REPRODUCTIVE MATURATION AND DIEL REPRODUCTIVE PERIODICITY IN WESTERN GULF OF MAINE HADDOCK

A Thesis Presented

by

KATIE A. ANDERSON

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DEDICATION

To my husband and best friend, Nicholas Stefan Burchard

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ABSTRACT

REPRODUCTIVE MATURATION AND DIEL REPRODUCTIVE PERIODICITY IN WESTERN GULF OF MAINE HADDOCK

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A new macroscopic ovarian reproductive maturity index for haddock, *Melanogrammus aeglefinus* L, was developed to improve field collection of reproductive stage data. The index was tested, validated and revised based on a comparison with a laboratory histological staging method. The comparison of field and histological observations helped to improve the field index and methodologies and provided useful insight into the reproductive biology of Haddock. Although laboratory staging based on histology is inherently more accurate than any macroscopic field staging method, field observations can reveal weaknesses in the laboratory approach due to sampling bias. The revised field index includes three new macroscopic stages that represent a progression in final oocyte maturation from early to late, which were found to be reliable for staging spawning readiness in the field. This index was then used to study a population of Haddock in the Gulf of Maine to determine if it exhibits diel spawning periodicity. Commercial fishing vessels were chartered for 25 dedicated longlining trips to collect sexually mature haddock in the Southwestern Gulf of Maine at locations identified by commercial fishers as having spawning aggregations. In order to examine diel effects on haddock reproduction, the change in catch per unit effort and percentage of male and

female haddock of all reproductive maturity stages together with the gonadosomatic index were observed across a 24 hour diel cycle. Only females in hydration stage 3 (defined as late final oocyte maturation stage ovaries with 50-75% of oocytes hydrated) were significantly affected by time of day with significant increases in both catch per unit effort and percentage of hydration stage 3 haddock during the night. Because H3 is the most advanced reproductive stage observed prior to a spawning event and therefore the best indicator of imminent spawning these results demonstrate that female haddock in Southwestern Gulf of Maine primarily spawn during night hours with a peak between 2100 and 0100 hours. No diel trend was observed for any male reproductive stages. Additionally, no diel trend was observed in male or female reproductive stages unrelated to spawning including immature, spent and resting.

PREFACE

Haddock, *Melanogrammus aeglefinus* support an important New England fishery with two major stocks, one located on Georges Bank and the other in the south-western Gulf of Maine (Clark et al. 1982). Both stocks have exhibited population collapses in the early to mid 1900's due to over-exploitation and bycatch of undersized fish (Clark et al. 1982). In 1976 the United States solely took responsibility for managing the Gulf of Maine haddock stock and share responsibility for Managing the Georges Bank stock through the New England Fishery Management Council (NEFMC), created under the Magnuson Stevens Act. Since this act, periodic strong year classes with high recruitment levels have enabled these stocks to improve (Brodziak and Traver 2005). The 2008 stock assessment concluded that both stocks were not in an overfished state and that they were not being overfished (Brooks et al. 2008; Palmer 2008). Despite recent monitoring and assessment of these commercially and recreationally valuable haddock stocks, limited information exists on their reproductive biology (Brodziak 2005). The lack of reproductive biology studies on haddock in the Gulf of Maine is an impediment to effective management.

Lab studies on haddock revealed that this species has complex spawning habits including males communicating through sound and displays to females (Casaretto and Hawkins 2002; Locascio et al. 2008). The sounds generated by haddock during courtship have been described as knocks (Rowe and Hutchings 2006). As the male proceeds through courtship with a female and nears spawning, it produces these knocks closer together in a repetitive manner. Sound production with a specific association to spawning behavior has been noted in many marine species (Guest and Lasswell 1978; Connaughton

and Taylor 1995). Haddock sounds have been recorded on known spawning grounds in the Jefferies Ledge-Stellwagen Bank region of the southwestern Gulf of Maine (Rountree and Goudey personal communication) as well as in Lindåpollene off the Norwegian coast (Langard et al. 2008) using passive acoustics techniques. Calls of males in the final stages of courtship and spawning have been recorded. This is an important achievement because, *in situ* recordings of haddock, and specifically the signature sound of spawning have never before been made in North American waters. These recordings suggest that there is a precise time of day when spawning takes place based on a peak in the number of courtship calls heard (Rountree and Goudey personal communication). A recent study by Langard et al (2008) reported that sound intensity was higher at dusk and night than at daytime with twice as many sounds per half-hour at night. This time-of-day correlation between sound production and spawning activity has been observed in other species of fish (Saucier et al. 1992; Connaughton and Taylor 1995; Hawkins et al. 2002; Rowe and Hutchings 2006; Locascio et al. 2008).

The lack of knowledge on haddock reproduction in the Gulf of Maine and the recordings of haddock spawning sounds in the Gulf of Maine made by Rountree and Goudey (personal communication) inspired the research for this thesis. A study was conducted on sciaenid fishes in the southeastern United States where passive acoustic technologies were used to identify spawning habitats and to quantify fecundity with a greater precision (Roumillat and Brouwer 2003). Passive acoustic technology was used to listen for the spawning and courtship calls of *Cynoscion nebulosus*. In this way both the location and time of spawning was determined. The researchers were then able to capture adult *C. nebulosus* on the spawning grounds while spawning activities were taking place

and were able to significantly improve fecundity estimates. I sought to determine if these methods utilizing passive acoustics technology could be successfully applied to haddock in a similar manner. The original primary objective of this study was to validate that there is a correlation between spawning activity and sound production through observations of change in catch per unit effort (CPUE) and percent of spawning individuals, and sound production (number of calls and peak power spectral density). An increase in mass of male sonic muscles with a simultaneous increase in sound production activity across the season would support the existence of a correlation between spawning activity and sound production. A simultaneous increase in number of spawning haddock caught with an increase in sound production level across season would also support this correlation.

A second objective was to determine if there is a precise time of day when haddock spawn. I planned to test for daily patterns in not only sound production but also in changes of maturity stages and gonadosomatic index of female haddock gonads. I would test for a correlation between daily patterns observed in sound production with those observed in maturity stages. If the correlation existed I would catch haddock with ovaries with oocytes in their final maturation stages during the same time of day I observe an increase in sound production levels. The third objective was to estimate potential annual fecundity which is defined as the total number of advanced yolked oocytes per mature female and year uncorrected by artesia (Hunter and Macewicz 1985; Murua et al. 2003). I anticipated identifying the spawning location and time of haddock using passive acoustics and then capture mature haddock on the spawning grounds to use in estimating fecundity.

Haddock are determinate spawners, where the number of yolked oocytes immediately prior to the onset of spawning can be considered equivalent to the potential annual fecundity of that fish (Murua et al 2003). Thus the number of oocytes destined to be spawned in a season is identifiable at the beginning of the season. The optimal development stage to which samples can be taken to determine fecundity is right before any spawning occurs when the most advanced oocytes in the ovary are in the late vitellogenesis stage. If sampling is conducted prior to this stage, all oocytes destined to be spawned may not be developed enough and would be left out, resulting in an underestimate of fecundity. If samples are taken from females that have already spawned, the number of eggs that have already been released are not detectable, also resulting in an underestimate. Thus it is important that the system used for determining maturity stage is accurate and unambiguous. Unfortunately, there are inconsistencies in the definitions of maturity stages of fishes among the existing indices in the literature. For example, O'Brien et al. (1993) defines a female developing ovary as “a mixture of less than 50% yolked eggs and hydrated eggs”, however, according to Murua et al. (2003) the presence of hydrated oocytes indicates that the spawning process has begun and the gonad is in a “spawning” stage, where ‘oocytes are either in migratory nucleus stage or hydration stage’. This discrepancy in the definition of a developing ovary between indices would produce very different estimates of fecundity. Furthermore, it is unclear whether accurate staging of a developing ovary can occur through macroscopic observations alone. During personal observations of mature female haddock gonads I noticed many had varying numbers of hydrated oocytes. I was interested in whether the increase in percentage of hydrated oocytes was detectable over time, and whether these stages may aid in

examining diel reproductive periodicity. I did not find an index in the literature that staged the progression in percentage of hydrated oocytes in a gonad. This led to a change the objective of my study to developing and testing a reproductive maturity index for female haddock instead of determining potential annual fecundity. Histological analysis was conducted on ovary tissues of a subsample of ovaries representative all field stages in the index to validate my ovary maturity stages.

Although I successfully recorded haddock sounds in the field in the proximity of spawning individuals, we did not collect sufficient data to examine diel periodicity of sounds and I decide to eliminate the passive acoustics portion of my study. The acoustic listening systems were deployed at the same time that fishing to collect samples was conducted. Consequently, there was often an unexpected amount of sound from the study vessel and other sampling that likely masked haddock sound. Therefore, the definitive objectives of this study include; Chapter 1: 1) develop a female haddock reproductive maturation stage index building on previous published indices (Homans and Vladykoy 1954; Robb 1982; Murua et al. 2003) and unpublished observations made in the field, and 2) testing, validating and revising this index based on a comparison with a laboratory histological staging method conducted on a sample of the same ovaries staged in the field; Chapter 2: 3) use what I learned on reproductive maturation through the developing, testing and validation of the maturation index to determine if natural populations of haddock exhibit diel reproductive periodicity in the wild by observing the incidences of male and female haddock of all reproductive maturity stages throughout the diel cycle.

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CHAPTER 1

STAGING HADDOCK *MELANOGRAMMUS AEGLEFINUS* L. OVARIES: IMPLICATION FOR MATURITY INDICES AND FIELD SAMPLING PRACTICES

1.1 Abstract

A new ovarian reproductive maturity index for haddock, *Melanogrammus aeglefinus* L ovaries, was developed to support field collection of reproductive stage data and provide guidance on sampling techniques for laboratory analysis. The index was tested and revised based on a comparison with a laboratory histological staging method. The comparison of field and histological observations helped me to improve the field index and methodologies and provided useful insight into the reproductive biology of haddock and other boreal determinate spawning fish. There was good agreement between field and histological methods except for 'spent' and 'resting' stages, which is the least important distinction for determining maturity or reproductive dynamics. In addition, staging of developing ovaries was problematic for both methods because haddock ovaries were found to be heterogeneous during the early stages of final oocyte maturation. This finding indicates that macroscopic staging alone is not sufficient to determine if an individual has begun spawning for the season. There were two cases where immature ovaries were miss-staged as resting. This error is important to note because it can lead to miscalculating spawning stock biomass and length at maturity ogives. Prior reports that haddock exhibits determinate fecundity were supported by evidence of last spawn in five percent of the histological samples. Post ovulatory follicles (POFs) in various degrees of atrophy were common in the same tissue sample indicating a prolonged period of

atrophy, and suggesting that POFs may not be useful indicators of temporal patterns in ovulation. However, the revised field index includes three new macroscopic stages that represent a batch's progression in final oocyte maturation from early to late, and were found to be reliable for staging spawning readiness in the field. The findings from this study highlight the problems of developing an accurate field maturation stage index based on macroscopic observation. Although laboratory staging based on histology is inherently more accurate than any macroscopic field staging method, I found that field observations revealed a weakness in the laboratory approach due to a sampling bias. Although a useful new macroscopic field index to assess haddock maturation stages is presented herein, it is recommend to use both field and histologically based indices for problematic stages to maximize accuracy in future studies.

1.2 Introduction

An important component of assessing and managing any fish stock is quantifying its productivity. The productivity of a fish stock is a function of survival, individual growth and reproductive success of fish in the population (Wootton 1998). To determine a fish stock's annual reproductive success, estimates of the spawning stock biomass and stock-recruitment relationships must be made (Jennings et al. 2001). Estimates of both spawning stock biomass and stock-recruitment relationships are reliant on the use of reproductive maturity indices on a sample of the population. Because the ability to accurately determine the reproductive maturity stage through macroscopic examination of the gonads alone is fallible, the validity of field reproductive indices has been questioned (Hilge 1977; Templeman et al. 1978; Vitale et al. 2006). A major criticism

has been that field determination of maturation stages is not dependable because different reproductive stages can look similar during gross assessment of the gonad. For example, studies seeking to estimate spawning stock biomass or mean length at maturity depend on an accurate distinction between adult fish with resting gonads and immature fish (Forberg 1982; West 1990). Similarly, estimates of fecundity in determinate spawning species such as cod, *Gadus morhua*, and haddock , require accurate identification of ovaries in pre-spawning stages (Murua et al. 2003). Thus it is important that the system used for determining maturity stage is accurate and unambiguous.

There are considerable inconsistencies in the definitions of maturity stages of fish among the existing indices in the literature. For example, O'Brien et al. (1993) defines a female developing ovary as “a mixture of less than 50% yolked eggs and hydrated eggs ”, however, according to Murua et al. (2003) the presence of hydrated oocytes indicate that the spawning process has begun and the gonad is in a “spawning” stage, where “oocytes are either in migratory nucleus stage or hydration stage”. This discrepancy in the definition of developing ovary between indices could result in different estimates of fecundity in determinate spawning species where the optimal stage in which samples can be taken is prespawning, when the most advanced oocytes in the ovary are in the late vitellogenesis stage. If sampling is conducted prior to this stage all oocytes destined to be spawned may not be developed enough and would be left out resulting in an underestimate of fecundity. If samples are taken from females that have already spawned one cannot detect how many eggs have already been released also resulting in an underestimate.

Another important difference between Murua et al's (2003) and O'Brein's (1993) maturation indices is the description of a resting ovary. O'Brien's (1993) definition is based off on Burnett (1989) and Kesteven (1960) and is similarly defined by Waiwood and Buzeta (1989), Tomkiewicz et al. (2003) and Vitale et al. (2006). All of these authors describe the resting maturation stage as occurring after the spent stage as an off season state of the ovary. Conversely, Murua et al's (2003) describes the resting stage as occurring before the spent stage and as an in-between batch state of the ovary where some remaining hydrated oocytes, from previous batch may appear and further batches of hydrated oocytes will be produced'. Therefore there is a need for greater consistency in definitions and standardization in terminology of reproductive maturity stages of fishes. Many maturity indices are general and used on all teleost fish. However, because fishes have different reproductive strategies with regard to oocyte development, ovary organization, recruitment of oocytes and spawning pattern (Murua and Saborido-Rey 2003), unique indices could be developed for these different strategies. Such indices would be more detailed and consequently produce more accurate results.

Haddock is a batch spawning species with group-synchronous ovary organization, and determinate fecundity (Murua and Saborido-Rey 2003). This collection of reproductive traits is common in demersal Northwest Atlantic fishes including but not limited to cod, *Gadus morhua*, yellowtail flounder, *Limanda ferruginea* and Atlantic halibut, *Hippoglossus hipoglassus* (Murua and Saborido-Rey 2003). The standard number of yolked oocytes immediately prior to the onset of spawning in determinate fecundity spawners can be considered equivalent to the potential annual fecundity of that

fish (Murua et al 2003). After the onset of spawning the individual will hydrate several batches of yolked oocytes throughout the spawning season.

The purpose of this study was to develop a standard maturation index for haddock to support the collection of reproductive stage data in the field and provide guidance on sampling techniques for collecting samples for laboratory analysis that could be used to accurately stage all boreal, batch spawning, group-synchronous, determinate fecundity species. Additionally, I wanted to identify stages that could be used to examine diel periodicity in spawning activity (Chapter 2). First a staging method developed from unpublished observations and a review of existing published data was used to stage female haddock ovaries in the field. Then, the resulting stage index was revised based on a comparison with a laboratory histological staging method. Finally, the relative strengths and weaknesses of both field and laboratory approaches were assessed.

1.3 Methods

1.3.1 Initial field and laboratory indices developed

A new macroscopic field staging index for reproductive maturity of female haddock was developed, building on previous published indices (Homans and Vladykoy 1954; Robb 1982; Murua et al. 2003) and unpublished observations made in the field (Table 1.1). The index consists of eight stages, progressing from immature or resting (in the case of mature females) to spent. It differs from previously published indices with the addition of three stages representing early to late progression of final oocyte maturation (FOM) based on the percentage of hydrated oocytes present (H1, H2, H3, Table 1.1, Figure 1.1). H1, H2 and H3 occur with each batch of oocytes prior to it being spawned

(Figure 1.1). Hydration stage 1 (H1) is an ovary where a batch of oocytes is in the early phase of FOM where less than 25 percent of the ovary's visible surface contains translucent, hydrated oocytes (Table 1.1). Hydration stage 2 (H2) is an ovary where a batch of oocytes is in the middle phase of FOM when 25-50 percent of the ovary's visible surface contains translucent, hydrated oocytes (Table 1.1). Hydration stage 3 (H3) is an ovary with a batch of oocytes in a late phase of FOM when 50-75 percent of the visible surface of the ovary contains translucent, hydrated oocytes (Table 1.1). The index also includes for each stage: 1) a macroscopically derived ratio of gonad/body cavity volume, as Robb (1982) included for some of the stages, 2) a physical description of the ovary membrane, as Homans and Vladykoy (1954) included for some of the stages, 3) grossly assessed oocyte development description, included by Homans and Vladykoy (1954), Robb (1982) and Murua et al. (2003; Table 1.1).

The histological staging method was derived independently of the field staging method (i.e. during analysis, field-based stages were not used by laboratory personnel in developing histological stages and *vice versa*) and based on previous work of Tomkiewicz et al. (2003) and Roumillat and Brouwer (2004, Table 1.2). To differentiate the processes of early versus later vitellogenic activity, two histological index stages 2.1 or 2.2 were used to define developing ovaries (Table 1.2). During these two stages, all oocytes that will be spawned that season develop, because haddock have been reported to demonstrate determinate fecundity (Murua et al. 2003). However, the developing stages 2.1 and 2.2 were grouped together as one developing stage (2.0) when the histology results were compared to the field staging results. Three phases (early, middle and late) of FOM were assigned in the histological index as 3.1, 3.2 and 3.3 (Table 1.2). Two

histological index stages (7.1 and 7.2) were defined to categorize ovaries that showed evidence of having recently spawned a batch of eggs by exhibiting post ovulatory follicles (POFs; Table 1.2). The 7.1 stage was assigned to samples that contained very recent POFs while 7.2 was assigned to samples that contained older POFs. If a sample contained POFs, but also exhibited characteristics of another stage, the alternative stage was assigned (e.g. if a sample primarily contained oocytes in stage 3.1, but also contained POFs, it was assigned 3.1).

1.3.2 Field Sampling

Commercial fishing vessels were chartered for 25 dedicated survey trips in the spring of 2006 (15) and 2007 (10) to collect biological samples of haddock in the south-western Gulf of Maine (National Marine Fisheries Service Statistical area 514, Figure 1.2). Sampling surveys were based on a fixed station design where sampling was conducted where haddock aggregations were known to previously exist. Sampling was conducted during the known spawning season of Gulf of Maine haddock, between January and June (Collette and Klein-MacPhee 2002).

Longlines were the preferred gear type because haddock could be targeted with few discards. Approximately ninety meters of longline was set and hauled three times in the area over a 12 hour period with the objective of having two consecutive trips represent sampling over a twenty-four hour period (0100 - 0000 h, Table 1.3). Sets were conducted within specific four-hour time bins (0100 - 0500 h, 0500 - 0900 h, 0900 - 1300 h, 1300 - 1700 h, 1700 - 2100 h, 2100 - 0000 h EST) with an average soak time of two hours. The longline was fished with 150 to 400 circle hooks set 2 m apart. The number of

hooks fished varied with catch, with the intent of sampling at least 50 haddock from each haul. All haddock were measured by fork length (FL, +/- 1mm), and examined externally for signs indicating if they were in a ripe and running (staged “RR”, Table 1.1) maturity stage. The first fifty haddock in each haul were sacrificed to determine the stage of development of the gonads. If the fish was observed to be ripe and running, the sex and maturation stage could be determined without excisions and was automatically staged as RR in the field.

A sample of female haddock representative of all reproductive stages from each longline haul were labeled and reserved on ice. Fish from each of the following length bins were collected from each haul if possible to have representation from as many cohorts possible: 30-40 cm, 40-50 cm, 50-60 cm, and > 60 cm FL.

1.3.3 Laboratory methods

Female samples were processed in the laboratory within 24 hours of the end of each trip. Total weight (+/- 0.1 kg) and ovary weight (+/- 0.01 kg) of each individual was recorded. The macroscopic based maturity stage of all samples was re-examined. Digital photographs of whole ovaries were taken from a random subsample of each field index stage. To determine the accuracy of macroscopic maturity staging using our maturation index, histological analysis was conducted on tissue samples of a subsample of 169 ovaries from 1706 staged fish representative of all eight stages. All histological tissue samples were taken from the forward right lobe of each ovary. It was assumed that this was appropriate because, according to Robb (1982), haddock ovaries are homogenous throughout both lobes with oocytes present in various stages from the walls to the centre

of the gonad. Samples of 10 g tissue sections were fixed for at least 14 days in 10 % neutral buffered formalin before being transferred to 50 % isopropyl alcohol. Staining was done with Gills hematoxylin, counterstained with eosin-Y. Laboratory procedures followed Humason (1972). Oocyte samples were staged by noting the occurrence of specific histological features representing progressive oocyte maturation (Table 1.2). Photomicrographs were taken on a random subsample of stained tissue section from each field index stage.

1.3.4 Statistics

A contingency table was used to compare the results using the field staging methods and the histology staging methods (Figure 1.3). The cell where the two equivalent stages cross equals the number of times the data agreed. Percent agreement was calculated two different ways. One way divided the agreed data by the histology stage sample size (last vertical column in table 1.3). The second percent agreement divided the agreed data by the field stage sample size (last horizontal row in table 1.3). We did not have enough observed frequencies in each cell to do chi-square statistical analysis.

1.4 Results

All but two of the eight samples assigned the immature (1.0) stage using the laboratory method were also assigned the immature (I) stage in field staging (Figure 1.3). Two samples staged as 1.0 in the laboratory were assigned a resting (RE) stage in the field.

Only four of the nine ovaries staged as developing (D) in the field were also identified as developing (2.0) during histological analysis (Figure 1.3). Two of the remaining ovaries staged as D in the field were assigned as the adjacent histological stage 3.1, while three samples contained POFs (stages 7.1 or 7.2). In contrast, seven of the 12 ovaries staged as 2.0 in the laboratory were staged as the adjacent hydration stage 1 (H1) in the field, while one sample was staged as RE.

Twelve of the 32 ovaries staged as HI in the field were also staged the equivalent 3.1 histological stage (Figure 1.3). Seven of the ovaries staged as H1 in the field were assigned as adjacent histological stage 2.0, while two were assigned as 3.2 and five as 3.3. Six H1 staged ovaries contained POFs (7.1 or 7.2). In contrast, two of the 16 samples staged as 3.1 in the laboratory were staged the adjacent D stage in the field, while one was staged as hydration stage 3 (H3), and one as spent (S).

Twenty one of the 33 ovaries staged as hydration stage 2 (H2) in the field were also staged the equivalent 3.2 in the laboratory (Figure 1.3). Nine H2 staged ovaries were assigned the adjacent 3.3 histological stage, and three contained POFs. In contrast, four of the 29 ovaries assigned the 3.2 stage in the laboratory were assigned adjacent field stages (H1 and H3) and four were recorded as S.

The H3 staged samples were most frequently assigned the equivalent histology stage 3.3 (n=22, Figure 1.3). Two H3 staged ovaries were assigned to the adjacent stage 3.2, while one was assigned as 3.1. In contrast 35 of the 57 ovaries assigned the 3.3 histology stage were staged differently in the field, with most assigned as H2 (n=9) or ripe and running (RR, n=17).

All but two RR (n=17) field staged ovaries were assigned to the histological stage 3.3 (Figure 1.3). One of those two was staged as 4.0 and the other contained POFs (7.1).

Four of the 12 ovaries staged as S in the field were assigned the equivalent histology stage 4.0 (Figure 1.3). Four additional ovaries staged as S in the field were assigned as histological stage 3.2, while two were assigned as 3.3, one as 3.1, and one as 5.0. In contrast, most of the 21 ovaries assigned as 4.0 in the histology index, were assigned as RE in the field index (n=16, 76%), however one was assigned as H3 (Figure 1.3).

Twelve of the ovary samples staged as RE in the field assigned the equivalent histology stage 5.0 (Figure 1.3). Sixteen samples staged as RE in the field were assigned the adjacent stage 4.0 in the histological index. Two additional RE field staged samples were histologically assigned 3.3, while two were assigned as 1.0, and one as 2.0. In contrast, all but one of the 13 ovaries staged as 5.0 in the histology index were also assigned as RE in the field.

A final maturation index was created, based on the findings of this study (Figure 1.4). The final index consists of seven ovary reproductive maturity stages distinguishable at sea. Each maturation stage includes an image of the whole ovary, a photomicrograph of equivalent histological tissue, and a macroscopic and microscopic physical description of the ovary. Notes are included to aid the user in correctly macroscopically identifying each stage. Sampling techniques for taking tissue samples are also included for problematic stages. Based on the comparison with the histological data it was concluded that H3 and RR field stages are identical and grouped them together as one stage (H3).

Using the revised H3 field stage, 39 of the 44 ovaries assigned H3 were assigned the equivalent 3.3 histological stage.

1.5 Discussion

The utility of the field maturity staging method in fisheries management is entirely dependent on its biological accuracy. The findings from this study highlight the problems of developing an accurate field maturation stage index based on macroscopic observation. However, the comparison of field and histologically-based staging methods of haddock ovaries presented in this study revealed the need to revise the field methods to increase accuracy of each staging method. Although laboratory staging based on histology is inherently more accurate than any macroscopic field staging method, there was indication that field observations can reveal weaknesses in the laboratory approach due to sampling bias. The strengths and weaknesses of both approaches for each maturation stage are discussed below, followed by recommendations for correctly identifying the stage, and a description of helpful sampling techniques for taking tissue samples of problematic stages. Although this field index was based off of haddock I feel it can be used to stage reproductive maturity of any boreal species with the same set of reproductive strategies (group-synchronous, determinate fecundity, batch spawning fish)

1.5.1 Immature

The immature (I) field index stage was equivalent to the 1.0 histology stage (Table 1.1 and 1.2). The only stage mistaken for immature in the field was resting (RE, Table 1.1). In both stages the ovary is small and firm. The resting ovary appeared to

be a little larger, less transparent, and greyer in color rather than the pink color of an immature ovary. However, in a young mature fish these differences are less detectable. Comparing the current mean length at maturity for haddock with the size of the specimen can help support either maturity stage in the field. However, in this study the smallest haddock caught was 35.5 cm FL, larger than the mean length at maturity recorded for this species in the Gulf of Maine (34.5 cm, Collette and Klein-MacPhee 2002). The gear type used for sampling selected for larger fish, and it is suspected that smaller fish avoided the longline hooks. Although to our knowledge skipped spawning has not been observed in haddock, it is not uncommon in long-lived iteroparous fishes including *G. morhua* (Jørgensen et al. 2006; Rideout et al. 2006). Therefore, one cannot assume that a female is immature if it does not show signs of sexual maturity during the spawning season, as was assumed by Waiwood and Buzeta (1989) because, there is the possibility that the fish has skipped spawning that year. Conducting microscopic analysis or histology on a tissue sample of the ovary can be a sure way to determine whether the ovary is immature or resting. Immature ovaries can be histologically distinguished from resting ovaries by the diameter of the primary oocytes (Roumillat, personal communication). Immature ovaries contained primary oocytes that were equal in diameter, while resting reproductively mature fish have primary oocytes that vary in diameter. Due to the size selectivity of the fishing gear for larger fish and a limited sampling period of this project the methods were bias in the collection of samples in resting or immature maturity stage. Further work should be focused on differentiating a resting ovary from an immature ovary where sampling is conducted year round with a less size selective gear. Properly identifying

immature ovaries would greatly reduce the error of miscalculating spawning biomass estimates, and improve length at maturity ogives.

1.5.2 Developing

There was disagreement between developing (D) and early FOM stage, hydration stage 1(H1, Table 1.1). It was observed that when a haddock ovary initially began FOM, some oocytes complete the process before others within the same ovulating batch. Although, haddock ovarian development has been reported to be homogeneous throughout (Templeman et al. 1978; Robb 1982), the observations in the present study suggest that it is not homogenous during this very early stage of FOM (H1). This result supported by Alekseyeva and Tormosova (1979) who report that formation of batches occurs through asynchronous maturation of individual groups of oocytes. Histology reproductive maturation staging method sometimes misclassified H1 ovaries as developing. Hydration stage 1 ovaries have so few fully hydrated oocytes that taking a small tissue sample from a central location was sometimes unsuccessful in representing all stages of oocytes present. The agreement between macroscopic and histological staging for developing and H1 ovaries could be improved if the methodology used to take tissue samples from the ovary were modified. When ovaries are staged as H1 in the field a larger tissue sample or samples taken from multiple places in the ovary should improve the accuracy of the histological results. Our observations demonstrate that determining the maturation of an ovary based on histological examination alone may not always be accurate. To reduce staging errors based on histological analysis in future studies, it is recommended that each tissue sample be documented with a photograph of the whole

ovary it was extracted from, along with an estimate of the percentage of hydrated oocytes observed on the visible surface of the ovary.

Some ovaries staged as developing in the field contained POFs when analysed histologically, and by our definition a developing ovary cannot have previously spawned that season (Table 1.1, Fig.1.1). Therefore, those specimens had spawned at least one batch of eggs but had not yet hydrated oocytes for the next batch, and the decrease in volume of the ovary after spawning a prior batch of eggs was not evident in field observations. A closely related species *G. morhua*, begins to hydrate a batch of oocytes 1-2 days before spawning (Kjesbu 1991). Final oocyte maturation in cold water, marine fish with pelagic eggs generally lasts 1-2 days (Thorsen and Fyhn 1996). Trippel and Neil (2004) reported that haddock have a mean interval of 5.4 days between batches of eggs released in the Northwest Atlantic while Hawkins et al. (1967) and Alekseyeva and Trmosova (1979) report an interval of 26 to 40 hours. This indicates that there is an inter-batch period between a batch being spawned and the next batch beginning to hydrate as described as Murua et al. (2003) resting stage noted earlier (Fig. 1.1). Consequently, there is the possibility that a mature ovary could be incorrectly staged as developing in the field if it was between ovulation events in this inter-batch period. Therefore, I conclude that it is not always possible to be certain that an individual has begun spawning for the season based on macroscopic observation alone. This can pose a problem for fecundity studies that use ovary weight as a factor in determining fecundity. For the same reason it is also concluded that it is not possible to accurately stage an ovary as developing macroscopically. This poses a problem for studies that use gravimetric counting of vitellogenic oocytes and oocyte density to determine fecundity. The developing stage

when the most advanced oocytes in the ovary are in the late vitellogenesis stage is the optimal stage from which samples can be taken to determine fecundity. Therefore I recommended that a tissue sample be taken from fish staged as developing in the field based on macroscopic analysis to confirm the ovary is in a pre-spawning state through microscopic or histological analysis.

1.5.3 Hydration stages

A challenge in using the field index was the subjective evaluation of the percent of hydrated oocytes in an ovary that is used to assign the consecutive H1, H2 and H3 stages. Thus, histological samples were often assigned to a stage adjacent to the stage that was reported in the field.

The histology based laboratory staging method underestimated the H1 stage because the heterogeneous ovary was not adequately represented in the tissue samples. An H1 staged ovary could be incorrectly identified as developing based on histological examination under these conditions. However, as an ovary matured further the oocytes appeared to hydrate in unison and evenly throughout the ovary. This reduced the bias in the sampling methodology in later phases of H1, and eliminated it in later hydration stages H2 and H3.

Histological analysis verified that H3 staged ovaries were in a state where the next batch of eggs to be spawned was in its final FOM phase, with most oocytes in a late hydration phase microscopically. This consistent result is important because both the field H3 and histological 3.3 stages can be confidently used to identify spawning

readiness, and therefore be used to investigate diel patterns in the haddock spawning activity.

1.5.4 Ripe and running

Haddock ovaries were staged as ripe and running in the field when eggs were observed to be running freely from the female with little pressure applied to the abdomen. When the ovaries of these females were examined macroscopically in the laboratory they exhibited characteristics of stage H3. Furthermore, the tissue samples from these ovaries were staged as 3.3 using histology-based methods. Based on the results of histological analysis conducted on RR field staged ovaries, and the portion of the RR ovary full of hydrated oocytes during macroscopic observation it was decided to combine the RR and H3 field stages into one stage in the final index (H3, Figure 1.4).

Use of the ripe and running field stage proved problematic due to the sampling method and caution its use in future studies is recommended. Homans and Vladykov (1954) reported that female haddock stop feeding during spawning which would make it difficult to catch fish actually spawning using baited gear, and possibly resulting in an underestimation in RR females in the population. In addition, RR may be overestimated due to premature ovulation induced by stress or barotrauma. It is hypothesized that the barotrauma caused by forcing the specimens to ascend to the surface from an average depth of 90 meters during sampling, could have caused premature ovulation of hydrated oocytes. An increased level of cortisol in fishes is an indication of severe stress, but is also involved in the natural process of ovulation in teleost fishes (Billard et al. 1981; Wendelaar Bonga 1997). The two hour average soak time of the hooks in this study could

have been enough time for the stress response to induce ovulation in a H3 staged fish before landed on board. I hypothesize that it is difficult to catch a haddock in the act of spawning especially using baited hooks. If this is correct than using H3 staged fish to estimate spawning ready would be more accurate. However the practice of macroscopically staging a RR haddock by applying pressure to the abdomen and observing the excretion of hydrated oocytes is a method that can be used to classify a female as spawning ready without needing to sacrifice the fish.

1.5.5 Spent

Macroscopic identification of spent ovaries was the most problematic stage. The spent condition is particularly difficult to detect in a species such as haddock with asynchronous development where batches of eggs are spawned over a prolonged season (Hickling and Rutenberg 1936; West 1990). Species with determinate fecundity complete a spawning season by the maturation and spawning of the entire cohort of oocytes developed that year. When only a single batch of oocytes was left in the ovary to be spawned it was termed ‘last spawn’. This stage was only evident during histological analysis. Fifty eight percent (N = 7) of the field staged spent ovaries were staged as being in one of the three FOM histology stages. The only explanation for this result, other than observational error, is that these particular specimens were maturing the last batch of eggs to be spawned that season (last spawn) and the ovary at this point had lost its rigidness causing it to look spent. Last spawn was observed in eight (5%) of the histology samples, five of which were staged as spent in the field. Last spawn was also observed in North Sea haddock (Alekseyeva and Tormosova 1979). Near the end of the spawning

season the ovary can lose its rigidness even though it still has a batch or two of oocytes to spawn and appear as spent. The outside membrane thickens which increases the difficulty of staging the ovary by examining just the outside as also indicated by Templeman et al. (1978). Staging based on the flabbiness of the ovary alone is not recommended, and the inside of the ovary should be examined for hydrated oocytes. If any number of oocytes in FOM remains the ovary is most likely not spent and could be in last spawn. Conducting histology on a sample of the ovary can be a sure way to determine if the ovary is spent.

1.5.6 Resting

The histological results for resting staged ovaries reflect the difficulty in distinguishing between a resting and spent ovary in the field, with 46 % of the field staged resting ovaries assigned as spent during histological analysis. The explanation for this result is observational error, which much can be attributed to the short seasonal sampling period because as the ovary regresses into resting it became easier to differentiate from spent. For future studies it is recommended that the sampling should be conducted across a period that encompasses time well before and after the known spawning season, and that a photograph of each ovary should be taken for comparison with histology based staging results. This will improve the ability of gauging the regression from spent to resting as well as the ability to distinguish between the two stages. Conducting histology on a sample of the ovary can be a sure way to determine if the ovary is in the resting stage.

If a resting ovary is observed from a fish near or larger in size than the mean length at maturity during the peak spawning period it is possible that it skipped that

year's spawning season. One mature resting female was observed during the peak of the spawning season. Skipped spawning is a response to various physiological and ecological conditions (Jørgensen et al. 2006) and often a trade-off between present reproduction and survival for future reproduction (Bull and Shine 1979; Rideout et al. 2005). The inability to determine the existence and frequency of skipped spawning and its effect on recruitment, makes it difficult to determine SSB and hence difficult to conduct stock assessments and manage such species (i.e. stock-recruitment models may overestimate recruitment and underestimate survival; Rideout et al. 2005).

1.5.7 Post ovulatory follicles

Post ovulatory follicles were commonly found in H1, H2, H3 and S field staged ovary samples, but often POFs were in various stages of atrophy. The observation of early and late staged POFs in the same ovary indicated that POFs from the two previous batches still existed during the FOM of the next batch to be spawned. Evidence suggests that the complete atrophy of a POF takes a long time. It is estimated that a POF could take up to 10 days to completely degrade, considering that haddock have an average interval of 5.4 days between spawned batches (Trippel and Neil 2004) and final oocyte maturation in marine fish with pelagic eggs generally last 1-2 days and ends with ovulation (Thorsen and Fyhn 1996). The atrophy of POFs for the spotted seatrout, *Cynoscion nebulosus*, occurs in 24-36 h in water greater than 25 °C (Roumillat and Brouwer 2004) and 48 h in northern anchovy, *Engraulis mordax* at 19°C (Hunter and Macewicz 1985). The atrophy of haddock POFs may take much longer because of their

preference to spawn in cold temperatures (4 - 7°C, Overholtz 1987), a finding that may be widespread in boreal fish.

Aging of POFs has been used in other species to determine spawning frequency or duration of time since the female last spawned a batch of eggs (Hunter and Macewicz 1985; Roumillat and Brouwer 2004). No definitive information on diurnal timing of spawning was gleaned from the inspection of haddock POFs, since none of them appeared to have been very recently created. Fish collections were concentrated in an area where active spawning took place, and those haddock that had finished spawning may not have been available for capture. Observing many ovaries in spawning condition that also showed many stages of POF atrophy indicated that these residual tissues have a very slow atrophy and are of little use in making accurate assessments of diel timing of ovulation.

There were no equivalent field index stage for histology stages 7.1 and 7.2. Samples staged as 7.1 or 7.2 are typically assigned to an ovary in a state between the last batch of oocytes spawned and the next batch to be spawned, a state that was not attempted to be identified in the field. In this state no oocytes for the next batch had yet progressed to FOM and the only oocytes present were in a vitellogenic developed stage and is equivalent to Murua et al.'s (2003) resting stage noted earlier. It was found during this study that this stage is not easily or accurately ascertainable through macroscopic observation of the ovary. A trained eye may be able to recognize a degree of flaccidity of an ovary that has spawned already. Many of the ovaries assigned 7.1 or 7.2 exhibited characteristics of a developing ovary in the field and were staged as such. Overestimating the developing stage suggests the need to conduct histology on at least a subsample of

developing field staged ovaries to assure there is no indication that the female has started spawning that season based on the presence of POFs.

1.6 Conclusion

Comparison of macroscopic and microscopic observations of ovaries helped me to improve the field index and methodologies and provide useful insight into the reproductive biology of haddock. Noting the apparent longevity of POFs helped me understand the duration and cyclical process of FOM in this species and potentially other boreal or cold water fishes. Because reproductive maturation occurred over a prolonged period of time FOM occurred throughout three distinct field (H1, H2, and H3) and histology (3.1, 3.2, and 3.3) stages. These findings support Alekseyeva and Tormosova (1979) conclusion that haddock exhibits asynchronous maturation of individual groups of oocytes. Contrary to previous work (Robb 1981) the findings also suggest that haddock ovaries do not develop homogenously during the earliest stages of FOM, although homogeneity was achieved by later development. Therefore, improvements for tissue sampling used in histological examination are recommended in future studies.

Further work should be focused on differentiating a resting ovary from an immature ovary where sampling is conducted year round. This is the most important distinction in determining maturity or reproductive dynamics of a stock because of the use of these numbers in determining spawning stock biomass.

It is anticipated that the revised field maturation staging index presented herein will be useful to haddock resource managers (Figure 1.4). The use of hydration stages H2 and H3 appears to be useful indicators of spawning readiness for haddock ovaries in the

field. I suspect that the progression of FOM is detectable in other boreal species with the same reproductive traits as haddock, and that the later stages could also be used to examine diel periodicity in these species. The revised field index includes pointers to help users stage ovaries and take appropriate samples (Figure 1.4). Although this revised field index will improve accuracy in the determination of the maturity stage of haddock in the field, evidence has shown that field indices alone may not be enough to correctly stage a fish for problematic stages. However, the observations in the present study also demonstrate that determining the maturation of an ovary based on histological examination alone may not always be accurate, highlighting the importance of field staging. In addition to field staging with the resulting index presented herein, appropriate tissue samples should be collected and analysed microscopically or histologically to ground truth problematic stages, especially when field data are used in assessing and managing a fish stock.

Table 1.1: Female haddock reproductive maturity field index developed and used in this study

Stage	Description
I	Ovaries small and firm, about 1/8 the volume of the body cavity. Membrane thin and transparent, grey to pink in color. Contents microscopic. Individual oocytes not visible to the naked eye.
D	Ovaries larger and plump about 1/3 to 1/2 the length of the body cavity. Membrane reddish, yellow with numerous blood vessels. Contents visible to the naked eye and consist of opaque eggs that give the ovaries a granular appearance.
H1	Ovaries well developed, reddish yellow in color, at least 2/3 length of body cavity. Membrane opaque with blood vessels conspicuous looking. Contents consist of mostly, yellow looking oocytes with less than 25 percent of the ovary's containing larger translucent oocytes. A batch of oocytes in early stages of final oocyte maturation where oocytes start to hydrate.
H2	Ovaries well developed, reddish yellow in color, at least 2/3 length of body cavity. Membrane opaque with blood vessels conspicuous looking. Visible surface of the ovary consist of 25 - 50 percent larger translucent oocytes. Further progression of a batch of eggs in final oocyte maturation
H3	Ovaries well developed, reddish yellow in color, at least 2/3 the volume of body cavity. Membrane opaque with blood vessels conspicuous looking. Visible surface of the ovary consists of 50-75 percent larger translucent oocytes. Ovaries may appear a little flabby indicating the previous release of batch(s) of eggs. Final stages of the maturation of a batch of oocytes prior to a spawning event.
RR	Ovaries very large, over 2/3 the volume of the body cavity. Contents consist of mostly large translucent eggs. Eggs running freely with little to no pressure on the abdomen.
S	Ovaries soft, and flabby, about 1/4 the volume of the body cavity. Membrane thick and tough, purplish in color and bloodshot. Contents empty, few eggs remain giving the gonad a patchy appearance.
RE	Ovaries small and firm, 1/3 the volume of the body cavity. Membrane thin but less transparent, yellowish-grey in color. Contents microscopic, opaque.

Table 1.2: Reproductive maturity index developed and used during histological processing with analogous macroscopic field index stage. NA=Not Applicable

Histology	Macroscopic	Description
1	I	Immature oocytes with no sign of development
2.1	D	early vitellogenic activity (early stage in developing)
2.2	D	late vitellogenic activity (later stage in developing)
3.1	H1	early final oocyte maturation (FOM)
3.2	H2	further advanced mid stage of FOM
3.3	H3	all FOM stages but most oocytes in late FOM stage
7.1	NA	early post ovulatory follicles (POFs) present
7.2	NA	older POFs present
4	S	spawning residue remaining in the ovary, but will not spawn any more this season
5	RE	little indication that spawning had occurred, only primary oocytes that will begin to develop for the next spawning season

Table 1.3: Dates sampled to encompass each 24-hour period

24- hour period	Year	Dates Sampled
1	2006	3/12, 3/28, 3/31
2	2006	4/7, 4/10, 4/28
3	2006	4/30, 5/4, 5/8
4	2006	5/8, 5/16
5	2007	3/26, 3/31, 4/10
6	2007	4/10, 4/21, 4/24
7	2007	5/1, 5/22
8	2007	5/24, 5/30

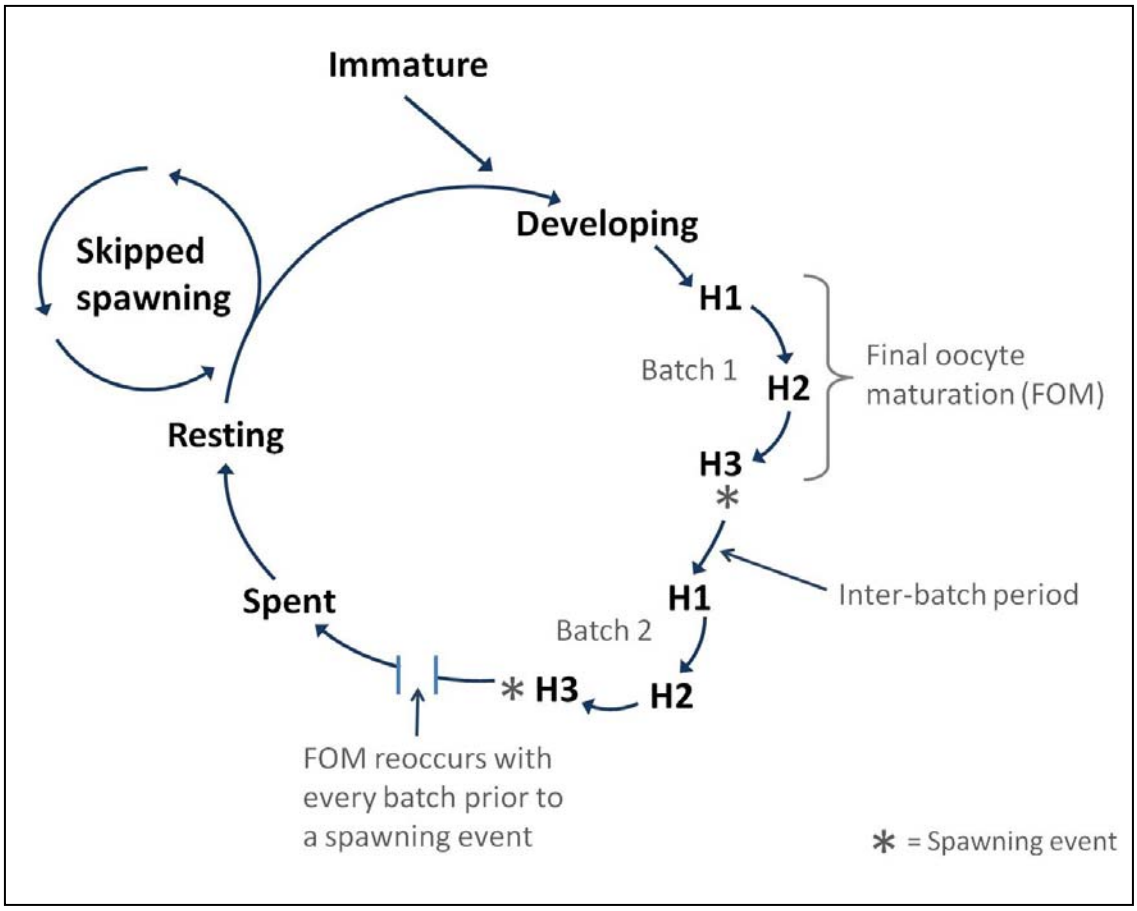


Figure 1.1: A diagram of the maturation cycle of the female haddock including three hydration stages introduced during this study. Hydration stage 1 (H1), hydration stage 2 (H2) and hydration stage 3 (H3) represent early to late progression of final oocyte maturation (FOM) respectively of a batch of oocytes, based on the percentage of hydrated oocytes present.

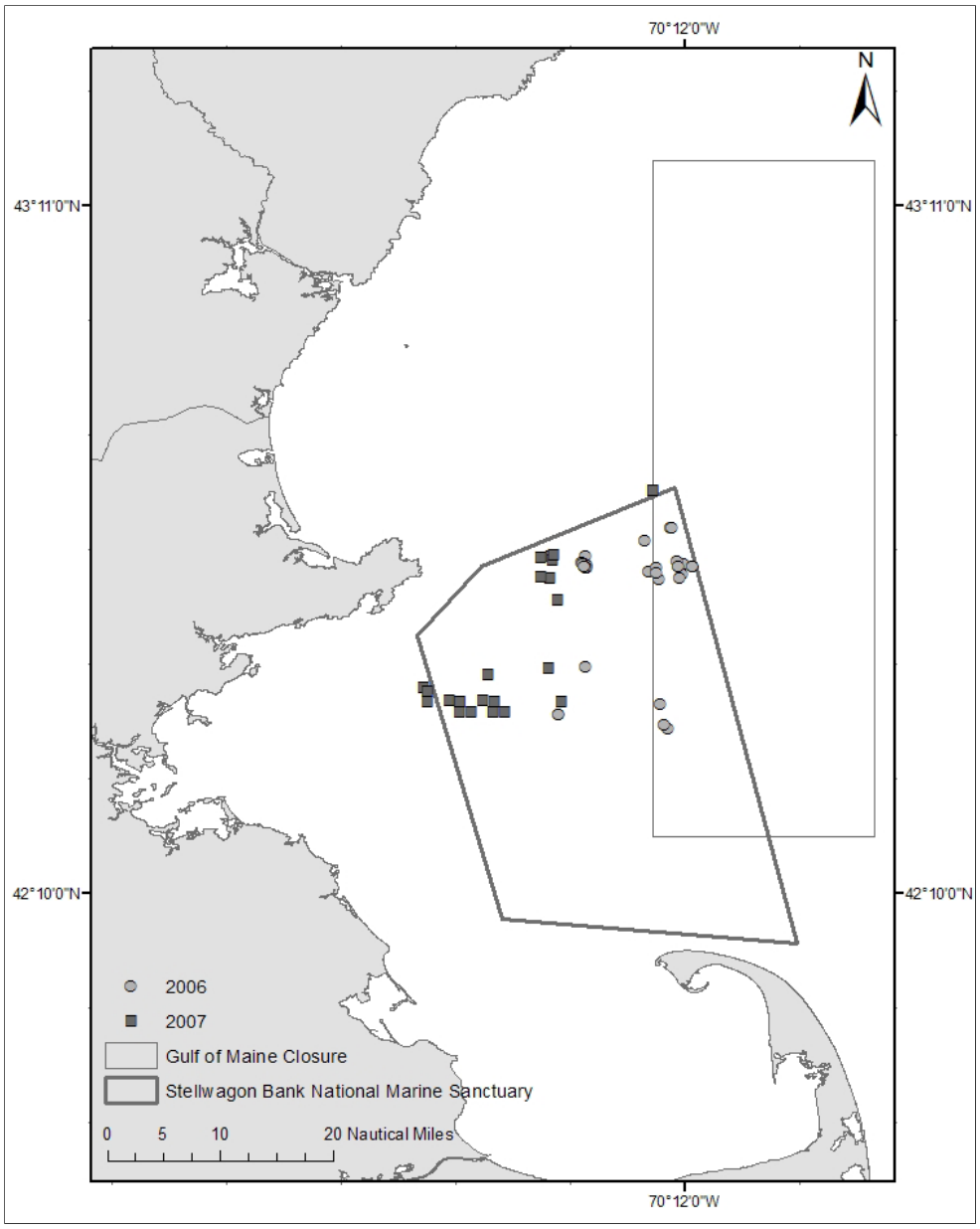


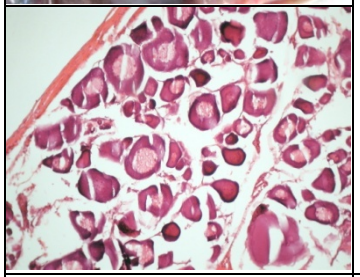
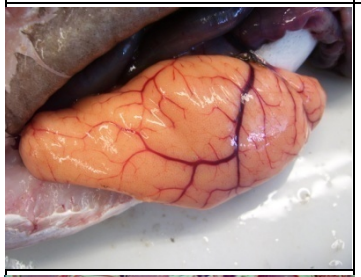
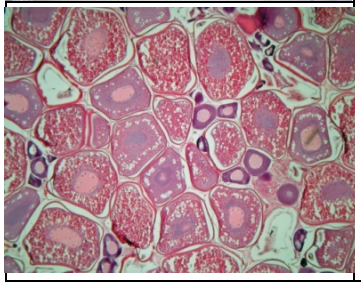
Figure 1.2: Study sampling location, southwest region of the Gulf of Maine.

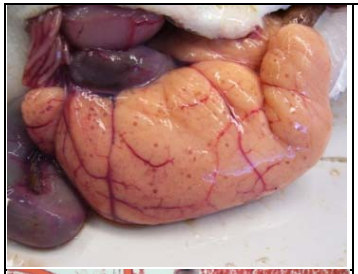
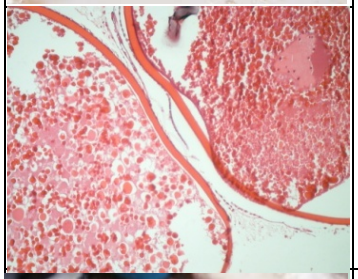
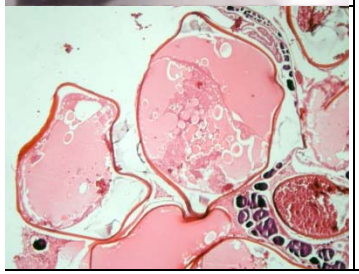
FIELD MATURITY INDEX STAGES

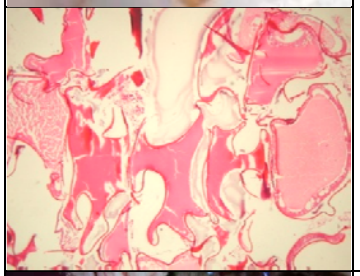
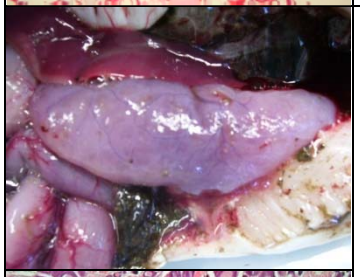
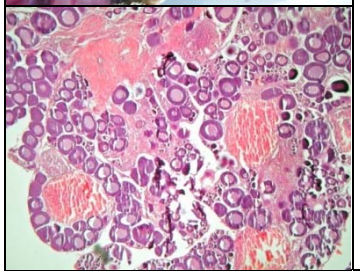
HISTOLOGY INDEX STAGES

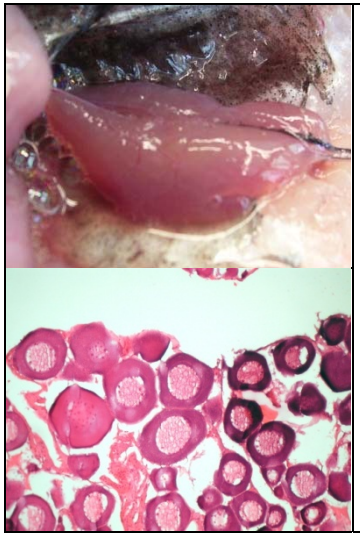
	I	D	H1	H2	H3	RR	S	RE	N	PA
1	6	0	0	0	0	0	0	2	8	75%
2	0	4	7	0	0	0	0	1	12	31%
3.1	0	2	12	0	1	0	1	0	16	75%
3.2	0	0	2	21	2	0	4	0	29	72%
3.3	0	0	5	9	22	17	2	2	57	39%
7.1	0	2	1	1	0	0	0	0	4	NA
7.2	0	1	5	2	0	1	0	0	9	NA
4	0	0	0	0	0	1	4	16	21	19%
5	0	0	0	0	0	0	1	12	13	92%
N	6	9	32	33	25	19	12	33		
PA	100%	44%	38%	64%	88%	NA	33%	36%		

Figure 1.3: Contingency table showing the results from the cross classification between histology maturity stages (vertical reading down) and field maturity stages (horizontal reading across). The grey squares represent where the cross classification is expected to have the highest frequencies. N, sample size; PA, percent agreement.

 	IMMATURE (I) Macroscopic: Ovaries small and firm, about 1/8 the volume of the body cavity. Membrane thin and transparent, grey to pink in color. Contents microscopic. Individual oocytes not visible to the naked eye. *Note: Can look similar to a resting ovary. Further support field staging with length of fish. Conducting microscopic analysis or histology on a sample of the ovary from fish close to mean length at maturity may be the only sure way to determine that the ovary is immature and not resting. Microscopic: The ovary contains germ cells, oogonia, and primary oocytes (oogonia that have undergone synopsis) that show no signs of development. The ovary wall is thin and the primary oocytes vary little in diameter. The nucleus is large with the most advanced oocyte having peripheral nucleoli.
 	DEVELOPING (D) Macroscopic: Ovaries larger and plump about 1/3 to 1/2 the length of the body cavity. Membrane reddish, yellow with numerous blood vessels. Contents visible to the naked eye and consist of opaque eggs that give the ovaries a granular appearance. *Note: Look carefully for any hydrated oocytes, which will be larger in diameter and translucent in color. If notice even one the ovary should be staged as H1 (see next stage below). A large tissue sample should be taken from all ovaries staged as developing in the field and analyzed microscopically to confirm that the ovary does not contain post ovulatory follicles and is in a pre-spawning state. Documenting the tissue sample with a photograph of the whole ovary may be helpful. Microscopic: Primary oocytes show signs of development increasing in diameter with the enlargement of the cytoplasm and accumulation of yolk.

 	HYDRATION STAGE 1 (H1) Macroscopic: Ovaries well developed, reddish yellow in color, at least 2/3 length of body cavity. Membrane opaque with prominent blood vessels. Contents consist of mostly, yellow looking oocytes with less than 25 percent of the ovary's containing larger translucent (hydrated) oocytes. *Note: In early H1 stage the ovary is not homogenous throughout. If microscopic analysis will be conducted on a subsample be extra careful in getting a representative tissue sample including translucent, hydrated oocytes. Take a large sample or multiple samples. Document with photograph if possible. Microscopic: Predominance of oocytes in early stage of final oocyte maturation (FOM), with very early yolk globule coalesence and the the beginning of oil droplet formation. Few oocytes will have completed FOM and are hydrated. Post ovulatory follicules (POFs) may be present from batch of occytes spawned early that season
 	HYDRATION STAGE 2 (H2) Macroscopic: Ovaries well developed, reddish yellow in color, at least 2/3 length of body cavity. Membrane opaque with blood vessels conspicuous looking. Visible surface of the ovary consist of 25-50 percent larger translucent oocytes. *Note: Naturally there is grey area between the consecutive H1 and H2 stage as well as the H2 and H3 stages, where it is difficult to assign one or the other stage. In these cases the ovary is at a state where it is either close to entering stage H2 or close to advancing to H3. In both cases the ovary is near if not in an intermediate phase of final oocyte maturation and most accurate if staged as H2. Microscopic: Predominance of oocytes in intermediate stage of final oocyte maturation (FOM) showing a greater amount of yolk coalescence, oil droplet completion and nuclear migration. A greater number of oocytes have completed FOM and are hydrated. Post ovulatory follicules (POFs) may be present .

 	HYDRATION STAGE 3 (H3) Macroscopic: Ovaries well developed, reddish yellow in color, at least 2/3 the volume of body cavity. Membrane opaque with blood vessels conspicuous looking. Visible surface of the ovary consist of 50-75 percent of larger translucent oocytes. Microscopic: Predominance of oocytes in last stages of final oocyte maturation (FOM), where yolk coalescence is complete and oocytes ready for ovulation. All other oocytes are in an intermediate stage of FOM. Post ovulatory follicules (POFs) may be present.
 	SPENT (S) Macroscopic: Ovaries soft, and flabby, about 1/4 the volume of the body cavity. Membrane thick and tough, purplish in color and bloodshot. Contents empty, few eggs remain giving the gonad a patchy appearance. *Note: Towards the end of the spawning season the ovary can lose its rigidness even though it still has a batch or two of oocytes to spawn. Be careful not to base your staging on the flabbiness alone. Look inside the ovary. If any oocytes in FOM remain it's probably not spent yet. Microscopic: An abundance of POFs are present. Germ cells and primary oocytes are more evident.



RESTING (R)

Macroscopic: Ovaries small and firm, 1/3 the volume of the body cavity. Membrane thin but less transparent, yellowish-grey in color. Contents microscopic, opaque.

*Note: If a resting ovary is observed from a fish greater in size than the mean length at maturity during the peak spawning period it is probable that she skipped that years spawning season and worth noting.

Microscopic: The ovary wall is thick. There is often indication of past spawning with reminiscence of undigested material that did not get reabsorbed back into the blood. Ovary contains previtellogenic oocytes that vary largely in diameter.

Figure 1.4: The final index developed from findings of the study. For each field stage an image of the complete whole ovary, stained microscopic image of a tissue sample of that stage, and descriptions of what is to be expected visually in each of the macroscopic and microscopic views are given. Notes are included to aid in field sampling.

CHAPTER 2

DIEL REPRODUCTIVE PERIODICITY OF M. AEGLEFINUS IN THE SOUTHWESTERN GULF OF MAINE

2.1 Abstract

Diel spawning periodicity has been increasingly studied in fish and has lead to better understanding early life history and recruitment variability of species. The awareness of diel reproductive periodicity in fish can contribute towards enhanced sampling methodology for additional reproductive studies or monitoring programs and species stock assessments. However, diel spawning periodicity has not been described for many commercially important groundfish in the Gulf of Maine. I studied a population of haddock *Melanogrammus aeglefinus* in the Gulf of Maine to determine if it exhibits diel spawning periodicity. Commercial fishing vessels were chartered for 25 dedicated longlining trips to collect sexually mature haddock in the southwestern Gulf of Maine at locations identified by commercial fishers as having spawning aggregations. In order to examine diel effects on haddock reproduction, the change in catch per unit effort (CPUE) and percentage of male and female haddock of all reproductive maturity stages and gonadosomatic index (GSI) were observed across a 24 hour diel cycle. Only females in hydration stage 3 (H3, defined as late final oocyte maturation stage ovaries with 50-75% of oocytes hydrated) were significantly affected by time of day with significant increases in both catch per unit effort and percentage of H3 staged haddock during the night. Diel patterns for H3 females was significantly different between years, in which only 2006

data exhibited significant a diel pattern. However, diel reproductive periodicity was also observed by a significantly higher female GSI during the night in 2007. Because H3 is the most advanced reproductive stage observed prior to a spawning event, and therefore the best indicator of imminent spawning, these results demonstrate that female haddock in Southwestern Gulf of Maine primarily spawn during night hours with a peak between 2100 and 0100 hours. The quadratic polynomial diel trend in CPUE of H1 females and FSR, and cubic polynomial trend in H2 staged females further supports that not only does spawning activity primarily occur during a preferential period of the day but suggests that female reproductive maturity processes leading up to spawning follow a diel cycle. No diel trend was observed for any male reproductive stages. Additionally, no diel trend was observed in male or female reproductive stages unrelated to spawning including immature, spent and resting.

2.2 Introduction

Diel spawning periodicity has been widely studied in marine fishes (e.g. Ferraro 1980; Walsh and Johnstone 1992; Wakefield 2010), freshwater fishes (e.g. Marshall 1967; Schwanck 1987; Marshall and Bielie 1996), estuarine fishes (Simpson 1971; e.g. Conover and Kynard 1984; Holt et al. 1985), and in tropical (e.g. Kohda 1988; Robertson 1991; Shibata and Kohda 2007), and temperate climates (e.g. Simpson 1971; Tzioumis and Kingsford 1995; Yamaguchi et al. 2006). Studies on diel reproductive periodicity provide details on the chronology of species reproductive processes. 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. 1300-1830h, *Lutjanus campechanus*, Jackson 2006) within a day. It has been suggested that diel spawning periodicity maximizes fish survival and reproductive success for reasons that include; predator avoidance for the adult or embryos, avoidance of harmful sunlight on the embryo, control of larval dispersal, reproductive synchronism between sexes, increased reproductive isolation (Ferraro 1980) and improved feeding efficiency (Shibata and Kohda 2007).

Diel reproductive periodicity has been examined by conducting ichythoplankton surveys (Gamulin and Hure 1956; e.g. Ferraro 1980; Wakefield 2010), observing spawning fish in the laboratory (e.g. Marshall 1967; Sherrill and Middaugh 1993) or in their natural habitat (e.g. 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). Ichthyoplankton surveys are the most common method used, in which fish eggs are collected and aged to determine an approximate time of day 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 hour 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, light intensity, and the lack of tidal cues or the disturbance of human activity

(Conover and Kynard 1984). Therefore, one advantage to observing fish in their natural environment is decreasing the likelihood of influencing endogenous cycles. However, observing fish in their natural habitat becomes increasingly difficult with an increase in depth and distance from shore. 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 if a species exhibits diel spawning periodicity (McBride et al. 2002; Yamaguchi et al. 2006; Anderson et al. in prep.). 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, etc.).

Three species in the Gadidae family have been reported to primarily spawn during specific times of the day (Ferraro 1980). 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, *Enchelopus cimbrius (*eggs obtained in planton tows, Battle 1930*)* and whiting, *Merluccius merluccius* (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 (Gadidae: *Melanogrammus aeglefinus)* inhabits both sides of the North Atlantic and is a highly valued commercial species (Clark et al. 1982; Bigelow and

Schroeder 2002; Schultz 2004). Haddock is a demersal fish most common at depths of 45-135 m (Brown 1998). Many studies have been conducted on various aspects of the reproductive biology of *Haddock* including; spawning behavior (e.g. , Casaretto and Hawkins 2002), maturation (e.g. Templeman et al. 1978; O'Brien et al. 1993; chapter 1), reproductive strategy (e.g., Murua and Saborido-Rey 2003) and fecundity (e.g. Raitt 1933; Alekseyeva and Tormosova 1979; Robb 1982; Clay 1989; Blanchard et al. 2003). Haddock is a group synchronous, determinate, batch spawner (Murua and Saborido-Rey 2003). Haddock spawn between January and June, with peak activity during late March and early April (Overholtz 1987; Brown 1998). To my knowledge, diel periodicity has not been directly studied in this species. However, Hawkins et al. (1967) reported anecdotal observations of one female haddock 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, information is not provided on the relative frequency of spawning between day and night, especially given the fact that night time observations were limited.

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 seasonal 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. Therefore, the objective of this study

was to determine if natural populations of haddock exhibit diel reproductive periodicity in the wild by observing the incidences of male and female haddock of all reproductive maturity stages throughout the diel cycle.

2.3 Methods

Commercial fishing vessels were chartered for 25 dedicated longlining trips to collect sexually mature haddock (length at first maturity 34.5 cm, Bigelow and Schroeder 2002) during late winter and spring of 2006 (N= 15) and 2007 (N= 10). Longlines were the preferred gear type because haddock could be targeted with little bycatch, and its selectivity for larger haddock greatly decreased bycatch of untargeted immature fish (Halliday 2002). A potential problem with using longline gear is the attraction of baited hooks biasing catch per unit effort. Sampling surveys were conducted within Southwestern Gulf of Maine in locations where spawning haddock aggregations had been found by collaborating commercial fishers (Fig. 1.2).

In order to examine diel effects on catch rates and reproductive maturity, longline sets were conducted within specific four-hour time bins (0100 - 0500h, 0500 - 0900h, 0900 - 1300h, 1300 - 1700h, 1700 - 2100h, 2100 - 0000h EST). However, due to vessel limitations sampling was limited to 12 h (3 consecutive time bins) for any given trip, so that it was necessary to pool trips within quasi-season spawning periods (early: mid March - early April, middle: mid April- early May, late: mid May –early June) to obtain a full 24 h sequence of samples (Table 2.1). For each set, 92 m of bottom longlines were fished with 150 to 400 round hooks set 1.8 m apart. The longlines sets were fished

for one to three hours (mean= 2 h 19 m). A total of 1702 haddock were caught from a total of 58 longline sets (mean= 29 fish per set, range 4 - 111).

All haddock caught were measured (Fork Length, FL, +/- 1mm). 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 body cavity of fish greater than 32 cm FL was then exposed to examine the gonads to determine sex and maturation stage (N= 627 and N= 643, in 2006 and 2007 respectively). Males were assigned standard maturation stages based on Northeast Fisheries Science Center maturity classification scheme (Burnett et al. 1989; O'Brien et al. 1993), and females were assigned to maturation stages based on a maturation stage index developed in chapter 1. The female maturation index developed in chapter 1 includes three stages representing the early to late progression of final oocyte maturation (FOM) of sexually mature ovaries based on the percentage of hydrated oocytes present: 1) hydration stage 1 (H1) when less than 25 percent of the ovary's visible surface contains translucent, hydrated oocytes, 2) hydration stage 2 (H2) when 25-50 percent of the ovary's visible surface contains translucent, hydrated oocytes, 3) hydration stage 3 (H3) when 50-75 percent of the visible surface of the ovary contains translucent, hydrated oocytes. One advantage of using the female-based field index developed in chapter 1 is that the hydration stages can be used to identify spawning readiness, with H3 being the closest to an actual spawning it is the best indicator of imminent spawning, and hence, most ideal for determining spawning diel periodicity. Because of the slow process of FOM in haddock the existence of any number of hydrated oocytes which categorizes the fish as spawning ready could not be used as an indication of spawning readiness because there would be little difference in

incidences of spawning ready female across time of day (chapter 1; Yamaguchi et al. 2006). The use of POFs used in other species to determine spawning periodicity cannot be used for haddock because they age too slowly (chapter 1).

To further quantify a time of day effect on maturation, in 2007 I measured gonadosomatic index (GSI) in a sample of mature haddock as an index of diel periodicity in spawning following methods of McBride et al. (2002), from a study on round scad (*Decapterus punctatus*). Samples of mature females (reproductive stages D, H1, H2, and H3) were labeled and preserved on ice to be processed in the laboratory to determine GSI. Sampled fish were processed in the laboratory within 24 hours of the end of each trip. Total weight (+/- 0.1 kg) and ovary weight (+/- 0.01 kg) of each individual was recorded. Female GSI was calculated using the formula GSI = Gonad Weight/ (Body Weight-Gonad Weight) x 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 catch-per-unit-effort (CPUE). Percent catch was constructed as a ratio of fish caught for each maturity stage, according to sex, to 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 same set). 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 **x** soak duration). An arcsin square root transformation was used to normalize the percent data and a natural logarithm transformation was used to normalize CPUE data.

A multifactor nested analysis of variance (ANOVA) programmed in SAS® was used to examine time of day effects on catch and maturation data. The ANOVA was performed on percent and CPUE data for all reproductive maturity stages sampled, as well as spawning ready females (FSR= H1, H2, H3 combined), spawning ready males (MSR= R and RR combined), total females, and total males (Table 2.2). Percent individuals within each set was the preferred index of catch because it is less influenced by location and season, thus having lower variance, and best representing any given sample of the population at any given time. Analysis of CPUE was an alternate approach to analysis of percent data and is presented herein for comparison purposes. The ANOVA model included year, season nested within year, and time of day main effects and their interactions. 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.

A priori linear (i.e. continual increase or decrease over time, Figure 2.1 A), quadratic (i.e. concave or convex U-shaped relationship with a signal change in direction over time, Figure 2.1 B), and cubic (i.e. S-shaped complex pattern with two changes of direction over time, Figure 2.1 C) polynomial contrasts tests (Quinn and Keough 2002) were used to further assess time of day trends in abundance or percent frequency of maturation stages. Additionally, an *a priori* means contrast test was conducted to examine difference between data grouped into day and night periods (time bins 0500-0900, 0900-1300, and 1300-1700 were grouped into a day period and time bins 0100-0500h, 1700-2100h and 2100-0100h were grouped into a night period) to assess whether there is any night- or day-specific trends in abundance or percent frequency of maturation

stages. In cases were variables exhibited a significant time by year interaction, diel trends were examined within each year separately using a reduced ANOVA model excluding the year factor.

Since GSI data were only collected in 2007, 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 an a priori contrast of data pooled within day and night periods were performed to test for a time of day main effect. A square root transformation was used to normalize GSI data.

2.4 Results

Mean percent females (F), males (M) and hydration stage 3 (H3) females were the only variables significantly affected by time of day indicated by either a significant main effect or significant quadratic and/or day versus night a priori contrast tests (Table 2.3). Percent F and stage H3 females exhibited a similar quadratic polynomial trend with values increasing to a high at night and then decreasing to low values during the day (Figure 2.2). On the contrary, percent M haddock exhibited a quadratic polynomial trend with values gradually decreasing to low values at night and then increasing to high values during the day (Table 2.3). Percent H3 staged female haddock also exhibited a day night difference with values significantly higher during the night (Figure 2.2). In contrast all other variables showed little consistent difference between day and night (Table 2.4).

Percent F, M, H3 staged females, ripe and running males (MRR) also exhibited a significant time interaction with year (Table 2.3, Figure 2.3). The diel trend for percent males in 2006 is u-shaped with values low at night and high during the day,

whereas no significant trend was observed in 2007 (Table 2.5, Figure 2.3 A). Although diel trends for percent H3 females were similar between years with higher values at night, in 2007 values were also high at 1300-1700h (Figure 2.3 B). The diel trend for F was the opposite of M with a u-shape of high values at night and low values during the day in 2006, but with no significant trend in 2007 (Table 2.5, Figure 2.3 C). The diel trend for MRR was opposite between years (Figure 2.3 D).

Percent F, M, H3, MRR, immature females (FI), resting females (Rowe and Hutchings), spent males (MS), resting males (MRE) and spawning ready females (FSR) all had significantly different season trends between 2006 and 2007 (Table 2.3, Table 2.6, Figure 2.4). One variable, immature males (MI), exhibited a very significantly year effect with percent MI values being higher in 2006 (2006 mean= 13 %, 2007 mean= 2 %).

In addition the percent MRR exhibited a significant time interaction with season within year (Table 2.3, Figure 2.5). In 2006, during the early season, percent MRR was greater during night and significantly lower during mid-day periods (0900-1300h, Figure 2.5 A). Conversely percent MRR was greater during the day during middle and late season in 2006 (Figure 2.5 A). In 2007 the trend in percent MRR was very different between seasons (Figure 2.5 B). Early season had means of 0% at 0500-0900h and 1700-2100h (Figure 2.5 B). Middle and late season was relatively steady in value with a slight peak in value mid-day (0900-1300h) during middle season and lower values at night during late season (Figure 2.5 B).

Analysis of CPUE data was consistent with percentage data except that Hydration stage 1 (H1), hydration stage 2 (H2) and females spawning ready (FSR) were also

influenced by time of day while M was not (Table 2.7, Figure 2.6). The CPUE of stage H1 females exhibited a quadratic polynomial trend with a gradual increase in values throughout the day, peaking between 1700-2100h and then decreasing until 1000-0500h (Table 2.7, Figure 2.6). The CPUE of stage H2 females exhibited a cubic polynomial trend with values increasing and decreasing twice across twenty-four hours. Hydration stage 2 values gradually decreased from 0100-0500 to mid day (0900-1300h) and then increased through 1700-2100h before decreasing again through 2100-0100h (Figure 2.6). The CPUE of FSR exhibited a quadratic polynomial trend similar to percent F and percent staged H3 females with values gradually increasing to high values at night and then decreasing to low values during the day (Figure 2.6).

Separate analyses of time of day effects were necessary for each survey year for variables that exhibited a significant interaction between time and year (Table 2.3, Table 2.5, and Figure 2.3). During 2006, percent F, M and H3 staged females were significantly affected by time category having a significant or highly significant quadratic polynomial trend as well as a significant or very significant day versus night difference with values gradually increasing to high values at night and then decreasing to low values during the day (Table 2.5, Figure 2.3 A-C). Percent H3 was significantly influenced by season in 2006 and 2007 (Table 2.5). In 2006 H3 values were high in early and late season and lowest during middle season, while in 2007, H3 values were high in early season and decreased through late season (Figure 2.4, H3). Additionally, percent F, M, and MRR were significantly influenced by season in 2007 (Table 2.5, Figure 2.4). Percent female values were highest in early season decreasing during the middle season and slightly increasing during the late season, while percent M had the exact opposite trend. Percent

MRR values were highest during the middle season with lower values during early and late season. Percent MRR (described earlier), M and F had significantly different diel trends across season in 2007 with either a considerably high or low value at 0500-0900h during the early season (Figure 2.5, Figure 2.7).

The mean GSI of mature females was significantly influenced by time of day ($P = <0.001$) exhibiting a linear ($P = 0.0275$), quadratic ($P = 0.0009$) and cubic polynomial response ($P = 0.0091$, Figure 2.8). Gonadosomatic index values covered a small range, however, values decreased from early morning (0100-0500h) to lowest during mid-day (0900-1300h), and then increased again into the night. Mean GSI also showed a highly significant day versus night difference ($P = 0.0001$) with GSI significantly higher during the night period (Figure 2.8).

2.5 Discussion

Total females and, most importantly, maturity stage H3 fish clearly exhibited diel periodicity in 2006 based on both CPUE and percent catch indices. Judging by the diel changes in proportion of females in stage H3 in 2006, and mean GSI in 2007, primary spawning activity occurred during the night categories (Figure 2.2, Figure 2.8). A recent study by Langard et al. (2008) presumed that the higher intensity and frequency of haddock sounds they recorded during dusk and night on the Norwegian coast were linked to reproduction. The combination of haddock having complex courtship calls leading up to spawning events (Casaretto and Hawkins 2002) and robust trends in higher percentage of H3 fish during night in this study support that the sounds Langard et al.(2008) presumption that the sounds they recorded were linked to reproduction.

The quadratic polynomial diel trend in CPUE of H1 females and FSR, and cubic polynomial trend in H2 staged females further support that spawning activity primarily occurs during a preferential period of the day and that female reproductive maturity processes leading up to spawning follow a diel cycle (Table 2.7). Conversely, I did not observe similar diel trends for spawning ready males MR and MRR (Table 2.3). No diel change in the presence of milt was observed, leading us to think that once male haddock become ripe they remain ripe for the duration of the spawning season. 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. As a result, a diel change in percent MRR or MSR will not be observed unless some other variable is influencing catch ability during certain times of day. Although percent males in 2006 showed a significant quadratic trend, this may only be because they are the reverse proportion to F in total percentage of haddock (1: 1.09 respectively) that year. Therefore, because F exhibited a quadratic convex, u-shaped trend, mathematically M exhibited the reverse concave down trend. CPUE of M had no significant diel trend.

As noted in chapter 1, a potential problem with using longline gear is the attraction of baited hooks biasing catch per unit effort. Lokkeborg et al. (1989) did not observe diel patterns in catch of North Sea haddock on baited hooks suggesting that haddock do not exhibit diel feeding periodicity. However, Temming et al (2004) reported that northern North Sea haddock stomach contents increased during the night. Reports from trawl surveys show various results in haddock diel catch rates. For instance,

Woodhead (1964) reported inconsistency between two sites 100 miles apart in the Artic; 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) report higher daytime catches for both juveniles and adult haddock in the North Sea and Northwest Atlantic, respectively. In this study I did not observe any consistent significant diel trend in catch of non-spawning stages (FI, MI, FD, MD, FS, MS, FRE and MRE) as I observed for spawning stages (Table 2.3, Figure 2.9). If a consistent diel trend had been observed across all maturity stages then the diel trend could be attributed to diel feeding periodicity. The lack of diel trend in non spawning stages in this study with the inconsistency in diel periodicity in catch rates and feeding periodicity in the literature strongly suggest that the diel periodicity shown in F and H3 staged females in this study is correlated with spawning behavior rather than feeding.

Diel patterns for F and M and spawning stages H3 and MRR were significantly different between years (Table 2.3, Table 2.5 and Figure 2.3) with only 2006 data exhibiting significant diel trends. Variation in diel spawning periodicity are 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. Pagrus auratus, Wakefield 2010), suggesting that it may be 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 in 2006 and 2007 diel trends. Most of 2006 sampling was conducted inside the Gulf of Maine closure, an area closed year-round to fishing (Federal Register 2004), while 2007

sampling was conducted in a location west of the closure (Fig.1). Environmental conditions including but not limited to fishing pressure, temperature and current could have been different between these two lotions causing trends in spawning to be different between years.

Nine variables exhibited a significantly different seasonal trend between years (Table 2.3, Figure 2.4). A comparison of the season trends between years for stages H3, FSR and MRR suggest that primary spawning occurred during different periods of the spawning season each year (Figure 2.4). In 2006 percentage of FSR, MRR, H3 were greater during early and late season with a decrease in value during middle season. This result suggests that there were two primary spawning periods across the entire 2006 spawning season, one during early and one during late spawning season where percentage of mature spawning ready fish were the greatest. In 2007 percentage of FSR and H3 peaked during the early season and gradually decreased in value through middle and late season suggesting that the primary spawning occurred during early 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 month shorter spawning season. In the western Gulf of Maine bottom temperatures during summer of 2007 Northeast Fisheries Science Center cruises were about 1.0° C below normal average, about 2.0° C cooler than in 2006 (Ecosystem Assessment Program in prep.; Fratantoni personal communication). Therefore, it is possible that the

spawning season of Western Gulf of Maine haddock peaked early and ended sooner in 2007 due to it being a colder year.

The ripe and running male stage was the only variable that exhibited seasonal differences in the diel trend (Table 2.3, Figure 2.5). When the ANOVA was conducted by year, MRR seasonal difference in diel trend only persisted in 2007 (Table 2.5). During the 2007 early season no MRR were caught during time categories 0500-0900h and 1700-2100h (Figure 2.5 B). There was little variation in percentage of MRR caught during all other time categories and season. I do not have data to investigate whether the lack of MRR during time categories 0500-0900 and 1700-2100 is due to natural variation or is a sampling artifact (Figure 2.5 B).

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 haddock do not exhibit diel feeding periodicity (Lokkeborg et al. 1989), diel spawning periodicity would not improve feeding efficiency. I can speculate that during the night reproductive synchronicity between sexes is best, 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 with mature haddock having few documented predators (Bigelow and Schroeder 2002). Further work examining Gulf of Maine haddock diel spawning activity across multiple years is needed to investigate annual differences in seasonal and diel patterns such as noted herein.

2.6 Conclusion

There is strong indication from the results of this study that haddock in the western Gulf of Maine exhibit diel reproductive periodicity based on my observation of a significant increase in percentage of female haddock in reproductive maturity stage H3 at night. However, the yearly difference in diel periodicity of H3 staged females is puzzling, particularly since I observed diel periodicity of GSI in female ovaries in 2007. I recommend future studies on the affect location, particularly fisheries management areas, have on diel reproductive periodicity. Results from this study also suggest that annual temperature differences can cause variation in the onset and duration of haddock spawning season. Findings from this study have increased our understanding of the reproductive biology of haddock. Future work to investigate additional environmental and intrinsic factors that may have an effect on spawning diel periodicity may reveal what factor or combination of factors cause haddock to primarily spawn at night. I have shown that final stages of oocyte maturation (stage H3) can be used to identify spawning readiness and investigate diel patterns in the haddock spawning activity. I recommend that similar studies be done on other boreal species with the same collection of reproductive traits.

Table 2.1: Number of longline sets fished in each time category by season within year. E= Early, M=Middle, L=Late

Year		Time of day					
Dates Sampled	Season	0100-0500h	0500-0900h	0900-1300h	1300-1700h	1700-2100h	2100-0100h
2006							
3/21, 3/28, 3/31	E	1	1	2	1	2	2
4/28, 4/30, 5/4, 5/8	M	2	1	2	2	1	1
5/16, 5/24, 6/13	L	1	2	2	2	1	1
2007							
3/26, 3/31, 4/10	E	1	1	2	2	1	1
4/21, 4/24, 4/25, 5/1	M	2	1	1	1	2	2
5/22, 5/24, 5/30	L	2	2	2	1	1	1

Table 2.2: The variables tested in the ANOVA model for a time of day effect

Variables Tested	Acronym
Females	F
Males	M
Female immature	FI
Female developed	FD
Female hydration stage 1	H1
Female hydration stage 2	H2
Female hydration stage 3	H3
Female spent	FS
Female resting	FRE
Male immature	MI
Male developed	MD
Male ripe	MR
Male ripe and running	MRR
Male spent	MS
Male resting	MRE
Female spawning ready	FSR
Male spawning ready	MSR

Table 2.3: Results of ANOVA performed on variables (percent, arcsin square root) with time of day as the main effect and polynomial contrast tests conducted on time of day. Linear and cubic polynomial contrast tests showed no significances and therefore are not reported. *=significant (<0.05), **= very significant (<0.01), ***= highly significant (<0.001)

Variable	Year	Season(yr)	Time	Year*Time	Time*Season(yr)	Quadratic	Day vs. Night
F	0.647	0.01*	0.0178*	0.0124*	0.3475	0.002**	0.1152
M	0.647	0.01*	0.0178*	0.0124*	0.3475	0.002**	0.1152
FI	0.5633	0.005**	0.5484	0.1084	0.8718	0.8542	0.7017
FD	0.117	0.598	0.3223	0.7263	0.8785	0.3751	0.72
H1	0.433	0.0703	0.4868	0.3961	0.4761	0.1076	0.2886
H2	0.557	0.0936	0.9546	0.1801	0.5818	0.7944	0.7965
H3	0.5347	0.0054*	0.0411*	0.0291*	0.096	0.0035**	0.0148*
FS	0.8177	0.394	0.6437	0.4253	0.9353	0.1489	0.5537
FRE	0.0966	<.0001***	0.6545	0.1007	0.0941	0.9983	0.6528
MI	0.0021**	0.9923	0.9505	0.9522	0.715	0.4633	0.5186
MD	0.5514	0.3563	0.4995	0.4035	0.3452	0.2932	0.2103
MR	0.5458	0.1333	0.5343	0.4115	0.1913	0.3018	0.8831

MRR	0.2412	0.0096*	0.7356	0.0064**	0.016*	0.1643	0.1411
MS	0.1711	0.0023**	0.831	0.7492	0.7134	0.8374	0.8821
MRE	0.3436	0.0006***	0.576	0.8209	0.9886	0.5022	0.3765
FSR	0.5267	0.0038**	0.9283	0.7314	0.4027	0.8291	0.9874
MSR	0.35	0.1404	0.5466	0.2214	0.1688	0.0778	0.2936

Table 2.4: Mean (μ) percent (arc sin square root) and standard error (se) of all variables by time of day.

	0100-0500h		0500-0900h		0900-1300h		1300-1700h		1700-2100h		2100-0100h	
Variable	μ	se	μ	se	μ	se	μ	se	μ	se	μ	se
F	0.81	0.11	0.83	0.14	0.60	0.10	0.72	0.05	0.52	0.05	0.85	0.13
FI	0.14	0.06	0.20	0.07	0.11	0.06	0.29	0.12	0.15	0.08	0.13	0.07
FD	0.29	0.09	0.08	0.08	0.25	0.10	0.26	0.09	0.04	0.04	0.21	0.09
H1	0.41	0.07	0.45	0.09	0.38	0.10	0.47	0.14	0.45	0.20	0.29	0.11
H2	0.26	0.09	0.24	0.08	0.19	0.07	0.21	0.08	0.25	0.11	0.16	0.08
H3	0.21	0.08	0.10	0.04	0.05	0.03	0.12	0.04	0.14	0.06	0.29	0.08
FS	0.20	0.07	0.23	0.08	0.21	0.09	0.14	0.08	0.09	0.06	0.14	0.06
FRE	0.29	0.12	0.32	0.12	0.20	0.10	0.25	0.09	0.41	0.22	0.40	0.14
FSR	0.88	0.11	0.93	0.11	0.70	0.16	0.75	0.16	0.95	0.18	0.89	0.17
M	0.76	0.11	0.74	0.14	0.98	0.10	0.86	0.05	1.05	0.05	0.72	0.13
MI	0.15	0.09	0.04	0.04	0.09	0.07	0.03	0.03	0.06	0.04	0.10	0.10
MD	0.15	0.09	0.00	0.00	0.22	0.13	0.48	0.20	0.11	0.06	0.10	0.07
MR	0.13	0.11	0.15	0.07	0.19	0.08	0.12	0.06	0.31	0.19	0.07	0.05

MRR	0.79	0.13	0.95	0.17	0.92	0.12	0.77	0.14	0.93	0.15	0.90	0.16
MS	0.28	0.10	0.28	0.11	0.17	0.07	0.20	0.07	0.21	0.08	0.14	0.09
MRE	0.20	0.11	0.14	0.07	0.08	0.07	0.08	0.05	0.11	0.07	0.09	0.09
MSR	0.90	0.15	1.01	0.17	1.09	0.15	0.87	0.17	1.19	0.09	0.98	0.19

Table 2.5: Results of ANOVA performed on variable (percent, arcsin square root) that exhibited a significant time by year interaction in the full ANOVA model (Table 2.3). Linear and Cubic polynomial contrast tests showed no significances and therefore are not reported here.
*= significant (<0.05), **= very significant (<0.01), ***= highly significant (<0.001)

Variable	Year	Season	Time	Time*Season	Quadratic	Day vs. Night
H3	2006	0.0291*	0.2264	0.1282	0.0272*	0.0454*
H3	2007	0.035*	0.0554	0.2982	0.0673	0.1945
M	2006	0.1215	0.0159*	0.6838	0.0007***	0.0039**
M	2007	0.001***	0.2288	0.02*	0.8464	0.1045
F	2006	0.1215	0.0159*	0.6838	0.0007***	0.0039**
F	2007	0.001***	0.2288	0.02*	0.8464	0.1045
MRR	2006	0.0885	0.3412	0.1782	0.0718	0.1055
MRR	2007	0.0064**	0.5537	0.0062**	0.883	0.8024

Table 2.6: 2006 and 2007 percent means (μ) and standard error for variables that exhibited a significant season effect in the full ANOVA model (Figure 2.4, Table 2.3).

		Early		Middle		Late	
Variable	Year	μ	se	μ	se	μ	se
F	2006	0.58	0.10	0.90	0.15	0.72	0.15
F	2007	0.82	0.12	0.53	0.05	0.56	0.05
FI	2006	0.03	0.03	0.28	0.06	0.20	0.09
FI	2007	0.00	0.00	0.23	0.08	0.03	0.03
H3	2006	0.25	0.07	0.09	0.04	0.26	0.07
H3	2007	0.24	0.08	0.07	0.04	0.06	0.03
FSR	2006	1.02	0.17	0.79	0.12	1.24	0.11
FSR	2007	1.29	0.11	0.68	0.09	0.58	0.09
FRE	2006	0.02	0.02	0.15	0.05	0.03	0.03
FRE	2007	0.13	0.10	0.63	0.12	0.87	0.10
M	2006	0.99	0.10	0.67	0.15	0.85	0.15
M	2007	0.75	0.12	1.05	0.05	1.01	0.05
MRR	2006	1.14	0.10	0.88	0.17	1.17	0.15
MRR	2007	0.81	0.18	1.02	0.05	0.76	0.05
MS	2006	0.12	0.07	0.07	0.04	0.04	0.04
MS	2007	0.07	0.04	0.39	0.08	0.51	0.03
MRE	2006	0.00	0.00	0.08	0.08	0.00	0.00
MRE	2007	0.00	0.00	0.07	0.05	0.52	0.06

Table 2.7: Results of the ANOVA on CPUE (logarithm) of variables influenced by time of day by either a significant main effect and/or significant time of day quadratic, cubic, or day vs. night a priori contrast test.
*=significant (<0.05), **= very significant (<0.01), ***= highly significant (<0.001)

Variable	Year	season(yr)	time	year*time	time*season(yr)	Quadratic	Cubic	Day vs. Night
F	0.0147*	0.5619	0.0263*	0.4078	0.9928	0.0012**	0.6519	0.01*
H1	0.0035**	0.9292	0.1343	0.3821	0.971	0.0147*	0.2955	0.1731
H2	0.7954	0.3329	0.0796	0.3075	0.9723	0.3658	0.0107*	0.4593
H3	0.5943	0.0229*	0.3863	0.2156	0.2973	0.0438*	0.5759	0.0571
FSR	0.0017**	0.8375	0.0278*	0.3338	0.9492	0.0017**	0.4925	0.0078**

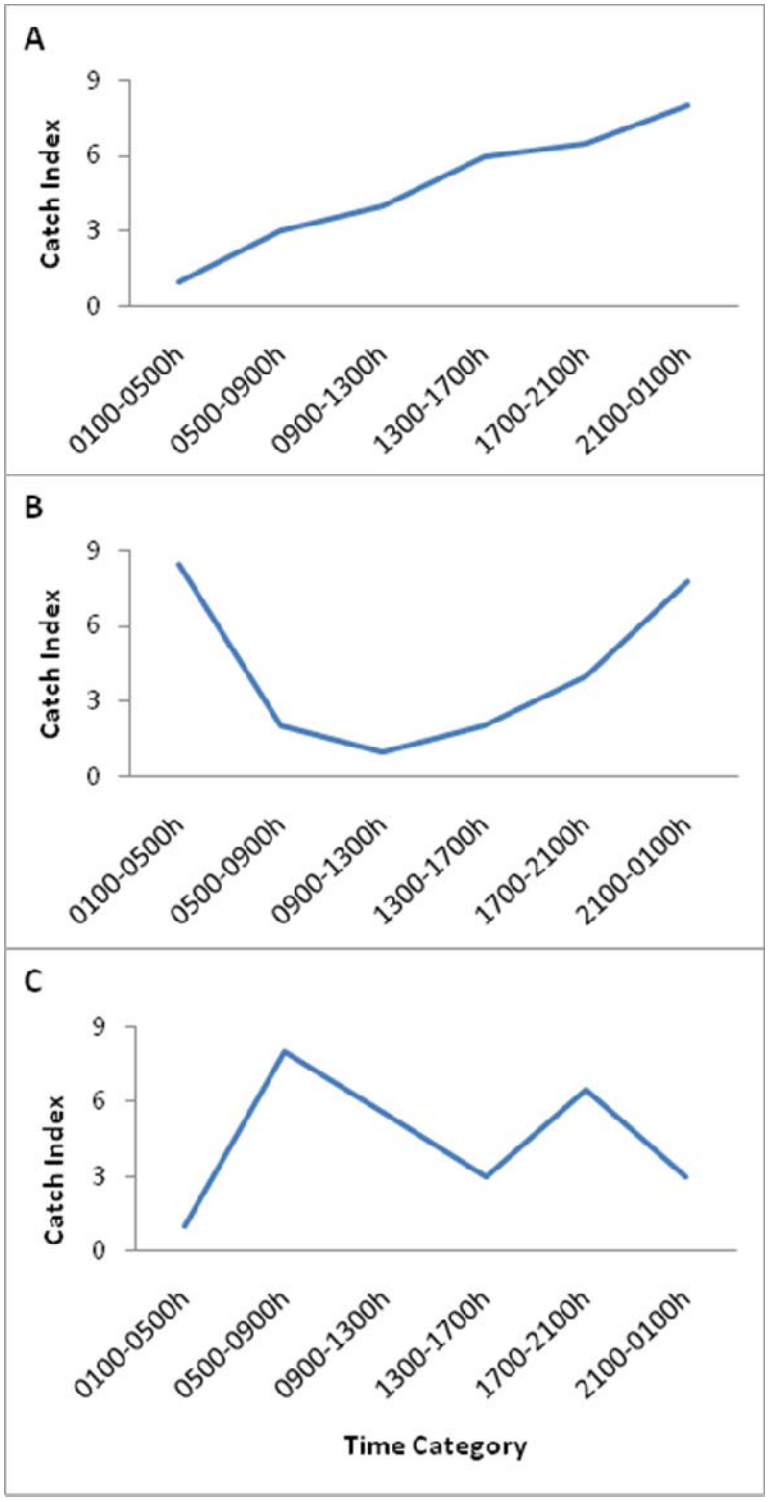


Figure 2.1: Examples of hypothetical linear (A), quadratic (B) and cubic polynomial (C) trends across time.

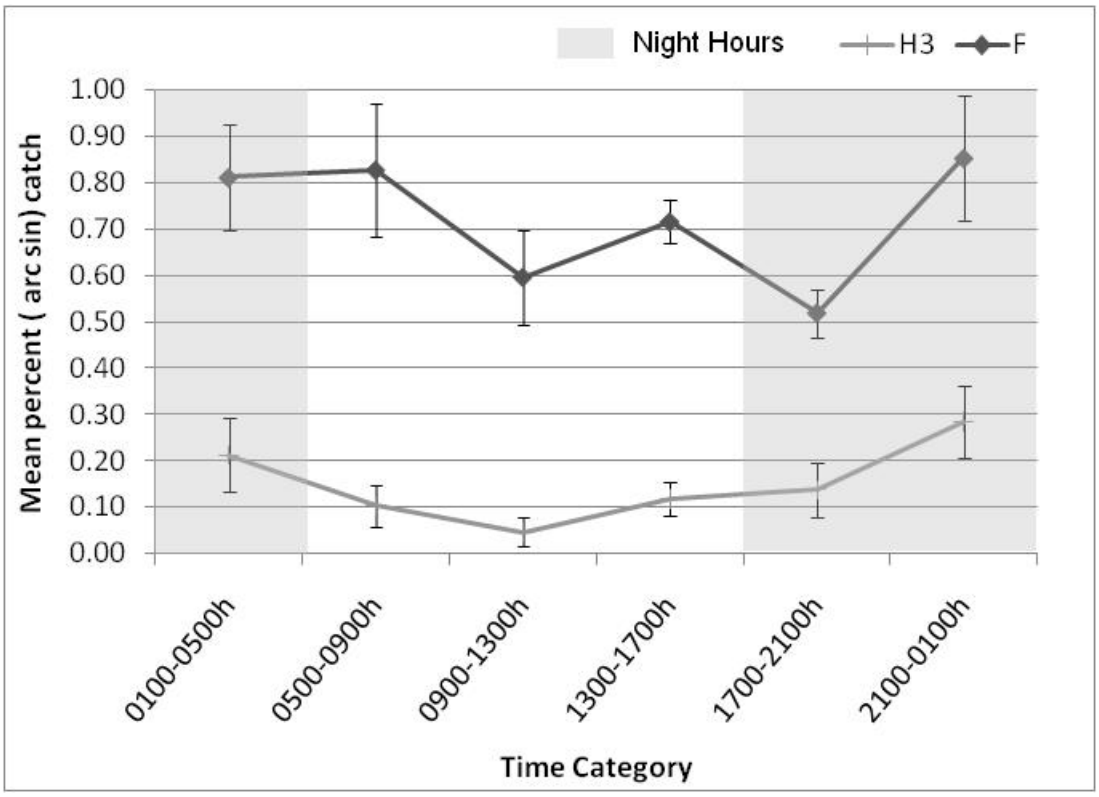


Figure 2.2: The diel trend of percent (arcsin square root) of females (F) and Hydration stage 3 (H3) female haddock. H3 females were significantly higher during the night (day vs. night contrast test, P= 0.0148, Table 3) and both H3 females and F exhibited a quadratic trend with values increasing at night and then declining to low values during the day (quadratic contrast test, Female P=0.002, H3 P= 0.0035,Table 2.3). Night hours are shaded. Vertical bars are one standard error of the mean.

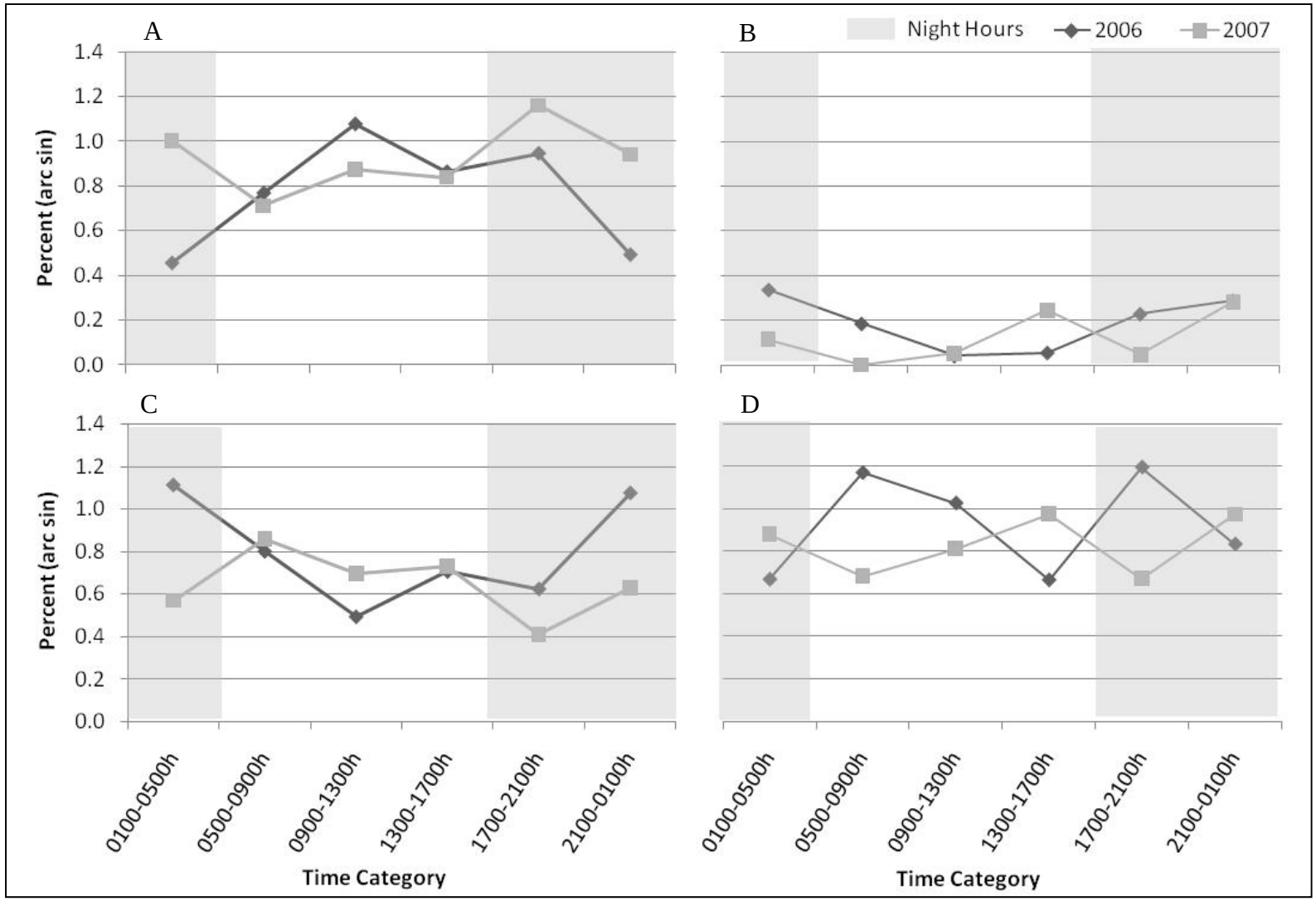


Figure 2.3: 2006 and 2007 diel trends of percent of Males (A), H3 (B), F (C) and MRR (D) that exhibited a significant time interaction with year (Table, 2.3, Table 2.5). Night hours are shaded.

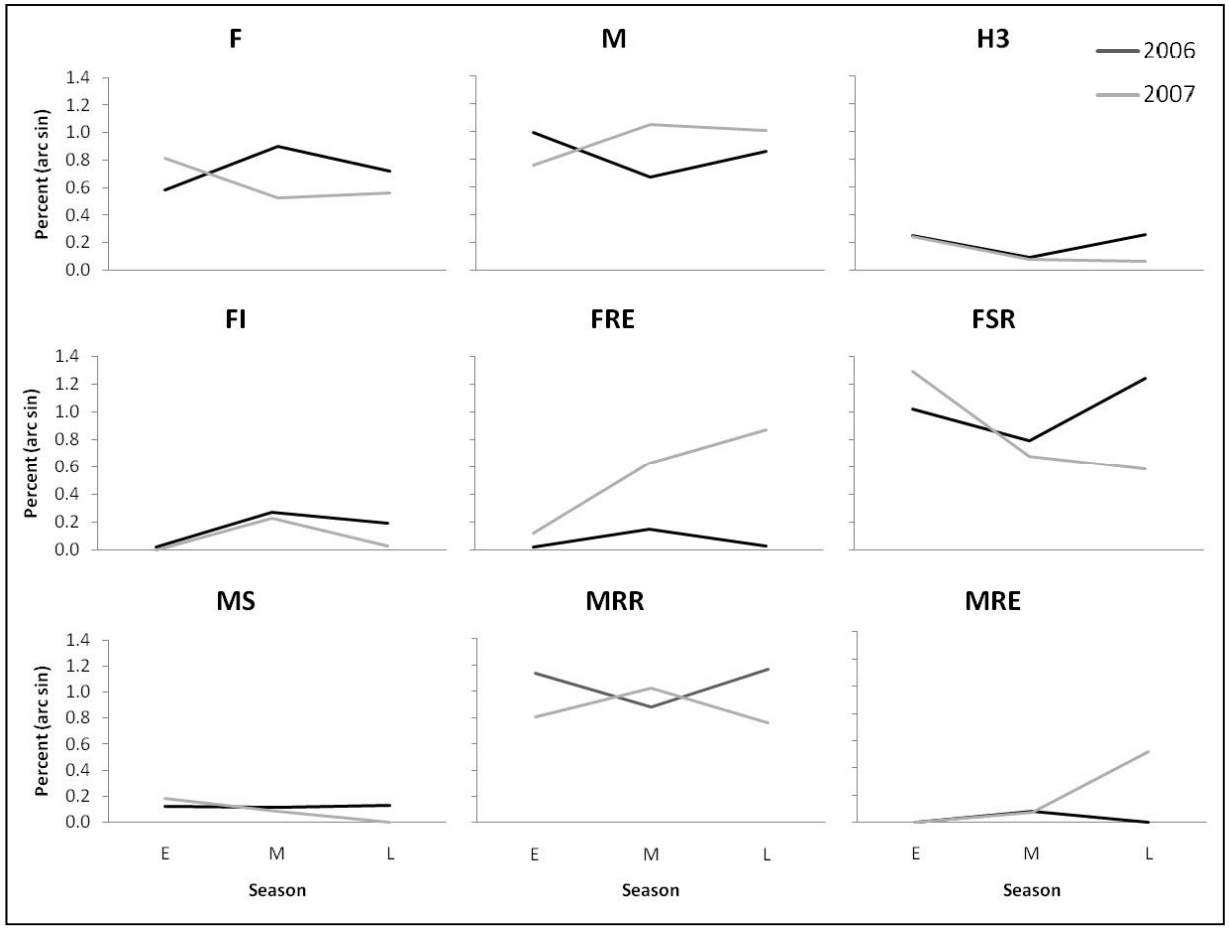


Figure 2.4: Season trends for 2006 and 2007 of variables which exhibited a significant season within year difference (Table 2.3).

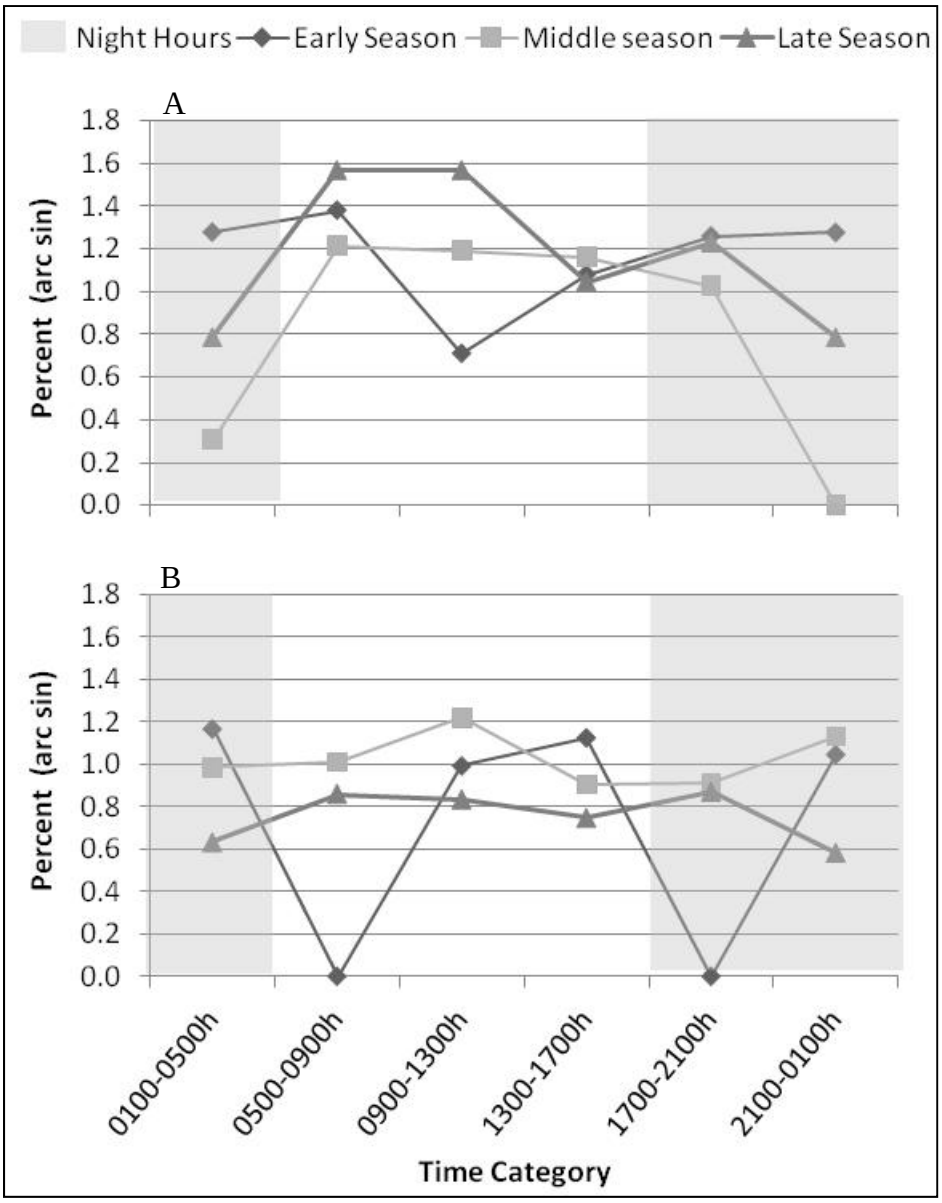


Figure 2.5: Diel patterns in 2006 (A) and 2007 (B) percent MRR across season. Night hours are shaded.

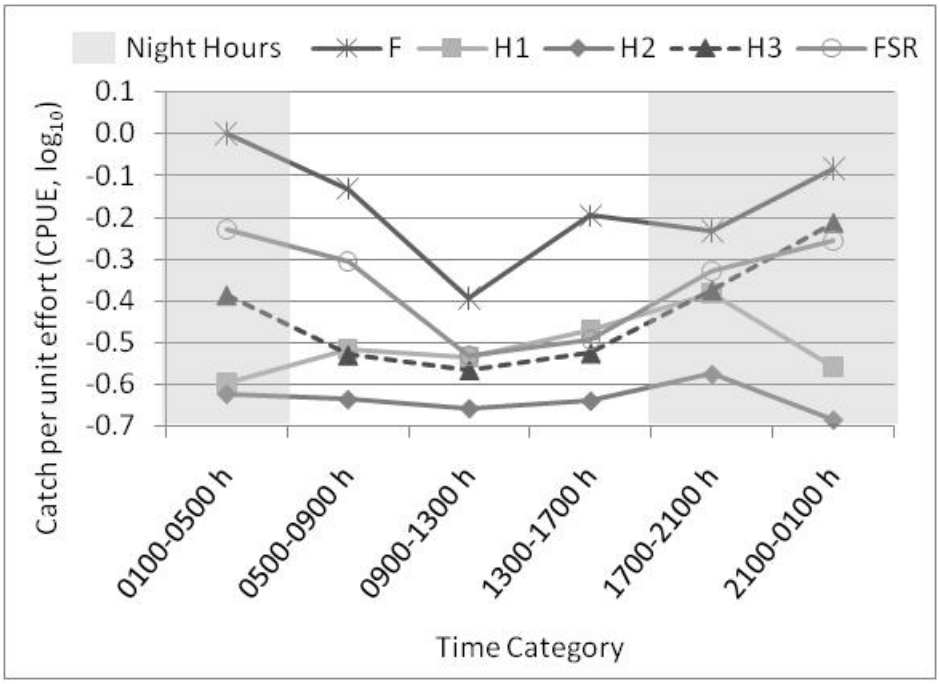


Figure 2.6: The diel trend in the mean CPUE (logarithm) of female (F), female maturity stages H1, H2, H3 and female spawning ready fish (FSR). The CPUE of stage H1 females exhibited a quadratic trend with values increasing during the day and decreasing at night (quadratic polynomial test, P= 0.0147, Table 2.7). The CPUE of stage H2 females exhibited a cubic polynomial trend with values changing twice across twenty-four hours. (cubic polynomial test, P=0.0107, Table 2.7). The CPUE of H3 females exhibited a quadratic trend with values significantly higher during the night (quadratic polynomial test, P= 0.0438, Table 2.7). The CPUE of FSR and F exhibited a quadratic trend and day night difference with values gradually increasing to high at night and then decreasing to low values during the day (FSR: quadratic polynomial test, P=0.0017, day vs. night contrast test, P= 0.0078, F: quadratic polynomial test, P=0.0012, day vs. night contrast test, P= 0.01, Table 2.7). Night hours are shaded.

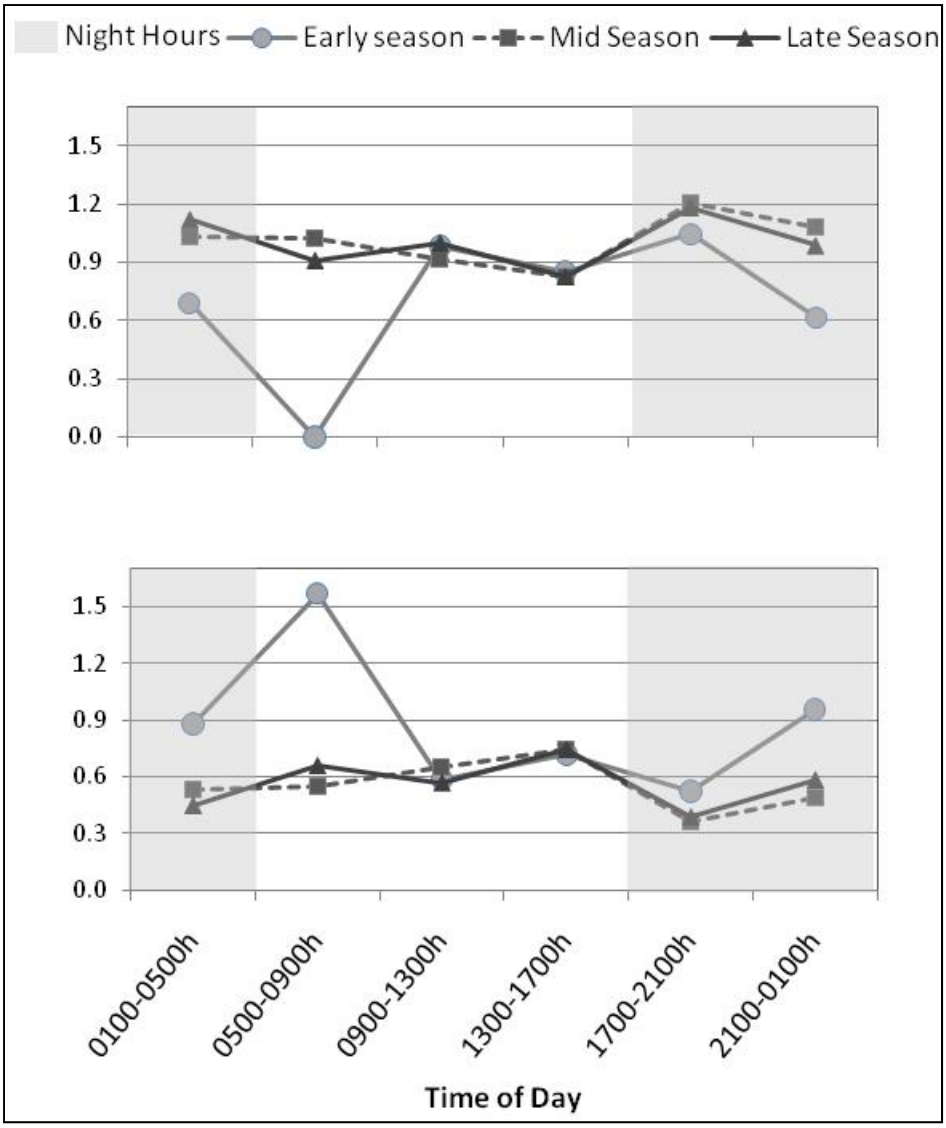


Figure 2.7: Diel trends by season for 2007 Males (A) and Females (B). Night hours are shaded.

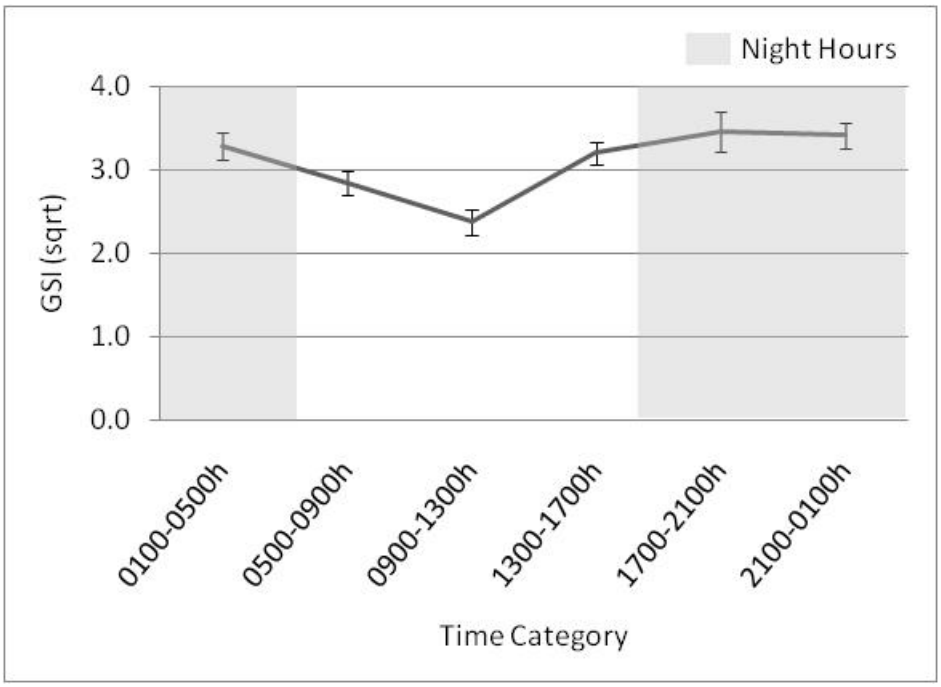


Figure 2.8: Mean GSI (square root) by time of day, based on samples from developing (D) and spawning ready females (H1, H2 and H3) for each time category. GSI was very significantly (<.0001) influenced by time category exhibiting a quadratic and cubic polynomial response as well as very significant day versus night difference with GSI values significantly higher in night time categories. Night hours are shaded. Vertical bars are one standard error of the mean.

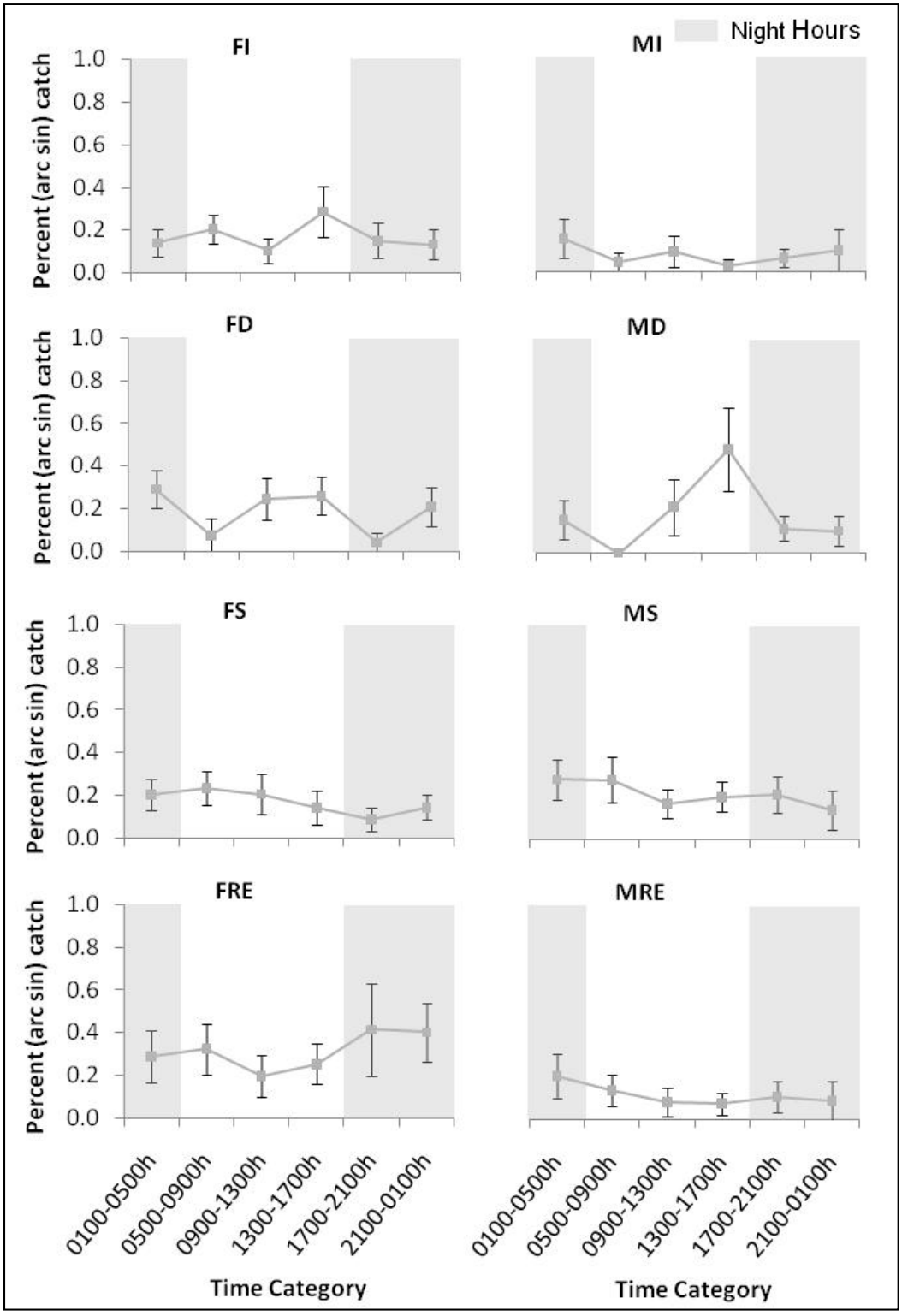


Figure 2.9: The diel trend of percent (arc sin square root) non-spawning maturity stages for both sampling years combined. No stage showed a significant result for any test for diel effects (Table 2.3).

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