Individual quality and boldness in male hermit crabs: Risk-averse individuals are the most fecund.

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Abstract

Biological variation is hierarchically structured and the fine scales of variation among and within individuals are responsible for the consistent among individual differences in behaviour currently described as animal personality. A potential explanation for animal personality is that different behavioural types derive from different life history strategies. Individuals that are highly fecund should also have high performance capacities and live life at a 'fast' pace showing high levels of boldness and risk taking, compared to less productive individuals that take life at a slower pace. Here we investigate individual differences in mean and intra-individual variation (IIV) of startle response duration of male hermit crabs in relation to two aspects of life history investment, aerobic scope (haemocyanin concentration) and fecundity (spermatophore size). Using doubly hierarchical generalized linear models to analyse longitudinal data on startle response durations we show that hermit crabs vary both in their mean response durations and in the amount of IIV that they show. Individuals that invested in large spermatophores also had high concentrations of the respiratory pigment haemocyanin. IIV was not influenced by haemocyanin or by spermatophore size but mean startle response duration increased with spermatophore size. This relationship was non-linear, such that the increase in startle response duration was greatest at the lower range of spermatophore sizes. Thus, counter to expectations it was the most risk-averse individuals, rather than the boldest and most risk prone, that were the most productive. We discuss our results in the context of the specific biology of hermit crabs, and discuss the potential for similar patterns in other species, where high quality individuals might be expected to avoid risky behaviour.

Introduction

Animal personality refers to among individual difference in behaviour that are consistent over time and in some cases across situations. Recent studies have addressed the fact that these consistent differences are nested within a hierarchy of scales over which biological variation occurs. Westneat et al. [1] emphasise variation among species, populations and individuals. Then there is a finer level of variation, often referred to as behavioural plasticity, within individuals that adjust their behaviour to match the current situation. Finally we can identify a yet finer level of residual variation within individuals that cannot be explained by plasticity. Briffa et al. [2] described six categories of behavioural variation but again arrived at residual variation within individuals as the finest level of behavioural organisation. This fine scale residual variation [3] has been described as consistency [4], stability [5,6] and recently as predictability and intra-individual variation (hereafter IIV) [2,7–10]. Although such hierarchies represent a useful framework for discussing the structure of behavioural variation, the levels are not isolated from one another. In-fact, animal personality, frequently quantified by calculating repeatability estimates, is dependent upon two of the levels described above. Significant repeatability is contingent on relatively high levels of among individual variance coupled with relatively low levels of within individual variance [11]. Assuming that observations of different individuals are not confounded by different environments or conditions [1], the aspect of within individual variance that is of interest becomes this residual variation or IIV. In order to fully understand the causes and consequences of animal personality attention should therefore be paid to both of these levels of variation that contribute to the observed personality effect.

While personality can be readily documented in a wide range of animal taxa, it is not immediately clear why animals should consistently differ in their behaviour and a range of approaches have been adopted to uncover the causes for personality. First we may look

directly or indirectly [12] at the heritability [13] and fitness consequences of personality variation [14–16]. Second, we might determine what environmental parameters contribute to animal personality, and under what conditions the personality effect tends to be eroded or promoted [2,17,18]. Indeed, plasticity can be demonstrated in both mean level responses and in IIV, so both components have the potential to vary among environments [2,8,19]. Another approach is to determine what other traits co-vary with personality. In some cases a personality trait might vary with another behavioural trait to form a behavioural syndrome [20]. Personality traits may also vary with physical traits including physiology (e.g. metabolic rate [21]) or morphology (e.g. size [22,23]). Understanding how a personality trait might vary with physical traits is an essential step in arriving at an adaptive explanation for personality because it is hypothesised that consistent behavioural differences derive from life-history trade-offs [22,24–26]. The application of the pace of life syndrome concept [25] to the question of animal personality suggests that these trade-offs could lead to divergent strategies represented by fast and slow individuals. Fast individuals are characterised by high metabolic rates and heavy investment in reproduction compared to slow individuals. In terms of behaviour, fast individuals are the more proactive, bold and aggressive, compared to reactive slow individuals. Such trade-offs could derive from the need to balance behaviours that minimise exposure to risk against behaviours that allow acquisition of the resources required to maintain high metabolic rates [27] or life-history productivity [28]. Productivity is defined as investment in biomass or fecundity and we might also include investment in any physiological system that enhances ongoing performance. Endurance capacity (stamina) for example requires investment in ventilation systems and oxygen transport systems, which define an individual's aerobic scope. This trait is known to influence success in demanding interactions [29–32]. In agonistic displays, for example, high quality individuals that win

fights tend to have greater stamina [31] and similar links between stamina and positive outcomes can also be seen in courtship behaviour [33] and foraging [29,30].

While life history *productivity* is best quantified by measuring rates (e.g. the rate of biomass accumulation) it is still possible to gain insights into the links between behaviour and life history by obtaining point estimates of traits that are indicative of productivity. Thus we might assess fecundity in spiders by counting the number of spiderlings emerging of the egg sacs of females, as a study on the jumping spider *Dolomedes triton* [34], where body mass (minus the egg sac) was similarly used as a proxy for investment in biomass. Similarly, fledgling success in relation to provisioning behaviour and other personality traits has been assessed in birds [12,35]. Fecundity, the potential for reproductive output, can also be assessed by quantifying investment in gametes, usually eggs in females. In the codling moth, *Cydia pomonella* the number of eggs shows negative correlation with activity rate, indicating a trade-off between these two traits [36]. In the Atlantic silverside, *Menidia menidia*, egg volume was the measure of fecundity and in this case there was a positive association with boldness [37].

Fecundity is most usually assessed in females but it can also vary between males and in both sexes it can be heavily influenced by costs associated with the production and packaging of gametes. Whilst spermatogenesis in males typically represents a marginal cost compared to oogenesis in females, in many cases males package their sperm up into costly spermatophores. These spermatophore costs can lead to sperm depletion following multiple matings as in the spiny lobster, *Panulirus argus*. The clutch size of females increases with the size of the sprematophore provided by the male such that the success of both parents is linked to spermatophore size [38]. Spermatophore size is also known to influence the fecundity of males in bush crickets such as *Gryllodes supplicans* where males distract the female from consuming the sperm carrying ampulla by providing a large spermatophylax that she

consumes first [39]. Interestingly, the links between gamete packaging and male fecundity are not restricted to animals. In sea beet, *Beta vulgaris* ssp. *maritima*, male fecundity is directly linked to the quality of their pollen [40], which contains the microscopic sperm producing haploid generation of spermatophyte plants. Thus, the question of how male fecundity might be traded-off against investment in other life history traits is relevant to a broad range of organisms.

We can therefore partition life history investment into traits that maximise day to day activity (enhancing growth and survival) and those that influence fecundity (enhancing reproductive success). Here we investigate the links between consistent among individual differences in boldness and markers of these two aspects of life history, in the hermit crab *Pagurus bernhardus*. These decapod crustaceans occupy empty gastropod shells and when threatened they withdraw rapidly into them before slowly re-emerging. The duration of this startle response is directly analogous to that shown by other animals that retreat into a refuge upon being disturbed. When repeated observations are made on the same individuals such responses are usually described as a measure of an individual's 'boldness' (see [28,37,41] for examples). Here, we first look at boldness in relation to investment in haemocyanin the respiratory pigment of crustaceans, which varies among individuals and contributes to their capacity for demanding aerobic activity [42]. Second, we investigate boldness in relation to investment in spermatophore production quantified by the size of spermatophores, which are produced continuously in males. Previous studies on *P. bernhardus* have shown that individual mean level boldness and IIV in boldness vary among individuals and that both are sensitive to environmental effects such as temperature [2] and predation risk [8,43]. However, it is not known how either aspect might vary with investment in physiological condition or reproductive potential. To the best of our knowledge this is the first time that the potential for links between personality and life history traits representing (i) investment in performance

capacity and (ii) investment in fecundity have been investigated simultaneously, at the levels of individual mean and IIV levels of variation. We also include crabs from two populations known to differ in mean startle response durations [43] such that the effects of the physical parameters that are of primary interest here can be compared to the known effects of an ecological variable.

Studies investigating the causation of personality at the level of both among individual differences and within-individual consistency (IIV) are still relatively rare [1,2,7–10,19]. In the following experiment, we ask whether haemocyanin and spermatophore size are positively or negatively associated. A positive correlation would indicate that both traits are related to individual quality, with high quality individuals able to invest heavily in both day to day performance and fecundity. A negative correlation would indicate that the two traits are traded off, such that short term performance is sacrificed for enhanced fecundity. Furthermore, if animal personality in hermit crabs represents underlying differences in pace of life we would expect to see a positive association between boldness and investment in large spermatophores and a positive association between boldness and investment in haemocyanin. Thus individuals that have high concentrations of haemocyanin and large spermatophores should recover from a startling stimulus more quickly than those that show lower investment.

Materials and methods

Collection of animals and behavioural observations

Hermit crabs were collected from the rocky intertidal at Mount Batten (SX 48718 52888) and Hannafore Point (SX 25792 52533), UK, during March 2011. The crabs from both locations

were bought to the laboratory at Plymouth and held in site-specific batches of 100 individuals in tanks containing aerated sea water maintained at 15°C in a constant temperature room. Crabs were then removed from their gastropod shells, by carefully cracking the shell in a bench vice. We selected 27 males from Hannafore (mean mass = 0.516 ± S.E. = 0.07g) and 26 males from Mount Batten (Mount Batten mean mass = 0.49 ± S.E. = 0.07g) that were free of obvious parasites and missing appendages. Females and males with parasites or missing appendages were supplied with new shells and returned to the sea. Each male selected for the experiment was weighed and supplied with a new *Littorina littorea* shell of 100% of its preferred shell mass. The appropriate shell mass was calculated from regression lines relating crab mass to preferred shell mass, obtained from a previous shell selection experiment [44]. This step is essential in studies of hermit crab behaviour because numerous experiments (e.g. see the review in [45]) have shown that shell optima affect the behaviour of hermit crabs, including their startle response durations [46]. Crabs were then housed individually in 20 cm diameter white plastic dishes containing continuously aerated sea water at 15°C to a depth of 5 cm, and fed white fish flesh *ad libitum*. During the experiment we did not collect dish-specific temperatures. However, we did assess temperature variation across the approximately 1.5m length of bench space over which dishes were distributed and found no significant variation in temperature across this space [2].

Following a 24 hour acclimation period, a startle response was induced in each crab every day for eight days. The startle response was induced by removing the crab from its tank, inverting the crab for five seconds, which causes it to withdraw into its shell and then returning it to the tank with the aperture facing upwards. Immediately prior to this, aeration was switched off so that the crab could be clearly observed and so that the air bubbles did not disturb the crab as it emerged from its shell. Aeration was switched back on again after observation. Startle responses were induced between 9am and 12pm each day and the

observation order was randomised between days. The duration of the startle response was timed from when the crab was replaced in the tank until the walking legs first contacted the floor of the dish [43].

Analysis of haemocyanin and spermatophores

After the eight day period a haemolymph sample was extracted from each crab. An insulin syringe was inserted into the infrabranchial sinus, via the arthrodial membrane at the base of the third pereiopod. A 10µl aliquot of haemolymph sample was immediately added to a semi-micro cuvette containing 690µl of double distilled water. Following mixing, the absorbance was measured at 337nm in a spectrophotometer. The haemocyanin concentration was then determined for each crab following Nickerson & Van Holde [47], using the extinction coefficient (*E*):

$E_{1\mathrm{cm}}^{\mathrm{mM/l}} = 17.26.$

After taking the haemolymph sample each crab was humanely euthanized by placing it in a saturated solution of magnesium chloride. The *vas deferens* was then dissected out and a distal section joined to the gonopore, of ca 1mm length, was cut away and placed on a microscope slide with a drop of seawater and broken up to release the spermatophores. In hermit crabs spermatophores consist of a cylindrical sperm containing ampulla, which is tapered towards the distal end, the proximal end being attached to a basal plate by a solid peduncle [48]. In *P. bernhardus* the basal plates usually carry a line of four to six spermatophores. For each crab five intact spermatophores, taken from five different basal plates were selected at random and photographed using a binocular confocal microscope (Leica MZ12) equipped with a Lumenera Infinity 1 camera connected to a computer. We

measured the spermatophore ampulla lengths (henceforth 'spermatophore length') using point-to-point measurements in Lumenera Infinity Analyze 5.0.3 software. An individual's spermatophore length was defined as the average ampulla length of the 5 selected spermatophores.

Statistical methods

In previous studies we assessed intra-individual variation (IIV) by first using a general linear mixed effects models to assess sample and individual mean level effects, and then deriving from this model a measure of residual individual standard deviation (riSD) [7]. An alternative approach taken by Westneat *et al*. [1] is to use a doubly hierarchical generalised linear model (DHGLM) fit using Bayesian methods via Markov chain Monte Carlo (MCMC) sampling. This method allows the simultaneous modelling of mean level effects and effects on variance, including both fixed and random factors. These two components are termed the mean and standard deviation (henceforth 'SD') models respectively. This approach has the advantages of (a) combining both steps into a single model such that uncertainty in our parameter estimates can be accounted for in each part of the analysis, and (b) the ability to cope with non-heterogeneous residual errors, allowing fixed effects to be assessed more robustly in comparison to LMM (although this is only likely to be of concern if there are greatly uneven sample sizes between groups). One of the differences between Bayesian statistics and the more familiar frequentist methods is that Bayesian analyses allow the formal incorporation of prior information into the statistical model. If information is available from previous experiments (or other sources) then this knowledge can be incorporated into the analysis by specifying informative prior distributions for the model parameters. Alternatively, non- or weakly-informative prior distributions may be specified in order that the prior distribution has little effect on the results [49] and we adopted this approach here.

We first specified a DHGLM as follows. For the mean model we included fixed effects for spermatophore length, haemocyanin concentration, crab mass, population and observation number. For each crab we allowed a random intercept effect and a random slope effect with respect to occasion. Data were Log_{10} transformed to improve normality. If Y_{ij} denotes the startle response of the i^{th} crab on the j^{th} occasion then we assume that $\log_{10}(Y_{ij})$ is normally distributed with mean μ_{ij} and standard deviation σ_i. The mean model can be expressed as:

$$\mu_{ij} = (\beta_1 + \delta_{0i}) + \beta_2 \text{Mass}_i + \beta_3 \text{Population}_i + \beta_4 \text{Spermatophore length}_i + \beta_5 \text{Haemocyanin}_i + (\beta_6 + \delta_{6i}) \text{Occasion}_{ij} \quad (1)$$

Parameter β_1 is the expected valve when all of the covariates are equal to zero and β_2 to β_6 represent fixed effects for the covariates as indicated. The value δ_{0i} represents the random intercept effect and δ_{6i} represents the random slope associated with the occasion effect. We assumed that the random effects were normally distributed with means of zero and unknown variances (see Supplement 1 for more details). The SD model can be expressed as:

$$\log_{10}(\sigma_i) = (\gamma_1 + \phi_{0i}) + \gamma_2 \text{Mass}_i + \gamma_3 \text{Population}_i + \gamma_4 \text{Spermatophore length}_i + \gamma_5 \text{Haemocyanin}_i \quad (2)$$

Here γ_1 represents the population mean and γ_2 to γ_5 are the fixed effects for the covariates as indicated. The value ϕ_{0i} denotes the random intercept, for which we assumed a mean of zero and standard deviation of $\tau\sigma,0$. The fixed effects allow us to ask, at the population level, whether IIV correlates with the morphological and physiological measures and whether it differs between the two populations. The random intercept allows us to ask whether individuals differ in IIV. Including a random slope effect in the SD model would have tested the idea that individuals might differ in how the level of IIV that they express varies across occasions. Given that there was only 1 observation per occasion this was not an appropriate

question to ask with this data set, although such an analysis could be attempted with a 'multiple burst' experiment.

Our initial mean model (Supplement 1) assumed a linear association between the predictors and the response. However, subsequent assessment of goodness of fit for this model (Supplement 1) suggests that the associations were more complex. Visual inspection of the data suggested a quadratic association would provide a better fit with the data, so the mean portion of the model was adjusted as follows:

$$E[\log_{10}(Y_{ij})] = (\beta_1 + \delta_{0i}) + \beta_2 \text{Mass}_i + \beta_3 \text{Population}_i + \beta_4 \text{Spermatophore length}_i + \beta_5 \text{Spermatophore length}_i^2 + \beta_6 \text{Haemocyanin}_i + \beta_7 \text{Haemocyanin}_i^2 + (\beta_8 + \delta_{1i})\text{Occasion}_{ij} \quad (3)$$

Sampling from the posterior distributions of the model parameters was conducted using the freely available software JAGS [50], which we controlled from within the R statistical computing environment using the package RJAGS (3.13) [51] (Plummer 2014). Following the usual MCMC setup, the parameters in each model were updated conditional on the remaining parameters to generate random draws from their posterior distribution. The standard deviations of the random effects and error terms in both the mean and SD models were assigned weakly informative scaled half-t prior distribution with 3 df [52] while the fixed effects parameters were assigned non-informative normal prior distributions (See supplement 1). Three chains were run in parallel so that convergence could be assessed and each chain was run with an adaptive phase ('burn in') of 10000 iterations and a sampling phase of 15000 iterations. Convergence was assessed using the Gelman-Rubin diagnostic, which was <1.1 for each model parameter, indicating that the adaptive phase was adequate. Here, we made inferences about the parameters in each model based on their posterior means and 95% credible intervals. As in the study by Westneat et al. [1], we based the primary assessment of the significance of each predictor on whether or not the 95% credible intervals

for the corresponding parameter covered zero. In the case of fixed effects we are also able to judge significance by generating values analogous to classical *P*-values. These pseudo *P*-values (hereafter '*P*') are obtained by calculating the tail probability for each fixed parameter. They express, as a value between 0 and 1, the probability over the set of all equal tailed credible intervals that cover zero. Thus if the 95% CI abuts zero, $P = 0.05$ but if the 95.5% CI abuts zero, $P = 0.045$ indicating a significant effect. Smaller values indicate that the posterior mean is further from zero (relative to the level of uncertainty) and provide stronger evidence for a significant effect. This approach was not available for random effects as they are constrained to be positive. In cases where the posterior mean effect size was very low (< 0.02) we considered that there was no evidence of a biologically meaningful effect, irrespective of whether the 95% credible intervals crossed or abutted zero.

Results

There was no difference between populations in crab weight ($t_{51} = 0.34$, $P = 0.74$), haemocyanin concentration ($t_{51} = 0.91$, $P = 0.37$) or average spermatophore length ($t_{51} = 1.6$, $P = 0.11$). There was no correlation between crab mass and average spermatophore length ($r_{51} = -0.2$, $P = 0.15$) or haemocyanin concentration ($r_{51} = -0.148$, $P = 0.28$) but there was as significant correlation between haemocyanin concentration and average spermatophore length ($r_{51} = 0.7$, $P < 0.0001$) (figure 1). Note that it is still valid to include both of these covariates in the main DHGLM analysis reported below. Unlike classical stepwise procedures, *P*-values are not computed by removing variables and the order of variable entry into the model has no bearing on the calculation of parameter estimates or probability. By retaining both predictors and allowing them to compete in the same model we may assess the

contribution of each conditional on the contribution of the other and thus assess which is more strongly associated with variation in startle response durations.

The parameter estimates from the DHGLM and their 95% credible intervals are presented in Table 1 and illustrated graphically in **Figure 2**. The fixed effects component of the mean model provided strong evidence that mean startle response duration was greater in crabs from Mount Batten compared to those at Hannafore ($P < 0.0001$) and that, on average, startle response duration increased with spermatophore length ($P < 0.0001$) (**Figure 3**). This positive association was non-linear, the effect of changes in spermatophore length on startle response duration being more marked in crabs with small spermatophores than in crabs with large spermatphores. There was no significant effect of haemocyanin concentration after contains for Spermatophore length (linear $P = 0.14$; quadratic $P = 0.9$) indicating that although haemocyanin and spermatophores co-vary, it is variation in spermatophore length that is most closely associated with startle response duration. In the case of occasion there was a very small effect size and the upper 95% credible interval abutted zero, indicating no significant sample mean level change with occasion ($P = 0.11$). There was no evidence that mean startle time varied with mass ($P = 0.32$). The random effects components of the mean model provide strong evidence for variation on the mean startle response duration among individuals that is not explained by the covariates in the model (see Figure 1A, Crab ID effect). This effect denotes significant repeatability in startle response duration. There was no evidence, however, that the pattern of change in mean startle time across occasions varies among individuals. Here the lower 95% credible interval abutted zero and the effect size was very small.

The fixed effects components of the SD model indicate that IIV in startle response duration increased with crab mass ($P = 0.03$). In the case of population ($P = 0.96$), spermatophopre length ($P = 0.17$), and haemocyanin concentration ($P = 0.44$) the 95%

credible intervals clearly overlapped zero and thus there was no evidence that IIV was influenced by these predictors. However, the 95% credible intervals for the random effects component were clearly distinct from zero providing strong evidence for significant variation in the standard deviations of the startle time among individuals that was not explained by the covariates in the model (see Figure 1B, Crab ID effect). In other words, there were significant differences among individuals in intra-individual variation (IIV) in startle response duration.

Discussion

As in previous studies the data clearly show that hermit crabs exhibit animal personality in terms not only of consistent among individual differences in behaviour but also in terms of significant among individual differences in IIV. We also found, similar to previous studies, that crab mass had no effect on the mean duration of startle response durations [43]. Although we have controlled for mass in previous analyses of IIV [2,7,8] this is the first time that we have tested the possibility that IIV varies with mass and there was a clear effect for larger crabs to be less consistent in their behaviour than smaller ones. The main aim of this study, however, was to ask whether these patterns of among individual variation in startle responses might be linked to variation in investment in spermatophores and haemocyanin. These traits did not vary with crab mass but were positively correlated with one another, indicating that individuals that invest in high fecundity also have high haemocyanin, a result which we would expect if highly fecund animals are also those with the greatest performance capacity as predicted by the pace of life hypothesis. Surprisingly, however, spermatophore length traits showed a negative association with boldness. That is, individuals with high fecundity (and therefore high haemocyanin) are on average slower to recover from the startling stimulus than are those that invest less spermatophores and haemocyanin. Although we did

not measure metabolic rate, a key process for the pace of life syndrome, this result seems counter to the idea that the most productive individuals should also be the boldest [25]. The links between behaviour and pace of life are not necessarily straightforward and there are various ecological factors that may disrupt the expected associations between behaviour and fecundity [25,28,53]. Thus, while some studies have provided clear support for the pace of life explanation for animal personality (e.g. *M. menidia* [37], domestic dogs, *Canis lupus familiaris* [54]) others have been more equivocal. Two recent studies indicate a tendency for physiological but not behavioural traits to form syndromes [55,56]. Below we interpret our results with reference to the specific biology of hermit crabs, and discuss the extent to which similar associations might be expected in other study systems.

Long startle responses reduce the time available for other activities but provide a mechanism for avoiding risk. Thus, in hermit crabs startle responses increase in duration when the ambient level of risk is high due to the presence of a predator [8,43,46]. Similar positive associations between risk level and hiding time are also seen in other species (e.g. grey mouse lemur, *Microcebus murinus* [57], rock agama *Agama planiceps* [58], nutmeg manikin, *Lonchura punctulata* [59]) suggesting that, despite the presence of personality variation, plasticity in boldness is a widespread coping strategy for animals that encounter changing risk levels. In hermit crabs long hiding times can also be advantageous in the context of fighting behaviour. For crabs defending their shell against an attacker, long startle responses are associated with a greater chance of resisting eviction whereas boldness has no effect on crabs playing the attacker role [60]. While long hiding times may be beneficial, there is a potential physical constraint on the maximum time for which a hermit crab may remain withdrawn into its shell. In the withdrawn position, a crab's access to fresh well-aerated seawater is restricted, which can constrain ventilation and hence aerobic respiration [61]. Some oxygen, however, will be available as oxyhaemocyanin (i.e. the oxygenated form

of the respiratory pigment) and the amount available will be proportional to an individual's haemocyanin concentration. Therefore an individual's aerobic scope might contribute to hiding times by imposing an upper limit on the duration of hiding.

If variation in boldness in hermit crabs is driven in part by an underlying constraint on maximum hiding times, to what extent is it analogous with boldness in other animals? As noted above, hiding will constrain the time available for essential activities such as foraging. Indeed, the presence of among individual differences in the requirement to return to ongoing activities has previously been assumed as a driver of personality variation. Rands et al. [62], for example, describe how differences in hunger levels should lead to variation in maximum hiding time prior to the resumption of foraging. The requirement to extract oxygen from the environment (and to dissipate carbon dioxide), and differences in the maximum time before ventilation must be resumed, is an analogous situation. In other examples of aquatic organisms where boldness is measured by timing the latency to emerge form a refuge (e.g. [41]) water movement within the refuge is likely to be restricted and oxygen availability may decline with hiding time if aerated water is depleted more quickly than it is replenished. Thus, we suggest that in other examples when boldness is assessed by latency to emerge from a shelter, this hiding behaviour could similarly be influenced by a mixture decision-making (based on the perception of risk) and constraints on hiding time.

In studies of aggression and reproductive investment, individuals that show enhanced performance capacity and high fecundity are typically regarded as being of high quality (see [63] for a review) with the implication that they achieve enhanced fitness. In this definition of quality, high levels of boldness in hermit crabs are negatively associated with individual quality. It appears that in hermit crabs, individuals that have invested more in future reproduction by making large spermatophores are the most risk averse. Until their spermatophores are provided to females the pay-off from their investment has not been

secured. This effect, where males that expect high reproductive success in the future take fewer risks, has also been demonstrated in the three spine stickleback, *Gasterosteus aculeatus* [64]. If high quality males do emerge slowly because they are attempting to optimise the benefits of making large spermatophores, it may be their high levels of haemocyanin that facilitate this strategy. Low quality males, on the other hand, may emerge more quickly in an attempt to maximise their access to resources or chance of mating, or because their ability to hide for a prolonged time is constrained by low haemocyanin, or through a combination of these causes. Interestingly, variation in haemocyanin among individuals is thought to be partly driven by exposure to environmental conditions such as food availability and long term exposure to hypoxia [65], which could in turn derive from differences in individual behaviour, such that there is the potential for feedback between haemocyanin and boldness. In this experiment, however, all animals were maintained in identical conditions in the laboratory and there was no difference in haemocyanin concentration between animals from the two populations.

In contrast to the significant mean level effect of spermatophore length, there was no evidence that IIV is linked to spermatophore length or to haemocyanin concentration, even though it showed significant variation among individuals. Previous studies indicate that IIV in hermit crab hiding times varies with ambient risk level [2,8]. We suggested that individuals might have an optimal way of behaving but depart from this at times of high risk [8], in an attempt to reduce predictability (see also [66–68]). Therefore we might also expect IIV to co-vary with individual traits if these traits influence the amount of risk that individual are likely to be exposed to. Perhaps then, the traits measured here do not directly affect risk exposure and IIV co-varies with other traits not analysed in the current study. Although haemocyanin concentration is indicative of high performance, we did not measure metabolic rate directly and IIV might co-vary with metabolic rate since high activity rates are expected to correlate

with risk exposure [21,25,27]. Another possibility is that IIV variation might reflect among individual differences in ability to accurately perceive risk levels [3]. Further studies focussing on how internal state, individual quality and environmental situation interact to influence IIV could help further elucidate these issues. Thus, the data currently available suggest that IIV is sensitive to environmental information [2,8] but not to internal state. This suggests a plastic trait that allows animals to adjust their behaviour in order to cope with heterogeneous environments.

Our results highlight the complexities of unpicking the underlying causes of personality in animals. An overall trend seen across study systems seems to be that high boldness enhances reproductive success at the cost of elevated mortality risk [15], a pattern that clearly matches explanations such as the pace of life syndrome. Here we have uncovered a different pattern where, although investment in aerobic scope and fecundity are positively associated, the more fecund individuals take longer to recover form a startling stimulus compared to low quality individuals. The link between fecundity and startle response duration was very clear and of a similar magnitude to the difference in mean startle responses seen between the two populations. Although aerobic scope might help explain why these highly fecund individuals can hide for longer, this effect was weak by comparison. Thus, there is still the possibility that variation in life style, as well as physical constraints contribute to hiding times. Further analyses integrating fecundity, eventual reproductive success, performance capacity and personality are warranted. We suggest that low boldness might represent a strategy whereby the most fecund individuals attempt to safeguard their investment in future reproduction.

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Table 1: Posterior summary statistics for the mean model.

Parameter name		Mean	SD	95% CI Lower	95% CI Upper	*P*
Intercept	β_1	0.11	0.09	-0.06	0.30	0.14
Crab mass	β_2	-0.04	0.04	-0.13	0.04	0.32
Population	β_3	0.40	0.09	0.22	0.56	<0.0001
Spermatophore length	β_4	0.70	0.08	0.56	0.87	0.08
Spermatophore length^2	B_5	-0.31	0.05	-0.42	-0.20	<0.0001
Haemocyanin concentration	B_6	0.11	0.07	-0.05	0.24	0.14
Haemocyanin concentration^2	B_7	0.00	0.05	-0.09	0.11	0.91
Occasion	B_8	-0.01	0.01	-0.02	0.00	0.11
Crab ID (intercept)	$\tau_{\mu,0}$	0.28	0.03	0.22	0.34	
Crab ID (occasion)	$\tau_{\mu,1}$	0.02	0.01	0.00	0.03	

Mean effect sizes of the standardised factor and covariates with their standard deviations and lower and upper 95% credible intervals.

Table 2: Posterior summary statistics for the standard deviation model, used to assess intra-individual variation (IIV) in startle response durations.

Parameter name		Mean	SD	95% CI Lower	95% CI Upper	*P*
Intercept	γ_1	-2.06	0.14	-2.33	-1.78	<0.0001
Crab mass	γ_2	0.21	0.10	0.02	0.41	0.03
Population	γ_3	0.01	0.20	-0.38	0.40	0.96
Spermatophore length	γ_4	-0.22	0.15	-0.52	0.08	0.17
Haemocyanin concentration	γ_5	0.12	0.15	-0.18	0.42	0.44
Crab ID (intercept)	$\tau_{\sigma,0}$	0.64	0.08	0.50	0.81	

Mean effect sizes of the standardised factor and covariates with their standard deviations and lower and upper 95% credible intervals.

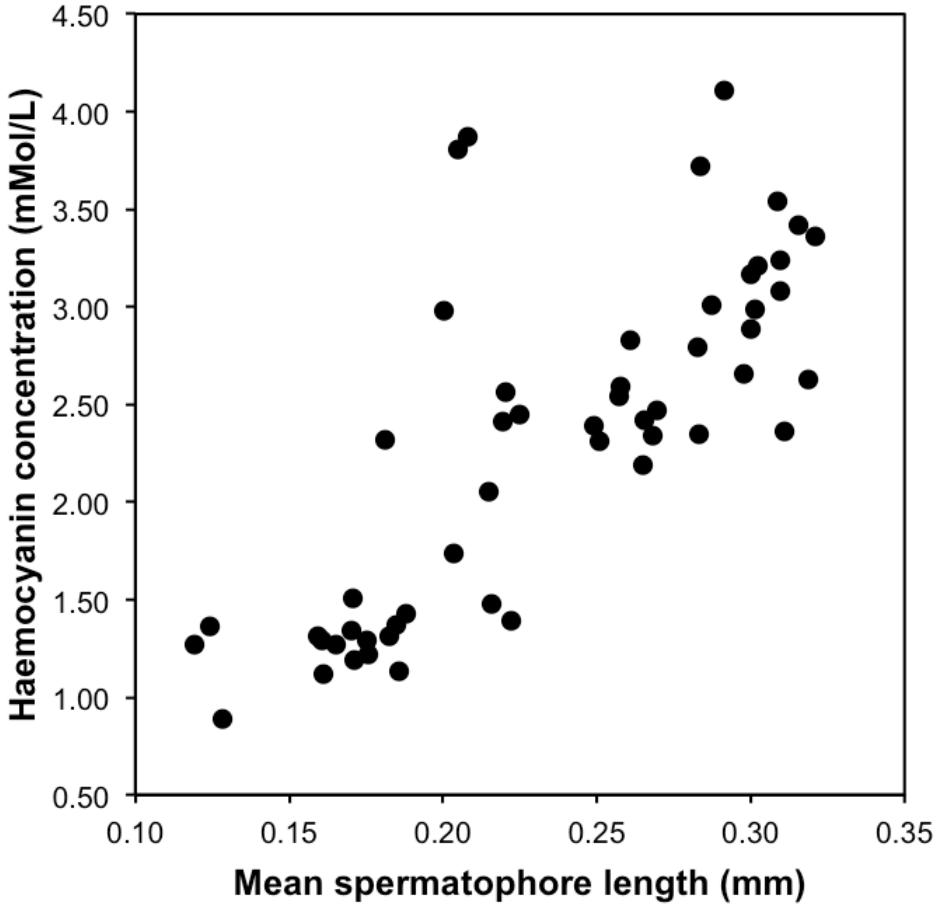

Figure 1: The positive association between mean spermatocphore length and haemocyanin concentration.

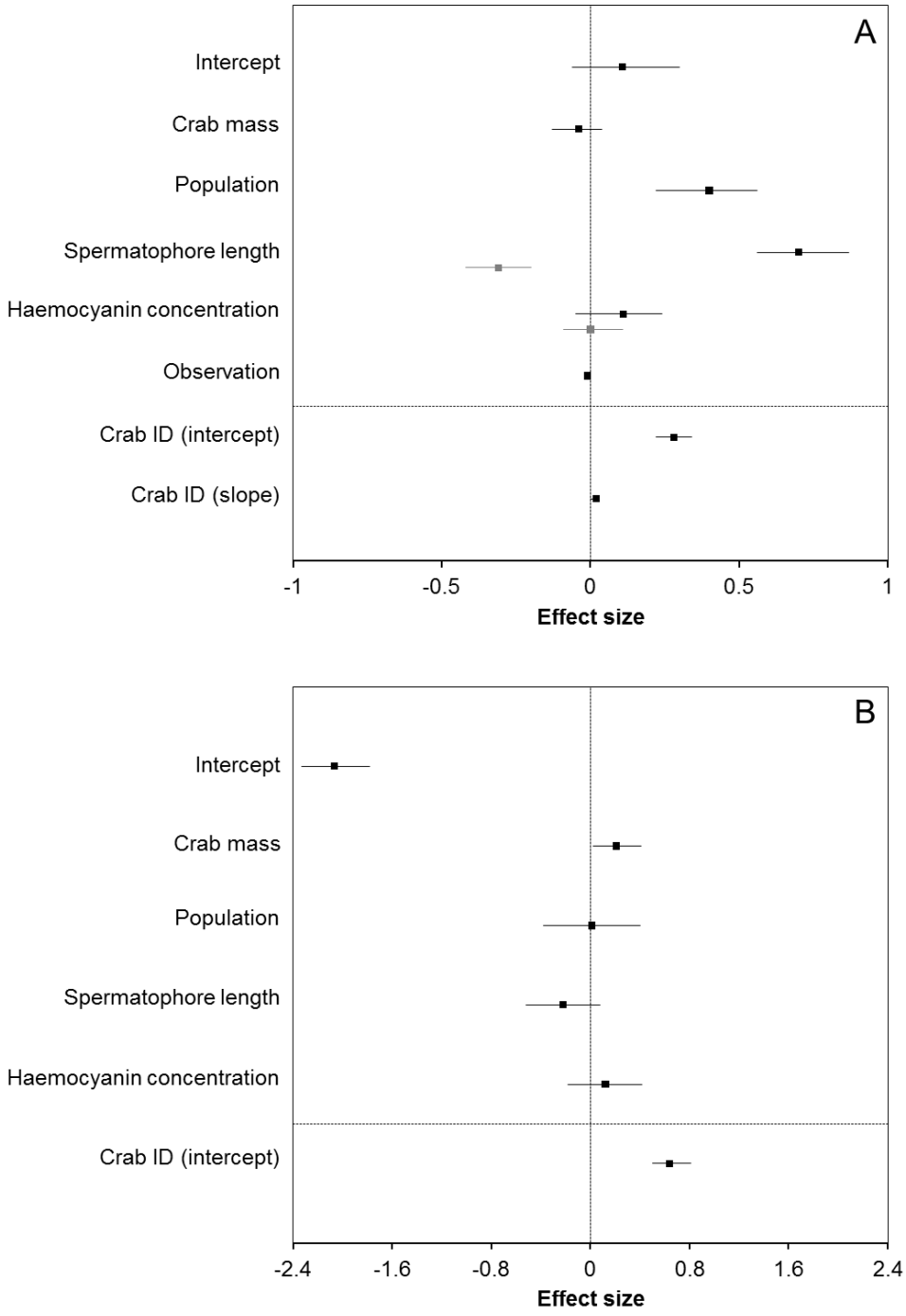

Figure 2: Standardized parameter estimates for the double hierarchical linear mixed model. (A) Results from the mean model assuming quadratic relationships(Equation 3) and (B) for the SD model (Equation 2). For each parameter, the thin lines span the 95% credible interval and the central dot corresponds to the mean. In part A, black dots and lines represent estimates of linear effects and grey dots and lines show quadratic effects.

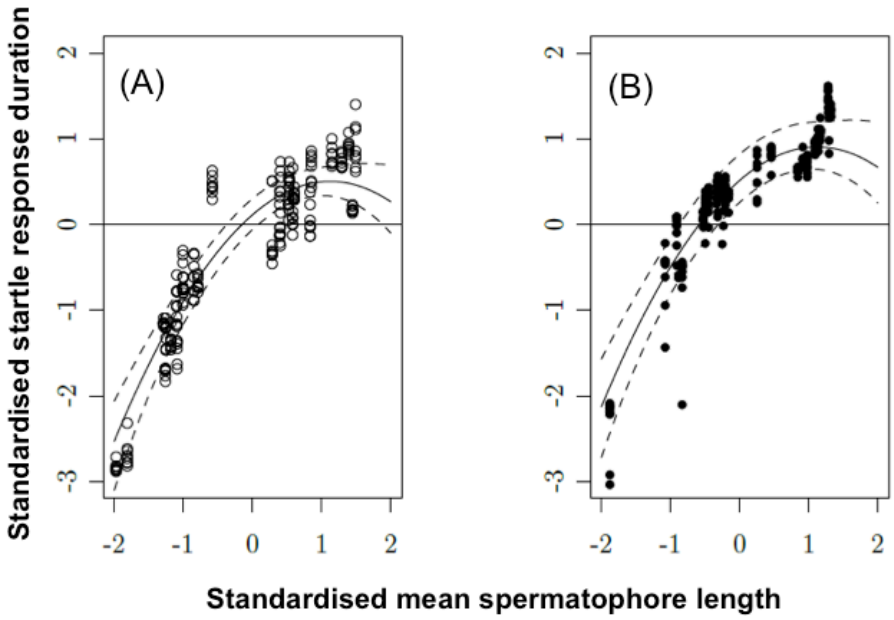

Figure 3: The quadratic relationships fitted to the association between standardised mean spermatophore length and standardised startle response duration, for data from (a) Mount Batten and (b) Hannafore populations.