins.

5.4.3. Graphical and statistical analyses

Data quality checks

Alongside initial normalisation checks, further data quality checks were also carried out. Overall proteomic profiles were plotted for both the temperature stress and control treatment to ensure coverage of a full spectrum of normalised abundances had been achieved. Further, to confirm that any protein differentiation between treatments was not caused by methodological discrepancies, the normalised abundances of the full protein profiles within each treatment were plotted against each other. A Pearson's correlation test was then run to ensure a tight, linear relationship. For all identified proteins whose BLAST results are discussed directly here, BLAST-identified sequences were compared with original *N. vectensis* database sequences using the EMBOSS (Rice et al., 2000) program dotmatcher (available: https://www.bioinformatics.nl/cgi-bin/emboss/dotmatcher). Dot plots used a

window size of 10 and a similarity threshold value of 23. Upon visual inspection, any BLAST-identified proteins that did not show clear alignment with the original sequence were discounted and labelled as "uncharacterized" (Appendix A5.B. for examples).

Structural groupings

To determine structural groupings of proteins for analytical purposes, functional labels were generated by inputting the original *N. vectensis* accession numbers into the enrichment tool g:profiler (Raudvere et al., 2019). Using g:profiler's KEGG pathway database, it was determined which structures were significantly represented in the overall *A. equina* protein profile, and which proteins were components of those structures. The ribosome, spliceosome, and proteasome were all significantly represented. A search for BLAST-identified proteins whose names contained structural terms was also carried out: for the ribosome, searches were carried out for "ribosome" and "ribosomal"; for the spliceosome, searches were "spliceosome" and "spliceosomal"; for the proteasome, searches were "proteasome" and "proteasomal". Any proteins deemed to have been missed by g:profiler were added to structural analyses. Proteins forming part of the mitochondrial ribosome were not added.

Protein expression between treatments

To determine the significance of fold-changes between temperatures, exploratory between-subject one-way ANOVAs were first run on each protein, comparing protein expression in temperature stressed with control individuals. Where differences in expression between treatments had a false discovery rate (q; Storey, 2003) of less than 0.15, proteins were deemed to be differentially expressed to a level where they should be included in further analyses.

Functional groupings between treatments

To investigate functional groupings in differentially expressed proteins, it was first determined which proteins were shown by initial ANOVAs to be significantly up or down-regulated under temperature stress. These groups were split into two separate datasets and g:profiler functional enrichment was carried out on each using GO: Molecular Function, Biological Process and Cellular Component databases, alongside the KEGG pathway database.

Proteomic profiles between treatments

To visualise how proteomic profiles changed between treatments, and whether there was a general trend for the up or down-regulation of proteins under heat stress, those proteins that were significantly differentiated between treatments in the initial ANOVAs were plotted in a cluster heatmap in R version 4.0.4 (R Core Team, 2020), using the package heatmap3 (Zhao et al., 2014). Hierarchical clustering was used to group proteomic profiles and determine whether they differed between treatments. Because structural components were of particular interest, as changes in their expression would, by extension, indicate changes in the rates of different cellular processes, further heatmaps were plotted for those significantly differentiated proteins that formed part of the ribosome, spliceosome, and proteasome.

Specific proteins between treatments

To investigate those proteins that were most clearly differentiated between treatments, the full identified protein list was visualised as a volcano plot, and those significantly differentiated proteins showing the lowest q values or largest fold-changes were identified and functionally investigated.

Protein expression of different personality-types between treatments

To investigate if there was a general interaction between personality-type and proteomic temperature response, data was first subset by personality-type and further exploratory ANOVAs were run using four "personality x treatment" categories. As in the previous ANOVAs, differential expression worthy of further analysis was determined using a threshold of $q < 0.15$. Due to lower numbers of significantly differentiated proteins shown by these ANOVAs, coupled with four grouping factors instead of two, g:profiler functional enrichment was not carried out.

Proteomic profiles of personality-types between treatments

To investigate whether separate personality-types showed differing proteomic profiles under temperature stress, a cluster heatmap was first plotted using all the significantly differentiated proteins from the personality x treatment ANOVAs. Hierarchical clustering was this time used to determine profile differences between treatments and between personality-types. A PCA analysis was also run on the same data to determine the key axes of variation driving any differences between individuals in their proteomic expression, and whether any groupings on these axes could be attributed to the effects of treatment or personality-type.

Associations between personality-type, treatment, and specific proteins

To determine which proteins showed strong interactions between personality-type and heat stress, and to explore the direction of these relationships, protein-specific GLMs were run in R on all the significantly differentiated proteins from the second ANOVAs. Protein abundance was the response variable and treatment, personality-type, and their interaction were initially specified as fixed effects. Stepwise model simplification was used to determine significant fixed effects at $P< 0.05$.

Associations between personality-type, treatment, and complex structures

The differential expression of proteins forming part of the three significantly represented structures (ribosome, spliceosome, proteasome) was of particular interest. Discrepancies between personality-types in these structures at different temperatures would indicate personality-level variation in structure-related processes under heat stress. As such, proteins forming parts of these structures (whether significantly differentiated in the initial ANOVA or not) were investigated via structure-specific general linear models (GLMs) in R. Z-transformed (within-protein) protein abundance was the response variable and temperature, personality-type, and the interaction between the two were set as fixed effects. Stepwise model simplification was again used to determine significant fixed effects at $P< 0.05$.

Associations between personality-type, treatment, and HSPs

Further, because proteins related to stress resistance were also of particular interest, as a personality-type exhibiting a more stress-resistant proteomic profile might be more robust to chronic high temperatures, a GLM was run on the seven identified heat-shock proteins (HSPs) present in the full proteomic profile. This model was specified and simplified in the same way as the GLMs described above.

5.5. RESULTS

5.5.1. Quality checks

Visual inspection of the proteomic data utilised in this study found the quality of the data to be high. 606 of the 976 identified proteins were identified by a single unique peptide, but figures 5.1a and 5.1b show the expected coverage of normalised abundances across a wide range of values in both treatments. Further, single peptide-identified proteins encompassed much of the lower end of abundance values and their standard deviations were not unusually high, other than at the lowest of abundances ($n \approx 20$), when compared with proteins identified by multiple peptides. As such, the removal of single peptide-identified proteins from analyses would have risked hiding much of the proteome and thus weakened the exploratory value of the dataset. Figure 5.1c further shows that the proteomic profiles of stressed and control individuals, while different, were still highly correlated with one-another as expected ($r = 0.98$), indicating that observed differences between the two treatments were due to treatment conditions, rather than any experimental error.

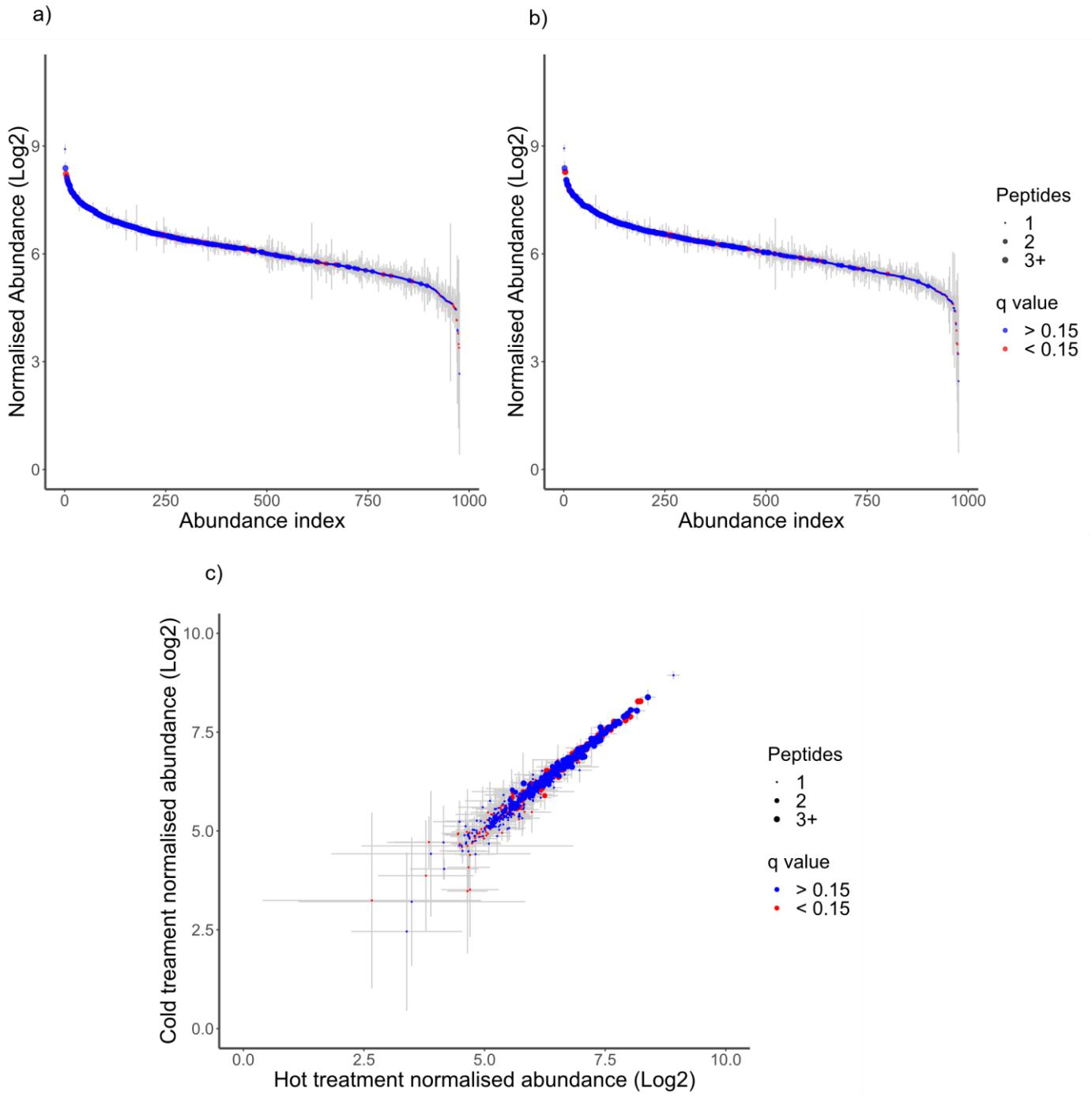


Figure 5.1. Protein abundances (±SD) showing a) abundance s-curve for the full identified protein list for the temperature stressed treatment and b) abundance s-curve for the full identified protein list for the control treatment. Panel c) shows the relationship between identified proteins between the two treatments. For all panels, proteins are sized based on the number of unique peptides and coloured based on significantly different expression across treatments.

5.5.2. Protein expression and functional groupings between treatments

Initial ANOVAs showed substantial differences in the protein expression of temperature stressed individuals as compared to controls. Of the 976 identified proteins, 323 were differentially expressed between treatments ($q < 0.15$). Of these, 226 were down-regulated under heat stress, and g:profiler functional enrichment analysis found that 124 significant ($p_{adj} < 0.05$; Figure 5.2a) functional groupings were present across this profile. Most significantly represented in this group ($p_{adj} < 1x10^{-16}$) were proteins related to the biosynthesis and metabolism of amides and peptides, ribosomal components, and translational and cytoplasmic proteins. All of these groupings indicate a general down-regulation of the biosynthesis of new molecules under heat stress. The 97 proteins that were up-regulated under heat stress (Figure 5.2b) showed only two weakly significant functional groupings. As such, there was no clear up-regulation of stress resistance proteins (e.g. those involved in homeostasis, detoxification, oxidative stress response) indicated by this analysis.

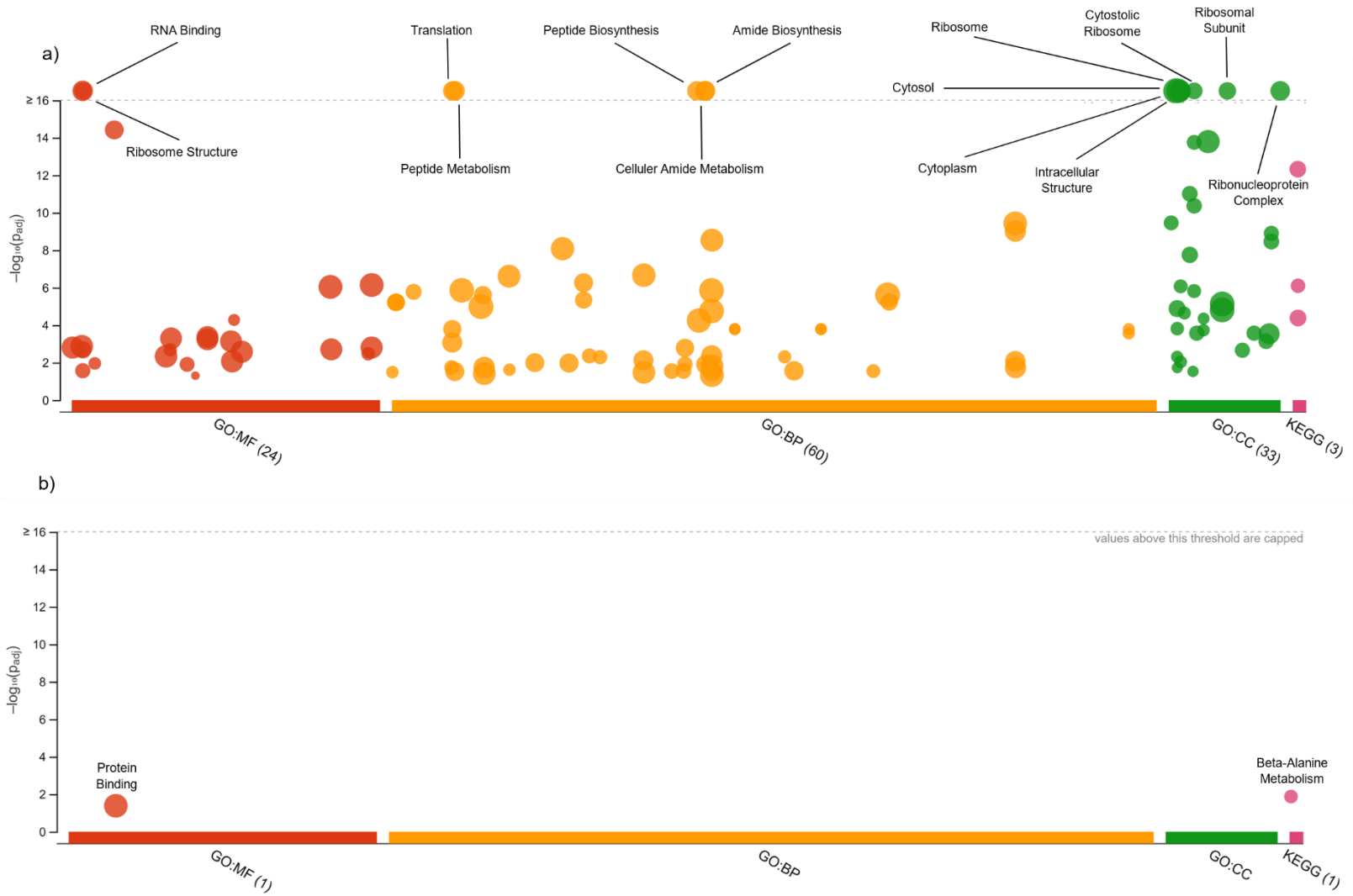


Figure 5.2. G:Profiler functional profile plots, showing significant functional clusters (using GO: Molecular Function, Biological Process and Cellular Component databases, alongside the KEGG pathway database) that were a) significantly more abundant in control anemones than temperature stressed anemones, and b) significantly more abundant in temperature stressed anemones than control anemones. Significant clusters above the dotted line have an adjusted p value of less than $1x10^{-16}$.

5.5.3. Proteomic profiles between treatments

Figure 5.3a further corroborates the clear difference between the proteomes of individuals in the two treatments, showing a general trend towards down-regulation of many proteins under heat stress and clear groupings of stressed and control individuals (but note four outliers). Both the ribosome (Figure 5.3b) and the spliceosome (Figure 5.3c) also show clear groupings, with components of both structures down-regulated in heat-stressed individuals, again indicating a reduction in biosynthetic processes. Groupings are not so clear for the proteasome (Figure 5.3d), whose function is more varied and includes protein recycling, apoptotic initiation, and responding to oxidative stress. It is, however, worth noting that for all proteasomal components across the full protein profile, mean normalised abundance was higher under control conditions. A possible reason for the lack of clear treatment grouping in the proteasome may simply have been its smaller protein sample size compared with the other structures (proteasome n= 12; spliceosome n= 18; ribosome n= 28).

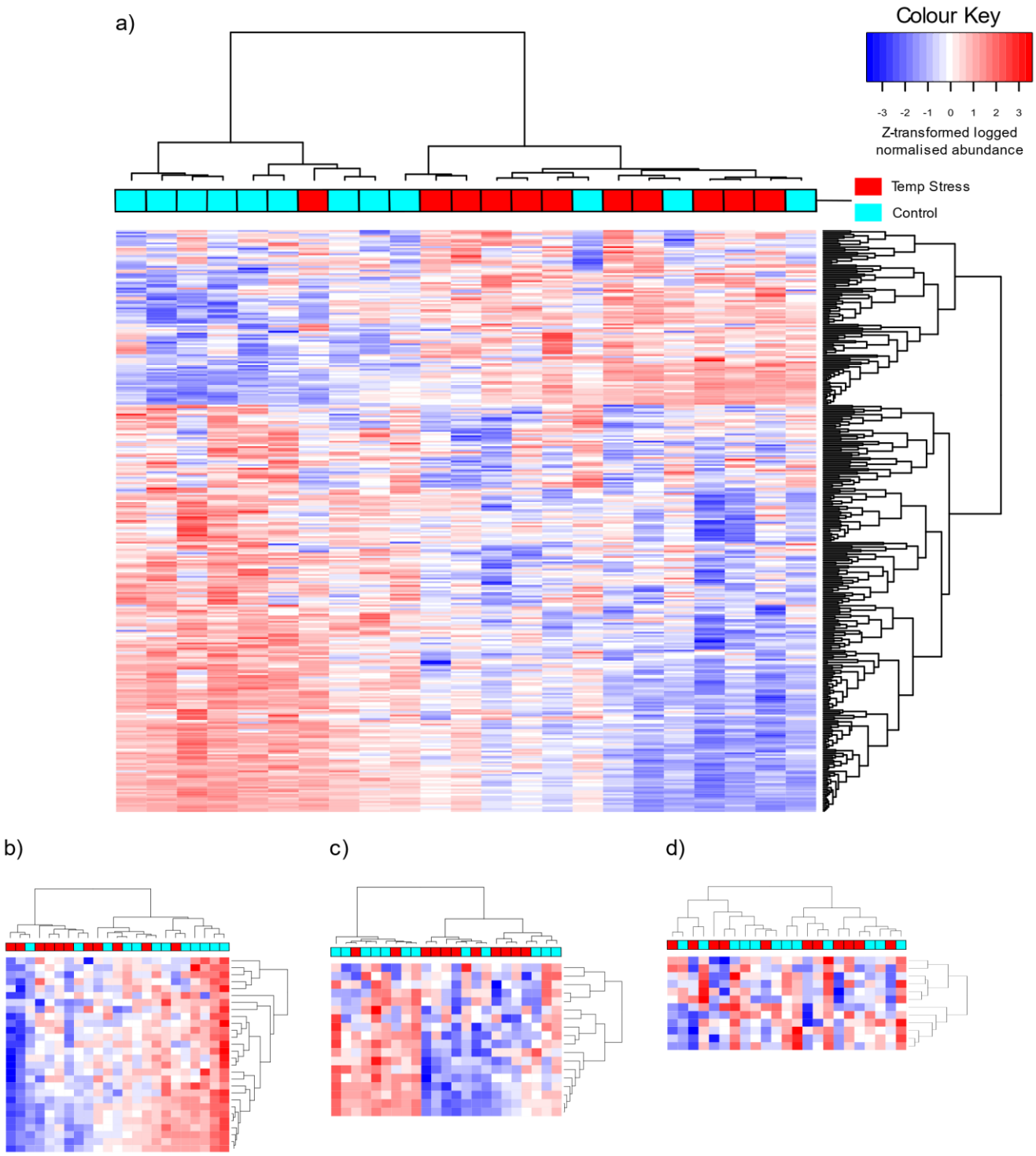


Figure 5.3. Heatmaps showing; a) hierarchical clustering of all 323 proteins significantly differentially expressed (q< 0.15) across temperatures, calculated by between-subject one-factor ANOVAs; b) hierarchical clustering of all 28 significantly differentiated ribosomal proteins; c) hierarchical clustering of all 18 significantly differentiated spliceosomal proteins; d) hierarchical clustering of all 12 significantly differentiated proteasomal proteins. All panels are labelled by temperature-treatment above columns.

5.5.4. Specific proteins between treatments

Many of those proteins showing the highest down-regulation or the most clearly significant down-regulation under heat stress, visualised in figure 5.4, were also involved in biosynthetic processes and protein recycling (e.g. ATP-dependent RNA helicase dbp2; components of the spliceosome; the 26S proteasome pathway; cathepsin L). This pattern again indicates a reduction in the rates of many of these processes under high temperatures. Further, figure 5.4 gives a clearer indication that a number of proteins involved in apoptotic pathways were also down-regulated under heat stress (caspase 3; the 26S proteasome pathway; adenosine receptor A3), while proteins whose functions included stress resistance or mitigation were most clearly up-regulated (e.g. asparagine synthetase; acetyl-coenzyme A synthetase YngI; NLRC4; mothers against decapentaplegic homolog 3). It should be noted that asparagine synthetase, which was the most strongly up-regulated protein and functions in the stress response of the endoplasmic reticulum, showed very low normalised abundances and very wide standard deviations, which overlapped between the two treatments (Hot mean normalised abundance= 94007, Hot SD= 78470; Cold mean normalised abundance= 26237, Cold SD= 56892). Nonetheless, figure 5.4 provides much clearer evidence that anemones under heat stress were indeed up-regulating stress-mitigation proteins, while down-regulating proteins that might have driven stress-induced cell death.

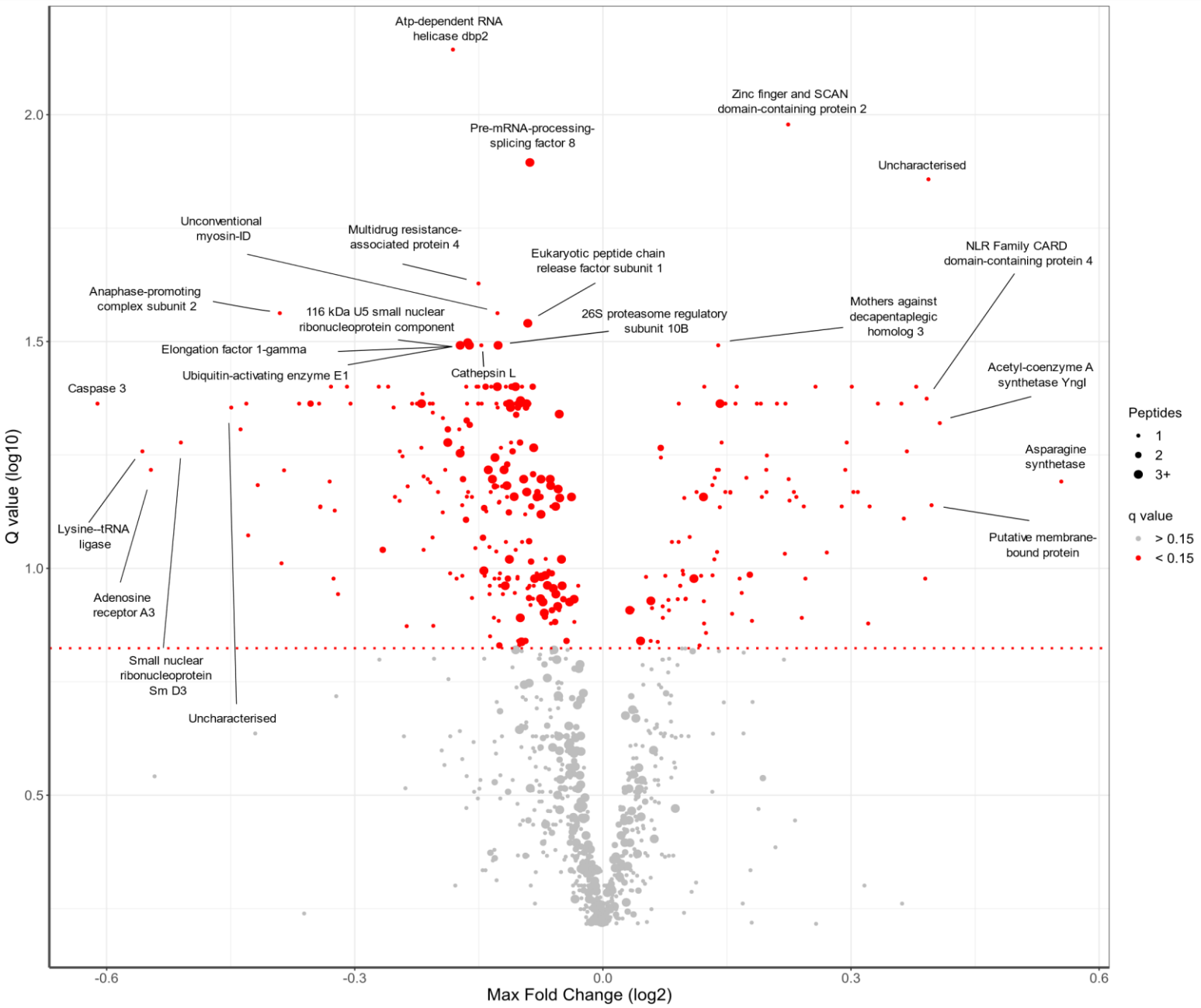


Figure 5.4. Volcano plot showing all 976 identified proteins, sized based on their number of unique peptides and coloured based on whether they were significantly differentially expressed between temperature-treatments at a threshold of q< 0.15. Labelled are the 15 proteins with the lowest q values, and the proteins showing the greatest positive or negative fold-changes (n= 5 for each). As some of these categories overlapped, 22 proteins are labelled. Fold-change values are based on whether proteins showed increased or decreased expression in the temperature stress treatment as compared with the control treatment.

5.5.5. Proteomic profiles of personality-types between treatments

Splitting the full dataset into within-treatment personality-types yielded significant differential expression in 112 proteins ($q < 0.15$). Upon inspection of figure 5.5a, it becomes clear that this was being driven by the interaction between temperature stress and personality-type, with bold temperature stressed individuals showing the clearest clustering, and four of the five shy temperature stressed individuals also clustering together. Figure 5.5b further solidifies the evidence for this interaction, with personality-types grouping away from one-another, but only when under heat stress. No evidence is apparent in figures 5.5a or 5.5b for a whole-proteome effect of personality-type alone, with no clustering in control individuals in figure 5.5a, and no grouping in figure 5.5b. While control individuals show no clear grouping by personality-type, they do appear to group differently on PC1 than their temperature stressed counterparts in figure 5.5b (but note very wide errors), indicating that the substantial effect of this axis is likely to denote temperature. Although PC2 is necessarily dependent on PC1, and should thus be treated with caution, the grouping of bold and shy individuals on figure 5.5b appears dependent on an interaction between both axes, suggesting that PC2 may be indicative of the more modest effect of personality-type.

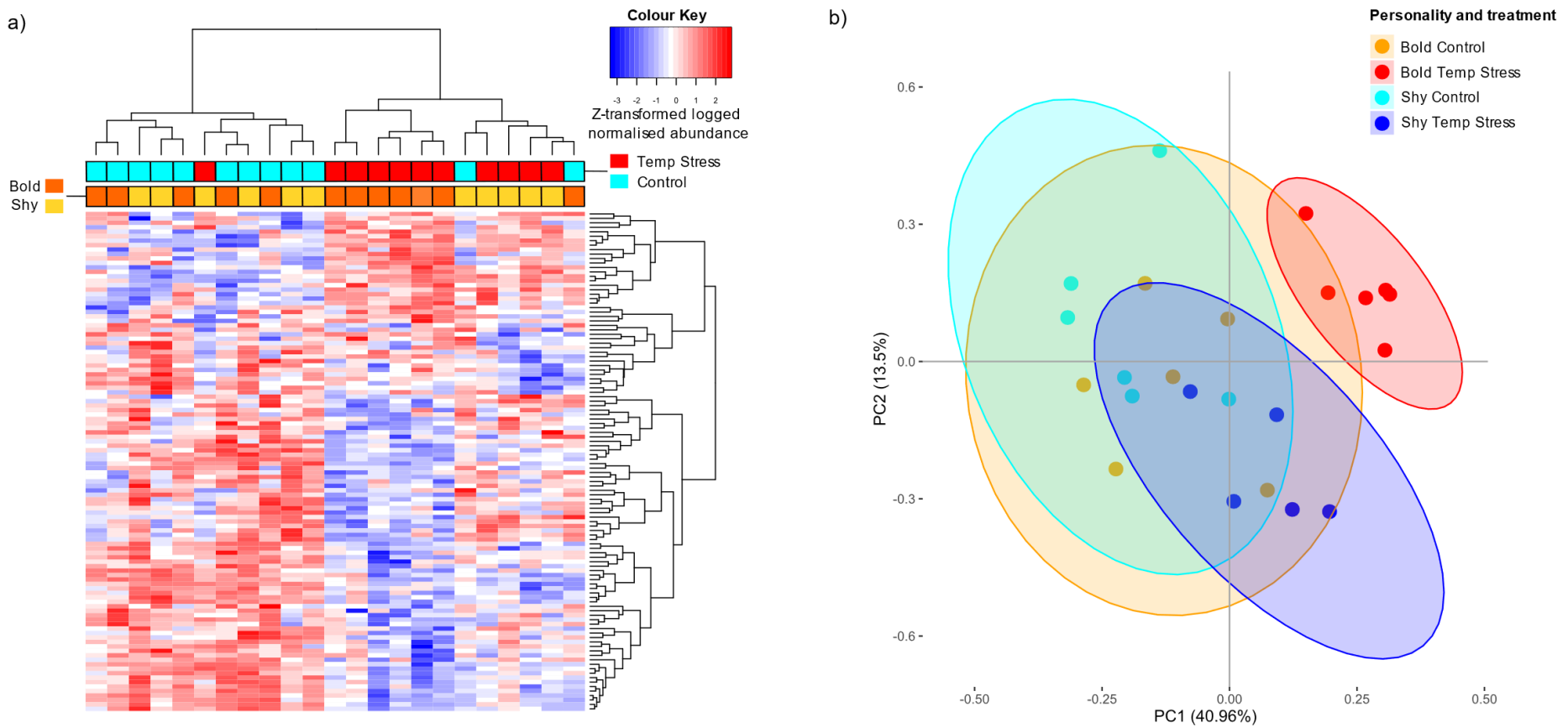


Figure 5.5. a) Heatmap showing hierarchical clustering of all 112 proteins identified as significantly differentially expressed ($q < 0.15$) between different personality-types at different temperatures by a one-way between-subject ANOVA, with treatment and personality-type shown above columns and b) the results of a principal-components analysis on the same 112 proteins, showing the loadings of each individual on each axis and ellipses surrounding each personality-type under each treatment, calculated on a multivariate distribution.

5.5.6. Associations between personality-type, treatment, and specific proteins

Protein-specific GLMs found 29 proteins where a significant ($P< 0.05$) interaction between treatment and personality-type was associated with expression. 16 of these were identified by a single unique peptide (Figure 5.6). While the proteins represented have a diverse range of functions, even at the within-protein level, several patterns of interest arise from inspection of figure 5.6. Bold individuals may have been up-regulating proteins involved in stress mitigation (e.g. E3 ubiquitin-protein ligase RNF213, Phospholipid-transporting ATPase IA), alongside proteins related to cellular proliferation (e.g. protein kinase C, tubulin). Shy individuals appeared to show less of a stress response in terms of mitigation and cellular replacement, and in most instances showed a down-regulation of these proteins. Shy individuals also showed more down-regulation of proteins involved in biosynthetic processes and protein recycling (e.g. spliceosomal and ribosomal components, importin 5), while many of these proteins showed no change or even up-regulation in bold individuals. There is also some indication that different personality-types may have been favouring different pathways towards programmed cell death (although note that the analysis of heat stress alone suggested an overall reduction in apoptosis): bold individuals showed increased expression of inositol polyphosphate 5-phosphatase K and an undescribed death-effector protein under heat stress, while shy individuals showed up-regulation of cytochrome c and maintenance of P2X purinoceptor 7 expression.

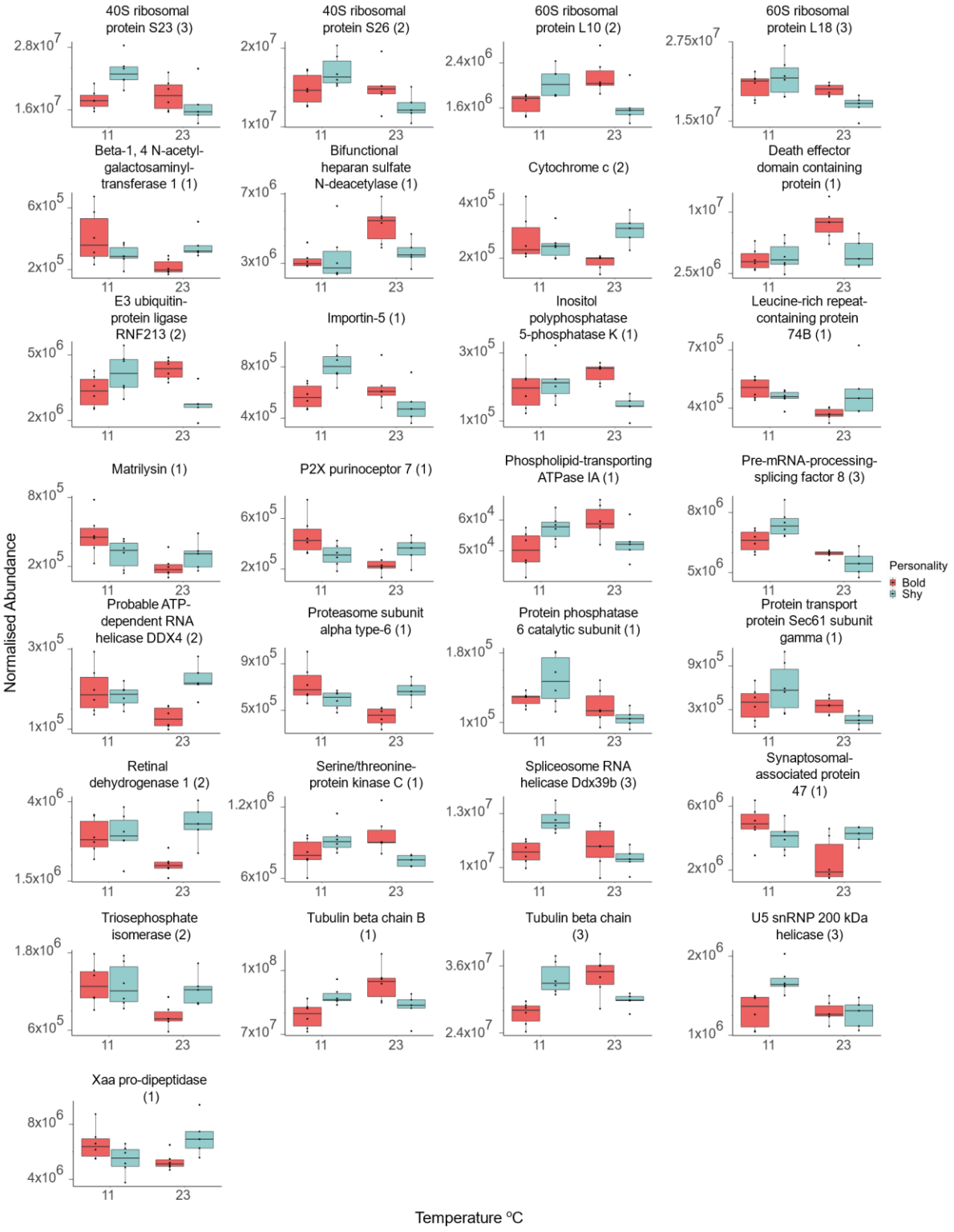


Figure 5.6. Box and whisker plots of proteomic expression at each temperature for each personality-type. Shown are the 29 proteins whose expression was significantly ($P < 0.05$) influenced by the interaction effect between personality-type and temperature in protein-specific GLMs. Where two proteins were identified as the same, the less significant is labelled as "B". The number of peptides a protein was identified from (1, 2, 3 or more) are shown in brackets.

5.5.7. Associations between personality-type, treatment, and complex structures

Structure-specific GLMs further corroborate the discrepancy between personality-types in their temperature responses. They indicate that shy individuals may have down-regulated biosynthetic processes (associated with the ribosome and the spliceosome), but not protein recycling and apoptosis (associated with the proteasome), more than bold individuals. Both the ribosome ($t= -6.1_{1,1353}$, $P< 0.001$) and the spliceosome ($t= -3.98_{1,893}$, $P< 0.001$) showed highly significant interactions between temperature and personality-type. The proteasome showed no significant interaction ($t= 1.5_{1,502}$, $P= 0.13$), but showed clear effects of both personality-type ($t= 5.86_{1,503}$, $P< 0.001$) and temperature ($t= -9.08_{1,503}$, $P< 0.001$) independently. The effect sizes of all three models were moderately low (ribosome $r^2= 0.12$; spliceosome $r^2= 0.12$; proteasome $r^2= 0.19$), but figure 5.7 shows that there were visible differences in the expression of the three structures related to both personality-type and temperature. In line with the protein-specific analysis above, shy individuals down-regulated ribosomal proteins under heat stress, while bolder individuals showed more similar expression of these proteins in both treatments. Spliceosomal expression showed similar discrepancies between personality-types, such that shy individuals showed higher expression in the control treatment, but down-regulated spliceosomal proteins more than bold individuals, leading to similar expression under heat stress for both personality-types. Finally, the proteasome showed clear down-regulation under heat stress in both personality-types, further indicating that the lack of temperature clustering in figure 5.3 was related to a smaller sample size. Shy individuals exhibited higher levels of proteasomal expression at both temperatures.

5.5.8. Associations between personality-type, treatment, and HSPs

The expression of HSPs also differed between personality-types (GLM; $t= 2.23_{1,159}$, $P= 0.03$), but not between temperatures ($t= -0.22_{1,158}$, $P= 0.83$), and the interaction between the two phenotypic levels was not significant ($t= 0.79_{1,157}$, $P= 0.43$). This indicates that while two HSPs, heat shock cognate 71 kDa (down-regulated under heat stress; q= 0.04) and alpha-crystallin B (up-regulated under heat stress; q= 0.12), were differentiated between temperatures in the first of our ANOVAs, this difference did not extent to HSPs as a whole. The overall effect of personality-type on HSP expression was small (model $r^2= 0.03$), and while the mean expression of both personality-types remained similar in both treatments, figure 5.7 suggests that differences between personality-types could have been driven by

temperature. The expression of HSPs in bold individuals under stress appears to have clustered lower, and more uniformly, than under no stress, while shy individuals maintained HSP expression at a similar level in both treatments.

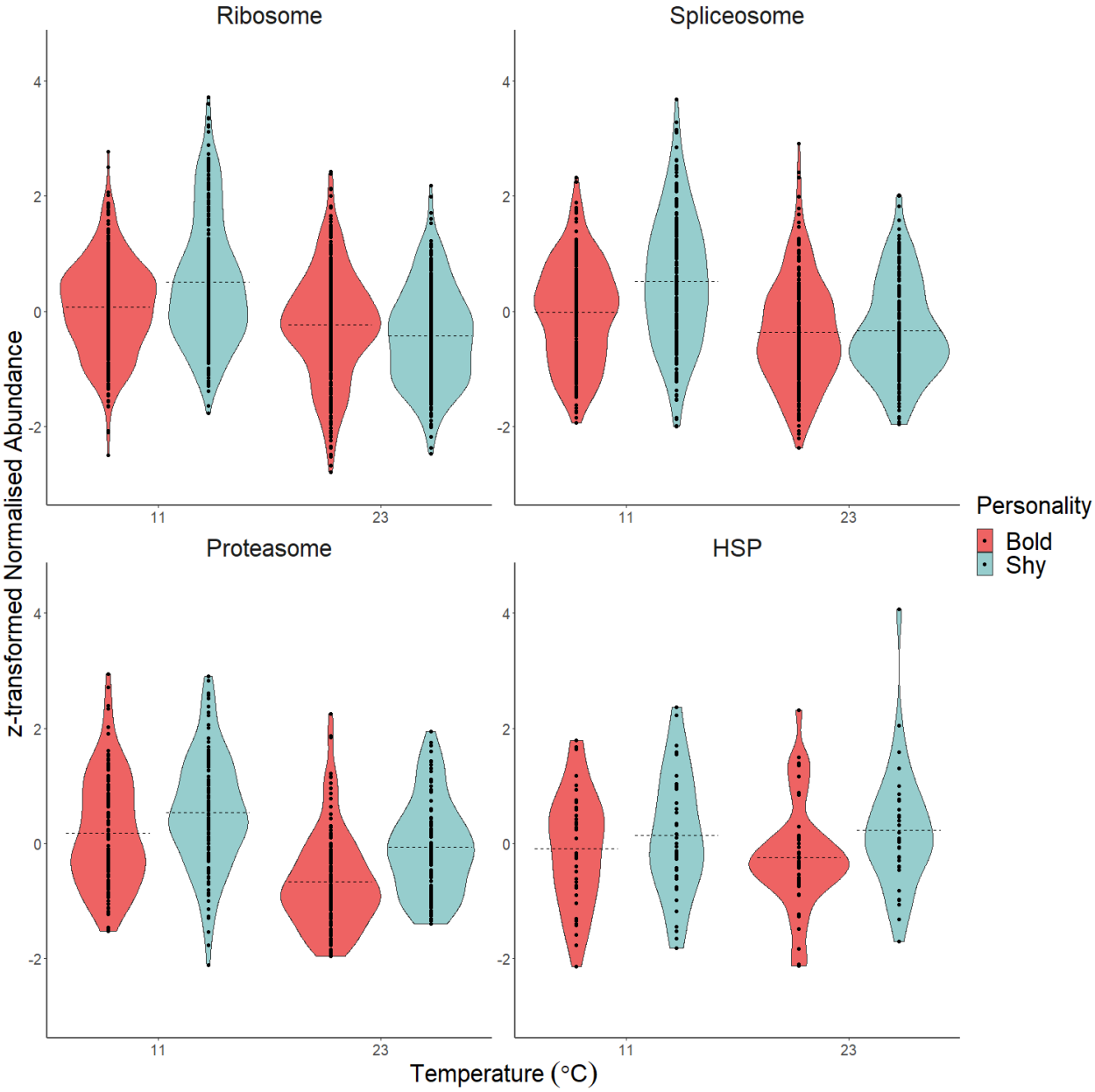


Figure 5.7. Violin plots showing the expression of different groups of proteins at each temperature for each personality-type. The top left panel shows components of the ribosome, the top right panel shows components of the spliceosome, the bottom left panel shows components of the proteasome, and the bottom right panel shows heat shock proteins (HSPs). Dotted lines denote mean values for each personality-type at each temperature.

5.6. DISCUSSION

Intertidal invertebrates display complex behavioural (Briffa et al., 2013; Chapter 2) and proteomic (Choresh et al., 2007; Dong et al., 2008) responses to heat stress, but the interplay between these two phenotypic levels remains poorly understood. Considering changes in proteins, which actively induce physiological stress responses through their roles in all cellular processes (Feder & Walser, 2005), in relation to behaviour could indicate which animals might be least robust to climate change-induced thermal perturbation (Geffroy et al., 2020; Sih et al., 2011; Tomanek, 2011). Here, in the first study of its kind in an invertebrate, we found clear proteomic changes in *Actinia equina* under near-lethal heat stress, which differed depending upon the personality-type of the individual. Indeed, it was only under thermal stress that differences in proteomic expression between personality-types became obvious. Overall proteomic changes were, as expected, centred on both stress response proteins and proteins involved with biosynthetic processes and apoptosis. Bold individuals showed a more uniform proteomic response under heat stress than shy individuals, but each personality-type showed a different profile of proteomic change. While shy individuals down-regulated biosynthetic proteins, as expected, it was bold individuals that showed more apparent up-regulation of stress resistance proteins, and each personality-type showed some induction of specific apoptotic pathways. This study points to the coexistence of multiple proteomic thermal stress response profiles in this population and indicates that these are related to individual personality.

As predicted, one of the clearest differences anemones under thermal stress exhibited, as compared with control individuals, was in proteins involved in molecular synthesis. Specifically, many biosynthetic proteins showed lower expression in thermally stressed animals. Previous work has suggested that the expression of these processes can be up or down-regulated under heat stress. In many marine invertebrates, including some from closely related taxa (Onyango et al., 2021), biosynthetic proteins may be up-regulated during temporarily stressful periods as a method to stabilise ribosomal structure, increase the efficiency of protein-folding, and retain cellular function (Ning et al., 2021; Teranishi & Stillman, 2007; Truebano et al., 2010). Equally, down-regulating these processes can come with its own benefits. Because biosynthetic pathways are energetically costly (Hawkins, 1995), limiting their expression could reduce metabolic demand at high temperatures, allowing anemones to keep their metabolic rates at lower levels than would otherwise be possible (evidenced in: Chapter 4), and to avoid energetic deficits emerging (Pörtner, 2012).

This strategy for biosynthetic suppression has been documented in other anemone species (Richier et al., 2008) and is so extreme in some intertidal invertebrates that protein synthesis ceases completely past a threshold temperature (Tomanek & Somero, 1999). Down-regulation of biosynthetic processes also provides the scope to up-regulate other proteins such as those involved in stress resistance (e.g. Choresh et al., 2007; Dong et al., 2008). The increased expression of stress resistance proteins under heat stress observed in this study could have allowed animals to mitigate against irreversible cellular damage and to down-regulate apoptotic initiation (Gleason & Burton, 2015; Ning et al., 2021). However, as the frequency of intertidal heatwaves increases (Weitzman et al., 2021), greater lengths of time spent under extreme heat stress may cause irreversible cellular damage which cannot be prevented or alleviated via proteomic stress-mitigation (Erguler et al., 2013; Yang et al., 2017). Future work should explore the responses of *A. equina* to longer-term chronic high temperatures, to ascertain whether sustained heat stress might cause severe damage and lead to increased induction of apoptotic pathways. Further, time series studies are needed to determine the nature of the proteomic stress response during a temperature increase and establish when near-lethal temperatures move from an acute to a chronic stressor in these anemones.

In accordance, to some extent, with our predictions of a more prominent proteomic stress response in shy animals, they exhibited more profound reductions in biosynthetic proteins at 23^{o}C than bold animals. In turn, they showed comparatively higher expression of these proteins at 11^{o}C. One explanation for this may be that shy anemones were investing more in maintenance and growth when not under stress (previously suggested in Chapter 4) than their bold counterparts. Increased investment in body condition when not under stress (e.g. Fürtbauer, 2015) could benefit shy anemones by allowing them to mitigate against partial consumption by nudibranch predators (*Aeolidia papillosa*: Edmunds et al., 1976), and by providing them with more scope to reduce that investment when under stress. However, periods where shy anemones can invest in their condition will become fewer and further between as heatwaves become more frequent (Pörtner, 2012; Vajedsamiei et al., 2021). Elevated HSP expression at both temperatures could further suggest high baseline levels of stress in shy animals. Anemones living in highly fluctuating environments are likely to exhibit raised HSPs even under baseline conditions (Choresh et al., 2007), and laboratory-related stress (e.g. Martins et al., 2011) could have exacerbated the differences between shy and bold animals. Shyer personality-types exhibit raised stress markers under moderate or

baseline stressors in other taxa including fish (Alfonso et al., 2019) and birds (Pusch et al., 2018), and this pattern could suggest that these anemones too were more prone to responding to mild stress than their bolder counterparts (Coppens et al., 2010; Koolhaas et al., 2010). On one hand, a propensity to respond to milder stressors and a stress mitigation strategy predicated on less stressful recovery spells could put shy animals at a selective detriment under increasingly frequent heatwaves. On the other, increased responsiveness to stress could drive shy animals towards exhibiting greater behavioural plasticity and a higher tendency to disperse (Geffroy et al., 2020), which might improve their chances of survival under continued periods of perturbation (Wong & Candolin, 2015).

Although shy individuals showed a more apparent reduction in biosynthetic proteins under heat stress, bold individuals displayed greater overall consistency across individuals in their proteomic stress responses, leading them to group closer in both the PCA and heatmap analyses. This may have been driven by up-regulation of proteins related to cellular maintenance and structure, the replacement of irreparably damaged cells, and stress resistance. Bold animals could have been exhibiting a strategy more in keeping with maintaining the efficiency of protein synthetic pathways (Ning et al., 2021; Teranishi & Stillman, 2007) and cellular structure (Truebano et al., 2010). In turn, although the overall pattern appeared to be for anemones to down-regulate apoptotic initiators under heat-stress, bold animals may still have up-regulated some apoptotic pathways to ensure efficient cellular turnover where maintenance was impossible (Erguler et al., 2013). It is worth noting here that this pattern did not extend to the proteasome, which is heavily involved in protein recycling and is thus important for both cellular maintenance and turnover (Sorokin et al., 2009). However, the proteasome is also particularly susceptible to oxidative stress (Reinheckel et al., 1998), which can be driven by high temperatures in anemone species (Perez et al., 2001; Richier et al., 2006). As such, what may have appeared to be down-regulation could have simply been driven by the denaturing (or alteration; Wang et al., 2010) of some proteasomal machinery. HSPs were also not up-regulated in bold individuals at high temperatures. Despite their common role in thermal stress resistance in intertidal invertebrates (Choresh et al., 2007; Dong et al., 2008; Ravaux et al., 2016), HSP expression is particularly energetically costly, and thus rapidly declines in many organisms after initial exposure to acute heat shock (Bowler, 2005). While bold individuals may have been expressing a more stress-resistant proteomic profile, it is possible that the energetic costs of HSP expression in *A. equina* outweigh the benefits during longer periods of thermal perturbation (Brahim et al., 2019).

Despite these caveats, a stress response that focuses on the maintenance of cellular function, rather than suppressing biosynthetic machinery in the hope of recovery in the future, could make bold individuals more robust than their shy counterparts to thermal stress. In turn, greater resistance to thermal stress could allow bold anemones to remain more rigid in their SRTs at high temperatures (Chapter 2).

While bold and shy individuals occupy behavioural extremes, and it might thus be predicted that their proteomic responses would follow suit, in this study we did not investigate the proteomic stress responses of more intermediate individuals. Previous research has provided a mixed picture of the relationships between intermediate personality-types and molecular stress responses. In sea bass (*Dicentrarchus labrax*), for instance, intermediate animals show stress response profiles similar to less plastic, proactive animals (Ferrari et al., 2020). Alternatively, theoreticians have suggested that since intermediate individuals should occupy the centre of any bell-curve of behavioural or physiological variation (Réale et al., 2007, 2010), they might show more pronounced stress responses than either behavioural extreme (Rey et al., 2016). In *A. equina*, intermediate animals are unique amongst the three personality-types in lengthening their SRTs under extreme high temperatures (Chapter 2), and how this behavioural response might translate into proteomic changes remains an open question. It is also worth noting that the data presented here do not indicate a whole-proteome effect of personality-type in the absence of a stressor. Given that physiological discrepancies between personality-types in the absence of stress are evidenced in *A. equina* (Chapter 4), and that a key proximate driver of physiological variation is proteomic differences (Tomanek, 2011), it would seem unusual for personality-types not to show baseline differences in their proteomic profiles. It is possible that these differences in *A. equina* are more subtle, such that the sample size of this study was too small to detect them outside of specific proteins, structures, or functional groupings. As such, another useful direction for future research could be to run larger-scale studies to gain a more complete picture of proteomic discrepancies between personality-types.

This study shows a clear temperature-induced proteomic stress response in *A. equina* and advocates for increased focus on the associations between behavioural and whole-proteome stress responses in understudied taxa. The data presented here indicate that at least two proteomic strategies for dealing with thermal perturbation are present in this population of *A. equina* and that these are related to personality-type. Bold individuals may directly mitigate against temperature stress via proteomic changes, allowing them to maintain a level of

normal cellular function at thermal extremes. Higher stress-levels in shy individuals, meanwhile, might lead them to disperse more readily under increasingly frequent perturbation. If some personality-types in a species display inferior strategies to mitigate against environmental changes, they could be placed under increased risk of perishing, which could drive a decrease in genetic diversity and population-level robustness to future climatic shifts. The proteins identified here could be further investigated in *A. equina* and other animal groups. By using whole-proteome or candidate protein approaches, future studies could enhance our knowledge of the relationships between climate change-driven thermal perturbation and intraspecific variation in behavioural and molecular phenotypes.

5.7. ACKNOWLEDGEMENTS

Thanks to Leslie Connor for technical support with anemone husbandry and to Stephen Crowley for assistance with tentacle sampling and flash-freezing. Thanks also to Rosemary Maher for hugely helpful contributions to the analysis and visualization of the data presented here. Finally, thanks to Guillermo Garcia-Gomez for invaluable assistance during anemone collection, without which this study would not have been possible. DKM was funded by a NERC ACCE PhD studentship (ref: 1950009) and Blue Planet Aquarium

APPENDIX TO CHAPTER 5: Different proteomic responses to temperature stress in bold and shy beadlet sea anemones

A5.A. Sampling order

Table A5.1. Order of the injection of the 24 samples (randomly assigned)

Injection order	Date Sampled	Within-Date Sampling Order	Treatment	Personality
1	09/07/2018	2	Temperature stress	Bold
2	09/07/2018	6	Temperature stress	Bold
3	09/07/2018	1	Temperature stress	Shy
4	09/07/2018	3	Temperature stress	Shy
5	11/06/2018	2	Control	Shy
6	26/04/2018	6	Temperature stress	Shy
7	11/06/2018	5	Control	Bold
8	09/07/2018	4	Temperature stress	Bold
9	11/06/2018	3	No-stress	Shy
10	08/08/2018	3	No-stress	Shy
11	26/04/2018	4	Temperature stress	Bold
12	09/07/2018	5	Temperature stress	Shy
13	08/08/2018	1	Control	Shy
14	11/06/2018	1	Control	Shy
15	11/06/2018	6	Control	Bold
16	26/04/2018	3	Temperature stress	Shy
17	26/04/2018	1	Temperature stress	Bold
18	26/04/2018	5	Temperature stress	Bold
19	11/06/2018	4	Control	Bold
20	08/08/2018	4	Control	Bold
21	26/04/2018	2	Temperature stress	Shy
22	08/08/2018	2	Control	Shy
23	08/08/2018	6	Control	Bold
24	08/08/2018	5	Control	Bold

A5.B. BLAST homology

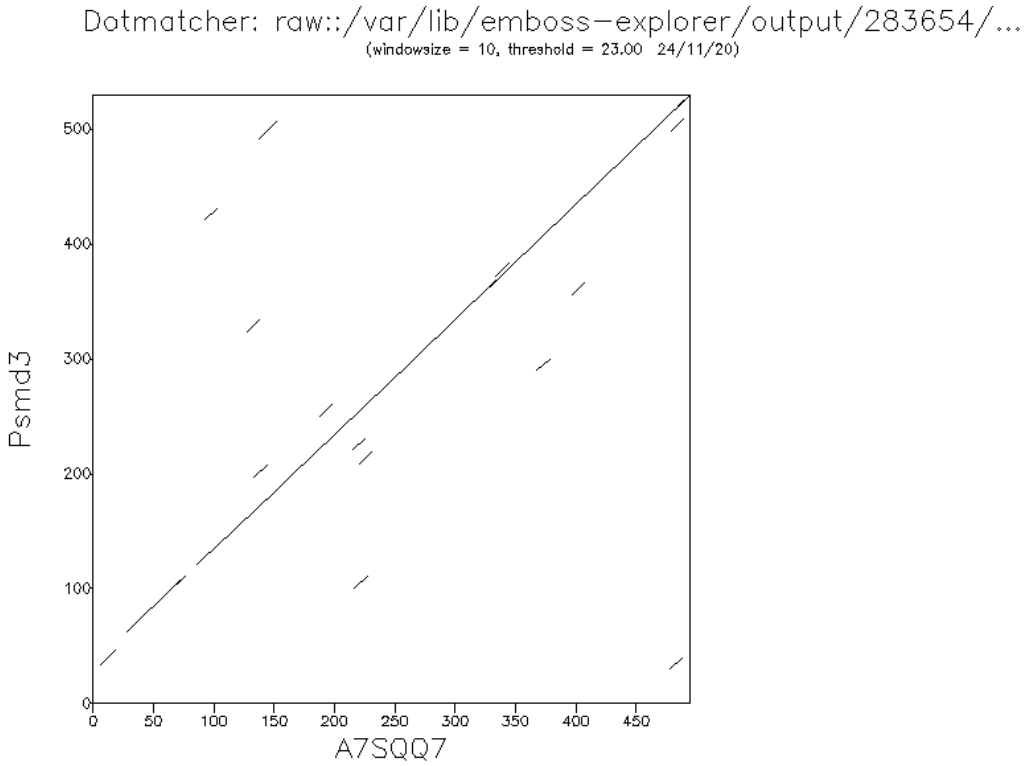


Figure A5.1. Dot plot with acceptable homology to BLAST ID.

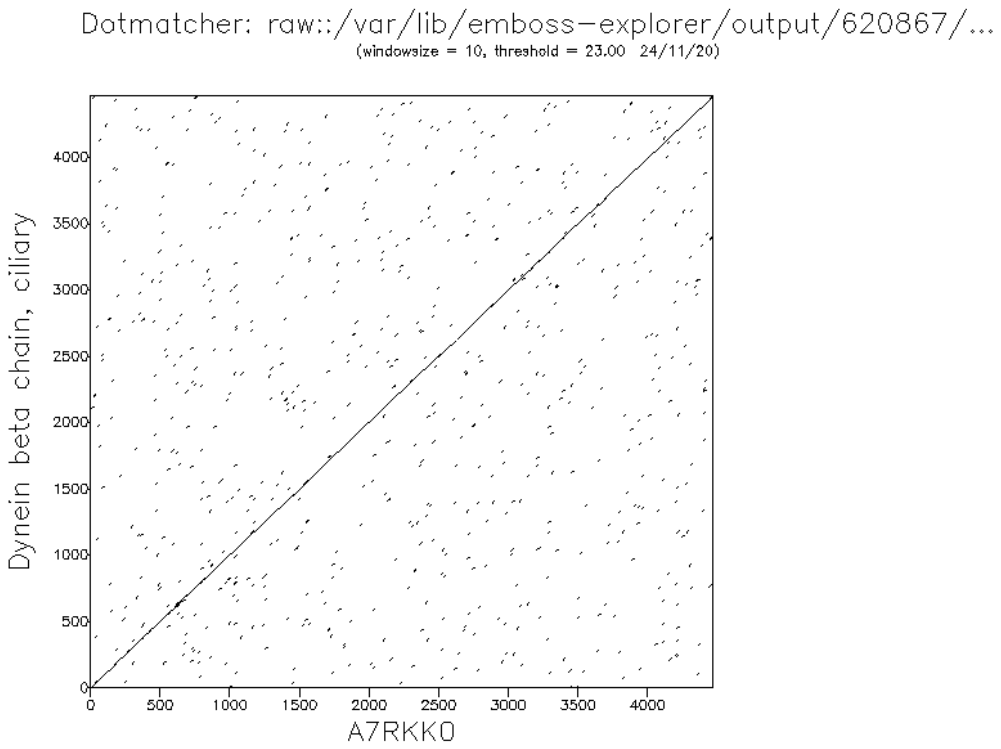


Figure A5.2. Dot plot with acceptable homology to BLAST ID.

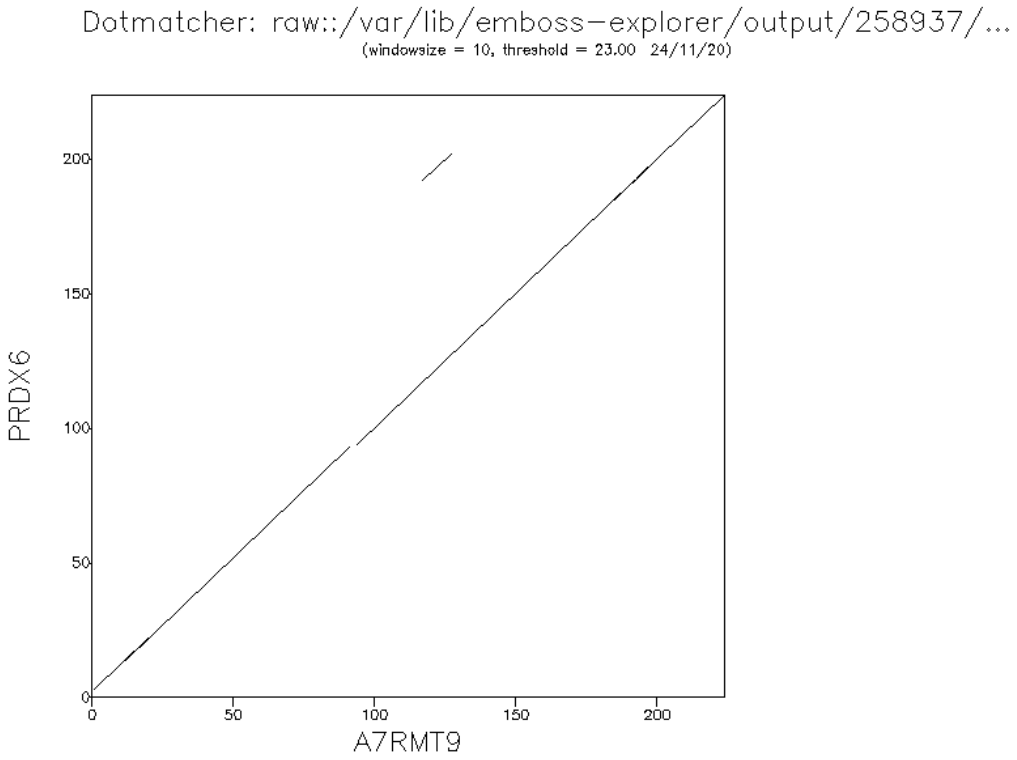


Figure A5.3. Dot plot with acceptable homology to BLAST ID.

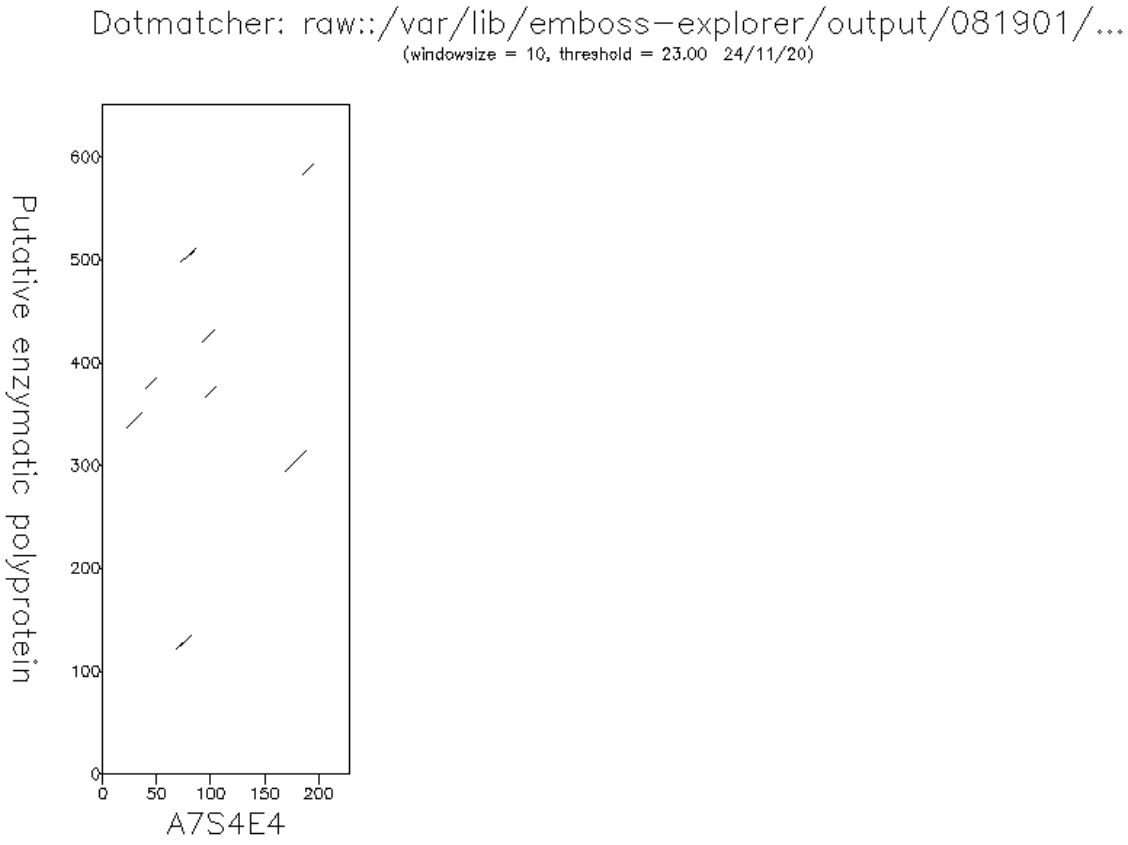


Figure A5.4. Dot plot without acceptable homology to BLAST ID which would be deemed 'uncharacterised'.

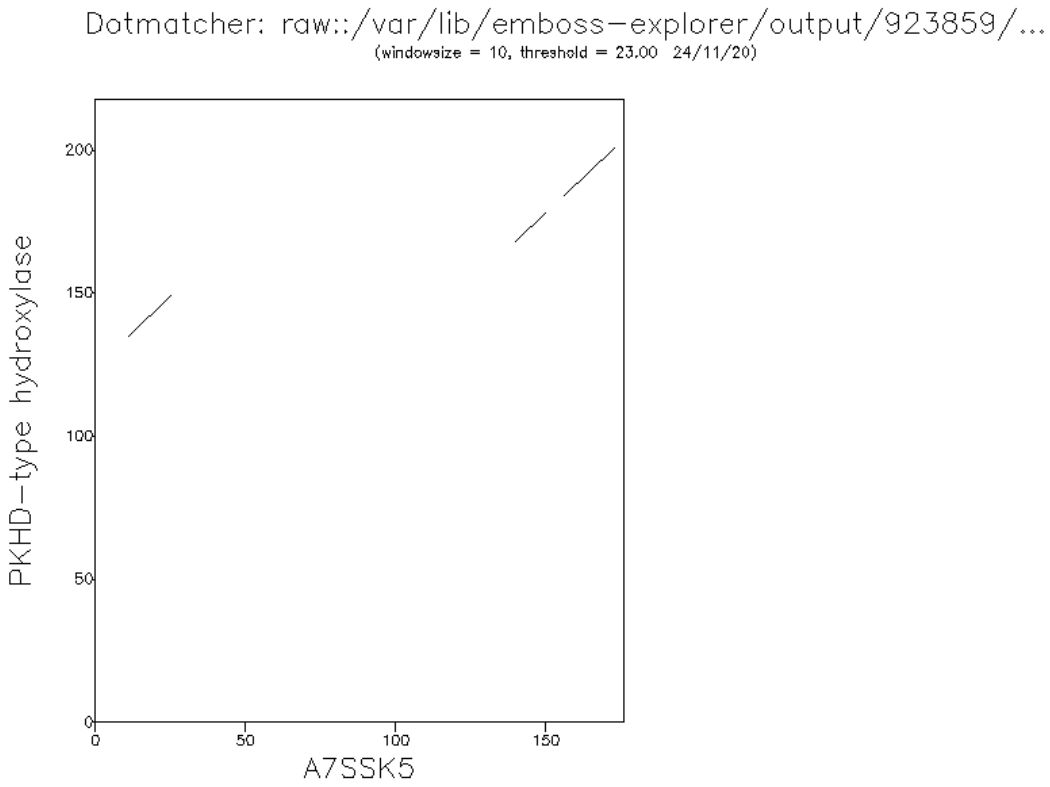


Figure A5.5. Dot plot without acceptable homology to BLAST ID which would be deemed 'uncharacterised'.

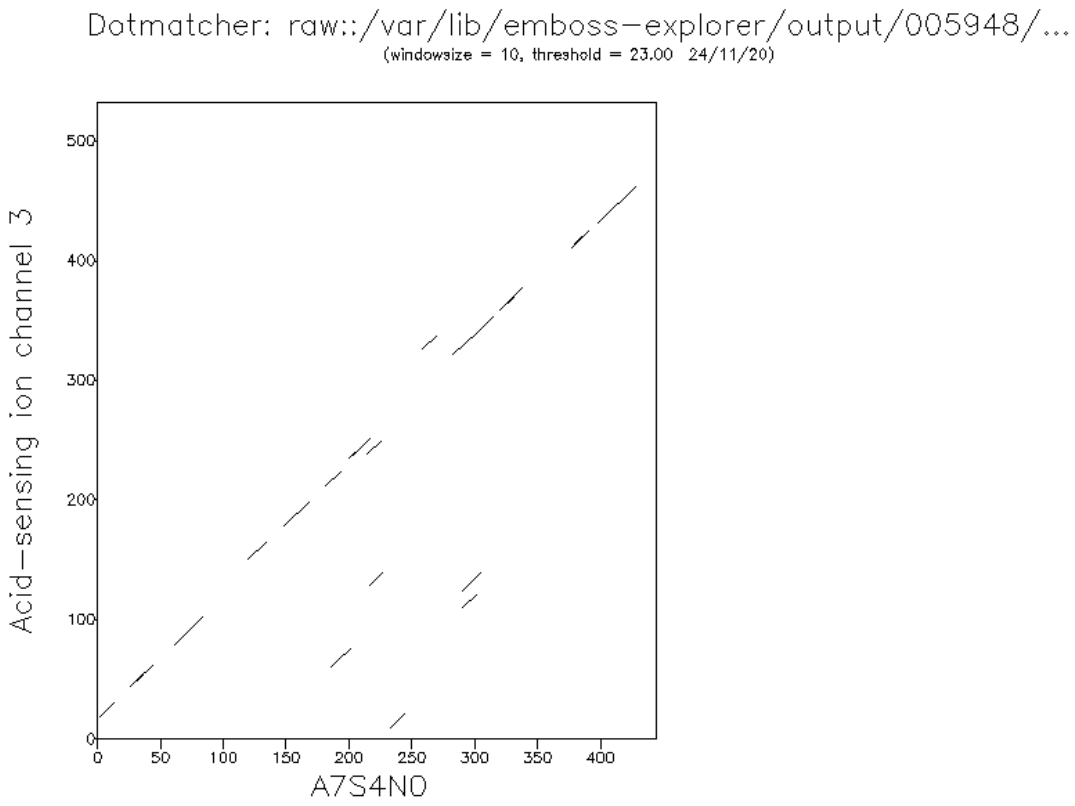


Figure A5.6. Dot plot without acceptable homology to BLAST ID which would be deemed 'uncharacterised'.

[Page intentionally left blank]

Chapter 6:

General Discussion

[Page intentionally left blank]

6.1. KEY FINDINGS

Understanding the implications of animal personality in a changing climate is an emerging goal for many biologists, including ecologists and conservationists (Brooker et al., 2016; de Azevedo & Young, 2021; Sih, 2013; Tuomainen & Candolin, 2011). In much the same way as different behavioural traits are often correlated to form a behavioural syndrome (Adriaenssens & Johnsson, 2013; Bell, 2007; Sih et al., 2012), recent evidence indicates that separate levels of an animal's phenotype, such as behaviour, physiology, and molecular expression, are also associated with one another (Coppens et al., 2010; Cornwell et al., 2020; Debecker & Stoks, 2019; Koolhaas et al., 2010; Polverino et al., 2018). In turn, between-individual differences in behavioural plasticity, which is an important and rapid mechanism by which animals can respond to environmental change (Mery & Burns, 2010; Snell-Rood, 2013; van Baaren & Candolin, 2018), should be correlated with intraspecific variation in plasticity at other phenotypic levels (Dammhahn et al., 2018; Montiglio et al., 2018). Multi-faceted plastic responses are an important mechanism by which animals can avoid mortality under climatic shifts (Acasuso-Rivero et al., 2019; Ducatez et al., 2020; Seebacher et al., 2015), so knowledge of how different personality-types will respond to climate change at multiple phenotypic levels could be of great value in identifying the most vulnerable individuals in a population.

In this thesis, I took a multidisciplinary approach to investigate the phenotypic plasticity of different beadlet anemones (*Actinia equina*) when exposed to stressful temperatures that they might experience during climate change-induced heatwaves or cold snaps. Over the course of four data chapters, I tested three hypotheses:

1. "Different *A. equina* individuals will show consistent differences in their startle response-times (SRTs) and immersion response-times (IRTs), and individuals will vary in how those behaviours change across temperatures. The morphotype and environmental history of an individual will also affect these patterns."
2. "Anemones will show consistent between-individual differences in their metabolic rates both within and across temperatures. These patterns will be influenced by morphotypic differences and will covary with an individual's boldness."
3. "Anemones will show a strong proteomic thermal stress response which will be associated with their boldness."

Complex relationships between behaviour, metabolism, and proteomic expression emerged, indicating that different personality-types employ different multi-level plastic strategies for dealing with thermal stress.

6.1.1. Hypothesis 1: Results

In my first two studies, I investigated different aspects of behavioural plasticity under thermal stress. In **Chapter 2**, I addressed this at the mean level and showed that different anemones, of different morphotypes and from different shore heights, varied in their behavioural responses to rising temperatures. I found that two ecologically relevant boldness-related behaviours, startle response-time (SRT) and immersion response-time (IRT), were only partially associated in *A. equina*, and that they showed very different plastic changes as the temperature increased. For SRT, individuals from the low-shore showed longer response-times at higher temperatures than those from other shore heights, possibly in line with predictions, but morphotypic differences remained consistent across temperatures. At the between-individual level, as predicted, bold individuals showed the most rigid SRTs, while shy individuals shortened their SRTs as the temperature rose and individuals with intermediate personality-types lengthened theirs. For IRT, the shore height anemones were collected from was unrelated to behaviour, but the two morphotypes showed opposite patterns of change as the temperature rose. The red morphotype, which favours the high-shore, exhibited what appeared to be a more appropriate behavioural response to high temperatures. Between individuals, IRT variation was reduced at high temperatures, such that both bold and shy animals showed similar magnitudes of behavioural change away from behavioural extremes towards intermediate response-times. This was contrary to expectations that bold animals would show less behavioural plasticity than shy animals.

In **Chapter 3** I expanded my behavioural investigation to incorporate the plasticity of unpredictability under temperature change. The unpredictability of both SRT and IRT varied between *A. equina* individuals, and this was related to mean level behaviour. For both behaviours, bold animals showed low levels of unpredictability as expected, but so too did the shyest individuals. For SRT, intermediate personality-types exhibited the highest unpredictability, while moderately shy individuals were most unpredictable for IRT. Patterns of unpredictability were further affected by temperature. Shore height was again a predictor of SRT, as individuals from lower down the shore became more unpredictable at 21^{o}C than those from other shore heights. For IRT, unpredictability was significantly higher at 21^{o}C

than 6ºC, and that difference was more apparent in animals from the cooling treatment, first tested at 21ºC, than those from the heating treatment, first tested at 6ºC. Individuals further varied in how the unpredictability of their IRTs changed between temperatures, exhibiting what appeared to be several coexisting strategies that were associated with mean level behavioural change.

6.1.2. Hypothesis 2: Results

Having investigated temperature-driven plasticity at multiple behavioural levels, I then moved on to incorporate other phenotypic traits. In **Chapter 4**, I tested how between-individual variation in plastic strategies might influence adherence to the pace of life syndrome hypothesis (POLS; Réale et al., 2010) by measuring the relationship between routine metabolic rate (RMR;Metcalfe et al., 2016; Velasque & Briffa, 2016) and IRT in the same individuals at different temperatures. I found that the association between RMR and IRT at 13ºC ran unexpectedly counter to POLS, such that shy individuals showed higher RMRs than bold. This pattern reversed at 21ºC, where individuals that were bolder at either temperature had higher RMRs. Individual differences were the most important variable in predicting RMR change between temperatures, and contrary to predictions, morphotypic differences remained stable across both temperatures, with red individuals exhibiting lower RMRs than green individuals.

6.1.3. Hypothesis 3: Results

Finally, in **Chapter 5,** I addressed the molecular underpinnings of responses to temperature stress and how these related to boldness. I found a clear proteomic thermal stress response in *A. equina* as predicted, with 323 proteins showing differential expression in anemones under heat stress when compared with controls. Most prominent amongst these were biosynthetic proteins and proteins involved with apoptotic initiation, both of which were down-regulated, alongside stress-mitigating proteins, which were up-regulated. Unexpectedly, the expression of heat shock proteins (HSPs) did not show any overall change in temperature stressed anemones. As I also anticipated, personality-types differed in their proteomic responses to thermal stress. Shy individuals showed more evidence of a reduction in biosynthetic processes, while bold individuals retained higher levels of biosynthetic proteins and up-regulated proteins related to cellular structure, proliferation, and stress resistance.

6.2. IMPLICATIONS

Phenotypic plasticity, in the form of behavioural, physiological, and molecular changes, has important implications for the survival of animal populations in the face of climate change (Kelly, 2019; Seebacher et al., 2015; Sih et al., 2011). Within species, particularly those living in highly heterogeneous environments such as the intertidal zone (Bockelmann et al., 2002; Brahim et al., 2019), different strategies for addressing perturbation coexist with one-another (Data Chapters; Chapperon et al., 2016; Cornwell et al., 2019). These strategies may be maintained by delicately balanced selective processes (Wolf et al., 2007; Wolf & McNamara, 2012), allowing different individuals of the same species to occupy subtly different environmental niches (Bolnick et al., 2003; e.g. Harris et al., 2020; Hensley et al., 2012). Many intertidal invertebrates, including *A. equina*, display patterns of niche differentiation across different habitats (Chapperon et al., 2016; Cornwell et al., 2019; Quicke et al., 1985). However, alterations to environmental gradients, driven by climatic changes such as more regular heatwaves and cold snaps (IPCC, 2013), could upset the selective balance, leading some plastic strategies to no longer be of selective value. Further, if extreme weather events change the habitability of the preferred habitats of some phenotypes (e.g. Suryan et al., 2021; Weitzman et al., 2021), animals from different levels on the shore may be forced into direct conflict with one-another.

In the case of *A. equina*, temperature fluctuations caused by climatic change should be more acutely felt further up the seashore (Huggett & Griffiths, 1986; Somero, 2002). As such, anemones from the high-shore might be driven towards the low-tide line, altering the selective equilibrium of a population and placing some phenotypes at risk of mortality. At the morphotypic level, the red morphotype, which favours the high-shore (Quicke et al., 1985), is competitively superior to the green morphotype in both direct competitive interactions (Brace et al., 1979; Brace & Reynolds, 1989) and in dealing with extreme weather. The red morphotype is more adhesive (Quicke et al., 1983), meaning it is less likely to be dislodged under tidal exposures driven by climate change-induced storms (Cheng et al., 2019; Hoegh-Guldberg & Bruno, 2010), and the results of Chapters 2 and 4 contend that it is also likely to be more robust to heatwaves, foraging more than the green morphotype at high temperatures (Chapter 2) and exhibiting lower metabolic demand even as the temperature increases (Chapter 4). Alongside morphotypic differences, individuals originally from the low-shore may also be placed under greater stress by regular thermal perturbation than anemones from other shore heights (Chapter 2; 3). High stress levels can lead to selectively detrimental

phenotypic changes (Ma et al., 2018; Seuront et al., 2019), particularly if an animal surpasses its critical thermal maximum (Abram et al., 2017; Schulte, 2015). As such, anemones of the green morphotype and from lower down the shore could be at risk of perishing under increasingly frequent thermal perturbation (IPCC, 2013). These findings have implications for the diversity of *A. equina* and for other organisms that live across the gradient of the shore. Through forcing conspecifics into direct or indirect conflict, increasingly frequent temperature extremes could have important consequences for the genetic diversity, and in turn future survival, of a number of intertidal species.

Temperature-driven changes to the selective landscape will also have consequences at the between-individual level (Geffroy et al., 2020; Merrick & Koprowski, 2017), but this thesis suggests that the nature of those consequences in *A. equina* is likely to be highly complex. Chapters 2 and 3 indicate multiple coexisting behavioural strategies for dealing with thermal perturbation, and Chapters 4 and 5 demonstrate that temperature-driven changes in behaviour are closely related to other phenotypic traits. In each case the interplay between behavioural plasticity and metabolic or proteomic changes could drive selective outcomes, and in each case it is unclear which personality-types these outcomes might favour. While individuals exhibiting shy tentacle extension behaviours at high temperatures (Chapter 2) might appear to place themselves at a selective disadvantage by limiting their foraging opportunities (Bell et al., 2006), Chapters 3, 4, and 5 indicate that the situation is not so simple. Other behavioural levels might provide greater strategic flexibility for risk-averse animals (Chapter 3), and physiological and molecular changes, such as lower metabolic and biosynthetic demand at high temperatures, could reduce the energetic deficits incurred by shy individuals during thermal perturbation (Chapters 4; 5). Further, energetically costly molecular mitigations, such as up-regulating proteins involved with stress resistance (Brahim et al., 2019), could underpin bold animals' ability to retain their behavioural phenotype under thermal stress (Chapters 2; 5). As such, the inferred fitness benefits or costs of behavioural changes in the face of environmental challenges may be less clear than they initially seem. This could be an especially important consideration in species such as *A. equina*, whose life-histories and body plans diverge substantially from well-studied vertebrate taxa and which might therefore be under different selective pressures. The phenotypic underpinnings and fitness consequences of behavioural plasticity remain very poorly understood (Buchholz et al., 2019; Killen et al., 2013; Montiglio et al., 2018; van Baaren & Candolin, 2018), and studies empirically determining selectively optimal plastic phenotypes are currently rare (but see: Ducatez et al.,

2020; Hall & Chalfoun, 2019). Without more detailed investigations of both the proximate drivers and selective outcomes associated with behavioural plasticity, ecologists cannot hope to fully understand how different populations, in the intertidal zone and elsewhere, will respond to climate change.

6.3. LIMITATIONS

The data chapters in this thesis are not without their limitations, and where these are specific to a given chapter they are discussed therein. However, some more overarching limitations are also present, and I outline these below.

6.3.1. Over-representation of bold animals

Although care was taken in Chapters 2-5 to collect population-representative samples, the techniques used were still necessarily not truly probability-based (Biro, 2013). Non-random sampling can bias studies towards collecting bolder animals because they are less likely than shy animals to avoid collection (Merrick & Koprowski, 2017). On the seashore, shy anemones could completely evade collection by placing themselves in unreachable tide pools or crevices (Biro et al., 2013). Further, some shore heights might offer more favourable locations for individuals to avoid certain types of risk, such as exposure (McQuaid et al., 2000) or predation (Bucklin, 1987). As such, samples collected from different shore heights might contain different proportions of risk-averse individuals. Over-representation of bold animals could influence the patterns presented in this thesis. For example, if the high and mid-shore offer a more favourable environment for evading collection, the relationship between SRT and shore height in Chapters 2 and 3 could be artificially enhanced. Further, given that different phenotypic levels are correlated in many species (Chapters 4, 5; Cornwell et al., 2020; Debecker & Stoks, 2019; Ferrari et al., 2020; Pusch et al., 2018), over-representation of bold animals could impact the results of any study investigating intraspecific phenotypic variation (Biro, 2013). Future investigators should attempt to design methods to mitigate against the discrepancies inherent in convenience sampling to produce more reliable pictures of phenotypic variation within and across populations.

6.3.2. A species complex

It is currently accepted that *A. equina* exhibits several genetically distinct morphotypes (Collins et al., 2017; Quicke et al., 1983). However, this assertion is contentious. Species that exhibit very similar physiological and behavioural characteristics to *A. equina*, such as the

strawberry anemone, *Actinia fragacea* (Turner et al., 2003), and the green anemone, *Actinia prasina* (Collins et al., 2017), were previously thought to be morphotypes themselves (Carter & Thorpe, 1981; Solé-Cava & Thorpe, 1987). Indeed, the morphotypes as they are currently defined may genetically diverge further from one-another than they do from *A. prasina* (Schama et al., 2005). As such, there is much debate concerning whether the different morphotypes of *A. equina* may actually represent separate species (Perrin et al., 1999; Watts et al., 2000). Recently, further genotypic evidence has more strongly suggested that the green morphotype may represent a different species from the red morphotype (Wilding & Weedall, 2019). If true, this could indicate that some of the phenotypic variation presented in this thesis may be interspecific, rather than intraspecific. While this would not change the overall implications of climate change placing the two morphotypes in direct conflict, it could influence the interpretation of some other results. For example, rather than the heterogeneity of the seashore leading to greater intraspecific phenotypic variation (Dingemanse & Wolf, 2013; Quicke et al., 1983), the intertidal zone may in fact be so heterogeneous that it has driven *Actinia* towards sympatric speciation (Haylor et al., 1984; Wolf & Weissing, 2012). The implications of the results contained herein may thus be moderately altered in the future as the nature of the *Actinia* species complex continues to become clearer.

6.3.3. An incomplete picture of boldness

Predation risk to *A. equina* is inherently linked to foraging tentacle extension. A fully expanded anemone occupies a much larger space in the water, and is thus more likely to come into contact with a predator (Edmunds et al., 1974, 1976). Even so, SRT and IRT may not present a complete picture of boldness in this species. The two are only moderately correlated, and that correlation decouples at higher temperatures (Chapter 2), indicating that multiple risk-related behavioural axes may be present in these anemones (e.g. Houslay et al., 2018; Maskrey et al., 2018; Moran et al., 2016). Furthermore, although measured under different conditions, both SRT and IRT only represent a specific aspect of boldness: emergence (Beckmann & Biro, 2013). Researchers could expand future investigations to encompass other behaviours that might be related to risk-taking, and use multivariate approaches such as eigen decomposition (Houslay & Wilson, 2017) to build a more complete picture of their related axes. For example, future studies could quantify dispersal and exploration (e.g. Maskrey et al., 2018; Mouchet et al., 2021), the risks of which encompass not only predation, but also selectively detrimental environmental perturbation (e.g. Ma et al., 2018). For *A. equina*, these risks are particularly stark, as individuals often place themselves

at the mercy of the tides when they disperse (Edmunds et al., 1976). As such, dispersal and exploration behaviour could elucidate a different aspect of risk-taking than SRT and IRT. Future work could also consider foraging effort (e.g. Hall & Chalfoun, 2019; Lei et al., 2019), which might be defined as the time an animal spends with its foraging tentacles extended in a given period. Animals behave differently when not under stress (Killen et al., 2016; Killen et al., 2013) or reacting to different stimuli (Beckmann & Biro, 2013; Carter et al., 2013), so foraging activity is also likely to encompass a subtly different aspect of risk-taking than SRT or IRT. Studies could also incorporate other known repeatable behaviours in *A. equina*, which themselves show intraspecific variation in plasticity, such as aggression and competition (Lane & Briffa, 2017; Rudin & Briffa, 2011). Defining and investigating a wider range of behaviours in future studies of *A. equina* could provide useful insights into behavioural syndromes in this species and indicate how these might influence individual responses to climate change.

6.4. FUTURE DIRECTIONS

Widening the scope of behavioural assays of boldness is one of several avenues for future research raised by this thesis. I outline some other possible future directions below.

6.4.1. Boldness, metabolism, and the proteome

Expanded behavioural investigations, to build a more complete picture of boldness, could drive further research into the interplay between personality, metabolic rate, and the proteome in *A. equina*. Chapter 5 indicates that the lack of flexibility bold individuals showed in their SRTs in Chapter 2 may have been partly facilitated via proteomic changes. This is in contrast to established theories of coping styles, which argue that bolder, "proactive", individuals should exhibit a less prominent molecular stress response and reduced behavioural plasticity when compared with shyer, "reactive" individuals (Coppens et al., 2010; Koolhaas et al., 2010). Although emergence forms part of proactive syndromes in some species (Alfonso et al., 2019; Skov et al., 2019), these syndromes also often incorporate exploration and foraging (Ruiz-Gomez et al., 2011; Wong et al., 2019). Investigations of the proteome in relation to a more diverse array of risk-related behaviours could thus illuminate relationships between different behavioural axes and molecular stress responses. Similarly, while IRT may be a partial proxy for both boldness and foraging, incorporating foraging effort and SRT into future investigations of POLS across temperatures could provide clearer information as to

whether energetic demand or risk avoidance was driving the unexpected relationships presented in Chapter 4.

6.4.2. Personality and the proteome at baseline temperatures

While investigations of POLS frequently fail to account for phenotypic plasticity (Dammhahn et al., 2018; Montiglio et al., 2018), investigations of the molecular underpinnings of personality often suffer from the opposite problem by measuring molecular traits only when animals are under stress (e.g. Alfonso et al., 2019; Ferrari et al., 2020; Pusch et al., 2018; Chapter 5). Given that molecular expression should be an important proximate driver of behavioural variation (Bell & Aubin-Horth, 2010) and metabolic rate (Tomanek, 2011), discrepancies in behaviour or metabolism at baseline temperatures would be expected to be reflected in an individual's proteomic profile. Chapter 5 does not provide strong evidence that this is the case in *A. equina*, but it does show that the expression of specific structures and proteins may differ between personality-types. As such, the presence (or absence) of a relationship between personality and the proteome at baseline temperatures warrants further investigation. One way to address this could simply be to use a larger sample size in a follow-up whole-proteome study. While this might seem the intuitive solution, proteomic investigations are both expensive and labour-intensive (Torson et al., 2020), and there would be no guarantee that a whole-proteome approach, even with a larger sample, would pick up subtle associations between specific proteins and personality. Alternatively, researchers could firstly run a transcriptomic study as a follow-up. While still costly, this would be more affordable than the proteomic alternative, as transcriptomic approaches allow the analysis of multiple samples simultaneously (Torson et al., 2020). Multi-'omics' approaches can provide a more comprehensive picture of the complexity of an animal's biology (Cavigelli et al., 2021; Zhang et al., 2010), and using broad transcriptomic analyses could allow the production of reference transcriptomes for bold and shy anemones (e.g. Stefanik et al., 2014; Tulin et al., 2013). In turn, researchers could use these transcriptomes and data from Chapter 5 to identify specific candidate genes for proteomic follow-up (e.g. Bellis et al., 2016; Zhu et al., 2016) and clarify the results contained in this thesis. For instance, if HSP expression were higher in shy animals under baseline conditions as suggested by Chapter 5, it could also indicate that higher baseline stress-levels, not investment in growth, was driving those animals' RMRs at baseline temperatures in Chapter 4. By taking an initially broader-scale approach, future studies might ultimately facilitate a

more comprehensive, but simultaneously finer-scale, understanding of the molecular underpinnings of personality variation in *A. equina*.

6.4.3. Chronic Heat Stress

Though not strongly associated in control animals, personality and proteomic expression were much more clearly related under heat stress in Chapter 5. However, although Chapters 2 and 5 in tandem provide a strong indication that behavioural plasticity and proteomic stress responses are likely to be associated in *A. equina*, neither chapter explicitly tests this. Time-series approaches (Nieto-Barajas et al., 2012; Wilkins, 2009) could address this knowledge gap, investigating how an anemone's behavioural phenotype and proteomic expression shift over the course of heat-shock. At the purely proteomic level, a time-series study could shed light on some of the unexpected patterns shown in Chapter 5, such as determining whether HSP expression is up-regulated during the initial acute phase of heat stress (Bowler, 2005; Brahim et al., 2019). Further, repeated measurements of proteomic expression and behaviour could help provide insights into sustainable strategies for dealing with chronic heat stress, and indicate whether behavioural mitigations, such as increasing foraging effort (e.g. Chiba et al., 2007) or reducing exposed surface area (e.g. Ng et al., 2017), might facilitate those strategies. A truly comprehensive study would also take metabolic rate measurements at different points during heat shock. This could establish how different proteomic stress responses influence metabolic outcomes (Tomanek, 2011), and determine the validity of hypotheses presented in the discussion of Chapter 5, such as proteomic stress resistance driving a rise in a bold animal's metabolic rate at high temperatures, or biosynthetic suppression facilitating metabolic suppression in shyer animals. Despite the logistical challenges it would present, combining all three phenotypic levels in a single study could provide a unique impression of how between-individual differences in plasticity at one level might potentially affect between-individual differences at another.

6.4.4. Life-history implications: inference vs. evidence

Continued investigation of the physiological and molecular underpinnings of personality and plasticity is crucial to understanding the different strategies that individuals might employ to deal with climate change. However, another key direction that arises from this thesis, as mentioned earlier in this chapter, is to explore the ultimate outcomes of different plastic strategies. Despite a general acceptance that behavioural change is an important tool with which animals can respond to climatic shifts, the actual selective implications of behavioural

plasticity remain very under-explored (Ducatez et al., 2020; Maspons et al., 2019). While informed inferences as to the fitness benefits or costs of different strategies can be made, there is no substitute for empirical study, without which the true conservation implications of between-individual variation in behavioural plasticity will remain opaque (Montiglio et al., 2018; van Baaren & Candolin, 2018). Indeed, investigations of the fitness outcomes of personality often find unexpected results. For example, shy animals, traditionally hypothesised as favouring risk avoidance over investment into short-term life-history advantages (Réale et al., 2010; Smith & Blumstein, 2008), exhibit higher body condition (Fürtbauer, 2015), greater resource holding potential (Courtene-Jones & Briffa, 2014; Maskrey et al., 2018), and improved survival at high population densities (Nicolaus et al., 2016) in some species. As such, it is no great leap to anticipate that the fitness outcomes of different plastic behavioural strategies for dealing with climate change may also diverge from expectations in some species.

The current lack of study means that possible avenues of research abound in this area, but one of particular interest in relation to this thesis concerns the adaptive value of unpredictability. Although simulations (Scott-Samuel et al., 2015), and studies using humans as a proxy for prey (Jones et al., 2011; Richardson et al., 2018) have suggested that unpredictability may reduce predation risk, thus far only one study to my knowledge has empirically investigated this relationship in an animal model (Chang et al., 2017). The results of this study were mixed: while high levels of unpredictability improved the survival of the jumping spider *Cosmophasis umbratica* under predation by docile predators, this pattern was reversed when predators were aggressive. Chapter 3 provides some of the first evidence of between-individual variation in the plasticity of unpredictability (but see: Cornwell et al., 2019; Mitchell & Biro, 2017; Nakayama et al., 2016), but the adaptive value of these plastic changes remains to be explored. One method of investigating this could be to incorporate a nudibranch predator, *Aeolidia papillosa* (Edmunds et al., 1974), into future investigations. By measuring between-individual variation in mean level behaviour, unpredictability, and predator avoidance at different temperatures, future work could explicitly define any trade-off between predation risk and foraging in *A. equina*, and describe how that trade-off may be altered by thermal perturbation. In turn, it could provide just the second true empirical measure of the value of unpredictability in avoiding predators, expanding on the results of Chang et al (2017) to show how prey and predator behaviour might alter the adaptive value of different strategies under different environmental contexts. For example, while ectothermic

predator activity should be expected to increase at high temperatures (Miller et al., 2014; Twardochleb et al., 2020), evidence suggests that nudibranch foraging effort rapidly declines beyond a thermal threshold (Pires, 2012). If temperatures exceed the thermal maximum of *A. papillosa*, energetically costly predator avoidance in the form of shyness and unpredictability could become entirely maladaptive for *A. equina*. Incorporating measures of fitness into future work is thus essential, both in *A. equina* and beyond, to truly address the adaptive value of different behavioural responses to anthropogenic climate change.

6.5. CONCLUSIONS

This thesis highlights the multi-faceted nature of behavioural plasticity in *A. equina* and advocates that future work take account of physiological and molecular correlates of behavioural variation when investigating individual susceptibility to climate change. Alongside showing temperature-driven plasticity at multiple behavioural levels, it offers the first evidence that individual level relationships between boldness and metabolic rate are not static across temperatures, and demonstrates clear differences in proteomic thermal stress responses between personality-types. Under increasingly frequent extreme weather events, the selective equilibrium maintaining the expression of different responses to environmental perturbation may be upset, leading some animals to perish. This could drive a loss of diversity in populations of intertidal invertebrates living across the gradient of the seashore. Future studies should expand investigations of the proximate drivers of behavioural plasticity, in conjunction with addressing the selective implications of different responses to thermal perturbation by incorporating measurements of individual fitness outcomes.

THESIS REFERENCES

Abram, P. K., Boivin, G., Moiroux, J., & Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity. *Biological Reviews*, *92*(4), 1859–1876. https://doi.org/10.1111/brv.12312

Acasuso-Rivero, C., Murren, C. J., Schlichting, C. D., & Steiner, U. K. (2019). Adaptive phenotypic plasticity for life-history and less fitness-related traits. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1904), 1–9. https://doi.org/10.1098/rspb.2019.0653

Adriaenssens, B., & Johnsson, J. I. (2013). Natural selection, plasticity and the emergence of a behavioural syndrome in the wild. *Ecology Letters*, *16*(1), 47–55. https://doi.org/10.1111/ele.12011

Alfonso, S., Gesto, M., & Sadoul, B. (2020). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, *98*(6), 1496–1508. https://doi.org/10.1111/jfb.14599

Alfonso, S., Sadoul, B., Gesto, M., Joassard, L., Chatain, B., Geffroy, B., & Bégout, M. L. (2019). Coping styles in European sea bass: The link between boldness, stress response and neurogenesis. *Physiology and Behavior*, *207*(4), 76–85. https://doi.org/10.1016/j.physbeh.2019.04.020

Allcock, A. L., Watts, P. C., & Thorpe, J. P. (1998). Divergence of Nematocysts in Two Colour Morphs of the Intertidal Beadlet Anemone Actinia Equina . *Journal of the Marine Biological Association of the United Kingdom*, *78*(3), 821–828. https://doi.org/10.1017/s0025315400044805

Andrew, N. R., Hart, R. A., Jung, M. P., Hemmings, Z., & Terblanche, J. S. (2013). Can temperate insects take the heat? A case study of the physiological and behavioural responses in a common ant, Iridomyrmex purpureus (Formicidae), with potential climate change. *Journal of Insect Physiology*, *59*(9), 870–880. https://doi.org/10.1016/j.jinsphys.2013.06.003

Angilletta, M. J., & Dunham, A. E. (2003). The Temperature-Size Rule in Ectotherms: Simple Evolutionary Explanations May Not Be General. *American Naturalist*, *162*(3), 332–342. https://doi.org/10.1086/377187

Angilletta, M. J., Niewiarowski, P. H., & Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology*, *27*(4), 249–268. https://doi.org/https://doi.org/10.1016/S0306-4565(01)00094-8

Angilletta, M. J., Steury, T. D., & Sears, M. W. (2004). Temperature, growth rate, and body size in ectotherms: Fitting pieces of a life-history puzzle. *Integrative and Comparative Biology*, *44*(6), 498–509. https://doi.org/10.1093/icb/44.6.498

Angiulli, E., Pagliara, V., Cioni, C., Frabetti, F., Pizzetti, F., Alleva, E., & Toni, M. (2020). Increase in environmental temperature affects exploratory behaviour, anxiety and social preference in Danio rerio. *Scientific Reports*, *10*, 5385. https://doi.org/10.1038/s41598-020-62331-1

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, *14*(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Arnell, N. W., Lowe, J. A., Challinor, A. J., & Osborn, T. J. (2019). Global and regional impacts of climate change at different levels of global temperature increase. *Climatic Change*, *155*(3), 377–391. https://doi.org/10.1007/s10584-019-02464-z

Auld, J. R., Agrawal, A. A., & Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1681), 503–511. https://doi.org/10.1098/rspb.2009.1355

Ballew, N. G., Mittelbach, G. G., & Scribner, K. T. (2017). Fitness Consequences of Boldness in Juvenile and Adult Largemouth Bass. *The American Naturalist*, *189*(4), 396–406. https://doi.org/10.1086/690909

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Baugh, A. T., Schaper, S. V., Hau, M., Cockrem, J. F., de Goede, P., & Oers, K. van. (2012). Corticosterone responses differ between lines of great tits (Parus major) selected for divergent personalities. *General and Comparative Endocrinology*, *175*(3), 488–494. https://doi.org/10.1016/j.ygcen.2011.12.012

Beckmann, C., & Biro, P. A. (2013). On the Validity of a Single (Boldness) Assay in

Personality Research. *Ethology*, *119*(11), 937–947. https://doi.org/10.1111/eth.12137

Beever, E. A., Hall, L. E., Varner, J., Loosen, A. E., Dunham, J. B., Gahl, M. K., Smith, F. A., & Lawler, J. J. (2017). Behavioral flexibility as a mechanism for coping with climate change. *Frontiers in Ecology and the Environment*, *15*(6), 299–308. https://doi.org/10.1002/fee.1502

Belgrad, B. A., Karan, J., & Griffen, B. D. (2017). Individual personality associated with interactions between physiological condition and the environment. *Animal Behaviour*, *123*, 277–284. https://doi.org/10.1016/j.anbehav.2016.11.008

Bell, A. M. (2007). Future directions in behavioural syndromes research. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 755–761. https://doi.org/10.1098/rspb.2006.0199

Bell, A. M., & Aubin-Horth, N. (2010). What can whole genome expression data tell us about the ecology and evolution of personality? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4001–4012. https://doi.org/10.1098/rstb.2010.0185

Bell, A. M., Hankison, S. J., & Laskowski, K. L. (2009). The repeatability of behaviour: a meta-analysis. *Animal Behaviour*, *77*(4), 771–783. https://doi.org/10.1016/j.anbehav.2008.12.022

Bell, A. M., & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). *Ecology Letters*, *10*(9), 828–834. https://doi.org/10.1111/j.1461-0248.2007.01081.x

Bell, J. J., Shaw, C., & Turner, J. R. (2006). Factors controlling the tentacle and polyp expansion behaviour of selected temperate Anthozoa. *Journal of the Marine Biological Association of the United Kingdom*, *86*(5), 977–992. https://doi.org/10.1017/S0025315406013956

Bellis, E. S., Howe, D. K., & Denver, D. R. (2016). Genome-wide polymorphism and signatures of selection in the symbiotic sea anemone Aiptasia. *BMC Genomics*, *17*(1), 1–14. https://doi.org/10.1186/s12864-016-2488-6

Bennett, A. F. (1988). Structural and functional determinates of metabolic rate. *Integrative and Comparative Biology*, *28*(2), 699–708. https://doi.org/10.1093/icb/28.2.699

Bierbach, D., Laskowski, K. L., & Wolf, M. (2017). Behavioural individuality in clonal fish arises despite near-identical rearing conditions. *Nature Communications*, *8*(1), 1–7. https://doi.org/10.1038/ncomms15361

Birnie-Gauvin, K., Costantini, D., Cooke, S. J., & Willmore, W. G. (2017). A comparative and evolutionary approach to oxidative stress in fish: A review. *Fish and Fisheries*, *18*(5), 928–942. https://doi.org/10.1111/faf.12215

Biro, P. A. (2013). Are most samples of animals systematically biased? Consistent individual trait differences bias samples despite random sampling. *Oecologia*, *171*(2), 339–345. https://doi.org/10.1007/s00442-012-2426-5

Biro, P. A., & Adriaenssens, B. (2013). Predictability as a Personality Trait: Consistent Differences in Intraindividual Behavioral Variation. *The American Naturalist*, *182*(5), 621–629. https://doi.org/10.1086/673213

Biro, P. A., Beckmann, C., & Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1678), 71–77. https://doi.org/10.1098/rspb.2009.1346

Biro, P. A., Garland, T., Beckmann, C., Ujvari, B., Thomas, F., & Post, J. R. (2018). Metabolic scope as a proximate constraint on individual behavioral variation: Effects on personality, plasticity, and predictability. *American Naturalist*, *192*(2), 142–154. https://doi.org/10.1086/697963

Biro, P. A., & Stamps, J. A. (2010). Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? *Trends in Ecology and Evolution*, *25*(11), 653–659. https://doi.org/10.1016/j.tree.2010.08.003

Biro, P. A., & Stamps, J. A. (2015). Using repeatability to study physiological and behavioural traits: Ignore time-related change at your peril. *Animal Behaviour*, *105*, 223–230. https://doi.org/10.1016/j.anbehav.2015.04.008

Biro, P., O'Connor, J., Pedini, L., & Gribben, P. E. (2013). Personality and plasticity: Consistent responses within-, but not across-temperature situations in crabs. *Behaviour*, *150*(7), 799–811. https://doi.org/10.1163/1568539X-00003081

Black, N. A., Voellmy, R., & Szmant, A. M. (2007). Heat Shock Protein Induction in

Montastraea faveolata and Aiptasia pallida Exposed to Elevated Temperatures. *The Biological Bulletin*, *188*(3), 234–240. https://doi.org/10.2307/1542301

Bockelmann, A. C., Bakker, J. P., Neuhaus, R., & Lage, J. (2002). The relation between vegetation zonation, elevation and inundation frequency in a Wadden Sea salt marsh. *Aquatic Botany*, *73*(3), 211–221. https://doi.org/10.1016/S0304-3770(02)00022-0

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist*, *161*(1), 1–28. https://doi.org/10.1086/343878

Bowler, K. (2005). Acclimation, heat shock and hardening. *Journal of Thermal Biology*, *30*(2), 125–130. https://doi.org/10.1016/j.jtherbio.2004.09.001

Boyd, C. E., & Pillai, V. K. (1985). Water Quality Management in Aquaculture. *CMFRI Special Publication 22*, 1–44.

Brace, R. C., Pavey, J., & Quicke, D. L. J. (1979). Intraspecific aggression in the colour morphs of the anemone Actinia equina: The "convention" governing dominance ranking. *Animal Behaviour*, *27*, 553–561. https://doi.org/10.1016/0003-3472(79)90190-8

Brace, R. C., & Quicke, D. L. J. (1986). Seasonal changes in dispersion within an aggregation of the anemone, Actinia equina, with a reappraisal of the role of intraspecific aggression. *Journal of the Marine Biological Association of the United Kingdom*, *66*(1), 49–70.

Brace, R. C., & Reynolds, H. A. (1989). Relative intraspecific aggressiveness of pedal disc colour phenotypes of the beadlet anemone, Actinia equina. *Journal of the Marine Biological Association of the United Kingdom*, *69*(2), 273–278. https://doi.org/10.1017/S0025315400029398

Brahim, A., Mustapha, N., & Marshall, D. J. (2019). Non-reversible and reversible heat tolerance plasticity in tropical intertidal animals: Responding to habitat temperature heterogeneity. *Frontiers in Physiology*, *9*, 1909. https://doi.org/10.3389/fphys.2018.01909

Bridger, D., Bonner, S. J., & Briffa, M. (2015). Individual quality and personality: Bolder males are less fecund in the hermit crab Pagurus bernhardus. *Proceedings of the Royal*

Society B: Biological Sciences, *282*(1803), 20142492. https://doi.org/10.1098/rspb.2014.2492

Briffa, M. (2013). Plastic proteans: Reduced predictability in the face of predation risk in hermit crabs. *Biology Letters*, *9*(5), 20130592. https://doi.org/10.1098/rsbl.2013.0592

Briffa, M., Bridger, D., & Biro, P. A. (2013). How does temperature affect behaviour? Multilevel analysis of plasticity, personality and predictability in hermit crabs. *Animal Behaviour*, *86*(1), 47–54. https://doi.org/10.1016/j.anbehav.2013.04.009

Briffa, M., & Greenaway, J. (2011). High in situ repeatability of behaviour indicates animal personality in the beadlet anemone Actinia equina (Cnidaria). *PLoS ONE*, *6*(7), e21963. https://doi.org/10.1371/journal.pone.0021963

Brommer, J. E. (2013). Phenotypic plasticity of labile traits in the wild. *Current Zoology*, *59*(4), 485–505. https://doi.org/10.1093/czoolo/59.4.485

Brooker, R. M., Feeney, W. E., White, J. R., Manassa, R. P., Johansen, J. L., & Dixson, D. L. (2016). Using insights from animal behaviour and behavioural ecology to inform marine conservation initiatives. *Animal Behaviour*, *120*, 211–221. https://doi.org/10.1016/j.anbehav.2016.03.012

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Buchholz, R., Banusiewicz, J. D., Burgess, S., Crocker-Buta, S., Eveland, L., & Fuller, L. (2019). Behavioural research priorities for the study of animal response to climate change. *Animal Behaviour*, *150*, 127–137. https://doi.org/10.1016/j.anbehav.2019.02.005

Buckley, L. B., & Kingsolver, J. G. (2019). Environmental variability shapes evolution, plasticity and biogeographic responses to climate change. *Global Ecology and Biogeography*, *28*(10), 1456–1468. https://doi.org/10.1111/geb.12953

Bucklin, A. (1987). Adaptive advantages of patterns of growth and asexual reproduction of the sea anemone Metridium senile (L.) in intertidal and submerged populations. *Journal of Experimental Marine Biology and Ecology*, *110*(3), 225–243. https://doi.org/10.1016/0022-0981(87)90003-7

Busby, A. T., Ayllón, N., Kocan, K. M., Blouin, E. F., de La Fuente, G., Galindo, R. C., Villar, M., & de la Fuente, J. (2012). Expression of heat shock proteins and subolesin affects stress responses, Anaplasma phagocytophilum infection and questing behaviour in the tick, Ixodes scapularis. *Medical and Veterinary Entomology*, *26*(1), 92–102. https://doi.org/10.1111/j.1365-2915.2011.00973.x

Caldwell, A. J., While, G. M., & Wapstra, E. (2017). Plasticity of thermoregulatory behaviour in response to the thermal environment by widespread and alpine reptile species. *Animal Behaviour*, *132*, 217–227. https://doi.org/10.1016/j.anbehav.2017.07.025

Careau, V., Thomas, D., Humphries, M. M., & Réale, D. (2008). Energy Metabolism and Animal Personality. *Oikos*, *117*(5), 641–653. https://doi.org/10.1111/j.2008.0030-1299.16513.x

Caro, T. (2016). Behavior and conservation, conservation and behavior. *Current Opinion in Behavioral Sciences*, *12*, 97–102. https://doi.org/10.1016/j.cobeha.2016.09.008

Carter, A. J., & Feeney, W. E. (2012). Taking a comparative approach: Analysing personality as a multivariate behavioural response across species. *PLoS ONE*, *7*(7), e42440. https://doi.org/10.1371/journal.pone.0042440

Carter, A. J., Feeney, W. E., Marshall, H. H., Cowlishaw, G., & Heinsohn, R. (2013). Animal personality: What are behavioural ecologists measuring? *Biological Reviews*, *88*(2), 465–475. https://doi.org/10.1111/brv.12007

Carter, M. A., & Thorpe, J. P. (1981). Reproductive, genetic and ecological evidence that actinia equina var. mesembryanthemum and var. fragacea are not conspecific. *Journal of the Marine Biological Association of the United Kingdom*, *61*(1), 79–93. https://doi.org/10.1017/S0025315400045926

Castanheira, M. F., Conceição, L. E. C., Millot, S., Rey, S., Bégout, M. L., Damsgård, B., Kristiansen, T., Höglund, E., Øverli, Ø., & Martins, C. I. M. (2017). Coping styles in farmed fish: consequences for aquaculture. *Reviews in Aquaculture*, *9*(1), 23–41. https://doi.org/10.1111/raq.12100

Cavigelli, S., Leips, J., Xiang, Q.-Y. (Jenny), Lemke, D., & Konow, N. (2021). Next Steps in Integrative Biology: Mapping Interactive Processes Across Levels of Biological

Organization. *Integrative and Comparative Biology*, icab161. https://doi.org/10.1093/icb/icab161

Cerqueira, M., Rey, S., Silva, T., Featherstone, Z., Crumlish, M., & MacKenzie, S. (2016). Thermal preference predicts animal personality in Nile tilapia Oreochromis niloticus. *The Journal of Animal Ecology*, *85*(5), 1389–1400. https://doi.org/10.1111/1365-2656.12555

Chang, C. C., Teo, H. Y., Norma-Rashid, Y., & Li, D. (2017). Predator personality and prey behavioural predictability jointly determine foraging performance. *Scientific Reports*, *7*, 40734. https://doi.org/10.1038/srep40734

Chapperon, C., Volkenborn, N., Clavier, J., Séité, S., Seabra, R., & Lima, F. P. (2016). Exposure to solar radiation drives organismal vulnerability to climate: Evidence from an intertidal limpet. *Journal of Thermal Biology*, *57*, 92–100. https://doi.org/10.1016/j.jtherbio.2016.03.002

Cheng, L., Zhu, J., Abraham, J., Trenberth, K. E., Fasullo, J. T., Zhang, B., Yu, F., Wan, L., Chen, X., & Song, X. (2019). 2018 Continues Record Global Ocean Warming. *Advances in Atmospheric Sciences*, *36*(3), 249–252. https://doi.org/10.1007/s00376-019-8276-x

Chiba, S., Arnott, S. A., & Conover, D. O. (2007). Coevolution of foraging behavior with intrinsic growth rate: Risk-taking in naturally and artificially selected growth genotypes of Menidia menidia. *Oecologia*, *154*(1), 237–246. https://doi.org/10.1007/s00442-007-0825-9

Chintiroglou, C., & Koukouras, A. (1992). The feeding habits of three Mediterranean sea anemone species, Anemonia viridis (Forskål), Actinia equina (Linnaeus) and Cereus pedunculatus (Pennant). *Helgoländer Meeresuntersuchungen*, *46*(1), 53–68. https://doi.org/10.1007/BF02366212

Chomsky, O., Kamenir, Y., Hyams, M., Dubinsky, Z., & Chadwick-Furman, N. E. (2004a). Effects of feeding regime on growth rate in the Mediterranean Sea anemone Actinia equina (Linnaeus). *Journal of Experimental Marine Biology and Ecology*, *299*(2), 217–229. https://doi.org/10.1016/j.jembe.2003.09.009

Chomsky, O., Kamenir, Y., Hyams, M., Dubinsky, Z., & Chadwick-Furman, N. E. (2004b).

Effects of temperature on growth rate and body size in the Mediterranean Sea anemone Actinia equina. *Journal of Experimental Marine Biology and Ecology*, *313*(1), 63–73. https://doi.org/10.1016/j.jembe.2004.07.017

Choresh, O., Azem, A., & Loya, Y. (2007). Over-expression of highly conserved mitochondrial 70-kDa heat-shock protein in the sea anemone Anemonia viridis. *Journal of Thermal Biology*, *32*(7–8), 367–373. https://doi.org/10.1016/j.jtherbio.2007.04.006

Choresh, O., Ron, E., & Loya, Y. (2001). The 60-kDa heat shock protein (HSP60) of the sea anemone Anemonia viridis: A potential early warning system for environmental changes. *Marine Biotechnology*, *3*(5), 501–508. https://doi.org/10.1007/s10126-001-0007-4

Clarke, A., & Fraser, K. P. P. (2004). Why does metabolism scale with temperature? *Functional Ecology*, *18*(2), 243–251. https://doi.org/10.1111/j.0269-8463.2004.00841.x

Clarke, A., & Johnston, N. M. (1999). Scaling of metabolic rate with body mass and temperature in teleost fish. *Journal of Animal Ecology*, *68*(5), 893–905. https://doi.org/10.1046/j.1365-2656.1999.00337.x

Cleasby, I. R., Nakagawa, S., & Schielzeth, H. (2015). Quantifying the predictability of behaviour: Statistical approaches for the study of between-individual variation in the within-individual variance. *Methods in Ecology and Evolution*, *6*(1), 27–37. https://doi.org/10.1111/2041-210X.12281

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(2), 169–178. https://doi.org/10.1007/s10336-007-0175-8

Collins, J. R., Vernon, E. L., & Thomson, J. S. (2017). Variation in risk-taking and aggression in morphotypes of the beadlet anemone, Actinia equina (L.), and the green anemone, Actinia prasina (Gosse). *Journal of Experimental Marine Biology and Ecology*, *496*, 29–36. https://doi.org/10.1016/j.jembe.2017.07.011

Coppens, C. M., de Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: towards underlying mechanisms. *Philosophical Transactions: Biological Sciences*, *365*(1560), 4021–4028.

Cornwell, T. O., McCarthy, I. D., & Biro, P. A. (2020). Integration of physiology, behaviour and life history traits: personality and pace of life in a marine gastropod. *Animal*

Behaviour, *163*, 155–162. https://doi.org/10.1016/j.anbehav.2020.03.009

Cornwell, T. O., McCarthy, I. D., Snyder, C. 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*(4), 511–520. https://doi.org/10.1111/1365-2656.12935

Costantini, D., Ferrari, C., Pasquaretta, C., Cavallone, E., Carere, C., Von Hardenberg, A., & Réale, D. (2012). Interplay between plasma oxidative status, cortisol and coping styles in wild alpine marmots, Marmota marmota. *Journal of Experimental Biology*, *215*(2), 374–383. https://doi.org/10.1242/jeb.062034

Courtene-Jones, W., & Briffa, M. (2014). Boldness and asymmetric contests: Role- and outcome-dependent effects of fighting in hermit crabs. *Behavioral Ecology*, *25*(5), 1073–1082. https://doi.org/10.1093/beheco/aru085

Crickenberger, S., Hui, T. Y., Landry Yuan, F., Bonebrake, T. C., & Williams, G. A. (2020). Preferred temperature of intertidal ectotherms: Broad patterns and methodological approaches. *Journal of Thermal Biology*, *87*, 102468. https://doi.org/10.1016/j.jtherbio.2019.102468

Dall, S. R. X., Bell, A. M., Bolnick, D. I., & Ratnieks, F. L. W. (2012). An evolutionary ecology of individual differences. *Ecology Letters*, *15*(10), 1189–1198. https://doi.org/10.1111/j.1461-0248.2012.01846.x

Dall, S. R. X., Houston, A. I., & McNamara, J. M. (2004). The behavioural ecology of personality: Consistent individual differences from an adaptive perspective. *Ecology Letters*, *7*(8), 734–739. https://doi.org/10.1111/j.1461-0248.2004.00618.x

Dalvi, R. S., Das, T., Debnath, D., Yengkokpam, S., Baruah, K., Tiwari, L. R., & Pal, A. K. (2017). Metabolic and cellular stress responses of catfish, Horabagrus brachysoma (Günther) acclimated to increasing temperatures. *Journal of Thermal Biology*, *65*, 32–40. https://doi.org/10.1016/j.jtherbio.2017.02.003

Dammhahn, M., Dingemanse, N. J., Niemelä, P. T., & Réale, D. (2018). Pace-of-life syndromes: a framework for the adaptive integration of behaviour, physiology and life history. *Behavioral Ecology and Sociobiology*, *72*(3). https://doi.org/10.1007/s00265-018-2473-y

Darmaraki, S., Somot, S., Sevault, F., Nabat, P., Cabos Narvaez, W. D., Cavicchia, L., Djurdjevic, V., Li, L., Sannino, G., & Sein, D. V. (2019). Future evolution of Marine Heatwaves in the Mediterranean Sea. *Climate Dynamics*, *53*(3), 1371–1392. https://doi.org/10.1007/s00382-019-04661-z

de Azevedo, C. S., & Young, R. J. (2021). Animal personality and conservation: Basics for inspiring new research. *Animals*, *11*(4), 1–13. https://doi.org/10.3390/ani11041019

De Bruijn, R., & Romero, L. M. (2011). Behavioral and physiological responses of wild-caught European starlings (Sturnus vulgaris) to a minor, rapid change in ambient temperature. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *160*(2), 260–266. https://doi.org/10.1016/j.cbpa.2011.06.011

Debecker, S., & Stoks, R. (2019). Pace of life syndrome under warming and pollution: integrating life history, behavior, and physiology across latitudes. *Ecological Monographs*, *89*(1), 1–22. https://doi.org/10.1002/ecm.1332

Debeffe, L., Lemaître, J. F., Bergvall, U. A., Hewison, A. J. M., Gaillard, J. M., Morellet, N., Goulard, M., Monestier, C., David, M., Verheyden-Tixier, H., Jäderberg, L., Vanpé, C., & Kjellander, P. (2015). Short- and long-term repeatability of docility in the roe deer: Sex and age matter. *Animal Behaviour*, *109*, 53–63. https://doi.org/10.1016/j.anbehav.2015.08.003

Dell, A. I., Pawar, S., & Savage, V. M. (2014). Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. *Journal of Animal Ecology*, *83*(1), 70–84. https://doi.org/10.1111/1365-2656.12081

Deutsch, C., Ferrel, A., Seibel, B., Pörtner, H. O., & Huey, R. B. (2015). Climate change tightens a metabolic constraint on marine habitats. *Science*, *348*(6239), 1132–1136. https://doi.org/DOI: 10.1126/science.aaa1605

Dewitt, T. J., Sih, A., & Sloan Wilson, D. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology and Evolution*, *13*(2), 77–81. https://doi.org/https://doi.org/10.1016/S0169-5347(97)01274-3

Dias, G. M., Christofoletti, R. A., Kitazawa, K., & Jenkins, S. R. (2018). Environmental heterogeneity at small spatial scales affects population and community dynamics on intertidal rocky shores of a threatened bay system. *Ocean and Coastal Management*,

164, 52–59. https://doi.org/10.1016/j.ocecoaman.2017.12.001

Dijkstra, J. A., Westerman, E. L., & Harris, L. G. (2011). The effects of climate change on species composition, succession and phenology: A case study. *Global Change Biology*, *17*(7), 2360–2369. https://doi.org/10.1111/j.1365-2486.2010.02371.x

Dingemanse, N. J., & Dochtermann, N. A. (2013). Quantifying individual variation in behaviour: Mixed-effect modelling approaches. *Journal of Animal Ecology*, *82*(1), 39–54. https://doi.org/10.1111/1365-2656.12013

Dingemanse, N. J., Kazem, A. J. N., Réale, D., & Wright, J. (2010). Behavioural reaction norms: animal personality meets individual plasticity. *Trends in Ecology and Evolution*, *25*(2), 81–89. https://doi.org/10.1016/j.tree.2009.07.013

Dingemanse, N. J., & Wolf, M. (2013). Between-individual differences in behavioural plasticity within populations: Causes and consequences. *Animal Behaviour*, *85*(5), 1031–1039. https://doi.org/10.1016/j.anbehav.2012.12.032

Dingemanse, N. J., Wright, J., Kazem, A. J. N., Thomas, D. K., Hickling, R., & Dawnay, N. (2007). Behavioural syndromes differ predictably between 12 populations of three-spined stickleback. *Journal of Animal Ecology*, *76*(6), 1128–1138. https://doi.org/10.1111/j.1365-2656.2007.01284.x

Diz, A. P., Martínez-Fernández, M., & Rolán-Alvarez, E. (2012). Proteomics in evolutionary ecology: Linking the genotype with the phenotype. *Molecular Ecology*, *21*(5), 1060–1080. https://doi.org/10.1111/j.1365-294X.2011.05426.x

Dochtermann, N. A., Schwab, T., Anderson Berdal, M., Dalos, J., & Royauté, R. (2019). The Heritability of Behavior: A Meta-analysis. *Journal of Heredity*, *110*(4), 403–410. https://doi.org/10.1093/jhered/esz023

Dochtermann, N. A., Schwab, T., & Sih, A. (2014). The contribution of additive genetic variation to personality variation: Heritability of personality. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1798), 20142201. https://doi.org/10.1098/rspb.2014.2201

Dodd, L. F., Grabowski, J. H., Piehler, M. F., Westfield, I., & Ries, J. B. (2015). Ocean acidification impairs crab foraging behaviour. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1810), 20150333. https://doi.org/10.1098/rspb.2015.0333

Dohm, M. R. (2003). Repeatability estimates do not always set an upper limit to heritability. *Functional Ecology*, *16*(2), 273–280. https://doi.org/10.1046/j.1365-2435.2002.00621.x

Doney, S. C., Ruckelshaus, M., Emmett Duffy, J., Barry, J. P., Chan, F., English, C. A., Galindo, H. M., Grebmeier, J. M., Hollowed, A. B., Knowlton, N., Polovina, J., Rabalais, N. N., Sydeman, W. J., & Talley, L. D. (2012). Climate change impacts on marine ecosystems. *Annual Review of Marine Science*, *4*, 11–37. https://doi.org/10.1146/annurev-marine-041911-111611

Dong, Y., Miller, L. P., Sanders, J. G., Somero, G. N., Dong, Y., Miller, L. P., Sanders, J. O. N. G., & Somero, G. N. (2008). Heat-Shock Protein 70 (Hsp70) Expression in Four Limpets of the Genus Lottia: Interspecific Variation in Constitutive and Inducible Synthesis Correlates with in situ Exposure to Heat Stress. *Biological Bulletin*, *215*(2), 173–181. https://doi.org/https://doi.org/10.2307/25470698

Ducatez, S., Sol, D., Sayol, F., & Lefebvre, L. (2020). Behavioural plasticity is associated with reduced extinction risk in birds. *Nature Ecology and Evolution*, *4*(6), 788–793. https://doi.org/10.1038/s41559-020-1168-8

Duckworth, R. A. (2010). Evolution of Personality: Developmental Constraints on Behavioral Flexibility. *The Auk*, *127*(4), 752–758. https://doi.org/10.1525/auk.2010.127.4.752

Eckerström-Liedholm, S., Sowersby, W., Morozov, S., van der Bijl, W., Rowiński, P. K., Gonzalez-Voyer, A., & Rogell, B. (2019). Macroevolutionary evidence suggests trait-dependent coevolution between behavior and life-history. *Evolution*, *73*(11), 2312–2323. https://doi.org/10.1111/evo.13845

Edmunds, M., Potts, G. W., Swinfen, R. C., & Waters, V. L. (1974). The feeding preferences of Aeolidia papillosa (L.) (Mollusca, Nudibranchia). *Journal of the Marine Biological Association of the United Kingdom*, *54*(4), 939–947. https://doi.org/10.1017/S0025315400057660

Edmunds, M., Potts, G. W., Swinfen, R. C., & Waters, V. L. (1976). Defensive behaviour of sea anemones in response to predation by the opisthobranch mollusc Aeolidia papillosa (L.). *Journal of the Marine Biological Association of the United Kingdom*, *56*(1), 65–83. https://doi.org/10.1017/s0025315400020440

Edwards, H. A., Winney, I. S., Schroeder, J., & Dugdale, H. L. (2013). Do rapid assays predict repeatability in labile (behavioural) traits? A reply to Biro. *Animal Behaviour*, *85*(3), e1. https://doi.org/10.1016/j.anbehav.2012.12.021

Erguler, K., Pieri, M., & Deltas, C. (2013). A mathematical model of the unfolded protein stress response reveals the decision mechanism for recovery, adaptation and apoptosis. *BMC Systems Biology*, *7*. https://doi.org/10.1186/1752-0509-7-16

Feder, M. E., & Walser, J. C. (2005). The biological limitations of transcriptomics in elucidating stress and stress responses. *Journal of Evolutionary Biology*, *18*(4), 901–910. https://doi.org/10.1111/j.1420-9101.2005.00921.x

Ferrari, C., Pasquaretta, C., Carere, C., Cavallone, E., von Hardenberg, A., & Réale, D. (2013). Testing for the presence of coping styles in a wild mammal. *Animal Behaviour*, *85*(6), 1385–1396. https://doi.org/10.1016/j.anbehav.2013.03.030

Ferrari, S., Rey, S., Høglund, E., Øverli, Ø., Chatain, B., MacKenzie, S., & Bégout, M. L. (2020). Physiological responses during acute stress recovery depend on stress coping style in European sea bass, Dicentrarchus labrax. *Physiology and Behavior*, *216*, 112801. https://doi.org/10.1016/j.physbeh.2020.112801

Fordyce, A. J., Ainsworth, T. D., Heron, S. F., & Leggat, W. (2019). Marine heatwave hotspots in coral reef environments: Physical drivers, ecophysiological outcomes and impact upon structural complexity. *Frontiers in Marine Science*, *6*, 498. https://doi.org/10.3389/fmars.2019.00498

Forsatkar, M. N., Nematollahi, M. A., Biro, P. A., & Beckmann, C. (2016). Individual boldness traits influenced by temperature in male Siamese fighting fish. *Physiology and Behavior*, *165*, 267–272. https://doi.org/10.1016/j.physbeh.2016.08.007

Foster, N. L., & Briffa, M. (2014). Familial strife on the seashore: Aggression increases with relatedness in the sea anemone Actinia equina. *Behavioural Processes*, *103*, 243–245. https://doi.org/10.1016/j.beproc.2014.01.009

Fox, R. J., Donelson, J. M., Schunter, C., Ravasi, T., & Gaitán-Espitia, J. D. (2019). Beyond buying time: The role of plasticity in phenotypic adaptation to rapid environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180174. https://doi.org/10.1098/rstb.2018.0174

Fürtbauer, I. (2015). Consistent individual differences in haemolymph density reflect risk propensity in a marine invertebrate. *Royal Society Open Science*, *2*(6), 0–5. https://doi.org/10.1098/rsos.140482

Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., Harmelin, J. G., Gambi, M. C., Kersting, D. K., Ledoux, J. B., Lejeusne, C., Linares, C., Marschal, C., Pérez, T., Ribes, M., Romano, J. C., Serrano, E., Teixido, N., … Cerrano, C. (2009). Mass mortality in Northwestern Mediterranean rocky benthic communities: Effects of the 2003 heat wave. *Global Change Biology*, *15*(5), 1090–1103. https://doi.org/10.1111/j.1365-2486.2008.01823.x

Gashout, S. E., & Ormond, R. F. G. (1979). Evidence for Parthenogenetic Reproduction in the Sea Anemone Actinia Equina L. *Journal of the Marine Biological Association of the United Kingdom*, *59*(4), 975–987. https://doi.org/10.1017/S0025315400036973

Gasparini, C., Speechley, E. M., & Polverino, G. (2019). The bold and the sperm: Positive association between boldness and sperm number in the guppy. *Royal Society Open Science*, *6*(7), 1–6. https://doi.org/10.1098/rsos.190474

Geffroy, B., Alfonso, S., Sadoul, B., & Blumstein, D. T. (2020). A World for Reactive Phenotypes. *Frontiers in Conservation Science*, *1*, 1–5. https://doi.org/10.3389/fcosc.2020.611919

Ghalambor, C. K., Angeloni, L. M., & Carroll, S. P. (2010). Behaviour as phenotypic plasticity. In D. F. Westneat & C. W. Fox (Eds.), *Evolutionary Behavioural Ecology*. Oxford University Press.

Glazier, D. S. (2005). Beyond the "3/4-power law": Variation in the intra- and interspecific scaling of metabolic rate in animals. *Biological Reviews of the Cambridge Philosophical Society*, *80*(4), 611–662. https://doi.org/10.1017/S1464793105006834

Gleason, L. U., & Burton, R. S. (2015). RNA-seq reveals regional differences in transcriptome response to heat stress in the marine snail Chlorostoma funebralis. *Molecular Ecology*, *24*(3), 610–627. https://doi.org/10.1111/mec.13047

Goold, C., & Newberry, R. C. (2017). Modelling personality, plasticity and predictability in shelter dogs. *Royal Society Open Science*, *4*(9), 170618. https://doi.org/10.1098/rsos.170618

Gormally, B. M. G., & Romero, L. M. (2020). What are you actually measuring? A review of techniques that integrate the stress response on distinct time-scales. *Functional Ecology*, *34*(10), 2030–2044. https://doi.org/10.1111/1365-2435.13648

Gosling, S. D. (2008). Personality in Non-human Animals. *Social and Personality Psychology Compass*, *2*(2), 985–1001. https://doi.org/10.1111/j.1751-9004.2008.00087.x

Goulet, C. T., Michelangeli, M., Chung, M., Riley, J. L., Wong, B. B. M., Thompson, M. B., & Chapple, D. G. (2018). Evaluating cognition and thermal physiology as components of the pace-of-life syndrome. *Evolutionary Ecology*, *32*(5), 469–488. https://doi.org/10.1007/s10682-018-9948-1

Goulet, C. T., Thompson, M. B., Michelangeli, M., Wong, B. B. M., & Chapple, D. G. (2017). Thermal physiology: A new dimension of the pace-of-life syndrome. *Journal of Animal Ecology*, *86*(5), 1269–1280. https://doi.org/10.1111/1365-2656.12718

Griffiths, R. J. (1977a). Temperature acclimation in Actinia equina L. (Anthozoa). *Journal of Experimental Marine Biology and Ecology*, *28*(3), 285–292. https://doi.org/10.1016/0022-0981(77)90097-1

Griffiths, R. J. (1977b). Thermal stress and the biology of Actinia equina L. (Anthozoa). *Journal of Experimental Marine Biology and Ecology*, *27*(2), 141–154. https://doi.org/10.1016/0022-0981(77)90134-4

Grilo, T. F., Lopes, A. R., Sampaio, E., Rosa, R., & Cardoso, P. G. (2018). Sex differences in oxidative stress responses of tropical topshells (Trochus histrio) to increased temperature and high pCO2. *Marine Pollution Bulletin*, *131*, 252–259. https://doi.org/10.1016/j.marpolbul.2018.04.031

Hadfield, J. D. (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. *Journal of Statistical Software*, *33*(2). https://doi.org/10.18637/jss.v033.i02

Hall, L. E., & Chalfoun, A. D. (2019). Behavioural plasticity modulates temperature-related constraints on foraging time for a montane mammal. *Journal of Animal Ecology*, *88*(3), 363–375. https://doi.org/10.1111/1365-2656.12925

Hall, M. L., van Asten, T., Katsis, A. C., Dingemanse, N. J., Magrath, M. J. L., & Mulder, R.

A. (2015). Animal personality and pace-of-life syndromes: do fast-exploring fairy-wrens die young? *Frontiers in Ecology and Evolution*, *3*, 28. https://doi.org/10.3389/fevo.2015.00028

Hall, S. J., Todd, C. D., & Gordon, A. D. (1982). The Influence of Ingestive Conditioning on the Prey Species Selection in Aeolidia papillosa (Mollusca: Nudibranchia). *The Journal of Animal Ecology*, *51*(3), 907. https://doi.org/10.2307/4013

Hansen, J. E. (2006). Can we still avoid dangerous human-made climate change? *Social Research*, *73*(3), 949–971.

Harianto, J., Carey, N., & Byrne, M. (2019). respR—An R package for the manipulation and analysis of respirometry data. *Methods in Ecology and Evolution*, *10*(6), 912–920. https://doi.org/10.1111/2041-210X.13162

Harley, C. D. G., Hughes, A. R., Hultgren, K. M., Miner, B. G., Sorte, C. J. B., Thornber, C. S., Rodriguez, L. F., Tomanek, L., & Williams, S. L. (2006). The impacts of climate change in coastal marine systems. *Ecology Letters*, *9*(2), 228–241. https://doi.org/10.1111/j.1461-0248.2005.00871.x

Harris, S. M., Descamps, S., Sneddon, L. U., Bertrand, P., Chastel, O., & Patrick, S. C. (2020). Personality predicts foraging site fidelity and trip repeatability in a marine predator. *Journal of Animal Ecology*, *89*(1), 68–79. https://doi.org/10.1111/1365-2656.13106

Hartenstein, V. (2006). The neuroendocrine system of invertebrates: A developmental and evolutionary perspective. *Journal of Endocrinology*, *190*(3), 555–570. https://doi.org/10.1677/joe.1.06964

Hawkins, A. J. S. (1995). Effects of temperature change on ectotherm metabolism and evolution: Metabolic and physiological interrelations underlying the superiority of multi-locus heterozygotes in heterogeneous environments. *Journal of Thermal Biology*, *20*(1–2), 23–33. https://doi.org/10.1016/0306-4565(94)00023-C

Haylor, G., Thorpe, J., & Carter, M. (1984). Genetic and ecological differentiation between sympatric colour morphs of the common intertidal sea anemone Actinia equina. *Marine Ecology Progress Series*, *16*, 281–289. https://doi.org/10.3354/meps016281

He, R., Pagani-Núñez, E., Chevallier, C., & Barnett, C. R. A. (2017). To be so bold: boldness

is repeatable and related to within individual behavioural variability in North Island robins. *Behavioural Processes*, *140*, 144–149. https://doi.org/10.1016/j.beproc.2017.04.014

Helmuth, B., Mieszkowska, N., Moore, P., & Hawkins, S. J. (2006). Living on the edge of two changing worlds: Forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 373–404. https://doi.org/10.1146/annurev.ecolsys.37.091305.110149

Hemraj, D. A., Posnett, N. C., Minuti, J. J., Firth, L. B., & Russell, B. D. (2020). Survived but not safe: Marine heatwave hinders metabolism in two gastropod survivors. *Marine Environmental Research*, *162*, 105117. https://doi.org/10.1016/j.marenvres.2020.105117

Hensley, N. M., Cook, T. C., Lang, M., Petelle, M. B., & Blumstein, D. T. (2012). Personality and habitat segregation in giant sea anemones (Condylactis gigantea). *Journal of Experimental Marine Biology and Ecology*, *426–427*, 1–4. https://doi.org/10.1016/j.jembe.2012.05.011

Herborn, K. A., Heidinger, B. J., Alexander, L., & Arnold, K. E. (2014). Personality predicts behavioral flexibility in a fluctuating, natural environment. *Behavioral Ecology*, *25*(6), 1374–1379. https://doi.org/10.1093/beheco/aru131

Highcock, L., & Carter, A. J. (2014). Intraindividual variability of boldness is repeatable across contexts in a wild lizard. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0095179

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *Science*, *328*(5985), 1523–1529. https://doi.org/10.1367/1539-4409(2003)003<0044:TIOCCO>2.0.CO;2

Holtmann, B., Santos, E. S. A., Lara, C. E., & Nakagawa, S. (2017). Personality-matching habitat choice, rather than behavioural plasticity, is a likely driver of a phenotype–environment covariance. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1864), 20170943. https://doi.org/10.1098/rspb.2017.0943

Horváth, G., Mészáros, B., Urszán, T. J., Bajer, K., Molnár, O., Garamszegi, L. Z., & Herczeg, G. (2017). Environment-dependence of behavioural consistency in adult male European green lizards (Lacerta viridis). *PLoS ONE*, *12*(11), e0187657.

https://doi.org/10.1371/journal.pone.0187657

Houslay, T. M., Earley, R. L., Young, A. J., & Wilson, A. J. (2019). Habituation and individual variation in the endocrine stress response in the Trinidadian guppy (Poecilia reticulata). *General and Comparative Endocrinology*, *270*, 113–122. https://doi.org/10.1016/j.ygcen.2018.10.013

Houslay, T. M., Vierbuchen, M., Grimmer, A. J., Young, A. J., & Wilson, A. J. (2018). Testing the stability of behavioural coping style across stress contexts in the Trinidadian guppy. *Functional Ecology*, *32*(2), 424–438. https://doi.org/10.1111/1365-2435.12981

Houslay, T. M., & Wilson, A. J. (2017). Avoiding the misuse of BLUP in behavioural ecology. *Behavioral Ecology*, *28*(4), 948–952. https://doi.org/10.1093/beheco/arx023

Houston, A. I. (2010). Evolutionary models of metabolism, behaviour and personality. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 3969–3975. https://doi.org/10.1098/rstb.2010.0161

Huggett, J., & Griffiths, C. (1986). Some relationships between elevation, physico-chemical variables and biota of intertidal rock pools. *Marine Ecology Progress Series*, *29*(2), 189–197. https://doi.org/10.3354/meps029189

IPCC. (2013). Summary for Policymakers. In Intergovernmental Panel on Climate Change (Ed.), *Climate Change 2013 - The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press. https://doi.org/10.1017/CBO9781107415324

IPCC. (2018). Summary for Policymakers. In Intergovernmental Panel on Climate Change (Ed.), *Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels.* Cambridge University Press. https://apps.ipcc.ch/outreach/documents/451/1551801374.pdf

Jennings, D. J., Hayden, T. J., & Gammell, M. P. (2013). Personality and predictability in fallow deer fighting behaviour: Therelationship with mating success. *Animal Behaviour*, *86*(5), 1041–1047. https://doi.org/10.1016/j.anbehav.2013.09.009

Jolles, J. W., Briggs, H. D., Araya-Ajoy, Y. G., & Boogert, N. J. (2019). Personality, plasticity and predictability in sticklebacks: bold fish are less plastic and more predictable than shy fish. *Animal Behaviour*, *154*, 193–202.

https://doi.org/10.1016/j.anbehav.2019.06.022

Jones, K. A., Jackson, A. L., & Ruxton, G. D. (2011). Prey jitters; Protean behaviour in grouped prey. *Behavioral Ecology*, *22*(4), 831–836. https://doi.org/10.1093/beheco/arr062

Kareklas, K., Arnott, G., Elwood, R. W., & Holland, R. A. (2016). Plasticity varies with boldness in a weakly electric fish. *Frontiers in Zoology*, *13*(1), 1–7. https://doi.org/10.1186/s12983-016-0154-0

Kearney, M., Shine, R., & Porter, W. P. (2009). The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences*, *106*(10), 3835–3840. https://doi.org/10.1073/pnas.0808913106

Kelly, M. (2019). Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1768), 20180176. https://doi.org/10.1098/rstb.2018.0176

Kilgour, R. J., McAdam, A. G., Betini, G. S., & Norris, D. R. (2018). Experimental evidence that density mediates negative frequency-dependent selection on aggression. *Journal of Animal Ecology*, *87*(4), 1091–1101. https://doi.org/10.1111/1365-2656.12813

Killen, S. S. (2014). Growth trajectory influences temperature preference in fish through an effect on metabolic rate. *Journal of Animal Ecology*, *83*(6), 1513–1522. https://doi.org/10.1111/1365-2656.12244

Killen, S. S., Adriaenssens, B., Marras, S., Claireaux, G., & Cooke, S. J. (2016). Context dependency of trait repeatability and its relevance for management and conservation of fish populations. *Conservation Physiology*, *4*(1), jeb242522. https://doi.org/10.1093/conphys/cow007

Killen, S. S., Christensen, E. A. F., Cortese, D., Závorka, L., Norin, T., Cotgrove, L., Crespel, A., Munson, A., Nati, J. J. H., Papatheodoulou, M., & McKenzie, D. J. (2021). Guidelines for reporting methods to estimate metabolic rates by aquatic intermittent-flow respirometry. *Journal of Experimental Biology*, *224*(18), jeb242522. https://doi.org/10.1242/jeb.242522

Killen, S. S., Marras, S., Metcalfe, N. B., McKenzie, D. J., & Domenici, P. (2013).

Environmental stressors alter relationships between physiology and behaviour. *Trends in Ecology and Evolution*, *28*(11), 651–658. https://doi.org/10.1016/j.tree.2013.05.005

Killen, S. S., Marras, S., Ryan, M. R., Domenici, P., & Mckenzie, D. J. (2012). A relationship between metabolic rate and risk-taking behaviour is revealed during hypoxia in juvenile European sea bass. *Functional Ecology*, *26*(1), 134–143. https://doi.org/10.1111/j.1365-2435.2011.01920.x

Kjærsgaard, A., Blanckenhorn, W. U., Pertoldi, C., Loeschcke, V., Kaufmann, C., Hald, B., Pagès, N., & Bahrndorff, S. (2015). Plasticity in behavioural responses and resistance to temperature stress in Musca domestica. *Animal Behaviour*, *99*, 123–130. https://doi.org/10.1016/j.anbehav.2014.11.003

Kniel, N., & Godin, J.-G. J. (2019). Characterizing the (co)variance of personality traits in female Trinidadian guppies (Poecilia reticulata). *Environmental Biology of Fishes*, *102*(11), 1351–1363. https://doi.org/10.1007/s10641-019-00911-5

Kolluru, G. R., Grether, G. F., & Contreras, H. (2007). Environmental and genetic influences on mating strategies along a replicated food availability gradient in guppies (Poecilia reticulata). *Behavioral Ecology and Sociobiology*, *61*(5), 689–701. https://doi.org/10.1007/s00265-006-0299-5

Koolhaas, J. M., de Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology*, *31*(3), 307–321. https://doi.org/10.1016/j.yfrne.2010.04.001

Koolhaas, J. M., Korte, S. M., Boer, S. F. De, Vegt, B. J. Van Der, & Reenen, C. G. Van. (1999). *Coping styles in animals: current status in behavior and stress-physiology*. *4*(99). https://doi.org/10.1016/S0149-7634(99)00026-3

Kralj-Fišer, S., & Schneider, J. M. (2012). Individual behavioural consistency and plasticity in an urban spider. *Animal Behaviour*, *84*(1), 197–204. https://doi.org/10.1016/j.anbehav.2012.04.032

Kralj-Fišer, S., & Schuett, W. (2014). Studying personality variation in invertebrates: Why bother? *Animal Behaviour*, *91*, 41–52. https://doi.org/10.1016/j.anbehav.2014.02.016

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, *82*(13), 1–26.

https://doi.org/10.18637/jss.v082.i13

Kvitt, H., Rosenfeld, H., & Tchernov, D. (2016). The regulation of thermal stress induced apoptosis in corals reveals high similarities in gene expression and function to higher animals. *Scientific Reports*, *6*, 30359. https://doi.org/10.1038/srep30359

Lane, S. M., & Briffa, M. (2017). Boldness is for rookies: prefight boldness and fighting success in a sea anemone. *Animal Behaviour*, *132*, 13–20. https://doi.org/10.1016/j.anbehav.2017.07.012

Larios-Soriano, E., Re-Araujo, A. D., Díaz, F., Sánchez, C. G., López-Galindo, L., Castro, L. I., & Ramírez, D. T. (2020). Effect of acclimation temperature on thermoregulatory behaviour, thermal tolerance and respiratory metabolism of Lutjanus guttatus and the response of heat shock protein 70 (Hsp70) and lactate dehydrogenase (Ldh-a) genes. *Aquaculture Research*, *51*(3), 1089–1100. https://doi.org/10.1111/are.14455

Laskowski, K. L., & Bell, A. M. (2013). Competition avoidance drives individual differences in response to a changing food resource in sticklebacks. *Ecology Letters*, *16*(6), 746–753. https://doi.org/10.1111/ele.12105

Le Galliard, J. F., Paquet, M., Cisel, M., & Montes-Poloni, L. (2013). Personality and the pace-of-life syndrome: Variation and selection on exploration, metabolism and locomotor performances. *Functional Ecology*, *27*(1), 136–144. https://doi.org/10.1111/1365-2435.12017

Le Lann, C., Wardziak, T., van Baaren, J., & van Alphen, J. J. M. (2011). Thermal plasticity of metabolic rates linked to life-history traits and foraging behaviour in a parasitic wasp. *Functional Ecology*, *25*(3), 641–651. https://doi.org/10.1111/j.1365-2435.2010.01813.x

Leal, M. C., Nunes, C., Engrola, S., Dinis, M. T., & Calado, R. (2012). Optimization of monoclonal production of the glass anemone Aiptasia pallida (Agassiz in Verrill, 1864). *Aquaculture*, *354–355*, 91–96. https://doi.org/10.1016/j.aquaculture.2012.03.035

Lee, Y., & Nelder, J. A. (2006). Double hierarchical generalized linear models. *Journal of the Royal Statistical Society. Series C: Applied Statistics*, *55*(2), 139–185. https://doi.org/10.1111/j.1467-9876.2006.00538.x

Leggat, W. P., Camp, E. F., Suggett, D. J., Heron, S. F., Fordyce, A. J., Gardner, S., Deakin, L., Turner, M., Beeching, L. J., Kuzhiumparambil, U., Eakin, C. M., & Ainsworth, T. D.

(2019). Rapid Coral Decay Is Associated with Marine Heatwave Mortality Events on Reefs. *Current Biology*, *29*(16), 2723–2730. https://doi.org/10.1016/j.cub.2019.06.077

Lei, J., Jia, Y., Wang, Y., Lei, G., Lu, C., Saintilan, N., & Wen, L. (2019). Behavioural plasticity and trophic niche shift: How wintering geese respond to habitat alteration. *Freshwater Biology*, *64*(6), 1183–1195. https://doi.org/10.1111/fwb.13294

Lesser, M. P., & Kruse, V. A. (2004). Seasonal temperature compensation in the horse mussel, Modiolus modiolus: Metabolic enzymes, oxidative stress and heat shock proteins. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *137*(3), 495–504. https://doi.org/10.1016/j.cbpb.2003.10.022

Letcher, B. H., Coombs, J. A., & Nislow, K. H. (2011). Maintenance of phenotypic variation: Repeatability, heritability and size-dependent processes in a wild brook trout population. *Evolutionary Applications*, *4*(4), 602–615. https://doi.org/10.1111/j.1752-4571.2011.00184.x

Lienart, G. D. H., Mitchell, M. D., Ferrari, M. C. O., & McCormick, M. I. (2014). Temperature and food availability affect risk assessment in an ectotherm. *Animal Behaviour*, *89*, 199–204. https://doi.org/10.1016/j.anbehav.2013.12.031

Lim, H. J., Kim, B. M., Hwang, I. J., Lee, J. S., Choi, I. Y., Kim, Y. J., & Rhee, J. S. (2016). Thermal stress induces a distinct transcriptome profile in the Pacific oyster Crassostrea gigas. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, *19*, 62–70. https://doi.org/10.1016/j.cbd.2016.06.006

Ma, G., Bai, C. M., Wang, X. J., Majeed, M. Z., & Ma, C. Sen. (2018). Behavioural thermoregulation alters microhabitat utilization and demographic rates in ectothermic invertebrates. *Animal Behaviour*, *142*, 49–57. https://doi.org/10.1016/j.anbehav.2018.06.003

Madeira, D., Narciso, L., Cabral, H. N., Vinagre, C., & Diniz, M. S. (2013). Influence of temperature in thermal and oxidative stress responses in estuarine fish. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *166*(2), 237–243. https://doi.org/10.1016/j.cbpa.2013.06.008

Malagoli, D., & Ottaviani, E. (2017). Cross-talk among immune and neuroendocrine systems in molluscs and other invertebrate models. *Hormones and Behavior*, *88*, 41–44.

https://doi.org/10.1016/j.yhbeh.2016.10.015

Martin, J. G. A., Nussey, D. H., Wilson, A. J., & Réale, D. (2011). Measuring individual differences in reaction norms in field and experimental studies: A power analysis of random regression models. *Methods in Ecology and Evolution*, *2*(4), 362–374. https://doi.org/10.1111/j.2041-210X.2010.00084.x

Martin, J. G. A., Pirotta, E., Petelle, M. B., & Blumstein, D. T. (2017). Genetic basis of between-individual and within-individual variance of docility. *Journal of Evolutionary Biology*, *30*(4), 796–805. https://doi.org/10.1111/jeb.13048

Martins, C. I. M., Castanheira, M. F., Engrola, S., Costas, B., & Conceição, L. E. C. (2011). Individual differences in metabolism predict coping styles in fish. *Applied Animal Behaviour Science*, *130*(3–4), 135–143. https://doi.org/10.1016/j.applanim.2010.12.007

Maskrey, D. K., Sneddon, L. U., Arnold, K. E., Wolfenden, D. C. C., & Thomson, J. S. (2020). The impact of personality, morphotype and shore height on temperature-mediated behavioural responses in the beadlet anemone Actinia equina. *Journal of Animal Ecology*, *89*(10), 2311–2324. https://doi.org/10.1111/1365-2656.13301

Maskrey, D. K., White, S. J., Wilson, A. J., & Houslay, T. M. (2018). Who dares does not always win: risk-averse rockpool prawns are better at controlling a limited food resource. *Animal Behaviour*, *140*. https://doi.org/10.1016/j.anbehav.2018.04.023

Maspons, J., Molowny-Horas, R., & Sol, D. (2019). Behaviour, life history and persistence in novel environments. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1781), 20180056. https://doi.org/10.1098/rstb.2018.0056

Matera, A. G., & Wang, Z. (2014). A day in the life of the spliceosome. *Nature Reviews Molecular Cell Biology*, *15*(2), 108–121. https://doi.org/10.1038/nrm3742

Mathot, K. J., Dingemanse, N. J., & Nakagawa, S. (2019). The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. *Biological Reviews*, *94*(3), 1056–1074. https://doi.org/10.1111/brv.12491

Mathot, K. J., Wright, J., Kempenaers, B., & Dingemanse, N. J. (2012). Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. *Oikos*, *121*(7), 1009–1020. https://doi.org/10.1111/j.1600-0706.2012.20339.x

Mbokodo, I., Bopape, M. J., Chikoore, H., Engelbrecht, F., & Nethengwe, N. (2020).

Heatwaves in the future warmer climate of South Africa. *Atmosphere*, *11*(7), 1–18. https://doi.org/10.3390/atmos11070712

McLean, S., & Morrell, L. J. (2020). Consistency in the strength of laterality in male, but not female, guppies across different behavioural contexts. *Biology Letters*, *16*(5). https://doi.org/10.1098/rsbl.2019.0870

McMahon, R. F., Russell-Hunter, W. D., & Aldridge, D. W. (1995). Lack of metabolic temperature compensation in the intertidal gastropods, Littorina saxatilis (Olivi) and L. obtusata (L.). In *Advances in Littorinid Biology* (pp. 89–100). Springer Netherlands. https://doi.org/10.1007/978-94-011-0435-7_9

McQuaid, C. D., Lindsay, J. R., & Lindsay, T. L. (2000). Interactive effects of wave exposure and tidal height on population structure of the mussel Perna perna. *Marine Biology*, *137*(5), 925–932. https://doi.org/10.1007/s002270000398

Mell, H., Josserand, R., Decencière, B., Artacho, P., Meylan, S., & Le Galliard, J. F. (2016). Do personalities co-vary with metabolic expenditure and glucocorticoid stress response in adult lizards? *Behavioral Ecology and Sociobiology*, *70*(6), 951–961. https://doi.org/10.1007/s00265-016-2117-z

Menge, B. A. (1976). Organization of the New England Rocky Intertidal Community: Role of Predation, Competition, and Environmental Heterogeneity. *Ecological Monographs*, *46*(4), 355–393. https://doi.org/10.2307/1942563

Merrick, M. J., & Koprowski, J. L. (2017). Should we consider individual behavior differences in applied wildlife conservation studies? *Biological Conservation*, *209*, 34–44. https://doi.org/10.1016/j.biocon.2017.01.021

Mery, F., & Burns, J. G. (2010). Behavioural plasticity: An interaction between evolution and experience. *Evolutionary Ecology*, *24*(3), 571–583. https://doi.org/10.1007/s10682-009-9336-y

Metcalfe, N. B., Van Leeuwen, T. E., & Killen, S. S. (2016). Does individual variation in metabolic phenotype predict fish behaviour and performance? *Journal of Fish Biology*, *88*(1), 298–321. https://doi.org/10.1111/jfb.12699

Michelangeli, M., Goulet, C. T., Kang, H. S., Wong, B. B. M., & Chapple, D. G. (2018). Integrating thermal physiology within a syndrome: Locomotion, personality and habitat

selection in an ectotherm. *Functional Ecology*, *32*(4), 970–981. https://doi.org/10.1111/1365-2435.13034

Miller, L. P., Matassa, C. M., & Trussell, G. C. (2014). Climate change enhances the negative effects of predation risk on an intermediate consumer. *Global Change Biology*, *20*(12), 3834–3844. https://doi.org/10.1111/gcb.12639

Mitchell, D. J., & Biro, P. A. (2017). Is behavioural plasticity consistent across different environmental gradients and through time? *Proceedings of the Royal Society B: Biological Sciences*, *284*(1860), 20170893. https://doi.org/10.1098/rspb.2017.0893

Mitchell, D. J., Dujon, A. M., Beckmann, C., & Biro, P. A. (2020). Temporal autocorrelation: A neglected factor in the study of behavioral repeatability and plasticity. *Behavioral Ecology*, *31*(1), 222–231. https://doi.org/10.1093/beheco/arz180

Mitchell, D. J., Fanson, B. G., Beckmann, C., & Biro, P. A. (2016). Towards powerful experimental and statistical approaches to study intraindividual variability in labile traits. *Royal Society Open Science*, *3*(10). https://doi.org/10.1098/rsos.160352

Mitchell, D. J., & Houslay, T. M. (2021). Context-dependent trait covariances: how plasticity shapes behavioral syndromes. *Behavioral Ecology*, *32*(1), 25–29. https://doi.org/10.1093/beheco/araa115

Moiron, M., Laskowski, K. L., & Niemelä, P. T. (2020). Individual differences in behaviour explain variation in survival: a meta-analysis. *Ecology Letters*, *23*(2), 399–408. https://doi.org/10.1111/ele.13438

Mondal, M., Rajkhowa, C., & Prakash, B. S. (2006). Relationship between plasma growth hormone concentrations and temperament in mithuns (Bos frontalis). *Hormones and Behavior*, *49*(2), 190–196. https://doi.org/10.1016/j.yhbeh.2005.06.006

Monteiro, F. A., Solé-Cava, A. M., & Thorpe, J. P. (1997). Extensive genetic divergence between populations of the common intertidal sea anemone Actinia equina from Britain, the Mediterranean and the Cape Verde Islands. *Marine Biology*, *129*(3), 425–433. https://doi.org/10.1007/s002270050183

Montiglio, P. O., Dammhahn, M., Dubuc Messier, G., & Réale, D. (2018). The pace-of-life syndrome revisited: the role of ecological conditions and natural history on the slow-fast continuum. *Behavioral Ecology and Sociobiology*, *72*(7), 1–9.

https://doi.org/10.1007/s00265-018-2526-2

Moore, P. B., & Steitz, T. A. (2002). The involvement of RNA in ribosome function. *Nature*, *418*(6894), 229–235. https://doi.org/10.1038/418229a

Moran, N. P., Mossop, K. D., Thompson, R. M., & Wong, B. B. M. (2016). Boldness in extreme environments: temperament divergence in a desert-dwelling fish. *Animal Behaviour*, *122*, 125–133. https://doi.org/10.1016/j.anbehav.2016.09.024

Mouchet, A., Cole, E. F., Matthysen, E., Nicolaus, M., Quinn, J. L., Roth, A. M., Tinbergen, J. M., van Oers, K., van Overveld, T., & Dingemanse, N. J. (2021). Heterogeneous selection on exploration behavior within and among West European populations of a passerine bird. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(28), 1–6. https://doi.org/10.1073/pnas.2024994118

Murray, J. J. (1972). *Genetic diversity and natural selection.* Oliver and Boyd. https://www.cabdirect.org/cabdirect/abstract/19720100862

Nakagawa, S., Gillespie, D. O. S., Hatchwell, B. J., & Burke, T. (2007). Predictable males and unpredictable females: Sex difference in repeatability of parental care in a wild bird population. *Journal of Evolutionary Biology*, *20*(5), 1674–1681. https://doi.org/10.1111/j.1420-9101.2007.01403.x

Nakayama, S., Laskowski, K. L., Klefoth, T., & Arlinghaus, R. (2016). Between- and within-individual variation in activity increases with water temperature in wild perch. *Behavioral Ecology*, *27*(6), 1676–1683. https://doi.org/10.1093/beheco/arw090

Nanninga, G. B., Horswill, C., Lane, S. M., Manica, A., & Briffa, M. (2020). Microplastic exposure increases predictability of predator avoidance strategies in hermit crabs. *Journal of Hazardous Materials Letters*, *1*, 100005. https://doi.org/10.1016/j.hazl.2020.100005

Navarro, E., Ortega, M. M., & Madariaga, J. M. (1981). Effect of body size, temperature and shore level on aquatic and aerial respiration of Actinia equina (L.) (Anthozoa). *Journal of Experimental Marine Biology and Ecology*, *53*(2–3), 153–162. https://doi.org/10.1016/0022-0981(81)90016-2

Ng, T. P. T., Lau, S. L. Y., Seuront, L., Davies, M. S., Stafford, R., Marshall, D. J., & Williams, G. A. (2017). Linking behaviour and climate change in intertidal ectotherms:

insights from littorinid snails. *Journal of Experimental Marine Biology and Ecology*, *492*, 121–131. https://doi.org/10.1016/j.jembe.2017.01.023

Nicolaus, M., Tinbergen, J. M., Bouwman, K. M., Michler, S. P. M., Ubels, R., Both, C., Kempenaers, B., & Dingemanse, N. J. (2012). Experimental evidence for adaptive personalities in a wild passerine bird. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1749), 4885–4892. https://doi.org/10.1098/rspb.2012.1936

Nicolaus, M., Tinbergen, J. M., Ubels, R., Both, C., & Dingemanse, N. J. (2016). Density fluctuations represent a key process maintaining personality variation in a wild passerine bird. *Ecology Letters*, *19*(4), 478–486. https://doi.org/10.1111/ele.12584

Niemelä, P. T., Vainikka, A., Hedrick, A. V., & Kortet, R. (2012). Integrating behaviour with life history: Boldness of the field cricket, Gryllus integer, during ontogeny. *Functional Ecology*, *26*(2), 450–456. https://doi.org/10.1111/j.1365-2435.2011.01939.x

Nieto-Barajas, L. E., Müller, P., Ji, Y., Lu, Y., & Mills, G. B. (2012). A Time-Series DDP for Functional Proteomics Profiles. *Biometrics*, *68*(3), 859–868. https://doi.org/10.1111/j.1541-0420.2011.01724.x

Ning, J., Zou, D., Lu, X., Cao, W., Chen, M., Liu, B., & Wang, C. (2021). Transcriptomic analyses provide insights into the adaptive responses to heat stress in the ark shells, Scapharca subcrenata. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, *38*, 100813. https://doi.org/10.1016/j.cbd.2021.100813

Nonnis, S., Angiulli, E., Maffioli, E., Frabetti, F., Negri, A., Cioni, C., Alleva, E., Romeo, V., Tedeschi, G., & Toni, M. (2021). Acute environmental temperature variation affects brain protein expression, anxiety and explorative behaviour in adult zebrafish. *Scientific Reports*, *11*, 2521. https://doi.org/10.1038/s41598-021-81804-5

Norin, T., Malte, H., & Clark, T. D. (2016). Differential plasticity of metabolic rate phenotypes in a tropical fish facing environmental change. *Functional Ecology*, *30*(3), 369–378. https://doi.org/10.1111/1365-2435.12503

Oakley, C. A., Durand, E., Wilkinson, S. P., Peng, L., Weis, V. M., Grossman, A. R., & Davy, S. K. (2017). Thermal Shock Induces Host Proteostasis Disruption and Endoplasmic Reticulum Stress in the Model Symbiotic Cnidarian Aiptasia. *Journal of Proteome Research*, *16*(6), 2121–2134. https://doi.org/10.1021/acs.jproteome.6b00797

Okuyama, T. (2015). Optimal foraging behavior with an explicit consideration of within-individual behavioral variation: an example of predation. *Evolutionary Ecology*, *29*(4), 599–607. https://doi.org/10.1007/s10682-015-9758-7

Ólafsdóttir, G. Á., & Magellan, K. (2016). Interactions between boldness, foraging performance and behavioural plasticity across social contexts. *Behavioral Ecology and Sociobiology*, *70*(11), 1879–1889. https://doi.org/10.1007/s00265-016-2193-0

Onyango, C. A., Glassom, D., & MacDonald, A. (2021). De novo assembly of the transcriptome of scleractinian coral, Anomastraea irregularis and analyses of its response to thermal stress. *Molecular Biology Reports*, *48*(3), 2083–2092. https://doi.org/10.1007/s11033-021-06184-5

Ortega, M. M., Iglesias, J. I. P., & Navarro, E. (1984). Acclimation to temperature in Actinia equina L.: Effects of season and shore level on aquatic oxygen consumption. *Journal of Experimental Marine Biology and Ecology*, *76*(1), 79–87. https://doi.org/10.1016/0022-0981(84)90018-2

Osborn, A., & Briffa, M. (2017). Does repeatable behaviour in the laboratory represent behaviour under natural conditions? A formal comparison in sea anemones. *Animal Behaviour*, *123*, 197–206. https://doi.org/10.1016/j.anbehav.2016.10.036

Palaoro, A. V., Velasque, M., Santos, S., & Briffa, M. (2017). How does environment influence fighting? the effects of tidal flow on resource value and fighting costs in sea anemones. *Biology Letters*, *13*(5), 20170011. https://doi.org/10.1098/rsbl.2017.0011

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Patrick, S. C., & Weimerskirch, H. (2014). Personality, foraging and fitness consequences in a long lived seabird. *PLoS ONE*, *9*(2), e87269. https://doi.org/10.1371/journal.pone.0087269

Perez, S. F., Cook, C. B., & Brooks, W. R. (2001). The role of symbiotic dinoflagellates in the temperature-induced bleaching response of the subtropical sea anemone Aiptasia pallida. *Journal of Experimental Marine Biology and Ecology*, *256*(1), 1–14. https://doi.org/10.1016/S0022-0981(00)00282-3

Perkins-Kirkpatrick, S. E., & Gibson, P. B. (2017). Changes in regional heatwave characteristics as a function of increasing global temperature. *Scientific Reports*, *7*, 12256. https://doi.org/10.1038/s41598-017-12520-2

Pernice, M., Dunn, S. R., Miard, T., Dufour, S., Dove, S., & Hoegh-Guldberg, O. (2011). Regulation of apoptotic mediators reveals dynamic responses to thermal stress in the reef building coral acropora millepora. *PLoS ONE*, *6*(1), e16095. https://doi.org/10.1371/journal.pone.0016095

Perrin, M. C., Thorpe, J. P., & Sole-Cava, A. M. (1999). Population structuring, gene dispersal and reproduction in the Actinia equina species group. In *Oceanography and Marine Biology, An Annual Review* (Vol. 37). CRC Press.

Pinsky, M. L., Eikeset, A. M., McCauley, D. J., Payne, J. L., & Sunday, J. M. (2019). Greater vulnerability to warming of marine versus terrestrial ectotherms. *Nature*, *569*(7754), 108–111. https://doi.org/10.1038/s41586-019-1132-4

Pires, V. L. (2012). *Impact of global warming on the spawning success, metabolism and embryogenesis of a specialized soft coral-feeding nudibranch, Armina maculata*. http://repositorio.ul.pt/handle/10451/7705

Plummer, M. (2014). *rjags: Bayesian graphical models using MCMC*. http://mcmc-jags.sourceforge.net

Polverino, G., Santostefano, F., Díaz-Gil, C., & Mehner, T. (2018). Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology and behaviour in two populations of Eastern mosquitofish. *Scientific Reports*, *8*, 14673. https://doi.org/10.1038/s41598-018-33047-0

Pörtner, H. O. (2012). Integrating climate-related stressor effects on marine organisms: Unifying principles linking molecule to ecosystem-level changes. *Marine Ecology Progress Series*, *470*, 273–290. https://doi.org/10.3354/meps10123

Prentice, P. M., Houslay, T. M., Martin, J. G. A., & Wilson, A. J. (2020). Genetic variance for behavioural 'predictability' of stress response. *Journal of Evolutionary Biology*, *33*(5), 642–652. https://doi.org/10.1111/jeb.13601

Pusch, E. A., Bentz, A. B., Becker, D. J., & Navara, K. J. (2018). Behavioral phenotype predicts physiological responses to chronic stress in proactive and reactive birds.

General and Comparative Endocrinology, *255*, 71–77. https://doi.org/10.1016/j.ygcen.2017.10.008

Quicke, D. L. J., & Brace, R. C. (1984). Evidence for the existence of a third, ecologically distinct morph of the anemone, Actinia equina. *Journal of the Marine Biological Association of the United Kingdom*, *64*(3), 531–534. https://doi.org/10.1017/S0025315400030216

Quicke, D. L. J., Donoghue, A. M., & Brace, R. C. (1983). Biochemical-genetic and ecological evidence that red/brown individuals of the anemone Actinia equina comprise two morphs in Britain. *Marine Biology*, *77*(1), 29–37. https://doi.org/10.1007/BF00393207

Quicke, D. L. J., Donoghue, A. M., Keeling, T. F., & Brace, R. C. (1985). Littoral distributions and evidence for differential post-settlement selection of the morphs of Actinia Equina. *Journal of the Marine Biological Association of the United Kingdom*, *65*(1), 1–20. https://doi.org/10.1017/S0025315400060768

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. http://www.r-project.org/

Ragazzola, F., Marchini, A., Adani, M., Bordone, A., Castelli, A., Cerrati, G., Kolzenburg, R., Langeneck, J., di Marzo, C., Nannini, M., Raiteri, G., Romanelli, E., Santos, M., Vasapollo, C., Pipitone, C., & Lombardi, C. (2021). An intertidal life: Combined effects of acidification and winter heatwaves on a coralline alga (Ellisolandia elongata) and its associated invertebrate community. *Marine Environmental Research*, *169*, 105342. https://doi.org/10.1016/j.marenvres.2021.105342

Raudvere, U., Kolberg, L., Kuzmin, I., Arak, T., Adler, P., Peterson, H., & Vilo, J. (2019). G:Profiler: A web server for functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Research*, *47*(W1), W191–W198. https://doi.org/10.1093/nar/gkz369

Ravaux, J., Léger, N., Rabet, N., Fourgous, C., Voland, G., Zbinden, M., & Shillito, B. (2016). Plasticity and acquisition of the thermal tolerance (upper thermal limit and heat shock response) in the intertidal species Palaemon elegans. *Journal of Experimental Marine Biology and Ecology*, *484*, 39–45. https://doi.org/10.1016/j.jembe.2016.07.003

Raven, J., Caldeira, K., Elderfield, H., Hoegh-Guldberg, O., Liss, P., Riebesell, U., Shepherd, J., Turley, C., & Watson, A. (2005). *Ocean acidification due to increasing atmospheric carbon dioxide*. http://eprints.ifm-geomar.de/7878/1/965_Raven_2005_OceanAcidificationDueToIncreasing_Monogr_pubid13120.pdf

Réale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V., & Montiglio, P. O. (2010). Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 4051–4063. https://doi.org/10.1098/rstb.2010.0208

Réale, D., Reader, S. M., Sol, D., McDougall, P. T., & Dingemanse, N. J. (2007). Integrating animal temperament within ecology and evolution. *Biological Reviews*, *82*(2), 291–318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Reinheckel, T., Sitte, N., Ullrich, O., Kuckelkorn, U., Davies, K. J. A., & Grune, T. (1998). Comparative resistance of the 20 S and 26 S proteasome to oxidative stress. *Biochemical Journal*, *335*(3), 637–642. https://doi.org/10.1042/bj3350637

Rey, S., Boltana, S., Vargas, R., Roher, N., & Mackenzie, S. (2013). Combining animal personalities with transcriptomics resolves individual variation within a wild-type zebrafish population and identifies underpinning molecular differences in brain function. *Molecular Ecology*, *22*(24), 6100–6115. https://doi.org/10.1111/mec.12556

Rey, S., Jin, X., Damsgård, B., Bégout, M. L., & Mackenzie, S. (2021). Analysis across diverse fish species highlights no conserved transcriptome signature for proactive behaviour. *BMC Genomics*, *22*(1), 1–17. https://doi.org/10.1186/s12864-020-07317-z

Rey, S., Ribas, L., Morera Capdevila, D., Callol, A., Huntingford, F. A., Pilarczyk, M., Kadri, S., & Mackenzie, S. (2016). Differential responses to environmental challenge by common carp Cyprinus carpio highlight the importance of coping style in integrative physiology. *Journal of Fish Biology*, *88*(3), 1056–1069. https://doi.org/10.1111/jfb.12877

Rice, P., Longden, L., & Bleasby, A. (2000). EMBOSS: The European Molecular Biology Open Software Suite. *Trends in Genetics*, *16*(6), 276–277. https://doi.org/10.1016/S0168-9525(00)02024-2

Richardson, G., Dickinson, P., Burman, O. H. P., & Pike, T. W. (2018). Unpredictable movement as an anti-predator strategy. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1885), 20181112. https://doi.org/10.1098/rspb.2018.1112

Richier, S., Rodriguez-Lanetty, M., Schnitzler, C. E., & Weis, V. M. (2008). Response of the symbiotic cnidarian Anthopleura elegantissima transcriptome to temperature and UV increase. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, *3*(4), 283–289. https://doi.org/10.1016/j.cbd.2008.08.001

Richier, S., Sabourault, C., Courtiade, J., Zucchini, N., Allemand, D., & Furla, P. (2006). Oxidative stress and apoptotic events during thermal stress in the symbiotic sea anemone, Anemonia viridis. *FEBS Journal*, *273*(18), 4186–4198. https://doi.org/10.1111/j.1742-4658.2006.05414.x

Rivera, H. E., Chen, C. Y., Gibson, M. C., & Tarrant, A. M. (2021). Plasticity in parental effects confers rapid larval thermal tolerance in the estuarine anemone Nematostella vectensis. *Journal of Experimental Biology*, *224*(5), jeb236745. https://doi.org/10.1242/jeb.236745

Rodriguez-Dominguez, A., Connell, S. D., Leung, J. Y. S., & Nagelkerken, I. (2019). Adaptive responses of fishes to climate change: Feedback between physiology and behaviour. *Science of the Total Environment*, *692*, 1242–1249. https://doi.org/10.1016/j.scitotenv.2019.07.226

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, *21*(9), 1425–1439. https://doi.org/10.1111/ele.13107

Ross, T., Jackson, J., & Hannah, C. (2021). The Northeast Pacific: Update on marine heatwave status and trends. *PICES Press*, *29*(1), 46–48. https://www.proquest.com/openview/08f8ec83600d3a651040b24099f0b795

Royauté, R., Berdal, M. A., Garrison, C. R., & Dochtermann, N. A. (2018). Paceless life? A meta-analysis of the pace-of-life syndrome hypothesis. *Behavioral Ecology and Sociobiology*, *72*(3), 1–10. https://doi.org/10.1007/s00265-018-2472-z

Rudin, F. S., & Briffa, M. (2011). The logical polyp: Assessments and decisions during contests in the beadlet anemone Actinia equina. *Behavioral Ecology*, *22*(6), 1278–1285.

https://doi.org/10.1093/beheco/arr125

Rudin, F. S., & Briffa, M. (2012). Is boldness a resource-holding potential trait? Fighting prowess and changes in startle response in the sea anemone, Actinia equina. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), 1904–1910. https://doi.org/10.1098/rspb.2011.2418

Ruiz-Gomez, M. de L., Huntingford, F. A., Øverli, Ø., Thörnqvist, P. O., & Höglund, E. (2011). Response to environmental change in rainbow trout selected for divergent stress coping styles. *Physiology and Behavior*, *102*(3–4), 317–322. https://doi.org/10.1016/j.physbeh.2010.11.023

Salt, L. A., Thomas, H., Prowe, A. E. F., Borges, A. V., Bozec, Y., & De Baar, H. J. W. (2013). Variability of North Sea pH and CO2 in response to North Atlantic Oscillation forcing. *Journal of Geophysical Research: Biogeosciences*, *118*(4), 1584–1592. https://doi.org/10.1002/2013JG002306

Santicchia, F., Gagnaison, C., Bisi, F., Martinoli, A., Matthysen, E., Bertolino, S., & Wauters, L. A. (2018). Habitat-dependent effects of personality on survival and reproduction in red squirrels. *Behavioral Ecology and Sociobiology*, *72*(8), 1–13. https://doi.org/10.1007/s00265-018-2546-y

Sasaki, T., Mann, R. P., Warren, K. N., Herbert, T., Wilson, T., & Biro, D. (2018). Personality and the collective: Bold homing pigeons occupy higher leadership ranks in flocks. *Philosophical Transactions of the Royal Society B: Biological Sciences, 373*(1746), 20170038. https://doi.org/10.1098/rstb.2017.0038

Scapini, F., Innocenti Degli, E., & Defeo, O. (2019). Behavioral adaptations of sandy beach macrofauna in face of climate change impacts: A conceptual framework. *Estuarine, Coastal and Shelf Science*, *225*, 106236. https://doi.org/10.1016/j.ecss.2019.05.018

Schama, R., Solé-Cava, A. M., & Thorpe, J. P. (2005). Genetic divergence between east and west Atlantic populations of Actinia spp. sea anemones (Cnidaria: Actiniidae). *Marine Biology*, *146*(3), 435–443. https://doi.org/10.1007/s00227-004-1462-z

Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, *24*(1), 35–68. https://doi.org/10.1146/annurev.es.24.110193.000343

Scheiner, S. M. (2013). The genetics of phenotypic plasticity: XII: Temporal and spatial heterogeneity. *Ecology and Evolution*, *3*(13), 4596–4609. https://doi.org/10.1002/ece3.792

Schulte, P. M. (2015). The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *Journal of Experimental Biology*, *218*(12), 1856–1866. https://doi.org/10.1242/jeb.118851

Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, *51*(5), 691–702. https://doi.org/10.1093/icb/icr097

Scott-Samuel, N. E., Holmes, G., Baddeley, R., & Cuthill, I. C. (2015). Moving in groups: how density and unpredictable motion affect predation risk. *Behavioral Ecology and Sociobiology*, *69*(6), 867–872. https://doi.org/10.1007/s00265-015-1885-1

Sears, M. W., Angilletta, M. J., Schuler, M. S., Borchert, J., Dilliplane, K. F., Stegman, M., Rusch, T. W., & Mitchell, W. A. (2016). Configuration of the thermal landscape determines thermoregulatory performance of ectotherms. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(38), 10595–10600. https://doi.org/10.1073/pnas.1604824113

Sebens, K. P. (1981). The allometry of feeding, energetics, and body size in three sea anemone species. *The Biological Bulletin*, *161*(1), 152–171. https://doi.org/10.2307/1541115

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), 61–66. https://doi.org/10.1038/nclimate2457

Segev, U., Burkert, L., Feldmeyer, B., & Foitzik, S. (2017). Pace-of-life in a social insect: behavioral syndromes in ants shift along a climatic gradient. *Behavioral Ecology*, *28*(4), 1149–1159. https://doi.org/10.1093/beheco/arx079

Seuront, L., Nicastro, K. R., Zardi, G. I., & Goberville, E. (2019). Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel Mytilus edulis. *Scientific Reports*, *9*, 17498. https://doi.org/10.1038/s41598-

019-53580-w

Seyfarth, R. M., Silk, J. B., & Cheney, D. L. (2012). Variation in personality and fitness in wild female baboons. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(42), 16980–16985. https://doi.org/10.1073/pnas.1210780109

Sih, A. (2013). Understanding variation in behavioural responses to human-induced rapid environmental change: A conceptual overview. *Animal Behaviour*, *85*(5), 1077–1088. https://doi.org/10.1016/j.anbehav.2013.02.017

Sih, A., Bell, A., & Johnson, J. C. (2004). Behavioral syndromes: An ecological and evolutionary overview. *Trends in Ecology and Evolution*, *19*(7), 372–378. https://doi.org/10.1016/j.tree.2004.04.009

Sih, A., Cote, J., Evans, M., Fogarty, S., & Pruitt, J. (2012). Ecological implications of behavioural syndromes. *Ecology Letters*, *15*(3), 278–289. https://doi.org/10.1111/j.1461-0248.2011.01731.x

Sih, A., Ferrari, M. C. O., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary Applications*, *4*(2), 367–387. https://doi.org/10.1111/j.1752-4571.2010.00166.x

Skov, P. V., de Jesus Gregersen, K. J., Gesto, M., & Jokumsen, A. (2019). Proactive coping style in early emerging rainbow trout carries a metabolic cost with no apparent return. *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology*, *231*, 104–110. https://doi.org/10.1016/j.cbpa.2019.01.016

Smith, B. R., & Blumstein, D. T. (2008). Fitness consequences of personality: A meta-analysis. *Behavioral Ecology*, *19*(2), 448–455. https://doi.org/10.1093/beheco/arm144

Smith, B. R., & Blumstein, D. T. (2010). Behavioral types as predictors of survival in Trinidadian guppies (Poecilia reticulata). *Behavioral Ecology*, *21*(5), 919–926. https://doi.org/10.1093/beheco/arq084

Sneddon, L. U., Margareto, J., & Cossins, A. R. (2005). The use of transcriptomics to address questions in behaviour: Production of a suppression subtractive hybridisation library from dominance hierarchies of rainbow trout. *Physiological and Biochemical Zoology*, *78*(5), 695–705. https://doi.org/10.1086/432141

Snell-Rood, E. C. (2013). An overview of the evolutionary causes and consequences of

behavioural plasticity. *Animal Behaviour*, *85*(5), 1004–1011. https://doi.org/10.1016/j.anbehav.2012.12.031

Solé-Cava, A., & Thorpe, J. (1987). Further genetic evidence for the reproductive isolation of green sea anemone Actinia prasina Gosse from common intertidal beadlet anemone Actinia equina (L.). *Marine Ecology Progress Series*, *38*(1860), 225–229. https://doi.org/10.3354/meps038225

Somero, G. N. (2002). Thermal physiology and vertical zonation of intertidal animals: Optima, limits, and costs of living. *Integrative and Comparative Biology*, *42*(4), 780–789. https://doi.org/10.1093/icb/42.4.780

Somero, G. N., & Hochachka, P. W. (1969). Isoenzymes and short-term temperature compensation in poikilotherms: Activation of lactate dehydrogenase isoenzymes by temperature decreases. *Nature*, *223*(5202), 194–195. https://doi.org/10.1038/223194a0

Sorokin, A. V., Kim, E. R., & Ovchinnikov, L. P. (2009). Proteasome system of protein degradation and processing. *Biochemistry (Moscow)*, *74*(13), 1411–1442. https://doi.org/10.1134/S000629790913001X

Spiegelhalter, D. J., Best, N. G., Carlin, B. P., & Van Der Linde, A. (1998). *Bayesian deviance, the effective number of parameters, and the comparison of arbitrarily complex models*. Research Report, 98-009.

Spiegelhalter, D. J., Best, N. G., Carlin, B. P., & Van Der Linde, A. (2002). Bayesian measures of model complexity and fit. *Journal of the Royal Statistical Society. Series B: Statistical Methodology*, *64*(4), 583–616. https://doi.org/10.1111/1467-9868.00353

Staes, N., Guevara, E. E., Helsen, P., Eens, M., & Stevens, J. M. G. (2021). The Pan social brain: An evolutionary history of neurochemical receptor genes and their potential impact on sociocognitive differences. *Journal of Human Evolution*, *152*, 102949. https://doi.org/10.1016/j.jhevol.2021.102949

Staes, N., Weiss, A., Helsen, P., Korody, M., Eens, M., & Stevens, J. M. G. (2016). Bonobo personality traits are heritable and associated with vasopressin receptor gene 1a variation. *Scientific Reports*, *6*, 38193. https://doi.org/10.1038/srep38193

Stamps, J. A. (2007). Growth-mortality tradeoffs and "personality traits" in animals. *Ecology Letters*, *10*(5), 355–363. https://doi.org/10.1111/j.1461-0248.2007.01034.x

Stamps, J. A. (2016). Individual differences in behavioural plasticities. *Biological Reviews*, *91*(2), 534–567. https://doi.org/10.1111/brv.12186

Stamps, J. A., & Biro, P. A. (2016). Personality and individual differences in plasticity. *Current Opinion in Behavioral Sciences*, *12*, 18–23. https://doi.org/10.1016/j.cobeha.2016.08.008

Stamps, J. A., Briffa, M., & Biro, P. A. (2012). Unpredictable animals: Individual differences in intraindividual variability (IIV). *Animal Behaviour*, *83*(6), 1325–1334. https://doi.org/10.1016/j.anbehav.2012.02.017

Stefanik, D. J., Lubinski, T. J., Granger, B. R., Byrd, A. L., Reitzel, A. M., DeFilippo, L., Lorenc, A., & Finnerty, J. R. (2014). Production of a reference transcriptome and transcriptomic database (EdwardsiellaBase) for the lined sea anemone, Edwardsiella lineata, a parasitic cnidarian. *BMC Genomics*, *15*(1), 1–20. https://doi.org/10.1186/1471-2164-15-71

Storey, J. D. (2003). The positive false discovery rate: A Bayesian interpretation and the q-value. *Annals of Statistics*, *31*(6), 2013–2035. https://doi.org/10.1214/aos/1074290335

Storey, K. B., & Storey, J. M. (2004). Metabolic rate depression in animals: Transcriptional and translational controls. *Biological Reviews of the Cambridge Philosophical Society*, *79*(1), 207–233. https://doi.org/10.1017/S1464793103006195

Suryan, R. M., Arimitsu, M. L., Coletti, H. A., Hopcroft, R. R., Lindeberg, M. R., Barbeaux, S. J., Batten, S. D., Burt, W. J., Bishop, M. A., Bodkin, J. L., Brenner, R., Campbell, R. W., Cushing, D. A., Danielson, S. L., Dorn, M. W., Drummond, B., Esler, D., Gelatt, T., Hanselman, D. H., … Zador, S. G. (2021). Ecosystem response persists after a prolonged marine heatwave. *Scientific Reports*, *11*, 6235. https://doi.org/10.1038/s41598-021-83818-5

Svendsen, M. B. S., Bushnell, P. G., & Steffensen, J. F. (2016). Design and setup of intermittent-flow respirometry system for aquatic organisms. *Journal of Fish Biology*, *88*(1), 26–50. https://doi.org/10.1111/jfb.12797

Teranishi, K. S., & Stillman, J. H. (2007). A cDNA microarray analysis of the response to heat stress in hepatopancreas tissue of the porcelain crab Petrolisthes cinctipes. *Comparative Biochemistry and Physiology - Part D: Genomics and Proteomics*, *2*(1),

53–62. https://doi.org/10.1016/j.cbd.2006.11.002

Thomson, J. S., Watts, P. C., Pottinger, T. G., & Sneddon, L. U. (2011). Physiological and genetic correlates of boldness: Characterising the mechanisms of behavioural variation in rainbow trout, Oncorhynchus mykiss. *Hormones and Behavior*, *59*(1), 67–74. https://doi.org/10.1016/j.yhbeh.2010.10.010

Thomson, J. S., Watts, P. C., Pottinger, T. G., & Sneddon, L. U. (2012). Plasticity of boldness in rainbow trout, Oncorhynchus mykiss: Do hunger and predation influence risk-taking behaviour? *Hormones and Behavior*, *61*(5), 750–757. https://doi.org/10.1016/j.yhbeh.2012.03.014

Tomanek, L. (2008). The importance of physiological limits in determining biogeographical range shifts due to global climate change: the heat-shock response. *Physiological and Biochemical Zoology*, *81*(6), 709–717. https://doi.org/10.1086/590163

Tomanek, L. (2010). Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *Journal of Experimental Biology*, *213*(6), 971–979. https://doi.org/10.1242/jeb.038034

Tomanek, L. (2011). Environmental proteomics: Changes in the proteome of marine organisms in response to environmental stress, pollutants, infection, symbiosis, and development. *Annual Review of Marine Science*, *3*, 373–399. https://doi.org/10.1146/annurev-marine-120709-142729

Tomanek, L., & Somero, G. N. (1999). Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus Tegula) from different thermal habitats: implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology*, *202*(21), 2925–2936. https://doi.org/10.1242/jeb.202.21.2925

Toni, M., Angiulli, E., Miccoli, G., Cioni, C., Alleva, E., Frabetti, F., Pizzetti, F., Grassi Scalvini, F., Nonnis, S., Negri, A., Tedeschi, G., & Maffioli, E. (2019). Environmental temperature variation affects brain protein expression and cognitive abilities in adult zebrafish (Danio rerio): A proteomic and behavioural study. *Journal of Proteomics*, *204*, 103396. https://doi.org/10.1016/j.jprot.2019.103396

Torson, A. S., Dong, Y., & Sinclair, B. J. (2020). Help, there are 'omics' in my comparative

physiology! *Journal of Experimental Biology*, *223*(24), 1–10. https://doi.org/10.1242/jeb.191262

Trainor, B. C., & Hofmann, H. A. (2006). Somatostatin Regulates aggressive behavior in an african cichlid fish. *Endocrinology*, *147*(11), 5119–5125. https://doi.org/10.1210/en.2006-0511

Truebano, M., Burns, G., Thorne, M. A. S., Hillyard, G., Peck, L. S., Skibinski, D. O. F., & Clark, M. S. (2010). Transcriptional response to heat stress in the Antarctic bivalve Laternula elliptica. *Journal of Experimental Marine Biology and Ecology*, *391*(1–2), 65–72. https://doi.org/10.1016/j.jembe.2010.06.011

Tulin, S., Aguiar, D., Istrail, S., & Smith, J. (2013). A quantitative reference transcriptome for Nematostella vectensis early embryonic development: A pipeline for de novo assembly in emerging model systems. *EvoDevo*, *4*(1), 1–15. https://doi.org/10.1186/2041-9139-4-16

Tuomainen, U., & Candolin, U. (2011). Behavioural responses to human-induced environmental change. *Biological Reviews*, *86*(3), 640–657. https://doi.org/10.1111/j.1469-185X.2010.00164.x

Turner, V. L. G., Lynch, S. M., Paterson, L., León-Cortés, J. L., & Thorpe, J. P. (2003). Aggression as a function of genetic relatedness in the sea anemone Actinia equina (Anthozoa: Actiniaria). *Marine Ecology Progress Series*, *247*, 85–92. https://doi.org/10.3354/meps247085

Twardochleb, L. A., Treakle, T. C., & Zarnetske, P. L. (2020). Foraging strategy mediates ectotherm predator–prey responses to climate warming. *Ecology*, *101*(11), 1–11. https://doi.org/10.1002/ecy.3146

Urszán, T. J., Török, J., Hettyey, A., Garamszegi, L. Z., & Herczeg, G. (2015). Behavioural consistency and life history of Rana dalmatina tadpoles. *Oecologia*, *178*(1), 129–140. https://doi.org/10.1007/s00442-014-3207-0

Vajedsamiei, J., Wahl, M., Schmidt, A. L., Yazdanpanahan, M., & Pansch, C. (2021). The Higher the Needs, the Lower the Tolerance: Extreme Events May Select Ectotherm Recruits With Lower Metabolic Demand and Heat Sensitivity. *Frontiers in Marine Science*, *8*, 264. https://doi.org/10.3389/fmars.2021.660427

van Baaren, J., & Candolin, U. (2018). Plasticity in a changing world: behavioural responses to human perturbations. *Current Opinion in Insect Science*, *27*, 21–25. https://doi.org/10.1016/j.cois.2018.02.003

Van Oers, K., De Jong, G., Van Noordwijk, A. J., Kempenaers, B., & Drent, P. J. (2005). Contribution of genetics to the study of animal personalities: A review of case studies. *Behaviour*, *142*(9), 1185–1206. https://doi.org/10.1163/156853905774539364

Velasque, M., & Briffa, M. (2016). The opposite effects of routine metabolic rate and metabolic rate during startle responses on variation in the predictability of behaviour in hermit crabs. *Behaviour*, *153*(13–14), 1545–1566. https://doi.org/10.1163/1568539X-00003371

Wang, Q., Liu, Y., Zheng, Z., Deng, Y., Jiao, Y., & Du, X. (2018). Adaptive response of pearl oyster Pinctada fucata martensii to low water temperature stress. *Fish and Shellfish Immunology*, *78*, 310–315. https://doi.org/10.1016/j.fsi.2018.04.049

Wang, X., Yen, J., Kaiser, P., & Huang, L. (2010). Regulation of the 26S proteasome complex during oxidative stress. *Science Signaling*, *3*(151), 1–10. https://doi.org/10.1126/scisignal.2001232

Watts, P. C., Louise Allcock, A., Lynch, S. M., & Thorpe, J. P. (2000). An analysis of the nematocysts of the beadlet anemone Actinia equina and the green sea anemone Actinia prasina. *Journal of the Marine Biological Association of the United Kingdom*, *80*(4), 719–724. https://doi.org/10.1017/S002531540000254X

Weitzman, B., Konar, B., Iken, K., Coletti, H., Monson, D., Suryan, R., Dean, T., Hondolero, D., & Lindeberg, M. (2021). Changes in Rocky Intertidal Community Structure During a Marine Heatwave in the Northern Gulf of Alaska. *Frontiers in Marine Science*, *8*, 115. https://doi.org/10.3389/fmars.2021.556820

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics.*, *20*(1), 249–278. https://doi.org/10.1146/annurev.es.20.110189.001341

Westneat, D. F., Wright, J., & Dingemanse, N. J. (2015). The biology hidden inside residual within-individual phenotypic variation. *Biological Reviews*, *90*(3), 729–743. https://doi.org/10.1111/brv.12131

White, S. J., & Briffa, M. (2017). How do anthropogenic contaminants (ACs) affect behaviour? Multi-level analysis of the effects of copper on boldness in hermit crabs. *Oecologia*, *183*(2), 391–400. https://doi.org/10.1007/s00442-016-3777-0

White, S. J., Kells, T. J., & Wilson, A. J. (2016). Metabolism, personality and pace of life in the Trinidadian guppy, Poecilia reticulata. *Behaviour*, *153*(13–14), 1517–1543. https://doi.org/10.1163/1568539x-00003375

Wickham, H. (2011). ggplot2. *Wiley Interdisciplinary Reviews: Computational Statistics*. https://doi.org/10.1002/wics.147

Wilding, C. S., & Weedall, G. D. (2019). Morphotypes of the common beadlet anemone Actinia equina (L.) are genetically distinct. *Journal of Experimental Marine Biology and Ecology*, *510*, 81–85. https://doi.org/10.1016/j.jembe.2018.10.001

Wilkins, M. (2009). Proteomics data mining. *Expert Review of Proteomics*, *6*(6), 599–603. https://doi.org/10.1586/epr.09.81

Williams, L. J., King, A. J., & Mettke-Hofmann, C. (2012). Colourful characters: Head colour reflects personality in a social bird, the Gouldian finch, Erythrura gouldiae. *Animal Behaviour*, *84*(1), 159–165. https://doi.org/10.1016/j.anbehav.2012.04.025

Wilson, A. J. (2018). How should we interpret estimates of individual repeatability? *Evolution Letters*, *2*(1), 4–8. https://doi.org/10.1002/evl3.40

Wolf, M., & McNamara, J. M. (2012). On the Evolution of Personalities via Frequency-Dependent Selection. *The American Naturalist*, *179*(6), 679–692. https://doi.org/10.1086/665656

Wolf, M., Van Doorn, G. S., Leimar, O., & Weissing, F. J. (2007). Life-history trade-offs favour the evolution of animal personalities. *Nature*, *447*(7144), 581–584. https://doi.org/10.1038/nature05835

Wolf, M., & Weissing, F. J. (2010). An explanatory framework for adaptive personality differences. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1560), 3959–3968. https://doi.org/10.1098/rstb.2010.0215

Wolf, M., & Weissing, F. J. (2012). Animal personalities: Consequences for ecology and evolution. *Trends in Ecology and Evolution*, *27*(8), 452–461. https://doi.org/10.1016/j.tree.2012.05.001

Wong, B. B. M., & Candolin, U. (2015). Behavioral responses to changing environments. *Behavioral Ecology*, *26*(3), 665–673. https://doi.org/10.1093/beheco/aru183

Wong, M. Y. L., Medina, A., Uppaluri, C., Arnold, S., Seymour, J. R., & Buston, P. M. (2013). Consistent behavioural traits and behavioural syndromes in pairs of the false clown anemonefish Amphiprion ocellaris. *Journal of Fish Biology*, *83*(1), 207–213. https://doi.org/10.1111/jfb.12133

Wong, R. Y., French, J., & Russ, J. B. (2019). Differences in stress reactivity between zebrafish with alternative stress coping styles. *Royal Society Open Science*, *6*(5), 181797. https://doi.org/10.1098/rsos.181797

www.seatemperature.org. (2020). *Llandudno Water Temperature | United Kingdom Sea Temperatures*. https://www.seatemperature.org/europe/united-kingdom/llandudno.htm

Yamane, L., & Gilman, S. E. (2009). Opposite responses by an intertidal predator to increasing aquatic and aerial temperatures. *Marine Ecology Progress Series*, *393*, 27–36. https://doi.org/10.3354/meps08276

Yang, C., Gao, Q., Liu, C., Wang, L., Zhou, Z., Gong, C., Zhang, A., Zhang, H., Qiu, L., & Song, L. (2017). The transcriptional response of the Pacific oyster Crassostrea gigas against acute heat stress. *Fish and Shellfish Immunology*, *68*, 132–143. https://doi.org/10.1016/j.fsi.2017.07.016

Zhang, W., Li, F., & Nie, L. (2010). Integrating multiple “omics” analysis for microbial biology: Application and methodologies. *Microbiology*, *156*(2), 287–301. https://doi.org/10.1099/mic.0.034793-0

Zhao, S., Guo, Y., Sheng, Q., & Shyr, Y. (2014). Heatmap3: An improved heatmap package with more powerful and convenient features. *BMC Bioinformatics*, *15*(10), 15–16. https://doi.org/10.1186/1471-2105-15-S10-P16

Zhu, W., Wang, L., Dong, Z., Chen, X., Song, F., Liu, N., Yang, H., & Fu, J. (2016). Comparative Transcriptome Analysis Identifies Candidate Genes Related to Skin Color Differentiation in Red Tilapia. *Scientific Reports*, *6*, 31347. https://doi.org/10.1038/srep31347

[Page intentionally left blank]

[Page intentionally left blank]