Manipulations of signalling environment affect male competitive success in three-spined sticklebacks

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Abstract. Breeding adult male three-spined sticklebacks, *Gasterosteus aculeatus* L., develop an elaborate breeding signal, the primary component of which is ventral red coloration. In laboratory dominance tests staged under illumination conditions that maintained or eliminated male perceived coloration, this study examined (1) the relationship between male coloration and fighting ability and (2) the applicability of three hypotheses for the current function of male coloration in male–male competitive interactions. In pairs of males from a Long Island, NY, population matched for size and prior dominance experience, the more brightly coloured males were more likely to initiate and win under white light. The effect of colour on dominance success was lost under replicates illuminated by coloured-light treatments that masked the signal by eliminating its visual contrast. The loss of competitive advantage associated with more conspicuous coloration was not an artefact of illumination manipulations, since the effects seen under white light were maintained under a coloured-light treatment that maintained male coloration. These data suggest that red belly coloration of males functions as a threat signal.

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Aggressive behaviour plays an important role in the reproductive biology of many animal species. The male often establishes a territory from which conspecific males are aggressively excluded and to which conspecific females are attracted. To gain or expand a territory, males must assess the resource holding power of rivals, e.g. their willingness or ability to defend a territory. Visual cues can play a role in the decision to threaten, escalate to combat or avoid confrontation, with the choice of tactics weighed against energetic costs and risks of injury (Parker 1974).

The features used to determine the outcome of territorial encounters are sometimes arbitrary (Dawkins & Krebs 1978), but selection most often favours the evolution of visual signals that honestly reveal a male's actual fighting ability. Otherwise, some individuals in a population may bluff the expression of the signal to exaggerate real fighting ability (Parker 1974; Dawkins & Krebs 1978).

Studies in invertebrate and vertebrate species demonstrate that male visual signals correlate with territorial defence, fighting ability and, ultimately, dominance rank (Andersson 1994). Manipulations reveal the importance of visual signals in territorial interactions in males. In birds, the elimination of male signals by, for example, the trimming of tufts in a malachite sunbird, *Nectarinia johnstoni* (Evans & Hatchwell 1992), or by masking coloured patches with dyes or paints in the red-winged blackbird, *Agelaius phoeniceus* (Smith 1972; Peek 1972; Roskaft & Rohwer 1987) and an African warbler, *Phylloscopus inornatus* (Marchetti 1993), adversely affects territorial retention by treated males. Similarly, the painting of dewlaps of male lizards significantly reduces their success in staged dominance interactions (Hover 1985; Thompson & Moore 1991).

Breeding adult male three-spined sticklebacks, *Gasterosteus aculeatus*, establish a territory from which they exclude conspecific intruders. Males

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develop a nuptial signal that in most populations is composed of a blue eye and red belly coloration (but see Reimchen 1989; Blouw & Hagen 1990). The red coloration varies from pale orange to deep red, and in extent from the lining of the mouth to the throat, operculum and across the flank. Red belly coloration functions as a signal in both male–male competitive and female choice contexts, with the latter probably more important for the evolution of the feature (Bakker & Milinski 1993; Bakker 1994; Rowland 1994).

Tinbergen and co-workers (ter Pelkwijk & Tinbergen 1937; Tinbergen 1948, 1951) suggested that red belly coloration acts in male–male interactions as a 'sign stimulus' that provokes aggression in rivals. They observed that territorial males presented with a dummy painted to mimic a nuptially coloured rival elicited more attacks than a dummy lacking red.

Attempts to replicate Tinbergen's findings have not always been successful (e.g. Chauvin-Muckensturm 1979; Rowland & Sevenster 1985; but see Collias 1990). In one such experiment, Chauvin-Muckinsturm (1979) paired males and allowed them to compete for a single territory under different illumination conditions. She hypothesized that white light would maintain, and that blue and red light would disrupt, a male's ability to detect the colour of a rival. Although the results were inconsistent, she did conclude that males would attack rivals whose belly coloration had been eliminated by illumination manipulation.

More intense red belly coloration in three-spined stickleback males is associated with greater reproductive activity (Rowland 1984; Bakker & Milinski 1991), lower parasite loads (Milinski & Bakker 1990), higher aggression levels (Rowland 1984; McLennan & McPhail 1989b) and greater social dominance (Bakker & Sevenster 1983; Bakker 1986), although some of these relationships are not always present (e.g. FitzGerald & Kedney 1987; Rowland 1989; Hosking 1996). These observations, along with the difficulties in replicating Tinbergen's initial findings, led Rowland and co-workers (Rowland & Sevenster 1985; Rowland 1994; Rowland et al. 1995) to suggest that red belly coloration acts as a threat signal that 'intimidates' rivals. The expression of red coloration demonstrates territorial status and the resource holding power of its bearer.

In reconciling the two seemingly contradictory functions for male red belly coloration in male–male interactions, that is provocation versus intimidation, Rowland (1994; Rowland et al. 1995) pointed out that the context in which the signal is presented is critical to understanding its function. He suggested a 'dual function' hypothesis in which the context in which a signal is presented affects the relative activation of fear and aggressive behavioural tendencies that compete for expression in rivals. When presented with a red belly signal close to his nest, a territorial male's aggressive tendency is heightened and fear is reduced. The result is more attacks to red stimuli (e.g. Tinbergen 1951; Collias 1990). In contrast, the same red signal presented nearer to the periphery of a male's territory elicits less aggression (see also van Iersel 1958) and heightened fear, resulting in fewer attacks (e.g. Rowland & Sevenster 1985).

The sign stimulus and intimidation hypotheses both predict that elimination of male colour by manipulation, such as by the use of coloured illumination (Chauvin-Muckinsturm 1979), will also eliminate the function, i.e. stimulation or intimidation.

The expression of red belly coloration may also be more broadly interpreted as an index, or 'indicator' (Grafen 1990; Collins 1993), of overall male condition. One possible correlate of male condition that can have significant fitness consequences is fighting ability. If more intense red belly coloration evolved as an indicator of various aspects of heritable variation in male quality, such as fighting ability, the expression of that signal evolved to reliably signal heritable variation in male fighting ability.

Much is known about the mechanisms of colour processing of the three-spined stickleback, including the spectral sensitivity of retinal photoreceptors (Ali & Anctil 1976; Lythgoe 1979; C. L. Baube & E. R. Loew, unpublished data), the frequency distributions of each receptor class (E. R. Loew, unpublished data) and the identification of neural mechanisms providing what appears to be true colour vision (i.e. colour opponent processing; Cronly-Dillon & Sharma 1968; Baube 1994; McDonald & Hawryshyn 1995). Since true colour vision requires inputs from at least two regions of the visible spectrum to function (Lythgoe 1979; Endler 1990), coloured illumination manipulations that eliminate light

input to visual pigment classes crucial to colour opponent processing should adversely affect the perception and function of a coloured signal.

I used dyadic staged territorial encounters over a limited resource (nesting space in test aquaria of sufficient size for only one territory) and illumination manipulations to investigate the relationships between fighting success and various measures of male phenotype, including colour. The goal was to disrupt the perception of the red belly signal without otherwise affecting the behaviour of competing males to test the extent to which red belly coloration elicits attack and/or reliably signals the willingness and ability of males to defend a territory. This approach provides the opportunity to re-evaluate current hypotheses for the function of red belly coloration during male–male competitive interactions.

A second goal of these experiments was to gain insight into the mechanisms of colour assessment by sticklebacks and the resulting consequences for colour signal evolution. If three-spined sticklebacks rely on colour vision to assess conspecifics, then manipulations of the signalling environment that impair visual functioning should also affect signal perception.

METHODS

Subject Collection and Maintenance

Sticklebacks were collected in March and April 1992, 1994 and 1995 by seining brackish-water pools in salt marshes on Long Island, New York, shortly after the fish migrated from adjacent marine waters. Subjects were transported to Omaha, Nebraska, and maintained under conditions simulating those of the pools from which they were collected ($6.0 \pm 0.5°C$; 8:16 h light:dark photoperiod; in brackish seawater of approximately 12 g/litre commercial artificial sea salts). All fish were fed frozen *Artemia salina* and/or live *Tubifex* worms once daily throughout the study.

Groups of 8–16 males expressing some development of nuptial coloration (blue eye, red mouth lining and/or belly) were slowly acclimated to the simulated spring conditions of the laboratory ($22.0 \pm 2°C$; 16:8 h L:D photoperiod). Males were individually placed into 40-litre ($50 \times 30 \times 25$ cm) brackish water (12–15 g/litre) tanks with a sand-filled dish ($14 \times 14 \times 3$ cm deep) and filamentous algae (*Rhizoclonium* sp.) for nesting. Males were

given sufficient time to build a nest and develop full nuptial coloration before the territorial contests were begun.

Separate aquaria were set up to be as similar to the subjects' maintenance aquaria as possible. These aquaria served as the neutral test arenas in which the contests were conducted.

Illumination Treatments

The three-spined stickleback retina has at least three photoreceptor classes with approximate maximum sensitivities to light of wavelengths 435 nm (short wavelength, S), 530 nm (mid wavelength, M) and 605 nm (long wavelength, L) regions of the light spectrum (Ali & Anctil 1976; Lythgoe 1979; C. L. Baube & E. R. Loew, unpublished data). The M and L classes numerically dominate the retinal mosaic (E. R. Loew, unpublished data) and interact neurally to provide what appears to be colour opponent processing (Cronly-Dillon & Sharma 1968; Baube 1994; McDonald & Hawryshyn 1995).

Combinations of theatrical gels (Lee filters, Andover, U.K.; GamColor, The Great American Market, Hollywood, CA, U.S.A.) were selected to optimize the stimulation of desired photopigments while minimizing stimulation, when desired, of the remaining photopigments. I hypothesized that illumination treatments that stimulated both L and M photopigment classes will maintain male visual functioning, since both provide input to the photoreceptor classes responsible for colour processing. Alternatively, I hypothesized that illumination treatments that stimulate only one of the two primary photoreceptor classes will eliminate male coloration, since neither provides stimulation to both components of the L–M colour opponent mechanism necessary for colour processing.

Illumination was provided by two 52-W tungsten incandescent lights (G.E. SuperSaver) in metal aquarium hoods suspended 15 cm over pairs of aquaria. Colour environments were created by wrapping theatrical gels around the hoods so that no white light escaped. Overhead lights in the experimental room were turned off, so that the only light reaching subjects passed through the gel filters.

Treatment SM provided stimulation to the S sensitive and M sensitive regions of the visible spectrum and was produced by two layers of blue

gel (Lee #079, Just Blue). Treatment L provided stimulation to only the L sensitive region of the visible spectrum and was produced by one layer of bright red gel (Lee #019, Fire) and one layer of red-orange gel (Lee #022, Dark Amber). I hypothesized that these two illumination treatments would eliminate a male's ability to detect the colour status of rivals. Treatment (SM)L provided S, M and L wavelength light and was produced by one layer of blue gel (Lee #079, Just Blue) and one layer of deep purple gel (Lee #180, Dark Lavender). I hypothesized that this treatment would maintain the ability of three-spined stickleback males to assess the colour state of rivals.

The total number of incident photons (the quantal flux) in the span 400–700 nm were measured using a calibrated spectroradiometer (Photo Research, SpectraScan PR714, 2° sample area, 4 nm bandwidth). Layers of Log 0.15 neutral density gel (GamColor #1514) were added to the brighter treatments to equalize all three to the dimmest. The intensity selected for tests exceeded the minimum ambient illumination necessary for three-spined stickleback cone photoreceptors to operate and provide colour vision, that is the 'photopic threshold', as determined experimentally by Milinski & Bakker (1990). A GamColor #1516 gel (Log 0.6 neutral density) and GamColor #1517 gel (Log 0.9 neutral density) were added to unmanipulated aquarium hoods to create a white light (treatment W) of the same intensity as the other treatments.

The spectral distributions for all illumination treatments are shown in Fig. 1. Preliminary inspection demonstrated that, to human observers, they had the desired effect of maintaining (W and (SM)L treatments) or eliminating (SM and L treatments) male red coloration. A white card appeared deep blue (SM), blue-purple ((SM)L), white (W) and deep red (L) and the red of brightly coloured males appeared blue-black, deep red, orange-red and red (against a red flank and red background), respectively.

The effectiveness of each illumination treatment in uniformly (W, (SM)L) or differentially (SM, L) stimulating the stickleback L–M colour opponent unit was determined empirically by calculating the relative quantum catch (ranging from 0.0–1.0) of each photopigment class. In simplest terms, a photopigment's relative quantum catch is the proportion of all photons striking a subject's
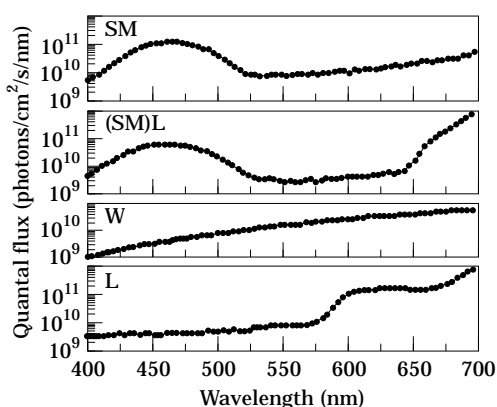
Figure 1. Spectral quantal flux (in quanta/cm²/s/nm) of light sources used to illuminate staged territorial encounters.

retina that will be absorbed by the particular photopigment considered. Relative quantum catch for each of the three photopigment classes was calculated as follows.

$$P_i = \frac{Q_i}{Q_1 + Q_2 + Q_3}$$

where,

$$Q_i = \sum_{400}^{700} S_{i,\lambda} \times F_\lambda \times d_\lambda$$

P_i is the relative quantum catch for pigment i ($i_1 = S$, $i_2 = M$, $i_3 = L$), Q_i the quantum catch of each photopigment, $S_{i,\lambda}$ relative spectral absorbance (ranging from 0.0–1.0) of photopigment i at wavelength λ (corrected for intraocular absorbance; Lythgoe 1979), and F_λ the quantum flux of the illumination source at wavelength λ (as determined above). The relative absorbance spectrum of each photopigment ($S_{1-3,\lambda}$) was based on microspectrophotometry using sticklebacks from Long Island (data provided by E. R. Loew).

Staged Pair-wise Territorial Encounters

Preliminary assessments

Before I determined opponent pairings, a number of features that might influence competitive ability were measured for each male. Standard length and mass were measured to the nearest millimeter and hundredth of a gramme,

respectively. These measures were also used to calculate the condition factor, which quantifies a subject's physical condition relative to other members of its population (Bolger & Connolly 1989). This factor was determined for each male using:

$$\text{Condition factor} = \frac{100 \times \text{mass (g)}}{\text{standard length (cm)}^{1.828}}$$

where the exponential is the slope of Log mass (g) versus Log standard length (cm) of the subjects used in this study ($N=170$, $r^2=0.409$).

The time taken by a male to complete construction of a nest (days to nest) was used to estimate male reproductive activity. Males completing nests more quickly might be more reproductively active, and thus more likely to initiate or win a territorial encounter.

I also measured the number of bites that male subjects delivered in a 4 min period to a male of intermediate colour enclosed in a glass flask and placed 10 cm from the subject's nest (van Iersel 1958; Wootton 1971; FitzGerald & Kedney 1987). Each male's bite score was assessed in the male's home tank half an hour prior to a staged territorial encounter.

The coloration of each subject was assessed on two occasions and the colour score value used in analyses is the average, rounded to the nearest whole number. The first assessment was made on the day prior to pairing in a staged territorial encounter, immediately after the subject was paired with a gravid female for 15 min. The second colour assessment was made immediately after the bite score assay.

Subjects were assigned a colour score ranging from 1 to 9 by visual estimation using the following criteria: 1–3: some red colour in the mouth and on the lower jaw; 4–6: red colour across lower jaw and underside; 7–9: red colour throughout lower jaw and underside, extending across the side of the body. Colour assessments for males under manipulated illumination were done under momentary illumination with white light provided by a hand-held 54-W incandescent bulb.

Pairings

After I assessed a group of males for bite score and colour score, I assigned each male an opponent. Pairs were drawn from the group of 8–16 males brought from the laboratory and placed under the illumination treatment on the same day. Males were paired based on two priorities. Rowland (1989) showed that the larger of two males paired in a staged territorial encounter will have a competitive advantage if the size asymmetry between them is 15% or more. Because the effect of colour, independent of size, was the subject of experiments, mass and standard length asymmetries between males within a pair were kept to less than 10% whenever possible. Since condition factor is a compound variable that is determined by both mass and standard length, equalizing these two variables also tended to equalize condition factor. The second priority was to maximize the asymmetry in colour scores between males. Pairings were made at random with respect to the remaining parameters, days to nest and bite score.

After I determined pairings, I chose one member of each pair at random and clipped a small portion of the second dorsal spine to facilitate identification. The other male of the pair was handled in an identical manner, except that the instrument used to clip marked males was only gently closed on, but not strongly enough to cut, the erected dorsal spine.

Males were netted within 15 s of each other in random order and placed simultaneously at opposite ends of the previously prepared neutral test arena. In early trials, a stopwatch was started upon subject introduction and the time to the first overt aggressive act (bite, nip, chase) and the identity of the fish that performed it (fight initiator) was noted. It soon became clear that males differed in the time taken to acclimate to introduction to the neutral test arena, thus affecting the reliability of the measurement of time to initiation. In later trials, the stopwatch was not started until the two males were in a line of sight and one of the two approached the other. The fight latency was defined as the time from first sight and approach by one male to the first overt aggressive act by either.

Territorial contests were monitored at half-hour intervals and allowed to proceed until a fight winner was determined. The winner of a territorial contest of this type is always readily identifiable as the individual swimming around the neutral test arena unmolested and digging vigorously in the sand of the nest dish. The loser often lingered in an upper corner of the aquarium or under a lip of the nesting dish out of the line of sight of the

winner. The fight was terminated as soon as the winner began to build a nest and the losing male failed to interfere. The fight duration was noted to the nearest half hour.

In early tests ($N=102$ males), I used subjects in only a single territorial encounter. In later trials ($N=68$ males), I paired all males in a group twice. After the first encounter, I changed the illumination treatment for all males and maintained them under this new illumination for a minimum of 4 days, during which the preliminary morphological, aggression score and colour score assessments were redone. Each male was paired with a second opponent, a winner determined and both males removed from further testing.

Prior fighting success increases the probability of a subsequent win and prior loss increases the probability of subsequent loss (Bakker & Sevenster 1983; Bakker 1986; Bakker et al. 1989). This effect probably persists for no more than 24 h, however (Bakker et al. 1989). Second trials were conducted at least 4 days after the first, and males were paired based on similar prior competitive success (winners with winners, losers with losers). Thus, subjects that were tested twice were never paired with the same opponent twice, and the second opponent had comparable competitive experience.

Statistical Analyses

Since all staged territorial encounters were between pairs of individuals, the effect of various phenotypic characters on competitive ability can be partitioned in two ways, (1) absolute and (2) relative. The analysis of absolute effects assumes that the probability a male will initiate and/or win an encounter is based on the absolute value of the variable, regardless of his opponent's value for that variable. The analysis of the relative effects assumes that the probability a male will initiate and/or win a contest depends on the male's expression of a variable relative to that of his opponent in a given encounter.

Comparisons between all individuals tested (absolute effects on competitive ability)

I used logistic regression analysis (/fstep option, default values; SPSS v.4.0; Norusis 1990) to identify the variable(s) associated with the probability that males will (1) initiate or (2) win staged

Table I. Relative quantum catches of the primary photopigment classes present in the three-spined stickleback retinal mosaic under each illumination treatment (see text for calculation formulas)

Illumination treatment	Photopigment classes	
	1 and 2	3
SM	0.726	0.274
(SM)L	0.501	0.499
W	0.353	0.647
L	0.183	0.817

Photopigment classes: 1: S, P435 nm; 2: M, P530 nm; 3: L, P605 nm.

territorial encounters. In the first analysis (initiate), the independent variables were standard length, mass, bite score, condition factor and colour score. In the second analysis (win), fight initiator was added to the previous variables.

Comparisons between individuals in a staged encounter (relative effects on competitive ability)

I used the binomial test (Siegel & Castellan 1988; with table-wide corrections for multiple comparisons; Rice 1989) to determine the probability that males with particular morphological and behavioural characteristics were more likely to initiate and/or win a staged territorial encounter.

RESULTS

Illumination Treatments

Because the spectral sensitivities of photopigments present in the stickleback retina are similar to human retinas (Lythgoe 1979), the colour of illumination treatments and of colour-manipulated sticklebacks perceived by humans and sticklebacks are likely to be similar. The illumination treatments differed in the relative distribution of photons (Fig. 1) absorbed by each of these photopigments. The (SM)L and W treatments nearly uniformly stimulated the three photopigment classes, and L and SM more differentially stimulated the two (Table I).

Bite scores and colour scores of subjects differed between treatments, presumably due to the colour of illumination (two-tailed Kruskal–Wallis one-way ANOVA by ranks, corrected for ties (Siegel

Table II. Mean ± SE and range of morphological and behavioural features of males used in statistical analyses (except where indicated)

	Illumination treatment				
	SM	(SM)L	W	L	All subjects[a]
Number of males	$N=54$	$N=60$	$N=66$	$N=58$	$N=170$
Standard length (cm)	5.296 (0.025)	5.240 (0.029)	5.211 (0.021)	5.338 (0.025)	5.253 (0.015)
	4.8–5.7	4.8–5.8	4.8–5.6	4.9–5.8	4.8–5.8
Mass (g)	1.810 (0.024)	1.684 (0.027)	1.777 (0.022)	1.762 (0.028)	1.745 (0.014)
	1.49–2.25	1.22–2.37	1.40–2.33	1.22–2.25	1.22–2.37
Condition factor	8.557 (0.080)	8.120 (0.109)	8.653 (0.073)	8.207 (0.100)	8.374 (0.053)
	7.244–9.938	5.971–10.833	7.489–10.643	5.971–9.690	5.971–10.833
Colour score[b]	3.981 (0.295)	4.233 (0.282)	3.348 (0.224)	4.397 (0.283)	3.766 (0.152)
	1–8	1–8	1–8	1–9	1–8
Bite score[c]	153.889 (14.802)	109.300 (13.027)[d]	174.106 (13.226)[d]	72.707 (8.505)	151.550 (7.894)
	1–380	0–393	2–379	0–233	0–380
Days to nest[d]	1.667 (0.171)	1.950 (0.143)	2.031 (0.213)	1.707 (0.128)	1.834 (0.106)
	1–7	1–6	1–7	1–6	1–7

[a]The values for individuals used in two treatments were averaged prior to inclusion (68 of 170 males).
[b]Square-root (X) transformed prior to logistic regression analysis.
[c]Square-root ($X+0.5$) transformed prior to logistic regression analysis.
[d]Omitted from logistic regression analysis.

& Castellan 1988); bite score: $H_{c\,3}=34.245$, $P<0.001$; colour score: $H_{c\,3}=8.051$, $P<0.05$, Table II). Bite scores were highest and colour scores lowest under W, followed in sequence by SM, (SM)L then L. The number of days to nest completion was unaffected by illumination treatment ($H_{c\,3}=5.649$, $P>0.10$).

Colour Assessments

Colour scores were generated by a single observer, so a measure of observer reliability was assessed to ensure the reliability and consistency of these estimates. Colour scores generated by the judge were often compared with the colour scores assigned to the same male sticklebacks by two additional independent judges. The agreement between the observer used here and the two independent judges was consistently high (Kendall's coefficient of concordance: $W=0.7$–0.9, $P<0.01$ for all events; Siegel & Castellan 1988; C. L. Baube, unpublished data), with colour scores rarely differing by more than two increments of the nine-increment scale.

I also assessed the temporal variability in subjects' colour scores. Colour scores assigned 2 days apart to the same 58 male sticklebacks were reasonably consistent (Spearman's rank order correlation: $r_{adj}=0.647$, $P<0.0001$; C. L. Baube,

unpublished data). The colour scores of only 16 males stayed the same. However, 90% of all males remained within two colour score increments and, of all males changing colour intensity over this period, an equal number became redder ($N=19$) as duller ($N=23$; Binomial test; $z=0.463$, $P>0.20$).

Staged Territorial Encounters

I used 170 subjects in 119 staged competitive interactions that resulted in a winner, as defined above (SM: $N=27$ pairs; (SM)L: $N=30$ pairs; W: $N=33$ pairs; L: $N=29$ pairs). The distributions of phenotypic and behavioural characters of all males used in tests are presented in Table II.

In nearly all of the 119 trials the asymmetry in standard length was less than 10% ($\bar{X} \pm \text{SE}= 3.20 \pm 0.373\%$, range=0.00–10.42, $N=119$). The asymmetry was greater than 10% in only one trial, and the longer fish was not at a competitive advantage, neither initiating nor winning. The mean mass asymmetry was $6.05 \pm 0.502\%$ (range=0.00–22.95). In 14 trials, the difference exceeded 10%, but heavier males initiated and won at random (Binomial tests, Siegel & Castellan 1988: initiated: $P=0.212$, $N=5$ trials; won: $P=0.395$, $N=6$ trials). The mean asymmetry in condition factor was $5.17 \pm 0.420\%$ (range= 0.00–22.95). In 14 trials one subject had a
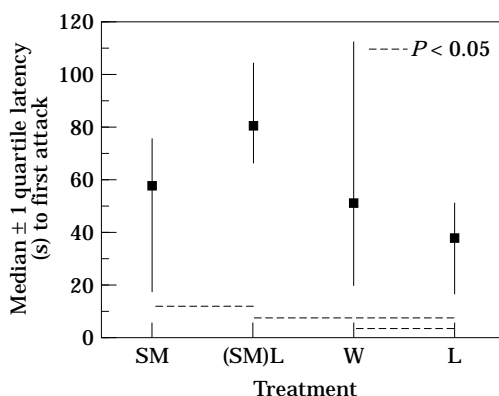
Figure 2. Median (± 1 quartile) fight latency (time (s) to first nip, bite or chase by either male) in trials illuminated by the treatment indicated. Data from treatments connected by a horizontal dashed line are significantly different *P*<0.025; two-tailed Wilcoxon Mann–Whitney tests.

condition factor 10% or greater than the other but these males initiated and won at random (initiated: *P*=0.395, *N*=6 trials; won: *P*=0.605, *N*=7 trials).

Spine clipping had no discernible effect on male behaviour patterns, because such males initiated (*P*=0.337) and won (*P*=0.166) territorial encounters as often as unclipped males.

The latency to the first aggressive behaviour (nip, chase or bite) was shorter in treatments predicted to disrupt the males' ability to detect the red of opponents. Fight latencies under SM and L illumination were similar to each other and significantly shorter ($H_{c\,3}$=12.518, *P*<0.01) than those under (SM)L and W (Fig. 2).

I found no relationship between illumination treatment and fight duration ($H_{c\,3}$=4.882, *P*>0.10). Under W and (SM)L, however, fight duration was inversely related to the difference in colour score between the participants (two-tailed Spearman's rank-order correlation, colour score D_i versus fight duration, corrected for ties, W: r_{adj}= − 0.480, *P*=0.007, *N*=33; (SM)L: r_{adj}= − 0.445, *P*=0.017, *N*=30), but not under SM and L (SM: r_{adj}= − 0.269, *P*=0.171, *N*=27; L: r_{adj}=0.231, *P*=0.222, *N*=29; Fig. 3). Thus, encounters involving males that were more closely matched for colour were longer in duration, but only under illumination where males can discriminate the intensity of their opponent's belly coloration.

Comparisons between all individuals tested (absolute effects on competitive ability)

I used forward step-wise multiple logistic regression analysis to identify the variable(s) most closely related to the probability of initiating and winning encounters. Colour score and bite score were transformed to normalized skewed distributions prior to analysis. I omitted bite score from W and (SM)L treatments analyses and days to nest completion for all treatments because these data were not normally distributed, even with transformation.

These analyses reveal that male colour score is the only variable consistently and significantly associated with the probability of initiating and winning a fight. Under W illumination, logistic regression models incorporating only colour score predict the probability of fight initiation (Wald χ_1^2=10.741, *P*=0.001) and marginally the probability of fight winning (χ_1^2=3.412, *P*=0.065). The logistic regression goodness-of-fit probability indicates that each model is appropriate for its data set (χ_{64}^2=67.154, *P*=0.370; χ_{64}^2=66.315, *P*=0.397). Under (SM)L illumination, the effect of colour score on fight initiation is similar to that seen under W illumination and the logistic regression is marginally significant (colour score: χ_1^2= 2.759, *P*=0.096; model: χ_{58}^2=60.079, *P*=0.400). Together with the variable fight initiator, colour score predicts fight winning under (SM)L illumination (colour score: χ_1^2=3.884, *P*=0.049; fight initiator: χ_1^2=12.007, *P*=0.0005; model: χ_{57}^2=59.542, *P*=0.383). The resulting logistic regression functions are shown in Fig. 4. In contrast, no variable or combination of variables creates significant logistic regression models for fight initiation or fight winning under SM or L illumination.

In three of four cases, the resulting logistic regression models seem to overestimate the probabilities associated with the brightest males (colour scores 8–9; Fig. 4b, c, f, g). Under W and (SM)L illumination, the brightest males tend to initiate and win fights disproportionately less frequently than suggested by the trend for males with colour scores 1–7. Recalculating logistic regressions (Fig. 4) with the data from males with colour scores 8 or 9 omitted (*N*=15) reconfirms the effect of male colour score on the probability of initiating (χ_1^2=8.553, *P*=0.003; model: χ_{61}^2=64.925, *P*=0.342) and winning (χ_1^2=5.628, *P*=0.018; model: χ_{61}^2=62.880, *P*=0.410) under W
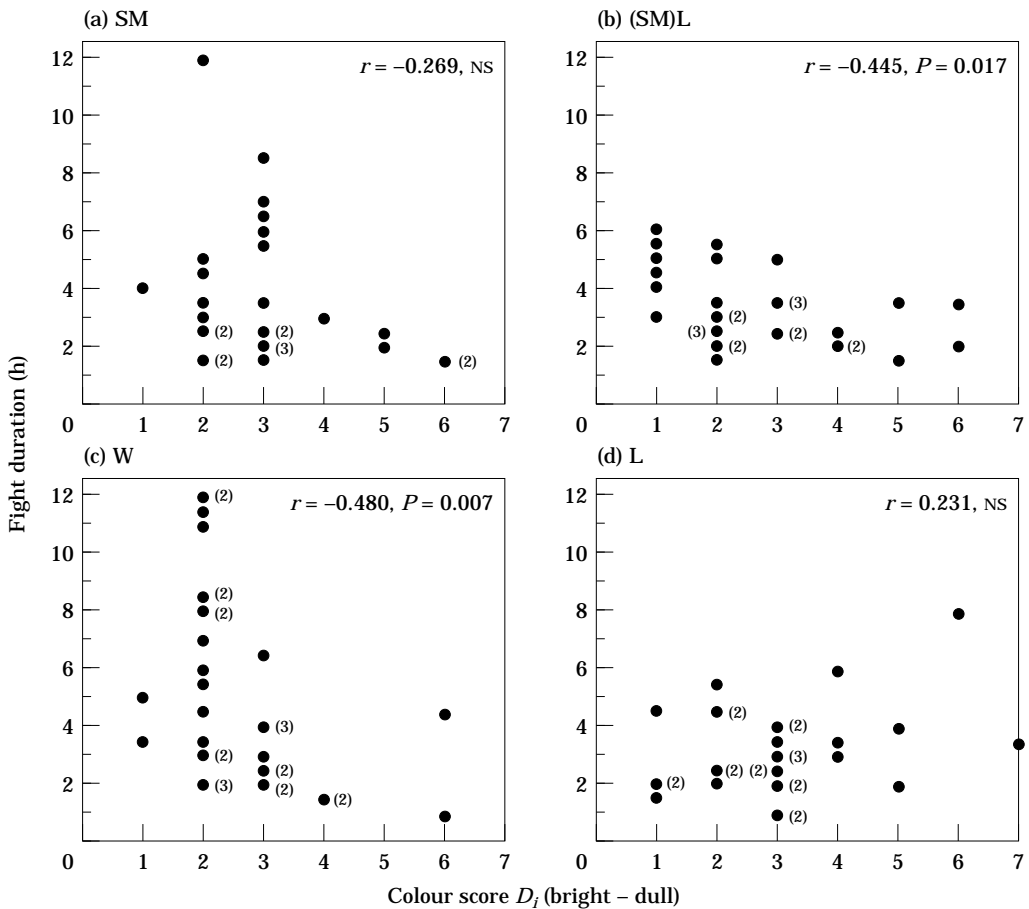
Figure 3. Relationship between coloration (colour score) asymmetry between competing males (bright–dull) and the duration of the territorial dominance encounter (fight duration) under illumination treatments: (a) SM, (b) (SM)L, (c) W, (d) L. Correlation coefficients and statistical probabilities are for two-tailed Spearman rank-order correlations.

and demonstrates a similar association under (SM)L (initiation: colour score: $\chi_1^2=7.057$, $P=0.008$; model: $\chi_{54}^2=56.076$, $P=0.397$; win: colour score: $\chi_1^2=6.565$, $P=0.010$; fight initiator: $\chi_1^2=11.042$, $P=0.001$; model: $\chi_{53}^2=55.830$, $P=0.369$). As with the complete data set, no variable or combination of variables creates significant logistic regression models for fight initiation or fight winning under SM or L illumination.
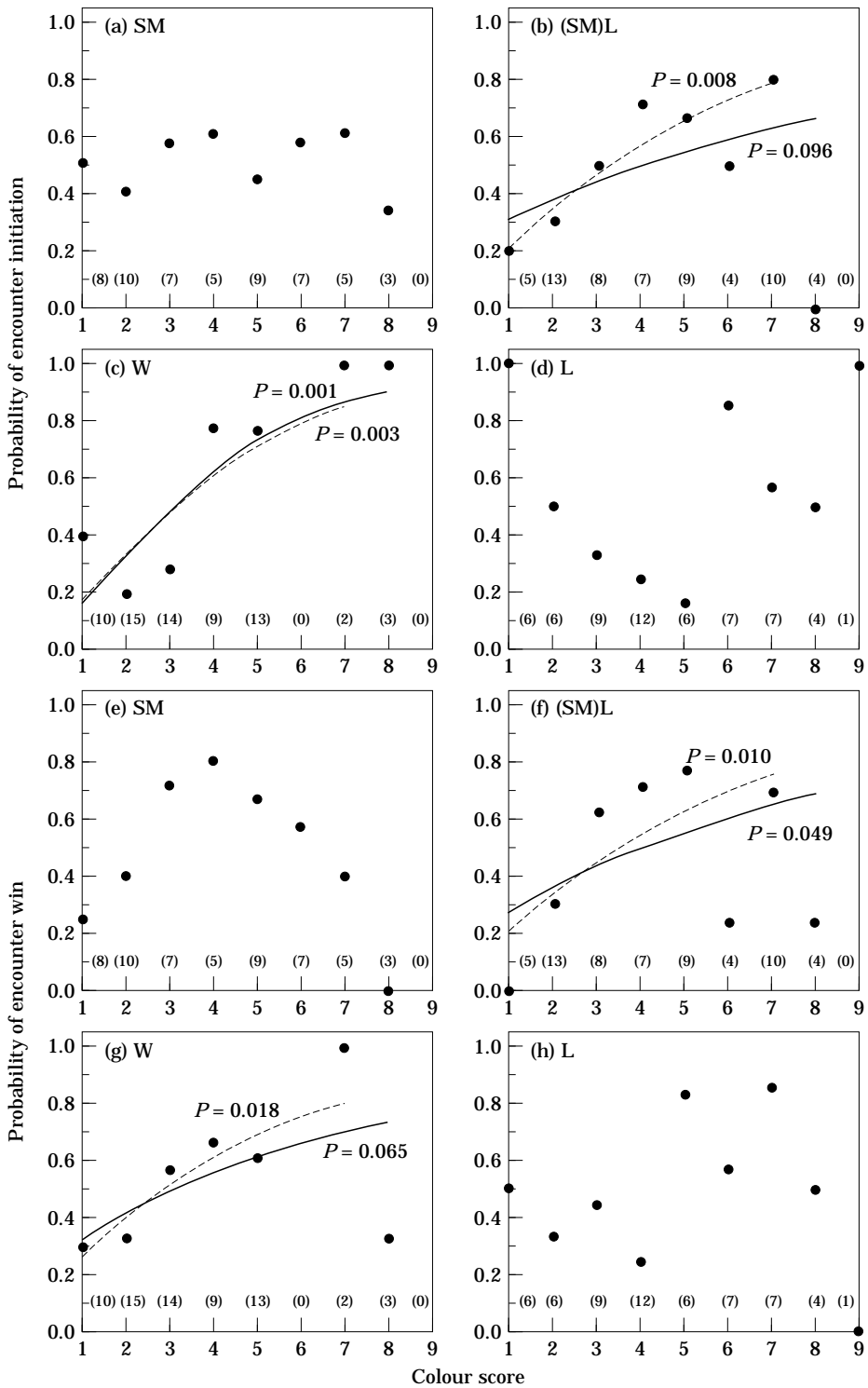
Comparisons between individuals in a staged encounter (relative effects on competitive ability)

For this analysis, I used just condition factor in place of its components, standard length and mass. The data omitted from the logistic

regression (days to nest and bite score; partial) were replaced to the list of independent variables analysed.

The individual initiating was also more likely to win a fight (one-tailed Binomial test; $z=2.20$, $N=119$, $P=0.014$).

As in the logistic regression analyses, colour score best predicts the likelihood of fight initiation and winning, but only under illumination in which males can discriminate the colour state of opponents (Table III). Under W, the brighter male in a trial was more likely to initiate (one-tailed Binomial test; $P=0.002$) and win ($P=0.009$). This effect is maintained under (SM)L (fight initiator: $P=0.026$; win: $P=0.026$), but

Figure: Panels (a)–(h) plot probability of encounter initiation (a–d) and probability of encounter win (e–h) against colour score (x axis, 1–9) for categories SM, (SM)L, W and L. Sample sizes in parentheses along the x axis.

(a) SM

(b) (SM)L — *P* = 0.008, *P* = 0.096
(5) (13) (8) (7) (9) (4) (10) (4) (0)

(c) W — *P* = 0.001, *P* = 0.003
(10) (15) (14) (9) (13) (0) (2) (3) (0)

(d) L
(6) (6) (9) (12) (6) (7) (7) (4) (1)

(e) SM
(8) (10) (7) (5) (9) (7) (5) (3) (0)

(f) (SM)L — *P* = 0.010, *P* = 0.049
(5) (13) (8) (7) (9) (4) (10) (4) (0)

(g) W — *P* = 0.018, *P* = 0.065
(10) (15) (14) (9) (13) (0) (2) (3) (0)

(h) L
(6) (6) (9) (12) (6) (7) (7) (4) (1)

Probability of encounter initiation

Probability of encounter win

Colour score

Table III. Statistical probabilities (ties omitted) associated with the likelihood of a staged territorial encounter being initiated or won by the male of the pair with the indicated morphological or behavioural attribute

	Illumination treatment (*P*)			
Variable	SM (*N*=27 pairs)	(SM)L (*N*=30 pairs)	W (*N*=33 pairs)	L (*N*=29 pairs)
Initiation				
Greater condition factor	0.361	0.354	0.182	0.250
Greater bite score	0.325	0.410	0.008*	0.357
Fewer days to nest completion	0.484	0.294	0.417	0.083
Higher colour score	0.325	0.026*	0.002*	0.322
Win				
Greater condition factor	0.212	0.091	0.182	0.322
Greater bite score	0.325	0.410	0.180	0.357
Fewer days to nest completion	0.484	0.434	0.488	0.417
Higher colour score	0.325	0.026*	0.009*	0.067
Fight initiator	0.349	0.009*	0.242	0.356

P-values for one-tailed binomial tests, all males (colour score 1–9) included.
*P<0.10 after correction for multiple comparisons.

eliminated under SM (initiate: *P*=0.325; win: *P*=0.325). Under L illumination, males initiated at random (*P*=0.322), but brighter males were marginally more likely to win (*P*=0.067). Under W, males with a higher bite score were more likely to initiate the encounter (*P*=0.008).

DISCUSSION

In nature, fights between three-spined stickleback males are common (Black & Wootton 1970; Kynard 1978) and only males successfully defending a territory realize significant reproductive success (with the exception of males that steal fertilizations; Kynard 1978). In this study, I used the probability of fight initiation as a measure of male willingness to establish and defend a territory and ability to deter attack. Territorial encounters in nature are brief and unlikely to continue until one combatant is exhausted (Chellappa & Huntingford 1989).

Here I allowed two males to fight until one gave up to provide an estimate of the opponent's relative physical condition in a context reflecting fighting skill, which is crucial to reproductive success.

Colour Score, Illumination and Dominance

By creating an experimental situation in which two males matched for size and prior competitive experience fight for a single neutral territory, I demonstrated a significant effect of male colour expression on competitive success. Under W, males with higher colour scores (brighter coloration) and higher bite scores were more likely to initiate staged territorial encounters; and brighter males were more likely to win (Table III, Fig. 4). The relationship seen between colour and the likelihood of initiation is not surprising, in light of similar observations by Rowland (1984) and McLennan & McPhail (1989a, 1990; but see Hosking 1996). Similarly, the observation that the

Figure 4. Relationship between male coloration (colour score) and the probability of (a–d) fight initiation and (e–h) fight win in staged territorial encounters. Numbers in parentheses indicate sample sizes. Lines and statistical probabilities are for logistic regression for males with colour scores 1–9 (solid) and 1–7 (dashed). Logistic functions $Y=1/[1+e^{-z}]$ where, (b) $z=-1.614+(0.814 \sqrt{colour\ score})$ (solid line), $z=-2.858+(1.571 \sqrt{colour\ score})$ (dashed line); (c) $z=-3.757+(2.138 \sqrt{colour\ score})$ (solid line), $z=-3.528+(1.994 \sqrt{colour\ score})$ (dashed line); (f) $z=-1.954+(0.985 \sqrt{colour\ score})$ (solid line), $z=-2.797+(1.496 \sqrt{colour\ score})$ (dashed line); (g) $z=-1.737+(0.987 \sqrt{colour\ score})$ (solid line), $z=-2.530+(1.498 \sqrt{colour\ score})$ (dashed line).

individual initiating a fight is also more likely to win is a general property of dyadic aggressive encounters and has been shown in three-spined sticklebacks by Bakker & Sevenster (1983) and FitzGerald & Kedney (1987).

Bakker & Sevenster (1983) and Bakker (1986, 1994) showed that brighter males of some Dutch populations are more likely to win staged encounters similar to those conducted here. Experiments with males from other populations in the Netherlands (Bakker 1994) and North America (FitzGerald & Kedney 1987; Rowland 1989) failed to demonstrate an effect of colour. Whoriskey & FitzGerald (1987), Rowland (1994) and Bakker (1994) provided several explanations for this discrepancy. First, colour effects are probably subtle and undetected by studies using small sample sizes and males that differ little in red coloration. The outcomes of dominance inter-actions may also be influenced by factors other than colour such as body size, age, aggressiveness and prior competitive success. The present study used large sample sizes and males that differed widely in colour. Moreover, I controlled for the other factors noted by Whoriskey & FitzGerald (1987), Bakker (1994), and Rowland (1989, 1994) that can influence fighting success. Thus, I tested the influence of male coloration on dominance success directly and under a variety of illumination manipulations.

Manipulation of illumination per se does not appear to adversely affect male competitive ability. (SM)L illumination, which largely maintains male red belly coloration, retains the relationships between male red coloration and increased likelihood of fight initiation and winning (Table III, Fig. 4). Similarly, the effects of male colour score on fight latency (Fig. 2) and fight duration (Fig. 3) are nearly identical to those seen under W illumination.

In contrast, illumination that disrupts visual functioning (i.e. SM or L) decreases fight latency (Fig. 2), eliminates the association between colour score difference and fight duration (Fig. 3) and eliminates the positive relationship between colour score and the probability of win (Table III, Fig. 4).

Interpretation of Signal Function of Red Belly Coloration

The often cited hypothesis that red belly color-ation stimulates or 'provokes' aggression fails to explain the data presented here. Under W and (SM)L illumination, males with greater absolute and relative colour scores are less, rather than more, likely to be attacked. Moreover, the latency to first attack should be shorter when the putative stimulatory stimulus is present than when it is absent. The pooled latencies to first attack under W and (SM)L (median=74.0 s, $N=31$) is significantly greater than under SM and L (42.0 s, $N=41$; one-tailed Wilcoxon Mann–Whitney U, corrected for ties: $z_{adj}=3.05$, $P=0.001$; Siegel & Castellan 1988), rather than less, as this hypothesis would predict. These data suggest that red belly coloration may stimulate more avoidance than aggression by rivals in the context of these experiments.

The results of the experiments reported here also argue against a hypothesis that brighter males may dominate dull ones because brighter males are physically superior and of higher overall viability. Although fighting success is a crude measure of physical quality, it is likely to have significant fitness consequences and thus is more likely to possess some measure of heritable genetic variability. The elimination of the competitive advantage of redder males under SM and L suggests that colour is not correlated with male condition as measured by dominance success (Table III, Fig. 4). Otherwise, the redder (hence more inherently viable) male should still win when red cues are masked. A test of the correlation between colour score and the condition factor (a measure of male relative condition; e.g. Bolger & Connolly 1989; Milinski & Bakker 1990) of the males used in my study is not significant ($r=-0.487$, $P=0.626$, $N=170$; see also Rowland 1994). Signals found in a variety of animal species such as guppies, *Poecilia reticulata* (Nicoletto 1991), a European three-spined stickleback *G. aculeatus* (Milinski & Bakker 1990; Frischknecht 1993), crested newts, *Triturus* spp. (Green 1991; Baker 1992) and the house finch, *Carpodacus mexicanus* (Hill 1991) reflect the possessor's physical condition, and may or may not have heritable genetic variability. This does not appear to be the case for male belly coloration in this population of three-spined sticklebacks.

Here I provide experimental evidence to suggest that red belly coloration of male sticklebacks acts as a threat display that discourages territorial intrusions. In an experiment in which males compete in pairs for a limited resource of equal value

to each, the intimidation hypothesis makes several predictions. First, duller red males should be less likely to initiate staged territorial encounters than brighter opponents since they are, on average, likely to be the more threatened or intimidated. Under W and (SM)L, males with higher colour scores (Fig. 4), and the redder in each pair (Table III), are more likely to initiate fights. In contrast, this relationship disappears under illumination in which the threat signal is eliminated (SM and L; Fig. 4, Table III). Second, the latency to first attack decreases under illumination where the colour threat is eliminated (Fig. 2). Finally, greater difference between signals of competitors results in a greater asymmetry in threat, hence a quicker resolution of the fight (Fig. 3; Bakker & Sevenster 1983; see also Robertson 1986).

This hypothesis also explains Tinbergen's (1948) observation that a red dummy placed in an artificial territory was more effective in preventing territory intrusions than a non-coloured dummy and why territorial males aborted more attacks directed towards a red-bellied dummy than a silver dummy (Rowland & Sevenster 1985).

In this study, masking of male red belly coloration eliminates male competitive ability, suggesting that male red belly coloration acts to intimidate rivals. This conclusion suggests a role for a visual signal similar to that observed in a variety of avian species (e.g. Peek 1972; Hansen & Rohwer 1986; Roskaft & Rohwer 1987; Marchetti 1993) in which natural or artificial increases in signal expression reduce the incidence of territory intrusions.

Male Coloration and the Possibility of 'Deception'

Under (SM)L and W, the brightest males (colour score 8–9) initiated and won encounters less often than duller males (colour score 1–7; Fig. 4). The statistically significant increase in competitive success by redder males (Fig. 4) is due to the behaviour patterns of intermediate-coloured males (colour score 3–7). In contrast, the reddest males are relatively less successful, even though the overall pattern favours redder males. This effect was independent of illumination, since these brightest males initiated and won less frequently than expected under nearly all illumination treatments.

Perhaps the physical condition of many of these extremely bright males is poorer than their signal indicates. Hosking (1996) reported that a subset of stickleback males placed on a low-quality (i.e. low caloric content) diet increased in colour score and decreased in condition over the first 2 weeks. This result suggests that restricted caloric intake may cause a short-term increase (a flush) of colour in some individuals, even though they are in relatively poor physical condition. This hypothesis is supported by the observation that the condition factors of intermediate-coloured males (colour score 6–7; 8.388 ± 0.127, $N=42$) are greater, rather than less, than those of the brightest males (colour score 8–9; 8.023 ± 0.149, $N=15$), although this difference is not significant (one-tailed Wilcoxon Mann–Whitney U: $z_{adj}=1.03$, $P=0.152$). Males in relatively poor physical condition (whether by parasite infection and/or lower nutritional status) and consequently with a lower expected future reproductive success, may invest more heavily in coloration and thus realize higher short-term mating success because of their heightened attractiveness to females (Milinski & Bakker 1990; Bakker & Milinski 1991; Baube et al. 1995). This tactic may provide little or no long-term reproductive success, however, because such males might not have sufficient energy to defend a territory long enough to raise offspring to independence. Whether a subset of stickleback males of poor physical condition could bluff their true condition by diverting their scarce nutritional resources to produce a signal that effectively intimidates rivals and attracts females warrants further study.

stimulus radiance measurements. All subjects were maintained, and experiments conducted, in approved facilities and in accordance with the Creighton University and Animal Behavior Society guidelines for the use of animals in research.

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