

Patterns in Male Breeding Behaviors in the Bluefin Killifish, *Lucania goodei*: A Field Study (Cyprinodontiformes: Fundulidae)

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The determinants of male reproductive success in natural populations have not been measured for any fundulid species in North America. In this study, spawnings, courtship of females, and aggression toward other males, females, and heterospecifics were recorded during daylight hours in a natural population of bluefin killifish, *Lucania goodei*. Three main findings emerge. First, spawning success is correlated with male aggression toward other males and with male courtship of females, indicating that both male/male competition and female choice are likely to be operating. This result implies that both male/male competition and female choice must be considered in studies of sexual selection (as opposed to dichotomous choice tests, which preclude male/male competition). Second, males exhibited substantial levels of aggression toward both nonbreeding females and heterospecifics. Males may be guarding eggs, guarding food items, or trying to keep an open area around them so that they can be seen by gravid females. Third, a polymorphism is present where males had either yellow or red anal fins. Males of both morphs were in breeding condition and spawned with females. Potential mechanisms for the maintenance of the polymorphism are discussed.

IN recent years, there have been numerous studies of female mate choice in fishes (Andersson, 1994; Johnstone, 1995). Fishes are attractive organisms for studying mate choice because of the ease with which dichotomous choice tests can be used where male/male competition is completely eliminated (Basolo, 1990, 1995). However, the results from such studies may not be indicative of sexual selection under field conditions. This is because both male/male competition and female choice can affect male mating success and sexual selection. To properly design and interpret mate choice studies, we must understand the degree to which male mating success is correlated with male competitive behaviors, male courtship behaviors, and female choice. In addition, we must know the degree to which males can force (or sneak) matings, thereby bypassing female choice. In this study, I address these issues by measuring male breeding behaviors in a natural population of the bluefin killifish (*Lucania goodei*).

Lucania goodei is a common hard water fundulid in Florida (Loftus and Kushlan, 1987; Page and Burr, 1991). Several aquarium studies have examined its breeding behavior (Breder and Rosen, 1966; Foster, 1967), physiology (Dunson and Travis, 1991), and larval development (Crawford and Balon, 1994). However, there have been no quantitative descriptions of *L. goodei* breeding behavior in field settings despite the fact that it is one of the most common fish species in Florida (Loftus and Kushlan,

1987). In this paper, I describe the breeding behavior of *L. goodei* in a natural population, examine correlations between male behaviors and spawning success, and compare the breeding behaviors of males of two color morphs.

MATERIALS AND METHODS

Lucania goodei was observed on 10 separate days at Wacissa Springs, Jefferson County, Florida, between 9 June and 14 August 1998. This site has a moderate level of boat activity that drastically lowers alligator abundance but, unfortunately, entails high levels of human activities in the late afternoon. Observations were made by snorkeling on an inflatable air raft. This allowed me to keep my face in the water while remaining relatively immobile. Fish were observed for 30 min once every 2 h from roughly 0630 to 1830 h. Observations were recorded on a water tablet.

During each 30-min observation period, a haphazardly chosen male was identified and watched. If a male swam out of view for more than 3 min, I continued the observation period on another male. Observations were made in areas that were at least 10 m from other human activity. During each observation period, I recorded aggressive male behaviors: the number of times the male flared his dorsal and anal fins when in close proximity to a male (hereafter referred to as "fin flares toward males"), the number of times he chased or attacked a male (hereafter referred to as "chases/attacks toward

TABLE 1. SPEARMAN CORRELATION COEFFICIENTS BETWEEN MALE TRAITS AND SPAWNING SUCCESS. Unadjusted probability values are shown. $n = 48$ for all correlations.

Trait	Spearman correlation coefficient	Unadjusted probability value
Fin flares to males	0.469	0.001*
Chase males	0.386	0.007
Fin flares to females	0.078	0.599
Chase females	0.042	0.777
Fin flares to minnows	0.050	0.730
Chase minnows	0.037	0.804
Head flicks	0.321	0.026
Visit from females	-0.036	0.806

* Only the correlation between fin flares to males and spawning success is significant after a sequential bonferroni correction which controls for 36 comparisons.

males"), the number of fin flares toward females, the number of chases/attacks toward females, the number of fin flares toward heterospecifics, and the number of chases/attacks toward heterospecifics. I also recorded the number of times a male courted a female by performing a bout of "head flicks," the number of times a male spawned with a female (hereafter referred to as "spawning success"), and the number of times a male was visited by a female. Visits occurred when a male was oriented toward a female that swam within roughly 40 cm. This measure cannot distinguish between random approaches by nongravid females and mate inspection visits by gravid females. After the first two days of observations, the color of the male's anal fin was also recorded.

Response variables were compared between the observation periods using analysis of variance or Kruskal-Wallis test. The Kruskal-Wallis test was used when Bartlett's test for homogeneity of variance indicated significant heteroscedasticity (Sokal and Rohlf, 1995). Correlation coefficients between response variables were also determined. Pearson correlation coefficients were used if both variables were normally distributed as indicated by Kolmogorov-Smirnov Lilliefors test. For all other correlations, Spearman correlation coefficients were used. Because of the large number of correlations, a sequential Bonferroni correction was employed (Sokal and Rohlf, 1995). Because detecting patterns in spawning behavior was the main objective of this study, both the unadjusted probability value and the Bonferroni corrected probability value were considered for correlations between spawning success and other response variables (Table 1). For all other correlations, I considered only the Bonferroni

corrected probability value. I present results in the text as means ± 1 SE. All statistical tests were conducted using Systat statistical package (vers. 5.2, L. Wilkinson, SYSTAT, Inc., Evanston, IL, 1992, unpubl.). All probability tests are two-tailed.

RESULTS

Description of behavior.—Large males (estimated > 32 mm SL) in nuptial coloration tended to remain in a small area associated with a patch of vegetation where they were visited by females. Males chased and nipped at each other, especially when guarding females. During these bouts, they also flared their fins by erecting their dorsal and anal fins. Males courted females by swimming around them and by performing head flicks where they quickly twitched their heads back and forth while positioned either in front or directly beneath the female's head (Foster, 1967). The actual spawning involved the male and female vibrating while positioned alongside each other with the posterior third of their bodies in contact. While in contact, they pressed against vegetation and deposited an egg. Eggs were deposited on vegetation throughout the water column ranging from floating vegetation to bottom substrate (< 1.5 m depth).

Males also chased and attacked females and heterospecifics (mainly *Notropis harperi*, *Notropis petersoni*, and *Elassoma* sp.). Females visited male territories both in assemblages and as single individuals. Females frequently associated with heterospecifics, *N. harperi*. Occasionally, small, drab colored *L. goodiei* males were in the schools as well.

Patterns in behavior.—Twenty-nine spawning events were observed; 13 spawning females were observed in total, and some females spawned multiple times with the same male during a 30-min observation period. No group spawnings were observed. In one spawning event, a second male dashed toward a spawning pair as if attempting to join the spawn surreptitiously, but this attempt did not appear successful. Spawning events were observed throughout the study period (June–August). Fin flares toward males, chases/attacks toward males, and head flicks were positively associated with spawning success (Fig. 1, Table 1).

Lucania goodiei engaged in spawning behaviors throughout the day with the exception that none were observed between 1630–1700 h. Spawning events were most commonly observed at 0830–0900, 1230–1300, and 1830–1900 h al-

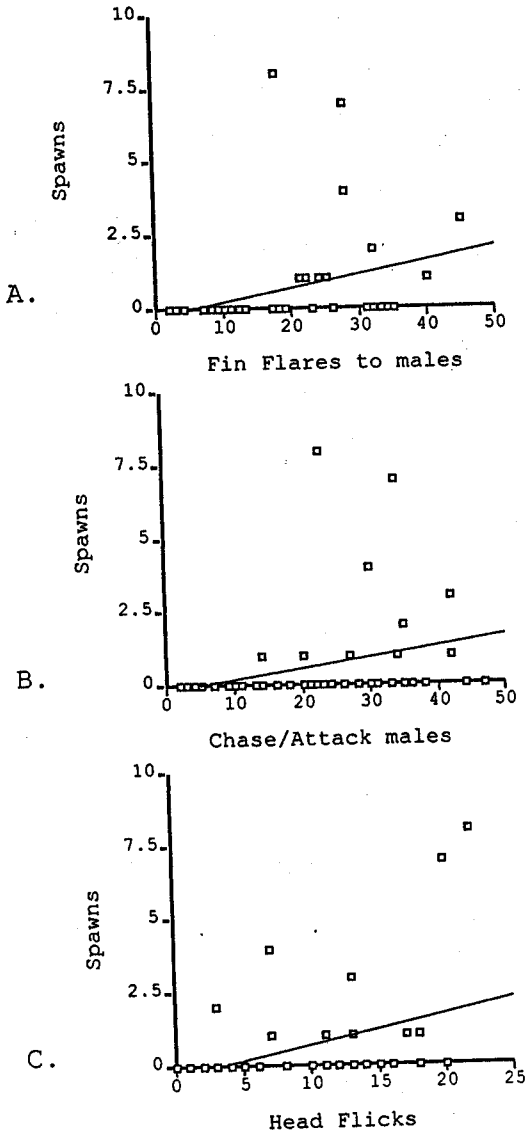


Fig. 1. The relationship between male behaviors—(A) fin flares, (B) Chases, (C) Head flicks—and spawning success.

though differences among the observation periods were not statistically significant (Kruskal-Wallis Test Statistic = 7.848, $P = 0.249$, average number of spawns 0630–0700 h: 0.167 ± 0.167 $n = 6$, 0830–0900 h: 1.286 ± 0.606 $n = 7$, 1030–1100 h: 0.125 ± 0.125 $n = 8$, 1230–1300 h: 1.125 ± 0.990 $n = 8$, 1430–15:00 h: 0.286 ± 0.286 $n = 7$, 1630–1700 h: 0 ± 0 $n = 7$, 1830–1900 h: 1.4 ± 1.4 $n = 5$). Number of fin flares and chases/attacks toward other males varied significantly among the observation periods (Fig. 2A, $F_{6,41} = 2.463$, $P = 0.040$; $F_{6,41} = 2.923$, $P = 0.018$,

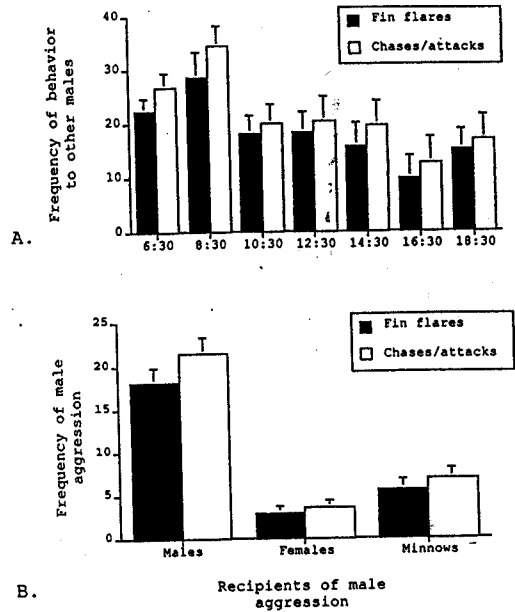


Fig. 2. (A) Male/male aggression (fin flares and chases) at 2-h intervals throughout the day (0630–1900 h). Mean and standard errors are shown. Sample sizes are as follows: 0630–0700 h, $n = 6$; 0830–0900 h, $n = 7$; 1030–1100 h, $n = 8$; 1230–1300 h, $n = 8$; 1430–1500 h, $n = 7$; 1630–1700 h, $n = 7$; 1830–1900 h, $n = 5$. (B) Male aggression (fin flares and chases) toward males, females, and minnows. Means and standard errors are shown. $n = 48$ for all values shown.

respectively). Males performed significantly more fin flares toward other males and engaged in more attacks/chases with other males at 0830–0900 h than at 1630–1700 h (Tukey HSD, $P = 0.016$, $P = 0.009$, respectively, Fig. 2A). These patterns are most likely attributable to human activities. Late afternoon (1630–1700 h) coincides with high levels of human activity (boating, swimming, fishing). On one occasion, the 1630–1700 h observation could not be completed because the visibility was so poor as a result of high boat and jetski activity. No other behavioral variable differed among the observation periods.

Fin flares appear to be used in aggression because they were highly correlated with chases/attacks. Fin flares toward males were correlated with chases/attacks toward males (Pearson correlation coefficient, $R = 0.930$, $P < 0.001$, $n = 48$). Fin flares toward females were correlated with chases/attacks towards females (Spearman correlation coefficient, $R_s = 0.864$, $P < 0.001$, $n = 48$). Fin flares toward heterospecifics were correlated with chases/attacks toward heterospecifics ($R_s = 0.589$, $P < 0.001$, $n = 48$).

Males were also aggressive toward females and heterospecifics. Male aggression toward females was correlated with male aggression toward heterospecifics (i.e., when an individual male frequently attacked females he was also likely to attack heterospecifics in the same observation period). Fin flares toward females were correlated with fin flares toward heterospecifics ($R_s = 0.449$, $P = 0.028$, $n = 48$). Chases/attacks toward females were correlated with chases/attacks toward heterospecifics ($R_s = 0.536$, $P = 0.003$, $n = 48$). However, male-male aggression was not correlated with aggression toward females or heterospecifics. There was no relationship between fin flares toward males and fin flares toward females or heterospecifics nor between chases/attacks toward males and chases/attacks toward females or heterospecifics ($P > 0.500$, for all correlations).

Overall, males were more aggressive toward other males than they were toward females or heterospecifics (Fig. 2B). Fin flares towards males were significantly more frequent than fin flares towards females or heterospecifics (Fig. 2B, Wilcoxon Signed Ranks Test $Z = -5.92$, $P < 0.001$, $Z = -5.36$, $P < 0.001$, respectively). Similarly, males chased/attacked other males more often than females or heterospecifics (Fig. 2B, $Z = -5.72$, $P < 0.001$, $Z = -5.29$, $P < 0.001$, respectively). Males were slightly more aggressive towards heterospecifics than they were toward females (Fig. 2B, fin flares: $Z = 3.03$, $P = 0.0024$, chases/attacks $Z = 3.74$, $P = 0.0002$).

Male anal fin coloration was polymorphic. Twenty-six males had yellow anal fins. Eight males had red anal fins. There were no behavioral differences between these two groups of males ($P > 0.500$ in all tests). Two males with red anal fins spawned. Three of 26 males with yellow anal fins spawned. These differences were not statistically significant.

DISCUSSION

Male-male competition and female choice are likely to be operating in this mating system as spawning success is positively correlated with the frequency of male-male aggression and male courtship of females. Visiting females appeared free to leave male territories and were never forced to spawn with a male. Males never harassed the females they courted. Group/sneaky spawnings were absent. These findings indicate that there is relatively little male harassment to constrain potential female mating preferences. Anecdotal accounts indicate that male/male competition and female choice si-

multaneously occur in many other fundulid species (Foster, 1967; Taylor and Burr, 1997).

Given that both male/male competition and female choice are operating in this system, how can we best discern the relative importance of each mechanism? Houde (1997) suggests the use of open aquarium experiments where animals are allowed to freely interact. By recording the behaviors of individual males and females, the correlations between female choice, male competition, and mating success can be examined. Furthermore, the potential for male/male competition and female choice can be manipulated by varying the ratios of males to females (Kvarnemo et al., 1995) or by varying the amount of spawning substrate available to each male (Forsgren et al., 1996). Such experiments allow us to examine the importance of each mechanism under a variety of conditions and should be more reflective of the mating dynamics taking place under field conditions.

Male aggression functioned not only against competing males but also against females and heterospecifics. Several hypotheses are consistent with this pattern. First, males may be guarding their eggs from egg predators. Females and heterospecifics both tried to forage in a male territory. Males may guard their eggs either to increase their reproductive success or use them as a food resource for their own consumption. Previous aquarium experiments indicate that males initially guard their eggs from females but later join the female in eating the eggs (Breder and Rosen, 1966).

Males of both color morphs engaged in reproductive behaviors and spawned with females. Previously Foster (1967) suggested that males with yellow anal fins are not in full nuptial color. In this study, males with both yellow and red anal fins were observed spawning throughout this study. In an aquarium study (R. C. Fuller and J. Travis, unpubl.), fertilization success did not differ and was high for both morphs (fertilization success: red morph 100%, yellow morph 99.6%). This yellow/red color polymorphism has been found in other populations of Florida (Wakulla Springs, Wakulla County, FL; Manatee Springs, Levy County, FL; R. C. Fuller unpubl. data). Males with blue anal fins such as those depicted in most field guides (Page and Burr, 1991) exist in other populations along with yellow and red morphs (for Everglades populations, see Loftus and Kushlan, 1987; Tower Pond, St. Marks National Wildlife Refuge, Wakulla County, FL, R. C. Fuller, unpubl. data). The selective forces that allow these color polymorphisms to coexist are unknown.

There are four main ways in which multiple

color morphs could be maintained in a population: heterozygote advantage, negative frequency dependence, environmental heterogeneity, and/or trade-offs in life-history components (Roff, 1992). Any of these mechanisms could conceivably allow multiple color morphs to exist in *L. goodei*. Before these alternatives can be investigated, it is necessary to know the function of the color pattern. Research on other fishes has demonstrated that color patterns serve in a variety of functions. Research on both guppies (*Poecilia reticulata*) and sticklebacks (*Gasterosteus aculeatus*) indicate that females base their choice of mates on color patterns (Milinski and Bakker, 1990; Bakker, 1993; Houde, 1997). Males also use color patterns in mediating fights between males (Evans and Norris, 1996). Male color patterns may also function in species recognition (Seehausen et al., 1997). Finally, color patterns can also be used for crypsis (Endler, 1978, 1983), thermoregulation (although this is less applicable for fishes, Endler, 1978), and coordinating fish shoals (Pitcher and Parrish, 1993). However, these latter functions should not promote sexually dimorphic color patterns.

The functional significance of color patterns in fundulids is poorly understood. We do not understand the use of color patterns within a species nor the reason for coloration differences among species. *Lucania goodei* is interesting because males can have yellow, red, or blue anal and posterior dorsal fins. Breeding males of both *Lucania parva* and *Fundulus zebrius* (close phylogenetic relatives of *L. goodei*) use red or orange median fins as do several other fundulids including *Fundulus chrysotus*, *Fundulus cingulatus*, *Fundulus seminolis*, *Fundulus sciadicus*, *Fundulus julisia*, *Fundulus catenatus*, *Fundulus bifax*, and *Fundulus stelliifer* (Foster, 1967; Wiley, 1986; Page and Burr, 1991). In contrast, males in both *Fundulus rathbuni* and *Fundulus diaphanus* have yellow fins. Differences in sexual selection, predation, environmental lighting regimes, or carotenoid abundance could potentially explain these differences. Further studies into the maintenance and evolution of this color polymorphism in *L. goodei* may shed light on patterns found throughout the family.

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