A test for male parental care in a fundulid, the bluefin killifish, *Lucania goodei*

Rebecca C. Fuller & Joseph Travis  
*Florida State University, Department of Biological Sciences, Tallahassee, FL 32306-4340, U.S.A.*  
(e-mail: fuller@neuro.fsu.edu)

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**Synopsis**

Under field conditions, breeding male bluefin killifish, *Lucania goodei*, have been observed aggressively defending territories from other breeding males, non-breeding females, and minnows (mainly *Notropis harperi*). We performed an aquarium experiment to test whether male aggression serves to protect newly deposited eggs from predation. We allowed a male and a female to spawn in a yarn mop, removed the female, and exposed the eggs to one of four treatments (spawning male present, two minnows present, spawning male + two minnows present, no adult fish present). Mops were censused daily for seven days. Egg predation rates were highest in the male + minnows and male only treatments. Egg predation rates in the male + minnows treatment did not differ from the predicted predation rate (sum of male only and minnows only treatments). Hence, there is no evidence for male parental care in *L. goodei*. In addition, we compared the egg predation rates (filial cannibalism) between males of 3 different color morphs and found no evidence for differential egg cannibalism.

**Introduction**

The evolutionary transition in fishes from no parental care to male parental care is thought to be common (Blumer 1982, Gross & Sargents 1985). Male parental care in fishes is often diagnosed in part by observations that males can be territorial around oviposition sites (Gross & Sargents 1985, Clutton-Brock 1991). However, distinguishing between male territoriality around oviposition sites and genuine parental care through guarding eggs simply on the basis of male behavior is problematic. Clutton-Brock (1991, p. 8) defines parental care as any form of parental behavior that appears likely to increase the fitness of a parent’s offspring. On the one hand, one could argue from this definition that consistent male defense of oviposition sites constitutes male parental care. For example, male fantail darters, *Etheostoma flabellare*, defend breeding stones where they spawn with females, and this has been considered as parental care for offspring (Page 1983). On the other hand, filial cannibalism is high in this system. In one experiment, all males ate some of their eggs and 56% of males ate all of their eggs (Lindström & Sargent 1997). Whether or not offspring fitness is higher in the presence of males is unclear. This example highlights the need to determine experimentally whether male behaviors after fertilization actually increase offspring fitness.

Fishes in the order Cyprinodontiformes provide an informative group in which to study parental care (see Costa 1998 for a recent phylogeny) because its incidence varies markedly among families. Male parental care is present in the Cyprinodontidae (e.g. *Jordanella floriae*) where males build, defend, and fan nests of eggs (Breder & Rosen 1966, Foster 1967). In contrast, male parental care is absent (or at least primitive) among killifish (Fundulidae) (Breder & Rosen 1966, Foster 1967). Males in this group are often territorial around spawning sites but do not exhibit the well-defined behaviors associated with parental care such as preparing nests or fanning eggs (Breder & Rosen 1966, Foster 1967). Given the possibility of filial cannibalism, it is unclear whether spawning site defense ought to be considered a primitive form of
parental care. Parental care could be absent in which case males guard spawning substrates but this provides no protection for the eggs. In contrast, parental care could be primitive in that male territoriality around spawning substrates also serves to reduce predation on eggs. This distinction is critical because many theories posit that the evolutionary transition from no care to male care is predisposed in groups where males guard spawning substrates (Baylis 1981, Gross & Sargent 1985, Crawford & Balon 1996).

The bluefin killfish, *Lucania goodei*, is a freshwater fundulid found throughout Florida. Several aquarium studies have examined its physiology (Dunson & Travis 1991) and ontogenic development (Crawford & Balon 1994a,b). Males defend areas of aquatic vegetation on which spawning and egg attachment occurs. Males exhibit aggression not only towards competing males but also towards minnows and females that attempt to feed on male territories (Fuller 2001). Whether this aggression constitutes genuine parental care is unknown.

*Lucania goodei* is also unique because a color polymorphism exists amongst males with multiple color morphs (at least 3 morphs) coexisting in many populations. The anterior three-quarters of the dorsal fin are blue in all males. The posterior quarter of the dorsal fin can be yellow, red, or blue. The anal fin also exhibits a variety of colors: yellow, red, blue, a combination of yellow and blue, or a combination of red and blue (Fuller unpublished data). In a snorkeling study, Fuller found no differences in aggressive behavior between males with red versus males with yellow anal fins. The question of how many color morphs can co-exist within a population has not been answered. Differential parental care and/or egg cannibalism among morphs could play a role in the maintenance of these morphs.

In this paper, we report results of two experiments designed to test whether male *L. goodei* aggressive behavior constitutes parental care in the form of egg defense against minnows *Notropis harperi*. We also compare filial cannibalism among color morphs to ascertain whether differential egg cannibalism can explain the maintenance of this polymorphism.

**Materials and methods**

**Animal husbandry**

We collected *L. goodei* and *N. harperi* with a dip-net and seine and brought them to the laboratory at Florida State University. *L. goodei* were maintained in 19 liter aquaria with well water and foam filters. *N. harperi* were housed in 76 liter aquaria. Water temperatures ranged from 22–24°C. Light ratios operated on 14L : 10D. We fed animals frozen brine shrimp and chironomids 1–2 times each day. *N. harperi* also received an occasional pinch of flake food. During the experiment, focal animals received 2 frozen chironomids each day.

**Experiment 1: test for male parental care**

In this experiment, we measured the rate at which eggs died or disappeared under four treatments: no adult fish present, spawning male *L. goodei* present, two minnows present, spawning male *L. goodei* + two minnows present. We used animals collected from the Wakulla River, Wakulla Co., FL. For each trial, we placed a male and a female in a clean 19 liter aquarium with a yarn mop and allowed them to spawn for 30–120 min. Taylor & Burr (1997) showed that killifish readily use mops as spawning substrates. We used clutches if there were more than 5 eggs in the mop. Occasionally, we found eggs on the bottom of the aquarium and placed them in the mop. We then removed the female and added 5 drops of 1% methylene blue to the aquarium to prevent fungus infection of the eggs. For the control treatment, we removed the male from the aquarium. For the male only treatment, the male remained in the aquarium. For the minnows only treatment, we removed the male and added two *N. harperi* individuals to the aquarium. For the male + minnows treatment, we left the male in the aquarium and added two *N. harperi* individuals. We censused mops daily for one week, or until we counted zero eggs on two consecutive days.

We used each of 20 males once in each of the four treatments and randomized the order of the treatments. Half of these males had yellow posterior dorsal and yellow anal fins (yellow–yellow males), and the other half had red posterior dorsal and red anal fins (red–red males). We measured male standard length before the first trial and after the last trial. Male anal and rear dorsal fin color remained constant throughout all 4 trials. We only used minnows once. Minnow standard length was determined after their trial was completed. We reused females throughout the study. We measured female standard length each time after she deposited a usable clutch. We report all standard lengths as body length. This experiment
was conducted from 29 October 1998 through 4 May 1999.

Experiment 2: test for differential cannibalism among morphs within a second stock

In this experiment, we measured filial egg cannibalism by males of 3 color morphs collected from Wacissa Springs, Jefferson Co., FL, to see if morph differences found in the first experiment were repeatable in another population. We used the same procedures as those described above except that only one treatment (male only) was used. For each trial, we placed a male and a female in a 38-liter aquarium and allowed them to spawn. We then removed the female from the aquarium, and treated the water with methylene blue. To reduce the variance in initial clutch size, we removed eggs from large clutches so that 12 eggs was the maximum initial clutch size. Again, the minimum allowable clutch size was six eggs. We censused mops daily for one week or until complete clutch failure. Ten focal males had red posterior dorsal and red anal fins (red–red males), ten focal males had yellow posterior dorsal and yellow anal fins (yellow–yellow males), eight had red posterior dorsal and yellow anal fins (red–yellow males). This experiment ran from 20 July 1999 until 23 August 1999.

Statistical analysis

To test whether males provide parental care, we compared egg survival over time among the treatments. We performed repeated measures analysis on the log-transformed data (ln(eggs in mop + 1)). We also calculated the predation rate for each trial as the number of eggs eaten divided by the number of days until all eggs were eaten. If eggs were still present in the mop on day 7, then we let days until complete clutch failure equal eight. This is a conservative measure as it potentially reduces the range of differences in predation rate between treatments and thereby reduces our power to detect real differences. We used non-parametric Kruskal–Wallis and post-hoc Mann–Whitney U tests for pair-wise comparisons of predation rate among treatments. Finally, we compared the predation rate in the male + minnows treatment with the expected predation rate if males do not defend their eggs. In this case the predation rate should equal the sum of the predation rates from the male only and minnows only treatments.

We tested whether our results were attributable to aquarium artifacts by examining the effects of initial clutch size and male body length and growth on male cannibalism. Theory predicts that initial clutch size has large effects on egg cannibalism (Sargent 1992, Hoelzer 1995). Kvarnemo et al. (1998) have shown that male sand gobies, Pomatoschistus minutus, will eat 100% of their eggs when clutches are extremely small. We investigated this possibility by examining the correlations between egg cannibalism and initial clutch size and by examining the effects of the treatments on egg predation on day 1 (number of eggs eaten in first 24 h) and on the proportion of eggs eaten on day 1. We also examined the correlations between egg cannibalism and male body length and male growth.

To compare male cannibalism between color morphs, we compared predation rates and egg survival over time. For the Wakulla sample, all eggs had been eaten by day 5. We therefore performed repeated measures analysis on the natural log transformed (number of eggs in the mop + 1) on days 0–4, treating color morph as a categorical variable. For the Wacissa sample, we performed repeated measures analysis on the natural log transformed (number of eggs in the mop + 1) for days 0–7. All statistical tests were conducted using Systat statistical package Version 5.2. All probabilities are two-tailed.

Results

Test for male parental care: Wakulla sample

Initial clutch size ranged from 6 to 41 eggs and did not differ between treatments (Figure 1a, F_{21,76} = 0.487, p = 0.692). Average initial clutch size was 16.3 eggs. Neither female nor minnow body lengths varied among treatments (females: F_{21,75} = 2.293, p = 0.085, minnows: F_{21,75} = 1.080, p = 0.306).

Egg survival and egg predation rates differed significantly among treatments (Figure 1a,b, repeated measures: treatment * days F_{21,532} = 30.783, p = 0.000, Kruskal–Wallis test statistic = 60.00, df = 3, p < 0.001). Egg predation was substantially and significantly higher in both the male only and male + minnows treatments (~8 eggs per day) than in the minnows only (~2 eggs per day) and control treatments (no loss) (male + minnows vs. minnows only: Mann–Whitney U = 365.5, df = 1, p < 0.001, male + minnows vs. control: Mann–Whitney U = 400,
in the nest. In fact, the number of days until complete clutch failure did differ between these two treatments (independent t-test $t = 2.486$, df = 38, $p = 0.0174$, male + minnow treatment = $2.000 \pm 0.241$ days, male only treatment = $2.900 \pm 0.271$ days). However, there are no statistically significant differences between the egg predation rate for the male + minnow treatment and the expected rate (male only predation rate plus minnows only predation rate) (Wilcoxon signed ranks $Z = 0.075$, $p = 0.941$, $n = 20$). Thus, there is no evidence in these data to indicate that offspring fitness is higher in the presence of guarding males.

Aquarium artifacts?

Clutch sizes were similar to those that males experience in the field only on day 1 of the experiment (Figure 1a). Fuller estimates that males should receive approximately 16 eggs per day (unpublished data, based on average number of matings during 30 min observation periods and assumes animals spawn throughout the day, 14 h). Restricting the analysis to day 1 does not change the results qualitatively. Egg predation on day 1 differed significantly among treatments (Figure 2a,b, number of eggs eaten on day 1: Kruskal–Wallis test statistic = 52.71, df = 3, $p < 0.0001$; proportion of eggs eaten on day 1: Kruskal–Wallis test statistic = 54.95, df = 3, $p < 0.0001$). Again, egg predation was higher in the male only and male + minnows treatments than in the minnow only or control treatments (Figure 2a,b). There were no statistically significant differences between the male + minnows treatment and the expected predation rate (male only predation rate + minnows only predation rate) (Wilcoxon signed ranks $Z = -1.2785$, $p = 0.2011$, $n = 20$).

There was no evidence to suggest that males were more likely to eat all of their eggs when clutches were small. On the contrary, there was a positive relationship between initial clutch size and predation rate ($r = 0.539$, $p = 0.022$, $n = 20$). This correlation is not attributable to the fact that some males ate all their eggs in one day. In this case, the predation rate (number of eggs eaten/number of days eggs present) would equal the initial clutch size. However, exclusion of these data points does not change the sign of the correlation between initial clutch size and predation rate (excluding replicates in which males ate all of the eggs in 1 day: $r = 0.799$, $p = 0.0001$, $n = 17$; excluding replicates in which mates ate all of the eggs in 1
Figure 2. Box-plots of predation rates across treatments on day 1. a – Number of eggs eaten on the first day of the experiment. b – Proportion of eggs eaten on the first day of the experiment. Letters denote statistically significant differences. See legend 1b for an explanation of box-plot symbols.

or 2 days: \( r = 0.935, p < 0.0001, n = 13 \). Finally, there was no correlation between initial clutch size and the proportion of eggs eaten on day 1 (\( r = -0.022, p = 0.927, n = 20 \)).

Finally, there is no evidence to suggest that egg cannibalism rates were inflated because males were not fed sufficiently. First, no males died in this experiment, and all males retained nuptial coloration throughout the experiment. Male body length was generally correlated with egg cannibalism (overall predation: \( r = 0.462, p < 0.05 \), predation on day 1: \( r = 0.285, p > 0.05 \), proportion eaten on day 1: \( r = 0.307, p > 0.05 \), n = 20). Male growth was negatively correlated with egg cannibalism although these correlations were not statistically significant (overall predation: \( r = -0.270 \), predation on day 1: \( r = -0.316 \), proportion eaten on day 1: \( r = -0.307, p > 0.05 \) and n = 20 for all tests). Male growth was not statistically different from zero (one-sample t-test, \( t = 1.00, df = 19, p = 0.330 \)).

**Egg cannibalism: Wakulla and Wacissa samples**

For the Wakulla males, there was some evidence to suggest that the two color morphs differed in rates of egg cannibalism. Although overall predation rates did not differ (Mann–Whitney \( U = 63.5, df = 1, p = 0.306 \)), the temporal course of egg survival differed between the two color morphs (Figure 3a, repeated measures: color morph * days \( F_{7,72} = 6.393, p = 0.002 \)). The number of surviving eggs in mops initially declined more rapidly for red–red males than for yellow–yellow males. Analysis of variance indicated that the initial number of eggs in mops (day 0) did not differ between the two morphs (\( F_{1,18} = 0.607, p = 0.446 \)). The number of eggs in the mops did differ on day 1 and day 2 (day 1: \( F_{1,18} = 11.786, p = 0.003 \), day 2: \( F_{1,18} = 5.884, p = 0.026 \)). In addition, the number of days until complete clutch failure differed between the two color morphs (Mann–Whitney \( U = 20.0, df = 1, p = 0.0193 \), red–red males = 2.3 ± 0.42 SE, yellow–yellow males = 3.5 ± 0.224 SE). These patterns may be attributed to the fact that there are slightly more eggs in mops of yellow–yellow males on day 1.

No pattern of differential egg cannibalism among color morphs was found for Wacissa males. There were no differences in predation rates (Kruskal–Wallis \( U = 0.080, df = 2, p = 0.961 \)) nor were there differences in egg survival over time (Figure 3b, repeated measures: color morph * days \( F_{14,175} = 0.452, p = 0.954 \)). There were no differences in the number of days until complete clutch failure (Kruskal–Wallis = 0.260, \( p = 0.878 \)). The same results were obtained when only red–red and yellow–yellow males were included in the analysis.

For the Wacissa males, there was no evidence to indicate higher egg cannibalism rates on small clutches. There was no statistically significant relationship between initial clutch size and overall predation rate nor with the proportion of eggs eaten on day 1 (overall predation rate: \( r = 0.0150, p = 0.447, n = 28 \), proportion of eggs eaten on day 1: \( r = 0.028, p = 0.888, n = 28 \)). There was a positive relationship between predation on day 1 and initial clutch size (\( r = 0.387, p = 0.042, n = 28 \)). In contrast to the Wakulla sample, there was a negative relationship
between male body length and egg cannibalism (overall predation rate: $r = -0.355$, $p = 0.064$, predation on day 1: $r = -0.356$, $p = 0.0628$, proportion of eggs eaten on day 1: $r = -0.386$, $p = 0.0423$). However, the effects of color morph on egg cannibalism do not change when male body length is included in the model.

**Discussion**

Male territoriality around oviposition sites may be difficult to distinguish from male parental care simply on the basis of male behavior. In this study, we found no evidence to suggest that male aggression towards minnows serves as a form of parental care in *L. goodei*. Is this difficulty in discerning between territoriality around oviposition sites and genuine egg guarding a problem for biologists studying other fish species? Males of most species in which male parental care has been studied exhibit behaviors other than egg guarding such as fanning (Forsgren 1997, Östlund & Ahnesjö 1998), rubbing eggs with a protective mucus (Unger & Sargent 1988, Sargent 1989), or remain with the eggs even after females have stopped spawning (Bisazza & Marconato 1988). However, cryptic forms of male parental care may be common. For example, males in the genus *Cyprinodon* defend static territories from other males, non-breeding females and heterospecifics for many days (Echelle 1973, Kodric-Brown 1977). Echelle (1973) posited that territoriality functions in both egg defense and attainment of mates, whereas Kodric-Brown (1977) proposed that territoriality only functions in mate acquisition. The question of whether or not male defense of oviposition sites increases egg survival can only be answered experimentally.

The drawback to the experimental approach is the potential for experimental artifacts. The results of this experiment appear robust. We found no evidence that small initial clutch sizes were associated with high egg cannibalism in either the Wakulla or Wacissa samples (see Sargent 1992, Hoelzer 1995). The results of this experiment did not change when we limited the analysis to egg predation on day 1 (when clutch sizes were most similar to those experienced under field conditions). In addition, there is little evidence to suggest that male condition unduly affected egg cannibalism. There was no male mortality, and males remained in breeding coloration (and presumably breeding condition) throughout the experiment.

The question remains: why are male *L. goodei* aggressive towards females and heterospecifics? Males may be guarding food items. Guarding males may be under high energetic stress and limited to foraging on the few food items occurring on their territories. In many fishes, males that engage in filial cannibalism are in poor condition and/or have reduced food intake (Rowher 1978, Marconato & Bisazza 1988, Linstrom & Sargent 1997, Kvarnemo et al. 1998; however see Lindstrom 1998). Another possibility is that breeding males need to maintain an open area around themselves in order to see and be seen by females.
Endler & Thery (1996) have shown that male birds of some lekking species fight over well-exposed light patches where they can effectively signal to females. Finally, the act of spawning may inform surrounding fish of egg location. Males may aggressively defend eggs directly after spawning but stop guarding a few hours later. This phenomenon would explain male aggression in the field, but the lack of male parental care when measured over multiple days. Such a phenomenon may be particularly likely if male territories change location between successive days.

Finally, this study found negligible evidence for differential cannibalism rates among different color morphs. Although a slight difference was found in egg survival curves in the Wakulla sample, this pattern could not be duplicated with males from the Wacissa sample. These findings underscore the importance of studying multiple populations. Other phenomena such as variation in female mating preferences, tradeoffs with predation, and environmental heterogeneity in lighting environments may play a role in maintaining this color polymorphism.

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References cited


