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# Techniques for improving estimates of maturity ogives in groundfish using double-reads and measurement error models

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ABSTRACT

The reproductive output of a population depends upon physiological factors, including maturation rates and fecundity -at-size and -at-age, as well as the rate at which post-maturation females fail to spawn (i.e. skipped spawning). These rates are increasingly included in stock assessment models, and are thought to change over time due to harvest and environmental factors. Thus, it is important to accurately estimate maturation and skipped spawning rates while also including information on imprecision. For this task, we developed a new double-read and measurement-error modeling protocol for estimating maturity that is based on the use of multiple histological reads of ovaries to account for reader error caused by poorly prepared slides, nuclear smear, and early yolk development. Application to three U.S. West Coast groundfishes (Pacific hake *Merluccius productus*, darkblotched rockfish *Sebastes crameri*, and canary rockfish *Sebastes pinniger*) indicates that reader uncertainty is strongly predictive of reader error rates. Results also show differences in rates of skipped spawning among species, which should be further investigated. We recommend that future maturity studies record reader certainty, use models that incorporate covariates into the analysis, and conduct an initial double reader analysis. If readers exhibit little variation, then double reads may not be necessary. In addition, slide quality should also be recorded, so that future studies do not confuse this with reader imprecision. This improved protocol will assist in estimating life history, as well as environmental, and anthropogenic effects on maturity.

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## 1. Introduction

Reproduction is a basic demographic process in population dynamics, and density-independent and -dependent changes in reproductive success contribute to a large portion of variability in marine populations. Timing of sexual maturity will vary among individuals according to individual size, age, season, location, and other factors (Jørgensen and Fiksen, 2006). Maturity is a function of length and age, and fast growing fish generally mature at much younger ages. Growth rates slow during reproduction, with immature fish growing faster than mature fish, and increase following skipped spawning events (Folkvord et al., 2014). These factors, paired with errors in correctly identifying maturity stages, influence our ability to accurately estimate the probability of sexual maturity as a function of length and/or age (termed the "maturity ogive"). In addition, since many individuals do not spawn annually following initial maturation, the average skipped spawning rate may be poorly estimated. Skipped spawning and reader error remain understudied and often go unaccounted for in fisheries models (Rideout et al., 2000; Kennedy et al., 2011). Understanding how shifting marine environments and anthropogenic impacts affect these life history processes and our ability to accurately quantify them is vital to the accuracy of stock recruitment models used in fisheries management (Burton, 1999; Kennedy et al., 2011).

In recent years, improvements in survey design and statistical methods have revolutionized how other demographic processes are estimated. For example, estimation of growth curves now uses methods that explicitly account for misreading of individual ages (e.g., Cope and Punt, 2007), while estimates of natural mortality involve state-space modeling of individual tagged fishes (e.g., Schaub and Royle, 2013). Estimating reader error variability for aging fish is routinely based on double reads of a single otolith by different individuals, along with validation methods that confirm the accuracy of the results or indicate the degree of bias (Campana, 2001; Hamel, 2008; Punt et al., 2008). Hierarchical models are used to process the results of double-reads, allowing error

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to propagate and be accurately reported (Royle and Dorazio, 2008) when estimating emergent biological process such as shifts in size- or age-at-maturity and skipped spawning. Research has revealed maturity ogives shift over time, e.g. Atlantic cod (Olsen et al., 2004). Skipped spawning and incidence of high intensity atresia (breakdown and reabsorption of vitellogenic oocytes) are correlated with nutrient availability and temperature, factors that are highly variable over space and time (Rideout et al., 2006). Including spatial and temporal variables in hierarchical maturity models allows differences in sampling to be 'controlled' when estimating decadal changes in maturity. Recent research has proposed using mixed-effects models or covariates to smooth time-variation in the maturity ogive given sparse annual sampling of fish maturity (Cadigan et al., 2014; Wright et al., 2011; Xu et al., 2015).

Reader variability is often calculated for age reading, but to the authors' knowledge it has not been incorporated into maturity models. In studies on age, re-aging or double reading otoliths is considered important for data reliability (Hare, 2007). Maturity studies based on macroscopic examination of ovaries found high variability in the determination of mature and immature fish (Costa, 2009). Histological analysis allows for greater accuracy and insight into skipped spawning events, but the effect of reader error on variability of microscopic determination of maturity states is unclear. Accounting for variability in measurements of maturity (either using histological or macroscopic methods) also allows analysts to more accurately characterize uncertainty regarding estimates of the maturity ogive. Uncertainty in these maturity analyses can then be incorporated in stock assessment models and resulting management advice (Stewart et al., 2013).

Here, we use a measurement error model to estimate maturity ogives for three species inhabiting the California Current system: Pacific hake (*Merluccius productus*), darkblotched rockfish (*Sebastes crameri*), and canary rockfish (*Sebastes pinniger)*, and also incorporate a double read analysis. These species were selected based upon the availability of samples, variations in life history, and importance along the U.S. West Coast. The objectives of this study are to: (1) to identify and reduce the main sources of reader error in estimating maturity of fish, (2) to add uncertainty in maturity reads and reader precision to model analysis and (3) to select the best fit maturity ogive model for each species. We noted that reader uncertainty was a strong predictor of reader error rates, implying that reader certainty should be documented during histological analysis.

## 2. Methods

### *2.1. Study species*

The life history strategies for the three species studied varied greatly, but all are commercially important either economically or as catch-limiting species along the West Coast. Pacific hake occur from 25°N to 55°N on the West Coast of North America, and are typically found from southern California waters to the Queen Charlotte Sound. The coastal Pacific hake stock is the most abundant groundfish species in the California Current system. A study conducted from 1990–1992 visually assessed maturity for female Pacific hake, with length-at-50%-maturity estimated as 37.8 cm (Dorn and Saunders, 1997). Spawning reportedly takes place off the coast of California from January to March, but more recent studies suggest spawning may occur in multiple batches throughout the year (Taylor et al., 2015). It is uncertain whether batches spawned outside of the winter spawning season are viable. Pacific hake are estimated to live up to 20 years of age (Hesler and Alade, 2012).

Canary rockfish (*S. pinniger)* are distributed from Baja California to the Gulf of Alaska with the highest concentrations between British Columbia, Canada and central California to Bodega Bay (Love et al., 2002; Miller and Lee, 1972). This species has an estimated lifespan of 75–95 years and a maximum female size of 61 cm; though, females are rarely observed over 30 years of age. Recent histological analysis of ovaries collected by the Oregon Department of Fish and Wildlife (ODFW) and the West Coast groundfish bottom trawl (WCGBT) survey estimated the length-at-50%-maturity to be approximately 42 cm (Thorson and Wetzel, 2015). Canary rockfish, like all *Sebastes* are viviparous, live bearing fish, and spawn in the winter months (Love, 1996; Thorson and Wetzel, 2015).

Darkblotched rockfish (*S. crameri)* are found in the southeast Bering Sea and Aleutian Islands to Santa Catalina Island, California, and are commercially important along the U.S. west coast from the Canadian border to Northern California. Darkblotched rockfish have an estimated maximum lifespan of 105 years and size of 58 cm (Gertseva and Thorson, 2014; Love, 1996). Length-at-50%-maturity was recently assessed for darkblotched rockfish using ovaries collected during the 2011–2012 WCGBT survey, and found to be 30 cm (Frey et al., 2015). Similar to canary rockfish, they are viviparous and spawning occurs during the winter months (Nichol and Pikitch, 1994; Frey et al., 2015).

### *2.2. Data collection and histological analysis*

Pacific hake, canary rockfish, and darkblotched rockfish were sampled during the Northwest Fisheries Science Center's (NWFSC) annual fishery-independent bottom trawl (WCGBT) survey off the U.S. West Coast from May–October (see Bradburn et al., 2011 for sampling methods). A random subsample of each species was selected from the catch at each site to determine sex, fork length (cm), weight (kg), and age (yr). Age was subsequently determined from extracted otoliths using the break-and-burn method (Beamish and Chilton, 1982). From 2009–2011, ovaries were collected opportunistically from females with associated age samples. Since 2012, collections were based on length bins to ensure a broad size range of fish for inclusion in maturity analysis. Ovaries were stored in cloth sampling bags and stored in 10% neutral buffered formalin (sodium bicarbonate). Pacific hake ($n = 329$), darkblotched rockfish ($n = 307$), and canary rockfish ($n = 131$) ovaries were histologically examined by two independent readers (R1 and R2). Four hake samples were excluded from the model analysis due to missing ages.

Tissue samples from individual ovaries were embedded in paraffin, thin-sectioned to 4-μm, mounted on slides, and stained with hematoxylin and eosin (H&E) stain (Sheehan and Hrapchak, 1980). To determine maturity, each prepared ovary section was examined using a Leica DM1000 binocular microscope at 40×–400× magnification, equipped with a Leica DFC295 camera and imaging software (Leica Microsystems LAS EZ 4.0).

Ovaries with oocytes containing dark-stained vitellogenin yolk (eosin positive) and more advanced stages (hydration and/or embryonic development) were classified as mature. We used an oocyte development Table to assess each sample following similar methods developed to stage maturity for *Sebastes aleutianus* and *S. borealis* (McDermott, 1994), *S. crameri* (Nichol and Pikitch, 1994), and *Pleurogrammus monoptergius* (McDermott and Lowe, 1997). Oocyte developmental stage 4 and maturity stage 3 were defined as mature for all three species (Tables S1 and S2). Samples estimated with over 25% oocytes in an atretic state were marked as high intensity atresia following McDermott (1994). Oocytes exhibiting atresia were not used for maturity determination; therefore, only non-atretic oocytes were used in maturity analysis. Ovary samples were identified as mature solely by the presence of yolk and characteristics associated with more advanced maturity stages, for the purposes of identifying fish that would be contributing to the overall spawning biomass in any one year (Figs. S1 and S2). Determination of maturity was independent of size and age. Post spawning samples (termed "spent") were characterized by the