



# Lateralization in response to social stimuli in a cooperatively breeding cichlid fish

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## ABSTRACT

Cerebral lateralization, an evolutionarily ancient and widespread phenomenon among vertebrates, is thought to bestow cognitive advantages. The advantages of lateralization at the individual-level do not necessarily require that the entire population share the same pattern of lateralization. In fact, directional bias in lateralization may lead to behavioural predictability and enhanced predator success or prey evasion. Recent theory has suggested that population-level lateralization may be favored if individuals are better able to perform coordinated behaviours, providing a distinct advantage in cooperative contexts. Here we test whether the highly social, cooperatively breeding cichlid fish *Neolamprologus pulcher* shows lateralized responses to a social stimulus. We found population-level biases in males; on average male *N. pulcher* use their right eye/left hemisphere to view their mirror image. Individual females had a preferred hemisphere, but these preferences appeared not to be directionally aligned among females. We discuss these results in the context of coordinated social behaviour and suggest future research directions.

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## 1. Introduction

Lateralization of cerebral function, a widespread vertebrate phenomenon, is thought to be an evolutionarily ancient adaptation (Vallortigara et al., 1999; Rogers and Andrew, 2002; MacNeilage et al., 2009). Cerebral lateralization can confer certain cognitive benefits upon those individuals that possess it, for example, individuals with lateralized nervous systems are better able to attend to multiple stimuli simultaneously (Rogers et al., 2004; Dadda and Bisazza, 2006) and may have generally superior cognitive functioning (Magat and Brown, 2009). The demonstrated advantages of cerebral lateralization do not require that the direction of lateralization be aligned at the population-level, and would accrue to an individual regardless of direction (Vallortigara and Rogers, 2005; Vallortigara, 2006). In the absence of additional selective forces, there should be an equal number of left and right lateralized individuals in the population.

Directional alignment of lateralization at the population-level (a ratio of left lateralized to right lateralized individuals that departs from 1:1) may in fact carry certain disadvantages (Vallortigara and Rogers, 2005; MacNeilage et al., 2009). The resulting sensory or behavioural biases may lead to predictable behaviour at the population-level that could be exploited by predators or prey (Vallortigara, 2006). For example, toads (*Bufo spp.*) show stronger escape responses to a simulated predator presented to their left

visual field (Lippolis et al., 2002). This bias may leave them susceptible to attack from the right side, resulting in a vulnerability that a predator may learn to exploit. Nevertheless, population-level directional biases in cognitive processing are common (Rogers, 2002), and require an additional explanation over and above the existence of advantages of lateralization at the individual-level (Vallortigara and Rogers, 2005).

Ghirlanda and Vallortigara (2004) tackled this problem with a game theoretical model and showed that population-level biases in cerebral lateralization are selected when individuals interact regularly with conspecifics that share the same direction of lateralization (Vallortigara and Rogers, 2005). Animals may be better able to coordinate actions with conspecifics that are lateralized in the same way. For example, fish that shoal may be able to form more cohesive groups if most of the shoal members are similarly lateralized in terms of the hemisphere used to process social information. Consistent with this idea, Bisazza et al. (2000) found that among 16 taxonomically diverse species of fish, species that shoal tended to be lateralized at the population-level. However, species that did not readily form shoals were more likely to be lateralized at the individual-level only. In addition, Anfora et al. (2009) have shown that a hive forming social bee (*Apis mellifera*) exhibits population-level lateralization, while a related solitary bee (*Osmia cornuta*) has individual-level laterality only. If Ghirlanda and Vallortigara's model is generally applicable, then it ought to apply to a wide variety of social organizations.

*Neolamprologus pulcher* are a substrate spawning cichlid fish endemic to Lake Tanganyika, Africa. Although *N. pulcher* do not swim in shoals, they live and breed in permanent social groups

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(Taborsky and Limberger, 1981). Each group consists of a single breeding pair, who monopolize most or all of the reproduction in the group, and 1–20 subordinate group members who help at the nest (Balshine et al., 2001; Heg et al., 2005; Stiver et al., 2009). Helpers assist the dominant pair's breeding efforts by repelling intruders, clearing debris away from the breeding site and assisting in direct brood care (Taborsky and Limberger, 1981). These activities often require the coordinated actions of multiple group members. Repelling intruding conspecifics, space competitors or predators, in particular, is likely to be most effective when individuals act together, as these actions are both energetically costly and dangerous (Grantner and Taborsky, 1998).

In this study, we examined eye use lateralization using *N. pulcher* viewing a simulated social stimulus. We predicted that the remarkable social complexity of this species will have led to the evolution of population-level lateralization and that the fish would preferentially use one eye over the other to view social stimuli.

## 2. Materials and methods

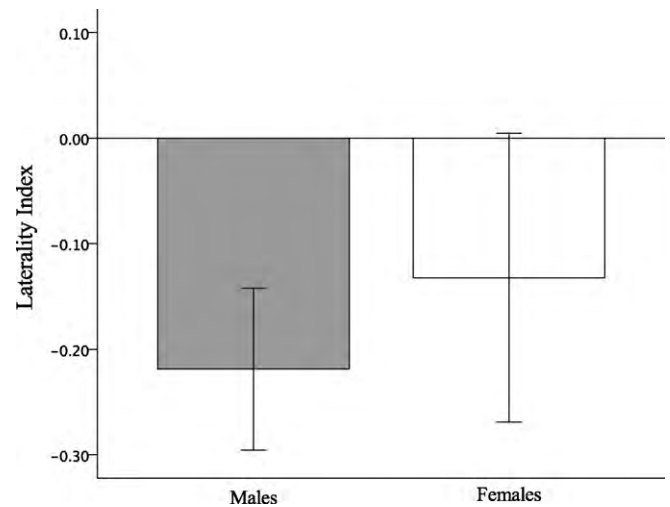
Forty adult *N. pulcher* (24 males, 16 females) were used for this study, all were laboratory reared descendants of animals collected from Lake Tanganyika, Africa. Fish were housed in 200 L glass aquaria, each of which contained a single social group. Social groups consisted of a breeding pair and several (mean = 11.6; range = 5–16) helpers of varying size (SL range = 1.0–7.0 cm), mimicking the social conditions observed in the wild (Balshine-Earn et al., 1998; Balshine et al., 2001). As focal fish, we used the four largest fish in each of 10 social groups (both breeders and the largest two helpers). All fish were sexually mature (mean SL  $\pm$  SEM = 6.0  $\pm$  1.1 cm; range = 4.5–8.6 cm). Water was maintained at 26  $\pm$  1 °C and within the chemical parameters found in the species' natural habitat. A 14L:10D light cycle was maintained in the lab. Fish were fed *ad libitum* six times a week with dried and frozen prepared cichlid food.

Subjects were tested for lateralization using a large octagonal mirror arena (70 cm  $\times$  70 cm  $\times$  30 cm; Sovrano et al., 2001) filled with water to a depth of 11 cm. A series of eight square mirrors (30 cm  $\times$  30 cm) were aligned in an octagonal arrangement creating an unbroken mirrored surface. An octagonal opaque plastic start box (35 cm across) with a single removable door was situated in the center of the test arena, equidistant from all mirrors.

Each trial began by gently netting a fish out of its social group and placing it in the start box. Each fish was given a 5 min acclimation period in this start box before the door was removed allowing the fish access to the mirror arena. If the fish had not exited the start box within 5 min of the door being raised ( $n = 33$ ), it was gently coaxed towards the opening using a net. All fish readily responded to the mirrors upon leaving the start box and there was no obvious difference in behaviour between the fish that were and were not coaxed. Once the fish began interacting with its mirror image, it was allowed to do so for a period of 8 min. Trials were filmed from above for subsequent analysis. Following the 8 min trial, the fish were removed, sexed by examination of external genital morphology and returned to their social group. Each fish was tested only once.

We scored the tapes for time spent in monocular fixation during the trial. Like most fish, *N. pulcher* have laterally placed eyes with very little overlap in their optic fields. In fish, each eye projects almost entirely to the contralateral hemisphere and measuring asymmetry in eye use is a well established method for assessing lateralized processing in the brain (reviewed in Vallortigara and Bisazza, 2002). We calculated a laterality index (LI) for each fish using the following formula:

$$LI = \frac{\text{time left eye} - \text{time right eye}}{\text{time left eye} + \text{time right eye}}$$



**Fig. 1.** Mean  $\pm$  S.E. laterality index for male (grey bar) and female (white bar) *Neolamprologus pulcher*. Positive scores indicate a preponderance of left eye/right hemisphere use, negative scores indicate a preponderance of right eye/left hemisphere use.

We also calculated the absolute value of LI, as extreme values of LI in opposite directions will cancel out when averaging, which may obscure individual strength of lateralization (Brown et al., 2007). Neither LI nor absolute LI differed significantly from normality (Kolmogorov–Smirnov tests:  $Z_s = 0.99, 1.31$  respectively,  $p > .05$ ) so the data were analyzed using parametric statistics.

## 3. Results

There were no differences among the social groups in average LI or average absolute LI (LI:  $F_{(9,30)} = 1.15, p = 0.36$ ; absolute LI:  $F_{(9,30)} = 0.44, p = 0.90$ ) and no differences were observed between breeding fish and helpers for LI or absolute LI measures (LI:  $t = -1.07, df = 38, p = 0.29$ ; absolute LI:  $t = 0.19, df = 38, p = 0.85$ ) so we combined fish of different social status and from different social groups for all subsequent analyses. Males and females did not differ in the laterality index ( $t = 0.6, df = 38, p = 0.57$ ). The LI for males and females combined was significantly left biased (one-sample  $t = -2.6, df = 39, p = 0.013$ ) indicating that *N. pulcher* preferred to use their right eye (left hemisphere) to view their mirror image. Visual inspection of the data suggested that leftward bias in males might have driven this result. When we examined the sexes separately the laterality index for males remained on average significantly left biased (one-sample  $t = -2.85, df = 23, p = 0.009$ ; Fig. 1) while average LI for females showed no such directional bias (one-sample  $t = -0.97, df = 15, p = 0.35$ ; Fig. 1). Female *N. pulcher* may not be lateralized at the population-level when inspecting their mirror image, although they did show a non-significant trend in the same direction.

Males and females did not differ significantly in absolute LI ( $t = 0.83, df = 38, p = 0.41$ ) and the average absolute LI for males and females combined significantly differed from zero (one-sample  $t = 6.19, df = 39, p < 0.001$ ). When examined separately both male and female fish had absolute LI scores that differed from zero (males: one-sample  $t = 4.7, df = 23, p < 0.001$ ; females: one-sample  $t = 4.0, df = 15, p = 0.001$ ; Fig. 2) indicating that both sexes show significant individual-level lateralization for viewing social stimuli. There was no correlation between LI or absolute LI and body size as measured by either standard length (LI:  $r = 0.06, df = 38, p = 0.69$ ; absolute LI:  $r = 0.03, df = 38, p = 0.28$ ) or body mass (LI:  $r = 0.01, df = 38, p = 0.94$ ; absolute LI:  $r = 0.08, df = 38, p = 0.61$ ).

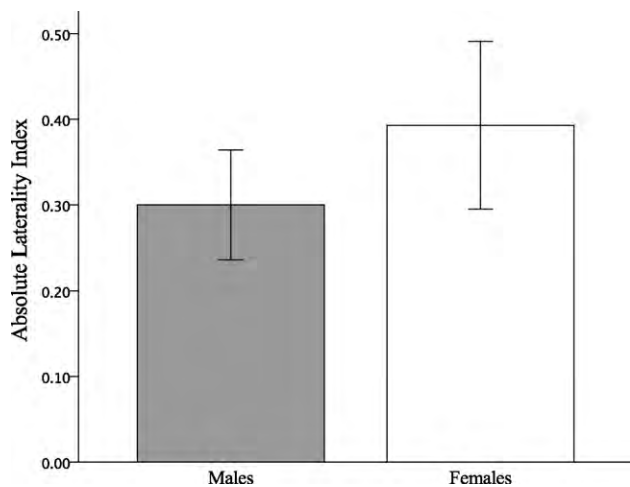


Fig. 2. Mean  $\pm$  S.E. absolute laterality index for male (grey bar) and female (white bar) *Neolamprologus pulcher*.

#### 4. Discussion

Males of the highly social cooperatively breeding cichlid fish *N. pulcher* showed a significant population-level lateralization for viewing social stimuli. Males preferentially used their right eye and hence their left hemisphere when viewing their mirror image. Females may not have a significant population-level preference for eye use, however, both sexes showed biases at the individual-level. In other words, individual fish of both sexes prefer using a particular eye for viewing their mirror image, but these preferences appear to be aligned at the population-level in males only.

Most of the other cichlid fishes tested to date have been shown to have individual-level lateralization only and do not exhibit shoaling behaviour (e.g. *Archocentrus nigrofasciatus*, Reddon and Hurd, 2009a; *Geophagus brasiliensis*, Reddon et al., 2009; *Pelvicachromis pulcher*, Reddon and Hurd, unpublished data). However, the one cichlid included in the Bisazza et al.'s (2000) study, *Pterophyllum scalare*, did exhibit shoaling behaviour and was significantly right hemisphere biased when viewing a model predator. Among those fish that do show directional lateralization for viewing social stimuli, the left eye (right hemisphere) is more commonly favored (Sovrano et al., 1999, 2001). However, there are some exceptions (e.g. De Santi et al., 2000; Brown et al., 2007) notably, Bisazza and De Santi (2003) found that three different species of fish showed a preference for their right eye (left hemisphere) when displaying aggressively to their mirror image.

Sex differences in lateralization are a common finding (Bianki and Filippova, 2001), and have been observed in other studies on cichlid fishes (Reddon and Hurd, 2008, 2009b; Reddon et al., 2009). Nevertheless, it is somewhat puzzling that males exhibit the predicted population-level directionality while females appear to be lateralized at the individual-level only. There are a number of potential explanations for this finding. First, it is possible that females too tend to use their right eye and hence left hemisphere to inspect their mirror image, and this bias is simply weaker in females. Our data do show a pattern in that direction, however this trend did not approach significance ( $p=0.35$ ) and our sample size for females ( $n=16$ ) was comparable to the samples used in Bisazza et al. (2000), albeit with different methods. However, when tested directly against each other, male and female *N. pulcher* did not differ significantly in LI and when males and females were analyzed together, the whole sample showed a significant leftward bias, lending support to this interpretation.

It is also possible that the laterality task was interpreted differently by males and females. Bisazza et al. (1998) found that in

two species of poeciliid fish (*Gambusia hoolbroki* and *Girardinus falcatus*), males and females did not differ in their lateralization when viewing non-social stimuli, however, sex differences emerged when social stimuli were used. Males of both species showed directional biases to view female but not male conspecifics. Females by contrast, showed directional tendencies to view female conspecifics, but showed directional tendencies to view males only after a period of sexual deprivation. Bisazza et al. (1998) suggest that this sex difference is a result of differences in the social and sexual motivations of males versus females in those species. It is possible that female and male *N. pulcher* differ in their motivation to interact with a simulated same sex conspecific (their mirror image), and this asymmetry in motivation is responsible for the differences we observed.

A third possibility is that the observed differences in lateralization are a result of ecological/life history differences between the sexes. In *N. pulcher*, females are typically the philopatric sex and are more likely to inherit the breeding position in their natal group. Males tend to be the dispersing sex and are less likely to inherit the breeding position in their natal group (Stiver et al., 2004, 2006). Perhaps population-level lateralization is beneficial to males but not females because males are more likely to join or form new social groups, and hence be thrust into cooperative situations with unfamiliar individuals. *N. pulcher* from different social groups will also infrequently form temporary loose aggregations and move together between territories in a colony (S. Balshine, *per obs*). Directional alignment of lateralization may be valuable in such situations. These potential explanations are not necessarily mutually exclusive. For example, differences in the life history trajectory of males and females may give rise to motivational differences that produce the observed pattern of results.

Our data for males fits well with the predictions of Ghirlanda and Vallortigara's model (2004). As predicted, males on average have a directional lateralization for viewing simulated social fellows. Furthermore, some males did show a less typical left eye (right hemisphere) bias. When a population includes some individuals that are lateralized in the converse way, the costs of being less able to efficiently interact with the majority phenotype may be counteracted by the intrinsic virtue of being less common (Ghirlanda and Vallortigara, 2004). These conversely lateralized animals would still enjoy all of the privileges of lateralization at the individual-level and may additionally have an advantage in dealing with predators or prey animals, as predators and prey would have less experience coping with their phenotype. Recently, Ghirlanda et al. (2009) have expanded the initial Ghirlanda and Vallortigara model to include competitive interactions with conspecifics. In this situation, having the rarer lateralization phenotype may also be advantageous, as their behaviour will be less familiar to competitors.

In conclusion males but perhaps not females of the highly social, cooperatively breeding cichlid fish, *N. pulcher* are lateralized at the population-level. Males and females both show individual-level lateralization. Future research should strive to uncover the ecological consequences of lateralized functioning in complex social systems, in particular, the effect of lateralization on the stability and structure of social groups. A fruitful direction for future research should be to examine the relationship between individual variation in direction and strength of lateralization and the individual variation in social behaviour in this species.

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