Animal Behaviour xxx (2013) 1–6

Contents lists available at ScienceDirect

Animal Behaviour

journal homepage: www.elsevier.com/locate/anbehav

Aggression and sex differences in lateralization in the zebrafish

Tolulope O. Ariyomo*, Penelope J. Watt

Department of Animal and Plant Sciences, University of Sheffield, Sheffield, U.K.

ARTICLE INFO

Article history:
Received 29 January 2013
Initial acceptance 21 March 2013
Final acceptance 12 June 2013
Available online xxx
MS. number: 13-00094R

Keywords:
aggressive behaviour
Danio rerio
eye use
lateralization
zebrafish

Aggression plays an important role in survival and reproduction. It can be measured using mirror and dyadic tests, but there is some debate about whether interactions with a mirror image and with a real opponent measure the same aspects of aggressiveness. Variation in aggressiveness among individuals has been linked to behavioural lateralization. Lateralization, the preference for one side of the body over the other, has been reported widely in vertebrates. During aggression, individuals may use their right or left eye to view their opponent, but results vary among vertebrates; while some show a left-eye preference, others show a right-eye preference, with some individuals being more strongly lateralized than others within a population. In this study, we determined whether adult male and female zebrafish, *Danio rerio*, showed similar levels of aggression towards a mirror image as towards an opponent, and whether there were differences in eye use when the fish displayed aggressive behaviours. We found no difference in the rate of aggression shown towards a mirror image and an opponent, indicating that both tests are representative of the same measure. Furthermore, the sex of the zebrafish and the aggression test they experienced had a significant effect on eye use. Eye use by the females when viewing their opponent was similar to that of the males when they viewed an image and an opponent, but males used their left eye more.

Aggression plays an important role in reproduction and survival, and can involve actual or potential harm to another individual (Larson et al. 2006; Spence et al. 2008; Ariyomo & Watt 2012). Aggressive interactions are used to maintain dominance, defend territories, monopolize resources and mates, and protect offspring (Davies & Houston 1981; Stamps 1994; Larson et al. 2006; Spence et al. 2008; Nephew et al. 2010; Davis et al. 2011; Ariyomo & Watt 2012). Aggression towards a mirror image, assessed using the mirror test (Gerlai et al. 2000; Moretz et al. 2007; Hirschenhauser et al. 2008; Ariyomo et al. 2013), and towards an opponent, assessed via a dyadic contest (Larson et al. 2006; Oliveira et al. 2011), can be used to measure the rate of aggression. However, because the dyadic test measures the interaction of an animal with a conspecific, whereas in the mirror test the interaction is with an image, there has been some debate about whether individuals react in the same way and, then, whether these tests measure the same aspects of behaviour. Indeed, research has indicated that both brain activity and hormonal level can vary during aggression, depending on which test is used (Oliveira et al. 2005; Hirschenhauser et al. 2008; Desjardins & Fernald 2010; Oliveira & Canário 2011; Dijkstra et al. 2012), and so the relationship between the two tests remains inconclusive. Furthermore, there is little information on how the sex of an individual may influence its response to an image or opponent.

Variation in aggressiveness among individuals has been linked to differences in behavioural lateralization (Reddon & Hurd 2008). Lateralization, the preference for one side of the body over the other, is a phenomenon that is not peculiar to humans (Vallortigara & Bisazza 2002) but is also common in other vertebrates and in invertebrates, suggesting that the specialization of the left and right hemispheres of the brain for particular tasks is a taxonomically widespread characteristic (Vallortigara et al. 1999, 2011; Vallortigara & Rogers 2005; Sovrano & Andrew 2006; Frasnelli et al. 2012; Rogers et al. 2013). Furthermore, lateralization may be influenced by the sex of an animal because of underlying differences in the behaviour of males and females that may have selected for different patterns of cerebral lateralization (Reddon & Hurd 2008). Lateralization may manifest itself, for example, as the preferential use of the left and right eye for different tasks, and might confer on lateralized individuals the ability to do more than one task at once (Rogers et al. 2004; Dadda & Bisazza 2006a, b). Many vertebrates, both tetrapods and nontetrapods, share the same pattern of lateralization during aggressive behaviours towards conspecifics, with preferential use of the left side or left eye (Bisazza et al. 1998b; Rogers 2002). However, variability has been observed in the strength and the direction of eye use within and among populations when performing tasks (Brown et al. 2004; Bisazza & Dadda 2005; Reddon & Hurd 2008, 2009b), with some

* Correspondence: T. O. Ariyomo, Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, U.K.
E-mail address: tolulope.ariyomo@gmail.com (T. O. Ariyomo).

0003-3472/$38.00 © 2013 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.anbehav.2013.06.019

individuals being more strongly lateralized than others within a population. For example, studies of different populations have documented left-eye/right-hemisphere bias during aggressive encounters (Sovrano et al. 1999) while others have shown a right-eye/left-hemisphere bias (Bisazza & de Santi 2003). Additionally, in some species, the direction of lateralization in aggressive situations may vary among individuals within populations (Cantalupo et al. 1996; Vallortigara & Rogers 2005; Clotfelter & Kuperberg 2007; Arcadi & Wallauer 2011).

Individual variation in lateralization may occur within a population if there are frequency-dependent fitness costs and benefits associated with having left- or right-eye use bias in, for example, predator–prey interactions and shoaling (Bisazza et al. 2000; Vallortigara & Bisazza 2002; Vallortigara & Rogers 2005). Theoretically, alignment at the population level may occur as an evolutionarily stable strategy when individually asymmetric individuals benefit by coordinating some aspect of their overt behaviour with others within a population (Ghirlanda & Vallortigara 2004; Ghirlanda et al. 2009).

Zebrafish form dominance hierarchies and both sexes show aggressive behaviour (Spence et al. 2008; Paull et al. 2010) towards conspecifics. Aggressive interactions include chases, fast bouts of swimming towards an opponent (which usually precede biting), bites and displays with erect fins (Gerlai et al. 2000; Larson et al. 2006; Spence et al. 2008). The degree of expression of aggression may be influenced by factors such as size (Paull et al. 2010), rearing conditions (Marks et al. 2005) and habitat complexity (Basquill & Grant 1998), thus suggesting that aggression may be plastic, although it is often consistent within individuals (Ariyomo & Watt 2012). Lateralization in behaviour has been documented in both larval (Watkins et al. 2004; Sovrano & Andrew 2006) and adult zebrafish (Miklósi et al. 1998, 2001; Miklósi & Andrew 1999), but there is limited knowledge of whether and how this behaviour may differ between the sexes. Males and females have been found to show differences in the direction of lateralization in other species of fish (Reddon & Hurd 2008), and these differences may be influenced by external cues. For example, Reddon & Hurd (2009b) found that female convict cichlids, *Archocentrus nigrofasciatus*, were more strongly lateralized when responding to an appetitive stimulus (i.e. food) than an alarm cue, but the reverse was the case for the males. Consistency in eye use has been shown in the zebrafish when viewing different stimuli such as strange objects, plants, other fish species, a mirror and empty space (Miklósi et al. 1998), with initial right-eye use and switching to the left eye during subsequent viewing (Miklósi & Andrew 1999). However, little is known about eye use during aggressive encounters and whether this varies between males and females. In this study, we tested whether adult male and female zebrafish showed similar levels of aggression towards a mirror image as towards an opponent, and whether there were differences in left- and right-eye use when fish displayed aggressive behaviours towards a mirror image and an opponent. Given that both male and female zebrafish show aggression towards conspecifics (Spence et al. 2008; Paull et al. 2010), we expected the two sexes to show similar aggressive responses to their image and an opponent, and for their eye use during these interactions to be the same.

METHODS

Fish were obtained from stock maintained in the Department of Animal and Plant Sciences at the University of Sheffield, U.K. The zebrafish used in this study were adults over 10 months old. Females were separated from the males and the sexes were housed in 10-litre tanks (30 × 15 cm and 24 cm deep) in groups of 20 individuals per tank in a continuously filtered circulatory system. Water was centrally filtered and heated, kept at 27 °C with a 12:12 h light/dark photoperiod. Fish were fed with commercial food flakes and brine shrimp twice daily. The pH, temperature, nitrate, nitrite, ammonia and dissolved oxygen levels were checked routinely: pH was 6.8–7.2 mg/litre, temperature was at 26 ± 1 °C, while nitrate, nitrite, ammonia and dissolved oxygen levels were 24–26.4 mg/litre, 0.056–0.066 mg/litre, 0–0.1 mg/litre and 84–95%, respectively (Ariyomo & Watt 2012).

Aggression

Fish were sexed and standard length (standard length, measured from the tip of the snout to the caudal peduncle) was measured using callipers (Mitutoyo Digimatic CD6' CSX). Fish were then housed singly in their home tanks for 24 h prior to the start of the trials. Males and females were tested for aggression when presented with a mirror image and when presented with an opponent of the same sex (Desjardins & Fernald 2010). We tested 40 males and 40 females, assigning 20 individuals of each sex to either the mirror test group (20 males and 20 females, mean ± SE standard length $= 30.29 \pm 0.34$ mm and 29.24 ± 0.52 mm, respectively) or the opponent fish group (20 males and 20 females, mean ± SE standard length $= 30.40 \pm 0.37$ mm and 29.22 ± 0.49 mm, respectively). For the mirror test, a mirror (18.0 × 6.5 cm) was placed at the side of a rectangular tank (17.1 × 11.6 cm and 5.0 cm deep) filled with 600 ml of water (see Appendix Fig. A1a). A fish was added to the tank and left to acclimatize for 60 s. A piece of opaque Perspex was used to cover the mirror during acclimatization. Once the fish had acclimatized, the Perspex was removed and the aggressive interactions (displays with erect fins and fast bouts of swimming towards the image) that a fish conducted towards its mirror image were recorded using a digital camera over a period of 10 min. Fish were returned to their individual tanks once they had been tested.

Fish were left for 24 h and then tested in a second experiment for aggression towards an opponent of the same sex as the test fish (see Appendix Fig. A1b). Test fish were paired with opponent fish, which were the fish used in the mirror test, with similar rates of aggression (paired *t* test: females: $t_{19} = 0.56$, $P = 0.58$; males: $t_{19} = 0.58$, $P = 0.57$) and size (paired *t* test: females: $t_{19} = 0.29$, $P = 0.77$; males: $t_{19} = 0.67$, $P = 0.50$). This ensured that the test fish did not respond submissively to the opponent. The test fish and the opponent were each placed in rectangular tanks (17.1 × 11.6 cm and 5.0 cm deep) side by side, but the tanks were separated by opaque Perspex during the acclimatization period (60 s), after which the Perspex was removed and the number of aggressive interactions was recorded using a digital camera, as before. To ensure uniformity in hunger level, fish were fed only after the experimental trials on the day they were tested. Each opponent fish was used only once.

Trials were recorded for 10 min periods in both studies, in line with recommendations for testing aggression in adult zebrafish (ZFIN; http://zfin.org/).

None of the methods involved any regulated procedures and so ethical approval was not needed for the experiments. There was no contact between any of the test fish and the opponent fish during the experiments. Fish occasionally pushed against the side of the tank where the mirror was placed or where the opponent fish was visible, but none of the fish showed any signs of stress that would cause them harm or sustained any injury. Fish were returned to the stock tanks once all the trials had been completed.

Lateralization

The digital recordings of each of the male and female test fish interacting with a mirror image and an opponent were viewed

Please cite this article in press as: Ariyomo, T. O., Watt, P. J., Aggression and sex differences in lateralization in the zebrafish, Animal Behaviour (2013), http://dx.doi.org/10.1016/j.anbehav.2013.06.019