

Population variation in lateralized eye use in the poeciliid *Brachyraphis episcopi*

C. Brown*, C. Gardner and V. A. Braithwaite

*Institute of Cell, Animal and Population Biology,
University of Edinburgh, Edinburgh EH9 3JT, UK*
* Author for correspondence (culum.brown@ed.ac.uk).

Recd 03.05.04; Accptd 24.05.04; Published online 20.07.04

Differential use of each hemisphere of the brain for specific tasks is a widespread phenomenon that appears to have arisen in the early history of tetrapod lineage. Despite a high degree of conformity in the development of lateralization among the tetrapods, some variation exists. The mechanisms underlying this variation remain largely unresolved. We exposed fish from regions of high and low predation pressure to a series of visual experiences, including viewing an empty compartment, a novel object and a live predator. Fish from each region differed in their preferential use of each eye to view the scenes. For example, fish from high predation regions viewed a live predator by using their right eye, whereas fish from low predation sites showed no eye preference. These results suggest that the degree of lateralization varies between populations of the same species that have been exposed to different ecological/evolutionary pressures.

Keywords: lateralization; behaviour; predation; poeciliids

1. INTRODUCTION

Differential use of either side of the brain in a variety of cognitive functions (lateralization) was once thought to be unique to humans, but has now been traced to the very routes of the tetrapod lineage. Preferential eye use is ubiquitous among birds, reptiles, amphibians and fish (Deckel 1995; McKenzie *et al.* 1998; Sovrano *et al.* 1999; Dadda *et al.* 2003; see Andrew 2002 for a review). Current theories suggest that lateralization initially arose in response to the development of laterally placed eyes with little binocular overlap in the visual field and complete decussation at the optic chiasma. Lateralization persists today even in animals that have strongly binocular vision, suggesting that it conveys some selective advantage.

There have been several approaches to investigate lateralized behaviour in fish, all of which reveal consistent results (Facchin *et al.* 1999). The most commonly adopted approach is the 'detour test' where subjects are required to swim down a corridor as they approach an open field and detour to the left or the right to view a scene partly obscured behind an obstacle. When the scene consists of a shoal of the opposite sex or a predator, most individuals show a preference to turn to the left thereby using the right eye to fixate on the scene. When fish are

faced with a shoal of individuals of the same sex, or if the detour barrier causes them to lose sight of the stimulus goal, they turn to the right (Bisazza *et al.* 1999). Furthermore, lateralization is enhanced in females viewing a shoal of males if they have been deprived of male contact for two months (Bisazza *et al.* 1998).

A second approach is to record the angle at which fish view objects. Miklosi *et al.* (1997) reports that zebrafish use the right eye to inspect novel scenes but swap to the left eye on subsequent viewing. When mosquito fish, *Gambusia holbrooki*, are placed in a round arena with a predator in the middle they tend to swim in a clockwise direction, enabling them to fixate on the predator with the right eye (Bisazza *et al.* 1997). When their mirror image is on the left side, mosquito fish are more likely to inspect predators than when a mirror is placed on their right (Bisazza *et al.* 1999). Likewise, female poeciliids tend to fixate on conspecifics using the left eye (Sovrano *et al.* 1999). Male mosquito fish can also be induced to fixate on shoal mates with the left eye but only shortly after capture in a net when the motivation to shoal is highest (Sovrano *et al.* 1999). Taken together, these results indicate that the preferential use of either eye is not necessarily fixed even with an individual. However, stimuli resulting in an emotive response generally cause fish to view them using the right eye whereas other scenes are generally observed using the left eye (Bisazza *et al.* 1998).

There appears to be intriguing variation between species within the family Poeciliidae in the propensity for lateralization (Bisazza *et al.* 1997) and more widely among other families (Bisazza *et al.* 2000). Eye preference within a population is not absolute, rather a bell curve (shifted to the left or right) of the tendency use each eye exists (Facchin *et al.* 1999). One possible benefit of this variation is that it may prevent predators from guessing from which side prey are likely to approach or flee. Additionally, variation in eye preference would enable both predator and social fixation to be optimized within a shoal (Rogers 1989). This notion is supported by theoretical models exploring situations where groups of asymmetrical organisms must coordinate their behaviour (Ghirlanda & Vallortigara 2004). Although there is no evidence of population variation in lateralization due to geographical distribution of predators, this remains a tantalizing possibility. Left-side bias has been observed in the Mauthner neurons in goldfish (Moulton & Barron 1967), which is associated with the fast start anti-predator response. Lateralization of trunk muscle volume and predictable lateralized anti-predator responses, have been observed in other species (Cantalupo *et al.* 1995; Heuts 1999). There are documented cases where laterality fluctuates over time because of predator-prey interactions owing to frequency-dependent selection (Takahashi & Mori 1994). It follows that lateralization with respect to viewing of predators ought to fluctuate between species (Heuts 1999; Bisazza *et al.* 2000; Vallortigara 2000) and between populations of the same species that have been exposed to differing levels of predation pressure.

We exposed poeciliid fish from regions of high or low predation pressure to a test consisting of a control (an empty compartment), a novel object or a live predator placed behind a clear Perspex screen and determined which eye the fish relied on to view each scene. We proposed that fish from high predation areas view predators with their right eye and the novel and control scenes with

the left eye. Fish from low predation sites were expected to view all three scenes with their left eye.

2. MATERIAL AND METHODS

Fish were collected from four streams that run independently into the Panama Canal. Each of these streams contains a barrier waterfall. Above these falls only two species exist: the poeciliid *Brachyraphis episcopi* and killifish *Rivulus brunneus*. Below the falls a full complement of the fish fauna exists, including a multitude of predators (see Brown & Braithwaite 2004 for further details). We collected *B. episcopi* from high and low predation regions in each of the four streams. The fish were transported to the University of Edinburgh where they were housed in standard 90 cm × 30 cm × 30 cm aquaria. The benthos comprised river gravel, and filtration was achieved by use of a power filter. The fish were fed once a day using commercial flake food with occasional live food supplements. Lighting was provided by overhead fluorescent tubes maintaining a 12 L : 12 D cycle and room temperature was maintained at 26 °C.

The test apparatus consisted of an aquarium of identical size to that of the housing tank. At one end of the tank, a clear Perspex partition was placed. Directly behind this clear partition a second opaque partition was positioned so that it could be raised and lowered by means of a remote pulley. The partitions were located 20 cm from the end of the aquarium.

Four female fish were removed from a housing tank chosen at random, placed in the experimental tank and allowed to adjust to the new surroundings. Each group was then exposed to three treatments; an empty compartment ('control'), a live blue acara cichlid (*Aequidens pulcher*—'predator') and a large cross, made out of two sheets of bright green plastic ('novel object'). The treatment order was randomized and we allowed a 5 min rest interval between exposures. For each exposure, the opaque partition was raised for 5 min and lowered again, during which time a snapshot was taken of the arena every 20 s using a digital camera mounted overhead. The images were viewed on a computer screen and the angles of the fish relative to the Perspex partition were noted. We classified any fish orientated between 0 and 90° to be viewing the scene with the left eye and fish between 270 and 360° to be using the right eye (Sovrano *et al.* 1999). Only fish within 10 cm (3–4 body lengths) of the partition were included in the analysis, a distance that is consistent with predator inspection behaviour (Magurran & Seghers 1994). From the snapshot data we calculated the proportion of time that all fish within a group used their left eye to view each scene. A total of 36 replicate groups were tested.

Owing to the fact that we could not control the number of fish entering the viewing zone, the data were not distributed normally and had to be analysed by using non-parametric statistics. Thus, we combined the data for high and low predation areas from all four streams. Kruskal–Wallis tests were conducted to examine the effect of treatment (exposure to the blank novel object or cichlid) within each location (high or low predation). Comparisons between locations for each treatment were conducted using Mann–Whitney *U*-tests, as were deviations from the expectation of equal left and right-eye use.

3. RESULTS

Fish from high and low predation sites differed in their visual responses to the three treatment scenes. A significant effect of the three treatments was detected in the high predation fish (Kruskal–Wallis: d.f. = 2, $H = 7.331$, $p = 0.0256$), whereas no effect was evident in the low predation fish (d.f. = 2, $H = 3.786$, $p = 0.1566$). Fish from high predation sites viewed the blank scene more often using their left eye than low predation fish (Mann–Whitney *U*-test: $U = 4$, $Z = 2.013$, $p = 0.0441$). There was no difference in which eye was used to view the novel object ($U = 5.5$, $Z = 1.625$, $p = 0.1042$), and low predation fish used their left eye to view the predator more often than high predation fish ($U = 54$, $Z = 2.006$, $p = 0.0449$).

The proportion of time that fish from high predation regions relied on their left eye to view the novel object and the right eye to view the predator differed significantly

from random (Mann–Whitney *U*-tests: $U = 4$, $Z = 2.675$, $p = 0.0075$; $U = 52.5$, $Z = 2.702$, $p = 0.0069$, respectively). The use of the left eye to view the blank scene did not differ from random. The low predation fish only showed a significant preferential use of the right eye to view the blank scene ($U = 8$, $Z = 2.006$, $p = 0.0448$). Generally, fish from high predation areas tended to use their left eye to view the control and novel object whereas they used their right eye to view the cichlid predator. The low predation showed the opposite trend (figure 1).

4. DISCUSSION

Fish that have experienced either high or low predation pressure differed in their preferential eye use to view objects behind a clear partition. This is the first documented case of population variation in lateralized behaviour in any animal and our results highlight the considerable importance of predation pressure on the development of lateralized behaviour patterns. Only fish from high predation regions showed significant responses to the three treatments (control, novel object and live predator). Fish from high predation areas viewed the live predator as a potential threat, invoking an emotive response causing them to assess the predator using the right eye. They showed no significant preference for either eye while viewing a control scene and a left-eye preference for viewing the novel object. The predator should not evoke such a response in predator-naïve populations who ought to view them as novel objects (Brown & Warburton 1997) and consequently view them using the left eye. Contrary to our expectations, fish from low predation regions showed a significant preference for the right eye when viewing a blank scene; the reason for this remains unresolved. No significant preference for either eye was shown when viewing the novel object and the predator.

Heuts (1999) suggested that differential exposure to predators owing to the occupation of varying habitats (benthic versus limnetic) might explain interspecific variation in lateralized escape responses in fish. Likewise, intraspecific variation in eye use has also been explained by shoaling versus non-shoaling habits (Rogers 1989; Bisazza *et al.* 2000), which may also vary with predation pressure and other environmental variables (Brown & Warburton 1997). Our results strongly suggest that variation in predation pressure plays a key role in the evolution of lateralized behaviour in fish. In shoaling species, individuals must be able to simultaneously monitor both predators and shoal mates. Such responses are unlikely to evolve in populations that have had no contact with predators. Furthermore, predators are unlikely to result in an emotive response in these predator naïve populations. Lateralization may enable animals to concentrate on two tasks simultaneously such as foraging and predator vigilance (Rogers *et al.* 2004). If lateralization initially evolved to cope with different information coming from each eye, resulting in left and right hemisphere specialization of information processing and a reduction of the potential negative effects of divided attention (Griffiths *et al.* 2004), then variation in the level of exposure to predators may have affected the degree of specialization in the two brain hemispheres. Future studies using a detour test may help clarify this point further.

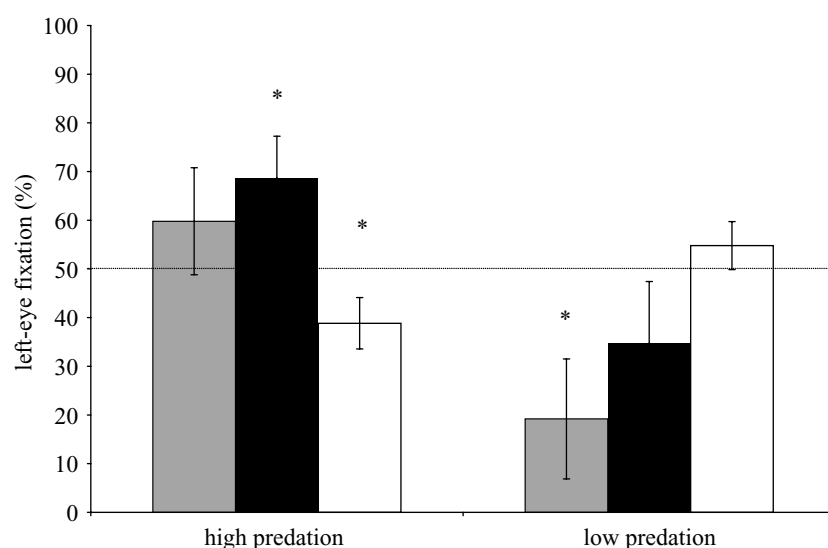


Figure 1. The average ($\pm$ s.e.m.) proportion of time that fish from areas of high and low predation pressure spent fixating on the blank scene (grey bars; control), a novel object (black bars) and live predator (open bars) by using the right eye. Values greater than 50% represent preferential left-eye use, whereas those less than 50% represent preferential right-eye use. Asterisks represent significant deviations from random.

Acknowledgements

This research was conducted under ANAM permit no. 31503 and under Home Office licence number PPL60/2954. The authors thank the Smithsonian Tropical Research Institute and NERC (A/S/01/00608) for their continued support and funding. They thank Biff Birmingham for his help in identifying fish species, Michael Jennions for his aid in identifying field sites, Fiona Burgess for her help with husbandry, and Felicity Brown for her help collecting fish and other field data.

- Andrew, R. J. 2002 The earliest origins and subsequent evolution of lateralisation. In *Comparative vertebrate lateralisation* (ed. L. J. Rogers & R. J. Andrew), pp. 70–93. Cambridge University Press.
- Bisazza, A., Pignatti, R. & Vallortigara, G. 1997 Laterality in detour behaviour: interspecific variation in poeciliid fish. *Anim. Behav.* **54**, 1273–1281.
- Bisazza, A., Facchin, L., Pignatti, R. & Vallortigara, G. 1998 Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behav. Brain Res.* **91**, 157–164.
- Bisazza, A., De Santi, A. & Vallortigara, G. 1999 Laterality and cooperation: mosquito fish move closer to a predator when the companion is on the left side. *Anim. Behav.* **57**, 1145–1149.
- Bisazza, A., Cantalupo, C., Capocchiano, M. & Vallortigara, G. 2000 Population lateralisation and social behaviour: a study with 16 species of fish. *Laterality* **5**, 269–284.
- Brown, C. & Braithwaite, V. 2004 Size matters: a test of boldness in eight populations of the poeciliid, *Brachyraphis episcopi*. *Anim. Behav.* (In the press.)
- Brown, C. & Warburton, K. 1997 Predator recognition and anti-predator responses in the rainbowfish *Melanotaenia eachamensis*. *Behav. Ecol. Sociobiol.* **41**, 61–68.
- Cantalupo, C., Bisazza, A. & Vallortigara, G. 1995 Lateralization of predator-evasion response in a teleost fish (*Girardinus falcatus*). *Neuropsychologia* **33**, 1637–1646.
- Dadda, M., Sovrano, V. A. & Bisazza, A. 2003 Temporal pattern of social aggregation in tadpoles and its influence on the measurements of lateralised responses to social stimuli. *Physiol. Behav.* **78**, 337–341.

- Deckel, A. W. 1995 Lateralisation of aggressive responses in *Anolis*. *J. Exp. Zool.* **272**, 194–200.
- Facchin, L., Bisazza, A. & Vallortigara, G. 1999 What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behav. Brain Res.* **103**, 229–234.
- Ghirlanda, S. & Vallortigara, G. 2004 The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proc. R. Soc. Lond. B* **271**, 853–857. (DOI 10.1098/rspb.2003.2669.)
- Griffiths, S. W., Brockmark, S., Höjesjö, J. & Johnsson, J. I. 2004 Coping with divided attention: the advantage of familiarity. *Proc. R. Soc. Lond. B* **271**, 695–699. (DOI 10.1098/rspb.2003.2648.)
- Heuts, B. A. 1999 Lateralization of trunk muscle volume, and lateralization of swimming turns of fish responding to external stimuli. *Behav. Process.* **47**, 113–124.
- McKenzie, R., Andrew, R. J. & Jones, R. B. 1998 Lateralisation in chicks and hens: new evidence for control of response by the right eye system. *Neuropsychologia* **36**, 51–58.
- Magurran, A. E. & Seghers, B. H. 1994 Predator inspection covaries with schooling tendency amongst wild guppy, *Poecilia reticulata*, populations in Trinidad. *Behaviour* **128**, 121–134.
- Miklosi, A., Andrew, R. J. & Savage, H. 1997 Behavioural lateralisation of the tetrapod type in the zebrafish (*Brachydanio rerio*). *Physiol. Behav.* **63**, 127–135.
- Moulton, J. M. & Barron, S. E. 1967 Asymmetry in the Mauthner cells of the goldfish brain. *Copeia* **4**, 836–837.
- Rogers, L. J. 1989 Laterality in animals. *Int. J. Comp. Psychol.* **3**, 5–25.
- Rogers, L. J., Zucca, P. & Vallortigara, G. 2004 Advantages of having a lateralized brain. *Proc. R. Soc. Lond. B* (Suppl.) Published online 14 May 2004. (DOI 10.1098/rsbl.2004.0200.)
- Sovrano, V. A., Rainoldi, C., Bisazza, A. & Vallortigara, G. 1999 Roots of brain specializations: preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behav. Brain Res.* **106**, 175–180.
- Takahashi, S. & Mori, M. 1994 Unstable evolutionary strategy and oscillation: a model of lateral asymmetry in scale eating cichlids. *Am. Nat.* **144**, 1001–1020.
- Vallortigara, G. 2000 Comparative neuropsychology of the dual brain: a stroll through animals' left and right perceptual worlds. *Brain Lang.* **73**, 189–219. (DOI 10.1066/brln.2000.2303.)