1 **Title:** Spermatogenesis, reproductive maturation, and spawning seasonality of male winter
2 flounder, *Pseudopleuronectes americanus*: comparisons among fishery stocks

3

4 **Authors:** Emilee K. Tholke[1, 2], W. David McElroy[1, 2], and Richard S. McBride[2]

5 [1]Integrated Statistics, Woods Hole, MA, under contract with NOAA Fisheries (National
6 Marine Fisheries Service)

7 [2]National Marine Fisheries Service, Northeast Fisheries Science Center, 166 Water Street,
8 Woods Hole, MA 02543 USA

9 Corresponding author: Emilee.Tholke@noaa.gov, *(508) 495-2079*

Abstract

Winter flounder, *Pseudopleuronectes americanus*, were collected from three fishery stocks in the United States (U.S.) during 2009-2013 to describe spermatogenesis. Spermatogenesis included rapid, synchronous transitions during autumn from mitotic spermatogonia to meiotic spermatocytes, then to haploid spermatids, and the release of spermatozoa into lobule lumen. As these transitions occurred several months prior to the spring spawning season, maturity of the testes are a poor indicator of spawning season, however, individuals can be readily evaluated prior to the spawning season for evidence of skipped spawning. Throughout this process, the gonad weight increased dramatically, with spawning-season maxima from 11-17% relative to individual somatic weight, suggesting considerable reproductive investment relative to other flatfishes. Fish condition also cycled seasonally, which indicated that males follow a capital breeding pattern that has been reported for female winter flounder. The timing of spermiogenesis was very similar in the fish among each U.S. stock (Southern New England [SNE], Georges Bank [GB], and the Gulf of Maine [GOM]) and published data from coastal Newfoundland, even though U.S. winter flounder spawned earlier in spring than fish in the Canadian stock. Male maturity varied in relation to both size and age, and over time, in patterns similar to those reported for female winter flounder, but at smaller and younger sizes than females. These intra-specific variations in reproductive seasonality, maturation, and skipped spawning suggest that winter flounder have the potential to adaptively respond to a dynamic environment in a region where ocean warming is occurring rapidly.

Keywords: North Atlantic Ocean; flatfish; male maturity; geographic variation; skipped spawning; gonad histology

1. Introduction

Understanding fish gametogenesis, the order and timing of mitotic and meiotic events, has widespread relevance to fisheries science. In aquaculture, such basic knowledge is essential for improving current practices or expanding the diversity of cultured species (Alavi et al., 2012; Chauvigné et al., 2017). In harvest fisheries, management advice is often based on an interpretation of a population's reproductive potential, typically using a maturation schedule to characterize female spawning stock biomass (SSB) (Lowerre-Barbieri et al., 2016; Saborido-Rey and Trippel, 2013). The frequent use of female SSB proxy estimates may occur for the sake of model simplicity, but more legitimately because it is assumed that producing eggs is more costly than producing sperm, and thereby more likely to be a limiting condition (Bateman's principle). It may also occur because of a lack of evidence regarding a complex mating system or sex-specific traits, but when this is not true or when no evidence exists, then this should warrant greater attention to males (McBride et al., 2015; Morgan, 2008). For flatfishes, attention to the reproductive traits of both sexes appears justified and may improve predictions about fisheries productivity (Morgan et al., 2011; Trippel, 2003).

Sex-specific traits are well described among flatfishes. Size dimorphism is evident in nearly all flatfishes, such that females grow faster and to larger size than males (Cunningham, 1900; Imsland et al., 1997; Rijnsdorp et al., 2015). Although it is accepted that males mature at a smaller size and younger age than females, there are few data to support this (Rijnsdorp et al., 2015). Other morphological differences between sexes, such as scale development, have

been observed and postulated to be adaptive for spawning (Cunningham, 1900; Tomiyama, 2013). Sexual dimorphism in flatfish can result in sex-specific exploitation rates and in fisheries-induced changes to life-history characters, which can have implications for the biological realism of SSB estimates, our understanding of mating behavior, and predictions of recruitment (Rijnsdorp et al., 2010).

Knowledge about fish reproductive biology is concentrated among the more economically important fishery species, particularly at northern latitudes (McBride et al., 2015; Rijnsdorp et al., 2015). Among these well-studied flatfishes is the winter flounder *Pseudopleuronectes americanus*, which supports commercial and recreational fisheries in the western North Atlantic Ocean. The United States (U.S.) manages this species as three stocks – Gulf of Maine (GOM), Southern New England/Mid-Atlantic (SNE), and Georges Bank (GB) (NEFSC, 2017). The GB stock is a shared stock managed jointly with Canada. Canada manages three additional stocks (DeCelles and Cadrin, 2011; McBride, 2014). A broad framework of knowledge, across a broad latitudinal range, exists regarding winter flounder: in terms of seasonal migrations, habitat use, spawning behavior, and sex-specific reproductive traits (Fairchild et al., 2013; Kennedy and Steele, 1971; Pereira et al., 1999; Press et al., 2014; McElroy et al., 2013; Stoner et al., 1999; Wuenschel et al., 2009).

Annual oocyte development, spawning seasonality and hormonal cycles have been well researched for winter flounder (Burton and Idler, 1984; Dunn and Tyler, 1969; Dunn, 1970; Harmin et al., 1995; Ng et al., 1980; Press et al., 2014), and at least relative to other species, male winter flounder traits are fairly well understood. Some characterization of the spawning behavior of both sexes, such as promiscuous mating from sunset to midnight, has also been described (Kennedy and Steele, 1971; Stoner et al., 1999). Moulton and Burton

(1999) present cytological details of the testis structure and the rapid process of spermiogenesis (the transformation of spermatids into spermatozoa) and spermiation (the release of spermatozoa into the lobular lumen) during autumn, which occurs several months prior to spawning. For both sexes, maturation schedules have been described, but there has not been any recent detailed (~25 years) study of males in U.S. waters (O'Brien et al., 1993; Witherell and Burnett, 1993).

Of particular interest when characterizing reproductive potential for winter flounder is the frequency of reproductively mature but inactive spawners (skipped spawning), which has been recognized to occur in both sexes (Burton, 1994; Tyler and Dunn, 1976). Winter flounder skipped spawning was experimentally demonstrated as a response to energetic status in the post-spawning period, for mature females (Burton 1994; reviewed by Rideout and Tomkiewicz, 2011). Female skipped spawning rates in U.S. stocks, typically less than 2%, are lower than the northern GOM (11.5%; McBride et al., 2013; McElroy et al., 2013; Press et al., 2014). Skipped spawning rates for male winter flounder in Newfoundland waters range from 5- 35% (Burton, 1999), but none have been examined for U.S. waters for males, which we address herein.

Although specific data gaps exists, this level of information makes male winter flounder an excellent candidate for broad geographic comparisons, which could lead to better predictions about how marine species respond to dynamic environments (Brander, 2015; Pankhurst and Munday, 2011). Plasticity in maturity schedules among fish stocks and geographic regions has been previously documented for female winter flounder (McBride et al., 2013; Winton et al., 2014) as well as other species like, American shad, *Alosa sapidissima* and Hogfish, *Lachnolaimus maximus* (Collins and McBride, 2015; Leggett and Carscadden,

1978). Recent use of gonad histology to study U.S. female winter flounder stocks has facilitated comparisons among stock regions, demonstrating that (1) spawning season occurs earlier for southern stocks, (2) maturity is more related to size rather than age, being younger in the south, (3) fecundity is highest in the most southern stock, and (4), skipped spawning rates are lower for females in southern stocks (McBride et al., 2013; McElroy et al., 2013; Press et al., 2014; Winton et al., 2014). Such spatial variation in age at maturity and skipped spawning rates suggest that these life history traits are plastic for female winter flounder. Documenting such plasticity at a stock-specific level is the first step to understanding if such plasticity could be adaptive to climate change.

Paternity can matter, so this study attempts to describe male reproductive traits in a complementary manner as has been done for females (Press et al., 2014). A gonad histology approach is emphasized to describe: the testis structure of winter flounder, its year-round seasonal reproductive cycle at the cytological and population level, and relative rates of maturity and skipped spawning among two U.S. managed stocks and the jointly managed GB stock. Although the sampling domain is largely limited to U.S. shelf waters, where possible, these new results will be compared to published results for Canadian stocks. We also consider how such plasticity should allow winter flounder to respond successfully to climate change, at least within portions of its range but not throughout its historical range.

2. Methods

2.1 Fish collection and processing

A total of 602 male winter flounder were collected (total length, TL range 101 – 543 mm) monthly from December 2009 to May 2011(Table 1.) from the following sources: the

Northeast Fisheries Science Center, Northeast Cooperative Research Program (NEFSC-NCRP) Study Fleet (n = 264) and other NEFSC – NCRP studies (n = 66); the NEFSC Ecosystem Survey Branch bottom trawl survey (n = 107); the Massachusetts Department of Fish and Game, Division of Marine Fisheries bottom trawl survey (n = 132); the Connecticut Department of Environmental Protection bottom trawl survey (n = 23); and the University of Rhode Island, Graduate School of Oceanography bottom trawl survey (n = 10). Supplemental samples were used to fill in specific temporal gaps (May 2012 and March, April and May 2013). Geographically, fish were from three stock areas: GOM, GB, and SNE (Figure 1). Due to logistical constraints, fish were unable to be collected from GB during the months of February, September, and December.

Some fish (n = 483) were saved at sea and transferred back to the laboratory where they were processed within 24 hours to insure the freshness of the reproductive tissue. Total length (TL ± 1mm), total body mass (BM ± 0.001g), total gonad (testis) mass (GM ±0.001g), and a macroscopic maturity stage were recorded. A subset (n = 119) of the total amount of fish used were processed while at sea (TL ± 5 mm, BM and GM ± 1g). Otoliths were removed and stored dry in envelopes until processed for age determination. A section of gonad tissue, no larger than 1 cm^3, was excised from the middle of one testis, fixed in 10% buffered formalin for histological processing.

The gonadosomatic index (GSI) was calculated for each fish as: GSI = GM / (BM-GM) × 100. Relative condition (K_n) was calculated for each fish as the ratio of the observed mass over the predicted body mass (Le Cren, 1951) using a reference TL-BM equation determined from all males sampled. This predicted equation was fit using a log-transformed least squares regression: $\ln(BM_{TF}) = -10.773 + 2.892 \ln(TL)$, ($n$ = 566, SE a = 0.130, SE b

144 = 0.023, r^2 = 0.97), where a testis-free body mass (BM_{TF}) was used to examine changes in
145 condition independent of testicular development.

146 ***2.2 Aging Methods***

147 Ages for 426 males were determined. Whole otoliths were examined in water against a
148 black background through a dissecting microscope within a range of 1.25 - 1.6× magnification
149 using reflected light. Ages were determined by counting alternate hyaline (translucent) and
150 opaque zones from the otolith core to the margin (outer edge). Winter (translucent) bands
151 appeared dark while summer (opaque) bands appeared white (Penttila and Dery, 1988).

152 For whole otoliths in which the determined age was 5 years or greater, thin sections
153 were also made. Otoliths were mounted in a wax medium with the core marked, and a
154 transverse section was taken using a low speed saw with double diamond blades. Sections
155 were read under a dissecting microscope at a magnification range of 3.2- 4.0× using reflected
156 light. Using the methodology noted by Penttila and Dery (1988), age determination was made
157 counting hyaline and opaque zones from the core.

158 ***2.3 Histology processing and staging***

159 The fixed testis sample were removed from formalin after one month and prepared
160 using standard paraffin embedding techniques (McBride et al., 2013). The tissue was
161 sectioned using a rotary microtome set to 5µm, stained with Schiffs-Mallory trichrome, and
162 mounted on microscope slides. In the lab, slides were projected onto a monitor with a digital
163 camera and viewed (40 – 100x).

164 Terminology and description of stages of spermatogenesis followed Grier et al. (2009)
165 and previous work on winter flounder (Moulton and Burton, 1999). Six germ cell stages were

observed: primary (Spg1) and secondary (Spg2) spermatogonia, primary (Spcy1) and secondary (Spcy2) spermatocytes, spermatids (Sptd), and spermatozoa (Sp). The "most advanced sperm cell stage" (MASS), along with earlier spermatogenic stages present, were all recorded. Later, it was determined that distinguishing between primary and secondary – for both spermatogonia and spermatocytes – did not affect assignment of class or characterization of seasonality, so these were grouped for all analyses. Additional histological observations recorded to determine individual maturity were: amount of interstitial tissue (referred to as an 'interlobular region' by Moulton and Burton [1999]); presence of a sperm duct and sperm within the duct; and tunica thickness (µm) and complexity (Table 2). The tunica was categorized as simple or complex. Simple tunica lacked defined smooth muscle, did not have notable vascularization, and typically ranged from 10 µm to 35 µm (n = 45 measurements). Complex tunica had a multi-layered appearance with defined smooth muscle, visible vascularization within, and it was typically >15µm during the spring, when maturity classes were readily distinguished.

2.4 Maturation and skipped spawning

Maturation schedules were estimated by logistic regression to produce a predictive maturity ogive. Only fish from January to June were used, as this is when spawning fish were frequently observed across all stocks and mature fish were readily distinguished from immature fish. Assignment of maturity, which was based on histology and macroscopic characters, were simplified to a binary condition of immature or mature (mature = all classes not immature; Table 2). A generalized linear model was fit to this binary maturity data with a logit link function in R software (v.3.3.0, www.r-project.org) for both TL and age, including reporting the median age (A_{50}) and length (L_{50}) at 50% maturity. Stock differences were

explored by adding stock as just a main effect and with an interaction term. These models were evaluated relative to a base model with just length or age using second order Akaike information criterion, AICc (Anderson, 2008; using the aictab function in the AICcmodavg package). Model fit was also compared by looking at the proportional deviance explained, and specifically the adjusted form (adjusted D^2) accounting for the number of parameters and sample size (Guisan and Zimmermann, 2000; Dsquared function in modEvA package).

Skipped spawning rates were calculated by examining mature fish in months prior to the spawning season, and assigning individuals that had not entered spermiogenesis. This criterion was consistent with Moulton and Burton (1999). The months used to evaluate potential skipped spawners for all stocks were December through March (number of mature males during months examined for skipped spawners: GOM n = 34, SNE n = 67, and GB n = 23). These months were selected as spawning active fish could be readily distinguished from non-spawning individuals; nearly all fish had sperm present and the median GSI was elevated (approximately 5% or above). As skipped spawners are mature but inactive, they would have complex tunica, spermatogenic cells not advanced past primary or secondary spermatogonia, and no sperm present in lobules or the duct.

3. Results

3.1 Spermatogenic stages

All stages of spermatogenesis were observed, from mitotic proliferation of spermatogonia to spermiation. Primary spermatogonia were the largest germ cell type (range 5.4µm – 10.4µm, mean 6.9µm,n = 50). They appeared in all maturity classes; whereas, secondary spermatogonia were an ephemeral stage and were of limited use for characterizing

maturity classes. Spermatogonia initially appeared as single cells, and later, organized in cysts surrounded by interstitial tissue (Figure 2, A1). Seminiferous development was relatively homogeneous within the testes of all maturity classes, except late in the developing class, where many cell stages were present (Figure 2, B). Spermatozoa were widespread in the lobule lumen and the efferent duct, among spawning classes (Figure 2, C and D). Residual sperm was commonly observed in the lobules among post-spawning classes (Figure 2, E and F1). Spermatozoa were present in nearly all mature classes, but absent in later stage resting fish (Figure 2, F2).

3.2 Maturity classification

The MASS and additional histological criteria alone were not enough to distinguish all mature classes. Therefore, macroscopic characters were incorporated in the definitions of some mature classes (Table 2); however, immature males were defined using only microscopic criteria. Throughout the testes of immature males, primary spermatogonia dominated the testes, the tunica was simple, and a continuous matrix of interstitial tissue occurred (Figure 2, A1 and A2).

The developing class was dynamic, and best defined by the presence of spermatocytes (Table 2). Depending on where in the developing stage the male was captured, the testes were either dominated by spermatocytes, or contained a mosaic of all developmental cell stages (Figure 2, B). The crypt structure containing the meiotic cells was continuous along the germinal epithelium lining the interstitial tissue, and the tunica appeared simple, as the gonad size rapidly expanded.

Differentiating ripe from ripe and running microscopically posed a challenge. In both classes, spermatids and fully developed spermatozoa dominated the testes (Figure 2, C and D). Small nests of primary spermatogonia were apparent, but crypts of meiotic germ cell stages no longer lined the interstitial tissue. The sperm duct was not always present in the histology section; therefore, using sperm presence in the duct could not be used as a defining characteristic. Ripe and running was best differentiated only by macroscopic evaluation, i.e. the flow of milt from the vent (Table 2), from ripe fish. The limited presence of non-continuous interstitial tissue and a high GSI discriminated ripe fish from the spent class.

Spent testes had an open lumen and lots of continuous interstitial tissue (Figure 2, E). Unreleased residual sperm was still the MASS, but nests of primary spermatogonia were clearly evident. The intermediate GSI of spent fish, relative to ripe and resting fish, further discriminated them (Table 2). At the beginning of the resting stage (Figure 2, F1); residual spermatozoa in the lumen were still evident but greatly reduced. Later in the resting stage (Figure 2, F2) the testes had continuous interstitial tissue, primary spermatogonia were the most abundant germ cell stage, the GSI was low compared to spent fish, and the tunica was complex distinguishing it from immature fish.

3.3 Seasonality

At the cellular level, spermatogonia were present in fish year round for all stocks (Figure 3 and 4). Spermatocytes, evidence of meiotic division, were observed in few males in spring but became more common as the MASS by summer, as early as July, and declined in frequency by October or November. Spermatids appeared starting in October or November

253 coincident with a rising GSI (Figure 4), and were followed quickly by spermiogenesis and
254 spermiation, with spermatozoa found in most males until the following summer.

255 At the class level, developing males dominated mature classes in autumn; spawning
256 classes (ripe, ripe and running) dominated in winter. Spent and resting fish were present
257 starting in winter, and resting fish dominate in spring and into summer (Figure 3).

258 The GSI followed the seasonal cycle of spermatogenesis, rising rapidly in late autumn,
259 staying high in winter, and falling later, winter into the spring (Figure 4 and 5). The GSI's
260 remained low from June to September in all stocks. For all stocks, median GSI was variable
261 but near peak levels in January and February, with few fish below 5% GSI (Figure 4).
262 Medians peaked at 6-9% across stocks, but fewer male fish in the GOM appeared to exceed
263 10%. The median GSI dropped starting in March, but the distribution of GSI's became broad
264 and bimodal as spawning itself peaked and the number of post-spawning fish increased
265 (Figure 3 and 5). By April the majority of fish had low GSI's in all stocks, as spent and resting
266 fish increased. The decline appeared slightly earlier and steeper for fish in SNE compared to
267 the fish in the GOM, as some ripe fish with elevated GSI's continued as late as May and June
268 in the GOM. From April to September the median GSI for all stocks was $\leq$ 2%.

269 The morphometric condition cycle reflected the seasonal build up and depletion of
270 energy related to the reproductive cycle. Condition achieved minima in spring (March-May,
271 later for GOM males) following spawning: median K_n = 0.91 in GB, 0.93 in GOM, 0.95 in
272 SNE (Figure 5). The fish in SNE declined in condition earlier (beginning in February and
273 March) and improved sooner (K_n > 1.0 in May) than the male fish in the GOM, which had K_n
274 < 1.0 from March until June and lowest in May. GB data were more limited, but were closer

to SNE fish in timing with median condition improving rapidly to above 1.0 in May. Fish from all three stocks achieved highest condition (median $K_n > 1.1$) in July and August, and remained high throughout the fall.

3.4 *Maturity and skipped spawning rates*

The size at maturity varied by stock: the L_{50} was smallest for males from the GOM (204 mm TL), higher in SNE fish (236 mm TL), and largest for GB fish (270 mm TL) (Figure 6). Very few mature fish below 200 mm TL were observed in the GOM and SNE, or below 250 mm in the GB stock. Among all stocks only one fish > 300 mm was classified as immature. Smaller sample size on GB, particularly for immature fish, contributed to lower confidence in parameter estimation and broader confidence intervals (Figure 6). Model evaluations demonstrated that including stock as a main effect was the best length model (Table 3, A); therefore individual stock-specific models and parameters were subsequently generated. The overall fit of the stock-specific length regressions were reasonable, and the fish in the GB stock had the steepest slope but highest standard errors around its parameters (Figure 6; Table 4, B). The SNE maturity at length was the most variable and the length model had the lowest amount of variation explained (D^2=0.40), the GB male model explained more variation (D^2 = 0.63), and the fish in the GOM was intermediate (D^2 = 0.50).

Stock-specific values of A_{50} ranged from 2.05 to 2.11 years, and few fish matured at age 1 or were immature past age 3 (Table 5; Figure 6). There was no evidence for stock improving the age model (Table 3, B). However, stock-specific age models are presented for comparison and to provide stock-specific information for use in assessments (Table 4, B). Age models explained less of the variation in maturity than length models. The fish in the three

297 stocks had similar maturity schedules, 99% mature by age 5, but there was some variation
298 around age 3 with a higher percentage maturity for the male fish in GB compared to the fish
299 in the two other stocks (Table 5).

300 No skipped spawners were found in any of the U.S. stocks. Some resting fish were
301 identified during those months, but residual sperm were present in the lobules or the sperm
302 duct so therefore were not classified as skipped spawners.

303 **4. Discussion**

304 Winter flounder have an unrestricted testis type, characterized by spermatogonia
305 distributed throughout the length of the lobules (Uribe et al., 2014). Spermiation in an
306 unrestricted testis type results in a non-continuous lining of interstitial tissue as the haploid
307 cells release from germinal crypts into each lobule lumen. This is a common testes type
308 among teleosts, including flatfish (García-López et al., 2005; Uribe et al., 2014; Weltzien et
309 al., 2002). Our description of lobule structure, as well as the strong synchrony of meiotic
310 divisions, agrees with Moulton and Burton (1999), who examined winter flounder from July-
311 October in coastal Newfoundland. Our year-round and more southerly collections afford new
312 temporal and spatial comparisons.

313 Most strikingly, male winter flounder in both Canada and U.S. stocks start the annual
314 cycle of testes development well in advance of spawning. Meiotic divisions were evident as
315 soon as the spawning season ended in spring, and spermatids were present with elevated
316 hormone levels by October and November (Harmin et al., 1995; Moulton and Burton, 1999).
317 Sperm within lobules was first detected in the fourth quarter (October-December) in both U.S.
318 (data herein) and Canadian (Moulton and Burton, 1999) stocks. Burton and Idler (1984), also

in Newfoundland, indicated that sperm was present as early as November and could be activated in January, even though winter flounder spawn later there than in the U.S. stocks. Sperm can be observed microscopically nearly year-round because residual sperm was observed with decreasing frequency in the post-spawning period until autumn, overlapping with the transition back to meiotic divisions of spermatocytes starting in late summer. Therefore, the presence of sperm is not a precise proxy for spawning season or maturity class. Although our terminology refers to a post-spawning 'resting' class, this is only in reference to spawning activity itself, because within the resting gonad, mitotic activity of spermatogonia is quite active (Uribe et al., 2014).

Winter flounder spawn at some of the lowest temperatures of the year (Rogers, 1976). Ripe individuals occurred as early as November, spawning peaked roughly February-April in all three U.S. stocks, and some males were ripe as late as June. The seasonality was offset later in the GOM relative to the other stocks. Ripe males overlapped, even extending before and after, the appearance of ripe females in these stock areas (January-May; Press et al., 2014). Although Moulton and Burton (1999) reported a rapid, early (autumnal) transition of spermiogenesis and spermiation, spawning in Newfoundland occurs later than the SNE and GB fish and is closer to the colder-water GOM stock in timing (March-June; Burton and Idler, 1984; Kennedy and Steele, 1971;). Moulton and Burton (1999) hypothesized that early and rapid meiotic development of the testes was adaptive, occurring prior to the winter fast, for energetic reasons. We are not aware of a rigorous test of such a hypothesis, but this pattern of spermatogenesis is similar in Newfoundland yellowtail flounder, *Limanda ferruginea*, a sympatric summer spawner that spermiates the previous autumn (Manning et al., 2004).

If GSI is a proxy for reproductive investment then it is interesting to note that male winter flounder are heaviest just prior to spawning, peaking at about 15% on average (data herein; Harmin et al., 1995; Stoner et al., 1999), whereas other male flatfishes have GSIs at 5% or less when spawning (Manning et al., 2004; Rijnsdorp et al., 2015). Copious amounts of sperm may be related to the spawning behavior of this species, which has been observed to involve multiple males per female (Stoner et al., 1999) and the volume of sperm needed to fertilize the eggs produced by a female, which ovulates all at once (i.e., total spawner; Burton and Idler, 1984; Press et al., 2014). Flatfish spawning behavior varies considerably between species, including courtship and territorial defense (Carvalho et al., 2003; Manabe et al., 2000), and a review of sex-specific GSI in relation to mating systems could benefit our understanding of these differences. The high values of male GSI are also an aspect of winter flounder being capital breeders (i.e., they store energy during summer and use that energy during the winter spawning period; McBride et al., 2015). Capital breeding is evident in males from cyclic and inverse patterns of male condition and GSI. Wuenschel et al. (2009) also report seasonal cycles in percent dry muscle mass (a proxy for energy density) and a hepatosomatic index in male and female winter flounder offshore of New Jersey, and such cycles are evident for female winter flounder in other regions (Burton and Idler, 1984; Harmin et al., 1995).

Winter flounder size and age at maturity vary by sex, when adjusted by stock (Table 6). Sex effects have been noted in many flatfish (Fitzhugh et al., 2008; Imsland et al., 1997; Rijnsdorp et al., 2015), including winter flounder. Kennedy and Steele (1971) reported that male winter flounder in coastal waters of Newfoundland mature at a smaller size and at a younger age than females. However, elsewhere in Canada, Beacham (1982) found limited

differences between the sexes, particularly in the southern most region examined in that study (Northwest Atlantic Fisheries Organization [NAFO] region 4X). Our histology-based results can be compared well to other recent, histology-based studies of female winter flounder from U.S. stocks, indicating that males mature at a smaller size and age than females when separated by stock area (McBride et al., 2013; Winton et al., 2014). Macroscopic assessments of maturity reported limited sex-specific differences, < 20 mm TL, in the 1980s (SNE and GOM: Witherell and Burnett, 1993; SNE, GOM, and GB: O'Brien et al., 1993).

Winter flounder size and age at maturity also varies over time. In the 1980s, male fish in SNE and GOM had larger L_{50}s (~40-80 mm) and were older (~ 1yr) than now. For male fish on GB, the L_{50}s were smaller and younger compared to fish in SNE and GOM in the 1980's; similar L_{50}s are evident today (Table 6). Beacham (1982) found a declining trend in the size at maturity in southern Canada (NAFO region 4X) between 1959-1964 and 1975-1979 (Table 6). At a practical level, these changes indicate the dynamic nature of maturity schedules, and why maturity for some fishery stocks is modeled as a moving average, e.g. in the GB winter flounder assessment (NEFSC, 2011). At a more mechanistic level, changes in the size at maturity could be related to year-class strength, fishing pressure, environmental conditions, and habitat quality (Kulaw et al., 2017; McBride et al., 2015; Roff, 1982). Species like winter flounder have the time-series of data to evaluate how these multiple factors influence both short- and long-term changes in these traits. The data-rich situation for winter flounder suggest that a strategic approach to continued monitoring of this species – across sexes and stocks – may yield insight into these population dynamics.

Size at maturity was related to U.S. stock of origin, whereas age at maturity was not. This result is consistent with the earlier studies for the two inshore U.S. stocks, which had

nearly identical A_{50}'s in the 1980's (Table 6). The maturity differences by fish length reflect the differences in growth among the stocks: slower in the GOM and faster on GB (NEFSC, 2011). Comparison among regional studies is complicated by temporal change and methodological differences. In general, most of the Canadian studies have L_{50}'s closer to the GOM stock currently, but at an older age (Table 6), consistent with slower growth in the colder waters. Beacham (1982) compared three regions within Canada in the later 1970's, and also found the smallest length at maturity in the northern most region (Gulf of St. Lawrence) at 188 mm (Table 6). Differences in age at maturity among stocks of male and female winter flounder have been attributed to lower available energy (i.e., shorter growing season) at higher latitudes (Burton and Idler, 1984; Moulton and Burton, 1999). However, Rijnsdorp et al. (2015) provides examples of flatfish maturation schedules correlating and not correlating with latitude. Such dynamics are of considerable interest in a warming ocean, but their interpretation requires simultaneous consideration of growth and mortality rates, best examined with a probabilistic maturation reaction norm approach (Rijnsdorp et al., 2015), for which these data in the current study was insufficient.

Sub-stock level variation in the size at maturity for male winter flounder is likely. A few unusual fish were noted, particularly at the extreme northern and southern ends of the stock region, but the data is insufficient to examine for intra-stock variation, as done for female winter flounder in U.S. waters (McBride et al., 2013; Winton et al., 2014). Winton et al. (2014) uses generalized additive models to show female maturity varying considerably within both the SNE (by 84 mm) and GOM (by 122 mm) stocks. A spatial cluster analysis indicates three sub-regions within the SNE stock and six within the GOM stock region. A

more comprehensive data set for males would likely reveal a more fine-scale spatial model of maturation schedules comparable to that reported for females by Winton et al. (2014).

Because spermiation occurs well in advance of spawning, this creates a period preceding or early in spawning to distinguish delayed spawning from skipped spawning (Rideout and Tomkiewicz, 2011). The presence of residual spermatozoa, months after the spawning season, also distinguishes post-spawn fish from resting fish during the spawning season. We looked but skipped spawning by male winter flounder was not observed for any of the three U.S. stocks examined here. As reported elsewhere, male skipped spawning rates fluctuate widely across this species' range, as high as 35% in coastal waters of Newfoundland (Burton, 1994; Burton, 1999) to none observed in U.S. stocks (herein; Wuesnchel et al., 2009). Experimental studies linked skipped spawning to poor condition and reduced feeding during the period immediately after spawning (Burton, 1994; Burton, 1999), and condition measures for U.S. stocks have been found to be higher than in the colder water Canadian stocks (Plante et al., 2005; Wuenschel et al., 2009). We might expect the GOM stock was the most likely U.S. stock to exhibit male skipped spawning because it had the lowest condition and coldest waters of the U.S. stocks. However, none was observed currently; therefore we would expect with warming temperatures it will remain at or near zero in the foreseeable future. However, female skipped spawning rates were higher (11.5%) in the northern GOM than the southern GOM (2.7%, McBride et al. 2013), so we might expect changes with temperature in male skipped spawning could vary within this basin.

In terms of geographic stock dynamics, Nye et al. (2009) reports a conflicting pattern of shifting distributions of winter flounder stocks. Specifically, the center of distribution of the fish in the SNE stock has moved northward whereas the fish in the GOM stock has moved

southward. Nye et al. (2009) suggests that the geography and bathymetry of the GOM, as well as migratory behavior (from southern to northern stocks) could have contributed to the southward movement of the biomass centroid for some species, including winter flounder. From our perspective focused on reproductive biology of male and female winter flounder, we suggest changes in vital rates and therefore productivity as the primary mechanism. Warming in the southern region could be causing physiological stress for the portion of winter flounder at its southern distributional limit, such as offshore of New York and New Jersey, thereby decreasing growth or reproductive success (Bell et al., 2018; Wuenschel et al., 2009). This decreased productivity at the stock's southern range makes it look like the stock's center of distribution is moving northward, rather than the actual fish moving northward. The opposite response could be operating in the GOM where growth and maturity rates, along with other vital rates, may actually be improving with warming trends. Increased productivity could be driving the center of the biomass distribution southward, rather than the fish moving southward. The effects of temperature on productivity can be difficult to disentangle from other well recognized drivers, such as fishing pressure, larval dispersal, mortality, and recruitment (Adams et al., 2018; Baudron et al., 2014; Morgan et al., 2014), but here, we make two points: 1) demographic dynamics means that not all fish need migratory behaviors or large home ranges for their populations distributions to 'move,' and 2) the distributions and demographics of the 'cold-water' species may be independent between stocks. The maturity schedules presented herein, and elsewhere for females (Winton et al., 2014), serve as a basis to monitor the potentially adaptive stock-specific vital rates in response to a warming ocean.

Acknowledgements

We dedicate this paper to the memory of Harry Grier (Florida Fish & Wildlife Cons. Comm.) who inspired our interest in male fish reproduction and offered comments on an earlier draft. This study was funded by the National Oceanic and Atmospheric Administration (NOAA), Northeast Fisheries Science Center (NEFSC) and Northeast Cooperative Research Program (NCRP). Most samples were provided by the NCRP's Study Fleet. We appreciate the work of all the Study Fleet staff (especially J. Hoey, J. Moser, M. Ball, D. St. Amand, and G. Gianesin) and all the participating fishermen. We thank all the staff members of these various programs: the officers and crew of the NOAA research vessel H. B. Bigelow and the scientific staff of the Ecosystems Surveys Branch of the NEFSC; J. King, M. Camisa, V. Manfredi, and M. Symanski of the MADMF; J. Collie and A. Malek of URI; and K. Gottschall and D. Pacileo of the CTDEP. Finally, we thank M. Wuenschel (NEFSC) for contributions to the study design, data collection, condition analyses, and histological interpretations; Y. Press, M. Winton, and J. Dayton (NEFSC) for their contributions to data collection, protocol development, and histology processing; J. Burnett, G. Thornton, and E. Robillard (NEFSC) for ageing the samples; and E. Trippel (Canada-DFO) for sharing information about interpreting gonad histology, Professor Kenneth Oliveira from the University of Massachusetts Dartmouth for constructive feedback on an earlier draft and to the two anonymous reviewers for their constructive suggestions. Mention of any products is for descriptive purposes and does not indicate endorsement by NOAA Fisheries.

References

Adams, C. F., L. A. Alade, C. M. Legault, L. O'Brien, M. C. Palmer, K. A. Sosebee, M. L. Traver, 2018. Relative importance of population size, fishing pressure and temperature on the spatial distribution of nine Northwest Atlantic groundfish stocks. PLoS One. 13(4). https://doi.org/10.1371/journal.pone.0196583.

Alavi, S.M.H., Rodina, M., Gela, D., Linhart, O., 2012. Sperm biology and control of reproduction in sturgeon: (I) testicular development, sperm maturation and seminal plasma characteristics. Rev. Fish Biol. Fish. 22, 695-717. Doi: 10.1007/s11160-012-9268-4

Anderson, D.R., 2008. Model based inference in the life sciences: a primer on evidence. Springer Science & Business Media LLC, New York.

Baudron, A.R., Needle, C.L., Rijnsdorp, A.D., Tara Marshall, C., 2014. Warming temperatures and smaller body sizes: synchronous changes in growth of North Sea fishes. Global Change Biol. 20, 1023-1031. http://dx.doi.org/10.1111/gcb.12514.

Bell, R. J., A. Wood, J. A. Hare, D. E. Richardson, J. P. Manderson, T. J. Miller, 2018. Rebuilding in the face of climate change. Can. J. Fish Aquat. Sci. 75, 1405-1414. https://doi.org/10.1139/cjfas-2017-0085.

Beacham, T.D., 1982. Biology and exploitation of winter flounder (*Pseudopleuronectes americanus*) in the Canadian Maritimes area of the Northwest Atlantic Ocean. Can. Tech. Rep. Fish. Aquat. Sci. No.1113.

Brander, K., 2015. Improving the reliability of fishery Predictions under climate change. Curr. Clim. Change Rep. 1, 40-48. Doi: 10.1007/s40641-015-0005-7.

Burnett, J., O'Brien, L., Mayo, R.K., Darde, J.A., Bohan, M., 1989. Finfish maturity sampling and classification schemes used during Northeast Fisheries Center bottom trawl surveys, 1963-89. NOAA Tech. Mem. NMFS-F/NEC-76, 14 p. http://nefsc.noaa.gov/publications/tm/pdfs/tmfnec76.pdf.

Burton, M.P.M., 1994. A critical period for nutritional control of early gametogenesis in female winter flounder, *Pseudopleuronectes americanus* (Pisces: Teleostei). J. Zool., London. 233, 405-415. Doi: 10.1111/j.1469-7998.1994.tb05273.x.

Burton, M.P.M., 1999. Notes on potential errors in estimating spawning stock biomass: determining the effect of non-participatory adults for some groundfish species. J. Northwest Atl. Fish. Sci. 25, 205-213.

Burton, M.P., Idler, D.R., 1984. The reproductive cycle in winter flounder, *Pseudopleuronectes americanus* (Walbaum). Can. J. Zoolog. 62, 2563-2567. https://doi.org/10.1139/z84-374.

Carvalho, N., Afonso, P., Serrao Santos, R., 2003. The haremic mating system and mate choice in the wide-eyed flounder, *Bothus podas*. Environ. Biol. Fisheries. 66, 249-258.

Chauvigné, F., Ollé, J., González, W., Duncan, N., Giménez, I., Cerdà, J., 2017. Toward

developing recombinant gonadotropin-based hormone therapies for increasing fertility in the flatfish *Senegalese sole*. PLoS One. 12(3). https://doi.org/10.1371/journal.pone.0174387.

Collins, A.B., McBride, R.S., 2015. Variations in reproductive potential between nearshore and off shore spawning contingents of hogfish in the eastern Gulf of Mexico. Fisheries Manag. Ecol. 22, 113–124. Doi:10.1111/fme.12102.

Cunningham, J., 1900. Sexual Dimorphism in the Animal Kingdom. Adam and Charles Black, London.

DeCelles, G.R., Cadrin, S.X., 2011. An interdisciplinary assessment of winter flounder (*Pseudopleuronectes americanus*) stock structure. J. Northwest Atl. Fish. Sci. 43, 103-120. Doi: 10.2960/J.v43.m673.

Department of Fisheries and Oceans Canada (DFO), 2010. Size at sexual maturity and catch characteristics of the yellowtail and winter flounder fishery in the Magdalen Islands. DFO Can. Sci. Advis. Sec. Sci. Resp. 2009/020, 19.

Dunn, R.S., 1970. Further evidence for a three-year oocyte maturation time in the winter flounder (*Pseudopleuronectes americanus*). J. Fish. Res. Board Can. 27, 957-960.

Dunn, R.S., Tyler, A.V., 1969. Aspects of the anatomy of the winter flounder ovary with hypotheses on oocyte maturation time. J. Fish. Res. Board Can. 26, 1943-1947.

Fairchild, E.A., Siceloff, L., Howell, W.H., Hoffman, B., Armstrong, M.P., 2013. Coastal spawning by winter flounder and a reassessment of essential fish habitat in the Gulf of Maine. Fish. Res. 141, 118-129. Doi: 10.1016/j.fishres.2012.05.007.

Fitzhugh, G.R., William A. Fable, Jr, Trent, W.L., 2008. Life history parameters of gulf flounder (*Paralichthys albigutta*) from northwest Florida. Gulf Mex. Sci. 26, 100-117.

García-López, A., Martìnez Rodríguez, G., Saraquete, C., 2005. Male reproductive system in Senegalese sole *Solea senegalensis* (Kaup): Anatomy, histology and histochemistry. Hist. Histopathol. 20, 1179-1189.

Grier, H.J., Uribe Aranzábal, M.C., 2009. The testis and spermatogenesis in teleosts, in: Jamieson B.G.M., ed, Reproductive Biology and Phylogeny of Fishes (Agnathans and Bony Fishes) Volume 8, Part A of Series: Reproductive Biology and Phylogeny. Science Publishers, Enfield, New Hampshire, pp. 119-142.

Guisan, A., Zimmermann, N.E., 2000. Predictive habitat distribution models in ecology. Ecol. Modell. 135, 147-186. https://doi.org/10.1016/S0304-3800(00)00354-9

572
573 Harmin, S.A., Crim, L.W., Wiegand, M.D., 1995. Plasma sex steroid profiles and the seasonal
574 reproductive-cycle in male and female winter flounder, *Pseudopleuronectes*
575 *americanus*. Mar. Biol. 121, 601-610.
576 Doi: 10.1007/BF00349295.
577
578 Imsland, A.K., Folkvord, A., Grung, G.L., Stefansson, S.O., Taranger, G.L., 1997. Sexual
579 dimorphism in growth and maturation of turbot, *Scophthalmus maximus* (Rafinesque,
580 1810). Aquat. Res. 28, 101-114.
581
582 Kennedy, V., Steele, D., 1971. The winter flounder (*Pseudopleuronectes americanus*) in Long
583 Pond, Conception Bay, Newfoundland. J. Fish. Res. Board. Can. 28, 1153-1165.
584
585 Kulaw, D.H., Cowan, J.H., Jackson, M.W., 2017. Temporal and spatial comparisons of the
586 reproductive biology of northern Gulf of Mexico (USA) red snapper (*Lutjanus*
587 *campechanus*) collected a decade apart. PLoS One. 12(3).
588 https://doi.org/10.1371/journal.pone.0172360.
589
590 Le Cren, E.D., 1951. The length-weight relationship and seasonal cycle in gonad weight and
591 condition in the perch (*Perca fluviatilis*). J. Anim. Ecol. 20, 201-219.
592
593 Leggett, W.C., Carscadden, J.E., 1978. Latitudinal variation in reproductive characteristics of
594 American shad (*Alosa sapidissima*): Evidence for population specific life history
595 strategies in fish. J. Fish. Res. Board. Can. 35, 1469-1478.
596
597 Lowerre-Barbieri, S., DeCelles, G., Pepin, P., Catalán, I.A., Muhling, B., Erisman, B., Cadrin,
598 S.X., Alós, J., Ospina-Alvarez, A., Stachura, M.M., Tringali, M.D., Burnsed, S.W.,
599 Paris, C.B., 2016. Reproductive resilience: a paradigm shift in understanding spawner-
600 recruit systems in exploited marine fish. Fish Fish. 18, 285-312.
601 http://dx.doi.org/10.1111/faf.12180.
602
603 Manabe, H., Ide, M., Shinomiya, A., 2000. Mating system of the lefteye flounder,
604 *Engyprosopon grandisquama*. Ichthyol. Res. 47, 69-74.
605 Doi: 10.1007/bf02674315.
606
607 Manning, A.J., M. P. M. Burton, Crim, L.W., 2004. Puberty and recrudescence in cultured
608 male yellowtail flounder: plasma androgens and spermatogenesis. J. Fish Bio. 65,
609 1026-1046.
610 Doi:10.1111/j.0022-1112.2004.00507.x.
611
612 McBride, R.S., 2014. Managing a marine stock portfolio: stock identification, structure, and
613 management of 25 fishery species along the Atlantic coast of the United States. North
614 Am. J. Fish. Manage. 34, 710-734.
615 http://dx.doi.org/10.1080/02755947.2014.902408
616
617 McBride, R.S., Somarakis, S., Fitzhugh, G.R., Albert, A., Yaragina, N.A., Wuenschel, M.J.,

Alonso-Fernández, A., Basilone, G., 2015. Energy acquisition and allocation to egg production in relation to fish reproductive strategies. Fish Fish. 16, 23-57. Doi:10.1111/faf.12043.

McBride, R.S., Wuenschel, M.J., Nitschke, P., Thornton, G., King, J.R., 2013. Latitudinal and stock-specific variation in size- and age-at-maturity of female winter flounder, *Pseudopleuronectes americanus*, as determined with gonad histology. J. Sea Res. 75, 41-51. http://dx.doi.org/10.1016/j.seares.2012.04.005.

McElroy, W.D., Wuenschel, M.J., Press, Y.K., Towle, E.K., McBride, R.S., 2013. Differences in female individual reproductive potential among three stocks of winter flounder, *Pseudopleuronectes americanus*. J. Sea Res. 75, 52-61. Doi: 10.1016/j.seares.2012.05.018.

Morgan, M.J., 2008. Integrating reproductive biology into scientific advice for fisheries management. J. Northwest Atl. Fish. Sci. 41, 37-51. Dio:10.2960/J.v41.m615

Morgan, M.J., Perez-Rodriguez, A., Saborido-Rey, F., 2011. Does increased information about reproductive potential result in better prediction of recruitment? Can. J. Fish. Aquat. Sci. 68, 1361–1368. https://doi.org/10.1139/f2011-049.

Morgan, M.J., Shelton, P.A., Rideout, R.M., 2014. Varying components of productivity and their impact on fishing mortality reference points for Grand Bank Atlantic cod and American plaice. Fish. Res. 155, 64-73. Doi: 10.1016/j.fishres.2014.02.019.

Moulton, S., Burton, M., 1999. Histological observations on spermatogenesis in winter flounder, *Pleuronectes americanus*, Walbaum, from Conception Bay, Newfoundland, Canada. Can. J. Zool. 77, 1682-1689. https://doi.org/10.1139/z99-159

Northeast Fisheries Science Center (NEFSC), 2011. 52nd Northeast Reginal Stock Assessmnet Workshop: Assessment Report. NEFSC Reference Document, Woods Hole, MA. 11-17, 962pp.

Northeast Fisheries Science Center (NEFSC), 2017. Operational Assessment of 19 Northeast Groundfish Stocks, updated through 2016, US Dept. Commer. Northeast Fish Sci Cent Ref Doc. 17-17, 259pp. Doi: 10.7289/V5/RD-NEFSC-17-17

Ng, T.B., Idler, D.R., Burton, M.P., 1980. Effects of teleost gonadotropins and their antibodies on gonadal histology in winter flounder. Gen Comp Endocrinol. 42, 355-364.

Nye, J.A., Link, J.S., Hare, J.A., Overholtz, W.J., 2009. Changing spatial distribution of fish

stocks in relation to climate and population size on the Northeast United States continental shelf. Mar. Ecol-Prog. Ser. 393, 111-129.

O'Brien, L., Burnett, J., Mayo, R.K., 1993. Maturation of nineteen species of finfish off the northeast coast of the United States, 1985 - 1990. NOAA Tech. Rep. NMFS 113, pp. 72.

Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. Mar. Freshwater Res. 62, 1015-1026. Dio: 10.1071/MF10269.

Penttila, J., Dery, L.M., 1988. Age determination methods for northwest Atlantic species. NOAA Tech. Rep. NMFS. 72(25 September 2009), 135 pp.

Pereira, J.J., Goldberg, R., Ziskowski, J.J., Berrien, P.L., Morse, W.W., Johnson, D.L., 1999. Essential Fish Habitat Source Document: Winter flounder, *Pseudopleuronectes americanus*, life history and habitat characteristics. NOAA Tech. Mem. NMFS-NE-138, 39 pp.

Plante, S., Audet, C., Lambert, Y., de la Noüe, J., 2005. Alternative methods for measuring energy content in winter flounder. North Am. J. Fish. Manage. 25, 1-6. Dio: 10.1577/m03-104.1.

Press, Y.K., Wuenschel, M.J., McBride, R.S., 2014. Time course of oocyte development in winter flounder (*Pseudopleuronectes americanus*) and spawning seasonality for the Gulf of Maine, Georges Bank, and Southern New England stocks. J. Fish. Biol. 85, 421-445. Doi: http://dx.doi.org/10.1111/jfb.12431.

Rideout, R.M., Tomkiewicz, J., 2011. Skipped spawning in fishes: more common than you might think. Mar. Coast Fish. 3, 176–189. https://doi.org/10.1080/19425120.2011.556943.

Rijnsdorp, A.D., van Damme, C.J.G., Witthames, P.R., 2010. Implications of fisheries induced changes in stock structure and reproductive potential for stock recovery of a sex-dimorphic species, North Sea plaice. ICES J. Mar. Sci. 67, 1931-1938. http://dx.doi.org/10.1093/icesjms/fsq049.

Rijnsdorp, A.D., van Damme, C.J.G., Witthames, P.R., 2015. Ecology of reproduction, in: Gibson R.N., Nash R.D.M., Audrey J. Geffen, Veer H.W.v.d., eds, Flatfishes: Biology and Exploitation (2nd Ed). John Wiley & Sons, Ltd., UK, pp. 101-131.

Roff, D.A., 1982. Reproductive strategies in flatfish: a first synthesis. Can. J. Fish. Aquat. Sci. 39, 1686-1698.

Rogers, C.A., 1976. Effects of temperature and salinity on the survival of winter flounder embryos. Fish. Bull. 74, 52-58.

Saborido-Rey, F., Trippel, E.A., 2013. Fish reproduction and fisheries. Fish. Res. 138, 1-4. http://dx.doi.org/10.1016/j.fishres.2012.11.003.

Stoner, A.W., Bejda, A.J., Manderson, J.P., Phelan, B.A., Stehlik, L.L., Pessutti, J.P., 1999. Behavior of winter flounder, *Pseudopleuronectes americanus*, during the reproductive season: laboratory and field observations on spawning, feeding, and locomotion. Fish. Bull., U S. 97, 999-1016.

Tomiyama, T., 2013. Sexual dimorphism in scales of marbled flounder, *Pseudopleuronectes Yokohamae*, (Pleuronectiformes: Pleuronectidae), with comments on the relevance to their spawning behavior. J. Fish. Biol. 83, 1334-1343. Doi: 10.1111/jfb.12232.

Trippel, E. A. 2003. Estimation of male reproductive success of marine fishes. Journal of Northwest Atlantic Fishery Science **33**:81-113. http://journal.nafo.int/Portals/0/2003-Vol33/trippel.pdf

Tyler, A.V., Dunn, R.S., 1976. Ration, growth, and measures of somatic and organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis. J. Fish. Res. Board. Can. 33, 63-75.

Uribe, M.C., Grier, H.J., Mejía-Roa, V., 2014. Comparative testicular structure and spermatogenesis in bony fishes. Spermatogenesis. 4. Doi: 10.4161/21565562.2014.983400.

Weltzien, F.-A., Taranger, G.L., Karlsen, Ø., Norberg, B., 2002. Spermatogenesis and related plasma androgen levels in Atlantic halibut (*Hippoglossus hippoglossus L.*). Comp. Biochem. Physiol. A 132, 567–575.

Winton, M.V., Wuenschel, M.J., McBride, R.S., 2014. Investigating spatial variation and temperature effects on maturity of female winter flounder (*Pseudopleuronectes americanus*) using generalized additive models. Can. J. Fish. Aquat. Sci. 71, 1279-1290. Doi: 10.1139/cjfas-2013-0617.

Witherell, D.B., Burnett, J., 1993. Growth and maturation of winter flounder, *Pleuronectes americanus*, in Massachusetts. Fish. Bull., U.S. 91, 816-820.

Wuenschel, M.J., Able, K.W., Byrne, D., 2009. Seasonal patterns of winter flounder *Pseudopleuronectes americanus* abundance and reproductive condition on the New York Bight continental shelf. J. Fish. Biol. 74, 1508-1524. Doi: 10.1111/j.1095-8649.2009.02217.x.

753 **Table 1:** Immature and mature male winter flounder collected by month and stock area (Gulf of Maine [GOM], Georges Bank [GB] and southern
754 New England [SNE]). Sample size (*n*), total fish length (TL), and total body mass (BM) are summarized.

Stock Maturity		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total (*n*)
GOM	*n*	0	0	0	1	38	0	0	0	11	0	1	0	**51**
Immature	TL range (mm)				130	101-273				125-235		244		
	BM range (g)				24	13-257				24-174		201		
	n	8	11	12	30	65	2	6	2	11	12	14	3	**176**
Mature	TL range (mm)	279-366	305-351	265-365	190-347	116-419	288-298	276-365	337-345	223-353	170-338	270-368	320-346	
	BM range (g)	237-715	345-526	201-555	70-495	18-889	238-303	227-735	523-553	144-624	63-478	100-764	398-482	
GB	*n*	0	0	0	9	0	1	0	1	0	2	1	0	**14**
Immature	TL range (mm)				140-290		274		323		250-380	327		
	BM range (g)				29-284		271		421		160-655	442		
	n	6	0	17	20	11	21	3	6	0	9	6	0	**99**
Mature	TL range (mm)	396-437		338-503	230-530	330-384	310-543	406-473	354-480		290-490	345-420		
	BM range (g)	781-1159		456-1628	130-1590	438-643	359-1625	820-1033	527-1379		275-1355	505-915		
SNE	*n*	1	0	9	18	25	3	11	7	3	3	0	2	**82**
Immature	TL range (mm)	274		119-280	129-291	143-307	220-272	155-217	185-244	131-216	213-226		245-246	
	BM range (g)	238		17-255	24-328	35-438	152-277	46-134	84-180	29-118	107-131		174-184	
	n	9	11	39	21	38	18	6	3	5	13	9	8	**180**
Mature	TL range (mm)	259-357	308-358	170-403	167-355	212-369	236-398	180-340	208-394	238-295	209-310	225-321	209-310	
	BM range (g)	222-565	349-580	61-776	45-525	118-545	169-685	80-180	126-306	181-327	119-374	139-422	99-374	

Table 2: Macroscopic and microscopic characteristics of male winter flounder testes, specifying the criteria used to assign a maturity class. Macroscopic characters are modified from Burnette et al., (1989) and microscopic characters are adapted from Moulton and Burton (1999). (GSI, gonadosomatic index).

Maturity Class	Macroscopic characteristics	GSI	Microscopic characteristics	Staging criteria using macroscopic and microscopic characteristics
Immature (Im)	Testes triangular shape, endpoint is rounded and flattened. Extends back into body cavity. Translucent to gray in color.	mean: 0.3 range: 0.1 - 3.6 stdev: 0.5	Nests of primary spermatogonia. Continuous interstitial tissue throughout and a simple tunica.	No spermatozoa present and simple tunica.
Developing (De)	Testes enlarged and firm, very little or no milt present. Off white to gray in color.	mean: 2.85 range: 0.02 - 16.1 stdev: 3.67	Several germ cell stages present within the teste: Primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids and sometimes spermatozoa. Continuous interstitial tissue.	Primary and secondary spermatocytes present.
Ripe (Ri)	Testes now very large and robust, with a thick liquid consistency. Chalky white in color.	mean: 8.9 range: 2.1 - 28.3 stdev: 4.2	Spermatozoa encompass most of lobule area and some spermatids may be present. Small nests of primary spermatogonia present. Thin sections of non-continuous interstitial tissue and a simple tunica.	GSI >2% and spermatozoa present. Some or little non-continuous interstitial tissue.
Ripe and Running (RR)	Milt flows easily from vent with little or no pressure, once cut open milt flows easily. Very “jello- like” consistency. Chalky white in color.	mean: 6.4 range: 1.5 - 13.3 stdev: 3.2	Very similar to ripe stage. Mostly spermatozoa throughout, some spermatids still visible. Nests of primary spermatogonia present and a simple tunica.	Assigned using macroscopic criteria.
Spent (St)	Testes flaccid, not as robust as in ripe stage. Will contain residual milt. Edges and other parts of testes starting to turn gray in color.	mean: 1.6 range: 0.8 - 4.2 stdev: 0.98	Residual spermatozoa within lobules and the duct (if duct is present in section). Nests of primary spermatogonia more abundant than ripe and ripe and running stages. Open spaces with lots of thick interstitial tissue throughout. Complex tunica.	GSI between 0.8 and 2%. Or GSI > 2% if lots of continuous interstitial tissue present.
Resting (Re)	Testes shrunken in size and tightened up. Little to no residual milt. Yellowish, brownish, or gray in color.	mean: 0.4 range: 0.02 - 0.8 stdev: 0.2	Nests of primary spermatogonia more abundant. Sections of residual spermatozoa often present within lobules and the duct (if duct is present in section). Lots of interstitial tissue and complex tunica.	GSI <0.8% and complex multi-layered tunica.

Table 3: AICc model analysis comparing logistic regressions of male winter flounder maturity at length (A) and age (B). The base model (just length or age) was compared to models including stock both as a main (+) effect or an interaction term (*). The model evaluation output includes the number of estimable parameters (K), the second order Akaike's Information Criterion (AICc), change in the AICc between models (ΔAICc), the AICc weight (AICc Wt.), log-likelihood (LL), and adjusted proportion of the deviance explained (adjusted D^2).

A	Model Names	K	AICc	ΔAICc	AICc Wt.	LL	Residual Deviance	Adjusted D^2
	model TL + Stock	4	261.43	--	0.9997	-126.67	253.34	0.4749
	model TL * Stock	6	263.91	2.48	0.2244	-125.86	251.72	0.4759
	model TL	2	277.71	16.28	0.0003	-136.84	273.68	0.4353
B								
	model AGE	2	279.42	--	0.7984	-137.70	275.39	0.3895
	model AGE + Stock	4	282.18	2.75	0.2016	-137.04	274.08	0.3895
	model AGE * Stock	6	283.41	3.99	0.0981	-135.61	271.21	0.3930

766 **Table 4:** Coefficients and standard errors presented for stock-specific logistic regressions of male winter flounder maturity at length (A) and age
767 (B) and for overall models of all stocks combined. Measures of model fit include the residual deviance, degrees of freedom (DF), second
768 order Akaike's Information Criterion (AICc), and adjusted proportion of the deviance explained (adjusted D^2).

A	Stock	Intercept	Intercept SE	Slope	Slope SE	Residual Deviance	DF	AICc	Adjusted D^2
	GOM	-8.055	1.452	0.039	0.006	91.01	165	95.08	0.496
	GB	-15.228	5.299	0.056	0.018	22.82	83	26.97	0.625
	SNE	-8.368	1.338	0.036	0.005	137.89	190	141.95	0.402
	ALL	-7.523	0.866	0.034	0.003	273.68	442	277.71	0.435
B									
	GOM	-3.483	0.799	1.647	0.302	94.72	154	98.80	0.408
	GB	-6.556	2.433	3.199	1.090	28.80	81	32.95	0.523
	SNE	-3.482	0.697	1.659	0.277	147.69	185	151.76	0.328
	ALL	-3.661	0.512	1.758	0.202	275.39	424	279.42	0.389

Table 5: The percent mature at age estimated using the stock-specific models from Table 4B.

AGE	GOM	GB	SNE
1	13.8	3.4	13.9
2	45.3	46	45.9
3	81.1	95.4	81.7
4	95.7	99.8	95.9
5	99.1	100	99.2
6	99.8	100	99.8
7	100	100	100

Table 6: Reported estimates of length (L_{50}, mm) and age (A_{50}, yr) at 50% maturity for male winter flounder from different regions and stock time periods. Estimates were fitted to data assuming a logistic cumulative density function unless noted. Canadian stocks listed by NAFO divisions.

Region	Stock	L_{50}	A_{50}	Time period	Citation
Newfoundland, Canada	3L	210	5	1962 - 1963	Kennedy & Steele (1971)*
Gulf of St. Lawrence	4T	188	3.7	1975 - 1979	Beacham (1982)*
Magdalen Islands, Canada	4T	207	-	2006 - 2008	DFO (2010)
Scotian Shelf (north)	4W	229	-	1975 - 1979	Beacham (1982)*
Scotian Shelf (south)	4X	292	-	1959 - 1964	Beacham (1982)*
Scotian Shelf (south)	4X	254	-	1975 - 1979	Beacham (1982)*
Massachussetts, U.S.	GOM	272	3.3	1983 - 1991	Witherell & Burnett (1993)
Massachussetts, U.S.	GOM	276	3.3	1987 - 1990	O'Brien et al. (1993)
Northeast U.S. coast	GOM	204	2.1	2009 - 2013	Current study
Georges Bank	GB	256	1.8	1986 - 1990	O'Brien et al. (1993)
Georges Bank	GB	270	2.1	2009 - 2013	Current study
Massachussetts, U.S.	SNE	280	3.1	1983 - 1991	Witherell & Burnett (1993)
Massachussetts, U.S.	SNE	290	3.3	1985 - 1990	O'Brien et al. (1993)
Northeast U.S. coast	SNE	236	2.1	2009 - 2013	Current study

* Used a probit link to estimate a median size-at-maturity parameter but this probably accounts for only minor differences from logit link estimates. Kennedy and Steel (1971) did not report an A_{50} value so was estimated from their tabulated data.

Figure 1: Map of the study region along the North American east coast (inset). The three stock areas are marked by thick black lines: Gulf of Maine (GOM), Georges Bank (GB), and Southern New England (SNE). Crosses (+) indicate where samples were collected and their size is scaled to the number of male winter flounder sampled per location. The black dashed line indicates the U.S./Canadian exclusive economic zone boundary.

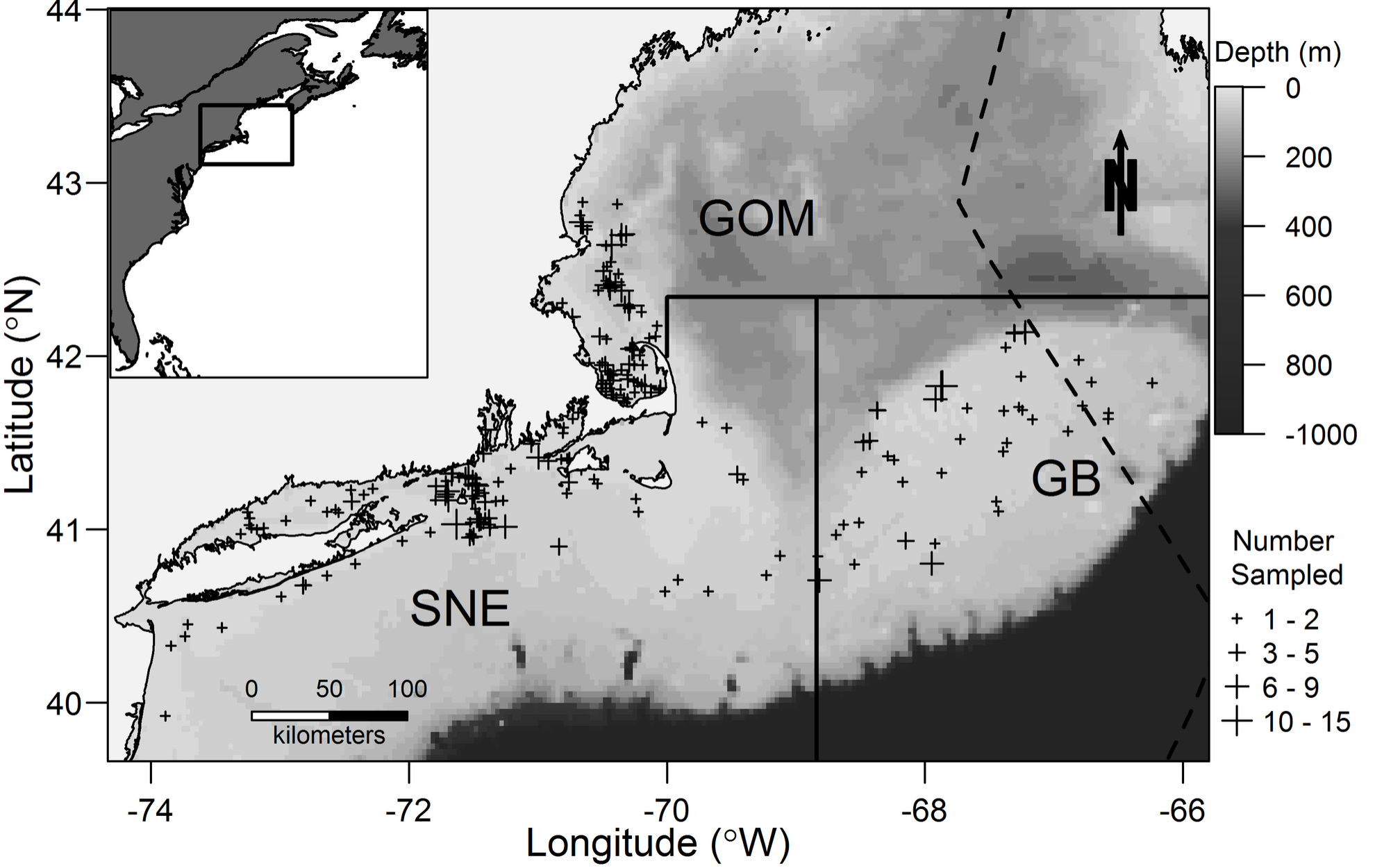
Figure 2: Testicular tissue of winter flounder, *Pseudopleuronectes americanus*, showing different spermatogenic cell types and structures seen in the maturity classes. **Immature (A1 and A2):** Primary spermatogonia grouped in nests, continuous interstitial tissue, and simple tunica, is shown. **Developing (B):** A mosaic of several stages at once, continuously along the interstitial tissue. **Ripe (C):** Spermatozoa dominate the lobules, thin sections of non-continuous interstitial tissue, and isolated nests of primary spermatogonia. **Ripe and running (D):** Very similar to ripe stage microscopically with simple tunica shown. **Spent (E):** Primary spermatogonia nests, thick continuous interstitial tissue, and residual spermatozoa within lobules. **Resting (F1):** Early in this stage residual spermatozoa still present, complex tunica and recrudescing primary spermatogonia. Later in this stage **(F2)**, tightly packed primary spermatogonia and continuous interstitial tissue. Labeled features include: primary spermatogonia (Spg1); primary spermatocytes (Spcy1); spermatids (Sptd); spermatozoa (Sp); simple tunica (sTN); complex tunica (cTN); continuous interstitial tissue (cIT); non-continuous interstitial tissue (nIT). Scale bar for images A1, A2, C, D, F1 and F2 = 50µm; for images B and E = 25µm.

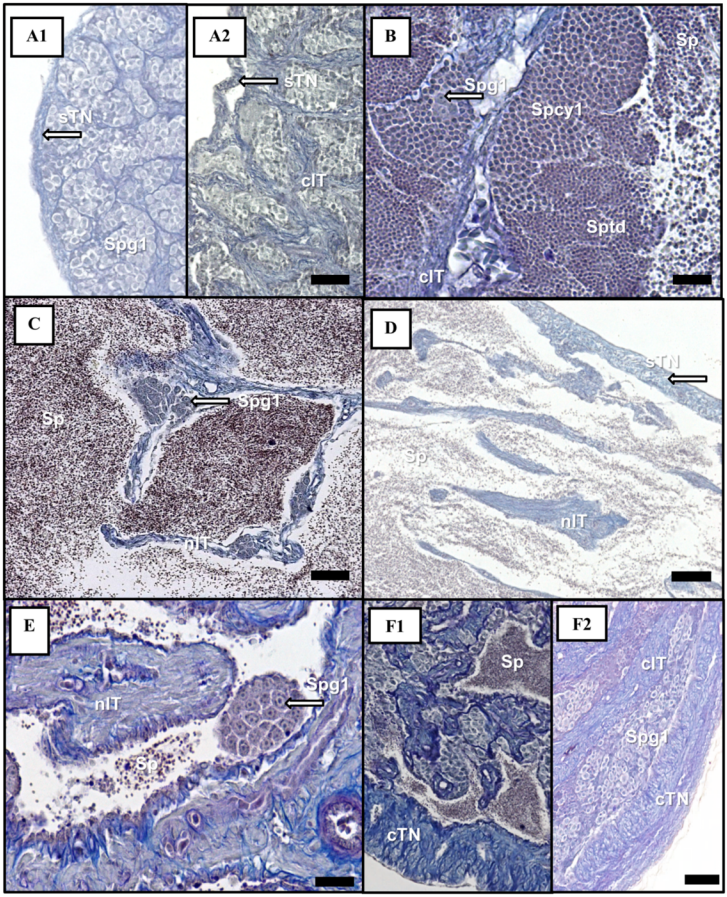
Figure 3: Monthly trends in assigned maturity class (Re, resting; St, spent; RR, ripe and running; Ri, ripe; De, developing) (top panel) and most advanced stage of spermatogenesis (Sp, sperm; Sptd, spermatids; Spcy, spermatocytes; Spg, spermatogonia) (bottom panel) compared by stock (see Figure 1 for stock definitions). Only mature male winter flounder are included. The numbers above the bars represent sample sizes. No fish were collected in February, September, and December for GB. Data not plotted for months with less than 3 fish.

Figure 4: Seasonality of spermatogenesis (circles) with the median gonadosomatic index (GSI, gray line) on the secondary y-axis compared by stock of male winter flounder (see Figure 1 for stock definitions). The black circles are scaled as a proportion of males each month a sperm stage (Sp, sperm; Sptd, spermatids; Spcy, spermatocytes; Spg, spermatogonia) was observed. Crosses indicate germ cell stage not observed in that month. Immature males not included.

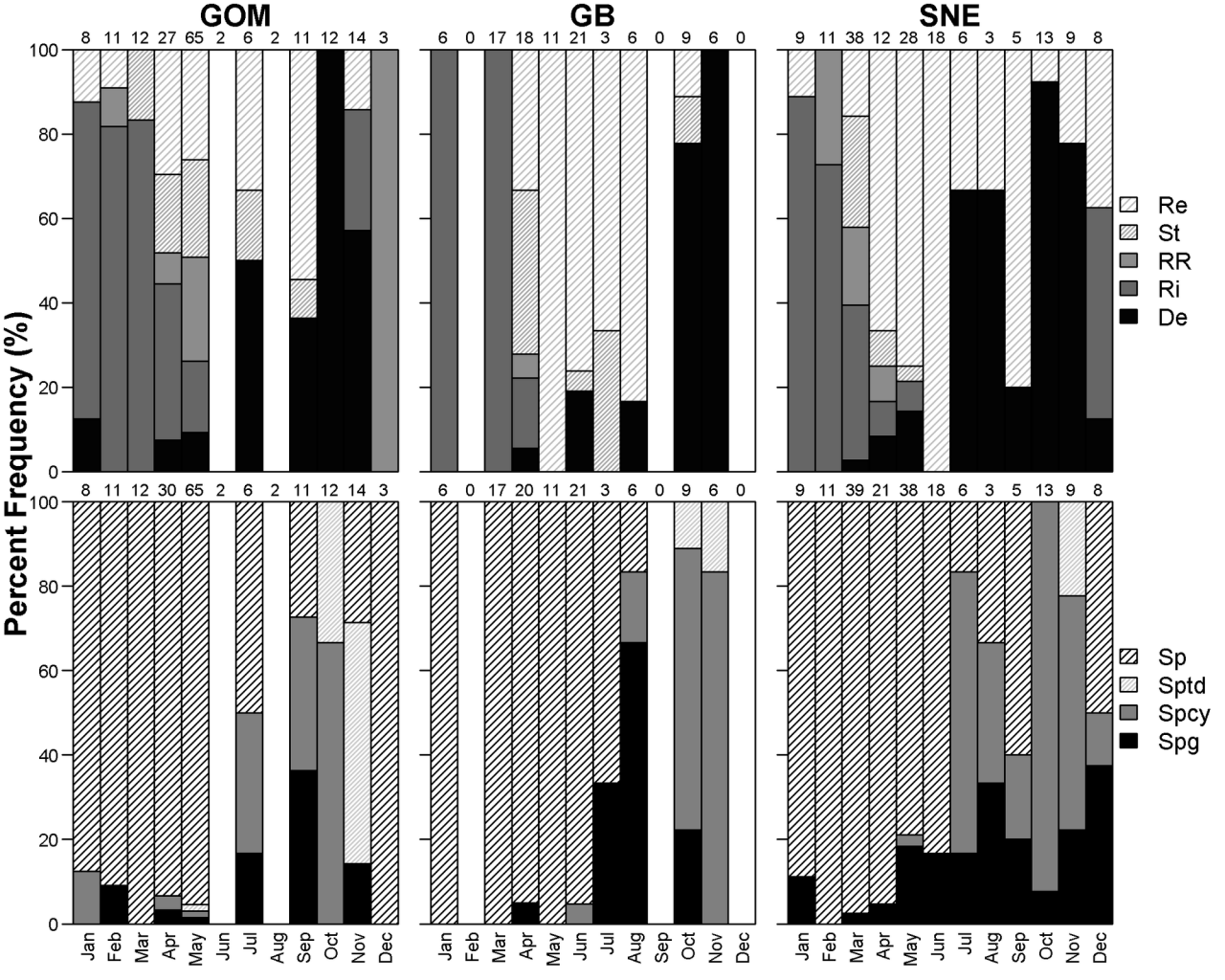
Figure 5: Monthly trends in gonadosomatic index (%GSI) and condition (K_n) compared by stock of male winter flounder (see Figure 1 for stock definitions). Numbers above represent sample size. Immature males were not included. Horizontal line indicates 'average' condition ($K_n = 1$), and plus signs indicate outliers.

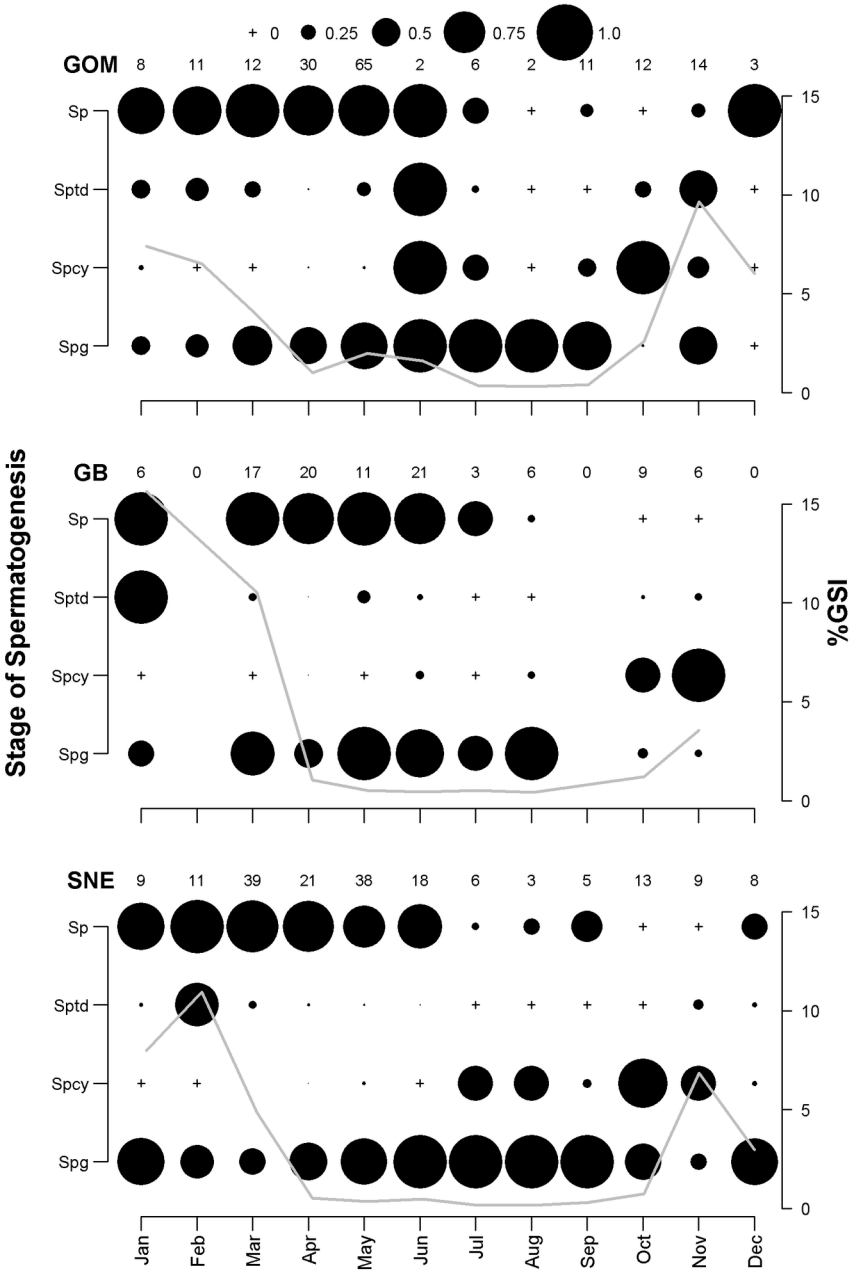
Figure 6: Length-based (left) and age-based (right) maturity ogives for male winter flounder by stock (see Figure 1 for stock definitions). The solid line is the predicted fit, and dashed lines are the 95% confidence intervals. Points along the axes indicate sizes or ages of immature (bottom axes) or mature (top axes) males (ages are jiggered to show the density of individuals). Sample size (*n)*, and point estimates for the size (mm) or age (yr, year) at 50% maturity are listed on each panel.

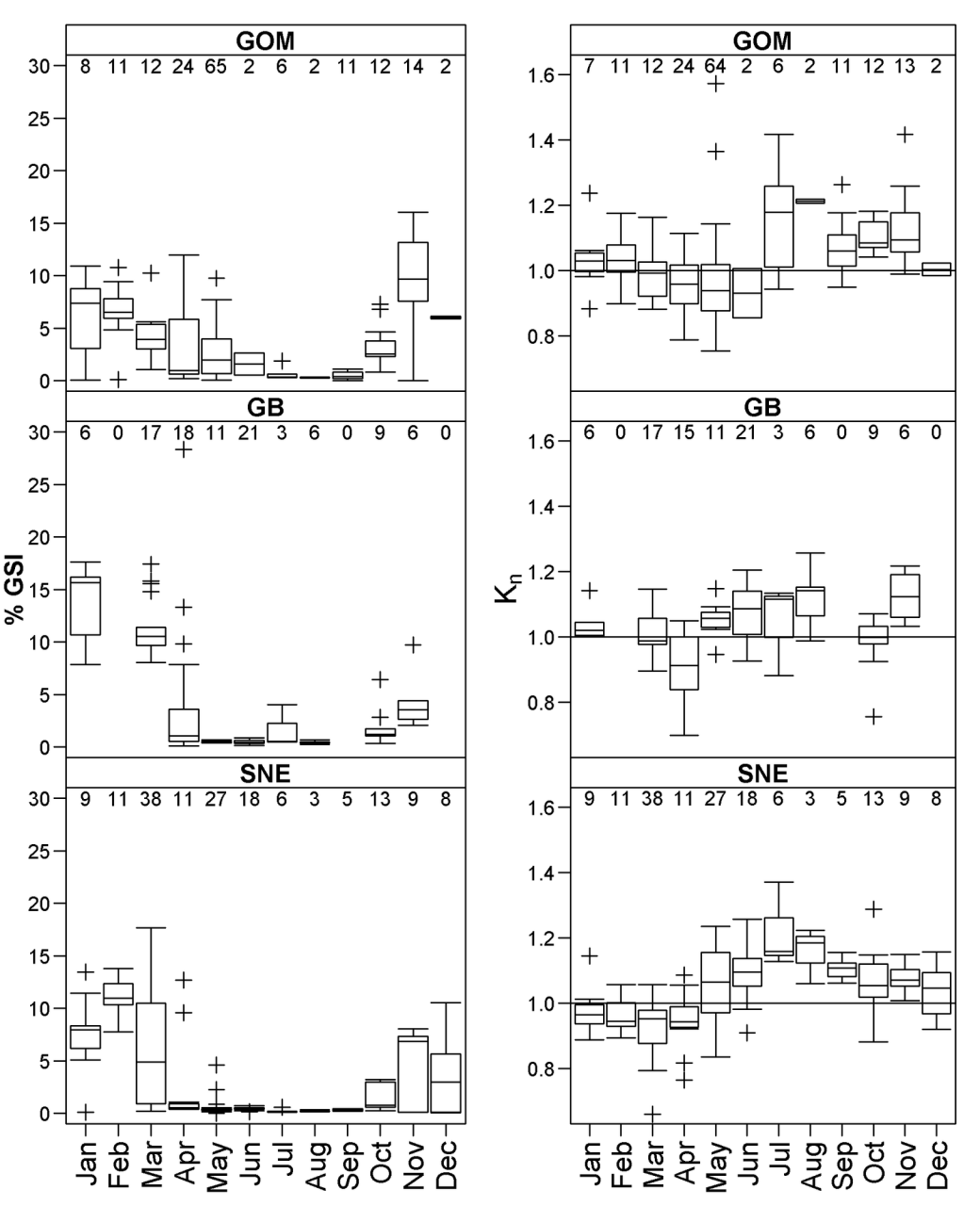

GOM
GB
SNE
N
Depth (m)
0
200
400
600
800
-1000
Number Sampled
1 - 2
3 - 5
6 - 9
10 - 15
0
50
100
kilometers
Latitude (°N)
44
43
42
41
40
-74
-72
-70
-68
-66
Longitude (°W)

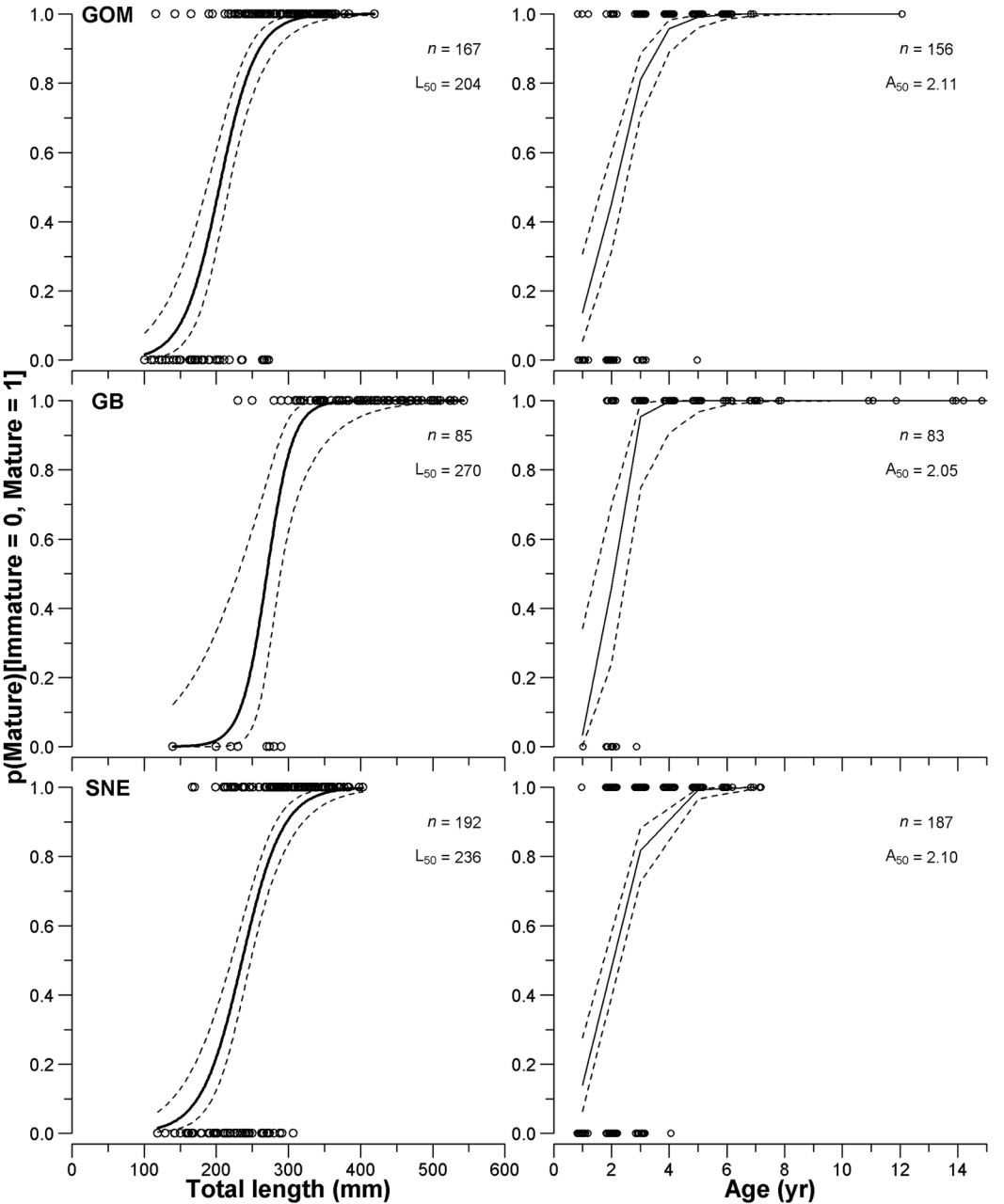

A1
sTN
Spg1
A2
sTN
clT
B
Sp
Spg1
Spcy1
Sptd
clT
C
Sp
Spg1
nlT
D
sTN
Sp
nlT
E
Spg1
nlT
Sp
F1
Sp
cTN
F2
clT
Spg1
cTN

sp


GOM
GB
SNE
8 11 12 27 65 2 6 2 11 12 14 3
6 0 17 18 11 21 3 6 0 9 6 0
9 11 38 12 28 18 6 3 5 13 9 8
Re
St
RR
Ri
De
8 11 12 30 65 2 6 2 11 12 14 3
6 0 17 20 11 21 3 6 0 9 6 0
9 11 39 21 38 18 6 3 5 13 9 8
Sp
Sptd
Spcy
Spg
Percent Frequency (%)
0
20
40
60
80
100
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec


+ 0
0.25
0.5
0.75
1.0
GOM
8 11 12 30 65 2 6 2 11 12 14 3
GB
6 0 17 20 11 21 3 6 0 9 6 0
SNE
9 11 39 21 38 18 6 3 5 13 9 8
Sp
Sptd
Spcy
Spg
Stage of Spermatogenesis
%GSI
0
5
10
15
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec


GOM
8 11 12 24 65 2 6 2 11 12 14 2
GB
6 0 17 18 11 21 3 6 0 9 6 0
SNE
9 11 38 11 27 18 6 3 5 13 9 8
% GSI
0 5 10 15 20 25 30
GOM
7 11 12 24 64 2 6 2 11 12 13 2
GB
6 0 17 15 11 21 3 6 0 9 6 0
SNE
9 11 38 11 27 18 6 3 5 13 9 8
K_n
0.8 1.0 1.2 1.4 1.6
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec


GOM
n = 167
L50 = 204
n = 156
A50 = 2.11
GB
n = 85
L50 = 270
n = 83
A50 = 2.05
SNE
n = 192
L50 = 236
n = 187
A50 = 2.10
p(Mature)[Immature = 0, Mature = 1]
Total length (mm)
Age (yr)