

Nuptial coloration varies with ambient light environment in a freshwater fish

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Abstract

Visual signals play a vital role in many animal communication systems. Signal design, however, often varies within species, raising evolutionarily important questions concerning the maintenance of phenotypic diversity. We analysed nuptial colour variation within and among nine populations of southern pygmy perch (*Nannoperca australis* Günther) along an environmental light gradient. Within populations, larger males were redder and blacker, and better-condition males were blacker. Among populations, red colour was positively correlated with the amount of orange-red light present, suggesting that males are likely optimizing signal conspicuousness by producing proportionally larger and redder patches in broad spectrum environments with more orange-red light. Signal contrast, in this regard, is maximized when red colour, appearing bright because of the prevalence of red wavelengths, is viewed against the water-column background. Together, our results are concordant with the sensory drive hypothesis; selection favours signal adaptations or signal plasticity to ensure communication efficacy is maximized in different light environments.

Introduction

Animals have evolved a remarkably diverse range of signals to communicate reproductive information. These signals are often the result of antagonistic sexual and natural selection: traits that are favoured in male–male competition or female mate choice can have associated costs that affect longevity and survivorship (Andersson, 1994). Although previous studies have explored this trade-off in terms of changing predation regimes (e.g. Endler, 1980) and physiological costs (e.g. Folstad & Karter, 1992), we know much less about how the contextual environment can influence the evolution of, and variability in, reproductive signals. However, as recent studies suggest, the contextual environment can

strongly influence signal diversity. For example, male great tits (*Parus major*) living in urban environments modify their songs to be heard above noisy conditions (Slabbekoorn & den Boer-Visser, 2006), and populations of *Anolis* lizards (*Anolis cristatellus*) inhabiting different light environments exhibit variability in the coloration of their dewlaps (throat fans) to ensure detection by conspecifics (Leal & Fleishman, 2004). The efficacy of these signals is dependent on the interplay among properties intrinsic to the signal itself, the environment through which it is transmitted, and the reception and processing abilities of the receiver (Endler, 1990).

The sensory drive hypothesis (Endler, 1992) proposes that natural selection will favour variations to signal design and sensory systems that maximize the effectiveness of communication given particular environmental conditions. This variation can be either adaptive or plastic and drives spatial and temporal signal variation. In visual communication systems, signal conspicuousness can be optimized through increasing achromatic brightness

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contrast (reflectance and transmission of signal differ strongly from that of the surrounding background) or chromatic contrast (signals rich in wavelengths that are poorly reflected by the background). These, in turn, can be tailored to match the perceptual sensitivities or biases of the individuals receiving the signals within a given environment (Endler, 1992, 1993). Identifying and understanding patterns in signal variation along environmental gradients is important in helping elucidate the particular selective pressures experienced by an organism and contributes evidence towards our understanding of the mechanisms through which communication operates.

Many fish communicate using visual signals. The perception of these signals is in part dependent on the physical properties of the ambient light spectra and the degree of attenuation, absorption and scattering of the transmission medium (Lythgoe, 1979; Reimchen, 1989). Clear waters are generally illuminated by broad-spectrum, high-intensity light. Objects reflecting this ambient light are viewed against a low-intensity blue/green-shifted background because of long wavelength attenuation over distance. In tannin and turbid waters, organic compounds and suspended particles attenuate shorter wavelengths of light such that objects are viewed against a red-shifted background of lower intensity. These effects are intensified with increasing depth or path length. Some fish, such as Lake Victoria haplochromine cichlids (Seehausen *et al.*, 1997; Maan *et al.*, 2006), maximize conspicuousness in broad spectrum environments by utilizing the most abundant wavelengths available in their signal design: blue and red coloration appear bright when viewed under blue and red light, respectively. Other species, such as bluefin killifish (*Lucania goodei*), display the reverse pattern with the proportion of blue and red colour morphs in a population inversely related to the amount of blue/UV and red light available in the environment. Against a blue/green-shifted background, red males will be more conspicuous than blue males because of colour contrast (Fuller, 2002). Likewise, in red-shifted environments, male sticklebacks (*Gasterosteus* spp.) display less red colour and thus maximize signal contrast because females are less sensitive to red light and exhibit less preference for red males in these environments (Boughman, 2001). Conversely, female guppies (*Poecilia reticulata*) from red-shifted environments exhibit a stronger preference for orange males, but male colour is not affected by the light environment (Endler & Houde, 1995).

In this study, we explored the relative importance of signal content and ambient environmental conditions in determining variation in male nuptial coloration within and across populations of an Australian freshwater fish, the southern pygmy perch, *Nannoperca australis*. First, we considered variation in nuptial colour relative to body size and condition to explore whether these visual signals convey information about male quality or status (signal

content). Next, we explored spatial variation in nuptial colour along a light gradient to test an aspect of the sensory drive hypothesis that states signal properties should vary predictably with changing environmental conditions to maximize signal efficacy.

Methods

Study species

Nannoperca australis is a small-bodied (< 80 mm) freshwater fish found throughout south-eastern Australia. This species inhabits perennial streams, large rivers, ephemeral creeks and wetlands. Within these habitats, fish are exposed to a range of environmental lighting conditions from clear to turbid and tannin-stained water. Males develop red and black nuptial coloration on their fins and body (Fig. 1a) from May to July prior to breeding at the end of the austral winter and throughout spring (July–November). Aquarium studies suggest that males aggressively defend territories of aquatic vegetation, with larger males being dominant over smaller males (Mitchell, 1976).

Sample collection

We collected 174 sexually mature *N. australis* from nine sites across Victoria, south-eastern Australia, using fyke nets and bait traps (Fig. 1b; Table 1). As male coloration might seasonally vary within a site, five sites were sampled on two occasions. There was no consistent directional pattern in colour change over time, and intraclass correlation coefficients (ICC) for sample averages (McGraw & Wong, 1996) indicated that they were temporally repeatable [ICC(C,2) range 0.49–0.87 for coloration measures (definitions below)]. We therefore pooled data to give an integrated measure of a site's male coloration characteristics.

Photography and image analysis

Colour data were derived from standardized digital photographs as this method is relatively quick, preserves spatial information and can accurately quantify colour patterns (reviewed in Stevens *et al.*, 2007). After capture, males were immediately photographed in a portable field 'dark box'. Males were placed in a small glass holding aquarium (8 × 4 × 1.5 cm) filled with distilled water, which in turn was mounted into a bracket 23 cm from the camera lens. The dark box (35 × 31 × 30 cm) interior was painted matte black and the roof white. Standardized photographs of fish were taken with a Nikon D80 camera (Nikon Inc., Tokyo, Japan) connected to a stereo macroflash orientated towards the dark box roof to avoid reflection off the glass. Photographic conditions were kept constant with regard to shutter speed (1/125 s), aperture (f16), ISO settings (ISO 100) and flash intensity

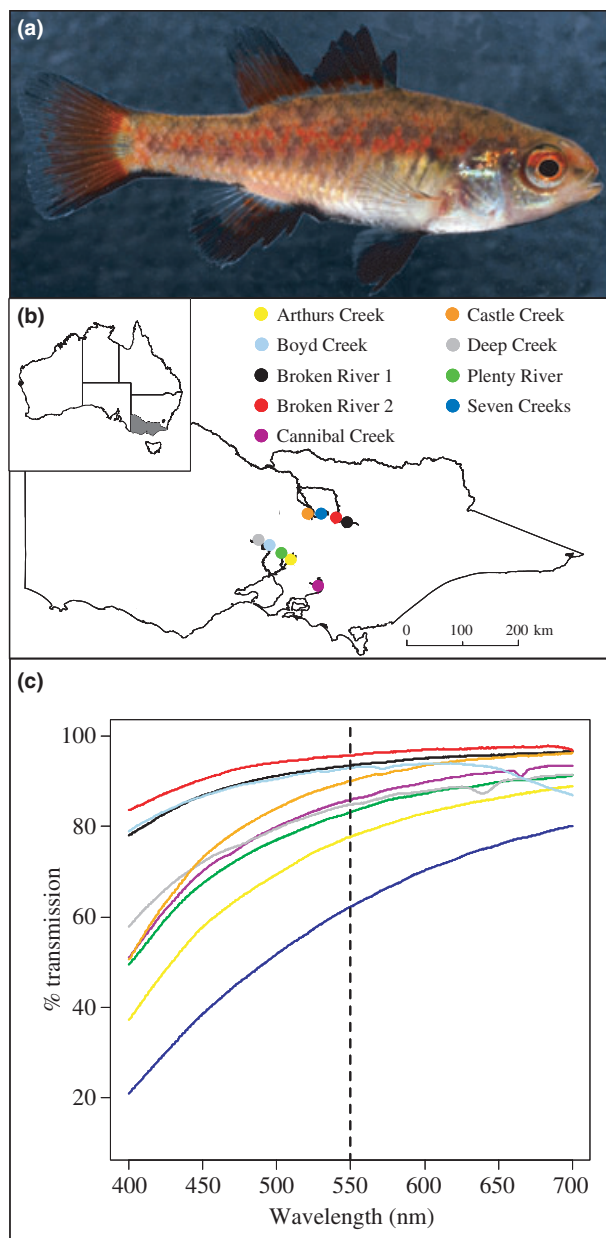


Fig. 1 (a) Male southern pygmy perch *Nannoperca australis*, (b) location map of the nine populations and (c) transmission spectral profiles for each site. Colours used in (c) correspond to population colours denoted in (b). The vertical dashed line in (c) indicates the 550-nm threshold used to calculate the orange integral.

(Svensson *et al.*, 2005). Furthermore, all photographs included a series of white, grey and black reflectance standards. As grey has a flat reflectance spectrum, the use of this reflectance standard facilitated linearization of the camera's response to changes in light intensity and equality of colour reflectance (Stevens *et al.*, 2007). Photographs were saved in RAW file format to ensure

no device-specific image alterations occurred (Stevens *et al.*, 2007). After photographing, fish weight (g) and total length (mm) were recorded, and the ordinary least squares (OLS) residuals from population-specific linear models of $\log(\text{weight}) \sim \log(\text{length})$ were used as an index of male condition.

Male photographs were analysed using Adobe Photoshop CS3 Extended 10.0 (Adobe Systems Inc., San Jose, CA, USA). RAW images were converted into 16-bit TIFF files and an $L^*a^*b^*$ colour space was employed [Commission International de l'Eclairage (CIE)]. CIE $L^*a^*b^*$ is a perceptually uniform and device-independent colour space that has previously been used in analyses of fish colour (e.g. Craig & Foote, 2001; Svensson *et al.*, 2006); L^* values correspond to the relative blackness of an image ranging from absolute black to absolute white, a^* values represent the 'redness' (balance between magenta and green) of an image and b^* values represent the 'yellowness' (balance between yellow and blue). Red and black components of nuptial coloration were analysed using their 'redness' (a^*) and 'blackness' (L^*) value, respectively. Redness measures were corrected by dividing a^* values from the fish with a^* values from the grey reflectance standard (these grey values themselves standardized by calculating the ratio of known grey reflectance to the recorded grey reflectance) in each photograph (Stevens *et al.*, 2007; Bergman & Beehner, 2008). There was less variation among a^* values for grey standards (CV = 0.5) compared to those of fish (CV = 1.9). Blackness measures did not need to be corrected as L^* channel was standardized from previous linearization.

Each fish was removed from the background image using the magnetic lasso tool, and average redness and blackness of the whole fish were measured using the histogram function. Dorsal, caudal, anal and pelvic fins were then selected (regions that develop significant nuptial colour), and total fin area and the proportion of fins red and black were estimated. The redness and blackness of these colour patches were then quantified.

Estimation of water transmission properties

Each site's water spectral properties were measured from a 1-L water sample collected at the time of fish capture and returned to the laboratory for analysis on a Cary 50 UV-Vis spectrophotometer (Varian Inc., Walnut Creek, CA, USA). Samples were immediately chilled and analysed within 3 days of collection. Samples were agitated to re-suspend settled particulate matter, and water was transferred to a 5-cm glass cuvette for analysis. A 5-cm cuvette was chosen as it had the longest path length available and roughly corresponds to the distance over which most male–male aggression and male–female courtship occurs (Mitchell, 1976). Although this is not a standard measure of light transmission, it does provide a relative estimate of environmental lighting conditions

Table 1 Summary of data used in this study.

Site	Sampling date (2007)	Longitude	Latitude	# Fish	Mean length (range) (mm)
Arthurs Creek	15 August	145°12'23"E	37°34'54"S	4	49.00 (43–58)
Boyd Creek	26 September and 30 October	144°53'42"E	37°23'20"S	34	50.47 (36–75)
Broken River site 1	9 November	146°06'28"E	36°58'33"S	19	55.32 (42–74)
Broken River site 2	28 August	146°01'43"E	36°58'53"S	15	68.40 (64–75)
Cannibal Creek	24 September and 8 October	145°44'16"E	38°03'57"S	13	52.92 (35–71)
Castle Creek	13 August and 23 September	145°35'09"E	36°51'58"S	31	42.53 (34–53)
Deep Creek	26 September	144°46'39"E	37°17'08"S	18	48.56 (30–67)
Plenty River	15 August and 9 October	145°07'36"E	37°29'01"S	17	46.77 (38–55)
Seven Creeks	27 August and 22 September	145°45'44"E	36°50'46"S	23	48.26 (36–66)

and facilitates among population comparisons. The transmission spectrum from 400 to 700 nm was recorded at 0.46-nm intervals and standardized against a distilled water spectral profile. The 'orange integral' (integral of 550–700 nm transmission) was calculated to measure the absolute amount of long light wavelengths available in an environment (Fig. 1c). Replicate orange integrals from five sites showed that this measure was repeatable through time [ICC(C,2) = 0.95], and therefore, they were averaged to produce one value per population.

Statistical analyses

Linear mixed-effects models were developed to investigate the relationships between each of the coloration properties (*fish redness*, *fish blackness*, *red fin proportion* or *black fin proportion*) and explanatory variables (*orange integral*, *length*, *condition* and *population*). For each coloration property model, *length*, *condition* and *orange integral* were treated as fixed effects, *population* treated as a grouping random effect (intercept) and the nine *length*, or *condition*, by coloration property slopes allowed to randomly vary among *populations*. Mixed-effects modelling allows for hierarchical structuring in the data: here, we assume that our populations are a random representation of all populations and within these are nested the observed data. This approach allows us to test for the effects of factors and covariates across populations while allowing for the intercept and slope of covariate by response relationships to randomly vary among populations (after Zuur *et al.*, 2009). Random effect structures were explored using restricted maximum likelihood estimation (REML) and the most parsimonious selected using Akaike's information criterion (AIC), corrected for small sample size (AICc). Candidate models were compared using Δ AICc (Burnham & Anderson, 2002). For all coloration property models, a random intercept (*population*) with fixed slopes for *length* and *condition* performed best, and this random effect structure was adopted in subsequent exploration of fixed effects.

We fitted 21 models of increasing fixed effect complexity to each of our coloration property data using maximum likelihood estimates of error (ML). *Orange*

integral, *length* and *condition* were centred to enable fitting of interaction terms among explanatory variables (Quinn & Keough, 2002), and *black fin* and *red fin proportion* data were arcsine-square-root-transformed to ensure homogeneity of errors. The most parsimonious models were selected using AICc and then re-analysed using REML to produce unbiased parameter estimates reported here (Zuur *et al.*, 2009).

The effect of inter-population differences in average *length* and *condition* on coloration properties and the relationship between *fin redness* and *fin blackness* (representing discrete colour patches found side by side) were analysed using simple linear regression. *Fin blackness* data were natural-log-transformed to meet model assumptions. All statistical analyses were conducted in R 2.4.1 (R Development Core Team, 2006) with linear mixed-effects models fitted using the nlme package (Pinheiro & Bates, 2000).

Results

Fish redness and red fin proportion

The most parsimonious model explaining variation in *fish redness* included the fixed effects *length* and *orange integral* (Table 2). Overall, larger fish were redder than smaller fish (Table 3), and there was no evidence to suggest that the strength of this relationship varied among populations (random slope and *orange integral* by *length* interaction non-significant). The average *fish redness* of a population was significantly and positively correlated with the amount of orange-red light (*orange integral*) (Fig. 2a; Table 3). We identified Seven Creeks as a potentially influential population with relatively low values of *orange integral* and *fish redness*, but its removal did not alter the analysis results (mixed-effects model: *orange integral* estimate 0.157, $t_6 = 2.67$, $P = 0.037$).

Red fin proportion was best explained by a model including just the predictor *orange integral* (Table 2), with both *length* ($t_{163} = 0.17$, $P = 0.87$) and *condition* ($t_{163} = 0.37$, $P = 0.71$) being non-significant. Fish inhabiting streams with more orange light had, on average, proportionally more of their fins covered in

Table 2 Results of model selection procedure for 21 combinations of explanatory variables fitted coloration property data. Shown are models with similar levels of support ($\Delta\text{AICc} < 2$, Burnham & Anderson, 2002).

Model	d.f.	AIC	AICc	ΔAICc
Fish redness				
<i>Orange integral</i> + <i>length</i>	5	720.90	721.26	0.00
<i>Orange integral</i> \times <i>length</i>	6	722.60	723.10	1.84
Red fin proportion				
<i>Orange integral</i>	4	1005.06	1005.30	0.00
<i>Orange integral</i> \times <i>condition</i>	6	1006.07	1006.57	1.27
<i>Orange integral</i> + <i>condition</i>	5	1006.93	1007.28	1.99
Fish blackness				
<i>Length</i> + <i>condition</i>	5	1474.71	1475.07	0.00
<i>Length</i> \times <i>condition</i>	6	1475.19	1475.69	0.63
Black fin proportion				
<i>Length</i> + <i>orange integral</i>	7	1260.94	1261.62	0.00
<i>orange integral</i> \times <i>condition</i>	8	1261.94	1262.82	1.20
<i>orange integral</i> + <i>orange integral</i> \times <i>length</i>				

AIC, Akaike's information criterion.

red pigmentation than those inhabiting streams with less orange light (Fig. 2b, Table 3). This relationship, however, broke down with the removal of the Seven Creeks population (mixed-effects model: orange integral estimate 0.140, $t_6 = 1.00$, $P = 0.36$). On an inter-population level, neither average *fish redness* nor average *red fin proportion* was related to average *length* (*fish redness*: $F_{1,6} = 0.59$, $P = 0.47$; *red fin proportion*: $F_{1,6} = 0.82$, $P = 0.40$) or average *condition* (*fish redness*: $F_{1,6} = 0.21$, $P = 0.67$; *red fin proportion*: $F_{1,6} = 1.01$, $P = 0.36$).

Fish blackness and black fin proportion

The best model explaining variation in *fish blackness* included the predictors *length* and *condition* (Table 2),

with bigger and better conditioned individuals being blacker (Table 3). The slope of these relationships did not differ among populations. *Black fin proportion* was best explained by a model including *length* and the interaction between *orange integral* and *condition* (Table 2). Bigger, and in general better conditioned individuals, had more of their fins covered in black pigmentation (Table 3). However, at very low *orange integral* levels (namely Sevens Creek), better conditioned individuals had relatively less black pigmentation on their fins. Among-population differences in average *length* and average *condition* did not correlate with differences in average *fish blackness* (*length*: $F_{1,6} = 3.22$, $P = 0.123$; *condition*: $F_{1,6} = 0.30$, $P = 0.60$) or *proportion fins black* (*length*: $F_{1,6} = 1.34$, $P = 0.29$; *condition*: $F_{1,6} = 0.01$, $P = 0.94$).

Fin redness vs. fin blackness

There was a significant, albeit weak (as based on r^2), negative relationship between *fin redness* and *fin blackness* ($F_{1,172} = 21.01$, $P < 0.001$; Fig. 3). Fish with redder (higher a^*) patches on their fins also had blacker (lower L^*) patches adjacent to them.

Discussion

We found that coloration patterns in male *N. australis* are strongly associated with relative environmental lighting conditions, body size and condition. Within individuals, adjacent colour patch properties potentially help optimize conspicuousness through increasing contrast. The results also suggest that within a population, nuptial coloration communicates information about male dominance status (i.e. body size) and quality (i.e. condition). Among populations, red colour varies predictably with changing light environment to maximize signal efficacy.

Table 3 Restricted maximum likelihood estimates and 95% confidence intervals of four linear mixed-effects models describing changes in coloration properties as a function of light environment, fish length and condition.

Parameter	Estimate (SE)*	t-value	d.f.	P-value	95% Confidence interval
Fish redness					
<i>Orange integral</i>	0.174 (0.022)	7.983	7	< 0.001	0.122, 0.225
<i>Length</i>	0.066 (0.015)	4.367	164	< 0.001	0.036, 0.096
Red fin proportion					
<i>Orange integral</i>	0.135 (0.054)	2.515	7	0.040	0.008, 0.263
Fish blackness					
<i>Length</i>	-0.77 (0.139)	5.566	163	< 0.001	-53.146, -25.237
<i>Condition</i>	-18.657 (6.618)	2.819	163	0.005	-31.725, -5.589
Black fin proportion					
<i>Orange integral</i>	-0.125 (0.344)	0.364	7	0.727	-0.938, 0.688
<i>Length</i>	0.323 (0.075)	4.320	162	< 0.001	0.175, 0.470
<i>Condition</i>	7.837 (3.586)	2.185	162	0.030	0.756, 14.918
<i>Orange integral</i> \times <i>condition</i>	0.927 (0.345)	2.686	162	0.008	0.245, 1.608

*Parameter estimates for centred variables.

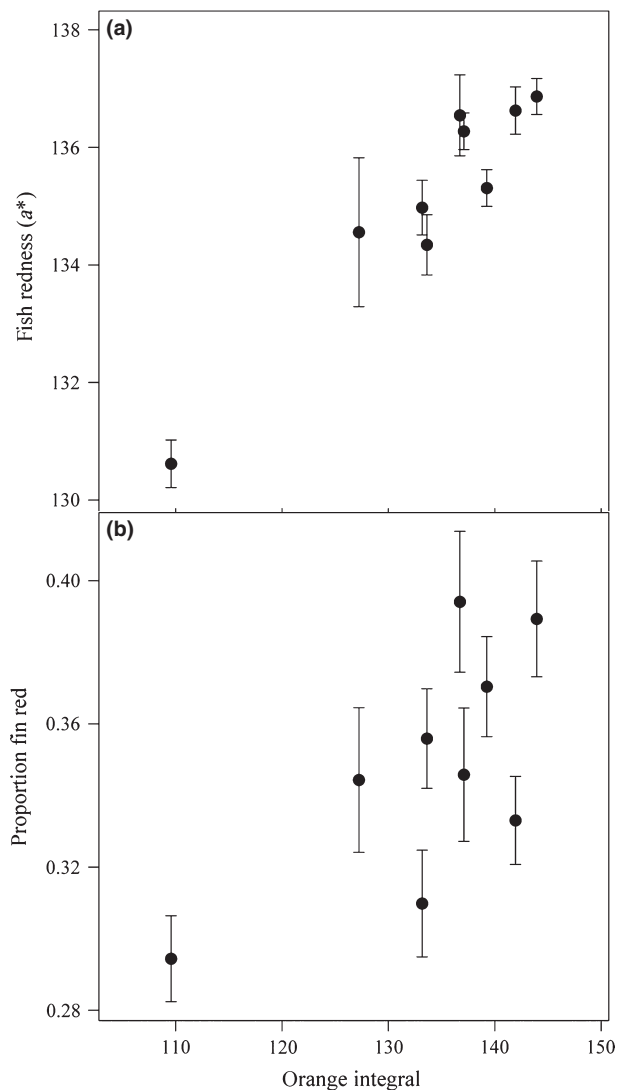


Fig. 2 Relationship between the orange integral and (a) mean (\pm SE) fish redness (a^*) and (b) mean (\pm SE) proportion of fins covered in red pigmentation for each population.

Previous coloration studies across taxa have found that colour can vary with the light environment via two mechanisms: chromatic contrast (e.g. Indian warblers *Phylloscopus Marchetti*, 1993) and brightness contrast (e.g. *Anolis* lizards Leal & Fleishman, 2004). Some studies have found that both factors are important in closely related species (e.g. dwarf *Bradypodion* chameleons Stuart-Fox *et al.*, 2007). Among-population variation in *N. australis* orange-red colour (redness and patch size) was positively related to the amount of red light available. This pattern is consistent with fish maximizing their chromatic contrast when viewed against a water-column background. As in earlier studies, we did not measure habitat spectral properties (e.g. Seehausen *et al.*,

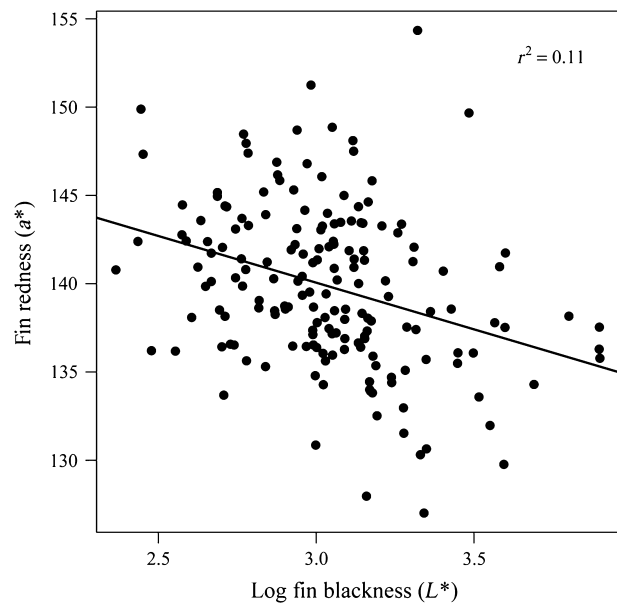


Fig. 3 Relationship between log-transformed fin blackness (L^*) and fin redness (a^*) for 174 individuals.

1997, 2008; Boughman, 2001; Fuller, 2002; Maan *et al.*, 2006), attributable in part to the relative importance of the water-column 'space-light' (ambient lighting environment) in aquatic systems (Lythgoe, 1979). Nonetheless, conspicuousness via chromatic contrast would likely be maintained against a habitat background as the male's red pigmentation and the green of aquatic vegetation in their territories are 'complementary', with few wavelengths in common (Endler, 1992).

Red nuptial colour was most strongly related to available orange-red light, but it is possible that fish are also responding to changes in other regions of the ambient spectra as the transmission of orange light in a stream positively correlated with total light and blue light transmission. Reimchen (1989) found a positive correlation between red throat pigmentation in threespine sticklebacks (*Gasterosteus aculeatus*) and the transmission of blue light ($\%_{T400}$ nm). He concluded that red males inhabiting lakes with broad spectrum downwelling light would be more conspicuous when viewed against a blue-green background, whereas black-throated males would be more conspicuous when viewed against tannin-stained, red-shifted backgrounds. Seehausen *et al.* (1997) also reported positive correlations between the blueness and redness of Lake Victoria haplochromine cichlids and the width of the transmission spectrum; males increased their signal conspicuousness in environments rich in blue and red light to enhance contrast against yellowish side-welling light.

Male *N. australis* inhabiting clear water streams (broad-spectrum and high-intensity light) should appear conspicuous because the available red light increases patch

brightness (red coloration appears brighter in redder light Endler, 1992), which in turn maximizes chromatic contrast against a blue-green-shifted water-column background. As the water becomes more turbid or tannin-stained (such as occurs in Sevens Creek), the available light spectrum and intensity diminish and red nuptial coloration appears duller and no longer contrasts against a now red-shifted water-column background. In these low-light environments, black colour, which is conspicuous in all lighting conditions (Endler, 1992, 1993), may become a more efficient signal and be employed by males.

Although not tested in this study, it is important to acknowledge that differential predation regimes and population phylogeny can influence spatial variation in communication signals. For example, the presence of an acoustically orientating parasitoid fly resulted in the localized loss of song in male field crickets (*Teleogryllus oceanicus*) (Zuk *et al.*, 2006), and bark beetles (*Ips pini*) spatially and temporally varied the chemistry of, and their preference for, aggregating pheromones depending on the type and abundance of eavesdropping predators present (Raffa *et al.*, 2007). Likewise, spatial biases in visual sensitivities (Seehausen *et al.*, 2008), mate preferences (Boughman, 2001) or colour expression (Lewandowski & Boughman, 2008) may be genetically controlled, resulting in phylogenetic patterns of coloration. However, in *N. australis*, we believe that predation and population phylogeny are unlikely to completely override the influence of lighting environment on coloration. Firstly, the suite of sympatric piscivorous species to which *N. australis* is exposed varied among sites but was not correlated with red coloration (qualitatively based on the presence of piscivorous fish at time of sampling, J. Morrongiello, unpublished data). Secondly, despite the nine populations sampled spanning different drainage basins (Fig. 1b) and this species displaying localized and large-scale genetic differentiation (Hammer, 2001; Cook *et al.*, 2007), spatial patterns in red coloration were not related to geographic separation. Future studies, however, would do well to consider both these possibilities in more detail.

Within individuals, the conspicuousness of a colour patch is dependent not only on properties intrinsic to the patch itself, but on those of the entire colour pattern (Endler & Mielke, 2005). We found that males with redder fin patches also had blacker fin patches nearby, and this enhances conspicuousness as patch contrast is maximized when those adjacent vary greatly in their brightness (total reflectance) or chroma (saturation) (Lythgoe, 1979; Endler, 1990). Within populations, larger males were both redder and blacker, and this is consistent with other studies that show larger or more dominant males generally display more conspicuous colour signals (reviewed in Berglund *et al.*, 1996). Signals that convey information regarding status or male quality are likely to be 'honest': trait variation is dependent on

trade-offs between sexual signalling and physiological demands (Milinski & Bakker, 1990) or social enforcement (Candolin, 1999). Although size-colour variation may reflect an underlying link with fish age or maturity, this is unlikely as all individuals were reproductively mature, the majority of *N. australis* spawn after their first year (Humphries, 1995) and length–frequency histograms showed no clear cohort structure within populations.

Average male size and condition differed markedly among populations, but average coloration measures did not vary concordantly. This suggests that spatial variation in red colour is primarily a response to changes in the light environment rather than differences in male size or condition-related effects. Variation in black coloration properties, however, was only related to the within-population variables body size and condition. As black is highly conspicuous in all light environments (Endler, 1992, 1993), varying its expression with changes in the light environment should not alter signal efficacy. Interestingly, only black coloration properties were correlated with condition. These results reinforce conclusions drawn from size data, indicating that black colour plays an important role in honest signalling of status or quality among males within a population.

In conclusion, our study supports an important aspect of the sensory drive hypothesis. Specifically, male *N. australis* predictably vary their red nuptial colour with the light environment to maximize signal efficacy through optimizing patch brightness when red light is plentiful, which in turn optimizes contrast when viewed against a water-column background. Further work is needed to explore whether this pattern is an adaptive or plastic response to environmental variation. Within-population coloration patterns suggest that both red and black nuptial colours are signals of dominance and condition. Taken together, these findings contribute to our understanding of the importance of signal efficacy in reproductive systems under variable environmental conditions.

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