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Animal Cognition

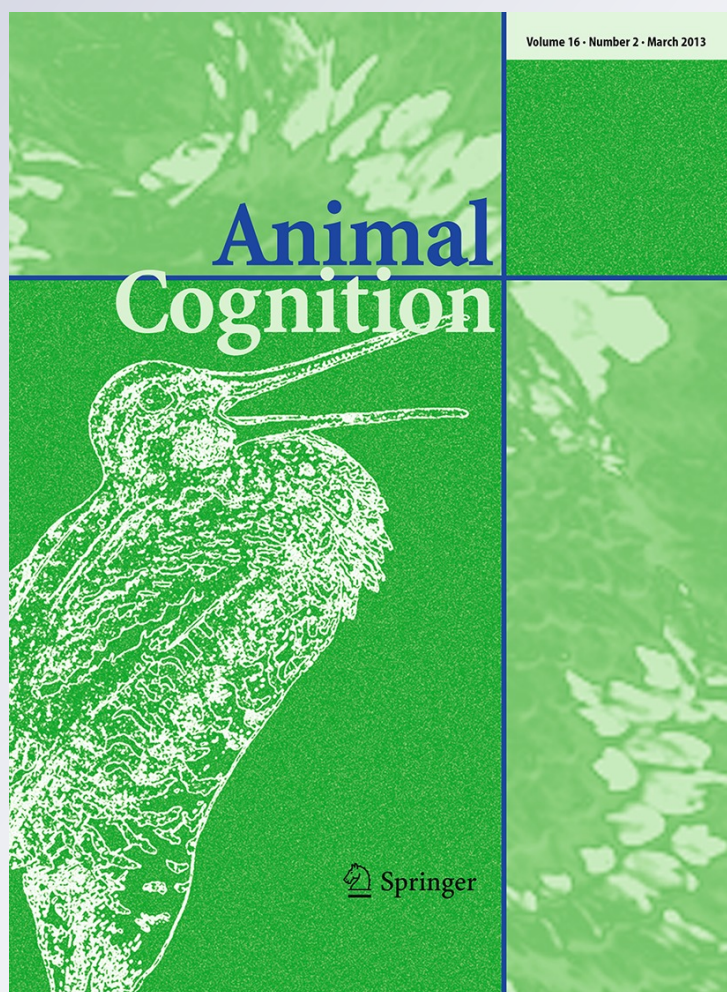
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Eye as a key element of conspecific image eliciting lateralized response in fish

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Abstract Visual lateralization in different aspects of social behaviour has been found for numerous species of vertebrates ranging from fish to mammals. For inspection of a shoal mate, many fishes show a left eye–right hemisphere preference. Here, we tested the hypothesis that in fish, there is a key cue in the conspecific appearance, which elicits lateralized response to the whole image of the conspecific. In a series of eight experiments, we explored eye preferences in cryptic-coloured Amur sleeper, *Percottus glenii*, fry. Fish displayed left-eye preferences at the population level for inspection of a group of conspecifics, their own mirror image, and a motionless flat model of a conspecific. In contrast, no population bias was found for scrutinizing an empty environment or a moving cylinder. When fry were showed a model of a conspecific in a lateral view with the eye displaced from the head to the tail, they again showed a significant preference for left-eye use. On the other hand, ‘eyeless’ conspecific model elicited no lateralized viewing in fry. Finally, the left-eye preference was revealed for scrutiny of the image of a conspecific eye alone. We argue that in Amur sleeper fry, eye is the element of the conspecific image, which can serve as a ‘key’ for the initiation of lateralized social response. This key element may serve as a trigger for the rapid recognition of

conspecifics in the left eye–right hemisphere system. Possible causes and advantages of lateralized perception of social stimuli and their key elements are discussed in the context of current theories of brain lateralization.

Keywords Lateralization · Left eye–right hemisphere · Schooling fish · Social stimulus · Sign stimulus · Conspecific recognition

I am a mirror. I am a mirror. Looking at me you see yourself

I am a mirror. I am a mirror. Every face is someone else.

Look at me smile and you're the clown, and if I dance you turn around.

Look in my eyes and see your tears until the music disappears.

(The Alan Parsons Project, Freudiana, ‘I am a mirror’)

Introduction

Cerebral lateralization, that is, division of cognitive functions by the two hemispheres, is a common characteristic of the animal brain (Rogers and Andrew 2002; Vallortigara and Rogers 2005; Vallortigara et al. 2011). Generally, the left side of the brain is responsible for the behaviour in routine situations, while the right hemisphere is predominantly involved in response to unpredictable changes in the environment (MacNeilage et al. 2009). The need to maintain coordination among asymmetrical individuals in social behaviours was argued to be an important factor in the origin of population-level lateralization (Rogers 1989;

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Vallortigara 2006a, b; Vallortigara and Rogers 2005; MacNeillage et al. 2009; Rosa Salva et al. 2012). For instance, extensive study of sixteen species of teleost fish found population lateralization in all gregarious species compared with only 40 % of the non-gregarious fish (Bisazza et al. 2000). What was less expected, but intuitively predicted (Giljov et al. 2009), it appeared that even invertebrates support the rule of a higher lateralization in social versus solitary species. As so far the only example, a solitary mason bee showed no asymmetry in antennal use if compared to a highly social honeybee (Anfora et al. 2010) in the study, which conclusively confirmed also the previous work on social insects (see Malashichev 2006; Frasnelli et al. 2012 for complementary reviews). This hypothesis is further supported by the results of mathematical modelling showing that in conditions of both prey–predator and high degree of intraspecific interactions, populations consisting of left- and right-type individuals in unequal numbers can be evolutionarily stable (Ghirlanda and Vallortigara 2004; Ghirlanda et al. 2009). Not surprisingly, lateralization in social responses of different nature was found in a large number of vertebrate species from fish to mammals. The preferential use of either the left or the right eye (visual lateralization) was described for agonistic and sexual interactions, individual recognition, schooling, etc. (reviewed in Vallortigara and Rogers 2005; Rosa Salva et al. 2012). Just one of the recent examples of population level one-sided alignment of behavioural response in a real social situation is a preference of beluga whale, *Delphinapterus leucas*, calves to keep their mothers in the left visual hemifield, suggesting the preferential involvement of the right brain hemisphere in social recognition (Karenina et al. 2010).

For studying social lateralization in aquatic anamniotes, the mirror test is widely used and the results reveal a notable conformity across species. Eight species of fish belonging to five teleost orders prefer to examine monocularly their own reflection in a mirror with the left eye that indicates preferential role of the right brain in perception of such a stimulus (Sovrano et al. 1999, 2001). The same is true for fish (Sovrano and Andrew 2006) and anuran (Bisazza et al. 2002; Dadda et al. 2003) larvae. It was considered that in anamniotes, the own mirror image is identified by an individual as a conspecific and in case of gregarious fish as a shoal mate. Indeed, experimentation on young guppies, *Poecilia reticulata*, showed that when choosing between real companions and their own mirror images, fish did not exhibit a preference for one of these stimuli (Miletto Petrazzini et al. 2012). Zebrafish larvae, *Brachydanio rerio*, display the same lateralization when viewing the own reflection and a group of conspecific fry (Sovrano and Andrew 2006). Notably, these are fish normally living in groups, which show lateralized inspection

of the mirror image (Sovrano et al. 2001). In addition, schools of lateralized fish are characterized by a better cohesion and coordination in a new environment, than schools of non-lateralized fish (Bisazza and Dadda 2005). Visual information is important for social learning of schooling fish and is widely used for communication between shoal mates, for example, in antipredator responses (reviewed in Pitcher and Parrish 1993; Brown and Laland 2003). Apparently, rapid recognition of social partners using visual cues in surrounding environment is crucial for individual survival in gregarious species.

One of the central postulates in classical ethology is that in some situations animals react not to the stimulus as a whole, but only to a certain part of it—the idea stated by Konrad Lorenz and developed by Nikolaas Tinbergen (Tinbergen 1948, 1951). In social context, this means that particular features in the conspecifics' appearance, called 'sign stimuli' or 'releasers', often serve as socially significant signals and produce a specific behavioural response, or a fixed action pattern. N. Tinbergen supported this conception with a number of well-known examples of experiments in which more or less schematic models were used to evoke species-typical reactions in animals (see, e.g., Rowland 2000; ten Cate 2009 for more recent studies). One may, therefore, expect that when shoaling, a fish could be preferably responsive to a specific part of social stimulus, that is, a particular element of the whole social partner image could be enough for successful shoal mate recognition and initiation of a certain reaction to it.

Indeed, in experimental conditions, zebrafish prefer to shoal with companions having the same pigment pattern (horizontal black stripes) and ignore the fish with a radically different pigment pattern (Engeszer et al. 2007a; Saverino and Gerlai 2008). However, in the wild, zebrafish form mixed shoals with several congener species and closely related *Devario* species (Engeszer et al. 2007b), which have vertical rather than horizontal stripes or have no stripes at all. This indicates that some other visual traits of companions, besides body colour pattern, could be used by a fish in gregarious behaviour. It could be assumed that the eye of the conspecific can serve as such a visual cue for social recognition, especially in species with cryptic coloration, due to eyes usually contrasting to the rest of the body appearance (e.g. Martin and Hengstebeck 1981; Miyai et al. 2011).

The principal aim of the present study is to test the hypothesis that there is a key cue in the conspecific appearance, which elicits lateralized response to the whole social stimulus, and that in cryptic-coloured fish, an eye can serve as such a cue. To test this assumption, schooling Amur sleeper, *Perccottus glenii*, larvae ('fry') (Odontobutidae, Teleostei) have been chosen as a model for our

experimentation, because of their cryptic coloration, which makes the potential use of pigment pattern for social recognition in this species unlikely. Since the left-eye preference was found for scrutinizing of the whole conspecific image in fish (Sovrano et al. 2001), we expected that observation of the most significant key cue of this social stimulus would elicit the same lateralized response.

In a series of experiments, Amur sleeper fry were shown social stimuli of different completeness, and eye preferences (the use of the lateral (monocular) parts of the right vs. left visual fields) for the inspection of such stimuli were estimated using standard experimental protocol developed in previous work on fish visual lateralization (e.g. Sovrano et al. 1999, 2001; Sovrano and Andrew 2006). In all experiments, the design of the testing apparatus restricted the use of other sensory modalities, besides vision, for the perception of the stimulus. Firstly, we examined the visual lateralization in Amur sleeper fry in response to the social stimulus as a whole: when viewing a group of conspecifics of the same age. Afterwards, the fish were shown incomplete social stimuli: their own mirror reflection, motionless flat model of a conspecific, model of a conspecific with an eye displaced from the head to the tail, 'eyeless' model of a conspecific and a model of a conspecific eye alone. As control experiments, the fish were exposed to the empty environment without any specific stimuli, and a moving object of the shape drastically differing from that of a conspecific.

Methods

Subjects

Amur sleeper is a native Russian Far East species introduced into the European part of Russia at the beginning of the last century (Dmitriev 1971). We used *P. glenii* fry about 5 months old (body length 22–30 mm) caught from a wild population (outskirts of Saint-Petersburg, Northwest Russia). No other fish species inhabited the subjects' native pond; however, the fry have been exposed to predation pressure from adults of their own species (Dmitriev 1971). Subjects were maintained in the laboratory for 15–20 days prior to the beginning of the experiments. We used two samples of fish of the same age caught from the same pond with an interval of about a year. Experimental Set I (Experiments 1–4) was conducted with the sample 1 ($N = 16$) and Experimental Set II (Experiments 2', 5–8) with the sample 2 ($N = 15$). The mirror test (Experiments 2 and 2') was conducted with both samples to assess their conformity as a standard test for social laterality in fish. Fish were kept solitarily in vegetation-rich (*Ceratophyllum* sp.) opaque plastic tanks with water at a temperature of

20 ± 2 °C under a 14/10-h light/dark cycle. Animals were fed daily with live bloodworm larvae (*Chironomus* sp.). All fish were returned to the native pond after the end of the experiments.

Apparatus

All experiments were carried out in the rectangular plastic tank ($16 \times 7 \times 7$ cm) with white opaque walls and bottom. A barrier transparent when viewing from either side divided the tank into two compartments of equal size (Fig. 1). The transparent barrier fully isolated the compartments from each other and thus excluded the olfactory or mechanical perception of the stimuli by fish. Lateral edges of the barrier were covered with white plastic stripes. During the experiments, an individual fish was placed in the 'testing compartment' of the apparatus, whereas the 'stimulus compartment' contained a visual stimulus, which depended on the experiment (see the description of each experiment below for details). Each compartment was used as the testing one or the stimulus one in random order across experiments and subjects. Preliminary testing showed that relatively narrow space for subjects induced less stress in fry as compared to the bigger tanks, but is enough for free movement. Both compartments were filled in with water up to 6 cm deep and evenly lit by a fluorescent lamp (11 W) placed above the apparatus. The water in both compartments was changed after testing of each individual. A video camera (Sony DCR-HC-17E) was mounted above the apparatus in order to videotape the fish behaviour.

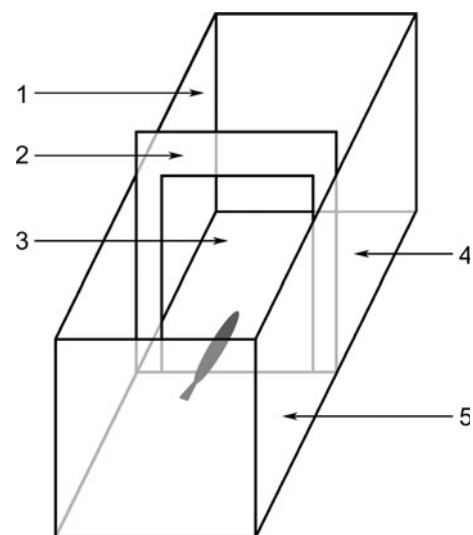


Fig. 1 Schematic representation of the testing apparatus. (1) All four walls are white and opaque; (2) white opaque frame; (3) transparent barrier; (4) 'stimulus compartment'; (5) 'testing compartment' with an experimental fish

Procedure and analysis

Before the beginning of an experiment, a visual stimulus was installed into the stimulus compartment of the experimental apparatus. Then, the test fish was gently removed from its home tank into the testing compartment and, immediately after, the video recording from the top of the testing apparatus began. When the test fish was placed in the apparatus, it already contained a stimulus. The behaviour of each fish was video recorded for 1 h. The procedure of video analysis was based on that used by Sovrano and co-authors (Sovrano et al. 1999, 2001). When the video was played back in slow mode, the individual position of the fish was noted every 2 s; the use of the right or left monocular visual field was assessed basing on the angle between the head part of the fish's body and the transparent intercompartmental barrier. The monocular eye use was scored as such only when the distance between the barrier and the fish's head was no more than ca. 1.5 cm. The data were discarded when the animal observed the barrier binocularly (i.e. formed nearly a perpendicular angle with the barrier) or when the tail was closer to the barrier than the head (i.e. the fish formed angles larger than 90° with respect to the barrier). The mean frequency of the monocular inspection in each experiment reflected the mean number of data points used for the statistical analysis. We analysed the whole time the fish were exposed to the stimuli (1 h).

To assess the inter-rater reliability of the method described above, a subset of ten one-hour video recordings (1 h = one subject) of mirror experiments (Experiments 2 and 2') was scored independently by two raters both blind to the conditions of the study. Five random videos from the sample 1 and five random videos from the sample 2 were used. Analysis showed that scores from the two raters correlated (Pearson's product-moment correlation: $r_8 = 0.99$ for the left-eye use and $r_8 = 0.97$ for the right-eye use). Thus, the method of eye use scoring was considered to be reliable.

In both of the samples, the order of experiments was randomized for each individual. Thus, Experiments 1–4 were randomly conducted with the subjects from the sample 1 and Experiments 2', 5–8 with the subjects from the sample 2. It should be noted that the sample size varied slightly between the experiments, since several subjects did not approach the transparent intercompartmental barrier, that is, no data from these subjects were obtained in some experiments.

The frequency of the use of monocular visual field (of the left (L) and the right (R) eye separately) for each individual in each test was converted into a laterality index: $LI = (R - L)/(R + L)$. LI is a continuous variable ranging from -1 to $+1$. Positive scores of LI represent a bias for

use of the right eye, negative scores represent a bias for the use of the left eye, and scores of zero have no bias in the eye use. Binomial z scores, based on a total number of stimulus-inspection responses with the left and the right eye in each test, were calculated to determine the individual preferences.

Individual z scores were used to categorize subjects as left-eye preferent ($z = -1.96$) and right-eye preferent ($z = 1.96$). The fish with individual z scores ranging between -1.96 and 1.96 were classified as having no preference. Normality of LI data was assessed using one-sample Kolmogorov–Smirnov test. Since the assumptions of normality were not violated, one-sample t test based on LI was used to assess the preference in eye use at the population level in each test. The G test (log-likelihood ratio, chi-square test) was used to estimate whether the distribution of lateralized and non-lateralized individuals differed from chance. The data for *right*-sided and *left*-sided models inspection were compared with paired t test. Finally, Mann–Whitney U test was performed to assess whether the order in which the subjects were tested to observe *right*-sided and *left*-sided model images affected the lateralized eye use. We adopted an alpha value of 0.05 for all analyses.

Experimental Set I

All experiments in Experimental Set I were conducted with the fish from the sample 1 (see Subjects for details).

Inspection of the conspecifics and own mirror reflection (left-eye preference)

For the gregarious fish, one of the most natural social stimuli seems to be a group of shoal mates. The Amur sleeper fry normally form small flocks in natural habitats (Dmitriev 1971). Therefore, in the first experiment (Experiment 1), each fish was demonstrated a group of three conspecifics of the same age. That is, a group of fry was placed into the stimulus compartment of the experimental tank ca. 5 min before placing a test fish into the testing compartment. The stimulus fish for Experiment 1 ($N = 10$) were caught together with the test subjects in the same pond. These fish were kept separately from the test subjects, but were housed in the same conditions and were also maintained in the laboratory for 15–20 days prior to the beginning of the experiments. In random order, a group of three stimulus fish was formed for conducting the Experiment 1 with each test fish, that is, different groups of stimulus fish were demonstrated to different test subjects.

The group of conspecifics was assumed to be the fullest social visual stimulus used in the present study. To

demonstrate reduced social object as well as to make our results comparable with data from previous studies of social lateralization in fish (Sovrano et al. 1999, 2001; Sovrano and Andrew 2006), we further tested visual laterality in Amur sleeper fry when inspecting their own mirror image (Experiment 2). Here, the test subject could see not a group of shoal mates, but a single fish, which moved only when the subject itself moved (Sovrano and Andrew 2006); hence, the stimulus could be considered reduced. In contrast to the original studies, we used a single mirror in order to maintain a common plan of all our experimental protocols, so the mirror was placed by the transparent barrier in the stimulus compartment of the testing apparatus.

Results and discussion

The mean frequency of use of the monocular visual field for Experiment 1 (a group of conspecifics) was 52 ± 6 times per hour. A significant population-level left-eye preference during inspection of a group of conspecifics was revealed (mean LI \pm SEM = -0.48 ± 0.05 ; one-sample t test: $t_{13} = 9.49$, $P < 0.001$; Fig. 2). This preference arose from lateralization at the individual level: based on individual z scores, 11 fish were lateralized, all showing the left-eye preference and three fish did not display a preference for one eye. Significantly, more fish were lateralized than non-lateralized (G test: $G_1 = 4.86$, $P = 0.028$; Table 1).

In Experiment 2, the mean frequency of monocular mirror inspection was 237 ± 20.3 times per hour. Here, also a significant population-level left-eye preference was found (mean LI \pm SEM = -0.45 ± 0.03 ; one-sample

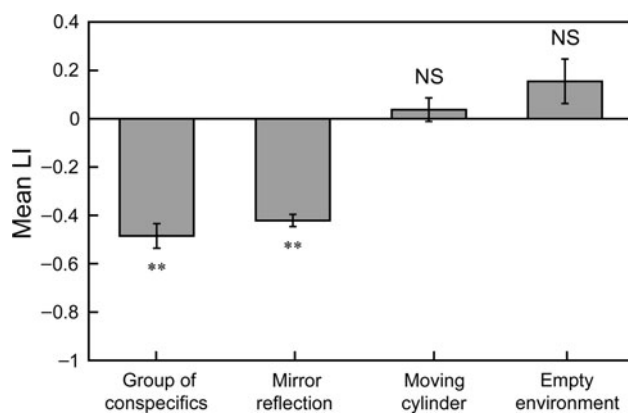


Fig. 2 Direction of eye preferences for Experiments 1–4. Mean LI (laterality index) scores \pm SEM (positive values indicate *right lateral bias*, negative values indicate *left lateral bias*) for inspection of a group of conspecifics ($N = 14$), mirror reflection ($N = 31$), moving cylinder ($N = 14$) and empty environment ($N = 15$). ** $P < 0.005$

t test: $t_{15} = 13.83$, $P < 0.001$). All subjects showed the preference for the left-eye use in this experiment (Table 1).

The results of Experiment 1 and 2 indicated that Amur sleeper fry preferred to use the left eye for viewing both a group of conspecifics of the same age and their own mirror image. Similarly, zebrafish fry demonstrated left-eye preference in mirror test and when observing a group of conspecific fry (Sovrano and Andrew 2006). In addition, when aggregating in shoals, anuran tadpoles showed a higher probability of approaching a conspecific visible in their left visual hemifield rather than in their right hemifield (Dadda et al. 2003). The preference to use the monocular field of their left eye for inspection of the own reflection in a mirror has been also found in adults of eight gregarious teleost species (Sovrano et al. 1999, 2001), with the exception of rainbowfish, *Melanotaenia duboulayi* and *M. nigrans*, which preferred the right eye while schooling with their mirror image (Bisazza and Brown 2011). Thus, it could be assumed that Amur sleeper display social lateralization typical for the most of the gregarious fish studied. It has been suggested that the asymmetrical inspection of mirror reflection is associated with lateralized reaction to a more general category—novelty (e.g. Sovrano and Andrew 2006). Indeed, in the mirror test, the experimental subject observed a relatively novel stimulus—unknown conspecific—which moved only when the subject itself moved. This unusual movement pattern could, potentially, be a reason for heightened frequency of monocular viewing for mirror inspection compared to viewing a group of ‘normal’ conspecifics. Nevertheless, it was previously shown that Sarasins minnows, *Xenopoeilus sarsasinorum*, preferred to inspect their own mirror image using the monocular visual field of the left eye even if they had been kept for 20 days in a tank with a mirror wall, that is, had been very familiar with such a stimulus (Sovrano 2004). Thus, it is unlikely that the left-eye preference for mirror inspection in Amur sleeper fry is caused only by its novelty. The group of conspecifics seems to be much more natural and familiar for gregarious Amur sleeper fry; therefore, it could be more quickly recognized and categorized by the experimental fish and, hence, provoke less interest and monocular viewing.

Since cannibalism is typical for the Amur sleeper (Dmitriev 1971), there is a possibility that a conspecific is perceived by the fry as a potential predator. However, only adults of *P. glenii*, which are several times bigger than the fry used in the study, normally predate on larvae of their own species. Hence, schooling fry most likely recognize a predator only in much larger conspecifics, whereas a conspecific of the same size (like observed by fry in experiments with a group of conspecifics of the same age or a mirror reflection) are perceived as a potential shoal mate.

Table 1 Individual preferences in eye use for the sample 1

Subject	Group of conspecifics			Mirror reflection			Moving object			Empty environment		
	LI	<i>z</i> score	pref	LI	<i>z</i> score	pref	LI	<i>z</i> score	pref	LI	<i>z</i> score	pref
1	-0.53	-2.79	L	-0.51	-7.73	L	-0.08	-0.33	NP	-0.14	-0.77	NP
2	-0.47	-4.99	L	-0.49	-7.14	L	-0.07	-0.62	NP	-0.07	-0.30	NP
3	-0.75	-5.20	L	-0.62	-7.56	L	-0.12	-0.71	NP	0.50	1.45	NP
4	-0.28	-1.93	NP	-0.29	-5.21	L	0.30	2.39	R	0.58	3.48	R
5	-0.54	-3.11	L	-0.45	-8.29	L	0.25	1.74	NP	0.00	0.00	NP
6	-	-	-	-0.20	-2.95	L	-	-	-	0.09	0.35	NP
7	-0.32	-1.79	NP	-0.19	-4.02	L	0.18	1.14	NP	-	-	-
8	-0.38	-2.41	L	-0.46	-6.92	L	0.18	1.20	NP	0.50	1.45	NP
9	-0.92	-8.21	L	-0.62	-7.62	L	-0.37	-2.61	L	-0.25	-1.02	NP
10	-0.44	-3.01	L	-0.52	-7.16	L	0.05	0.34	NP	0.20	0.61	NP
11	-0.45	-2.26	L	-0.43	-6.00	L	0.10	0.46	NP	0.20	0.61	NP
12	-	-	-	-0.40	-5.25	L	-	-	-	0.00	0.00	NP
13	-0.47	-2.41	L	-0.54	-9.35	L	0.07	0.49	NP	-0.20	-0.91	NP
14	-0.15	-1.02	NP	-0.41	-5.03	L	0.06	0.27	NP	1.00	3.46	R
15	-0.56	-5.08	L	-0.55	-9.02	L	0.16	0.91	NP	0.17	0.83	NP
16	-0.53	-3.14	L	-0.56	-8.25	L	-0.17	-1.10	NP	-0.25	-1.02	NP

LI laterality index, *z* score binomial *z* score, positive values indicate rightward bias, negative values indicate leftward bias; *pref* preference in eye use, *L* left-eye preference, *R* right-eye preference, *NP* no preference

Inspection of the moving object and the empty environment (no preference)

The aim of the following experiments was to examine whether the movement of an unfamiliar object or the empty environment in the stimulus compartment of the testing apparatus, both of which were the components of the visual stimuli used in Experiments 1 and 2, per se could facilitate the lateralized response in the studied subjects. In Experiment 3, the stimulus compartment of the apparatus contained a grey–brown cylinder of 12 mm in diameter and 20-cm height (i.e. the object of the shape differing from a fish) suspended via a thread on the electric motor, which moved it both in the horizontal and the vertical planes. The motor was connected with the cylinder via a system of blocks and was not visible to an experimental fish. When moving, the cylinder did not touch the walls of the apparatus, nor did it rise above the water surface. In Experiment 4, the stimulus compartment of the experimental tank contained only water.

Results and discussion

The mean frequency of monocular inspection was 57 ± 4 times per hour in Experiment 3 (moving object) and 23 ± 3 times per hour in Experiment 4 (empty environment). The population-level lateralization in eye use was not revealed in other experiment (Experiment 3: mean $LI \pm SEM = 0.04 \pm 0.05$, one-sample *t* test: $t_{13} = 0.78$,

$P = 0.448$; Experiment 4: mean $LI \pm SEM = 0.16 \pm 0.09$; $t_{14} = 1.69$, $P = 0.114$; Fig. 2). Moreover, the great majority of subjects did not show any preference for the particular eye: 13 out of 15 individuals were not lateralized at all, and only two displayed right-eye preference for the inspection of the empty environment (*G* test: $G_1 = 9.01$, $P = 0.003$; Table 1). For the inspection of the moving object, 12 out of 14 individuals showed no preference and two fish were lateralized in opposite directions (*G* test: $G_1 = 7.92$, $P = 0.005$; Table 1).

The results of Experiments 3 and 4 are straightforward. The movement of the object drastically differing from the shape of the conspecific as well as the empty environment did not evoke any visual lateralization in Amur sleeper fry. Hence, the left-eye preferences in previous experiments could not be caused by a lateralized perception of these components of the complex visual environment per se.

Interestingly, males of other teleost species, *Gambusia holbrooki* and *Xenotoca eiseni*, also showed no laterality biases when viewing the space free of any objects (Bisazza et al. 1997; Bisazza and De Santi 2003). Brown et al. (2004) revealed intriguing variation between the poeciliids *Brachyraphis episcopi* from the regions of high or low predation pressure in their lateralized response to empty scene, the test serving as a ‘control’ in this study. Fish from high predation areas showed no significant eye preference, whereas fish from low predation regions viewed an empty compartment preferably with their right eye. Thus, the

Table 2 Individual preferences in eye use for the sample 2 (experiments with mirror and 'normal' conspecific model)

Subject	Mirror reflection			'Normal' conspecific model					
				Right-sided			Left-sided		
	LI	z score	pref	LI	z score	pref	LI	z score	pref
1	-0.43	-4.43	L	-0.30	-3.96	L	-0.39	-5.69	L
2	-0.46	-5.19	L	-0.25	-3.88	L	-0.44	-3.32	L
3	-0.54	-7.49	L	-0.33	-3.89	L	-0.44	-4.89	L
4	-0.34	-4.67	L	-0.34	-4.73	L	-0.48	-7.69	L
5	-0.39	-5.82	L	-0.21	-3.16	L	-0.38	-4.16	L
6	-0.39	-7.80	L	-0.43	-6.03	L	-0.50	-7.66	L
7	-0.15	-3.05	L	-0.11	-1.73	NP	-0.43	-1.56	NP
8	-0.64	-4.52	L	-0.46	-6.25	L	-0.54	-6.71	L
9	-0.23	-3.62	L	-0.19	-2.60	L	-0.32	-5.37	L
10	-0.33	-6.45	L	-0.46	-6.41	L	-0.49	-8.16	L
11	-0.31	-6.95	L	-0.14	-1.74	NP	-0.22	-2.97	L
12	-0.67	-10.15	L	-0.50	-5.36	L	-0.55	-7.90	L
13	-0.41	-4.23	L	-0.44	-5.99	L	-0.46	-6.62	L
14	-0.26	-4.57	L	-0.01	-0.07	NP	-0.36	-1.38	NP
15	-0.24	-3.97	L	-0.18	-2.96	L	-0.35	-2.87	L

LI laterality index, z score binomial z score, positive values indicate rightward bias, negative values indicate leftward bias, pref preference in eye use, L left-eye preference, R right-eye preference, NP no preference

lateralized perception of an empty environment seems to be a variable characteristic in fish, which may be caused by methodological differences between the studies as well as differences in subjects' experience and temperament.

Consistency across experiments

Analysis revealed significant positive correlations between LI scores in Experiments 1 and 2 ($r_{12} = 0.74$, $p = 0.002$), Experiments 1 and 3 ($r_{12} = 0.70$, $p = 0.006$), Experiments 1 and 4 ($r_{11} = 0.60$, $p = 0.029$) and Experiments 2 and 3 ($r_{12} = 0.69$, $p = 0.007$). LI scores between Experiments 2 and 4, as well as between Experiments 3 and 4, in contrast, did not correlate ($r_{13} = 0.32$, $p = 0.244$ and $r_{11} = 0.47$, $p = 0.108$, respectively).

Experimental Set II

All experiments in Experimental Set II were conducted with the fish from the sample 2 (see Subjects for details).

To test whether the individuals from two samples used in the study showed a consistency of lateralization in response to a standard social stimulus (mirror reflection), we conducted Experiment 2' with the experimental animals from the sample 2. The plan of this experiment was identical to Experiment 2 (inspection of the own mirror image) conducted with the sample 1.

In the sample 2, the mean frequency of monocular inspection of the mirror reflection was 250 ± 33.8 times per hour. The fry demonstrated preferential left-eye use (mean LI \pm SEM = -0.39 ± 0.04 , $t_{14} = 10.14$, $P < 0.001$). Notably, no significant difference was found between the two samples (Mann–Whitney U test: $U = 81.00$, $N_1 = 16$, $N_2 = 15$, $P = 0.128$). The leftward population-level bias was also present in case of the combined data from two samples (mean LI \pm SEM = -0.42 ± 0.03 , $t_{30} = 16.61$, $P < 0.001$; Fig. 2). All 31 individuals were lateralized during own mirror image inspection with the preferred use of the left eye (Tables 1, 2). This meant that both samples equally represented the population in respect to social lateralization and allowed us to use the fry from the sample 2 in further experiments.

Inspection of the 'normal' and the 'eye on the tail' conspecific models (left-eye preference)

In the following two experiments, we explored the visual lateralization in *P. glenii* fry when inspecting artificial model of a conspecific of the same age. The aim of Experiment 5 was to test whether experimental fry would show the lateralized response to a motionless and flat visual image of a conspecific (= 'normal' conspecific model), that is, is such a stimulus enough to evoke left-eye preference in fish? For this purpose, we used a coloured side-view Amur sleeper fry photo (taken with Sony DSC-H5 video camera)

cut out along the contour and laminated by waterproof transparent film. The size of the model was identical to the mean size of the experimental animal. The model was placed by the transparent barrier in the 'stimulus compartment' of the testing apparatus.

In Experiment 6, the fry were tested in reaction to conspecific model with an eye displaced from the head to the tail ('eye on the tail' conspecific model). The aim of this experiment was to explore whether the eye is the most important element of conspecific image for the initiation of the left-eye preference or the complex of conspecific traits (e.g. correct positioning of the eye as regard to other parts of the body) is required. A similar model as in Experiment 5 was used, but before printing the photo, it was modified using the Adobe Photoshop. In particular, the eye was cut out along a contour and laid on the tail fin on the same height like on a 'normal' conspecific model. The distance from the tip of the tail fin to the eye was identical to the distance from the tip of the head to the eye on a 'normal' conspecific image. The place where the eye was previously situated was substituted with a copied piece of the nearest head area of the eye-size.

Each fish was tested in two variants of Experiments 5 and 6, where we used identical, but mirrored in Adobe Photoshop images: *right-sided* and *left-sided*. A half of the individuals were tested with the *right-sided* image firstly (*left-sided*—secondly), and a half were tested with the *left-sided* image firstly (*right-sided*—secondly).

Results and discussion

The analysis revealed no effect of the order of testing with two variants of the 'normal' conspecific model

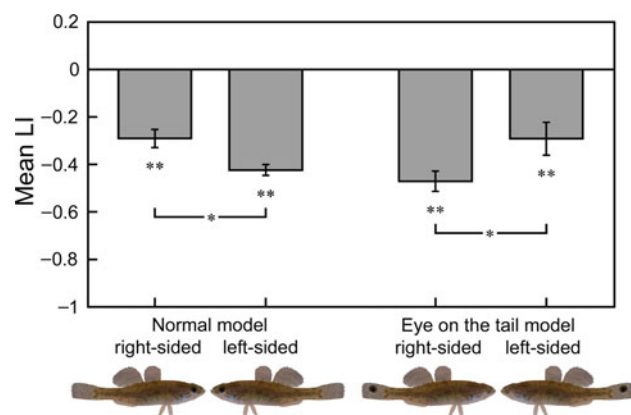


Fig. 3 Direction of eye preferences for Experiments 5 and 6. Mean LI (laterality index) scores \pm SEM (positive values indicate *right lateral bias*, negative values indicate *left lateral bias*) for inspection of 'normal' conspecific model ($N = 15$) and 'eye on the tail' conspecific model ($N = 15$). Both models were demonstrated in *right-sided* and *left-sided* variants. * $P < 0.05$, ** $P < 0.005$

(*right-sided/left-sided*) either in the *right-sided* image inspection (Mann–Whitney U test: $U = 26.00$, $N_1 = 8$, $N_2 = 7$, $P = 0.867$) or in the *left-sided* image inspection ($U = 26.00$, $N_1 = 8$, $N_2 = 7$, $P = 0.867$). Similarly, the Mann–Whitney U tests showed no effect of the testing order in the 'eye on the tail' conspecific model demonstrations either in the case of the *right-sided* model ($U = 25.00$, $N_1 = 8$, $N_2 = 7$, $P = 0.779$) or in the case of the *left-sided* model ($U = 26.00$, $N_1 = 8$, $N_2 = 7$, $P = 0.867$).

In Experiment 5 ('normal' conspecific model), the mean frequency of monocular inspection was 195 ± 10 times per hour in the case of the *right-sided* model and 200 ± 13 times per hour in the case of the *left-sided* model.

The Amur sleeper fry inspected the *right-sided* 'normal' conspecific model predominantly with their left eye (mean LI \pm SEM = -0.29 ± 0.04 ; one-sample t test: $t_{14} = 7.53$, $P < 0.001$; Fig. 3). Significantly more individuals were lateralized than that displayed no preference (G test: $G_1 = 5.78$, $P = 0.016$); lateralization at the population level arose from a high portion of individuals preferred to use their left eye (12 fish), in contrast to only three subjects, which had no any preference (Table 2).

A significant left-eye preference during inspection of the *left-sided* 'normal' conspecific model was also found (mean LI \pm SEM = -0.42 ± 0.02 ; one-sample t test: $t_{14} = 18.64$, $P < 0.001$; Fig. 3). The majority of fish were lateralized (G test: $G_1 = 9.01$, $P = 0.003$) with 13 out of 15 individuals showing left-eye preference and two individuals displaying no preference for one eye over the other (Table 2).

The analysis revealed that preference in eye use was more leftward when fish inspected the *left-sided* normal conspecific model than the *right-sided* one (paired t test: $t_{14} = 3.92$, $P = 0.002$).

The mean frequency of monocular inspection in Experiment 6 ('eye on the tail' conspecific model) was 97 ± 5 times per hour in case of the *right-sided* model inspection and 155 ± 16 times per hour in case of the *left-sided* model. When viewing the *right-sided* 'eye on the tail' model, fry showed a significant population-level left-eye preference (mean LI \pm SEM = -0.47 ± 0.04 ; one-sample t test: $t_{14} = 10.94$, $P < 0.001$; Fig. 3). All 15 individuals were lateralized in this test, and all of them preferred to use the left eye (Table 3).

Preferential use of the left eye at the population level was also revealed in the inspection of the *left-sided* 'eye on the tail' conspecific model (mean LI \pm SEM = -0.29 ± 0.07 ; one-sample t test: $t_{14} = 4.18$, $P < 0.001$; Fig. 3). Significantly, more individuals were lateralized than non-lateralized (G test: $G_1 = 9.01$, $P = 0.003$), with majority of subjects displaying preference for the left-eye use (G test: $G_1 = 6.86$, $P = 0.009$): 11 fish out of 15

Table 3 Individual preferences in eye use for the sample 2 (experiments with ‘eye on the tail’ and ‘eyeless’ conspecific model, ‘eye’ model)

Subject	‘Eye on the tail’ conspecific model						‘Eyeless’ conspecific model						‘Eye’ model		
	Right-sided			Left-sided			Right-sided			Left-sided			LI	z score	pref
	LI	z score	pref	LI	z score	pref	LI	z score	pref	LI	z score	pref			
1	-0.47	-4.01	L	-0.37	-5.84	L	-0.05	-0.69	NP	0.03	0.38	NP	-0.51	-3.72	L
2	-0.46	-3.72	L	-0.35	-4.31	L	0.04	0.48	NP	0.07	1.09	NP	-0.38	-2.72	L
3	-0.51	-4.21	L	-0.38	-5.44	L	-0.02	-0.09	NP	0.06	0.67	NP	-0.40	-3.88	L
4	-0.56	-6.22	L	-0.35	-4.54	L	0.05	0.47	NP	-0.02	-0.22	NP	-0.41	-2.83	L
5	-0.49	-4.85	L	-0.47	-5.60	L	-0.01	-0.08	NP	0.04	0.22	NP	-0.43	-3.48	L
6	-0.60	-6.77	L	-0.59	-6.45	L	0.03	0.39	NP	-0.03	-0.36	NP	-0.47	-3.88	L
7	-0.25	-2.06	L	0.05	0.35	NP	-0.11	-1.29	NP	0.08	0.43	NP	-0.05	-0.35	NP
8	-0.47	-4.51	L	-0.23	-2.41	L	-0.05	-0.58	NP	0.03	0.12	NP	-0.61	-5.10	L
9	-0.31	-3.26	L	-0.30	-5.29	L	0.07	0.70	NP	0.08	0.87	NP	-0.53	-3.91	L
10	-0.60	-6.15	L	-0.49	-6.36	L	0.00	0.00	NP	-0.03	-0.11	NP	-0.56	-4.41	L
11	-0.35	-3.39	L	0.19	2.19	R	0.01	0.08	NP	0.10	1.17	NP	-0.25	-1.43	NP
12	-0.88	-8.97	L	-0.59	-6.17	L	-0.15	-1.17	NP	-0.27	-3.72	L	-0.67	-4.62	L
13	-0.55	-5.94	L	-0.57	-5.98	L	-0.11	-0.95	NP	-0.12	-1.05	NP	-0.54	-3.75	L
14	-0.27	-2.53	L	-0.21	-1.89	NP	0.07	0.65	NP	-0.03	-0.32	NP	-0.18	-1.12	NP
15	-0.27	-2.72	L	0.27	3.43	R	0.10	1.14	NP	0.21	2.22	R	-0.37	-3.02	L

LI laterality index, z score binomial z score, positive values indicate rightward bias, negative values indicate leftward bias, pref preference in eye use, L left-eye preference, R right-eye preference, NP no preference

preferred their left eye, two individuals preferred their right eye, and two had no preference for one eye over the other (Table 3).

The analysis revealed that preference in eye use was more leftward when fish inspected the right-sided ‘eye on the tail’ conspecific model than the left-sided model (paired *t* test: $t_{14} = 2.55, P = 0.023$).

The results of Experiment 5 show that when viewing a motionless and flat visual image of the conspecifics, Amur sleeper fry exhibit the same bias in eye use as when viewing their own mirror reflection or a group of conspecifics. Together with the results of previous experiments, this indicates that some specific features of conspecific appearance (shape, coloration or their association) induce the left-eye inspection in fry. Furthermore, the population preference to use the left eye for viewing ‘eye on the tail’ conspecific model (Experiment 6) provides evidence of the eye to be an important element of conspecific image for the initiation of specific visual lateralization. This result also indicates that a particular position of the eye on the conspecific body is not a crucial variable for the lateral bias to occur.

An intriguing tendency was found—the preference for left-eye use was stronger when fry inspected the left-sided ‘normal’ conspecific model if compared to the right-sided model, whereas the reverse was true for the ‘eye on the tail’ conspecific model. The most likely interpretation of this phenomenon is that the left side of a conspecific induced a

stronger lateral bias; and in case of the ‘eye on the tail’ model, the right-sided image evoked more leftward eye use because it was recognized by fish as the left side of a conspecific. If the latter is true, it could be suggested that fry are able to assess the position of an eye as regard to the rest of the body to recognize which side of the conspecific’s body is turned to them. Potentially, the eye position may be used by fish for distinguishing between the ‘head’ and the ‘tail’ parts of the conspecific body.

Interestingly, convict cichlids, *Amatitlania nigrofasciata*, have been found to exhibit a population-level preference for showing the right side of the body during aggressive lateral displays (Arnott et al. 2011), which seem to be consistent with the right-eye preference found in agonistic behaviour in three other fish species (Bisazza and De Santi 2003). This fact underlines the importance of recognition of conspecific’s side (left/right) for fish. We can hypothesize that generally the right side of a conspecific is perceived by a fish as a precondition for aggression, whereas the left body side could serve as a signal for shoaling or other non-aggressive interactions. It is important to note that in non-experimental conditions, the left-sided conspecific viewed by a fish is the conspecifics that look at the focal fish with the left eye; therefore, it is unlikely to display aggressiveness. Hence, the left side viewed conspecific could be more likely perceived by a fish as a potential shoal mate in comparison with the right side

viewed conspecific. This could be a possible explanation for a more leftward reaction to the *left*-sided models in Amur sleeper fry.

Inspection of the ‘eyeless’ conspecific model (no preference) and the ‘eye’ model (left-eye preference)

Experiments 7 and 8 were conducted to test the assumption that the eye is a crucial component of a conspecific image for the left-eye bias to occur. In Experiment 7, we used a conspecific image in a lateral view, but with the eye displaced from the head. It was cut out along a contour from the same conspecific photo used in previous experiments via the Adobe Photoshop, with the space it occupied before substituted with the nearest head image area. Two variants of the experiment with mirrored images (*right*-sided/*left*-sided) were carried out in a similar way as in Experiments 5 and 6. To explore whether an eye image alone can evoke left-eye preference, in Experiment 8 we tested fry in reaction to the ‘eye’ model. For this aim, the eye was cut out along a contour from the photo of a fry. The obtained eye image was laminated and placed on the same height like on the other conspecific models, but in the middle of the transparent barrier between the compartments.

Results and discussion

No effect of the order of the ‘eyeless’ conspecific model (*right*-sided/*left*-sided) demonstration was found either

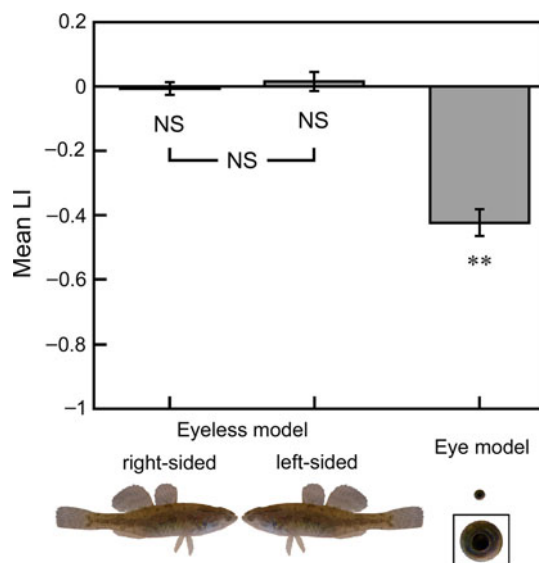


Fig. 4 Direction of eye preferences for Experiments 7 and 8. Mean LI (laterality index) scores \pm SEM (positive values indicate *right* lateral bias, negative values indicate *left* lateral bias) for inspection of *right*-sided and *left*-sided ‘eyeless’ conspecific models ($N = 15$) and ‘eye’ model ($N = 15$). The ‘eye’ model in the square frame is illustrated enlarged; in fact, the size of this model was equal to the eye on the ‘normal’ conspecific model. $**P < 0.005$

during the *right*-sided model inspection (Mann–Whitney U test: $U = 23.00$, $N_1 = 8$, $N_2 = 7$, $P = 0.613$) or during observing the *left*-sided model ($U = 25.00$, $N_1 = 8$, $N_2 = 7$, $P = 0.779$).

The mean frequency of monocular inspection of the ‘eyeless’ conspecific model was 155 ± 14 times per hour in case of the *right*-sided model and 141 ± 16 times per hour in case of the *left*-sided model.

No population-level preference during the *right*-sided ‘eyeless’ conspecific model inspection was revealed (mean $LI \pm SEM = -0.01 \pm 0.02$; one-sample t test: $t_{14} = 0.44$, $P = 0.667$; Fig. 4). The lack of lateralization at the population level was stressed by the absence of any lateralized individuals (Table 3).

No preference in eye use was revealed also for the *left*-sided ‘eyeless’ conspecific model inspection (mean $LI \pm SEM = 0.01 \pm 0.03$; one-sample t test: $t_{14} = 0.49$, $P = 0.635$; Fig. 4). The majority of subjects did not show any preference in the eye use (G test: $G_1 = 9.01$, $P = 0.003$); 13 out of 15 individuals were not lateralized with two individuals displaying the right-eye preference.

In Experiment 8 (‘eye’ model), the mean frequency of monocular inspection was 61 ± 4 times per hour. Analysis revealed a significant left-eye preference during observing the ‘eye’ model (mean $LI \pm SEM = -0.42 \pm 0.04$; one-sample t test: $t_{14} = 9.99$, $P < 0.001$; Fig. 4). This preference arose from the lateralization at the individual level: the majority of fish were lateralized (G test: $G_1 = 5.78$, $P = 0.016$), 12 out of 15 subjects demonstrated a leftward bias, and three fish did not show a preference for one eye over the other (Table 3).

The results of Experiment 7 clearly demonstrate that the image of conspecific without an eye does not evoke any visual lateralization in the Amur sleeper fry at both the individual and the population levels. Also, it seems that the fry cannot distinguish between the sides of the fish’s image, which is lacking eyes. Hence, we can conclude that any other features of conspecific appearance except the eye do not cause the left-eye preference for inspection of the full conspecific image (live, mirror reflection, or model). The results of Experiment 8 fully support this: the eye image per se evokes a significant bias for the left-eye use in fish. These data confirm the hypothesis that in Amur sleeper fry, the eye is a crucial ‘key’ component of a conspecific image for the left-eye bias to occur and potentially for the recognition of a shoal mate in general. However, the incompleteness of the ‘eye’ model as a social stimulus could be a potential explanation of relatively low frequency of monocular viewing.

Consistency across experiments

For subjects from the sample 2, a significant positive correlation was revealed for the majority (21 out of 28) of

between-experiment comparisons ($r_{13} > 0.52$, $P < 0.05$), while correlations between Experiments 2 and 7 (*right-sided model*), Experiments 5 (*right-sided model*) and 7 (*right-sided model*), Experiments 5 (*left-sided model*) and 7 (*right-sided model*), Experiments 6 (*right-sided model*) and 7 (*right-sided model*), Experiments 6 (*left-sided model*) and 7 (*right-sided model*), Experiments 7 (*right-sided model*) and 8, and Experiments 7 (*left-sided model*) and 8 did not reach significance ($r_{13} < 0.50$, $P < 0.05$).

General discussion

The main results from the series of experiments in the present study were the following: (1) Amur sleeper fry displayed left-eye preference at the population level for inspection of a group of conspecifics as well as their own mirror image, which is consistent with the data on the majority of other gregarious fish studied to date (Sovrano et al. 1999, 2001; Sovrano and Andrew 2006; Brown et al. 2007); (2) the left-eyed preference was not found in situations when fish viewed an unfamiliar moving object or the free environment, but existed when fish inspected the motionless and flat visual image of a conspecific; (3) eye is the component of a conspecific image which alone can evoke the left-eye preference in fry; the particular position of the eye on the conspecific's body is not a crucial variable for such a lateral bias to occur, but is likely important for the recognition of the body side (left side vs. right side) of conspecifics by fish.

Thus, we can assume that in Amur sleeper fry, eye is the element of the conspecific image, which can serve as the 'key' for the initiation of the lateralization typical for the perception of social stimuli. The absence of a lateralized reaction to the 'eyeless' conspecific model suggests that any other traits of a conspecific image, such as coloration or shape, are not involved in the initiation of social lateralization. In female guppies, visual preferences in response to a sexual stimulus were showed to be affected by the coloration of demonstrated males: deeply orange males elicited more intense lateral biases than the dull ones (Kaarthigeyan and Dharmaretnam 2005). Here, also the presence of the particular cue (orange coloration) in the conspecific appearance influenced the expression of lateralized reaction to a socially relevant stimulus. In our case, however, the cue (an eye) not only strengthened the laterality, but predetermined the presence of lateralized response itself.

It could be further hypothesized that in the Amur sleeper, an eye may be used in gregarious behaviour for the identification of shoal mates, because this species has cryptic coloration without any bright stripes or spots, and the most contrasting and, therefore, the most noticeable detail of a conspecific image is a dark-coloured eye. Visual

recognition of companions plays a valuable role for survival of an individual in gregarious fish and develops during early social learning to the visual traits of shoal mates (reviewed in Brown and Laland 2003; Gómez-Laplaza and Gil-Carnicero 2008). Amur sleeper fry used in the present study were caught from a wild population at the age of about 4 months, that is, they had a social experience prior to the testing and could learn the visual traits of companions. In many fish species, colour patterns have been shown to help an individual to identify appropriate shoal mates, either within or among species (reviewed in Price et al. 2008). The species that are highly engaged in shoaling behaviour tended to have longitudinal stripes on the body sides, which can be used by fish for the establishment of the shoaling preferences (Price et al. 2008; Engeszer et al. 2004). Amur sleepers, however, are gregarious only on the early stages of life while are solitary as adults, and have cryptic-coloured body. Thus, for the fry of this species, the eye is the most probable and perhaps the only possible cue for quick visual recognition of conspecifics in the surrounding environment. There are a number of examples of how the eye as a visual stimulus can be used in social behaviour of fish. For instance, in some species, the eye coloration reflects the social status of the individual, and in the pearl cichlid, *Geophagus brasiliensis*, the eye colour has been found to serve as a signal shaping the intensity of the aggressive interactions between the rivals (Miyai et al. 2011). In guppies also, the eye colour can play a signalling function in aggressive interactions (Martin and Hengstebeck 1981). The other example is 'eyespot'—eye-shaped coloration patterns, which are believed to play an important role in fish social interactions (Beeching 1993). It was showed that in cichlid fishes, eyespots can be used as conspicuous visual cues in different aspects of intraspecific communication (Schroder and Zaret 1979; Beeching 1993). In other vertebrates, the eye region can serve as a target in aggression. In toads, for instance, agonistic tongue strikes are usually directed to the rival's eye and such 'eye' strikes seem to reflect more intense agonistic behaviour than strikes to other parts of the body (Robins et al. 1998). Notably, the eyes were found to be a target of agonistic strikes more frequently when the attacker had the conspecific in the left visual hemifield.

Why do the Amur sleeper fry preferred to use their left eye for viewing a social image?

In fish, the monocular visual fields are known to project almost exclusively to the contralateral side of the brain, that is, a stimulus seen with the left eye is largely analysed by the right brain (Vanegas and Ito 1983). In many vertebrate species from fish to mammals, the general evidence of left eye–right brain dominance for social recognition and

guiding social responses has been found (reviewed in Rogers 2002; Vallortigara and Rogers 2005; Rosa Salva et al. 2012; Bisazza and Brown 2011). For instance, in birds, left-eye system has been shown to be more effective in control of social pecking and processing information, which allows recognition of familiar and unfamiliar conspecifics (Vallortigara 1992; Vallortigara et al. 2001). Thus, the left bias showed in the Amur sleeper fry is in line with the general pattern of social lateralization in fish and other vertebrates, suggesting it is ancient in vertebrate lineages.

It has been suggested that the left-eye preference in fish is caused by more general characteristics of the perceived stimulus than its social significance per se (Sovrano 2004; Rosa Salva et al. 2012). The left-eye bias in fish could potentially result from the lateralized assessment of the stimulus' novelty (Sovrano and Andrew 2006). However, some degree of novelty could be found in the most of the stimuli demonstrated to the fry in the present study; however, the preferential use of the left monocular visual field was expressed selectively. In addition, the, apparently, novel stimuli, such as moving cylinder or eyeless conspecific, did not evoke any lateralization at the population level. Alternatively, it was argued that the left-eye preference in fish could be linked with the establishment of identity of familiar stimuli, either social or not social, realized predominantly by the right hemisphere (Sovrano 2004; Rosa Salva et al. 2012). In other words, the right hemisphere dominance for processing of conspecific image seems to be caused by familiarity of such a stimulus, that is, it is associated with the evaluation of stimulus familiarity (Rosa Salva et al. 2012). In case of the Amur sleeper fry, this could explain the left-eye bias not only in inspection of mirror and a group of shoal mates, but also in viewing flat and motionless conspecific image as well as an eye alone, since all these images are the elements of a familiar stimulus—a real conspecific. Thus, the need to establish identity between the experimental stimuli and one previously experienced may have determined the left visual hemifield bias. Hence, the lateralization in social recognition found here in fry is, potentially, the reflection of the right brain hemisphere specialization for more general processes.

The evolution of brain lateralization has been argued to be associated with the need of simultaneous parallel processing of information of different types (Andrew 1991; Rogers 2000, 2002, but see Hirnstein et al. 2008 for controversial evidence in humans). Separation of labour between the two hemispheres could potentially favour allocating attention to different activities simultaneously. A number of empirical data support this hypothesis (e.g. De Santi et al. 2002; Dadda and Bisazza 2006a, b; Rogers et al. 2004). In fish, lateralized individuals have been showed to

be significantly more efficient than non-lateralized in situations, which require sharing the attention between vigilance and prey capture (Dadda and Bisazza 2006b) or food searching and avoiding harassing male (Dadda and Bisazza 2006a), suggesting that they are able to process in parallel concurrent visual information. A further example of how complementary visual lateralizations impact on fish behaviour is that during predator inspection, mosquitofish get closer to the predator when their own mirror images are visible on the left rather than on the right side (Bisazza et al. 1999). A later study demonstrated that two opposite specializations working in parallel are involved in this phenomenon—right-eye preference for predator inspection and left-eye preference for scrutiny of a companion (own mirror reflection) (De Santi et al. 2001). The division of general functions between the two hemispheres seems to be true also for the Amur sleeper. Previously, we have found that this fish is significantly more reactive to a live prey visible in the visual field of the right eye than in the field of the left eye (Giljov et al. 2009). In the present study, we showed a significant left-eye preference for the inspection of social stimuli. These complementary specializations in visual processing, comparable to those for most other fishes (Nepomnyashchikh and Izvekov 2006; Bisazza and Brown 2011) and vertebrates in general (Vallortigara and Rogers 2005), may confer a benefit in the efficient performance of concurrent cognitive tasks. It can be suggested that in Amur sleeper, opposite lateral preferences in feeding and social responses favour to a better performance in schooling behaviour and parallel food searching, owing to partition of these two processes in different parts of the brain. In addition to being advantageous for multitasking, lateralization could enhance the success of schooling per se. Bisazza and Dadda (2005) showed the schools of lateralized fish display better cohesion and coordination in a new environment as compared to the schools of non-lateralized fish. Furthermore, in mixed shoals with both lateralized and non-lateralized individuals, the former ones more often occupied the safer and energetically more beneficial position—the core of the shoal—than the latter ones. Thus, lateralization seems to have a great impact on individual fitness, especially in social contexts (Ghirlanda et al. 2009; MacNeilage et al. 2009; Rosa Salva et al. 2012), and this may be the principal reason for the evolution of pronounced lateralized reaction not only to a social stimulus as a whole, but even to a single key element of it.

According to the 'sign stimuli' conception (Tinbergen 1948, 1951), specific traits in animals' appearance serve as socially significant signals and produce specific behavioural responses in conspecifics. The results of the present study indicate that the eye is the sign component of the whole conspecific image for eliciting specific lateralized response (preferential left-eye use) in the Amur sleeper fry.

In terms of behavioural lateralization in general, this means that there are particular ‘keys’ perceived by an individual, which determine the lateralized response to a social stimulus, and the left-eye–right hemisphere system could be responsible for perception of such key elements. It is well-established that rapid, species-typical responses are under the control of the right brain hemisphere (Vallortigara and Rogers 2005; George 2010). For instance, the right hemisphere in humans was showed to be faster than the left in performing emotional face processing (Pizzagalli et al. 1999). Prerogative of the right brain hemisphere is a typically environmentally motivated behaviour calling for the immediate reaction from the animal, such as conspecific recognition and control of social responses (MacNeilage et al. 2009). Apparently, key or sign elements of conspecific appearance, allowing avoiding longer analysis of the whole stimulus, serve well for quick and easy recognition of and responding to a social partner. Thus, the major role of the right hemisphere in analysing of the key elements of social image can be explained by the need for producing a rapid response to the stimulus. Furthermore, the preference for the left eye in non-aggressive social interactions, particularly in scrutiny of potential shoal mates, in fishes (Sovrano et al. 1999, 2001; Sovrano and Andrew 2006) and anurans (Bisazza et al. 2002; Dadda et al. 2003) could be due to the preferential role of the right hemisphere in the rapid monitoring of companions in the environment with the use of social sign stimuli. It is probable that this way of analysis is characteristic for other animals, as well.

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References

- Andrew RJ (1991) The nature of behavioural lateralization in the chick. In: Andrew RJ (ed) Neural and behavioural plasticity: the use of the chick as a model. Oxford University Press, Oxford, pp 536–554
- Anfora G, Frasnelli E, Maccagnani B, Rogers LJ, Vallortigara G (2010) Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee. *Behav Brain Res* 206(2):236–239
- Amott G, Ashton C, Elwood RW (2011) Lateralization of lateral displays in convict cichlids. *Biol Lett* 7:683–685
- Beeching SC (1993) Eyespots as visual cues in the intraspecific behavior of the cichlid fish *Astronotus ocellatus*. *Copeia* 4:1154–1157
- Bisazza A, Brown C (2011) Lateralization of cognitive functions in fish. In: Brown C, Laland K, Krause J (eds) Fish cognition and behavior. Wiley, Oxford, pp 298–324
- Bisazza A, Dadda M (2005) Enhanced schooling performance in lateralized fishes. *P Roy Soc B* 272:1677–1681
- Bisazza A, De Santi A (2003) Lateralization of aggression in fish. *Behav Brain Res* 141:131–136
- Bisazza A, Pignatti R, Vallortigara G (1997) Laterality in detour behaviour: interspecific variation in poeciliid fish. *Anim Behav* 54(5):1273–1281
- Bisazza A, De Santi A, Vallortigara G (1999) Laterality and cooperation: Mosquitofish move closer to a predator when the companion is on their 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
- Bisazza A, De Santi A, Bonso S, Sovrano VA (2002) Frogs and toads in front of a mirror: lateralization of response to social stimuli in five tadpole amphibians. *Behav Brain Res* 134:417–424
- Brown C, Laland KN (2003) Social learning in fishes: a review. *Fish Fish* 4:280–288 (special edition)
- Brown C, Gardner C, Braithwaite VA (2004) Population variation in lateralized eye use in the poeciliid *Brachyrhaphis episcopi*. *Proc R Soc Lond B* 271:S455–S457
- Brown C, Western J, Braithwaite VA (2007) The influence of early experience on, and inheritance of, cerebral lateralization. *Anim Behav* 74:231–238
- Dadda M, Bisazza A (2006a) Lateralized female topminnows can forage and attend to a harassing male simultaneously. *Behav Ecol* 17:358–363
- Dadda M, Bisazza A (2006b) Does brain asymmetry allow efficient performance of simultaneous tasks? *Anim Behav* 72:523–529
- Dadda M, Sovrano VA, Bisazza A (2003) Temporal pattern of social aggregation in tadpoles and its influence on the measurement of lateralised response to social stimuli. *Physiol Behav* 78:337–341
- De Santi A, Sovrano VA, Bisazza A, Vallortigara G (2001) Mosquitofish display differential left- and right-eye use during mirror-image scrutiny and predator-inspection responses. *Anim Behav* 61:305–310
- De Santi A, Bisazza A, Vallortigara G (2002) Complementary left and right eye use during predator inspection and shoal-mate scrutiny in minnows. *J Fish Biol* 60:1116–1125
- Dmitriev MA (1971) Look out, rotan. *Rybovodstvo i Rybolovstvo* 1:26–27 (in Russian)
- Engeszer RE, Ryan MJ, Parichy DM (2004) Learned social preference in zebrafish. *Curr Biol* 14:881–884
- Engeszer RE, Da Barbiano LA, Ryan MJ, Parichy DM (2007a) Timing and plasticity of shoaling behaviour in the zebrafish, *Danio rerio*. *Anim Behav* 74(5):1269–1275
- Engeszer RE, Patterson LB, Rao AA, Parichy DM (2007b) Zebrafish in the Wild: a review of natural history and new notes from the field. *Zebrafish* 4:21–40
- Frasnelli E, Vallortigara G, Rogers LJ (2012) Left–right asymmetries of behaviour and nervous system in invertebrates. *Neurosci Biobehav Rev* 36(4):1273–1291
- George I (2010) Hemispheric asymmetry of Songbirds. In: Hugdahl K, Westerhausen R (eds) The two halves of the brain: information processing in the cerebral hemispheres. MIT Press, Cambridge, pp 91–120
- Ghirlanda S, Vallortigara G (2004) The evolution of brain lateralization: a game theoretical analysis of population structure. *P Roy Soc B-Biol Sci* 271:853–857
- Ghirlanda S, Frasnelli E, Vallortigara G (2009) Intraspecific competition and coordination in the evolution of lateralization. *Philos T Roy Soc B* 364:861–866
- Giljov AN, Karenina KA, Malashichev YB (2009) An eye for a worm: Lateralisation of feeding behaviour in aquatic annelids. *Laterality* 14(3):273–286
- Gómez-Laplaza LM, Gil-Carnicero P (2008) Imprinting in fish: a little explored phenomenon with possible implications for fish welfare. *Ann Rev Biomed Sci* 10:51–62

- Hirnstain M, Hausmann M, Güntürkün O (2008) The evolutionary origins of functional cerebral asymmetries in humans: does lateralization enhance parallel processing? *Behav Brain Res* 187:297–303
- Kaarthigeyan J, Dharmaretnam M (2005) Relative levels of motivation and asymmetries of viewing and detour task in guppies (*Poecilia reticulata*). *Behav Brain Res* 159:37–41
- Karenina K, Giljov A, Baranov V, Osipova L, Krasnova V, Malashichev Y (2010) Visual laterality of calf–mother interactions in wild whales. *PLoS One* 5(11):e13787
- MacNeilage PF, Rogers LJ, Vallortigara G (2009) Origins of the left and right brain. *Sci Am* 301:60–67
- Malashichev Y (2006) Is there a link between visceral and neurobehavioural asymmetries in development and evolution? In: Malashichev YB, Deckel W (eds) *Behavioural and morphological asymmetries in vertebrates*. Georgetown, Landes Bioscience, pp 33–44
- Martin FD, Hengstebeck MF (1981) Eye colour and aggression in juvenile guppies, *Poecilia reticulata peters* (Pisces: Poeciliidae). *Anim Behav* 29(2):325–331
- Miletto Petrazzini ME, Agrillo C, Piffer L, Dadda M, Bisazza A (2012) Development and application of a new method to investigate cognition in newborn guppies. *Behav Brain Res* 233(2):443–449
- Miyai CA, Carretero Sanches FH, Tânia MC, Colpo KD, Volpato GL, Barreto RE (2011) The correlation between subordinate fish eye colour and received attacks: a negative social feedback mechanism for the reduction of aggression during the formation of dominance hierarchies. *Zool* 114:335–339
- Nepomnyashchikh VA, Izvekov EI (2006) Variability of the behavioural laterality in Teleostei (Pisces). *J Ichthyol* 46(S2):S235–S242
- Pitcher TJ, Parrish JK (1993) Functions of shoaling behaviour in teleosts. In: Pitcher TJ (ed) *Behaviour of teleost fishes*. Chapman & Hall, London, pp 363–439
- Pizzagalli D, REGARD M, Lehmann D (1999) Rapid emotional face processing in the human right and left brain hemispheres: an ERP study. *NeuroReport* 10(13):2691–2698
- Price AC, Weadick CJ, Shim J, Rodd FH (2008) Pigments, patterns, and fish behavior. *Zebrafish* 5:297–307
- Robins A, Lipollis G, Bisazza A, Vallortigara G, Rogers LJ (1998) Lateralized agonistic responses and hindlimb use in toads. *Anim Behav* 56:875–881
- Rogers LJ (1989) Laterality in animals. *Int J Comp Psychol* 3:5–25
- Rogers LJ (2000) Evolution of hemispheric specialization: advantages and disadvantages. *Brain Lang* 73(2):236–253
- Rogers LJ (2002) Lateralization in vertebrates: its early evolution, general pattern, and development. In: Slater PJB, Rosenblatt J, Snowden C, Roper T (eds) *Advances in the study of behavior*, vol 31. Academic Press, New York, pp 107–162
- Rogers LJ, Andrew RJ (2002) *Comparative vertebrate lateralization*. Cambridge University Press, Cambridge
- Rogers LJ, Zucca P, Vallortigara G (2004) Advantages of having a lateralized brain. *Proc R Soc Lond B* 271(suppl.):420–422
- Rosa Salva O, Regolin L, Mascalon E, Vallortigara G (2012) Cerebral and behavioural asymmetries in animal social recognition. *Comp Cogn Behav Rev* 7:110–138
- Rowland WJ (2000) Habituation and development of response specificity to a sign stimulus: male preference for female courtship posture in stickleback. *Anim Behav* 60:63–68
- Saverino C, Gerlai R (2008) The social zebrafish: behavioral responses to con-specific, heterospecific, and computer animated fish. *Behav Brain Res* 191:77–87
- Schroder S, Zaret T (1979) The adaptive significance of color patterns in *Cichla ocellaris*. *Copeia* 1:43–47
- Sovrano VA (2004) Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behav Brain Res* 152:385–391
- Sovrano V, Andrew R (2006) Eye use during viewing a reflection: behavioral lateralization in zebrafish larvae. *Behav Brain Res* 167:226–231
- Sovrano V, 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
- Sovrano V, Bisazza A, Vallortigara G (2001) Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiol Brain* 74:237–244
- ten Cate C (2009) Niko Tinbergen and the red patch on the herring gull's beak. *Anim Behav* 77:785–794
- Tinbergen N (1948) Social releasers and the experimental method required for their study. *Wils Bull* 60(1):6–51
- Tinbergen N (1951) *The study of instinct*. Oxford University Press, London
- Vallortigara G (1992) Right hemisphere advantage for social recognition in the chick. *Neuropsychologia* 30:761–768
- Vallortigara G (2006a) The evolution of behavioural and brain asymmetries: bridging together neuropsychology and evolutionary biology. In: Malashichev Y, Deckel W (eds) *Behavioral and morphological asymmetries in vertebrates*. Landes Bioscience, Austin, pp 1–20
- Vallortigara G (2006b) The evolutionary psychology of left and right: costs and benefits of lateralization. *Dev Psychobiol* 48:418–427
- Vallortigara G, Rogers LJ (2005) Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav Brain Sci* 28:575–589
- Vallortigara G, Cozzutti C, Tommasi L, Rogers LJ (2001) How birds use their eyes: opposite left–right specialisation for the lateral and frontal visual hemifield in the domestic chick. *Curr Biol* 11:29–33
- Vallortigara G, Chiandetti C, Sovrano VA (2011) Brain asymmetry (animal). *WIREs Cogn Sci* 2:146–157
- Vanegas H, Ito H (1983) Morphological aspects of the teleostean visual system: a review. *Brain Res Rev* 6:117–137