Nuptial colouration and courtship behaviour during induced breeding of the swamp barb *Puntius chola*, a freshwater fish

Moncey Vincent and K. John Thomas*

Animal Behaviour and Wetland Research Laboratory,
Department of Zoology, Christ College, Irinjalakuda 680 125, India

In *Puntius chola*, scarlet bands appear on the lateral sides in both sexes during reproductive season. Sex-specific reproductive behaviour patterns, development and disappearance of nuptial colouration were studied following injection of Ovaprim. In females, intensity of the colour of the lateral bands increased with the advancement of courtship activity, reached a peak just before spawning and then faded away. In males, intense nuptial colouration developed earlier than the females and disappeared 40 h after spawning. Vent kissing, circling and synchronized movement were some of the characteristic behaviours of the males, while the females often remained motionless in the column of water and occasionally exhibited trailing behaviour to a vigorously displaying male. The results show that the male *P. chola* have a low threshold for sexual arousal. They attain nuptial colouration earlier than the females and also retain the colouration for a longer period than the females.

Keywords: Courtship behaviour, induced breeding, nuptial colouration, Ovaprim, *Puntius chola*.

THE social context of expression of nuptial colouration and variations in the strategies adopted by the male and female through visual communication is not fully elucidated in fishes. It is generally believed that colour patterns and courtship behaviours act as a mechanism of reproductive isolation. In cichlids, evolution of colour pattern is driven by sexual selection and colour display forms the basis of mate choice[1]. A recent molecular phylogenetic study involving 700 species of African cichlids has corroborated the hypothesis that sexual selection is the major force in the evolution of nuptial colouration and rapid speciation[2].

Several species of the genus *Puntius* are known to exhibit spectacular colour changes during courtship and spawning[3]. The nuptial colouration may vary from different hues of red and yellow depending on the species. *Puntius chola* is endemic to the Western Ghats and is listed as a vulnerable species by the National Bureau of Fish Genetic Resources[4,5]. It inhabits freshwater ponds, streams and small canals associated with paddy fields. The fish exhibits sexually dimorphic colouration during the southwest monsoon – its normal breeding season. During this period pelvic and anal fins of males appear tinged with orange colour, while in females they are pale yellow[3]. The operculum of adult male and female shows an orange-red mark throughout the year. In both sexes prominent scarlet horizontal bands appear on the lateral sides with the onset of the southwest monsoon. The present study records sequential colour changes and courtship behaviour in male and female *P. chola*.

Healthy mature fishes were collected from canals associated with paddy fields at Irinjalakuda, Thrissur, Kerala in May 2005 and stocked in three cement tanks (125 × 60 × 60 cm), and acclimated to the laboratory conditions for three or four weeks. Each tank housed five males and 15 females. A pair of fish (SL, 6.23 ± 0.3 cm) was isolated and transferred into an aquarium (60 × 30 × 30 cm) containing pond water for further studies. A layer of river sand at the bottom and enough vegetation of *Hydrilla* were provided to create a suitable substratum. All experiments were done during June–July. The male and female fish were injected with single doses of Ovaprim (salmon gonadotropin releasing hormone analogue), 1 ml (i.e. 20 μg)/kg body wt intramuscularly at 6.00 p.m. Since *P. chola* usually breeds at the onset of monsoon, a shower of water was given to simulate rain for 15 min soon after the injection. Sterile water-injected pairs of fish were kept as control. The experiment was repeated using 18 pairs of fish (n = 18). The courtship behaviour and nuptial colouration of the fish were recorded using a digital video camera (Coolpix 4500; Nikon) at intervals of 15 min. The behavioural patterns were studied with frame-by-frame analysis of the video footages using the program 'Image Ready' in Adobe Photoshop. Variations in the intensity of red colour in the scarlet nuptial band were measured using a facility in Adobe Photoshop. Still photographs recorded during different phases of courtship activity were arranged sequentially. In photographs of the male and female fish, five points on the mid region along the lateral line were sampled using the colour picker tool of the computer program. Intensity of red, green and blue colours was determined from the 'colour window' in the software. The mean of the percentage intensity of red colour was taken as the measure of the intensity of the scarlet band. Data were plotted on a graph to compare the development and disappearance of colour patterns.

Prior to hormone injection, both male and female were bright, steel-grey in appearance (Figure 1 *a* and *b*). Within 45 min after Ovaprim administration, the orange-red colour spot on the operculum of both male and female fish became prominent and the fishes started moving in pairs. Four hours after hormone administration, the male started exhibiting active movements around the female, with occasional synchronized movements along with the female and on both flanks of the male an iridescent scarlet band appeared (Figure 1 *c*). However, in females a less intense, narrow, red band appeared at about 5 h after hormone injection. Seven hours after injection the male exhibited in-

*For correspondence. (e-mail: jkurishinkal@rediffmail.com)

tense movements and was seen pushing the protruded belly region of the female with its lip. After 10 h, the pairs frequently moved up to the surface of the water. After 12 h, the female oriented in a head-down posture, while the male continued nipping at the entire ventral region of the body of the female starting from the operculum (Figure 2 *a*). During this period the female exhibited circular movements with curved body, and started nipping at the caudal peduncle of the male. At the end of the 12th hour, the frequency of nipping or butting activity by the male increased. The nuptial colouration of both male and female became intense at 15 h after injection (Figure 3 *a*). The male pushed the belly region of the female with its lip, resulting in the release of a few eggs from the vent of the female (Figure 2 *b*). Then the male suddenly turned around, brought its head near the head of the female and stroked her on the opercular region with its operculum. At times, the male exhibited a slow and continuous movement pattern in an elliptical path around the female (Figure 2 *c*). At the 16th hour after Ovaprim treatment, both male and female showed more intense colouration (Figure 3 *b*). Approximately 23 h after Ovaprim admini-

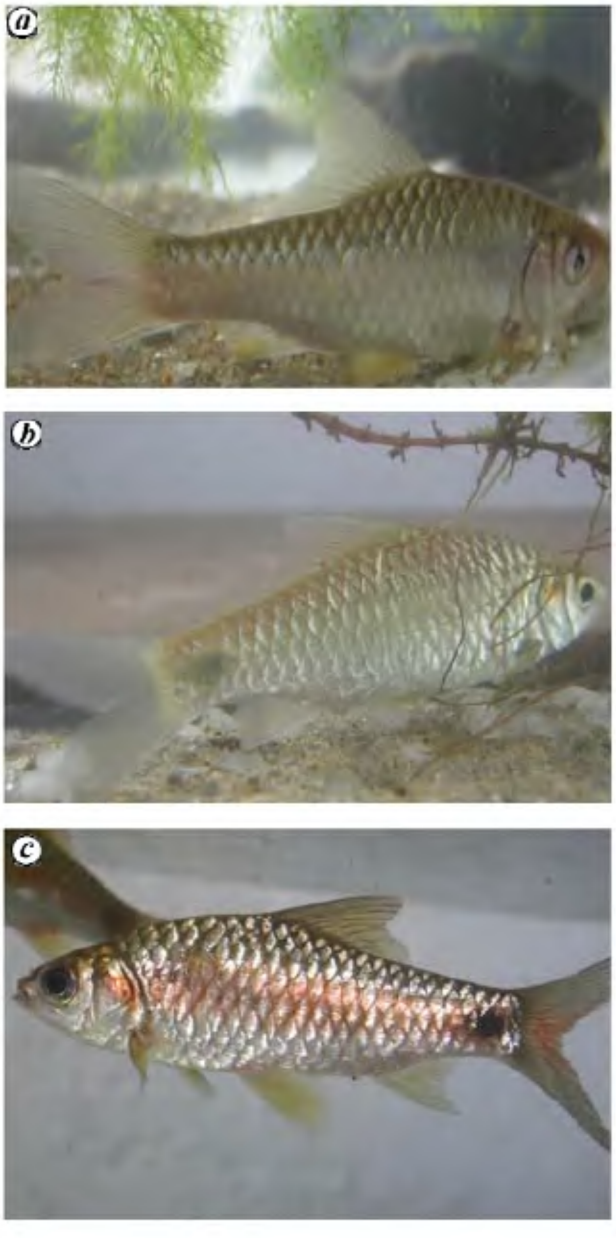

Figure 1. Male (***a***) and female (***b***) fish before ovaprim treatment. ***c***, Development of nuptial colouration in the male.

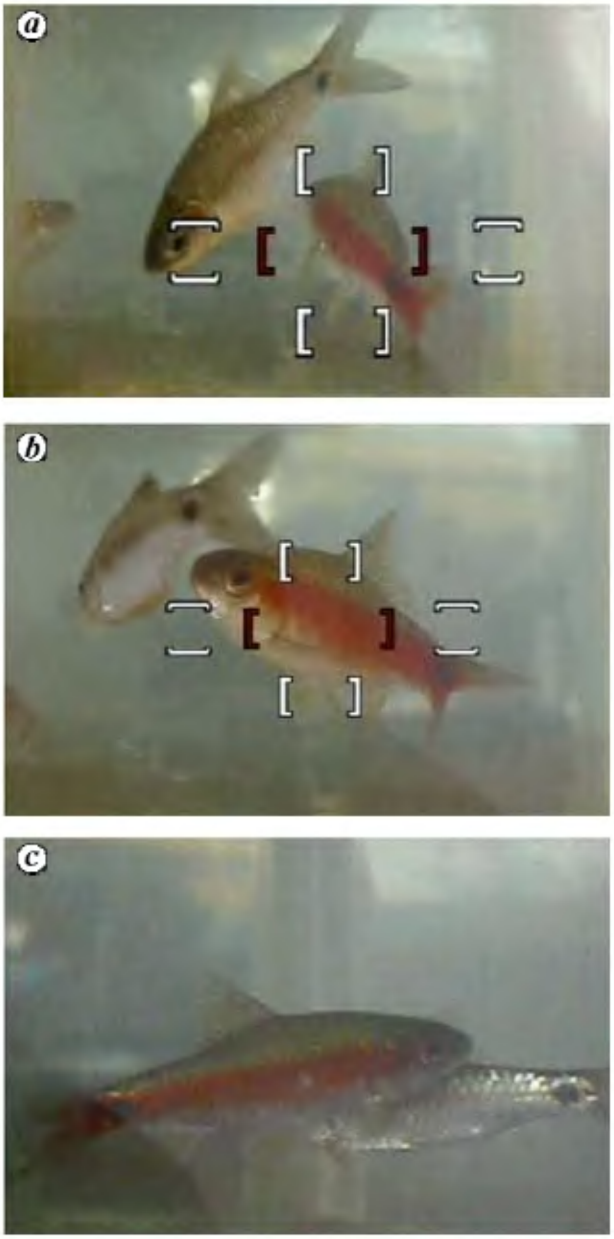

Figure 2. ***a***, Head-down posture of the female. ***b***, ***c***, Vent kiss (***b***) and circling movement (***c***) by the male.

stration, spawning was completed and the nuptial colouration of the female gradually faded away (Figure 3 *c*), whereas body colouration of the male remained intense until 40 h. The swimming movements of the female became slow at the end of the spawning activity.

Cyprinid fishes in general, exhibit some common patterns in their courtship and reproductive behaviour[6,7]. *P. chola* exhibited swimming movements in pairs, circling and pushing the female on the abdomen during courtship, as reported in *Danio aequipinnatus*[8]. However, temporal sequences of each behaviour pattern may vary widely in different genera and/or between species. In the fish *Corynopoma riisei*, courting behavioural sequences occur randomly, with variable durations and probability of occurrence of a particular behaviour depends on the preceding pattern. During spawning, behavioural sequences become more determinate, as females are influenced by the cumulative effect of male actions[9,10]. In *P. chola* the sequence of behavioural patterns is well organized and characterized by several circling movements around the female during courtship activity.

There are striking similarities in body colouration and behaviour patterns among several species under the genus *Puntius*, such as *P. parrah*, *P. amphibius* and *P. chola*, that occupy the same habitat. However, there are also several differences in the onset and disappearance of nuptial colouration (unpublished obs.). It is possible that the differences in courtship behaviour of the fish may function as a mechanism for the ethological isolation among closely related species[11].

The present study shows that a scarlet band of less intense colour develops in the female fish during courtship behaviour. The peak colour intensity of this lateral band is seen at the beginning of spawning and remains till the completion of ovulation, and it fades away soon after spawning (Figure 4). Interestingly, the scarlet lateral band develops in the male earlier than the female, and also persists longer compared to females.

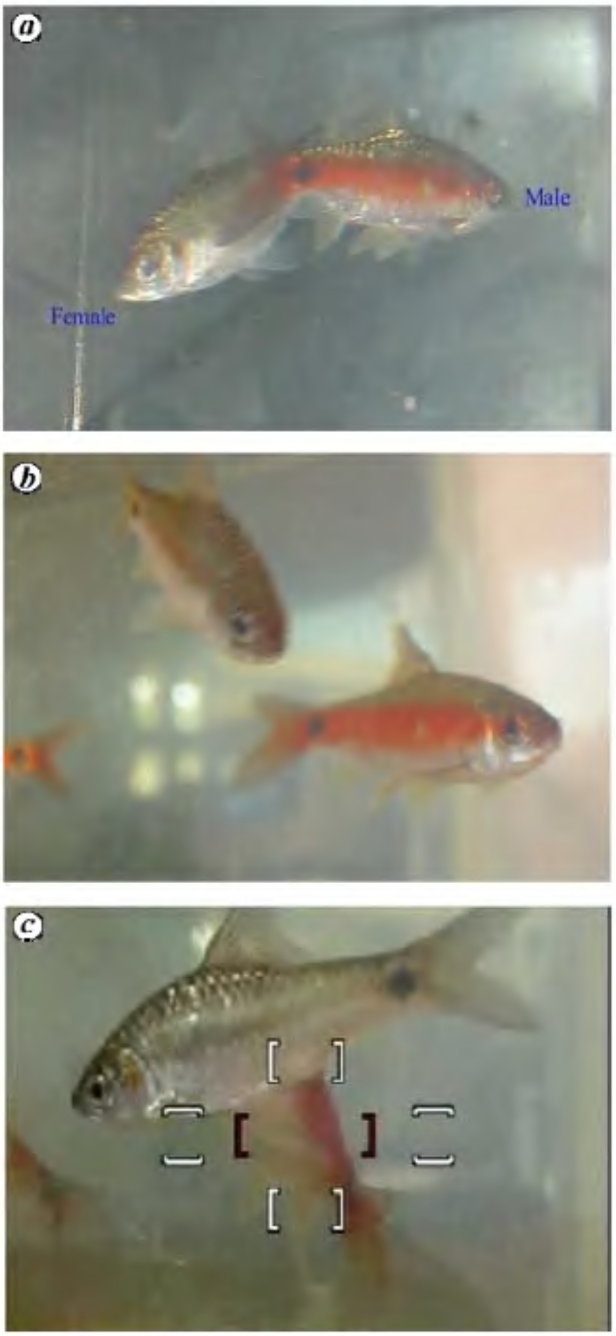

Figure 3 *a–c*. ***a***, Nuptial colouration at the beginning of spawning. ***b***, Colouration at 16 h after Ovaprim treatment. ***c***, Female at the end of spawning.

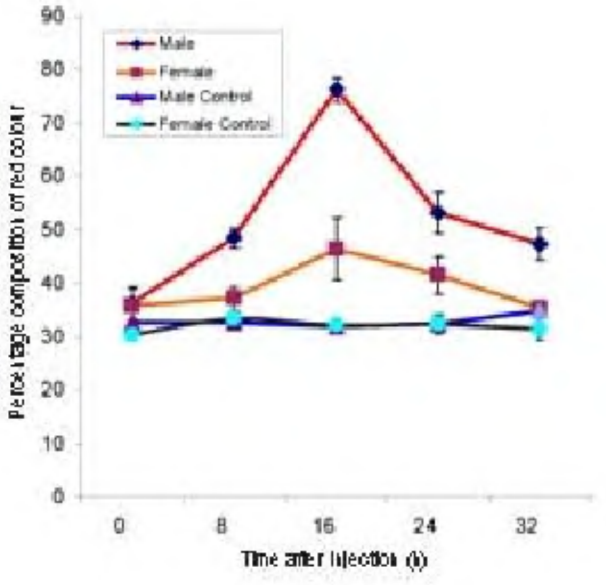

Figure 4. Variation in intensity of nuptial colouration in male and female at different intervals after Ovaprim injection (controls injected with sterile water).

The exact significance of this colour modulation and the difference observed in the male and the female is not clear. The state of arousal of a courting male is reflected in coloured skin patches and change in body colouration is one of the reliable indications of the motivational status of an organism[11]. Laboratory studies have demonstrated that females of sticklebacks, guppies and cichlids, when presented with males differing in body colouration preferred brighter males with brighter colouration[6], indicating sexual selection. Female mate choice can also be a powerful source of selection on male secondary sexual characteristics[12], and a potential agent for rapid population differentiation and speciation[13].

Goodenough *et al.*[14] suggested that despite costs associated with mating, males of most species appear to follow a strategy of copulating with as many females as possible. The results indicate that the males have a low threshold for sexual arousal and attain nuptial colouration earlier than the females. Thus, as it is observed in the present study, the persistent colour of the lateral band of the males may act as an attractive stimulus for the unmated females.

1. Dominey, W., Effects of sexual selection and life history on speciation: Species flocks in African cichlids and Hawaiian Drosophila. In *Evolution of Fish Species Flocks* (eds Echelle, A. A. and Kornfield, I.), University of Maine Press, Orono, 1984, pp. 231–249.
2. Seehausen, O., Mayhew, P. J. and Van Alphen, J. J. M., Evolution of colour pattern in East African cichlid fish. *J. Evol. Biol.*, 1999, **12**, 514–534.
3. Talwar, P. K. and Jhingran, A. G., *Inland Fishes of India and Adjacent Countries*, Oxford and IBH Publishing Co Pvt Ltd, New Delhi, 1991, vol. 1.
4. Radhakrishnan, K. V. and Kurup, B. M., Distribution, habitat preference and biodiversity status of potential freshwater ornamental fishes of Kerala. In Proceedings of the Riverine and Reservoir Fisheries of India (eds Boopendranath, M. R. *et al.*), Society of Fisheries Technologists (India), Cochin, 2002, pp. 162–171.
5. Kurup, B. M. and Radhakrishnan, K. V., Freshwater fish biodiversity of Kerala; Status and utilization for commercial fishing, food security and livelihood. *Fish. Chimes*, 2006, **25**, 111–122.
6. Turner, G. F., Teleost mating behaviour. *Behaviour of Teleost Fishes* (ed. Pitcher, T. J.), Chapman and Hall, London, 1993, pp. 307–331.
7. Anna Mercy, T. V., Jacob, E. and Thomas, R. K., Studies on the reproductive behaviour of the common catopra, *Pristolepis marginata* Jerdon (Nandidae–Perciformes) under captive conditions. *Curr. Sci.*, 2003, **84**, 1468–1473.
8. Kharbuli, S., Dey, S., Nongkynrih, J. and Goswami, U. C., Ovaprim induced spawning and subsequent development in an aquarium fish, *Danio aequipinnatus*. *J. Inland Fish. Soc. India*, 2004, **36**, 58–62.
9. Nelson, K., The temporal patterning of courtship behaviour in the glandulocaudine fishes (Ostariophysi, Characidae). *Behaviour*, 1964, **124**, 90–144.
10. Nelson, K., After-effects of courtship in the male three-spined stickle back. *Z. Vgl. Physiol.*, 1965, **50**, 569–597.
11. Colgan, P., The motivational basis of fish behaviour. In *Behaviour of Teleost Fishes* (ed. Pitcher, T. J.), Chapman and Hall, London, 1996, pp. 39–41.
12. Andersson, M., *Sexual Selection*, Princeton University Press, Princeton, NJ, 1994.
13. Haesler, M. P. and Seehausen, O., Inheritance of female mating preference in a sympatric sibling species pair of Lake Victoria cichlids: Implications for speciation. *Proc. R. Soc. London, Ser. B.*, 2005, **272**, 237–245.
14. Goodenough, J., McGuire, B. and Wallace, R. A., *Perspectives on Animal Behaviour*, John Wiley, New York, 1993, p. 471.

ACKNOWLEDGEMENTS. We are grateful to the Department of Science and Technology, New Delhi for financial assistance. We thank the Principal and the management of Christ College for providing necessary facilities. We express our gratitude to Prof. C. K. G. Nayar, President, Limnological Association of Kerala for critical reading and comments on the draft of the paper.

Received 18 December 2006; revised accepted 19 February 2008

Observations on tool use in captive lion-tailed macaque (*Macaca silenus*)

Shanthala Kumar[1,2], H. N. Kumara[3] and Mewa Singh[4,*]

[1]Sri Chamarajendra Zoological Garden, Mysore 570 001, India
[2]Present address: 'KUMARAGIRI', Lakshmisha Nagara, Deepa Nursing Home Opposite Road, Chikmagalur 577 101, India
[3]National Institute of Advanced Studies, Indian Institute of Science Campus, Bangalore 560 012, India
[4]Biopsychology Laboratory, University of Mysore, Mysore 570 006, India

Lion-tailed macaques in Sri Chamarajendra Zoological Garden, Mysore, India were observed to use several tools (plastic piece, candy wrapper, leaf, vegetable leaf, egg-shell and coconut shell) to drink water from the pool. Here we report the simple multiple tool use in the species. The behaviour was first observed in an adult male. This male was confiscated and was probably hand-reared. It started using tools within a short period of introduction. After some time, the other captive monkeys also started to use multiple tools in a similar way to drink water. This may be an example of social learning.

Keywords: Captive monkeys, lion-tailed macaque, social learning, tool use.

A TOOL has been defined as 'an external object free of any fixed attachment to the substrate, which can be held, carried or manipulated by the user'[1]. Similarly, tool use can be defined as 'the use of such a tool to attain a particular goal, the user must hold or carry the tool during or just prior to use and is responsible for the proper and effective orientation of the tool'[1]. Tool use is interesting,

*For correspondence. (e-mail: msingh@psychology.uni-mysore.ac.in)