

RESEARCH ARTICLE

Role of the iridescent eye in stickleback female mate choice

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SUMMARY

Many vertebrates exhibit prominent body colours that are used in courtship and territorial communication. Some fishes also have an eye whose iris becomes iridescent during the mating season, as in the threespine stickleback. Behavioural studies in this species have focused on the redness of the throat/jaw as the primary determinant of female mate choice. Unlike the iridescent eye, however, the red throat/jaw is not present in all stickleback populations, suggesting that the colour of the eye may be equally important for female mate choice. Here, we used data on photoreceptors and environmental light to assess body conspicuousness and the colour contrast of courtship signals for stickleback populations living in a range of waters, from clear (mesotrophic) to red light shifted (dystrophic). This analysis indicated that the redness of the throat/jaw is expressed to enhance the contrast of the eye. To test the importance of eye colour as a courtship signal, we carried out mate choice experiments in which females were presented with identical videos of a courting male but for the colour of the eye and/or the throat/jaw. Females did not choose based on differences in throat/jaw redness between videos, but preferred males with the highest contrast between the eye and the throat/jaw. This result points to the blue iridescent eye as a primary courtship signal in stickleback female mate choice.

Key words: body reflection, nuptial signal, colour contrast, sexual selection, behaviour.

Received 2 January 2013; Accepted 2 April 2013

INTRODUCTION

Colour is a widespread signal used by males of many species for various types of communication including sexual display and territoriality (Endler et al., 2005; Doucet and Meadows, 2009). Such colour, which is commonly found in prominent areas of the body, like the wings, tails and crowns of birds (Hunt et al., 1998; Pryke et al., 2001; Endler et al., 2005; Loyau et al., 2007), the faces and flanks of fishes (Siebeck et al., 2010; Kline et al., 2011) and the dewlaps of lizards (Fleishman et al., 2011), constitutes, for the most part, an honest signal that indicates male fitness and status. In addition, several fishes have irises that are iridescent (Denton and Nicol, 1966), or that become so during the mating period, as is the case in the threespine stickleback (Albert et al., 2007; Pike et al., 2011). Although eye colour has been linked to social rank in some fish species (Miyai et al., 2011), the significance of this signal in mate choice remains unknown.

The threespine stickleback, *Gasterosteus aculeatus*, has long been a model of ecological and evolutionary research to explore the role of colour in sexual selection (Rowland, 1994; Smith et al., 2004; Boulcott and Braithwaite, 2007; Lewandowski and Boughman, 2008). During the breeding season, males from most stickleback populations develop red colour on the throat, jaw and anterior part of the belly, and iridescent blue irises (Albert et al., 2007; Pike et al., 2011). This ‘nuptial’ (courtship) colour is aimed at attracting reproductive females to deposit their eggs in the male’s nest, as females often (but not always) (see Rowland, 1994) prefer males with the most intense and largest red throat coloured area (Lewandowski and Boughman, 2008). Indeed, red colour is

positively correlated with physical condition, parasite resistance, courtship effort, nest defence during parental care, and mating success (see Smith et al., 2004; Albert et al., 2007). Some stickleback populations, however, do not express the red nuptial colour (Reimchen, 1989). As opposed to sticklebacks from clear waters, these animals have melanic (black) bodies and live in dystrophic lakes where the light spectrum is shifted to long (red) wavelengths (Reimchen, 1989; McDonald et al., 1995). In these populations, only the iridescent iris is present during the mating season.

Traditionally, stickleback literature has focused on the redness of the throat/jaw as the major nuptial signal driving female mate choice (Rowland, 1994; Lewandowski and Boughman, 2008). The omnipresence of the blue iris in all populations, and the lack of a red throat/jaw in some dystrophic lake populations, suggests that perhaps the iris plays at least as important a role as the throat/jaw in female mate choice. This hypothesis has been put forward in the context of colour contrast between the eye and the throat/jaw, whereby the redness of the latter may have evolved to enhance the contrast of the former (Rush et al., 2003; Rowe et al., 2004). Under such a scenario, it is expected that the redness would disappear if it did not enhance the contrast of the iris beyond that provided by the colour of the body. This is because the red nuptial colour is costly both energetically, in the procurement and redistribution of carotenoids within the body (Pike et al., 2011), and in promoting potentially deleterious encounters by enhancing conspicuousness to predators and rival males (Rowland, 1994).

In this study, we combined reflectance spectra of sticklebacks from different photic regimes with light measurements from the

environment and data on stickleback visual pigments and photoreceptor densities (Novales Flamarique et al., 2013) to evaluate colour contrast perceived by female sticklebacks when observing males from different populations. This analysis revealed the effect of body colour on conspicuousness, and the contrast of the two courtship signals: the iridescent eye and the red of the throat/jaw. Guided by these results, we performed female mate choice experiments to test whether the iridescent blue eye plays a role in stickleback female mate choice.

MATERIALS AND METHODS

Animals

Wild adult threespine stickleback (*G. aculeatus* L.) were obtained from seven locations in British Columbia, Canada: Doogan's Lake, Swan Lake, Mayer Lake, Mayer Pond, Drizzle Lake, Drizzle Pond and the Broughton Archipelago (coastal ocean) (Novales Flamarique et al., 2013). Of these locations, Mayer Lake and Drizzle Lake are dystrophic lakes, Swan Lake is a eutrophic lake, and the rest are mesotrophic water bodies. The dystrophic lakes have no transmission below 500 nm at 1 m depth and no light at 4 m depth (McDonald et al., 1995), whereas the mesotrophic systems are characterized by a full light spectrum in surface waters (320–750 nm) and peak transmission in the range 560–565 nm with depth (Novales Flamarique and Hawryshyn, 1993; McDonald and Hawryshyn, 1995; Novales Flamarique et al., 2013). Swan Lake exhibits a full light spectrum (320–750 nm) in surface waters but is long wavelength dominated with depth (McDonald and Hawryshyn, 1995; Novales Flamarique et al., 2013). During the reproductive season, stickleback nests are found near the shores of these water bodies spanning a ~0.1–1 m depth range.

Sticklebacks from the various locations were transported live to the aquatic facility of Simon Fraser University where reflectance measurements were obtained using the source waters. All holding and experimental procedures were approved by the Animal Care Committee of Simon Fraser University, which follows the guidelines set by the Canadian Council for Animal Care.

Reflectance measurements

Reflectance measurements were acquired from 10 reproductive males from both dystrophic and mesotrophic lakes. These measurements were conducted in the laboratory on live animals, anaesthetized using a solution of 25 mg l⁻¹ buffered MS-222, and positioned in an underwater holder that allowed repeatable and equivalent measurements between individuals (Novales Flamarique et al., 2007). Illumination was provided by a light guide connected to a tungsten-halogen source and directed at 45 deg to the normal to the surface measured. Radiance measurements were obtained using a USB-2000 spectroradiometer equipped with a 600 µm diameter input, 0.22 NA, liquid light guide (Ocean Optics, Dunedin, FL, USA), with the light guide oriented along the normal to the surface, as in other studies (Rowe et al., 2004). Reflectance measurements were generated by comparison with equivalent radiance measurements from a barium sulphate standard. Mean reflectance spectra were obtained for the following body parts: red throat/jaw, blue iris, melanic flank and non-melanic flank. Fish from dystrophic lakes were melanic (black) in colour while those from non-dystrophic systems were silvery on the belly and some combination of blue-green-brown colour on the side (flank) (Novales Flamarique et al., 2013).

Because of the limited spectrum emitted by the illumination source, reflectance measurements were restricted to the range 400–700 nm. Our measurements closely resembled those obtained by other authors in the human visible range (i.e. λ of 400–750 nm),

so we decided to extrapolate the curves into the ultraviolet range (λ: 300–400 nm) by mathematically modelling the mean curves published in this region of the spectrum for non-melanic fish flank, red throat/jaw and blue iris (Rush et al., 2003; Rowe et al., 2004). The melanic flank had no reflection in the ultraviolet. Reflectance measurements from several laboratories using a variety of clear water adult sticklebacks from Europe and North America (Rush et al., 2003; Rowe et al., 2004; Rick et al., 2004) have shown similar mean reflectance curves across the spectrum 300–750 nm. These extrapolations were performed to test whether absorbance by the UV cone had any effect on the visual contrast calculations (see 'Photon catch estimates and visual contrast calculations'), i.e. we aimed to assess whether this cone type could play a role in female mate choice or conspecific detection.

Photon catch estimates and visual contrast calculations

Threespine sticklebacks have four spectral types of cone that are maximally sensitive to ultraviolet (UV), short (S, blue), middle (M, green) or long (L, red) wavelength light (Novales Flamarique et al., 2013). We used the mean wavelength of maximum absorbance (λ_{max}) of each cone visual pigment in each population [see table 1 in Novales Flamarique et al. (Novales Flamarique et al., 2013)] to derive visual pigment absorbance spectra based on polynomial templates (Palacios et al., 1996). The use of template-derived absorbance spectra, as opposed to those obtained experimentally, ensures proper β-band representation, which is not always the case from microspectrophotometry records. The derived spectra served to compute absorbance using the equation: $\text{absorbance} = 1 - 10^{-\text{absorbance} \times S \times l}$, where S is the transverse specific density and l is the average outer segment length of each cone type in threespine stickleback [obtained from table 2 in Novales Flamarique et al. (Novales Flamarique et al., 2013)]. For a given spectral cone type, the absorbance term was calculated in the case of opsin conjugation to vitamin A₂ or A₁ chromophore, as indicated by visual pigment measurements from fish in reproductive and non-reproductive condition, respectively (see Novales Flamarique et al., 2013).

Photon catch estimates for each spectral cone type (UV, S, M or L) over the entire retina were calculated as follows:

$$P = \text{cone fraction} \int (\text{absorbance} \times \text{illumination} \times \text{reflectance}) d\lambda, \quad (1)$$

where P is the photon catch, cone fraction is the ratio of the mean density of a given spectral cone type to that of the sum of all cone types, illumination is the spectral irradiance or radiance of the water body in the relevant direction (sidewelling or downwelling), and reflectance (when relevant) is that of a fish body part, i.e. the throat/jaw, iris or flank. With the exception of the reflectance measurements, all the other parameters were derived from fig. 1 (spectral irradiance and related radiance measurements) and fig. 8 (cone densities) in Novales Flamarique et al. (Novales Flamarique et al., 2013).

The photon catch estimates were part of two types of calculation. First, they were used to evaluate the adaptation state of each cone mechanism (i.e. the neural pathway sub-served by a particular cone type) in a given visual scenario. Second, they were used to compute the contrast between two adjacent body parts or between the body flank and the background (sidewelling radiance). The adaptation state of each cone mechanism was computed as the photon catch (with the reflectance term equal to 1) of the associated cone type with respect to that of the cone type with the lowest photon catch. In the case of contrast calculations between two body parts, these

normalized values were derived from photon catches in which the adapting illumination was the horizontal radiance, as this is the background against which the female observes a courting male. In the case of contrast calculations between body flank and background (sidewelling radiance), the adapting illumination was the mean irradiance from scans in the downwelling and sidewelling orientations, as these are the directions along which sticklebacks normally view their conspecifics, thus constituting the 'veiling' illumination. The normalized photon catches were used as multiplicative factors to correct the photon catches from targets (body reflections) or background (sidewelling radiance) in the contrast calculations. To our knowledge, there are no available data on threespine stickleback lens transmission but the lens of a related species, the fifteen-spine stickleback (*Spinachia spinachia*), is highly transmissive in the UV to visible range (drop to 50% transmission at 320 nm) (Thorpe et al., 1993). As such, we did not consider this factor in the photon catch calculations.

Colour contrast between a body part and the water background (sidewelling radiance) or between two body parts was computed as the difference in photon catch between the two visual targets (or target and background) divided by the sum of photon catches from all cone types involved. There were seven sets of colour contrast calculations performed that compared adjacent visual features, as follows: (1) melanic flank and sidewelling light, (2) non-melanic flank and sidewelling light, (3) red throat/jaw and melanic flank, (4) red throat/jaw and non-melanic flank, (5) blue iris and melanic flank, (6) blue iris and non-melanic flank, and (7) blue iris and red throat/jaw. The comparisons were guided by the most likely contrast features that would be apparent to a female when observing the ritualized postures of the male stickleback during the mating dance, such as the vertical fanning of the nest and the oblique zig-zag movements (Rowland, 1994).

Comparisons 3–7 involved the analysis of nuptial features and, as such, the calculations were based on vitamin A2 visual pigment spectra, because these are the pigments present at the time of reproduction (Novales Flamarique et al., 2013). These comparisons revealed the contrast between different body parts under the various sidewelling adapting backgrounds [measured at 40 cm depth (Novales Flamarique et al., 2013), i.e. close to the mid-range of nest deposition] and as a function of whether the fish was melanic or non-melanic. Comparisons 1 and 2 revealed the relative conspicuousness of the melanic *versus* the non-melanic body against the various sidewelling backgrounds, for both vitamin A1- and vitamin A2-dominated retinas. We assumed that the source of light for body reflections was downwelling because the male often fans the nest or swims obliquely during courtship, thereby reflecting downwelling light. Contrast calculations involving an L–M cone interaction (see next paragraph) were restricted to comparisons 3–7 because such a term has been linked to the analysis of carotenoid content in courtship colour of sticklebacks (Pike et al., 2011).

Perceived contrasts were evaluated based on photon catch estimates for various combinations of targets and backgrounds following published interactions of cone mechanisms in fishes, reptiles and mammals (Rowe et al., 2004; Risner et al., 2006; Connaughton and Nelson, 2010). These interactions were based on the following photon catch sums: (1) all cone types, (2) middle wavelength (M) and long wavelength (L) cones minus ultraviolet (UV) and short wavelength (S) cones [i.e. (L+M)–(UV+S)], (3) S–L cones, (4) L–M cones, (5) L–M–S cones and (6) L–M–UV cones. The first interaction is a comparison of perceived luminance (summed output from all cones), while the second and third, removing the UV cone contribution from the second, constitute the

main colour information channels present in humans, which are also assumed to occur in sticklebacks (Rowe et al., 2004). The remaining interactions have a basis in the retinas of other fishes (Connaughton and Nelson, 2010) and the L–M interaction is also assumed to occur in sticklebacks (Pike et al., 2011).

Behavioural experiments

Reproductive female sticklebacks from Swan Lake (total $N=149$) were used in experiments designed to test the role of the male's colour contrast between body parts (e.g. iris and throat/jaw) or between body parts and background (e.g. iris and background), in female mate choice preference. A role for iris–throat/jaw contrast in mate selection of sticklebacks has been hypothesized previously (Rush et al., 2003; Rowe et al., 2004) but never tested, and our mathematical estimates and fish colour observations suggested that such contrast could be of importance to Swan Lake fish.

These experiments were carried out at the Nature Conservancy House of Swan Lake using water collected from the lake. Live females were caught using minnow traps and screened for responsiveness to a video showing a male performing the courting dance. Responsive females were left to acclimate to the holding aquarium and tested for mate preference over the next 2–3 days. The experimental set-up consisted of a 40 l aquarium with a line painted across its bottom surface bisecting it into two equal halves. Three of the four aquarium side walls were covered with black mat tape; the remaining side had a computer screen placed immediately next to it, on the outside. The screen was divided into two equal halves, each projecting identical looping videos of a reproductive male performing the ritual mating dance near its nest. The dividing line on the screen was matched to that on the aquarium bottom surface such that the two videos projected onto corresponding halves of the aquarium. The aquarium was filmed from above using a digital camera (Canon FS10).

Each experiment consisted of placing a reproductive, gravid female in the aquarium and filming its movements for 15 min following 5 min of acclimation. The female was simultaneously exposed to the two videos in which only the colour of the throat/jaw (including the opercula) and/or iris had been digitally manipulated using Adobe Photoshop. The videos were 11 s long and re-looped for the duration of the experiment. To avoid spatial bias in female choice, the two videos swapped sides every other experiment. Fourteen videos containing different combinations of iris and throat/jaw colours were produced and two of them paired at a time for a given experiment. The pairings were arranged to have an overall range of contrasts between iris and jaw/throats and between throats that resembled those found in nature. In total, seven video combinations were tested and between 21 and 22 females were used for each. Each female was used only once.

The spectral emissions of the iris, throat/jaw, flank and background (average of three locations) on each video were measured from the computer screen, through the glass aquarium wall, using the USB-2000 spectroradiometer and the liquid light guide. None of these emissions contained UV wavelengths. Perceived contrast was calculated between the following visual targets: (1) iris and throat/jaw per video, (2) iris and flank per video, (3) iris and background per video, (4) throat and flank per video, (5) throat and background per video, (6) irises between videos and (7) throats between videos. These contrasts were computed from photon catches (corrected for veiling light in the aquarium and with the reflectance term set to 1) using the cone interactions described previously. The range of contrasts between eye and throat/jaw and between throat and flank from the videos was 0.004–0.89 and

0.005–0.98, respectively. For the L–M and S–L cone interactions, which were statistically significantly correlated with female choice (see Results), these ranges were: 0.006–0.56 (L–M, iris to throat/jaw contrast), 0.015–0.95 (S–L, iris to throat/jaw contrast), 0.31–0.58 (L–M, throat to flank contrast) and 0.27–0.98 (S–L, throat to flank contrast). The range of contrasts between eye and throat/jaw and between throat and flank computed from live stickleback measurements was 0.005–0.85 and 0.003–0.81, respectively. For the L–M and S–L cone interactions, these contrasts were: 0.005–0.61 (L–M, iris to throat/jaw contrast), 0.05–0.72 (S–L, iris to throat/jaw contrast), 0.13–0.80 (L–M, throat to flank contrast) and 0.22–0.84 (S–L, throat to flank contrast). Thus, the contrasts computed from the videos were mostly within the ranges predicted for sticklebacks in nature, and the contrasts for the two nuptial features (i.e. eye to throat/jaw and throat/jaw to flank) spanned similar ranges (<3% difference overall from all cone interactions). These comparisons suggest that the characteristics of nuptial features in the videos would not have led to an inherent bias toward a particular feature by the female. Female interest in a given combination of parameters (e.g. iris–throat/jaw) was determined by the average length of time that a female faced toward a video per visit. We then computed the difference in mean female visit time between videos per experiment as a measure of female preference.

The data obtained on differences in video contrast and differences in mean female visit time were not normally distributed, even after multiple transformations, so parametric tests could not be used in the analysis. As a result, the analysis was based on a non-parametric test (Kendall's rank correlation) performed on the ranked values of the data. In the analysis of iris–throat/jaw combinations, for instance, the experiment with the smallest difference in colour contrast between iris and throat/jaw between videos was ranked '1' and the experiment with the greatest difference was ranked '7', with intermediate contrasts between videos assuming corresponding values within that range. Likewise, the lowest mean female visit time difference between videos was ranked '1' and the highest was ranked '7'.

RESULTS

Fish reflectance

The sticklebacks inhabiting dystrophic lakes had melanic (black) bodies and exhibited little to no red nuptial colour (Novales Flamarique et al., 2013). Similar to fish from other water bodies, however, melanic sticklebacks had a blue iridescent iris during the mating season. The iris reflectance was the spectral complement of the red from the throat/jaw exhibited by fish from non-dystrophic systems (Fig. 1). As has been previously observed in other stickleback populations (Reimchen, 1989), the area of red colour was most extensive for sticklebacks from the clearest freshwater bodies (e.g. Doogan's Lake) where the throat, jaw (including the opercula) and belly (including the pectoral fins) were often prominently coloured (Novales Flamarique et al., 2013). The typical flank of non-melanic fish had highest reflectance in the long wavelengths whereas that of melanic fish showed very little reflectance (Fig. 1).

Visual contrast of conspecifics

All cone interactions considered showed that the melanic flank would exhibit low contrast (<0.25) in the red light-shifted waters of dystrophic lakes when compared with other water types (contrasts >0.4) (Fig. 2A). The non-melanic flank would, conversely, show highest contrast (>0.5) in dystrophic waters and generally much less contrast (<0.4) in mesotrophic and eutrophic waters (Fig. 2B). Variability in these results arose primarily from differences in the ratio of downwelling to sidewelling light and the spectral profile of

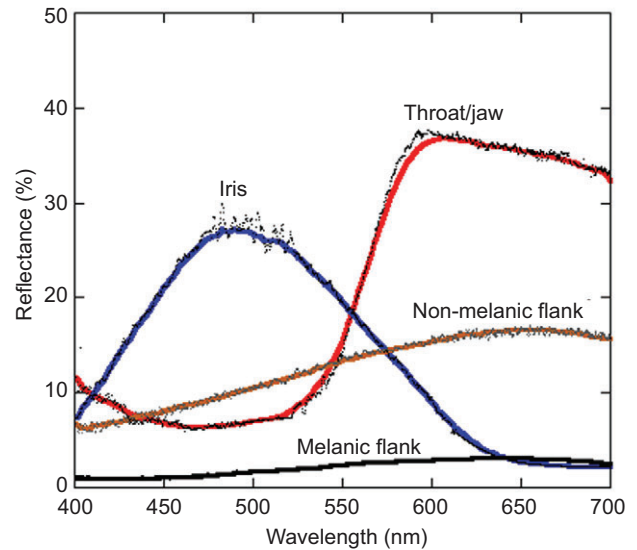


Fig. 1. Mean spectral reflectance of stickleback body parts ($N=10$). The iris showed a mean reflectance curve that was the spectral complement of that of the throat/jaw. The spectral trends of the melanic (black) and non-melanic flanks were similar, but the melanic flank had almost no reflectance.

transmitted light between water bodies (Novales Flamarique et al., 2013). Although higher contrasts were obtained for vitamin A2- as opposed to vitamin A1-based visual pigments, chromophore type altered the results by less than 0.05 (Fig. 2A,B). In general, computed contrasts were in line with our snorkelling observations: darker and lighter sticklebacks were harder to detect in dystrophic and mesotrophic systems, respectively. The correspondence between our human observations and the modelling results is supported by previous research in which the perception of stickleback throat colour hues was equal when human rankings were compared with those produced by a neural model based on predicted sensitivities of the S, M and L cone mechanisms of sticklebacks (Rowe et al., 2006).

Our modelling results also indicated that the red colour of the throat/jaw and the blue of the iris would exhibit more contrast against the melanic than the non-melanic flank, regardless of water type (Fig. 2C–F). The lowest contrasts found were for spectrally opponent mechanisms involving an L–M interaction. This is because the photon catch of the L and M cone types approximated each other for the various body parts, but especially for the iris, under various spectral regimes. Contrast magnitudes between the iris and throat/jaw were highest for the opponent mechanisms: S–L, L–M and L–M–S (Fig. 2G). It is noteworthy that the contribution of the UV cone to the above results was minor (e.g. compare L–M and L–M–UV contrasts), altering values by less than 0.025.

Female mate preference

When exposed to two videos differing only in the male's iris and/or throat/jaw colours, females spent significantly more time in front of the male with the highest iris to throat/jaw contrast. This was found for both the L–M (Kendall's $\tau=0.82$, $P=0.01$) and S–L (Kendall's $\tau=0.62$, $P=0.05$) cone interactions (Fig. 3A,B). In comparison, differences in throat/jaw colour between videos did not significantly affect female choice at $\alpha=0.05$ (Kendall's $\tau=0.55$, $P=0.09$; Fig. 3A,B). None of the other contrasting features (i.e. differences in eye colour, eye-to-flank, eye-to-background, throat-to-flank or throat-to-background) analysed between videos had any effect on female mate choice ($P>0.05$).

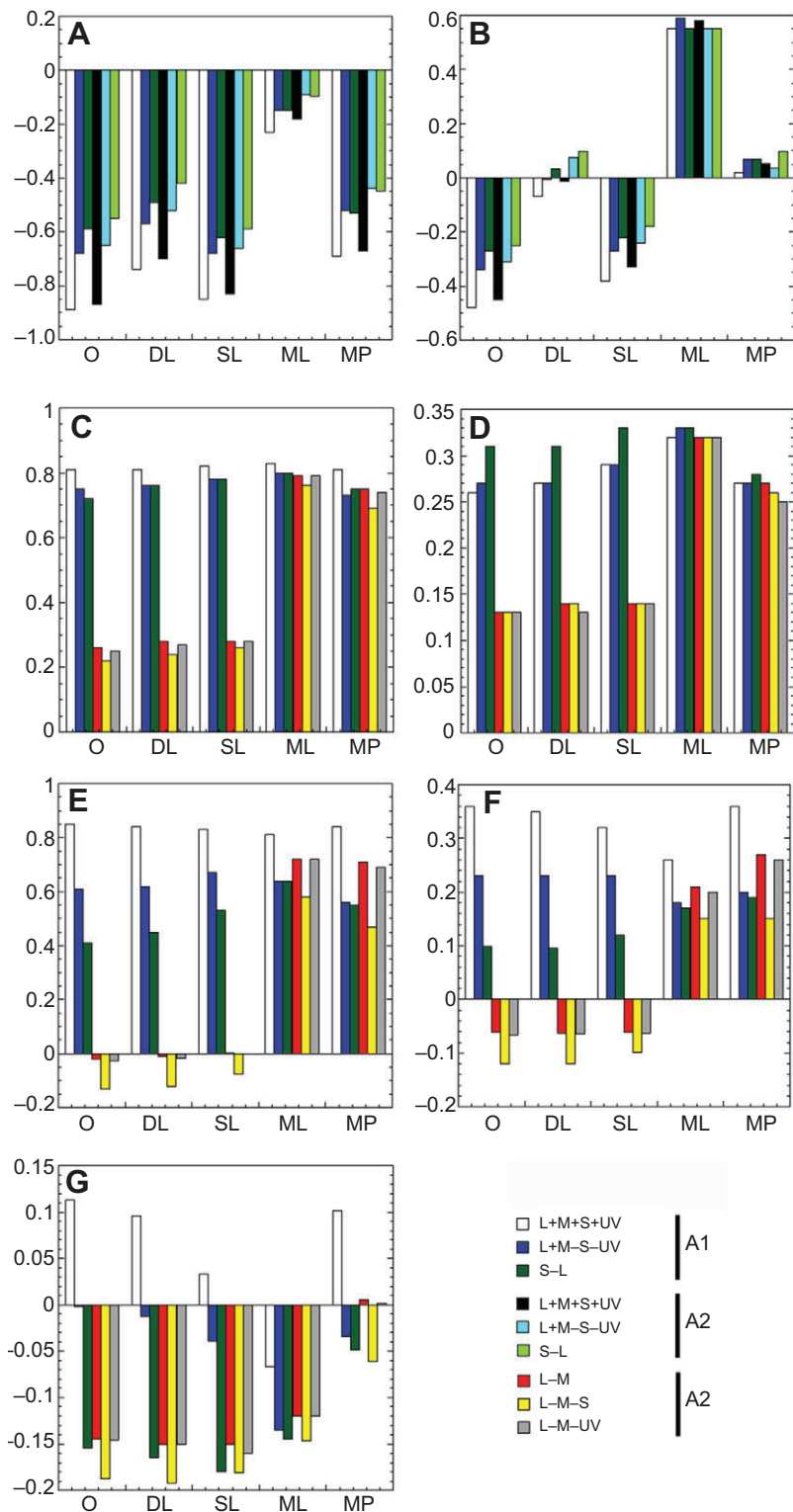


Fig. 2. Average contrasts between body parts and between body flank and background computed for various combinations of cone mechanisms, visual pigments and representative water types (see Novales Flamarique et al., 2013). The body parts or flank–background combinations involved in the computations are as follows: (A) melanic flank–sidewelling light; (B) non-melanic flank–sidewelling light; (C) red throat/jaw–melanic flank; (D) red throat/jaw–non-melanic flank; (E) blue iris–melanic flank; (F) blue iris–non-melanic flank; (G) blue iris–red throat/jaw. The cone mechanisms and visual pigment types considered (A1 or A2 chromophore based) are detailed in the key; UV, ultraviolet; S, short; M, middle; L, long wavelength visual pigment. Negative and positive contrasts reflect a bigger photon catch magnitude for the first or the second term being compared, respectively. O, ocean (Broughton Archipelago); DL, Doogan’s Lake; SL, Swan Lake; ML, Mayer Lake; MP, Mayer Pond.

DISCUSSION

Evolution of stickleback nuptial colour

Our modelling results suggest an interplay between two colour-dependent attributes, conspicuousness and camouflage, in the evolution of stickleback nuptial colour. Accordingly, the red throat/jaw exhibits high contrast on a melanic stickleback (Fig. 2C), but this nuptial feature would also disrupt its camouflage in dystrophic waters (Fig. 2A), perhaps inducing its disappearance over time (Reimchen, 1989). In comparison, the

red throat/jaw shows less contrast next to a non-melanic flank (Fig. 2D), which is the preferred colour for camouflage in mesotrophic and eutrophic waters (Fig. 2B). In fact, in the ocean and in Swan Lake, the contrast between the red throat/jaw and the flank is of similar or smaller magnitude to that between the flank and the spectral background (Fig. 2B,D). This suggests that the extra conspicuousness gained from a red throat/jaw for the purposes of sexual display does not significantly alter overall camouflage.

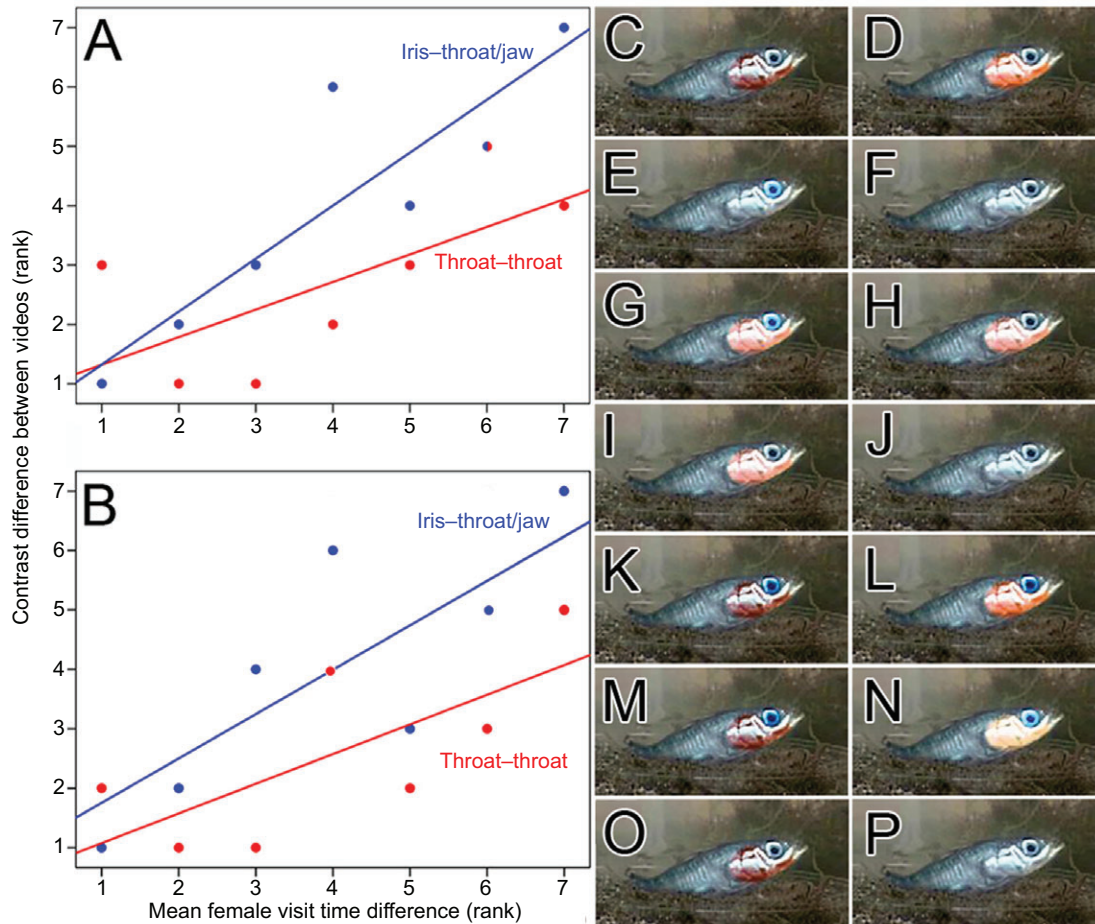


Fig. 3. Female choice between paired videos of a reproductive male performing the ritual mating dance in which only the colour of the eye and/or the throat/jaw varied between videos. (A,B) Correlations between female preference, expressed as the mean visit time difference between videos, and contrast difference of the iris–throat/jaw between videos (blue) or between throats between videos (red) for the L–M (A) or S–L (B) cone interactions. Only the correlations involving iris–throat/jaw contrasts were statistically significant at $\alpha=0.05$. (C–P) The same video frame, illustrating the male's iris and throat/jaw colours, for the seven paired combinations of videos presented to the female. Paired frames represent videos with the lowest (C,D) to highest (O,P) mean female visit time difference between videos. Video frames that are the same: C and O; F, J and P; H and I; K and M.

The blue iris also shows higher contrast against a melanic than a non-melanic flank (Fig. 2E,F) and its contrast against the red throat/jaw is lower than that against a melanic flank (Fig. 2E,G). This provides another reason for the loss of the red throat/jaw colour in melanic sticklebacks, i.e. the redness does not enhance the contrast of the iris beyond that provided by the melanic flank. In keeping the blue iris alone as a courtship feature, melanic males can provide information to the female about their quality at close range, as required during mating displays, while avoiding signal detection by predators located further away.

In non-dystrophic waters, however, the blue iris exhibits contrasts against the red throat/jaw that are similar to or improved over those against a non-melanic flank for the S–L and L–M cone interactions (Fig. 2F,G). Coincidentally, it is these neural pathways that may be linked with the analysis of concentration and relative proportion of carotenoids, the skin pigments that produce the nuptial colours (Pike et al., 2011). Thus, based on these neural processing pathways, non-melanic sticklebacks should retain the red throat/jaw as it provides additional contrast against the iris beyond that provided by the flank.

Our behavioural results support the above evolutionary scenario whereby the red throat/jaw would be maintained to enhance the contrast of the iris, the only nuptial feature present in all stickleback

populations. Females preferentially chose males based on differences in contrast between the iris and the throat/jaw (Fig. 3). This unexpected result points to the iris as the main nuptial signal in stickleback mating behaviour and illustrates the importance of colour contrast in female mate choice. Because other lower vertebrates exhibit adjacent courtship features of contrasting colours (e.g. Meyers et al., 2006), our results should be of relevance to understanding the evolution of mating signals and mating behaviour in a variety of taxa.

Potential roles of the UV cone in stickleback vision

Both our modelling and behavioural results indicate that, among single cones, S cones should play the major role in the colour vision of sticklebacks during mate selection or conspecific detection. UV cones, instead, are smaller and fewer in number (Novales Flamarique et al., 2013), leading to contrast calculations that are, overall, unaffected by UV cone input. It is only when computing throat/jaw and iris contrasts under the L–M–UV interaction that UV cone input alters the results by up to 0.023 (Fig. 7D–G). This is because, under some of the lighting conditions considered, the L and M cones had a similar photon catch for these body parts. Thus, an antagonistic interaction between L and M cone mechanisms, as previously documented in salmonid and

cyprinid fishes (e.g. Novales Flamarique and Hawryshyn, 1997; Risner et al., 2006), would leave the S and UV cones as the main determinants of the L–M–S and L–M–UV pathways, respectively. The dependence of the latter interaction on UV cone input is expected to increase during crepuscular periods, times when the ambient spectrum shifts to shorter wavelengths further approaching L and M photon catches (Novales Flamarique and Hawryshyn, 1997; Novales Flamarique and Hárosi, 2000). It is also at these times of the day that the relative sensitivity of the UV cone increases with respect to that of other cone types (Novales Flamarique et al., 1992; Novales Flamarique and Hawryshyn, 1997). Thus, our modelling results suggest that the UV cone, if exerting a meaningful visual role in sticklebacks, is specialized for target detection as part of a pathway involving L–M input. It is of relevance that the greatest variation in reflectance of the throat/jaw occurs in the UV wavelengths (Rowe et al., 2004) and that such reflections are correlated with physical condition in many animals including sticklebacks (Rick et al., 2004). It is therefore possible, though unlikely given the minute influence on the contrast calculations, that the UV cone could contribute to the discrimination of throat quality. The same conclusion was reached by Rowe et al. (Rowe et al., 2004) who showed, using independent analyses, that the inclusion of UV cones or UV wavelengths in the calculations did not alter discriminability of throat colours or overall detection capabilities beyond those provided by the S, M and L cones.

A more plausible role for the stickleback UV cone may be in prey detection. UV vision improves the foraging performance of some zooplanktivorous fishes because the crustaceans that they prey upon differentially absorb or scatter light in the UV and short wavelengths with respect to the water background (Browman et al., 1994; Novales Flamarique and Browman, 2001). Evidence for the role of a UV cone in enhancing the foraging performance of juvenile, zooplanktivorous, rainbow trout has recently been found (Novales Flamarique, 2013) and it is likely that the same target contrast enhancement mechanism, mediated by the UV cone, operates in sticklebacks. In support of this idea, recent estimates of stickleback foraging performance indicate increased activity when light backgrounds contain UV wavelengths (Rick et al., 2012).

ACKNOWLEDGEMENTS

We thank Lisa Grebinsky for generating the stickleback videos and for laboratory and field assistance, and Terry Morrison, director of Swan Lake Christmas Hill Nature Sanctuary, for the use of sticklebacks and facilities at the Nature Conservancy House of Swan Lake (Victoria, BC, Canada).

AUTHOR CONTRIBUTIONS

I.N.F. performed the reflectance measurements and mathematical modelling, prepared the figures and wrote the manuscript. C.B. performed the behavioural experiments and their analysis. C.L.C. carried out previous opsin expression work needed for the modelling. T.E.R. carried out previous light measurements from dystrophic lakes needed for the modelling. C.B., C.L.C. and T.E.R. commented on the manuscript.

COMPETING INTERESTS

No competing interests declared.

FUNDING

This research was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant [no. 238886] to I.N.F.

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