

2022-06-25

Size specific boldness associated with differences in resource requirements and habitat use: A cross-sectional study in hermit crabs

Briffa, M

<http://hdl.handle.net/10026.1/19358>

10.1093/cz/zoac049

Current Zoology

Oxford University Press (OUP)

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Size specific boldness associated with differences in resource requirements and habitat use: A cross-sectional study in hermit crabs.

Mark Briffa and Rose Archer

School of Biological and Marine Sciences,

University of Plymouth,

Drake Circus,

Plymouth PL4 8AA.

U.K.

Correspondence: mark.briffa@plymouth.ac.uk

Running head: Size specific boldness in hermit crabs

Keywords: hermit crab, boldness, personality, development, resources, habitat

Accepted manuscript. Version of record (open access) at doi: [10.1093/cz/zoac049](https://doi.org/10.1093/cz/zoac049)

Abstract

Animal personality is often studied within compressed periods of observation that represent narrow windows in comparison to animal lifespans. While much is known about the relations between repeatable personality traits and cross-situational behavioural plasticity, less is known about how such traits might differ across age classes or life-history transitions. We conducted a cross-sectional study of startle response duration in three size classes of *Pagurus bernhardus*, the common European hermit crab. We defined size classes using transitions in the preferred species of gastropod shells that accompany growth, and this change in preference is in turn associated with a transition from intertidal to subtidal habitats. Compared to small and medium sized intertidal individuals the larger subtidal hermit crabs behaved cautiously by showing startle responses of greater duration following disturbance. Startle responses were also repeatable within all three size classes, confirming the presence of animal personality in intertidal hermit crabs and demonstrating that this pattern is retained within the largest size classes, that have undergone the transition from intertidal to subtidal habitat. Interestingly, there was a trend for the pattern of repeatable startle response durations to increase with size class, with the highest value for repeatability and greatest range of startle response durations being present within the large subtidal population. The greater range of startle responses indicates that the longer startle response durations in some larger individuals are more likely due to developmental changes with age and habitat use than reflecting selection against the boldest individuals during earlier stages of life.

Introduction

Animal personality is the presence of behavioural repeatability (Nakagawa & Schielzeth 2010), i.e. between-individual behavioural differences that are consistent across repeated sampling occasions (Dall *et al.* 2004, Sih *et al.* 2004, Dingemanse & Réale 2005, Dingemanse *et al.* 2010). Thus, animal personality defined in this way is dependent on two sources of behavioural variation, between individual variance (which increases repeatability) and within individual variance (IIV, also described as residual variance, which decreases repeatability). Animal personality is present in a wide range of animals. Initial studies on chordates (e.g. see Gosling 2001) were soon followed by studies of personality in animals across a range of phyla, including molluscs (e.g. Sinn *et al.* 2008), cnidarians (e.g. Briffa & Greenaway 2011) and arthropods (e.g. Pratt *et al.* 2005; Johnson & Sih, 2007; Reaney & Backwell, 2007; Briffa *et al.* 2008; see Kralj-Fišer & Schuett 2014 for a general review of personality in invertebrates). Typically, the longitudinal data needed for animal personality studies are collected within short windows relative to animal lifespans. This approach is sufficient to investigate repeatability itself (although the number of samples per individual and sampling interval can influence the results, see Bell *et al.* 2009). Such data can also allow investigation of a range of ancillary questions relating to animal personality. These include the interrelations between repeatability and behavioural plasticity (where conditions naturally change in the short term, can be experimentally manipulated, or where individuals can be tracked as they encounter heterogeneous habitats). We might also be interested in the presence of among individual differences in that behavioural plasticity (i.e. differences in behavioural reaction norm or the individual x environment interaction, Dingemanse *et al.* 2010). Finally, longitudinal data can be used to assess the balance between behavioural variation between and within individuals (Stamps *et al.* 2012) and the circumstances where this may change (e.g. Briffa *et al.* 2013, Maskrey *et al.* 2021). In contrast with data collected

in relatively restricted (relative to lifespans) time windows, less attention has been given to investigating animal personality over the longer term, including over developmental timescales and how it might be affected by key life history transitions. Such studies could contribute to our understanding of why animal personality is present (e.g. Stamps & Groothuis 2010, Wilson & Krause 2012). For instance, mean level behavioural differences among cohorts could occur if some behavioural types have enhanced survivorship over others, which are selected against at earlier developmental stages. This scenario underpins the pace of life syndrome (POLS) hypothesis (see Montiglio *et al.* 2018 for an overview), which suggests that animal personalities derive from among individual differences in lifetime fitness strategies. Proactive individuals should show high boldness and prioritise rapid growth, early maturation and early reproduction at the cost of greater risk exposure and reduced survival. In contrast, shy reactive individuals resolve this trade-off through slower growth and delayed reproduction but with enhanced longevity. Alternatively, developmental changes in resource requirements and in prioritising current versus future fitness could also lead to differences in behavioural type among cohorts. In this case, individuals might additionally show different behavioural trajectories during development, as in behavioural developmental reaction norms (i.e. individual \times age interaction effects, encompassing individual \times environment and genotype \times environment effects if different environments are experienced during development), which could then produce different behavioural types within cohorts (see Stamps & Groothuis 2010).

Examples of studies on the development of personality include vertebrates (e.g. see Stamps & Groothuis 2010), molluscs (e.g. Sinn *et al.* 2008, Dahirrel *et al.* 2017) and insects (Amat *et al.* 2018). In the case of insects, the metamorphoses that mark major life history transitions provide clear opportunities for investigation of potential causes and underlying mechanisms of personality (Wilson & Krause 2012). Studies of insects show that behavioural

types and behavioural syndromes can persist across these major life history transitions (Brodin 2009, Gyuris *et al.* 2012, Niemelä *et al.* 2012, Stanley *et al.* 2017) at least in heterometabolous species (but see Amat *et al.* 2018 for a discussion of exceptions). At the same time, average trait values can vary across ontogeny, with adults typically being less bold than larvae (Gyuris *et al.* 2012, Niemelä *et al.* 2012, Stanley *et al.* 2017). Nevertheless, boldness can still change within a life history phase, as in the cricket *Gryllus campestris* where boldness increased during observations over the course of adulthood (ca. 45-50 days) (Fisher *et al.* 2015). In contrast to the situation in insects, little is known about the ontogeny of personality in other arthropods including crustaceans.

As outlined above, when individuals can be followed through ontogeny (e.g. Sinn *et al.* 2008, Fisher *et al.* 2015), this approach can potentially yield rich information, such as the presence of developmental plasticity and among individual differences in developmental reaction norms, as well as signs of niche construction and niche picking (Bergmüller & Taborsky 2010). In many cases, however, there are barriers to collecting longitudinal behavioural data over developmental time-scales, particularly in non-captive animals, such as wild crustaceans. For example, individuals sampled from a wild population may not be amenable to marking and recapture. An alternative approach is to conduct cross-sectional investigations comparing behaviour, already known to be repeatable, across individuals of different life-history stages (Dowling & Godin 2002, Brown & Braithwaite 2004; Magnhagen & Borcharding 2008, Wallis *et al.* 2020). Although such an approach cannot directly address differences in individual developmental trajectories it can show us (a) whether mean level expression of personality traits differ and (b) whether the personality effect size (i.e. repeatability) differs between life stages / cohorts. In human populations for example, behavioural repeatability increases with age because individuals become more predictable (reduced IIV) in their behaviour (Roberts & DeVuccio 2000), whereas in *G. campestris*

repeatability increases with age due to higher among individual variance (Fisher *et al.* 2015). In contrast, in ectothermic vertebrates there is an overall pattern for greater behavioural repeatability in samples of juveniles compared with samples of adults (Bell *et al.* 2009). These examples illustrate the importance of understanding the contribution of population demography in studies of repeatable behaviour. First, if samples of individuals encompass a wide range of size / age classes, then developmental changes in behaviour (if present) could contribute to overall repeatability. Second, one could investigate the potential causes of such differences in personality traits between cohorts. In general, though, studies (longitudinal or cross-sectional) investigating across cohort differences in repeatable behaviour are still relatively rare outside of vertebrates (Stamps & Groothuis 2010, Amat *et al.* 2018).

In hermit crabs, it is not possible to mark and recapture individuals with much success because they are small animals that do not seem to show much fidelity to a particular territory. Intertidal individuals in particular may undergo some spatial redistribution during each tidal cycle. Additionally, they do not possess body parts that can reliably retain marks in the long term, due to periodic moulting of the exoskeleton associated with growth, and marking their gastropod shells is not feasible as they can change shells (see below). Furthermore, it is not possible to age individuals directly because they do not retain hard body parts that can provide an index of age. Nevertheless, adult body size correlates with age (Lancaster 1988) and growth in hermit crabs is associated with stark changes in their natural history. These changes offer an opportunity to study the links between personality and life history transitions additional to those marked by metamorphoses studied in insects. First, there is a change in resource requirements concerning the empty gastropod shells that they occupy and use as portable shelters. As the size limits of a particular shell species are exceeded, there is a change in preference to a larger species of shell. Second, while smaller individuals inhabit the intertidal zone, the largest size class are only found subtidally, a

change which in part is likely to be due to the presence of larger gastropod species and hence their empty shells. In the European hermit crab, *Pagurus bernhardus*, individuals fall into at least three size classes defined by the species of gastropod shell that they preferentially occupy. Small intertidal crabs typically occupy shells of the smooth periwinkle, *Littorina obtusata*, which they will select in preference to other shells of similar size (various species of top shell such as *Steromphala* (= *Gibbula*) *cineraria*, for example). *L. obtusata* shells, however, are not large enough for all intertidal hermit crabs and as growth continues their preferences switch to shells of the common periwinkle, *Littorina littorea*. Subtidal *P. bernhardus* individuals are substantially larger than the two size classes already described. They occupy shells of the common whelk, *Buccinum undatum* a much larger species snail at maturity. Thus, in *P. bernhardus*, changing shell requirements provide a context for investigating differences in repeatable behaviour across an important life-history transition.

When threatened, hermit crabs of all sizes show a startle response of rapidly withdrawing into the shell and the duration until re-emergence can be analysed for variation in mean level responses across conditions and for repeatability (Briffa *et al.* 2008; Briffa & Bibost 2009; Briffa & Twyman 2011; Briffa *et al.* 2013; Bridger *et al.* 2015). Within the two intertidal size classes that have been analysed in previous studies, startle response durations are repeatable, but we have yet to compare boldness across size classes. An increasingly common approach to animal personality studies has been to obtain many repeated observations (i.e. $n > 2$) from each individual such that overall repeatability can be decomposed into the within and between individual variance components mentioned above (e.g. Stamps *et al.* 2012). In the current study, however, our primary aim is simply to determine whether larger (and hence older) individuals differ in mean boldness compared to smaller individuals. We nevertheless observed each individual twice to provide a basic index of repeatability, in order to establish the extent to which boldness is a personality trait in the

hitherto unanalysed subtidal hermit crabs. We thus assess the possibility that personality in hermit crabs varies across a key life-history transition – the changes in resource requirement that occur with increased size and the transition from life in intertidal to subtidal habitats. Studies of other arthropods have shown that boldness often decreases across life history stages. These changes may reflect either decreased survival of bold individuals in early life, or a developmental reduction in boldness, for example due to a reduced need to perform risky behaviours such as dispersal and resource acquisition. In the case of the former we would expect to see both bold and shy individuals at the early life stages but if shyer individuals only appear at later stages this would more likely represent a developmental shift away from boldness. Additionally, a greater diversity of behavioural types across individuals at later stages would indicate the presence of variation in developmental reaction norms. On the other hand, if boldness increases with age this is more likely to represent a developmental shift away from prioritising future survival over current resource acquisition at early life stages.

Materials and Methods

Small individuals occupying *L. obtusata* shells (size class A: 0.08g to 0.32g, mean = 0.219 ±SE = 0.014g) and intermediate size individuals occupying *L. littorina* shells (size class B: 0.41g to 1.78g, mean = 1.121 ±SE = 0.08g) were collected by hand inter-tidally from Hannafore point, Cornwall, UK between September and November 2009. Large subtidal individuals occupying *B. undatum* shells (size class C: 7.12 to 46.4g, mean = 23.05 ±SE = 2.26g) were obtained from trawls of Plymouth Sound, UK also between September and November 2009. Unsurprisingly, there was clear variation in crab mass across the three size classes as defined by the species of shell occupied (1-way ANOVA: $F_{2,47} = 838.1$, $P < 0.0001$), with *post hoc* Tukey tests indicating significant differences between each pair of

adjacent size classes (A versus B, B versus C; $P < 0.0001$ in each case). For each size class, crabs were transported to the lab and housed individually either in 12cm diameter crystalizing dishes (size classes A and B) or in 1L tanks (size class C) containing aerated seawater at 15°C. For all three size classes the individual housing conditions were sufficient for each crab to move around freely without constraint. Each crab was startled using a handling procedure described previously (Briffa *et al.* 2008), causing the crab to tightly withdraw into its shell. Briefly, crabs were lifted out of the seawater by hand and inverted for 5s, which causes them to withdraw into the shell. They were then replaced on the base of the container, with the aperture facing upwards so that their re-emergence could be clearly observed without the observer needing to overshadow the subject. The duration of withdrawal (henceforth startle response duration) was timed until the crabs re-emerged and made first contact with the container base with the walking legs, to the nearest 0.01s. There was no maximum observation time, i.e. no censoring of the data. Startle response durations were obtained from each crab on two occasions, seven days apart. After behavioural data collection was completed, crabs were removed from their shells by crushing in a vice, the shell fragments were dried and weighed to obtain shell mass (SM) and the crab was weighed and sexed. Data from female crabs were not included in the analyses to avoid sex-specific variation in shell optima associated with reproductive state (Elwood *et al.* 1979), which can affect startle response durations (Briffa & Bibost 2009). We used the mass of each male crab to estimate its optimal shell mass (OSM) using regression lines from previous shell selection studies (Briffa & Elwood 2005, 2007), and calculated the proportional deviation from this (DOSM) for each crab's actual shell mass (SM) as follows:

$$DOSM = \left(\frac{|OSM - SM|}{OSM} \right)$$

Since deviation from optimal shell mass might affect startle response duration (since it will determine the amount of protection afforded by retreating into the shell), DOSM was included as a covariate in the analysis. However, DOSM did not vary among size classes ($F_{2,47} = 2.1$, $P = 0.14$). We obtained two startle responses from the following number of males in each size class: A, $n = 17$; B, $n = 17$; C, $n = 16$, producing 100 observations across 50 individuals. Sample sizes for classes A and B were chosen to match the number of individuals in size class C that were obtained from the trawl (+1 in each case to bring N up to 50 individuals). At the end of the experiment all male crabs were supplied with a new shell and returned to the sea.

In order to determine the effect of size class on startle response duration, we used a linear mixed effects model, estimated by restricted maximum likelihood (REML). The fixed predictors were size class (A, B or C), occasion (startle response 1 or 2) and proportional deviation from mass-specific optimal shell mass. We allowed individual specific random intercepts but did not attempt to fit random slopes across occasions as there were only two occasions. Initial analyses indicated that there was no interaction between size class and occasion so these were omitted from the final model (model 1). We also found that Log_{10} transforming the startle response data improved the model fit, moving the normality of residuals and the homogeneity of variance in residuals across individuals closer to the underlying model assumptions (see Schielzeth *et al.* 2020). We confirmed that the single random effect in the model was justified by re-fitting the model using maximum likelihood estimation (ML) and comparing this refitted model to a linear model that contained only fixed effects. Significance testing for fixed effects was then assessed via F -ratios, using Satterthwaite's method to calculate the degrees of freedom. We further investigated the among-individual differences accounted for in the random intercept by calculating the repeatability of startle responses, for all data combined and for each size class separately.

Analyses were performed in R 3.6.1 (R Core Team 2019) using the packages lme4 (Bates *et al.* 2015), lmerTest (Kuznetsova *et al.* 2017) and rptR (Stoffel *et al.* 2017). Model code is provided in Appendix 1.

Results

Startle responses were unaffected by deviation from optimal shell size ($F_{1,46} = 0.01$, $P = 0.9$) but were greater on occasion 2 compared with occasion 1 ($F_{1,49} = 12.5$, $P < 0.0001$) and in large crabs in size class C compared with smaller crabs in size classes A and B ($F_{2,46} = 4.9$, $P = 0.012$) (Figure 1A, B, C; See table 1 for the model parameters). Post-hoc tests show that duration was not different between size classes A and B ($P = 0.93$) but for crabs in class C the duration was greater than for crabs in classes B ($P = 0.03$) and A ($P = 0.015$). While dividing crab masses into discrete size classes is an intuitive approach given the natural history of hermit crabs an alternative way of analysing the data would be to treat crab mass as a continuous predictor (Figure 1D). This approach yielded similar results, reported in Appendix 2 (model 2). It was evident (see Figures 1 and 2) that eight outlying data points were present in the data. To check that the patterns reported above were not driven by these observations, both models (1 and 2) were re-run with these data omitted, which returned qualitatively identical results (see Appendix 2). While it is clear that startle response duration varies with crab mass it is unclear whether this represents a discontinuous pattern of variation between size classes (i.e. Figure 1, A – C) or a pattern of continuous variation over the range of crab masses (Figure 1 D). In the latter case we would expect to see a significant positive slopes within each size class so to test this possibility model 2 was re-run separately for each size class. There was no correlation between mass and startle response duration for size classes A and C (A; $\beta = -0.12$, $F_{1,14} = 0.0008$, $P = 0.92$, C ; $\beta = -0.01$, $F_{1,13} = 0.54$, $P = 0.47$) but within size class B a positive correlation was present ($\beta = 0.74$, $F_{1,13} = 5.87$, $P = 0.03$; Figure 2) .

The random intercepts model where size class was used as a categorical predictor (model 1) provided a better fit with the data than an equivalent general linear model that lacked random intercepts (see Appendix 1.2), indicating significant among individual differences in startle response duration ($\chi^2_1 = 28.24$, $P < 0.0001$; Figure 1A, B, C). To quantify these differences we calculated the repeatability of startle responses across the two observations, adjusted on proportional deviation from optimal shell size. For all size classes combined startle responses were highly repeatable ($R = 0.61$, 95% CIs = [0.42, 0.77], $P < 0.0001$). When repeatability was estimated specifically for each size class, there was a trend for much greater repeatability in the largest size class C compared with A and B on the basis of non-overlapping 80% confidence intervals. However there was no significant repeatability difference on the basis of 95% confidence intervals (A: $R = 0.40$, 95% CIs = [<0.01 , 0.725], 80% CIs = [0.088, 0.625]; B: $R = 0.41$, 95% CIs = [<0.01 , 0.73], 80% CIs = [0.142, 0.676] ; C: $R = 0.88$, 95% CIs = [0.70, 0.96], 80% CIs = [0.788, 0.942]; $P < 0.0001$ in each case. Note that for size classes A and B, the lower 95% CIs are constrained not to cross zero).

Table 1: Variance and parameter estimates for the mixed effects model of startle response durations.

Random intercept	Variance	SD
ID	0.10974	0.3313
Residual	0.05297	0.2302
Fixed effects	Estimate	SE
(Intercept)	0.84295	0.11381
Size class B	0.04503	0.12662
Size class C	0.38646	0.13313
Occasion 2	0.16303	0.04603
DOSM	0.02646	0.28526

Discussion

As in previous studies of hermit crabs, consistent between-individual variation in startle response duration was present. Here we found that as well as being present in the previously studied intertidal samples of hermit crabs, animal personality was also present in the sample of the larger-sized individuals of the subtidal population. Indeed the greatest repeatability value was found for those larger crabs in comparison with the smaller size classes. In addition to significant repeatability within each size class, we also found mean level differences in startle response duration between size classes. Size specific mean level boldness is seen in a range of study species, but the direction of the effect differs between examples. In several studies on insects (Gyuris *et al.* 2012, Niemelä *et al.* 2012, Stanley *et al.* 2017) and in gastropods (Dahirel *et al.* 2017) younger individuals were bolder than older ones, similar to the current results, but results in other taxa are more mixed. In killifish, *Fundulus diaphanous*, small individuals show the longest startle responses, possibly behaving cautiously because they are easy for predators to handle and therefore subject to high predation risk (Dowling & Godin 2002). In other examples, such as the poeciliid fish *Brachyrhaphis episcopi* (Brown & Braithwaite 2004) and perch *Perca fluviatilis* (Magnhagen & Borcherdig 2008) it was the larger individuals that behaved more cautiously. In these vertebrate examples, boldness varies continuously with body size but the pattern of variation in the hermit crabs studied here was different. Only intermediate sized crabs in *L. littorea* shells showed continuous variation in startle response with mass but there were also clear differences across size categories, with the small and intermediate sized intertidal individuals being bolder on average (shorter startle response durations) than the large subtidal individuals in *B. undatum* shells.

For all three size-classes, startle responses increased, on average, between occasions one and two. This may represent a mean level pattern of sensitisation to the startle procedure.

In previous studies we found an approximately equal number of individuals that increased and decreased their startle response duration over repeated observations (see Stamps *et al.* 2012) but the current sample contained more individuals that increased the duration (see Figure 1). The differences in startle response between size classes were unlikely to have been influenced by occasion, however, or by physical constraints resulting from different shell morphologies. Responses were timed up to the point where the crab first contacted the substrate with its walking legs in order to make a righting attempt, rather than until the crab successfully righted its position. A previous study in which shell size was manipulated to be much too small (Briffa & Bibost 2009) demonstrated that the size of the gastropod shell relative to the size of the crab can influence startle response durations. In contrast, adequacy was far less variable in the present study based on naturally occupied shells, and there was no difference in POSM between size classes and no effect of POSM on startle response durations. There are two general explanations remaining for the longer startle responses in larger crabs. First, the pace of life syndrome hypothesis (POLS) implies that declining boldness with age would arise because bolder individuals with short startle responses may have lower early survival such that fewer of them attain large size. Second, there might be a developmental shift in boldness, with crabs becoming less bold with age. While it is not possible to directly distinguish between contributions from these two processes using the current cross-sectional data, the following can be noted: Short startle responses (i.e. high boldness) are still present in size class C, even though the mean duration is high. Furthermore, all of the startle responses in size class A are at the lower end of range seen across the whole dataset, whereas the longest startle responses are present in size class C. These observations seem less compatible with the first explanation, leaving the possibility of developmental change in startle response duration open for further investigation.

There are a number of reasons why, for hermit crabs, behaving more cautiously as size /age increases, could be beneficial. First, the shift in boldness could be due to differences between the intertidal and subtidal habitats. In the intertidal, hermit crabs frequently utilise cover from seaweed at the fringes of tide pools, in crevices and underneath loose stones. Sandy bottomed subtidal areas often lack such cover and general rugosity, perhaps elevating the level of predation risk (e.g. from predatory crustaceans or fishes) that hermit crabs are exposed to. Second, throughout growth hermit crabs must obtain new gastropod shells of increasing size, to maintain adequate protection. Those hermit crabs that have reached the largest size class however are less likely to require a change of shell, and larger shells are in any case less available as they already occupy shells at the upper range of available sizes. Thus, they will not need to allocate as much time to prospecting for new shells compared with smaller individuals. Given that boldness as measured with startle responses correlates with shell investigation behaviour (Mowles *et al.* 2012) it is likely that the chance of obtaining new shells correlates with boldness. Hence, the benefits of short startle responses would decline with crab size and age, such that an age related decline in boldness would be adaptive. Although the shift in boldness would be associated with access to a resource this possibility seems analogous to other situations where the requirement to perform risky behaviours such as dispersal declines with age.

The mechanisms underlying developmental personality change also warrant further investigation. One possibility is that developmental change occurs as a result of social learning from observing the behaviour of others. Social learning has not been directly demonstrated in hermit crabs but several studies have shown that isolation leads to changes in social behaviour in other crustaceans (e.g. Hemsworth *et al.* 2007, Duffield *et al.* 2015). If present, social learning could produce an increase in mean startle responses, if most individuals learned to act cautiously. In the current data we found (albeit tentatively) greater

repeatability within the largest size class. Although it is not possible to formally decompose variance components in the current data, the greater spread of startle responses in group C suggests that greater among individual variance might contribute to increased repeatability in older individuals, similar to the case of *G.campestris* crickets, although boldness increased rather than decreased across adulthood in that example (Fisher *et al.* 2015). This wider range of startle response durations compared to the smaller intertidal crabs, might reflect differences in experience and the opportunity to learn among individuals (e.g. see Frost *et al.* 2007). Alternatively, development of differences in behaviour might be driven by different experiences of social conflict (Bergmüller & Taborsky 2010). In animals that frequently engage in aggression (e.g. over gastropod shells in the case of hermit crabs), winner and loser effects could result in a form of niche specialization associated with distinct behavioural strategies. Indeed, startle responses in *P. bernhardus* can increase after fighting in individuals that have defended their gastropod shell from an attacker (Courtene Jones & Briffa 2014). Boldness and variation in boldness could also change during development independently of social experiences, as a result of other experiential differences and differences in developmental reaction norms (e.g. Stamps & Groothuis 2010; Curley *et al.* 2013).

The pattern of increasing caution with size indicates the possibility of developmental changes in boldness in hermit crabs. The primary cause of this mean shift in boldness does not appear to be reduced survivorship in less bold individuals. The decline in boldness indicates that being more risk averse with increasing size class and age is beneficial, either because of higher risk levels in the subtidal environment or because the benefits of behaving in a risk prone way reduce with age. The proximate causes of this change could involve processes such as social learning and social conflict but these questions still need to be resolved. Lifetime studies on dumpling squid, *Euprymna tasmanica*, show that size specific boldness can be plastic and vary with habitat as well as developmental stage (Sinn *et al.*

2008). Lifetime studies on hermit crabs would most likely need to be conducted in the laboratory due to the difficulties of long-term marking and recapturing crustaceans. Here, however, we show that the expression of a personality trait in a natural population of arthropods varies across size classes and appears to be subject to developmental change associated with a key life-history transition between habitats and associated resource requirements.

Acknowledgements

We are grateful to two anonymous reviewers for their insightful comments, which have helped us to improve the manuscript.

References

- Amat I, Desouhant E, Gomes E, Moreau J, Monceau K, 2018. Insect personality: what can we learn from metamorphosis? *Curr Opin Insect Sci* **27**: 46-51.
- Bates D, Maechler M, Bolker B, Walker S, 2015. Fitting linear mixed-effects models using lme4. *J Stat Software* **67**: 1-48.
- Bell AM, Hankison SJ, Laskowski KL, 2009. The repeatability of behaviour: a meta-analysis. *Anim Behav*, **77**: 771-783.
- Bergmüller R, Taborsky M, 2010. Animal personality due to social niche specialisation. *Trends Ecol Evol* **25**: 504–511.
- Bridger D, Bonner SJ, Briffa M, 2015. Individual quality and personality: bolder males are less fecund in the hermit crab *Pagurus bernhardus*. *Proc R Soc B* **282**: 20142492.

- Briffa M, Bibost A-L, 2009. Effects of shell size on behavioural consistency and flexibility in hermit crabs. *Can J Zool* **87**: 597-603.
- Briffa M, Bridger D, Biro PA, 2013. How does temperature affect behaviour? Multilevel analysis of plasticity, personality and predictability in hermit crabs. *Anim Behav* **86**: 47-54.
- Briffa M, Elwood RW, 2005. Metabolic consequences of shell choice in *Pagurus bernhardus*: do hermit crabs prefer cryptic or portable shells? *Behav Ecol Sociobiol* **59**: 143-148.
- Briffa M, Elwood RW, 2007. Monoamines and decision making during contests in the hermit crab *Pagurus bernhardus*. *Anim Behav* **73**: 605-612.
- Briffa M, Greenaway J, 2011. High In Situ Repeatability of Behaviour Indicates Animal Personality in the Beadlet Anemone *Actinia equina* (Cnidaria). *PLoS ONE* **6**: e21963.
- Briffa M, Rundle SD, Fryer A, 2008. Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proc R Soc B* **275**: 1305-1311.
- Briffa M, Twyman C, 2011. Do I stand out or blend in? Conspicuousness awareness and consistent behavioural differences in hermit crabs. *Biol Letts* **7**: 330-332.
- Brodin, T, 2009. Behavioral syndrome over the boundaries of life—carryovers from larvae to adult damselfly. *Behav Ecol* **20**: 30-37.
- Brown C, Braithwaite VA, 2004. Size matters: a test of boldness in eight populations of bishop, *Brachyrhaphis episcopi*. *Anim Behav* **68**: 1325-1329.
- Courtene-Jones W, Briffa M, 2014. Boldness and asymmetric contests: role- and outcome-dependent effects of fighting in hermit crabs. *Behav Ecol* **25**: 1073-1082.

- Curley JP, Branchi I, 2013. Ontogeny of stable individual differences; gene, environment and epigenetic mechanisms. In: Carere C, Maestriperi D, editors. *Animal Personalities; Behaviour, Physiology and Evolution*. Chicago: University of Chicago Press.
- Dahirel M, Vong A, Ansart A, Madec L, 2017. Individual boldness is life stage-dependent and linked to dispersal in a hermaphrodite land snail. *Ecol Res* **32**: 751-755.
- Dall SRX, Houston AI, McNamara JM, 2004. The behavioural ecology of personality: Consistent individual differences from an adaptive perspective. *Ecol Letts* **7**: 734-739.
- Dingemanse NJ, Kazem AJ, Réale D, Wright J, 2010. Behavioural reaction norms: animal personality meets individual plasticity. *Trends Ecol Evo.* **25**: 81–89.
- Dingemanse NJ, Réale D, 2005. Natural selection and animal personality. *Behaviour* **142**: 1165-1190.
- Dowling LM, Godin JGJ, 2002. Refuge use in a killifish: influence of body size and nutritional state. *Can J Zool* **4**: 782-788.
- Duffield C, Wilson AJ, Thornton A 2015. Desperate prawns: Drivers of behavioural innovation vary across social contexts in rock pool crustaceans. *PLoS ONE* **10**: e0139050.
- Elwood RW, McClean A, Webb L, 1979. The development of shell preferences by the hermit crab *Pagurus bernhardus*. *Anim Behav* **27**: 940-946.
- Fisher DN, David M, Tregenza T, Rodríguez-Muñoz R, 2015. Dynamics of among-individual behavioral variation over adult lifespan in a wild insect. *Behav Ecol*, **26**: 975-985.
- Frost AJ, Winrow-Giffen A, Ashley PJ, Sneddon LU, 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proc R Soc Lond B* **274**: 333–339.

- Gosling S, 2001. From mice to men: what we can learn about personality from animal research. *Psychol Bull* **127**: 45–86.
- Gyuris E, Feró O, Barta Z, 2012. Personality traits across ontogeny in firebugs, *Pyrrhocoris apterus*. *Anim Behav* **84**: 103-109.
- Hemsworth R, Villareal W, Patullo BW, MacMillan DL, 2007. Crustacean social behavioral changes in response to isolation. *Biol Bull* **213**: 187-195.
- Johnson JC, Sih A, 2007. Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, *Dolomedes triton*. *Anim Behav*, **74**: 1131-1138.
- Kuznetsova A, Brockhoff PB, Christensen RHB, 2017. lmerTest Package: Tests in linear mixed effects models. *J Stat Soft* **82**: 1-26.
- Kralj-Fišer S, Schuett W, 2014. Studying personality variation in invertebrates: why bother? *Anim Behav* **91**: 41-52.
- Lancaster I, 1988. *Pagurus bernhardus* (L.) – An introduction to the natural history of hermit crabs. *Field Stud* **7**: 189-238.
- Magnhagen C, Borcherdig J, 2008. Risk-taking behaviour in foraging perch: does predation pressure influence age-specific boldness? *Anim Behav* **75**: 509-517.
- Maskrey DK, Sneddon LU, Arnold KE, Wolfenden DCC, Thomson JS, 2021. Temperature-driven changes in behavioural unpredictability and personality in the beadlet sea anemone, *Actinia equina*. *Anim Behav* **181**: 13-27.
- Montiglio PO, Dammhahn M, Dubuc Messier G, Réale D, 2018. The pace-of-life syndrome revisited: the role of ecological conditions and natural history on the slow-fast continuum. *Behav Ecol Sociobiol* **72**: 116.

- Mowles SL, Cotton PA, Briffa M, 2012. Consistent crustaceans: the identification of stable behavioural syndromes in hermit crabs. *Behav Ecol Sociobiol* **66**: 1087–1094.
- Nakagawa S, Schielzeth H, 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev* **85**: 935–956.
- Niemelä PT, Vainikka A, Hedrick AV, Kortet R, 2012. Integrating behaviour with life history: boldness of the field cricket, *Gryllus integer*, during ontogeny. *Funct Ecol*, **26**: 450-456.
- Pratt AE, McLain DK, Berry AS, 2005. Variation in the boldness of courting sand fiddler crabs (*Uca pugilator*). *Ethology* **111**: 63-76.
- R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Reaney LT, Backwell PRY, 2007. Risk-taking behavior predicts aggression and mating success in a fiddler crab. *Behav Ecol* **18**: 521-525.
- Roberts BW, DelVecchio WF, 2000. The rank-order consistency of personality traits from childhood to old age: A quantitative review of longitudinal studies. *Psychol Bull* **126**: 3-25.
- Schielzeth H, Dingemanse NJ, Nakagawa S, Westneat DF, Allogue C, Teplitsky C, Réale D, Dochtermann NA, Zsolt Garamszegi L, Araya-Ajoy YG, 2020. Robustness of linear mixed-effects models to violations of distributional assumptions. *Methods Ecol Evol* **11**: 1141– 1152.
- Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends Ecol Evol* **19**: 372-378.

- Sinn DL, Gosling SD, Moltschaniwskyj NA, 2008. Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Anim. Behav* 75: 433-442.
- Stamps JA, Briffa M, Biro PA, 2012. Unpredictable animals: individual differences in intraindividual variability (IIV). *Anim Behav* **83**: 1325-1334.
- Stamps JA, Groothuis TGG, 2010. The development of animal personality: relevance, concepts and perspectives. *Biol Rev* **85**: 301–325.
- Stanley CR, Mettke-Hofmann C, Preziosi RF 2017. Personality in the cockroach *Diploptera punctata*: evidence for stability across developmental stages despite age effects on boldness *PLoS ONE*, **12**: 1-23.
- Stoffel MA, Nakagawa S, Schielzeth H, 2017. rptR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol Evol.* **8**: 1639-1644.
- Wallis LJ, Szabó D, Kubinyi E, 2020. Cross-sectional age differences in canine personality traits; Influence of breed, sex, previous trauma, and dog obedience tasks. *Front Vet Sci* **6**: 493.
- Wilson ADM, Krause J, 2012. Metamorphosis and animal personality: a neglected opportunity. *Trends Ecol Evol* **27**: 529-531.

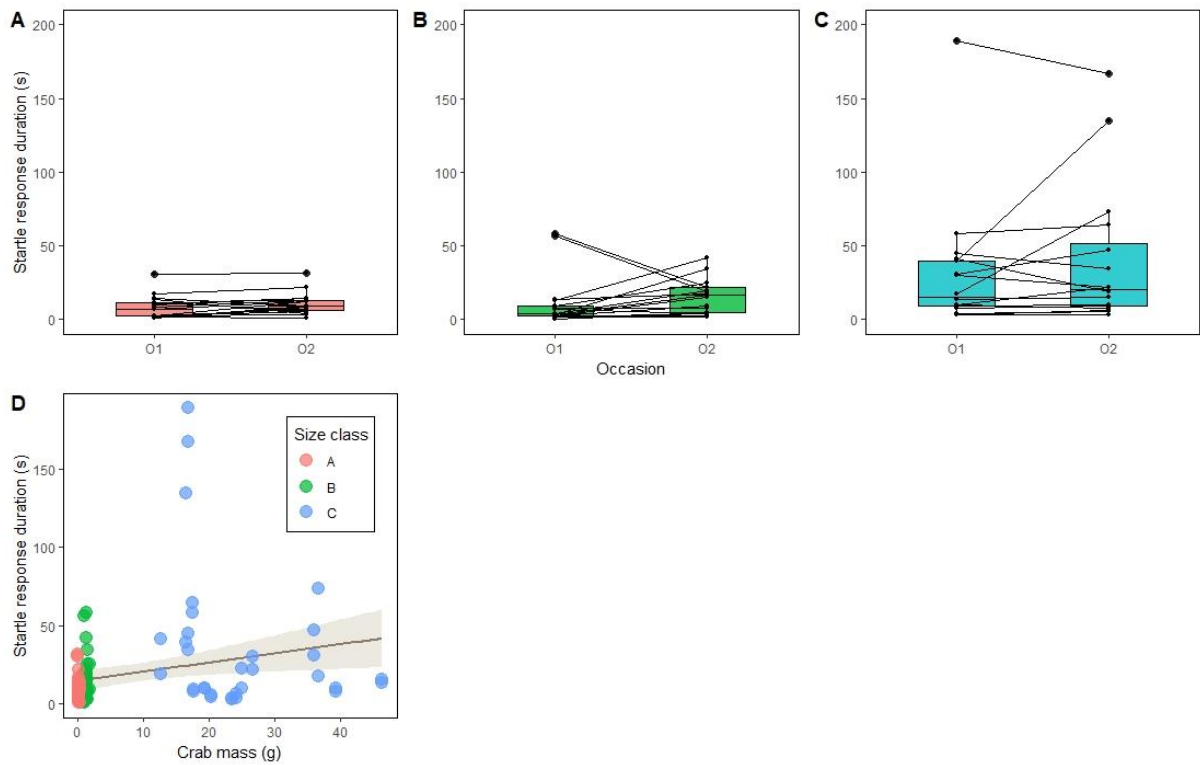


Figure 1: Startle responses in occasion one and two for different sized hermit crabs. Box and whisker plots for size classes A ($n = 17$), B ($n = 17$) and C ($n = 16$), based on species of occupied gastropod shell (*Littorina obtusata*, *L. littorea* and *Buccinum undatum* respectively). Within each plot startle responses durations are indicated by black dots and the two responses from each individual are linked by black lines. Larger black dots denote outlying data points that were excluded from the supplementary re-analysis (see Appendix 2 for details). Part D shows the same startle responses plotted against crab mass ($N = 50$) with a fitted regression line (shaded area shows the standard error of the regression).

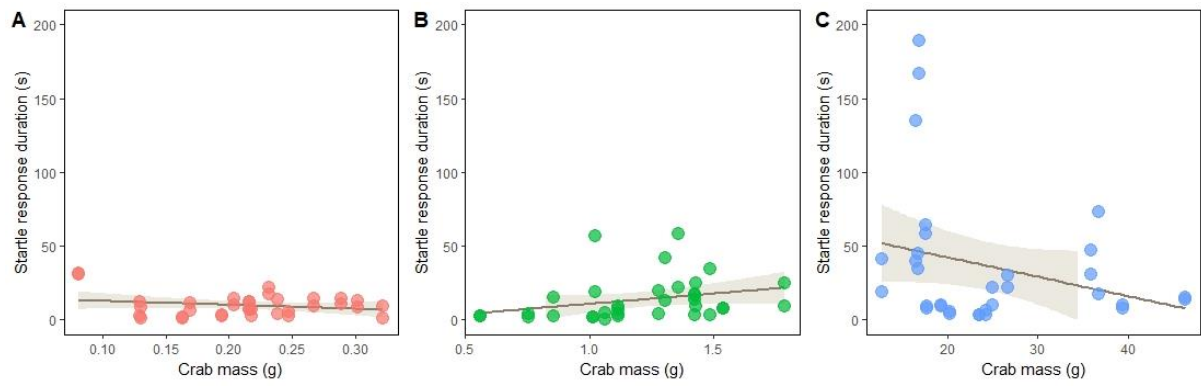


Figure 2: Intra-size class correlations between crab mass and startle response durations, for size classes A ($n = 17$), B ($n = 17$) and C ($n = 16$). Regression lines fitted for illustration (shaded area shows the standard error of the regression).

Appendix 1: R code

Variable codes

ID = hermit crab ID

Class = size class

Mass = crab mass

Occ = occasion

DOSM = deviation from optimal shell mass

Dur = startle response duration (s)

Packages

lme4, lmerTest, emmeans

1.1 R code for LMM using size class as a categorical predictor

```
model.1 <- lmer(log10(1+Dur) ~ Class + Occ + DOSM + (1|ID))

summary(model.1) #parameters
anova(model.1) #stats tests
emmeans(model.1, list(pairwise ~ Class), adjust = "tukey") #posthocs
```

1.2 R code for LMM using a crab mass as a continuous predictor

```
model.2 <- lmer(log10(1+Dur) ~ Mass + Occ + DOSM + (1|ID))
```

Further versions of this model were used to obtain estimates for the correlation between crab mass and duration within each specific size class.

```
A.data <- data [which(Class=='A'), ]
```

```
B.data <- data [which(Class=='B'), ]
```

```
C.data <- data [which(Class=='C'), ]
```

```
m2A<-lmer(log10(1+Dur) ~ Mass + Occ + DOSM + (1|ID), data= A.data)
```

```
m2B<-lmer(log10(1+Dur) ~ Mass + Occ + DOSM + (1|ID), data= B.data)
```

```
m2C<-lmer(log10(1+Dur) ~ Mass + Occ + DOSM + (1|ID), data= C.data)
```

1.2 R code for testing the random effects part of model 1

```
model.1.ML <-lmer(log10(1+Dur) ~ Class + Occ + DOSM + (1|ID), REML = F)
```

```
model.1.FEO <-lm(log10(1+Dur) ~ Class + Occ + DOSM)
```

```
anova(model1.ML, model1.FEO)
```

1.3 R code for estimating repeatability

```
A.data <- data [which(Class=='A'), ]
```

```
B.data <- data [which(Class=='B'), ]
```

```
C.data <- data [which(Class=='C'), ]
```

```
#All data
```

```
rep.All<-rpt(log10(1+Dur) ~ DOSM + (1 | ID), grname = "ID", data = data, datatype =  
"Gaussian", nboot = 1000, npermut = 0)
```

```
#A
```

```
rep.A <-rpt(log10(1+Dur) ~ DOSM + (1 | ID), grname = "ID", data = A.data, datatype =  
"Gaussian", nboot = 1000, npermut = 0)
```

```
#B
```

```
rep.B <-rpt(log10(1+Dur) ~ DOSM + (1 | ID), grname = "ID", data = B.data, datatype =
"Gaussian", nboot = 1000, npermut = 0)
```

```
#C
```

```
rep.C <-rpt(log10(1+Dur) ~ DOSM + (1 | ID), grname = "ID", data = C.data, datatype =
"Gaussian", nboot = 1000, npermut = 0)
```

```
#To change the CIs from the 95% default to 80%, add CI = 0.8 to the formula
```

Appendix 2: Results of supplementary analyses

Table A2.1 Model using mass as a continuous predictor. The second and third columns show variance and parameter estimates, the remaining columns report the results of significance testing.

Random intercept	Variance	SD	χ^2	df	P
ID	0.11812	0.3437	30.95	1	<0.0001
Residual	0.05297	0.2302			
Fixed effects	Estimate	SE	F	df	P
(Intercept)	0.85934	0.09944			
Mass	0.01041	0.00440	5.60	1,47	0.022
Occasion			12.54	1,49	0.0009
Occasion 2	0.16303	0.04603			
DOSM	0.15442	0.28674	0.29	1,47	0.60

Table A2.2 Model using size class as a categorical predictor omitting outliers. The second and third columns show variance and parameter estimates, the remaining columns report the results of significance testing

Random intercept	Variance	SD	χ^2	df	P
ID	0.003233	0.05686	14.8	1	<0.0002
Residual	0.002570	0.05070			
Fixed effects	Estimate	SE	F	df	P
(Intercept)	0.238685	0.021360			
Size class			3.8484	2, 43.5	0.03
Size class B	0.003886	0.023609			
Size class C	0.064200	0.025659			
Occasion			17.3	1, 44.9	<0.0002
Occasion 2	0.044161	0.010617			
DOSM	0.051625	0.054473	0.90	1, 43.1	0.35

Table A2.3 Model using mass as a continuous predictor omitting outliers. The second and third columns show variance and parameter estimates, the remaining columns report the results of significance testing

Random intercept	Variance	SD	χ^2	df	P
ID	0.003214	0.05670	1	14.9	<0.0002
Residual	0.002581	0.05081			
Fixed effects	Estimate	SE	F	df	P
(Intercept)	0.236	0.018			
Mass	0.002	0.0007	6.65	1, 43.5	0.013
Occasion			17.1	1,44.6	<0.0002
Occasion 2	0.044	0.011			
DOSM	0.074	0.052	1,43.8	2.02	0.16