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

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Received 29 January 2021

Initial acceptance 12 May 2021

Final acceptance 19 July 2021

MS number 21-00077R

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***Keywords****- behavioural plasticity, boldness, climate change, marine invertebrate, temperature fluctuation, unpredictability*

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 oC and one at 21 oC in two crossed-over treatments, and two at 13 oC 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 oC than those from more stochastic environments. For the immersion response, animals were more unpredictable at 21 oC than at 6 oC; 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.

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 (Maskrey et al., 2020). Further, temperature shifts in *A. equina* drive changes in metabolic rate (Chomsky et al., 2004), proteomic expression (Choresh et al., 2001) and boldness-related behaviours (Maskrey et al., 2020). 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; Maskrey et al., 2020). 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 (Maskrey et al., 2020). *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., 2004; 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.

**<H1>Methods**

***<H2>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 (latitude 53.330359, longitude 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; Maskrey et al., 2020). 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 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 (80x45 cm and 40 cm deep), filled to a depth of ca. 12 cmwith artificial sea water (RO water and Tropic Marin, Wartenberg, Germany, Pro Reef Salt) and situated in a 13 oC (±1 oC) 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 6 oC (±1 oC) and 21 oC (±1 oC), 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 13 oC (±1 oC) 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 (7.5 oC–16.25 oC; 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 6 oC, a temperature at the very lower bound of the normal range of extremes this population might experience (www.seatemperature.org, 2020), was chosen to reflect this. The other extreme, 21 oC, is nearing the thermal maximum for this population (Maskrey et al., 2020). It is well above the normal high sea temperature extremes experienced by Lllandudno (17.3 oC), but it is possible that isolated tide pools could reach this temperature during a heatwave. We chose 13 oC 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 libitumon brine shrimp (Monkfield Nutrition, Ely, U.K.) 48 h into their 60 h acclimation period (as per Maskrey et al., 2020).

***<H2>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 1, Figs A1–A3). 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 Maskrey et al., 2020). 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 2, Fig. A4), 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 2, Fig. A4) and only 25% of the collar was still visible, or as when an anemone returned to its prestartle 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 (Maskrey et al., 2020).

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 (Maskrey et al., 2020). For logistical purposes, the upper bound of SRT was lowered from 5400 s (Maskrey et al., 2020) to 4800 s. Preliminary analyses of data from that previous paper 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 oC/h, to account for the influence of treatment order (as per Briffa et al., 2013). The temperature in the control system remained at 13 oC. 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.

***<H2>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 data sets, allowing for the direct comparison of model outputs.

***<H2>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 data set. 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 data set allowed for the specification of further models for our experimental and control data sets, 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 a 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 data set. 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 data set 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 3 for a full discussion of the decision to retain maximal SRT values.

***<H2>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 (Rint) 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; Ru). 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; Rc). 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 individual consistency in unpredictability was reduced across temperatures as compared with across timepoints, across-context CVp (the coefficient of variation in predictability; Cleasby et al., 2015) values were calculated. For IRT these extended to within-context CVp 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.

***<H2>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.

***<H2>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, 2020) and high (Maskrey et al., 2020) 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.

**<H1>Results**

***<H2>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 (Fig. 1a). This contrasted with controls, where both estimates were significant (Fig. 1a). Conditional repeatability, meanwhile, was significant and similar in both treatments (Fig. 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 (Fig. 1a), 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 (Fig. 1a). 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.

***<H2>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 21 oC (estimate=0.13, 95% CI=0.07, 0.20) than at 13 oC, and when they were first tested at 6 oC (cold–hot) rather than 21 oC (hot–cold; estimate=0.21, 95% CI=0.08, 0.34). Figure 2 shows this effect clearly, and further indicates that while population level unpredictability was significantly higher at 21 oC than at 6 oC 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.

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).

***<H2>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 CVp and across-timepoint CVp (Fig. 1b) 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 CVp significantly higher than the across-temperature estimate (Fig. 1b).

The reduced analysis of SRT did not indicate an effect of temperature on between-individual differences in unpredictability. Moderate but significant across-context CVp estimateswere extracted from both models (Fig. 1b), indicating that between-individual variation in unpredictability was present for this behaviour. Across-context CVp was lower in the experimental model than the control (Fig. 1b), but not significantly so. As such, no temperature effect could be inferred.

***<H2>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 (Fig. 1c, Fig. 3a,b,c). 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 (Fig. 1c). 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 (Fig. 1c). 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 (Fig. 3c) 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 (Fig. 1c). Figure 3d 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.

***<H2>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 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 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 oC and thus showed the lowest unpredictability at that temperature became more unpredictable and shyer at 21 oC. Similarly, many of the shyer, more unpredictable individuals at 6 oC became bolder and less unpredictable at 21 oC, 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.

***<H2>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 (Maskrey et al., 2020) but for full results and discussion in relation to the models presented here, see Appendix 4, Table A1.

**<H1>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 oC. 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 oC than 6 oC 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 oC, 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 (Maskrey et al., 2020; Appendix 4). 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 oC 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 oC in the hot–cold treatment, further decreasing unpredictability. While this pattern was not present in the opposite direction (i.e. greater unpredictability at 21 oC after heating than before cooling due to higher metabolic demands at 21 oC 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.

**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.

**Acknowledgments**

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.

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**Appendix 1: Experimental schedule**

**Appendix 2: Anemone anatomy**

**Appendix 3: Censoring response times and retaining right-censored values**

It was deemed inappropriate to remove those individuals exhibiting maximal values from either SRT data set as (1) it cut the effective sample size of our control data set by 27% and of our experimental data set 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 data set 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 data sets 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 could be argued to 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 (Maskrey et al., 2020). 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 Maskrey et al., 2020).

**Appendix 4: Fixed effects on mean behaviour**

*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 oC than at 21 oC. 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 oC. 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.

*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 (Maskrey et al., 2020). These data also substantiate that shore height may be an important predictor of SRT but not of IRT at the mean level (Maskrey et al., 2020). 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.

**Table A1.** Table of fixed and random effect estimates taken from the mean level models of double-hierarchical GLMs

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 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) |

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 sea 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 bold text. 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.

**Figure Legends**

**Figure 1.** Individual level behavioural estimates (± 95% credible intervals, CI) from double-hierarchical GLMMs in the sea anemone, *Actinia equina*, showing: (a) mean level individual behavioural intercepts across contexts (Rint), unconditional repeatability (Ru) and conditional repeatability (Rc) for immersion response time (IRT); (b) Rint, Ru and Rc for startle response time (SRT); (c) the coefficient of between-individual variation in unpredictability (CVp) across and within contexts for IRT; (d) CVp 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.

**Figure 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.

**Figure 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 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 13 oC; (f) experimental IRTs within 21 oC; (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.

**Figure 4.** Plasticity of individual level unpredictability, here measured as within-environment point estimates of residual individual standard deviation (riSD), estimates 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) 6 oC and 21 oC 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.

**Figure A1.** (a)The full experimental timeline for a single data collection block and (b) the daily behavioural schedule.

**Figure A2.** The crossed-over temperature schedule of a data collection block.

**Figure A3.** 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.

**Figure A4.** The basic anatomy of *A. equina*.