THE ROLES OF ACOUSTIC AND VISUAL SIGNALS IN THE REPRODUCTIVE BEHAVIOR OF THE FEDERALLY THREATENED PYGMY SCULPIN, *COTTUS PAULUS*, (COTTIDAE)

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Nicole Catherine Kierl was born in Kansas City, MO, on August 23, 1984, and was raised in Paola, Kansas. She attended Southwestern College in Winfield, Kansas and graduated with bachelor's degrees in Marine Biology and Biology with a minor in New Media Communications. During her undergraduate, she received a NSF-funded Research Experience for Undergraduates (REU) at Mote Marine Laboratory in Sarasota, FL. After graduation, she returned to Mote Marine Laboratory for a three month internship. In July 2007, she started a Master of Science at Auburn University in the Department of Fisheries and Allied Aquaculture.

THESIS ABSTRACT

THE ROLES OF ACOUSTIC AND VISUAL SIGNALS IN THE REPRODUCTIVE BEHAVIOR OF THE FEDERALLY THREATENED PYGMY SCULPIN, *COTTUS PAULUS*, (COTTIDAE)

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Animals use many modes of communication (i.e. tactile, visual, acoustic and chemical) for territory defense, resource defense, and/or courtship. These communication systems can be used singularly or combined together, such as visual courtship displays can be used to circulate pheromones secreted into the water by fishes. Animals may use modes depending on the context and content of the intended signal and the use of multiple signals can increase the chance of the complete signal reaching the intended receiver. The objectives of this thesis were to investigate two modes of communication, acoustic and visual, in the federally threatened pygmy sculpin, *Cottus paulus*. Acoustic communication was documented in breeding male *C. paulus* during agonistic and courtship contexts. There are two distinct call types, a single knock and a knock train, and both were produced during courtship and agonistic behaviors. Acoustic parameters were significantly different between contexts. Calls are similar to those documented for the European sculpin, *Cottus gobio*, and the mottled sculpin, *Cottus bairdi*. Coloration is used in crypsis in most sculpin, with colors ranging from tan, rust, olive, brown to black, with some sculpin exhibit an orange margin on the 1st dorsal fin. Breeding male pygmy sculpin, *C. paulus*, exhibit a black and orange coloration on their fins as well as their body. The male body condition is positively correlated with orange hue as well as the average number of eggs and clutches. However, there is no correlation between coloration and eggs. This suggests that females are choosing males based on condition but are not using coloration as an indicator for condition but some other trait not tested in this thesis.

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Style manual or journal used: Journal of Fish Biology

Computer software used: Microsoft Word, Microsoft Excel, Raven 1.2 (Cornell University), SPSS 11.0

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CHAPTER 1

SOUND PRODUCTION IN THE PYGMY SCULPIN, *COTTUS PAULUS* (COTTIDAE), DURING COURTSHIP AND AGONISTIC BEHAVIORS

Many signals, such as vocalizations or coloration, produced by fishes are used for assessment and mate choice. The acoustic signal structure and the behavioral context in which calls were produced during were investigated in the federally threatened pygmy sculpin, *Cottus paulus*. Breeding male pygmy sculpin produce two distinct call types: a single knock and a knock train. Single knocks were not significantly different between courtship and agonistic contexts. However, knock train call duration is significantly longer during courtship than agonistic contexts. Signal structure for *C. paulus* is consistent with sounds produced by other sculpin, *Cottus gobio* and *Cottus bairdi*. All species in the Cottidae family that are known to produce sound, produce broadband pulses and generally are low in frequency (50 – 500 Hz).

INTRODUCTION

There are several modes of communication used by a diversity of animals (i.e. visual, vocal and chemical). Vocal or acoustic signals have been well studied in many terrestrial (for reviews see: birds, Slater 2003; frogs, Narins et al., 2007; mammals, McComb & Reby, 2005; and aquatic vertebrates (marine mammals, Janik, 2005; fishes, Ladich, 2004). According to Nelson (2006), fishes account for 51% (27,977) of all recognized living vertebrate species. Considering this, the function of sound communication in fishes is under represented in behavioral studies relative to their diversity.

Fishes have been documented to produce sound during courtship, spawning and agonistic behaviors. Sound production can function as the primary courtship display to attract females to a nest or only be part of a collection of courtship displays (Myrberg & Lugli, 2006). Calls usually occur during early phases of courtship displays and increase as the female approaches the nest (Torricelli et al., 1986; Lugli et al., 1997). Parental care in fishes is done mainly by the male, therefore, females should select for a quality male that will take good care of the offspring (Ridley, 1979; Gross & Sargent, 1985). Therefore, courtship calls may be used as an indicator of male quality or size.

Fishes engage in a range of aggressive behaviors (or displays) towards conspecifics while establishing and defending territories, in particular nesting sites (Ladich & Myrberg, 2006). In numerous fish species, these acts of aggression are often accompanied by sound production. Aggressive contests usually begin with less costly displays, such as visual threats and/or sounds, escalating to more costly displays, such as circle swims, biting and ramming, which may lead to injury and/or death (Ladich & Myrberg, 2006; Phillips & Johnston, 2008). Sounds have been found to be associated with visual displays in the low cost phase of the contest, which is thought to be a way to gain knowledge of an opponent's fighting abilities and solve the contest before escalating to more costly displays (Ladich, 1990; Krebs & Davies, 1993; Ladich & Myrberg, 2006)

A relationship between body size and call dominant frequency has been found for some soniferous fishes. However, the results vary among species; dominant frequency decreases with body size in mormyrids, drums and damselfish (Mann & Lobel, 1995; Crawford et al., 1997; Connaughton et al., 2000); dominant frequency is inversely correlated to body mass in croaking gouramis (Ladich et al., 1992) and there is no correlation at all for doradid and pimelodid catfishes (Ladich, 1997). A relationship between body size and dominant frequency of acoustic signals would provide receivers of such signals with information on body size before engaging in contests. Any information on size of a potential opponent or mate would allow an individual to decide chances of a winning a contest or choosing a good mate.

The family Cottidae is dominated by marine species (73%) with a few freshwater species (27%). Sound production has been documented for many of the marine species however, there are only two freshwater species known to produce sound. Ladich (1989, 1990) found that both sexes of the European river bullhead, *Cottus gobio* L., produce knocking sounds with threat displays to defend territories of this solitary fish. Calls are always accompanied by a "head nod" action. Morris (1954) observed nodding movements in *C. gobio* during aggressive and courtship behaviors. This suggests that calls maybe used in both contexts but courtship behavior was not investigated. “Head nods” have also been observed in mottled sculpin, *Cottus bairdi* (Girard), during courtship (Savage, 1963; Jivoff, 1986). Whang and Janssen (1994) found that these head nod motions of *C. bairdi* are also associated with knocking sounds.

The pygmy sculpin, *Cottus paulus* (Williams), is divergent from other members of the family Cottidae by its small size, rarely exceeding 45mm in total length, a contiguous dorsal fin, an incomplete lateral line, a diurnal behavior and contrasting black/orange male breeding coloration (Fig. 1; McCaleb, 1973; Williams, 1968). Like most cottids, *C. paulus* males establish cavities for nesting, guard eggs until hatching and have allopaternal care (Johnston, 2000). Reproduction occurs throughout the year with higher intensity April through August (McCaleb, 1973; Johnston, 2000; Boschung & Mayden, 2004), rather than limited to a few months as in other freshwater sculpins.

The species description of Williams’ (1968) placed *C. paulus* in the *C. bairdi* species-group based on morphologic similarities. Recent genetic studies on the family Cottidae show *C. paulus* to be more closely related to the banded sculpin, *Cottus carolinae* (Gill) species-group (Kinziger et al., 2005). However, Neely et al. (2007) states that *C. paulus* possesses traits of both species group without being very closely related to either. The recent Cottidae phylogeny generated by Kinziger et al. (2005) shows *C. gobio* as a more basal or ancestral species with *C. bairdi* and *C. paulus* being more derived species. This suggests that sound production is a basal trait and will most likely found in many other cottids with further investigation. The role of sound production among cottids could be an important part of their life history and retained through time, with little change in the “head nod motion” or the behavioral context.

This field study investigated sound production during courtship and agonistic behavioral contexts in the pygmy sculpin, *Cottus paulus*. The objectives of this study were to document sound production in the federally threatened pygmy sculpin, *Cottus paulus*, and describe the signal characteristics of the calls including the behavioral context in which they were produced. Context-dependent differences and correlations between body size and dominant frequency were investigated.

METHODS

SPECIES ACCOUNT

Cottus paulus has been on the federally threatened species list since 1989, solely because of its limited distribution. The species is only known to inhabit the Coldwater Spring and its corresponding run near Anniston, Alabama (McCaleb, 1973, Fig. 2). The range of *C. paulus* in the run only extends approximately 150 m downstream, at which point the run is joined by Dry Creek and they become Coldwater Creek. Dry Creek was quite polluted and had a much higher temperature than the spring run in the 1970s (McCaleb, 1973) but the stream's condition has noticeably improved. Many have suggested that pygmy sculpins are intolerant to higher water temperatures because *C. paulus* is not found downstream of the Dry Creek junction (Williams, 1968; McCaleb, 1973; Walsh et al., 1997).

STUDY SITE DESCRIPTION

The Coldwater Spring and Coldwater Spring Run is 4.8 km west of the city of Anniston, in Calhoun County, Alabama (McCaleb, 1973; Fig. 1-1). The city of Anniston uses the spring as a water source which increases the protection *C. paulus* receives. The pool and run fed by Coldwater Spring have a constant temperature of 17^{o}C (+/-) 1^{o}C year round (Williams, 1968; McCaleb, 1973). The spring pool has a surface area of approximately 4.05 m^2 and has been impounded by a weir. The spring run is sustained by the water flowing over the weir. Both the spring pool and run have a gravel/sand substrate with the occasional cobble or boulder, the latter more associated with the run (McCaleb, 1973).

EXPERIMENTAL PROCEDURE

All experiments took place at the Coldwater Spring under field conditions. Guarding males chosen for observation met the following criteria: male exhibited breeding coloration (orange and black), established a nest/territory, territory defense (chasing intruders), and courtship displays with females nearby. Guarding males were found using snorkeling observations. After finding an appropriate focal male, a circular wire pen (52.07 cm diameter x 30.48 cm height) was placed around the male and his nest. The pen was used to ensure that focal males stayed within close proximity of the hydrophone and video camera. The pen did not completely enclose the focal male because the substrate made it difficult, therefore some females and intruding males were free to come and go.

After pen placement, there was an acclimation period of 20 minutes, during which the recording equipment was setup on the bank. Randomly selected individuals (males and females) were added into the pen to ensure some interaction. The trial started with the addition of fish and for approximately 60 mins. Trials were ended if no interactions occurred for 15 minutes. For all behaviors observed the duration, context, movement, and whether sound was produced or not was recorded. The standard length was recorded for focal males and pictures were taken of males and eggs if there were any.

ACOUSTICAL METHODS

Sound recordings were made using a HTI-96- MIN hydrophone with a built in preamp (High Tech Inc.). A splitter was used to record sounds from the hydrophone to a Dell Latitude D510 Laptop and a Sony model TC-D5 Pro II stereo cassette recorder simultaneously. Sounds recordings were made on the laptop using the software program Raven Lite (Cornell University, Ithaca, NY). Sound recordings were analyzed using Raven ver. 1.2.1. Trials were videotaped with a waterproof video camera attached to the wire pen. Sound recordings were synchronized with the video recordings in the laboratory, then manually reviewed for acoustic calls.

DATA ANALYSIS

Each call was first classified as one of two call types: a single knock or a knock trains, then by context (agonistic or courtship). Temporal parameters measured for each call were total call duration, pulse interval (time between pulses), pulse duration and number of pulses using waveforms and spectrograms created by Raven ver. 1.2.1. The dominant and secondary frequencies were determined using power spectrums. Descriptions of these acoustic parameters are listed in Table 1-1.

All statistical analyses were conducted using the SPSS software package (SPSS ver. 11.0, SPSS Inc, Chicago, IL.) The means of all signal variables were be calculated for each individual and analysis of variances (ANOVA) were performed on means comparing 1) agonistic single knocks vs courtship single knocks and 2) agonistic knock trains vs courtship knock trains. An ANOVA was also used to test for significance between call types. A regression analysis was conducted to explore the effect of male body size on the dominant frequency of both call types during each context. Statistical analyses are adapted from Phillips and Johnston (2008) and Ladich (1990

RESULTS

Fourteen, one hour trials were conducted at Coldwater Spring near Anniston, Alabama; one trial was omitted because of noise contamination. Nine focal males produced sound during both courtship and agonistic, 4 focal males produced sound in only one context. A total of 331 calls were analyzed. Male *C. paulus* produced two call types: a single pulse knock and a knock train (two or more pulses strung together) (Fig. 1-2 and 1-3). Call types were based on previous cottid sound production literature (Ladich 1989, 1990). Both call types were produced in courtship and agonistic behaviors. Single knocks and knock trains produced by male *C. paulus* have acoustic parameters that are significantly different from each other in both behavioral contexts. These parameters are total call duration, pulse number and pulse interval (all $p < 0.001$, Table 1-2). The parameters that were not significantly different are pulse duration, dominant frequency.

BEHAVIOR

Focal males engage in agonistic behaviors when an intruder came too close to the focal male's nest cavity. Agonistic behaviors were grouped into sedentary aggression, frontal displays, chases, fights and post aggression (Table 1-3). Sedentary aggression was sometimes the start of an interaction, often before an intruding male came into the camera view. Frontal displays were face offs between two males or the act of sizing each other up. Chases occurred often immediately after sight of intruder and were fast and abrupt. During fights, males engaged in repeated physical contact including hitting and bites. Post aggression occurred after the intruder has left. This behavior was categorized as the focal male would either patrol the area to make sure the intruder had left or return to his nest perhaps to check on eggs. Sedentary aggression, frontal displays, chases, fights and post aggression occurred 51.1%, 1.3%, 27.5%, 7.0% and 13.1% respectively.

Courtship behaviors began when a female entered the area surrounding the nest cavity and were categorized as sedentary courtship, courting, fin wiggle, pursuit and post female (Table 1-3). Sedentary courtship was when there was no movement from the focal male but his orientation was towards a potential female. Courting behavior included males perching at nest opening and wiggling pectoral fins and lateral displays. The fin wiggle behavior is strictly movement of the pectoral fins at a semi constant rate for long periods of time. Pursuits were described as slower chases or males pursuing females as they left. The actions of a focal male after a female had left such as, searching area and repeatedly entering and exiting the nest cavity are categorized as post female behaviors. Sedentary courtship, courting, fin wiggle, pursuits and post female occurred 50.8%, 23.5%, 3.3%, 13.1% and 9.3% respectively.

SOUNDS

Male *C. paulus* produced sounds during 37.6% of sedentary aggression (n=117, observed number of occurrences), 66.7% of frontal displays (n=3), 19.1% of chases (n=64), 18.7% of fights (n=16) and 66.7% of post aggression (Table 1-4). When bites occurred during fights the occurrence of sounds was only 4.5% (1 out of 22). During courtship, sounds were produced 34.4% of sedentary courtship (n=93), 32.6% of courting (n=43), 50% of fin wiggling (n=6), 25.0% of pursuits (n=24) and 64.7% of post female (n=17) (Table 1-4). Overall, single knocks occurred more often than knock trains in both contexts 64.6% vs 35.4% in agonistic and in courtship 66.8% vs 33.2% (Table 1-5). However, when broken down by behavior, call types are more evenly produced during chases (single: 56.5% and trains: 43.5%), fights (50% and 50%) and pursuit (52% and 48%) (Table 1-5). In the case of the behaviors fin wiggle and courting, knock trains occur more frequently than single knocks (fw: 40% vs 60%; crt: 43.9% vs 56.1%) (Table 1-5).

AGONISTIC VS COURTSHIP SIGNALS

Acoustic parameters of agonistic and courtship signals were compared for single knocks and knock trains (Table 1-6). The only parameter that was significantly different between contexts was knock train call duration (p = 0.037, Table 1-6). The total call duration for knock trains was longer during courtship behaviors (459.36±253.95, n=12) than during agonistic behaviors (263.13±150.30, n=11). However, total call duration for single knocks did not differ significantly between contexts, courtship (43.96±34.87, n=11) and agonistic (33.38±13.72, n=11). All other acoustic parameters were not significantly different between contexts (Table 1-6).

A regression analysis of male body size and dominant frequency showed a significant effect during agonistic calls for single knocks and knock trains ($r^2 = 0.549$ and

$r^2 = 0.601$ respectively, all $p < 0.05$; Fig. 1-4 and 1-5). No such effect was seen in courtship calls in either single knocks or knock trains ($r^2 = 0.120$ and $r^2 = 0.055$ respectively, $p > 0.05$). Due to this significant relationship, male size became a covariate in the comparison of dominant frequencies between contexts. Accounting for male size, dominant frequency did not differ significantly for either call type between contexts (Table 1-6).

DISCUSSION

Male *C. paulus* produce sounds during courtship and agonistic behaviors. Two distinct call types are produced in both contexts: the single knock and the knock train. These call types are quite similar in call structure to sounds produced by *C. gobio* (Ladich, 1989). Single knock duration is shorter in duration in *C. paulus*. However, knock train call duration is longer in duration in *C. paulus*. Call dominant frequencies for *C. paulus* are consistent with the dominant frequency range documented for *C. gobio*. Unlike *C. gobio*, female *C. paulus* do not produce sounds. There could not be a direct acoustic comparison between close relatives, *C. bairdi* and *C. paulus*, because measurements were obtained through different acoustic devices. However, sounds produced by all three cottid species are broadband and generally low in frequency.

Knock trains produced during courtship behaviors were significantly longer than those produced during agonistic behaviors. Courtship sounds are generally longer in duration than agonistic behaviors in fishes (Myrberg, 1972; Kaatz & Lobel, 1999; Bass & McKibben, 2001; Ladich & Myrberg, 2006; Phillips & Johnston 2008). This may be due to the higher reward of successfully courting a female (i.e. more eggs). In contrast, male agonistic encounters tend to be short in duration. The benefit to ending agonistic encounters quickly would be to spend less time and energy protecting the nest from intruders. Due to the high population density of *C. paulus* and close proximity of other active nests, encounters with intruding males and juveniles are very common. Thus, males who draw out these encounters may have less time to court females and care for eggs as well as appear weak to other males.

Total call duration of courtship knock trains may also be increased by the trend for longer pulse durations in courtship calls than agonistic calls, although not statistically significant. It is not uncommon for acoustic calls to differ in call structure between contexts in fishes (*Cyprinella galactura*, Phillips & Johnston 2008; *Porichthys notatus*, Bass & McKibben, 2001; genus *Stegastes*, Myrberg, 1972; catfish, Kaatz & Lobel, 1999). Calls can carry different types of information to different receivers depending on the context or motivation (Bradbury & Vehrencamp, 1998). The weak distinction between courtship and agonistic call in this study may be due to a small sample of focal males.

Male *C. paulus* were less likely to produce sounds during highly aggressive behaviors such as chases and fights than sedentary aggression and frontal displays. This is a reoccurring relationship in fishes (Stout, 1975; Ladich, 1989, 1998; Phillips & Johnston, 2008). The whitetail shiner, *Cyprinella galactura* (Cope), readily produced sounds during behaviors of low aggression levels, such as chases, lateral displays and parallel swims, but did not during highly aggressive circle swims and lip locks. This relationship seems to be a trade off between the cost of calling and letting the agonistic encounter escalate in aggression level. Ladich (1990) suggested that acoustically assessing an opponent would prevent energy cost and the risks of further fighting for both parties. In *C. gobio*, dominant frequencies are lower for larger males suggesting that opponents may assess size through acoustics. Vocalizations seemed to be very effective threats for *C. gobio* because encounters rarely ended in physical fights (Ladich, 1990). However, they may not be as effective for *C. paulus* as aggressive encounters quickly became chases and fights, during which vocalizations still occurred roughly 20% of the time. Perhaps, vocalizations have become less reliable for *C. paulus* than reported for *C. gobio*, due to small variation in body size. If there is less variation in the population for male body size, vocalizations may not be very good predictors of opponent size. Vocalizations may play a more important role for other sculpins because they are nocturnal and are limited in the modes of communication they can use to attract a mate. Pygmy sculpin are diurnal and live in very clear water, increasing the potential for visual communication, which is used in conjunction with sounds quite frequently in fishes (Ladich & Myrberg, 2006).

Male *C. paulus* used acoustic signals during all courtship behaviors. It is common in fishes for the number of calls generally increases as the female approaches a nest or territory (Torricelli et al., 1986; Lugli et al., 1997; Myrberg & Lugli, 2006). Vocalizations have been reported during the act of spawning for some freshwater fishes (Phillips & Johnston, 2008; Lugli et al., 1997; Johnston & Vives, 2003). However, spawning was not observed so the role of sound production cannot be assessed for the actual act. Unlike aggression, sound production could play an important role in mate choice and therefore occurs at varying levels of courtship for *C. paulus* as well as *Cyprinella sp.* (Phillips & Johnston, 2008) and *Codoma ornata* (Johnston & Vives, 2003).

The mechanism of sound production in *C. paulus* is unknown however, it is known for a marine counterpart, the longhorn sculpin, *Myoxocephalus octodecimspinosus* (Mitchill). Barber and Mowbray (1956) used electrophysiological techniques to identify that contractions of deep cranioclavicular muscles produce the sounds emitted by *M.*

octodecimspinosus and corresponding pectoral girdle movements produce the sound vibrations of surrounding medium. A head nodding behavior has been observed for *C. gobio* and *C. bairdi*, a closer relative to *C. paulus.* Ladich (1989) concluded that the swift movements of the skull and pectoral girdle during the head nod were similar to the mechanism of sound production in *M. octodecimspinosus*. He also concluded that family Cottidae has a characteristic sound-producing mechanism (Ladich, 1989). However, the head nod motion was not observed during production of single knocks possibly due to the constraints of video recording in the field. A forward lurching or jerking motion was observed during knock trains produced at high levels of motivation. The characteristic head nod could also be harder to perceive due to the small size of *C. paulus* in relation to the other studied sculpins.

The relationship between body size and call dominant frequency has been studied for many soniferous fishes. The results vary among species; dominant frequency decreases with body size (Mann & Lobel, 1995; Crawford et al., 1997; Connaughton et al., 2000); dominant frequency is inversely correlated to body mass (Ladich et al., 1992), or no correlation at all (Ladich, 1997). The relationship found in this study was an increase in dominant frequency with body size for only agonistic calls. This study's effect of body size on the dominant frequency of agonistic calls and not courtship calls could be because dominant frequency is more important in male-male interactions, providing size information before a contest to allow lesser quality male to avoid injury. The dominant frequency of calls can differ between courtship and agonistic context, such a relationship was found for the ornate minnow, *Codoma ornata* (Girard) (Johnston and Vives, 2003).

This study is one of the few field studies on sound production in freshwater fish (Lepomis: Gerald, 1971; Johnson, 2001) and the first field study of its kind for a member of the Cottidae family. The pygmy sculpin, *C. paulus*, is a unique representative of the Cottidae family and its use of acoustic signals and associated behaviors can provide insight into the importance of sound production in the family Cottidae.

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Table 1-1 Signal parameter terminology and definitions

Parameters	Definitions
Temporal:	
Knock call duration	Time from the beginning to end of a single knock call.
Train call duration	Time from the beginning to end of a knock train call.
Train pulse number	The number of pulses per knock train.
Train pulse duration	Time from the beginning to end of one pulse within a knock train call.
Train pulse interval	The interval between pulses, measured from the end of one pulse to the beginning of the next pulse.
Spectral:	
Knock dominant frequency	The frequency measured from a single knock containing the greatest energy.
Train dominant frequency	The frequency measured from a knock train containing the greatest energy.

All temporal parameters were measured from the waveform. All spectral parameters were measured from the power spectrum.

Table 1-2 MANOVA comparison of acoustic parameters by call type in *Cottus paulus*

Parameter	Single Knock		Knock Train		*p* value
	Mean	N	Mean	N	
Agonistic:					
Call duration (ms)	37.29 ± 27.12	96	310.87 ± 388.75	52	0.000[a]
Pulse number	1.00 ± 0.00	96	3.08 ± 1.62	52	0.000[a]
Pulse duration (ms)	37.29 ± 27.12	96	34.59 ± 29.42	52	0.575
Pulse interval (ms)	0.00 ± 0.00	96	113.75 ± 117.88	52	0.000[a]
Dominant frequency (Hz)	137.78 ± 60.53	96	157.19 ± 87.58	52	0.115
Courtship:					
Call duration (ms)	35.51 ± 20.77	123	539.05 ± 529.60	60	0.000[a]
Pulse number	1.00 ± 0.00	123	2.97 ± 1.35	60	0.000[a]
Pulse duration (ms)	35.51 ± 20.77	123	40.88 ± 23.57	60	0.118
Pulse interval (ms)	0.00 ± 0.00	123	211.76 ± 142.39	60	0.000[a]
Dominant frequency (Hz)	150.62 ± 84.04	123	132.63 ± 69.39	60	0.153

[a] Significant *p* values from MANOVA

Table 1-3 Behavior descriptions observed in *Cottus paulus* in order of escalation

Behavior	Description
Agonistic:	
Sedentary aggression	No movement from male, orientation is towards intruding male
Frontal Display	Male is stationary near his nest facing another male.
Post aggression	Male may patrol area looking for intruders or enter nest
Chase	Male abruptly chases male away from nest; some physical contact may occur, hitting most common
Fight	Male physically attacks male; biting and circle swims very common, hitting less common
Courtship:	
Sedentary courtship	No movement from male, orientation is toward female
Courting	Categorized by male body undulations, pectoral fin wiggling, lurches (short movements toward female) and lateral orientation to female.
Fin wiggling	Special case of nest perching in which males wiggle pectoral fins for long periods of time w/o female visible.
Post female	Male continues to look for female or repeatedly enters and exits nest.
Pursuit	Male follows female a short distance.

Table 1-4 Percent occurrence of sounds by behavioral context by male *Cottus paulus*

Courtship	% Sound (n)	Agonistic	% Sound (n)
Sedentary Courtship	34.4 (93)	Sedentary Aggression	37.6 (117)
Courting	32.6 (43)	Frontal display	66.7 (3)
Fin wiggle	50.0 (6)	Post aggression	66.7 (30)
Post female	64.7 (17)	Chase	19.1 (63)
Pursuit	25.0 (24)	Fight	18.8 (16)

Table 1-5 Percent occurrence of call type by behavior of male *Cottus paulus*

	% Single Knock (n)	% Knock Train (n)
All Agonistic	**64.6 (144)**	**35.4 (144)**
Sedentary Aggression	67.5 (77)	32.5 (77)
Frontal display	71.4 (7)	28.6 (7)
Chase	56.5 (23)	43.5 (23)
Fight	50.0 (4)	50.0 (4)
Post Fight	63.6 (33)	36.4 (33)
All Courtship	**66.8 (187)**	**33.2 (187)**
Sedentary Courtship	81.3 (91)	18.7 (91)
Fin wiggle	40.0 (5)	60.0 (5)
Courting	43.9 (41)	56.1 (41)
Pursuit	52.0 (25)	48.0 (25)
Post Female	72.0 (25)	28.0 (25)

Table 1-6 ANOVA comparisons between courtship and agonistic acoustic parameters in *Cottus paulus*

Parameter	Courtship		Agonistic		*p* value
	Mean	N	Mean	N	
Knock call duration (ms)	43.96 ± 34.87	11	33.38 ± 13.72	11	0.360
Knock dominant frequency (Hz)	138.38 ± 59.77	8	134.89 ± 35.24	7	0.992
Train call duration (ms)	459.36 ± 253.95	12	263.13 ± 150.30	11	0.037[a]
Train pulse duration (ms)	39.64 ± 17.67	12	28.91 ± 14.52	11	0.128
Train pulse interval (ms)	189.37 ± 98.56	12	129.95 ± 89.83	11	0.147
Train pulse number	2.88 ± 0.52	12	2.74 ± 0.81	11	0.646
Train dominant frequency (Hz)	106.68 ± 28.23	8	145.17 ± 66.49	7	0.144

[a] Significant *p* values from ANOVA

Fig 1-1 An aerial photo of the study site, Coldwater Spring, near Anniston, Alabama. Courtesy of Google Earth.

Fig. 1-2 A representative waveform (a), spectrogram (b) and power spectrum (c) of a single knock emitted by a guarding male as it chases away an intruding male. The waveform (a) and spectrogram (b) illustrate a distinct knock. The power spectrum (c) shows that most of the call energy (120 dB) is down in 200 Hz.

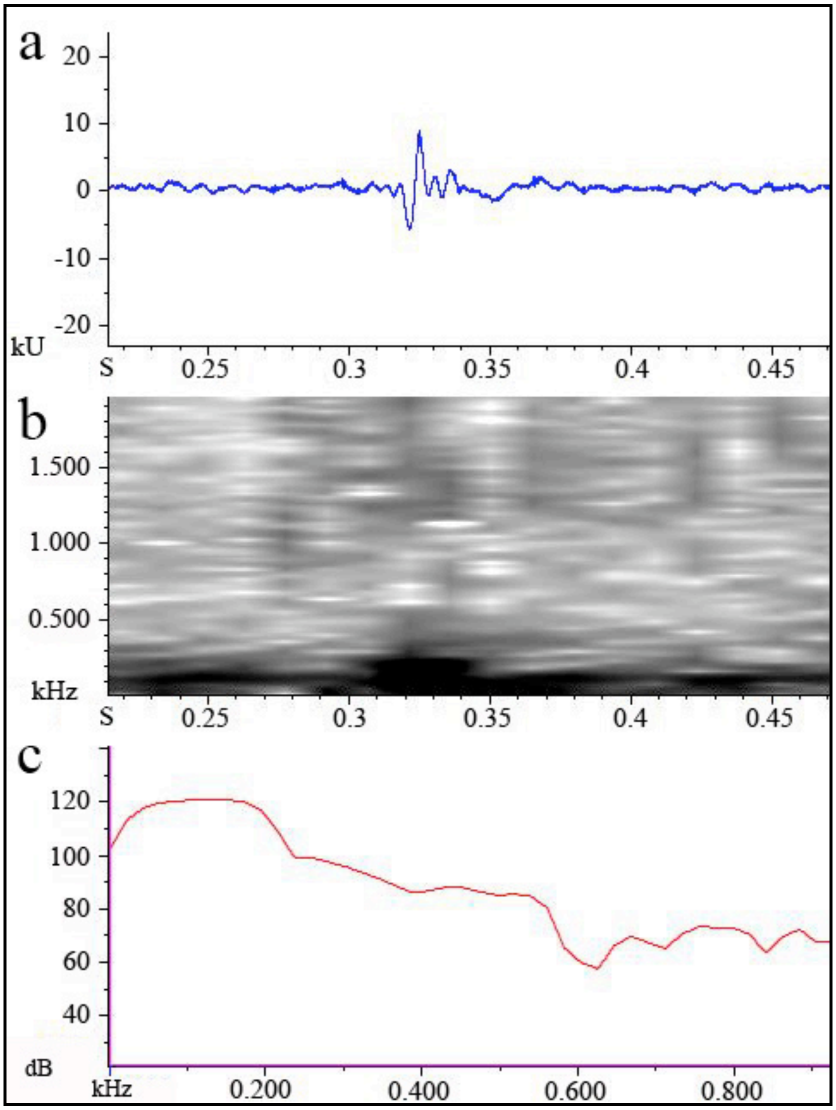


Fig. 1-3 A representative waveform (a), spectrogram (b) and power spectrum (c) of a knock train emitted by a guarding male as it chases away an intruding male. The waveform (a) and spectrogram (b) illustrate the distinct knocks that make up the call series. The power spectrum (c) shows that most of the call energy (120 dB) is down in 200 Hz.

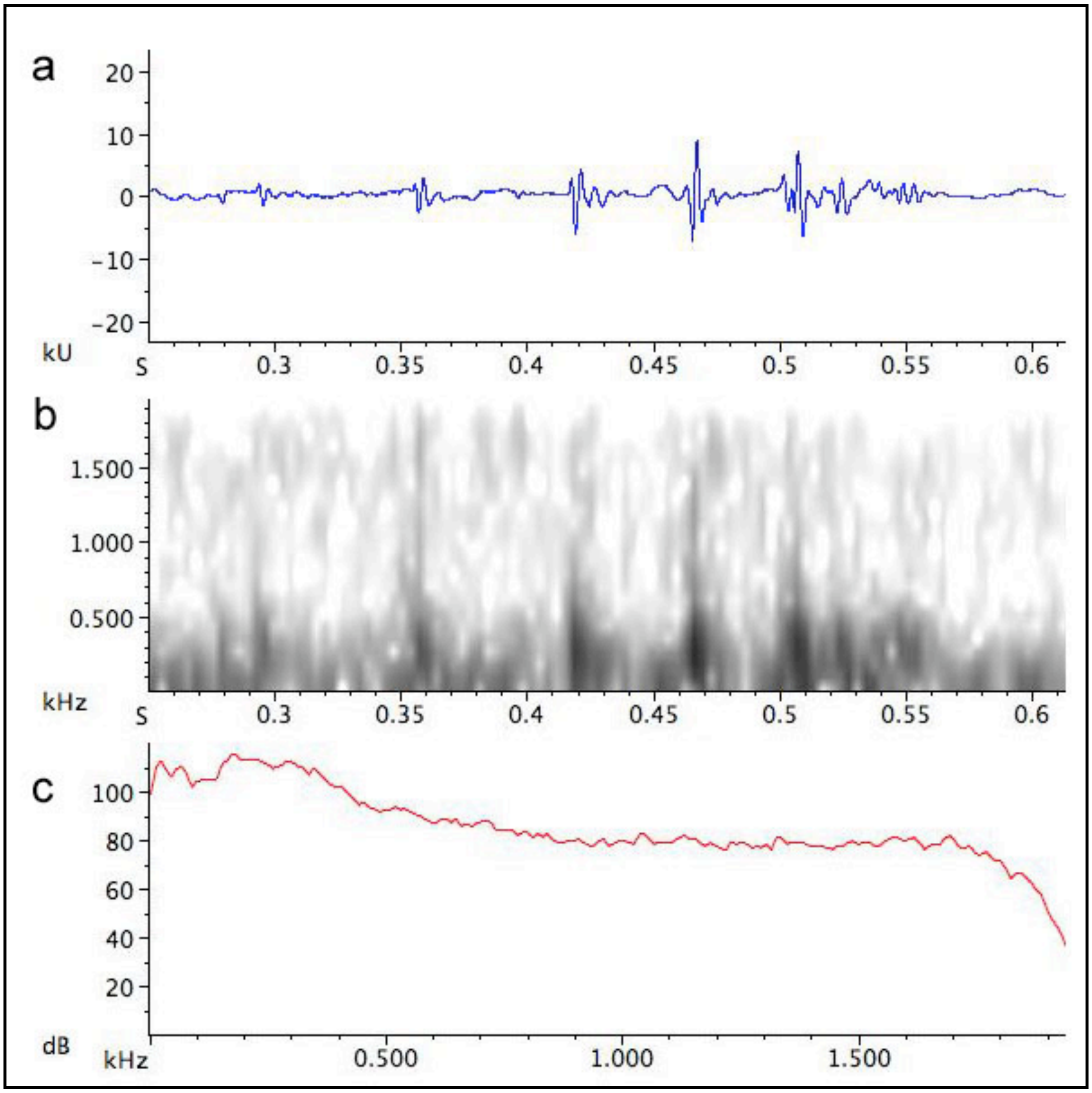


Fig. 1-4 Regression of male size and call dominant frequency for agonistic single knocks ($r^2 = 0.549$, $p < 0.05$).

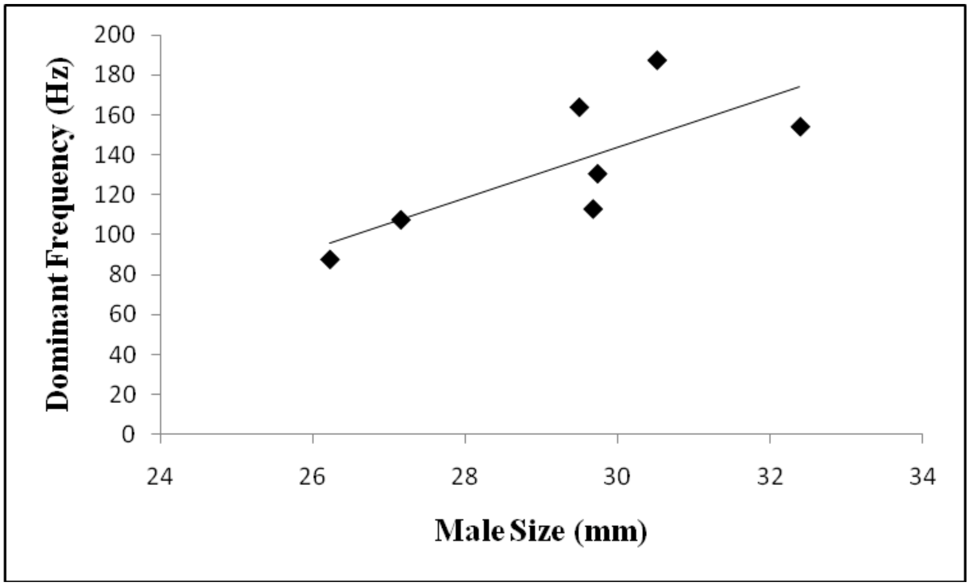
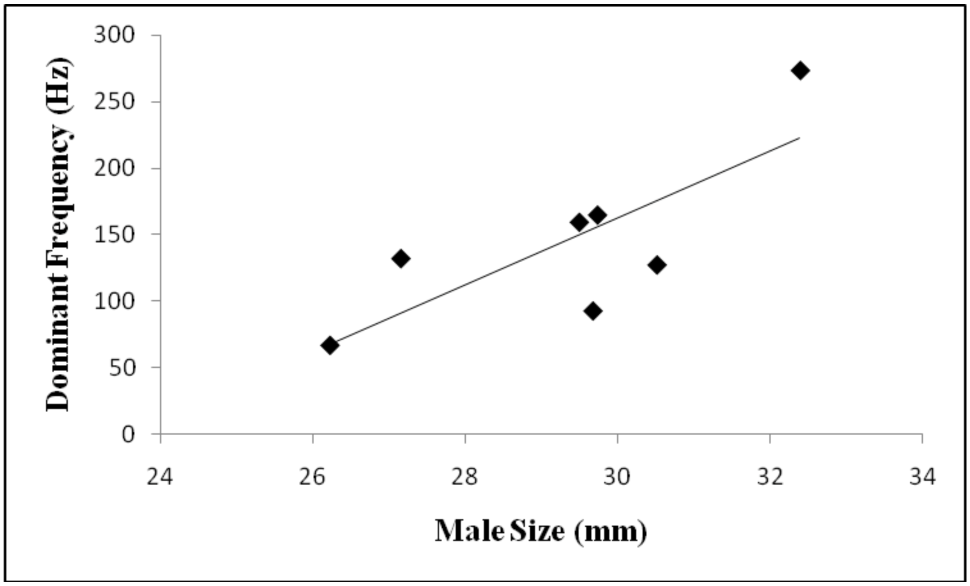


Fig. 1-5 Regression of male size and call dominant frequency for agonistic knock trains ($r^2 = 0.601$, $p < 0.05$).



CHAPTER TWO

BREEDING COLORATION OF MALE *COTTUS PAULUS* AS INDICATOR OF BODY CONDITION

Carotenoid-based coloration is well studied in birds and fishes because carotenoid pigments must be acquired from the diet, it may be used as an honest signal of condition. Females may select males based on the color and intensity of the carotenoid pigments found in a high quality male. The orange patches on the pectoral fins and body of male pygmy sculpin, my study species, are positively correlated with body condition. However, coloration did not correlate with average eggs gained or average egg clutch gained but male body condition did. This unexpected relationship may be because sculpin generally lack color except a thin, orange margin on the first dorsal fin and are nocturnal. Coloration in pygmy sculpin is not yet a trait linked to female choice, despite its association with body condition. However, it is clear that female *Cottus paulus* prefer males in good condition and receive numerous benefits such as effective brood defense, decreased filial cannibalism and fungal infections.

INTRODUCTION

Mate choice is most often up to the female sex due to the high energetic cost of egg production. Therefore, females should choose a male of high quality and it is how they make that choice that has sparked numerous studies in female mate choice (Kirkpatrick, 1987; Jennions & Petrie, 1997; Kokko et al., 2003). Females cue into male traits that can provide them information about male quality. Zahavi's handicap principle suggested that males of high quality can afford elaborate ornamental traits (i.e. frog mating calls, antlers of deer or moose, peacock feathers, and courtship displays of some fish) while males lesser quality cannot (Zahavi & Zahavi, 1997). It has been shown that the longer swords (elongated caudal fin ray) on male green swordtails, *Xiphophorus helleri*, are preferred over males with shorter swords by females (Basolo, 1990). Basolo (1990) suggested that the elongation of this ornamental trait could increase conspicuousness to predators, decrease mobility and have higher energetic costs of swimming. Studies on male ornamental traits have been shown to be condition-dependent signals of individual quality (Andersson, 1994). Such traits are considered as "indicator" traits that can divulge aspects of male condition, information that would greatly help a female choose a quality mate (Andersson, 1994).

Most notable studies of condition-dependent signals have focused on conspicuous, brilliant carotenoid based coloration seen in birds (see Hill & McGraw, 2006a) and fishes (Kodric-Brown, 1989; Houde and Torio, 1992; Frischknecht, 1993;

Nicoletto, 1993; Barber et al., 2000; Grether, 2000). Carotenoids are responsible for most of the red, yellow and orange coloration found in the integuments of animals (Goodwin, 1984). These pigments have been shown to provide beneficial properties acting as free radical scavengers, immune system enhancers and stimulants, cancer inhibitors and antioxidants (Bendich, 1989, 1993; Mayne, 1996; Matsuno, 2001). Animals cannot synthesize their own carotenoids and must obtain them by eating plants, algae or carotenoid sequestering animals (Goodwin, 1984). Coloration may be an "honest" indicator of foraging ability, swimming ability, a male's current health status, health history, or potential resistance to future infections (Endler, 1980; Kodric-Brown & Brown, 1984; Nicoletto, 1991; Olson & Owens, 1998; Braithwaite & Barber, 2000; Møller et al., 2000).

Different pigments and ratios of pigments can have an effect on the coloration of ornaments as well as differences in the absorption, metabolic and deposition rates (Hill 2002; Hill & McGraw, 2006b). Due to these differences, ornaments on different parts of the body may respond differently than each other, thus producing multiple signals. The red body coloration of sticklebacks (*Gasterosteus aculeatus*) and blue eye coloration respond differently to change diet quantities (Frischknecht, 1993). The yellow coloration on feathers and the bill of American goldfinches (*Carduelis tristis*) respond differently to carotenoid access, food intake and parasite load (Hill et al., 2009). This suggests that coloration may signal differently depending on the hue or body location.

Sculpins (Cottidae) are cryptically colored. Most sculpin match the surrounding substrate, similar to the trend in other scorpaeniform fishes (Jenkins & Burkhead, 1993). Breeding males in some sculpin species have color on the margin of the first dorsal fin that can be reddish, orange, pale to white, which contrasts against the typical brown, olive, black overall coloration, but otherwise lack breeding colors (Jenkins & Burkhead, 1993; Boschung & Mayden, 2004; Rohde et al., 2009). Most sculpin are nocturnal and probably breed at night, making visual signals unlikely. The only study to investigate color in sculpin looked at color change plasticity and background matching in the coastrange sculpin, *Cottus aleuticus* (Whitely et al., 2009).

Unlike other sculpins, breeding male pygmy sculpin, *Cottus paulus*, exhibit bright orange and black coloration. The colorful margin on the first dorsal is still present, but less prominent in comparison to the bright orange patches on the pectoral fins and body (Figure 2-1). This species can also be distinguished from other sculpin by its small size (rarely > 45mm TL), contiguous dorsal fin, incomplete lateral line, diurnal behavior and contrasting black/orange male breeding coloration (Williams, 1968; McCaleb, 1973). It is thought that *C. paulus* was isolated in one spring because of a contraction of the range of its ancestor, *Cottus bairdi* or *Cottus carolinae*, during the pre-Wisconsin interglacial period over 70,000 years ago (Williams, 1968). When a cyptic appearance is changed into a more colorful one, it will be less efficient as camouflage (Endler, 1983), therefore either *C. paulus* lacks the need to be cryptic, or the colorful display may play a role in courtship behavior. The change to a diurnal activity period possibly has allowed *C. paulus* the potential use visual signals, therefore explaining their more colorful appearance.

Male pygmy sculpin establish nests and provide parental care such as predator protection and egg fanning. Males also exhibit allopaternal care (Johnston, 2000). Egg fanning requires the male to remain halfway in the nest, leaving only his head and pectoral fins visible, and during courtship males wiggle the pectoral fins towards females (pers. obs.). This wiggling behavior makes the orange coloration on the pectoral fins quite conspicuous to females while the orange coloration on the body is only visible when a male exits the nest. Thus, slight differences between body and pectoral coloration may reflect different signals.

The objective of this study was to investigate the potential role of orange coloration in male pygmy sculpin mating success by: (1) examining the variation between pectoral color and body color; (2) examining the correlation between coloration and body condition and between condition and number of eggs and clutches obtained; (3) Assessing the relationship between male coloration and the number of eggs and clutches obtained

METHODS

In order to follow mating success of individual males, 38 artificial nesting tiles (11cm x 11cm x 1.5 high) were placed in the spring run of Coldwater Spring two weeks before breeding observations began in order to allow ample time for males to colonize them. Breeding male *C. paulus* were followed from April 21 to May 1, observations occurring every other day. On observation days, all nests were checked for breeding males and eggs using a mask and snorkel. Males were captured at the tile using a small dip net, then weighed to the nearest 0.1 g and placed in a clear, plexiglass view box for photographs and length measurements to the nearest 0.001 mm. Photographs were taken of the male's left and right sides by an Olympus stylus 720SW digital camera, with a orange and black color standard in view to adjust for differences in lighting.

At male removal, the nest tile was moved into a tray of water to prevent egg predation. Photographs were also taken of the eggs attached to the underside of the nest tile. Nest location was recorded for each male measured in order to track movement. Any marks, such as tears or scars on the fins, were noted for identification purposes. Identification of males from day to day was also done by reviewing photographs for matching body patterns. Intensity of black body coloration varied however, the underlying pattern did not.

DATA ANALYSIS

Only males that were observed consecutively for two or more times were used for analysis. Male length was measured from digital photographs using the measure tool in ‘Image J’ software (National Institutes of Health, Bethesda, MD, USA); measurements were standardized to a 1 cm ruler in each photograph. A condition factor for each male was calculated using male weight (W) and standard length (L) in Fulton’s condition factor equation (Murphy & Willis 1996):

$$K = \frac{100{,}000\ W}{L^3}$$

The number of eggs each male had was recorded for each observation. Due to the presence of allopaternal care behavior in *C. paulus*, all eggs present on the first observation of each male were excluded because they may be from a prior breeding male that was displaced by the current residing male. Therefore, eggs gained or lost from the first observation were averaged for an overall egg score. The average number of clutches gained was also quantified.

The color quality of orange spots on the pectoral fins and body of breeding male *C. paulus* were measured using digital images with a color standard. Images were taken within five mins of capture because the stress of handling can influence coloration associated with a change in melanin distribution within chromatophores (Donald, 1998; Höglund et al., 2000, 2002; Parsons, 2001). The Adobe Photoshop color sampler tool (5 by 5 pixel average)(Adobe Photoshop CS4 extended, v.11.0.1, Adobe Systems Incorporated, San Jose, CA, USA) was used to quantify orange hue, saturation and brightness at four points on both pectoral fins, each side of the body and the orange color standard.

Two photographs were measured for each observation, a right and left picture (i.e. a male followed for three days would have six photographs). The assistant who measured these points did not know how many eggs a male had and was instructed to sample the most intense areas of orange pigmentation within the pectoral fins and on the body, thereby eliminating the possibility of quantifying regions with glare or water spots as could have happened by choosing random points (modeled from Hill et al. 2009). All color measurements for a single male were averaged to get a single orange hue, saturation and brightness for the pectoral and body. Color standard scores from all photographs were averaged to get a single overall standard score, which was used to standardize all photographs based on the deviation of a photograph's color standard score from the overall standard score. Because field lighting conditions (sunny vs cloudy) varied between observation days, photos in which the standardization adjustment was larger than average were excluded.

In Photoshop, hue is a degree measure on a color wheel (0-360^{o}); low values closer to red wavelengths and higher values closer to green wavelength. Chroma (saturation) is measured using a percentage scale with 0% dull and 100% fully saturated. Brightness is also uses a percentage scale with 0% equaling black and 100% equaling white (Adobe Photoshop CS4 extended, ver. 11.0 manual). Digital photographs only record human visible coloration, therefore no analysis of UV coloration was possible.

STATISTICS

All statistical analyses were conducted using the SPSS software package (SPSS ver. 11.0, SPSS Inc, Chicago, IL.). Color differences between body and pectoral patches were tested for using a paired t-test. All relationships between male coloration, body condition and courtship success were examined by linear regression analyses.

RESULTS

Sixty-one individual males were identified at the artificial nesting tiles, twenty-eight of the males were seen two consecutive observation periods or more. However, six males already had 3 or more clutches on the first observation periods and only lost eggs through all subsequent observations due to development. These males were presumed past the courtship stage and focusing on the parental care stage, therefore were excluded from the analyses.

PECTORAL VS BODY PATCH COLORATION

The hue of the pectoral patches is significantly more orange than body patches (paired t-test, n=22: $p < 0.05$, Table 2-1). The mean hue of pectoral is 23.36 ± 4.83 while the mean hue of body is 24.93 ± 4.29. The chroma of the pectoral patches is significantly less saturated than body patches (paired t-test, n=22; $p < 0.01$). The mean chroma of pectoral and body patches is 64.69 ± 8.43 and 70.34 ± 6.96, respectively. The brightness of pectoral and body patches did not differ significantly (paired t-test, n=22; $p > 0.05$). The mean brightness of pectoral and body patches is 55.35 ±10.40 and 56.91 ± 12.84, respectively.

COLORATION AND BODY CONDITION

The coloration of pectoral patches and body condition were correlated for 2 out of 3 color parameters (linear regression, n = 22; hue: $p < 0.01$; chroma: $p > 0.05$; brightness: $p < 0.05$). Pectoral hue had a strong correlation with body condition ($r^2 = 0.378$; Fig. 2-2). Pectoral brightness was negatively correlated with body condition also ($r^2 = 0.204$; Fig. 2-3). Pectoral chroma was not correlated with body condition ($r^2 = 0.079$). Only the hue of body patches were correlated with body condition (linear regression, n = 22; hue: $r^2 = 0.180$, $p < 0.05$, Fig. 2-4; chroma: $r^2 = 0.009$, $p > 0.05$; brightness: $r^2 = 0.115$, $p > 0.05$).

COLORATION AND COURTSHIP SUCCESS

The coloration of pectoral patches was not correlated with the average number of eggs gained by a male (linear regression, n = 22; hue: $r^2 = 0.045$; chroma: $r^2 = 0.029$; brightness: $r^2 = 0.087$, all $p > 0.05$). The coloration of body patches were not correlated with the average number of eggs gained by a male (linear regression, n = 22; hue: $r^2 = 0.031$; chroma: $r^2 = 0.000$; brightness: $r^2 = 0.041$, all $p > 0.05$).

The coloration of pectoral patches was not correlated with the average egg clutches gained by a male (linear regression, n = 22; hue: $r^2 = 0.053$; chroma: $r^2 = 0.003$; brightness: $r^2 = 0.149$, all $p > 0.05$). The coloration of body patches was not correlated with the average egg clutches gained by a male (linear regression, n = 22; hue: $r^2 = 0.004$; chroma: $r^2 = 0.001$; brightness: $r^2 = 0.096$, all $p > 0.05$).

BODY CONDITION AND COURTSHIP SUCCESS

Body condition of male pygmy sculpins was correlated with both measures of courtship success. Body condition and average egg gained were positively correlated (linear regression, $n = 22$, $r^2 = 0.184$, $p < 0.05$, Fig. 2-5). Body condition was also positively correlated with the average clutches gained (linear regression, $n = 22$, $r^2 = 0.221$, $p < 0.05$, Fig. 2-6).

DISCUSSION

The orange coloration of pectoral and body patches is correlated with body condition in male breeding *C. paulus*. Coloration did not translate into more eggs or clutches. However, males with better body condition gained more clutches/eggs. The hue of both pectoral and body patches and the chroma of pectoral patches is correlated with body condition. Hue, chroma and brightness have been shown to be affected differently by factors such as carotenoid availability and parasite load (Candolin, 2000; Grether, 2000; Hill et al., 2009). Therefore, within a color patch, chroma and brightness may signal different information than hue or the overall color of the patch. However, female pygmy sculpin seem to be selecting males based on body condition independent of coloration.

Coloration in fishes is quite flexible and commonly change with social interactions or stressful encounters (Candolin, 2000; Höglund, 2000). Red coloration in male sticklebacks (*Gasterosteus aculeatus*) acts as a more honest indicator for male quality after exposure to conspecific males (Candolin, 2000). Lesser quality males reduce their red areas to escape aggressive encounters with males of higher quality. It is possible that a change in brightness may make males appear duller after a fight; such a change in coloration was not tested in this study but still may play a role in social status signaling. Hue and chroma make up the overall color and color intensity and are more likely to be correlated to condition because the kind of carotenoid determines the color and carotenoid concentration affects the chroma or saturation of the color (Hill, 2002; Hill & McGraw, 2006b).

Carotenoids have not been shown to be responsible for the orange coloration in breeding male *C. paulus*. However, they have been shown to responsible for the orange and red coloration in fishes (Evans & Norris, 1996; Wedekind et al., 1998; Barber et al., 2000; Grether et al., 2001). Chemical analysis of the orange coloration is unlikely to occur due to the constraints of the *C. paulus*'s federally threatened status. There is sufficient supporting evidence from other fishes and their relationship between coloration and carotenoids and a similar relationship in *C. paulus* to presume that the orange breeding coloration may be carotenoid-based.

The orange coloration of the patches on the pectoral fins is significantly different than orange coloration on the body of male breeding *C. paulus*. The pectoral hue was more orange than the body hue. This suggests that the pectoral patches may signal different information than patches on the body. The body patches play more of a role during lateral display behavior during courtship. However, when a male is attending his nest only his head and pectoral fins are visible to an approaching female, therefore signals using pectoral color patches may be slightly better indicators than using color patches on the body. The red colour in the jaw and throat of male three-spined sticklebacks (*Gasterosteus aculeatus*) has been hypothesized to signal recent changes in condition factor while the blue irises in the eyes signal absolute condition or condition history (Frischknecht, 1993). It was also hypothesized that the two colours could act as indicators for different parasite resistances (Frischknecht, 1993). The coloration of feathers and bill in American goldfinches, *Carduelis tristis*, has been shown respond differently to carotenoid access, food intake and parasite load (Hill et al., 2009). This supports the idea that feather and bill coloration are significantly different traits in songbirds, even though carotenoids are responsible for both pigmentations (Hill et al., 2009). Therefore, it is possible that pectoral and body patches of orange coloration may signal different traits linked to condition as well.

It is also been suggested that the color and pattern on the pectoral fins resemble egg clutches (L. Page and C. Johnston, pers. comm.). Females have been showed to prefer mates that already have eggs in sculpin (Marconato & Bisazza, 1986), darters (Page, 1974, 1975; Knapp & Sargent, 1989; Porter et al., 2002), sticklebacks (Ridley & Retchen, 1981), fathead minnow (Unger & Sargent, 1988), and sand goby (Forsgren et al., 1996). It has been hypothesized that females prefer eggs because it indicates good parenting behavior (Sargent, 1988) or for less predation due to the dilution effect (Whoriskey & Fitzgerald, 1994). Some populations of striped darter, *Etheostoma virgatum*, get conspicuous white spots on their pectoral eggs (Porter et al. 2002). Female preferred to spawn with males that had more white spots (Porter et al. 2002). This further supports the conclusion that pectoral and body patches in *C. paulus* may function as different signals and that pectoral patches may be used by males to enhance reproductive success.

Pectoral patch coloration was not correlated with average eggs gained or average clutch, neither was body patch coloration. This unexpected result could be due to a number of reasons. Orange pigmentation may function for dominance and status signaling purposes, rather than female mate choice. Female mate choice is based on male size in other sculpins, thus there is no basal preference for coloration in sculpins

(Downhower & Brown, 1980; Bisazza & Marconato, 1988; Downhower & Lank, 1994). Coloration may be linked to condition due to carotenoids in the diet but selection has not made this a linked signal.

Male body condition was correlated with both measures of courtship success. This suggests that females are choosing males in better condition based on some other trait than the orange color patches. Filial cannibalism is less likely to occur when the male is in good condition (Manica, 2002). Partial filial cannibalism has been shown to enhance body condition in clingfish, where male continuously breed for up to four months (Gomangano & Kohda, 2008). Filial cannibalism occurred more frequently as male body condition deteriorates in male river bullheads (*Cottus gobio*) (Marconato & Bisazza, 1988; Marconato et al., 1993). Egg guarding males have less time to feed thus a male may choose to eat some eggs in order to prevent starvation. Egg fanning is a common part of egg care in fishes that helps prevent fungus from growing on the eggs (Côté & Gross, 1993). Males in bad condition may be less successful in keeping fungus off eggs. Male *C. gobio* fan eggs almost constantly for four weeks coupled with limited feeding opportunities, leads to body condition deterioration (Marconato & Bisazza, 1988; Marconato et al., 1993). Brood defense is also very important for male reproductive success in *C. gobio*. Larger males are more effective at preventing conspecifics cannibalism (Marconato & Bisazza, 1988). In pygmy sculpin, male size is less variable therefore male body condition may be the determining factor in effective brood defense. Therefore, females gain many benefits from choosing a male in good condition.

Female mate choice has also been linked to previous experience, where the size of a previous male affects the likelihood that a female will spawn with the next male she visits (Downhower & Lank, 1994). In the larger sculpin species, movement is limited and energetically costly, so females may not spend large amounts of time visiting (Petty & Grossman, 2004). Previous experience may cut down on the energetic cost of visiting a lot of males. This behavior may also occur with female *C. paulus*, however the close proximity of nests should allow ample mate comparison.

The lack of correlation between breeding coloration and mating success may be because lower quality males are never able to maintain a territory. There is less suitable nesting substrate in the spring run than in the spring pool so competition for nest tiles is probably high. There were males seen at multiple nests, never with eggs, throughout the study. It is possible these males were displaced by better quality males and forced to reestablish at a different nest. This species reproduces year-round with the peak in July (McCaleb, 1973; Johnston 2000). Lower quality males may be limited to less than desirable months for egg survival. It is uncertain whether an individual female reproduces multiple times in a year or if the population is large enough that females are always becoming reproductively active.

There is no doubt that potential for visual signaling has risen for *C. paulus*. Coldwater spring produces clear water that rarely has turbidity issue. *Cottus paulus* prefer shallow, moderate current with rocky substrate, which makes up a relatively small fraction of the available pool habitat. The orange pigmentation in breeding male *C. paulus* is correlated with body condition. Body condition is correlated with courtship success, while coloration is not. Females do not appear to prefer males based on the orange coloration of the pectoral fins or body but male body condition. Exactly how female *C. paulus* are assessing body condition was not tested in this study. Female *C.*

paulus may be selecting mates by size primarily and color secondary because of the preferences of their close sculpin relatives. The unique breeding coloration in *C. paulus* is yet another thing that distinguishes it from other sculpin species. The purpose of this coloration is still largely unknown. It may be that selection has not yet made coloration a signal linked to female choice in the pygmy sculpin.

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Table 2-1 Paired T-test comparison between pectoral and body patch coloration

Color Variable	**Pectoral Patch**	**Body Patch**	***P* value**
Hue (n = 22)	23.37 ± 4.83	24.93 ± 4.29	0.033[a]
Chroma (n = 22)	64.69 ± 8.43	70.34 ± 6.96	0.006[a]
Brightness (n = 22)	55.35 ± 10.40	56.91 ± 12.84	0.274

[a]Significant *p* values for paired t-test

Fig. 2-1 Breeding Coloration of a male pygmy sculpin, *Cottus paulus*

Fig. 2-2 Regression of Pectoral Hue and Body Condition of Male *C. paulus* ($r^2 = 0.378$, $p < 0.01$)

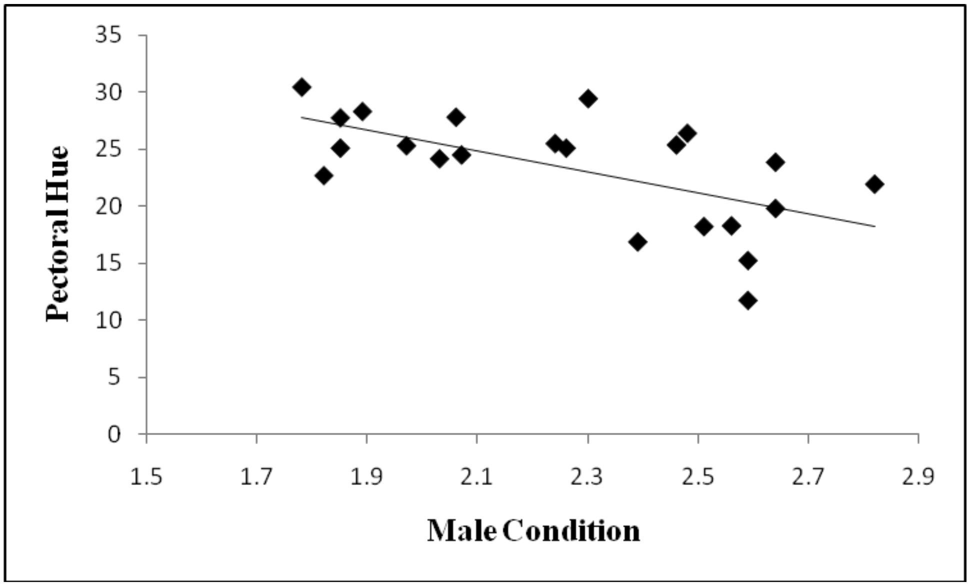

Fig. 2-3 Regression of Pectoral Brightness and Body Condition of Male *C. paulus* ($r^2 = 0.204$, $p < 0.05$)

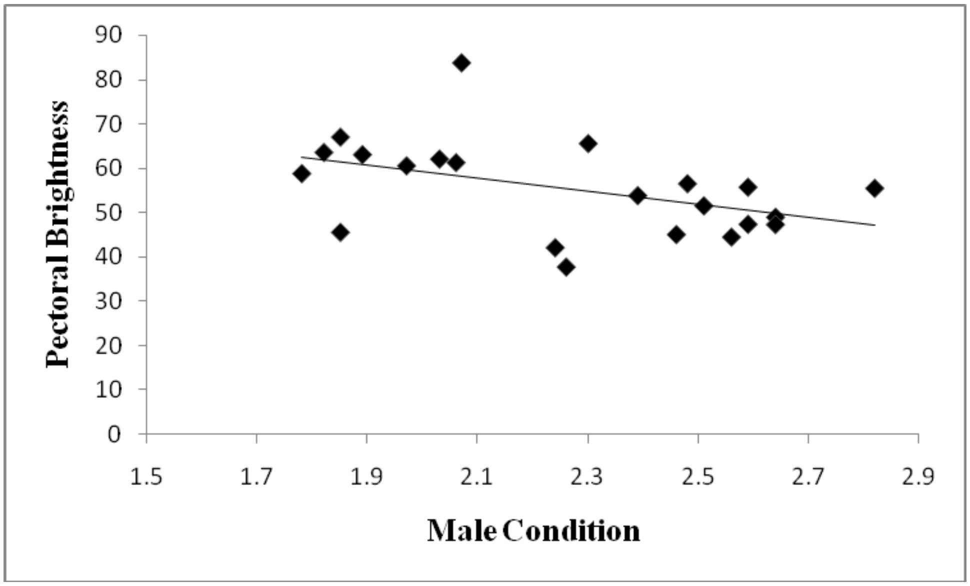

Fig. 2-4 Regression of Body Hue and Body Condition of Male *C. paulus* ($r^2 = 0.180$, $p < 0.05$)

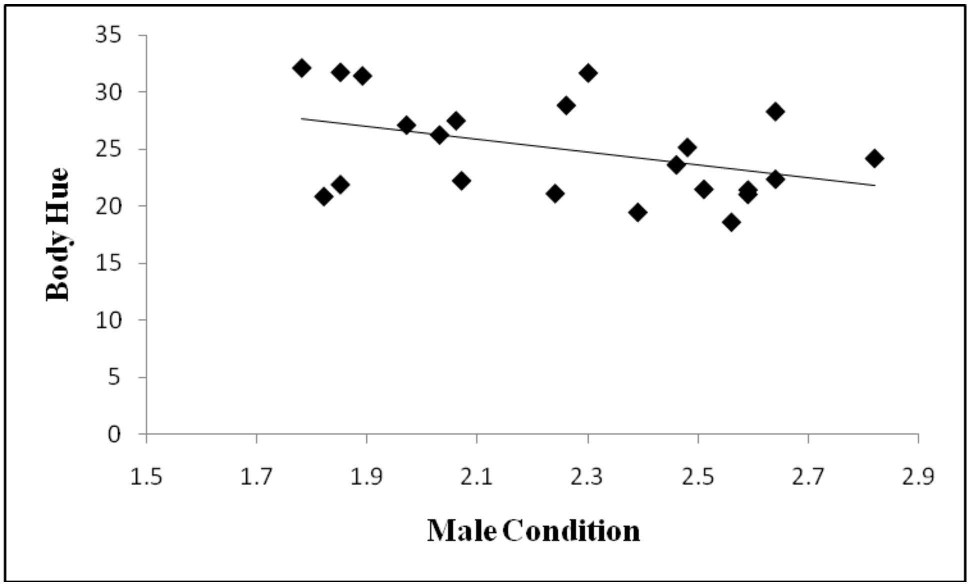

Fig. 2-5 Regression of Male Body Condition and Average Eggs Gained ($r^2 = 0.184$, $p < 0.05$)

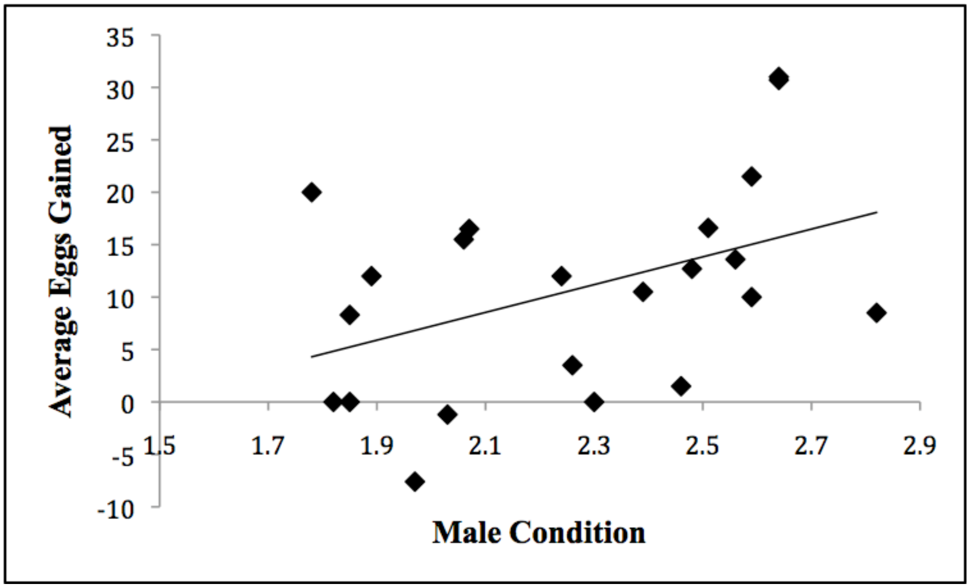

Fig. 2-6 Regression of Male Body Condition and Average Clutches Gained ($r^2 = 0.221$, $p < 0.05$)