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Bluegill Coloration as a Sexual Ornament: Evidence From Ontogeny, Sexual Dichromatism, and Condition Dependence

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Abstract

In aquatic environments, visual communication is expected in animals that inhabit clear, shallow waters. Here, we investigate variation in the colorful traits of bluegills, *Lepomis macrochirus*, to elucidate their possible function. Bluegills use alternative mating tactics whereby males develop into one of two irreversible phenotypes termed parental and cuckolder. Parentals build and defend nests and care for offspring whereas cuckolders obtain matings by sneaking copulations. We hypothesized that bluegill coloration might function as a sexual ornament in parental males and that ornamental coloration might serve as an honest indicator of male quality. We predicted that coloration should be more pronounced in parental males than in females and immature males and should be more pronounced during the breeding season. We also predicted that males in better condition should be more intensely colored than fish in poor condition. To test our predictions, we sampled 510 bluegills during the breeding and post-breeding seasons at nine lakes in southern Ontario, Canada, in 2007. We used reflectance spectrometry to quantify the coloration of five body regions, aged and sexed each fish, and calculated Fulton's condition factor from morphological measurements. A separate experiment showed that color did not fade several minutes post capture, suggesting that coloration could be measured reliably and consistently. We found that color was influenced by maturity, sex, and season, in the predicted direction, for three body regions (breast, cheek, and opercular flap). We also found that color varied with the condition of males such that males in better condition were darker for the sexually dichromatic ventral and facial regions. Our findings therefore suggest that some colorful traits in bluegills may serve as condition-dependent sexual signals during the breeding season. Our research contributes to a growing appreciation of the importance of visual signaling in aquatic environments.

Introduction

In typical mating systems, male–male competition and female preference for male traits are the basis of sexual selection (Andersson 1994). Traits used in competition are considered weapons, whereas those used in mate choice are considered ornaments (Andersson 1994). One common type of sexual ornament is the vibrant breeding coloration displayed by males of many species. In fishes, ornamental coloration is known to influence female mate choice and male–male competition in both tropical and temperate species (Kodric-Brown 1998). For example, females prefer orange coloration in guppies, *Poecilia reticulata*, (Endler 1980, 1983) and coloration is known to influence male–male interactions in

three-spined sticklebacks, *Gasterosteus aculeatus* (Rowland 1989; Candolin 1999). Despite long-standing interest in sexual ornaments of fishes, more research is warranted on this topic. A number of studies have demonstrated that ornamental coloration can serve as a sexually selected trait in fishes (e.g. Endler 1983, 1991; Seehausen & van Alphen 1998; Rick et al. 2006; Maan et al. 2006; Millar et al. 2006). However, further research is needed to determine whether these findings are applicable across a diversity of species that vary in distribution, ecology, life history, and mating system. Moreover, until recently, most studies of fish sexual ornaments focused on quantifying the number and area of colorful patches or quantifying color using visual assessments rather than spectrophotometric techniques (but see, e.g., Grether et al. 2001; Rick et al. 2004; Cheney et al. 2009). Finally, studies have often focused exclusively on assessing coloration in breeding males. However, the incorporation of ontogenetic, sexual, seasonal, or geographic variation can provide a more comprehensive perspective on the selective factors that affect sexual ornamentation (Endler 1980, 1991; Miller & Brooks 2005; Millar et al. 2006). In this study, we used reflectance spectrometry to investigate the influence of ontogeny, sex, and season on coloration and its possible role as a sexual ornament in the bluegill, *Lepomis macrochirus*.

Bluegills are an abundant freshwater species. They are generally greenish in coloration with yellow or orange breasts, iridescent blue cheeks and chins, and black opercular flaps (Becker 1983). Bluegills breed colonially, typically spawning in Jun. at northern latitudes. Male bluegills follow one of two irreversible alternative life history strategies termed parental and cuckolder (Gross & Charnov 1980). Parental males delay reproduction, typically maturing at age 7 in Ontario, Canada, and establish nesting colonies where each male builds and defends a single nest (Gross 1982, 1991). Cuckolders include young sneakers (age 2) that steal fertilizations (Neff et al. 2004) and older satellite males (age 4) that mimic the appearance and behavior of females to enter the nests of parental males, typically while a female is present, and steal fertilizations (Dominey 1981). Cuckolders never become parental males and they never reach the age or size of parental males (Gross & Charnov 1980; Gross 1982). Once parental males have congregated at a colony and constructed their nests, reproductively mature females (age 4) arrive at the spawning grounds and deposit eggs into nests (Dominey 1981; Gross 1982, 1991). Spawning within a colony typically occurs over a single day (Gross 1982), although parental males remain to guard eggs and fry for an additional 7–10 days after spawning (Jennings et al. 1997).

Because parental males are solely responsible for egg and offspring care, females could benefit by choosing males based on traits that honestly reveal male quality. Elaborate sexual ornaments that advertise quality should be costly so that they cannot be faked by those unfit to produce them (Zahavi 1975; Grafen 1990; Getty 2006). In bluegills, such honest indicator traits could potentially provide females with direct benefits through improved parental care, as suggested by the good parent hypothesis (Hoelzer 1989), or indirect benefits through heritable good genes (Zahavi 1975; Hamilton & Zuk 1982). Despite these potential benefits to females, sexual ornaments have never been investigated in bluegills. Recently, we found that both female preferences and male reproductive success were related to male coloration in a wild population of bluegills, suggesting that male color may play a role in sexual selection in this species (Cogliati et al., in press).

Our objectives in this study were twofold. First, we sought to determine whether ornamental coloration might function as a sexually selected trait in bluegills. We tested this hypothesis indirectly by examining ontogenetic, sexual, and seasonal variation in color. Because conspicuous colors can be costly to produce (see Olson & Owens 1998) and often increase predation risk (Endler 1980, 1991; Forsgren 1992), they should be restricted to those individuals that stand to benefit from displaying them. If coloration functions as a sexual ornament in bluegills, we predicted that ornamental coloration should be more pronounced in males than in females, in parental males than in immature males, and in the breeding season than during post-breeding. For our second hypothesis, we proposed that ornamental coloration is an honest indicator of quality in bluegills. If bluegill coloration reveals quality, we predicted that fish in better condition would be more intensely colored than fish in poor condition.

Methods

Fieldwork

In 2007, we captured 510 bluegills by angling and cast netting at nine lakes in southern Ontario, Canada. We sampled Rondeau Bay, Lake Erie (42°16′N, 81°56′W; N = 52), Lake St. Clair (42°19′N, 82°28′W; N = 40), Lake Scugog (44°10′N, 78°55′W; N = 59), Pigeon Lake (44°29′N, 78°31′W; N = 67),

Sharbot Lake (44°46′N, 76°41′W; N = 30), Desert Lake (44°31′N, 76°36′W; N = 54), Devil Lake (44°34′N, 76°26′W; N = 61), Buck Lake (44°33′N, 76°27′W; N = 45), and Eagle Lake (44°40′N, 76°42′W; N = 53). We sampled each site in Jun. (breeding) and Aug. (post-breeding) to investigate possible seasonal variation in coloration. We aimed to capture approx. 30 fish from each lake during both breeding and post-breeding sampling. For each fish, we measured spectral reflectance (see below), total length (L_T), and mass (W), and then euthanized the specimen using clove oil.

Sex, Season, Age and Body Condition

In the laboratory, we dissected each fish to determine sex and to weigh the gonads. We calculated the gonadosomatic index (GSI) as gonad mass as a percentage of total body mass. We also extracted both otoliths from each fish for aging following Devries & Frie (1996). Two readers independently counted annual bands using a light microscope and resolved discrepancies by mutual examination. Age estimates using otoliths have been previously validated for bluegills (Schramm 1989), and provide more precise age estimates than scales (Hoxmeier et al. 2001). Altogether, our arbitrary sampling at each lake resulted in a total of 300 males (mean age 3.7 yrs, range 1–9) and 155 females (mean age 3.6 yrs, range 1–9).

Although we wanted to investigate ontogenetic variation in color in this species, we knew at the outset that color was unlikely to vary linearly with age since males adopt different reproductive strategies that vary with size and age, and parental males and females only reach reproductive maturity at a given age. Thus, we assigned each fish to an age category (hereafter referred to as maturity) using separate criteria for males and females. We classified males as parental if they were 7 yrs or older or longer than 175 mm and as immature if they did not meet these criteria (Gross 1982, 1991). Our sample of younger males (breeding season N = 110) likely consisted primarily of immature parental males, since cuckolder males make up a maximum of 21% of males at age 2 and 3% at age 6 (Gross 1982). Because cuckolders likely differ in appearance from immature parental males due to their different life strategies (i.e. satellite males mimic female appearance and behavior), we decided to separate these two groups based on GSI values. Cuckolders have much higher GSI values than parental males (Neff et al. 2003), so we placed immature males with a GSI value at or above that of parental males (mean ± SE: 0.91 ± 0.11%) less 1 standard error in a 'cuckolder' group. Unfortunately, this group only contained seven males and we therefore excluded these fish from our dataset and did not include them in any analyses. The remainder of immature males had a mean GSI value of 0.08 ± 0.01% (mean - SE). We categorized females as mature if they were 4 yrs or older and immature if 3 yrs or younger (Gross 1982, 1991). Our GSI estimates also confirmed that we sampled fish during breeding and post-breeding seasons, since GSI values were much higher during the breeding season in both mature females (t-test: $t_{73} = -8.33$, $p < 0.0001$) and parental males (t-test: $t_{41} = -4.67$, $p < 0.0001$).

We calculated Fulton's condition factor for each fish captured as W/L_T^3 (Ricker 1975). Previous work has shown Fulton's condition factor is highly correlated with non-polar lipid density in bluegills and is therefore a useful indicator of body condition in this species (Neff & Cargnelli 2004).

Spectral Reflectance

Immediately after the fish were captured and prior to morphological measurements and euthanasia, we measured their spectral reflectance using an Ocean Optics USB4000 spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA). We removed the fish from water and placed them on a flat surface to measure their reflectance using a bifurcated 400 μm fiber optic cable mounted in a probe that transmitted broad spectrum light to the surface of the fish (R-400 SR; Ocean Optics). The probe then transmitted the reflected light back to the spectrometer, where data were collected with OOIBase32 software on a laptop computer. We maintained a fixed distance from the tip of the probe, perpendicular to the measurement surface, using a matte black rubber sheath; this sheath also excluded external light from the measurement area. All reflectance measurements were expressed as the percentage of the total spectral reflectance from a Spectralon white standard (WS-1; Ocean Optics). We measured spectral reflectance on five landmarked body regions from each fish: the orange breast, the green caudal peduncle, the green cheek directly below the eye, the green lateral side of the fish directly above the highest portion of the lateral line, and the black opercular flap (Figs 1 and 2a–e). We collected five readings from each region, each of which was comprised of 20 measurements averaged by OOIBase32, and averaged these to obtain one mean spectral reflectance spectrum per

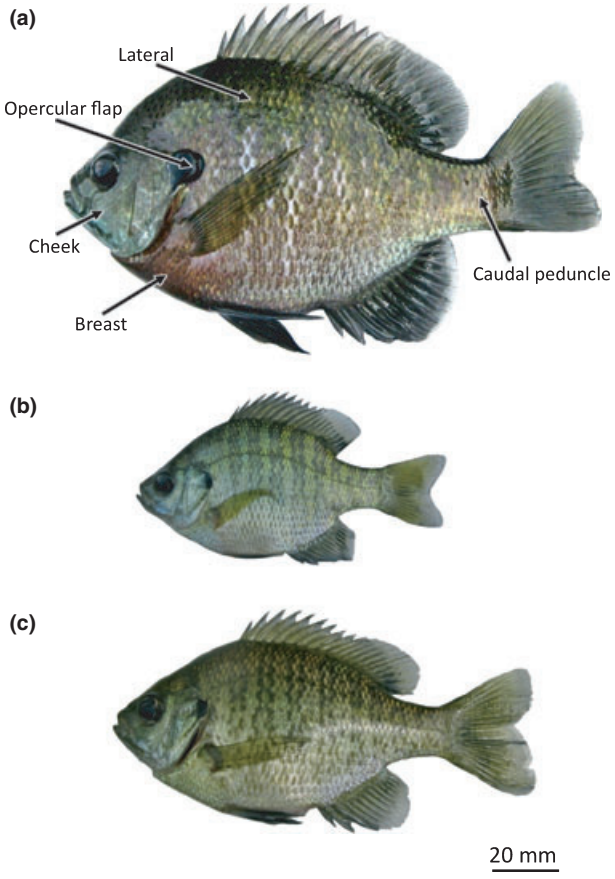

Fig. 1: Representative photographs of bluegills (*Lepomis macrochirus*). (a) Parental male (186 mm TL) – labels indicate body regions from which spectral reflectance was measured; (b) immature male (108 mm TL); (c) mature female (146 mm TL).

body region per fish. Readings were always collected in the same order, and the entire process was completed in approx. 1 min.

Color Time Series Experiment

To determine whether bluegill color changed over time after capture, we conducted a time series experiment on a separate group of 20 fish (five from Desert Lake, five from Pigeon Lake, and ten from Rondeau Bay). We captured fish by angling and measured their spectral reflectance as described above. We then re-measured each fish every 10 min until 50 min post-capture. Between sets of reflectance measurements, fish were held in white buckets filled with fresh lake water and equipped with an air stone from a portable aerator.

We restricted spectral analyses to wavelengths between 300 and 700 nm because it encompassed the red (620 nm) and green (536 nm) peak cone photopigment sensitivities of bluegills (Hawryshyn et al. 1988) and part of the ultraviolet (UV) spectrum. Although bluegills apparently do not have a cone photoreceptor with maximum sensitivity in the ultraviolet (UV), their ocular media transmit some UV wavelengths (Hawryshyn et al. 1988), and juvenile bluegills respond to flashes of ultraviolet radiation, suggesting that they may be sensitive to UV wavelengths (Leech & Johnsen 2006). Furthermore, since many fish are UV-sensitive (Losey et al. 1999), bluegill predators may also detect UV wavelengths and thereby exert selective pressure on this aspect of bluegill coloration.

Statistical Analyses

To summarize overall variation in spectral reflectance, we performed principal components analysis (PCA) on reflectance spectra for each body region (Endler 1990). We averaged reflectance data in 10-nm increments using CLR color analysis software (Montgomerie 2008), and used these as variables in our analyses. We performed separate analyses for each body region, and each individual represented an observation in these analyses (Endler 1990; Montgomerie 2006). We ran separate principal components analyses on fish in our time series experiment.

We analyzed the data from our time series experiment using repeated measures ANOVAs on spectral reflectance PC scores for each body region to determine whether the color of bluegills changes over time after capture.

To evaluate our hypotheses and predictions, we used a mixed model ANOVA to determine which factors might influence bluegill coloration. In this analysis, we used PC scores for each region as dependent variables, and our model effects were sex, maturity (parental vs. immature in males and mature vs. immature in females), season and their interactions. All non-significant interactions were removed in our final models. We also included sampling site as a random effect in each model. Although sampling site significantly influenced the coloration of each body region, interpreting these differences is beyond the scope of the current study and will be assessed elsewhere. For all analyses on Fulton condition, we separated males and females, and ran analyses that included Fulton condition, maturity, season, and sampling site to control for the variation that each of these also plays on coloration.

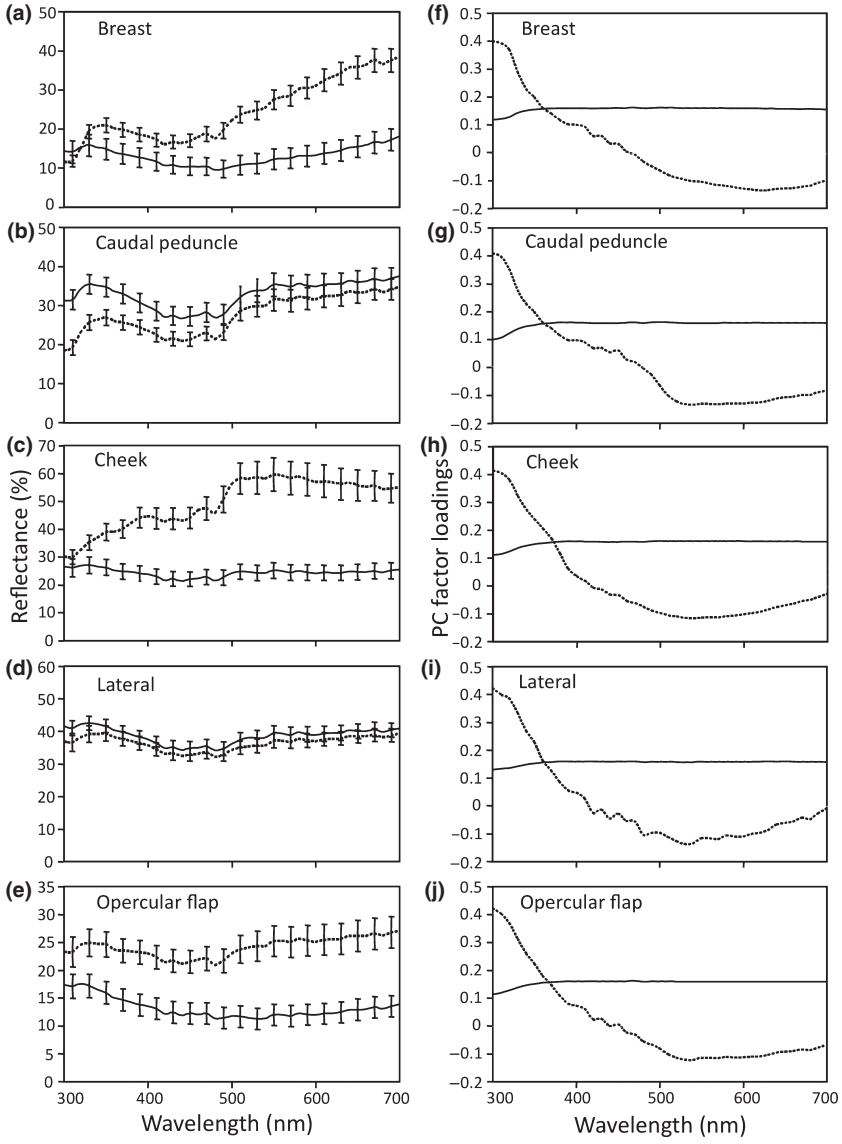

Fig. 2: Reflectance spectra (a–e) and factor loadings from principal components analyses on reflectance spectra (f–j) for bluegills. Mean spectra (±SE) of parental males (solid lines; N = 29) and mature females (dashed lines; N = 41) measured during the breeding season for those same body regions (a–e). *Y*-axis spectral reflectance (%) values differ among body regions. PC1 (solid line) and PC2 (dotted line) loadings in relation to wavelength for these same regions, with origin axis (light dashed line). Principal components analysis included all individuals sampled (N = 460).

Results

Principal Components Analyses

Our analyses resulted in two principal components for each body region with eigenvalues >1 that together explained 96.0%–98.8% of the variation in bluegill spectral reflectance. The first principal component (PC1) explained 88.4%–94.3% of the variation in spectral reflectance and the second principal component (PC2) explained 4.5%–8.2% of the variation. For each of the five body regions, PC1 had moderate positive loadings across all wavelengths (Fig. 2f–j), suggesting that PC1 indicates variation in brightness, as is typical of PCA performed on reflectance data (Endler 1990). Therefore, fish with high PC1 scores are relatively lighter in coloration than fish with low (or negative) PC1 scores. The second principal component factor loadings were variable across wavelengths in both magnitude and direction, suggesting an association with hue and saturation (Endler 1990). For each region, PC2 had high positive loadings for short wavelengths below the range of 420–480 nm, and moderate negative associations for long wavelengths above the range of 420–480 nm (Fig. 2f–j). Thus, fish with high PC2 scores reflected proportionally more at short wavelengths (UV, blue, and green; 300–500 nm), whereas fish with low PC2 scores reflected proportionally more at

long wavelengths (yellow, orange, and red; 500–700 nm). These scores represent proportional variation between individuals based on the reflectance of the entire spectrum. In absolute terms, most fish tend to reflect more at long wavelengths (Fig. 2a–e). For example, breeding parental males have deep red breast coloration (Figs 1 and 2a). Relative to females, they have higher proportional spectral reflectance at shorter wavelengths compared to long wavelengths, and therefore higher PC2 scores. However, in absolute terms, females reflect more at shorter wavelengths than males, but have lower proportional short wavelength reflectance when compared to long wavelength reflectance, and both males and females reflect long wavelength colors (Fig. 2a). Our separate PCA for fish in the time series experiment generated similar loadings.

Color Time Series Experiment

Data from repeated measures ANOVAs revealed that bluegill color did not change with time after capture for any body region for PC1 (all $p > 0.07$) or PC2 (all $p > 0.14$) except for PC2 operculum ($p = 0.03$). For the operculum, there was a slight decrease in PC2 at 20 min, although no overall fading trend was noticeable.

Variation in Spectral Reflectance Across Body Regions

Bluegill coloration varied by body region. The breast, which ranged in color from yellow to red (Figs 1 and 2a), reflected more strongly at longer wavelengths. For breeding-season parental males, breast coloration was deep orange/red, whereas mature, breeding females were lighter yellow/orange (Fig. 1). That is, brighter regions likely have less absorbing pigmentation, and thus have higher reflectance spectra, as in the breast regions of females (Fig. 2a). For the caudal peduncle and lateral regions, both males and females had low spectral reflectance in the 400–500 nm range, and steady, high spectral reflectance at longer wavelengths, giving an olive-brown appearance to these regions (Figs 1 and 2b,d). The cheek was greenish in color for both males and females (Fig. 1), peaking in spectral reflectance near 500 nm, although males tended to be darker than females (Fig. 2c). Finally, the opercular flap was black in both sexes (Fig. 1). This black region was heavily pigmented, and therefore exhibited low spectral reflectance at most wavelengths (Fig. 2e). Males had darker opercular flaps than females (Fig. 2e).

Ontogenetic, Sexual, and Seasonal Variation in Color

The breast region was significantly influenced by sex, maturity and season for PC1 (a measure of brightness; Table 1). Males had lower PC1 scores than females, parental males and mature females had lower PC1 scores than immature fish, and breeding fish had lower PC1 scores than post-breeding fish (Table 1; Fig. 3a). Overall, this indicates that males, parental males and mature females, and breeding fish were darker (more pigmented) for the breast region (Figs 1 and 2a). For PC2 (a measure of hue and saturation), there was a significant interaction between sex and maturity (Table 1). When analyzing the two sexes separately, parental males had higher PC2 scores than immature males ($F_{1,289} = 16.60$, $p < 0.0001$), whereas maturity had no effect on color in females ($F_{1,145} = 1.77$, $p = 0.19$; Fig. 3b). Conversely, when separated by maturity, males had significantly higher PC2 scores than females for both immature ($F_{1,326} = 11.06$, $p = 0.001$) and mature ($F_{1,108} = 47.97$, $p < 0.0001$) fish.

The caudal peduncle region showed no significant variation for PC1 (Table 1; Fig. 3c), but was significantly influenced by the interaction between sex and maturity and between sex and season for PC2 (Table 1). When analyzing the two sexes separately, PC2 increased with maturity in males ($F_{1,288} = 12.51$, $p = 0.0005$) but not females ($F_{1,124} = 0.68$, $p = 0.41$) and post-breeding females had higher PC2 scores than breeding females ($F_{1,144} = 9.28$, $p = 0.003$; Fig. 3d). When separating the data based on maturity, males had higher PC2 scores than females in mature fish only ($F_{1,108} = 10.60$, $p = 0.002$). Furthermore, when analyzed by season, males had higher PC2 scores only during the breeding season ($F_{1,189} = 4.80$, $p = 0.03$).

There was a significant interaction between sex and maturity that influenced cheek PC1 (Table 1). When males and females were analyzed separately, PC1 scores decreased with maturity in males ($F_{1,289} = 21.11$, $p < 0.0001$) but not females ($F_{1,145} = 0.001$, $p = 0.97$; Fig. 3e). When separating by maturity, males had lower scores for both immature ($F_{1,326} = 5.93$, $p = 0.02$) and mature ($F_{1,108} = 23.69$, $p < 0.0001$) age categories, indicating that males were darker for this region. Also, breeding fish, in general, had lower PC1 scores than post-breeding fish ($F_{1,441} = 56.56$, $p < 0.0001$; Fig. 3e). For PC2, the three-way interaction between sex, maturity, and season significantly influenced cheek coloration (Table 1). When analyzing the data separately for

Table 1: The influence of sex, age, season, and their interactions on PC1 and PC2 color scores in bluegills

		PC1				PC2			
Region	Source	R^2	df	F	p	R^2	df	F	p
Breast	Whole model	0.20	11,442	10.02	<0.0001	0.30	12,441	16.10	<0.0001
	Sex		1,442	23.37	<0.0001		1,441	57.08	<0.0001
	Age		1,442	9.14	0.003		1,441	3.45	0.06
	Season		1,442	19.48	<0.0001		1,441	0.53	0.47
	Sex × Age				ns		1,441	15.17	0.0001
	Sex × Season				ns				ns
	Age × Season				ns				ns
	Age × Season × Sex				ns				ns
	Site		8,441	3.79	0.0003		8,441	18.28	<0.0001
Caudal peduncle	Whole model	0.11	11,442	4.75	<0.0001	0.29	13,440	13.76	<0.0001
	Sex		1,442	0.47	0.49		1,440	13.98	0.0002
	Age		1,442	0.58	0.45		1,440	4.39	0.04
	Season		1,442	0.001	0.98		1,440	9.83	0.002
	Sex × Age				ns		1,440	7.00	0.008
	Sex × Season				ns		1,440	6.86	0.009
	Age × Season				ns				ns
	Age × Season × Sex				ns				ns
	Site		8,442	6.15	<0.0001		8,440	18.95	<0.0001
Cheek	Whole model	0.27	12,441	13.82	<0.0001	0.27	15,438	11.01	<0.0001
	Sex		1,441	26.07	<0.0001		1,438	27.26	<0.0001
	Age		1,441	2.60	0.11		1,438	4.60	0.03
	Season		1,441	56.56	<0.0001		1,438	2.02	0.05
	Sex × Age		1,441	10.66	0.001		1,438	7.16	0.008
	Sex × Season				ns		1,438	0.21	0.64
	Age × Season				ns		1,438	1.56	0.21
	Age × Season × Sex				ns		1,438	3.96	0.05
	Site		8,441	5.52	<0.0001		8,438	16.08	<0.0001
Lateral	Whole model	0.18	12,441	8.19	<0.0001	0.29	11,442	16.28	<0.0001
	Sex		1,441	1.29	0.26		1,442	1.93	0.17
	Age		1,441	4.67	0.03		1,442	1.81	0.18
	Season		1,441	6.55	0.01		1,442	26.57	<0.0001
	Sex × Age				ns				ns
	Sex × Season				ns				ns
	Age × Season		1,441	10.75	0.001				ns
	Age × Season × Sex				ns				ns
	Site		8,441	6.30	<0.0001		8,442	16.89	<0.0001
Opercular flap	Whole model	0.29	15,438	11.64	<0.0001	0.18	11,442	8.85	<0.0001
	Sex		1,438	0.14	0.71		1,442	4.15	0.04
	Age		1,438	0.51	0.48		1,442	1.81	0.18
	Season		1,438	21.16	<0.0001		1,442	1.21	0.27
	Sex × Age		1,438	1.88	0.17				ns
	Sex × Season		1,438	0.43	0.51				ns
	Age × Season		1,438	7.09	0.008				ns
	Age × Season × Sex		1,438	7.44	0.007				ns
	Site		8,438	10.78	<0.0001		8,442	11.45	<0.0001

PC1, first principal component; PC2, second principal component.
Data are from mixed-model ANOVA's. Sample site was included as a random effect. Separate analyses were run for each PC score for each body region. Non significant (ns) interaction terms were omitted from the final model. Main effects were only considered when no interaction was observed. See Methods and Fig. 2 for interpretation of PC scores.

each season, there was a significant interaction between sex and maturity only during the breeding season ($F_{1,187} = 8.26$, $p = 0.005$). When we further separated breeding season fish by sex, only males were significantly influenced by maturity, such that mature males had higher PC2 scores than immature

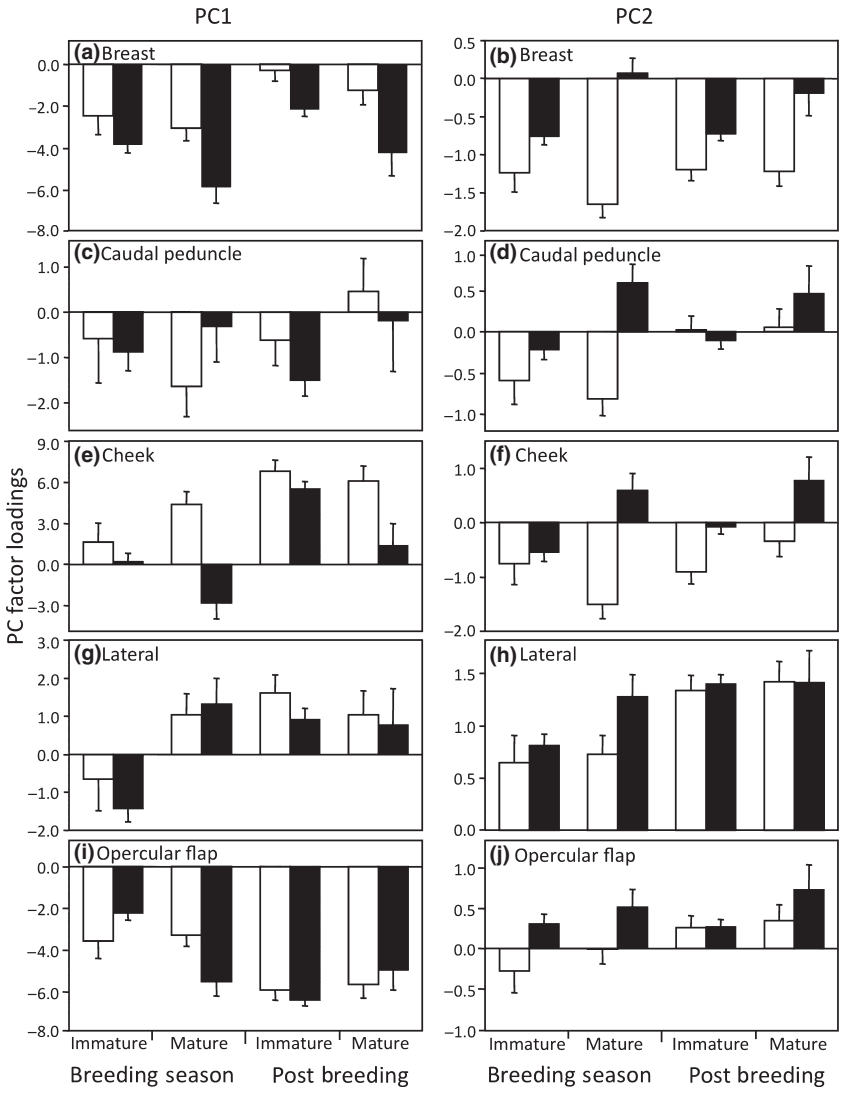

Fig. 3: Principal component scores on reflectance spectra for male (solid black bars) and female (white bars) bluegills separated by season and maturity (Mature includes parental males and mature females). Data are least squares mean (±SE) calculated from a mixed-model ANOVA that also controlled for sampling site. High PC1 scores indicate a light region, whereas low PC1 sores indicate a dark region. Similarly, high PC2 scores indicate a proportional increase of short wavelength spectral reflectance, and low PC2 scores indicate a proportional increase of long wavelength spectral reflectance. See text for further explanation.

males ($F_{1,129}$ = 0.13.68, p = 0.0003; Fig. 3f). When we analyzed breeding season fish separated by maturity, only mature fish were significantly influenced by sex ($F_{1,61}$ = 13.36, p = 0.0005), such that mature males had higher PC2 scores than mature females. During the post-breeding season, both sex and maturity influenced cheek PC2. Mature fish ($F_{1,245}$ = 0.12.80, p = 0.0004) and males ($F_{1,245}$ = 23.16, p < 0.0001) had higher PC2 scores. When analyzing data from the three-way interaction separated by maturity, only sex significantly influenced coloration for both immature ($F_{1,325}$ = 10.22, p = 0.002) and mature ($F_{1,107}$ = 20.11, p < 0.0001) fish, such that males had higher PC2 scores than females in both age categories. Finally, when the data were analyzed separated by sex, only male coloration was significantly influenced by maturity ($F_{1,288}$ = 16.14, p < 0.0001) and season ($F_{1,288}$ = 5.98, p = 0.02), such that mature males and post-breeding season males had higher PC2 scores.

There was a significant interaction between maturity and season in PC1 of the lateral region (Table 1). When analyzing each season separately, color significantly varied with maturity only during the breeding season ($F_{1,189}$ = 21.78, p < 0.0001) such that older, mature fish had higher PC1 scores than immature fish (Fig. 3g).When maturity was analyzed separately, only immature fish varied with season ($F_{1,326}$ = 31.67, p < 0.0001) such that they had lower PC1 scores during the breeding season. There were also significant effects of season on PC2 for the lateral region, where post-breeding fish had higher PC2 scores than breeding fish (Table 1; Fig. 3h).

The brightness (PC1) of the opercular flap was significantly influenced by the three-way interaction among maturity, sex, and season (Table 1). When

running separate analyses for each sex, PC1 scores decreased post-breeding in females ($F_{1,144} = 20.30$, $p < 0.0001$) with no differences observed in maturity ($F_{1,144} = 0.09$, $p = 0.76$; Fig. 3i). For males, there was a significant interaction between maturity and season ($F_{1,287} = 13.60$, $p = 0.0003$). With males further divided by maturity, our data suggest that immature fish experienced the greatest change from breeding to post-breeding seasons, where they were the lightest during breeding and darkest post-breeding ($F_{1,246} = 69.14$, $p < 0.0001$; Fig. 3i). With males divided by season, maturity only significantly influenced coloration during the breeding season ($F_{1,129} = 11.37$, $p = 0.001$), where mature parental males had lower scores than immature males. When analyzing data from the three-way interaction separated by season, only the breeding season was significantly influenced by the interaction between sex and maturity ($F_{1,187} = 6.75$, $p = 0.01$). When these data were further separated by sex, only breeding season males were significantly influenced by maturity ($F_{1,129} = 11.37$, $p = 0.001$), where mature males had lower scores. Conversely, when these data were separated by maturity, only mature breeding season fish were influenced by sex ($F_{1,61} = 9.19$, $p = 0.004$), such that mature males had lower scores than mature females. When the data from the three-way interaction were separated by maturity, immature fish were only significantly influenced by season ($F_{1,325} = 81.15$, $p < 0.0001$), where post-breeding fish had lower scores. Mature fish were influenced by the interaction between sex and season ($F_{1,106} = 6.07$, $p = 0.02$). When these data were further separated by season, breeding season fish were significantly influenced by sex ($F_{1,61} = 9.19$, $p = 0.004$), where parental males had lower scores than mature females. When separated by sex, only mature females were influenced by the effects of season ($F_{1,65} = 9.86$, $p = 0.003$), such that breeding season mature females had higher PC1 scores than post-breeding mature females. For PC2, only sex significantly influenced opercular flap coloration, where males had higher PC2 scores than females (Table 1; Fig. 3j).

Overall, three of the five regions showed similar patterns for PC1. For the breast, cheek, and opercular flap, males were typically darker than females, parental males were darker than immature males, and breeding season fish were darker than post-breeding season fish. The exception to these patterns is that breeding season fish have lighter opercular flaps compared to post-breeding. The lateral region showed opposite patterns, such that mature fish were lighter than immature fish. For PC2, the breast, cheek, and opercular flap exhibited sexual dichromatism, where males had higher scores than females. Ontogenetic changes were observed for breast, caudal peduncle, and cheek regions, such that mature fish had higher scores than immature fish. For seasonal effects, breeding season males had lower PC2 scores for the cheek region. This was opposite to the patterns observed for the caudal peduncle where males had higher PC2 score during the breeding season.

Fulton Condition

We tested for possible condition-dependence of coloration separately in males and females. In analyses controlling for maturity, season, and sampling site, coloration did not show evidence of condition-dependence for any body region in females (all $p > 0.24$). Using the same analyses in males, we found evidence for condition dependence of the breast, cheek and opercular flap. In particular, breast PC2 increased with condition, such that males in better condition had breasts that reflected proportionally more at short wavelengths ($F_{1,291} = 20.63$, $p < 0.0001$). Similarly, cheek PC2 increased with condition, where males in better condition had cheeks with proportionally more short wavelength spectral reflectance ($F_{1,291} = 22.53$, $p < 0.0001$). For the opercular flap, both PC1 and PC2 varied with condition. The first principal component decreased with an increase in condition, such that males in better condition had opercular flaps that were darker ($F_{1,291} = 11.04$, $p = 0.001$). The second principal component increased with condition for the opercular flap such that males in better condition reflected proportionally more at short wavelengths ($F_{1,291} = 7.57$, $p = 0.0063$). There was no evidence for condition dependence of coloration for the remaining body regions and PC scores (all $p > 0.18$).

Discussion

Bluegill coloration is likely produced by a combination of pigmentary and structural mechanisms. Overall, melanin and carotenoid pigments are found in cutaneous layers throughout the body of centrarchids, partly giving rise to the range of colors observed in bluegills (Czeczuga 1981; Mabee 1995). Thus, the black opercular flap and lateral banding is likely produced by melanin pigmentation (Mabee 1995), whereas the orange breast is likely produced by the concentrated deposition of carotenoid

pigments (Matsuno & Hirao 1989). Bluegills also have iridescent patches of color, which are likely produced structurally by iridophores (Denton & Nicol 1966; Hawkes 1974; Losey et al. 1999; Doucet & Meadows 2009). Fish are also capable of temporal changes in color due to neuroendocrine control of their chromatophores (Hawkes 1974; Kodric-Brown 1998). However, our time-series experiment revealed that bluegill coloration did not change significantly for at least 50 min post-capture, suggesting that the findings we describe below are unlikely to be confounded by fading or other types of changes in color.

Our study documented significant influences of maturity, sex, season and condition on coloration in bluegills. Although all body regions were affected by at least one of these factors, the direction of color variation was not consistent among regions, suggesting that different body regions may be influenced by different selective factors. Our ontogenetic analyses revealed that males typically underwent the greatest change in coloration with maturity. Parental male bluegills became darker for the breast, cheek and opercular flaps and lighter in lateral coloration. From an ultimate perspective, these ontogenetic color changes may result from a combination of increasing sexual selection pressure in adults, particularly males, and differential age-specific selective factors (i.e. predation) on younger fish. Milinski (1993) suggested that predation risk may be the primary selective factor acting on coloration in young bluegills, as their small size makes them more vulnerable to a large suite of potential predators. Parental males and mature females, on the other hand, may experience reduced predation pressure due to their large size, with sexual selection favouring more conspicuous coloration to facilitate mate choice. Miller & Brooks (2005) found that as male guppies grow older, they increase their allocation of resources to ornamental traits. In bluegills, parental males should invest more time and energy into mate attraction than immature males, supporting the role of age-dependent sexual advertisement in bluegills (Kokko 1997, 1998).

Previous authors have suggested that bluegills are sexually dichromatic during the breeding season (Gross & Charnov 1980; Gross 1982). Our spectral analyses confirmed that bluegills are strongly sexually dichromatic. In general, males had much darker breast and cheek regions, and breeding parental males had darker opercular flaps. For all regions, males expressed deeper pigmentation and a proportional increase in short wavelength spectral reflectance when compared to females (Fig. 2a–e). Sexual dichromatism is widespread in fishes, where males are typically the more ornamented sex (Kodric-Brown 1998). In bluegills, male breast and cheek coloration are important traits that may influence female preferences during mate choice (Cogliati et al., in press). Other mate choice experiments have confirmed that such dimorphic coloration is the target of female choice in a variety of species, including the upland bully, *Gobiomorphus breviceps* (Hamilton & Poulin 1999), the sand goby, *Pomatoschistus minutus* (Forsgren 1992) and the Trinidadian guppy (Kodric-Brown 1985; Houde 1987).

Our study also revealed that bluegill coloration varies seasonally. Mirroring ontogenetic patterns, all bluegills were generally darker for the breast and cheek, and parental males and mature females had lighter lateral regions during the breeding season. Our analyses indicate that sex differences in coloration for the breast and cheek region, particularly in parental males and mature females, persisted during the post-breeding season, suggesting that some permanent dichromatism is likely in bluegills. Interestingly, permanent dichromatism is usually more prevalent in fishes that have prolonged breeding seasons or that spawn throughout the year, such as tropical species (Kodric-Brown 1998). For fishes where reproduction is seasonally restricted, seasonal sexual dichromatism is more common (Kodric-Brown 1998). In the threespine stickleback, for example, the intensity of red coloration decreases over the course of the breeding season (Bakker & Mundwiler 1994). However, some studies suggest that male ornamental color also is expressed during the parental care phase. McLennan (1991) showed that the intensity of nuptial coloration in the threespine stickleback exhibited a secondary peak post-mating, during male fry guarding.

While female coloration did not vary with condition, we found that males in better condition reflected proportionally more short wavelengths in the breast and cheek regions, and had darker opercular flaps. Our findings support honest indicator models of sexual selection for these body regions. Previous work has shown that Fulton condition predicts parasite load, relative paternity, and probability of egg and larval cannibalism in this species (Neff 2003; Neff & Cargnelli 2004). Given that coloration relates to condition in male bluegills, this trait may reveal multiple aspects of male quality. The condition dependence of carotenoid-based color in particular could be maintained by limited carotenoid availability in the environment (Endler 1980; Grether et al. 2001), parasite limitation (Houde &

Torio 1992; Maan et al. 2006), antioxidant properties of carotenoids (reviewed in McGraw 2006), or some combination of these mechanisms. If coloration reveals heritable parasite resistance or immune health, females could obtain indirect genetic benefits for their offspring by mating with colorful males (Hamilton & Zuk 1982). Alternatively, bright male coloration may reveal current condition and parental quality, allowing females to benefit directly from their choice of mates. Given that our study is largely descriptive, we cannot provide direct support for these hypotheses; however, our findings suggest that this may be a promising avenue for future research.

We also found that bluegill opercular flaps are sexually dichromatic, most notably between parental males and mature females, suggesting some function as a sexual ornament. Parental male bluegills are known to flare their flaps during aggressive interactions (Neff et al. 2004), and Gross (1982) suggested that this trait might be an intrasexually selected character. We found that individuals in better condition had darker, more deeply pigmented opercular flaps; thus, flap coloration might honestly reveal a male's ability to win an aggressive interaction.

To conclude, our findings suggest that multiple factors influence coloration in bluegills. In particular, both the breast and cheek regions may function as sexually selected traits, as they are sexually dichromatic and their coloration is positively correlated with condition. The opercular flap may also serve as a sexual ornament as it is sexually dichromatic, condition dependent in males, and used in intrasexual aggressive interactions (Gross 1982; Neff et al. 2004). Because the caudal peduncle and lateral regions do not appear to be involved in sexual signaling, they are likely controlled by different selective pressures such as predation. These regions showed less variation than is typical of sexually selected traits and the lateral region was lighter in parental males and during the breeding season. Thus, variation in bluegill color may be induced by age-specific selective factors, seasonal and ontogenetic changes in diet, and seasonal variation in lake characteristics. Importantly, our findings identify probable sexual ornaments in a species that has become a model system for behavioral studies in temperate freshwater ecosystems. Together with another recent study (Cogliati et al., in press), our findings suggest that ornamental coloration plays a significant role in the mating system of bluegills. Future studies of intrasexual aggression, mate choice, condition dependence, and age-specific selection factors will provide valuable insights on the role of ornamental coloration in bluegills and other temperate, freshwater fishes.

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