Shyness and behavioural asymmetries in larval zebrafish (*Brachydanio rerio*) developed in light and dark

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Summary

This is the first study analysing individual differences in shyness–boldness and behavioural asymmetries in young zebrafish larvae (fry, 7 days post-fertilisation). Individual differences were more stable in tests with predator model (crude image of a fish face) than with an arbitrary novel stimulus (vertical black stripe). Principal component analysis revealed a dimension of 'shyness' that involved the tendency of fry to avoid a predator model and reduced locomotion in its presence. The fry took longer to enter a novel environment and kept at greater distance when the stimulus was first seen with the left rather than right eye. Individual differences in eye use were consistent with either novel stimulus or predator model, but there was no correlation between these two contexts. Shyness correlated with left eye bias for viewing novel stimulus but not predator model. Development of eggs and larvae in darkness during the first six days after fertilisation increased shyness and reduced behavioural asymmetries in response to the predator model.

Keywords: boldness, shyness, fear, laterality, asymmetry.

Introduction

Individual differences were documented in a wide variety of animal species and in different contexts (Wilson et al., 1994; Sih et al., 2004). Individual differences in responses to novel and potentially dangerous stimuli, which have

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especially important fitness consequences, can be subsumed by such constructs as fearfulness, anxiety (Boissy, 1995), alternative coping strategies (Benus et al., 1991; van Oers et al., 2005; Carere et al., 2005), and shyness–boldness continuum (Wilson et al., 1994). These constructs have both heritable and experiential components (e.g., van Oers et al., 2005; Brown et al., 2007a; Frost et al., 2007), but their development remains poorly understood. Previous studies used adults or juveniles. It was unknown whether individual differences have any degree of consistency across measures and contexts at very early (larval) stages.

There is a growing interest in the relations between lateralisation, fear, and shyness–boldness. Both animal (Andrew & Rogers, 2002; Rogers, 2002; Vallortigara & Rogers, 2005) and human (e.g., Murphy et al., 2003) studies indicated that the right brain hemisphere is involved in fear. Lateralisation of the shyness–boldness continuum is, therefore, expected. However, the current evidence is contradictory, especially for fishes. For example, Dadda et al. (2007) did not find any relation between boldness and laterality in strains of a poeciliid fish *Girardinus falcatus* that were selectively bred for left, right or no turning bias in a detour test. In contrast, studies of another poeciliid *Brachyraphis episcopi*, which compared populations with high and low predation pressures, revealed a covariation between boldness (Brown et al., 2005) and laterality (Brown et al., 2007b).

Early exposure to light may represent an important non-genetic factor affecting behavioural and neural development. For example, in birds it causes the development of behavioural asymmetries (Rogers, 2002). Recently, similar effects were documented in larval zebrafish (Andrew et al., 2009): development of eggs and larvae until day 6 post-fertilisation reduced eye use asymmetries while viewing a mirror reflection. It would be interesting to know how early exposure to light may affect shyness–boldness and behavioural laterality in larval zebrafish.

This is, to our knowledge, the first study linking boldness and lateral asymmetries in larval zebrafish. The zebrafish is an important model for the study of brain and behaviour, and especially their development. Adult zebrafish have consistent individual and population differences in risk-taking (e.g., Moretz et al., 2007). Several lateral asymmetries were documented in both adults (Miklósi et al., 2001) and larvae (Watkins et al., 2004; Barth et al., 2005). Here we assessed (1) the consistency of boldness in response to arbitrary novel stimulus and predator model, (2) lateralised eye use and

response asymmetries, (3) relationships between boldness and laterality, and (4) possible effects of early light exposure on them.

Methods

Breeding zebrafish came from a pet store (Brighton, UK) and were maintained at 28°C on a 14 : 10 light/dark cycle. Three hours after fertilization, eggs were removed from the parental aquarium and transferred to white plastic boxes (140 × 80 × 50 mm). The eggs and larvae (fry) were maintained in these boxes at 28°C in groups of about 20. Six hours after fertilization eggs were divided into two experimental groups. The first group ('Light group', $N = 14$) was maintained under the normal 14 : 10 h light/dark cycle. The second group ('Dark group', $N = 14$) developed in darkness (<0.01 lux, Extech EasyView EA30 digital light meter). The eggs and fry of the Dark group were taken to light only for a short time (less than 2 min each time) every second day for maintenance, inspection and cleaning.

Development in the dark for the first 6 days after fertilisation meant that Dark fry had less sensory experience than Light. We believe that this had little or no effect on visual abilities. Bilotta (2000) showed that raising zebrafish in complete darkness until day 6 post-fertilization (dpf) had no effect on vision beyond a minor reduction in acuity at days 12–14 dpf. Constant dark rearing to 6 dpf has almost no effect on retinal anatomy and only slight effects on physiology (Saszik & Bilotta, 1999). Finally, in this and other studies (Andrew et al., 2009), Dark fry responded in different ways to different visual stimuli as readily as Light.

Experimental procedure

All tests used a white swim-way (320 × 125 mm) with seven compartments (50 × 40 mm) filled with water to a depth of 25 mm (Figure 1a). All adjacent compartments were connected by vertical 5 mm slits in the middle of the connecting walls. Two plastic bars were attached at each side of the slit to create a 9 mm long corridor (Figure 1b). Each compartment contained two lamps, which could not be directly seen by the fry. The lighting of each compartment was controlled by switches and a rheostat. A video camera could be slid along a glass sheet covering all the compartments and monitor each in turn. The whole apparatus was covered by black cloth to exclude

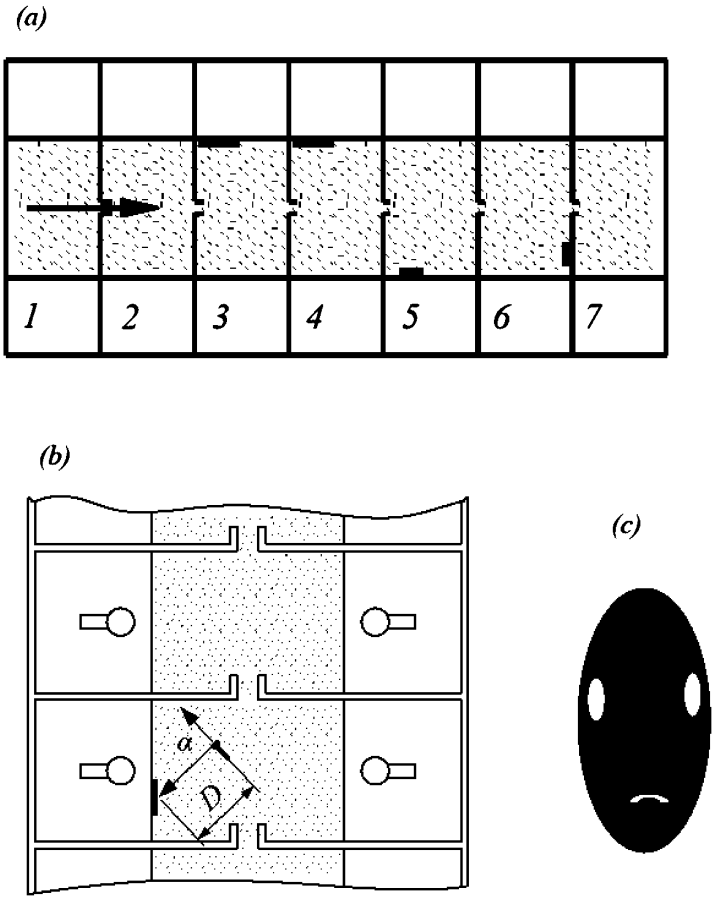

Figure 1. An outline of the experimental swim-way (a); a close-up view of individual compartments (b), with the position of the stimulus and a scheme of the measurements of the distances (D) and angles (α); (c) the predator model. Black bars in (a) and (b) show the position of the stimuli. Numbers and arrow in (a) point to the succession of tests.

light from other sources. Behavioural testing was on 7 dpf, when the yolk sac is fully absorbed. The dark developed fry were tested 4 hours after they were transferred to the light. The fry were not fed before testing. One fry was sucked into a large pipette (entrance diameter 6 mm), together with an adequate amount of water, and released gently into the first compartment of the swim-way, which was lit by the lamps. This procedure was not stressful and did not evoke unusual behaviour or elevated mortality.

At release, all compartments other than start were darkened. The fry was left undisturbed for two min. It was then video recorded in the compartment for 2 min. Subsequently the light in the start compartment was dimmed to darkness over 20 s. The video camera was immediately shifted to monitor the second compartment. Illumination was then similarly raised in this second compartment, which the fry entered under positive phototaxis.

After entry, the fry was left to explore for a further 1 min. Thereafter, the same sequence of changes in lighting was used to attract the fry into subsequent compartments. The next compartment (first stripe test) had a vertical black stripe (17 × 42 mm) at the closest end of the entry wall on the left or right side. The large size, high contrast against the white background and novelty made it a potentially dangerous object. The stripe was placed so that it could be seen with one eye before entry (Figure 1b), once the fry had arrived at the end of the short between-compartment corridor. After entry the fry was left to explore it for 1 min. A second similar stripe test followed.

The next test (fifth compartment) presented a crude predator model, which was a black oval with white eyes and mouth (10 × 20 mm, Figure 1c), placed similarly to the previous tests. Teleost fish respond to key stimuli of eyes and mouth as to a live fish predator (e.g., Altbäcker & Csányi, 1990). The model was also highly contrast against the background but had a different appearance than the black stripe. Its size and key features of a fish made it a potential predator for small zebrafish fry. However, it was still comparable to the stripe in the overall size, contrast and orientation (although did not present presumably more efficient cues like naturalistic appearance and movement). We expected that the fry would respond to this model differently than to the black stripe but not in a grossly dissimilar way (e.g., with strong escape or freezing).

The next compartment contained the same predator model, but it was positioned at the end wall of the compartment, so that the fry could see it from within the compartment, if appropriately positioned. Viewing before entry was of necessity more frontal. In the final test, the fry entered the seventh compartment, which was empty. Each fry was tested only once with stimuli placed either on left or right side in a random order. The water in the apparatus was at home tank temperature and was changed after each fry.

Because these tests were administered in a single array, they cannot be considered completely independent, but it is hardly possible to repeatedly

test very young and fragile larvae without seriously affecting them (parenthetically, individual marking is also hardly possible). The rate of development in fry is high and retesting them after several days would involve significant morphological and behavioural change. We, therefore, argue that the correlations across our tests provide reliable assessment of short-term consistency.

Behavioural measures and statistical analysis

We recorded two measures from videos, locomotion score (the number of 1×1 cm coordinate grid lines crossed per min), and the latency to emerge fully in each compartment. In the stripe and predator model tests, two measures were recorded every 10 s: the distance between the fry and the stimulus and the angle between the longitudinal body axis and a line joining the midpoint of the stripe or predator model and the eye viewing the stimulus (Figure 1). We subsequently used the minimum distance achieved and two different laterality indices. LI was given by the formula: $\mathrm{LI} = (R - L)/(R + L)$, where R and L are the number of occurrences of the stimulus on the right and left side of the fry. Calculation of the LI was limited to cases where the body axis angle was 15–150° (based on the angular density distribution). LI was positive when bias was to the right eye and negative for left eye bias. The absolute index $\mathrm{LI}_{\mathrm{abs}}$ was calculated to characterize the strength of the asymmetries irrespective of the side. In two cases (Light fry), full deciphering of the videos was impossible because of mist forming on the glass covering the swim-way, which resulted in a reduced sample size ($N = 12$).

Most p-values are two-tailed. One-tailed p-values were used in in the analysis of behavioural consistency to confirm predicted positive relationships in Dark and Light fry (any negative relationship would be equivalent to no consistency). We used ANOVA (exponentially distributed latencies were log-transformed) and permutation tests for comparisons between experimental groups.

Separate ANOVAs were calculated within each test because their interpretation was much simpler than possible repeated measures analysis across all tests that would involve complex higher-order interactions. This also avoided more complicated calculation of the error term because individuals were tested once with random right or left stimulus (incomplete design). Such an analysis did not account for behavioural changes across tests within the

same individuals, but this was not the main focus of the study. Also, no adjustment for multiple comparisons was made. It was justified because in this exploratory study we did not test a specific hypothesis. There is currently no consensus if, when and how Bonferroni adjustments should be performed, but most statisticians agree that adjustment for multiple tests is impossible in exploratory analysis with no pre-specified hypothesis (Perenger, 1998). We, however, used the Truncated Product Method (TPM) to assess the overall probability of Type-I error among significant p-values (Neuhaüser, 2004) and calculated the effect size ($r_{\text{equivalent}}$, Rosenthal & Rubin, 2003).

To assess whether the correlation matrix was suitable for the principal component analysis we calculated the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy (Dziuban & Shirkey, 1974). We used parallel analysis of random data (involving 100 random samples) for estimation of the number of principal components (Zwick & Velicer, 1986). In ANOVAs, an approximate calculation of power with our sample size allows detection of medium to large effects with the power exceeding 0.8. A correlation equal to 0.5 can be detected with the power of 0.8. The R software package (http://www.r-project.org) was applied for the data analysis.

Results

Behavioural asymmetries in Light and Dark fry

Two-way ANOVAs revealed behavioural asymmetries in the fry (Figure 2). At first encounter with the black stripe, the fry hesitated to enter the novel compartment more when the stimulus was on the left. They also avoided the left stripe (higher minimum distance). At second encounter, Dark fry avoided, whereas Light approached, the stripe on the left, and responded in the opposite way to the stripe on the right (Figure 2c). In predator model test (Figure 2d), Light fry avoided left stimulus (left/right difference in minimum distance: $p = 0.006$, permutation test) whereas Dark showed no asymmetry ($p = 0.66$, permutation test).

There was no population bias in eye use for viewing any stimulus: LI did not differ from 0.0 (all $p > 0.2$, one-sample t-tests). The only exception was a low (average LI $= 0.28$) but significant (one sample t-test: $t_{13} = 2.2$, $p = 0.048$) tendency for Dark fry to use the right eye during the first predator model test.

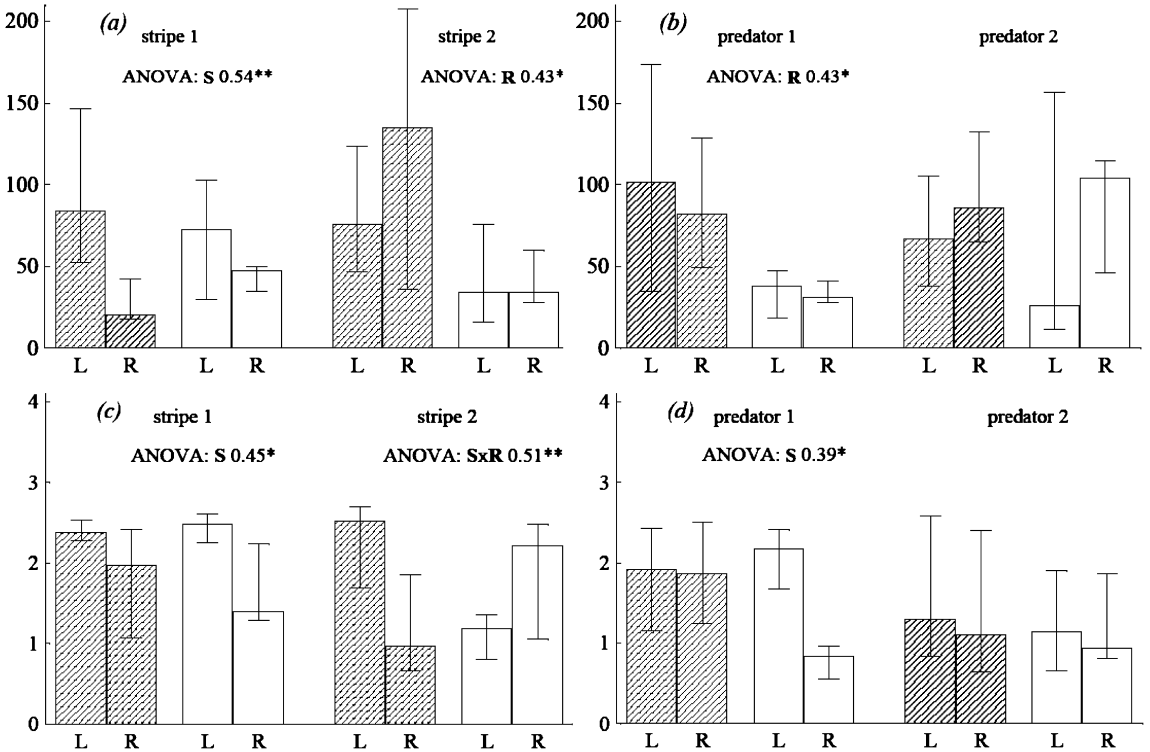

Figure 2. Behavioural asymmetries in the stripe (a, c) and predator model tests (b, d). Medians (bars) and 25–75% quartiles (whiskers) are shown. Hatched bars present Dark, open bars, Light fry. Symbols on the abscissa: L, left stimulus; R, right stimulus. Significant ANOVA effects: S = side, Left/Right position of the stimulus, R = regime of development (Light/Dark). TPM $p < 0.0009$, it is unlikely that all p-values are significant by chance. Numbers represent the effect size ($r_{\text{equivalent}}$) for significant effects; $^{*}p < 0.05$; $^{**}p < 0.01$.

Behavioural consistency

Locomotion scores were intercorrelated across the tests (standardised Cronbach $\alpha = 0.90$), which was true of Light ($\alpha = 0.77$) and Dark ($\alpha = 0.82$) fry. Consistency of other measures differed between tests (Table 1): latency to emerge and minimum distance were significantly correlated only in predator model tests. LI and LI_{abs} were correlated within both stripe and predator model tests.

Shyness in Light and Dark fry

Behavioural measures that significantly correlated across the same tests were subjected to the principal components analysis. These measures included: locomotion in the three open field tests, stripe test and predator model test, minimum distances to the predator model in two predator model tests, and latencies (log-transformed) to enter the novel compartment in the predator model tests. Parallel analysis indicated that only one component (accounting for 44.8% of the total variance) was necessary. We interpreted this factor as 'Shyness' (Table 2).

We also conducted two separate principal component analyses limited to behavioural measures (latency to emerge, locomotion and minimum distance) either from stripe or predator model tests. Analysis of the correlation matrix from the stripe tests indicated that no meaningful components analysis could be performed (KMO = 0.39, unacceptably low level according to

Table 1. Correlations between the two stripe and predator tests.

Measure	Total	Light[a]	Dark[a]
Stripe tests			
Latency to emerge	−0.12	0.36	−0.39
Minimum distance	−0.02	−0.21	0.11
LI	0.67***	0.57**	0.71***
LI_{abs}	0.41**	0.19	0.48**
Predator tests			
Latency to emerge	0.39**	0.24	0.53**
Minimum distance	0.45**	0.43**	0.46**
LI	0.35*	0.66**	0.28
LI_{abs}	0.41**	0.45*	0.45**

* $p < 0.07$; ** $p < 0.05$; *** $p < 0.01$; [a]one-tailed p-values.

Table 2. Principal component loadings (KMO = 0.75).

Test, measure	Shyness
Empty compartment	
Locomotion[1]	−0.42
Locomotion[2]	−0.24
Locomotion[3]	−0.67*
Stripe tests	
Locomotion[1]	−0.62*
Locomotion[2]	−0.77*
Predator model tests	
Locomotion[1]	−0.92*
Locomotion[2]	−0.87*
Minimum distance[1]	0.65*
Minimum distance[2]	0.66*
Latency to emerge[1]	0.74*
Latency to emerge[2]	0.49*

Superscripts show the test number; *interpretable loadings.

Dziuban & Shirkey, 1974). In contrast, the matrix coming from the predator model tests had good sampling adequacy (KMO = 0.80). Parallel analysis revealed a single factor (accounted for 58% of variance), with loadings almost identical to those in the main analysis.

Separate component analyses conducted in Light and Dark fry revealed almost identical patterns of factor loadings with the same single factor: the congruence coefficient between the two factor solutions was 0.89. Dark fry were characterized by significantly higher scores on the Shyness factor than Light ($t_{24} = 3.22$, $p = 0.003$).

Shyness and lateralised eye use

Correlated laterality indices were averaged across stripe and predator model tests, producing (1) laterality index LI in the stripe tests; (2) LI in the predator model test; (3) LI_{abs} in the stripe test; (4) LI_{abs} in the predator model test. There was no relationship between LI in the stripe and predator model tests; the same was true for LI_{abs} (all correlations $r < 0.2$, $p > 0.1$). Thus, whereas individual lateral asymmetries in eye use were consistent within each of the two behavioural domains (novel stimulus and predator model), there was no relation between them.

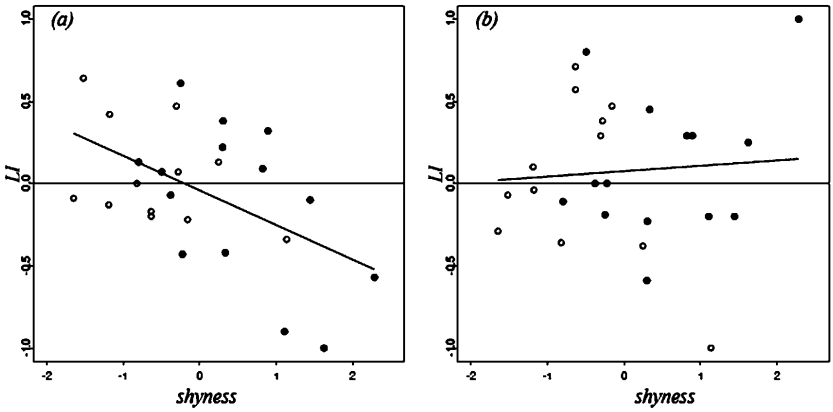

Figure 3. Relationships between *Shyness* factor and laterality index LI in the stripe (a) and predator model (b) tests. Open circles depict Light, black circles, Dark fry.

Shyness significantly correlated with the averaged LI in stripe ($r = -0.51$, $N = 26$, $p = 0.008$) but not predator tests ($r = 0.07$, $N = 26$, $p > 0.5$, Figure 3). It also correlated with LI_{abs} in both stripe ($r = 0.41$, $N = 26$, $p = 0.039$) and predator ($r = 0.41$, $N = 26$, $p = 0.040$) tests. The same pattern was characteristic of both Light and Dark fry. However, such correlations might be caused by higher locomotion and more random placement of bolder fry. To test this we calculated the principal components analysis excluding all locomotion scores (the results were virtually identical to the full analysis) and partial correlation coefficients between the factor and the laterality indices, adjusting for all locomotion measures and the effect of the light or dark development. Shyness still significantly correlated with LI in stripe (partial $r_{20} = -0.42$, $p = 0.05$) but not predator test (partial $r_{20} = 0.08$, $p > 0.1$). Relationships between LI_{abs} and Shyness were nonsignificant (stripe test: partial $r_{20} = -0.04$, predator test partial $r_{20} = 0.21$, both $p > 0.1$).

The correlations between Shyness and LI might also be inflated by a carryover effect of the left or right position of the stimulus. Partial correlation between Shyness and LI in the stripe test, adjusting for the left or right position of the stimulus revealed significant relationship (partial $r_{25} = -0.60$, $p = 0.001$), whereas partial correlation between Shyness and LI in the predator model tests adjusting for the left or right positions of the model was again nonsignificant (partial $r_{24} = 0.11$, $p > 0.1$). Thus, shyer individuals have a significant tendency to use their left eye when viewing the novel

black stripe. Bolder fry might use their right eye in this context, although this is masked by their higher locomotion.

Discussion

Individual differences

Overall, we found moderate correlations between the same measures scored in similar tests separated by only a few minutes in very young zebrafish fry. Our results indicated that behavioural consistency in larval zebrafish increases in situations involving predator risk. The results of Bell & Sih (2007) are interesting: exposure to predatory trout induced the correlation between boldness and aggression that was previously absent in sticklebacks (*Gasterosteus aculeatus*) from a low predation population. In our study, young zebrafish fry also responded differently to a black stripe and a comparable fish-like model: inter-trial correlations were not significant in the stripe but significant in the predator model test.

To our knowledge, this study is the first to provide evidence for shyness–boldness continuum in very young larval fish. However, this continuum was primarily expressed in situations involving predator features. There was no coherent factor pattern in stripe tests, which is not surprising given their inconsistency.

Shyness, the opposite of boldness (Wilson et al., 1994), is seen as the propensity to avoid risk: approaching a large fish-like object is risky, as is locomotion in this context. In our experiments, shy fry were characterized by lower locomotion, took longer to emerge in a novel compartment with predator model and did not approach it. However, the overall swimming pattern of the fry was qualitatively random, no unambiguous inspection behaviour to the stimuli were observed. Thus, the Shyness factor as found in very young zebrafish fry seems to involve a varying tendency to avoid rather than to approach dangerous stimuli. This probably reflects high vulnerability of fish larvae (with relatively underdeveloped sensory and cognitive apparatus and limited behavioural repertoire) to predators.

Behavioural asymmetries

This study adds to the growing literature documenting lateral asymmetries very early in zebrafish development (Watkins et al., 2004; Barth et al., 2005).

When a large novel stimulus was located on the left, zebrafish fry took longer to emerge in novel environment and kept at greater distance than when it was on the right. This pattern is consistent with the involvement of the left eye system (right hemisphere) in fear responses (see Andrew & Rogers, 2002; Rogers, 2002; Vallortigara & Rogers, 2005, for reviews). Human studies show that involvement of the right cerebral hemisphere is more likely to give intense emotional responses, especially if they are negative (e.g., Murphy et al., 2003).

A significant link was found between Shyness and the left eye system asymmetry in viewing of the black stripe. This would be expected if Shyness (the propensity to avoid risks) is associated with an internal state of fear, involving the left eye system. A similar correlation was found in horses: individuals which were more emotional and aroused in presence of novel objects, tended to preferentially use the left eye for novel object viewing (Larose et al., 2006). If this relationship is mediated by fear, it should remain high with predator model. However, we found no significant correlation between Shyness and left eye viewing of the predator model. One explanation may be that cognitive and emotional mechanisms of fear responses have not yet developed fully in very young larvae but the left eye system is still involved in assessment of novelty (Andrew & Rogers, 2002). Assessment of novelty might, therefore, be a more basic (or even evolutionary more ancient) function of the left eye system than fear.

There may be a link between consistent eye use asymmetries and coping strategies. If shyer individuals use passive coping (cf. Benus et al., 1991; also see Carere et al., 2005), they would be characterised by slow and thorough exploration involving the left eye system. Bold zebrafish may be fast and superficial explorers, less interested in novel stimuli (Benus et al., 1991; Carere et al., 2005). If bold fry tend to use the right eye for viewing, this could reflect sustaining a readiness to perform a planned response (Miklósi et al., 2001).

Effects of development in dark

Our study provides evidence that development in dark until 7 dpf significantly affects shyness in young zebrafish fry. The patterns of behavioural consistency and intercorrelations were almost identical in Light and Dark fry, but development in dark brought about an increase of Shyness. We found

some differences in behavioural asymmetries between Light and Dark fry. In the first stripe test, both Light and Dark fry avoided the left stimulus (although this seems more pronounced in Light). In the second test, Dark fry avoided the stripe on the left whereas Light, approached. We hypothesise that after assessment, Light fry may use the stripe as a landmark or refuge, with left eye dominance (left eye system is involved in spatial processing, Vallortigara & Rogers, 2005). Dark fry had no significant behavioural asymmetries in response to the predator model. This is unlikely to be a mere consequence of habituation because reduced behavioural asymmetries have been documented in Dark fry in the mirror test (Andrew et al., 2009) and in a single predator model test (unpublished data). Interestingly, Dark fry, showed a significant right eye viewing bias, indicating that lateralised eye use may be unrelated to response asymmetries.

The mechanism of light effects on zebrafish embryos is unknown. However, it is certainly different from that in chicks, in which embryonic head posture constrains light entering through the shell to the right eye (Rogers, 2002). Such constraint is impossible in largely transparent zebrafish embryos. We speculate that early epithalamic (especially parapineal) sensitivity to light is involved, since some behavioural asymmetries are reversed by epithalamic reversal (Barth et al., 2005). In any event, environmental factors such as light levels following egg laying that affect shyness and lateral asymmetries are potentially a source of inter-individual variation. It will be interesting to learn more about variations in the amount of light reaching eggs in natural habitats and, hence, possible ecological consequences of these effects.

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References

Altbäcker, V. & Csányi, V. (1990). The role of eyespots in predator recognition and antipredator behaviour in the paradise fish. *Macropodus opercularis* L. — Ethology 85: 51-57.

Andrew, R.J., Dharmaretnam, M., Gyori, B., Miklósi, A., Watkins, J. & Sovrano, V.A. (2009). Precise endogenous control of involvement of right and left visual structures in assessment by zebrafish. — Behav. Brain Res. 196: 99-105.

Andrew, R.J. & Rogers, L.J. (2002). The nature of lateralisation in tetrapods. — In: Comparative vertebrate lateralisation (Rogers, L.J. & Andrew, R.J., eds). Cambridge University Press, Cambridge, p. 94-125.

Barth, K.A., Miklósi, A., Watkins, J., Bianco, I.H., Wilson, S.W. & Andrew, R.J. (2005). *fsi* zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses. — Curr. Biol. 15: 844-850.

Bell, A.M. & Sih, A. (2007). Exposure to predation generates personality in threespined sticklebacks (*Gasterosteus aculeatus*). — Ecol. Lett. 10: 828-834.

Benus, R.F., Bohus, B., Koolhaas, J.M. & van Oortmerssen, G.A. (1991). Heritable variation for aggression as a reflection of individual coping strategies. — Experientia 47: 1008-1019.

Bilotta, J. (2000). Effects of abnormal lighting on the development of zebrafish visual behavior. — Behav. Brain Res. 116: 81-87.

Boissy, A. (1995). Fear and fearfulness in animals. — Q. Rev. Biol. 70: 165-191.

Brown, C., Jones, F. & Braithwaite, V. (2005). In situ examination of boldness–shyness traits in the tropical poeciliid, *Brachyraphis episcopi*. — Anim. Behav. 70: 1003-1009.

Brown, C., Burgess, F. & Braithwaite, V.A. (2007a). Heritable and experiential effects on boldness in a tropical peociliid. — Behav. Ecol. Sociobiol. 62: 237-243.

Brown, C., Western, J. & Braithwaite, V.A. (2007b). The influence of early experience on, and inheritance of, cerebral lateralization. — Anim. Behav. 74: 231-238.

Carere, C., Drent, P.J., Privitera, L., Koolhaas, J.M. & Groothuis, T.G.G. (2005). Personalities in great tits, *Parus major*: stability and consistency. — Anim. Behav. 70: 795-805.

Dadda, M., Zandonà, E. & Bisazza, A. (2007). Emotional responsiveness in fish from lines artificially selected for a high or low degree of laterality. — Physiol. Behav. 92: 764-772.

Dziuban, C.D. & Shirkey, E.S. (1974). When is a correlation matrix appropriate for factor analysis? Some decision rules. — Psychol. Bull. 81: 358-361.

Frost, A.J., Winrow-Giffen, A., Ashley, P.J. & Sneddon, L.U. (2007). Plasticity in animal personality traits: does prior experience alter the degree of boldness? — Proc. Roy. Soc. London B 274: 333-339.

Larose, C., Richard-Yris, M.-A., Hausberger, M. & Rogers, L.J. (2006). Laterality of horses associated with emotionality in novel situations. — Laterality 11: 355-367.

Miklósi, A., Andrew, R.J. & Gasparini, S. (2001). Role of right hemifield in visual control of approach to target in zebrafish. — Behav. Brain Res. 122: 57-65.

Moretz, J.A., Martins, E.P. & Robison, B.D. (2007). Behavioral syndromes and the evolution of correlated behavior in zebrafish. — Behav. Ecol. 18: 556-562.

Murphy, F.C., Nimmo-Smith, I. & Lawrence, A.D. (2003). Functional neuroanatomy of emotions: a meta-analysis. — Cogn., Affect. Behav. Neurosci. 3: 207-233.

Neuhaüser, M. (2004). Testing whether any of the significant tests within a table are indeed significant. — Oikos 106: 409-410.

van Oers, K., de Jong, G., van Noordwijk, A.J., Kempenaers, B. & Drent, P.J. (2005). Contribution of genetics to the study of animal personalities: a review of case studies. — Behaviour 142: 1185-1206.

Perenger, P.V. (1998). What's wrong with Bonferroni adjustments. — Br. Med. J. 316: 1236-1238.

Rogers, L.J. (2002). Lateralization in vertebrates: its early evolution, general pattern, and development. — Adv. Stud. Behav. 31: 107-161.

Rosenthal, R. & Rubin, D.B. (2003). $r_{\text{equivalent}}$: a simple effect size indicator. — Psychol. Methods 8: 492-496.

Saszik, S. & Bilotta, J. (1999). Effects of abnormal light-rearing conditions on retinal physiology in larvae zebrafish. — Invest. Ophthalmol. Vis. Sci. 40: 3026-3031.

Sih, A., Bell, A.M., Johnson, J.C. & Ziemba, R.E. (2004). Behavioral syndromes: an integrative overview. — Q. Rev. Biol. 79: 241-277.

Vallortigara, J. & Rogers, L.J. (2005). Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. — Behav. Brain Sci. 28: 575-633.

Watkins, J., Miklósi, A. & Andrew, R.J. (2004). Early asymmetries in the behaviour of zebrafish larvae. — Behav. Brain Res. 151: 177-183.

Wilson, D.S., Clark, A.B., Coleman, K. & Dearstyne, T. (1994). Shyness and boldness in humans and other animals. — Trends Ecol. Evol. 9: 442-446.

Zwick, W.R. & Velicer, W.F. (1986). Comparison of five rules for determining the number of components to retain. — Psychol. Bull. 99: 452-442.