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Female Sablefish Age-at-Maturity in Alaska Applicable to Stock Assessment

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ABSTRACT

This report is a summary of results from sablefish maturity studies conducted in December 2011, July through August 2015, and December 2015. More thorough investigations and descriptions of these studies have been published or are in preparation for publication. Included here are descriptions of ovarian development, definitions used for microscopic and macroscopic maturity classification, and age-at-maturity results that may be applicable to sablefish stock assessment in Alaska. In winter 2011 and 2015, microscopic methods were used to categorize maturity. In summer 2015, maturity was classified three ways: macroscopically at-sea by several scientists, macroscopically from photographs by a single expert observer (standardized macroscopic), and microscopically (with histology slides). Macroscopic methods, used during the summer, resulted in estimates of the age-at-50% maturity (A_{50}) that were younger than histological and standardized macroscopic methods. Because the macroscopic method could not be validated by the microscopic method, classifications made at-sea may not accurately represent the current oocyte development. For the summer months, oocytes were in later stages of development in the second half of August and so this time period provides the most accurate predictions whether fish will spawn in the coming season. December proved to be an ideal time to sample for maturity because fish that would spawn were in advanced stages of vitellogenesis. In winter 2011, skipped spawning was more prevalent than in the summer or winter of 2015. For the 2011 samples, incorporating skip spawning into age-at-maturity models had a large effect on age-at-maturity.

CONTENTS

INTRODUCTION

The correct classification of a fish as spent, will spawn (maturing), immature, or skip spawning depends on 1) an understanding of the reproductive cycle, 2) when fish are sampled relative to their reproductive cycle, and 3) and what characteristics are used for classifications. Misclassification of maturity stage can occur for a number of reasons: 1) gonad samples are collected too early in development; 2) maturing oocytes are not yet present in all fish that will spawn; 3) fish are resting and no longer show evidence of past spawning; and 4) macroscopic methods are used for classification but have not been validated microscopically (e.g., Vitale et al. 2006). A microscopic evaluation of ovarian tissue allows for a detailed account of ovarian structures and oocyte developmental stages and is considered a more accurate method of classifying ovarian development and maturity than macroscopic methods. If macroscopic methods can be validated by microscopic evaluations, the macroscopic method can be used with confidence in place of the more expensive and time-consuming microscopic method.

Sablefish are batch spawners with group synchronous oocyte development and determinate fecundity (a fixed number of oocytes mature as a single cohort, which are spawned in batches) (Hunter et al. 1989). For sablefish, fish that will skip spawning can be identified when all fish that will spawn have progressed into vitellogenesis. Although the timing of the initiation of vitellogenesis in Alaska is not known, maturity sampling in December in the Gulf of Alaska proved to be an ideal time for classifying maturity status because by December fish that will spawn in the late winter or early spring (within 2–3 months) were in late stages of vitellogenesis (Rodgveller et al. 2016).

The objectives for this report are to 1) describe sablefish oocyte development during July and August, when surveys regularly occur in the Gulf of Alaska, 2) compare age-at-maturity during the summer using three methods for classifying maturity: macroscopic at-sea, macroscopically by one observer from photographs of fresh ovaries, and microscopic, and 3) provide age-at-maturity models for data collected in winter 2011, summer 2015, and winter 2015 in a single document as a reference for sablefish stock assessment.

METHODS

Sampling

The annual AFSC summer longline survey extends throughout the Gulf of Alaska (GOA) and into the Eastern Bering Sea in odd years and the Aleutian Islands in even years (Rutecki et al. 2016). In 2015, sampling occurred in the Eastern and Central GOA only (legs 3–7 of the survey, Fig. 1). During the 2015 longline survey, ovaries and livers were collected from all females sampled for otoliths on legs 3–7. Samples were not collected on legs 1 and 2, which occur in June in the Eastern Bering Sea and the Western Gulf of Alaska because it was likely too early in the reproductive cycle for fish to show signs of development toward future spawning. Leg 3 begins on 5 July at the southern-most station in the East Yakutat management area (EYAK) (Fig. 1). Leg 4 is 2 days long and occurs nearby Yakutat Bay on 21-22 June in the West Yakutat management area (WYAK). Leg 5 begins on 24 June and includes all stations in WYAK. Leg 6 begins on 5 August in the eastern side of the CGOA. Leg 7 starts on 17 August and includes stations in the western side of the CGOA; the last day of sampling is on 26 August. Ovarian development on all legs (3-7) is reported; however, age-at-maturity results from legs 6 and 7 only are included because ovaries from these legs were further along in development than on earlier survey legs. Length at maturity is not reported because it is less likely to be used for stock assessment purposes.

In a special study during December 2011, specimens were collected from the continental slope and cross-shelf gullies nearby Kodiak Island (Rodgveller et al. 2016). The same areas were sampled in December 2015 (Fig. 1).

Defining Maturity Classification

In December 2011 and 2015 microscopic methods were used to categorize maturity. In summer 2015 maturity was classified three ways: 1) macroscopically at-sea by several scientists, 2) macroscopically from photographs by a single scientist (the author) with expertise in sablefish maturity (termed "standardized macroscopic" in this report), and 3) microscopically (with histology slides) by the same scientist as method 2. While at-sea, ovaries were categorized macroscopically into five ovarian development phases that have been used since 1996: immature, maturing, spawning, spent, and resting (Table 1, "Macroscopic maturity classification"). There is no category for skip spawning because 1) skip spawning sablefish were first documented in 2011, and 2) skip spawning is difficult to reliably identify without histology. Annual training is used to standardize maturity classifications. However, there is some subjectivity when classifying maturity macroscopically during the summer because 1) the summer samples were collected ~6-8 months prior to spawning, 2) the timing of ovarian development may vary annually, and 3) ovarian development progresses throughout the course of the 3-month survey.

Histologically, ovaries containing oocytes in late-stage cortical alveoli or vitellogenic stages (Table 2, oocytes stages 3 – 7) were categorized as maturing (i.e., will spawn in the coming spawning season) (Tables 1 and 2). Although oocytes are not in the most advanced stages of vitellogenesis this time of year, the vitellogenic stages were broken down into multiple stages to track fine-scale oocyte development throughout the survey. If ovaries had perinucleolar and/or early cortical alveolar oocytes (Tables 1 and 2, oocytes stages 1 – 2) accompanied by evidence of past spawning, which included 1) thick stroma and more space between the lamellae (loose structure with tissue surrounding oocytes), 2) blood vessels within the lamellae, and 3) a thick ovarian wall, they were classified as skip spawning (Table 1). In December 2011, the time of year when spawning sablefish contain advanced vitellogenic oocytes, skip spawning fish contained oocytes in stages 1 and 2 (Rodgveller et al. 2016). Therefore, I assumed that fish that showed evidence of past spawning, but had no oocytes in stages 3 – 7 would skip spawning.

Age-at-Maturity Analysis

Age-at-maturity was estimated using the logistic regression formula,

$$\hat{p}_a = 1/\left(1 + e^{-\delta(a - a_{50\%})}\right), \tag{1}$$

where $\hat{p}_a$ is the estimate of the proportion mature at age, δ is the parameter that describes the slope of the logistic curve (the speed at which maturity approaches 100%), and $a_{50\%}$ is the parameter that describes the age at which 50% of the fish are mature. The observed proportion mature at each age was calculated as

$$p_a = \frac{m_a}{n_a}, \tag{2}$$

where m_a was the number of mature fish observed at age-a and n_a was the total number of fish at age-a. We used the binomial likelihood to fit the observed proportion mature at age with the logistic model given in equation 1, with an additional penalty that accounted for maturity at length or maturity at age-0 being 0%. Age at maturity was estimated for the winter samples in two ways. In the first, fish determined to be skip spawning were classified as mature and in the second they were classified as immature.

RESULTS

Oocyte Development on Legs 3–7

When oocyte development was staged microscopically, the proportion of fish with early stage vitellogenic oocytes (stage 4, Tables 1 and 2) decreased throughout the survey and the proportion of fish with more developed oocytes (oocyte stages 6 and 7) were proportionally higher on legs 6 and 7 dates, particularly on leg 7 (Fig. 2). This progression indicates that ovaries progress in development from July to August and there are fewer fish entering the early vitellogenic oocyte stage later in the survey (i.e., there is a low probability that fish have not initiated vitellogenesis by leg 7). Therefore, confidence in maturity classifications, at least microscopically, can be highest on leg 7. The proportion of immature fish (those with stage 1 or 2 oocytes) was higher on legs 6 and 7 than on earlier legs (Table 3, Fig. 2).

Summer 2015: Agreement Between Histology and Standardized Macroscopic Classifications

Agreement between microscopic and at-sea macroscopic classification methods varied by leg (Table 3). The highest disagreement was on legs 6 and 7. The source of disagreement on legs 6 and 7 was that fish were classified as maturing (will spawn) macroscopically and as immature microscopically. On leg 3 dates the disagreement occurred for both categories (immature and will spawn). There was low disagreement on leg 5 dates and none on leg 4 dates (2-day leg).

Skip Spawning

In summer 2015, fish classified as skip spawning using microscopic methods were found on all legs except leg 6 (Table 3) (N = 11). In most cases, the skip spawning fish were classified macroscopically at-sea as maturing, one was classified as immature, and one as resting. The majority of skip spawning fish were sampled from the slope (8 of 11). The proportion of spawning capable fish (maturing + skip spawning fish) that identified to be skip spawning on all legs was 2.5%.

Skip spawning was more prevalent in December 2011 (21% of spawning capable fish) and had a noticeable effect on age-at-maturity model results (Table 4) (Rodgveller et al. 2016). There were fewer skip spawning fish in winter 2015 (6%) and this rate had a minor impact on age-at-maturity models (Table 4).

Age-at-Maturity

Because there was higher confidence in microscopic maturity classification on legs 6 and 7 than legs 3 through 5, the results from legs 6 and 7 are presented as potential sources of data for age-at-maturity models for stock assessment. At-sea macroscopic methods resulted in estimates of fish maturing at significantly younger ages than histological methods (Table 4, Figs. 3 and 4). The at-sea macroscopic method of classifying maturity on leg 6 resulted in an A_{50} that was 2.09 years younger than the microscopic method (4.78 vs. 6.87 years) (Table 4, Fig. 3); there were no skip spawners on leg 6. The "standardized macro" results, where maturity was classified from photographs from a single observer, were almost identical to the model that used data from microscopy (Table 4, Fig. 3).

There were few skip spawners on leg 7 and they had little influence on model parameters (Table 4, Fig. 4). Similar to leg 6, the "standardized macro" was very similar to histology. The at-sea macroscopic method resulted in an age-at-50% maturity that was 2.26 or 2.47 years younger than with microscopic methods (Table 4, Fig. 4). The "standardized macro" was very similar to the models using data collected with microscopic classification.

In winter 2011, skipped spawning was more prevalent than in other data sets. The classification of skip spawners as immature or mature had large effects on age-at-maturity models (Table 4, Fig. 5). Skipped spawning was less prevalent in 2015 (both winter and summer) and so there were small differences between models where skipped spawning fish were classified as mature or immature (Table 4, Fig. 5). In all but one case, data collected in the winter resulted in older ages-at-maturity than in the summer (summer leg 6 data resulted in slightly older ages-at-maturity than winter 2011, when skip spawners were classified as mature) (Table 4).

DISCUSSION

This document contains a summary of the best data available for female sablefish maturity in Alaska. The 2015 results indicate that macroscopic determinations of maturity at-sea do not always match well with microscopic or standardized macroscopic methods, indicating that classifications made at-sea may not accurately represent the current oocyte development. Samples collected on the last leg of the survey, during the second half of August, likely provide the most accurate predictions of which fish will spawn, if classifying maturity microscopically or using the standardized macroscopic method. On leg 7, at-sea macroscopic staging was biased towards identifying more fish as mature, resulting in a lower age-at-maturity. Although survey staff are all trained how to classify maturity macroscopically at-sea in the same training session, the ovarian development stage can be difficult to classify during the summer without extensive experience. This is because there are not discrete differences in ovary sizes and macroscopic characteristics between fish that are immature, skip spawning, and maturing during the summer. Therefore, microscopic or standardized macroscopic methods from leg 7 may provide the most accurate maturity classifications in the summer. Although there was closer agreement between classification methods on legs 3–5, the similarity may not be consistent. The observer effect seen on legs 6 and 7 could occur on other legs in other years and all ovaries may not show signs of maturation during those legs of the survey.

Skipped spawning is difficult to determine without histology and it should not be added to the summer at-sea classification scheme. To identify skipped spawning, a microscopic evaluation of histology slides will likely be required because, even with expertise, skip spawners will be difficult to distinguish macroscopically. A combination of standardized and microscopic methods could be used, where histology slides are only produced for fish that have moderately sized ovaries, which are the most difficult to classify, or when the at-sea observer has uncertainty in their classification. Although the identification of skip spawners did not affect age-at-maturity curves in 2015, with higher skip spawning rates misidentification can cause bias in age-at-maturity estimates (Rodgveller et al. 2016)

Accurate maturity classifications for Atlantic cod can be determined ~6 months prior to spawning, histologically (Burton et al. 1997, Skjæraasen et al. 2009). For a fish spawning in February or March, as sablefish likely do in the GOA, this time period would be in August or September. This aligns with our data where microscopic methods appear to be more dependable starting in the second half of August, at least in the Central GOA in 2015.

Because later stages of oocyte development are more prevalent in August, there is potential that fishery collections in the fall could be used for accurate assessments of maturity. I hesitate to promote macroscopic classifications in late summer/early fall by port samplers or observers because of the bias observed in our survey. However, macroscopic observations later in the season may provide more accurate results. Macroscopic observations made from September through November (the tail end of the Federal sablefish fishing season) would need to be validated with histology. We attempted to collect ovarian samples at deliveries in Sitka, Alaska in October and November over the course of two years and there were few deliveries during that period. All samples obtained were from mature fish, likely because the fishery targets larger fish than the survey, and so fishery samples in the fall may not be useful for estimation of age-at-maturity. Therefore, fall fishery samples may not be fruitful.

There are some caveats to the data that are important to consider. Samples from December 2011 and 2015 were both collected in the same areas, but not at the same stations and are not a representative sample of the entire Alaska sablefish stock. However, both surveys covered slope and gully habitats. During summer sampling, leg 6 was on the eastern side of the central GOA and leg 7 stations were closer to those on the winter surveys. A bottom trawl vessel was used to collect samples in the winter surveys rather than a longline vessel as in the summer and the depths sampled were shallower than in the summer. Trawl gear is likely to not sample the larger adult females as well as longline gear (Sigler 1999). However, the sex ratio was heavily skewed towards males (K. Echave, AFSC, pers. comm.) in the winter, whereas the ratio was near 1:1 in the summer (D. Hanselman, AFSC, pers. comm.). This may be because females are found in deeper water in the winter. The specific influence of gear on selectivity or sampling depths on availability in the winter and summer are unknown.

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Table 1. -- Sablefish ovarian maturity and accompanying oocyte development stages identified with either histology (top table) or macroscopically (bottom table) during July and August in the Gulf of Alaska. Definitions of oocyte stages can be found in Table 2. Oocyte stages under “macroscopic maturity classification” were identified using histology after macroscopic maturity classifications were assigned.

Structures defining maturity	Maturity	Oocyte stage
Histological ovarian maturity classification		
Oocytes with multiple nucleoli and/or perinucleolar; thin ovarian wall.	Immature*	1
Oocytes with multiple nucleoli and/or perinucleolar; may also contain oocytes in early cortical alveoli stage; thick ovarian wall; thick stroma; blood vessels present.	Skip spawning*	1 and possibly 2
Early cortical alveoli stage	Immature*	2
Late cortical alveoli stage	Maturing*	3
Yolk accumulated within eosinophylic spheres (vitellogenesis) (broken down into 4 stages for summer)	Maturing*	1 or more of stages 4 through 7
Macroscopic maturity classification		
Ovaries thin and tubular, no oocytes visible	Immature	1
Ovaries tubular; oocytes are indistinct through ovary wall and may be noticeable in ovarian tissue.	Immature*	1 and possibly 2 and/or 3
Ovaries distended; oocytes opaque white and clearly discernible.	Maturing*	One or more of stages 4-7
Ovaries engorged with free-flowing, translucent	Spawning	N/A
Ovaries large, flaccid, and may be bloodshot or red in color.	Spent*	N/A
Ovaries large and firm, but no oocytes are discernable	Resting	1 and possibly 2

* Indicates that the stage was identified on surveys included in this report.

Table 2. -- Sablefish oocyte development stages identified with histology during July and August in the Gulf of Alaska.

Oocyte development	Oocyte stage	Maturing
Multiple nucleoli and/or perinucleolar	1	no
Early cortical alveoli stage	2	no
Late cortical alveoli	3	yes
vitellogenesis 1	4	yes
vitellogenesis 2	5	yes
vitellogenesis 3	6	yes
vitellogenesis 4	7	yes

Table 3. -- The number of female sablefish sampled per leg on the AFSC groundfish longline survey on legs 3-7 for maturity. The matrix of values shows the maturity stage classified using macroscopic (macro) or microscopic (micro) methods, and whether the two methods agreed or were dissimilar. The cells in grey denote that there was disagreement between methods and the “% dissimilar for leg” represents the proportion of samples where there was disagreement for each leg of the survey. Also included are the number of fish per leg that were classified microscopically as skip spawning and whether they were classified macroscopically as “will spawn” (mature) or immature.

		Micro				
Leg	Macro	Immature	Will spawn	% dissimilar	% dissimilar for leg	Skip spawn
3	Immature	22	4	15%		
	Will Spawn	20	169	11%	9%	6
4	Immature	2	0	0%		
	Will Spawn	0	34	0%	0%	1
5	Immature	38	2	5%		1
	Will Spawn	5	100	5%	4%	1
6	Immature	20	2	9%		
	Will Spawn	33	64	34%	29%	
7	Immature	13	0	0%		
	Will Spawn	27	58	32%	26%	2

Table 4. -- Logistic model parameter estimates fit to the proportion of female sablefish mature at age from samples collected on leg 6 (6), leg 7 (7), and legs 6/7 combined (6/7) of the summer longline survey, and December 2011 (W11) or December 2015 (W15). Maturity was classified using either microscopic methods (Micro), while at-sea (Macro), or a standardized macroscopic method, where maturity was classified from photographs by a single observed (Standardized Macro). For the models, skip spawning fish were classified as either immature (SSImm) or mature (SSMat) to examine the effect of skip spawning classification on parameter estimates. The model for macroscopic data on leg 7 would not converge (C Issue). There were no skip spawning (SS) fish on leg 6 and the model output is listed under Micro SSImm. There were no macroscopic classifications in the winter of 2011 or 2015.

	Micro SSImm		Micro SSMat		Macro		Standardized Macro	
Survey	slope	50% maturity	slope	50% maturity	slope	50% maturity	slope	50% maturity
6	0.93	6.87			1.35	4.78	0.95	6.85
6/7	0.86	6.56	0.89	6.46	1.42	4.32	0.86	6.54
7	0.97	6.23	1.06	6.02	1.99	3.76	0.98	6.14
W11	0.56	9.84	1.17	6.79				
W15	0.67	7.94	0.89	7.27				

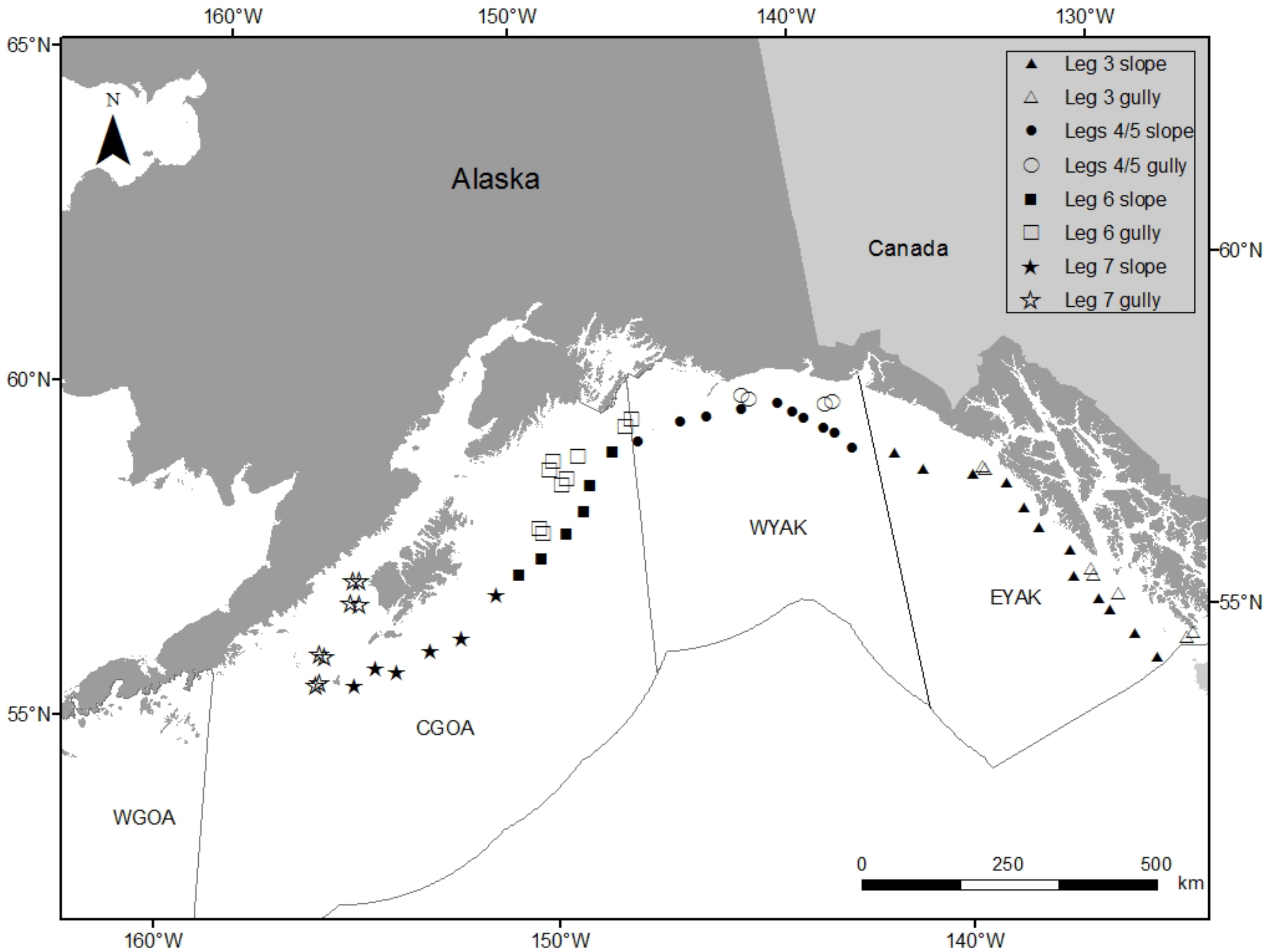

Figure 1. -- Stations sampled on the AFSC longline survey on legs 3–7 in cross-shelf gullies or on the continental slope. North Pacific Fishery Management Council sablefish management areas are delineated: East Yakutat = EYAK, West Yakutat = WYAK, Central Gulf of Alaska = CGOA, and Western Gulf of Alaska = WGOA.

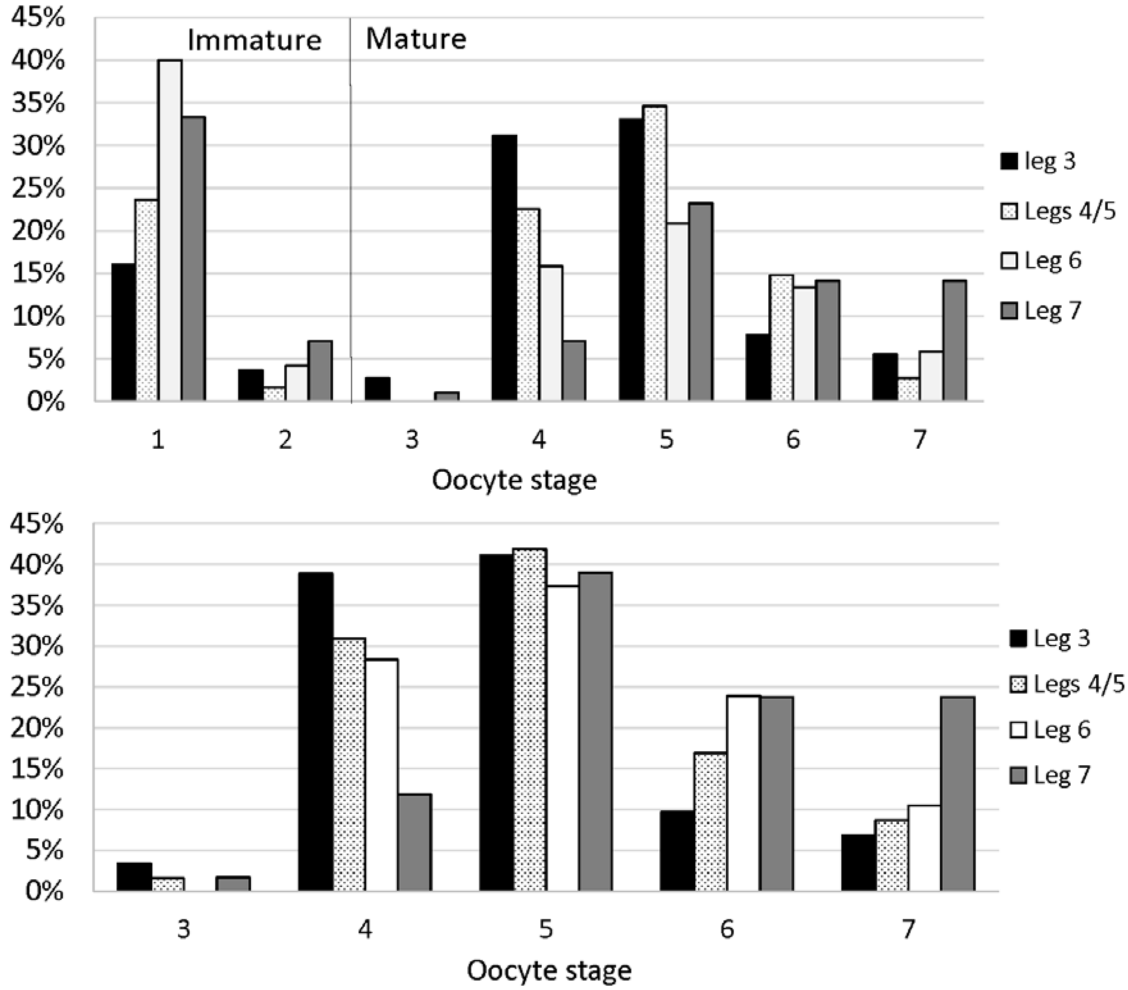

Figure 2. -- Proportion of each oocyte development stage by leg of the AFSC longline survey, where the developemental stage is the most advanced oocyte stage in the ovary,   gauged using microsopic methods. The top pannel includes all samples. The bottom pannel displays proportions of fish with ovaries containing maturing oocytes only (only specimens with oocyte stages 3-7 are included in proportions).

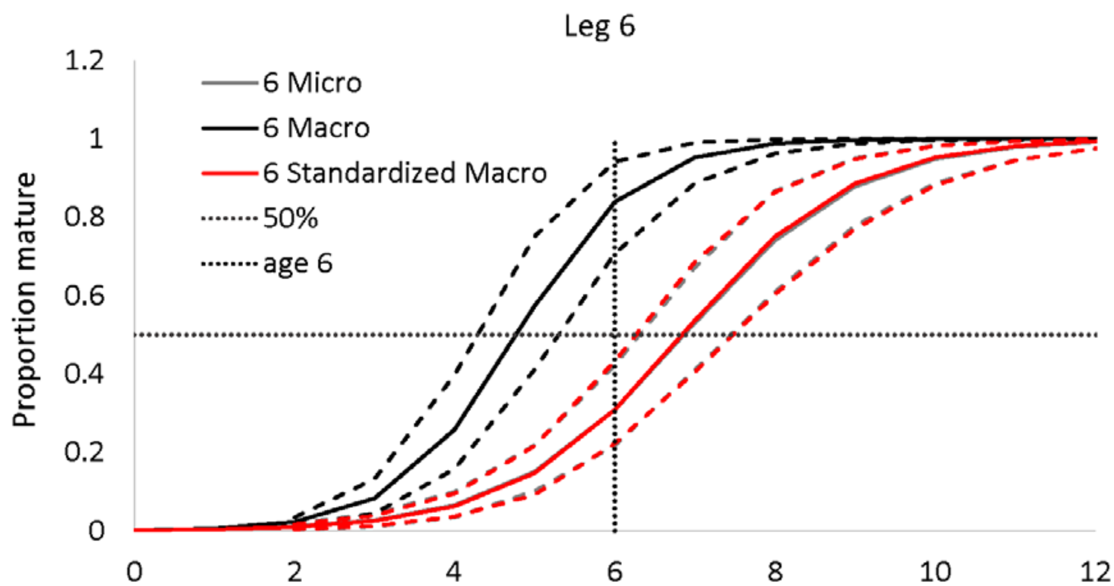

Figure 3. -- Logistic age-at-maturity models fit to data collected on leg 6 of the longline survey in 2015. Maturity was either classified using microscopic methods (Micro), macroscopic methods while at-sea (Macro), or a standardized macroscopic method (Standardized Macro), where maturity was classified from photographs by a single expert observer. Confidence intervals (95%) are dashed lines. There were no skip spawning (SS) fish on leg 6.

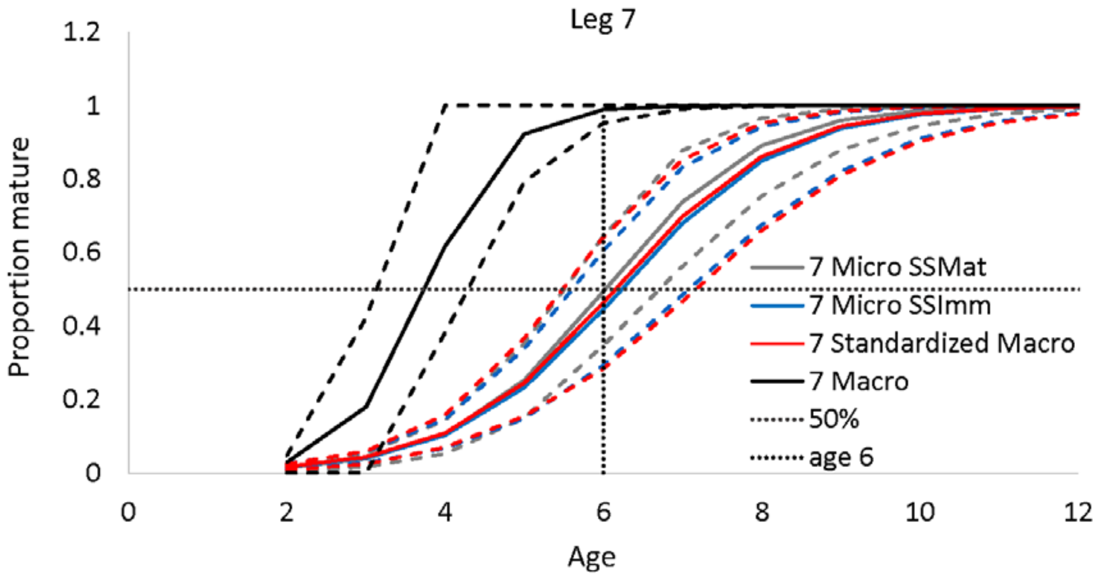

Figure 4. -- Logistic age-at-maturity models fit to data collected on leg 7 of the longline survey in 2015. Maturity was either classified using microscopic methods (Micro), macroscopic methods while at-sea (Macro), or a standardized macroscopic method (Standardized Macro), where maturity was classified from photographs by a single expert observer. Skip spawning fish were classified as either immature (SSImm) or mature (SSMat). Confidence intervals (95%) are dashed lines. The model for macroscopic data on leg 7 would not converge.

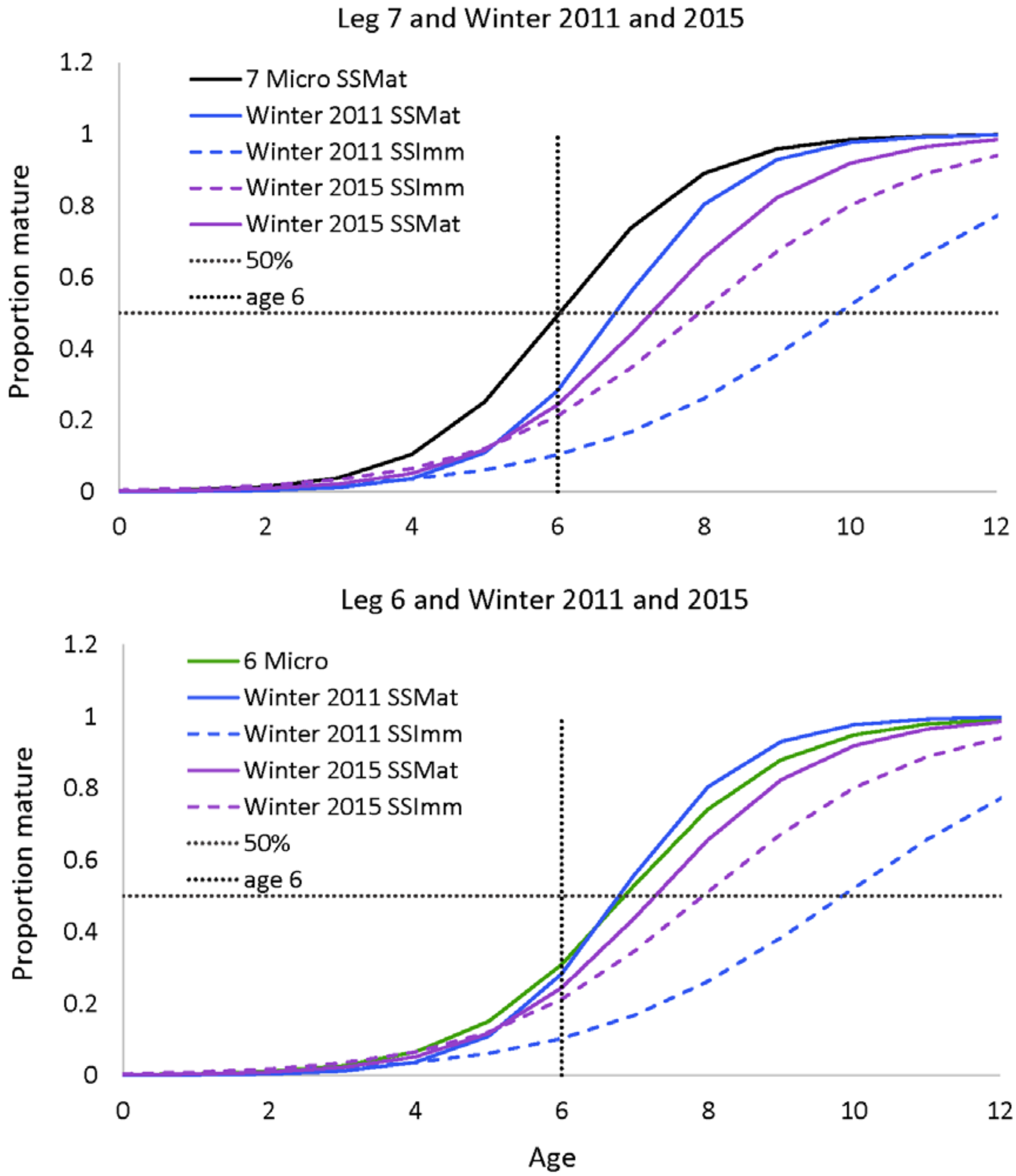

Figure 5. -- Logistic age-at-maturity models fit to data collected during winter 2011, winter 2015, and summer 2015 (on legs 6 or 7). Data in all panels were collected using microscopic methods. Skip spawning fish were classified as immature (SSImm) or mature (SSMat) in the winter data. Because there were very few skip spawners on leg 7; that is, both curves were nearly identical and there were no skip spawning fish on leg 6, only one curve is presented for leg 7 and one for legs 6 and 7 combined (7 Micro SSMat and 6/7 Micro SSMat, respectively).