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
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