

Spawning Periodicity and Reproductive Behavior of *Fundulus heteroclitus* in a New England Salt Marsh

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The Mummichog, *Fundulus heteroclitus*, is a widely distributed fish that has been extensively researched in the southern portion of its range (south of Cape Cod, MA). During the summers of 2003 and 2004, we studied the reproductive ecology of *F. heteroclitus* in a northern population (Northeast Creek, Mount Desert Island, Maine). Our direct observations show that unlike more southern populations, northern *F. heteroclitus* spawns daily during its two-month spawning season, with no preference for spring tides over the entire season. However, within consecutive semidiurnal tides significantly more spawning was associated with the higher high tide. Spawning occurred on bare gravel and on mud associated with the grass *Spartina patens*. Spawning was highly promiscuous with males typically spawning in groups with females in very shallow water during receding tides. These temporal and spatial patterns of oviposition caused eggs to be deposited in a much broader range of habitats than in southern populations of this species. We present and evaluate critically several hypotheses that may explain the variation in spawning patterns observed in this species.

INTERTIDAL spawning is a well-documented pattern of reproduction in marine and estuarine fishes, occurring in at least nine orders and 26 families of fishes (DeMartini, 1999). Many of these fishes appear to spawn on lunar or tidal cycles, including many temperate species that spawn in salt marshes such as the Atlantic Silverside, *Menidia menidia* (Middaugh, 1981; Conover and Kynard, 1984; Conover and Heins, 1987; Conover, 1992), and the Mummichog, *Fundulus heteroclitus* (reviewed in DeMartini, 1999; Taylor, 1999). These temporal patterns of lunar or tidal spawning will influence spatial patterns of egg deposition in intertidal spawners. Spawning in the intertidal may be adaptive for several reasons, including predation refuge for embryos, increased development rates due to either increased oxygen availability or increased temperature, or as a predation refuge for spawning adults from aquatic predators (reviewed in DeMartini, 1999; Martin et al., 2004). Intertidal spawning can also increase the risks of physiological stress on spawning adults or embryos, and increase the risk of predation from terrestrial predators for both adults and embryos.

A large literature has been amassed detailing several aspects of the intertidal spawning of *F. heteroclitus*, including a semi-lunar (14.5 d) spawning periodicity (Taylor et al., 1979; Taylor and DiMichele, 1980; Taylor, 1984, 1986), substrate preferences for oviposition (Taylor et al., 1977, 1979; Taylor, 1986), hatching cues (DiMichele and Taylor, 1981; DiMichele and Powers, 1984), survivorship of eggs in the intertidal (reviewed in Taylor, 1999), and patterns of gamete and egg viability over a range of salinities (Palmer and Able, 1987; Able and Palmer, 1988). In addition, tidally mediated patterns of spawning have been observed in other species of *Fundulus* (Greeley and MacGregor, 1983). This wealth of studies makes *F. heteroclitus* a major example of an intertidally spawning fish.

Fundulus heteroclitus occur over a wide range of latitudes, from Nova Scotia to Florida (Able, 2002). The eastern shore

of North America exhibits a relatively steep latitudinal gradient in physical characteristics, especially temperature (Conover, 1992). This gradient provides the potential environmental variation for studying intraspecific variation and adaptation in reproductive traits of *F. heteroclitus*. In *F. heteroclitus*, clinal variation has been documented for molecular, morphological, life-history, and reproductive traits (Place and Powers, 1979; Morin and Able, 1983; Able and Felley, 1986; Taylor, 1986; Marteinsdottir and Able, 1988; González-Villaseñor and Powers, 1990; Ropson et al., 1990; DiMichele and Westerman, 1997; Adams et al., 2006; Haney et al., 2009). These data all support the designation of two subspecies, a northern *F. heteroclitus macrolepidotus* and a southern *F. heteroclitus heteroclitus* (Morin and Able, 1983; Able and Felley, 1986; Schultz et al., 1996; Haney et al., 2009), with the major demarcation between the two subspecies near the Hudson River and northern New Jersey. Several authors have taken advantage of this variation to study physiological adaptation (Place and Powers, 1979; Ropson et al., 1990; Powers et al., 1993), but most of the studies documenting variation in reproductive traits have been limited to descriptive differences among populations.

Two aspects of the reproductive biology of *F. heteroclitus* that have been studied less extensively are the spawning periodicity of northern populations and spawning behavior in the wild. Several studies suggest that spawning periodicity may be reduced or absent in northern populations (Wallace and Selman, 1981; Conover and Kynard, 1984; Able, 2002; but see Taylor, 1986). At the same time, there are conflicting reports regarding spawning behavior in this species. Although records of spawning in the field are minimal, the laboratory observations by Newman (1907) and limited field observations from Able and Hata (1984) suggest that pair spawning occurs in this species. However, the relatively high male gonosomatic index (GSI; the percentage of body mass devoted to gonad) observed in all cases where it has been reported (2% to over 4%; Taylor, 1986) combined with

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Submitted: 4 December 2008. Accepted: 18 November 2009. Associate Editor: E. Schultz.

© 2010 by the American Society of Ichthyologists and Herpetologists DOI: 10.1643/CP-08-229

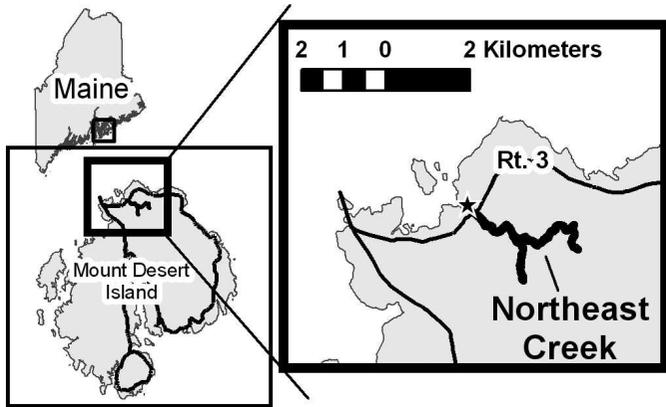


Fig. 1. Location of the study site. The study site is shown within the state of Maine, as well as its location on Mount Desert Island. The star represents the location of the study site on the inset of Northeast Creek.

incidental observations of spawning by Conover and Kynard (1984) suggest that some form of multi-male mating and sperm competition is probably common in this species.

In this paper, we examined the spatial and temporal patterns of spawning in a population of *F. heteroclitus* in a salt marsh in Maine. We estimated spawning activity using several measures, including direct observations of spawning, which also allowed us to give a detailed description of natural spawning in this species. We compare our results with those of other populations in this species, and examine these patterns in light of hypotheses for the adaptive significance of intertidal spawning.

MATERIALS AND METHODS

Fundulus heteroclitus was studied in Northeast Creek (also known as King's Creek, Fig. 1), Mount Desert Island, Maine, USA during the summers of 2003 and 2004. Northeast Creek is a small salt marsh that is crossed by a road approximately 600 m from the mouth of the estuary, and continues for several kilometers where salinity intrusions can be regularly measured (Nielsen, 2002). The main study site was located on the oceanic side of the road, including both a small area (approximately 30 m of shoreline) of rock and cobble area adjacent to the road and bridge as well as the vegetated habitat that makes up the majority of the shoreline in this part of the marsh. In this brackish portion of the marsh, vegetation is dominated by *Spartina alterniflora* in the low to mid intertidal zone and by *Spartina patens* in the upper intertidal zone. The rock cobble habitat at this location is available for fish at all intertidal heights. Fish species in the marsh included American Eel (*Anguilla rostrata*), three species of sticklebacks (Gasterosteiformes), and Atlantic Silverside (*Menidia menidia*), although Mummichogs were clearly the numerically dominant fish species. Mummichogs are found in abundance throughout the estuary in a wide range of salinities, ranging from 1–30 ppt (Fundis et al., 2003). Tides at this site are mixed semidiurnal.

Temporal patterns of spawning.—Temporal patterns of reproductive activity over the season were characterized in two ways, relying on estimates of female batch fecundity as well as through direct observations of spawning in the field. Data were collected after the spawning season had begun in 2003, from 12 June for fecundity estimates, and from 30 June for spawning observations through 31 July. In 2004,

sampling included the entire reproductive season, with fecundity estimates from 10 May to 16 August and spawning observations from 15 May to 13 August. For data on spawning periodicity over the entire season, we restricted our analysis to the more systematically collected and complete data set from 2004.

To estimate batch fecundity, individuals were collected using minnow traps or a seine approximately 1–2 hours before high tide, and 50 mature females (>35 mm standard length) were examined for the presence of mature eggs. Eggs were expressed by gently squeezing the abdomen of the fish, and all mature eggs were counted. Females did not release eggs until we applied pressure to their abdomen. We believe that the number of mature eggs released represented the number of eggs that a female had available for oviposition, although we do not know if all of these eggs would have been released during the subsequent high tide. Reproductive activity from the sample was estimated as either the proportion of females with mature eggs or as the average number of eggs per female. Mature eggs were larger and more transparent than the smaller white immature eggs. Fish were sampled at least three times a week over the spawning season, for a total of 24 fecundity checks in 2003 and 40 in 2004. In addition, once in each year a sample of males was sacrificed to determine male GSI.

To record direct observations of spawning, censuses for spawning activity were made during all tidal periods, but soon focused on the time around high tide, when all spawning was observed. In 2004, two observers placed themselves on either side of the estuary channel and sat above the intertidal, noting any spawning activity they observed in an area up to 3 m wide beneath them. For high-tide observations at night, observers used flashlights and headlamps, to which the fish appeared to quickly acclimate. Every ten minutes, the tidal height at the site was recorded using reference marks drawn on a concrete wall in the channel. Spawns were defined as involving a female and one to several males quivering while in close contact with the bottom. Sex of an individual was determined by coloration (Able, 2002). Virtually all males were identified by the presence of a black ocellus on the first dorsal fin; males also tended to have darker and thicker bars and brighter yellow to green bodies ventrally. Females tended to have no distinct markings and were gray in color. At times, males were observed quivering in a group, but our view of a female was obstructed either by males or rocks; these were recorded as possible spawns. Spawning intensity for a tide was estimated as the Spawning index = Number of spawns + (Number of possible spawns/2). In addition, we periodically estimated the sex ratio of individuals at the spawning site.

To test for semi-lunar and lunar cycles in spawning activity, non-parametric circular statistics were used. Because the data were not collected every day, we first tested the null hypothesis that spawning observations were distributed evenly over the semi-lunar period using a Rayleigh test (Zar, 1999). We then tested for a periodic distribution of spawning intensity among samples by using the Moore modification of the Rayleigh test (Zar, 1999). The null hypothesis of this test is that the net magnitude of spawning during a tidal period is unbiased, so that spawning during all phases of the cycle are equally likely. Rejection of the null indicates a bias in spawning, and if spawning is biased for extreme tides, we expect the largest values to occur during these tides. We tested several potential

Table 1. Relative Number of Spawns Observed with Differing Number of Males for the Entire 2004 Season.

	Number of males per spawn			
	1 ♂	2 ♂	3 ♂	4+ ♂
Total number of spawns	291	292	194	1,193
Percentage of all spawns	14.8	14.8	9.8	60.6

estimates of spawning intensity for semi-lunar and lunar periodicity, including raw data on percent females fecund and number of eggs per female from the batch-fecundity checks, and the estimate of spawning intensity from the spawning observations. We corrected for seasonal patterns of spawning intensity by using the residuals of those variables after fitting linear regressions to both the pre- and post-peak reproductive intensities in 2004. We also tested for spawning periodicity by examining temporal autocorrelation patterns using autocorrelation function (ACF) analysis (Systat v. 11). We investigated lag times from one to 30 days for all measures of spawning intensity, using the same residuals used in the circular statistics.

Although previous reports have stated that semi-lunar spawning periodicity in *F. heteroclitus* occurs at other locations, and many of the figures in previously published papers seem to show semi-lunar or lunar periodicity, these data were never statistically tested using any form of circular statistics or time-series analysis. Taylor et al. (1979) do show that some estimates of reproductive activity are higher around spring tides than at other times, and combined with peaks in fry emergence supported a semi-lunar spawning periodicity. Using the data graphically displayed in Taylor et al. (1979) and Taylor (1986), we tested for semi-lunar spawning periodicity using the same Moore non-parametric circular statistic we used on our own data set. Data were extracted with xyExtract (v. 2.5).

We also used our data set to test for differences in spawning intensity between two consecutive high tides within a 24-hour period to see if either light or height of the high tide influenced spawning intensity between consecutive high tides ($n = 32$ pairs). Within a tide, we examined the relative time of spawning relative to the high tide and the absolute tidal height. If there was strong selection for fishes to spawn only above a certain tidal height, then we expected that spawning would have a shorter duration during less extreme tides, and that spawning would finish at a consistent absolute tidal level.

Spatial patterns of spawning.—We were able to regularly observe spawning in the rock/cobble study area, but it also appeared that fish were actively spawning in the mudflats dominated by *Spartina patens*. To determine the existence and intensity of spawning in this habitat, we took samples of substrate from various tidal heights in both the vegetated and unvegetated study areas and examined them visually unaided and under a dissecting microscope for the presence of *F. heteroclitus* eggs. Samples were taken using circular cores (cross-sectional area either 45 or 144 cm²) that removed the substrate to a depth of approximately 5 cm, although eggs were always found in the top cm. We also examined any emergent vegetation in a core for the presence of eggs.

RESULTS

Spawning behavior.—Fish spawned in the intertidal both within mudflats of *Spartina patens* and in natural and man-made rock and gravel habitat bare of vegetation along the banks of the estuary. However, spawning was difficult to observe in mudflats with *S. patens*, as the fish quickly fled when we approached. In contrast, observers could sit within 2 m of spawning individuals in areas with rock and cobble substrate and observe spawning without obstructions.

Spawning almost always occurred in very shallow water at the shoreline. Individuals were typically observed spawning in less than 5 cm of water, and both males and females were often observed spawning with the dorsal parts of their bodies out of the water. On two occasions during particularly intense spawning bouts, we observed large numbers of fish apparently spawning in approximately 20 cm of water within one meter of the water's edge along the bottom, but in all other occasions spawning occurred in extremely shallow water.

Spawning behavior was similar to that reported for other externally fertilizing teleosts, and similar to descriptions in Newman (1907) and Able and Hata (1984), with the exception that pair spawns were seldom observed. The female would come into the shallow water and push her abdomen down along cobbles or on the side of rocks, and one to several males would come alongside the female and actively quiver while the female appeared to deposit eggs. At the beginning of the season, we confirmed that we were observing successful oviposition by checking locations where spawning had been observed, and always found newly laid eggs. Individual females were seen spawning up to 12 times during a several minute period, suggesting that only a few eggs are released per spawning bout.

Males appeared to engage in intense sperm competition when spawning. Although males sometimes spawned alone with females, it was much more common for multiple males to spawn with a female (Table 1). These data undoubtedly underestimate the degree of sperm competition in spawns, since it did not include the possible spawns that almost always involved several males that obscured the female, and were approximately half as common as definitive spawns. The sex ratio at the water's edge during spawning was heavily male-biased, with estimates ranging from a male:female ratio of 4:1 to 10:1 on different dates. The gonosomatic index of males was quite high (2003: mean GSI = 6.5%, s.d. = 0.2, $n = 52$; 2004: mean GSI = 4.6%, s.d. = 0.1, $n = 20$). Males were similar in size between the two years (two sample t-test, $t = 1.09$, $P = 0.28$; 2003: mean standard length = 46.86, s.d. = 9.5; 2004 mean standard length = 49.74, s.d. = 10.26). There was no significant effect of individual size on GSI, although in both years the trend was negative (ANCOVA, $F_{1,68} = 2.54$, $P = 0.11$). GSI was significantly higher in 2003 than in 2004 (two-sample t-test, $t = 5.32$, $P < 0.001$), possibly because the samples were taken at different days in the calendar year (17 June in 2003, 30 June in 2004).

Spawning occurred over a wide range of salinities (0.2–27.1 ppt) and temperatures (10.6–27.8°C), and we observed spawning at high tides at virtually all times of day. Spawning appeared to be size-assortative, but we did not collect any data on this pattern. Males did show some aggressive behavior, and at times attempted to chase other males away from the shallows, especially when densities were lower at the beginning of the spawning period.

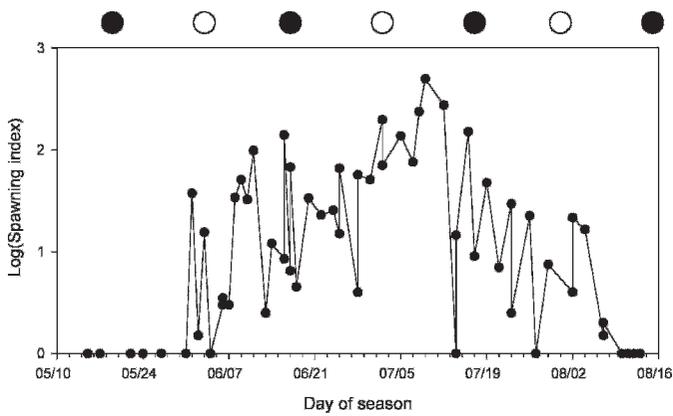


Fig. 2. Temporal pattern of spawning for 2004 (see text for definition of Spawning index). Full and new moons are indicated by open and filled circles, respectively.

Temporal patterns of spawning.—In 2003 spawning was already underway when observations began on 30 June, with the last fecund females found in collections on 23 July and the last spawning observed 28 July. Spawning in 2004 was first observed on 1 June and last observed on 4 August (Fig. 2). Spawning intensity varied seasonally, with a peak of activity in early July for both years. Spawning was observed in the vast majority of high-tide observations (42 of 47) during the spawning season.

There was no pattern of semi-lunar periodicity in the reproductive activity of *F. heteroclitus* in Northeast Creek (Figs. 2, 3). The failure to find any semi-lunar periodicity applied to all three measurements of reproductive activity (spawning intensity, percent females with mature eggs, number of mature eggs per female) and whether the raw data or seasonally corrected residuals were used (Moore tests, $P > 0.5$ for all six comparisons). In addition, there were no significant autocorrelations of approximately 14 days with the corrected data, which would be expected if the population were reproducing on a semi-lunar period (ACF analysis, $P > 0.05$).

Using the same statistical techniques, we did find a significant pattern of lunar periodicity from published reports of reproductive activity from a southern population in Delaware. For this Delaware population (1976 data from Taylor et al., 1979), the percentage of females that were ripe showed significant semi-lunar periodicity (Moore test: $R' =$

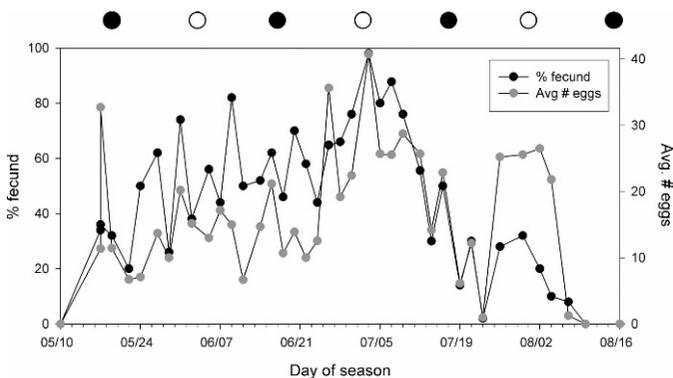


Fig. 3. Temporal patterns of female reproductive readiness for 2004 using either percentage of females collected that released ovulated eggs (black dots and line) or the average number of ovulated eggs per female (gray dots and line).

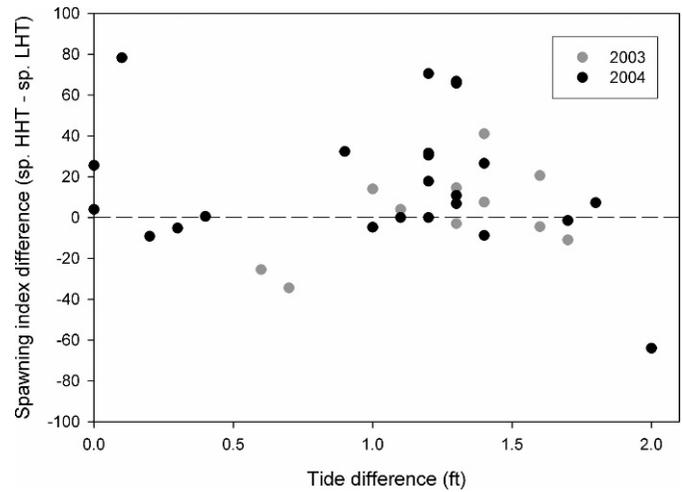


Fig. 4. Comparison of consecutive (within a 24-hour period) high tide observations for 2003 and 2004.

1.12, $P < 0.025$) peaking just before full and new moons, and female GSI showed significant lunar periodicity peaking around full moon (Moore test: $R' = 1.28$, $P < 0.01$). Similarly, data from other years showed a significant periodicity in female GSI (semi-lunar periodicity, $R' = 1.104$, $P < 0.05$; 1978 data from Taylor [1984], lunar periodicity, $R' = 1.18$, $P < 0.025$; 1981 data from Taylor [1986]; both cases using residuals from regression to control for seasonal pattern). These analyses consisted of similar or smaller data sets, so the difference in significance between populations is not due to differences in statistical power between studies. A smaller data set from Waquoit Bay, MA showed a slight but non-significant trend between multiple measures of female spawning activity and semi-lunar periodicity (eggs/0.1 m substrate, $R' = 0.967$, $0.1 > P > 0.05$, Female GSI; $R' = 0.7$, $P > 0.1$; Taylor, 1986:figs. 1, 4, residuals from regression). A second small data set from Sapelo Island, GA had peaks of female GSI near new moon but overall showed no lunar or semilunar spawning periodicity ($P > 0.5$ for all comparisons; Taylor, 1986:fig. 3). Overall, the previously published populations appear to show some peak in reproduction around new or full moon, but varied in the degree that spawning was exclusively tied to the highest spring tides.

Although individuals spawned over an entire range of high tides at our study site, and did not show a preference for the highest high tides over the reproductive season, they did show a preference for the higher of the two high tides within the 24.8 h cycle (Wilcoxon paired signed-rank test: $P = 0.021$, $n = 32$; Fig. 4). However, at this location higher high tides for a day typically occur at night, so the preference for high tides could also be a preference for spawning in the dark. In the four cases where consecutive high tides both occurred during daylight hours, the level of spawning activity did not appear to be related to tidal height (47% of the combined activity occurred during HHT, 53% during LHT). The later high tide of the day tended to have more spawning, with the average percentage of spawning during the afternoon approximately 68%, with approximately 31% in the morning. The two indirect measures of spawning intensity, the percentage of gravid females and the number of ovulated eggs per female leading up to a high tide, were highly correlated with direct observations of spawning (linear regression log spawning intensity vs.

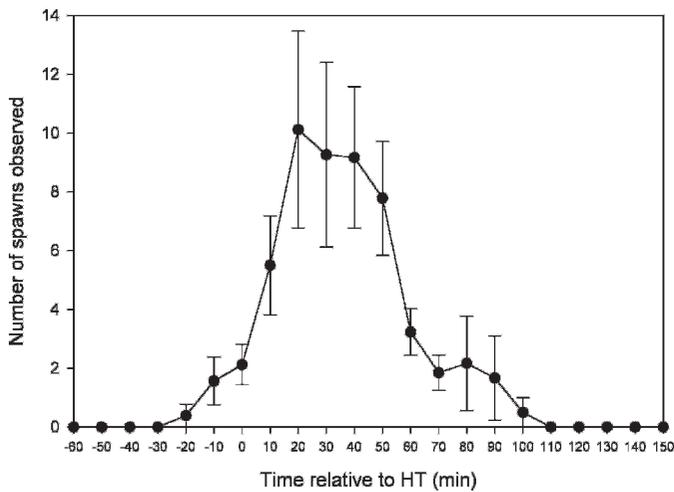


Fig. 5. Spawning intensity versus time relative to high tide. Data include all 2004 observations.

%fecund: $r = 0.81$, $P = 0.001$; log spawning intensity vs. avg. number eggs, $r = 0.77$, $P = 0.003$.

During a tide, virtually all of the spawning occurred once the tide had reached its peak and was receding (Fig. 5). The duration of spawning averaged 48.6 minutes (s.d. = 24.9, $n = 42$). There was no relationship between spawning duration and tidal height (linear regression: $r = 0.001$, $P = 0.868$).

Spatial patterns of spawning.—Spawning was observed over a total tidal range of 1.2 m (approximately 2.9 to 4.1 m [9.6 to 13.3 ft] above mean lower low water [MLLW]), which is the majority of the intertidal zone at this level in the salt marsh. At this location, water input from upstream and a limited outflow kept the water level above approximately 2.65 m [8.7 ft] above MLLW. Eggs were found both in the mud around *S. patens* and under small cobble and rocks in the intertidal zone. Eggs were found singly, not in clumps. Some eggs in the upper intertidal, especially in the cobble/rock area appeared desiccated or infected with a fungus. Within a spawning period, spawning occurred over a vertical range of 1 to 60 cm ($n = 42$). Substrate samples yielded highly variable densities of eggs in both the mudflats of *S. patens* (mean = 0.22 eggs·cm⁻², s.d. = 0.5, range = 1.77, $n = 15$) and in the rock/cobble area (mean = 0.78 eggs·cm⁻², s.d. = 1.21, range = 4.11, $n = 21$).

DISCUSSION

Spawning behavior.—Spawning in *F. heteroclitus* occurs in a relatively narrow time window centered immediately after high tide when the water begins to recede. In *Menidia menidia*, fish spawn in very shallow water during the receding tide, virtually identical to the behavior observed in *F. heteroclitus* in this study. Conover and Kynard (1984) hypothesized that this behavior reduced the time that eggs were vulnerable to predation from *F. heteroclitus*, which is a major egg predator of both *M. menidia* and eggs of its own species. Eggs of *Menidia menidia* appear to be more vulnerable to predation immediately after they are laid; after a few hours eggs become water-hardened and are capable of passing through the digestive tract of Mummichogs intact (Conover, unpubl. data, cited in Conover and Kynard, 1984). We observed Mummichogs eating eggs immediately

after spawning, and our data on timing and location of spawning in *F. heteroclitus* are consistent with the egg-predation hypothesis of Conover and Kynard.

Male *F. heteroclitus* at our site engage in intense sperm competition, with several males often releasing sperm at the same time near the same female. Promiscuous spawning, with multiple males typically spawning with individual females in very shallow water, is very similar to the pattern described for *Menidia menidia* by Conover and Kynard (1984). The pair-spawning behavior recorded by Newman (1907) does occur, but only when fish densities are lower, most often at the beginning of the spawning period. During this period, we also observed males attempting to exclude other males from the shallow spawning sites. However, the majority of spawns we observed were classified as group spawns, with multiple males releasing sperm with a female (Table 1). During periods of intense spawning, we observed very little or no male territorial aggressive behavior. The high densities of individuals in a limited spawning area appear to have led to a highly promiscuous mating system in *F. heteroclitus*, with multiple-male matings as the rule. Thus, the high male GSI values are reflected in a mating system that is characterized by intense sperm competition, as is predicted and has been observed in other fishes (Stockley et al., 1997; Petersen and Warner, 1998). Petersen and Warner (1998) report ranges of up to 4% for species in three families of tropical reef fishes with the highest level of sperm competition, while species with pair spawning and low levels of sperm competition had males with GSI levels always below 0.5%. The high male allocation to testicular tissue in this population (4.6 and 6.5%) is similar to or slightly higher than that reported for other populations of *F. heteroclitus* (2–4%, Taylor et al., 1979; Taylor, 1986), suggesting that sperm competition is common throughout the geographic range of this species.

These spawning behaviors are from individuals spawning on bare gravel and sand. We were not able to observe spawning in the areas dominated by *Spartina patens*, and it is possible that the different structure of the habitat will change the degree of multiple matings and sperm competition. However, the high male GSI in this species suggests that sperm competition is a chronic condition in this and other populations of this species.

There was no evidence that fish responded to salinity or temperature within the spawning season. Spawning occurred over a wide range of physical variables, and previous work has suggested that gametes and embryos are capable of functioning over a wide range of these variables (Palmer and Able, 1987; Able and Palmer, 1988). The dissolved oxygen readings at this site were always close to saturation levels, suggesting that oxygen levels in the water were not a major factor limiting oviposition at this location.

Temporal and spatial patterns of spawning.—*Fundulus heteroclitus* at this northern New England site clearly have a different temporal pattern of spawning, compared with the southern populations studied by Taylor and others (reviewed in Taylor, 1999). The relatively shorter breeding season (approximately two months) is expected given the higher latitude of this population, but the lack of semi-lunar periodicity differs from the typical pattern reported for this species. Other authors have suggested a lack of spawning periodicity in northern populations from southern New England (Wallace and Selman, 1981; Conover and Kynard,

1984), but these patterns were not well documented, and Taylor (1986) observed semi-lunar periodicity in nearby populations. Despite the lack of a preference within a lunar month for extreme spring tides, individuals do appear to prefer the higher of consecutive high tides. There are several potential differences between populations that could influence this spawning pattern. There could be a difference in the magnitude of physical differences between sites, a difference in the biology of the fishes, or a difference that is associated with the more limited spawning season in the northern population.

The main cue for timing in southern populations, the height of the tides, does not seem to differ between the northern and southern population. Tidal variation and the variance in high tides over a lunar cycle do not appear to differ between the northern and southern locations where this species has been studied (Sapelo Island, GA: approx. range [highest high tide to lowest low tide] = 9.2 ft, highest tide level = 8.4 ft; Waquoit Bay, MA: approx. range = 12.7 ft, highest tide level = 11.6 ft). Although there are differences in the species composition of the salt marshes that these species spawn in, all data were collected from salt marshes along the Atlantic coast of the United States, where *Spartina* species dominate as the ground cover within the marsh.

One potential problem with comparing results from different studies is that the methodologies specific to a study or site might have biases that produce a false pattern of differences among studies. This was the first study to directly measure reproductive activity in the field for *F. heteroclitus* by observing spawning. However, there was strong covariance between spawning intensity and the estimates of reproductive activity using proportion of ripe females collected prior to high tide, which is a common method used in other studies. This suggests that the documentation of spawning for other populations that has included changes in adult anatomy and physiology over the spawning season (Taylor et al., 1979; Taylor and DiMichele, 1980; Taylor, 1984; Cochran et al., 1988; Hsiao et al., 1996) and temporal patterns of egg deposition in the field (Taylor et al., 1977) are probably both accurate and comparable among populations, and that differences among populations are probably not due to any methodological differences among studies.

There are three additional reasons that might help explain this change in spawning periodicity: that the dynamics of egg survivorship versus tidal height change with latitude in this species, that the restricted spawning season selects against spawning periodicity, or that higher productivity and adult energy intake reduces the temporal selectivity of spawning in more northern populations. These potential explanations are explored below.

Is there differential embryo survivorship for intertidal spawners?—Spawning during high spring tides has been hypothesized to be adaptive in *F. heteroclitus*, possibly to reduce predation on eggs or to keep eggs out of anoxic habitats in the lower intertidal and subtidal (Taylor and DiMichele, 1983). However, in the Taylor and DiMichele study, eggs had equally high survivorship from the highest high tide down to the point where eggs were inundated at least once every day (the lowest higher high tide level). Given the shallow spawning of this species, this lower location would be available as a spawning location on a daily basis. Thus, previous experimental results do not demonstrate an egg

survivorship advantage for limiting spawning only during spring tides on the highest high tides.

Development rate of embryos in the intertidal can be accelerated by both the higher oxygen content of air versus water and the typically higher air temperatures in the intertidal compared to adjacent ocean temperatures during the reproductive season (DeMartini, 1999; Strathmann and Hess, 1999). Several recent studies have shown that development rate can be faster in the intertidal as opposed to embryo development in adjacent subtidal habitat (MacDonald et al., 1995; Yamahira, 1996). It is also possible that more frequent immersion of eggs laid lower in the intertidal may increase the risk of predation from aquatic predators, as first suggested for *Hypomesus pretiosus* by Loosanoff (1937). Tewksbury and Conover (1987) concluded that increasing egg survivorship in the higher intertidal of experimentally placed embryos of *Menidia menidia* was probably due to increased embryo predation by *Fundulus* at lower elevations. However, one study has found opposing gradients in embryo survivorship. Yamahira (1996) found that although eggs of *Takifugu niphobles* developed faster in the upper intertidal, they had increased mortality due to desiccation, and suggested that embryos would have higher survivorship if laid in the lower intertidal.

The best approach to untangle these potential costs and benefits is the experimental approach taken by Tewksbury and Conover (1987), who outplanted embryos at various tidal heights in caged and uncaged containers. Integrating this kind of experimental design across habitat types (rock/cobble and *Spartina* at this site) over the spawning season should help to determine both the relative costs and benefits and the range of processes affecting egg development and survival.

Could a reduced reproductive season select against spawning periodicity?—If there were times when spawning gave higher embryo fitness, we would expect organisms to be selected to pick those times to spawn. However, the cost of not spawning over the intervening time period may be a reduction in total seasonal fecundity. In a population with a short spawning period, where all of the year's reproductive effort is released over a short time, short peaks of a few semi-lunar spawning periods might severely limit seasonal reproductive effort if clutch size is limited by anatomical or physiological constraints. If these costs are not outweighed by the benefits of enhanced survivorship of eggs deposited at particular times (and places), we might expect them to spawn at some rate during the intervening period. The higher levels of spawning at the higher of two consecutive high tides (Fig. 4) suggest that within a day, individuals prefer to spawn on higher high tides, but the lack of spawning peaks during spring tides (Figs. 2, 3) suggests that individuals do not take longer breaks in spawning during the short spawning season.

This hypothesis assumes that the lack of lunar periodicity in this population is part of a larger clinal pattern of less spawning periodicity in more northern populations with reduced spawning seasons. Although anecdotal data from other studies (Wallace and Selman, 1981; Conover and Kynard, 1984) support this idea, it is important to verify this pattern with quantitative data from other northern populations.

Taylor (1986) noted that spawning appeared to be more continuous and less cyclic in the early part of the spawning

season, and hypothesized that this might explain the difference among the Georgia, Massachusetts, and Delaware populations, which were sampled at different points in their spawning season. The even shorter spawning season in our study population may resemble the less cyclic pattern of early spawning in other populations, when females have large amounts of energy devoted to egg production and waiting to spawn at the next high tide would cause significant reductions in seasonal egg output. The temporal selectivity of spawning would also be reduced if individuals had increased energy available to reproduction due to higher productivity in the environment or higher energy intake or energetic conversion rates. Northern populations have a higher growth capacity (Schultz et al., 1996). This combination of factors might lead to northern populations having more eggs to release per time for their reproductive season. It would be interesting to compare spawning seasons and total fecundity of fishes from different latitudes. A higher rate of egg release for more northern populations and an inverse correlation between the rate of egg release and the degree of spawning periodicity would support this hypothesis. This hypothesis also predicts that if the energy intake of fishes were increased, that individuals would spawn more frequently and the pattern of semi-lunar spawning would diminish.

Intraspecific variation in timing of spawning has been found in several species, including fishes that switch from lunar or semi-lunar spawning to acyclic spawning at sites with a shorter reproductive season. Both *Abudefduf troschelii* and *Ophioblennius steindachneri* have lunar spawning cycles in Pacific Panama, but lose this lunar periodicity in more seasonal populations in the Gulf of California (Robertson et al., 1990). On the other hand, a sympatric intertidal spawner, the Atlantic Silverside *Menidia menidia*, appears to maintain its semi-lunar spawning periodicity throughout its range, which is similar to the range and habitat used by *F. heteroclitus*. A more systematic examination of clinal variation among widely dispersed species could determine the generality of any latitudinal patterns of spawning periodicity.

The intraspecific variation in spawning periodicity reported here adds to the already rich literature on intraspecific variation in *F. heteroclitus* in several other aspects of its biology. As a wide-ranging species with potentially limited dispersal, *F. heteroclitus* offers the continued possibility to study reproductive characteristics of natural populations where there is an extensive literature on the species physiology, clinal variation, and local adaptation.

ACKNOWLEDGMENTS

We thank R. Anderson, J. Baldwin, Y. Brandvain, J. Childers, K. Kocot, J. Marty, R. Petersen, and N. Therkildsen for assistance in the field. G. Longworth made the map shown in Figure 1. H. Hess made helpful comments that substantially improved the manuscript. This work was supported by NSF grant C-RUI 011860. All animal use was approved by the Mount Desert Island Biological Laboratory Animal Care Committee.

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