Role of male spatial distribution and condition-dependent colouration on female spawning behaviour and reproductive success in bluegills

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Summary

Female choice for male ornamental colouration has been demonstrated in a number of different taxa. Among fishes, most studies have been conducted in a laboratory setting and show that females prefer more colourful male ornaments. In this study, we observed female bluegills (*Lepomis macrochirus*) spawning in their natural environment and compared spawning behaviours to male traits and position within a colony. We observed spawning activities of 76 parental males in Lake Opinicon, Ontario. We captured each male and used reflectance spectrometry to objectively quantify the colour of six body regions and measured morphological characteristics. Our results show that female spawning behaviours did not significantly differ between central and peripheral males, although egg scores were higher in central nests. During spawning, females appeared to enter the nests of parental males haphazardly. However, our results suggest that male cheek colouration influenced the number of females spawning, the number of eggs they released, and the amount of time they spent in the nest. Moreover, male breast colouration significantly predicted reproductive success as quantified through egg scores. Together, our findings suggest that females may use male cheek and breast colouration, condition-dependent sexual ornaments, as key traits on which to base their mate choice decisions.

Keywords: bluegill, female preference, spawning behaviours, male ornamental colour, nest location/

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Introduction

Female choice for elaborate male traits can evolve through several mechanisms (reviewed in Andersson & Simmons, 2006). In general, females exhibit preferences for particular traits if they stand to gain direct or indirect benefits by evaluating these traits in mate choice (Andersson, 1994a). Females may benefit directly from their choice of mates if male ornaments reflect their ability to provide resources such as high quality territories, food, parental care, or protection. Alternatively, female choice for elaborate male traits may yield indirect (genetic) benefits. For example, the sexy son hypothesis proposes that females benefit from choosing attractive males because their male offspring will also bear these attractive traits (Weatherhead & Robertson, 1979). Moreover, because their daughters will also inherit preferences for these same traits, male traits and female preferences can spread rapidly through the runaway process in subsequent generations (Fisher, 1930). Female choice for male ornaments may also yield indirect genetic benefits if these traits honestly reveal heritable aspects of male quality, such as heritable parasite resistance (Hamilton & Zuk, 1982). Finally, models of mate choice based on genetic compatibility suggest that females obtain reproductive benefits by choosing males that are genetically dissimilar from themselves (Brown, 1997; Mays & Hill, 2004; Neff & Pitcher, 2005; Andersson & Simmons, 2006). Male traits that reveal the possibility of direct benefits, indirect benefits, and compatible genes are not mutually exclusive, and all may play a role in female assessment of male traits (Kokko et al., 2002; Andersson & Simmons, 2006).

The vibrant ornamental colouration displayed by males of many species provides a striking example of traits involved in female mate choice. Classic examples include female preferences for redder plumage in house finches (*Carpodacus mexicanus*) (Hill, 1990), and female preferences for orange spots in Trinidadian guppies (*Poecilia reticulata*) (Endler, 1980, 1983). In fishes, ornamental colouration has been studied on a comparatively small number of species, most notably guppies (e.g., Endler, 1980, 1983; Kodric-Brown, 1985; Houde, 1987) and the threespine stickleback (*Gasterosteus aculeatus*; e.g., Rowland, 1989; Milinski & Bakker, 1990). Many of these studies have convincingly demonstrated a role of colour in female mate choice in a laboratory environment (Nicoletto, 1995; Brooks & Endler, 2001; Karino & Urano, 2008); however, lab experiments do not always correspond to mate choice patterns in more natural settings (e.g., Wilson et al., 2008). In addition, several studies assessing the function of colourful ornaments in fishes have focused on quantifying the size of the ornament rather than the colouration, or have relied on visual assessments of colour rather than spectrophotometric techniques. Spectrophotometric techniques provide a more objective and accurate representation of colour and allow a better comparison among studies than traditional measures (reviewed in Andersson & Prager, 2006). In this study, we conducted behavioural observations of bluegills (*Lepomis macrochirus*) in their natural environment to evaluate whether female spawning behaviour and male reproductive success are influenced by male traits and position within the breeding colony.

Bluegills are colonial breeders that nest in shallow waters during the spring and summer (Gross & MacMillan, 1981). Male bluegills follow one of two irreversible alternative life history strategies termed parental and cuckolder (Gross & Charnov, 1980). Parental males are responsible for building and guarding nests and will delay reproduction until they reach age seven in Ontario, Canada (Gross, 1982, 1991; Neff, 2001). Among cuckolders, two polymorphic forms exist: sneaker and satellite. Sneaker males first mature at age two (Gross, 1982) and they obtain sneak fertilizations by darting through the nest of a parental male while a female is spawning (Fu et al., 2001; Neff et al., 2004). In subsequent years, sneakers become satellite males (Gross, 1982), and these satellites obtain fertilizations by mimicking the appearance and behaviour of females (Dominey, 1981a). This mimicry allows satellite males to enter the nests of parental males with minimal aggressive interactions.

Parental male bluegills establish a colony prior to the arrival of reproductively mature females (Dominey, 1981a). Within three days of the initial establishment of males within a colony, females arrive to spawn with the males over the course of a single day, and males may receive eggs from multiple females (Gross, 1982; Neff, 2003). Once females depart, parental males are responsible for guarding eggs and fry for an additional 7–10 days (Jennings et al., 1997). Due to high predation of eggs in peripheral nests from greater exposure and less mutual defence from neighbours (Gross & MacMillan, 1981), females may benefit from choosing males that are positioned in the center of the colony.

The bluegill system provides an interesting avenue for research on ornamentation because parental and cuckolder males should experience divergent selective pressures, and only parental males are expected to use courtship and ornamentation to attract females. To date, male sexual ornaments remain poorly studied in bluegills. Noble (1934) suggested that colouration in sunfishes may be used in sexual selection, an idea that was quickly discounted by Breder (1936). More recent mention of sexual ornaments in bluegills include the opercular flaps used in intrasexual aggression (Gross, 1982; Neff et al., 2004), and sexual dichromatism during the breeding season (Gross & Charnov, 1980; Dominey, 1981a). In a recent study, we found that bluegill colouration was sexually dichromatic, changed ontogenetically and seasonally, and varied with condition in parental males (breast, cheek, and opercular flap colouration), suggesting that male colouration might function as a sexually selected trait in this species (Cogliati et al., 2010).

Our objective in this study was to evaluate whether male spatial and morphological characteristics influenced female spawning behaviour and reproductive success in naturally-spawning bluegill colonies. If a male's position within a colony is important in spawning, we predicted that centrally-nesting males would attract a greater number of spawning females and a larger number of eggs in their nests. If male ornamental colouration is an important mate choice signal in this species, we predicted that the more colourful males, regardless of nest location, would attract more spawning females laying a larger number of eggs in their nests.

Methods

Spawning observations

Between May 31 and June 27, 2008, we observed bluegill spawning activities at three colonies of similar depth (1.4–1.7 m) in Lake Opinicon, ON, Canada (44°34′N, 76°19′W). At the beginning of the season, we monitored previously used nesting colonies (indicated by clusters of saucer-like depressions in the substrate) throughout the lake for the arrival of parental males. If spawning within a colony commenced, we stayed and observed this colony for the day. When we arrived at a spawning colony, we marked each active nest with a numbered tile for identification. We defined an active nest as one that already contained eggs and where the parental male was actively courting and guarding. This distinction is important as the females may prefer nests where eggs are already present (e.g., Forsgren et al., 1996 and references within). We observed each nest within the colony for 30 min, 2–3 times throughout the day (morning and afternoon) to account for any possible diel variation in activity, for a total of 60–90 min of observations per male. Observation times were converted into a 5-point scale, where each point represented a range of two hours, starting from 0800 and ending at 1800 h. During the 30-min observation period, each observer was responsible for documenting the activities of 1–4 nests within the colony. Previously, Colgan et al. (1979) showed that the presence of an observer does not cause large disturbances in the spawning activities of bluegills.

Observations were made by 2–3 snorkelers and were supplemented with an underwater video camera (Sony® Handycam HDR-SR8, 100GB HDD housed in Amphico® DiveBuddy evoHD elite). The video unit was positioned on a tripod to record activities at multiple nests, and video recordings were analyzed by two separate observers. For each nest, we recorded male location (central or peripheral) in the colony, number of females that entered the nest area, number of females that spawned with the parental male, number of tilts (release of approx. 10–30 eggs; Neff et al., 2003) performed by each female, duration of the spawning event, and the number of aggressive behaviours performed by the parental male toward actual or potential intruders (other parental males and sneaker males). We defined central males as those that had at least one nest between themselves and the edge of the colony, whereas peripheral males had at least one edge of the nest exposed and adjacent to no other nest (Gross & MacMillan, 1981). We converted each observation measurement to a rate in minutes based on the total observation time (typically 60–90 min). Other studies have investigated mate choice in lekking species using similar methods to those described here (e.g., McKaye et al., 1990; Young et al., 2009).

Reflectance measurements

Once spawning activity was completed, we collected each observed parental male from his nest using a dip net, either the evening of or the morning following spawning, for a total of 76 parental males. Previous studies on bluegills suggest that nest takeovers during or after spawning are unlikely (Gross, 1982; Neff, 2001; Neff et al., 2004). To prevent egg loss by predators, we placed a mesh cover over the nest for the brief time (maximum 5 min) that the male was being handled and measured. We performed reflectance measurements on a boat anchored near the colony. We measured reflectance using an Ocean Optics USB4000 portable spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, USA). We used a bifurcated fiber-optic cable mounted with a probe that transmitted broad spectrum light to the surface of the fish and reflected light back to the spectrometer, where data were collected with OOIBase32 software (Ocean Optics) on a PC 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 reflectance from a Spectralon white standard (WS-1; Ocean Optics). To prevent specular reflectance (glare) from affecting our measurements, we gently patted the surface of each fish with a paper cloth prior to measuring reflectance. We measured reflectance on six landmarked body regions: orange breast, green caudal peduncle, green part of the cheek directly below the eye, iridescent part of the cheek at the bottom of the preorpercle, green lateral side of the fish directly above the highest portion of the lateral line, and black opercular flap (Cogliati et al., 2010). We took five readings from each region, where each consisted of an average of 20 readings conducted by the OOIBase 32 software. We then averaged our data into one reflectance spectrum per body region per fish. We restricted spectral analyses to wavelengths between 300 and 700 nm, which encompass the red (620 nm) and green (536 nm) peak cone photopigment sensitivities of bluegills (Hawryshyn et al., 1988).

Male characteristics and egg score

Once we completed our reflectance measurements, we photographed each male and measured his total length (L_T), weight (W), and girth (measured from directly anterior to the dorsal fin). We calculated Fulton condition as W/L_T^3 (Ricker, 1975). We then released each male and removed the mesh covering his nest. Finally, we quantified the total number of eggs in each nest by means of egg scores ranked on a 5-point scale (Claussen, 1991; Côté & Gross, 1993; Cargnelli & Gross, 1996; Neff et al., 2004; Cargnelli & Neff, 2006). Because bluegill eggs are so small and numerous (individual nests can contain more than 1×10^5 eggs), these egg scores were developed to facilitate egg quantification based on visual assessment of overall egg coverage, and have been shown to be highly correlated with the actual number of eggs in nests (Claussen, 1991). We recorded egg scores for all nests the morning following spawning to both ensure that spawning activities in the colony were complete and to reduce potential bias from egg predation (Bain & Helfrich, 1983).

Statistical analyses

Principal components analyses

To summarize variation in male reflectance, we performed principal components analysis (PCA) on reflectance spectra for each body region (Endler, 1990; Montgomerie, 2008). Our analyses resulted in two principal components that together explained 98.4–99.8% of the variation in reflectance. The first principal component (PC1) explained 93.6–97.7% of the variation in reflectance and the second principal component (PC2) explained 1.6–4.9% of the variation. For each region, PC1 had moderate positive loadings across all wavelengths, suggesting that PC1 indicates variation in brightness, where high scores indicate lighter regions. PC2 factor loadings were variable in both magnitude and direction across wavelengths, indicating an association with hue and saturation (see Cogliati et al., 2010). For each region, PC2 had moderate to high positive factor loadings for both short wavelengths (below 375–410 nm) and long wavelengths (550–640 nm), and negative associations in the middle of the spectrum (410–550 nm). Thus, males with high PC2 scores reflected proportionally more at short and long wavelengths (short, UV and violet, 300–450 nm; long, yellow, orange and red, 500–700 nm), whereas males with low PC2 scores reflected proportionally more at wavelengths in the middle of the spectrum (blue and green, 400–570 nm).

Sampling effects

Because we sampled three colonies on two different dates, we evaluated whether female spawning behaviours varied by date or colony. We found no differences in behaviours (number of females that entered and spawned, number of tilts, total time spent spawning, and number of cuckolders) across dates sampled (all $F_{1,74} < 2.84$, $p > 0.1$) or across colonies (all $F_{2,73} < 2.7$, $p > 0.07$). Because we conducted behavioural observations on the same parental males in both the morning and afternoon, we tested for differences in female behaviours between the different observation periods (based on the 5-point scale described above) and found that female nest entry rate ($F_{4,154} = 4.87$, $p = 0.0001$) and female spawning rate ($F_{4,154} = 2.75$, $p = 0.03$) decreased with time of day (more spawning occurred in the morning). We, therefore, used the residuals of regressions between these two spawning behaviours and time of day (rank) to control for time of day effects in all subsequent analyses. The remaining female spawning behaviours observed did not significantly differ between observation times (all $F_{4,154} < 1.31$, $p > 0.27$).

Additional statistical tests

We used unpaired t-tests to test for an influence of male spatial distribution on both male characteristics and female spawning behaviours. To determine if male colour was influenced by male morphology, we used generalized linear regression models, with our colour PC scores as dependent variables and male morphological traits as independent variables. Finally, to determine whether female spawning behaviours were influenced by male characteristics, we used a stepwise regression analysis with a backward elimination for each of the female behaviours. We set the probability to leave the model at 0.055.

Results

Male characteristics

Central vs. peripheral

Prior to the arrival of females, males establish their position within a colony in either a central or peripheral location. We found that peripheral males were significantly heavier than central males (t-test: $t_{71} = 2.16$, $p = 0.03$) and had marginally significant larger girth sizes (t-test: $t_{71} = 1.96$, $p = 0.05$). Fulton condition did not differ between central and peripheral males (t-test: $t_{71} < 1.09$, $p = 0.28$); although total length varied in the same direction (t-test: $t_{71} = 1.76$, $p = 0.08$). With respect to colouration, only the iridescent cheeks of males were significantly different between central and peripheral males (t-test: $t_{71} = -2.08$, $p = 0.04$; PC2), where peripheral males had lower PC2 scores. Opercular flap PC1 and breast PC2 showed similar trends, where peripheral males had lower scores than central males (opercular flap PC1, t-test: $t_{71} = -1.81$, $p = 0.07$; breast PC2, t-test: $t_{71} = -1.74$, $p = 0.09$). None of the other regions differed significantly between central and peripheral males (t-tests: $t_{71} < 1.6$, $p > 0.12$).

Colour vs. morphology

Generalized linear regression models revealed that male girth was significantly related to PC1 (brightness) for both the breast and the cheek region, such that males with a larger girth had significantly lower PC1 scores for both regions (Table 1). For five of the six body regions measured (breast,

Table 1. Male colour characteristics in relation to morphology and condition. Results are from generalized linear regression models for each body region.

Region	Colour Component	R^2	df	F	Std. β	p
Breast	PC1 whole model	0.11	4	2.28		0.07
	Girth		1	7.61	−0.82	0.007
	PC2 whole model	0.14	4	2.92		0.03
	Total length		1	7.38	−3.49	0.008
	Weight		1	7.52	3.79	0.008
	Fulton condition		1	7.31	−2.26	0.009
Caudal	PC1 whole model	0.05	4	0.91		0.46
	No significant effects					
	PC2 whole model	0.09	4	1.81		0.14
	No significant effects					
Cheek	PC1 whole model	0.18	4	3.84		0.007
	Girth		1	11.85	−0.98	0.001
	PC2 whole model	0.13	4	2.73		0.04
	Total length		1	7.97	−3.65	0.006
	Weight		1	7.54	3.81	0.008
	Fulton condition		1	7.58	−2.31	0.008
Iridescent Cheek	PC1 whole model	0.05	4	0.90		0.47
	No significant effects					
	PC2 whole model	0.12	4	2.44		0.06
	Total length		1	5.94	−3.17	0.02
	Weight		1	5.02	3.13	0.03
	Fulton condition		1	5.91	−2.06	0.02
Lateral	PC1 whole model	0.08	4	1.59		0.19
	No significant effects					
	PC2 whole model	0.13	4	2.73		0.04
	Total length		1	6.44	−3.23	0.01
	Weight		1	6.33	3.49	0.01
	Fulton condition		1	6.39	−2.12	0.01
Opercular Flap	PC1 whole model	0.04	4	0.76		0.56
	No significant effects					
	PC2 whole model	0.14	4	3.01		0.02
	Total length		1	7.25	−3.45	0.009
	Weight		1	7.60	3.80	0.007
	Fulton condition		1	7.35	−2.26	0.008

cheek, iridescent cheek, lateral, and opercular flap), colour PC2 scores (hue and saturation) were significantly related to total length (TL), weight, and Fulton condition (Table 1). Each of these body regions followed the same pattern, where males with a longer total length and a higher Fulton condition factor had lower PC2 scores, and males that were heavier had higher PC2 scores.

Female spawning behaviour

Central vs. peripheral

We found no significant effect of a male's position within a colony on any of our observed female spawning behaviours (Table 2). However, we did find that central nests had significantly higher egg scores than peripheral nests (Table 2).

Female spawning behaviour vs. male characteristics

Using stepwise regression analyses with a backward elimination procedure, we found that female spawning behaviours were significantly influenced by both male morphology and colour (Table 3). Although none of the male characteristics predicted the number of females that entered the nest, the total length, weight, Fulton condition and PC1 of the iridescent cheek significantly predicted the number of females that remained in the nest and spawned with the parental male (Table 3). In particular, shorter, heavier males with a low

Table 2. Female spawning behaviours in relation to male location within a colony.

Behaviour	t	Central ($N = 30$)	Peripheral ($N = 43$)	p
Number of females entering/h[a]	−1.23	5.07 ± 0.55	4.18 ± 0.46	0.22
Number of females spawning/h[a]	−1.31	2.54 ± 0.32	1.94 ± 0.27	0.20
Number of tilts/h	−0.77	89.55 ± 13.80	75.79 ± 11.53	0.45
Amount of time (min) spent spawning/h	−0.39	11.95 ± 1.93	10.98 ± 1.61	0.70
Number of cuckolders/h	1.32	28.18 ± 5.88	38.29 ± 4.91	0.19
Egg score	−3.04	2.37 ± 0.15[b]	1.79 ± 0.12[c]	0.004

Data shown are from t-tests. Values for central and peripheral males are given as means per h ± SE.

[a] t-test performed on residual data controlling for time of day, means ± SE shown are from original data.

[b] $N = 27$.

[c] $N = 39$.

Table 3. Male characteristics and colour influence female spawning behaviours.

Female spawning behaviour	Model effects	R^2	df	F	Std. β	p
Number of females entering the nest[a]	No significant effects					
Number of females spawning[a]	Whole model	0.11	4	2.12		0.09
	Total length		1	4.11	−2.60	0.05
	Weight		1	4.36	2.99	0.04
	Fulton condition		1	4.14	−1.73	0.05
	PC1 iridescent cheek		1	5.14	−0.26	0.03
Number of tilts	Whole model	0.17	3	4.97		0.004
	PC1 cheek		1	3.90	0.22	0.05
	PC1 iridescent cheek		1	4.13	−0.23	0.05
	PC1 lateral		1	7.22	0.30	0.009
Time spent spawning	Whole model	0.15	2	6.54		0.002
	PC1 caudal		1	5.22	0.26	0.03
	PC1 cheek		1	3.93	0.22	0.05
Egg score	Whole model	0.30	3	8.82		<0.0001
	Fulton condition		1	6.16	−0.27	0.02
	PC2 breast		1	16.66	0.97	0.0001
	PC2 caudal		1	10.84	−0.78	0.002
Number of cuckolders	Whole model	0.16	3	4.71		0.005
	PC1 cheek		1	13.50	0.46	0.0005
	PC2 cheek		1	6.33	1.44	0.01
	PC2 operculum		1	6.19	−1.44	0.02

Results are from reverse stepwise regression analyses with a probability to leave the model set to 0.055. Female spawning behaviours are rates per min based on the total observation time.

[a] Residuals controlling for diel variation were used in these analyses.

Fulton condition and low PC1 score (iridescent cheek) had significantly more females spawning in their nests. In addition, PC1 scores for the cheek, iridescent cheek, and lateral region significantly predicted the number of tilts by females (egg deposition; Table 3), such that males with higher PC1 scores for the cheek and lateral region received significantly more tilts. Males with higher PC1 scores for the iridescent cheek region received significantly fewer tilts by females. For both the caudal peduncle and the cheek, PC1 significantly influenced the time that males spent spawning (Table 3), such that males with higher PC1 scores for both regions spawned for a significantly longer amount of time.

We also found that male cheek and opercular flap colouration predicted the number of cuckolders that enter a male's nest (Table 3). Males that had a higher PC1 and PC2 score for the cheek region had significantly more cuckolders, and males with a higher PC2 score for the opercular flap had significantly fewer cuckolders. Finally, we found that a male's Fulton condition, as well as the colouration of his breast and caudal peduncle, significantly predicted his eggs scores (Table 3). Males with a low Fulton condition factor, high PC2 scores for the breast, and low PC2 scores for the caudal peduncle had significantly higher egg scores.

Discussion

In this study, we observed a population of bluegills spawning in their natural environment to investigate whether male morphology, ornamental colour, and position in the breeding colony influence female spawning behaviour and male reproductive success. We used female spawning behaviour as an indirect measure of female choice, and used egg scores as a measure of male reproductive success (Cargnelli & Gross, 1996; Neff et al., 2003, 2004). Our findings suggest that multiple aspects of male morphology and colour influence female preference and reproductive success in bluegills. Furthermore, our findings suggest that although nest location within the colony is important for male reproductive success, it does not appear to strongly influence female spawning behaviour.

In colonial breeding systems, central positions in the colony are thought to be preferred because predation rates should be higher at the periphery (Gross & MacMillan, 1981; Dominey, 1981b). Females of lek-based mating systems also prefer central males that are found in dense clusters, where male mating success positively correlates with proximity to the centre of the lek (Wiley, 1991; Bradburry & Gibson, 1983). Previous work has shown that in bluegill colonies, central and peripheral males did not differ in their number of egg predation attempts (often from cuckolders) during spawning (Gross & MacMillan, 1981) nor did they differ in differ in Fulton condition, length, and age (Neff et al., 2004). In this study, only weight and the iridescent colour of the cheek differed between central and peripheral males. Weight may fluctuate readily because males do not forage while establishing and protecting their nests; therefore, the differences we observed may not reflect male size at the time of colony establishment. Alternatively, males may be heavier at the periphery if they were late to arrive at the colony during nest establishment because they were spending more time foraging and, thus, had to settle with a peripheral nest.

In addition to these differences in male characteristics, we found that egg scores were higher in central nests. It is possible that overnight predation from bullheads and catfishes contributed to these differences because we recorded egg scores the morning following spawning (Dominey, 1981a; Gross & MacMillan, 1981). Filial cannibalism has also been shown to be high during the egg phase of parental care, where males in better condition have lower rates of cannibalism (Neff, 2001). However, our data show that egg scores and Fulton condition are inversely related, suggesting that cannibalism did not contribute to our egg score differences. Moreover, differences in male morphology did not explain the higher eggs scores in central nests. On the other hand, breast colouration strongly predicted egg score values. These findings suggest that central males have higher reproductive success than peripheral males, which is consistent with previous studies on bluegills and closely related species (Jennings & Philipp, 1992; Neff et al., 2004).

Despite finding significant effects of nest location on egg scores, female spawning behaviour did not significantly differ between central and peripheral males, although the relationships were in the same direction as egg scores with regards to centrality. Given that the relationships follow the expected direction, it is worthwhile noting that our data for female behaviours were much more variable than our egg scores. High variance in female behaviour may have resulted from the fact that we only observed each male for 60 to 90 minutes. Had we been able to observe each male for the entire duration of spawning, we might have been able to obtain a clearer picture of female preference for male traits. Additionally, this mismatch between female spawning behaviour and egg scores could have resulted from higher predation on peripheral nests as mentioned above. Another possibility is that a female’s spawning behaviour may not directly correlate with the number of eggs she releases. Ultimately, our findings highlight the importance of considering multiple measures of male reproductive success in this type of study.

With regard to the influence of male characteristics on female spawning behaviours, our findings indicate that male colour and morphology did not significantly predict the number of females that entered a nest. Interestingly, not all females that entered nests spawned. Females often entered a nest but left before releasing any eggs, suggesting that females enter the nests of parental males haphazardly and evaluate the characteristics of multiple males before choosing one with which to spawn, a finding consistent with various mate choice models (Gibson & Langen, 1996; Widemo & Saether, 1999). Kraak (1996) found similar patterns in Mediterranean blennies (*Aidablennius sphynx*), where not all sexual interactions in the nest led to female egg depositions.

Once a female remained in a nest to spawn, multiple male characteristics appeared to influence the number of tilts produced by females, the total amount of time spent spawning by females, and males' final egg scores. In particular, the cheek and iridescent cheek regions significantly influenced female spawning behaviours, and the breast region significantly predicted male egg scores. When a female enters a male's nest, she primarily receives visual stimulus from the anterior portion of the male, most notably his breast, cheek, and operculum, as the pair usually swims in parallel while circling the nest. Possibly, these regions could stimulate the female to remain in the nest and release more eggs. Visual signals that stimulate reproduction have also been documented in other species such as collared lizards (*Crotophytus collaris*; Baird, 2004) and Nile tilapia (*Oreochromis niloticus*; Castro et al., 2009). Alternatively, male cheek and breast colouration may serve as honest indicators of male quality. Data from a previous study suggest that both of these traits are condition-dependent (Cogliati et al., 2010). As such, cheek and breast colouration could reveal a male's parental abilities through the good parent hypothesis (Hoelzer, 1989), or could indicate some heritable aspect of male quality (Andersson, 1994a). Studies have shown that female choice can be influenced by the presence of eggs or other females (e.g., Ridley & Rechton, 1981; Kraak & Groothuis, 1994; Forsgren et al., 1996); however, we assume that this had minimal impact on our findings because all of our observations took place on nests that already contained eggs, and because we rarely observed females spawning in the same nest simultaneously. However, it is possible that females cued in on the total number of eggs in the nest or the quality of those eggs. Although our data seem to suggest that female spawning behaviours are influenced by male colour and morphology, the percent variation in female spawning behaviour explained by male traits was relatively low. As with differences between female behaviour and egg scores, these analyses may have been influenced by the large variation in female spawning behaviour, which may have resulted from the length of our observation periods. Alternatively, this pattern may reflect real variation in female preference for different male traits, or the possible influence of multiple traits on female preference, including traits we did not measure such as genetic compatibility (Mays & Hill, 2004; Neff & Pitcher, 2005). Our analyses are comparable to those of Warner and Schultz (1992), who conducted experimental field studies on the bluehead wrasse (*Thalossoma bifasciatum*) to determine which characteristics may be sexually selected through multiple regression techniques.

A male's colour was also related to the number of cuckolders entering his nest. A number of non-mutually exclusive explanations could explain this finding. First, this pattern could simply be a correlation effect, such that cuckolders intrude more often in nests that have spawning females. An alternative possibility is that cuckolders select males to cuckold based on the same traits that are preferred by females. Finally, with cuckolders strategically positioned around the colony, females may choose nests that are more likely to be cuckolded to increase genetic benefits (Alonzo & Warner, 2000; Neff, 2008).

Our study revealed surprising relationships between male characteristics and ornamental colour. We found that males with a larger girth had significantly darker breasts and cheeks, as might be expected. However, contrary to our expectations, we found that males with higher Fulton condition had a proportional decrease in short and long wavelength reflectance for five of six regions. In a previous study based on a large sample of males from multiple lakes, breast, cheek, and opercular flap colouration were condition-dependent traits in parental males, with more colourful individuals being in better condition (Cogliati et al., 2010) — a relationship opposite to that found in this study. Although the synoptic field study was conducted during the breeding season, it did not include spawning individuals (Cogliati et al., 2010), whereas the current study focused exclusively on spawning parental males. Fulton condition may be a poor measure of body condition at the time of spawning as it readily fluctuates based on energy spent on nest defence and maintenance. During the spawning period, parental males do not forage and can lose up to 20% of their body mass (average 11%;

Coleman & Fischer, 1991). Thus, males that were more active in attracting females and defending nests may have had a lower body condition index at the time of measurement. Similar patterns have been documented in lek-mating species, where high quality males spend considerable time and energy advertising for females (Vehrencamp et al., 1989), and often experience a reduction in condition as a consequence (e.g., Höglund et al., 1992; Andersson, 1994b). This interpretation is anecdotally supported by our study, where we found that the most colourful males were more attractive to females and achieved higher reproductive success but were in poorer condition, and that males in poor condition had higher egg scores than males in good condition.

The opercular flap has been proposed to be an intrasexually selected trait in this species, as males often flare out their opercular flaps during aggressive interactions (Gross, 1982; Côté, 1993). However, Neff et al. (2004) showed that opercular flap size did not differ between central and peripheral males. We previously suggested that opercular flap colouration, a condition dependent ornament, may be important in intrasexual aggression and be used in establishing a colony (Cogliati, 2009). However, opercular flap colouration did not significantly vary between central and peripheral males, nor did it significantly predict any of our observed female spawning behaviours.

In conclusion, our investigation of female spawning behaviour, male traits, and male reproductive success suggests that female bluegills do exhibit mate choice preferences, and that those preferences may be mediated in part by male ornamental colour. In contrast, male nesting position does not appear to influence the observable spawning behaviours of females, although central males experience higher reproductive success quantified through egg scores. These findings suggest that male traits and position within the colony are both important during spawning. Although other factors likely contribute to the overall picture of sexual selection in bluegills, we have identified condition-dependent colour traits that appear to be important in naturally spawning colonies, largely in terms of overall reproductive success. Our findings suggest that multiple factors may influence bluegill reproductive success, and that even in a species with alternative reproductive tactics, particular male strategies may involve the type of condition-dependent signaling that has been documented in species with more traditional breeding systems.

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