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Environment-contingent sexual selection in a colour polymorphic fish

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Sexual selection could be a driving force in the maintenance of intraspecific variation, but supporting observations from nature are limited. Here, we test the hypothesis that spatial heterogeneity of the visual environment can influence sexual selection on colourful male secondary traits such that selective advantage is environment contingent. Using a small fish endemic to Sulawesi, Indonesia (*Telmatherina sarasinorum*) that has five male colour morphs varying in frequency between two visually distinct mating habitats, we used direct behavioural observations to test the environment-contingent selection hypothesis. These observations were combined with measurements of the visual environment, fish coloration and the sensitivity of visual photopigments to determine whether differential morph conspicuousness was associated with reproductive success across habitats. We found that blue and yellow males are most conspicuous in different habitats, where they also have the highest reproductive fitness. A less conspicuous grey morph also gained high reproductive success in both habitats, raising the possibility that alternative behaviours may also contribute to reproductive success. In a comprehensive analysis, conspicuousness was strongly correlated with reproductive success across morphs and environments. Our results suggest an important role for spatially heterogeneous environments in the maintenance of male colour polymorphism.

Keywords: colour polymorphism; sexual selection; environmental heterogeneity; *Telmatherina sarasinorum*; Telmatherinidae; Malili Lakes

1. INTRODUCTION

Understanding how intraspecific diversity is maintained in nature is a fundamental problem in biology (Darwin 1869). For decades, researchers have used easily observable colour polymorphisms, defined as the occurrence of two or more interbreeding colour morphs in the same population (Huxley 1955), to investigate this problem. The role of spatially heterogeneous environments in the maintenance of polymorphism has been discussed extensively (e.g. Hedrick 2006). Using a broad definition of 'environment', there are many examples of how selection across a heterogeneous environment influences the maintenance of colour polymorphisms. For example, male throat colour polymorphism in side-blotched lizards is maintained within variable social environments via frequency- and density-dependent selection (Sinervo *et al.* 2001; Svensson & Sinervo 2004); *Heliconius cydno* butterfly morphs with different wing colour patterns are maintained by diversifying natural selection across mimicry rings (Kapan 2001) and population-level variation in guppy male colour pattern is maintained, in part, by natural and sexual selection across different visual environments (Endler 1983, 1991; Gamble *et al.* 2003).

In recent work, the role of sexual selection in maintaining male colour polymorphisms (Endler 1992; Andersson 1994; Sinervo *et al.* 2001; Kingston *et al.* 2003; Chunco *et al.* 2007; Gray & McKinnon 2007) and in generating new species (Seehausen *et al.* 1999; Seehausen & Schluter 2004; Carleton *et al.* 2005) has been especially emphasized. Owing to the inextricable link between colour signals and the light environment (Lythgoe 1979; Endler 1990), the visual environment should be important in shaping the direction of such selection. The conspicuousness and hence attractiveness of a male's colour pattern depends upon various aspects of the viewing environment (e.g. the spectral composition of the medium, complexity of the substrate) and the visual sensitivity of the signal receiver (Lythgoe 1979; Endler 1990; Endler & Mielke 2005). In a heterogeneous environment, a male could be alternately conspicuous and cryptic, depending on where he is viewed. A recent model that includes natural and sexual selection across different visual microhabitats predicts the maintenance of multiple male phenotypes under such a scenario (Chunco *et al.* 2007). Chunco *et al.* (2007) showed that strong sexual selection favouring conspicuous male coloration in environments that vary in perception of conspicuousness can lead to a stable

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polymorphism, even in the absence of natural selection. Supporting empirical work is still lacking, although studies are accumulating that document relationships between the visual background and the predominant colour pattern within a population, with putatively sexually selected traits often showing higher contrast and conspicuousness (e.g. sticklebacks *Gasterosteus aculeatus*: Boughman 2001; bluefin killifish *Lucania goodei*: Fuller 2002; manakins *Macanus vitellinus*: Uy & Endler 2004). However, no field studies have been done, which link, across different visual habitats, measurements of natural light environments, an assessment of conspicuousness that incorporates the visual system of the taxon in question, and observations of mating success in nature. Here, we combine these approaches to test key predictions of the hypothesis that environment-contingent sexual selection contributes to colour polymorphism maintenance.

We studied *Telmatherina sarasinorum*, a member of the Lake Matano (Malili Lakes; figure S1 in the electronic supplementary material) sharpfin complex (Gray & McKinnon 2006; Herder *et al.* 2006*a*). The taxonomy of this group remains an area of active study (Herder *et al.* 2006*a*,*b*), and some evidence suggests gene flow throughout the complex, including stream-dwelling populations (Herder *et al.* 2006*a*). The complex mating behaviour of *T. sarasinorum* has only recently been described (Gray & McKinnon 2006). There are two mating tactics through which males can potentially gain reproductive success. First, a male can be paired with a female: spawning takes place on the substrate with no parental care. During spawning, males can be cuckolded by several other males, which often leads to cannibalism of the eggs (Gray *et al.* 2007). Second, if a male is not paired with a female, he can attempt to sneak spawnings (i.e. cuckold other males). Individual males of all colour morphs have been observed using both the courting and sneaking tactics. Although sexual selection theory predicts that males with the most conspicuous traits will be favoured (Andersson 1994), when behavioural tactics such as sneaking can be employed, matings may be obtained by less conspicuous males. It is even possible that more conspicuous males may more often be seen and targeted by sneakers.

The colour patterns of five *T. sarasinorum* male morphs have been qualitatively described using body, head and fin coloration (Gray & McKinnon 2006): blue males are all blue; yellow males are all yellow; blue-yellow males have blue bodies with a yellow head patch and fins; grey males are grey all over, sometimes with a black/white fin; and grey-yellow males are grey bodied and have a yellow head patch. We found *T. sarasinorum* mating in various habitats (Gray & McKinnon 2006). Here we have analysed two extremes of the environment: shallow beach sites are composed of sand and cobble upon which the fish spawn, and root sites drop off steeply from the shore and spawning takes place on over-hanging, algae-covered roots (figure S2 in the electronic supplementary material). Beach habitat appears yellow, whereas root habitat appears bluer, at least qualitatively based on the human visual system. Each habitat therefore provides a different visual background against which fish are viewed. Although distinct, these two habitats are often adjacent and movement of individuals between habitats occurs; thus ‘habitat’ is not equivalent to ‘population’.

We tested whether environmental heterogeneity leads to environment-contingent sexual selection, with the prediction that differential conspicuousness of morphs between habitats will influence mating success across habitats. We also tested for differential reproductive fitness between morphs within a habitat, with the expectation that less conspicuous morphs also gain some reproductive fitness since all morphs are found in both habitats. We measured both morph conspicuousness and reproductive success directly in the lake under natural conditions.

2. MATERIAL AND METHODS

(a) *Collection of observational data*

We visited Lake Matano, Sulawesi, Indonesia, during two wet (January to March 2003 and January to May 2004) and one dry season (October to December 2004), and established eight permanent sampling sites around the lake (see figure S1 and table S1 in the electronic supplementary material). At four of these sites, we placed two transects (80 m long by 1–2 m deep), one each in a root and beach habitat, separated by a minimum distance of 100 m and a maximum distance of 500 m. The remaining four sites had a single transect placed along the shore in the centre of either root (two sites) or beach habitat (two sites) and not in proximity to the alternate habitat, making a total of six root and six beach transects. Each site was visited between two and six times per field season (table S1 in the electronic supplementary material), at least one week apart with the order of site visits chosen randomly. On each visit to a site, the day was divided into five, 2 hour time periods between 06.30 and 16.30 hours. During each period, we counted the number and colour of males on the transect(s), and collected mating behaviour data by following individual females and males. All observations were made near the transect by two or three observers using snorkel gear (see table S1 in the electronic supplementary material, for details).

Transect data were collected by a single observer snorkelling at a slow, constant pace along a transect, counting only the male fish 2.0 m in front and 1.0 m on either side of the transect line. Where the depth of the transect varied, we only counted fish to a depth of approximately 1.0 m, since most courting activity takes place in that space, regardless of depth. Male colour morphs were identified and whether or not they were paired with a female was recorded (see table S1 in the electronic supplementary material, for sample sizes per site). A male was considered paired with a female if he was actively courting her and defending her from approaching males (Gray & McKinnon 2006). We estimated the probability that a male of a given morph was paired (P_p) or unpaired (P_u) with a female at any given time and place (e.g. no. of blue males paired/total no. of blue males counted). Morph frequency was determined for each time period that males were counted along transects by dividing the total number of males counted for each morph by the total number of all males counted.

A focal follow was performed by haphazardly selecting either a female or a paired male along a transect and following that individual for 4–10 min. Female follows were recorded directly on underwater paper by an observer who noted each apparent spawning, the colour of the paired male and (if present) the number of cuckolders and apparent cannibals. The same data were collected for each successive spawning event, even if a different male was paired with the focal female. From the female follow data ($n = 570$), we calculated the total number of spawnings each morph had within a habitat and site. Each spawning event could have a particular outcome, depending on a number of factors, such as if there were cuckolders or cannibals present at the time of spawning. Therefore, we estimated the probability that if a male spawned he did so in the absence of cuckolders and cannibals (P_n); in the presence of cannibals (including himself—if he cannibalized—and non-mating males) but the absence of cuckolders (P_c); in the presence of one cuckolder (no cannibals; P_{k_1}); in the presence of two or more cuckolders (no cannibals; P_{k_2}) and in the presence of both cuckolders and cannibals (including himself, sneakers and non-mating males; P_b).

Male follows were recorded with an underwater video camera (Sony miniDV camcorder, Ocean Images underwater video housing). Males were followed regardless of whether they lost the original female, providing data on male behaviour, both while paired and unpaired. When the male was paired with a female, the observer recorded the number of apparent spawnings. When the male was not paired with a female, the observer recorded the number of attempted sneak fertilizations he made. From individual male follows ($n=285$), we determined the mean spawning rate (no. of spawns per minute per female) when paired and the mean sneaking rate (no. of sneaks per minute) when unpaired for each site and transect. All probabilities were calculated within a single morph, so that differences in morph frequency are removed from our estimations. Sample sizes (e.g. male follows, spawnings within female follows) were too small to calculate probabilities per time period per day, and so were pooled for a site and habitat (i.e. there is one value for each morph in each site/habitat, $n=60$).

(b) *Calculation of reproductive fitness*

We estimated the relative reproductive fitness of morphs within each habitat at each of 12 transects (six root, six beach) by building a simple fitness equation that sums the estimated reproductive success gained by a male when paired with a female through spawning (2.1*a*), and that gained while unpaired through sneaking behaviour (2.1*b*).

$$W = \{P_p \times \text{spawning rate} \times [(P_n \times k_n) + (P_c \times k_c) + (P_{k_1} \times k_{k_1}) + (P_{k_2} \times k_{k_2}) + (P_b \times k_b)]\} \qquad (2.1a)$$

$$+ \{P_u \times \text{sneaking rate} \times k_s\}. \qquad (2.1b)$$

The estimated fitness values (k_i) for each possible means of gaining fitness (i) were assigned according to a set of basic assumptions. We assume that in the absence of cuckolders and cannibals a spawning event is successful, so the male gains a full unit of fitness ($k_n=1$). Any spawning event that involved cannibalism yields no fitness because it is assumed that all of the egg(s) are eaten; therefore the value of spawning in the presence of cannibals (k_c) and cannibals and cuckolders (k_b) is zero. The presence of one cuckolder at a spawning event reduces the paired male's chance of fertilizing the egg(s) by half ($k_{k_1}=0.5$), because two males are competing for fertilization. Likewise, the presence of two or more cuckolders would reduce the male's chance of a successful spawning event by two-thirds ($k_{k_2}=0.33$). Although we have observed up to 10 males cuckolding a single spawning event, this happens rarely compared with cuckoldry by one or two sneakers at a time (median no. of cuckolders = 1.0). When unpaired and acting as a sneaker (cuckolding paired males), we assume a male has only half a chance of fertilizing the egg(s) ($k_s=0.5$). Without further work on the determinants of the actual fertilization success of sneakers (e.g. the distance and position of the cuckolder relative to the female may contribute to the chance of fertilizing an egg when sneaking), this is our best approximation of the possibility of gaining fitness from this behaviour. Sensitivity analyses on each variable suggest our approximations are robust (see the electronic supplementary material for details).

(c) *Measurement and calculation of colour and brightness contrast*

We used a radiometer (Ocean Optics USB2000) with a radiance probe (HOBI Labs) to measure the colour reflected from the fish and the background against which it was viewed under ambient photic conditions in the field. The radiometer was calibrated in the Loew laboratory using a Gamma Scientific irradiance standard, calibrated over the wavelength range of 250–2600 nm, and a magnesium sulphate white reflectance standard. All radiance measurements were taken on transects between 10.00 and 12.00 hours, the time interval when mating behaviour peaks. Individual fish were captured and immediately killed in an overdose of clove oil (5.0 ppt). The fish was placed on a mechanical frame that holds it suspended in the water (at 1.0 m depth) and maintains a constant distance (10 cm) between the fish and the radiance probe, with a circular patch size of 0.4 cm diameter. The measurements were taken with the probe pointing towards the shore, thus representing what a female would see if she viewed a male fish from the side. Since most spawning takes place very close to the shore, this was our best approximation of a realistic situation. Two patches on each fish were measured: mid-body below the anterior insertion of the second dorsal fin, and on the anterior basal portion of the second dorsal fin itself. After the patches were measured, the fish was moved out of the field of the probe and a measurement of the ambient background light was taken. The entire procedure for one fish was completed in less than 10 min to minimize post-mortem loss of colour intensity.

Radiance values were standardized following Endler (1990; see the electronic supplementary material for details). The predominant spectral component of ambient light (i.e. predominant ambient colour) was determined using the spectral index (λp50), calculated as the wavelength that halves the total photon count (i.e. the area under the spectral radiance curve; McDonald & Hawryshyn 1995). Although a single value cannot fully explain the variation in a spectral curve (e.g. breadth, shape), the λp50 at least provides a single wavelength around which the majority of photons are likely to be (McFarland & Munz 1975) and which can be compared statistically between environments. We used one-way ANOVA to test for a difference in λp50 between beach ($n=29$) and root ($n=33$) habitats (samples from different sites within a habitat were pooled owing to limited samples). In a similar analysis, we also evaluated the maximum wavelength at which the spectra for beach and root habitats peaked.

Colour patches that differ spectrally from the visual background should have higher perceived contrast than patches that more closely match the background (Lythgoe 1979). Chromatic and brightness contrast were calculated by taking the difference between patch and background (Uy & Endler 2004) using the spectral sensitivities of retinal photopigments of a closely related congener (table S3 in the electronic supplementary material), so that contrast values are based on a fish's perception of the optical signal under natural conditions. From the mean $\lambda_{\max}$ for each cone class, we generated nomograms that were used in combination with the corrected radiance spectra for each measurement to determine the Euclidean contrast values (Endler 1990). Radiance spectra for the patch and the background were each multiplied by the nomogram spectrum (standardized for cone sensitivity) for a cone class and summed over all wavelengths. To determine chromatic contrast, the difference between the summed patch and background values for each cone class was squared; the squared values were summed among cone types and the square root taken of the sum. Brightness contrast was calculated by taking the difference between the summed patch values across all cone types and the summed background values across all cone types, divided by the sum of patch and background values. This gives a value ranging from $+1.0$ to -1.0, where positive values mean the patch is brighter than the background, negative values mean the background is brighter than the patch and values around 0 mean the patch and background are similar with respect to brightness. Both procedures were repeated for all morphs (each colour patch separately) in both the beach and root habitats.

(d) *Statistical analyses*

Frequency data and all probabilities were arcsine square root transformed for normality (Field 2005). We used two-way ANOVAs with habitat and morph as independent variables to test whether the variable of interest (i.e. morph frequency, contrast, reproductive fitness) differed between habitats. ANOVAs were also used to determine how each variable differed between morphs within each habitat. *Post hoc* Tukey's HSD tests were used to make pairwise comparisons between each morph within a habitat if the ANOVA test was significant. For contrast analyses, we also performed individual *t*-tests (two-tailed), one for each morph, to test whether contrast in a morph varied significantly between habitats. Tests for homogeneity of variance were conducted prior to analyses. All other assumptions of were met. All statistical analyses were performed using SPSS v. 15.0.

We used a simple regression analysis to determine whether chromatic contrast predicts reproductive fitness. A multiple regression analysis was used to test if contrast and morph frequency, combined, predict reproductive fitness. In addition to the ANOVAs used to test for between- and within-habitat differences in morph reproductive fitness, we also performed a more conservative test using only the two morphs, blue and yellow, which had the highest contrast compared with all other morphs, as we expected them to also have the greatest difference in fitness. We took the difference in fitness between blue and yellow males at each site and performed a *t*-test (two-tailed) with habitat as the independent variable and site as the unit of replication ($n=12$).

3. RESULTS

(a) *Visual background and morph contrast*

The visual background of the two mating habitats differed spectrally, and correspondingly so did morph colour contrast. The beach habitat had higher λp50 (ANOVA: $F_{1,59}=112.2, p<0.0001$) than did root habitat, indicating more yellow shifted light in beach compared with root habitat (table S2 in the electronic supplementary material). This is also evident by the difference in wavelength at which radiance peaked (mean beach: 578 ± 1.1 nm; mean root: 563 ± 0.01 nm; ANOVA: $F_{1,59}=80.41, p<0.0001$).

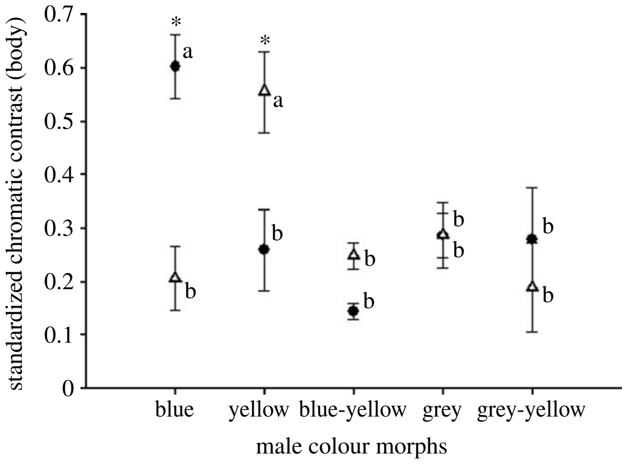

Figure 1. Mean chromatic body contrast for five male colour morphs in two mating habitats. Morphs vary in mean chromatic body contrast (error bars are ±1 s.e.) between and within beach (filled circles) and root (open triangles) mating habitats. Within a habitat different letters indicate morphs that are significantly different from each other, as determined by Tukey's HSD ($q_{\text{beach}}=2.94$, $q_{\text{root}}=2.91$, $\alpha=0.05$). Asterisks indicate where a morph differs significantly between beach and root sites (two-tailed *t*-tests).

In the beach habitat, blue male bodies contrasted chromatically with the background more than any other morph (ANOVA: $F_{4,28}=5.48, p=0.003$; figure 1). In the root habitat, the yellow males contrasted more with the background than any other morph (ANOVA: $F_{4,33}=5.405, p=0.002$; figure 1). The blue and yellow morphs were also differentially conspicuous between habitats (*t*-tests between habitats for each morph separately: blue, $t_{16}=4.23, p=0.0004$; yellow, $t_{16}=2.733, p=0.015$). The other three morphs had similar relative body chromatic contrast in both habitats. There was no difference between fin chromatic contrast, or body and fin brightness contrast between morphs within or between habitats within a morph, except for body brightness in the blue morph: blue males have higher brightness contrast in the root relative to the beach habitat ($t_{16}=3.96, p=0.001$).

(b) *Morph frequency*

All five *T. sarasinorum* male colour morphs were found mating in both habitats, but at varying frequencies (two-way ANOVA: $F_{4,50}=37.0$, $p<0.0001$; separate ANOVAs: beach $F_{4,29}=29.6$, $p<0.0001$; root $F_{4,29}=68.1$, $p<0.0001$; figure S3 in the electronic supplementary material). Blue morphs were significantly more frequent than any other morph in the beach habitat, whereas yellow males were more frequent than all other morphs in the root habitat (Tukey's HSD $\alpha=0.05$; figure S3 in the electronic supplementary material).

We wanted to test whether morph frequency was influenced by the spatial and temporal observational units used to collect the data. A five-way ANOVA with morph frequency (arcsine square root transformed) as the dependent variable, and male colour morph, habitat, time period, season and site as independent variables showed that only habitat (morph × habitat: $p<0.001$) and site (morph × site: $p<0.001$) influenced differences in

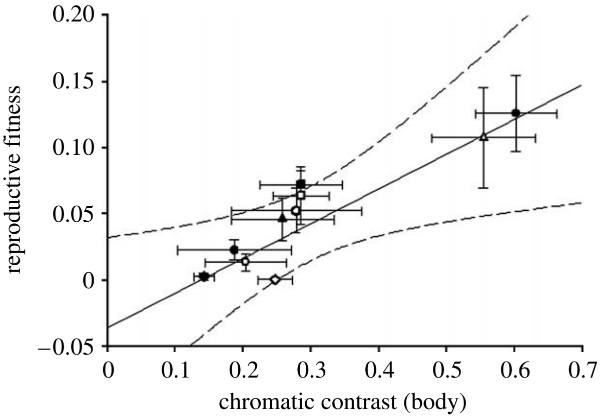

Figure 2. Regression of chromatic body contrast on reproductive fitness across habitats. Chromatic contrast significantly predicts reproductive fitness across habitats (filled symbols, beach; open symbols, root). Male colour morphs are distinguished by symbols: circles, blue; triangles, yellow; diamonds, blue-yellow; squares, grey; hexagons, grey-yellow. Error bars are ±1 s.e. for mean body chromatic contrast (vertical) and mean reproductive fitness (horizontal).

frequency between morphs. In a secondary analysis that removed all non-significant interaction terms and those terms that did not include 'morph', the morph×period term became significant ($p=0.01$), while the other terms remained significant. Limited sample sizes of colour and behavioural data necessitated using values pooled for each morph and habitat, and we therefore lacked the power to test site and time period effects in our main analyses of colour contrast and reproductive fitness.

(c) *Reproductive fitness*

We found that conspicuousness accounts for 84.1% of the variation in reproductive fitness ($r^2=0.841$, $p<0.0001$; figure 2). When morph frequency was added as a second predictor to the regression analysis, the variation in fitness explained by the model increased by 8.5% ($r^2=0.926$, $p<0.0001$). The change in r^2 was significant ($p=0.03$). This suggests that sexual selection favours higher contrast as predicted by theory (Andersson 1994); however, in this case, contrast varies across a spatially heterogeneous environment such that alternate morphs are favoured dependent upon the environment (two-way ANOVA: habitat×morph $F_{4,50}=4.93$, $p=0.002$). Moreover, in a more conservative analysis based on the key prediction emerging from our contrast analyses, the difference in fitness between blue and yellow males (i.e. blue morphs were the most conspicuous morph in the beach habitat and yellow males in the root habitat) varies significantly across habitats (t-test, $t_{10}=3.63$, $p=0.004$, $\text{mean}_{\text{beach}}=0.08$ (0.03 s.e.), $\text{mean}_{\text{root}}=-0.09$ (0.03 s.e.)).

Although sexual selection appears to favour conspicuousness across habitats, we found that within a habitat less conspicuous morphs also realize some reproductive fitness. In the beach habitat, where blue males are the most conspicuous morph, both blue and grey males shared the highest mating success (ANOVA: $F_{4,29}=6.78$, $p=0.0008$; *post hoc* Tukey's HSD, $q=2.94$, $\alpha=0.05$; figure 3), while in the root habitat, where yellow males are more conspicuous than all other morphs, both yellow and grey males had similarly high mating success (ANOVA: $F_{4,29}=4.82$, $p=0.005$; *post hoc* Tukey's HSD, $q=2.94$, $\alpha=0.05$; figure 3).

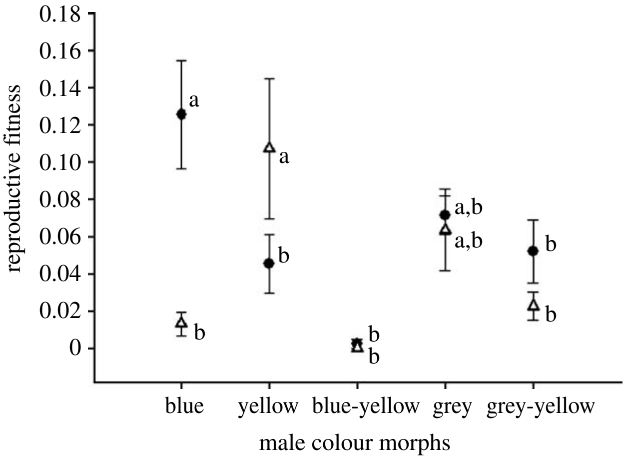

Figure 3. Estimated reproductive fitness of five male colour morphs in two mating habitats. Morphs vary in mean reproductive fitness (error bars are ±1 s.e.) between and within beach (filled circles) and root (open triangles) mating habitats. Within a habitat different letters indicate morphs that are significantly different from each other, as determined by Tukey's HSD ($q_{\text{beach}}=2.94$, $q_{\text{root}}=2.94$, $\alpha=0.05$).

4. DISCUSSION

Male reproductive fitness was significantly positively predicted by colour contrast, and the direction of selection varied between habitats. Blue males were favoured in the beach habitat and yellow males in the root habitat, where those morphs are, respectively, the most conspicuous and the most abundant. To the best of our knowledge, this is the first time that observations of reproductive success in nature, with replicated habitat classes, have been combined with field spectral measurements and perceptual models of conspicuousness to test the hypothesis of environment-contingent sexual selection. Our results suggest that diversifying selection in the form of differential reproductive success across visual environments contributes to the maintenance of this polymorphism. Alternative, within-habitat mechanisms may be needed to explain the observation that the grey morph, which is always less conspicuous than blue or yellow fish, shared high reproductive fitness within each habitat (further analyses of reproductive success variables will be presented elsewhere). In both habitats, the grey morph had lower reproductive success than the most conspicuous morph, although not significantly so. It is possible that with a larger sample size (or the use of a less conservative pairwise comparison) this trend would become significant.

It is clear from the literature that rarely does one mechanism maintain polymorphism in nature (e.g. Gray & McKinnon 2007) and mechanisms other than straightforward sexual or natural selection may operate (e.g. Magurran 1998; Sinervo & Svensson 2002; Blows *et al.* 2003). Here, we show that diversifying selection acts strongly on the two most conspicuous male colour morphs: blue and yellow. The blue-yellow and grey-yellow morphs that have more complex colour patterns were not particularly conspicuous in either habitat, nor were they successful at achieving reproductive success. This result is suggestive of disruptive selection, especially for blue-yellow males. These morphs may represent 'intermediate' forms (e.g. heterozygotes) that are at a mating disadvantage in both habitats, although behavioural tactics such as sneaking may facilitate their persistence. Little evidence for disruptive sexual selection maintaining a colour polymorphism exists (although assortative mating is well documented in some cichlids: Seehausen & van Alphen 1998). Greene *et al.* (2000) have documented disruptive selection on male colour in lazuli buntings (*Passerina amoena*). More recently, van der Sluijs *et al.* (2008) showed the potential for disruptive sexual selection via a female preference polymorphism in a cichlid fish that is currently constrained by a homogeneous visual environment. More detailed studies of within-habitat processes are necessary to understand if this mechanism is acting in *T. sarasinorum*.

The spatial scale at which animals experience environmental heterogeneity is important (Joron & Iwasa 2005; Gray & McKinnon 2007). Here, we measured only a snapshot of the background against which the fish are viewed. The root habitat is structurally more complex than the beach habitat, and further inspection of fine-scale variation in microhabitat lighting properties may be enlightening. For example, under very specific conditions the blue-yellow and grey-yellow morphs may be more conspicuous and may garner some reproductive success in those times and places where the background against which they are viewed makes them perceptually more attractive to females. Again, this points to within-site processes that help to maintain colour polymorphism in this species.

A recent resurgence of work based on natural populations has suggested an important role for both negative frequency-dependent natural (Nosil 2006; Olendorf *et al.* 2006) and sexual (Hughes *et al.* 1999; Eakley & Houde 2004) selection in maintaining colour polymorphisms. In *T. sarasinorum*, morph frequency is positively correlated with reproductive fitness, suggesting a limited role for negative frequency-dependent selection, at least at the spatial and temporal scales at which we estimated fitness. The results of the multiple regression analysis suggest that morph frequency is somewhat less important than visual habitat, at least across habitats. Morph frequencies remained the same over three field seasons. This consistency possibly results from frequency dependence that is difficult to detect owing to minimal variation in frequency within sites. Frequency-dependent male–male competition appears to be important in the diversification of male nuptial coloration in territorial Lake Victoria cichlid fishes, especially in interspecies interactions (Seehausen & Schluter 2004; Dijkstra *et al.* 2005). Until more is known about male–male competition between the five *T. sarasinorum* male colour morphs in each of the two habitats, negative frequency-dependent selection through such a mechanism cannot be ruled out.

In many cases, directional sexual selection is balanced by natural selection in the form of predation against conspicuous colour patterns, such as in the guppy (*Poecilia reticulata*; Endler 1983; also see Stuart-Fox *et al.* 2004). Little is known about major predators on adult telmatherinids in Lake Matano and attempts to determine if the only large fish, *Glossogobius* sp., eats telmatherinids, were inconclusive. It is very possible that predation on the fertilized eggs of conspicuous males is a factor in the maintenance of this polymorphism; however, further investigation will be required to test this hypothesis.

The Malili Lakes are home to several adaptive radiations of diverse taxa (von Rintelen *et al.* 2004; Herder *et al.* 2006*a*), including the endemic telmatherinids, most of which are male colour polymorphic (Gray & McKinnon 2006; Herder *et al.* 2006*b*). This presents an opportunity to examine parallel mechanisms of polymorphism maintenance. The system as a whole also currently offers the opportunity to study a relatively pristine environment compared with other systems, such as Lake Victoria where eutrophication has led to the loss of colour diversity in some cichlid fishes (Seehausen *et al.* 1997). Unfortunately, the condition of the Malili Lakes could change drastically in the next few years if land development and mining activities in the area are not controlled.

The study protocol was approved by SFU's Animal Care Committee, and met the CCAC guidelines.

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