



Journal of Fish Biology (2011) **78**, 967–972

doi:10.1111/j.1095-8649.2011.02909.x, available online at wileyonlinelibrary.com

The effects of water depth and light on oviposition and egg cannibalism in the bluefin killifish *Lucania goodei*

B. A. SANDKAM* AND R. C. FULLER

Department of Animal Biology, School of Integrative Biology, University of Illinois, Champaign, IL 61820, U.S.A.

(Received 23 September 2010, Accepted 11 January 2011)

This study showed that sex and depth had strong effects on egg cannibalism, whereas water clarity (clear *v.* tea-stained) had no effect on cannibalism or oviposition in the bluefin killifish *Lucania goodei*. These results are consistent with the extreme levels of iteroparity in *L. goodei* where females appear to spread their eggs across multiple locations and depths presumably to avoid egg predation.

© 2011 The Authors

Journal of Fish Biology © 2011 The Fisheries Society of the British Isles

Key words: Cyprinodontiformes; Fundulidae; iteroparity; light attenuation; mating strategies; visual ecology.

Aquatic habitats are highly variable with respect to light. Light is attenuated as a function of depth and can vary according to the quantity of dissolved material in the water column (Effler *et al.*, 2002; Loiselle *et al.*, 2005). Both depth and water clarity can affect bluefin killifish *Lucania goodei* Jordan, which occur in both springs and swamps (Fuller, 2002), and can spawn at a variety of depths (Fuller, 2001). This study focused on the effects of depth, water clarity and sex on oviposition and egg cannibalism in *L. goodei*.

The extent to which variation in depth and water clarity affects oviposition behaviour and subsequent egg survival in *L. goodei* is unclear. Eggs located at the surface will be exposed to greater levels of light, which may influence egg predation. Different egg predators also occur at different depths [potential surface predators, *e.g.* eastern mosquitofish *Gambusia holbrooki* Girard and potential predators lower in the water column, *e.g.* *L. goodei*, redeye chub *Notropis harperi* Fowler and weed shiner *Notropis texanus* (Girard)], which may lead to differences in egg predation. Depth may affect the detection of eggs because they are viewed against different backgrounds, thereby altering predation susceptibility (Cerri, 1983; Aksnes & Giske, 1993; Ruxton, 2009).

Springs are clear and have high transmission of all wavelengths, whereas swamp water contains tannins that decrease the transmission of ultraviolet (UV) and blue wavelengths as well as overall transmission (Meyer, 1990; Fuller & Travis, 2004;

*Author to whom correspondence should be addressed at present address: Simon Fraser University, 8888 University Drive, Burnaby, BC, V5A 1S6 Canada. Tel.: +1 778 782 5641; email: bsandkam@sfu.ca

Chimney & Jordan, 2008). These different light environments have been implicated in the evolution of male colour patterns (Fuller & Travis, 2004), the visual system (Fuller *et al.*, 2004, 2005), and foraging and mating behaviours (Fuller *et al.*, 2010; Fuller & Noa, 2010). The effects of these different light environments on oviposition and egg survival are less clear. Light penetrating tea-stained water has a spectrum with the greatest intensity between *c.* 550 and 620 nm (Fuller & Travis, 2004). Tea-stained water appears yellow (at least to humans). *Lucania goodei* eggs also appear yellow to humans raising the possibility that eggs are more cryptic in tea-stained habitats. In this study, two experiments were used to examine the effects of clear *v.* tea-stained water and depth on oviposition preferences and sex-specific egg cannibalism.

Full grown animals (mean size 32 mm standard length, L_S) were collected using seines and dip-nets from two populations in Florida, U.S.A.: Upper Bridge of the Wakulla River (30° 12' 47" N; 84° 15' 41" W) and the Santa Fe River close to Blue Springs (29° 49' 55" N; 82° 40' 54" W). The Wakulla River is a spring-fed river, which runs clear. The Santa Fe River is highly variable in water clarity. During dry years, the river is fed by springs and runs clear, but during wet years, the river is quite tannin-stained due to the input of dissolved organic material. Animals were returned to the laboratory and housed in aquaria in a climate-controlled green house at the University of Illinois, Urbana-Champaign (UIUC). Animals were fed to satiation daily with frozen bloodworms *Chironomus* sp. and adult brine shrimp *Artemia* sp. (*c.* 2 ml per individual).

Three experimental treatments were established to examine egg cannibalism: sex (males *v.* females), lighting (clear *v.* tea-stained water) and egg position (top *v.* bottom). Fish were allowed to cannibalize eggs that had been laid in mops under a variety of conditions. Mops are bundles of 0.10 m strands of green yarn that *L. goodei* readily use for spawning substrata (Arndt, 1971; McGhee *et al.*, 2007). For the egg position treatment, animals had access to floating mops (attached to positively buoyant Styrofoam balls) that contained eggs and floated at the surface of the water as well as bottom mops (attached to negatively buoyant PVC pieces) that contained eggs and rested on the bottom of the aquarium. Mops with newly laid conspecific eggs were placed in 115 l testing aquaria so that there were at least 10 eggs in the top mops and 10 eggs in the bottom mops (all eggs were taken from clear water stock tanks of a spring population). For the sex treatment, either two males or two females were placed in a 115 l aquarium (0.45 m high by 0.30 m wide) in the greenhouse, where they were exposed to natural sunlight. For the lighting treatment, the testing aquaria were filled with either clear water or tea-stained water. Clear water mimics spring-water conditions and was produced in the laboratory using dechlorinated tap water. Tea-stained water mimics swamp water conditions and was produced in the laboratory using unsweetened decaffeinated iced tea to stain the water. This technique accurately mimics lighting characteristics of naturally occurring swamp environments by reducing light in the UV and blue region of the spectrum (350–450 nm) (Fuller & Travis, 2004; Fuller *et al.*, 2005).

For each trial, two same-sex size-matched individuals were placed in the testing aquarium with a known number of eggs in the top and bottom mops at 0900 hours. The numbers of eggs remaining were then counted at 6, 24, 30, 48 and 54 h. Fish were fed a 4 ml mix of frozen *Artemia* sp. and dry fish food each day at 0900 hours

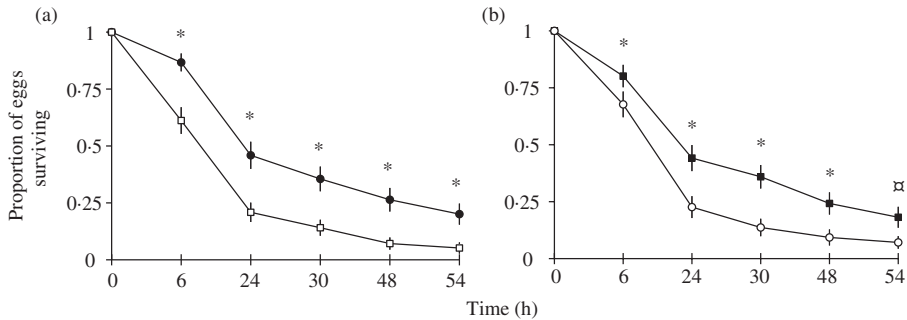


FIG. 1. (a) *Lucania goodei* cannibalism rates differed with respect to location in the water column with eggs near the bottom being cannibalized more rapidly than eggs near the surface ($P < 0.001$) (●, top mops; □, bottom mops). (b) Cannibalism rates also differed with respect to sex with females (○) cannibalizing eggs more rapidly than males (■) ($P < 0.001$). *, significant differences ($P < 0.05$). ✕, a marginally non-significant difference ($P > 0.05$). Values are means \pm s.e.

(equivalent to being fed to satiation). An initial analysis indicated no statistically significant effects of population. Hence, this variable was removed from subsequent analyses. Ten replicates were performed for each combination of sex and water treatment (total of 40 fish). In the analysis, the proportions of eggs surviving in top and bottom mops were treated as separate observations. A repeated-measures ANOVA was used to determine whether sex, light and location affected egg cannibalization and whether these factors varied over time.

Light had little effect on egg cannibalism rates. There were strong effects of position in the water column ($F_{1,72} = 17.97$, $P < 0.001$). Eggs located in the bottom mops were cannibalized $\times 1.37$ faster than eggs located at the surface [Fig. 1(a)]. Cannibalism rates also differed by sex ($F_{1,72} = 12.50$, $P < 0.001$). Females cannibalized eggs $\times 1.33$ faster than males [Fig. 1(b)]. There was no significant interaction between sex and egg position ($F_{1,72} = 0.00$, $P > 0.05$) or between sex and lighting environment ($F_{1,72} = 0.00$, $P > 0.05$).

The goals of the second experiment were to determine (1) whether female *L. goodei* oviposit their eggs non-randomly between floater v. bottom mops and (2) whether egg placement is affected by the light environment. To do this, the number of spawns (and eggs) on floater v. bottom mops in either clear or tea-stained water was observed. Fish were allowed to spawn in tall, hexagonal aquaria (40 l, 0.45 m tall) that contained both floater and bottom mops. Fifteen trials were conducted in aquaria with tea-stained water and 16 trials were conducted in aquaria with clear water. The night before a female's trial, she was placed in the trial tank to acclimate. Five minutes prior to the start of each trial, the female was fed a 1 ml mix of frozen *Artemia* sp. shrimp and TetraMin flake food (Tetra Holdings; www.tetra-fish.com). One male was introduced to the aquarium and the pair was observed for 1 h.

Male territoriality is not a problem in regard to restricting the female's choice of oviposition substrata. Hundreds of spawning events have been observed in aquaria. In the absence of competition, males readily follow females to the spawning substrata and switch between floater and sinker mops. In nature, males face the duel problem of following and courting females while defending females from nearby males. In this experiment, however, there was a single male and a single female. Hence,

there were few impediments to females exerting preferences to spawn on particular substrata.

After 1 h, the mops were removed and the numbers of eggs in floater and bottom mops were counted. Trials were discarded if cannibalization occurred or if fewer than five eggs were spawned. Fish that failed to spawn any eggs were returned to their holding tanks and tested later. Trials were conducted between 0900 and 1500 hours. From these data, the proportion of eggs in the floater mop was calculated for each trial. A Wilcoxon signed ranks test was used to determine whether the proportion of eggs laid in the floater mop differed from a null expectation of 0.5. A Mann–Whitney *U*-test was used to compare the proportion of eggs in the floater mop between clear *v.* tea-stained water. All analyses were performed using SAS statistical software (www.sas.com).

Lucania goodei had no clear preference for spawning on either the floater or the bottom mops. The proportion of eggs spawned on floater *v.* bottom mops did not differ from a null expectation of 0.5 (mean \pm S.E. proportion of total spawned on floater mops = 0.49 ± 0.25 , Wilcoxon signed ranks $Z = 0.80$, $P > 0.05$, $n = 18$). Light environment (clear *v.* tea-stained water) also had no effects on placement of eggs (Mann–Whitney *U*-test = 51, $P > 0.05$; mean \pm S.E. clear water = 0.53 ± 0.12 ; tea water = 0.34 ± 0.17). Obviously, the power to detect a significant difference between these two treatments (if one should exist) was low at 0.26. The experiment, however, did have the power to detect a difference of 0.4 (*i.e.* 30 *v.* 70% of eggs eaten) between the two proportions 80% of the time. Hence, there was reasonable power to detect biologically meaningful patterns.

This study found little evidence that clear *v.* tea-stained habitats affect oviposition behaviour or egg cannibalism. Similarly, there was no indication that oviposition or egg cannibalism was affected by an interaction between depth and water clarity (clear *v.* tea-stained habitats). These results are surprising because these habitats differ widely in their light environments and, in nature, differ with respect to exposure to egg predators. Instead, *L. goodei* displayed no distributional preference for oviposition across habitat types. This tendency to widely distribute eggs across space is consistent with both the temporal allocation of eggs and the general mating behaviour of *L. goodei* females. Female *L. goodei* engage in extreme iteroparity. Females can spawn for multiple days in a row (Breder & Rosen, 1966). Each day the female spawns 10 to 20 eggs but only releases one to three eggs per individual spawning event (Breder & Rosen, 1966; McGhee *et al.*, 2007). Each spawning event occurs between only one male and one female. In the field, females appear to spawn sequentially with multiple males, hence distributing their eggs across multiple territories. The observation here that adults spawn in equal proportions in both floating and bottom substrata is consistent with this theme of widely dispersing eggs over time and space.

Water depth did have a strong effect upon egg cannibalism. Conspecifics were more likely to cannibalize eggs located near the bottom of the water column in close proximity to adults. *Lucania goodei* typically spend more time foraging deeper in the water column and only approach shallower depths when spawning. Higher cannibalism on bottom mops obviously results in a higher proportion of eggs developing near the surface. Two methodological questions arise. First, were cannibalism rates unduly high due to the fact that this was an aquarium experiment? The data do not address this question. Field studies of egg predation in other fishes, however, have shown that

conspecific egg predation can be quite high (Marconato & Bisazza, 1988; Frommen *et al.*, 2007). A recent review found that at least 15 species of fishes engage in cannibalism to the point of consuming entire clutches (Manica, 2002). The adults used in the above experiments were always in good condition and well fed. The second question is whether predation on eggs located on floating vegetation would be higher under field conditions, where surface-oriented fishes such as *G. holbrooki* are present. Obviously, the results of this study are dependent on the types of predators present, which in this case were *L. goodei*. Snorkelling observations suggest a higher density of potential predators at greater depths in the field. *Lucania* spp., *Heterandria* spp. and a variety of conspecific minnows such as *N. harperi*, *N. texanus* and sailfin shiner *Pteronotopsis hypselopterus* (Günther) opportunistically forage on *L. goodei* spawning substrata (Fuller, 2001). The extent to which egg predation alters the location of eggs in the field, however, is a question that requires field experimentation.

Finally, egg cannibalism rates differed between males and females, with females cannibalizing eggs more readily than males. Female egg cannibalism rates are frequently higher than those of males in species with male parental care (Marconato *et al.*, 1993; Lion & van Baalen, 2007). There have been few (if any) studies, however, comparing cannibalization between the sexes in species lacking male parental care. It might be tempting to conclude that *L. goodei* possess a form of rudimentary parental care due to the fact that males (1) engaged in less egg cannibalism than females and (2) are somewhat territorial (Ah-King *et al.*, 2005). The eggs of *L. goodei*, however, do not benefit from the presence of males. Males do not significantly decrease egg consumption by either heterospecifics (Fuller & Travis, 2001) or conspecifics (unpubl. data). Males aggressively defend spawning substrata and can be particularly aggressive immediately after spawning, but these behaviours do not decrease egg cannibalism over longer periods of time. Instead, the sex difference in egg cannibalism may be attributable to high metabolic demands on females due to egg production, as seen in species such as sockeye salmon *Oncorhynchus nerka* (Walbaum) (Hendry & Berg, 1999), causing them to forage more (and hence consume more eggs) than males.

Overall, this study shows that *L. goodei* do not preferentially spawn on surface *v.* bottom spawning substrata. This behaviour is not affected by clear *v.* tea-stained water conditions. Instead, *L. goodei* appear to distribute their eggs widely. *Lucania goodei* do consume more eggs from bottom substrata with which they are in close proximity. This results in a higher proportion of eggs being located at the water surface.

The authors thank D. Welsh and E. Berdan for assistance in field collection, N. Prior for assistance with the cannibalization experiment and K. Danielson and S. Gehrig for assistance with oviposition observations. This work was ethically approved by the University of Illinois IACUC (No. 05153) and partially supported through an REU (Research Experience for Undergraduates) grant provided by NSF (IOB 0445127).

References

- Ah-King, M., Kvammen, C. & Tullberg, B. S. (2005). The influence of territoriality and mating system on the evolution of male care: a phylogenetic study on fish. *Journal of Evolutionary Biology* **18**, 371–382.
- Aksnes, D. L. & Giske, J. (1993). A theoretical model of aquatic visual feeding. *Ecological Modelling* **67**, 233–250.

- Arndt, R. G. E. (1971). Ecology and behavior of the cyprinodont fishes *Adinia xenica*, *Lucania parva*, *Lucania goodei* and *Leptolucania ommata*. *Dissertation Abstracts International B* **32**, 1917–B–1918–B.
- Breder, C. M. & Rosen, D. E. (1966). *Modes of Reproduction in Fishes*. Garden City, NY: Natural History Press.
- Cerri, R. D. (1983). The effect of light-intensity on predator and prey behavior in cyprinid fish-factors that influence prey risk. *Animal Behaviour* **31**, 736–742.
- Chimney, M. J. & Jordan, F. (2008). The fish assemblage of a constructed treatment wetland in South Florida. *Florida Scientist* **71**, 246–264.
- Effler, S. W., Perkins, M., Ohrazda, N., Matthews, D. A., Gelda, R., Peng, F., Johnson, D. L. & Stepchuk, C. L. (2002). Tripton, transparency and light penetration in seven New York reservoirs. *Hydrobiologia* **468**, 213–232.
- Frommen, J. G., Brendler, C. & Bakker, T. C. M. (2007). The tale of the bad stepfather: male three-spined sticklebacks *Gasterosteus aculeatus* L. recognize foreign eggs in their manipulated nest by egg cues alone. *Journal of Fish Biology* **70**, 1295–1301.
- Fuller, R. C. (2001). Patterns in male breeding behaviors in the bluefin killifish, *Lucania goodei*: a field study (Cyprinodontiformes: Fundulidae). *Copeia* **2001**, 823–828.
- Fuller, R. C. (2002). Lighting environment predicts relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. *Proceedings of the Royal Society B* **269**, 1457–1465.
- Fuller, R. C. & Noa, L. A. (2010). Female mating preferences, lighting environment, and a test of sensory bias in bluefin killifish. *Animal Behavior* **80**, 23–35.
- Fuller, R. C. & Travis, J. (2001). A test for male parental care in a fundulid, the bluefin killifish, *Lucania goodei*. *Environmental Biology of Fishes* **61**, 419–426.
- Fuller, R. C. & Travis, J. (2004). Genetics, lighting environment, and heritable responses to lighting environment affect male color morph expression in bluefin killifish, *Lucania goodei*. *Evolution* **58**, 1086–1098.
- Fuller, R. C., Carleton, K. L., Fadool, J. M., Spady, T. C. & Travis, J. (2004). Population variation in opsin expression in the bluefin killifish, *Lucania goodei*: a real-time PCR study. *Journal of Comparative Physiology A* **190**, 147–154.
- Fuller, R. C., Carleton, K. L., Fadool, J. M., Spady, T. C. & Travis, J. (2005). Genetic and environmental variation in the visual properties of bluefin killifish, *Lucania goodei*. *Journal of Evolutionary Biology* **18**, 516–523.
- Fuller, R. C., Noa, L. A. & Strellner, R. S. (2010). Teasing apart the many effects of lighting environment on opsin expression and foraging preference in bluefin killifish. *The American Naturalist* **176**, 1–13.
- Hendry, A. P. & Berg, O. K. (1999). Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. *Canadian Journal of Zoology* **77**, 1663–1675.
- Lion, S. & van Baalen, M. (2007). From infanticide to parental care: why spatial structure can help adults be good parents. *American Naturalist* **170**, E26–E46.
- Loiselle, S. A., Bracchini, L., Cozar, A., Dattilo, A. M. & Rossi, C. (2005). Extensive spatial analysis of the light environment in a subtropical shallow lake, Laguna Ibera, Argentina. *Hydrobiologia* **534**, 181–191.
- Manica, A. (2002). Filial cannibalism in teleost fish. *Biological Reviews* **77**, 261–277.
- Marconato, A. & Bisazza, A. (1988). Mate choice, egg cannibalism and reproductive success in the river bullhead, *Cottus gobio* L. *Journal of Fish Biology* **33**, 905–916.
- Marconato, A., Bisazza, A. & Fabris, M. (1993). The cost of parental care and egg cannibalism in the river bullhead, *Cottus gobio* L. (Pisces, Cottidae). *Behavioral Ecology and Sociobiology* **32**, 229–237.
- McGhee, K. E., Fuller, R. C. & Travis, J. (2007). Male competition and female choice interact to determine mating success in the bluefin killifish. *Behavioral Ecology* **18**, 822–830.
- Meyer, J. L. (1990). A blackwater perspective on riverine ecosystems. *Bioscience* **40**, 643–651.
- Ruxton, G. D. (2009). Non-visual crypsis: a review of the empirical evidence for camouflage to senses other than vision. *Philosophical Transactions of the Royal Society B* **364**, 549–557.