Computer animation as a tool to study preferences in the cichlid *Pelvicachromis taeniatus*

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Four choice experiments were conducted with both sexes of the cichlid *Pelvicachromis taeniatus* using computer-manipulated stimuli of digital images differing in movement, body shape or colouration. The results show that computer animations can be useful and flexible tools in studying preferences of a cichlid with complex and variable preferences for different visual cues.

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Visual cues play a decisive signalling role in many fish species. Colour patterns, the visual perception of body size, symmetry and other individual characteristics transfer information that is relevant in many behavioural contexts, like species recognition (Seehausen & van Alphen, 1998) and sexual selection (Milinski & Bakker, 1990). Cichlids in particular show a huge variation in colouration and body shapes (Keenleyside, 1991; Barlow, 2002). The experimental investigation of visual signals, such as colour patterns in fishes, however, is often difficult (Kocher, 2004).

Computer-manipulated stimuli provide an opportunity to manipulate single or combined visual traits selectively (Künzler & Bakker, 1998), while keeping all other potentially confounding variables constant (Baldauf *et al.*, 2008). Until now, presentation of virtual stimuli has not been applied to study cichlid behaviour, despite their potential to answer a wide array of questions in this rapidly evolving group (Kocher, 2004). Yet it is unclear whether computer animations will work properly in cichlids at all due to differences in cone absorbance colour perception varies between species. Thus, it depends on the physiology of a species' visual system whether a computer-manipulated stimulus is reliably perceived. In several cichlid species, three cone classes are described, one for short, one for medium and one for long wavelengths, *e.g. Aequidens pulcher* (Gill): blue 453 nm, green 530 nm and red 570 nm (Kroeger *et al.*, 1999; Parry *et al.*, 2005) which is comparable to the human visual system (blue 435 nm, green 534 nm and red 560 nm; Boynton, 1979).

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Thus, computer-manipulated stimuli may be an appropriate method in the study of visual signals in cichlids.

The aim of this study was to investigate whether the cichlid *Pelvicachromis taeniatus* (Boulenger) reliably responds to computer stimuli. *Pelvicachromis taeniatus* is a small, cave-breeding cichlid, that inhabits small rivers in western Africa. The sexes show a distinct dichromatism (Thünken *et al.*, 2007a). The most conspicuous part of the male's nuptial colouration is the yellow-coloured ventral body region, while females develop a violet ventral colouration. Sub-adult or subdominant males and females do not show conspicuous nuptial colouration, thus dull individuals of both sexes can also be found in the natural environment. Until now it is unclear whether the conspicuous colouration of both sexes has a function in intersexual communication or is rather used as status signalling in intrasexual dominance.

Four different experiments were conducted to test the response of fish to two-dimensional (2D) digital images differing in movement, body shape or colouration. Experiment 1 examined whether test fish differentiate between dull and nuptially coloured stationary images of the opposite sex. In experiment 2, the response of fish to a moving (animation) or a stationary image of the other sex showing the same nuptial colouration was investigated. Experiment 3 examined whether test fish reliably distinguished between body shapes of conspecific and heterospecific animations. Lastly, experiment 4 determined whether females distinguish between animations of nuptially coloured males and dull males.

Digital images from a nuptially coloured, courting, medium-sized male (5·5 cm standard length L_S) and female (4·0 cm L_S) were taken to prepare artificial stimuli using an Olympus Camedia Widezoom 5060 camera (www.olympus.com). Pictures were saved in RAW-format to avoid the loss of colouration data due to algorithmic compression and were imported in Adobe Photoshop 5·5 (www.adobe.com). Here, they were white-balanced to ensure adjusted colour reproduction (Stevens *et al.*, 2007; Baldauf *et al.*, 2008). The pictures were manipulated for each experiment using Adobe Photoshop 5·5 in RGB colour mode. The body outline was separated from the background and inserted in a blank, bright grey (RGB: 238, 238, 238) background. This picture was imported in Microsoft PowerPoint 2003 (www.microsoft.com), using a resolution of 1024 × 768 pixels for the screen. The stimuli were positioned 2 cm above the bottom of the screen. The stimuli for the experiments were prepared as described below.

Individuals used in the experiments were the laboratory-raised F1 generation of fish that had been caught from the River Moliwe, Cameroon (04°04′ N; 09°16′ E). Test individuals were raised in mixed-sex family tanks (80 × 30 × 30 cm), which were surrounded with opaque plastic sheets to avoid visual contact with other aquariums. The water temperature was kept at 25° C, range ± 1° C and natural light conditions were provided (12L:12D). Fish were fed once a day with a mixture of frozen chironomid *Chironomus* sp. larvae and *Artemia* spp. Test fish were 1−2 years old. Sexual maturity of each sex was determined visually on the basis of the ventral colouration (Thünken *et al.*, 2007a, b).

Experiment 1 (dull stationary image *v.* nuptially coloured stationary image): a dull stationary image was produced by removing the nuptial colouration from the digital model. The nuptially coloured model was not additionally manipulated, as a picture taken by a digital device is a reduction of a continuous spectrum into three data points (RGB), thus already represents a form of manipulation. Thus, one stimulus

showed the colouration of subdominant males or females, whereas the other stimulus showed a nuptially coloured fish. Each stationary model was separately placed in the middle of the grey background. Twelve females and 12 males from 12 different families were used in this experiment.

Experiment 2 (nuptially coloured animation *v.* nuptially coloured stationary image): to achieve an animation of a nuptially coloured fish the tool 'animation paths' in PowerPoint 2003 was used to let the model move from one side of the monitor to the other. The stimulus moved its pathway for a period of 15 s. After that, it reoccurred horizontally flipped and took another 15 s to move its way back. This cycle was set to loop until the end of each experimental trail. The other stimulus showed the stationary image of the nuptially coloured fish. Twelve female and 12 male test fish originating from 12 different families were used.

Experiment 3 (conspecific animation *v.* heterospecific animation): a picture of a non-sympatric cichlid, *Tilapia bakossiorum* Stiassny, Schliewen & Dominey was taken from an internet source (www.blackwaterfish.com) to get the shape of a different cichlid fish of comparable body size which does not prey upon *P. taeniatus*. The red ventral colouration of the body was replaced with skin patterns of the nuptial colouration of *P. taeniatus*. Stimuli displayed to male subjects showed the foreign species with violet colouration, whereas stimuli for female test fish showed the nuptial yellow colouration of males. The paired stimulus was a nuptially coloured model of the opposite sex of *P. taeniatus*. The animation paths and speed of movement of both stimuli were identical to the pathway of the animation in experiment 2. Twelve female and 12 male test fish originating from 12 different families were used during this experiment.

Experiment 4 (nuptially coloured male animation v. dull male animation): an animation of the dull male model from experiment 1 was produced. Animation settings complied with the methods used in experiments 1 and 2. The paired stimulus was the animation of the nuptially coloured male model. Sixteen females from 16 different families were tested in this experiment.

All experiments were conducted between 27 November 2006 and 20 April 2007. Before starting the experiments, randomly chosen *P. taeniatus* from different families were individually isolated in separate tanks ($30 \times 20 \times 20$ cm) for a minimum period of 2 days. The isolation tanks were surrounded by bright, grey paper (R G B: 238, 238, 238) at the broad sides and opaque, grey partitions at the longer sides, ensuring that fish could habituate to a grey background used in the trials. Java moss *Vesicularia dubyana* was added to provide shelter for the females, whereas male tanks were equipped with a standardized breeding cave. All other conditions were similar to those of the mixed-sex tanks.

To reduce stress, test fish remained in their isolation tanks during the experiments. The Java moss in the females' tank was replaced by a plastic plant in the middle of the tank to provide a fixed shelter. The isolation tanks containing the test fish were placed without grey background partitions between two cathode ray tube (CRT) monitors of the same model (EIZO Flex Scan F520, 85 Hz, connected to a Matrox G550 PCIe graphic board; www.eizo.com; Fig. 1). The bottom of the tank was justified so that it coincided with the lower margins of the monitor screens. An association zone of 5 cm in front of each monitor was marked on the white styrofoam under the tank creating a 15 cm neutral zone in between (Fig. 1). Previous experiments indicated that the time test fish spend in an association zone near a stimulus of
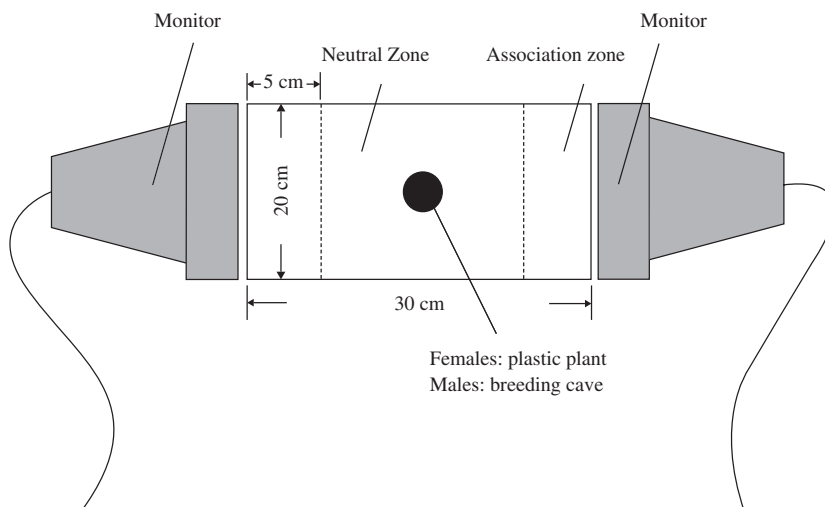
FIG. 1. The experimental set-up. Individual *Pelvicachromis taeniatus* were tested in their isolation tank that was placed between two cathode ray tube monitors. A plastic plant (for female test fish) or a cave (for male test fish) was situated in the middle of the tank. An association zone of 5 cm was marked on the bottom of the tank in front of each monitor.

the opposite sex reliably predicts mating decisions in both males and females of *P. taeniatus* (Thünken *et al*., 2007a, T. Thünken, T. C. M. Bakker, N. Henning and H. Kullmann unpubl. data). White Styrofoam separated the long sides of the tank from the surroundings. The set-up was illuminated by a fluorescent tube (37 W) installed 1 m above the middle of the tank.

A webcam connected to a computer was installed 50 cm above the middle of the tank to record the behaviour of the test fish. During an acclimatization period of 15 min both screens showed the grey background. After acclimatisation both stimuli appeared simultaneously (MacLaren & Rowland, 2006). It was determined randomly which stimulus was presented on which side. After the test fish had entered one association zone, its behaviour was recorded for 2 min (trial 1). After recording, the empty background was shown again for 7 min. The trial was then repeated with changed sides of the stimuli (trial 2). In cases where the test fish did not enter an association zone within 30 min after the stimulus presentation started, the experiment was stopped. Thus in the various experiments, a different number of test fish thus was excluded, so that sample sizes varied among experiments.

A naïve observer analysed the video recordings. In the analyses, relative proportion of time (T_{rel}) spent in association zones on the left$_{(l)}$ or the right$_{(r)}$, $T_{rel} = 100$ $T_l(T_l + T_r)^{-1}$, for both consecutive trials was calculated. For each test fish, the mean relative time (mean T_{rel}) spent in front of each stimulus in the first and the second trial was calculated, thus reducing the effect of possible side biases. Test fish were used only once within single experiments. Females from experiment 1, however, were also used in experiment 3 and males in experiment 1 were also used in experiment 2. In these cases, the experiments for the test fish were allocated randomly.

Parametric statistics were used in case of normally distributed data according to Kolmogorov–Smirnov tests with Lilliefors' correction. Given test probabilities

are two-tailed. Analyses were performed using SPSS 12·0 statistical package (www.spss.com).

Experiment 1 (dull stationary image v. nuptially coloured stationary image): seven out of 12 females completed both consecutive trials. Females showed no significant preference for a stationary photograph of a nuptially coloured (mean $\pm$ s.d. $T_{rel} = 60·97 \pm 20·60\%$) or a dull male stimulus (mean $\pm$ s.d. $T_{rel} = 39·02 \pm 20·60\%$) [paired t-test, d.f. $= 6$, $P > 0·05$, Fig. 2(b)]. Seven out of 12 males completed both consecutive trials. Males showed no significant preference for a stationary photograph of a nuptially coloured (mean $\pm$ s.d. $T_{rel} = 60·02 \pm 43·16\%$) or dull stimulus (mean $\pm$ s.d. $T_{rel} = 39·98 \pm 43·16\%$) of the opposite sex [paired t-test, d.f. $= 6$, $P > 0·05$, Fig. 2(b)].

Experiment 2 (nuptially coloured animation v. nuptially coloured stationary image): 11 out of 12 females completed both consecutive trials. Females showed a significant preference for the moving stimulus male (mean $\pm$ s.d. $T_{rel} = 71·85 \pm$
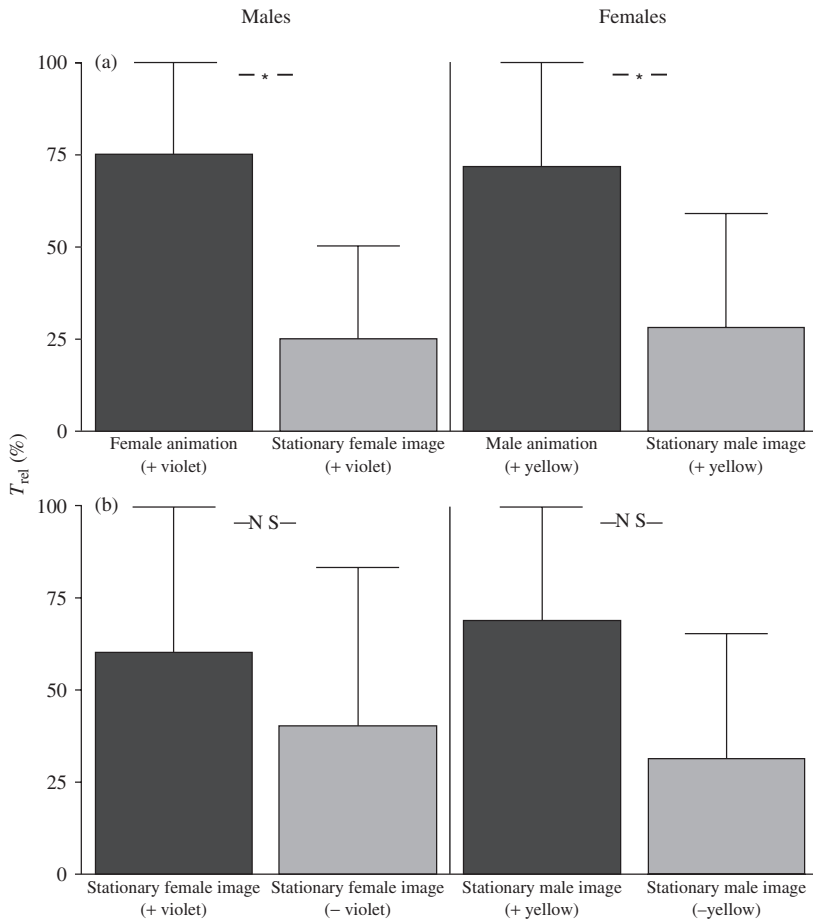

FIG. 2. Mean + s.d. relative amount of time (T_{rel}) males and females spent within the association zones in front of (a) an animation and a stationary image of the other sex or (b) stationary images of the other sex differing in nuptial colouration. *, $P < 0·05$; NS $P > 0·05$.

30·95%) over the stationary image (mean ± S.D. $T_{rel} = 28{\cdot}15 \pm 30{\cdot}95\%$) of a nuptially coloured male [paired t-test, d.f. = 10, $P < 0{\cdot}05$, Fig. 2(a)]. Ten out of 12 males completed both consecutive trials. Males significantly preferred a moving stimulus (mean ± S.D. $T_{rel} = 74{\cdot}98 \pm 25{\cdot}20\%$) over a stationary stimulus (mean ± S.D. $T_{rel} = 25{\cdot}02 \pm 25{\cdot}20\%$) of the same image of a nuptially coloured female [paired t-test, d.f. = 9, $P < 0{\cdot}05$, Fig. 2(a)].

Experiment 3 (conspecific animation $v.$ heterospecific animation): eight out of 12 females completed both consecutive trials. Females significantly preferred a conspecific male stimulus (mean ± S.D. $T_{rel} = 57{\cdot}55 \pm 4{\cdot}85\%$) over a heterospecific stimulus (mean ± S.D. $T_{rel} = 42{\cdot}45 \pm 4{\cdot}85\%$) (paired t-test, d.f. = 7, $P < 0{\cdot}01$, Fig. 3). Ten out of 12 males completed both consecutive trials. Males likewise preferred a conspecific female stimulus (median, quartiles $T_{rel} = 94{\cdot}43\%$, 61·3 and 100%) over the heterospecific stimulus (median, quartiles $T_{rel} = 5{\cdot}57\%$, 0 and 38·7%) (Wilcoxon test, $n = 10$, $P < 0{\cdot}05$, Fig. 3).

Experiment 4 (nuptially coloured male animation $v.$ dull male animation): 13 out of 16 females completed both consecutive trials. Females stayed significantly longer (paired t-test, d.f. = 12, $P < 0{\cdot}05$, Fig. 4) with a nuptially coloured male animation (mean ± S.D. $T_{rel} = 68{\cdot}73 \pm 22{\cdot}71\%$) than with the dull animation (mean ± S.D. $T_{rel} = 31{\cdot}27 \pm 22{\cdot}71\%$).

The results show that both sexes of *P. taeniatus* did not discriminate significantly between stationary images of a dull and nuptially coloured stimulus of the opposite sex. First, colouration might not play a role in *P. taeniatus* when choosing partners, which is a counter-intuitive hypothesis with regard to the results of recent studies on the role of cichlid colouration (Seehausen *et al.*, 1999; Dijkstra *et al.*, 2007), and contradicts the results of experiment 4. Second, there may be a methodological problem concerning the use of stationary images as a stimulus for this cichlid. Earlier studies using live stimuli have already shown the effect of movement and speed on the perception of predators (Brown & Warburton, 1997, 1999). Thus, a stationary image that
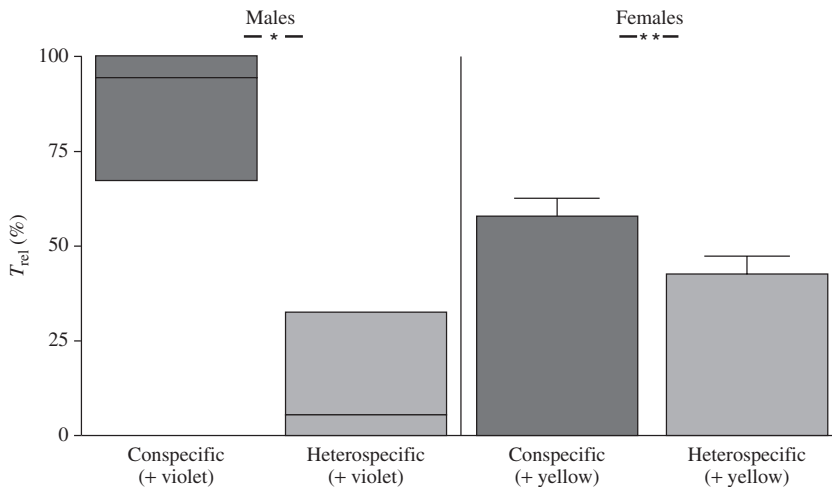

FIG. 3. Relative amount of time (T_{rel}) males (median ± quartiles) and females (mean + S.D.) spent in the association zones in front of a stimulus of a conspecific and an equally coloured heterospecific stimulus of the other sex. *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$.
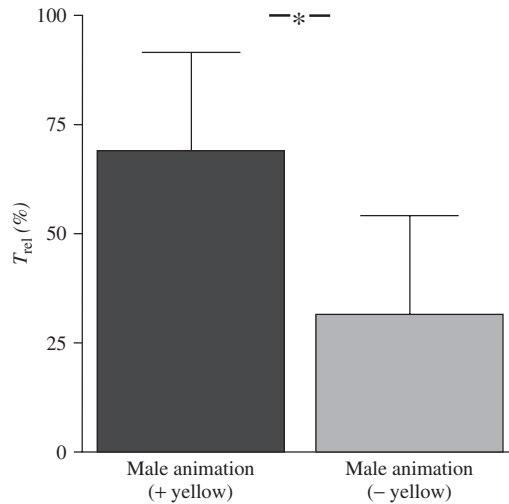
FIG. 4. Mean + S.D. relative amount of time (T_{rel}) females spent in the association zones in front of a nuptially coloured or dull male animation. *, $P < 0.05$.

displays shape and colouration but lacks movement may be a less effective stimulus than a moving animation. The experimental results of experiment 2 (animation *v.* stationary image) support that the addition of movement leads to stronger response in test fish when presenting computer stimuli. Furthermore, in mate-choice experiments using animations with three-spined sticklebacks *Gasterosteus aculeatus* L., stimuli with multiple cues caused stronger female preferences (Künzler & Bakker, 2001). In contrast to this, earlier studies using stationary but three-dimensional dummies were quite successful in measuring preferences based on colouration, body posture or body dimensions of sailfin molly *Poecilia latipinna* (Lesueur) (MacLaren & Rowland, 2006) and *G. aculeatus* (Rowland & Sevenster, 1985; Rowland, 1989; Bakker & Rowland, 1995). In experiment 1, however, only seven out of 12 males and seven out of 12 females showed a response to the stationary stimuli at all, suggesting that movement plays a crucial role in the visual perception of this species. This suggestion is strengthened by the results of experiment 2, in which both sexes preferred the animation over the stationary image. This result agrees with the finding of Rowland (1995) that gravid females of *G. aculeatus* show a weaker preference for slow moving than for faster moving males. Thus, presenting a moving animation seems to be a better method for presenting computer-manipulated stimuli in *P. taeniatus* than stationary images.

In the 'conspecific *v.* heterospecific' experiment (experiment 3) both sexes reliably recognized the body shape of their own species, even when the foreign species emitted the same colour signal as *P. taeniatus*. Thus, the fish did not only recognize the species' colour signal of the opposite sex (experiment 4) but were also able to recognize body shapes presented by the computer animation. This corresponds to findings in other species, for example, damselfishes (Thresher, 1976; Katzir, 1981), in which body shape plays an important role in species recognition.

When a dull male animation was presented against a nuptially coloured male animation, females significantly preferred the nuptially coloured male (experiment 4).

Thus, female *P. taeniatus* are able to discriminate between differently coloured male animations, suggesting that the radiance of colour information emitted by the CRT is perceived adequately. Thus, computer animations are suitable tools to further investigate the role of colouration in inter and intrasexual communication in this species.

In general, computer systems can provide a very high degree of standardization of visual stimuli. Computer displays, however, are tailored to human vision (D'Eath, 1998; Baldauf *et al*., 2008) and do not emit wavelengths such as ultraviolet or polarized light, which may play an important role in the vision of shallow cichlid species (Seehausen *et al*., 2008). Hence, it is possible that the colours of the animations were perceived differently from the way real fishes would appear in the natural environment. The results of experiment 4, however, suggest that female *P. taeniatus* reliably perceived the male's nuptial colouration shown by the CRT display.

Overall, the results suggest that computer stimuli like animated 2D images seem to be a simple, effective and efficient ethological tool to quantify the behavioural preferences in this cichlid. Depending on the species and the task, however, it may be necessary to apply 3D-animations when depth cues are needed (Baldauf *et al*., 2008). Other cichlid species may require the addition of olfactory (Plenderleith *et al*., 2005) or acoustic cues (Amorim *et al*., 2004; Simoes *et al*., 2008) into the set-up to measure their preferences. These cues are initially not available, but can be embedded into an experimental set-up in addition to the visual stimulus presentation (Mehlis *et al*., 2008). Altogether, computer animation can be a useful and effective tool in studying preferences in a cichlid that shows complex and variable nuptial colouration.

References

Amorim, M. C. P., Knight, M. E., Stratoudakis, Y. & Turner, G. F. (2004). Differences in sounds made by courting males of three closely related Lake Malawi cichlid species. *Journal of Fish Biology* **65,** 1358–1371.

Bakker, T. C. M. & Rowland, W. J. (1995). Male mating preference in sticklebacks: effects of repeated testing and own attractiveness. *Behaviour* **132,** 935–949.

Baldauf, S. A., Kullmann, H. & Bakker, T. C. M. (2008). Technical restrictions of computer-manipulated visual stimuli and display units for studying animal behaviour. *Ethology* **114,** 737–751.

Barlow, G. W. (2002). *The Cichlid Fishes - Nature's Grand Experiment in Evolution*. Cambridge: Perseus.

Boynton, R. M. (1979). *Human Color Vision* New York, NY: Holt, Rinehart and Winston.

Brown, C. & Warburton, K. (1997). Predator recognition and anti-predator responses in the rainbowfish, *Melanotaenia eachamensis*. *Behavioural Ecology and Sociobiology* **41,** 61–68.

Brown, C. & Warburton, K. (1999). Differences in timidity and escape responses between predator-naive and predator-sympatric rainbowfish populations. *Ethology* **105,** 491–502.

D'Eath, R. B. (1998). Can video images imitate real stimuli in animal behaviour experiments? *Biological Reviews* **73,** 267–292.

Dijkstra, P. D., Hekman, R., Schulz, R. W. & Groothuis, T. G. G. (2007). Social stimulation, nuptial colouration, androgens and immunocompetence in a sexual dimorphic cichlid fish. *Behavioral Ecology and Sociobiology* **61,** 599–609.

Katzir, G. (1981). Visual aspects of species recognition in the damselfish *Dascyllus aruanus* L. (Pisces, Pomacentridae). *Animal Behaviour* **29,** 842–849.

Keenleyside, M. H. A. (1991). *Cichlid fishes - Behaviour, Ecology and Evolution*. London: Chapman & Hall.

Kocher, T. D. (2004). Adaptive evolution and explosive speciation: the cichlid fish model. *Nature Reviews Genetics* **5,** 288–298.

Kroeger, R. H. H., Bowmaker, J. K. & Wagner, H. J. (1999). Morphological changes in the retina of *Aequidens pulcher* (Cichlidae) after rearing in monochromatic light. *Vision Research* **39,** 2441–2448.

Künzler, R. & Bakker, T. C. M. (1998). Computer animations as a tool in the study of mating preferences. *Behaviour* **135,** 1137–1159.

Künzler, R. & Bakker, T. C. M. (2001). Female preference for single and combined traits in computer animated stickleback males. *Behavioral Ecology* **12,** 681–685.

MacLaren, R. D. & Rowland, W. J. (2006). Differences in female preference for male body size in *Poecilia latipinna* using simultaneous versus sequential stimulus presentation designs. *Behaviour* **143,** 273–292.

Mehlis, M., Bakker, T. C. M. & Frommen, J. G. (2008). Smells like sib spirit: kin recognition in three-spined sticklebacks (*Gasterosteus aculeatus*) is mediated by olfactory cues. *Animal Cognition* **11,** 643–650.

Milinski, M. & Bakker, T. C. M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. *Nature* **344,** 330–333.

Parry, J. W. L., Carleton, K. L., Spady, T., Carboo, A., Hunt, D. M. & Bowmaker, J. K. (2005). Mix and match color vision: tuning spectral sensitivity by differential opsin gene expression in Lake Malawi Cichlids. *Current Biology* **15,** 1734–1739.

Plenderleith, M., van Oosterhout, C., Robinson, R. L. & Turner, G. F. (2005). Female preference for conspecific males based on olfactory cues in a Lake Malawi cichlid fish. *Biology Letters* **1,** 411–414.

Rowland, W. J. (1989). Mate choice and the supernormality effect in female sticklebacks (*Gasterosteus aculeatus*). *Behavioral Ecology and Sociobiology* **24,** 433–438.

Rowland, W. J. (1995). Do female sticklebacks care about male courtship vigour? Manipulation of display tempo using video playback. *Behaviour* **432,** 951–961.

Rowland, W. J. & Sevenster, P. (1985). Sign stimuli in the 3-spine stickleback (*Gasterosteus aculeatus*) – a re-examination and extension of some classic experiments. *Behaviour* **93,** 241–257.

Seehausen, O. & van Alphen, J. J. M. (1998). The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei* complex). *Behavioral Ecology and Sociobiology* **42,** 1–8.

Seehausen, O., Mayhew, P. J. & van Alphen, J. J. M. (1999). Evolution of colour patterns in East African cichlid fish. *Journal of Evolutionary Biology* **12,** 514–534.

Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., van der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H. & Okada, N. (2008). Speciation through sensory drive in cichlid fish. *Nature* **455,** 620–623.

Simoes, J. M., Fonseca, P. J., Turner, G. F. & Amorim, M. C. P. (2008). African cichlid *Pseudotropheus* spp. males moan to females during foreplay. *Journal of Fish Biology* **72,** 2689–2694.

Stevens, M., Parraga, C. A., Cuthill, I. C., Partridge, J. C. & Troscianko, T. S. (2007). Using digital photography to study animal coloration. *Biological Journal of the Linnean Society* **90,** 211–237.

Thresher, R. E. (1976). Field experiments on species recognition by the threespot damselfish, *Eupomacentrus planifrons*, (Pisces: Pomacentridae). *Animal Behaviour* **24,** 562–569.

Thünken, T., Bakker, T. C. M., Baldauf, S. A. & Kullmann, H. (2007*a*). Active inbreeding in a cichlid fish and its adaptive significance. *Current Biology* **17,** 225–229.

Thünken, T., Bakker, T. C. M., Baldauf, S. A. & Kullmann, H. (2007*b*). Direct familiarity does not alter mating preferences for sisters in male *Pelvicachromis taeniatus* (Cichlidae). *Ethology* **113,** 1107–1112.