An eye for a worm: Lateralisation of feeding behaviour in aquatic anamniotes
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Some animals, notably birds, preferentially approach and capture food items in their right visual field. However, this lateralised behaviour has not been studied extensively in anamniotes. Here we test eye preference during feeding for a fish, \( \textit{Perccottus glenii} \); Teleostei, Perciformes), a newt, \( \textit{Pleurodeles walti} \); Amphibia, Caudata), and a frog, \( \textit{Xenopus laevis} \); Amphibia, Anura) using a test chamber that assesses reaction to visual stimuli while blocking olfactory and mechanical input. Both the fish and the newt showed right preferences in reactions to food items, but the frog did not. Our data extend our knowledge of the lateralised behaviours of vertebrates and are the first record of lateralised prey capture in a caudate amphibian. This finding dates back the history of the common pattern for visual lateralisation in vertebrates to Devonian, when the fish and quadruped lineages diverged.

Keywords: Lateralisation; Eye preference; Prey-catching behaviour; Fish; Amphibians; Caudata.

Lateralised reactions to various stimuli positioned on the left or on the right of the animal have been recorded for a number of vertebrate species (reviewed in Vallortigara & Rogers, 2005). One-sided preferences in eye use during perception of visual stimuli, e.g., social objects, predator, or food, were demonstrated first and explored most intensively in the domestic chick (Andrew & Brennan, 1983; Rogers & Anson, 1979; Vallortigara & Andrew, 1991, 1994). For example, visual lateralisation of feeding behaviour is well...
studied in a variety of bird species both in the laboratory (Alonso, 1998; Güntürkün & Kesch, 1987; Mench & Andrew, 1986; Valenti, Sovrano, Zucca, & Vallortigara, 2003) and in the field (Rogers & Kaplan, 2006; Ventolini et al., 2005). For classes other than birds, lateralisation of feeding responses is known only for one teleost fish (zebrafish, *Brachydanio rerio*; Miklosi & Andrew, 1999), three anuran amphibians—all three are toads of the genus *Bufo* (Robins & Rogers, 2004; Vallortigara, Rogers, Bisazza, Lippolis, & Robins, 1998)—and one reptile (the ornate dragon lizard, *Ctenophorus ornatus*; Robins, Chen, Beazley, & Dunlop, 2005). With such a small sample the evolutionary history of lateralised responses to prey items cannot easily be resolved.

Among lower vertebrates, lateralised responses to potential food items were recorded in *B. rerio*, when the fish were presented with small coloured beads. The fish initially fixated on the objects preferentially with the right eye before trying to bite them (Miklosi & Andrew, 1999). Responses in this test were likely to be predatory, because the fish was presented with some food at the place of testing just before being presented with a bead. Interestingly the right eye preference, as well as biting activity, gradually disappeared over further trials and was restored when the bead colour was changed, similar to a phenomenon of learning food items described for birds (Aoki, Izawa, Kago, Shin, & Matsushima, 2000). In subsequent experiments (Miklosi, Andrew, & Gasparini, 2001) fish could see a bead through a barrier of vertical bars and preferentially approached the bead by turning round the edge of the barrier with their right eye close to the barrier. It was thus probable that the right eye/left hemisphere system was also responsible for the choice of the route to the food. In another test, fish observed two identical beads and then swam towards one of them from the corridor ending with inclined (to the left or to the right) glass walls. After leaving the corridor pointing to the right, the fish maintained the preference to bite the nearest (right) target, whereas fish forced to swim in the left-inclined corridor demonstrated left preferences, but quickly lost them in subsequent trials, again suggesting a greater importance of the target in the right visual hemifield.

The right side preference in prey catching has also been demonstrated for a few species of *Bufo* toads (Vallortigara et al., 1998), by placing them in a transparent cylinder while a live insect was rotated outside the cylinder in a clockwise or counterclockwise direction starting from behind the toad. Both *B. bufo* and *B. viridis* directed most strikes at the prey when it was moving clockwise and entered the right visual hemifield. When the prey was moving counterclockwise there was no significant side bias, although a tendency for more strikes to the right of the toad’s midline remained for *B. viridis*. *Bufo marinus* also showed a right side preference, but not as strong as in the other two *Bufo* species, although later studies using a dummy prey revealed
a significant right eye bias in this species as well (Robins & Rogers, 2004).
Lastly B. marinus showed significant right eye preference in direction of predatory tongue strikes when tested in an open field with freely moving live crickets as prey (Robins & Rogers, 2006a).
In the present paper we used a common test protocol for assessing lateralised responses to potential prey for various aquatic lower vertebrates. Our protocol excludes non-visual sensory inputs, thus strictly revealing visual lateralisation. We report here on the lateralised prey-catching behaviour of the fish, the Amur sleeper, Percottus glenii (Pisces, Teleostei, Perciformes), a representative of a most advanced clade of teleost fishes. We also show for the first time visually guided lateralised behaviour in response to a food stimulus in a caudate amphibian, the ribbed newt, Pleurodeles walti (Amphibia, Caudata), while in an aquatic frog species, the African smooth clawed frog, Xenopus laevis (Amphibia, Anura), lateralisation was not found. These results extend our knowledge of lateralisation in the visual system of vertebrates and allow speculation on its evolutionary history.

METHOD
Immature 5–6-month-old P. glenii fry (N = 12) were collected from a wild population (outskirts of Saint Petersburg, north-west Russia). This native of the Russian Far East was introduced into the European part of Russia at the beginning of the last century (Dmitriev, 1971). Fish were kept singly in opaque plastic tanks (3 l) containing plants (Ceratophyllum sp.) and maintained in the laboratory 40 days prior to the start of the experiment. Animals were maintained in a 14-h/10-h light/dark period with water temperature at 20 ± 2°C. They were fed live bloodworm (Chironomus sp.) larvae every other day.

Juvenile frogs (metamorphosed, but not older than 2 months), X. laevis (n = 14), and newts, P. walti (N = 19), were obtained from several pairs of parents from a local supplier (the number of generations in captivity is not known) and kept in the laboratory for 3 months before testing. Individuals of both species were kept singly in opaque plastic tanks (2 l), which were lit from above under a 12-h/12-h photoperiod with water temperature maintained at 25 ± 2°C. Xenopus laevis were fed every 3 days and P. walti every 2 days with live Chironomus sp. larvae. Thus all tested animals were familiar with bloodworms as a prey well before the experiment.

Each animal was tested in the same apparatus (Figure 1). The design of this testing apparatus excluded the possibility that olfactory or mechanical cues coming from the Chironomus larvae could be detected by the animal in the testing corridor, while visual information could be easily processed.
Animals were placed singly in the blunt end of the corridor (temporarily separated from the rest of the apparatus by an opaque barrier) and allowed to settle for 1 minute. At this time the testing individual was given a few *Chironomus* larvae to stimulate feeding reaction in the apparatus. After the barrier was lifted the animal was allowed to move forward along the corridor, attracted by a frontal presentation of *Chironomus* larva in pincers moving exactly along the central dashed line marked on the floor of the testing apparatus.

**Figure 1.** A photo (A) and a schematic representation (B) of the testing apparatus (view from above) made of transparent Plexiglas. It constitutes a long rectangular (21 × 4 × 5 cm) corridor (1) with walls converging to a sharp angle at one end and symmetrical transparent cuvettes (2) of 3 × 1 × 5 cm suspended outside the corridor closer to its pointed end. The testing apparatus is placed inside a larger tank with white opaque walls and is evenly illuminated from above. Both the testing corridor and the cuvettes are filled with water up to 3 cm deep. The laterally placed cuvettes with their front opaque wall (3) contain the bloodworms (10 ± 2 mm length, N = 20 in each cuvette); hence the food was hidden from the animal’s view until the animal reached the level of the cuvettes. The arrow indicates the direction of the animal’s movement during the testing trial along the dashed line (4) on the bottom of the testing corridor.

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corridor. This procedure was carried out using both arms of the experimenter (the right arm holding the pincers was supported by the left arm in such a way that the pincers with food were positioned without any lateral bias). When the animal reached the level of the cuvettes, the experimenter cautiously (in order not to scare the animal) pulled away the pincers with the food. By this time the tested animal was situated exactly between the two cuvettes. After noticing the food it chose the left or the right cuvette with food to attack; i.e., it turned to it and tried to catch the larvae.

The direction of the predatory turn was recorded. The trials when the animal was positioned asymmetrically (closer or half turned to one of the cuvettes) prior to the attack were discarded. Ten successful trials for each individual animal were performed with each trial conducted subsequently every 5 days. In between the trials the animals were deprived of food, hence eliciting a higher responsiveness to larvae during the testing procedure, and were fed only once in 5 days between trials. The number of right side trials was scored for each animal. To reveal the preferential use of the left or the right eye for each species, paired Student’s t-test and log-likelihood ratio chi-square test (G-test) were used.

RESULTS

A significant rightward bias in predatory biting was found in *P. glenii* (mean percentage right ± MSE was 75.8 ± 5.6; \( t_{11} = 4.63, p = .0007 \); Figure 2A). For 12 fish the total of 29 leftward and 91 rightward bites was observed, \( G_1 = 33.64, p < .0001 \). The majority of individuals preferred to bite more often on one side (8 or more out of 10 trials). Eight fish were therefore scored right-eyed and only one fish was scored left-eyed (Figure 2B); hence the lateralisation at the group level arises from a high percentage (67%) of individuals lateralised to one and the same side.

A non-significant right-sided preference was indicated in *X. laevis* (mean percentage right ± MSE was 55.0 ± 5.3; \( t_{13} = 0.94, p = .36, ns \); Figure 2C). Frogs (\( N = 14 \)) showed a total of 63 left-sided and 77 right-sided responses (\( G_2 = 1.4, p = .2367, ns \)). Only few frogs were asymmetric, demonstrating individual rightward (\( N = 2 \)) or leftward (\( N = 1 \)) biases (Figure 2D). Therefore, the overall distribution is symmetrical, with few individuals evenly lateralised to either side or not lateralised at all.

*P. walti*, in contrast, demonstrated highly lateralised feeding behaviour (mean percentage right ± MSE was 84.4 ± 3.1; \( t_{17} = 11.3, p < .0001 \); Figure 2E). For 18 newts a total of 28 leftward and 152 rightward bites was observed, \( G_1 = 93.93, p < .0001 \). It is worth noting that a majority of individuals (14 out of 18) were more likely to bite to the right side (8 or more out of 10 trials) and thus they were scored right eyed (Figure 2F). Moreover,
four individuals showed 10 rightward bites on all 10 testing trials (individuals of other species never showed such a high ratio of right to left bites). Therefore, the lateralisation at the group level arises from the fact of a
DISCUSSION

Our data show the existence of a right-sided group-level visual lateralisation for feeding in a fish species, \textit{P. glenii}. This lateralisation corresponds to that of the zebrafish, which shows significant group-level lateralisation for fixation and biting on a dummy prey object (Miklosi & Andrew, 1999; Miklosi et al., 2001). However, in the zebrafish the right eye preference at a first presentation decreased during further trials. In our study the fish could select between equivalent live food that appeared simultaneously on both sides and the right-sided choices were shown repeatedly from trial to trial. Individual trials spaced 5 days apart reduced the possibility of learning the procedure and the apparatus. Our apparatus further excluded any possibility that non-visual sensory information was used. Therefore we can conclude that \textit{P. glenii} is clearly lateralised in prey-catching behaviour mediated by the visual analyser.

The Amur sleeper belongs to a more advanced order of teleost fishes, the Perciformes, while the zebrafish represents a more basal clade, the Cypriniformes. Since these two species belonging to phylogenetically divergent groups of teleost fishes both have lateralised feeding behaviour, this suggests that such a laterality might be common to teleosts in general at the individual and group levels, particularly in social species (Bisazza, Cantalupo, Capocchiano, & Vallortigara, 2000; Ghirlanda & Vallortigara, 2004). Indeed, there are several other perciform fish from Lake Tanganyika demonstrating behavioural laterality when feeding, e.g., hunting on shrimps (Takeuchi & Hori, 2008). However, in the latter and other similar examples with Tanganyikan cichlids there is a pronounced morphological asymmetry of the jaw apparatus (skewed mouths), which attributes to the observed behavioural laterality. In our experiment with the Amur sleeper, it is more likely that the fish showed behavioural lateralisation not connected to morphological asymmetry in the jaw mechanics.

In \textit{X. laevis} we found no significant group-level preference in eye use during feeding, although there was a slight tendency of the frogs to preferentially attack food on the right side. In our experiments recently metamorphosed juveniles were used because in this species the eyes are still positioned laterally at this age; later they move on to the dorsal side of the head and the lateral hemifields of vision are minimal in mature frogs (for more details see Elepfandt, 1996). As a result the adult frogs, with the gaze directed above the head and not to the lateral cuvettes, would probably not see the food, or the lateral monocular fields would be so small that...
additional factors (e.g., morphological asymmetry of the head) could influence the probabilities of detecting food on either side. Hence visual lateralisation in adult *Xenopus* with dorsally, but not laterally (as in juveniles), placed eyes would likely not be easily and correctly assessed with a kind of testing procedure such as we used for our experiments.

Despite the absence of the group-level lateralisation, three individuals showed a preference (8 or more out of 10 trials) to either the left or right side. The ability to reveal individual asymmetries suggests that the method described in this study is appropriate for this species, whereas the nonsignificant lateralisation at the group level is explained by a low number of highly lateralised individuals. Other authors (Gouchie, Roberts, & Wassersug, 2008) also found low visual lateralisation in *X. laevis* tadpoles, which observed their own mirror images, being significant only in a few age stages and directed right-about to that in other anuran species studied so far (Bisazza, De Santi, Bonso, & Sovrano, 2002). However, in our case *X. laevis*’s slight rightward tendency to attack prey was similar to that of *Bufo* (Vallortigara et al., 1998). Interestingly, a nonsignificant, but still right-sided, tendency in predatory responses was found in the Australian green tree frog (*Litoria caerulea*; Robins & Rogers, 2006b), suggesting that the right-sided preference for prey catching may be a universal for anurans with its level of expression varying among species.

Our data are the first record of visual lateralisation in a caudate amphibian. Compared to the other species we studied, *P. walti* were least stressed when placed in the testing apparatus and always reacted strongly to food. These reactions were biased to the right, revealing highly significant lateralisation of feeding behaviour at the group level as well as at the level of individuals. A behavioural lateralisation was found previously during the sexual display in *Triturus vulgaris* (Green, 1997). However, the author related the bias for left-sided courtship not to lateralisation in the visual system but to the lateralised tail use, i.e., motor lateralisation, in this species. In contrast, a congener (*T. alpestris*) showed no preference in similar experiments (Marzona & Giacomo, 2002). Although we believe that in our experiment the visual lateralisation played the main role, we cannot exclude that lateralised tail movements in newts (secondary to the initial sensory reaction to food) may coincide in direction to the lateralised feeding reaction, and thus enhance the sensory lateralisation revealed in our tests. Hence, the lateralisation here is so pronounced. Generalised tailed amphibians of the family Cryptobranchidae are known for their unusual asymmetric suction feeding, when the food is drawn in by the unilateral opening of the mouth (Cundall, Lorenz-Elwood, & Groves, 1987). The side of suction depended on where the food was situated prior to the attack and the lateralisation of the behaviour was not reported.
For our study we used a testing method to assess behavioural asymmetry in response to visual stimuli suitable for fully aquatic vertebrates. A similar protocol to ours is the so-called “choice-test” used for terrestrial anuran amphibians by Robins and Rogers (2006a). There, two novel prey stimuli (model insects) were attached to the ends of the Y-shaped wire fork and were presented from behind to the animal (*B. marinus*). At the moment when the toad was presented the choice, it had been fixed on a “zeroing” stimulus—a vertical bar moving vertically on a screen in front of the toad. In our apparatus food stimuli in two symmetrical cuvettes were analogous to the model insects on the Y-fork, while the *Chironomus* larva in pincers in front of the animal was similar to the “zeroing” stimulus in Robins and Rogers’ experiment. However, the prey we used was familiar to the animal (which was fed on it long before the tests) and the larva in the forceps was taken away when the animal reached the cuvettes. Apparently these principal distinctions (first of all, the familiar prey) may explain why the leftward bias was observed in toads in the choice test, while our data revealed rightward tendencies in all non-toad species studied.

Zebrafish and chicks, in tests with introduction of coloured beads, as well as toads in the choice test, demonstrated gradual disappearance of lateralisation due to habituation to the visual stimulus, thus showing true visual and not motor asymmetry (Aoki et al., 2000; Miklosi & Andrew, 1999; Miklosi et al., 2001; Robins & Rogers, 2006a). As our tests were designed in a very similar way to the choice test we can be sure that we also measured mainly the sensory reactions. We scored as reactions to food both turns to fixate the food with an eye and attempts to catch the prey without pre-fixation with an eye. Since the movements here differed between individuals and species, probably involving various muscles and motor neurons, it therefore supports the notion that the results are mainly due to lateralisation in the visual analyser. It is especially possible assuming strong motivation for food in conditions of food deprivation. However, complete discrimination of asymmetry in the visual analyser from a motor preference is hardly possible, at least due to a possibility of lateralised tail use in newts (see above) and fish.

Our data are completely in line with the pattern of eye/hemisphere specialisation common to other vertebrates, where the right eye/left hemisphere controls feeding responses, striking at prey, and non-fearful inspection of the environment, in contrast to the left eye/right hemisphere control of agonistic interactions (Koboroff, Kaplan, & Rogers, 2008; Rogers, 2002). In showing this right eye preference our data indicate that lateralisation of the predatory behaviour occurs in non-amniote aquatic lower vertebrates. They also raise the evolutionary question of how ancient in vertebrates is such a specialisation of the hemispheres for categorisation of objects, e.g., food.
Previously the lateralised response to food items, with preferential reactions to those on the right, has been demonstrated for several species of birds, some anurans, one lizard, and one teleost fish. We added another species of teleost to this list. However, the simple assumption that a trait present in bony fish must be ancestral is not fully correct. Indeed, although teleost fish are generally considered to be on a lower organisational grade than birds and amphibians, they emerged in Triassic, i.e., after the divergence of cartilaginous and osseous fish, including those leading to quadruped vertebrates. Birds and anurans are terminal specialised taxa within amphibian and reptilian grades. Therefore teleost fish, birds, and anurans could have developed lateralisation in food discrimination independently. Lateralisation in lizards only partially fills in the existing evolutionary gaps (Robins et al., 2005).

Our finding that a caudate amphibian is lateralised in visually guided response to food stimuli is therefore important from the evolutionary point of view. It is the first record of the lateralised function of the visual analyser in caudate amphibians, a group that dates back to Devonian. It suggests that the earliest tetrapods and fish possessed a common pattern of lateralisation of the visual system. However, it is premature to conclude that all the jawed vertebrates would preferentially attack food items on their right side. To support this proposition one should first examine visual lateralisation in primitive actinopterygian fishes, lung fishes, and sharks. To the best of our knowledge, there is only one paper showing partial asymmetry in jaw muscle contraction during feeding of the gar *Lepisosteus oculatus* (Holostei, Lepisosteiformes; Lauder & Norton, 1980). Lateralised behaviours in these groups are not known.

There is also an accumulating evidence of nervous system asymmetry in invertebrates including different types of worms and several species of insects and spiders (see for a review, Malashichev, 2006). A more recent paper clearly demonstrates behavioural laterality in a nematode, *Caenorhabditis elegans*, in response to salt concentrations due to asymmetric functioning of the contralateral taste neurons (Suzuki et al., 2008). Differential chemotaxes based on asymmetrical distribution of receptors in antennas or differential processing in brain hemispheres seem to be common in insects as well. Lateralised olfactory learning has been shown in honeybees, *Apis mellifera* (Letzku et al., 2006) requiring lateralisation of information processing and its lateral transfer between the sides of the brain (Rogers & Vallortigara, 2008; Sandoz & Menzel, 2001) similar to that found in vertebrates (Andrew, 1999). It has also been proposed that brain lateralisation in vertebrates and insects can be similar despite the striking differences in formation of the asymmetry of the rest of the body (Malashichev & Wassersug, 2004). It is not clear, however, whether brain lateralisations here are homologues and similar in all details. For example,
bees recall olfactory information better with the right antenna in use, and the recall is possible with the left antenna after some training (Rogers & Vallortigara, 2008). In contrast, toads react better when they use the left eye for the novel, and the right eye for the familiar, prey (Robins & Rogers, 2006a).

Bumblebees of four species consistently rotate either clockwise (*Bombus lapidarius*) or anticlockwise (*B. lucorum*, *B. pascuorum*), or show no preference (*B. terrestris*) in rotation around inflorescence (Kells & Goulson, 2001). Although they are related to honeybees, which are shown to possess visual lateralisation in memory of colours (Letzkus, Boeddeker, Wood, Zhang, & Srinivasan, 2008), it is not clear whether olfactory or visual cues are used here. Regardless of the particular sensory modality, the inconsistency in the direction of lateralisation in four species of bumblebees makes it difficult to judge whether lateralisation is universal even within Hymenoptera. It also does not support the proposition that the direction of circling around flowers depends on the delay period after initial learning and hence the left or right antenna in use (Rogers & Vallortigara, 2008), since the circling, in bumblebees at least, is species but not condition specific. Moreover, there is also no consistency in direction of lateralisation in searches for food in other insects studied so far. Although rightward population-level biases for the first turn and searches for food in a dichotomy maze were found in ants, *Mirmica rubra* (Udalova & Karas, 1986), no such lateralisation was found in foraging seven-spot ladybird, *Coccinella septempunctata*, with only 45% of animals lateralised to either side when walking along a branched Y-maze and thus showing an antisymmetrical distribution (Girling, Hassall, & Turner, 2007). However, it is not clear to what extent those insects could use the olfactory or other sensory cues in both experiments. Similarly, the cephalopod molluscs, as another example of invertebrate animals with laterally placed eyes used for prey determination, also demonstrated only individual asymmetries in eye use for food, while the population distribution is again antisymmetric with equal numbers of left-eyed and right-eyed animals (Byrne, Kuba, & Meisel, 2004). Therefore despite the enormous interest of such studies of lateralised behaviour in insects, so far they provide a complicated picture of its evolution and give us no precise idea on the evolutionary time when the specific pattern of visual lateralisation seen in most vertebrates—e.g., considering items on the right as prey—first appeared.

It is probable that the answer could come from the study of predatory and parasitoid insects. One such example could be the European beewolf, *Philanthus triangulum*. This solitary wasp is a specialised predator, which flies from flower to flower in search of its food but although reacting to all moving objects, attacks nothing but bees. Both visual and olfactory cues are used by *Philanthus* depending on the distance to the potential prey, first
vision and then olfaction (Herzner, Schmitt, Linsenmair, & Strohm, 2005; Tinbergen, 1935, 1975). Whether reactions to potential prey guided either by vision or olfaction are lateralised is not known. However, the very small amount of (Z)-11-eicosen-1-ol serves as a recognition cue to discriminate bees from other insects, the substance that is also specific to beewolf males and thus used by females to recognise a sexual partner. Interestingly, in other insects olfactory cues are used in recognition of a sexual partner as well, and the corresponding reactions are indeed lateralised in females of cockroaches, *Nauphoeta cinerea*, due to asymmetry in deuotocerebrum receiving inputs from the left and the right antennas (Streng, 2003). Therefore, it possible to surmise that in beewolfs the reaction to prey items, at least those guided by the olfactory cues, should be lateralised, while both olfactory and visual lateralisation is worth studying in this and other predatory invertebrates to paint a complete picture of the evolution of lateralised functions of the neural system.

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REFERENCES

PREY CATCHING IN AQUATIC ANAMNIOTES


