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UMI
MULTIPLE SOCIAL AND ECOLOGICAL FACTORS INFLUENCE COLORATION IN BLUEGILLS (*Lepomis macrochirus*)

By

KAREN MICHELLE COGLIATI

A Thesis
Submitted to the Faculty of Graduate Studies through Biological Sciences
in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2009

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DECLARATION OF CO-AUTHORSHIP

I hereby declare that this thesis incorporates material that is the result of joint research. All three data chapters are co-authored with my supervisors, Drs. Lynda Corkum and Stéphanie Doucet. My research was supported by both advisors, and each provided valuable feedback, helped with project design and statistical analyses, and provided editorial input during the writing of each manuscript. Chapter 2 was prepared as a manuscript, and has been submitted to Behaviour for publication. Chapter 3 has also been prepared submitted to Behavioral Ecology and Sociobiology. Finally, Chapter 4 was prepared as a manuscript for submission to Oikos.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from my co-authors to include the above materials in my thesis. I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work, completed during my registration as graduate student at the University of Windsor.

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Antagonistic interactions between sexual and natural selection influence the evolution of ornamental signals. I investigated possible functions of colourful traits in bluegills (*Lepomis macrochirus*). First, I conducted a descriptive study among eight lakes in Ontario to determine how colour traits varied with age, sex, season, and condition. My findings identified breast, cheek and opercular flap coloration as possible sexual ornaments. Subsequently, I investigated the role of sexual selection on coloration through observations of spawning bluegills. Results suggest that breast and cheek coloration influence female spawning behaviour and male reproductive success. Finally, I investigated effects of ecological factors on coloration. Vegetation type and density influenced coloration for all bluegills, and predator species richness influenced coloration of immature fish. These studies provide the first investigations of the function of coloration in bluegills and contribute to our understanding of the interacting influences of sexual and natural selection on the evolution of ornamental traits.
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CHAPTER 1: GENERAL INTRODUCTION

The evolution of ornamental signals may be driven, in part, by female preferences, and is often counteracted by natural selection (Andersson, 1994). The function of such signals in males may be to provide information to reproductive females regarding quality (Zahavi, 1975; Andersson, 1994), parental abilities (Hoelzer, 1989), and parasite resistance (Hamilton & Zuk, 1982), among others. Not all colourful traits function as ornaments, and these may have several other functions, including crypsis (Endler, 1990) and species recognition (Seehausen & van Alphen, 1998).

Intra- and inter-specific communication is an important process in the life of an animal. Animals can communicate using a diversity of signaling modalities, and the prominent sense organ used by a particular species will typically depend on the environment in which the signal is transmitted. For example, visual signals are often best suited for clear environments and at short distances, whereas chemical signals may be more effective in dark or turbid environments at a variety of distances (Bradbury & Vehrencamp, 1998). The evolution of animal signals is influenced by both signal content and signal efficacy (Guilford & Dawkins, 1991, 1993; Andersson, 2000). The content of the signal is simply the information that is being communicated, whereas the efficacy is how successful the signal is in altering the behaviour of the receiver. Signal efficacy is dependent on the receiver sensory system and the environmental conditions through which the signal is transmitted (Endler, 1992, 1993a,b). The content and efficacy of signals used in mating should be designed to increase the reproductive success of both sender and receiver. Secondary sexual signals are often shaped by the antagonistic interaction of sexual and natural selection (Andersson, 1994). In terms of visual signals
based on colour, sexual selection will tend to favour conspicuous coloration, whereas natural selection will tend to favour cryptic coloration (Endler, 1978, 1991).

**Sexual selection**

Darwin first proposed the theory of sexual selection in 1859 to explain the evolution of elaborate traits that appear to impede rather than enhance survival, and thereby apparently contradicted his theory of natural selection. Rather than enhancing survival, sexually selected traits could evolve through a struggle for access to mates, with successful competitors having more offspring. Such competition for access to mates should lead to differential reproductive success, giving rise to sexual selection (Darwin, 1871; Andersson, 1994). In many mating systems, females produce few costly gametes that are rich in energy and males have many inexpensive gametes that are highly motile (Andersson, 1994). This difference in sexual allocation (anisogamy) leads to the evolution of behavioural and morphological sex differences (Andersson, 1994). Furthermore, such differences create sexual conflict, whereby females are often the choosier of the two sexes due to their higher energetic investment in gamete production (Batemen, 1948; Trivers, 1972).

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 (Darwin, 1871; Andersson, 1994). However, these are not necessarily mutually distinct, as some male weapons may also be used by females when assessing mates (Fisher, 1930; Noble, 1934a). Secondary sexual traits may be evaluated by females during the mate
choice process, which can lead to the evolution of elaborate male ornaments (Darwin, 1871; Fisher, 1930; Andersson, 1994).

Theories of evolution of female mate choice

Controversy regarding how female preferences for male ornaments evolve has led to the proposition of several non-mutually exclusive mechanisms of female mate choice (Andersson, 1994; Kokko et al., 2002; Andersson & Simmons, 2006). Some mate choice models propose that females exhibit preferences for particular traits if they stand to gain direct or indirect benefits by evaluating these traits in mate choice (Andersson, 1994). According to mate choice models based on direct effects, females may benefit from their mate choice decision if male ornaments reflect their ability to directly provide resources to a female, such as parental care, protection, high-quality territories, or food resources (Møller & Jennions, 2001; Andersson & Simmons 2006). Another model that describes female preference based on direct benefits includes the good parent hypothesis (Hoelzer, 1989). Females may also gain indirect (genetic) benefits by selecting males based on secondary sexual traits. Secondary sexual traits may indicate genetic quality in males, where females may prefer such traits as they may suggest heritable parasite resistance (Hamilton & Zuk, 1982), or traits that indicate the genetic or phenotypic quality of the bearer as they may be costly to bear (Zahavi, 1975; Andersson, 1994). Other models that have emerged from indirect female benefits include the sexy son hypothesis, which proposes that females benefit from choosing attractive males because their male offspring will also bear these attractive traits (Weatherhead & Robertson, 1979; Kirkpatrick, 1982; Andersson, 1994). Any of these models, both with direct and indirect benefits, may lead
to the coevolution of traits. That is, since daughters of females with a preference gene will also inherit the preference for the same male traits, the genetic coevolution of male trait and female preference can spread rapidly through the runaway process (Fisher, 1930).

Although many models exist to describe female preferences for male traits, the origins of these preferences are not always known. Female preference for male secondary sexual traits may have evolved for reasons other than sexual selection. For example, female preference may have evolved through sensory drive (Endler & Basolo, 1998), which can precede the mechanisms of female mate choice mentioned previously. Here, females may have initially favoured a male trait through natural selection, such as in the context of foraging or predator avoidance, and males further evolved traits that exploited these female biases and ultimately became favoured by sexual selection (Endler & Basolo, 1998; Ryan, 1998).

A more recent view on the evolution of mate choice proposes that females may seek to increase non-additive genetic benefits through mating decisions based on genetic compatibility (Zeh & Zeh, 1996). That is, females may obtain reproductive benefits by choosing males that are genetically dissimilar from themselves (Brown, 1997; Mays & Hill, 2004; Neff & Pitcher, 2005; Andersson & Simmons, 2006). By choosing mates that are genetically dissimilar, females may increase the heterozygosity of their offspring, which has in turn been shown to increase offspring fitness (Amos et al., 2001; Hansson & Westerberg, 2002; Penn, 2002). Overall, there are many proposed mechanisms for the evolution of female preferences, many of which suggest that male ornaments may reveal the possibility of direct benefits, indirect benefits, or compatible genes. As mentioned
previously, these proposed mechanisms are not mutually exclusive and all may influence female assessment of male secondary sexual traits (Kokko et al., 2002; Andersson & Simmons, 2006).

**Influence of natural selection on sexually selected traits**

Sexual and natural selection often exert opposing selective pressures on the evolution of ornamental traits (Andersson, 1994). In a simplistic sense, this means that natural selection prevents the over-exaggeration of ornamental traits, which might otherwise occur if sexual selection were acting alone. A classic example of this antagonistic relationship is the influence of predation on the evolution of sexual ornaments. Male secondary sexual traits, which may be conspicuous in order to attract females, may also be conspicuous to potential predators (Andersson, 1994; Zuk & Kolluru, 1998). Thus, female preference for conspicuous traits should decrease with an increased cost of mate choice (Crowley et al., 1991). For example, the song of male field crickets (*Gryllus integer*) is attractive to females that are ready to mate but also attractive to female parasitoid flies, which end up killing the male crickets (Cade, 1975). In threespined sticklebacks (*Gasterosteus aculeatus*), females prefer males with redder throat patches (Bakker & Milinski, 1993); however, males with a bright red throat patch were twice as likely to be attacked by predators as males with dull throat patches (McPhail, 1969; Moodie, 1972). Similar effects of predation have been documented in Trinidadian guppies (*Poecilia reticulata*). Females from areas of high predation will exhibit weakened preferences for bright male coloration compared to those in areas of low
predation (Endler, 1978). Consequently, males in areas of high predation are not as ornamented and may appear more cryptic in their environment.

**Colour signaling in fishes**

Several studies have investigated the use of colourful ornaments in fishes, especially in the context of sexual selection. Male nuptial coloration is designed to both attract females and deter male competitors (Kodric-Brown 1990). Often, females will also display nuptial coloration as a courtship signal or as a way to reduce male harassment toward females that are not yet reproductive (Rowland et al. 1991, McLennan 1995). Studies have also demonstrated a role of honest advertising of ornamental coloration in fishes, as bright colour patches are often produced by carotenoid pigments which cannot be synthesized in vivo and must therefore be obtained from the diet (e.g., Hamilton & Poulin, 1999; Amundsen & Forsgren, 2001). In such cases, females may prefer males that display a higher intensity of carotenoid pigmentation because it honestly reveals mate quality by signaling their superior foraging success and general state of health (Kodric-Brown 1989). This is beneficial to the females since there is likely to be a heritable component involved in one’s ability to acquire and use carotenoids (e.g., Hill, 1991). Finally, many studies have also successfully demonstrated a role of ornamental coloration in female mate choice, for example, in Lake Malawi cichlids (Jordan et al., 2003), three-spined sticklebacks (*Gasterosteus aculeatus*; Bakker & Milinski, 1993; Boulcott et al., 2005), and Trinidadian guppies (*Poecilia reticulata*; Kodric-Brown, 2001; Karino & Urano, 2008).
Environmental influences on coloration in fishes have also been investigated in a number of species. As previously mentioned, predation has been shown to play a large role on female preferences for ornamental traits in three-spined sticklebacks (McPhail, 1969; Moodie, 1972) and in guppies (Endler, 1978). Also important is the visual environment in which fishes communicate. The visual environment comprises the amount and coloration of irradiant light in the water, which determines the light available for display and constitutes the visual background against which fishes are normally seen, as well the type and amount of vegetation in the habitat, which may also contribute to the visual background and influence signal contrast. In a study on bluefin killifish (*Lucania goodei*), for example, light environment strongly predicted the presence of two different male colour morphs (Fuller, 2002). Each colour morph was found in habitats that increased their conspicuousness against the ambient light background to enhance signal transmission (Fuller, 2002). Overall, although considerable research has been conducted on the function and evolution of coloration in fishes, there is still a great deal of progress to be made to further our understanding of colour signaling in a wider variety of species.

The next steps

To date, the majority of studies aiming to investigate ornamental coloration in fishes have focused on only a handful of species, most notably guppies (e.g., Endler, 1980, 1983; Kodric-Brown, 1985; Houde, 1987) and three-spined sticklebacks (e.g., Rowland, 1989; Milinski & Bakker, 1990). The majority of these studies have shown that colour is used by females during the mate choice process and may act as an honest indicator of male quality. However, these studies are often conducted in laboratory choice
tests, which do not always ideally represent behaviours in natural settings (e.g., Nicoletto, 1995; Brooks & Endler, 2001; Karino & Urano, 2008). Until recently, most studies investigating the role of fish coloration in mate choice used simplistic techniques to measure colour. These included quantifying the number and area of colour patches or using visual assessments rather than spectrophotometric techniques, which objectively measure colour (see below for description). By investigating coloration using spectrophotometric techniques on a greater number of fishes, we may be able to provide a more in-depth understanding of the diversity, function, and evolution of coloration.

Objective quantification of colour using reflectance spectrometry

Biologists have long been interested in the function of animal colour signals. Until recently, however, colours have been described using human-subjective methods, including colour charts and photography. Only in the last two decades have researchers begun to use objective assessments to measure animal colour patterns (mostly bird coloration) with reflectance spectrometers. The benefits of using reflectance spectrometers are that the entire spectrum (300 – 700 nm) can be repeatedly measured more accurately, allowing better comparison among studies (reviewed in Andersson & Prager, 2006). Also, reflectance spectrometers are not human biased and provide a more accurate and precise representation of colour. A reflectance spectrometer setup consists of a bifurcated fiber-optic probe, a broad spectrum light source, and a spectrometer. The broad spectrum light is transmitted through the fiber-optic cable to the surface of the specimen and the reflected light is then captured by the fiber-optic cable and sent to the spectrometer, which is connected to a computer (Andersson & Prager, 2006). With the
use of specialized software, researchers are able to visualize the reflected light using a spectrograph, where percent reflectance is shown on the y-axis, and wavelength on the x-axis (Andersson & Prager, 2006). From the saved reflectance data, researchers are able to calculate all three dimensions of colour (brightness, hue, and chroma), and use these variables in further statistical analyses (Endler, 1990; Montgomerie, 2006). The use of reflectance spectrometry for measuring fish coloration has lagged behind the use of spectrometry measuring bird coloration; however, more recent studies have begun using such techniques on fish (e.g., Grether et al., 2001; Fuller, 2002; White et al., 2003; Rick et al., 2004).

**Study species**

The bluegill, *Lepomis macrochirus* Rafinesque 1819 (Perciformes: Centrarchidae), is an abundant freshwater sunfish found throughout the United States of America and southern Canada (Becker, 1983). It is often found in vegetated lakes, ponds, and streams of low current velocity (Becker, 1983). Bluegills are also known to associate strongly with habitats of fine substrates and complex macrophytes (Lapointe et al., 2007). All sunfish in the family Centrarchidae are similar in shape with notable differences in colour. There is considerable individual variation in coloration among bluegills; however, they usually possess a solid black opercular flap, an iridescent blue chin, green cheeks, and an overall greenish coloration with yellow to orange breasts (Becker, 1983). Although the general colour of bluegills has been described, the function of their variation in colour has remained poorly studied. The idea that coloration in sunfishes could be used in sexual selection was first proposed by Noble (1934b), but was quickly
discounted by Breder (1936). Since then, few suggestions have been made as to the function of bluegill coloration, and suggestions include intrasexually selected opercular flaps (Gross, 1982; Neff et al. 2004) and sexual dichromatism during the breeding season (Gross & Charnov, 1980; Dominey, 1981).

Bluegills have long been used as a model system for studies of alternative reproductive tactics. In this system, males follow one of two irreversible life history strategies termed parental and cuckolder (Gross & Charnov, 1980). Parental males delay reproduction until approximately age seven, at which time they begin to build and defend nests (Gross, 1982, 1991; Neff, 2001). Cuckolder males become reproductively mature at a much younger age, typically around two years, at which time they are considered sneaker males (Gross, 1982). Because of their small size, these males can fertilize the eggs of spawning females by darting undetected through the nests of parental males (Fu et al., 2001; Neff et al., 2004). In subsequent years, typically around age four, sneaker males become satellites (Gross, 1982). As satellites, males mimic the behaviour and appearance of females in order to deceive parental males, enter the nest of spawning pairs, and fertilize eggs (Dominey, 1981). These two strategies are considered irreversible, since cuckolders never become parental males, nor do they approach them in age or size (Gross & Charnov, 1980; Gross, 1982).

As colonial breeders, parental males are responsible for establishing a dense colony in shallow waters during spring and summer (Gross & MacMillan, 1981). Within the colony, each parental male is also responsible for maintaining a single nest prior to the arrival of reproductively mature females (Dominey 1981). Within three days of the arrival of males, females arrive together to spawn over the course of a single day (Gross,
1982). At the end of the spawning day, the females leave the colony and parental males remain on the nests for an additional seven to ten days to guard eggs and fry (Jennings et al., 1997). Cuckolders typically remain at the colony and continue their attempts at intruding parental nests to consume eggs and fry.

The general ecology of bluegills is also well known. They are considered opportunistic feeders, eating almost anything that will fit in their mouth (Egertson & Downing, 2004). Like most fishes, bluegills become less vulnerable to natural predation with an increase in size. Bluegills have a diversity of predators that vary as they increase in size, with their principal predator being the largemouth bass (*Micropterus salmoides*). Other predators include the Northern pike, (*Esox lucius*), muskellunge (*Esox masquinongy*), walleye, (*Sander vetreus*), and yellow perch (*Perca flavescens*). Despite their abundance and distribution, bluegills are not typically preferred prey items because they have hard fin rays which they erect when attacked (Savitz & Janssen, 1982; Keast, 1978). Bluegills are also known to undergo an ontogenetic shift, where juveniles remain in the shallow vegetated, littoral-zone and feed on invertebrates, and adults shift to open-water and feed largely on zooplankton (Mittelbach, 1981, 1984; Ehlinger & Wilson, 1988; Werner & Hall, 1988).

**Determinants of colour signaling in bluegills**

A great deal of research has been conducted on bluegills (Spotte, 2007) including a detailed characterization of their mating system (Gross & Charnov, 1980; Gross, 1991; Neff et al., 2003; Neff et al., 2004). With such an extensive research focus on this species, it is surprising that their use of coloration has yet to be thoroughly investigated.
The focus of my thesis is to characterize colour variation in bluegills and to investigate possible functions of this variation in colour. In Chapter 2, I investigated how colour varied with age, sex, season, and condition. Such variation may provide some indication of which traits are likely to be under the influence of sexual selection. In Chapter 3, I directly assessed the possible influence of sexual selection on colour of parental males by observing the spawning activities of female bluegills in relation to male coloration. We did not consider the influences of cuckolder males in this study. In Chapter 4, I investigated how environmental conditions influenced both ornamental and non-ornamental coloration in bluegills during the breeding season. These studies provide the first in-depth investigations of the function of bluegill coloration, which enhances our understanding of mate choice in this species. This body of work also demonstrates the importance of considering multiple factors associated with mate selection (multiple ornaments) as well as multiple environmental factors that may interact to shape the evolution of coloration. Finally, my research provides some insight on the interaction between natural and sexual selection on the evolution of colourful traits.
References


CHAPTER 2: BLUEGILL COLORATION AS A SEXUAL ORNAMENT: EVIDENCE FROM ONTOGENY, SEXUAL DICHROMATISM, AND CONDITION DEPENDENCE

Synopsis

To date, relatively few studies have examined ornamental coloration in fishes. Here, we examine the function of colourful traits in a freshwater temperate fish, the bluegill, *Lepomis macrochirus*. In 2007, we sampled 510 bluegills during the breeding and post-breeding seasons at nine lakes. We used reflectance spectrometry to quantify colour in five body regions. We aged fish using otoliths, sexed them, and calculated Fulton’s condition factor. A time series experiment demonstrated that colour did not fade for 50 minutes post capture. Colour changed consistently with age; parental males and mature females were darker and proportionally more pigmented at longer wavelengths than immature fish. Bluegills were sexually dichromatic; males had darker and more saturated carotenoid-based ventral and iridescent facial coloration than females. During the breeding season, the breast and cheek regions were darker, whereas the opercular flap and lateral region were lighter than post breeding. Colour varied with the condition of males. Males in better condition were darker for the sexually dichromatic ventral and facial regions. Our findings suggest that some colourful traits in bluegills serve as condition-dependent sexual signals during the breeding season. Our research identifies a possible sexual ornament in this species that has an elaborate mating system.

Introduction

Sexually selected traits may evolve through multiple mechanisms of sexual selection, including direct benefits, indirect benefits, sensory drive, and sexually
antagonistic coevolution (Andersson, 1994; Partridge & Hurst, 1998; Chapman et al., 2003). Although these mechanisms are often studied in isolation, they are not mutually exclusive and a more integrative view of sexual selection has been advocated in recent years (Kokko et al., 2002). Thus, females could prefer males with elaborate secondary sexual traits because of a pre-existing sensory bias (Endler & Basolo, 1998) or because these traits honestly reveal the superior genetic or phenotypic quality of the bearer (Zahavi, 1975; Andresson, 1994). Moreover, these mechanisms can act together to shape the evolution of male traits, and can even be reinforced by the runaway process (Fisher, 1930).

One widespread type of sexual ornament is the vibrant colours displayed by males of many species during the breeding season. In fishes, ornamental coloration has been shown to influence female mate choice and male-male competition in both tropical and temperate species (Kodric-Brown, 1998). For example, females are known to prefer orange coloration in guppies, *Poecilia reticulata*, (Endler, 1980, 1983) and coloration is proposed to influence male-male interactions in three-spined sticklebacks, *Gasterosteus aculeatus* (Rowland, 1989). Despite long-standing interest in the sexual ornaments of fishes, however, research on this topic is lacking in three important respects. First, much of our understanding of the function of ornamental colour in fishes comes from studies conducted in only a handful of species. Although these studies have been instrumental in demonstrating that ornamental coloration can serve as a sexually-selected trait in fishes, more 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. Second, most studies to date have focused on quantifying the number and area of
colourful patches rather than the coloration of these patches, or on quantifying colour using human visual assessments rather than spectrophotometric techniques (but see, e.g., Grether et al., 2001; Fuller, 2002; White et al., 2003; Rick et al., 2004). Finally, studies often focus exclusively on assessing coloration in breeding males. However, exemplary research has shown that a more inclusive 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; Endler & Houde, 1995; Hamilton & Poulin, 1999; Miller & Brooks, 2005; Millar et al., 2006). In this study, we used reflectance spectrometry to investigate coloration and its possible role as a sexual ornament in the bluegill, *Lepomis macrochirus*.

Bluegills inhabit lakes, ponds, and streams across most of the United States and in southeastern Canada (Becker, 1983). 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, nesting near shore in shallow waters during the spring and summer. Male bluegills follow one of two irreversible alternative life history strategies termed parental and cuckolder (Gross & Charnov, 1980), and, as such, bluegills have been a model system for studies of alternative reproductive tactics. In Ontario, Canada, parental males delay reproduction until they reach age seven, when they begin to build and defend nests (Gross, 1982, 1991; Neff, 2001). This delay in sexual maturation allows parental males to attain large body sizes because they spend several years investing in growth rather than reproduction. Cuckolder males become reproductively mature at age two in Ontario (Gross, 1982). When cuckolder males first mature, they are much smaller than other males and mature females. They obtain sneak
fertilizations by darting through the nest of a parental male while a female is spawning and are therefore called sneaker males (Fu et al., 2001; Neff et al., 2004). These sneakers become satellite males in subsequent years (Gross, 1982). Satellite males obtain fertilizations by mimicking the appearance and behaviour of females, which allows them to enter the nests of parental males without being attacked (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 established a nesting colony during the breeding season, reproductively mature females arrive together at the spawning grounds and deposit eggs in nests (Dominey, 1981). In Ontario, females become reproductively mature at age four (Gross, 1982, 1991). Spawning within a colony occurs over a single day (Gross, 1982), at which time the females leave the colony while parental males guard eggs and fry for an additional seven to ten days (Jennings et al., 1997).

These features of the bluegill mating system make them likely candidates for sexual selection on parental male traits. 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. According to honest indicator models of sexual selection, elaborate sexual ornaments that advertise quality should be costly so that they cannot be faked by those unfit to produce them (Zahavi, 1975; Kodric-Brown & Brown, 1984; Grafen, 1990; Getty, 2006). In bluegills, 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. Despite these potential benefits to females, sexual ornaments have never been
investigated in bluegills, although there are anecdotal suggestions that the males' opercular flaps function in intrasexual aggression (Gross, 1982; Neff et al., 2004), and that there exist sex differences in colour, at least during the breeding season (Gross & Charnov, 1980; Dominey, 1981).

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 colour. Because conspicuous colours 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. Our second hypothesis proposes 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 coloured than fish in poor condition.

Methods

Field work

In 2007, we captured 510 bluegills by angling and cast netting at nine lakes in Eastern 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 both June (breeding) and August (post-breeding) to investigate possible seasonal variation on coloration. Although we did not conduct extensive behavioural observations, we did observe nesting males at our sampling sites in June but not in August. We aimed to capture approximately 30 fish from each lake during both breeding and post-breeding sampling. For each fish, we measured the total length (LT) and weight (W), recorded time of capture, photographed the fish, and then euthanized the specimen using clove oil. We placed each fish into a separate bag with a unique code for identification purposes.

Sexing and ageing

In the lab, we dissected each fish to determine sex and to weigh the gonads. Reproductively mature males have oblong, cream-coloured testes, while immature males have poorly developed thread-like testes (James, 1946; Gross, 1982). Reproductively mature females have large, globular, orange ovaries, while immature females have smaller, similarly-shaped, creamy-pink ovaries (James, 1946). These gonad differences allowed us to easily identify immature and reproductively mature males and females. 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 ageing. We mounted one of the two otoliths on a microscope slide to visualize the annulæ that depict the fish’s age. We accomplished this by sanding (GatorGrit 120-c waterproof paper, Mastercraft) and polishing (3M Lapping film, 261X, 30 micron, ID# 60-0700-0517-2) the otoliths from
both sides (anterior and posterior) of the core, so that only a slice of the otolith remained on the slide (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 sample included 305 males (mean age 3.7 years, range 1-9) and 155 females (mean age 3.6 years, range 1-9).

Although we wanted to investigate ontogenetic variation in colour in this species, we knew at the outset that colour 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. We therefore assigned each fish to an age category (hereafter referred to as age) using separate criteria for males and females. We classified males as parental if they were 7 years or older or longer than 175 mm and as immature males if they did not meet these criteria (Gross, 1982, 1991). Our sample of younger males (breeding season $N=17$) likely primarily consists of immature parental males, since cuckolder males only make up a maximum of 21% of males at age 2 and 3% at age 6 (Gross, 1982). In addition, our breeding season GSI data suggest that most of our samples consisted of immature parental males since their GSI values were low ($\text{mean} \pm \text{SE}: 0.16 \pm 0.03\%$). Only one male had a GSI value of 2.5, which is still below the GSI value for known sneaker ($\text{mean} \pm \text{SE}: 3.66 \pm 1.45\%$) and satellite ($\text{mean} \pm \text{SE}: 3.74 \pm 1.06\%$) males (Neff et al., 2003). We categorized females as mature if they were 4 years or older and immature if 3 years or younger (Gross, 1982, 1991).
Fulton condition

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).

Reflectance

Immediately after the fish were captured and prior to euthanasia, we measured their surface reflectance using an Ocean Optics USB4000 spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, Fl, U.S.A.). We used a bifurcated fiber optic cable mounted in a probe that transmitted broad spectrum light to the surface of the fish. The probe then transmitted the reflected light back to the spectrometer, where data were collected with OOIBase32 software 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). We measured 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 (Figure 2.1). We took five readings from each region, each of which was comprised of 20 measurements averaged by OOIBase 32, and averaged these to obtain one mean
reflectance spectrum per body region per fish. Readings were always collected in the same order, and the entire process was completed in approximately one minute.

*Colour time series experiment*

To determine whether bluegill colour changed over time after capture, we performed a separate time series experiment on a total of 20 fish (five from Desert Lake, five from Pigeon Lake, and ten from Rondeau Bay). We captured fish by angling and measured their reflectance as described above. We then re-measured each fish every 10 minutes until 50 minutes 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. We chose this range as it encompassed the green (536 nm) and red (620 nm) peak cone photopigment sensitivities of bluegills (Hawryshyn et al., 1988). 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 are sensitive to UV wavelengths (Leech & Johnsen, 2006). Thus, our wavelength range also included the ultraviolet region of the spectrum.

*Statistical analyses*

To summarize overall variation in reflectance, we performed principal components analysis (PCA) on reflectance spectra for each body region. To do this, we
averaged reflectance data in 10-nm bins (increments) using CLR colour analysis software (Montgomerie, 2008), and used these bins as variables in our analyses. We performed separate analyses for each body region, and each individual represented an observation in these analyses (Endler, 1990; Hunt et al., 1998; Cuthill et al., 1999; Montgomerie, 2006). Our analyses resulted in two principal components for each body region with eigenvalues greater than 1 that together explained 96.0% - 98.8% of the variation in bluegill reflectance. The first principal component (PCI) explained 88.4% - 94.3% of the variation in reflectance and the second principal component (PC2) explained 4.5% - 8.2% of the variation. For the cheek region, we obtained a third principal component with an eigenvalue greater than 1; however, this component explained relatively little of the variation in reflectance (3.1%) and was not included in our analyses to maintain consistency across regions.

For each of the five body regions, PCI had moderate positive loadings across all wavelengths (Figure 2a-e), suggesting that PCI indicates variation in brightness, as is typical of PCA performed on reflectance data (Endler, 1990). Therefore, fish with high PCI scores are relatively lighter in coloration than fish with low (or negative) PCI scores. In contrast with PCI loadings, PC2 factor loadings were variable across wavelengths in both magnitude and direction, which suggests 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 (Figure 2.2a-e). Although all PC2 factor loading curves were similar in shape, the wavelength at which the loadings became negative varied by body region, ranging between 420 nm and 480 nm (Figure 2.2a-e).
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). It is important to recognize that these scores represent proportional variation between individuals; in absolute terms, most fish tend to reflect more at long wavelengths (Figure 2.2f-j). For example, breeding parental males have deep red breast coloration (Figure 2.1, 2.2f). Relative to females, they have higher proportional reflectance at shorter wavelengths, and therefore higher PC2 scores, even though in absolute terms, females reflect more at shorter wavelengths, and both reflect long wavelength colours. We performed a separate PCA for fish in the time series experiment and obtained similar loadings.

**Results**

*Colour time series experiment*

To determine whether the colour of bluegills changes over time after capture, we performed repeated measures ANOVAs on reflectance PC scores for each body region (Figure 2.3). Bluegill colour 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 is a slight decrease in PC2 at 20 minutes; however, no overall fading trend is noticeable.

*Seasonal variation in GSI*

To confirm that the June and August sampling periods represented breeding and post-breeding seasons, we compared the mean GSI of males and females captured in each
of those time periods. We found highly significant differences between breeding and post-breeding GSI values for both mature females (t-test: $t_{73}=-8.33$, $P<0.0001$; breeding GSI (mean±SE): 5.23±0.40%, $N=41$, post-breeding GSI: 0.29±0.44%, $N=34$) and parental males (t-test: $t_{41}=-4.67$, $P<0.0001$; breeding GSI: 0.91±0.09%, $N=29$, post-breeding GSI: 0.16±0.13%, $N=14$). Indeed, August GSI values approached zero for both sexes, suggesting that June and August sampling represented breeding and post-breeding seasons, respectively.

**Variation in appearance and reflectance across body regions**

Bluegill coloration varied by body region. The breast, which ranged in colour from yellow to red (Figure 2.1, 2.2f), 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 for this region (Figure 2.1). For the caudal peduncle and lateral regions, both males and females had low reflectance in the 400-500 nm range, and steady, high reflectance at longer wavelengths, giving an olive-brown appearance to this region (Figure 2.1, 2g,i). The cheek was greenish in colour for both males and females (Figure 2.1), peaking in reflectance near 500 nm, although males tended to be darker than females (Figure 2.2h). Finally, the opercular flap was black in both sexes (Figure 2.1). This black region was heavily pigmented, and therefore exhibited low reflectance at most wavelengths (Figure 2.2j). Males had darker opercular flaps than females (Figure 2.2j).

**Ontogenetic, sexual, and seasonal variation in colour**
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, age category (parental vs. immature in males and mature vs. immature in females), season and their interactions; we also included sampling site as a random effect. Although sampling site significantly influenced coloration for four of five body regions, interpreting these differences is beyond the scope of the current study.

The breast region was significantly influenced by sex, age and season for PC1 (Table 2.1). Males had lower PC1 scores than females, parental/mature fish had lower PC1 scores than immature fish, and breeding fish had lower PC1 scores than post-breeding fish (Figure 2.4a). Overall, this indicates that males, parental/mature fish, and breeding fish were darker in coloration (more pigmented) for the breast region (Figure 2.1, 2.2f). For PC2, there was a significant influence of sex, age and their interaction on breast coloration (Table 2.1). Males had higher PC2 scores than females, and parental males had higher PC2 scores than immature males but not females (Figure 2.4b).

The caudal peduncle region showed no significant variation for PC1 (Table 2.1, Figure 2.4c), but was significantly influenced by sex, age, season and the interaction between sex and age and between sex and season for PC2 (Table 2.1). In general, males had higher PC2 scores than females, PC2 increased with age in males but not females, and post-breeding females had higher PC2 scores than breeding fish females (Figure 2.4d).

The cheek region was significantly influenced by sex, season and the interaction between sex and age for PC1 (Table 2.1). Overall, males had lower PC1 scores than
females and breeding fish had lower PC1 scores than post-breeding fish (Figure 2.4e). PC1 scores decreased with age in males but not females (Figure 2.4e). For PC2, sex, age, season and the interaction between sex and age significantly influenced cheek coloration (Table 2.1). Males and post-breeding fish had higher PC2 scores, and PC2 scores increased with age in males but not females (Figure 2.4f).

For the lateral region, PC1 varied significantly with age, season and their interaction (Table 2.1). In both males and females, parental/mature fish had higher PC1 scores than immature fish during the breeding season, but not in the post-breeding season (Figure 2.4g). There were also significant effects of sex and season on PC2 for the lateral region (Table 2.1). Males had higher PC2 scores than females, and post-breeding fish had higher PC2 scores than breeding fish (Figure 2.4h).

The brightness (PC1) of the opercular flap was significantly influenced by season, the interaction between age and season, and the three-way interaction among age, sex and season (Table 2.1). In general, post-breeding fish had lower PC1 scores than breeding fish (Figure 2.4i), with the exception of parental males. Immature fish experienced the greatest change from breeding to post-breeding seasons, where they were the lightest during breeding and darkest post-breeding (Figure 2.4i). Our data also suggest that breeding season parental males and mature females were the most divergent from one another, and that PC1 scores decreased post-breeding in mature females while they did not change in post-breeding parental males (Figure 2.4i). For PC2, sex and season significantly influenced opercular flap coloration (Table 2.1). Males had higher PC2 scores than females, and post-breeding fish had higher PC2 scores than breeding fish (Figure 2.4j).
Altogether, bluegills exhibited strong sexual dichromatism for the breast and cheek regions. Our data show that males had darker, more pigmented orange breasts and green cheeks (Figure 2.1a,c; 2.2f,h), although all body regions exhibited some degree of sexual dichromatism for at least one of the two PC scores. There was also significant ontogenetic variation in colour for the breast, cheek and lateral regions, with parental/mature fish having darker breasts and cheeks with higher proportional reflectance at short wavelengths, and lighter lateral regions. All bluegill body regions also exhibited seasonal variation in colour, although these effects were more pronounced in the breast, cheek, and opercular flap. The breast and cheek were both darker during the breeding season, whereas the opercular flap tended to be lighter during breeding, except in parental males. Finally, there were a number of significant interactions among the main effects of sex, age and season, suggesting that variation in colour was not consistent within each of these categories. Of particular note, ontogenetic variation in breast and cheek colour was more pronounced in males than in females, and ontogenetic differences in the colour of the lateral region were only apparent in the breeding season. In addition, opercular flap brightness exhibited strong ontogenetic variation in males and strong sexual dichromatism, but these effects were only apparent during the breeding season.

_Fulton condition_

We tested for possible condition-dependence of coloration separately in males and females. In analyses controlling for sampling site, season and age, coloration did not show evidence of condition-dependence for any body region in females (all \( P>0.24 \)). In males, we found evidence for the condition dependence of the breast, the cheek and the
opercular flap. In particular, breast PC2 increased with condition, such that males in better condition had breasts that reflected proportionally more short wavelengths than long wavelengths (whole model $R^2=0.32$, $F_{11,291}=12.45$, $P<0.0001$; condition $F_{1,291}=20.63$, $P<0.0001$). Similarly, the cheek PC2 increased with condition, where males in better condition had cheeks with proportionally more short wavelength reflectance (whole model $R^2=0.31$, $F_{11,291}=11.99$, $P<0.0001$; condition $F_{1,291}=22.53$, $P<0.0001$). For the opercular flap, both PC1 and PC2 varied with condition. PC1 decreased with an increase in condition, such that males in better condition had opercular flaps that were darker and more heavily pigmented (whole model $R^2=0.26$, $F_{11,291}=9.39$, $P<0.0001$; condition $F_{1,291}=11.04$, $P=0.001$). PC2 increased with condition for the opercular flap, such that males in better condition had a higher proportion of short wavelengths being reflected from this region (whole model $R^2=0.23$, $F_{11,291}=8.07$, $P<0.0001$; condition $F_{1,291}=7.57$, $P=0.0063$). There was no evidence for condition dependence of coloration for the remaining body regions and PC scores (all $P>0.18$).

**Discussion**

In this study, we documented significant influences of age, sex, season and condition on coloration in bluegills. Although all body regions were affected by at least one of these factors, the direction of colour variation was not consistent among regions, suggesting that different body regions may be influenced by different selective factors. Indeed, our data suggest that the coloration of the cheek, breast, and opercular flap may be sexually selected traits in bluegills. By contrast, the coloration of other body regions (caudal peduncle and lateral region) are likely influenced other selective factors.
Bluegill coloration is produced by a combination of pigmentary and structural mechanisms. Overall, melanin and carotenoid pigments are found throughout the body of centrarchids, giving rise to a range of colours observed in bluegills (Czeczuga, 1981; Mabee, 1995). Thus, the black opercular flap and lateral banding is produced by melanin pigmentation (Mabee, 1995), whereas the orange breast is likely produced by the concentrated deposition of carotenoid pigments (Matsuno & Hirao, 1989).

Our ontogenetic analyses revealed that males typically underwent the greatest change in coloration with increasing age. Parental male bluegills became darker for the breast, cheek and opercular flaps and lighter in lateral coloration. In general, immature male coloration fell between that of parental males and mature females, and more closely resembled mature female coloration for the breast and opercular flap. Ontogenetic colour changes may result from a combination of increasing sexual selection pressure in adults, particularly males, and differential age-specific selective factors (i.e. predators) 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. If this is the case, parental/mature fish 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, which helps to support 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 (Dominey, 1980; 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 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). Mate choice experiments have confirmed that dimorphic traits are targets of female choice in a variety of species, including the upland bully, *Gobiomorphus breviceps* (Hamilton & Poulin, 1999), the sand goby, *Pomatoschistus minutus* (Forsgren, 1992), the redtail notho, *Nothobranchius guentheri* (Haas, 1976) and the Trinidadian guppy (Kodric-Brown, 1985; Houde, 1987).

Our study also revealed that bluegill coloration varies seasonally. As with our documented 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/mature fish, persisted during the post-breeding season, suggesting that some permanent dichromatism is likely in bluegills. Interestingly, permanent dichromatism is much more prevalent in fishes that have prolonged breeding seasons or that spawn throughout the year, as is often found in tropical species (Kodric-Brown, 1998). For fishes where reproduction is limited to certain times of the year, seasonal sexual dichromatism is more common (Kodric-Brown, 1998). For example, in three-spined sticklebacks, the intensity of red coloration decreases over the course of the
breeding season (Bakker & Mundwiler, 1994). However, some studies suggest that parental care may also favor ornamental male colour. McLennan (1991) showed that the intensity of nuptial coloration in three-spined sticklebacks exhibited a secondary peak during male fry guarding. Moreover, Ward & McLennan (2006) found that nuptial coloration actually peaked during the parental stage rather than during courtship in a population of brook sticklebacks (Culaea inconstans).

In the final part of our study, we investigated the possible condition-dependence of coloration in bluegills. 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, paternity, and probability of egg and larvae cannibalism in this species (Neff, 2003; Neff & Cargnelli, 2004). Given that coloration relates to condition in male bluegills, this coloration may reveal multiple aspects of male quality. The condition dependence of carotenoid-based colour in particular could be maintained by limited carotenoid availability in the environment (Endler, 1980; Kodric-Brown, 1989; Grether et al., 2001), parasite limitation (Houde & Torio, 1992; Folstad et al., 1994; 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 colourful 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.
The quality-indicating potential of ornamental coloration has been well supported in some taxonomic groups such as birds (Hill, 2006), but studies have revealed only mixed support in fishes. In guppies, for example, sperm quality was related to the size of their carotenoid-based orange spots (Pitcher et al., 2007), and experimental infection with naturally occurring parasites decreased the saturation of their orange coloration (Houde & Torio, 1992). By contrast, a recent study found no association between carotenoid ornamentation and parasitism either within or among populations of wild guppies (Martin & Johnsen, 2007). In three-spined sticklebacks, Milinski & Bakker (1990) and Barber et al. (2000) found that the intensity of the red, carotenoid-based coloration was positively correlated with body condition, whereas Candolin (1999) found a curvilinear relationship between lipid content and red coloration. By contrast, Rowland (1994) found no relationship between red coloration and body condition. Despite these mixed findings, studies have focused on only a handful of species to date, and few have assessed coloration using reflectance spectrometry (but see Grether et al., 2001; Fuller, 2002; White et al., 2003; Rick et al., 2004).

The opercular flaps of bluegills 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. Flap coloration might also function as a species recognition mechanism. Although hybridization is common in the genus *Lepomis,*
sympatric sunfish can usually discriminate between heterospecifics and conspecifics (Keenleyside, 1967, 1978; Gerald, 1971). Sympatric sunfish tend to differ conspicuously in opercular flap coloration, and Childers (1967) showed that removal of the opercular flap of male redear sunfish (L. microlophus) increased the frequency of heterospecific mating with bluegill females. Thus, opercular flap coloration may serve as an important species recognition cue in both sexes, while also revealing condition in males.

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 be sexually selected as it too is sexually dichromatic, condition dependent in males, and functions in intrasexual aggressive interactions (Gross, 1982; Neff et al., 2004). The caudal peduncle and lateral regions are likely controlled by different selective pressures. These regions showed less variation typical of sexually selected traits and the lateral region was lighter in parental males and during the breeding season. In fact, these regions may enhance colour contrast with the darker regions for parental males (Endler, 1990). Thus, variation in bluegill colour 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 behavioural studies in temperate freshwater ecosystems. 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.
Acknowledgements

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References


Table 2.1 The influence of sex, age, season, and their interactions on PC1 and PC2 colour scores in bluegills.

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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. See Methods and Figure 2.2 for interpretation of PC scores. Table shows numerator df; denominator df is 445.
Figure 2.1 Representative photographs of bluegills (*Lepomis macrochirus*). (a) Parental male (186 mm TL) (b) Satellite male (108 mm TL) (c) Mature female (146 mm TL).
Figure 2.2 Factor loadings from principal components analyses on reflectance spectra (a-e) and reflectance spectra (f-j) for bluegills. PC1 (solid line) and PC2 (dotted line) loadings in relation to wavelength for the breast (a), caudal peduncle (b), cheek (c), lateral region (d), and opercular flap (e), with origin axis (light dashed line). 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 (f-j). Y-axis reflectance (%) values differ among body regions. PCA included all individuals sampled (N=460).
Figure 2.3 Effect of time on reflectance PC1 (dashed triangles) and PC2 (solid circles) for the breast (a), caudal peduncle (b), cheek (c), lateral region (d), and opercular flap (e) of 20 bluegills. Data are least squared means from repeated measures ANOVAs.
Figure 2.4 Principal component scores on reflectance spectra for male (solid black bars) and female (white bars) bluegills separated by season and age (Immat: immature males & females; Mat: parental males & mature females). Data are least squared means (±SE) calculated from a mixed-model ANOVA that also controlled for sampling site (see Table 2.1). Breast (a,b), caudal peduncle (c,d), cheek (e,f), lateral region (g,h), opercular flap (i,j). High PC1 scores indicate a light region, whereas low PC1 scores indicate a dark region. Similarly, high PC2 scores indicate a proportional increase of short wavelength reflectance, and low PC2 scores indicate a proportional increase of long wavelength reflectance. See text for further explanation.
CHAPTER 3: MALE MORPHOLOGY AND ORNAMENTAL COLOR, NOT POSITION WITHIN THE BREEDING COLONY, INFLUENCE FEMALE PREFERENCE AND REPRODUCTIVE SUCCESS IN BLUEGILLS

Synopsis

Female choice for male ornamental coloration has been demonstrated in a number of fish species. Most studies have been conducted in a laboratory setting and show that females prefer more colorful male ornaments. In this study, we observed female bluegills (Lepomis macrochirus) spawning in their natural environment and compared spawning behaviors to male position within a colony and male traits. We observed the 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, measured morphological characteristics, and calculated Fulton condition’s index. Our results show that female spawning behaviors 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, as none of our measured male traits influenced the number of females that entered the nest. However, our results suggest that male morphology significantly influenced the number of females spawning, and that male cheek coloration influenced the number of females spawning, the number of eggs they release, and the amount of time they spend in the nest. Moreover, male breast coloration significantly predicted reproductive success as quantified through egg scores. Finally, the number of cuckolders that intrude in a male’s nest was significantly predicted by cheek coloration. Together, our findings suggest that
females may use male cheek and breast coloration, condition-dependent sexual
ornaments, as key traits on which to base their mate choice decisions.

**Introduction**

In many mating systems, females are often the choosier of the two sexes due to
higher investment in gamete production (Trivers, 1972; Batemen, 1948). In these mating
systems, females may evaluate male secondary sex traits as part of the mate choice
process, which can lead to the evolution of elaborate male ornaments (Darwin, 1871;
Fisher, 1930; Andersson, 1994a). Such 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). For example,
females may benefit directly from their choice of mates if male ornaments reflect their
ability to provide resources to a female, 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, since 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 ornaments 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 coloration 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 coloration has been studied on a
comparatively small number of species, most notably guppies (e.g., Endler, 1980, 1983;
Kodric-Brown, 1985; Houde, 1987) and three-spined sticklebacks (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
However, most studies assessing the function of colorful ornaments in fishes have
focused on quantifying the size of the ornament rather than the coloration, or have relied
on visual assessments of colour rather than spectrophotometric techniques. In this study,
we conducted behavioral observations of bluegills (Lepomis macrochirus) in their natural
environment to evaluate whether female spawning behavior is influenced by male traits,
including ornamental coloration, morphology, and position within the breeding colony.
Bluegills are colonial breeders that nest in shallow waters during the spring and summer (Gross & MacMillan, 1981). They are found throughout the United States and southeastern Canada (Becker, 1983) and have been a model system for research on alternative reproductive tactics. 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 polymorphisms 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 behavior of females (Dominey, 1981a). This mimicry allows satellite males to enter the nests of parental males with minimal aggressive interactions.

Parental male bluegills arrive at nesting sites and 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 (Gross, 1982). Once females depart, parental males are responsible for guarding eggs and fry for an additional seven to ten days (Jennings et al., 1997). Due to high predation of eggs in peripheral nests (Gross & MacMillan, 1981), females may benefit from choosing males that are positioned in the center of the colony. Alternatively, females may benefit from choosing males based on traits that reveal quality, either through improved parental care or heritable good genes.
To date, male sexual ornaments remain poorly studied in bluegills. Noble (1934) suggested that coloration in sunfishes may be used in sexual selection, an idea that was quickly discounted by Breder (1936). More recent suggestions 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 coloration was sexually dichromatic, changed ontogenetically and seasonally, and varied with condition in males (breast, cheek, and opercular flap coloration), suggesting that male coloration might function as a sexually selected trait in this species (Chapter 2).

Our objective in this study was to evaluate whether male spatial and morphological characteristics influenced female spawning behavior in naturally-spawning bluegill colonies. If a male’s position within a colony is important in spawning, we predicted that centrally-nesting males would have a greater number of females spawning and a larger number of eggs in their nests. If male ornamental coloration is an important mate choice signal in this species, we predicted that the more colorful males, regardless of nest location, would attract more spawning females that lay 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, Ontario, Canada (44°34'N, 76°19'W). At the beginning of the season, we monitored previously used nesting colonies
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 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 minutes of observations per male. Observation times were converted into a 5-point scale, where each point represented a range of two hours, starting from 8 AM and ending at 6 PM. 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 eggs) performed by each female, duration of the spawning event, and the number of aggressive behaviors 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 hours.

**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. 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 preopercle, green lateral side of the fish directly above the highest portion of the lateral line, and black opercular flap (Cogliati et
al. in review). 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 green (536 nm) and red (620 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; Cargnelli & Gross, 1996). These egg scores 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 reduce potential bias from egg predation (Bain & Helfrich, 1983).

Statistical 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 in reflectance. For each of the body regions measured, 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 Chapter 2). 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), 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**

Since we sampled multiple colonies on different dates, we evaluated whether female spawning behaviors varied by date or colony. We found no differences in behaviors (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 $F_{2,73}<2.7$, $P>0.07$). Since we conducted behavioral observations on the same parental males in both the morning and afternoon, we tested for differences in female behaviors 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. We therefore
used the residuals of regressions between these two spawning behaviors and time of day (rank) to control for time of day effects in all subsequent analyses. The remaining female spawning behaviors observed did not significantly differ between observation times (all $F_{4,154}<1.31, P>0.27$).

**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 ($t_{71}=2.16, P=0.03$) and had marginally significant larger girth sizes ($t_{71}=1.96, P=0.05$). Other morphological traits did not differ between central and peripheral males (total length, $t$-test: $t_{71}=1.76, P=0.08$; Fulton Condition, $t$-test: $t_{71}<1.09, P=0.28$). With respect to coloration, only the iridescent cheek of males significantly differed between central and peripheral males ($t$-test: $t_{71}=-2.08, P=0.04$; PC2), where peripheral males had lower PC2 scores. None of the other regions differed significantly between central and peripheral males ($t$-tests: $t_{71}<1.8, P>0.07$).

**Colour vs. morphology**

General 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 3.1). For five of the six body regions measured (breast, 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 3.1). 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 behavior**

**Central vs. peripheral**

We found no significant effect of a male’s position within a colony on any of our observed female spawning behaviors (Table 3.2). However, we did find that central nests had significantly higher egg scores than peripheral nests (Table 3.2).

**Female spawning behavior vs. male characteristics**

Using stepwise regression analyses with a backward elimination procedure, we found that female spawning behaviors were significantly influenced by both male morphology and colour (Table 3.3). 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 3.3). In particular, shorter, heavier males with a low Fulton condition and low PC1 score (iridescent cheek) had significantly more females spawning. In addition, PC1 scores for the cheek, iridescent cheek, and lateral region significantly predicted the number of tilts by females (egg deposition; Table 3.3), 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 3.3), such that males with higher PC1 scores for both regions spawning for a significantly longer amount of time.

We also found that male cheek and opercular flap coloration predicted the number of cuckolders that enter a male’s nest (Table 3.3). 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 coloration of his breast and caudal peduncle, significantly predicted his eggs scores (Table 3.3). 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 color, and position in the breeding colony influence female mate choice. Female spawning may indicate preference for particular parental males, and egg scores often serve as a measure of male reproductive success (Cargnelli & Gross, 1996; Neff et al., 2003, 2004). Based on these measures of female preference, our findings suggest that multiple aspects of male morphology and colour influence female preference and reproductive success in bluegills.
In colonial breeding systems, central positions in the colony are thought to be preferred, since 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 during spawning (Gross & MacMillan, 1981) nor did they 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 since 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. In addition, we found that egg scores were higher in central nests, although any influence of differential predation should have been minimal since we measured eggs scores the morning following spawning (Gross & MacMillan, 1981). Moreover, differences in male morphology do not explain our higher eggs scores in central nests; instead, breast coloration 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). However, despite finding significant effects of nest location on egg scores, female spawning behavior did not significantly differ between central and peripheral males, although relationships were in the same direction as egg scores with regards to centrality. Perhaps this mismatch between female spawning behavior and egg scores results from
higher predation on peripheral than central nests both during and immediately following spawning, as we occasionally observed cuckolders and females cannibalizing eggs from parental male nests. Alternatively, a female’s spawning behavior may not directly correlate with the number of eggs she releases. Our findings highlight the importance of considering multiple measures of male reproductive success in this type of study.

With regards to the influence of male characteristics on female spawning behaviors, 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 enter 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 multiple males before choosing one with which to spawn, a finding consistent with various mate choice models (Gibson & Langen, 1996; Widemo & Saether, 1999).

Once a female remains in a nest to spawn, multiple male characteristics appear 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 behaviors, and the breast region significantly predicted male egg scores. When a female is in a male’s nest, the primary visual stimulus she receives comes from the anterior portion of the male, most notably his breast, cheek, and operculum, as the pair 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 collared lizards (Crotophytus collaris; Baird, 2004) and Nile tilapia (Oreochromis niloticus; Castro et al., 2009). Alternatively, male cheek and breast coloration may serve as honest
indicators of male quality. Our data from a previous study suggest that both of these traits are condition-dependent (Chapter 2). As such, cheek and breast coloration 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), although these possibilities remain to be investigated in more detail.

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 several surprising relationships between male characteristics and ornamental color. We found that males with a larger girth had significantly darker breasts and cheeks, as might be expected. However, we also 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 of 510 bluegills including both males and females and fish of multiples ages, we showed that breast, cheek, and opercular flap coloration are condition-dependent traits in males, with more colorful individuals being in better condition (Chapter 2), a relationship opposite to that found in this study. However, although our previous study was conducted during the breeding season, it did not include spawning individuals, 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 time spent at the nest and energy spent on nest defense 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 the fact that in our study, 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 coloration, a condition dependent ornament, may be important in intrasexual aggression and be used in establishing a colony (Chapter 2). However, opercular flap coloration did not significantly vary between central and peripheral males, nor did it significantly predict any of our observed female spawning behaviors.

In conclusion, our investigation of female spawning behavior and male reproductive success suggests that female bluegills do exhibit mate choice preferences,
and that those preferences are mediated in part by male ornamental color. In contrast, male nesting position does not appear to influence the spawning behaviors of females, although central males experience higher reproductive success quantified through egg scores. Although other factors may 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. 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.

Acknowledgements

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References


Table 3.1 Male colour characteristics in relation to morphology and condition. Results are from general linear regression models for each body region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Colour Component</th>
<th>$R^2$</th>
<th>df</th>
<th>$F$</th>
<th>Std. β</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>PC1 whole model</td>
<td>0.11</td>
<td>4</td>
<td>2.28</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girth</td>
<td></td>
<td>1</td>
<td>7.61</td>
<td>-0.82</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>PC2 whole model</td>
<td>0.14</td>
<td>4</td>
<td>2.92</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total length</td>
<td></td>
<td>1</td>
<td>7.38</td>
<td>-3.49</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
<td></td>
<td>1</td>
<td>7.52</td>
<td>3.79</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Fulton condition</td>
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<td>7.31</td>
<td>-2.26</td>
<td>0.009</td>
</tr>
<tr>
<td>Caudal</td>
<td>PC1 whole model</td>
<td>0.05</td>
<td>4</td>
<td>0.91</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No significant effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC2 whole model</td>
<td>0.09</td>
<td>4</td>
<td>1.81</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No significant effects</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>Cheek</td>
<td>PC1 whole model</td>
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<td>4</td>
<td>3.84</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Girth</td>
<td></td>
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<td>11.85</td>
<td>-0.98</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>PC2 whole model</td>
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<td>4</td>
<td>2.73</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total length</td>
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<td>7.97</td>
<td>-3.65</td>
<td>0.006</td>
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<tr>
<td></td>
<td>Weight</td>
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<td>7.54</td>
<td>3.81</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>Fulton condition</td>
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<td>7.58</td>
<td>-2.31</td>
<td>0.008</td>
</tr>
<tr>
<td>Iridescent Cheek</td>
<td>PC1 whole model</td>
<td>0.05</td>
<td>4</td>
<td>0.90</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No significant effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC2 whole model</td>
<td>0.12</td>
<td>4</td>
<td>2.44</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total length</td>
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<td>5.94</td>
<td>-3.17</td>
<td>0.02</td>
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<tr>
<td></td>
<td>Weight</td>
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<td>5.02</td>
<td>3.13</td>
<td>0.03</td>
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<tr>
<td></td>
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<td>1</td>
<td>5.91</td>
<td>-2.06</td>
<td>0.02</td>
</tr>
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<td>Lateral</td>
<td>PC1 whole model</td>
<td>0.08</td>
<td>4</td>
<td>1.59</td>
<td>0.19</td>
<td></td>
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<td></td>
<td>No significant effects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PC2 whole model</td>
<td>0.13</td>
<td>4</td>
<td>2.73</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
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<td>Total length</td>
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<td>6.44</td>
<td>-3.23</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
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<td>6.33</td>
<td>3.49</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Fulton condition</td>
<td></td>
<td>1</td>
<td>6.39</td>
<td>-2.12</td>
<td>0.01</td>
</tr>
<tr>
<td>Opercular Flap</td>
<td>PC1 whole model</td>
<td>0.04</td>
<td>4</td>
<td>0.76</td>
<td>0.56</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>PC2 whole model</td>
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<td>4</td>
<td>3.01</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total length</td>
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<td>7.25</td>
<td>-3.45</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Weight</td>
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<td>7.60</td>
<td>3.80</td>
<td>0.007</td>
</tr>
<tr>
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<td>Fulton condition</td>
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<td>1</td>
<td>7.35</td>
<td>-2.26</td>
<td>0.008</td>
</tr>
</tbody>
</table>
Table 3.2 Female spawning behaviors based on male location within a colony. Data shown are from t-tests. Values for central and peripheral males are given as means per hour ± standard error.

<table>
<thead>
<tr>
<th>Behavior</th>
<th></th>
<th>Central (N=30)</th>
<th>Peripheral (N=43)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of females entering/hour(a)</td>
<td>-1.23</td>
<td>5.07±0.55</td>
<td>4.18±0.46</td>
<td>0.22</td>
</tr>
<tr>
<td>Number of females spawning/hour(a)</td>
<td>-1.31</td>
<td>2.54±0.32</td>
<td>1.94±0.27</td>
<td>0.20</td>
</tr>
<tr>
<td>Number of tilts/hour</td>
<td>-0.77</td>
<td>89.55±13.80</td>
<td>75.79±11.53</td>
<td>0.45</td>
</tr>
<tr>
<td>Amount of time (min) spent spawning/hour</td>
<td>-0.39</td>
<td>11.95±1.93</td>
<td>10.98±1.61</td>
<td>0.70</td>
</tr>
<tr>
<td>Number of cuckolders/hour</td>
<td>1.32</td>
<td>28.18±5.88</td>
<td>38.29±4.91</td>
<td>0.19</td>
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<tr>
<td>Egg score</td>
<td>-3.04</td>
<td>2.37±0.15(b)</td>
<td>1.79±0.12(c)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

\(a\) 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 3.3 Male characteristics and colour affect female spawning behaviors. Results are from a reverse stepwise regression analysis with a probability to leave the model set to 0.055. Female spawning behaviors are rates per hour based on time spent observing. (ir. cheek = iridescent cheek).

<table>
<thead>
<tr>
<th>Female spawning behavior</th>
<th>Model effects</th>
<th>$R^2$</th>
<th>df</th>
<th>$F$</th>
<th>Std. β</th>
<th>$P$</th>
</tr>
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<tbody>
<tr>
<td>Number females entering the nest$^a$</td>
<td>No significant effects</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Number females spawning$^a$</td>
<td>Whole model</td>
<td>0.11</td>
<td>4</td>
<td>2.12</td>
<td>0.09</td>
<td></td>
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<tr>
<td></td>
<td>Total length</td>
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<td>4.11</td>
<td>-2.60</td>
<td>0.05</td>
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<td></td>
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<td>4.36</td>
<td>2.99</td>
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<td></td>
<td>Fulton condition</td>
<td>1</td>
<td>4.14</td>
<td>-1.73</td>
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<td></td>
<td>PC1 ir. Cheek</td>
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<td>5.14</td>
<td>-0.26</td>
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<tr>
<td>Number of tilts</td>
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<td>0.17</td>
<td>3</td>
<td>4.97</td>
<td>0.004</td>
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<tr>
<td></td>
<td>PC1 cheek</td>
<td>1</td>
<td>3.90</td>
<td>0.22</td>
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<td></td>
<td>PC1 ir. Cheek</td>
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<td>4.13</td>
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<td>PC1 lateral</td>
<td>1</td>
<td>7.22</td>
<td>0.30</td>
<td>0.009</td>
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<td>Time spent spawning</td>
<td>Whole model</td>
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<td>2</td>
<td>6.54</td>
<td>0.002</td>
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<tr>
<td></td>
<td>PC1 caudal</td>
<td>1</td>
<td>5.22</td>
<td>0.26</td>
<td>0.03</td>
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<tr>
<td></td>
<td>PC1 cheek</td>
<td>1</td>
<td>3.93</td>
<td>0.22</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Egg score</td>
<td>Whole model</td>
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<tr>
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<td></td>
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$^a$ Residuals controlling for diel variation were used in these analyses
CHAPTER 4: DIFFERENTIAL INFLUENCE OF ECOLOGICAL VARIABLES ON COLORATION IN DIFFERENT AGE CLASSES OF BLUEGILLS (LEPOMIS MACROCHIRUS)

Synopsis

Animal colour patterns are often shaped by the interaction between natural and sexual selection. Moreover, both ornamental and non-ornamental visual signals are influenced by characteristics of the sender, as well as by various components of the environment in which the signals are transmitted. In this study, we investigated the influence of ecological variables on coloration in different sex and age classes of bluegills (Lepomis macrochirus). During the breeding season, we captured 205 bluegills from eight lakes in Ontario that varied in environmental conditions. We used reflectance spectrometry to quantify the coloration of five body regions for each fish. Previous research has shown that three of these regions appear to be important sexual ornaments in parental males (cheek, opercular flap, and breast), whereas two of these regions do not appear to function as sexual ornaments (lateral and caudal peduncle). For each individual, we also collected morphological measurements, identified sex, and determined age using otoliths. We found that ecological variables influence reflectance patterns, with total amount of vegetation cover and predator species richness having the largest impact on coloration. Parental males, mature females, and immature fish were all influenced by vegetation in the same direction for the caudal peduncle and lateral region, where each group had an increase in short (ultraviolet to green) wavelength reflectance in lakes with greater amounts of vegetation cover. This pattern indicates that all fish may be increasing crypsis by reflecting proportionally more green wavelengths to match the background vegetation, suggesting a strong role of natural selection acting on each group of bluegills.
Predator species richness also strongly predicted the colour of immature fish in a
direction that decrease predator detection. Overall, our study suggests that natural
selection plays a role in shaping ornamental and non-ornamental coloration in bluegills
and that different age classes may be influenced both similarly and differentially by the
environment.

Introduction

Natural and sexual selection exert antagonistic selection pressures on signals, with
natural selection favouring crypsis and sexual selection favouring conspicuousness
(Endler, 1978, 1991; Andersson, 1994). Sexual selection may favour conspicuous traits,
as they may enhance reproductive success of the bearer because they are easier to
perceive and/or because they honestly reveal some aspect of the signaler’s quality
(Zahavi, 1975; Endler, 1992; Andersson, 1994). Natural selection, on the other hand,
tends to favour cryptic signals that allow animals escape the notice of predators. Research
on guppies (Poecilia reticulata) clearly demonstrates these opposing selection pressures,
where female generally prefer more colourful males, yet the strength of this preference,
and the extent of male ornamentation, both decrease with increasing predation risk

Whether or not a signal appears cryptic or conspicuous depends on a number of
factors, including the properties of the signal, the environment through which it is
transmitted, the perceptual abilities of the signal receiver, and receiver psychology
(Hailman, 1977; Endler, 1990, 1992, 1993a,b; Guilford & Dawkins, 1993; Kelber et al.,
2003). In aquatic habitats, both light environment (Reimchen, 1989; Boughman, 2001)
and background vegetation (Endler, 1982) can influence the transmission of visual
signals. For example, stream-specific ambient light conditions produce distinct colour morphs in the bluefin killifish (*Lucania goodei*), where each morph is optimally conspicuous in its particular environment (Fuller, 2002). Aside from the influence of the environment, the production of visual signals in fishes will depend on the specific selection pressures experienced by particular individuals such as differences between sex and age classes, or differences caused by variation in diet (e.g., Hill & Montgomerie, 1994; Hill et al., 2002), condition (e.g., Barber et al., 2000), and parasite load (e.g., Folstad et al., 1994). Finally, individual variation in selection pressure may also be mediated in part by ontogenetic niche shifts, where individuals in size structured populations shift to a new habitat or resource during their development (Werner & Gilliam, 1984). Corresponding changes in morphology, diet, visual background, predation risk, and life history stage could in turn influence visual signaling within a species for both naturally and sexually selected traits.

Many studies have investigated the impacts of environmental conditions on ornamental coloration (e.g., Forsgren, 1992; Godin & Briggs, 1996; Fuller, 2002), but few have shown how such conditions would influence non-ornamental colour traits, which are those that are not under the influence of sexual selection (but see, e.g., Millar et al., 2006). Furthermore, much is known about morphological changes that occur in species with ontogenetic shifts (Werner & Gilliam, 1984; Ehlinger & Wilson, 1988). However, the impact that these ontogenetic shifts have on signals used in communication is not well understood. In this study, we investigate the influence of ecological variables on coloration in different sex and age classes of bluegills (*Lepomis macrochirus*) using objective reflectance spectrometry.
Bluegills are freshwater sunfish (family Centrarchidae) that inhabit clear or turbid vegetated lakes, ponds, and streams across most of the United States and in southeastern Canada (Becker, 1983). Centrarchidae tend to be the dominant fish in habitats of low current velocities, fine substrates, and complex macrophytes (Lapointe et al., 2007). Although bluegills are largely opportunistic foragers (Egertson & Downing, 2004), they are known to undergo an ontogenetic niche shift. Juveniles, which are largely restricted by the presence of predators, remain in the vegetated littoral-zone and feed largely on invertebrates whereas adults shift to open-water habitats and feed mainly on zooplankton (Mittelbach, 1981, 1984; Ehlinger & Wilson, 1988; Werner & Hall, 1988). As prey items, bluegills become less vulnerable to predators as they grow until finally outgrowing all natural predators. Their principal predator is the largemouth bass (*Micropterus salmoides*), which can swallow bluegills up to a maximum size of 160 mm (Otis et al., 1998). Northern pike (*Esox lucius*), muskellunge (*Esox masquinongy*), and other esocids also consume bluegills of larger sizes; however, no esocid will prey heavily on bluegills if other options are available (Tomcko & Pierce, 2005). Walleye (*Sander vetreus*) may consume bluegills up to 127 mm long (Schneider & Lockwood, 2002), and yellow perch (*Perca flavescens*) will consume bluegills 20 to 30 mm in length (Keast, 1978). Despite their abundance, bluegills are not typically preferred prey (except by largemouth bass) due to their body morphology. The large depth of pan-shaped bluegills and their hard fin rays, which are erected when attacked, make them a difficult fish to swallow (Savitz & Janssen, 1982; Keast, 1978).

Bluegills have long been a model system for studies on alternative reproductive tactics. Male bluegills follow one of two irreversible alternative life history strategies
termed parental and cuckold (Gross & Charnov, 1980). Parental males build nests in dense colonies, whereas cuckolders try to obtain fertilizations using sneaking or female mimicry strategies (Dominey, 1981; Gross, 1982). Bluegills are typically greenish with yellow or orange breasts, iridescent blue cheeks and chins, and black opercular flaps (Becker, 1983). We have previously shown that opercular flap, breast, and cheek coloration are ontogenetically variable, sexually dichromatic, and condition-dependent traits in bluegills (Chapter 2). Moreover, breast and cheek coloration appear to influence female spawning behaviour and male reproductive success (Chapter 3). Two other body regions (caudal peduncle and lateral) varied with sex and ontogeny but did not correlate with condition or female spawning (Chapter 2, 3). Bluegills are ideal candidates to investigate the influence of ecological variables on coloration since they undergo ontogenetic shifts in both habitat and diet, experience reduced predation as they increase in size, and can be measured across multiple lakes that vary in environmental conditions.

We generate four main predictions to explain how ecological variables might influence coloration in bluegills. First, increasing brightness contrast is a common strategy for enhancing conspicuousness (e.g., Marchetti, 1993; Schultz et al., 2008). Thus, we predict that ornamental colour patches in parental male bluegills should be darker in clearer environments to enhance signal transmission. Conversely, mature females and immature fish should try to match the brightness of their background to increase crypsis, and should exhibit lighter coloration in clear environments. Second, short and long wavelengths dissipate sooner than intermediate wavelengths when moving down the water column, such that UV and red wavelengths are no longer present beyond 10 metres (Waterman, 1981). Thus, we predict that in deeper lakes, parental males should
have brighter coloration with a shift away from both short and long-wavelength reflectance, whereas mature females should have darker coloration to enhance crypsis. Since immature fish do not typically forage in deeper waters, their colours should not be influenced by lake depth. Third, bluegills prefer habitats with more complex vegetation (Lapointe et al., 2007), but grow better and consume more prey in habitats with intermediate vegetation density (Crowder & Cooper, 1982). Parental males should increase the chromatic contrast of their ornamental regions by reflecting more strongly at short and long wavelengths and avoiding the greenish wavelengths present in vegetation. Since mature females are typically large enough to avoid predation, and forage in more open habitats, we predict that background vegetation should have little influence on their colour. Also, since immature fish primarily forage in vegetated areas and are largely concerned with avoiding predation, we predict that their coloration should match that of the background vegetation, by reflecting more strongly at intermediate, greenish wavelengths. Finally, predators have maximum size limitations for their prey. Since the majority of parental males and mature females should exceed this maximum limitation, we predict that species richness should not influence their coloration. Conversely, since predators are more likely to affect immature fish, we predict that their coloration should become more cryptic with increasing predator species richness.

**Methods**

In June 2007, we sampled eight lakes in Ontario: Rondeau Bay, Lake St. Clair (Lighthouse Cove), Lake Scugog, Pigeon Lake, Desert Lake, Devil Lake, Buck Lake, and Eagle Lake (Fig. 4.1, Table 4.1). Our sampling period generally corresponds with the
breeding season for bluegills in that area. At each of the sampling sites, we estimated total percent vegetation cover based on visual assessment. We also collected aquatic macrophyte samples using an Ekman grab multiple times in multiple locations within each sampling site. We classified vegetation samples as simple or complex macrophytes, where we considered simple macrophytes as those with minimal branching (i.e. Vallisneria and Nymphaea spp.) and complex macrophytes those with extensive branching (i.e. Potamogeton and Myriophyllum spp.). We estimated the percentage of complex vegetation based on mean estimates of abundance levels in our samples and visual assessments during field sampling. We obtained the mean depth of each lake sampled from published data (Marleau, 2007a, 2007b), and used annual average ice-free Secchi depths for 2007 as a measure of lake turbidity for each lake (MOE, Lake Partner Program; Table 4.1). We determined predator species richness for each lake by considering the presence of northern pike, walleye, and muskellunge (Bolsenga & Herdendorf, 1993; Marleau, 2007a, 2007b; Land O’Lakes Tourism, 2008; R. Haas, pers. communication). For our analyses, each lake was assigned a value (up to three) based on the number of predator species present for our analyses. Because largemouth bass and yellow perch were present in all of the lakes, we did not include them in our analyses.

At each of these lakes, we captured approximately 30 bluegills by angling (Table 4.2). Each fish was euthanized using clove oil and placed individually in a uniquely coded bag. In the lab, we dissected each fish to determine sex, and aged each fish using otoliths (Schramm, 1989; see Chapter 2). We resolved discrepancies by mutual examination with an additional party. Sex and age were used to assign each fish to an age category (immature and parental/mature) using separate criteria for males and females.
We classified males as parental if they were 7 years or older or longer than 160 mm (maximum length consumed by largemouth bass) and as immature males if they did not meet these criteria (Gross, 1982, 1991; Otis et al., 1998). We categorized females as mature if they were 4 years or older and immature if 3 years or younger (Gross, 1982, 1991). For our analyses, we combined immature males and females, as these smaller individuals should experience similar selection pressures.

**Reflectance**

Once a bluegill was captured, we immediately measured the surface reflectance of the fish using an Ocean Optics USB4000 spectrometer and a PX-2 pulsed xenon lamp (Ocean Optics, Dunedin, FL, U.S.A.). Our setup consisted of a bifurcated fiber optic cable connected to a broad spectrum light source and a spectrometer. The cable, which was mounted in a probe, transmitted the generated light to the surface of the fish and the reflected light back to the spectrometer. The spectrometer was connected to a PC laptop computer, where reflectance data were collected using OOIBase32 software. A matte black rubber sheath on the probe maintained a fixed, perpendicular distance to the measurement surface and excluded external light from the measurement area. We used a Spectralon white standard (WS-1; Ocean Optics) to express all reflectance measurements as a percentage of the total reflectance. We collected five readings from each body region. Each of our five readings was averaged by OOIBase 32 software from 20 readings collected in rapid succession. We measured the following regions: the orange breast, the green cheek directly below the eye, the green caudal peduncle, the green lateral side of the fish directly above the highest portion of the lateral line, and the black
opercular flap. We averaged the five readings from each body region per fish to obtain an overall mean reflectance spectrum. We always collected our reflectance data in the same order, and we completed the entire process in approximately one minute. Colour does not change significantly for up to 50 minutes after capture (Chapter 2).

Statistical analyses

To summarize overall variation in reflectance, we performed principal components analyses (PCA) on reflectance spectra for each body region, where each individual represented an observation in our analyses. See Chapter 2 for a detailed description of these analyses. Briefly, our analyses resulted in two principal components with eigenvalues greater than 1 for each body region, which together explained between 96.0% - 98.8% of the variation in reflectance. The factor loadings for both principal components (PC1 and PC2) were similar across all five body regions. Based on these loadings, PC1 indicates variation in brightness, and PC2 indicates variation in hue and saturation (cf. Endler, 1990). In particular, fish with high PC1 scores have higher percent reflectance and are relatively lighter in coloration than fish with low (or negative) PC1 scores. In addition, 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).

We used multiple regression analyses to evaluate the effects of ecological variables on bluegill coloration expressed during the breeding season. We used PC scores for each body region as our dependent variables, producing a different model for each PC score. We used Secchi depth (2007 annual average), mean depth, percent complex
vegetation, total percent vegetation cover, and predator species richness as possible predictors in each model. We ran separate analyses for immature fish (both males and females), mature females, and parental males (Table 4.2).

**Results**

*Turbidity*

Secchi depth, our measure of turbidity, significantly influenced the colour of parental males and immature fish (Table 4.2). For parental males, breast PC2 was negatively influenced by Secchi depth, such that males from clearer lakes had a proportional increase in long wavelength reflectance (Table 4.2). For immature fish, the breast brightness (PC1) was positively predicted by Secchi depth (controlling for the other ecological variables), such that fish from clearer lakes (higher Secchi depths) had higher PC1 scores and so were lighter. Conversely, the caudal peduncle PC1 for immature fish was negatively predicted by Secchi depth. In addition, lateral and caudal peduncle PC2 scores were negatively predicted by Secchi depth in these fish (Table 4.2). These data partially support our predictions, as immature fish had lighter breasts in clearer lakes.

*Mean depth*

In contrast with our predictions, only the coloration of immature fish was influenced by lake depth (Table 4.2). Mean depth negatively predicted breast brightness, such that fish in lakes with a greater mean depth had darker breasts. Also, cheek PC2 was
negatively predicted by mean depth, such that proportional short wavelength reflectance decreased in deeper lakes (Table 4.2).

**Complex vegetation**

Relative amounts of complex vegetation significantly predicted coloration in parental males, mature females, and immature fish. In parental males, breast PC2, cheek PC2, and lateral PC2 were negatively correlated with the percentage of complex vegetation (Table 4.2). In mature females and immature fish, four and five body regions measured were influenced by complex vegetation, respectively (Table 4.2). Cheek PC2, caudal peduncle PC2, and lateral PC2 were all negatively correlated with complex vegetation for both mature females and immature fish, as was opercular flap PC2 in females and breast PC2 in immature fish (Table 4.2). In addition, breast PC1 was negatively correlated and opercular flap PC1 was positively correlated with complex vegetation in immature fish, such that fish had darker breasts and lighter opercular flaps in lakes with a greater percentage of complex vegetation.

**Vegetation cover**

In terms of total percentage of vegetation cover, lateral PC2 was positively correlated with vegetation cover in parental males and immature fish, as was opercular flap PC2 in parental males. In addition, breast PC1 was positively correlated with vegetation cover in parental males, such that males had brighter breasts in lakes with more vegetation cover (Table 4.2). In mature females, only the caudal peduncle region was influenced by the percentage of vegetation cover, such that vegetation cover
negatively predicted caudal peduncle PC1 and positively predicted caudal peduncle PC2 (Table 4.2). These results partially support our predictions for the effects of vegetation on colour. We had predicted that parental males and immature fish would differ in their relationship with vegetation, which is not the case. Also, we predicted that females would not be largely influenced by vegetation; however, only vegetation influenced female coloration.

**Predator species richness**

The number of bluegill predators significantly influenced coloration in immature fish only. Among immature fish, PC2 scores decreased in lakes with fewer predators for four of five body regions (Table 4.2; Fig. 4.2). That is, immature fish from lakes with more predators had a proportional decrease in short wavelength reflectance for the breast, cheek, lateral, and opercular flap regions. Parental male and mature female coloration were not significantly predicted by predator species richness for any body region. Interestingly, although mature female breast PC2 scores did not increase in lakes with more predator species, their PC2 scores did resemble those of immature fish from lakes with higher predator species richness (Fig. 4.2). Our results support our prediction in that parental male and mature female coloration should not be greatly affected by predators, and that the colour of immature fish should be influenced by predator species richness in a direction that would decrease detection by predators.

**Discussion**
Multiple ecological factors should be considered when investigating the evolution of colour patterns (Millar et al., 2006). In this study, we investigated a number of ecological factors that might explain the variation in bluegill coloration, namely turbidity, lake depth, percentage of complex vegetation, percentage of vegetation cover, and predator species richness. Each of these ecological factors significantly explained some of the variation in bluegill coloration. Turbidity has been shown to be an important selective factor in several other aquatic species (Endler, 1991; Seehausen et al., 1997; Granqvist & Mattila, 2004; Heubel and Schlupp, 2006). In our study, we show that turbidity predicted breast, caudal peduncle, and lateral region coloration among immature fish. As we predicted, breast coloration was lighter in clearer environments among these fish; however, the caudal peduncle was darker in clearer lakes. Although these relationships differ, the breast and caudal peduncle are thought to have different functions in bluegills (Chapter 2, 3). Contrary to our predictions, however, we did not find the opposite pattern among parental males. We offer two possible explanations for this lack of association between colour and turbidity. First, our turbidity measurement did not incorporate the spectral properties of the water, which may be more important for visual signaling than overall turbidity (Reimchen, 1989; Seehausen et al., 1997; Fuller, 2002). Second, many ecological factors, including rainfall, substrate composition, and the abundance of phytoplankton and algae, will vary throughout the season (Kalff 2001). In combination, these factors could lead to greater variation in turbidity within lakes than among lakes, which might reduce the selective pressure exerted by turbidity on coloration in bluegills. In the jeweled splitfin, *Xenotoca variatus*, turbidity likewise does not appear to influence colour (Moyaho et al., 2004).
Wavelengths are differentially absorbed as light descends through the water column (Waterman, 1981). Because short and long wavelengths are preferentially absorbed by water particles, UV and red coloration become more cryptic with increasing depth due to the absence of these wavelengths in deep water (Lythgoe, 1979; Johnsen, 2002). We predicted that parental males would become brighter, with a shift away from both short and long wavelengths, in deeper lakes and that lake depth would not affect the coloration of immature fish since these fish do not forage in deep water. Contrary to our predictions, mean depth only influenced the colour of immature fish. This pattern may result, in part, from our classification of immature fish. Some larger immatures are known to forage in open water habitats under increased competition for resources, or to seek out larger prey items appropriate for their size (Werner & Hall, 1988). We found that breast brightness decreased with lake depth among immature fish, which would result in more cryptic coloration in these darker environments (Endler, 1993b; Marchetti, 1993). We also found that the cheek region exhibited a proportional increase in long wavelength reflectance in these fish, which would increase crypsis in environments lacking long wavelengths (Marshall, 1971; McFall-Ngai, 1990).

Of the ecological variables we investigated, the one with the largest influence on coloration in bluegills was vegetation, both in terms of the percentage of complex vegetation and total percentage of vegetation cover (Table 4.2). We predicted that in lakes with greater amounts of vegetation, parental males should increase chromatic contrast of ornamental regions, mature female coloration should not change, and immature fish coloration should decrease chromatic contrast for all regions. The visual background, in this case aquatic vegetation, may be used by signaling individuals to
either minimize or maximize chromatic contrast (Endler, 1978, 1993b; Endler & Thery, 1996). Bluegills are known to prefer habitats with complex vegetation (Lapointe et al., 2007), and the amount of complex vegetation did influence multiple body regions, both ornamental and non-ornamental, for each group. In each case, there was a proportional decrease in short wavelength reflectance, suggesting that in high amounts of preferred macrophytes, all bluegills increase chromatic contrast for both ornamental and non-ornamental regions. However, the presence of complex macrophytes only increases the nature of background when it is present, not the overall amount of green vegetation comprising the visual background. The relative amount of complex macrophytes could enhance crypsis in two ways. First, complex macrophytes produce a more disruptive background than simple macrophytes (Merilaita, 2003), which could enhance visual crypsis from a signaling perspective. Second, greater relative amounts of complex vegetation could provide more hiding places for small, immature bluegills. Such context-dependent visual signaling has been demonstrated in Hawaiian reef fishes, where visual signals can be highly cryptic or conspicuous depending on the colour and complexity of the visual background (Marshall et al., 2003a, 2003b).

The total amount of vegetation cover significantly influenced the breast, lateral, and opercular flap region for parental males, the caudal peduncle for mature females, and the lateral region for immature fish. The coloration of these regions was influenced in the same direction for each group, with a proportional increase in short wavelength reflectance which would lead to more greenish coloration. Vegetation in the environment may provide a matching background against which prey can hide from predators (Endler, 1978). Given that the patterns were similar across sex and age groups, background
matching may be important throughout the life of bluegills. Although bluegills are known to prefer habitats of intermediate density (Crowder & Cooper, 1982), attack rates from largemouth bass decrease with increasing vegetation density (Savino & Stein, 1982). Thus, background matching may be especially important at intermediate densities.

We assumed at the outset that the role of predators in shaping the coloration patterns of bluegills would differ from that documented in other species, such as the guppy (Endler, 1980, 1991; Godin & Briggs, 1996) and the sand goby (Forsgren, 1992). Unlike these species that are under constant threat from predators, bluegills are nearly entirely freed from predation pressure once they exceed the maximum size their predators can handle (Otis et al., 1998). Parental male and mature female coloration did not differ among lakes based on predator species richness, supporting our prediction. Interestingly, regardless of predator species abundance, parental male breast coloration resembled that of immature fish from lakes with fewer predator species, whereas mature female breast coloration resembled that of immature fish from lakes with more predator species (Fig. 4.2). These observations suggest that parental males are minimally influenced by the presence of predators, which stands in contrast with studies on smaller species that experience continuous predation pressure (Endler, 1980, 1991; Forsgren, 1992; Godin & Briggs, 1996). On the other hand, mature females may still be influenced by the presence of predators, and apparently remain as cryptic as possible regardless of predator species abundance.

Our study revealed that the coloration of immature bluegills was highly influenced by predator species richness. For the breast, cheek, lateral, and opercular flap regions, immature bluegills had a proportional decrease in short wavelength reflectance
(UV, blue, and green) in lakes with more predator species. Many fish species are sensitive to short wavelengths, either throughout their lives or as juveniles only, including yellow perch (Loew & Wahl, 1991), rainbow trout (Salmo gairdneri; Hawryshyn et al., 1989), and brown trout (Salmo trutta; Bowmaker & Kunz, 1987). Thus, immature fish from lakes with greater predator species richness may be selected to decrease short wavelength reflectance to evade the visual system of their predators.

Although our study has revealed interesting patterns with regards to the influence of environmental factors on bluegill coloration, there are some limitations to our study. Although we collected similar numbers of fish from each lake, we were unable to determine sex and age until we conducted laboratory dissections and our sample sizes for each age and sex class are therefore uneven (Table 4.2). In addition, we collected fish from one location within each lake, which does not allow us to compare the coloration based on specific habitats used by different age classes and sexes (Mittelbach, 1981, 1984). Finally, our measure of predators is based on species richness and not abundance. Although we were not able to determine abundance of the predator species for each lake, this measure would be more accurate in determining predator pressures given low and high densities of fishes. It is also important to consider that although the ecological variables we included in our analyses were not significantly correlated, they could be indirectly linked via general biotic or abiotic processes. Thus, it is important to recognize that our analyses represent the relative influence of each of these ecological variables on bluegill coloration, controlling for the influence of the other variables.

In summary, a number of ecological variables appear to influence coloration patterns in bluegills, with vegetation and predators exhibiting the strongest influence.
Vegetation affected similar body regions in the same direction for parental males, mature females, and immature fish, suggesting that similar selective forces influence the coloration of bluegills in these different groups, even though their coloration differs among groups (Chapter 2). Predator species richness had a considerable influence on coloration in immature fish, with an apparent ontogenetic shift in selection, since parental male and mature female coloration did not vary with predator pressure. Together with other research, our study demonstrates the importance of considering multiple aspects of the environment when investigating the role of natural selection on ornamental and non-ornamental coloration (Millar et al., 2006). Although coloration does change with ontogeny (Chapter 2), we show that environmental factors may pose both similar and divergent selection pressures on coloration. These patterns imply that antagonistic selection pressures, such as those associated with survival and reproduction, can lead to complex signaling strategies within a species. Moreover, our findings suggest by considering both ecological and social influences, we can gain a better perspective on the evolution of communication signals.

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References


by eutrophication that curbs sexual selection. – Science 277:1808-1811.


Table 4.1 Morphological and ecological variables used in analyses for each lake sampled.

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<th>Latitude</th>
<th>Longitude</th>
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<th>Mean depth (m)</th>
<th>% complex vegetation</th>
<th>Total % vegetation</th>
<th>Predator richness*</th>
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<td>Eagle Lake</td>
<td>44°40'</td>
<td>76°42'</td>
<td>5.0</td>
<td>12.2</td>
<td>85</td>
<td>65</td>
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</tr>
</tbody>
</table>

*We only considered northern pike, walleye, and muskellunge since all lakes contained largemouth bass and yellow perch.
Table 4.2 Sample size and mean total length ± SE (mm) of bluegills used in our study for each lake sampled.

<table>
<thead>
<tr>
<th>Lake</th>
<th>Parental Males</th>
<th>Mature Females</th>
<th>Immature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean length ±SE</td>
<td>N</td>
</tr>
<tr>
<td>Rondeau Bay</td>
<td>5</td>
<td>174.2 ± 4.8 mm</td>
<td>8</td>
</tr>
<tr>
<td>Lake St. Clair</td>
<td>0</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Lake Scugog</td>
<td>7</td>
<td>178.3 ± 1.9 mm</td>
<td>2</td>
</tr>
<tr>
<td>Pigeon Lake</td>
<td>13</td>
<td>170.0 ± 2.4 mm</td>
<td>4</td>
</tr>
<tr>
<td>Desert Lake</td>
<td>5</td>
<td>175.8 ± 4.4 mm</td>
<td>5</td>
</tr>
<tr>
<td>Devil Lake</td>
<td>14</td>
<td>181.0 ± 3.8 mm</td>
<td>7</td>
</tr>
<tr>
<td>Buck Lake</td>
<td>5</td>
<td>192.0 ± 4.2 mm</td>
<td>4</td>
</tr>
<tr>
<td>Eagle Lake</td>
<td>2</td>
<td>172.5 ± 6.5 mm</td>
<td>10</td>
</tr>
<tr>
<td>All Lakes</td>
<td>51</td>
<td>177.4 ± 1.7 mm</td>
<td>41</td>
</tr>
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Table 4.3 Effects of ecological variables on bluegill colour body regions (PC1 and PC2) for parental males (N=51), mature females (N = 41), and immature males and females (N = 113; see text for categorization of fish into parental, mature, and immature age classes). Results are from multiple regression analyses.

<table>
<thead>
<tr>
<th>Region</th>
<th>Colour model effects</th>
<th>Parental Males</th>
<th>Mature Females</th>
<th>Immature</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>R²</td>
<td>F</td>
<td>Std. β</td>
</tr>
<tr>
<td>Breast</td>
<td>PC1 Whole model</td>
<td>0.08</td>
<td>0.78</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>Secchi depth</td>
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</tr>
<tr>
<td></td>
<td>Mean depth</td>
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</tr>
<tr>
<td></td>
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<td>0.44</td>
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<td></td>
<td>Percent vegetation cover</td>
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<td>0.23</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Predator species richness</td>
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<td>-0.17</td>
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</tr>
<tr>
<td></td>
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<td>2.68</td>
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<td>Secchi depth</td>
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</tr>
<tr>
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<td>Percent complex vegetation</td>
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<td>-0.34</td>
<td>0.03</td>
</tr>
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<td></td>
<td>Percent vegetation cover</td>
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<td>0.32</td>
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</table>
### Predator species richness

<table>
<thead>
<tr>
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<th>Lateral</th>
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<td>0.19</td>
</tr>
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<td>2.88</td>
</tr>
<tr>
<td>Percent complex vegetation</td>
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<td>3.11</td>
<td>2.47</td>
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<td>1.97</td>
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<table>
<thead>
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<th>Lateral</th>
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<td>0.15</td>
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<tr>
<td>Mean depth</td>
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<td>0.20</td>
<td>2.88</td>
</tr>
<tr>
<td>Percent complex vegetation</td>
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<td>2.47</td>
<td>2.23</td>
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<td>1.97</td>
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<table>
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### PC1 Whole model

<table>
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</tr>
<tr>
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<tr>
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<td>0.20</td>
<td>2.88</td>
</tr>
<tr>
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<td>2.47</td>
<td>2.23</td>
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<tr>
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<td>0.13</td>
<td>0.37</td>
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### Lateral

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### Significance

- **<.0001**
- **0.05**
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<th>Mean depth</th>
<th>Percent complex vegetation</th>
<th>Percent vegetation cover</th>
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<td>0.28</td>
<td>0.09</td>
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<td>0.32</td>
</tr>
<tr>
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<td>0.08</td>
<td>-0.07</td>
<td>0.78</td>
</tr>
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<td>0.004</td>
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<td>-0.70</td>
<td>&lt;.0001</td>
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<td>&lt;.0001</td>
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<td>0.26</td>
<td>0.07</td>
</tr>
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<td>1.71</td>
<td>-0.22</td>
<td>0.20</td>
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<tr>
<td>PC2 Whole model</td>
<td>0.002</td>
<td>0.32</td>
<td>9.95</td>
<td>&lt;.0001</td>
<td>16.73</td>
<td>-0.38</td>
</tr>
<tr>
<td>Percent complex vegetation</td>
<td>0.004</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.009</td>
<td>0.007</td>
<td>0.009</td>
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<tr>
<td>Percent vegetation cover</td>
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<td>0.36</td>
<td>3.90</td>
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<td>2.03</td>
</tr>
<tr>
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<td>-0.22</td>
<td>0.25</td>
<td>0.07</td>
<td>-0.05</td>
<td>0.80</td>
</tr>
</tbody>
</table>
Figure 4.1 Distribution of sampling locations in Ontario, Canada. Sites are represented by black dots, with major cities added for reference (Map contributed by L. Wang).
Figure 4.2 PC2 breast coloration from lakes with one predatory species (solid black bars) and from lakes with two predatory species (solid white bars) for parental males, mature females, and immature fish. Data are least square means (±SE) calculated from multiple regressions (Table 4.2).
CHAPTER 5: GENERAL DISCUSSION

Bluegills have long been a model system in a diversity of research areas (Spotte, 2007), and are particularly well known for their elaborate mating system based on alternative reproductive tactics (Gross & Charnov, 1980; Dominey, 1981; Gross, 1982, 1991; Neff et al., 2003; Neff et al., 2004), their interspecific interactions (Mittelbach, 1981, 1984; Werner & Hall, 1988), and their ontogenetic habitat shifts (Werner & Gilliam, 1984). Although the visual system of bluegills has been well characterized (Hawryshyn et al., 1988), visual communication remains poorly studied in this species. Furthermore, despite our current extensive understanding of the bluegill mating system, there are still aspects of this system that remain unanswered. For example, it is likely that females enter and spawn in the nests of multiple males; however, the mechanisms used by females to select males with which to spawn remain elusive.

The use of colour signals in mate selection has been well studied in fishes, albeit in a relatively small number of species (e.g. Endler, 1980, 1983; Kodric-Brown, 1989; Forsgren, 1992; Bakker & Milinski, 1993). Few studies have used spectrophotometric techniques to quantify colour, although there are notable exceptions (e.g., Grether et al., 2001; Fuller, 2002; White et al., 2003; Rick et al., 2004). Establishing the function of coloration in bluegills has been largely neglected, although some authors have speculated on possible sexual ornaments in this species (Gross & Charnov, 1980; Dominey, 1981; Gross, 1982; Neff et al., 2004). The goal of my study was to characterize colour variation in bluegills and investigate how variation in colour might be influenced by sexual and natural selection. In this thesis, I provided support for the use of ornamental colour
signals in bluegills (Chapter 2, 3). First, patterns of ontogenetic variation, sexual
dichromatism, seasonal differences, and condition-dependence revealed that the
coloration of certain body regions might function as sexual ornaments in this species.
Then, I used observations of naturally spawning bluegills to show that correlations
between female spawning behaviour, male reproductive success, and male colour
variation supported the role of bluegill coloration as a sexual ornament. Finally, I found
that environmental factors differentially influenced both ornamental and non-ornamental
regions in different sex and age groups in bluegills, highlighting the important
antagonistic interaction between sexual and natural selection.

**Ornamental coloration**

Based on my findings, I suggest that the breast, cheek, and opercular flap regions
may be sexual ornaments in bluegills. In Chapter 2 and 3, I support this notion for the
breast and cheek regions, but the function of the opercular flap remains in question
(discussed below). I propose that the breast and cheek regions are ornamental traits for
multiple reasons. First, both of these traits exhibit ontogenetic variation that may be the
result of age-dependent sexual advertisement (Kokko, 1997, 1998). This has been
demonstrated in guppies, where males will allocate more resources to ornamental traits
age they grow older (Miller & Brooks, 2005). Milinski (1993) suggests that predation
should be the primary selective force acting on young (immature) fish, which we
demonstrate in Chapter 4. Immature coloration, including the breast and cheek, was
largely affected by predator species richness. Next, I show that these two regions are
sexually dichromatic (Chapter 2), and such traits are often the target of female choice
(e.g., Forsgren, 1992; Hamilton & Poulin, 1999). Third, breast and cheek coloration are condition-dependent traits in males only (Chapter 2). Finally, I found that cheek coloration largely predicted female spawning behaviour and breast coloration predicted male egg scores. As condition-dependent signals, cheek and breast coloration may advertise a male’s parental abilities (Hoelzer, 1989) or genetic quality (Andersson, 1994b) to females.

**Opercular flap coloration**

In Chapter 2, I found that opercular flap coloration is sexually dichromatic, varies seasonally, and is condition-dependent in males only. Like the breast and the cheek regions, this may suggest a possible function as a sexual ornament. Goddard & Mathis (1997) showed that in related longear sunfish (*Lepomis megalotis*), females show preferences for males with experimentally elongated ear tabs. In bluegills, however, I found no association between female spawning behaviours and male opercular flap coloration, although I did not consider flap length. Alternatively, male bluegills will often flare their opercular flaps during aggressive interactions, suggesting a function in intrasexual selection (Gross, 1982; Côté, 1993). If opercular flap coloration mediates intrasexual aggression, central and peripheral males should differ with respect to their opercular flap colour if nest location is an important factor in bluegill mating. However, my results do not suggest this is the case, as coloration did not differ among males based on position within the colony, nor did female spawning behaviours. Furthermore, Neff et al. (2004) found no difference in opercular flap size between central and peripheral males. Another unusual feature of the opercular flap is that it was not largely influenced
by any of our measured environmental factors, suggesting that natural selection does not play a large role in shaping the coloration of this region. Another possible function of opercular flap coloration is that it may be used in species recognition. Hybridization among sunfishes (Lepomis spp.) is common (Becker, 1983), even though these fishes can usually discriminate between heterospecifics and conspecifics (Keenleyside, 1967, 1978; Gerald, 1971). However, these species usually differ in opercular flap coloration, and the removal of the opercular flap in male redear sunfish (Lepomis microlophus) has been shown to increase hybridization rates with female bluegills.

Non-ornamental coloration

Of the body regions I measured in bluegills, I suggest that the caudal peduncle and lateral regions may be considered non-ornamental. That is, these regions do not appear to be under the influence of sexual selection. Although both the caudal peduncle and lateral regions show some degree of ontogenetic and seasonal variation, as well as sexual dichromatism, there are other possible explanations for this variation, aside from sexual selection pressures. First, bluegills are known to undergo ontogenetic habitat shifts that result in a change in environment and diet (Mittelbach, 1981, 1984; Ehlinger & Wilson, 1988; Werner & Hall, 1988). Such significant habitat changes may lead to an ontogenetic change in coloration for both ornamental and non-ornamental regions. For example, Millar et al. (2006) showed that not only did orange coloration in male guppies decrease with predation pressure, but total overall coloration based on coloured area also decreased. Second, males and females may still appear sexually dichromatic for non-ornamental regions as the sexes are still under different selection pressures. For example,
parental males are forced to remain in colonies that are typically clear of vegetation for several days, whereas females are able to return to deeper, vegetated waters (Gross, 1982; Jennings et al., 1997). Finally, seasonal changes in coloration may result from intra-lake variation, such as an increase in macrophyte density or algal blooms over the summer months (Kalff, 2001). These natural selection forces will undoubtedly change the coloration of non-ornamental regions as well as ornamental ones.

In further support of the idea that the coloration of the caudal peduncle and lateral region are non-ornamental traits, I have shown that these two regions are not condition-dependent (Chapter 2), and do not explain female spawning behaviours or male reproductive success (Chapter 3). Although traits do not have to be condition-dependent to be preferred by females (e.g., sensory bias, Endler & Basalo, 1998; direct benefits, Møller & Jennions, 2001; indirect benefits, Weatherheard & Robertson, 1979), we have shown that some condition-dependent traits are found in bluegills and that these traits are preferred by females during spawning. Furthermore, these two regions were largely influenced by the presence of vegetation in the environment, for each sex and age group, which suggests a function of natural selection in shaping the coloration of the caudal peduncle and lateral regions (Endler, 1993; Marchetti, 1993; Merilaita, 2003).

**Summary and significance**

Through descriptive analyses and observations of mating patterns in the wild, I have identified two possible sexual ornaments (breast and cheek coloration) in bluegill sunfish. I also investigated the function of possible non-ornamental regions (caudal peduncle and lateral) and how these are influenced by ecological factors. Unfortunately,
the function of opercular flap coloration remains a mystery, although it does appear to be a condition-dependent trait in male bluegills. Overall, female preference appears to be an important factor in the evolution of coloration in bluegills, although more work is needed to understand whether females chose males based on direct benefits, indirect benefits, or other mechanisms of sexual selection.

These findings highlight the importance of considering multiple traits when investigating the possible influence of sexual selection (Candolin, 2003). That is, the breast and cheek regions are markedly different in coloration, but I found that they are both important in different aspects. The breast region was an important predictor of male reproductive success as quantified through egg scores, and the cheek region was an important predictor of female spawning behaviours. Furthermore, my study demonstrates the importance of considering multiple environmental factors when investigating the role of ecological selection, and contributes to our understanding of the antagonistic selection pressures that influence visual signals.

**Future Directions**

There are many opportunities for future research with this particular system in similar and related fields. First, future work could analyze the carotenoid concentration in bluegill skin to determine how this varies with diet, parasite load, condition, and numerous other health indicators. Such work can be carried out using high performance liquid chromatography (HPLC), which allows for the separation, identification and quantification of the compounds found in the skin of the fish. Similar work has been conducted in the guppy (Kodric-Brown, 1989) and threespine stickleback (Wedekind et
al., 1998). This would also allow us to directly characterize the relationship between carotenoid concentration and coloration for various body regions.

Another possible avenue of future research could be investigating the role of mutual mate choice in bluegills. Traditional resource-based mating systems, where males provide some direct resource to females, will often lead to female mate choice for male traits. Since parental male bluegills provide sole care for the offspring, females may be choosy based on male ornamental traits, which is what we have shown in Chapter 2. However, given that males are providing sole parental care, it is possible that they may also choose females that are more likely to provide large, nutritious eggs. Such mutual mate choice has been shown in the guppy, a non-resourced based mating system (Dosen & Montgomerie, 2004) and the threespine stickleback, a resource-based mating system (Kraak & Bakker, 1998). During our field observations of bluegill spawning, we noted that in many instances, females (or satellites perceived as females) would attempt to enter the nest of a parental male only to be chased away. Although this could be a form of courtship, our observations suggest that males appear to aggressively chase some females away. Just as females can easily assess multiple males in the colony, males may also be able to assess multiple spawning females and attempt to attract the largest and most fecund females. Even though it does not appear that males have a maximum number of eggs that may be deposited in their nests, they may still benefit from mutual mate choice. With an increase in spawning activity and subsequently egg numbers, nest protection may become more difficult leading to an increase in cuckolder rates which may cause a decrease in parental male paternity. Mutual mate choice could be investigated in this species by conducting controlled field observations using marked individuals to
determine male preferences (females that are accepted into the nest and females that are rejected). Egg weight and number could then be used as a measure of female fecundity. This line of research would contribute interesting information to our understanding of the bluegill mating system.

Finally, investigating mate choice based on genetic compatibility provides another possibility of future research on the bluegill mating system. The hypothesis for mate choice based on compatible genes suggests that females should choose males that are most dissimilar from themselves, often using the major histocompatibility complex (MHC) (Trivers, 1972; Zeh & Zeh, 1996, 1997). Although I have provided evidence supporting female choice for male morphology and colour, it is likely that other factors play a role in the mate choice process of bluegills (see Chapter 2). During our observations of spawning bluegills, we noticed that each parental male would release a large amount of urine when handled. MHC compatible cues may be mediate by urinary odours, as shown in mice (Roberts & Gosling, 2003). Thus, parental males may be releasing urine to provide females with genetic compatibility cues. As with studies of mutual mate choice, field observations of bluegill spawning behaviour could be conducted in a controlled setting on marked individuals. All marked fish would then be collected and their MHC genotypes determined using polymorphic microsatellites. Parental male and female compatibility would be determined and compared to field observations, specifically respect to female acceptance or rejection, egg hatching success, and fry quality. This area of research may provide a more thorough understanding of mate choice in bluegills, as well as revealing whether genetic compatibility plays a role in the evolution of female mate choice in this species.
References


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