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Improving macroscopic maturity determination in a pre-spawning flatfish through predictive modeling and whole mount methods

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ABSTRACT

Accurate maturity schedules are essential for informed management of many fishery resources. Although histological methods are generally acknowledged as the best approach to correctly assign maturity status of individual fish, the methods can be expensive and time consuming. We developed and tested a set of multivariable models to predict maturity of southern flounder, a valuable flatfish occupying estuarine and coastal systems in the southeastern US. We also evaluated the potential for whole mount methods to validate maturity assignments and help discriminate transitional oocyte stages. Lastly, we used one of the better performing models to conduct retrospective analysis of variability in southern flounder maturity schedules. Several models performed well in predicting southern flounder maturity; nearly half of the models we tested achieved ≥85% prediction success. We noted that the gonadosomatic index (GSI) was included in most of the higher performing models and, by itself, was a strong predictor of maturity for southern flounder. The addition of novel quantitative predictors, such as gonad color and dimensions, pushed model success above 90% in many cases. Whole mount methods showed a high level of agreement with histological methods, and should be investigated as an inexpensive alternative for validating maturity assignments. Retrospective analysis revealed the potential for interannual fluctuations in L_{50} of 2–5 cm for southern flounder, which can impact yearly estimates of SSB and target harvest rates. Multivariable predictive models using routinely collected fishery biological data can provide reliable estimates of fish maturity and, when coupled with whole mount methods, should represent an improvement of traditional macroscopic maturity assignment.

1. Introduction

Understanding temporal variability in the timing of maturity is essential for successful management of exploited fish stocks, yet maturity schedules are often incomplete or prone to error (Lowerre-Barbieri et al., 2011a). For many fishes, maturity assignments have traditionally been based on visual inspection of gross (macroscopic) features of whole gonads (West, 1990). However, macroscopic staging can be inaccurate since it is generally restricted to coarse and often subjective measures of gonad size, shape, or color that do not necessarily correspond with oocyte-level development (e.g., Vitale et al., 2006; Costa, 2009; Ferreri et al., 2009; McPherson et al., 2011; Midway and Scharf, 2012). Despite its limitations, macroscopic staging remains a common approach for assigning maturity status since it is inexpensive and relatively easy to complete. Alternatively, histological examination of gonadal tissue is widely considered to be the benchmark in fish reproductive biology as it provides oocyte-level information and a high degree of accuracy in distinguishing between immature and mature individuals (Hunter and Macewicz, 1985; Murua and Saborido-Rey, 2003). The major drawback to histology is that it is resource intensive, requiring considerable time and expense, and specialized training.

In some cases, accurate maturity assignments have been obtained using simple and inexpensive methods instead of histology (West, 1990; Neidig et al., 2000). Specifically, two alternative approaches that have promise include multivariable predictive modeling and whole mount procedures. Statistical models to predict maturity status for individual fish can take a number of forms, but recent models have generally taken advantage of routinely collected biological (e.g., length, weight, age, etc.) and environmental (e.g., location and date of capture, physical habitat attributes, etc.) variables to increase predictive success beyond simple macroscopic

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staging criteria. For example, Vitale et al. (2006) used a regression tree model to determine that gonadosomatic (GSI) and hepatosomatic (HSI) indices could correctly predict maturity of Kattegat Atlantic cod (*Gadus morhua*) for 95% of the individuals tested. Similarly, Schill et al. (2010) used environmental variables to generate maturity models with prediction success rates between 77 and 89% for Idaho redband trout (*Oncorhynchus mykiss gairdneri*). The ability to predict maturity status with high rates of success by supplementing macroscopic staging with information gained from multiple routinely collected variables could lessen the need for regular histological analysis.

Whole mounting of oocytes (also referred to as wet or squash mounts) is a simplified method to identify oocyte stages that requires only placing a sample of fresh or preserved ovarian tissue on a glass slide, compressing the sample with a cover slip, and then viewing using a stereo or compound microscope. Whole mount methods have been applied more commonly as part of fecundity studies (e.g., Thorsen and Kjesbu, 2001; Witthames et al., 2009) than for the development of maturity schedules; however, staging of oocytes from whole mounts has been successfully validated in cases where it was attempted (Kjesbu, 1991; Neidig et al., 2000). More specifically, both Forberg (1983) and West (1990) reported high agreement between whole mount and histological methods to stage oocytes of capelin (*Mallotus villosus villosus*) and brownstripe red snapper (*Lutjanus vittus*), respectively. West (1990) further suggested that, if validated, whole mounts can be more efficient than histology for generating maturity schedules. Similar to simple GSI calculations, whole mounting is rapid and inexpensive; however it can yield microscopically detailed information for some species, indicating its promise as a potential alternative to more extensive histological approaches.

The ability to produce rapid and accurate maturity schedules on a continual basis should increase our understanding of spatial and temporal variation in maturity and lead to more effective management (Morgan, 2008). Fish maturity schedules can vary for numerous reasons, including density-dependent responses to fluctuations in population abundance (Morgan and Colbourne, 1999; Hutchings and Baum, 2005), environmental variation (e.g., temperature; Dhillon and Fox, 2004; Tobin and Wright, 2011), and selective mortality (Law and Grey, 1989). Because changes in maturity are thought to be influenced more by changes in abundance and demographics (i.e., selective removal of individuals through fishing) and less by natural variation (Walsh and Morgan, 1999), exploited species are more likely to display temporal variability in maturation schedules. The ability to monitor temporal shifts in age- and size-specific maturity has the potential to serve as a warning of overfishing (Morgan, 2008), thus quantifying and understanding causes of variation in reproductive timing should be a research priority.

In this study, we attempt to improve upon existing macroscopic maturity staging for female southern flounder (*Paralicthys lethostigma*), an economically valuable flatfish in coastal waters of the U.S. South Atlantic and Gulf of Mexico. The latest assessment of the population harvested in North Carolina waters concluded that the stock was overfished (spawning stock biomass [SSB] below the threshold level; Takade-Heumaker and Batsavage, 2009). Recent findings (Midway and Scharf, 2012) also indicate that the macroscopic maturity classification system used in the assessment likely leads to overestimation of SSB and overly optimistic biological reference points. Overfished status combined with lower SSB than previously thought highlights the need for accurate maturity information so that future assessments and management decisions for southern flounder can be conducted with confidence.

Misclassification of southern flounder maturity likely stems from two sources, which together were suspected to be the cause of low agreement between macroscopic and histological maturity assignments for southern flounder in early stages of reproductive development (Midway and Scharf, 2012). The first source of misclassification is the application of mostly descriptive macroscopic staging criteria that have not been thoroughly validated. Second, southern flounder have historically been inaccessible during spawning, which is presumed to take place in deep offshore waters, meaning that most fish are collected during the months immediately preceding spawning and thus, only possess early oocyte developmental stages. Given the resources necessary to complete histological analyses, it is unlikely that they will become routine for southern flounder or other similar species managed by state and federal agencies. However, as shown for other teleosts, routinely collected biological variables (e.g., gonadosomatic indices, body mass, condition indices, age, date of capture) can enhance maturity predictions and are less time and cost intensive. Thus, there is strong potential to improve maturity predictions for southern flounder and thereby enhance future stock assessments and management decisions. To address this possibility, our objectives were: (1) to combine existing and novel macroscopic characters with additional biological variables to develop and evaluate multivariable models for maturity prediction; (2) to assess the potential for using whole mount methods to generate maturity schedules; and (3) to demonstrate the utility of multivariable predictive models by quantifying historic variability in size-based maturity of North Carolina southern flounder.

Table 1
Stages used to assess maturity in North Carolina southern flounder. Each stage and the description of its macroscopic features follow guides used by the North Carolina Division of Marine Fisheries. The most advanced oocyte stages are based upon histological examination of female gonadal sections and follow Brown-Peterson et al. (2011). CA = cortical alveolar; OM = oocyte maturation; PG = primary growth; POF = postovulatory follicle complex; Vtg1 = primary vitellogenesis; Vtg2 = secondary vitellogenesis; Vtg3 = tertiary vitellogenesis.

Macroscopic stage	Macroscopic features	Histologically most advanced stage oocyte
Immature	Ovaries small and thin—no oocytes visible	PG
Developing	Ovaries rotund, yellowish-orange and turgid	CA, Vtg1, Vtg2
Fully developed	Same as developing, but with oocytes visible	Vtg3
Ripe (running)	Ovaries large and soft with many large, free-flowing (with slight pressure) hydrated oocytes	Vtg3, OM, POF
Spent	Ovaries small and bloodshot; few hydrated oocytes, if any	POF, few Vtg
Resting	Ovaries small, flaccid, translucent with no visible oocytes	PG

2. Materials and methods

2.1. Fish collection and measured variables

Southern flounder were collected during the fall (Oct–Dec) of 2009 and 2010. A complete description of collection methods and data retrieval is contained in Midway and Scharf (2012). Briefly, fish were collected from both fishery-dependent and -independent sources, kept on ice, and returned to the laboratory for processing. Variables measured for all fish included total length (TL) in mm, weight (g), age (y), gonadosomatic index (GSI = [gonad weight/(body weight − gonad weight)] × 100), ordinal date of capture, and assigned macroscopic maturity stage (stages are those used by the North Carolina Division of Marine Fisheries [NCDMF] and are described in Table 1). Fish were randomly selected for histological analysis within each year (2009