

ARTICLE

## Diel Reproductive Periodicity of Haddock in the Southwestern Gulf of Maine

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### Abstract

We studied a population of Haddock *Melanogrammus aeglefinus* in the Gulf of Maine to determine whether it exhibits diel spawning periodicity. Commercial fishing vessels were chartered for 25 dedicated long-lining trips to collect sexually mature Haddock in the Stellwagen Bank National Marine Sanctuary at locations identified by commercial fishers as having spawning aggregations. In order to examine diel effects on Haddock reproduction, the changes in CPUE and percentages of male and female Haddock of all reproductive maturity stages, as well as gonadosomatic index (GSI), were observed across a 24-h diel cycle. The comparison of diel patterns among different maturation stages was used to differentiate diel periodicity due to spawning from feeding behavior. Diel periodicity was observed for the proportion of males and females in the catch, the proportion and CPUE of male and female maturation stages most indicative of imminent spawning, and for female GSI. Although spawning occurred throughout the diel cycle, peak spawning of females occurred during the evening hours (1700–2100 hours) and gradually declined to low values during the day at 0900 hours. In an apparent contradiction male spawning stages were uniformly high except for lows during the night between 2100 and 0100 hours. We hypothesize that this pattern is consistent with lekking behavior where strong competition among spawning-ready males during courtship would reduce their feeding activity during the peak spawning hours. Strong annual differences in diel periodicity may have resulted from different temporal patterns in the spawning season or density dependence, or both.

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Diel spawning periodicity has been widely studied in marine fishes from tropical (e.g., Kohda 1988; Robertson 1991; Kokita and Nakazono 2000; Shibata and Kohda 2007) and temperate climates (e.g., Simpson 1971; Ferraro 1980; Walsh and Johnstone 1992; Yamaguchi et al. 2006). Studies on diel reproductive periodicity provide details on the chronology of species reproductive processes and interest is largely focused around why species have evolved to spawn at particular periods in the day. Species have been documented to primarily spawn during certain periods or specific hours (e.g., 1300–1830 hours, Red Snapper *Lutjanus campechanus*: Jackson 2006) within a day.

Diel spawning periodicity can maximize fish survival and reproductive success for reasons that include predator avoidance for the adult or embryos (Ferraro 1980), avoidance of harmful sunlight on the embryo (Ferraro 1980), control of larval dispersal (Ferraro 1980), reproductive synchrony between sexes (Ferraro 1980), and increased reproductive isolation and improved feeding efficiency (Shibata and Kohda 2007).

Diel reproductive periodicity has been examined by conducting ichthyoplankton surveys (Gamulin and Hure 1956; Ferraro 1980; Wakefield 2010), observing spawning fish in the laboratory (Marshall 1967; Sherrill and Middaugh 1993) or in their

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natural habitat (Conover and Kynard 1984; Pennings 1991), and by examining changes in ovarian development and the gonadosomatic index (GSI) of mature female fish at different times of day (McBride et al. 2002; Yamaguchi et al. 2006). An ichthyoplankton survey in which fish eggs are collected and aged is the most common method used to determine an approximate time of day when the eggs were spawned. Some advantages to this method are being able to observe species-specific patterns in annual, seasonal, and spatial egg abundance, as well as being able to estimate the chronology of egg development through successive stages (Wakefield 2010). The advantages to directly observing fish spawning in laboratories is having the ability to observe the fish continuously over 24-h periods as well as controlling and manipulating the environment (e.g., control photoperiod: Robinson 1943; Marshall 1967; Campos-Mendoza et al. 2004). However, there is some evidence that endogenous cycles can fail to persist when fish are removed from their natural habitat due to changes in food availability or light intensity, the lack of tidal cues, or the disturbance by human activity (Conover and Kynard 1984). Therefore, one advantage to observing fish in their natural environment is that the likelihood of influencing endogenous cycles is decreased. However, observing fish in their natural habitat becomes increasingly difficult as depth and distance from shore are increased. Direct observation of fish behavior in both laboratory and field conditions is usually not possible in the dark without the aid of artificial lights, which can disrupt natural behavior, and infrared lights are ineffective at distances over 1 m (e.g., Rountree and Juanes 2010). Instead, examining ovary development and respective changes in the average GSI can provide a practical alternative to investigate whether a fish exhibits diel reproductive periodicity. In addition to diel fluctuations in average GSI, diel fluctuation in proportion of females with ovaries containing postovulatory follicles (POFs) and the aging of POFs to determine when eggs were previously spawned have been used to determine whether a species exhibits diel spawning periodicity (McBride et al. 2002; Yamaguchi et al. 2006; Anderson 2011; Burchard et al. 2013). However, one disadvantage of this method is that it requires sacrificing the fish, although that also provides the opportunity to collect additional data that otherwise could not be collected (e.g., fish condition, stomach content).

Three species in the family Gadidae have been reported to primarily spawn during specific times of the day (Ferraro 1980). Atlantic Cod *Gadus morhua* has been reported to primarily spawn during evening and night hours (appearance of eggs in a tank of captive cod: Meek 1911; observation of spawning cod in captivity: Brawn 1961). Fourbeard Rockling *Enchelyopus cimbrius* (eggs obtained in plankton tows: Battle 1930) and Whiting *Merlangius merlangus* (appearance of eggs in tanks of captive Whiting: Storrow 1913) have been reported to primarily spawn in the morning. There is a lack of studies on these and additional commercially important species in the Gulf of Maine.

Haddock *Melanogrammus aeglefinus* (Gadidae) inhabits both sides of the North Atlantic Ocean and is a highly val-

ued commercial species (Clark et al. 1982; Collette and Klein-MacPhee 2002; Schultz 2004:97). It is a demersal fish most common at depths of 45–135 m (Brown 1998). Many studies have been conducted on various aspects of their reproductive biology: spawning behavior (Casaretto and Hawkins 2002), maturation (Templeman et al. 1978; O'Brien et al. 1993; Burchard et al. 2013), and fecundity (Raitt 1933; Alekseyeva and Tormosova 1979; Robb 1982; Clay 1989; Alonso-Fernández et al. 2009). Haddock is a group-synchronous, determinate, batch spawner (Murua and Saborido-Rey 2003) and spawns between January and June, with peak activity occurring during late March and early April (Overholtz 1987; Brown 1998). To our knowledge, diel periodicity has not been directly studied in this species. However, Hawkins et al. (1967) reported anecdotal observations of one female Haddock that paired with three males spawning in captivity at various times of the day and night. Although these observations indicate that spawning can take place at any time of the day within a single female, nighttime observations were limited, and the influence of long-term captivity on spawning behavior is unknown.

Data on the timing of spawning can increase our understanding of biological success including early life history and recruitment variability of species (Jackson et al. 2006). The knowledge of diel reproductive periodicity in Haddock can be used in conjunction with information on annual variation in timing of sexual maturity and spawning frequency to assess annual and lifetime variability in reproductive timing and success among and within populations. Knowledge of the reproductive variability and the spatial and temporal elements and behavior that contribute to the variability will help in the assessment and management of these populations by improving estimation of parameters such as recruitment, spawning stock biomass, and fecundity used in stock assessment models (Lowerre-Barbieri et al. 2011). Therefore, the objective of this study was to determine whether a natural population of Haddock in the Gulf of Maine exhibits diel reproductive periodicity in the wild by observing the incidences of male and female Haddock of all reproductive maturity stages throughout the diel cycle.

## METHODS

Commercial fishing vessels were chartered for 25 dedicated survey trips in the spring of 2006 ( $n = 15$ ) and 2007 ( $n = 10$ ) to collect biological samples of Haddock in the southwestern Gulf of Maine (see Burchard et al. 2013 for a detailed sampling description). Sampling was conducted primarily within the Stellwagen Bank National Marine Sanctuary (SBNMS), although a few sites were sampled in immediately adjacent areas along the sanctuary border. Because the goal was to examine diel periodicity in spawning behavior, sampling targeted adult Haddock during the peak March to May spawning season in the Gulf of Maine (Brown 1998). Haddock were collected using longlines because they are known to be selective for adult Haddock, thus limiting capture of unwanted immature fish (Halliday 2002). In

TABLE 1. Sample sizes for data used in the ANOVA test of year, season, time of day, and interactions effects on Haddock catch (see Tables 4, 5). Sets = number of longline hauls (total  $N_{sets} = 51$ ), fish = number of fish staged (total  $N_{fish} = 1,214$ ), dates are given as month/day.

Dates sampled	Season	Time of day												Row totals	
		0100—0500		0500—0900		0900—1300		1300—1700		1700—2100		2100—0100			
		Sets	Fish	Sets	Fish	Sets	Fish	Sets	Fish	Sets	Fish	Sets	Fish	Sets	Fish
<b>2006</b>															
3/21, 3/28, 3/31	Early	1	25	1	31	2	15	1	11	2	56	2	86	9	224
4/28, 4/30, 5/4	Middle	2	47	1	28	1	16	1	39	1	24	1	10	7	164
5/8, 5/16, 5/24	Late	1	15	2	46	2	49	2	27	1	26	1	28	9	191
<b>2007</b>															
3/26, 3/31, 4/10	Early	1	32	1	6	2	26	2	55	1	4	1	12	8	135
4/21, 4/24, 4/25, 5/1	Middle	2	43	1	44	1	27	1	39	2	39	2	44	9	236
5/22, 5/24, 5/30	Late	2	86	2	55	2	52	1	24	1	14	1	33	9	264
Column totals		9	248	8	210	10	185	8	195	8	163	8	213	51	1,214

this study the smallest Haddock caught was 35.5 cm FL, which is larger than the mean length at maturity recorded for this species in the Gulf of Maine (34.5 cm according to Collette and Klein-MacPhee 2002). All longlines set were baited with squid.

To examine diel effects on catch rates and reproductive maturity, longline sets were conducted within specific 4-h time bins (0100–0500 hours, 0500–0900 hours, 0900–1300 hours, 1300–1700 hours, 1700–2100 hours, 2100–0100 hours eastern standard time). For convenience, time bins will be referred to by their starting time (e.g., 0100–0500 hours as 0100 hours) in all figures. However, due to vessel limitations, sampling was limited to 12 h (three consecutive time bins) for any given trip, so it was necessary to pool trips within quasi-season spawning periods: early (late March), middle (late April–early May), late (mid-May) to obtain a full 24-h sequence of samples (Table 1). For each set, 92 m of bottom longlines were fished with 150–400 circle hooks set 2 m apart. The number of hooks fished per line on each trip was dependent on the success of catching Haddock that day. With the intent of sampling at least 50 Haddock from each set, the number of hooks would increase if the sample size was not reached, or decreased if more than needed were caught. The longline sets were fished for 1–3 h (mean = 2 h 19 min). A total of 1,706 Haddock were caught from a total of 58 longline sets (mean = 29 fish per set; range, 4–111). However, only data from 51 longline sets were used in analysis (Table 1) because some trips were too temporally isolated to be reasonably included in one of the quasi-season periods (February and June).

All Haddock caught were measured (FL to nearest 1 mm). Additionally, light pressure was applied to the ventral surface of each fish to check for signs of a ripe and running (RR) condition (extrusion of milt or eggs). The first 50 Haddock in each set were sacrificed to determine sex and the maturation

stage of the gonads ( $N = 579$  and  $N = 635$  in 2006 and 2007, respectively; Table 1). A subsample of the sacrificed female Haddock representing all reproductive stages from each longline set was then labeled and reserved on ice. Fish from each of the following length bins were collected from each set, if possible, to have representation from as many cohorts as possible: 30–40 cm, 40–50 cm, 50–60 cm, and >60 cm.

The macroscopic field-staging methodology included histological validation and is described separately by Burchard et al. (2013). In brief, males were assigned standard maturation stages based on the Northeast Fisheries Science Center maturity classification scheme (Burnett et al. 1989; O’Brien et al. 1993), while a modified staging index designed for the examination of diel periodicity and other short-term temporal patterns in temperate climate fishes was used for the females (Burchard et al. 2013). This female (F) maturation index includes three sequential stages representing the early to late progression of final oocyte maturation (OM) of sexually mature ovaries based on the percentage of hydrated oocytes present during gross assessment of the ovary: (1) hydration stage 1 (F\_H1), when less than 25% of the ovary’s visible surface contains translucent, hydrated oocytes; (2) hydration stage 2 (F\_H2), when 25–50% of the ovary’s visible surface contains translucent, hydrated oocytes; (3) hydration stage 3 (F\_H3), when 50–75% of the visible surface of the ovary contains translucent, hydrated oocytes (Burchard et al. 2013). When a female reaches hydration stage 3 (F\_H3) most to all of the oocytes in the next batch of eggs to be spawned have completed OM. Because F\_H3 is found to be the maturity stage closest to actual spawning it is considered the best indicator of imminent spawning, and hence, most ideal for determining spawning diel periodicity (Burchard et al. 2013). Burchard et al. (2013) also concluded that POFs should not be

TABLE 2. Variables examined for diel periodicity for both CPUE and percent of catch of Haddock. Technical specifications for reproductive stages are described in Burchard et al. (2013).

Abbreviation	Variables tested
F_total	Female total: CPUE or proportion of the catch in a set that are females.
F_active	Female spawning active: combined CPUE or proportion of females in a set from stages F_D, F_H1, F_H2, and F_H3.
F_inactive	Female spawning inactive: combined CPUE or proportion of females in a set from stages F_I, F_S, and F_RE.
F_I	Female immature: CPUE or proportion of females in a set that are immature.
F_D	Female developed: CPUE or proportion of females in a set that are developed.
F_H1	Female hydration stage 1: CPUE or proportion of females in a set that are in the F_H1 stage.
F_H2	Female hydration stage 2: CPUE or proportion of females in a set that are in the F_H2 stage.
F_H3	Female hydration stage 3: CPUE or proportion of females in a set that are in the F_H3 stage.
F_S	Female spent: CPUE or proportion of females in a set that are spent.
F_RE	Female resting: CPUE or proportion of females in a set that are resting.
M_total	Male total: CPUE or proportion of the catch in a set that are male.
M_active	Male spawning active: combined CPUE or proportion of males in a set from stages M_D, M_R, and M_RR.
M_inactive	Male spawning inactive: combined CPUE or proportion of males in a set from stages M_I, M_S, and M_RE.
M_I	Male immature: CPUE or proportion of males in set that are immature.
M_D	Male developed: CPUE or proportion of males in a set that are developed.
M_R	Male ripe: CPUE or proportion of males in set that are ripe.
M_RR	Male ripe and running: CPUE or proportion of males in a set that are ripe and running.
M_S	Male spent: CPUE or proportion of males in a set that are spent.
M_RE	Male resting: CPUE or proportion of males in a set that are resting.

used to determine spawning periodicity in Haddock because the POFs age too slowly. For males (M) the ripe and running stage (M\_RR) is assumed to be the best indicator of spawning time. However, analysis of diel patterns for both spawning-active and nonspawning-active maturation stages (Table 2) was conducted in order to distinguish between spawning behavior and feeding behavior due to the potential behavioral bias inherent in baited longline sampling.

To further quantify a time of day effect on maturation, in 2007 we measured gonadosomatic index (GSI) in a subsample of 118 mature female Haddock as an index of diel periodicity in spawning following the methods of McBride et al. (2002). Samples of spawning-capable (actively spawning) mature females (F\_active, composed of reproductive stages: developed females [F\_D], F\_H1, F\_H2, and F\_H3; Table 2), were labeled and preserved on ice to be processed in the laboratory to determine GSI. Sampled fish were processed in the laboratory within 24 h of the end of each trip. Total weight (to nearest 0.1 kg) and ovary weight (to nearest 0.01 kg) of each individual were recorded. Female GSI was calculated using the formula:  $GSI = \text{gonad weight} / (\text{body weight} - \text{gonad weight}) \times 100$  (McBride et al. 2002).

The number of fish within each sex and maturation stage were enumerated for each longline set and then standardized in both percent and CPUE. To estimate the proportion of the local population in each maturation stage at any given time, percent catch was constructed as a ratio of fish caught for each maturity stage according to sex to the total catch for each set by sex (e.g., number of developed females caught in one set / total number of all female fish caught in the same set; i.e., F\_D /

F\_total). Additionally, we calculated percentages of spawning-ready fish by sex for F\_D, F\_H1, F\_H2, and F\_H3 for females and developed males (M\_D), ripe males (M\_R), and M\_RR for males, but do not report the results because they were redundant. The proportion of the total catch made up of males or females (percent M\_total and F\_total) was used to examine diel trends in the sex ratio. The CPUE metric was constructed for each maturity stage by sex for each set by dividing the number caught in the set by the number of hooks and duration of the set (e.g., number of developed females caught / number of hooks set  $\times$  soak duration). An arcsine-square-root transformation was used to normalize the percent data and a natural logarithm transformation [ $\ln(\text{CPUE} + 1)$ ] was used to normalize CPUE data. All references to percent and CPUE herein refer to the transformed variables.

A multifactor nested ANOVA programmed in SAS was used to examine effects of time of day on catch and maturation data. The ANOVA was performed on percent and CPUE data for all reproductive maturity stages sampled, as well as for the total males and females in spawning active and inactive stages (Table 2). Percent variables based on the proportion of the catch were assumed to be a better estimator of local population trends as they are less influenced by variability in catch size. However, CPUE variables provide information on diel periodicity in catchability and, hence, a comparison of CPUE and percent variable trends can provide better insight into behavioral patterns than either index alone can. The tests for percent males (M\_total) and females (F\_total) are identical because they are, by definition, reciprocal values with equal variances. Standard

Pearson's correlations (Quinn and Keough 2002) between all variables were computed.

The ANOVA model included main effects and their interactions of year, season nested within year, and time of day. The season-nested-within-year mean squares was used as the error term in the test for the year main effect, while the season-by-time-of-day-interaction mean squares was used as the error term for the test of the time-of-day main effect. The statistical design examined diel changes within the quasi-season and therefore partially accounted for unknown seasonal sources of variation. The quasi-season factor was nested within year because its levels (early, middle, and late) were not comparable between years due to annual differences in the timing of oceanographic and environmental seasons. For the purposes of this study we were not interested in the year or season main effects as the study was not designed to examine seasonal or annual variation in spawning. Although there was no intuitive reason to suspect that diel periodicity in spawning would be different among seasons or years, the ANOVA tests for year and season interactions with time of day were important, because they test that hypothesis. In cases where variables exhibited significant time-by-year or time-by-season interactions, least-squares means (lsmeans) tests of simple effects (Winer 1971) were used to examine time differences between years or among seasons. The main null hypothesis therefore is  $H_{01}$ : there are no time-of-day differences in the maturation stages. The secondary null hypothesis is  $H_{02}$ : there are no differences in the diel patterns for each maturation stage among seasons or years.

The results of the ANOVA main time effect together with several different a priori means contrast tests can be used to distinguish among the various possible diel temporal patterns (Figure 1; Table 3). These include an a priori "diel" contrast test defined herein and standard a priori polynomial contrasts typically used to determine whether response variables exhibit ordered structure (Quinn and Keough 2002). The diel contrast test was

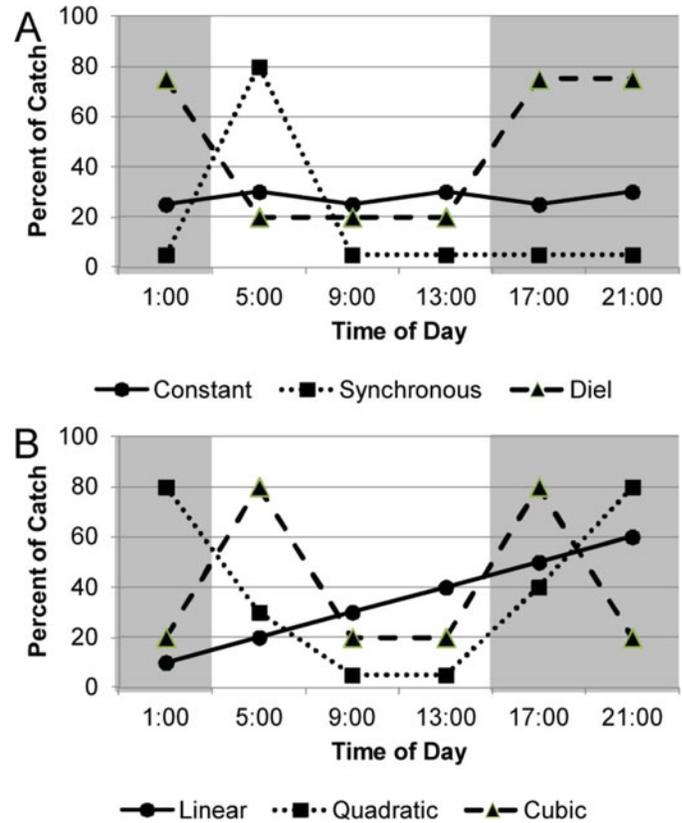


FIGURE 1. Comparison of expected hypothetical diel periodicity pattern types for Haddock (see Table 3 for definitions): (A) constant, synchronous, and diel; (B) linear, quadratic, cubic.

constructed by grouping the time bins 0500–0900 hours, 0900–1300 hours, and 1300–1700 hours into a day period and time bins 0100–0500 hours, 1700–2100 hours, and 2100–0100 hours into a night period for comparison. Constant (i.e., no pattern) and synchronous diel pattern types are relatively straightforward

TABLE 3. Definition of hypothetical diel spawning pattern types of Haddock and expected diagnostic statistical test.

Type of diel pattern	Description	Diagnostic test
None (constant or random)	Spawning is at equal levels throughout the day, or random, which would result in a constant pattern when data are pooled over long-term observations.	No significant time effects
Synchronous	All spawning occurs at approximately the same time of day.	Significant time main effect, no significant contrasts
Diel	Spawning occurs at different levels between day and night, but is constant within day or night (e.g., nocturnal or diurnal spawning).	Significant diel contrast
Linear	Spawning increases linearly from a low point to a high point crossing the diel boundaries.	Significant linear contrast
Quadratic	Spawning waxes and wanes gradually throughout the diel cycle, resulting in either a convex or concave response curve.	Significant quadratic contrast
Cubic	Spawning waxes and wanes gradually twice during the diel cycle (e.g., crepuscular spawning).	Significant cubic contrast

to diagnose (Table 3). A lack of diel periodicity (pattern type = none; Table 3) in spawning would result from either constant spawning through the diel cycle or random spawning, which itself would manifest as constant spawning over large data sets (Figure 1; Table 3). The lack of a diel cycle pattern would be indicated by a lack of significance of the time main effect and all of the means contrast tests, together with a flat response pattern in the data. A pattern where spawning is synchronized or otherwise occurs only at one time during the diel cycle would be indicated by a significant time main effect in the ANOVA, but have a lack of significance in any of the contrast tests (note that the contrast tests are more sensitive than the time main effect test).

The remaining four diel pattern types (diel, linear, quadratic, and cubic) can exhibit significant levels in multiple contrast tests and require inspection of the response trend to infer the most likely pattern, and in fact, may not always be mutually exclusive. A diel spawning pattern is when spawning occurs at different levels between day and night periods, but at constant levels within each period (Figure 1A; Table 3). Spawning at a constant level throughout the night and lack of spawning during the day (i.e., nocturnal spawning) would be an extreme example of this type of pattern. In this case we would expect a significant diel contrast effect, but the time-of-day main effect in the ANOVA may or may not be significant since the diel contrast is more sensitive. Although up to fifth-order polynomial contrasts are technically possible, we chose to examine only the linear, quadratic, and cubic polynomials that represent the most likely polynomial diel patterns (Figure 1B; Table 3). In cases where more than one polynomial contrast tests were significant, inspection of the data was used to determine the best-fitting diel pattern. It is also important to understand that since time of day is actually a circular statistic, different polynomial patterns can be obtained simply by sliding the starting time along the  $x$ -axis. This effect must be considered when inferring the diel pattern type based on a comparison of the statistical test results and inspection of the response pattern.

Since GSI data were only collected on a small subsample of females in 2007 ( $N = 118$ ), a single-factor ANOVA was used to test for time-of-day effects on mean GSI of mature fish. As with percent frequency and CPUE data, a priori polynomial contrasts and the a priori diel contrast were performed to determine the diel pattern type. A square-root transformation was used to normalize GSI data.

We recognize that there are many factors that produce confounding effects in any field sampling design, including spatial factors like location, depth, and environmental conditions, and temporal factors like lunar, tidal, and other sidereal factors such as time of sunset and sunrise. Tidal factors alone include many possible tidal constituents as well as tidal currents. Short-term direct and indirect effects can arise from lunar tides (changes in tide heights and currents associated with moon phase) or from changes in the amount of moonlight during the night hours. For our purposes we were only interested in factors that may

have affected the diel spawning periodicity. Unfortunately because lunar stage is completely confounded with season (i.e., pooling within lunar stages requires pooling across months and the quasi-seasons used herein), it was not possible to formally test for lunar effects. Further, lunar effects were partially controlled because all sampling was conducted on intermediate gibbous and crescent moon phases. No sampling occurred during either the full or new moons. However, we qualitatively examined the data by comparing trends among moon phases and by recoding the data to examine moonlight effects on the diel cycle. Means pooled into day samples were compared with night means broken out into night samples with high moonlight (gibbous moons), those with low light (crescent moons), and moonless "dark" night samples based on the time of moonrise and moonset relative to the actual sampling time. No statistical tests were performed because the proper error term could not be estimated.

Because tide stage is known to influence feeding and spawning behavior in many fishes and is also known to interact with the diel feeding cycle in fishes (e.g., see reviews in Rountree and Able 2007), a consideration of tide stage interaction with diel periodicity in spawning was attempted on a subsample of the data. Tides in the study area are semidiurnal (Chen et al. 2011) and the tidal cycle is independent of and statistically confounded with the diel cycle. It was not possible to partition the data to sample all four tide stages (high, ebb, low, and flood) for all six time-of-day periods even by pooling all data within each year (i.e., ignoring season). However, we were able to test tide and diel interactions in a reduced model three-way ANOVA performed separately for each year. The model contained season, diel, and tide effects where the diel factor was composed of time bins pooled into day and night periods ( $N_{\text{day}} = 13$  and  $13$  and  $N_{\text{night}} = 12$  and  $13$  sets for 2006 and 2007, respectively) and four tidal stages ( $N_{\text{high}} = 6$  and  $5$ ,  $N_{\text{low}} = 5$  and  $4$ ,  $N_{\text{ebb}} = 9$  and  $9$ , and  $N_{\text{flood}} = 5$  and  $8$  sets for 2006 and 2007, respectively).

## RESULTS

### Sex Ratio and Catch by Sex

The sex ratio represented by the proportion of the catch for each sex (percent M<sub>total</sub> and F<sub>total</sub>) exhibited strong diel periodicity and had both a significant time main effect and quadratic contrast (Table 4; Figure 2A). For all data combined, nearly equal proportions of males and females (i.e., a sex ratio  $\approx 1$ ) were captured at night, but catches were highly skewed towards males during the day (Figure 2A). However, because there was a highly significant time-by-year interaction in the ANOVA ( $P = 0.009$ ; Table 4), it was necessary to examine the time trends separately by year (Figure 2C, E). Catches were strongly skewed towards males during the day and females at night during 2006 (Ismeans:  $P = 0.0005$ ), but not during 2007 when males dominated over most of the diel period (Figure 2C, E).

While the total catch of Haddock lacked any significant time effects, the CPUE of females (F<sub>total</sub>) exhibited a significant

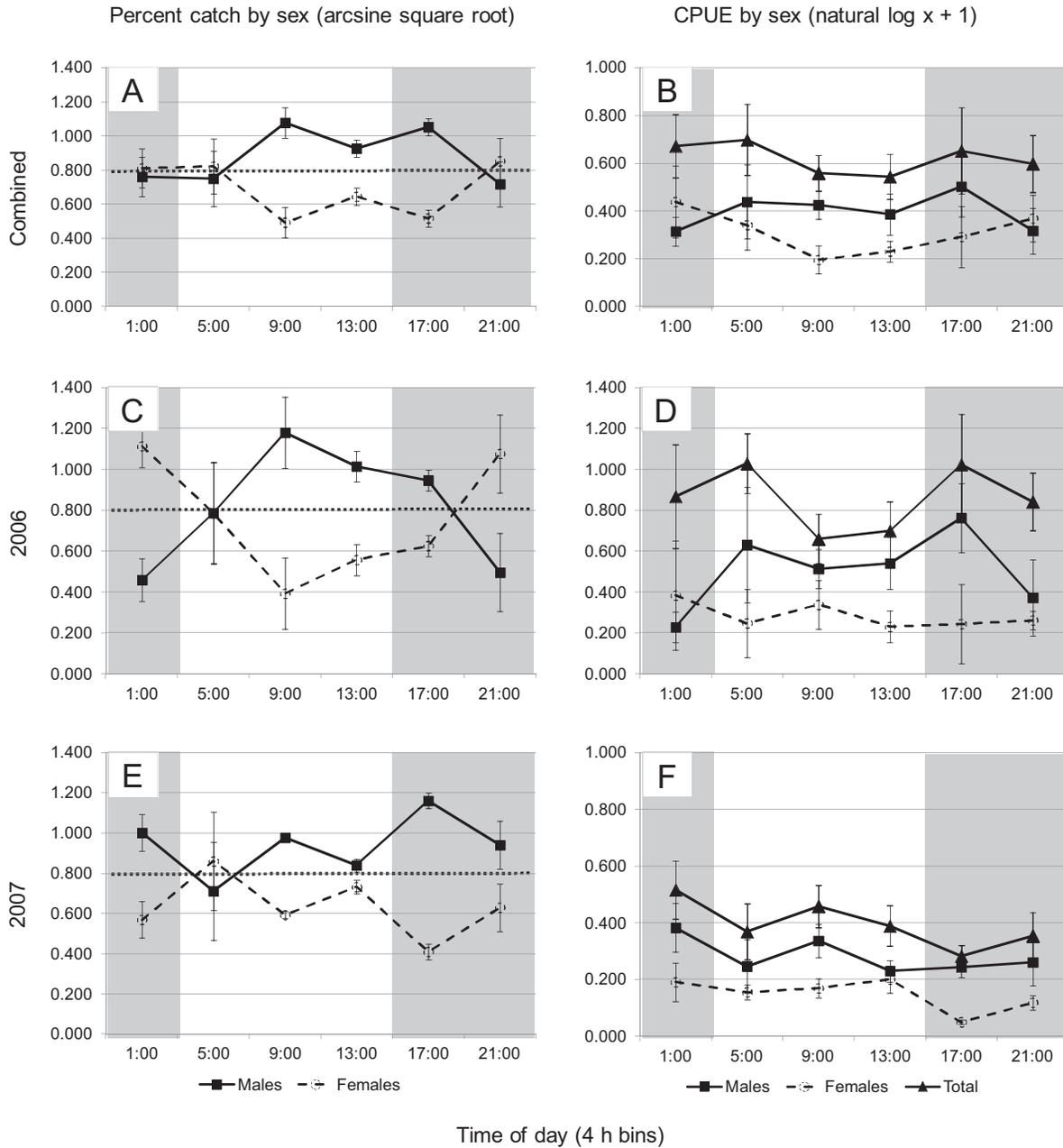


FIGURE 2. Observed diel patterns in catch sex composition of Haddock: (A, B) pooled years; (C, D) 2006; (E, F) 2007; (A, C, E) mean arcsine-square-root-transformed percent of males and females; (B, D, F) mean  $\ln(x + 1)$  CPUE. The horizontal dashed line indicates a 1:1 sex ratio reference, where means above the line are skewed towards males and means below are skewed towards females. Variables are defined in Table 2. Night hours are shaded. Vertical bars are  $\pm 1$  SE of the mean.

convex quadratic response and diel contrast (Table 5; Figures 2B, 3). The CPUE of males (M\_total) exhibited a weak concave quadratic response, in which highest abundances occurred during the day, as well as a potential time-by-year interaction (although only significant at  $\alpha = 0.1$ ; Table 5). The lsmeans test of the time effect within years on M\_total resulted in a signifi-

cant time effect during 2006 ( $P = 0.0220$ ) but not during 2007 (Figure 2D, F).

**Diel Periodicity by Maturation Stage**

The only maturation stages to exhibit time effects (Tables 4, 5; Figures 3, 4) were the spawning stages: percent M\_RR

TABLE 4. Results of the ANOVA and a priori contrast tests on arcsine-square-root-transformed percent catch by sex and maturation stage of Haddock. Significant ( $\alpha = 0.05$ )  $P$ -values are shown;  $P$ -values in bold italics were significant at  $\alpha = 0.1$ ; NS = not significant. Variables are defined in Table 2.

Variable	Year	Season	Time effects						
			Time	Season $\times$ Time	Year $\times$ Time	Linear	Diel	Quadratic	Cubic
<b>Males</b>									
M_total	NS	0.007	0.015	NS	0.009	NS	NS	0.004	NS
M_active	NS	<b>0.095</b>	NS	NS	NS	NS	NS	NS	NS
M_inactive	NS	0.012	NS	NS	NS	NS	NS	NS	NS
M_D	NS	NS	NS	NS	NS	NS	NS	NS	NS
M_R	NS	NS	NS	NS	NS	NS	NS	NS	NS
M_RR	NS	0.054	NS	0.014	0.006	NS	NS	NS	NS
M_S	NS	0.003	NS	NS	NS	NS	NS	NS	NS
M_RE	NS	0.001	NS	NS	NS	NS	NS	NS	NS
M_I	0.044	NS	NS	NS	NS	NS	NS	NS	NS
<b>Females</b>									
F_total	NS	0.007	0.015	NS	0.009	NS	NS	0.004	NS
F_active	NS	0.003	NS	NS	NS	NS	NS	NS	NS
F_inactive	NS	0.000	NS	NS	NS	NS	NS	NS	NS
F_D	NS	NS	NS	NS	NS	NS	NS	NS	NS
F_H1	NS	0.025	NS	NS	NS	NS	NS	NS	NS
F_H2	NS	<b>0.080</b>	NS	NS	NS	NS	NS	NS	NS
F_H3	NS	<b>0.078</b>	NS	NS	NS	NS	NS	0.028	NS
F_S	NS	NS	NS	NS	NS	NS	NS	NS	NS
F_RE	NS	0.000	NS	0.048	<b>0.084</b>	NS	NS	NS	NS
F_I	NS	0.010	NS	NS	NS	NS	NS	NS	NS

(time-by-year and time-by-season interactions), percent F\_H3 (quadratic contrast), CPUE F\_active (diel and quadratic contrasts), and CPUE F\_H3 (diel and quadratic contrasts); and the nonspawning (immature; I) stage: CPUE F\_I (year-by-time interaction). However, several other variables warranted a closer examination because they exhibited strong time-of-day trends that were significant at  $\alpha = 0.1$ , including the year-by-time inter-

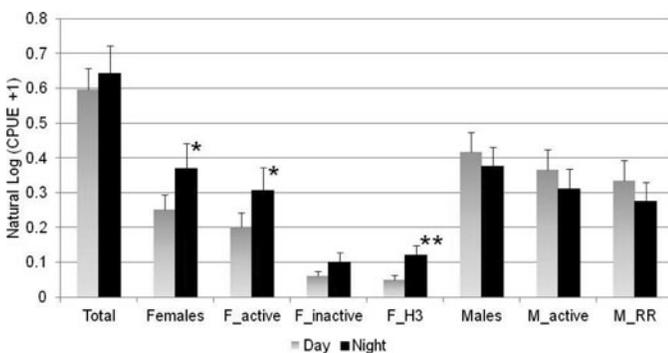


FIGURE 3. Comparison of day and night mean values for selected variables (see Table 2 for definitions of variables); asterisks indicate significant differences between means ( $P < 0.05^*$ ,  $P < 0.01^{**}$ ).

action for percent F\_RE, CPUE M\_active, and CPUE M\_RR, and the quadratic contrast test for CPUE F\_H2 (Table 4, 5; Figure 4).

The proportion of the females in the H3 stage (F\_H3) exhibited a significant convex quadratic response (Table 4; Figure 4A), in which lower proportions occurred during the day and higher proportions at night. The proportion of males in the running ripe stage (M\_RR) exhibited an opposite convex quadratic trend of higher proportions at night (Figure 4A), which was significant during 2006 (time-by-year interaction:  $P = 0.006$ ; lsmeans:  $P = 0.0121$ ) but not for 2007 (compare Figure 5A, C). Although the time-by-year interaction was not significant for the percent F\_H3, it showed a similar pattern of a strong quadratic diel pattern during 2006 (Figure 5A) and a weak response during 2007 (Figure 5C). The M\_RR stage also exhibited a significant time-by-season interaction ( $P = 0.014$ ; Table 4), in which significant time effects occurred only in the mid season during 2006 (lsmeans:  $P = 0.0008$ ; Figure 6B) and early season during 2007 (lsmeans:  $P = 0.0025$ ; Figure 6D).

The proportion of the females that were resting (F\_RE) was the only other percent variable to exhibit any significant time effects (Table 4; Figure 4A). It exhibited a significant time-by-season interaction ( $P = 0.048$ ; Table 4), in which time effects occurred in each season of 2007 (lsmeans:  $P = 0.0259$ ), but no time effects were observed in 2006 (Figure 6A, C). In 2007 the

TABLE 5. Results of the ANOVA and a priori contrast tests on  $\ln(x + 1)$ -transformed CPUE by sex and maturation stage. Significant ( $\alpha = 0.05$ )  $P$ -values are shown;  $P$ -values in bold italics were significant at  $\alpha = 0.1$ ; NS = not significant. Total catch is the CPUE of all Haddock captured per longline set; other variables are defined in Table 2.

Variable	Year	Season	Time effects							
			Time	Season $\times$ Time	Year $\times$ Time	Linear	Diel	Quadratic	Cubic	
<b>Combined</b>										
Total catch	0.005	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Males</b>										
M_total	NS	0.033	NS	NS	<b>0.061</b>	NS	NS	<b>0.097</b>	NS	NS
M_active	<b>0.056</b>	<b>0.056</b>	NS	NS	<b>0.095</b>	NS	NS	<b>0.071</b>	NS	NS
M_inactive	NS	0.002	NS	NS	NS	NS	NS	NS	NS	NS
M_D	0.021	NS	NS	NS	NS	NS	NS	NS	NS	NS
M_R	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M_RR	<b>0.072</b>	0.040	NS	NS	<b>0.069</b>	NS	NS	<b>0.089</b>	NS	NS
M_S	NS	0.013	NS	NS	NS	NS	NS	NS	NS	NS
M_RE	NS	0.000	NS	NS	NS	NS	NS	NS	NS	NS
M_I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
<b>Females</b>										
F_total	0.006	NS	0.054	NS	NS	NS	0.039	0.005	NS	NS
F_active	0.003	NS	0.044	NS	NS	NS	0.032	0.004	NS	NS
F_inactive	NS	<b>0.075</b>	NS	NS	NS	NS	NS	NS	NS	NS
F_D	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F_H1	0.005	NS	NS	NS	NS	NS	NS	NS	NS	NS
F_H2	NS	0.050	NS	NS	NS	NS	NS	<b>0.062</b>	NS	NS
F_H3	NS	0.029	0.028	NS	NS	NS	0.006	0.001	NS	NS
F_S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F_RE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
F_I	NS	0.002	NS	NS	0.005	NS	NS	NS	NS	NS

proportion of females in the resting stage increased significantly through the year ( $P < 0.0001$ ; Table 4), rising from a low of 6% during the early season, to 39% in the middle season and 55% in the late season (untransformed season means). More importantly, values remained low throughout the diel cycle during the early season, showed nocturnal increases during the middle season, and were consistently at or above 50% of the females during the late season except for lows at sunrise and sunset (Figure 6A, C).

In the analyses of the transformed CPUE data, only CPUE F\_active and F\_H3 had significant time effects without interactions, exhibiting significant quadratic and diel contrasts in which lows occurred during the day and peaks at night (Table 5; Figures 3, 4B). Trends for F\_active were likely driven by the stronger F\_H3 trend, which is reflected in the higher variance and lower significance level for CPUE F\_active. The only nonspawning stage to exhibit a time effect was the CPUE of immature females (F\_I), which had a significant time-by-year interaction (Table 5; Figure 4B) where the time effect was significant during 2006 (lsmeans:  $P = 0.0006$ ) but not during 2007 (Figure 5B, E). The F\_I trend for pooled years (Figure 4B) and

2006 (Figure 5B) included peaks at sunrise and sunset, suggesting a crepuscular pattern.

Three other spawning-stage variables might have exhibited time effects (although significant only at  $\alpha = 0.1$ ; Tables 4, 5), including CPUE F\_H2, M\_active, and M\_RR. The CPUE of females in the H2 spawning stage (F\_H2) exhibited a weak convex quadratic curve in which higher catches occurred between 0100 and 0500 hours (not shown). M\_active and M\_RR exhibited similar trends in which depressed abundance occurred between 2100 and 0100 hours (Figure 4B); however, both may have also exhibited an interaction between time and year (Table 5; Figure 5B, E) in which significant time effects occurred during 2006 (lsmeans:  $P = 0.0195$  and  $P = 0.0207$ , respectively) but not during 2007.

**Diel Periodicity in the GSI**

The mean GSI of mature females was significantly influenced by time of day (time main effect:  $P < 0.001$ ) and exhibited significant linear ( $P = 0.0275$ ), quadratic ( $P = 0.0009$ ), and cubic ( $P = 0.0091$ ; Figure 7) polynomial contrasts ( $N = 118$ ). The GSI was significantly higher (diel contrast:  $P = 0.0001$ ) at night

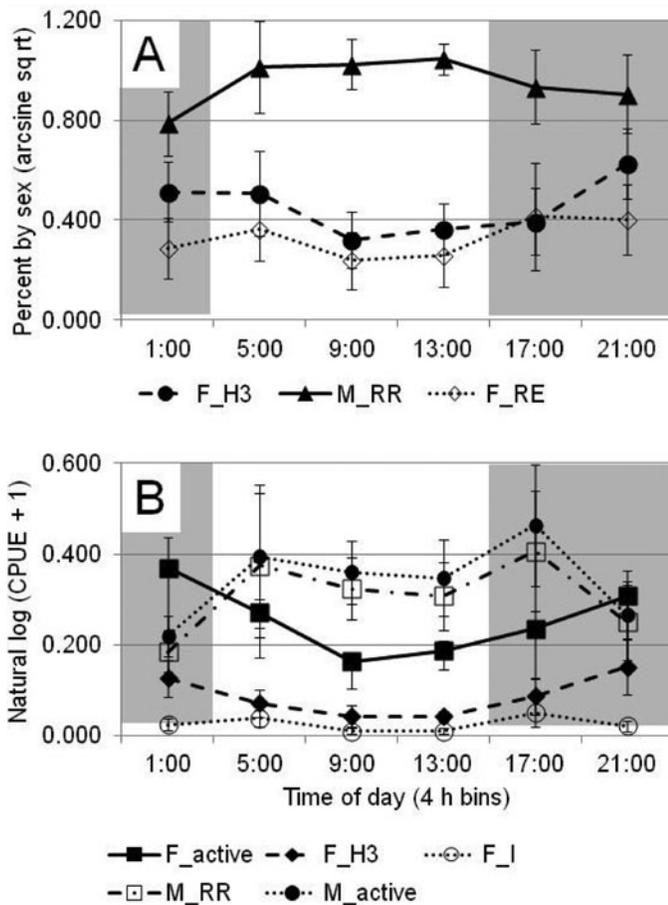


FIGURE 4. Diel trends for all Haddock maturation stages that exhibited significant diel trends at  $\alpha = 0.05$  or  $0.1$ : (A) mean arcsine square-root-transformed percent variables, and (B) mean  $\ln(x + 1)$  CPUE variables. Variables are defined in Table 2. Night hours are shaded. Vertical bars are  $\pm 1$ SE of the mean.

(mean = 3.36, SE = 0.10) than during the day (mean = 2.87, SE = 0.09). The GSI values were uniformly high during the evening (1700–0500 hours) but then waned and waxed gradually from a day low at 0900–1300 hours (Figure 7). The GSI of mature females exhibited a pattern most consistent with a quadratic diel pattern.

### Correlations

Most variables were only weakly correlated; therefore, we report only significant correlations of the most important spawning-stage variables, F\_H3 and M\_RR, with all other variables (Table 6). The percent of females in the H3 stage (F\_H3) was positively correlated with the percent of active females (F\_active,  $R^2 = 0.21$ ) and ripe and running males (M\_RR,  $R^2 = 0.09$ ), and the CPUE of F\_H3 ( $R^2 = 0.49$ ), M\_active ( $R^2 = 0.09$ ), and M\_RR ( $R^2 = 0.13$ ; Table 6). Weak negative correlations occurred with the percent F\_active ( $R^2 = 0.11$ ), F\_H1 ( $R^2 = 0.11$ ), and F\_RE ( $R^2 = 0.09$ ). The correlations of the percent F\_H3 with percent and CPUE of F\_active were trivial as F\_H3 made up a large component of the F\_active fish. Correlation of

percent F\_H3 with CPUE F\_H3 was also trivial. However, the weak correlations with percent and CPUE M\_RR is interesting as it suggests that female spawning is more closely related to the concentration of ripe and ready males (M\_RR) than to total males or total females.

### Tide and Lunar Effects

Inspection of the data plotted by date and lunar stage (data not presented) suggested that any potential lunar effect was masked by seasonal effects. That is, different lunar stages in the same season tended to have similar values, while the same lunar stage in different seasons tended to vary according to seasonal variation. Trends for F\_H3 data recoded into different moonlight conditions also suggested there was little or no lunar effect on spawning (Figure 8). Strong differences in CPUE F\_H3 between day and night were evident, while night catches of F\_H3 were not affected by the amount of moonlight during 2006. Percent F\_H3 exhibited greater variability. No diel or moonlight effects were evident for 2007, which is consistent with the full model diel analysis. Tidal effects were only observed for a few non-spawning active stages during 2006 (Figure 9). Most importantly evidence for tide and diel stage interactions were observed for the CPUE of inactive females (F\_inactive), immature females (F\_I), and inactive males (M\_inactive; Figure 9B). During 2007 most of the developing females (F\_D) that were collected were taken during ebb tide (data not presented). No other variables exhibited tidal effects in 2007.

### DISCUSSION

Haddock appear to exhibit strong diel periodicity in long-line catches, but their behavior is highly plastic. We observed differences between sexes, maturation stages, and years, which suggests an interaction between feeding and spawning behaviors. A potential problem with using longline gear in our study is that diel periodicity in feeding behavior may result in a false detection of diel periodicity in spawning. Lokkeborg et al. (1989) did not observe diel patterns in catches of North Sea Haddock on baited hooks suggesting that Haddock do not exhibit diel feeding periodicity. However, Temming et al. (2004) reported that the stomach contents of northern North Sea Haddock increased during the night (i.e., indicating increased nocturnal feeding activity). Reports from trawl surveys have shown various results in Haddock diel catch rates. For instance, Woodhead (1964) reported inconsistency between two sites 161 km (100 mi) apart in the Arctic; northern-site catches of juvenile Haddock were significantly higher during the day, and in the southern site, catches of juveniles were higher during the night. Casey and Myers (1998) and Petrakis et al. (2001) reported higher daytime catches for both juvenile and adult Haddock in the northwestern Atlantic Ocean and the North Sea, respectively. However, none of these studies examined catch patterns by sex or maturation stage, and our study, likewise, did not find a significant diel

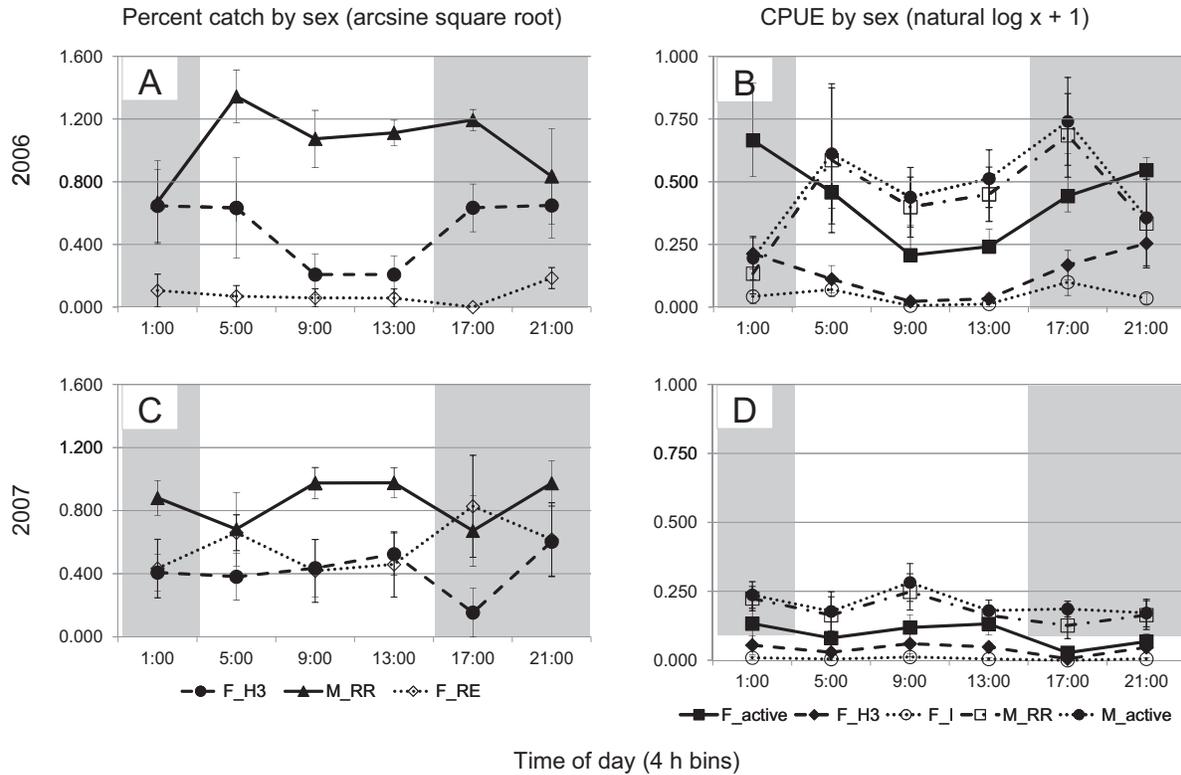


FIGURE 5. Comparison of diel patterns between sampling years for selected mean percent catch by sex (arcsine-square-root transformed) for: (A) 2006 and (C) 2007, and mean CPUE,  $[\ln(x + 1)]$  transformed for (B) 2006 and (D) 2007. Variables are defined in Table 2. Night hours are shaded. Vertical bars are  $\pm 1$  SE of the mean.

pattern in total catch (although there was a trend for lower day time catches (Figure 2B).

Examination of the influence of maturation stage on the diel catch patterns helps clarify the interaction between diel periodicity in feeding and spawning behaviors. Nonreproductive stages either exhibited no diel pattern (CPUE and percent F\_inactive, F\_S, M\_inactive, M\_S, M\_RE; CPUE F\_RE; and percent F\_I) or were possibly weakly crepuscular (proportion of resting females, F\_RE and CPUE of immature females, F\_I; Figure 4). The lack of diel trends in most nonspawning stages strongly suggests a lack of diel periodicity in feeding behavior, or at least that any diel periodicity in feeding is masked by diel periodicity in spawning. In fact, the H3 maturation stage for females and RR maturation stage for males appear to also drive the diel patterns for proportion and CPUE for total females and males, respectively. Therefore, we reject the primary null hypothesis ( $H_{01}$ ) and conclude that the diel patterns in F\_H3, M\_RR, and the sex ratio (M\_total and F\_total) result primarily from an underlying diel periodicity in spawning behavior of the quadratic type (Table 3). Although lunar and tidal effects were confounded with annual, seasonal, and diel effects in this study, there was little evidence that they had a strong effect on diel spawning periodicity in Haddock. The lack of evidence for lunar interac-

tions with diel behavior in our study is consistent with previous studies in temperate systems where lunar effects are thought to be uncommon in offshore fishes (Bye 1990). Tidal effects were limited to nonspawning stages and were specific to immature males and females. Weak diel periodicity and tidal effects on immature stages suggest some feeding periodicity for juveniles. Ontogenetic shifts in diel behavior patterns are common in fishes (see reviews in Rountree and Able 2007). The strong contrast in diel patterns among spawning and nonspawning maturation stages supports the hypothesis that Haddock exhibit diel spawning periodicity, which is further supported by strong agreement between F\_H3 and the GSI index.

However, our conclusion that the observed diel patterns in longline catches do in fact reflect spawning activity rather than feeding activity appears to be contradicted by the opposing patterns observed for males and females, which would, illogically, indicate increased spawning during the day for males and during the night for females (Figures 2, 3). Templeman et al. (1978) observed that milt was present in most mature male Haddock throughout the spawning season and that males had milt ready to be spawned several weeks before females had oocytes ready to be spawned. Consequently we would not expect to see a diel pattern in percent or CPUE for M\_RR, unless some

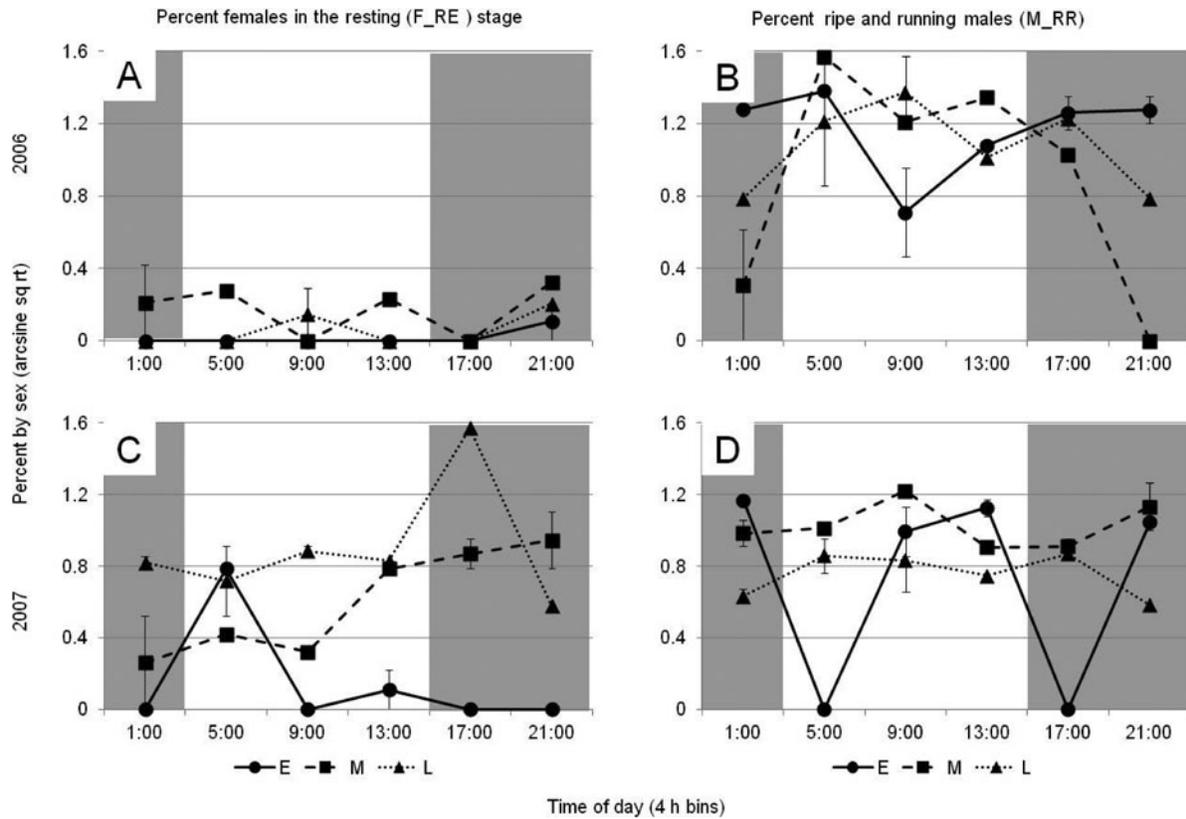


FIGURE 6. Comparison of seasonal and annual trends in diel patterns percent (arcsine-square-root transformed) for Haddock: resting stage females (F\_RE) for (A) 2006 and (C) 2007, and running males (M\_RR) for (B) 2006 and (D) 2007. Variables are defined in Table 2. Night hours are shaded. Vertical bars are  $\pm 1$  SE of the mean; E = early season; M = mid season; L = late season.

other variable is influencing catchability during certain times of day.

We suggest that reduced catches of ripe and running males at night is a consequence of male competitive behavior. Male Haddock are thought to exhibit lekking behavior (Casaretto 2007), and are known to compete for females through an elaborate

vocal courtship display (see review in Rountree et al. 2006). Under such conditions a reduction in feeding during spawning by ripe and running males (M\_RR) may be expected. Those males caught during the spawning time would be less likely to be in the ripe and running condition, and thus less distracted

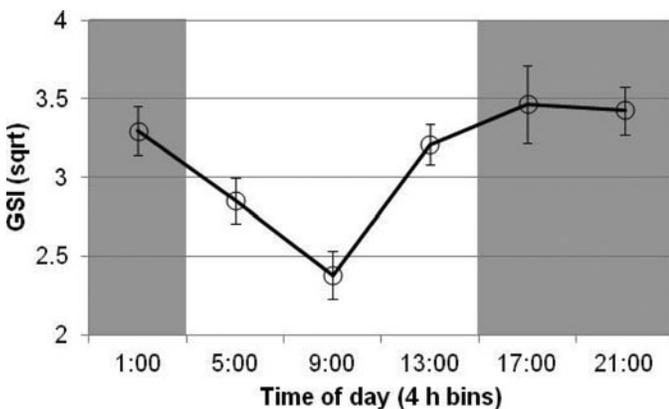


FIGURE 7. Mean GSI (square root) by time of day, based on samples from spawning active female Haddock (F\_active) for each 4-h time bin. Night hours are shaded. Vertical bars are  $\pm 1$  SE of the mean;  $N = 118$  mature females.

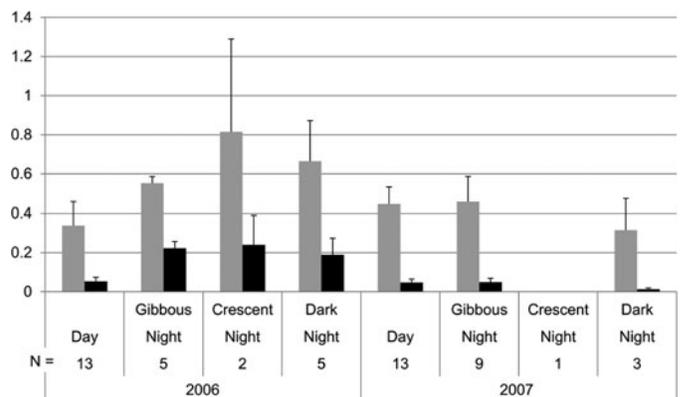


FIGURE 8. Comparison of mean arcsine-square-root-transformed percent F\_H3 (grey bars) and  $\ln(\text{CPUE} + 1)$  F\_H3 (black bars) among diel periods with night partitioned into high moonlight (gibbous moon), low moonlight (crescent moon), and dark night (samples occurring before or after moonrise or moonset). Vertical bars are  $+1$  SE of the mean;  $N =$  sample size for each group mean.

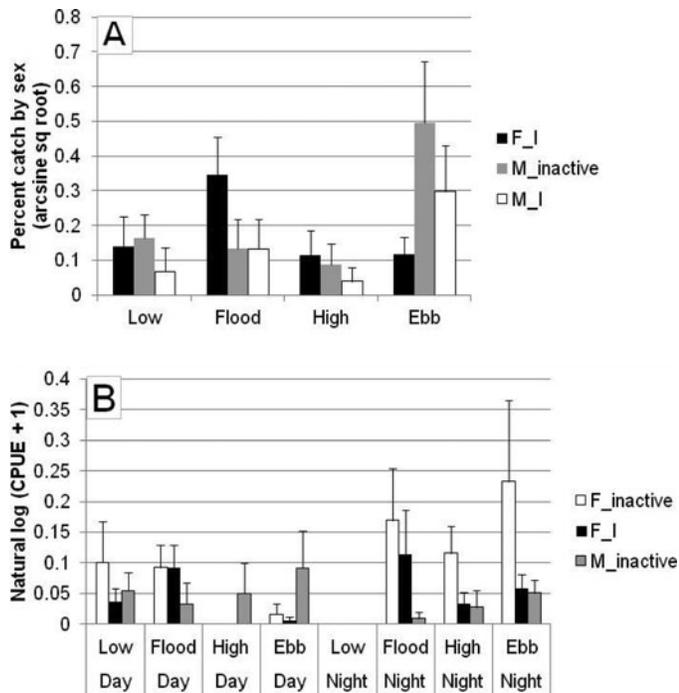


FIGURE 9. Comparison of tide stage differences for all maturation stages of Haddock that exhibited a significant (A) tide stage main effect or (B) tide stage by diel stage interaction ( $P < 0.05$ ) during 2006 ( $N = 25$  sets). Variables are defined in Table 2. Vertical bars are  $+1$  SE of the mean.

from feeding. Our observed pattern of high proportions and CPUE of ripe and running males (M<sub>RR</sub>) throughout the diel cycle, with slight reductions at night during the peak occurrence of spawning females (M<sub>H3</sub>), supports this scenario. In the lekking system females select the male of their choice when they are ready to spawn, so females may not face the same constraints on feeding. Thus, diel trends for F<sub>H3</sub> and GSI exhibited a similar pattern of wax and wane from a night peak indicating the convex quadratic diel pattern type (Figures 4, 7).

A recent study by Langård et al. (2008) presumed that the higher intensity and frequency of Haddock sounds they recorded on the Norwegian coast during dusk and night were linked to reproduction. The combination of Haddock having complex courtship calls leading up to spawning events (Casaretto and Hawkins 2002) and the trends in higher percentage of F<sub>H3</sub> fish during the night in this study supports the suggestion that the sounds Langård et al. (2008) recorded were linked to reproduction.

Diel patterns of several of the variables were unexpectedly found to be different between the 2 years (i.e., there was a significant time-by-year interaction), including sex ratio (percent M<sub>total</sub> and F<sub>total</sub>), percent M<sub>RR</sub>, and CPUE F<sub>RE</sub> (Tables 4, 5). In addition, other variables including CPUE M<sub>total</sub>, M<sub>active</sub>, M<sub>RR</sub>, and F<sub>I</sub> exhibited divergent time patterns between the years. In all cases strong diel patterns were observed during 2006 but not during 2007, except for percent F<sub>RE</sub> where the opposite trend occurred. Though the interaction

between time and year was not significant, percent and CPUE F<sub>H3</sub> also showed a similar pattern (Figure 4). Conducting separate ANOVAs for each year confirmed in all cases (except F<sub>RE</sub>) that the overall diel patterns were driven by strong quadratic diel patterns in 2006 and were absent except for a time-by-season interaction during 2007 (analysis not presented). The secondary null hypothesis ( $H_{02}$ ) of no differences in diel periodicity between years or among seasons is therefore rejected.

Variation in diel spawning periodicity is known to exist between species of the same family (e.g., gadids: Ferraro 1980; carangids: McBride et al. 2002) and between aggregations of the same species (e.g., Australasian Snapper *Pagrus auratus*: Wakefield 2010), suggesting that it is an adaptation to local conditions experienced by different species or populations (McBride et al. 2002; Yamaguchi et al. 2006). The ability of populations to adapt to short-term local conditions could explain the differences between 2006 and 2007 diel trends.

We identified two factors that might have contributed to the lack of evidence for diel periodicity during 2007: (1) annual differences in the seasonal timing of spawning and (2) density dependence. The influence of season can be seen in the comparison of significant seasonal differences in the time pattern between years for the percent resting females (F<sub>RE</sub>) and running ripe males (M<sub>RR</sub>; Figure 6). During 2006 few resting females were collected, while they made up to half the female catch during 2007 (Figure 6A, C). The proportion of ripe and running males (M<sub>RR</sub>) was higher during 2006 than 2007, and a weak diel trend was only observed for the early season during 2007 (Figure 6B, D). Similar patterns are seen for female spawning stages (not shown); therefore, we suspect that the lack of diel periodicity during 2007 occurred because we essentially missed the peak spawning season.

The direct relationship between Haddock distribution and the initiation of the spawning season with bottom temperature (Colton and Temple 1961; Marak and Livingstone 1970; Overholtz 1987) may explain the seasonal difference between years. Marak and Livingstone (1970) reported that during historic "cold" years "optimum spawning" was shorter than during "warm" years, and that a 1.5–2.0°C change in temperature can result in a spawning season that is a month shorter. In the western Gulf of Maine bottom temperatures during the summer of 2007 Northeast Fisheries Science Center cruises were about 1.0°C below average, and about 2.0°C cooler than in 2006 (P. Fratantoni, National Marine Fisheries Service, personal communication). Therefore, it is possible that the spawning season of western Gulf of Maine Haddock peaked early and ended sooner in 2007 because it was a colder year.

We suspect that density-dependent effects might have influenced the spawning pattern of Haddock because the proportion of spawning females (F<sub>H3</sub>) was significantly correlated with the CPUE of spawning males, but not to the CPUE of the total catch, total males (M<sub>total</sub>), or total females (F<sub>total</sub>) (Table 6). Correlation between the proportion of spawning fish and the abundance of spawning ready (M<sub>RR</sub>) males would

TABLE 6. Correlations of percent (arcsine-square-root) and CPUE [ $\ln(x + 1)$ ] for female Haddock hydration stage 3 (F.H3) and male Haddock ripe and running (M.RR) stage with percent and CPUE for all other stages (i.e., the first column shows the correlations of the percent F.H3 with the percents of all other stages, while the second column compares the percent F.H3 with CPUE of all other stages). The  $R^2$  values for significant ( $P < 0.05$ ) correlations are shown. Variables are defined in Table 2; na = not applicable; NS = not significant.

Variable Correlation with:	F_H3				M_RR			
	Percent		CPUE		Percent		CPUE	
	Percent	CPUE	Percent	CPUE	Percent	CPUE	Percent	CPUE
Total_catch	na	NS	na	0.29	na	0.09	-0.15	0.49
F_total	NS	NS	NS	0.23	-0.14	NS	NS	NS
F_active	0.21	NS	0.18	0.29	NS	NS	NS	NS
F_inactive	-0.11	NS	-0.12	NS	NS	NS	NS	NS
F_D	NS	NS	NS	NS	NS	NS	NS	NS
F_H1	-0.11	NS	NS	0.09	NS	NS	NS	0.10
F_H2	NS	NS	NS	NS	NS	NS	-0.08	NS
F_H3	na	0.49	0.49	na	0.09	0.08	0.13	0.22
F_S	NS	NS	NS	0.11	NS	NS	NS	NS
F_RE	-0.09	NS	-0.16	NS	NS	NS	-0.13	NS
F_I	NS	NS	NS	NS	NS	NS	NS	NS
M_total	NS	NS	NS	0.15	0.14	0.21	0.15	0.91
M_active	NS	0.09	NS	0.20	0.53	0.30	0.18	0.97
M_inactive	NS	NS	NS	NS	-0.18	-0.09	-0.10	NS
M_D	NS	NS	NS	NS	NS	NS	NS	NS
M_R	NS	NS	NS	NS	NS	NS	NS	NS
M_RR	0.09	0.13	0.08	0.22	na	0.36	0.36	na
M_S	NS	NS	-0.08	NS	NS	NS	NS	NS
M_RE	NS	NS	NS	NS	-0.17	-0.12	-0.09	NS
M_I	NS	NS	NS	NS	-0.09	NS	NS	NS

be expected in a lekking system where spawning-ready males aggregate to attract spawning-ready females, and may partially explain the observed differences in diel periodicity among seasons and ultimately between years. For example, why should the pattern of diel periodicity change seasonally unless it is a function of density? Otherwise one might expect to see the same diel pattern early in the season when few fish are spawning as during the peak spawning season. Because lekking fish, like the closely related Atlantic Cod, are thought to be particularly vulnerable to disturbance from fishing activity on their spawning grounds (Casaretto 2007), future studies are needed to examine the potential of closed management areas to protect Haddock spawning stocks.

Continued investigation of Haddock diel reproductive periodicity may provide some clues as to what drives Gulf of Maine Haddock to primarily spawn at night. Considering that adult Haddock do not appear to exhibit diel feeding periodicity (Lokkeborg et al. 1989), diel spawning periodicity would not improve feeding efficiency. We speculate that during the night reproductive synchrony between sexes is at its best, thereby maximizing reproductive success. The question left to answer is, why would reproductive synchronism be best at night? It

could be that males and females come together to spawn at night to minimize predation on newly spawned eggs (Ferraro 1980). It is less likely that they would spawn at night to minimize predation on themselves as mature Haddock have few documented predators (Collette and Klein-MacPhee 2002).

Future efforts to examine diel periodicity in Haddock or other fishes using methods similar to those in this study can best be improved by sampling throughout the 24-h cycle in as narrow a time window as possible (ideally within 24 h), which would greatly improve the sensitivity of the statistical tests. Using shorter time bins would also allow for better resolution of tidal stages and the transitional sunrise and sunset periods. In addition, conducting more 24-h time trials over the full prespawning, spawning, and postspawning seasons would be useful to examine seasonal changes in diel periodicity and possible density dependence together with interactions between spawning and feeding behaviors. Our conclusion that Haddock exhibit diel spawning periodicity is tempered by the possibility that interactions among other temporal factors such as tide and lunar stage might have resulted in a false detection of a diel pattern in the data. Additional studies are needed to examine the influence of these and

other temporal factors on spawning and feeding behaviors. However, incorporation of tidal and lunar effects into the sampling design is problematic because they are temporally confounded with each other, season, and time of day. Tidal and lunar interactions with the diel cycle may be best addressed in a focused study where samples are collected over the complete diel and tidal cycle every 24 h over enough consecutive days to obtain all combinations of tide stage, lunar stages, and time of day within each season. Alternative survey methods, such as trawl or gill-net sampling, may be useful since they reduce bias due to feeding behavior. However, the apparent interaction between feeding and spawning behavior suggested herein is in itself an interesting phenomenon that should be investigated further.

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