Reproductive ecology of two species of *Fundulus* in a Maine watershed

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Abstract

The mummichog, *Fundulus heteroclitus*, is a widely studied fish, yet little is known about its reproductive behavior in the field. The majority of what is known has been done using the southern subspecies, often in laboratory settings. Those studies have shown that *F. heteroclitus* exhibits a semilunar, pair spawning pattern; however, there exists anecdotal evidence that this is not the case in the northern subspecies. During 2003, we studied the reproductive biology of the northern subspecies of *F. heteroclitus* in a salt marsh habitat and a congener, *Fundulus diaphanus*, in a nearby freshwater lake. Both species spawn on a daily basis during the restricted breeding season from late May through mid-July. The lake species, *F. diaphanus*, spawns throughout the day, while *F. heteroclitus* restricted spawning to the highest tidal levels for approximately one hour during both day and night high tides. Although larger males attempt to defend territories in both species, in *F. heteroclitus* high male densities led to group spawning and intense sperm competition. There was no apparent effect of salinity on spawning-site preferences in *F. heteroclitus* over a wide range of salinities (2 – 32 ppt). Sperm were motile in both hyper- and hypoosmotic salinities, and only showed declines in motility at extreme salinities (<2 ppt, >27 ppt). In contrast, *F. diaphanus* showed strong decreases in motility at salinities over 10 ppt, and also showed reduced motility in freshwater compared to isoosmotic solutions. The effects of salinity on fertilization success were less dramatic than the sperm motility results; for *F. heteroclitus* fertilization was successful at all salinities, while for *F. diaphanus* fertilization dropped above 10 ppt. These results emphasize the reproductive adaptations in *F. heteroclitus* to variable salinities and an intertidal habitat, and also quantify several differences between this population and the more commonly studied southern subspecies.

Introduction

The killifish, *Fundulus heteroclitus*, has been studied intensively, yet little is known about its reproductive behavior in the field. The majority of what is known about its reproductive biology has been done using individuals from populations south of Cape Cod, often with the southern subspecies, and most of the work has been done in the laboratory. During 2003, we studied the reproductive biology of the northern subspecies of *F. heteroclitus* in a salt marsh habitat (Northeast Creek) and a congener, *F. diaphanus*, in a nearby freshwater lake (Lakewood). Specifically, we examined the mating systems of these species, factors affecting time and intensity of spawning, and salinity effects on sperm motility and fertilization ability.

http://www.eurnag.com/0602/fatal.html

Fundulus heteroclitus (♂)

http://fish.dnr.cornell.edu/myfish/Cyprinodontidae/bandedkillifishpic.html

Fundulus diaphanus (♂)

Methods

Field observations. Spawning activity was observed from the shoreline in Northeast Creek and snorkeling in Lakewood. Spawning intensity of *F. heteroclitus* was measured as the number of spawning events counted during the most active 30 minutes of observation. For both species, number of males per spawning event was recorded, as were time of day and salinity, and time relative to high tide for *F. heteroclitus*. We checked the fecundity of 50 female *F. heteroclitus* caught using a small seine before high tide during different phases of the lunar cycle. Both percent of gravid females and number of eggs per female were recorded as measures of reproductive activity within this species.

Deposition site of F. heteroclitus *eggs.*

Salinity effects on sperm motility. We qualitatively examined milt from 54 *F. heteroclitus* and 33 *F. diaphanus*. For each trial, milt was pipetted from a male directly to two microscope slides containing water with a salinity of 10 ppt (control) and water of one of the salinity trials. Artificial seawater was used for all salinity trials. Initial motility was evaluated in a scale of 0-5 and all runs in which sperm in the control slide scored less than 4 were discarded. Duration of sperm motility was recorded when the percentage of sperm showing forward movement was estimated as 50%, 5%, and 0%.

Collection of F. heteroclitus *in the field.*

Salinity effects on fertilization success. Eggs were stripped into a petri dish containing 10 ml of water (of one of the trial salinities) and simultaneously mixed with fresh sperm from 2-3 males. A sample of the sperm-containing water was taken shortly after to evaluate sperm concentration and to verify that sperm concentrations stayed roughly equal between trials. Eggs were considered successfully fertilized if they developed into clearly discernable embryos (stage 12)[1]. Once at this stage, all embryos we continued to follow through development successfully hatched.

Results

Environmental influences on spawning intensity

- *F. diaphanus* spawned during most daylight hours throughout the season. Spawning peaked in the early afternoon.
- Spawning intensity in *F. heteroclitus* steadily declined over the spawning season (r = -.49, p = .02).
- *F. heteroclitus* spawned over the entire range of salinities recorded (11.7 to 32.7 ppt) during both night and day, during both spring and neap tide series, and during all phases of the lunar cycle.
- Salinity did not affect spawning intensity of *F. heteroclitus* (p = .63 in a multiple regression with effect of date removed).
- There was no consistent difference in intensity of spawning of *F. heteroclitus* between the lower day high tides and the higher night high tides (paired t-test p = .31, n = 10 pairs).

Comparison of reproductive ecology

	F. heteroclitus	*F. diaphanus*
Mating system	*Group spawners.* ~80% of spawns had multiple ♂♂ (n > 500).	*Pair spawners.* Over 90% of spawns had one ♂ (n > 60).
Gonosomatic index (GSI)	*High GSI.* Testis 7.6% of body weight.	*Low GSI.* Testis 1% of body weight.
Salinity effects on sperm motility	Sperm were motile for extended periods of time at a broad range of salinities	Sperm have substantially reduced motility at salinities > 10 ppt.
Salinity effects on fert. success	High fertilization success over a wide range of salinities.	Fertilization did not occur in trials above 10ppt.

Salinity effects on sperm motility and fertilization success

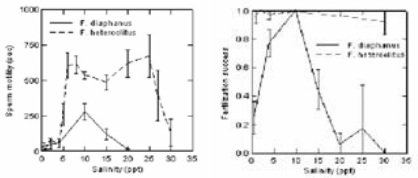

Figure 1. Sperm motility and fertilization success in F. heteroclitus *and* F. diaphanus. *Error bars are ± 1 SE. Sperm motility is time to no motility, and fertilization success is the percentage of eggs successfully developing. (Mann-Whitney U tests, 10, 15, 20 ppt F.h.> F.d.; 2 ppt F.h. = F.d.)*

Tidal and seasonal effects on *F. heteroclitus* fecundity

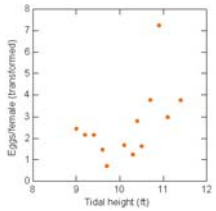

Figure 2. Tidal height does not affect female fecundity in F. heteroclitus. *(p = 0.06)*

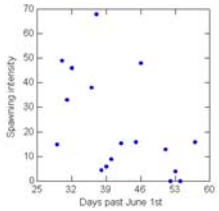

Figure 3. Spawning intensity shows no periodicity and decreases as season progresses in F. heteroclitus. *(p < 0.05)*

Conclusion

These results emphasize the differences in the reproductive biology of the closely-related *Fundulus species.*

The abundant estuarine species, *F. heteroclitus*:
- Spawns on all high tides
- Does not exhibit a marked semi-lunar periodicity in reproductive function, unlike the southern subspecies[2]
- Is predominantly group spawning (in contrast to aquarium observations[3])

The freshwater *F. diaphanus*:
- Spawns over a broad period during the day
- Exhibits less sperm competition, with large males defending territories

Our results also introduce *F. heteroclitus* as a unique system to study initiation of sperm motility. In most fish species studied, sperm motility is activated by a change in the relative osmolality of the external environment compared to the male reproductive tract. These cues work in opposite directions for freshwater and marine species. Since *F. heteroclitus* spawns over a broad range of salinities, the same unidirectional processes cannot occur. Understanding the mechanisms that allow for this tolerance in reproductive environment in *F. heteroclitus* may be important in understanding the success of this abundant estuarine fish.

References

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Acknowledgements

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