**LETTER**

Natural selection, plasticity and the emergence of a behavioural syndrome in the wild

**Abstract**

Individuals often show consistent behavioural differences where behaviours can form integrated units across functionally different contexts. However, the factors causing and maintaining behavioural syndromes in natural populations remain poorly understood. In this study, we provide evidence for the emergence of a behavioural syndrome during the first months of life in wild brown trout (*Salmo trutta*). Behavioural traits of trout were scored before and after a 2-month interval covering a major survival bottleneck, whereupon the consistency and covariance of behaviours were analysed. We found that selection favoured individuals with high activity levels in an open-field context, a personality trait consistent throughout the duration of the experiment. In addition, a behavioural syndrome emerged over the 2 months in the wild, linking activity to aggressiveness and exploration tendency. These novel results suggest that behavioural syndromes can emerge rapidly in nature from interaction between natural selection and behavioural plasticity affecting single behaviours.

**Keywords**

Activity, aggressiveness, behavioural syndromes, boldness, fitness, natural selection, personality traits, phenotypic plasticity, state-dependent behaviour, survival.

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**INTRODUCTION**

Studies of selection on phenotypic variation in natural populations are essential for our understanding of evolutionary processes. Until recently, however, behavioural traits have been under-represented in such studies, which mainly have focused on morphology or life-history traits (Kingsolver et al. 2001). To some degree, this may be due to the concern that behaviour is a plastic trait and likely to change according to its context (West-Eberhard 1989). Animal personality, i.e. consistent individual differences in behaviour, accounts nonetheless for a large proportion of the variance (on average, \(~37\%) in behaviour in a wide range of taxa (Bell et al. 2009). In addition, behavioural traits like aggression and boldness often covary across functionally different contexts and form behavioural syndromes (Clark & Ehlinger 1987; Sih et al. 2004). However, although behavioural syndromes have been frequently documented (see Sih et al. 2004 for a review), it remains a challenge to explain how they are generated and maintained by evolution (Sih et al. 2004; Dingemanse & Réale 2005; Dingemanse & Wolf 2010).

Proximate explanations for behavioural consistency build to a large extent on mechanistic constraints that slow down or impede changes in behaviour (*constraint hypothesis*, Lande 1979; Bell 2005). Slow turnover rates in hormonal mechanisms and pleiotropic gene effects governing several behaviours collectively could thus result in behavioural syndromes (McGlothlin & Ketterson 2008). Alternatively, behavioural syndromes may result from selection favouring behavioural trait combinations that are adaptive in the current environment (*adaptive hypothesis*, Lande & Arnold 1983; Bell 2005).

One method to formally test between these hypotheses is to compare behavioural syndrome structure between populations living in different environments (Bell 2005; Dingemanse et al. 2007; Pruitt et al. 2010). Such comparisons support the adaptive hypothesis if covariance between behaviours follows a pattern predicted by the environmental gradient. Depending on the model system, studies following this approach supported either the constraint hypothesis (the spider genus *Anelosimus*, Pruitt et al. 2010) or the adaptive hypothesis (sticklebacks, Bell 2005; Dingemanse et al. 2007). In sticklebacks, behavioural syndromes proved more tightly linked in populations that are sympatric with predators. However, the role of predation, and other environmental conditions, in shaping behavioural trait correlations remains poorly understood. Behavioural syndromes could be generated by correlational selection, i.e. selection favouring trait covariance (Lande & Arnold 1983). Studies measuring how behavioural syndrome structure responds to selection can thus provide insight into the mechanisms shaping variation in behavioural syndromes. Bell & Sih (2007), for instance, showed that exposure of sticklebacks to trout predation under artificial laboratory conditions results in a positive correlation between boldness and aggressiveness. However, it remains unknown whether selection in the wild has similar effects (Dingemanse et al. 2007). This question is highly relevant, as the limited information available on links between single behaviours and fitness suggest that selection pressures differ considerably among laboratory and natural environments (Smith & Blumstein 2008; Adriaenssens & Johnson 2009).

Associations between behaviours such as boldness and aggression, as discussed above, are in concordance with recent models of animal personality suggesting that consistent behaviours can result from adaptive processes involving life-history trade-offs (Biro & Stamps 2008; Réale et al. 2010). Put simply, increased body growth and reproduction requires a more risk-prone behaviour to secure more resources (e.g. food or territory) at a cost of increased mortality (e.g. exposure to predators, parasites) (Biro & Stamps 2008). Individuals within a population can therefore differ in their ‘pace-of-life’, which may affect a whole suite of behavioural, physiological and life-history traits (Réale et al. 2010). Such links have been reported regularly from studies under captive conditions (Biro &
Stamps 2008; Smith & Blumstein 2008; Réale et al. 2010). It remains, however, to be tested whether such associations occur also under more variable natural conditions often characterised by fluctuating resource abundance and competition levels (Adriaenssens & Johnsson 2009; Réale et al. 2010).

Consistent individual differences in behaviour or life history can result from genetic polymorphisms and/or phenotypic plasticity. Although genetic differences generally play an important role in the expression of personality traits (Sundström et al. 2004), maternal effects and gene–environment interactions can further shape an individual’s behaviour throughout its lifetime (Carere et al. 2005; Stamps & Groothuis 2010). In addition, differences in ecological niches between life stages may cause ontogenetic shifts in behavioural traits and their linkage (Groothuis & Trillmich 2011). Estimating behavioural consistency is therefore important to determining whether personality traits are consistent over critical periods of selection. Most often, however, behavioural consistency has been measured over short time periods in the laboratory, whereas scores of more long-term consistency in the wild are scarce (but see e.g. Wilson & Godin 2009; Dingemanse et al. 2012). As consistency in behaviour may differ between captivity and the wild (Bell et al. 2009), more field experiments are needed to investigate the general ecological and evolutionary relevance of behavioural syndromes.

During their first months of life, brown trout experience a major selection bottleneck where mortality rates often exceed 95% (Elliott 1994). Predation and starvation are the main mortality factors during this early period. In a field study on stream-living brown trout, Alexander (1979) found that 45% of the juveniles (age 0–1 year) were killed by predators, including cannibalism from larger conspecics (16%). In addition, energy depletion during the establishment of feeding territories and transition to new food resources is important in determining growth and survival during this bottleneck (Kennedy et al. 2008). Stream-living salmonids defend positions from where they feed on a diverse community of prey items. Heterogeneous environmental conditions affect both the abundance of drifting prey and the costs of excluding territorial intruders and maintaining position in the current. Dominant individuals should therefore compensate high costs of protecting high-value territories with aggression and active foraging behaviour (high-cost/high-return strategy, Metcalfe 1986), whereas subordinates may opt for a low-aggressive, energy-saving, strategy in low-quality territories. Thus, territory choice in salmonids has been suggested to affect the payoffs of entire suites of behaviours including activity budgets, boldness and agonistic behaviour (Metcalfe 1986).

In this study, we examined behavioural consistency and its relation to fitness over the first months of life in a wild cohort of brown trout (Salmo trutta). The following specific questions were addressed: (1) To what extent are behaviours consistent over a 2-month interval in the wild? (2) Do consistent behaviours covary into behavioural syndromes? and (3) How do behavioural variation and syndrome structure relate to fitness (i.e. growth, mortality)?

METHODOLOGY

Capture, scoring and release

Eighty-one yearling brown trout were caught on the 10th of August 2010 by electric fishing (± 2.5 months after emergence, mean body size ± SE: 52.25 ± 0.59 mm). When applied properly, electric fishing is a low-harm sampling method (Aarset & Solberg 1981), and fish were monitored continuously for any external signs of discomfort. Trout were caught in a 150-m section of Stenungeån, a small coastal stream in western Sweden (N 58° 4', E 11° 52'). In the laboratory, fish were housed in a large holding tank (120 L, 40 × 48 × 64 cm) and fed ad lib with fine-cut frozen calf liver. The holding tank provided shelter (rocks and plastic plants) and fresh water from a flow-through filtration system (flow rate 2 L min⁻¹). Photoperiod followed natural day–light cycles and temperature was kept constant throughout the experiment (12–14 °C). The 13th of August, all individuals were anaesthetised (0.5 mL 2-phenoxethanol/l) and provided with a unique combination of three visible implant elastomer colours (fluorescent yellow, orange and blue, Northwest Marine Technology, Olsen & Vollestad 2001). Colours were injected under the skin at a shallow angle at each of four positions (adipose tissue behind the eye and upper caudal region, both sides). Fish resumed foraging 1 day after tagging and no tag loss or mortality was observed during the week following tagging. Between the 16th and the 20th of August, we scored each individual for behaviour, and subsequently all fish were released in one batch within the initial capturing section (20th of August).

Recapture and rescoring

A section ranging from 150-m downstream to 100-m upstream of the release location was sampled four times by electric fishing on the 13th and 14th of October. A shorter upstream section was chosen because a waterfaill starting 60-m upstream prevented fish from moving further upstream. The first sampling yielded 23 individuals, the second 5, the third 1 and the last no individuals. Recapture positions of all marked individual were noted in metres (down (−) or upstream (+) the release location, ± 1 m). Upon recapture, each individual was again anaesthetised and measured for body length (60.32 ± 1.05 mm). One individual died shortly after recapture. The remaining fish were transferred to the laboratory where they were maintained under similar conditions as described above, and after a short period of acclimation, rescoring for behaviour on the 22nd and 23rd of October. Trout do not develop secondary sexual characters until maturation (age > 1 year) and sex is therefore not taken into account.

Scores of behaviour

Behavioural trials occurred in batches of 12–20 individuals that were scored on separate days until all individuals were tested. The evening before observations, individuals were each transferred to one of 20 available experimental tanks and allowed to settle down (opaque white plastic tanks, 27 × 19 cm, water level 5 cm). Tanks were positioned in five sets of four underneath a dim fluorescent light tube and a camera (Logitech webcam C120, Romanel-sur-Morges, Switzerland) and shielded from each other with plastic screens to avoid disturbance before and during observations. The next day, we scored the behaviour of each individual in each of the following contexts: (1) an open-field test, (2) a novel-object test and (3) a mirror-image test (Fig. 1). We chose these assays because they describe important aspects of the behavioural strategies of brown trout; their general activity patterns, tendency to explore new resources and aggressive competition. Previous experiments on
trout fry have shown high interindividual variation in these behaviours (Sundström et al. 2004; Höjesjö et al. 2011).

Open-field test

In the first experiment, we scored the undisturbed swimming patterns of trout fry for 20 min in an open-field test (Fig. 1a, Hall & Ballachey 1932).

Novel-object test

During the next trial, we scored the individual’s tendency to explore a novel object (Fig. 1b, Wilson et al. 1993). Novel objects consisted of a bright-green aquarium suction plug with aeration T-piece and washer attached to it (ballast). The novel object was gently attached to the bottom surface in the centre of each experimental tank. Starting the following minute, we recorded the position of each individual for 20 min.

Mirror-image test

During the last trial, we used mirror-image stimulation to score the aggression level of each individual (Fig. 1c, Johnsson et al. 2003). Mirrors were cut to the form of the shorter side of the tank (17 × 17 cm) and fitted gently in the tank at ca. 3 cm from one of two short sides of tank (alternating between tanks). Again, observations were started the following minute and the position of each individual was scored for 20 min.

All observations occurred between 8:00 am and 5:00 pm on the day following transfer to the experimental set-up. All individuals were subjected to the experimental sequence in the same order. The timing of all procedures was carefully chosen to minimise long-term effects of laboratory habituation while allowing sufficient time to fish to recover stress to baseline levels after handling (Peeke et al. 1979; Arnekleiv et al. 2004). Mirrors and novel objects were removed immediately after 20-min exposure and individuals were allowed at least 1-h recovery between each observation to minimise stress and habituation. Fish were not fed in the experimental tanks until shortly after conclusion of the experiments. One hour after feeding, fish were anaesthetised, scored for body length and transferred back to the holding tank. Experimental tanks were then cleaned, filled with fresh water and a new batch of fish was entered until all fish were scored. The order in which different individuals were scored and their allocation to each of the 20 experimental tanks was fully randomised throughout the experiments.

Tracking principles and scores of behaviour

We used motion-tracking software to detect the position of fish four times per second (LoliTrackQuattro v 1.00, Loligo systems, Denmark). This software uses background contrast to detect the position of the fish in the visual field of the camera. Each camera observed individuals in the four experimental tanks of each set simultaneously and video images were calibrated after each trial (pixels to cm). Novel objects and mirrors were masked in the tracking software to allow continuous tracking of the position of fish in the setup without interference from the contrast of these objects (see Lolitrack manual for further details).

From the raw position and time data, we first calculated four scores to describe general patterns of activity in each of the three different contexts: (1) the proportion of time spent active, (2) the average swimming velocity (cm/s), (3) the coefficient of variance of swimming velocity as a measure of changes in activity through time and (4) the maximum swimming velocity (cm/s). We then calculated scores to describe specific behaviours for the open-field context (2 scores), the novel-object test (2 scores) and the mirror test (3 scores). These were the proportion of time the individual spent in the middle of the tank (open-field test), close to the novel object (novel-object test) or close to the mirror (mirror test). We also scored the distance the individual moved in this area for each context. A last score was the time spent accelerating in proximity of the mirror. Pilot experiments on a separate batch of fish confirmed that this latter measure was a good proxy of the number of manually scored attacks against the mirror (Pearson correlation; r = 0.86, n = 18, P < 0.001). Table 1 summarises all scores.

Data handling and statistics

We used correlation tests to assess consistency between scores of behaviour in August and October and how these relate to body length. Pearson correlation tests were used when original variables followed bivariate normal distribution or when a common sqrt or ln transformation resulted in normality (Shapiro–Wilx test, all P > 0.05, Table 2). Otherwise, correlation tests were performed on ranks (Spearman rank correlation tests). Changes in behaviour between August and October were assessed accordingly with paired t-tests or nonparametric Wilcoxon signed-rank tests. We then calculated ANOVA-based intraclass correlation coefficients as estimates of repeatability in absolute values (R) and their statistical significance based upon 10 000 permutations. Regular intraclass correlations are sensitive to differences in average values across sampling events. To illustrate how these affect repeatability, we also calculated adjusted repeatabilities using the same method, but with data centred to a...
Table 1 Overview of the separate behavioural scores used in the experiment

<table>
<thead>
<tr>
<th>Context</th>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tests</td>
<td>% time active</td>
<td>Proportion of time with velocity greater than 0 cm/s</td>
</tr>
<tr>
<td></td>
<td>Average velocity (cm/s)</td>
<td>Length of the trajectory/1200 s</td>
</tr>
<tr>
<td></td>
<td>Max. velocity (cm/s)</td>
<td>Maximum recorded velocity</td>
</tr>
<tr>
<td></td>
<td>CV velocity (cm/s)</td>
<td>Coefficient of variance of velocity</td>
</tr>
<tr>
<td>Open test</td>
<td>% time in the middle</td>
<td>Proportion of time spent more than 5 cm from the side of the aquarium</td>
</tr>
<tr>
<td></td>
<td>Distance moved in the middle (cm)</td>
<td>Distance moved more than 5 cm from the sides of the aquarium (cm)</td>
</tr>
<tr>
<td>Novel Object test</td>
<td>% time in proximity of the novel object</td>
<td>Proportion of time spent within a radius of 5 cm of the novel object</td>
</tr>
<tr>
<td></td>
<td>Distance moved close to the novel object (cm)</td>
<td>Total distance moved within a radius of 5 cm from the novel (cm)</td>
</tr>
<tr>
<td>Mirror test</td>
<td>% time close to the mirror</td>
<td>Proportion of time spent within 5 cm of the mirror</td>
</tr>
<tr>
<td></td>
<td>Distance moved close to the mirror (cm)</td>
<td>Total distance moved within 5 cm of the mirror (cm)</td>
</tr>
<tr>
<td></td>
<td>Total time accelerating close to the mirror (s)</td>
<td>Summed time observed with positive acceleration within 5 cm of the mirror</td>
</tr>
</tbody>
</table>

Table 2 Descriptive values for scores of behaviour at the two sampling moments, their difference and consistency between August and October

<table>
<thead>
<tr>
<th>Context</th>
<th>Score</th>
<th>August (n=81)</th>
<th>October (n=28)</th>
<th>Transformation</th>
<th>t/Z</th>
<th>P</th>
<th>t/rho</th>
<th>P</th>
<th>Repeatability</th>
<th>P (p adj)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Field</td>
<td>% time active</td>
<td>36.25 ± 2.74</td>
<td>68.85 ± 3.49</td>
<td>x</td>
<td>−5.177 &lt;0.001</td>
<td>0.494</td>
<td>0.008</td>
<td>0.160 (0.474)</td>
<td>0.190 (0.005)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average velocity (cm/s)</td>
<td>1.30 ± 0.14</td>
<td>2.85 ± 0.23</td>
<td>sqrt(x)</td>
<td>−5.697 &lt;0.001</td>
<td>0.673</td>
<td>&lt;0.001</td>
<td>0.319 (0.620)</td>
<td>0.047 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. velocity (cm/s)</td>
<td>21.91 ± 1.39</td>
<td>31.98 ± 1.81</td>
<td>x</td>
<td>−2.676 0.013</td>
<td>0.490</td>
<td>0.008</td>
<td>0.393 (0.487)</td>
<td>0.014 (0.004)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV velocity (cm/s)</td>
<td>216.15 ± 11.76</td>
<td>109.82 ± 6.69</td>
<td>sqrt(x)</td>
<td>4.354  &lt;0.001</td>
<td>0.385</td>
<td>0.043</td>
<td>0.052 (0.308)</td>
<td>0.372 (0.056)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% time in the middle</td>
<td>15.35 ± 2.28</td>
<td>58.65 ± 11.74</td>
<td>ln(x)</td>
<td>−4.583 &lt;0.001</td>
<td>0.326</td>
<td>0.091</td>
<td>0.068 (0.342)</td>
<td>0.359 (0.035)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distance moved in the middle (cm)</td>
<td>218.24 ± 22.20</td>
<td>454.98 ± 58.84</td>
<td>sqrt(x + 0.5)</td>
<td>−3.338 0.002</td>
<td>0.563</td>
<td>0.002</td>
<td>0.449 (0.576)</td>
<td>0.006 (&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>Novel Object</td>
<td>% time active</td>
<td>46.01 ± 2.16</td>
<td>54.5 ± 4.3</td>
<td>x</td>
<td>−1.516 0.141</td>
<td>0.360</td>
<td>0.060</td>
<td>0.340 (0.376)</td>
<td>0.034 (0.020)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average velocity (cm/s)</td>
<td>1.64 ± 0.11</td>
<td>2.18 ± 0.25</td>
<td>sqrt(x)</td>
<td>−1.766 0.089</td>
<td>0.514</td>
<td>0.005</td>
<td>0.486 (0.526)</td>
<td>0.003 (0.002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. velocity (cm/s)</td>
<td>22.34 ± 1.05</td>
<td>27.56 ± 2.13</td>
<td>x</td>
<td>−1.760 0.090</td>
<td>0.302</td>
<td>0.118</td>
<td>0.267 (0.316)</td>
<td>0.081 (0.049)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV velocity (cm/s)</td>
<td>158.62 ± 7.01</td>
<td>146.69 ± 16.16</td>
<td>ln(x)</td>
<td>1.046 0.305</td>
<td>0.296</td>
<td>0.126</td>
<td>0.293 (0.311)</td>
<td>0.060 (0.048)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% time close to the novel object</td>
<td>21.95 ± 2.60</td>
<td>21.23 ± 4.12</td>
<td>ln(x + 1)</td>
<td>−1.135 0.266</td>
<td>−0.021</td>
<td>0.914</td>
<td>−0.027 (~0.003)</td>
<td>0.556 (0.500)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distance moved close to the novel object (cm)</td>
<td>347.28 ± 34.26</td>
<td>412.98 ± 66.52</td>
<td>sqrt(x + 0.5)</td>
<td>−1.859 0.074</td>
<td>0.103</td>
<td>0.602</td>
<td>0.061 (0.121)</td>
<td>0.377 (0.266)</td>
<td></td>
</tr>
<tr>
<td>Mirror test</td>
<td>% time active</td>
<td>14.79 ± 1.57</td>
<td>22.72 ± 4.2</td>
<td>ranks</td>
<td>−0.865 0.387</td>
<td>0.340</td>
<td>0.076</td>
<td>0.330 (0.341)</td>
<td>0.040 (0.033)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Average velocity (cm/s)</td>
<td>0.51 ± 0.08</td>
<td>0.92 ± 0.19</td>
<td>ranks</td>
<td>−1.366 0.172</td>
<td>0.263</td>
<td>0.176</td>
<td>0.289 (0.413)</td>
<td>0.061 (0.050)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Max. velocity (cm/s)</td>
<td>25.09 ± 1.58</td>
<td>25.75 ± 2.55</td>
<td>x</td>
<td>−0.919 0.366</td>
<td>0.178</td>
<td>0.365</td>
<td>0.180 (0.195)</td>
<td>0.180 (0.156)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CV velocity (cm/s)</td>
<td>442.21 ± 21.98</td>
<td>426.45 ± 55.39</td>
<td>sqrt(x)</td>
<td>0.344 0.733</td>
<td>0.301</td>
<td>0.120</td>
<td>0.296 (0.298)</td>
<td>0.056 (0.055)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% time close to the mirror</td>
<td>65.80 ± 3.97</td>
<td>74.95 ± 6.41</td>
<td>ranks</td>
<td>−2.027 0.043</td>
<td>0.341</td>
<td>0.076</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Distance moved close to the mirror (cm)</td>
<td>465.17 ± 96.75</td>
<td>966.1 ± 230.27</td>
<td>ranks</td>
<td>−1.548 0.122</td>
<td>0.183</td>
<td>0.350</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total time accelerating close to the mirror (s)</td>
<td>73.16 ± 10.37</td>
<td>131.12 ± 27.69</td>
<td>ranks</td>
<td>−2.163 0.031</td>
<td>0.381</td>
<td>0.046</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Descriptive values represent Mean ± SE of untransformed scores. Difference and correlation for rank-transformed variables was analysed with Wilcoxon signed-rank test (Z) or Spearman ranked correlation test (rho), for other variables with parametric t- and Pearson correlation tests (r). Repeatability and statistical significance were calculated for the original transformed data (R and p) and data centred to a mean of 0 within each trial (R adj and p adj). For calculation of R and R adj, % time active and average velocity in the mirror test were also ln-transformed. P-values in bold remain significant after correction for false discovery rate.

mean of 0 within each trial (R adj see Nakagawa & Schielzeth 2010 for both procedures). R and R adj were only calculated when the error term of the ANOVA model using transformed data followed normal distribution. This assumption was met for 15 of 19 variables after using transformations stated in Table 2, and ln-transformation of % time active and average velocity in the mirror test. As repeated testing procedures result in increased probability of making type-I errors, we only discuss P-values significant after adjusting the critical α-value for false discovery rate (FDR) according to Benjamini & Hochberg (1995).

We used correlation coefficients to select the single behavioural score that was most consistent through time to explore cross-context correlations in behavioural scores (behavioural syndrome structure) and covariance between behaviour and performance in the wild. This score, average velocity, likely also best described general activity in each context. We then used Spearman rank correlation tests to assess covariance in average velocity across all three contexts in August and October. We used Fisher’s Z transformation to evaluate differences in correlation coefficients (Zar 1999).

Changes between correlation coefficients before and after release could be due to natural selection causing lower survival of individuals with specific trait associations (correlational selection). To test this, we used linear regression analysis to estimate standardised directional, quadratic and correlational selection gradients for scores of average velocity in August in each context and body length on relative fitness (Lande & Arnold 1983). In this model, partial regression coefficients of single traits (βi) reveal the importance of directional
selection, their quadratic terms ($\gamma_4$), the importance of disruptive or stabilising selection and cross-product terms ($\gamma_5$), the importance of correlational selection. We did this analysis for each score of fitness (survival and growth). Because scores of survival were categorical, we used logistic regression to test for significance of partial regression estimates (Janzén & Stern 1998). To further meet assumptions of normal distribution of residuals, we sqrt-transformed scores of average velocity in the open-field and novel-object tests and In-transformed scores of length and average velocity in the mirror test. All traits were then standardised and relative fitness was calculated by dividing absolute fitness by the mean. Initial models showed that body length did not affect fitness and this variable was therefore removed from further analyses to gain power (Wald $\chi^2 = 1.172$, $P = 0.279$ and all nonlinear selection gradients, NS). Nonlinear selection coefficients ($\gamma_4$ and $\gamma_5$) were obtained from the full model whereas directional coefficients ($\beta$) were obtained from the model excluding quadratic and cross-product terms. Quadratic regression coefficients were doubled according to Stinchcombe et al. (2008). Multicollinearity can be problematic in models that include highly correlated traits (Lande & Arnold 1983). We therefore inspected variance inflation factors in all models and in addition, repeated the entire regression procedure with models including either only average velocity in the open-field test or in the novel-object test, which produced similar results (not presented).

We repeated this procedure to estimate selection acting via growth. Phenotypic traits were restandardised using only the subset of individuals that survived. As growth is a continuous variable, statistical significance was assessed with linear regression. Relative growth was calculated from scores of growth in g/day that were transformed scores of length and average velocity in the mirror test. Average velocity during the mirror test was not correlated with these two measures (Table 3a, Fig. 2e and f). However, in October, all three scores of average velocity were correlated forming a behavioural syndrome across contexts (Table 3b, Fig. 2g–i). This was due to an increase in the correlation coefficients between average velocity in the open-field test and the mirror test (Table 3, comparison 2 vs. 5), and average velocity in the novel-object test and the mirror test (Table 3, comparison 3 vs. 6).

We use recapture rate as a proxy of survival in our experiment and outline below how we ruled out alternatives. Differences in behavioural syndrome structure could be due to natural selection resulting in a lower survival of individuals that are ‘inconsistent’ in their behaviour across the three contexts and/or by changes in behaviour due to ontogeny or phenotypic plasticity. Below we explore these possibilities.

### Phenotypic plasticity/ontogeny

Survivors behaved more actively in the open-field test in October, scores of average velocity in the open-field test roughly doubled between August and October (Table 2). No other significant changes in behaviour occurred between August and October. Also, individuals that changed their behaviour in one trait did not show correlated shifts in other traits. Thus, behavioural correlations resulted from between- rather than within-individual effects (Table S1).

### Selection on behaviour

We observed directional selection for behaviour, where individuals with a higher average velocity in the open-field test were more likely to survive (Table 4a). In addition, individuals with higher average velocity in the mirror test grew slower (Table 4b). Average velocity in the novel-object test did not affect growth or survival. There was no evidence for quadratic or correlational selection on these behaviours through growth or survival (Table 4 and supplement section 2).

### Recapture rates as a proxy of survival

The steep reduction in the amount of fish recaptured in the four samplings in October suggests that capture efficiency was high.

#### Table 3

<table>
<thead>
<tr>
<th>Correlation test</th>
<th>Context 1</th>
<th>Context 2</th>
<th>rho</th>
<th>$P$</th>
<th>$n$</th>
<th>Z</th>
<th>Comparison</th>
<th>ts</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) August</td>
<td>1 Open field</td>
<td>Novel object</td>
<td>0.536</td>
<td>&lt;0.001</td>
<td>81</td>
<td>0.618</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 Open field</td>
<td>Mirror test</td>
<td>0.080</td>
<td>0.479</td>
<td>81</td>
<td>0.108</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 Novel object</td>
<td>Mirror test</td>
<td>0.179</td>
<td>0.110</td>
<td>81</td>
<td>0.202</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) October</td>
<td>4 Open field</td>
<td>Novel object</td>
<td>0.641</td>
<td>&lt;0.001</td>
<td>28</td>
<td>0.782</td>
<td>1 vs. 4</td>
<td>−0.701</td>
<td>0.242</td>
</tr>
<tr>
<td></td>
<td>5 Open field</td>
<td>Mirror test</td>
<td>0.556</td>
<td>0.002</td>
<td>28</td>
<td>0.730</td>
<td>2 vs. 5</td>
<td>−2.377</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>6 Novel object</td>
<td>Mirror test</td>
<td>0.568</td>
<td>0.002</td>
<td>28</td>
<td>0.730</td>
<td>3 vs. 6</td>
<td>−2.019</td>
<td>0.022</td>
</tr>
</tbody>
</table>

*P*-values in bold are significant after correction for FDR

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Nevertheless, there are two alternative explanations to non-random survival, which could potentially explain non-random presence of specific behavioural phenotypes amongst recaptured individuals.

First, recapture efficiency could have been biased towards individuals with a specific behavioural phenotype (Wilson et al. 1993). Assuming that activity in the open-field test relates positively to activity in the field (Wilson & McLaughlin 2007), we would then expect individuals with a high average velocity in the open-field test to be caught more frequently in earlier electric fishing trials compared to later fishing trials. However, we found the opposite, but non-significant, trend for less active individuals to be caught earlier (t-test first vs. later trials, \( t = -1.788, \text{df} = 26, P = 0.085 \)). If anything, our method is thus biased towards catching inactive individuals more easily, and our estimate of the survival effects of behaviour should, in this respect, be regarded conservative.

Second, non-random movement of individuals within the stream may have resulted in higher migration of less active individuals outside the catching area. This is intuitively unlikely because less active individuals, if anything, would be expected to move less in the stream than more active conspecifics. However, there was no effect of aver-

**Figure 2** Figure showing behavioural consistency and covariation for all scores of average velocity in August and October. Consistency of behavioural scores across time is shown in panels (a–c). Across-context covariance in behaviour in August is shown in panels (d–f) and in October in panels (g–i). Dark points represent individuals that were not recaptured. Dotted lines represent significant correlations.
age velocity in any context on downstream movement (supplement, section 3). In addition, a waterfall 60-m upstream of the release position further obstructed upstream movement of released juveniles and no tagged individuals were caught more than 67-m downstream of the release position. This is concordant with other studies on similarly-aged brown trout, suggesting that juvenile brown trout rarely move far away from their release position (Højesjø et al. 2011).

We therefore conclude that our recapture-based estimate of the survival effects of behaviour is robust.

**DISCUSSION**

Our results show that interindividual differences in activity (open field) are highly consistent and result in survival differences in juvenile brown trout, with less active individuals suffering the highest mortality. Moreover, a behavioural syndrome emerged linking activity (open field) to aggressive (mirror image) and exploratory (novel object) activities over the first critical months of life. Previous studies suggest that behavioural syndromes are more pronounced in predation-exposed populations than in predator-naïve populations (Bell 2005; Dingemanse et al. 2007). Bell & Sih (2007) demonstrated that exposure of sticklebacks to predation in captive settings results in stronger associations between boldness and aggressiveness. The present results provide the first evidence for a similar role of natural selection and phenotypic plasticity in the emergence of behavioural syndromes in the wild.

Dingemanse & Réale (2005) suggested that the application of standard methods to investigate the importance of correlational selection on behavioural traits may shed more light on the evolutionary forces shaping behavioural syndromes. Despite this, the few studies applying these techniques found no evidence that correlational selection generates behavioural trait combinations (Bell & Sih 2007; this study). One potential reason for this is a loss of power when nonlinear selection gradients are added to the regression model. Other studies of nonlinear selection including morphological or life-history traits did, however, detect moderate correlational selection with similar sample sizes as in our study (see e.g. Kingsolver et al. 2001; supplement references). Whereas our study cannot entirely rule out weak correlational selection, we did not find any evidence for large effects as predicted from the large observed shifts in behavioural correlations. Instead, our results suggest that a behavioural syndrome after recapture emerged from the joint action of natural selection and phenotypic plasticity affecting single behaviours. Though difficult to evaluate statistically (this variable was not normally distributed), there was also a tendency for a higher variance in average velocity in the mirror test in October. Even when behavioural covariance was scored with ranked correlations (Table 3), changes in individual variation of behaviour may have further contributed to the emerging behavioural syndrome in our study.

Active individuals in the open-field test had the highest survival, and also increased activity between August and October. We cannot entirely exclude the possibility that habituation to the experimental procedures from August affected behaviour in the October trials. However, results on other fish species suggest that such habituation effects are unlikely to be retained over 2 months (Peake et al. 1979; Arnekleiv et al. 2004). We therefore think it is more likely that changes in behaviour occurred as a result of ontogenetic changes and/or in environmental changes in the wild between capture and recapture. Increased activity may reflect an ontogenetic shift to a different life stage associated with new behavioural requirements (Stamps & Groothuis 2010; Groothuis & Trillmich 2011). Increasing size over the experimental period may for instance have released juvenile trout from the risks of some gape-limited predators, allowing for a more active lifestyle during October (Godin 1997). Alternatively, salmonids may have adjusted their aggression and activity over time, responding to the resources and risks they experience in their habitat (Frost et al. 2007). Changes in activity resulting in stronger covariance in behaviours conform with earlier suggestions that the payoffs of activity and aggressive strategies become dependent on each other in heterogeneous environments like salmonid streams (Metcalfe 1986).

Activity in the open-field and novel-object test proved to be the most consistent behaviours over 2 months in the wild. Even when single behaviours are to some extent repeatable, behavioural correlations may still have emerged as a result of parallel shifts in pairs of behaviours of individuals over time (Dingemanse et al. in press). Further analysis showed, however, that such ‘within-individual correlations’ were of little importance for the behavioural correlations in our study, which instead were explained by stable correlations between individuals in average levels of behaviour in each context (‘between-individual correlations’, supplement section 1). These stable differences are likely caused by additive genetic, permanent environmental and/or maternal effects. Consistency in these behaviours and their covariance may indicate either that these were consistent in the initial, non-selected, population or that consistent individuals had a higher chance of survival. Most adaptive models of behavio-

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**Table 4** Standardised directional ($\beta_d$), quadratic ($\gamma_d$) and correlational ($\gamma_{ij}$) selection gradients for selection on scores of average velocity in each context through survival (a) and growth (b)

<table>
<thead>
<tr>
<th>Fitness score</th>
<th>Predictor variables</th>
<th>$\beta/\gamma$</th>
<th>SE ($\beta/\gamma$)</th>
<th>Wald $\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Survival (0/1)</td>
<td>Open field</td>
<td>0.472</td>
<td>0.184</td>
<td>5.988</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>Novel object</td>
<td>-0.290</td>
<td>0.186</td>
<td>2.435</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>Mirror test</td>
<td>0.027</td>
<td>0.150</td>
<td>0.035</td>
<td>0.853</td>
</tr>
<tr>
<td></td>
<td>(Open field)$^2$</td>
<td>0.236</td>
<td>0.459</td>
<td>0.228</td>
<td>0.633</td>
</tr>
<tr>
<td></td>
<td>(Novel object)$^2$</td>
<td>0.342</td>
<td>0.359</td>
<td>0.728</td>
<td>0.393</td>
</tr>
<tr>
<td></td>
<td>(Mirror test)$^2$</td>
<td>-0.032</td>
<td>0.287</td>
<td>0.000</td>
<td>0.997</td>
</tr>
<tr>
<td></td>
<td>Open field</td>
<td>-0.018</td>
<td>0.202</td>
<td>0.002</td>
<td>0.965</td>
</tr>
<tr>
<td></td>
<td>* Mirror test</td>
<td>0.179</td>
<td>0.198</td>
<td>0.992</td>
<td>0.319</td>
</tr>
<tr>
<td></td>
<td>* Mirror test</td>
<td>-0.142</td>
<td>0.275</td>
<td>0.162</td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td>* novel object</td>
<td>0.024</td>
<td>0.142</td>
<td>0.275</td>
<td>0.636</td>
</tr>
<tr>
<td>b) Growth (sqrt g/day)</td>
<td>Open field</td>
<td>0.077</td>
<td>0.080</td>
<td>0.349</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Novel object</td>
<td>0.082</td>
<td>0.085</td>
<td>0.344</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mirror test</td>
<td>-0.159</td>
<td>0.066</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Open field)$^2$</td>
<td>-0.107</td>
<td>0.230</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Novel object)$^2$</td>
<td>-0.259</td>
<td>0.241</td>
<td>0.298</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Mirror test)$^2$</td>
<td>-0.065</td>
<td>0.155</td>
<td>0.680</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Open field</td>
<td>-0.194</td>
<td>0.151</td>
<td>0.215</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* Mirror test</td>
<td>0.178</td>
<td>0.144</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* novel object</td>
<td>0.262</td>
<td>0.171</td>
<td>0.144</td>
<td></td>
</tr>
</tbody>
</table>

* $P$-values calculated with logistic regression models for fitness gradients for survival and from linear regression models for fitness gradients for growth. $\beta$ and $\gamma$ values are derived from linear regression coefficients in which quadratic regression coefficients, and their standard errors, are doubled (Stinchcombe et al. 2008). Significant $P$-values are denoted in bold.
ural consistency build upon the idea that behaviour is to some extent linked to individual state (Dingemanse & Wolf 2010). State variables describe those features of an organism that are important to take into consideration during behavioural decisions (e.g. age, fat reserves). Under this scenario, we can expect that behaviours that show associations with more stable state variables will be most consistent through time (Wolf & Weissing 2010). However, not all state variables, or their association with specific behaviours, are stable across similar time scales (Wolf & Weissing 2010). We suggest that more long-term studies are needed that simultaneously measure trait consistency across batches of behavioural traits and state variables to gain insight into when and how state variable(s) influence the emergence of personality traits and behavioural syndromes.

Across-individual variation in behaviour can be explained if alternative (heritable) behavioural strategies result in similar fitness. This could be due to fluctuating selection pressures and/or frequency-dependent selection that promote variation rather than similarity (Sih et al. 2004; Dingemanse & Réale 2005). Our results suggest that selective forces on behavioural syndromes in nature can be considerable, and shape behaviour within the time frame of a single generation. This may be a common pattern also in other species that experience strong selection bottlenecks during specific life stages. It should be noted, however, that not all observed variation is necessarily attributed to heritable differences, and the contribution of early experience and maternal effects to behavioural variation may be more important than previously thought (Stamps & Groothuis 2010), as demonstrated for juvenile brown trout (Höjesjö et al. 2011). In addition, experience (e.g. with predators) may affect the expression of genetic variation in behaviour, suggesting that the causes of between-individual variation in behaviour in nature can be complex (Dingemanse et al. 2009).

Behavioural scores from two functionally different contexts correlated with two separate fitness measures: less active individuals suffered the highest mortality and low aggressiveness resulted in faster growth. Previous research suggests that mortality and starvation are the most important mortality factors in stream-living juvenile brown trout. Starvation risk is a major cause of mortality, especially during the very early stages where nutritional reserves are minimal (Elliott 1994; Kennedy et al. 2008). Adult brown trout, mink (Mustela vison) and heron (Ardea cinerea) are the main predators on juvenile trout in our study area. In a river field study by Alexander (1979), these three species accounted for 31% of the mortality of juvenile brown trout (age class 0–1). Opposite to Bell & Sih (2007), we found that more active behaviour resulted in increased survival. Superior predation survival by more active or bold individuals has previously been observed in other species and stimulated discussion on whether active behaviour is an honest signal of escape ability (Smith & Blumstein 2010). We speculate that this may occur also when predation targets individuals in poor condition from starvation. Whereas it is often assumed that higher food intake in active or bold individuals results in faster growth at the cost of increased mortality risk, our results as well as several other recent studies challenge the view of stable links between active/bold behaviour and growth or mortality in the wild (Dingemanse et al. 2004; Meekan et al. 2010; Adriaenssens & Johnsson 2011; Höjesjö et al. 2011). In contrast, associations between behaviour and life-history traits appear to differ between age classes and environmental conditions. We argue that the mechanisms shaping behavioural variation in the wild are still poorly understood, which should stimulate further theoretical and empirical studies in this intriguing field of research.

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AUTHORSHIP

BA & JIJ designed the experiment. BA carried out the behavioural experiments and analysed the data. BA wrote a first draft and both authors were closely involved in the revision process of the manuscript.

REFERENCES


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