

RESEARCH ARTICLE

Costs of colour change in fish: food intake and behavioural decisions

Gwendolen M. Rodgers^{1,*}, Nicholas W. Gladman², Hannah F. Corless¹ and Lesley J. Morrell²

¹Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, UK and ²Department of Biological Sciences, University of Hull, Hull HU6 7RX, UK

*Author for correspondence (groddgers@mnhn.fr)

SUMMARY

Many animals, particularly reptiles, amphibians, fish and cephalopods, have the ability to change their body colour, for functions including thermoregulation, signalling and predator avoidance. Many fish plastically darken their body colouration in response to dark visual backgrounds, and this functions to reduce predation risk. Here, we tested the hypotheses that colour change in fish (1) carries with it an energetic cost and (2) affects subsequent shoal and habitat choice decisions. We demonstrate that guppies (*Poecilia reticulata*) change colour in response to dark and light visual backgrounds, and that doing so carries an energetic cost in terms of food consumption. By increasing food intake, however, guppies are able to maintain growth rates and meet the energetic costs of changing colour. Following colour change, fish preferentially choose habitats and shoals that match their own body colouration, and maximise crypsis, thus avoiding the need for further colour change but also potentially paying an opportunity cost associated with restriction to particular habitats and social associates. Thus, colour change to match the background is complemented by behavioural strategies, which should act to maximise fitness in variable environments.

Key words: physiological colour change, morphological colour change, phenotypic plasticity, camouflage, background matching.

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INTRODUCTION

Animals such as reptiles, amphibians, fish and cephalopods can change their body colour for functions such as photoprotection, thermoregulation, social signalling and predator avoidance (reviewed in Sugimoto, 2002; Stuart-Fox and Moussalli, 2009; Leclercq et al., 2010). One way in which animals can avoid predators is by altering their colouration to become cryptic against the visual background (Ruxton et al., 2004). Classic examples include the rapid and dynamic background matching of octopuses (Hanlon et al., 1999; Hanlon, 2007; Hanlon et al., 2009) and chameleons (Stuart-Fox and Moussalli, 2009). Many fish darken their body colouration in response to dark visual backgrounds (Sugimoto, 2002; Mills and Patterson, 2009; Leclercq et al., 2010), which functions to facilitate predator avoidance and reduce predation risk (Sumner, 1935a; Sumner, 1935b; Whiteley et al., 2011), and is a plastic and reversible change (Sugimoto, 2002; Leclercq et al., 2010).

Body colour darkening in fish is associated with reversible responses of melanophores (Sugimoto, 2002; Mills and Patterson, 2009). Physiological colour change (controlled by the sympathetic nervous system) (Burton, 2008) occurs over short time scales (minutes to days) and involves two hormones: α -melanocyte stimulating hormone (α -MSH) and melanin-concentrating hormone (MCH). α -MSH causes melanosomes to disperse, making the fish appear darker, while MCH causes melanosomes to aggregate, lightening the appearance of the fish (Logan et al., 2006; Mills and Patterson, 2009). Over longer periods (days to weeks) the action of these hormones alters the morphology, density and distribution of melanophores in the dermal cells (Leclercq et al., 2010), known as morphological colour change (Sugimoto, 2002). Here, we will use ‘colour change’ as a general term encompassing either or both physiological and

morphological colour change, as we cannot distinguish between the two mechanisms in our study.

The acquisition and expression of colour by animals is known to carry a cost, as pigments must either be obtained through the diet (e.g. carotenoids) (Leclercq et al., 2010) or synthesised by the animal (e.g. melanin) (Mills and Patterson, 2009), and the expression of melanin-based traits is known to be condition dependent in many species [e.g. wasps (Tibbetts and Dale, 2004), butterflies (Talloen et al., 2004) and birds (Piault et al., 2012)] (reviewed in Stoehr, 2006). A significant cost to melanic colour change in fish has yet to be demonstrated (Stuart-Fox and Moussalli, 2009), although the suggestion is that there may be non-trivial costs associated with it, similar to those associated with other types of phenotypic plasticity (e.g. Relyea, 2002; Stuart-Fox and Moussalli, 2009).

Behavioural background matching (Garcia and Sih, 2003) is widespread in the animal kingdom, where prey animals select microhabitats that enhance their crypsis to avoid predation. Brown and green morphs of the Pacific tree frog *Hyla regilla* preferentially select brown and green substrates, respectively (Wente and Phillips, 2003; Wente and Phillips, 2005), and pale and dark morphs of the cichlid fish *Telmatochromis temporalis* defend territories in open (light) or shaded habitats, according to their body colour (Mboko and Kohda, 1995). For shoaling fish, the visual background may consist of other members of the shoal (Endler, 1978), and both western rainbowfish *Melanotaenia australis* (Rodgers et al., 2010) and the molly *Poecilia latipinna* (McRobert and Bradner, 1998) preferentially group with similarly coloured group mates, increasing their crypsis against the group and minimising the possibility of being selected by a predator for being phenotypically distinct (the oddity effect) (Landeau and Terborgh, 1986). By selecting habitats or group mates against which they are already colour adapted, fish

can simultaneously minimise both the energetic costs of colour change and the risk of predation.

Here, we investigated the energetic costs of colour change and the implications for subsequent behaviour decisions, using Trinidadian guppies (*Poecilia reticulata* Peters 1859) as a model system. Firstly, we document the ability of individual guppies to change colour in response to dark or light visual backgrounds, and explore the time scale on which this occurs. Secondly, we test the hypothesis that there is an energetic cost associated with repeated adaptation to the background, by assessing the food consumption of fish exposed to constantly coloured and changing habitats. Thirdly, we explore whether the energetic cost of repeated colour change has longer-term implications for growth. Finally, we test the prediction that fish select habitats and group mates that match their own colouration, reducing predation risk through behavioural background matching and avoiding the oddity effect, but potentially paying an opportunity cost through restriction to particular habitats and group mates.

MATERIALS AND METHODS

Study species and housing

We used the descendants of wild-caught (2003–2006) guppies (*P. reticulata*) that had been held in the aquarium facilities at the Universities of Hull (experiments 1 and 2) and Leeds (experiments 3 and 4) since capture. Hull fish were held in mixed-sex breeding groups in aquaria (200×340×390 mm) containing artificial substrate and plants. All aquaria were on a re-circulating aquarium system at 23–25°C on a 12h:12h light:dark cycle and fed daily on a mix of commercial flake and pellet food. Leeds fish were held in individually filtered aquaria (550×200 mm, filled to a depth of 140 mm) containing gravel to a depth of 1 cm and artificial plastic plants, at a temperature of 25°C, on a 12h:12h light:dark cycle, and were fed daily on commercial flake food. Where appropriate, experimental aquaria (described below) were furnished with artificial substrate (gravel) and/or artificial plants to mimic stock housing conditions. Details of conditions in individual aquaria are below.

Experiment 1: quantification of colour change

Sixty-eight fish, in groups of four consisting of two males and two females of differing body size were selected from the stock tanks. We selected fish of differing body size to allow for individual identification without marking (large female, 19–28.5 mm; small female, 15–22 mm; large male, 17–23.5 mm; small male, 15–20 mm; note that there is overlap between size classes but individuals of the same sex were identifiable in each group). Each group was photographed from above in a shallow dish of water (10 mm depth) using a digital camera (Canon Powershot G12), attached to a copy stand, with the camera positioned 270 mm above the dish. The dish was placed on a background of 1 mm graph paper (to allow for accurate calibration of photographs) with a black and white colour standard. The dish, copy stand and camera were positioned inside a light cube (EZCube 51 cm light tent) and illuminated with broad-spectrum lighting (2×5000 K 30 W 'Perfect Daylight' bulbs). No anaesthetic was used to photograph the fish as the procedure took only a few seconds, and anaesthesia is known to affect colouration in fish (Gray et al., 2011).

Following photography, each group of fish was housed in a tank (192×197 mm, filled to a depth of 141 mm) allocated to either 'black' or 'white' treatments. Black treatment tanks had black side walls, a black base and a black artificial plant. The rear wall of the tank was blue in colour, and the front wall was left uncovered to allow for observation of the fish. White treatment tanks had white side walls, base and plant, and again had a blue rear wall and uncovered

front. Tanks were held on a re-circulating aquarium system at 23–25°C on a 12h:12h light:dark cycle. Fish were held in these conditions for 24 h (black, $N=12$ fish; white, $N=12$), 48 h (black, $N=12$; white, $N=8$) or 2 weeks (black, $N=12$; white, $N=12$), and fed daily on a mix of flake and pellet food. Each group was then placed into a small plastic aquarium (197×125×120 mm, filled to a depth of 85 mm) covered on four sides with black or white plastic film to avoid any changes in colouration, and then photographed again following the procedure above. Fish were then returned to the stock tanks. There were no significant differences in body size in fish allocated to the different colour and time treatments (linear model: colour treatment effect, $F_{1,63}=1.913$, $P=0.172$; time effect, $F_{1,63}=2.198$, $P=0.143$; interaction, $F_{1,62}=0.373$, $P=0.543$).

All the digital images (saved as TIFF files) were analysed using ImageJ version 1.45 (<http://rsb.info.nih.gov/ij/index.html>). Images were scaled using the graph paper included in each photograph and then the body length of each fish was measured in millimetres. The proportion of melanin pigmentation present on the body of the fish was assessed using an adaptation of previous methods (Rodgers et al., 2010). Images were converted to greyscale, then each image was standardised for white balance by selecting five areas in the black and white colour standards included in the photographs, and setting the average value of each of these as the minimum and maximum pixel intensities, using the colour balance options in ImageJ. The outline of each fish was traced to measure the overall area of the visible dorsal surface (excluding the fins). We assessed colouration by setting the pixel threshold intensity to 70, and counted the number of pixels darker than this threshold to give an indication of the overall level of pigmentation. All images of all fish (before and after colour treatment) were analysed in this way. Fish could be individually identified by sex and size to give repeated-measures data. Over the course of the experiment, one fish died in each of the 14 day colour treatments, giving final sample sizes of $N=11$ fish for these treatments.

To establish whether 24 h was sufficient for colour change to occur when fish were moved from black to white tanks (and *vice versa*), we first placed mixed-sex groups of four to five fish into either black or white tanks (as above) for 24 h before photographing them and then placing them in tanks of the opposite colour for a further 24 h and photographing them again. Images were taken and analysed using the same methodology. One fish died in the white-to-black treatment, leaving $N=13$ fish in the black-to-white treatment and $N=11$ fish in the white-to-black treatment.

Experiment 2: food consumption

Seventy-four adult guppies (a mix of males and females; males, 14.5–21.5 mm, females, 14–26 mm) were removed from the stock tanks and placed in groups of three to five in small (192×197 mm, filled to a depth of 141 mm) aquaria as for experiment 1. Tanks were allocated to black, white and colour change treatments (black, $N=25$; white, $N=24$; colour change, $N=25$ fish). Black treatment tanks had a constant black background and white treatment tanks had a constant white background as in experiment 1. For the colour change treatment, the colour of the side walls, base and plant was changed from black to white (and *vice versa*) on a daily basis for a period of 9–11 days. To control for the disturbance caused by this colour change treatment, the side walls, base and plant in black and white treatment tanks were removed and replaced on a daily basis. Fish were fed *ad libitum* daily on small pellet fish food (ZM Systems, Winchester, Hants, UK) after the tanks colours were changed.

Fish were starved for 1 day before feeding trials commenced, to standardise their motivation to feed. Trials took place in a small

(192×197×183 mm) tank filled to a depth of 85 mm. Fish were placed individually into the tank and allowed 5 min to acclimatise. Ten individual food pellets (as above) were then added to the tank and a timer was started. If all pellets were eaten, an additional 10 pellets were added immediately following the consumption of the last pellet. The time interval between the consumption of one pellet and the consumption of the next was monitored, and when there was a delay of greater than 1 min, we considered that the fish had reached satiation, and the trial was terminated. The total number of food pellets consumed was then recorded. The sex and body size (measured to the nearest mm using callipers) of the fish was recorded, and individuals were returned to the stock tanks. There was no difference in size between fish allocated to the different treatments for either males ($F_{2,43}=0.653$, $P=0.525$) or females ($F_{2,25}=0.233$, $P=0.794$), and no fish died during the experiment.

Experiment 3: growth rate

Ninety newborn guppies (0–3 days old) were haphazardly selected from the stock tanks in groups of five. Groups were placed into a shallow dish of water (10 mm) and photographed from above using a Nikon D90 digital camera with a 105 mm Sigma DG macro lens under standardised conditions with daylight spectrum lighting. A scale was included in the image, and images were subsequently used to measure body length. Fish were then introduced to one of three colour treatment tanks in their groups (see below). There was no difference in body length between the fish assigned to the three treatments (linear model: $F_{2,87}=1.792$, $P=0.178$).

Treatment tanks were small plastic aquaria (200×140 mm, filled to a depth of 80 mm with aerated water) covered on all four sides with a sleeve of either black or white plastic, which could easily be removed and replaced without disturbing the water. Each tank contained an airstone to aerate and circulate the water, and 20% water changes were carried out on a fortnightly basis, to remove any uneaten food. No artificial plants were used to provide cover for the fish in this experiment, as the fish were very small, young individuals, and there was a risk of harming individuals hiding in the plants when we removed them. Three treatments were used: constant black, constant white and a colour change treatment. Every 2 days, the plastic sleeves were removed and replaced with a sleeve of the same colour for the constant treatments and of the opposite colour for the colour change treatment, thus controlling for disturbance between treatments. Half the tanks in the colour change treatment started with black and half started with white. Treatments were assigned randomly to tanks arranged in a 10×2 array, illuminated evenly from above by a daylight simulation strip light on a 12h:12h light:dark cycle.

Fry were fed daily *ad libitum* on fry food (ZM Systems; 80–200 µm particle size, 55% protein). Food was weighed to the nearest 0.001 g to ensure all tanks received the same amount of food, and portion size was increased every 2 weeks as the fish grew to ensure *ad libitum* feeding. After 65 days in the treatments, fish were removed from the tanks, killed with an overdose of MS-222 by a trained technician, weighed to the nearest mg, and photographed to measure length. Death was confirmed by destruction of the brain, according to Schedule 1 of the Animals (Scientific Procedures) Act, again by a trained technician. Prior to this, mortality over the course of the experiment was 6.7% (6/90 fish died – two in the black treatment, one in the white treatment and three in the colour change treatment). Because of the age of the fish, we were not able to reliably determine sex at the end of the experiment.

Experiment 4: habitat and shoal choice

Sixty-five adult female guppies (19–21 mm) were removed from the stock tanks and housed in groups of five in small plastic aquaria (200×140 mm, filled to a depth of 80 mm), assigned to either ‘black’ or ‘white’ treatments. For each treatment, the exterior walls were covered with either black or white film (as appropriate) and the base was covered with a 10 mm layer of either black or white gravel. Tanks were oxygenated *via* an airstone, and a full water change of conditioned water was carried out once a week. Tanks were held at 25°C and illuminated by daylight spectrum lighting on a 12h:12h light:dark cycle. Guppies were housed in these tanks for 1–2 weeks before being assessed in the habitat choice experiments, and then returned to the tanks for a further 2–3 weeks before shoal choice experiments were carried out. During this time, fish were fed *ad libitum* on commercial flake food. There was no difference in body size in fish allocated to the black and white treatments (*t*-test: $t=0.740$, d.f.=63, $P=0.462$). Mortality was not explicitly recorded during this experiment but was very low.

To assess preferences for differently coloured habitats, individual guppies were placed into a habitat choice tank. The choice tank (500×200 mm, filled to a depth of 100 mm with aerated water) was divided into three sections (habitat zones): a black end section (150×200 mm), a white end section (150×200 mm) and a neutral (brown) central section (200×200 mm). Fish could swim freely between the three sections. Each section was coated on the outside with coloured film (black, white or brown as appropriate), and the side facing the observer was left uncovered to allow observations to be made. The base of the tank was covered with a 10 mm depth of black, white or brown gravel. Gravel was contained within a watertight transparent plastic bag to eliminate potentially confounding effects of different chemical cues from the gravel within each habitat (Ward et al., 2004; Ward et al., 2005). Two tanks were used that were mirror images of each other, to remove any potentially confounding effects of side bias.

A single test fish (from either the black or white treatment tanks) was introduced to the test tank and allowed 2 min to acclimatise before observations began. Preliminary observations suggested that this time was sufficient to allow the majority of fish to begin swimming normally and exploring the tank. Any fish that remained motionless or had not swum into all three habitat zones after the 2 min period was excluded from the experiment (two fish were excluded – one from each treatment – giving final sample sizes of $N=25$ for black treatment fish and $N=24$ for white treatment fish). Over a 10 min observation period, we recorded the total time spent in each of the three habitat zones. Observations were made from behind a screen to minimise disturbance to the fish. After trials were complete, fish were returned to the black and white treatment tanks from which they had been taken.

Shoaling preferences were assessed using a standard binary choice design (Morrell et al., 2007). The choice tank (600×210 mm, filled to a depth of 100 mm with conditioned water) was covered on three sides with brown cloth and placed on a mid-brown base. The side facing the observer was left uncovered to allow observations to be made. The tank was divided into three sections by transparent, unperforated partitions to allow for transmission of visual but not olfactory cues (which may be associated with recent habitat) (Ward et al., 2004; Ward et al., 2005). Each end (‘stimulus’) compartment measured 210×150 mm, and contained a stimulus shoal of three black or white treatment fish (i.e. black- or white-adapted fish). The 60 mm adjoining each stimulus compartment were defined as ‘shoaling zones’, such that when the test fish entered the zone, it was

considered to be shoaling with the stimulus shoal. For each trial, one stimulus compartment contained a shoal of three black treatment fish, and the other contained a shoal of three white treatment fish. The side containing the black treatment fish was randomised between trials to control for any effects of side bias. Stimulus shoals were selected from different colour tanks to the test fish to avoid any confounding effects of familiarity (Griffiths and Magurran, 1997). Stimulus shoals were allowed 2 min to settle before the introduction of the test fish. Observations suggested this was sufficient for the shoals to begin swimming normally.

A single test fish was then introduced to the central compartment, and allowed 2 min to acclimatise before a 10 min observation period began. During the acclimatisation period, all test fish ($N=20$ for black treatment fish, $N=17$ for white treatment fish) began swimming and entered both shoaling zones, and thus no fish were excluded on the basis of inactivity. We recorded the cumulative time spent in each shoaling zone, which was then expressed as a proportion of the total shoaling time (i.e. the sum of the time spent in the two shoaling zones). After the trial was completed, test fish were returned to their colour tanks, and subsequently used as stimulus fish, but no fish that had been used as a stimulus fish was subsequently used as a test fish.

Statistical analysis

Statistical analysis was carried out using R version 2.13.0 (R Development Core Team, 2011). Visual inspection of residual and normal quantile–quantile plots was used to assess normality of data. Appropriate transformations or non-parametric tests were used where the assumption of normality was not supported.

Experiment 1

The proportion of black body colouration was arcsin transformed prior to analysis to meet assumptions of normality. We assessed the effect of stage (before or after exposure to the colour tanks) and sex, and their interaction on the proportion of black colouration for each time period (24 h, 48 h and 2 weeks) and colour treatment individually, using linear mixed effects models (Bates et al., 2011). Fish ID was included in the model as a random effect to account for the repeated-measures nature of the data. General linear models were used to confirm that there was no difference in the starting colour of fish allocated to the different colour or time treatments. There was no significant interaction and no significant effect of sex for any of the analyses, and so only the results of models including stage (before or after exposure to the colour tanks) are reported here.

Experiment 2

We assessed the effect of colour treatment (black, white or changing) and sex, and their interaction on the total number of food pellets consumed using a linear mixed effects model with body size as a random factor. The number of pellets consumed was log transformed to meet the assumptions of a normal error structure.

Experiment 3

Length and mass data conformed to the assumptions of normality, and so we used general linear mixed effects models to assess the effect of colour treatment on the final body length and mass of the fish, with tank ID as a random effect to control for potential non-independence of fish housed within the same tank.

Experiment 4

To test for an effect of colour treatment on habitat preference, and to account for the fact that the proportion of time spent in each colour

zone was not independent of the time in the other zones, we used a series of one-sample Wilcoxon tests combined with a correction for multiple testing [false discovery rate (FDR) control] (Benjamini and Hochberg, 1995). Data were split on the basis of fish colour and the proportion of time spent in each zone was tested against an expectation based on the size of the zone (expected proportions: black, 0.3; white, 0.3; neutral, 0.4). FDR control was applied against the three tests for each colour of fish. To test whether black treatment and white treatment fish showed a significant preference for shoaling with the colour-matched shoal over the unmatched shoal, we used non-parametric one-sample Wilcoxon tests, as the data were non-normal and could not be satisfactorily transformed to meet the assumptions of normality. We compared the proportion of time spent shoaling with the matched shoal against median value of 0.5, representing random association patterns (i.e. no preference for either shoal colour), for black and white treatment fish separately.

RESULTS

Experiment 1: quantification of colour change

Fish showed a significant darkening of body colouration after 48 h and 2 weeks in black treatment tanks (Table 1, Fig. 1A) but this was not evident after 24 h (Table 1). Fish in white treatment tanks showed a significant lightening of body colouration after all time periods (Table 1, Fig. 1A). When moved from a black to a white tank, fish showed a significant lightening in colour (Table 1, Fig. 1B), while fish moved from a white to a black tank showed a significant darkening (Table 1, Fig. 1B).

Experiment 2: food consumption

There was no significant interaction between colour treatment and sex ($F_{2,51}=2.319$, $P=0.108$), so this was removed from the model and only the main effects are presented here. There was a significant effect of colour treatment and sex (but no interaction) on the number of food pellets consumed (treatment, $F_{2,53}=13.288$, $P<0.0001$; sex, $F_{1,53}=10.767$, $P=0.0018$; Fig. 2). Fish in the colour change treatment consumed more pellets than fish in the static black and white treatments (Table 2). Male fish also consumed more than female fish across all treatments (Table 2, Fig. 2).

Experiment 3: growth rate

There was no significant effect of colour treatment (black, white or colour change) on the final length (linear mixed effects model: $F=1.494$, $P=0.256$, $N=84$ observations in 18 groups) or mass (linear mixed effects model: $F=1.031$, $P=0.381$, $N=84$ observations in 18 groups) of fish.

Table 1. Quantification of colour change

Time	Colour	d.f.	<i>t</i>	<i>P</i>
24 h	Black	11	0.432	0.674
	White	11	-3.388	0.006
48 h	Black	7	2.565	0.026
	White	7	-4.787	<0.001
2 weeks	Black	10	5.079	<0.001
	White	10	-6.016	<0.001
24 h	Black to white	12	-5.676	<0.001
	White to black	10	4.019	0.002

Results of linear mixed effects models assessing the effect of stage (before or after colour treatment) on the proportion of black pigmentation for the six combinations of time (24 h, 48 h and 2 weeks) and colour treatment (black and white), and for the follow-up black-to-white and white-to-black experiments. Significant *P*-values are presented in bold.

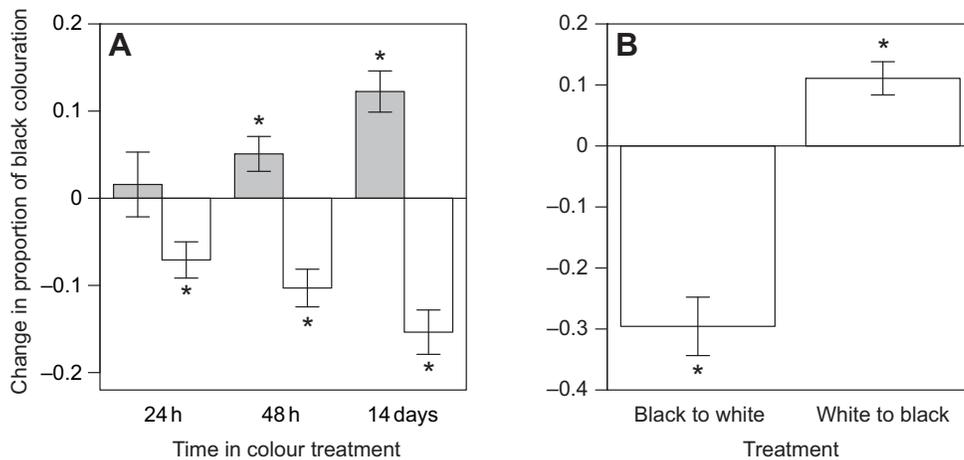


Fig. 1. (A) Change in the proportion of black body colouration for fish exposed to black (filled bars) and white (open bars) treatments for 24 h, 48 h and 14 days. (B) Change in the proportion of black body colouration for fish moved from black to white treatments (and vice versa) for 24 h. Error bars represent ±1 s.e.m. Asterisks indicate a significant change in colouration (see Table 1).

Experiment 4: habitat and shoal choice

Fish from the black treatment spent significantly more time in the black zone ($V=262$, adjusted $P=0.012$) and significantly less time in the white zone ($V=7$, adjusted $P<0.001$) than expected by chance (Fig. 3A). The proportion of time spent in the neutral zone did not differ from random expectation ($V=172$, adjusted $P=0.809$). Fish from the white treatment spent significantly more time in the neutral zone ($V=260$, adjusted $P=0.002$), and significantly less time in the black ($V=69$, $P=0.031$) and white ($V=78$, $P=0.040$) zones than expected (Fig. 3A). Both black and white treatment fish showed a significant preference for the colour-matched shoal (Wilcoxon signed rank test; black treatment fish: $V=170$, d.f.=19, $P=0.016$; white treatment fish: $V=131$, d.f.=16, $P=0.011$; Fig. 3B).

DISCUSSION

Here, we have demonstrated that there may be costs associated with colour change in guppies. Fish exposed to a changing environment changed their body colouration and increased their food consumption relative to those exposed to a constantly coloured environment. A cost to colour change in fish has been predicted (Stuart-Fox and Moussalli, 2009) but not previously demonstrated. However, we also found that when food was freely available, fish were able to meet this potential cost, and did not suffer negative consequences in terms of growth rate. It is well known that poor conditions, particularly food availability, can cause slower growth rates in fish (Metcalf and Monaghan, 2001; Lee et al., 2012), and that improved food availability can lead to compensatory (or catch-up) growth (Ali et al., 2003; Hector and Nakagawa, 2012). Thus, we would predict that if food supplies were limited, the costs associated with colour change that we identified in experiment 2 could lead to a reduction in growth rate, but this is yet to be tested. Further work is needed to conclusively demonstrate an energetic cost associated with colour change, which

may be linked to the mechanisms of colour change (physiological versus morphological).

In our experiments, fish were moved from the neutrally coloured stock tanks into the treatment tanks, and so those fish exposed to the constant black and white treatments would also have undergone some colour change, becoming darker or paler. In contrast to the fish in the colour change treatments, however, this change would have slowed and stabilised over time (e.g. Péan, 2012), while fish in the colour change treatment would have repeatedly undergone the initial rapid colour change. The large increase in food intake by fish in the colour change treatment strongly suggests that the initial rapid colour change has large associated energetic costs.

Our finding that males had an elevated food intake relative to females is unsurprising: sex differences in behaviour have been well documented in guppies (Houde, 1997; Magurran and Garcia, 2000; Magurran, 2005). Males are generally more active (Reader and Laland, 2000) and less risk averse (Magurran and Seghers, 1994) than females, and move between shoals more than females (Croft et al., 2003), as males attempt to maximise mate encounters and increase reproductive success. The increased activity levels of males provide a simple explanation for the increased food intake we observed.

It is possible that the increased food intake by fish from the colour change treatment could have been due to their being less risk averse. Juvenile cod (*Gadus morhua*) and guppies that experience unpredictable environments during early life are known to become

Table 2. Food consumption

	Value	s.e.m.	d.f.	t	P
Intercept	3.555	0.133			
Treatment: black	-0.741	0.150	53	-4.942	<0.0001
Treatment: white	-0.599	0.152	53	-3.940	0.0002
Sex: male	0.425	0.130	53	3.281	0.0018

Results of the linear mixed effects model investigating the effect of colour treatment (colour change, static black, static white) and sex on the number of food pellets consumed. The intercept refers to the value for female fish in the colour change treatment. Significant P-values are presented in bold.

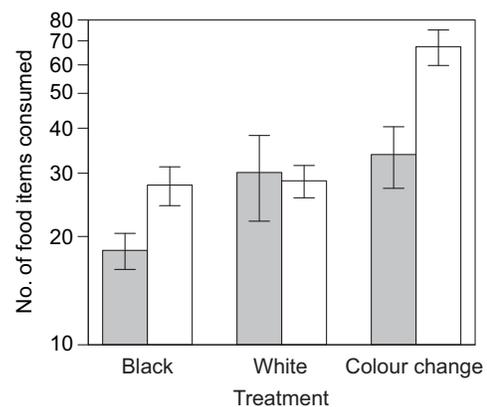


Fig. 2. Number of food pellets consumed by female (filled bars) and male (open bars) guppies from the three colour treatments. Error bars represent ±1 s.e.m.

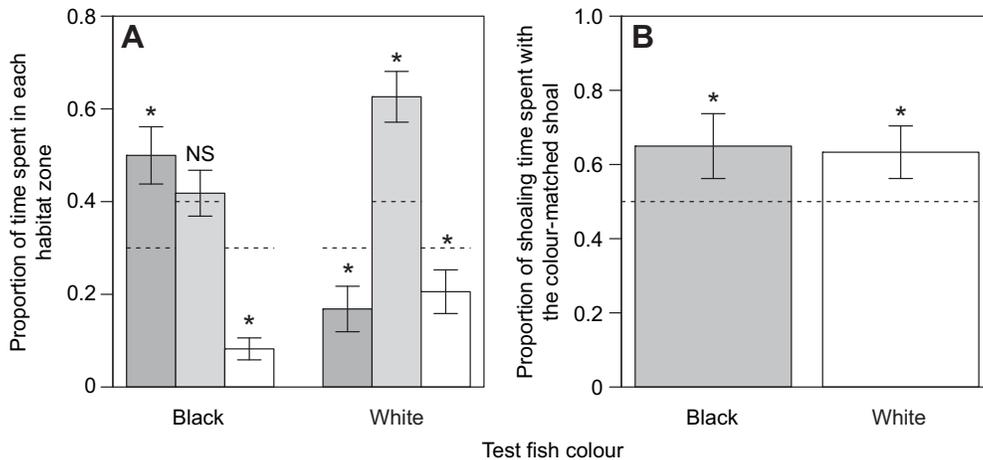


Fig. 3. (A) The proportion of time spent by black treatment and white treatment fish in each of the three habitat zones: black habitat (dark filled bars), neutral habitat (light filled bars) and white habitat (open bars). Asterisks indicate significant deviation from random expectation, indicated by the dashed line. (B) Mean proportion of time spent shoaling with the colour-matched shoal by black (filled bars) and white (open bars) treatment fish. The horizontal dashed line indicates no preference; asterisks denote a significant deviation from this. Error bars are ± 1 s.e.m.

'bolder' (Braithwaite and Salvanes, 2005; Chapman et al., 2010), leaving a refuge more quickly and being more explorative in a novel area, and this may cause them to forage more rapidly once introduced to the test tank. We did not specifically record latency to feed in the behavioural trials, but all fish were swimming normally by the time the first food pellet was added, and the majority of fish immediately attacked the first pellet, suggesting that differences in boldness due to recent experience may not have been important in determining the likelihood of foraging. Female fish did appear to take longer to settle during the 5 min acclimatisation period than males (N.W.G., personal observation), but we noted no differences in acclimatisation behaviour between colour treatments, and all fish experienced equal disturbance (changing of backgrounds) in a predictable manner during the short experimental period.

We also demonstrated that fish preferred both habitats and shoals that matched their own colouration: black-adapted fish showed a significant preference for the black-adapted shoal over the white-adapted one, and for darker habitats, and avoided white habitats. White-adapted fish also preferred colour-matched shoals, and neutrally coloured habitats, avoiding both black and white habitats. White habitats may be unattractive to fish for a range of reasons. The lighter background, and more reflected light, may mean that it is easier for a predator to identify a moving or camouflaged prey item (e.g. Strand et al., 2007). In guppies, the highest predation risk generally occurs at maximum light levels (Endler, 1987), and in salamanders (*Ambystoma barbouri* and *A. texanum*), the presence of olfactory cues from predatory fish resulted in a preference for dark substrates over light ones (Garcia and Sih, 2003). There is also evidence that living in light-coloured habitats may have other costly implications for fish: growth and survival in juvenile yellow seahorses (*Hippocampus kuda*), for example, were significantly reduced in light habitats compared with dark ones (Pawar et al., 2011).

Many animals are known to select habitats that best match their colouration (known as 'behavioural background matching') in order to increase crypsis and reduce predation (Garcia and Sih, 2003; Ruxton et al., 2004). Both black- and white-adapted fish avoided habitats of the opposite colour, where they would be most conspicuous, preferring instead those where they were more cryptic. White-adapted fish were not white in colour, and may have been more cryptic against the neutral background than against the white background, explaining their preference for the neutral background. The choice of neutral habitats by white-adapted fish also provides evidence that the preferences we observed were not based only on

familiarity with the habitat (Stamps and Swaisgood, 2007). White-adapted fish preferred neutral habitats to white ones, but a preference for white would be predicted if familiarity with recent habitat drove their decision making. Thus, we conclude that both black- and white-adapted fish are selecting habitats that maximise crypsis. Similarly, white morphs of black-and-white mollies (*Poecilia latipinna*) show no preference for white habitats over black ones, in contrast to their black counterparts, who prefer black backgrounds (Bradner and McRobert, 2001), suggesting that white habitats are not preferred even for those animals that would be most cryptic against them. 'Matching habitat choice' carries with it implications for gene flow and can promote population differentiation and adaptation (Edelaar et al., 2008), as individuals actively choose microhabitats that maximise fitness (Karpestam et al., 2011).

Both black- and white-adapted fish preferentially associated with colour-matched shoals. It is well established that fish assort into shoals based on phenotype: there is evidence for assortment by species (Keenleyside, 1955), body size (Krause et al., 1998; Ward and Krause, 2001) and colour (McRobert and Bradner, 1998; Rodgers et al., 2010). By shoaling with phenotypically matched fish (those that are similar in appearance), individuals can benefit from a reduced risk of predation *via* two interlinked mechanisms: the confusion (Krakauer, 1995) and oddity (Ohguchi, 1978; Landeau and Terborgh, 1986) effects. The oddity effect allows a predator to overcome the confusion caused by a moving group of phenotypically similar individuals (the confusion effect) by selecting one that is distinct ('odd') as the target, and together these mechanisms should lead to the evolution of phenotype-assorted groups.

The ability to associate with colour-matched fish and choose habitats that maximise crypsis suggests that a fish is able to judge its own body colouration. A fish may do this either through a self-referent matching process or by assessing the colour of those with whom it has recent experience and making decisions accordingly. While many fish learn their phenotype when young (Engeszer et al., 2004), there is evidence that recent experience can also contribute to this assessment (Mateo, 2004; Witte, 2006; Gómez-Laplaza, 2009). In sticklebacks (*Gasterosteus aculeatus*), self-referent phenotype matching is used in social decisions, and this, in common with much social behaviour in fish, is mediated by chemical rather than visual cues (Ward et al., 2005; Ward and Currie, 2013). Familiarity (associating with individuals of shared recent experience) is a key factor structuring fish shoals, supporting the notion that fish benefit from association with those that have experienced a similar recent environment (Ward and Hart, 2003).

By selecting habitats or shoals that match their current colouration, fish reduce their risk of predation *via* crypsis against the background (Ruxton et al., 2004) or shoal (Endler, 1978). Even though colour adjustment could be made in a matter of minutes in some species, it is never instant (Leclercq et al., 2010), and will be associated with an increased risk of predation before adaptation to the background is complete. Selecting matching habitats and shoals removes the need to adjust body colouration and pay the associated cost, but simultaneously may represent an opportunity cost (Ruxton et al., 2004) as it may act to restrict individuals to particular habitats or associates. Herbert and Emery suggest that the high cost associated with melanin synthesis restricts melanic morphs to areas of high UV radiation, for example (Herbert and Emery, 1990). All prey animals at risk from visual predators will face an opportunity cost while they remain in microhabitats with backgrounds that maximise their camouflage (Ruxton et al., 2004). This cost is reduced by the ability to change colour to match different backgrounds, and is thus inversely related to speed of colour change.

Colour change for background matching may have implications for other behaviours. Colour is widely used as a signal (Endler, 1992; Maynard Smith and Harper, 2003): melanin, for example, is linked to social signalling, particularly in advertising social status [e.g. house sparrows *Passer domesticus* (Møller, 1987), Atlantic salmon *Salmo salar* (O'Connor et al., 1999) and arctic charr *Salvelinus alpinus* (Höglund et al., 2002)]. Thus, there is a potential trade-off between colour change for background adaptation and colour change associated with other functions: colour change that is beneficial in the context of background matching may be detrimental in the context of social signalling, for example (Stuart-Fox and Moussalli, 2009). Colour change for background matching could also affect colour patterns used in sexual signals, masking the intensity of colour patches, or minimising variation between males, for example. The interaction between colour change for crypsis and colour change for thermoregulation presents another potential conflict for many species including reptiles and amphibians (Stuart-Fox and Moussalli, 2009), though this is likely to be of little importance to exclusively aquatic organisms.

Prey animals often have a repertoire of potential responses to increased predation risk, ranging from immediate behavioural responses to plastic morphological change to shifts in life history strategies (Garcia and Sih, 2003). We show here that colour change to match the visual background is complemented by behavioural strategies that minimise the need to pay the cost associated with colour change, but which may also represent an opportunity cost to the individual. Together, these strategies should act to maximise fitness in variable environments, but the extent to which these costs and decisions impact on fitness in wild populations is yet to be examined, and we know little about how colour change for crypsis interacts with other functions of colour (Stuart-Fox and Moussalli, 2009).

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AUTHOR CONTRIBUTIONS

G.M.R. and L.J.M. conceived and designed the experiments. All authors collected and analysed the data. G.M.R. and L.J.M. interpreted the findings. All authors drafted and revised the article.

COMPETING INTERESTS

No competing interests declared.

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