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Author for correspondence:
Sean T. Giery
e-mail: stgiery@gmail.com

†Present address: Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA.

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Dissolved organic carbon and unimodal variation in sexual signal coloration in mosquitofish: a role for light limitation?

Sean T. Giery† and Craig A. Layman

Department of Applied Ecology, North Carolina State University, Raleigh, NC 27695, USA

(iD) STG, 0000-0003-3774-5295

Natural selection plays an important role in the evolution of sexual communication systems. Here, we assess the effect of two well-known selection agents, transmission environment and predation, on interpopulation variation in sexual signals. Our model system is a series of 21 populations of Bahamian mosquitofish subjected to independent variation in optical conditions and predation risk. We show that optically diverse environments, caused by locally variable dissolved organic carbon concentrations, rather than spatial variation in predation, drove divergence in fin coloration (fin redness). We found a unimodal pattern of phenotypic variation along the optical gradient indicating a threshold-type response of visual signals to broad variation in optical conditions. We discuss evolutionary and ecological mechanisms that may drive such a pattern as well as the implications of non-monotonic clines for evolutionary differentiation.

1. Introduction

Optical variation among aquatic ecosystems has strong effects on fundamental ecological processes [1]. In shallow waters, a major driver of optical variation is the concentration of dissolved organic carbon (DOC) [2,3]. Essentially, by affecting the light environment, DOC drives a variety of ecological processes, from consumer foraging and habitat use [4], to the thermal stratification of lakes [5]. But perhaps the greatest effect of DOC is its effect on primary production. By strongly absorbing photosynthetically active radiation, DOC inhibits primary production at high concentrations [6–8] indirectly reducing zoobenthic production [9], the abundance of predatory fishes [6,10] and their somatic growth [11]. Yet, the effects of optical variation due to DOC on evolutionary processes are less well understood, despite the fact that many aquatic organisms employ visual signals during courtship and territorial displays.

Two types of mechanisms, evolutionary and ecological, appear likely to drive DOC-mediated effects on visual signals and sexual communication. First, light limitation can presumably reduce the effectiveness of visual signals, ultimately shaping their evolutionary trajectory through natural selection on signal performance (e.g. transmission). These selection-based explanations for signal–environment correlations typically draw upon evolutionary models such as sensory drive [12,13] to provide a mechanistic basis for divergence. Indeed, empirical evidence lends support to these evolutionary mechanisms by showing that spatially heterogeneous optical conditions can drive divergence in sensory systems and signal attributes [14–16]. Second, light conditions can also limit signal production and maintenance, representing a more proximate, ecological, basis for signal divergence. We believe this inhibitory effect can arise via two nutritional pathways, pigment limitation and energetic limitation. The development of many colourful signals in animals depends on the acquisition of plant-derived pigments (i.e. carotenoids) [17]. As discussed previously, DOC is likely to limit the availability of these algal-derived pigments by stifling primary production at high concentrations. Whether bottom-up limitation of sexual signals could be driven by DOC is unknown. However, existing evidence for canopy-driven limitations on carotenoid supply and signal development in

(a)

(b)

Figure 1. Male Bahamian *Gambusia* vary widely in coloration among populations. Individuals in panel (a) represent the diversity of coloration exhibited among populations on Abaco Island, The Bahamas. Panel (b) depicts the diversity of optical environments characterizing mosquitofish habitats. (Online version in colour.)

guppies suggests it may play an important role [18]. Alternatively, because signals are energetically costly to produce and maintain, light limitation may simply have negative effects on signal production by driving reduced male condition. Since most elaborate signals appear to have some degree of condition dependence, such a link between low mean condition and low mean signal elaboration seems likely.

Our primary objective in this study was to better understand the effects of optical variation on interpopulation divergence in sexually selected visual traits. We explored this effect by sampling a series of 21 Bahamian mosquitofish populations on Abaco Island (*Gambusia bahamasminimus*) across an optical gradient driven primarily by DOC. We focused on three male traits used during courtship displays and subject to female preference: dorsal fin redness, anal fin redness, and gonopodia length [19,20]. While DOC was the focal driver in our study, other ecological costs such as predation are potent evolutionary agents (reviewed in [21,22]) that also drive the adaptive evolution of sexual signals in this system [20,23,24], and many others [25–28]. Therefore, we included predation surveys in our study to assess this effect in concert with the spectral environment, and in doing so we offer an assessment of their individual and combined effects on sexual signals.

2. Methods

(a) Study system and sampling

This study was conducted on Abaco Island, The Bahamas. Mosquitofish populations on Abaco are of an undescribed species, tentatively named *Gambusia bahamasminimus* (previously *G. hubbsi*), within a Bahamian clade consisting of at least three phenotypically and ecologically similar species [29]. Bahamian mosquitofish are small, live-bearing, fish found in a variety of aquatic habitats [24,30,31]. Males exhibit enlarged, orange-red coloured, condition-dependent dorsal fins subject to sexual selection via female preference [20,23,24]. Males also possess anal fins with orange-coloured membranes and an elongate sperm-transfer organ formed from modified fin rays 3–5, the gonopodium [23]. Fin coloration in Bahamian mosquitofish has heritable components, but also seems affected by diet [23].

For this study we sampled *Gambusia* populations between June and August in 2011. To encompass a wide range of spectral conditions we sampled a variety of aquatic ecosystems throughout Abaco including blue holes, freshwater marshes and mangrove-lined tidal creeks. From each population we collected 4–71 adult males, measured their standard length (*SL*), gonopodium length (*GL*) and photographed them live for colour analysis following Giery & Layman [24]. Fin coloration was measured in RGB colour space using Adobe Photoshop CS5. RGB defines colour in three-dimensions where R indicates red, G indicates green and B
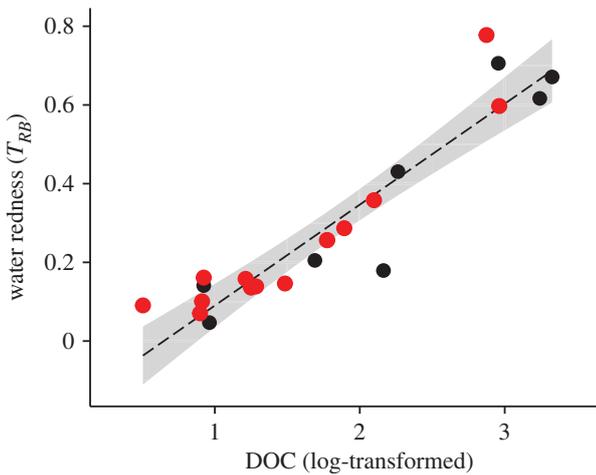
Figure 2. Dissolved organic carbon (DOC) strongly absorbs short and medium-wavelength light generating an asymptotic relationship between DOC and water redness (note the log-transformed x-axis). Non-predator populations are displayed in black predator populations in red (or grey). (Online version in colour.)

indicates blue. Following Endler [32] we use RG, an index of signal coloration $(R - G)/(R + G)$ that estimates colour (hue) along an axis from red (1) to yellow (0) and green (-1) which encompasses the range of colour (yellow-red) expressed in Bahamian *Gambusia* (figure 1; electronic supplementary material).

To describe the optical conditions in each site we passed a beam of light between two fibre optic cables fitted with co-limiting lenses positioned 10 cm apart and submerged in a basin of water collected from each site. Water colour was calculated as the amount of light at particular wavelengths reaching the spectrometer (Jaz, Ocean Optics, FL) after being transmitted through a sample, proportional to a distilled water standard (e.g. T = water sample/control). We restricted transmission measures to 370 and 570 nm because these wavelengths correspond with peak sensitivities for short and long-wavelength cones in poeciliid fishes (e.g. [33,34]), and piscivorous fishes ([35], electronic supplementary material). Gross patterns in water colour, i.e. the spectral shift that typifies our study system (reddening), were estimated with an index, T_{RB}. This index is the difference in transmission between long (red) and short (UV/violet) wavelengths $[(T_{570} - T_{370})/(T_{570} + T_{370})]$ with larger positive T_{RB} values indicating long wavelength dominated environments and large negative values indicating environments dominated by short wavelengths (hereafter called 'red' and 'blue' environments, respectively).

We sampled DOC concentrations between June and November 2012. Water samples were passed through precombusted (400°C for 4 h) Whatman GF/F filters. Filtered samples were analysed *in situ* with a Turner Aquaflor fluorometer and recorded in relative fluorescence units (RFU). RFUs were converted to mg C l^{-1} using field-collected samples measured for TOC concentrations following method 5310B [36]. Two to four samples were collected from each study site across the sample period. Repeatability was high among samples within sites ($r = 0.8$) indicating consistent DOC concentrations across the sampling period [37] so DOC measures were averaged for analysis (electronic supplementary material, table S1). Annual variation in DOC was not assessed in this study. However, previous study on Abaco shows that water colour, a product of DOC concentration in part [2], is largely consistent from year to year (see table S1 in [30]). To assess the contribution of DOC to site-level variation in optical environments we regressed T_{RB} against DOC (log-transformed). We also generated qualitative estimates of predator risk (predatory fish present/absent) for each site using visual surveys following Giery & Layman [24].

Table 1. Results of linear mixed models examining the effects of predation and water colour on signalling traits in *Gambusia*. Random effects (population and the interaction of population and SL) were included in both models.

trait	source	d.f.	F	p
fin redness (RGd)	SL(log)	1,14.5	31.6	<0.0001
	T_{RB}	1,16.9	3.8	0.0696
	T_{RB}^2	1,17.5	13.2	0.0020
fin redness (RGg)	SL(log)	1,13.6	92.7	<0.0001
	T_{RB}	1,18.1	0.5	0.5105
	T_{RB}^2	1,18.7	5.6	0.0290
gonopodium length (GL)	SL(log)	1,10.8	3863.5	<0.0001

(b) Data analysis

We began our analysis by checking for heterogeneous trait allometries between predation regimes [19,29]. We used linear mixed models (LMM) in which focal signalling traits were dependent variables. SL(log-transformed), predation, and their interaction were fixed effects. Population and population*SL were random effects in order to allow population-level heterogeneity in slopes and intercepts. There was no significant effect of predation on the slopes of the $SL - GL$, $SL - RGd$, or $SL - RGg$ relationships ($p = 0.4$; $p = 0.56$; $p = 0.72$, respectively). Predator-dependent allometry was not considered further.

To test for effects of water colour and predation on signal morphology we employed linear mixed models. We ran separate models for each focal trait: GL, RGd and RGg. Initial visual inspection of our raw data (e.g. RGd on T_{RB}) suggested a quadratic effect of T_{RB} for fin coloration. While not part of our original prediction, we chose to include quadratic T_{RB} terms in all models. Independent variables included log-transformed standard length (SL), water colour (T_{RB}), predator regime, the interaction between T_{RB} and predator regime, a quadratic term (T_{RB}^2), and its interaction with predation regime. Population and the interaction between population and SL were included as random terms to allow unique allometries for each population. Models were then reduced by sequential model comparison using log-likelihood comparisons [38]. Independent variables that did not significantly improve fit ($p > 0.05$) were removed. Variance inflation factors were greater than five for the predator*T_{RB}^2 term in all full models and removed prior to log-likelihood comparisons.

We also assessed the effect of geographical distances on the similarities of site conditions (T_{RB}, predator) and size-corrected morphological traits (RGd, RGg and GL) using Mantel tests. p-values were generated from 9999 simulations.

3. Results

DOC concentrations were highly variable among sites (electronic supplementary material, table S1), but typical for coastal ecosystems in the region [39]. Optical environments (T_{RB}) were also extremely variable among sites (electronic supplementary material, table S1) and strongly associated with DOC ($F_{1,19} = 129.9$, $p < 0.001$, $R^2 = 0.87$; figure 2). Piscivores such as snappers (*Lutjanus* spp.), needlefish (*Stongylura* spp.) and great barracuda (*Sphyraena barracuda*) were detected co-existing with 14 mosquitofish populations (electronic supplementary material, table S1). The predator status of each population has been maintained for at least several years, see

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Giery & Layman [24]. Importantly, a difference between predator and no-predator habitats in optical conditions T_{RB} was not detected ($t = -1.4$, d.f. = 19, $p = 0.20$).

We captured, photographed and subsequently measured 879 male mosquitofish (electronic supplementary material, table S2). Visual signals expressed by male mosquitofish varied considerably among populations (electronic supplementary material, table S2). Our reduced LMM explaining interpopulation variation in fin colour (RGd and RGg) included linear and quadratic water colour terms (table 1). Yet, the only significant effect in each model, besides the body size covariate (SL) was the quadratic term ($p < 0.03$) indicating a unimodal relationship between water colour and the coloration of dorsal and anal fins (figure 3). For gonopodium length, all focal terms besides SL were removed during model reduction indicating little-to-no effect of observed ecological variation on gonopodium length (figure 3 and table 1).

Geographical distance was not correlated with morphological traits ($r = 0.06$, $p = 0.26$) or ecological characters ($r = 0.01$ $p = 0.46$) indicating that phenotypic and ecological variation among sites are not driven by geographical proximity alone. Simple mantel tests performed for each morphological and ecological variable also failed to reveal a significant geographical effect (electronic supplementary material, table S3).

4. Discussion

High concentrations of DOC removed a substantial fraction of short-wavelength radiation from aquatic habitats and generated a strong gradient in water colour (figures 1 and 2). In turn, spatial heterogeneity in the optical environment appeared to drive phenotypic divergence in sexual signals used by Bahamian mosquitofish. We found no evidence for a predation effect in this study suggesting a relatively weak influence of predation pressure on sexual signals in our system—a result consistent with some of our previous findings [30], but inconsistent with others [23,24]. Despite this contribution, the most notable result from our study was not that optical environments are more important for sexual signal expression, but rather that this effect was non-monotonic (i.e. 'hump-shaped').

Most interpretations of light-mediated signal divergence in fishes favour evolutionary processes such as sensory drive to explain interpopulation variation in signal coloration (e.g. [40–42]). Although the direction of divergence appears to vary among species (negative or positive slopes along optical gradients), linear or near-linear relationships appear typical for continuous traits and are commonly regarded as indicators of an adaptive response to optical conditions [40,43]. However, the form of the response we found differs substantially from these existing empirical data and their underlying theoretical prediction. We believe that this difference is due, at least in part, to bottom-up ecological controls on signal elaboration, but we can only speculate without further examination of underlying mechanisms. Following, we discuss several hypotheses about how ecological variation might drive the observed pattern of phenotypic differentiation in order to stimulate new research in this field. We begin with evolutionary mechanisms and follow with ecological ones.

Our first two hypotheses invoke a role for functional thresholds in the evolution of visual communication. If phenotypic (signal) variation is constrained, selection on
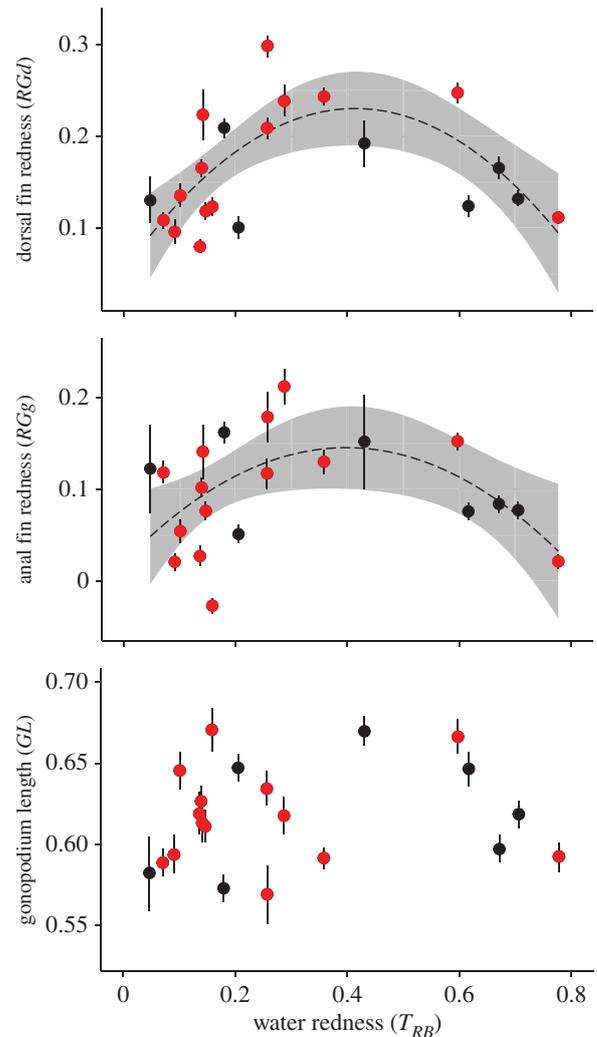
Figure 3. Water redness (T_{RB}) appears to have a strong nonlinear effect on mean (+ standard error) sexual signal coloration for 21 populations of Bahamian *Gambusia*: mean dorsal fin redness (RGd), top panel; and mean anal fin (RGg) redness, middle panel. No effect of water colour was detected for gonopodium length (GL), bottom panel. Predation was dropped from all models during model selection, however populations subject to predation are displayed here in red (or grey), non-predator populations in black. All traits are partialed for the effect of body size (Log SL). (Online version in colour.)

signal efficacy may produce thresholds at which selection for maximum conspicuity (e.g. via chromatic contrast) favours complex patterns of phenotypic divergence. For example, if signal coloration is constrained between yellow and red, then across a gradient of spectral conditions (e.g. blue to red), optimal signal design via chromatic contrast requires a nonlinear relationship between signal and environment to maximize conspicuity: e.g. yellow signals in blue environments, red signals in green environments, and yellow signals in red environments. The role of constraints in the adaptive diversification of visual signals has not been a focus of study, yet such an effect seems compatible with sensory drive hypotheses [13,44], with empirical studies lending support to this. For example, Fuller [45] shows that colour-polymorphic bluefin killifish (*Lucania goodei*) maximize conspicuity rather than maintain specific hues across a DOC-driven water colour gradient. The result is that blue-finned fish are common in red environments, and red-finned fish are common in blue environments. Such a pattern suggests that sexual selection may act on signal conspicuousness via chromatic contrast

when the optical environment is highly variable. While such a strategy seems well-established, it has never been shown for fishes that are not colour polymorphic, such as Bahamian mosquitofish. Therefore, a role for evolutionary constraints in these cases remains unexplored.

It is also plausible that the yellow coloration exhibited by fishes inhabiting the most red optical conditions simply reflects lowered investment in signal production. Disruptive transmission environments can relax selection on sexual signal production [46,47]. If the transmission environment is antagonistic to effective signalling, investment in signal production may constitute a substantial fitness cost [48] which could explain the predominance of yellow signals (i.e. less carotenoid rich) that we observe in disruptive signal environs. While we can only speculate as to whether divergent signal coloration across the DOC gradient is adaptive, the idea of colour signals 'tuned' to suit environmental variation generates appealing hypotheses with abundant support from a variety of study systems.

Alternatively, the effect of DOC on sexual signal coloration may be entirely ecological. That is, DOC-mediated light limitation may simply suppress sexual signal production via bottom-up controls. Light-limitation in oligotrophic aquatic ecosystems (like those in The Bahamas) strongly regulates productivity [6,9]. These effects can propagate up trophic levels, affecting zooplankton abundance, and reducing individual growth rates and population sizes of vertebrate consumers (i.e. fish; [6,10,11]). Therefore, at high DOC concentrations, a bottom-up reduction in primary productivity and pelagic zooplankton biomass could in turn limit the availability of carotenoids and/or the energy available for the production of red sexual signals [17,18]. Interestingly, several researchers also have shown unimodal responses in fish population density [10] and growth rate [11] across a gradient of DOC. They attributed this response to an ecological threshold at which DOC switches from a stimulator of lake productivity to an inhibitor via shading effects. Similarities in the response to increasing DOC, although superficial, point to a nonlinear, mechanistic link between light limitation, ecosystem productivity, growth, and the production of sexual signals in our system. Indeed, when we explored this link further using male *Gambusia* growth data collected previously from nine of our study sites [31], we found that growth rates also followed a unimodal pattern along the DOC gradient (electronic supplementary material, figure S1) providing corroborative evidence for bottom-up regulation of fish secondary production at high DOC concentrations. While merely suggestive, these supplemental data provide additional evidence for strong, bottom-up, ecological control of signal production via nutritional pathways. And while evolutionary hypotheses such as sensory drive provide attractive explanations for observed correlations between signal and optical environment, we suggest that one should not overlook the ecological effects that accompany variable optical conditions and the potential for bottom-up regulation of sexual signals.

What do these results, and those of previous studies on Bahamian mosquitofish, suggest about the importance of predation for the evolution of sexual signals in this system? Here, we find no significant effect of predators on interpopulation variation in sexual signals, a result shared with a previous archipelago-wide study [30], yet inconsistent with others [23,24]. These inconsistencies highlight heterogeneous prey responses to variable predation pressures in these systems, a

tendency seen in guppy sexual signals as well [49,50]. Underlying causes of variable responses to predation remain unclear, however several explanations seem likely. First, anti-predator adaptations may compensate for increased cost of signalling under high predation risk [22,51,52]. Second, predators might also regulate bottom-up drivers of trait variation [18] through trophic cascades — a hypothesis in line with the light limitation hypothesis detailed above. Essentially, these inconsistencies show that much about the effects of predators on sexual selected traits remains to be explained. Nevertheless, it seems clear that detectible effects of predators, in this system and others, are variable or at least hard to predict based on simplified scenarios of ecological costs [25,53].

At a more fundamental level, our results provide evidence that ecological gradients can generate complex patterns of phenotypic variation. In turn, nonlinear phenotypic responses to these gradients may have important evolutionary and ecological implications [54]. For example, non-monotonic divergence along environmental gradients could dampen local adaptation if phenotypes at disparate ends of ecological gradients are convergent, as in our system. Therefore, assortative mating could weaken local adaptation by facilitating gene flow among populations [55]. This suggests that adaptive divergence in sexual signals along environmental gradients may not necessarily favour reproductive isolation due to assortative mating by signal phenotype, a common phenomenon in fishes [56], and a hypothetically important mechanism in models of speciation with gene flow (e.g. [57,58]).

5. Conclusion

Despite a wealth of study on a suppressive role for predation regarding the elaboration of sexual traits, we found no such effect in our study. Rather, our data indicate that water colour was a strong driver of variation in the colour of a sexually selected trait. While these findings are important, we note that the more intriguing result is the complex relationship between sexual signal coloration in Bahamian mosquitofish and the optical properties of the environmental. In fact, this study is one of the first to identify non-monotic divergence in sexual signals due to environmental variation. Further study of the underlying mechanism(s) and evolutionary implications of this divergence pattern is needed. Nevertheless, our results clearly illustrate that the coupling of evolutionary and ecological dynamics are important for sexual signalling systems, and given the prevalence of optical heterogeneity in nature, other systems are also likely to exhibit complex divergence patterns.

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