Dietary Carotenoids Increase Yellow Nonpigment Coloration of Female Convict Cichlids (*Amantitlania nigrofasciata*)

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ABSTRACT

The carotenoid trade-off hypothesis states that diet-derived carotenoids are traded off among competing physiological demands, but this statement is rarely tested in ornamented females. In this study, reverse sexually dimorphic convict cichlids (*Amantitlania nigrofasciata*) were fed diets containing carotenoid supplementation at three biologically relevant levels for 12 wk. This treatment was followed by spectral, microscopic, and chemical analysis to determine how females allocated the pigments to tissues and how those decisions affected their ventral patch coloration. Yellow coloration of the integument increased with carotenoids in the diet, as did carotenoids deposited in ovaries, but diet did not change carotenoid allocation to skin. The results of this study suggest that females have the ability to modulate their expression of yellow coloration via an alternative coloration strategy. Gonadosomatic index and tank environment were also related to ventral patch color, supporting previous behavioral work highlighting the importance of social selection in reinforcing signal honesty.

Introduction

Melanins, pterins, and carotenoids are the three types of pigments used in integument coloration of vertebrates (Ferrer et al. 1999; Grether et al. 2001; Steffen and McGraw 2007), but while both melanins and pterins can be synthesized by animals, carotenoid pigments must be obtained from the diet (Palmer 1922; Goodwin 1984; Alonso-Alvarez et al. 2008; Vinkler and Albrecht 2010). Keratin-based or purine-based structural color can also contribute to coloration in some taxa (McGraw et al. 2011). The contributions of multiple color-producing factors may be difficult to disentangle in studies of animal ornamentation (Grether et al. 2004*b*).

Carotenoid pigments are the focus of many studies of animal ornamentation because they may also function as antioxidants and immunostimulants (Mougeot et al. 2010; Perez-Rodriguez et al. 2010; McGraw et al. 2011). The carotenoid trade-off hypothesis states that alternative physiological demands for carotenoids, such as immunity and antioxidant protection, can reinforce the honesty of carotenoid-based signals because only healthy animals can afford to allocate carotenoids to ornamentation (Lozano 1994). As a result, condition-dependent ornamentation can indicate competitive ability or mate quality (Andersson 1994; Cotton et al. 2004). Carotenoid-based color should correlate with immune status, which has been demonstrated in a wide range of birds and fish, including guppies (*Poecilia reticulata*; Grether et al. 2004*b*), Siamese fighting fish (*Betta splendens*; Clotfelter et al. 2007), greenfinches (*Carduelis chloris*; Aguilera and Amat 2007), and blackbirds (*Turdus merula*; Baeta et al. 2008). Recent studies also support the premise that carotenoids are important antioxidants in threespine sticklebacks (*Gasterosteus aculeatus*; Pike et al. 2007, 2010), although the antioxidant role in birds may have been overstated previously in the literature (Costantini and Moller 2008; Perez-Rodriguez 2009).

The carotenoid trade-off hypothesis also predicts that ornamented females will have a greater demand for carotenoids than their male counterparts if females need to also allocate carotenoids to their eggs (Fitzpatrick et al. 1995). In most cases, carotenoids in egg yolks confer fitness benefits to the parents by fortifying offspring health, growth, or number. Birds who place more carotenoids into their egg yolks produce larger offspring or offspring that survive better (Haga et al. 2008; Newbrey and Reed 2009), but the role of egg carotenoids in fishes is more variable. There is no link between egg carotenoid content and offspring survival or quantity in two-spotted gobies (*Gobiusculus flavescens*; Svensson et al. 2006) or guppies (Grether et al. 2008). In other fishes, a relationship exists between carotenoids in eggs and parental fitness: carotenoid content of eggs improves total egg and offspring quantity in striped jack (*Pseudocaranx dentex*; Vassallo-Agius et al. 2001) and enhances both offspring survival and offspring quantity in yellowtail (*Seriola quinqueradiata*; Verakunpiriya et al. 1997).

If the demands of ornamentation, somatic maintenance, and reproduction cannot be met by increased dietary carotenoid levels, ornamented females may evolve strategies to decrease carotenoid demands. For example, bird species often vary the

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carotenoid content of their eggs depending on laying order or environmental factors (Torok et al. 2007; Newbrey et al. 2008). Female two-spotted gobies (Svensson et al. 2005) and straight-tailed razorfish (*Xyrichtys martinicensis*; Baird 1988) have evolved a patch of translucent abdominal skin, allowing egg pigmentation to contribute to integument reflectance, while endogenously produced pterins are found to be the main pigments in the orange throat patches of female striped plateau lizards (*Sceloporus virgatus*; Weiss et al. 2011). Comparatively few studies to date have considered the carotenoid trade-off hypothesis in ornamented females, so we do not yet know the range of strategies employed by females to reduce the cost of visual signaling.

The question of how ornamented females allocate their dietary carotenoids is the focus of this study. We tested this question in Central American convict cichlids (*Amantitlania nigrofasciata*) using a three-level diet manipulation and high-performance liquid chromatography (HPLC) to determine the composition of the yellow ventral patch coloration and compare carotenoid amounts in integument and ovaries. Convict cichlids have been widely used in studies of physiology (Earley et al. 2004), sexual dichromatism (Noonan 1983; Beeching et al. 1998), effects of ultraviolet light exposure on growth (Fidhiany and Winckler 1999), mate choice (Beeching and Hopp 1999; Gumm and Itzkowitz 2007; Leese et al. 2010), aggression (Earley et al. 2006), and parental care (Lavery and Reebs 1994; Wisenden 1995; Wisenden et al. 1995; Galvani and Coleman 1998; Gagliardi-Seeley and Itzkowitz 2006). Several forms of sexual dimorphism occur in convict cichlids, including differences in color pattern (Noonan 1983), behavior (Mackereth and Keenleyside 1993), body size (McKaye 1977), and fin morphology (Paysan 1977). Most notably, sexually mature female convict cichlids have a yellow ventral patch that does not occur in males (fig. 1).

Convict cichlids are an excellent system in which to investigate the carotenoid trade-off hypothesis because the ornament occurs only in females. To date, there is no evidence that the yellow ventral patch is sexually selected by males. Instead, intrasexual social selection has been posited as the driving force behind female convict cichlid ornamentation (Beeching et al. 1998). In a previous study, increased dietary carotenoids increased the number of individual colored "flecks" in the ventral patch area of domestic female convict cichlids (Jackson 2003), but no chemical analyses of pigments were performed. It is still unclear whether the yellow ventral patch of this species is carotenoid based or whether it is condition dependent.

In this study, we hypothesized that a high level of carotenoids in the diet would allow females to meet all of their physiological demands but that a moderate dosage would force females to choose between allocating their carotenoids to integument or eggs. If this is the case, we predict that control and moderate diet females will show a decrease in coloration and carotenoid content of the integument paired with a concomitant increase in carotenoids in ovaries. If females can maintain coloration or pigment amounts in tissues independently of diet, this suggests that convict cichlid females may have evolved an alternative strategy to reduce their demands for carotenoid pigments.

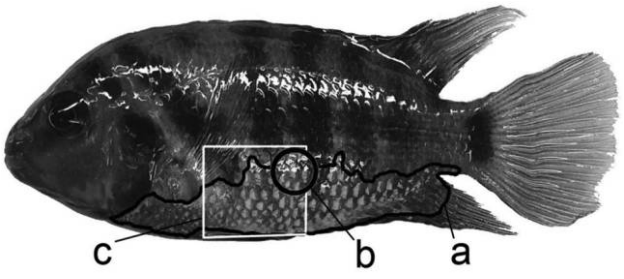

Figure 1. Female convict cichlid (*Amantitlania nigrofasciata*) showing sex-specific ventral coloration. *a*, Section of integument that was subjected to high-performance liquid chromatography is outlined in the square. *b*, Spectroscopic analysis was performed on the area inside the circle according to the methods outlined in the text. *c*, Irregular outline shows an example of how the area of the ventral patch was measured using scientific image-processing software. A color version of this figure is available in the online edition of *Physiological and Biochemical Zoology*.

Methods

Diets and Animals

Three treatment levels were fed to female *Amantitlania nigrofasciata* for 12 wk, which is adequate time to produce carotenoid-based color changes in fishes (Amar et al. 2004; Lin et al. 2010). The base recipe for all diets was a modified H440 diet composed of nutrient-free gelatin-dextrin-cellulose (Halver 1989; Kodric-Brown 1989) and a multivitamin premix (Pfizer, New York). After combining all the ingredients for the H440 base over heat, carotenoids were added and mixed thoroughly. Each diet treatment was refrigerated overnight and later processed manually into small chunks for storage at −80°C. Small batches of the diets were moved to 4°C for feeding each week to prevent carotenoid oxidation. No additional carotenoids were added to the control diet. Both the moderate- and high-carotenoid-supplemented diets contained lutein, zeaxanthin, and β-carotene from marigold (GNC, Pittsburgh, PA). The high-carotenoid diet contained, in addition to the above carotenoids, a high dose of β-carotene (Sigma-Aldrich C9750). Although the high group contained a concentration of carotenoids that was an order of magnitude higher than the moderate group, the dosage was within the range used in other studies of fish color (Garner et al. 2010) and within the natural range of carotenoid availability found in algae and aquatic insects of rocky freshwater habitats (Matsuno et al. 1999). Cichlids were fed to satiation twice daily, and any food remaining in the tank 10 min after the fish had stopped eating was removed with a dip net. HPLC analysis of the diets determined that the control diet contained trace levels of carotenoids ($\leq$1 ng g^{-1}), the moderate treatment contained 26.51 ng g^{-1} carotenoids, and the high diet contained 23 μg g^{-1} carotenoids.

Laboratory-reared F_1 offspring from multiple pairs of wild-

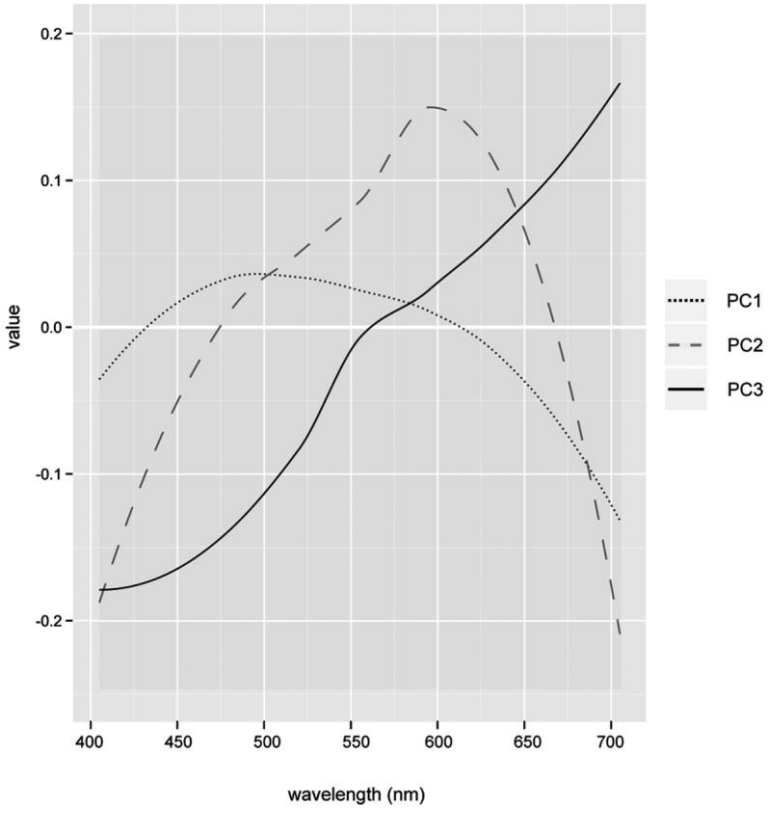

Figure 2. Principal component (PC) eigenvectors, or loadings. Three eigenvectors explained 97.54% of the variation in fish reflectance. PC1 explains that most of the variation in fish color (76.31%) is between 425 and 615 nm (violet-orange), with 470–510 nm being the area of greatest influence. PC2 indicates that variation in fish reflectance between 475 and 650 nm (green-orange) was the second-most variable (16.04%) aspect of the data, with the area between 570 and 610 nm (yellow) showing the most influence. The PC3 vector represents the variation in long (red) versus short (indigo) wavelengths. PC3 variation accounted for 5.19% of the total.

caught *A. nigrofasciata* (Río Cabuyo, Guanacaste Province, Costa Rica) were randomly assigned to control-, moderate-, or high-diet groups. All fish were sexually mature females weighing between 1.75 and 6.0 g, and none of them had produced eggs previously. Prior to the beginning of the experiment, each fish was individually marked with an injection of silastic elastomer (Northwest Marine Technology, Shaw Island, WA), which is commonly used to mark individual small fish, amphibians, and invertebrates (Buckley et al. 1994; Godin et al. 1996; Frederick 1997).

Due to space limitations, subjects could not be housed individually. Fish were housed in unisex groups of 10 in 38-L tanks on a 12L : 12D photoperiod at 25° ± 1°C (two tanks per diet). Convicts will attack and kill each other if housed at low densities in the laboratory, so a high density was used to decrease aggression. A 50% water change using deionized water reconstituted with electrolytes and other natural solids (Kent Marine, Franklin, WI) to a conductivity of 100 S m^{-1} was performed weekly or more often if needed. Filter cartridges with activated carbon and ammonia-removing resin (Aquarium Pharmaceuticals, Chalfont, PA) were changed weekly.

All fish were measured at the beginning of the feeding trial (week 0) and again at the end (week 12), following light anesthetization in a buffered solution of 1 g L^{-1} tricaine methane sulfonate (MS-222, Western Chemical, Ferndale, WA). Fish were individually weighed and photographed using a Nikon P-90 digital camera on a tripod under controlled lighting. A white standard and a metric ruler were in each frame for reference. Using ImageJ software (Rasband 1997), we measured standard body length, total body length, and ventral patch area (fig. 1*c*) from the images. All animal care and use protocols were approved by the Institutional Animal Care and Use Committee at Amherst College.

Spectral Analysis of Integument Color

At weeks 0 and 12, integument color was measured using a UV-VIS spectrophotometer (Ocean Optics, USB400, Dunedin, FL). Integration time was set by the probe software, and the boxcar smoothing was set to 50. Dark and white standards (Labsphere, North Sutton, NH) were used as references for 0% and 100% reflectivity. Reflectance was recorded using a 400-μm reflection probe (Ocean Optics R400-7) held at a 45° angle, 5 mm from the sample (Lahti 2006; Clotfelter et al. 2007). Readings were collected on the ventral patch region between the fourth and fifth stripes from the anterior (fig. 1*b*). We

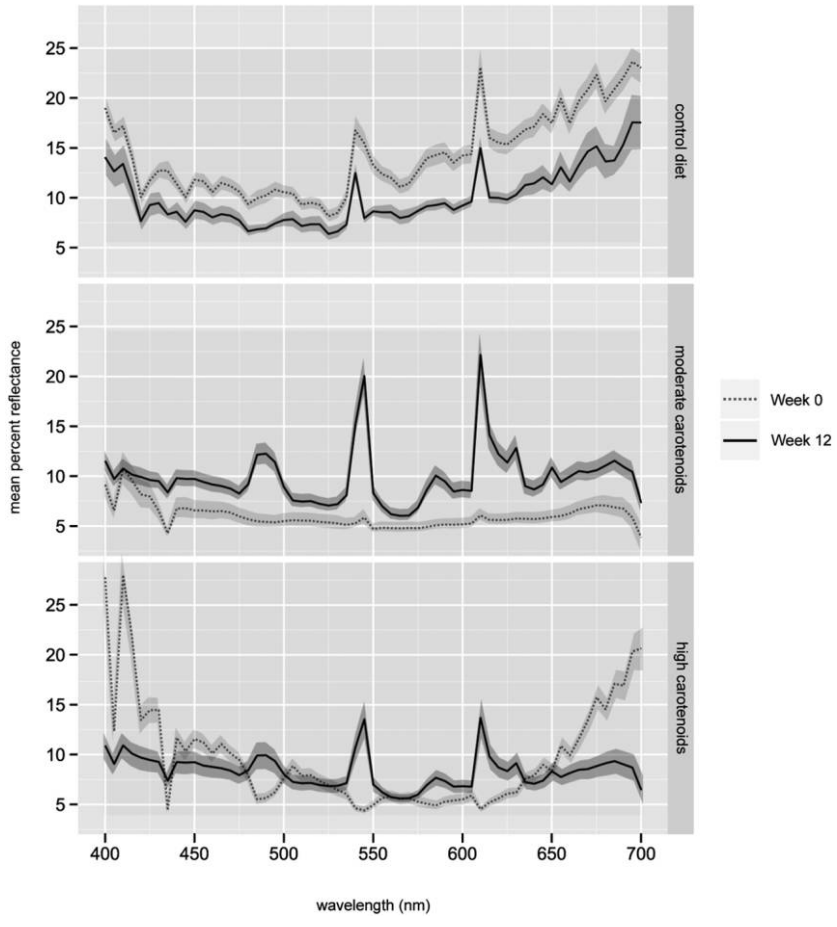

Figure 3. One of the potential drawbacks of principal components (PC) analysis is that some eigenvectors may not have a biologically meaningful interpretation (Jolliffe and Ebrary 2002), so we discuss the changes in PC values with respect to the average reflectance for fish in each diet group. Yellow ventral patches were composed of multiple peaks, with the largest at 540 (green) and 615 nm (yellow-orange). Peak height was enhanced with carotenoid supplementation, although peak location remained constant across the diets. The shaded ribbon represents the standard error.

restricted color and HPLC analysis in this study to the left side of each fish because a pilot experiment found that reflectance of left and right flanks is significantly correlated (Pearson's $r = 0.65$, $n = 51$, $P < 0.0001$).

Tissue Analysis

On week 12, following reflectance recordings, each fish was euthanized in a buffered solution of 2 g L^{-1} MS-222. Liver and ovaries were aseptically removed and weighed (± 0.001 g; Denver Instrument balance). Gonadosomatic index (GSI) was calculated as (ovary mass/body weight) × 100 (Hassanin et al. 2002). A 1.5-cm^2 epidermal sample was taken from the ventral patch area (fig. 1*c*) to be weighed and stored at −80°C until carotenoid extraction. Extractions were performed under nitrogen gas in a darkened fume hood at 4°C to prevent carotenoid oxidation. Carotenoid extraction and HPLC methods used in this study have been described previously (McGraw and Ardia 2003; Clotfelter et al. 2007). Pterins were extracted and analyzed according to methods described by Steffen and McGraw (2007).

To detect iridophores, integument from the right side of each fish was rinsed gently with ethanol and hexane and then mounted on glass slides using Shandon xylene substitute mountant (no. 1900231, Thermo Scientific, Waltham, MA). A coverslip was applied and microscopic evaluation of skin performed immediately. A movable light source allowed for samples to be lit from multiple angles (Meadows et al. 2011).

Diet Analyses

Samples from each diet were taken from freshly thawed fish food stock each week. These were weighed, frozen with liquid nitrogen, ground to powder, and extracted in hexane and acetone. Deionized water and sodium sulfate decahydrate were added to the solvent to force remaining nonpolar molecules into the solvent layer. Samples were vortexed vigorously, and the solvent layer was removed and dried under nitrogen. Sam-

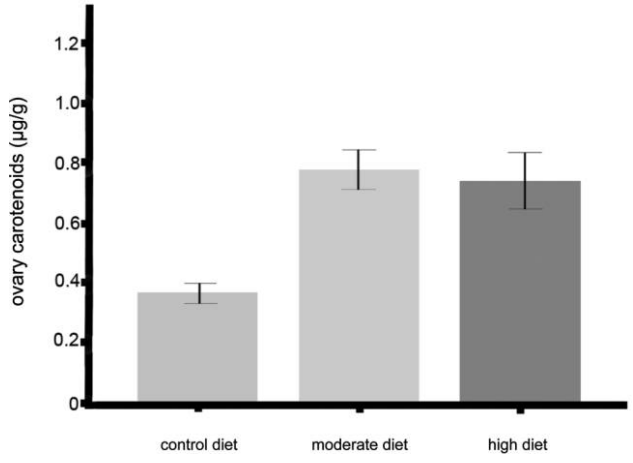

Figure 4. Fish that received moderate- or high-carotenoid diets allocated significantly more carotenoids to ovaries. Error bars represent the standard error of the mean. A color version of this figure is available in the online edition of *Physiological and Biochemical Zoology*.

ples were resuspended in 82 : 18 hexane : acetone mobile phase and run on Waters 600 HPLC at ambient temperature at a rate of 1 mL min^{-1} with a Waters dual-wavelength absorbance detector set to 474 nm. The column was 150 × 4.6 mm Luna 3u Silica (Chromadex 00F-4162-E0) with a 4 × 3.0-mm silica guard column (Chromadex AJ-4348).

Statistical Analysis

Analysis was conducted using R software (R Development Core Team 2010). Reflectance data were binned into 5-nm increments and averaged over 100 scans. Raw data were trimmed to the visible spectrum of *A. nigrofasciata* (400–700 nm; Jackson 2003) and centered before analysis (Cuthill et al. 1999). Reflectance spectra were compressed using principal components analysis (PCA). This process does not make any assumptions about the observer and can account for a large amount of variation in a data set with only a few components (Jolliffe and Ebrary 2002; Hill and McGraw 2006).

In brief, PCA calculates orthogonal eigenvectors to account for observed variance in a multidimensional space. Eigenvectors are calculated in order of decreasing variance explained until the cumulative variance explained equals 100% (an eigenvector calculated for each variable). The number of eigenvectors retained depends on the goals of the investigation—many ecological studies use the first two or three vectors (Lahti 2006; Clotfelter et al. 2007; Budaev 2010), whereas commercial color technology research may report as many as six (Tzeng and Berns 2005). Eigenvalues (also called PC values) represent the residual of each datum on the eigenvector, meaning that they cannot be compared across data sets or without a representation of the eigenvector (or "loading," a term borrowed from factor analysis).

Only eigenvectors that could explain >1% of the variance were retained in this study (fig. 2). Week 12 PCs were subtracted from week 0 components to determine the amount and direction of color change for each female. Analysis of carotenoid content of tissues was compared to PC values at week 12 only because this was the time at which tissue samples were collected.

A repeated-measures ANOVA was used to examine the effect of supplementation on integument color, tissue carotenoids, and patch area. We included tank as a random effect to account for tank effects. Tukey's tests were performed using the multcomp package for R (Hothorn et al. 2008). Multiple regression compared relationships between variables within diet treatments, and—due to influential outliers that could not be resolved with transformation robust—regression was used to test the relationships between GSI and tissues. Log transformation was applied when residuals were not normally distributed. P values were considered significant when $P < 0.05$.

Results

The experiment began with 20 fish in each diet group, for a total of 60 females. In tests where $n < 60$, data were missing because fish lost their silastic elastomer tags or samples were lost during the carotenoid extraction process.

Coloration Analysis

For PC1, fish fed moderate carotenoids gained reflectance between 420 and 615 nm (violet-orange)—significantly more than either controls or high-dose fish ($F_{5,43} = 31.35$, $P < 0.01$; fig. 3). There was also a marginally significant effect of housing ($F_{5,43} = 3.37$, $P = 0.04$). But when Tukey's tests were applied, the control and supplemented diets were not significantly different for PC1 ($P > 0.05$ for all contrasts).

Both supplemented groups gained reflectance between 475 and 650 nm (PC2, green-orange) compared to control animals ($F_{5,43} = 37.73$, $P < 0.001$; fig. 3), and again there was a significant tank effect ($F_{5,43} = 54.40$, $P < 0.001$). Tukey's tests showed that both supplemented groups had significantly increased reflectance over controls in this spectral region ($P < 0.001$ for both) but that moderate- and high-diet groups were not significantly different from each other ($P > 0.05$).

ANOVA detected an effect of treatment for PC3 ($F_{5,43} = 31.21$, $P < 0.001$), but there was no effect of housing ($F_{5,43} = 0.16$, $P > 0.05$). Tukey's tests reported that moderate-diet fish increased their reflectance above 650 more than controls or high-diet fish ($P < 0.05$ for both), but control- and high-diet fish did not significantly differ from each other ($P > 0.05$).

Integument and Ovary Analysis

We were unable to achieve sufficient separation with HPLC to identify individual carotenoid types due to esterification, so total carotenoids are reported. Integument carotenoids did not differ among diet groups ($F_{2,45} = 0.48$, $P = 0.62$), but fish in moderate and high diets had significantly more carotenoids in ovaries ($F_{2,42} = 3.66$, $P = 0.03$; Cohen's $d = 0.89$ and 0.90; fig.

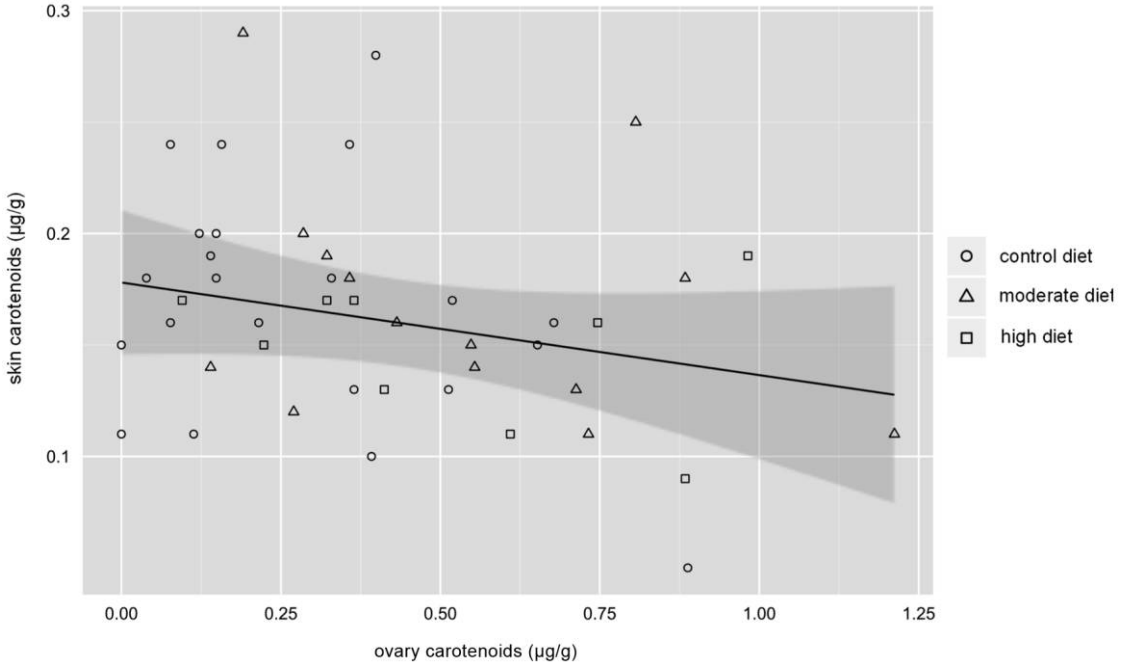

Figure 5. Carotenoids in ovaries are linked to the amounts found in the integument. Treatment group did not affect the slope of the relationship. Carotenoid amounts were log transformed to achieve normality, and the shaded area represents the standard error.

4). Skin reflectance was not significantly related to gonad carotenoids ($t_{1,42} = 0.80$, $P = 0.43$), but skin carotenoids were negatively correlated with gonad carotenoids ($t_{1,42} = -2.04$, $P = 0.04$; fig. 5). Supplementation did not affect GSI ($F_{2,45} < 2.53$, $P > 0.09$). GSI was not related to any aspect of reflectance ($P > 0.05$) but was significantly related to carotenoid content of both tissue types tested. Increasing GSI increased carotenoid allocation to ovaries ($t_{2,43} = 6.72$, $P = 0.001$; fig. 6), as well as decreased allocation to integument ($t_{2,43} = -3.48$, $P = 0.001$; fig. 6).

For a subset of 12 fish from the moderate and high diets, the pterin pigment content of skin was determined. Pterin content of skin was trace or undetectable (mean, 0.71 ± 0.19 ng g^{-1}) and not significantly different between treatments ($F_{1,11} = 0.92$, $P = 0.36$). Iridophores, chromophores, and melanocytes were observed in opaque integument filets under multiangle light microscopy (fig. 7) but were not quantified in this experiment.

Effects of Fish Size

The change in area of the ventral patch was not affected by carotenoid supplementation ($P > 0.05$), but patch area was positively correlated with fish mass ($R^2 = 0.54$, df = 47, $P < 0.001$). There were no significant treatment effects on body mass ($F_{2,45} = 1.26$, $P = 0.29$), nor was there a relationship between fish mass and skin carotenoids ($P \geq 0.24$ for all), but fish mass was a significant positive predictor of ovary carotenoids ($R^2 = 0.35$, df = 43, $P < 0.001$). Large control fish lost more yellow coloration (PC2) than their smaller counterparts within the same treatment ($R^2 = 0.27$, df = 24, $P = 0.008$), but this did not occur in either supplemented group (moderate carotenoids: $R^2 = 0.12$, df = 8, $P = 0.37$; high carotenoids: $R^2 = 0.14$, df = 13, $P = 0.19$).

Discussion

The first aim of this study was to show that the coloration of the ventral patch is mediated by dietary carotenoids. In support of this, we found that carotenoid content of diets affected the visible color of the fish, and we found carotenoids in integument with HPLC. Presence of carotenoids in the diet increased fish reflectance in the green, yellow, and orange portion of the spectrum as shown by increased PC2 values. In the nonsupplemented group, fish lost yellow coloration as a result of carotenoid deprivation. Although initially we detected an effect of diet on PC1 (green-orange) using ANOVA, after pairwise testing, PC1 was not significant. This could have occurred because of some initial differences in coloration between the groups or because melanin-based pigmentation is the main source of individual variation detected by the PCA (Cuthill et al. 1999).

Moderate carotenoid group fish also showed a significant increase in reflectance above 650 nm (PC3), as shown in figure 3, over the high and control groups. High doses of β-carotene may have suppressed reflectance of long wavelengths (red) and shorter wavelengths (blue) while increasing midrange wavelengths (green-orange), resulting in an overall decrease in PC3.

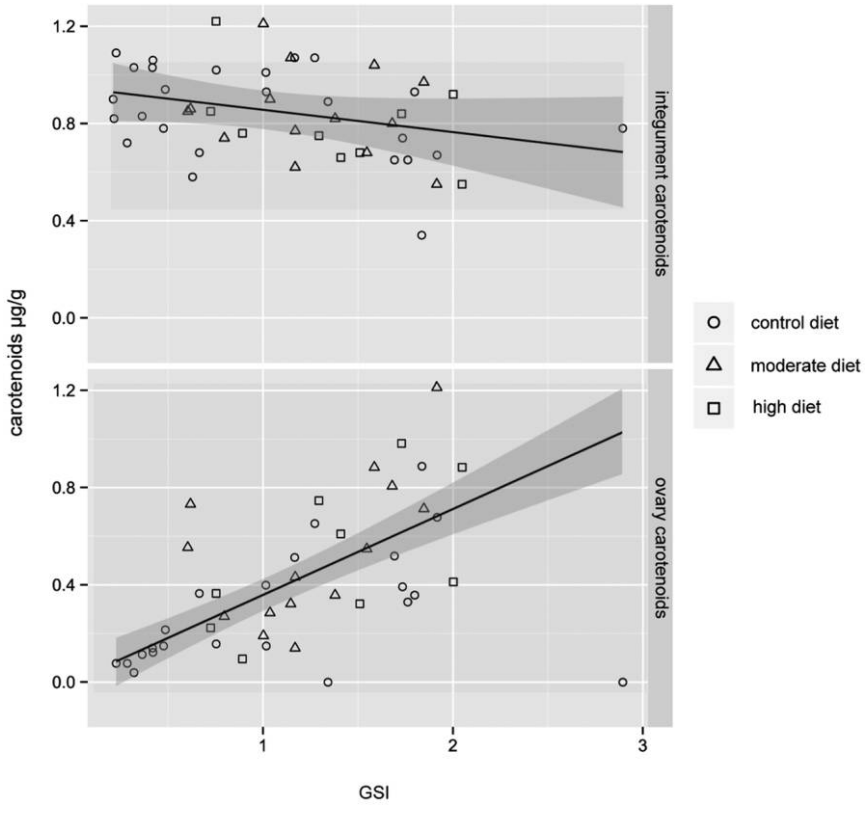

Figure 6. Carotenoid allocation to integument (*top*) and ovaries (*bottom*) was closely related to gonadosomatic index (GSI). Fish nearing breeding readiness may reallocate their pigment resources to offspring rather than integument, but this trend was not accompanied by a decline in color. Carotenoid amounts were log transformed to achieve normality. Shading represents the standard error.

This leaves the moderate group as the only diet to increase reflectance in the spectral region above 650. While the moderate group changed in the direction we predicted, the high group did not. This could be due to variations in melanophore dilation, skin thickness, mucus layer, or other physical changes in the integument. Because 650 is above the range at which carotenoids would reflect, we do not believe that PC3 is informative for the purposes of this work.

HPLC results indicate that carotenoids are the main pigment of yellow ventral patches, but carotenoids failed to account for a significant portion of the variance in fish color. As predicted, fish that ate both the moderate and high diets placed significantly more carotenoids in their ovaries than fish on the control diet. Carotenoid content in integument was also linked to ovary carotenoids, which is consistent with previous findings in female fishes (Svensson et al. 2006; Grether et al. 2008). We did not find that integument reflectance was linked to carotenoids in the ovaries, which supports previous work suggesting that the yellow ventral patch is not sexually selected by males (Beeching et al. 1998).

Patch coloration was not related to GSI, but carotenoid deposition in integument and ovaries appears to respond strongly to gonad maturation. As gonads mature, GSI increases, and so does the concentration of carotenoids. Gonad maturation was also associated with a decline in carotenoid allocation to the integument, and both of these effects appeared independently of diet group. This supports our alternate hypothesis that females have evolved some means of reducing the costs of carotenoid-based ornamentation in the integument, but the adaptive importance of our conclusions rest on the assumption that yolk carotenoids are important to offspring fitness. As noted in the introduction, this finding is variable in fishes. Multigenerational observations are currently under way in our laboratory to determine how yolk carotenoids might enhance offspring fitness in convict cichlids.

Fish could alter their coloration without changing the amount of carotenoids in their integument by aggregating or dispersing xanthophores (Leclercq et al. 2010), which can be triggered by various environmental factors, such as stress and light (Gray et al. 2011). However, any handling stress the fish may have experienced would have been present across all of the groups, and previous work does not suggest a link between dietary carotenoids and chromophore dilation. We also did not detect any indicators of long-term physiological stress in terms of reduced growth or GSI as a result of the diet treatment. The possibility that gonads are directly visible through a translucent patch of skin (Baird 1988; Svensson et al. 2009) can also be dismissed; convict cichlids possess an opaque gray or silver

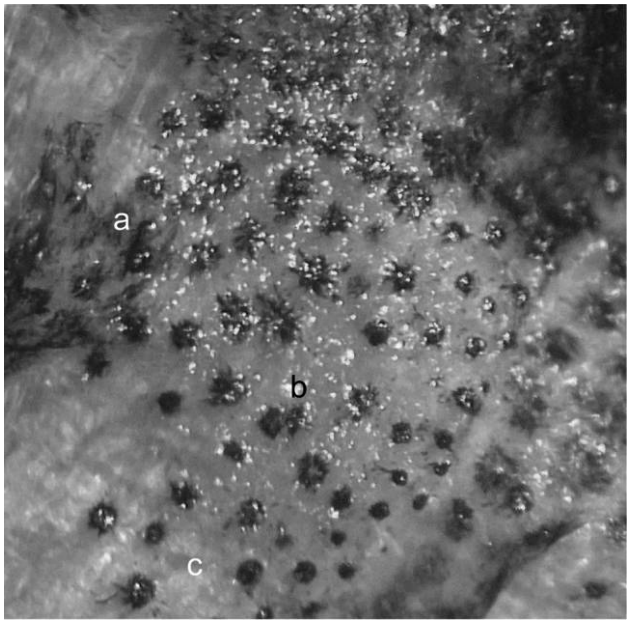

Figure 7. Female convict cichlid integument at 40× magnification. Melanin-based coloration regions (*a*), iridophores (*b*), and chromophores (*c*) interact to produce convict cichlid ventral patch color. Integument was mounted on a slide in this photograph; the pigmented layer lining the peritoneal cavity of convict cichlid skin rules out the possibility that gonads produce ventral patch color by showing through the skin. A color version of this figure is available in the online edition of *Physiological and Biochemical Zoology*.

lining of the peritoneal cavity that conceals all of the organs, including the gonads, from observation through the skin.

Iridophores can produce color in fishes, lizards, amphibians, and other taxa either through interactions with pigments (Grether et al. 2004*b*) or by altering the angle, dispersion, and orientation of purine platelets to produce matte white, silver, blue, brown, or yellow coloration in addition to others (Oshima and Kasai 2002; Bagnara et al. 2007; Mäthger et al. 2009). Under magnification, yellow-green light-reflecting iridophores were visible within the ventral patch region of convict cichlids. Iridophores were present on other areas of fish skin but appeared blue or blue-green in color. We were unable to quantify iridophores and their contribution to the spectral readings because these calculations require a priori knowledge of the underlying structure of the skin layers and any attenuating or amplifying effects of other color-producing molecules (Grether et al. 2004*a*). Further study will be necessary to determine how structural coloration is affected by dietary carotenoid availability in convict cichlids, but the results presented here are similar to a recent finding in common lizards (*Lacerta vivipara*) in which iridophores control chromatic variation of a carotenoid-based ornament (San-Jose et al. 2013).

Carotenoid limitation could be responsible for the relationship between increased body mass and decreased yellow coloration in the control-diet group. Larger animals may require more carotenoids in order to maintain the same tissue carotenoid concentrations as smaller fish, as signal detection theory predicts that signalers with larger ornaments experience an exponential, rather than linear, demand for color-reflecting molecules (Weber's law; Barlow and Mollon 1982). In addition, we found that larger fish from all the treatment groups placed more carotenoids in their ovaries than smaller fish. It is possible that larger fish became carotenoid limited before their smaller counterparts because their demand increases exponentially with respect to their body size.

However, the lack of a significant effect of mass on skin carotenoids casts doubt on this conclusion; it is possible that the effect size of mass was simply too small to be detected with our sampling methods or that social environment influences color expression. If yellow ventral patches have an intrasexual signaling function (Beeching et al. 1998), modulation of fish color should be based on social interactions. Furthermore, iridophores have been shown to be under neurological control and play an important role in agonistic and reproductive behaviors in squid (*Lolliguncula brevis*; Hanlon et al. 1990); the same could be true for convict cichlids. The importance of social environment in color expression is further supported by the detection of significant tank effects in the coloration analysis.

This study showed that dietary carotenoid content increases ventral patch coloration, which is not related to carotenoid content of the integument. Also, gonads sequestered carotenoids as they matured and reduced allocation of carotenoids to the skin independently of the diet groups. Yellow coloration may contain information about an individual's fitness or quality, but we cannot say that yellow ventral patches are condition dependent. This would require evidence for a proximate mechanism and a cost of signal production, which we did not detect.

This study found support for a strong influence of social environment on the expression of the ventral patch. Iridophores may perform the role of decreasing the cost of carotenoid allocation to the integument, as carotenoid content of skin did not account for a significant portion of variance in spectral reflectance. The results presented here do not support our initial hypothesis that ornamented females experience a high demand for carotenoids but instead offer some support for our alternative hypothesis that females may evolve a strategy to reduce the cost of carotenoid allocation. From a broader perspective, our results raise interesting questions about the importance of competition for resources as a selective pressure for female ornamentation, as well as how honesty is maintained. Future studies should address the social aspect of convict cichlid ventral patch color and consider the influence of structural color on ventral patch expression.

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