Express yourself: bold individuals induce enhanced morphological defences

Kaj Hulthén, Ben B. Chapman, P. Anders Nilsson, Johan Hollander and Christer Brönmark

Department of Biology, Aquatic Ecology, Lund University, Ecology Building, 223 62 Lund, Sweden

Organisms display an impressive array of defence strategies in nature. Inducible defences (changes in morphology and/or behaviour within a prey’s lifetime) allow prey to decrease vulnerability to predators and avoid unnecessary costs of expression. Many studies report considerable interindividual variation in the degree to which inducible defences are expressed, yet what underlies this variation is poorly understood. Here, we show that individuals differing in a key personality trait also differ in the magnitude of morphological defence expression. Crucian carp showing risky behaviours (bold individuals) expressed a significantly greater morphological defence response when exposed to a natural enemy than shy individuals. Furthermore, we show that fish of different personality types differ in their behavioural plasticity, with shy fish exhibiting greater absolute plasticity than bold fish. Our data suggest that individuals with bold personalities may be able to compensate for their risk-prone behavioural type by expressing enhanced morphological defences.

1. Introduction

Virtually all animals constitute potential prey; as such, they are under strong selection to avoid capture by their natural enemies. Consequently, a huge range of anti-predator defences is displayed in nature [1,2]. Constitutive defences are those always expressed by the host, regardless of the prevailing predation risk. However, many animals are under variable predation risk and have evolved plasticity in defensive traits [3,4]. Inducible defences constitute a widespread form of adaptive phenotypic plasticity, allowing prey organisms to express defences and reduce vulnerability to predators only when required, thus saving costs when enemies are absent [3,5]. Predator exposure for prey adopting an inducible defence strategy may elicit the expression of elaborate morphological defensive structures such as horns, spines, armour, elongated helmets or specific body morphologies [3,6].

Morphological defences are but one form of defence. Many animals also exhibit behavioural defences, which have been shown to covary with morphological defence both between and within species in a number of cases. Interspecific differences in defensive morphology between fish species (armoured and unarmoured) correlate with anti-predator behaviour, with the unarmoured species (fathead minnows) responding stronger than the armoured species (brook stickleback) to a predatory stimulus [7]. Intraspecific variation in morphological and behavioural defences has also been reported [8,9]. A classic example here is the northwestern garter snake, which exhibits diverse variation in a constitutive defence—colour pattern—within populations [10]. Individuals that are camouflaged exhibit different anti-predator behaviour (freezing) than those that are striped (flee behaviour [10]). Hence, in these examples, behaviour and constitutive defence strategy are correlated. Yet, perhaps surprisingly, given the links between constitutive defence strategy and behaviour, how behaviour is related to inducible defence strategy is not well studied. Behaviour has been suggested as a proximate mechanism shaping defence expression in snails [11] and fish [12], but whether individual variation in behaviour can predict individual differences in defence expression is not known.

All behaviours have both flexible and consistent components that vary in their relative importance [13]. Until recently, there has been little recognition that...
behaviour is often surprisingly consistent across time and context, a phenomenon known as ‘animal personality’ [14–16]. Individuals within a population, occupying the same spatial location in the wild, may thus behave differently in the same situation, and if individual behaviours are stable over time and/or contexts they are referred to as personalities [13]. Evidence from a growing body of literature suggests that animal personality is exceptionally widespread, and has been documented in taxa as diverse as mammals, birds, reptiles, fish, crustaceans and insects [15,17]. Considerable attention has been directed towards behaviours associated with risk taking and behavioural variation along the bold–shy continuum [18–23]. According to this well-studied axis of animal personality, individuals differ in their propensity to take risks, with bold individuals being consistently more prone to engage in risky behaviour, whereas shy individuals are consistently more risk-averse and cautious [23,24]. Meta-analysis has shown that bold individuals in many examples suffer a mortality cost for their risk-prone behaviour compared with shy individuals [25]. Hence, bold individuals may benefit more than shy individuals from investing in a potentially expensive inducible defence.

To the best of our knowledge, no previous study has investigated a link between individual personality traits and inducible defence expression; to date, the ecology and evolution of personality traits and inducible defences have been extensively studied, but in isolation. Here, we take an integrative approach and experimentally test whether underlying differences in a key personality trait (boldness) is linked to inducible defence expression in our model organism, the crucian carp Carassius carassius. Given that bold individuals are likely to have a higher mortality risk, we predict that they should compensate by investing more energy, compared with shy individuals, into a defensive morphological trait: increased body depth, known to reduce the risk of being predated by gape-limited predators [26]. Our secondary aim was to assess whether crucian carp behaviour was influenced by predator exposure. Here, we predicted that exposure to a predator would increase boldness (in line with previous studies [27–29]) and also that shy individuals would exhibit a great degree of behavioural plasticity (again in line with findings from previous studies [30,31]). To test these hypotheses, we first quantified the individual boldness of wild-caught fish and then performed a factorial experiment crossing personality (bold or shy) with predation exposure (presence or absence). After approximately four months of treatment exposure, we re-scored boldness, and the magnitude of inducible defence expression was quantified and related to initial boldness in each individual fish. In addition, we compared post-treatment boldness scores between predator-exposed and control individuals, and assessed whether variation in behavioural plasticity was related to initial boldness score.

2. Material and methods

(a) Study organism

Our model organism, the crucian carp (C. carassius), provided the first example of a vertebrate with an induced morphological defence and has remained an important model organism for studying phenotypic plasticity [12,32–36]. Laboratory and field studies have shown that crucian carp exhibit a spectacular inducible morphological defence: when exposed to chemical cues released by predators, such as pike (Esox lucius), they increase in body depth [36]. The morphologically defended phenotype constitutes less desirable prey for gape-limited predators, and the deep body improves escape performance via enhanced locomotor capacity [26,37].

(b) Subject collection and maintenance

We used wild-caught crucian carp (n = 160; size range 100–121 mm) collected with trap nets in a pond located in Lund, southern Sweden. Because the crucians were the only fish present in the pond, all subjects were naive to predation and consequently of a shallow-bodied morphology. Following Skov et al. [38], we surgically implanted passive integrated transponder tags into the body cavity of the subjects. These uniquely coded electronic tags allowed us to keep track of each individual during the entire experiment. Thereafter, fish were acclimatized for four weeks in a rearing tank (volume 385 l) equipped with a flow-through system. The crucian carp were fed commercial trout pellets and frozen chironomids five times weekly during the acclimatization period. Pike (size range 37–41 cm) were collected in lake Kranksjön, southern Sweden, maintained individually in holding tanks (volume 80 l) and fed two crucians weekly for at least three weeks prior to experiments.

(c) Bold–shy assessment

Following the acclimatization period, crucian carp were assayed for boldness. To obtain an index of individual boldness, we used an established refuge emergence protocol where the boldness score is defined as the latency to emerge from a refuge box [28]. The experimental arena consisted of a circular PVC tank (volume 70 l), lined with ScotchLite luminous tape in order to facilitate behavioural analysis by providing a strong background contrast to the fish. We monitored water temperature throughout the period to ensure that the holding and experimental tanks did not differ radically in temperature (mean ± s.d.: holding tank = 13.40 ± 0.51 °C; experimental arena = 13.33 ± 0.44 °C). Within the arena, we placed a refuge box (28 × 20 × 20 cm) made of grey PVC. To decrease environmental disturbance, a tarpaulin tent sheltered the experimental apparatus. Each trial started when one individual in the holding tank was haphazardly chosen and transferred to the refuge box. Each fish was given 5 min to acclimatize in the box, and when this time had elapsed, a vertically sliding trapdoor was slowly raised via a remote pulley system. We then observed refuge emergence behaviour via a video camera centrally mounted above the tank and linked to a monitor placed outside the tent. Observations were made with the criterion that the whole body of the fish had to be outside the box, and the time taken to emerge was recorded to the nearest 1 s. Each trial lasted for 20 min, and fish that had failed to emerge were given a ceiling value of 1200 s. All subjects were assayed twice with at least 6 days between trials, and boldness was consistent in this population (Spearman’s ρ = 0.237, p = 0.093). Boldness is a continuous trait, however, and in order to effectively assess the impact of boldness upon defence expression in our factorial experimental design, we compared defence expression in fish classified into behavioural phenotypes. To categorize fish into ‘bold’ or ‘shy’ groups, we used the refuge emergence scores from two boldness trials pre-exposure (figure 1a). Fish with two boldness scores of less than or equal to 25 s were defined as bold, whereas fish with two scores greater than or equal to 45 s were deemed shy. In total, four individuals (2.5% of fish assayed) failed to emerge from the refuge box and were excluded from the experiment. Sixty individuals were included in the experiment (mean ± s.d.: total length = 11.17 ± 0.50 cm; maximum body depth = 3.30 ± 0.22 cm; weight = 17.59 ± 2.91 g). These consistently bold (n = 30; mean ± s.d.: emergence time = 14.7 ± 4.03 s) and shy (n = 30; mean ± s.d.: emergence time = 122.1 ± 77.02 s) individuals were then assigned
to experimental tanks. We kept fish in exclusively bold or shy tanks to avoid the confounding effect of interindividual differences in food intake. Boldness is often correlated with competitive ability [18], and so having a mix of behavioural types within tanks may have led to a skew in the feeding rates of bold and shy fish, and therefore defence expression; hence we housed fish of a single behavioural type in each tank.

(d) Rearing experiment

The experiment was conducted in a controlled environmental room that provided a constant temperature of 18°C and a regime of light to darkness of 12:12 h. We set up 20 aquaria (volume 165 l each), each divided into two compartments of equal size by a PVC-framed plastic mesh partition. In order to prevent visual interactions between replicates, three sides of each aquarium were externally covered with a black plastic film. Each experimental tank had three crucian carp (either bold or shy), and the experiment was initiated when fish were introduced to one of the aquarium compartments. All test fish were laterally photographed for morphometric analysis, and weighed to the nearest 0.1 g prior to the onset of the experiment. Single pike were allocated to the other compartment in aquaria used for the predator treatment. Aquaria were randomly assigned to treatments in a 2 × 2 design with the factors pike (presence/absence) and personality (bold/shy), and each treatment combination was replicated five times. Crucian carp were fed frozen chironomids five times weekly, and the food delivery rate corresponded to 6% of the weight of the crucians in each replicate tank. After 122 days, crucian carp were removed from the tanks, acclimatized over night in a predator-free holding tank and then re-assayed for boldness the following day (figure 1b). All fish were photographed once again and weighed to assess morphological changes and body growth (final weight – initial weight).

(e) Data treatment and statistical analysis

Using the image analysis software ImageJ v. 1.43 (http://rsweb.nih.gov/ij/), we measured the total length and maximum body depth (measured in a 90° angle anterior to the dorsal fin) of all fish pre- and post-treatment exposure. All measurements were made blind. We then calculated the individual change in maximum body depth and weight as body depth and weight post-exposure minus body depth and weight pre-exposure. Similarly, we calculated the individual change in ln-transformed boldness scores for each individual using the mean value of the two boldness score derived pre-exposure minus the boldness score derived post-exposure. We additionally multiplied the ln-transformed refuge emergence value by −1 to aid interpretation and clarify. In this way, a high score indicates a bold individual and a low score indicates a shy individual. A Spearman’s rank correlation test was used to assess individual consistency in boldness over the course of the experiment (i.e. the 122-day predator/control exposure period). Similarly, correlations between boldness and body depth, length and weight before treatment exposure were assessed by Spearman rank analysis. We compared initial differences between bold and shy individuals in body length, body mass and body depth using t-tests. Similarly, we used a t-test to compare ln-transformed post-treatment boldness scores between control and predator-exposed fish. Because phenotypic traits may be correlated, we used nested MANOVAs and two separate models with maximum body depth, growth rate and ln-transformed boldness scores as dependent variables. For the first model, we investigated the relative trait change \( x \), where \( x = (y_2 - y_1) / y_1 \), with \( y_1 \) indicating pre-treatment trait values and \( y_2 \) post-treatment trait values. Analysing all values, including negative changes, allowed us to assess the actual consequences of exposure for each trait value. However, to analyse plasticity per se, one must quantify the degree of absolute change of the different trait values, which we analysed in our second model. In these models, initial personality type, predation and their interaction term were used as fixed factors, and tanks were nested within the predator × personality interaction term to compensate the degrees of freedom according to the experimental design. Subsequent univariate F-tests were used as a post hoc procedure to evaluate significant multivariate effects. One replicate tank (predator × bold) had to be excluded from analysis because the aquarium partition detached during the treatment period, and the subjects were predated. All statistics were performed using SPSS v. 21.0.0.0 for Mac OS X (SPSS Inc., Chicago, IL).

3. Results

Boldness was consistent over the 122-day period (Spearman’s \( p = 0.472, p < 0.001 \)). Boldness was not related to initial total length, maximum body depth or weight (length: \( r = 0.067, p = 0.609 \); depth: \( r = 0.040, p = 0.762 \); weight: \( r = 0.034, p = 0.797 \)). There were no significant initial differences between our bold and shy groups in body length \( (t_{20} = 0.492, p = 0.624) \), body mass \( (t_{20} = 0.560, p = 0.578) \) or body depth \( (t_{20} = 0.647, p = 0.520) \).
The two-way nested MANOVAs with relative and absolute change in body morphology, weight and boldness as response variables revealed significant effects of predation, personality and their interaction (table 1). In addition, the MANOVAs revealed a significant effect for tanks nested within predator and personality factor (table 1). Univariate tests showed that the interaction effect of predation exposure / personality type originated from a change in maximum body depth (table 1 and figure 2a); bold fish exposed to predators expressed a more pronounced morphological defence than fish with shy personalities in both relative and absolute terms. We also found a significant effect of predator presence on relative and absolute growth rate, with fish under predation risk putting on more weight than the control group over the 122-day exposure period (table 1 and figure 2b). Furthermore, we found marginally non-significant effects of personality type on relative and absolute growth rate, which indicates a trend towards bold individuals having a higher growth rate than shy fish (table 1 and figure 2b). Finally, both predator exposure and personality type significantly influenced change in boldness (table 1 and figure 1c). Predator exposure affected the relative but not the absolute change in boldness scores. Fish from the predator treatment became bolder than fish from the control, and we also report personality-dependent behavioural plasticity, with shy fish changing their boldness score to a greater degree than bold individuals. Hence, when re-assayed after treatment exposure boldness scores between behavioural types in control treatments remained considerably different ($t_{28} = 4.231, \ p = 0.001$), whereas differences between initially bold and shy fish exposed to predators were only

Table 1. Results of overall MANOVA and subsequent univariate ANOVAs examining relative and absolute (italic) change in maximum body depth, weight and boldness scores between treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Wilks's lambda</th>
<th>$F$-value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MANOVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>predation</td>
<td>3,36</td>
<td>0.139</td>
<td>74.387</td>
<td>$&lt;0.001$***</td>
</tr>
<tr>
<td>personality</td>
<td>3,36</td>
<td>0.582</td>
<td>8.627</td>
<td>$&lt;0.001$***</td>
</tr>
<tr>
<td>predation / personality</td>
<td>3,36</td>
<td>0.619</td>
<td>7.382</td>
<td>$0.001$**</td>
</tr>
<tr>
<td>tanks (predation / personality)</td>
<td>45,108</td>
<td>0.186</td>
<td>1.825</td>
<td>$0.066$**</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>mean square</th>
<th>$F$-value</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>change in maximum body depth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>predation</td>
<td>1,38</td>
<td>0.073</td>
<td>109.089</td>
<td>$&lt;0.001$***</td>
</tr>
<tr>
<td>personality</td>
<td>1,38</td>
<td>0.003</td>
<td>4.492</td>
<td>$0.041$**</td>
</tr>
<tr>
<td>predation / personality</td>
<td>1,38</td>
<td>0.005</td>
<td>8.026</td>
<td>$0.007$**</td>
</tr>
</tbody>
</table>

| change in weight | | | | |
| predation | 1,38 | 0.040 | 8.999 | 0.005** |
| personality | 1,38 | 0.012 | 2.686 | 0.109 |
| predation / personality | 1,38 | 0.000 | 0.098 | 0.756 |

| change in boldness score | | | | |
| predation | 1,38 | 0.437 | 6.004 | 0.019** |
| personality | 1,38 | 0.479 | 1.146 | 0.291 |
| predation / personality | 1,38 | 0.034 | 0.469 | 0.498 |

*p < 0.05, **p < 0.01, ***p < 0.001.
marginally non-significant ($t_{25} = 1.836, p = 0.078$). Mean trait values (as opposed to individual trait changes) are shown in the electronic supplementary material, figure S3.

4. Discussion

We found that the magnitude of change in predator-induced morphology differed among treatments such that bold individuals induced a significantly greater morphological defence than shy individuals when exposed to a natural enemy. Furthermore, predator exposure increased growth rate in crucian carp in general, and bold fish tended to have a higher growth rate than shy fish. We show that exposure to predators can influence prey behaviour, with exposed fish becoming bolder than control individuals, despite that the degree of the behavioural plasticity did not differ between predator treatments. Finally, we found that personality-dependent behavioural plasticity occurs, with shy fish showing a stronger (relative and absolute) shift in behaviour than bold fish.

Our first major result was in line with our initial prediction: bold fish exhibit stronger morphological plasticity and express more pronounced defences than fish with shy personalities following predator exposure. Prior to exposure, there were no morphological differences between shy and bold fish, which highlights that the interaction between behaviour and predation on inducible defence expression was triggered by exposure to live predators. One explanation for these data is that the links we report between personality and morphological plasticity are adaptive, and that individuals that have a risky lifestyle compensate by investing in a more pronounced morphological defence. Ample data from a range of species,

Figure 2. Behavioural and morphological responses (mean ± 1 s.e.) in relative (left panel) and absolute (right, shaded panel) terms over the experimental period for bold or shy (personality) crucians held in the presence or absence of pike (predator), showing (a) change in maximum body depth, (b) change in body weight and (c) change in boldness scores.
including crucian carp, have shown that inducible defences increase survival probability at various stages in the predation sequence. In crucian carp specifically, a deeper body morphology can enhance escape locomotion [37] and increase handling time when an individual is caught [26], and in choice trials deep-bodied prey were less predated than shallow-bodied morphs [26]. Hence, compensating for a bold personality by investing more in morphological defences is likely to increase survival probability in systems where crucian carp co-occur with predators. Other studies have shown that bold individuals have an increased vulnerability to predation [25], which suggests that bold individuals would benefit particularly from enhanced defence expression. Finally, as the expression of a morphologically defended phenotype commonly incurs costs (in crucian carp, see [39,40]), selection should favour the strongest expression of inducible morphological defences in individuals for whom the risk of predation is highest (i.e. where paying such costs is worthwhile). Conversely, following this rationale, individuals with a shy personality and a lower risk of predation should invest less in costly morphological defences, as was also found in our experiment. Together, these factors may drive the coevolution of behavioural type and inducible defences to produce the patterns of potentially adaptive behavioural and morphological correlations we report here.

A complementary, mechanistic explanation could be that the links between boldness and morphological defence expression are part of a broader syndrome that includes a high growth rate. A number of commentators have argued that boldness may be linked with intrinsic growth rate (e.g. [41,42]), and indeed our data show a marginally non-significant trend towards bold individuals having a higher growth rate over the course of our experiment. Future work could test this idea more rigorously by monitoring the growth rate of individuals of different behavioural types under controlled conditions prior to predator exposure.

The exact physiological mechanisms underlying the extreme plasticity in body morphology in crucian carp remain hotly debated. One possibility is that chemical cues released from natural enemies such as pike directly elicit a physiological response that redirects energy normally used for other processes into rapid growth in body depth [36]. This idea is supported by the fact that experimental manipulation of food availability has relatively little effect on growth in body depth when compared with predator exposure [36]. Another possibility is that the inducible defence is indirectly mediated via energy-conserving behaviours (such as reduced swimming activity) elicited by predator exposure [12,43].

Our data also suggest a novel mechanism (‘phenotypic compensation’) to explain the puzzle of how personality variation can evolve and be maintained in wild populations, as enhanced defences can potentially help reduce the costs of having a bold personality type. The notion that animals with bold personalities engage in compensatory strategies to minimize the costs associated with this personality type has yet to be explored in detail. However, a recent study of seasonal migration in freshwater fish showed that bold individuals migrated from predator-rich lakes during the winter, whereas shy fish had a higher probability of remaining resident [22]. Hence, in this example, bold individuals may be taking compensatory steps to minimize their predation risk by investing in a migratory strategy during periods of low resource availability.

We also show that boldness, a key personality trait, is plastic and responsive to experience. Given that bold individuals might be more at risk of predation than shy individuals, the shift in relative emergence scores towards increased boldness under predation risk is seemingly counterintuitive. However, a number of studies have previously documented increased boldness as a result of exposure to fright stimulus (repeated ‘chasing’ with a net) or high predation pressure in both laboratory and field settings [27–29]. One explanation for these findings is that fitness-enhancing activities such as foraging and mating must be maintained under a range of environmental conditions, and individuals exposed to intense predation hazard must be willing to take greater risks for any given reward, leading to increased levels of boldness in predator sympatric populations [29].

Furthermore, we show evidence for personality-dependent plasticity, with shy individuals being both more plastic in absolute terms, and also having a greater behavioural shift in relative terms than bold fish. Personality-dependent plasticity is well documented (reviewed in [44]). In many cases, individuals with a bold behavioural type (also known as having a proactive coping style [45]) have been reported as showing reduced behavioural plasticity compared with shy, reactive individuals [46]. Our data support that personality type relates to individual plasticity, with shy fish exhibiting a greater absolute plasticity than bold fish following experimental exposure. While our data do not distinguish between different hypotheses to explain the source of this variation [44], they add to the growing body of data linking personality and behavioural plasticity. We also show significant overall behavioural consistency over four months for test fish (i.e. bold and shy individuals used in the experiment). An interesting finding from our study is that following predator exposure, shy fish became significantly bolder, such that there was only a marginally non-significant difference between the two groups in their post-treatment behaviour. This raises the question, why do shy fish not induce greater morphological defences? We cannot conclusively answer this question with our data; however, a potential explanation is that behaviourally plastic shy fish can adjust their behaviour to maximize their survival chances in a temporally and spatially patchy predator landscape in the wild, whereas behaviourally inflexible bold individuals must induce strong morphological defences to increase their fitness against predators. A next exciting step here would be to experimentally quantify fitness outcomes of different behavioural and morphological strategies.

From our data, it is possible to make predictions about the distribution of behavioural types across ecological gradients. One could predict that crucian carp from high-predation habitats should be bolder than those from low-predation habitats, owing to the link between boldness and defence expression (which should be under strong selection in high predation ponds). While we have no data to test this hypothesis in this species, a number of studies have shown that animals from high-predation populations differ in personality type from those from low-predation origins [27,47,48]. Hence, the relationship that we describe here between inducible defence expression and personality type may contribute to our understanding of the distribution of behavioural types across a heterogeneous landscape. The next critical step in this research direction requires more knowledge about the costs and benefits of different behavioural strategies under different ecological conditions (predator abundance, low and high competition, habitat structure), which we hope future work will elucidate.

Furthermore, recent discussions have addressed the potential ecological implications of personality variation in animal
populations [49,50]. Contemporary work has provided potential links as personality is implicated in shaping dispersal [51–53] and migration [22] processes, which can have powerful ecological effects. Here, we suggest an additional pathway via which personality variation can have ecological consequences, through its link with inducible defence expression variation. Inducible defences specifically, and phenotypic plasticity more generally, are thought to be important ecologically [54,55]. Here, we provide a putative link between individual behaviour (personality) and ecological processes such as population and food chain dynamics via inducible defence variation [56]. Further work to experimentally address the role of inducible defences and personality variation upon ecological processes is a critical next step in the development of these two previously isolated fields.

Ethical concerns on care and use of experimental animals were followed under permission (M165-07) from the Malmö/Lund Ethical Committee. The study complies with the current laws in Sweden.

Acknowledgements. We gratefully acknowledge colleagues on the Aquatic ecology unit for help with feeding the fish.

References


