# The dual effect of stickleback nuptial coloration on rivals: manipulation of a graded signal using video playback

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**Abstract.** Reproductive male three-spined stickleback, *Gasterosteus aculeatus*, were presented with pairs of videotaped images of another male played back at three different colour intensities: brightly coloured, moderately coloured and dull. Males attacked all three images but the proportion of attacks each received depended on the other image with which it was simultaneously presented. The moderately coloured image received the majority of attacks when presented with the brightly coloured or dull image, whereas the latter two images were attacked equally when presented together. Thus, nuptial coloration increased the capacity of an opponent to elicit attack from males but this increase was not open-ended. A dual-effect explanation for these colour effects suggests that dull males stimulated relatively low levels of aggression and little fear, whereas brightly coloured ones stimulated a high level of aggression but a level of fear sufficient to inhibit full expression of attack. Moderately coloured males received the highest levels of attack presumably because they stimulated high levels of aggression but relatively little fear. This interpretation emphasizes the importance of intensity of nuptial coloration in stimulating aggression and fear in an opponent and the role that these tendencies may play in determining a male's response to rivals.
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More than a half-century ago, ter Pelkwijk & Tinbergen (1937) reported that crude dummies with red undersides evoked attack in territorial male sticklebacks, whereas dummies lacking red evoked little or no such response. From this observation, Tinbergen (1948, 1951) concluded that the nuptial coloration of male sticklebacks, especially the red underside, functioned as a sign-stimulus for aggression in this species. Repeated citing of this example in textbooks (e.g. Marler & Hamilton 1966; Hinde 1970; McFarland 1985; Drickamer & Vessey 1992) led students of animal behaviour to accept the generality of these findings. Subsequent studies, however, failed to confirm that red undersides are a crucial feature for eliciting attack from male three-spined sticklebacks, *Gasterosteus aculeatus* (Muckensturm 1967, 1969; Peeke et al. 1969; Wootton 1971; Rowland 1982; Rowland & Sevenster 1985).

Perhaps the most consistent finding from these studies is the variation in the male's response to nuptial coloration, even within the same study. Several explanations for this variation have been proposed, including differences in the intensity and extent of coloration of the dummies used, the illumination under which the fish were tested, the distance and method of dummy presentation, the experience and physiological state of the subjects, and genetically based differences among populations (Rowland 1982, 1994; Bakker & Sevenster 1983; Baerends 1985; Rowland & Sevenster 1985; Collias 1990). None of these hypotheses has been explicitly tested, however, and the question of how nuptial coloration affects the behaviour of male sticklebacks continues to draw attention (e.g. Wootton 1976; Reiss 1984; Baerends 1985; Archer 1988; Barlow 1989; Collias 1990; Domjan 1993; Rowland 1994).

The brightness of nuptial coloration in sticklebacks is positively correlated with the male's dominance status (Bakker & Sevenster 1983) and with his tendency to attack intruders (Rowland 1984; McLennan & McPhail 1989). If coloration reflects the male's threat potential or resource-holding power (Parker 1974), we would expect brighter males to be more intimidating than less bright ones. Thus, the lesser threat posed by dull males should induce less fear and aggression, at least in males not yet caring for spawn. The present study tests the effects of nuptial coloration

on stickleback aggression by presenting males with videotaped displays (see also Clark & Uetz 1992; Evans et al. 1993) of a rival male which, unlike dummies, reproduce the natural movements of live males. More specifically, the present study focuses on how the presence and intensity of a male's nuptial coloration may affect the agonistic behaviour that a rival male directs towards him. These results could provide insight into the communicatory function of nuptial coloration in the male stickleback and the role that intra-sexual selection may have played in its evolution.

## MATERIALS AND METHODS

### Subjects

Adult males of the marine form of *G. aculeatus* were collected by seining brackish marsh pools in northeastern Long Island, New York and southern Rhode Island at the onset of the breeding season (April–May). Fish were transported to the laboratory in Bloomington, Indiana and maintained under spring conditions (16:8 h light:dark cycle and 18°C) throughout the study period. Each male was held in a separate tank containing brackish water (15 g/l salinity), vegetation and a sand-filled nesting dish, and fed a daily diet of frozen *Artemia*. Within a day or two after introduction into tanks, each male built a nest and developed the red underside and blue eye coloration to an extent (i.e. 'moderately coloured') typical of territorial males in our study population. At this point, we began testing.

### Video Playback Images

A brightly coloured territorial male stickleback (51 mm standard length) was recorded against a light grey background with a S-VHS camera-recorder (Olympus VX-S405-KU), during which time he observed his own image displayed on a $27 \times 20$-cm high-resolution colour video monitor (Panasonic CT-1382Y) located outside the tank. As the male watched his image on the monitor, he displayed vigorously with charges, zigzag dancing (a display that males also perform when courting females), and biting attempts. The overall response was typical of one that males initially direct to male or female conspecifics presented in jars (Rowland 1984) or in adjacent aquaria

(Rowland 1988). In this way the male's behaviour was videotaped while he directed his activities towards the moving image on the video monitor located just alongside the camera-recorder. The resulting videotape displayed the male at approximately natural to somewhat larger than natural size, providing a 2-min sequence of his behaviour that appeared to be directed at the individual viewing it on the monitor.

### Testing Tank

Space constraints in our laboratory precluded placing monitors at the ends of the home tank of each male. Because reproductive males engage in territorial behaviour even in a tank to which they have just been transferred (Bakker & Sevenster 1983; Rowland 1989), we conducted the video playback experiments in a separate testing tank ($50 \times 25 \times 30$ cm) containing filtered brackish water, a sand bottom and tufts of filamentous algae (*Rhizoclonium* spp.). Video monitors, described above, were positioned 10 cm from each end of the test tank and linked to an S-VHS video camera-recorder (VCR), so that both monitors displayed the playback image simultaneously (Fig. 1). Males in the test tank were thus free to view and approach within 10 cm of either video playback during the test trial.

### Testing Procedure

We conducted three test series, each presenting males with a choice of two video displays. Using its digital colour intensity control, each monitor was adjusted to play the video sequence at one of three predetermined colour intensities: dull (D), moderately coloured (M) and brightly coloured (B). To our eyes, the D-image appeared greytone, the M-image like a male with average nuptial coloration, and the B-image much brighter than an average male. We measured overall luminance at each colour setting (SpectraScan PR714 photometer with a 2° acceptance angle; Photo Research) by averaging readings from the same points on the video display: the area under the male's opercula (most saturated red area), the area between his flank and dorsum, and the background on which he appeared. These luminances were found to be closely matched: $D=13 \cdot 54$, $M=13 \cdot 57$ and $B=13 \cdot 63$ log quanta/ cm²/s.
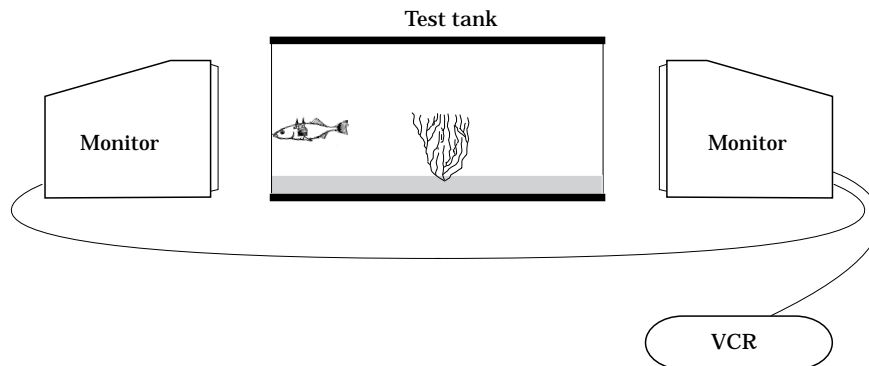
**Figure 1.** Experimental set-up used to test male response to video playback. The videotape of the stimulus male was played back via a video camera-recorder (VCR) through both monitors simultaneously. The male is depicted in position characteristic of the 'butt/bite' response to the playback image.

These test series used different groups of subjects, were carried out at least several days apart from each other, and tested different colour intensity pairs. Series I compared D versus B, series II compared D versus M, and series III compared M versus B.

Prior to testing, we gently transferred the subject from its home tank to the testing tank. After a brief acclimatization period ($\pm 1$ min), the video sequence was played back while the subject's response was recorded on a Hi-8 camera-recorder (Sony V-801) mounted in front of the test tank. At the end of the 2-min sequence the tape was rewound, the colour intensity settings of the two monitors switched to control for possible position bias of the test males, and the second half of the trial run. We attempted to begin playback with the subjects facing towards the back or front of the test tank, so that the subjects had a view of both monitors when the video images first appeared. To further control for position bias, we switched the starting position of a given colour setting from trial to trial. At the end of all test series an observer watched the videotaped tests and recorded each subject's activities, using the Observer event-recording programme. The activity of each subject was summed for both 2-min playback periods and the 4-min trial totals to each of the two colour settings compared.

### RESULTS

Although subjects were tested outside their home tanks, they attacked both video images in 41 of 48 trials; this was expressed by the subject charging a
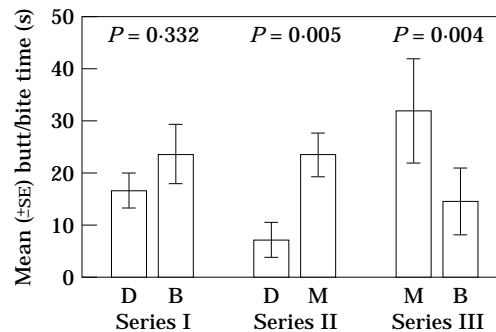


**Figure 2.** Subjects' responses to paired presentations of male video playback in test series I ($N=19$), II ($N=13$) and III ($N=9$). D: Dull (greytone) display; B: brightly coloured display; M: moderately coloured display. Wilcoxon matched-pairs signed-ranks test probabilities (two-tailed) appear above bars.

video image, often from one end of the tank to the other, and trying to butt or bite it as it moved across the monitor screen (Fig. 1). Because this activity usually occurred in bouts of several seconds, rather than in single, discrete units, we recorded it as 'butt/bite' time, the number of seconds per trial that a male spent thrusting his snout against the end wall in apparent pursuit of the moving image.

Analysis of data revealed no difference in butt/bite time to B and D displays (series I), but males did respond more to the M display when we presented it with either the D (series II) or the B (series III) display (Fig. 2).

When males were not butting/biting, they swam excitedly about the tank, sometimes stopping,

apparently to watch one or the other stimulus display. We recorded this behaviour as 'visit' time, the number of seconds per trial a male spent within one body length and oriented towards a given display. Visit time differed neither between D and B in series I (Wilcoxon matched-pairs signed-ranks test: two-tailed $P=0.189$) nor between M and B in series III (two-tailed $P=0.236$), but was higher to M than to D displays (two-tailed $P=0.024$).

The seven males that failed to attack either display spent nearly all of the test trial lying on or near the bottom, sometimes picking at the substrate.

## DISCUSSION

Social encounters may activate in an animal behavioural tendencies that compete for expression (Tinbergen 1952; Hinde 1970). For example, Archer (1988) discussed how fear and aggression often interact within an animal during an agonistic encounter. We can account for the present results on the basis of such interaction if we assume that nuptial coloration of male sticklebacks, like many signals used in territorial conflicts, induces both fear and aggression in an opponent (Tinbergen 1957). The relative levels of stimulation of these two factors would thus depend on the opponent's coloration.

The proposed interaction between aggression and fear is consistent with that hypothesized by Archer (1976, 1988) and is based on studies of humans (Berkowitz 1962), Siamese fighting fish, *Betta splendens* (Melvin & Anson 1969; Melvin & Ervey 1973) and other vertebrates. Those studies suggested that mildly threatening stimuli increase aggression more than fear, whereas intense stimuli have the opposite effect. The relatively low level of aggression induced by a dull-coloured fish is therefore readily expressed, because the fear it also induces is minimal. A bright, hence formidable, opponent strongly activates aggression, but this response would be partly blocked by the high level of fear that it also activates. The similar ratios of aggression:fear induced by bright- and dull-coloured opponents may therefore lead to similar levels of response, but by activating moderately strong aggression and relatively little fear, moderately coloured opponents would evoke the most attack.

If a male's coloration relative to that of his opponent is an important determinant of his response, we should expect brighter-coloured subjects to respond more aggressively than duller ones in our study. Inspection of the data, however, failed to reveal any such trend, but limited sample size and similarity in coloration among our males precluded a proper analysis of this point.

Although the opponent's location, size and behaviour may also affect the attack rate it elicits, for simplicity we assumed that these factors were constant. Our subjects were, however, tested in a 'neutral' tank, and a change in context could shift their relative levels of aggression and fear. Hence, if males experience relatively higher levels of aggression in their home tanks, they might have attacked the bright playback more than the moderately or dull-coloured one if they had been tested there.

The positive correlation of nuptial coloration with attack, dominance and courtship behaviour in male stickleback (Bakker & Sevenster 1983; Rowland 1984; McLennan & McPhail 1989) suggests that coloured opponents are more competitive than dull ones. If males respond in accordance with the challenge they perceive, relative to their own resource-holding potential, coloured males should induce more aggression in rivals than dull (i.e. non-reproductive) ones, an outcome that is also consistent with the proximal ('sign-stimulus') explanation for this effect (Tinbergen 1951). Excessive fear in response to very bright colour levels might, however, place an upper limit on this effect.

If bright males are more intimidating than less bright ones, intra-sexual selection should act to enhance coloration, whereas physiological costs (Milinski & Bakker 1990; Frischknecht 1993) and predation pressure (Moodie 1972) would have the opposite effect. If females were similarly intimidated by extremely bright males, this might provide an additional moderating influence on male colour intensity.

An alternative explanation as to why males responded less to the brightly coloured image is that they failed to recognize it as a conspecific. It is hard to rule out this possibility, but no species sympatric with the three-spined stickleback has a colour pattern resembling the bright video image, as conspecific males do. We speculate that without such counteracting selection pressure, a

perceptual mechanism in which moderate color-ation would stimulate aggressive tendencies more than supernormally bright coloration is unlikely.

Why males attacked nuptially-coloured op-ponents more than dull ones in the present study and in the original dummy experiment reported by Tinbergen, but not in all dummy experiments, is uncertain. The colour of even the brightest video image, however, appeared to us less satu-rated than that of the dummies used in studies in which some measure of colour (namely the Munsell value) was reported. It is thus possible that the coloration of these dummies was super-normal. Moreover, video images, unlike dummies, accurately reproduce conspecific movement cues, which also affect aggression in sticklebacks (Tinbergen 1951; Rowland 1994). Interaction between nuptial coloration and behaviour of an opponent may therefore have important conse-quences for a subject's response to it.

The present study demonstrates that video play-back can be an effective tool for the experimental analysis of visually mediated behaviour in fish. The method does not, however, enable subjects to approach the 'opponent' any closer than the monitor surface. For many studies, lack of direct contact with the stimulus may not be a serious disadvantage, and in this regard the method is no more limiting than presenting animals to op-ponents separated by a glass barrier, a method that has long proven useful in ethology.

The fixed nature of a playback sequence, being independent of the subject's behaviour, might impose further limitations on the technique. For example, male sticklebacks sometimes perform a head-down threat display when they encounter rivals near the boundary of their territory, pre-sumably because aggression and fear are balanced (Tinbergen & van Iersel 1947), but we never observed this display to video playback. This might reflect the non-interactive nature of video image stimulation, because male sticklebacks sometimes do direct head-down displays to their mirror image (see Plate I of Tinbergen 1953). We do not know how the dynamics of interaction might affect the male stickleback's response to opponents, but video and computer technology should soon enable experimenters to play back selected tape sequences according to the activity performed by a subject. This technique would permit interacting with a subject in a way that crudely approaches real encounters.

A final caution of relevance to the present study concerns an animal's colour perception of a given video image. Because video technology produces colour by methods based on human spectral sen-sitivity, we cannot be certain that a particular colour image appears the same to an animal whose spectral sensitivity differs from ours. Microspectrophotometric study reveals, however, that the absorbances of cones from the three-spined stickleback's retina do not differ markedly from ours (Lythgoe 1979; C. Baube & E. Loew, unpublished data; see also Frischknecht 1993). We therefore assume that the video image that we perceived to be more brightly coloured within a given pair also appeared so to our subjects. Nevertheless, if spectral sensitivities of stickleback and humans differ enough, the combination of red, green and blue pixels that appear greytone to humans might produce a strangely coloured image for sticklebacks. Continued improvement in the technology used to create and manipulate video images, and further understanding of ani-mal visual systems, should help to minimize this problem in future studies.

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