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'Handedness' in snakes? Lateralization of coiling behaviour in a cottonmouth, *Agkistrodon piscivorus leucostoma*, population

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Studies have documented the presence of behavioural lateralization in many groups of lower vertebrates, demonstrating that these behaviours are not limited to mammals and birds. These studies suggest that the evolution of brain lateralization, often linked to lateralized behaviours, may have occurred early in evolutionary history and may not have been the result of multiple independent evolutionary events as previously thought. The goal of this study was to further document behavioural lateralization in another group of lower vertebrates, snakes. Given the importance of the coiling posture to snakes, I examined the coiling behaviour of a cottonmouth population. Coiling asymmetries were found at both the individual and population levels. However, the adaptive significance and mechanisms influencing this behaviour remain undefined. Additional research is needed to explore these areas and to link the lateralized behaviours documented in this and other studies directly to brain asymmetries before the evolutionary history of brain lateralization can be further resolved.

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In higher vertebrates, such as mammals and birds, it has been well documented that asymmetrical sensory and motor representations within the brain are often linked to lateralized behaviours (reviewed by Bradshaw & Rogers 1993; Rogers & Andrew 2002). Behavioural lateralizations have also been documented in many lower vertebrates (reviewed by Bisazza et al. 1998; Vallortigara et al. 1999). Although it cannot be assumed that all lateralized behaviours are linked to brain lateralization, the strong links between brain and behavioural asymmetries established in birds and mammals suggest that lateralized behaviours in lower vertebrates are also likely to be representative of brain lateralizations.

Examples of lateralized behaviour in fish include preferential ventral fin use in the gourami, *Trichogaster trichopterus* (Bisazza et al. 2001), lateralization of pectoral stridulation sounds in the channel catfish, *Ictalurus punctatus* (Fine et al. 1996), and preferential eye use in a variety of fish species (Sovrano et al. 1999, 2001). Within tetrapods, amphibian examples include a turning bias in tadpoles (Wassersug et al. 1999; Yamashita et al. 2000; Oseen et al. 2001) and newts, *Triturus vulgaris* (Green 1997), lateralization of neural control for vocalization in frogs, *Rana pipiens* (Bauer 1993), and preferential use of hindlimbs (Robins et al. 1998), forelimbs (Bisazza et al.

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1996) and eyes (Vallortigara et al. 1998) in adult anurans. In reptiles, lateralized visual use associated with aggressive encounters has been observed in lizards (Deckel 1995, 1997; Deckel & Fuqua 1998; Deckel et al. 1998; Hews & Worthington 2001).

These studies of lower vertebrates suggest that the evolution of brain lateralization occurred earlier in vertebrate evolution than had been previously thought. In the past, it was commonly believed that 'handedness' seen in birds and mammals was a result of convergent evolution; however, more recent studies may suggest that 'handedness' pre-dates mammals and birds and may have evolved through the earliest of vertebrate ancestors (fish and early tetrapods). This view of brain lateralization as a highly conserved trait throughout evolutionary history has gained popularity, but is still contested (reviewed by Bisazza et al. 1998; Vallortigara et al. 1999). Additional studies documenting lateralized behaviours in lower vertebrates and establishing direct links between these behaviours and asymmetrical representations within the brain are needed to further clarify the evolutionary history of brain lateralization.

Despite the recent proliferation of studies documenting lateralities, the specific mechanisms influencing the proposed evolutionary success of brain lateralization are unclear. It has been hypothesized that brain lateralization was the evolutionary result of the need to break up complex tasks and perform them with highly specialized neuronal units to avoid functional overlap. In other

words, brain lateralization was the answer to the problem of 'functional incompatibility' (Sherry & Schachter 1987; Vallortigara et al. 1999). However, this explanation does not explain asymmetries at the population level, and Vallortigara (2000) suggested that it may be disadvantageous for an individual to show behavioural asymmetries different from the majority of the population.

In snakes, behavioural lateralization of hemipenis usage has been explored, suggesting asymmetric use at different temperatures (Shine et al. 2000). Heinrich & Klaassen (1985) suggested that behavioural lateralities also occur within constriction behaviours. The goal of this study was to evaluate evidence for other behavioural asymmetries at both the individual and population levels in snakes, by examining a population of western cottonmouths, *A. piscivorus leucostoma*.

The cottonmouth is a semiaquatic venomous snake that ranges throughout the southeastern U.S.A. (Conant & Collins 1991). Cottonmouths spend much of their time in coils. This coiled posture provides the structural foundation for defensive and offensive strikes used to envenomate predators and prey. In addition to processing sensory input for locating a target, the snake must have a high degree of motor control to execute a quick and accurate strike. This high degree of motor control and coordination of sensory input may be asymmetrically represented within the brain and functionally linked to lateralized coiling behaviours (i.e. 'handedness').

The possibility of lateralized coiling behaviour in snakes has been suggested (Amaral 1927; Davis 1978) but remains untested. Although Klauber (1997) claimed that he did not observe such patterns, Amaral (1927) stated, without providing any specific examples, observational frequencies or statistical tests, that rattlesnakes tend to coil with the left side of the body on the inside. If cottonmouths behave similarly to Amaral's suggested trend for other pit vipers, a clockwise coil preference for cottonmouths would be predicted.

METHODS

Subjects

I collected 30 cottonmouths during the summer of 2001 from areas in and around the Sam Houston State University, Center for Biological Field Studies in Huntsville, Texas, U.S.A. Each snake was assigned an identification number and classified based on sex and age. Individuals less than or approximately equal to 2 years of age were classified as juveniles. The snakes were housed individually in the laboratory in plastic cages ($51 \times 38 \times 18$ cm). The subjects were fed a diet of approximately one mouse per week and water was provided ad libitum. All subjects were permitted at least 1 week of acclimation to laboratory conditions before behavioural observations were initiated. To test for seasonal and temperature effects, the light:dark cycle and temperature were allowed to vary with the season. The light:dark cycle ranged from 14:10 h at the beginning of the study (June 2001) to approximately 12:12 h at the end of the
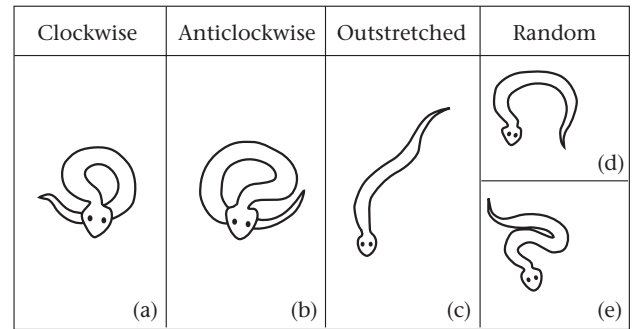

| Clockwise | Anticlockwise | Outstretched | Random |

Figure 1. Representative examples illustrating the categorization of posture observations.

study (November 2001). Laboratory temperatures ranged from 18 to 33°C. Upon completion of the study, all subjects were maintained in the laboratory for future observations.

Data Collection

Hours before data collection, I haphazardly determined a specific time at which observations would begin. At that predetermined time, I recorded the posture of each individual cottonmouth. No more than two observations were recorded in a 24-h period, and all observations were at least 7 h apart. Posture observations were categorized as clockwise (CW) coil, anticlockwise (AC) coil, outstretched or random (Fig. 1). The postures of the head and neck region were highly variable, so I categorized coils by examining only the coil direction of the posterior two-thirds of the body. Coil direction was determined by viewing the snakes from above and recording the direction of the coil beginning just inferior to the neck and continuing to the vent. Coils that were too open (Fig. 1d) or had an indeterminate dominant direction (Fig. 1e) were categorized as random. Date, time and temperature were recorded with each observation.

Analysis

I discarded observations categorized as outstretched or random before statistical analysis. The number of CW and AC observations for each individual were first examined with a chi-square test. For a population-level analysis, I formatted the data for analysis with the sign test. Thus, an individual with a majority of observations in the CW coil was labelled as having a CW coil preference and assigned a '+' value. Similarly, an individual with a majority of recorded observations in a AC coil was labelled as having a AC coil preference and assigned a '−' value. The sign test was then used to analyse population coil preferences (i.e. the frequency of CW and AC coil preferences). The population was then divided into subunits (e.g. males, females, adults and juveniles), and coil preferences were again analysed with the sign test. Due to relatively low power (<0.3) and the difficulty of interpreting statistical results based on small samples, sign tests were applied only to subunit samples with 15 or more individuals.

To quantify coil preference, I calculated a laterality index (LI) value for each individual by the following equation:

$$\mathrm{LI} = \frac{N\,\mathrm{CW\ observations}}{(N\,\mathrm{CW} + \mathrm{AC\ observations})}$$

All laterality index values were arcsine transformed before statistical analysis (Sokal & Rohlf 1995). I used a two-way analysis of variance (ANOVA) to compare LIs based on the age and sex of an individual and used a paired t test to examine LIs for seasonal differences. Seasons were classified as summer (June, July–August) and autumn (September–November). Repeated measures ANOVAs were used to test for LI differences due to temperature and time-of-day effects. Temperature observations were placed into three groups: 18–22°C, 23–27°C and 28–33°C. Likewise, time-of-day observations were classified as follows: morning (0600–1159 hours), afternoon (1200–1759 hours) and evening (1800–2359 hours).

RESULTS

Over the 6-month study period, I recorded 1813 observations ($\bar{X} \pm \mathrm{SE}=60.4 \pm 2.5$ observations per cottonmouth, $N=30$). At the individual level, only three of these snakes showed a significant coil preference (chi-square: χ_1^2: $P<0.05$; Table 1).

According to a sign test for a population-level analysis, 19 individuals had a clockwise bias, and 11 individuals had an anticlockwise bias. The sample population failed to show a significant trend for coil preference (sign test: $N=30$, $P=0.2$), but significant patterns emerged when the sample was classified into subunits (Fig. 2). In adult cottonmouths, the number of CW observations was greater for 16 individuals and less for four individuals (sign test: $N=20$, $P<0.02$). When only females were examined (adults and juveniles), a CW preference was again significant ($N=15$ CW, 5 AC; sign test: $P<0.05$).

To investigate further the effects of age and sex on coil preference, I performed a two-way ANOVA to compare LIs between the respective groups (Fig. 3). The main effect of age was significant, indicating coil-preference differences between adults and juveniles (ANOVA: $F_{1,26}=4.343$, $P<0.05$). There was no significant main effect of sex (ANOVA: $F_{1,26}=1.786$, $P=0.193$) or interaction (ANOVA: $F_{1,26}=0.660$, $P=0.424$).

No statistical differences were detected with respect to season (paired t test: $t_{29}=0.46$, $P=0.650$), time of day (repeated measures ANOVA: $F_{2,29}=1.30$, $P=0.280$) or temperature (repeated measures ANOVA: $F_{2,29}=0.853$, $P=0.431$).

DISCUSSION

At the individual level, only three out of 30 snakes showed a significant coil preference. It is unclear why a few individuals showed significant coil preferences. However, some similarities between these snakes were evident, because all three of these individuals were adult females and had a clockwise coil preference.

Table 1. Summary of the coil preference data for individual cottonmouths

Individual	CW	AC	Total	LI	Coil preference
Adult female	39	28	67	0.582	CW
	31	22	53	0.585	CW
	33	27	60	0.550	CW
	46	37	83	0.554	CW
	28	18	46	0.609	CW
	48	40	88	0.545	CW
	31	26	57	0.544	CW
	36	24	60	0.600	CW
	37	21	58	0.638	CW*
	42	22	64	0.656	CW*
	27	18	45	0.600	CW
	39	17	56	0.696	CW*
	25	34	59	0.424	AC
	36	37	73	0.493	AC
	27	28	55	0.491	AC
Juvenile female	21	20	41	0.512	CW
	35	28	63	0.556	CW
	39	30	69	0.565	CW
	20	28	48	0.417	AC
	18	24	42	0.429	AC
Adult male	36	28	64	0.563	CW
	40	32	72	0.556	CW
	24	22	46	0.522	CW
	20	17	37	0.541	CW
	17	26	43	0.395	AC
Juvenile male	36	38	74	0.486	AC
	29	30	59	0.492	AC
	38	42	80	0.475	AC
	39	45	84	0.464	AC
	33	34	67	0.493	AC

CW: number of clockwise observations. AC: number of anticlockwise observations. LI: calculated laterality index.
*Significant coil preference (chi-square: χ_1^2: $P<0.05$).

At the population level, similar results were obtained. Within population subunits (adults and females), I found a significant CW coil preference. The strength of coil preference varied between individuals. The magnitude of the preference was generally stronger for snakes that preferred the CW coil rotation. This was especially evident in adult females (Fig. 2). An ANOVA on the laterality indices showed a significant difference in coil preference between age groups but not between sexes. These results lead to two generalized conclusions. First, a subtle but significant CW coiling bias existed within select individuals and subunits of the population. Second, there was significant variance in coiling behaviour between subunits of the population, and factors related to age appeared to influence coiling biases.

Some studies have suggested that snakes may show behavioural lateralization of coil preference (Amaral 1927; Davis 1978). This study provides a specific example and statistical evidence supporting that suggestion. Subunits (adults and females) of this cottonmouth population showed a significant bias for the clockwise coil rotation. Similarly, Amaral (1927) claimed that rattlesnakes tend to coil with the left side of the body towards the inside.
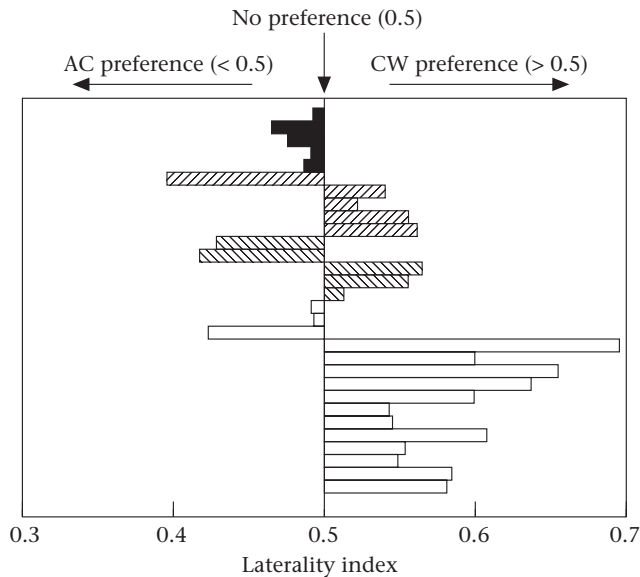
Figure 2. Distribution of individual cottonmouth laterality index values grouped by population subunits. ■: Juvenile male; ▨: adult male, ▨: juvenile female; □: adult female.
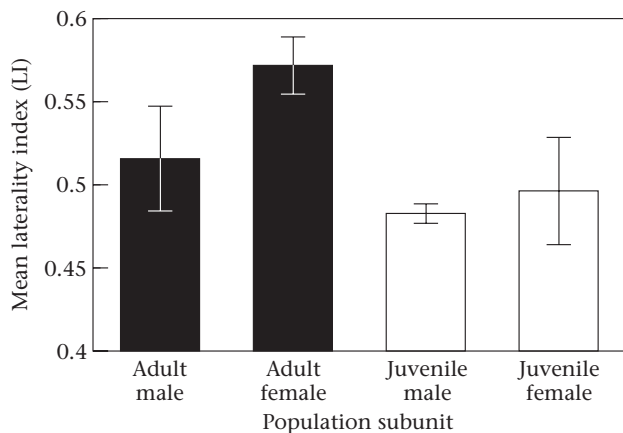

Figure 3. Mean±SD laterality index (LI) for coil preference grouped by population subunits.

This study documents the lateralization of coiling behaviour, but this behaviour cannot be definitively linked to brain lateralization without further research. The adaptive significance and specific mechanisms associated with the behavioural lateralization of coil preference among cottonmouths remain undetermined.

Asymmetrical internal visceral anatomy has been suggested as a possible factor influencing the various types of behavioural lateralization observed in other animals (Dill 1977; Davis 1978; Naitoh & Wassersug 1996; Green 1997; Petersen et al. 2001). The internal anatomy of most snakes is more asymmetrical (Greene 1997) than that of most other tetrapods. In derived snakes, the right lung is commonly offset by the stomach, and the left lung is reduced or lost. Paired viscera such as the gonads and kidneys are also asymmetrically aligned. Thus, coil preferences may reflect the need for snakes to

orient specific visceral structures towards the inside or outside of the coil for enhanced regional thermoregulation, protection or application of a variety of physiological processes. However, it is unclear how this explanation would explain coil preference differences within the population.

The observed behavioural lateralization of coil preference may be related to physiological and anatomical asymmetries within the brain. Behavioural lateralizations, such as preferential limb use and rotational biases, are often related to brain lateralization (reviewed by Bradshaw & Rogers 1993; Bisazza et al. 1998; Rogers & Andrew 2002). In humans, the hand motor cortex is expanded in the dominant hemisphere of the brain. This may allow for additional space of neural encoding, thus enhancing the motor skills of the dominant hand (Volkmann et al. 1998). It is plausible that similar mechanisms exist in the snake, allowing for finer motor control of important behaviours, such as a strike from a coiled posture.

Although these mechanisms could influence the coiling behaviour of each individual, I have no evidence directly supporting a specific mechanism, and it is unclear how these suggested mechanisms may account for the significant differences in laterality index values observed between age groups. To explain the adaptive significance and mechanisms influencing coiling behaviour, future studies may be aimed at exploring these observed differences. One avenue of future research may focus on dopamine concentrations in the brain. In humans and rats, a turning bias (i.e. the tendency to rotate the torso in the same direction) has been well documented (reviewed in Mead & Hampson 1997). Asymmetric dopamine concentrations can be correlated with rotational biases (Zimmerberg et al. 1974), and these turning biases may vary with respect to hormone levels (Robinson et al. 1982; Mead & Hampson 1996, 1997). Although these turning biases are associated with a preferred rotational movement, as opposed to a static posture, a similar mechanism may influence coiling behaviour (torso rotation) in snakes.

Despite the lack of knowledge regarding the adaptive significance or influential mechanisms, a prominent pattern of behavioural lateralization is appearing throughout the evolutionary history of vertebrates. This study provides an example of ophidian behavioural lateralization at the individual and population levels. This is further possible evidence of an early evolution of brain lateralization. However, without additional knowledge of the adaptive significance and mechanisms, including the genetic and developmental basis for brain lateralization, it is premature to conclude that brain lateralization is a highly conserved trait resulting from an early evolutionary event. Lateralized behaviours and brain lateralization may simply be the result of a common selection pressure leading to multiple independent origins. Regardless, a strong pattern of lateralization has been established, and future discoveries of brain and behavioural asymmetries in lower vertebrates should be expected. Of greater interest, future studies failing to document asymmetries in lower vertebrates may warrant additional research, as

they would provide exceptions to this developing pattern.

Acknowledgments

I thank L. D. Devenport, M. E. Kaspari, W. I. Lutterschmidt, H. E. Roth, T. L. Roth, L. J. Vitt and D. A. Wilson for comments and suggestions during the preparation of the manuscript. I thank M. L. Thies and the Sam Houston State University for access to and housing at the SHSU Center for Biological Field Studies. I thank C. McCallister for the graphics displayed in Fig. 1. I also thank W. Ginn for his assistance in the field and laboratory. Financial support was received from the Graduate Student Senate at the University of Oklahoma. This study was conducted under the University of Oklahoma Animal Care and Use Committee permit (A3240-01).

References

Amaral, A. 1927. Notes on nearctic poisonous snakes and treatment of their bites. *Bulletin of the Antivenin Institute of America*, **1**, 61–76.

Bauer, R. H. 1993. Lateralization of neural control for vocalization by the frog (*Rana pipiens*). *Psychobiology*, **21**, 243–248.

Bisazza, A., Cantalupo, C., Robins, A., Rogers, L. J. & Vallortigara, G. 1996. Right-pawedness in toads. *Nature*, **379**, 408.

Bisazza, A., Rogers, L. J. & Vallortigara, G. 1998. The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience and Biobehavioral Reviews*, **22**, 411–426.

Bisazza, A., Lippolis, G. & Vallortigara, G. 2001. Lateralization of ventral fins use during object exploration in the blue gourami (*Trichogaster trichopterus*). *Physiology & Behavior*, **72**, 575–578.

Bradshaw, J. L. & Rogers, L. J. 1993. *The Evolution of Lateral Asymmetries, Language, Tool Use and Intellect*. San Diego: Academic Press.

Conant, R. & Collins, J. T. 1991. *Reptiles and Amphibians: Eastern/Central North America*. New York: Houghton Mifflin.

Davis, T. A. 1978. Reversible and irreversible lateralities in some animals. *Behavioral and Brain Sciences*, **1**, 291–293.

Deckel, A. W. 1995. Laterality of aggressive responses in *Anolis*. *Journal of Experimental Zoology*, **272**, 194–200.

Deckel, A. W. 1997. Effects of alcohol consumption on lateralized aggression in *Anolis carolinensis*. *Brain Research*, **756**, 96–105.

Deckel, A. W. & Fuqua, L. 1998. Effects of serotonergic drugs on lateralized aggression and aggressive displays in *Anolis carolinensis*. *Behavioural Brain Research*, **95**, 227–232.

Deckel, A. W., Lillaney, R., Ronan, P. J. & Summers, C. H. 1998. Lateralized effects of ethanol on aggression and serotonergic systems in *Anolis carolinensis*. *Brain Research*, **807**, 38–46.

Dill, L. M. 1977. Handedness in the Pacific tree frog (*Hyla regilla*). *Canadian Journal of Zoology*, **55**, 1926–1929.

Fine, M. L., McElroy, D., Rafi, J., King, C. B., Loesser, K. E. & Newton, S. 1996. Lateralization of pectoral stridulation sound production in the channel catfish. *Physiology & Behavior*, **60**, 753–757.

Green, A. J. 1997. Asymmetrical turning during spermatophore transfer in the male smooth newt. *Animal Behaviour*, **54**, 343–348.

Greene, H. W. (Ed.) 1997. Classification and general biology. In: *Snakes: the Evolution of Mystery in Nature*, pp. 11–33. Los Angeles: University of California Press.

Heinrich, M. L. & Klaassen, H. E. 1985. Side dominance in constricting snakes. *Journal of Herpetology*, **19**, 531–533.

Hews, D. K. & Worthington, R. A. 2001. Fighting from the right side of the brain: left visual field preference during aggression in free-ranging male tree lizards (*Urosaurus ornatus*). *Brain, Behavior and Evolution*, **58**, 356–361.

Klauber, L. M. 1997. *Rattlesnakes: Their Habits, Life Histories, and Influence on Mankind*. 2nd edn. Los Angeles: University of California Press.

Mead, L. A. & Hampson, E. 1996. A sex difference in turning bias in humans. *Behavioural Brain Research*, **78**, 73–79.

Mead, L. A. & Hampson, E. 1997. Turning bias in humans is influenced by phase of the menstrual cycle. *Hormones and Behavior*, **31**, 65–74.

Naitoh, T. & Wassersug, R. 1996. Why are toads right-handed? *Nature*, **380**, 30–31.

Oseen, K. L., Newhook, L. K. D. & Wassersug, R. J. 2001. Turning bias in woodfrog (*Rana sylvatica*) tadpoles. *Herpetologica*, **57**, 432–437.

Petersen, A. D., Lombardo, M. P. & Power, H. W. 2001. Left-sided directional bias of cloacal contacts during tree swallow copulations. *Animal Behaviour*, **62**, 739–741.

Robins, A., Lippolis, G., Bisazza, A., Vallortigara, G. & Rogers, L. J. 1998. Lateralized agonistic responses and hindlimb use in toads. *Animal Behaviour*, **56**, 875–881.

Robinson, T. E., Camp, D. M., Jacknow, D. S. & Becker, J. B. 1982. Sex differences and estrous cycle dependent variation in rotational behavior elicited by electrical stimulation of the mesostriatal dopamine system. *Behavioural Brain Research*, **6**, 273–287.

Rogers, L. J. & Andrew, R. J. (Eds) 2002. *Comparative Vertebrate Lateralization*. Cambridge: Cambridge University Press.

Sherry, D. F. & Schacter, D. L. 1987. The evolution of multiple memory systems. *Psychological Review*, **94**, 439–454.

Shine, R., Olsson, M. M., LeMaster, M. P., Moore, I. T. & Mason, R. T. 2000. Are snakes right-handed? Asymmetry in hemipenis size and usage in garter snakes (*Thamnophis sirtalis*). *Behavioral Ecology*, **11**, 411–415.

Sokal, R. R. & Rohlf, F. J. 1995. *Biometry: the Principles and Practice of Statistics in Biological Research*. 3rd edn. New York: W. H. Freeman.

Sovrano, V. A., Rainoldi, C., Bisazza, A. & Vallortigara, G. 1999. Roots of brain specializations: preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behavioural Brain Research*, **106**, 175–180.

Sovrano, V. A., Bisazza, A. & Vallortigara, G. 2001. Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiology & Behavior*, **74**, 237–244.

Vallortigara, G. 2000. Comparative neuropsychology of the dual brain: a stroll through animals' left and right perceptual worlds. *Brain and Language*, **73**, 189–219.

Vallortigara, G., Rogers, L. J., Bisazza, A., Lippolis, G. & Robins, A. 1998. Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *NeuroReport*, **9**, 3341–3344.

Vallortigara, G., Rogers, L. J. & Bisazza, A. 1999. Possible evolutionary origins of cognitive brain lateralization. *Brain Research Reviews*, **30**, 164–175.

Volkmann, J., Schnitzler, A., Witte, O. W. & Freund, H.-J. 1998. Handedness and asymmetry of hand representation in human motor cortex. *Journal of Neurophysiology*, **79**, 2149–2154.

Wassersug, R. J., Naitoh, T. & Yamashita, M. 1999. Turning bias in tadpoles. *Journal of Herpetology*, **33**, 543–548.

Yamashita, M., Naitoh, T. & Wassersug, R. J. 2000. Startle response and turning bias in *Microhyla* tadpoles. *Zoological Science*, **17**, 185–189.

Zimmerberg, B., Glick, S. D. & Jerussi, T. P. 1974. Neurochemical correlate of a spatial preference in rats. *Science*, **185**, 623–625.