Evolutionary Ecology Research, 2016, **17**: 279–290

Knights in shining armour are not necessarily bold: defensive morphology correlates negatively with boldness, but positively with activity, in wild threespine stickleback, *Gasterosteus aculeatus*

Gunnar De Winter[1], Henrique Ramalho Martins[1,2],
Rafael Arnoni Trovo[1,3] and Ben B. Chapman[1]

[1]*Ecology and Evolution Group, School of Life Sciences, University of Nottingham, Nottingham, UK,*
[2]*Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
and [3]*Instituto de Biociências, University of São Paulo, São Paulo, Brazil*

ABSTRACT

Background: The threespine stickleback (*Gasterosteus aculeatus*) varies in armour phenotype from fully armoured to non-armoured morphs.

Aim: Investigate the connection between individual variation in behaviour and armour morphology within and across populations.

Study organism and site: During the Spring of 2015, we assayed the behaviour of 250 sticklebacks from seven populations on the Scottish island of North Uist, located in the Outer Hebrides.

Hypotheses: The 'phenotypic compensation' hypothesis holds that individuals with poorer defences should behave more timidly. Yet the energetic costs associated with bearing armour should reduce both the activity and exploration behaviour of more heavily armoured individuals.

Methods: We assayed individual stickleback for boldness, exploration, and activity. We then stained the fish with Alizarin red, which allowed the characterization of the armour phenotype of each fish.

Results: Across populations, bold fish were less armoured than their shy counterparts and active fish were more armoured. Exploratory behaviour was not correlated with armour phenotype.

Keywords: activity, armour, behaviour, boldness, exploration, *Gasterosteus aculeatus*, threespine stickleback.

Correspondence: G. De Winter, Ecology and Evolution Group, School of Life Sciences, University of Nottingham, Nottingham NG7 2UH, UK. e-mail: mbxgd@nottingham.ac.uk

INTRODUCTION

Recent research has highlighted the existence of individual behavioural consistency across a wide range of traits such as boldness (Brown and Braithwaite, 2004; Ward *et al.*, 2004; Chapman *et al.*, 2011a), exploration (Fraser *et al.*, 2001; Dingemanse *et al.*, 2002; Careau *et al.*, 2011), and mating behaviour (Chapman *et al.*, 2009). This has been observed in a wide variety of taxa, including mammals (Réale *et al.*, 2000; English *et al.*, 2010; Careau *et al.*, 2011), birds (Dingemanse *et al.*, 2002; Foltz *et al.*, 2015; McCowan *et al.*, 2015), fish (Brown and Braithwaite, 2004; Ward *et al.*, 2004; Chapman *et al.*, 2011a), and invertebrates (Chapman *et al.*, 2011b; Grinsted *et al.*, 2013; Planas-Sitjà *et al.*, 2015). This phenomenon, known as 'animal personality' (Dall *et al.*, 2004; Dingemanse and Réale, 2005), is perhaps surprising given that behavioural plasticity is beneficial to maximize individual fitness in the heterogeneous environments that most organisms experience during their lifetimes (Dingemanse and Réale, 2005). Hence a key question in contemporary behavioural and evolutionary biology is to address the existence and prevalence of animal personality within wild populations of varying ecology (Dingemanse and Réale, 2005).

Many models of animal personality are derived from the link between behaviour and state, where the concept of state refers to the various features of an animal that are relevant factors in behavioural decisions that affect fitness. One of these features is morphology (Wolf and Weissing, 2010). If morphology contributes to an animal's state, which, in turn, affects behaviour, one could predict that certain behaviours and morphological features may covary. One empirical example of this is the link between boldness and defence morphology in aquatic snails (*Radix balthica*), whereby bold snails have more defensible shells (i.e. rounder shells with a wider aperture) (Ahlgren *et al.*, 2015). Similarly, bold brown anole lizards (*Anolis sagrei*) autotomize their tails more readily than shy ones when subjected to an unrestricted diet (Kuo *et al.*, 2015), and bold crucian carp (*Carassius carassius*) express more pronounced inducible defences in response to sustained exposure to a piscivorous predator (Hulthén *et al.*, 2013). These findings are suggestive of 'phenotypic compensation', whereby bold individuals compensate for their risky lifestyle by exhibiting more pronounced anti-predation morphological features (Ahlgren *et al.*, 2015). In broad terms, this hypothesis suggests that the morphology of organisms is attuned to optimize the cost–benefit ratio of displaying certain behaviours. Hence, if an organism consistently displays a certain level of behaviour (being bold, exploratory, active, etc.), we expect that its morphology is such that it maximizes the benefits and minimizes the cost of performing that behaviour. Thus, bold, risk-taking individuals are expected to possess morphological features that allow them to deal with these risks. Since both morphology and behaviour can possess a certain plasticity, the direction of causality can run both ways in this hypothesis. Behaviour can be adapted to morphology, but the reverse is also possible. Given this hypothesis, we expect threespine stickleback (*Gasterosteus aculeatus*) that are more prone to predation (i.e. bolder fish) to be more heavily armoured. The large variation in armour phenotypes among threespine stickleback, from fully armoured marine fish to freshwater populations with greatly reduced armour, provides a great resource to address this hypothesis. Since armour is likely less plastic than behaviour, we focus on the direction of causality that implies that the armour of an individual influences its behaviour, rather than the reverse.

Furthermore, being heavily armoured may impact the energy requirements and hydrodynamics of swimming. Movement-related behaviours such as exploration and activity might therefore also be affected by armour phenotype. For example, a trade-off between armour and swimming hydrodynamics means that heavily armoured fish cannot reach the fast-start performance and fleeing velocity less armoured fish can (Andraso, 1997; Bergstrom, 2002). Findings in lizard species of the Cordylidae family support this. The degree of armature among and within these species is highly variable and correlates negatively with distance run from a predator (Losos *et al.*, 2002), leading to the assertion that active flight and armoured defence are incompatible anti-predator strategies. While we did not assess escape behaviour, we propose that similar considerations could apply to exploratory behaviour and activity. In a more general context, metabolism and growth rate, both of which are intimately tied to energetic requirements, underlie a set of explanations concerning the occurrence of behavioural types and syndromes (e.g. Biro and Stamps, 2008; Careau *et al.*, 2008). Therefore, the energetic costs of armour can affect the behaviour of an individual via a direct effect on movement efficiency, as well as indirectly via its effect on metabolism and/or growth rate. With regards to activity and exploration, we expect fish with more developed armour to display lower levels of movement-related behaviours due to energetic costs.

The link between morphology and behaviour might also shed light on the existence and maintenance of trait correlations. Regardless of the specific proximate mechanism(s), the existence of behaviour–morphology covariation can (but need not) indicate selection pressures that favour and reinforce certain trait correlations.

Here, we report a test of the ‘phenotypic compensation’ and ‘energetic cost’ hypotheses through an investigation of the highly variable armour morphology of wild stickleback populations on the Scottish island of North Uist. We predict that bolder individuals should be more armoured than shy ones, both across and within populations. Furthermore, we extend our investigation to include exploratory behaviour and activity, and postulate that both are negatively influenced by the degree of armature.

METHODS

Fish capture and maintenance

Over a 4-week period in April and May 2015, 250 threespine stickleback were collected from seven freshwater lakes on the Scottish island of North Uist (Table 1). Traps were set late afternoon and the fish collected the next morning. Such catch methods may result in non-random capture of behavioural types (Biro and Dingemanse, 2009), but this is difficult to avoid in natural conditions.

Table 1. Sample sites

Loch	Code	Coordinates	Sampled individuals (females, F; males, M)	Trout present?
na Buaile	BUAI	57°38′N, 7°11′W	40 (33 F, 7 M)	No
Chadha Ruaidh	CHRU	57°36′N, 7°12′W	40 (24 F, 16 M)	No
an Daimh	DAIM	57°35′N, 7°12′W	20 (11 F, 9 M)	Yes
na Reival	REIV	57°37′N, 7°31′W	30 (17 F, 13 M)	No
Scadavay	SCAD	57°35′N, 7°14′W	40 (23 F, 17 M)	Yes
nan Struban	STRU	57°34′N, 7°21′W	40 (20 F, 20 M)	Yes
Tormasad	TORM	57°33′N, 7°19′W	40 (40 F, 0 M)	Yes

During the behavioural experiments, the fish were kept in individual tanks (0.12 × 0.20 × 0.12 m) in the field station (Drumcharry House, Lochmaddy, 57°61′N, 7°17′W). The behavioural assays started after one day of acclimatization and lasted for 4 days with one trial performed each day. The water in the experimental and individual tanks was obtained from a nearby fishless loch (57°61′W, 7°17′W; pH, 6.68; electrical conductivity, 243 $\mu\text{S} \cdot \text{cm}^{-1}$), and its temperature was monitored daily (average, 6.85°C; range, 6–8°C). The fish were exposed to a natural photoperiod (approximately 10/14 hours light/dark) and not fed during the experimental period. Following each assay, half of the water in the individual tanks was refreshed to prevent the build-up of waste and remove possible chemical cues from previous fish.

All of the performed research adhered to the Association for the Study of Animal Behaviour Guidelines for the Use of Animals in Research, the legal requirements of the country in which the work was carried out, and all institutional guidelines.

Behavioural trials

Boldness

Each fish was tested twice on consecutive days to assess responsiveness to risk (i.e. individual boldness). Behavioural observations took place remotely using a Panasonic HX-A100 camera and the Panasonic Image app to ensure a limited influence of the observer on the behaviour of the fish. At the beginning of the trial, each focal individual was placed in an experimental tank (0.20 × 0.40 × 0.30 m) and allowed to acclimatize for 3 minutes. After this time had elapsed, the tank was rapidly covered with a cardboard plaque to elicit a fright response. The cardboard was removed in a single quick motion upwards after 3 seconds. Fish characteristically froze, and individuals varied in the time taken to return to normal movement. We chose this latency to return to movement after a fright stimulus as our measure of boldness (Chapman *et al.*, 2010; Piyapong *et al.*, 2010) and calculated the mean time taken to return to movement (defined as continuous motion for a distance equal or greater than one body length) over both trials. Each trial had a ceiling value of 300 seconds. To generate a ‘boldness score’ in which bold individuals had a high score and shy individuals a low score, we subtracted the mean latency to move from the ceiling value.

Exploration and activity

To assess exploratory behaviour and activity, we carried out a further series of trials. The boldness and exploration/activity trials were separated by approximately 24 hours. One focal individual was placed in an experimental tank (0.55 × 0.45 × 0.20 m). After an acclimatization period of 5 minutes, recording and live tracking of the movement of the experimental individuals was initiated using four mounted cameras (Panasonic WV-CP314E CCTV cameras equipped with Computar T4Z2813CS-IR 2.8–12 mm lenses) and specialized software (Ethovision XT recording, Noldus Information Technology, Wageningen, Netherlands). One refuge and three other habitat structures, all made out of white plastic cups to prevent unwanted interactions with the tracking software, were present in each tank. Testing took place under standardized light conditions comparable to natural twilight, which allowed optimal tracking and prevented reflections.

The Ethovision software recorded the coordinates of each fish in its randomly assigned experimental tank. Based on these data, activity was quantified as the total time spent

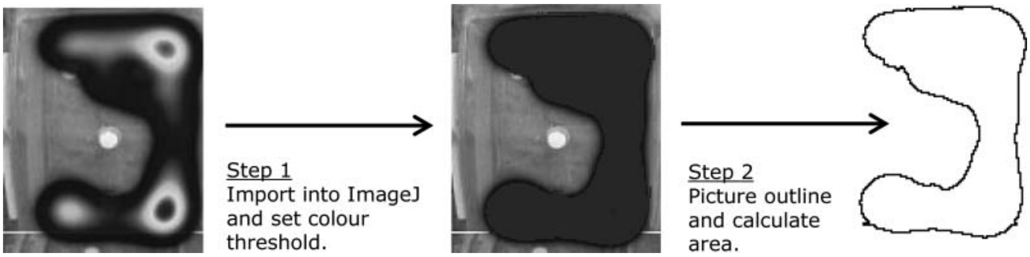

Fig. 1. The heatmaps generated by Ethovision (left) were imported into ImageJ, where they were processed (middle and right) in order to calculate the size of the area visited by the focal fish.

moving and exploratory behaviour as the area of the tank that the focal fish visited during the 5-minute trial. The exploration and activity scores used in the analysis are the means of both trials. The size of the explored area in square metres was obtained through processing the heatmaps generated by the Ethovision software in ImageJ (http://imagej.nih.gov/ij) (Fig. 1).

Quantifying defensive morphology

Following completion of the behavioural assays, fish were euthanized with MS222 (tricaine methanesulfonate). Next, they were rehydrated and fixated in a solution of 10% buffered formalin. After 2 weeks of fixation, all fish were washed with distilled water and incubated in a 1% KOH solution for one hour. Subsequently, they were bleached (5 mL of H_2O_2 in 1 L of KOH solution) overnight. Their bones and armour were then stained with 0.5% Alizarin red solution. After washing the stained fish with distilled water and 1% KOH solution, they were laterally photographed (Pentax K-50, smc DA 18–55 mm F3.5–5.6 lens) and stored in 40% isopropyl alcohol.

The photographs were analysed using ImageJ and as measures of armour we quantified the number of lateral plates, the height of the largest of these plates, the total number of spines (dorsal and pelvic), and the length of the first dorsal spine (if present).

Data analyses

The morphological parameters were summarized into a single armour score (PC Armour) using principal components analysis (PCA). Next, we assessed the presence of a correlation between armour score and boldness, exploration, and activity (all three behaviour scores are the means of the two trials), both among and within populations. Since exploration was normally distributed, we used a Pearson product–moment correlation; for boldness and activity (not normally distributed), we performed a Spearman's rank-order test.

Behavioural differences between the sexes (as determined via dissection) were assessed using a two-sample *t*-test in case of normal data (exploration), and a Wilcoxon rank sum test for non-normal data (i.e. boldness and activity).

Finally, to assess sources of variation for each of the three behaviours, we formulated general linear models with PC Armour, body length, sex, population, and all possible two-way interactions as fixed effects. When the residuals were not normally distributed (for boldness), we used a gamma error distribution. The models for exploration and activity had normal residuals and were run with a Gaussian error distribution.

All analyses were performed using R i386 v.3.2.1.

RESULTS

The PCA performed on the morphological traits resulted in a first principal component that explained 73.84% of the variance (Table 2). All morphological variables loaded negatively on this component; therefore, a high score reflects a low armour phenotype. In all further analyses, this PC1 score was inverted (multiplied by −1) so that a high score (i.e. high 'PC Armour') reflects a more armoured phenotype.

Across populations, boldness was significantly negatively correlated with PC Armour, meaning that individuals in bold populations were less armoured than those in shy ones (Fig. 2a, Table 3). No significant within-population correlations were found. Exploration was not correlated with armour phenotype across populations (Table 3). In one population (CHRU), however, exploratory behaviour did show a negative correlation with armour (Table 3). Activity was significantly positively correlated with armour so that armoured fish were also more active (Fig. 2b, Table 3). The same positive relationship between activity and armour was also found in CHRU and REIV (Fig. 2b, Table 3).

Across populations, none of the three behaviours differed significantly between the sexes (boldness: $W = 6071$, $P = 0.219$; exploration: $t = 0.488$, $P = 0.626$; activity: $W = 6478$, $P = 0.647$). Within populations, only DAIM ($t = -3.963$, $P = 0.001$), REIV ($t = 3.211$, $P = 0.004$), and SCAD ($t = -2.114$, $P = 0.042$) displayed significant behavioural differences between the sexes, all in exploratory behaviour (Fig. 3). In REIV, males were less exploratory than females; in DAIM and SCAD, this pattern was reversed.

All behaviours were significantly repeatable (boldness: 0.531 ± 0.022, $P < 0.0001$; exploration: 0.366 ± 0.021, $P < 0.0001$; activity: 0.383 ± 0.0211, $P < 0.0001$) (following Lessells and Boag, 1987; Nakagawa and Schielzeth, 2010).

The models revealed that population is a main source of variation for all behaviours (Table 4). Furthermore, body length had a significant effect on exploration and boldness, with smaller individuals being both less exploratory and less active than their larger conspecifics. According to our models, activity is also influenced by sex, but this is not borne out by our earlier analyses (see above). Significant interactions were between body length and population for boldness, between sex and population for exploration, and between PC Armour and population, as well as between PC Armour and body length, for activity (Table 4).

Table 2. Results of principal components analysis on the morphological parameters

Residuals	PC1 loadings
Number of lateral plates	−0.530
Height of largest plate	−0.515
Number of spines	−0.546
Length of first dorsal spine	−0.395
Proportion of variance explained	73.84%

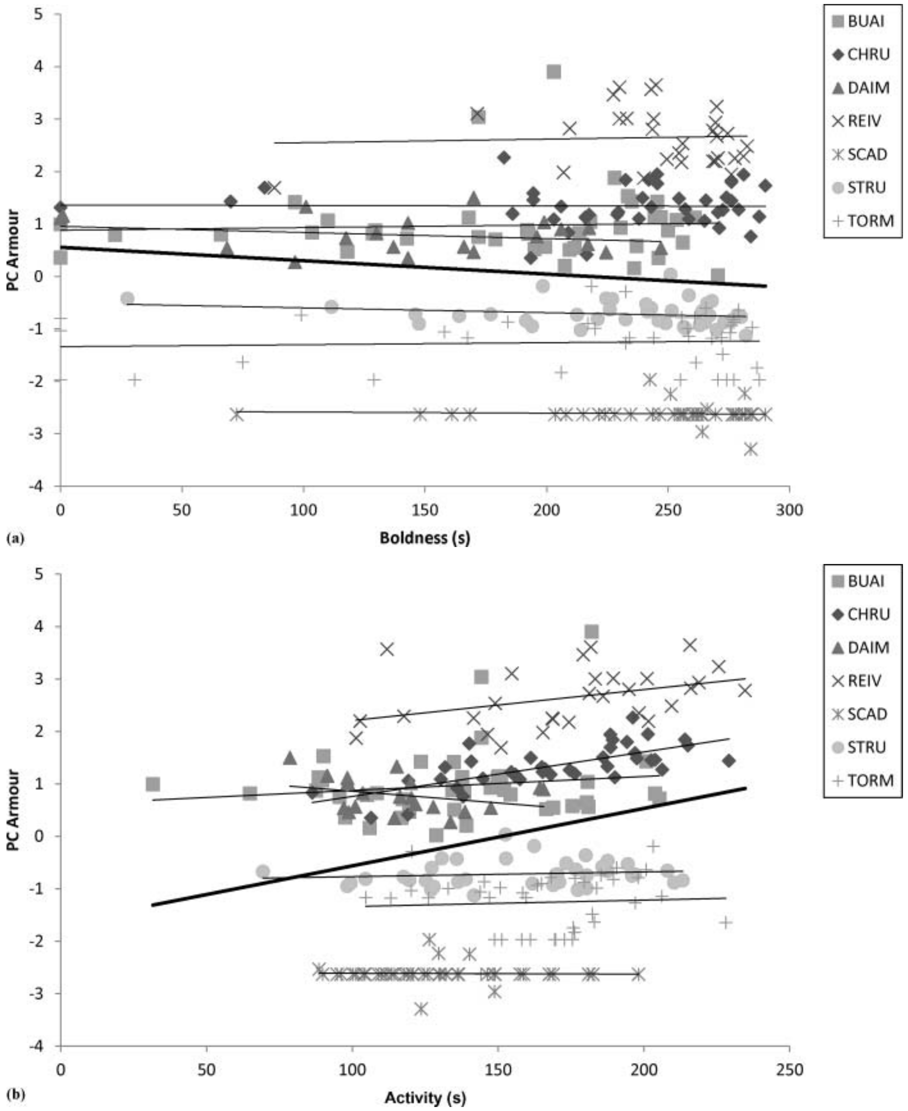

Fig. 2. Correlations between behaviour and armour phenotype across populations for (a) boldness and (b) activity. Each population has its own symbol and the overall correlation is illustrated by the bold black line.

DISCUSSION

Our results directly contradict our stated hypotheses. Bold fish are less armoured, and more active fish are more armoured. Armour phenotype as characterized here did not correlate with exploratory behaviour.

The phenotypic compensation hypothesis led us to suggest that risk-taking individuals possess a more pronounced anti-predator morphology; hence we proposed that bolder stickleback would be more armoured. Instead, we found the opposite to be the case.

Table 3. Correlations between armour phenotype and boldness, exploration, and activity

	Boldness		Exploration		Activity	
	ρ	P	r	P	ρ	P
Across populations	**−0.155**	**0.014**	−0.076	0.235	**0.254**	**<0.001***
Within populations:						
BUAI	0.054	0.740	−0.142	0.382	0.057	0.725
CHRU	0.053	0.745	**−0.360**	**0.024**	**0.707**	**<0.001***
DAIM	−0.157	0.508	0.145	0.543	−0.343	0.139
REIV	−0.247	0.197	0.061	0.753	**0.453**	**0.014***
SCAD	−0.082	0.615	−0.032	0.844	−0.090	0.579
STRU	−0.231	0.151	0.176	0.276	0.193	0.233
TORM	−0.016	0.923	−0.164	0.313	0.217	0.179

Note: Significant *P*-values in **bold**. **P*-values significant following false discovery rate for multiple testing.

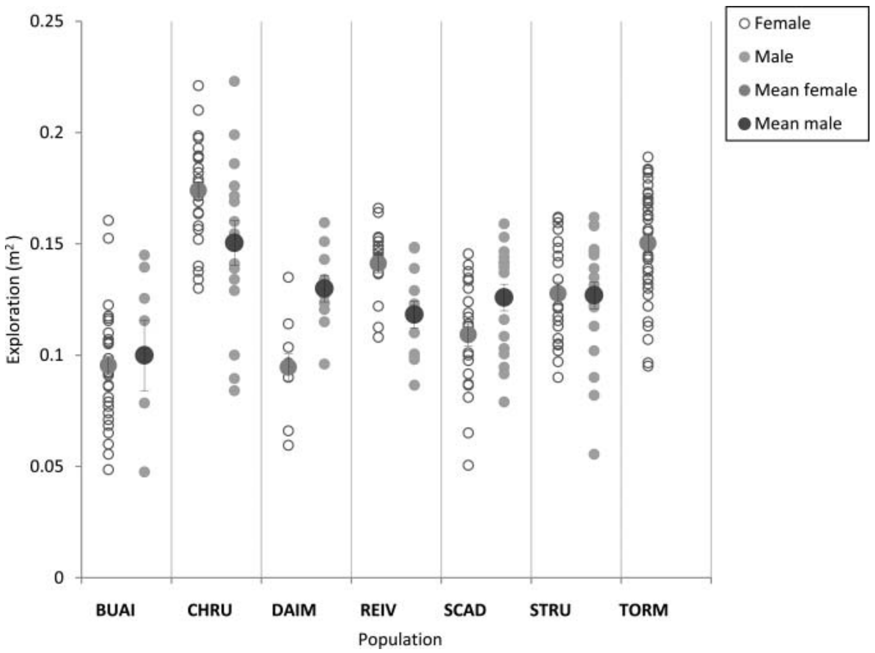

Fig. 3. Differences in exploratory behaviour between males and females by population (only females were caught in TORM).

However, our finding is not without precedent. Comparing threespine stickleback that differed in pelvic phenotype from two Canadian lakes did not yield an across-population correlation between risk-taking behaviour and anti-predator morphology but did find such a relation within one of the populations (Grand, 2000). In addition, in juvenile, laboratory-reared stickleback there is a strong (but non-significant) suggestion that boldness correlates negatively with armour (Lacasse and Aubin-Horth, 2012). Our results suggest that this might hold in natural conditions as well, prompting a reconsideration of the extent to which the

Table 4. Results from the general linear model for each of the tested behaviours

	Boldness		Exploration		Activity	
	χ^2_{df}	*P*	χ^2_{df}	*P*	χ^2_{df}	*P*
PC Armour	0.228_1	0.633	0.001_1	0.983	1.793_1	0.181
Body length	0.726_1	0.394	8.883_1	**0.003**	15.482_1	**<0.0001**
Sex	0.136_1	0.712	0.606_1	0.436	4.787_1	**0.029**
Population	46.726_6	**<0.0001**	137.110_6	**<0.0001**	65.436_6	**<0.0001**
PC Armour × Body length	0.708_1	0.400	3.166_1	0.075	4.748_1	**0.029**
PC Armour × Sex	0.209_1	0.647	0.015_1	0.904	0.891_1	0.345
PC Armour × Population	2.073_6	0.913	3.916_6	0.688	14.252_6	**0.027**
Body length × Sex	2.361_1	0.124	0.019_1	0.890	0.574_1	0.448
Body length × Population	15.394_6	**0.017**	4.974_6	0.547	12.583_6	0.050
Sex × Population	9.624_5	0.087	17.985_5	**0.003**	3.848_5	0.571

Note: Significant *P*-values in **bold**.

phenotypic compensation hypothesis can explain behaviour–morphology correlations in natural conditions. Within populations, however, we found no clear correlation between boldness and armour phenotype, which suggests that the boldness–armour correlation we observed is likely the result of ecological factors that differ substantially between populations, but not within them (predation, food availability, etc.), that affect both boldness and armour development. The possibility of co-specialization should also be considered. In the freshwater snail *Physa gyrina*, for example, it has been shown that predation can lead to both anti-predator behaviour and morphological defence (Dewitt *et al.*, 1999). If we consider our measure of boldness as an anti-predator behaviour, this might mean that predation leads to the development of more robust armour or increased boldness, which would lead to the boldness–armour correlation we observed. An alternative, or complementary, consideration is that the sample size for the across-population test was substantially larger than those of the within-populations tests. As such, the power of the former is higher than that of the latter.

We found a positive correlation between activity and armour, contradicting our energetic cost hypothesis. This suggests that the energetic costs of armour are either negligible or mitigated by potential benefits of being active. In two populations we also observed a positive relation between armour and activity. In the other populations we did not find a significant correlation. The same consideration as with boldness might apply here: the ecological factors a population is subjected to are more significant influences on behaviour than armour phenotype. Hence, if mitigation of the energetic costs of armour occurs, the extent of it is likely to be influenced by environmental factors. The fitness consequences of exploratory behaviour in great tits (*Parus major*), for example, fluctuate in a natural changing environment (Dingemanse *et al.*, 2004).

Perhaps the fish in certain populations are subjected to one or several specific environmental influences, or combinations thereof, that allow sufficient mitigation (and, in some cases, even a reversal compared with what would be expected under the energetic cost hypothesis). One likely option is food availability. Previous work, both theoretical and experimental, has shown that both food availability and predictability can influence boldness (e.g. Chapman *et al.*, 2010; Luttbeg and Sih, 2010) and exploratory behaviour (Chapman *et al.*, 2010).

Our results suggest that this might also be the case for activity. When ample food is available, fish might be able to acquire sufficient energetic resources to maintain high activity levels even with well-developed armour.

Furthermore, possessing armour could lead to a higher metabolism, which, in turn, affects hunger levels, possibly leading to more active behaviour in order to discover food patches. This idea is in line with proposals coupling metabolism to behavioural variation (Careau *et al.*, 2008, 2011; Careau and Garland, 2014). We did not, however, find a correlation between exploration and armour phenotype. One reason for this might be that interpreting behaviour is notoriously difficult. Our measure for exploration, the proportion of a novel environment explored, involves a certain aspect of risk and might thus also involve a certain aspect of boldness. In fact, our quantification for exploratory behaviour combines elements of risk (i.e. boldness) with elements of movement (i.e. activity) (the behaviours are all positively correlated with ρ = roughly 0.25 for the two boldness correlations and $r = \pm 0.5$ for the activity–exploration correlation). Maybe the negative correlation between armour and boldness and the positive one between armour and activity negate each other in our exploration assay, but this is, of course, highly speculative.

The overruling effect of population ecology is not limited to behaviour. For example, when investigating the stress reactivity of marine and freshwater stickleback, the ecological factors are highly significant for both cortisol levels and stress reactivity (Di Poi *et al.*, 2016). Di Poi *et al.* suggest that the divergence in stress reactivity could have its roots in different natural selection pressures (e.g. predation) between the sampled populations, mirroring our suggestions concerning population differences in behaviour.

We did not find behavioural differences between males and females across populations, and significant differences between the sexes were only found in three populations for exploratory behaviour. This contrasts with other studies which found that males of guppies (*Poecilia reticulata*), threespine stickleback, and the live-bearing fish genus *Brachyraphis* tend to be bolder and more exploratory than females (Harris *et al.*, 2010; King *et al.*, 2013; Ingley *et al.*, 2014). The differences we found did not show a consistent pattern, so we surmise that behavioural differences between the sexes depend on the prevailing (or past) ecological conditions of the specific population or reproductive status at the time of testing.

Our work involved wild-caught fish. This means that the variation we observed in both behaviour and morphology arises via a complex web of interactions including genetic and plastic effects. Even maternal effects cannot be ignored (see, for example, Lancaster *et al.*, 2010). As such, our results should be interpreted with caution and extrapolation to underlying causal mechanisms should be done with care. Nevertheless, the relationships we observe point the way towards future work that may help unravel the complex causal web in which individual variation in behaviour is embedded. A fruitful research avenue, for example, would be to investigate the ecology of these populations in more detail. This may provide more information and could help to better our understanding of the behaviour–morphology–ecology triad.

Overall, we found that, across wild populations of threespine stickleback, bolder fish are less armoured and higher activity is linked with a more pronounced armour phenotype. There is no clear correlation between armour phenotype and exploratory behaviour. Within-population correlations between any of the three tested behaviours and armour are rare and are likely the result of specific (combinations of) environmental factors. Such potential dependence of behaviour–morphology covariation on ecological facets cautions against extrapolating findings from one or a few populations to establish a general hypothesis.

ACKNOWLEDGEMENTS

G.D.W. is funded by a PhD studentship from the University of Nottingham's School of Life Sciences. B.B.C. is funded by a University of Nottingham Research Fellowship. H.R.M. and R.A.T. were supported by the Science Without Borders program. Thanks to Andrew MacColl, Talib Chitheer, and Abdul Rahman for sharing their knowledge of the North Uist lochs and their inhabitants. Another burst of gratitude goes to the editors and reviewers whose numerous comments markedly improved the final manuscript.

REFERENCES

Ahlgren, J., Chapman, B.B. and Nilsson, P.A. 2015. Individual boldness is linked to protective shell shape in aquatic snails. *Biol. Lett.*, **11**: 20150029.

Andraso, G.. 1997. A comparison of startle response in two morphs of the brook stickleback (*Culaea inconstans*): further evidence for a trade-off between defensive morphology and swimming ability. *Evol. Ecol.*, **11**: 83–90.

Bergstrom, C.A. 2002. Fast-start swimming performance and reduction in lateral plate number in threespine stickleback. *Can. J. Zool.*, **80**: 207–213.

Biro, P.A. and Dingemanse, N.J. 2009. Sampling bias resulting from animal personality. *Trends Ecol. Evol.*, **24**: 63–66.

Biro, P.A. and Stamps, J.A. 2008. Are animal personality traits linked to life-history productivity? *Trends Ecol. Evol.*, **23**: 361–368.

Brown, C. and Braithwaite, V.A. 2004. Size matters: a test of boldness in eight populations of the poeciliid *Brachyraphis episcopi. Anim. Behav.*, **68**: 1325–1329.

Careau, V. and Garland, T. 2014. Performance, personality, and energetics: correlation, causation, and mechanism. *Physiol. Biochem. Zool.*, **85**: 543–571.

Careau, V., Thomas, D.K., Humphries, M.M., and Réale, D. 2008. Energy metabolism and animal personality. *Oikos*, **117**: 641–653.

Careau, V., Thomas, D., Pelletier, F., Turki, L., Landry, F., Garant, D. *et al.* 2011. Genetic correlation between resting metabolic rate and exploratory behaviour in deer mice (*Peromyscus maniculatus*). *J. Evol. Biol.*, **24**: 2153–2163.

Chapman, B.B., Morrell, L.J. and Krause, J. 2009. Plasticity in male courtship behaviour as a function of light intensity in guppies. *Behav. Ecol. Sociobiol.*, **63**: 1757–1763.

Chapman, B.B., Morrell, L.J. and Krause, J. 2010. Unpredictability in food supply during early life influences boldness in fish. *Behav. Ecol.*, **21**: 501–506.

Chapman, B.B., Hulthén, K., Blomqvist, D.R., Hansson, L.-A., Nilsson, J.-Å., Brodersen, J. *et al.* 2011a. To boldly go: individual differences in boldness influence migratory tendency. *Ecol. Lett.*, **14**: 871–876.

Chapman, B.B., Thain, H., Coughlin, J. and Hughes, W.O.H. 2011b. Behavioural syndromes at multiple scales in *Myrmica* ants. *Anim. Behav.*, **82**: 391–397.

Dall, S.R.X., Houston, A.I. and McNamara, J.M. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecol. Lett.*, **7**: 734–739.

Dewitt, T.J., Sih A. and Hucko, J.A. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Anim. Behav.*, **58**: 397–407.

Dingemanse, N.J. and Réale, D. 2005. Natural selection and animal personality. *Behaviour*, **142**: 1165–1190.

Dingemanse, N.J., Both, C., Drent, P.J., van Oers, K. and Van Noordwijk, A.J. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. *Anim. Behav.*, **64**: 929–938.

Dingemanse, N.J., Both, C., Drent, P.J. and Tinbergen, J.M. 2004. Fitness consequences of avian personalities in a fluctuating environment. *Proc. R. Soc. Lond. B*, **271**: 847–852.

Di Poi, C., Lacasse, J., Roger, S.M. and Aubin-Horth, N. 2016. Evolution of stress reactivity in stickleback. *Evol. Ecol. Res.*, **17**: 395–405.

English, S., Nakagawa, S. and Clutton-Brock, T.H. 2010. Consistent individual differences in cooperative behaviour in meerkats (Suricata suricatta). *J. Evol. Biol.*, **23**: 1597–1604.

Foltz, S.L., Ross, A.E., Laing, B.T., Rock, R.P., Battle, K.E. and Moore, I.T. 2015. Get off my lawn: increased aggression in urban song sparrows is related to resource availability. *Behav. Ecol.*, **26**: 1548–1557.

Fraser, D.F., Gilliam, J.F., Daley, M.J., Le, A.N. and Skalski, G.T. 2001. Explaining leptokurtic movement distributions: intrapopulation variation in boldness and exploration. *Am. Nat.*, **158**: 124–135.

Grand T. 2000. Risk-taking by threespine stickleback (*Gasterosteus aculeatus*) pelvic phenotypes: does morphology predict behaviour? *Behaviour*, **137**: 889–906.

Grinsted, L., Pruitt, J.N., Settepani, V. and Bilde, T. 2013. Individual personalities shape task differentiation in a social spider. *Proc. R. Soc. Lond. B*, **280**: 20131407.

Harris, S., Ramnarine, I.W., Smith, H.G. and Pettersson, L.B. 2010. Picking personalities apart: estimating the influence of predation, sex and body size on boldness in the guppy *Poecilia reticulata*. *Oikos*, **119**: 1711–1718.

Hulthén, K., Chapman, B.B., Nilsson, P.A., Hollander, J. and Brönmark, C. 2013. Express yourself: bold individuals induce enhanced morphological defences. *Proc. R. Soc. Lond. B*, **281**: 20132703.

Ingley, S.J., Rehm, J. and Johnson, J.B. 2014. Size doesn't matter, sex does: a test for boldness in sister species of *Brachyrhaphis* fishes. *Ecol. Evol.*, **4**: 4361–4369

King, A.J., Fürtbauer, I., Mamuneas, D., James, C. and Manica, A. 2013. Sex-differences and temporal consistency in stickleback fish boldness. *PLoS One*, **8**: e81116.

Kuo, C.-Y., Irschick, D.J. and Lailvaux, S.P. 2015. Trait compensation between boldness and the propensity for tail autotomy under different food availabilities in similarly aged brown anole lizards. *Funct. Ecol.*, **29**: 385–392.

Lacasse, J. and Aubin-Horth, N. 2012. A test of the coupling of predator defense morphology and behavior variation in two threespine stickleback populations. *Curr. Zool.*, **58**: 53–65.

Lancaster, L.T., McAdam, A.G. and Sinervo, B. 2010. Maternal adjustment of egg size organizes alternative escape behaviours, promoting adaptive phenotypic integration. *Evolution*, **64**: 1607–1621.

Lessells, C.M. and Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk*, **104**: 116–121.

Losos, J.B., Mouton, P.L.F.N., Bickel, R., Cornelius, I. and Ruddock, L. 2002. The effect of body armature on escape behaviour in cordylid lizards. *Anim. Behav.*, **64**: 313–321.

Luttbeg, B. and Sih, A. 2010. Risk, resources and state-dependent adaptive behavioural syndromes. *Phil. Trans. R. Soc. Lond. B*, **365**: 3977–3990.

McCowan, L.S.C., Mainwaring, M.C., Prior, N.H. and Griffith, S.C. 2015. Personality in the wild zebra finch: exploration, sociality, and reproduction. *Behav. Ecol.*, **26**: 735–746.

Nakagawa, S. and Schielzeth, H. 2010. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol. Rev.*, **85**: 935–956.

Piyapong, C., Krause, J., Chapman, B.B., Ramnarine, I.W., Louca, V. and Croft, D.P. 2010. Sex matters: a social context to boldness in guppies (*Poecilia reticulata*). *Behav. Ecol.*, **21**: 3–8.

Planas-Sitjà, I., Deneubourg, J.-L., Gibon, C. and Sempo, G. 2015. Group personality during collective decision-making: a multi-level approach. *Proc. R. Soc. Lond. B*, **282**: 20142515.

Réale, D., Gallant, B., Leblanc, M. and Festa-Bianchet, M.. 2000. Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Anim. Behav.*. **60**: 589–597.

Ward, A.J.W., Thomas, P., Hart, P.J.B. and Krause, J. 2004. Correlates of boldness in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.*, **55**: 561–568.

Wolf, M. and Weissing, F.J. 2010. An explanatory framework for adaptive personality differences. *Phil. Trans. R. Soc. Lond. B*, **365**: 3959–3968.