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A Comparison of Methods for Classifying Female Sablefish Maturity and Skip Spawning Outside the Spawning Season

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Abstract

Ovarian development in Sablefish *Anoplopoma fimbria* was classified using three methods for samples collected during July and August in the Gulf of Alaska, approximately 6–8 months prior to spawning. Although not an ideal time for predicting future spawning, this is when survey platforms, such as longline and trawl vessels, are available annually. The three maturity classification methods included (1) macroscopic classification at sea by trained scientists but with these personnel varying throughout the survey period, (2) macroscopic classification after the survey from photographs by a single, highly trained scientist (standardized macroscopic), and (3) a microscopic evaluation of ovarian structures from histological slides. Based on certain oocyte development characteristics, the second half of August was identified as the time period when maturity could be the most accurately classified in the Gulf of Alaska. Age and length at maturity were estimated to be earlier or smaller, respectively, on some portions of the survey, when macroscopic at-sea methods were used as opposed to standardized macroscopic or microscopic methods. Skip spawning was documented throughout the survey but at a lower rate than was reported in other studies (2% versus 6% and 21%), indicating that the rate of skip spawning is likely variable. The results demonstrate that accurate maturity classifications may be determined from collections during nonpreferable months when histology or the standardized macroscopic method is used. Identifying skip spawning is likely reliant on microscopic analysis, and so a combination of the standardized macroscopic method along with limited histological sampling, to identify skip spawning rates or to classify maturity when there is uncertainty at sea, may be the most time- and cost-effective option for species similar to Sablefish that skip spawn.

The correct classification of a fish as spent, will spawn (mature), immature, or skip spawning (those that have spawned previously but are not spawning in the current year) depends on (1) an understanding of the reproductive cycle, (2) when fish are sampled relative to their reproductive cycle, (3) what ovarian structures are used for classifications, and (4) if using a macroscopic method, that the macroscopic classifications have been validated using microscopic methods. Errors or unknown bias in maturity classification are most likely to

arise when either gonad samples are collected early in development when advanced oocytes (those with vitellogenin) are not yet present in all fish that will spawn (Hunter et al. 1992), when early stage vitellogenic oocytes do not continue to develop towards spawning, or when unvalidated macroscopic methods are used (e.g., Vitale et al. 2006). The microscopic structures and the macroscopic classifications used to define maturity may vary depending on the species and a combination of the time of year and the geographic area.

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Sablefish *Anoplopoma fimbria* inhabit the northeastern Pacific Ocean from northern Mexico to the Gulf of Alaska, westward to the Aleutian Islands, and into the Bering Sea (Wolotira et al. 1993). They are a commercially important species off of Alaska, the U.S. West Coast, and British Columbia, Canada. The annual commercial ex-vessel value in the federal fisheries in Alaska in 2016 was US\$93 million (Hanselman et al. 2017). In Alaska, fish age 2+ reside in waters approximately 150–1,000 m deep along the continental slope, in cross-shelf gullies, and in nearshore, deep channels (Rutecki et al. 2016). They spawn in the late winter or early spring in Alaska (Sigler et al. 2001; Rodgveller et al. 2016) and are batch spawners with group-synchronous oocyte development and determinate fecundity (a fixed number of oocytes mature as a single cohort and immature oocytes are not recruited throughout ovarian development) (Hunter et al. 1989). Sablefish are highly migratory and are managed as a single population in Alaska (Hanselman et al. 2014). The direct distance between tag and recapture locations can be over 2,000 km (Hanselman et al. 2014). They are long-lived and regularly are aged over 40 years old (Kimura et al. 1993), and the maximum age that has been found in Alaska is 94 years old (Kimura et al. 1998).

Sablefish ovaries have previously been collected in December 2011 in the central Gulf of Alaska for a study of Sablefish age at maturity and fecundity (Rodgveller et al. 2016). This proved to be an ideal time to sample because fish had not yet started to spawn, and in fish that would spawn, oocytes were at advanced stages of vitellogenesis, clearly separating them from immature or skip-spawning fish. Using microscopic methods, skip spawning in female Sablefish was identified for the first time. They exhibited the resting type of skip spawning, where oocytes do not enter vitellogenesis (Rideout and Tomkiewicz 2011). Estimates of age at maturity and spawning stock biomass were affected by whether or not these fish were classified as mature or immature (Rodgveller et al. 2016).

The December 2011 sampling event was a rare opportunity to collect maturity information just prior to spawning because routine scientific surveys are uncommon or absent during this time in Alaska due to inclement weather. Commercial fisheries are closed during the winter months, from mid-November through mid-March, which encompasses the spawning season. Due to inclement weather, the fisheries often obtain much of their quota prior to the end of the season, making it difficult to obtain fishery-dependent samples close to the spawning season. In fact, this is a problem for many commercially important species at extreme latitudes (e.g., Pacific Halibut *Hippoglossus stenolepis* [Loher and Seitz 2008], Pacific Ocean Perch *Sebastes alutus* and Northern Rockfish *S. polyspinis* [TenBrink and Spencer 2013], and Pacific Cod *Gadus macrocephalus* [Neidetcher et al. 2014]). Since 1996, macroscopic maturity

classifications have been collected annually from June through August during the Alaska Fisheries Science Center (AFSC) longline survey. However, these collections have not been used for stock assessment purposes because they have not been validated with microscopy. The age-at-maturity curve currently used in the Alaska Sablefish stock assessment model was calculated using maturity classifications from the early 1980s. This data set suffers from the same issues; the samples were collected during the summer and classified using macroscopic methods. The age-at-maturity curve from the 2011 study is not currently used because it included samples from a restricted geographic area and the data set had a high number of skip-spawning fish. If this skip-spawning rate is not consistent, the 2011 data would provide biased estimates of age at maturity in other years. If a method for accurately classifying female Sablefish maturity on summer surveys was validated and up to date, then annual estimates of age at maturity would be available for the stock assessment.

The goal of this study was to determine if maturity classifications collected during summer surveys in Alaska could be used to accurately predict if fish would spawn in the coming spawning season and whether histology was required to accurately classify ovarian development. The specific objectives were to (1) describe the ovarian development of female Sablefish collected during the summer longline survey using microscopic methods, (2) compare microscopic maturity classifications to macroscopic observations made at sea and to macroscopic classifications based on photographs staged by a single, experienced scientist (someone who specializes in fish reproductive biology and has experience ground-truthing macroscopic classifications with histology for Sablefish), and (3) define sampling dates during the summer that are late enough in the reproductive cycle to accurately gauge whether a fish will spawn in the coming spawning season.

METHODS

Survey design and procedure.—The annual AFSC longline survey extends throughout the continental slope and select deep, cross-shelf gullies in the Gulf of Alaska and into the eastern Bering Sea in odd years and the Aleutian Islands in even years (Rutecki et al. 2016). For this study, samples were collected in 2015 in the eastern and central Gulf of Alaska only (legs 3–7 of the survey; Figure 1). As part of the survey design, stations were placed systematically 37–56 km apart along the continental shelf and in cross-shelf gullies. Gear was set at depths from approximately 150 to 1,000 m. Sablefish ages 0–2 inhabit shallower, inshore waters and the continental shelf. Generally at age-3 Sablefish migrate to habitat on the continental slope and in cross-shelf gullies. Starting at age 3, Sablefish are caught on the longline survey, but they are caught in larger numbers at

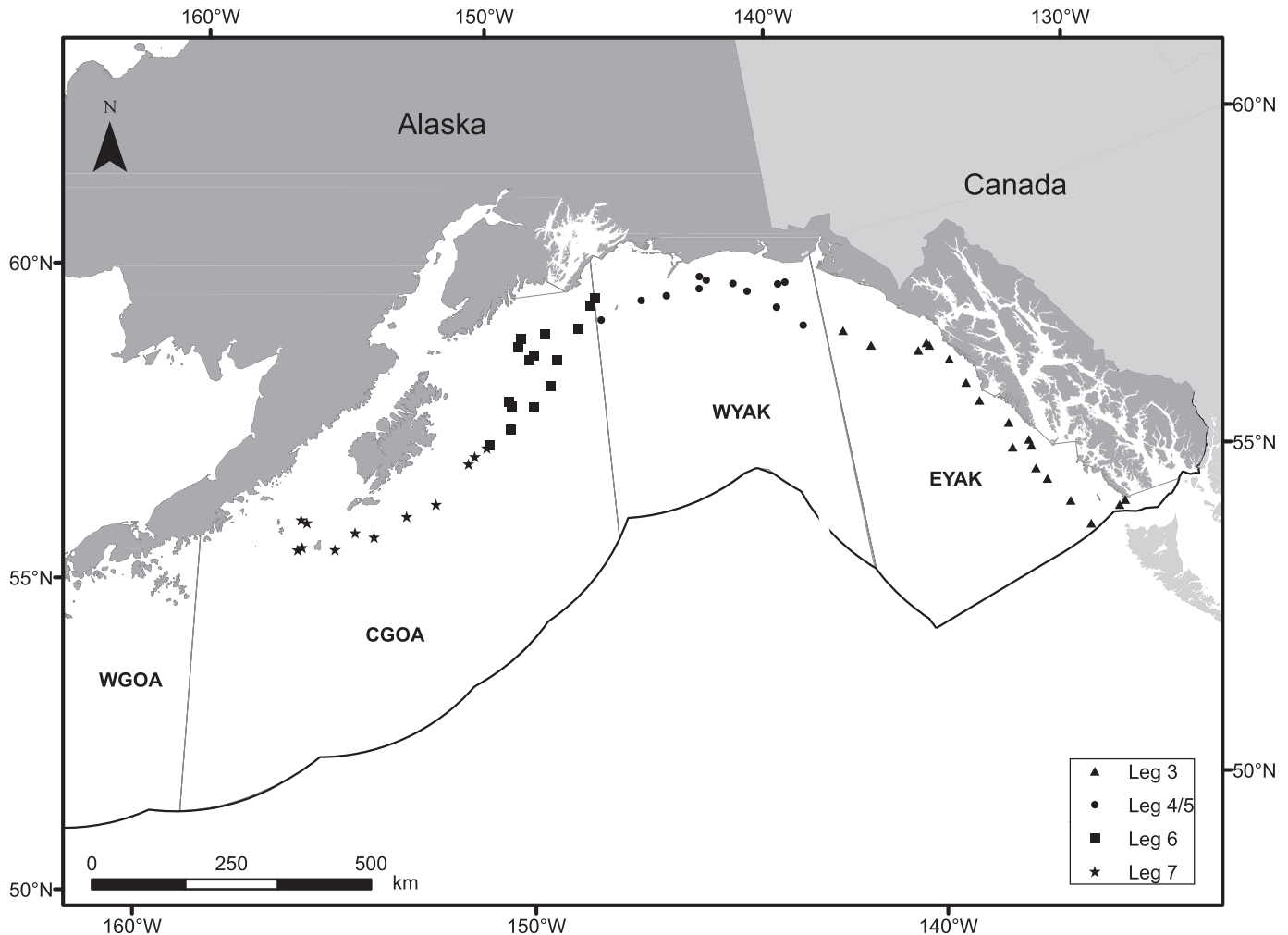


FIGURE 1. Stations sampled in 2015 on the Alaska Fisheries Science Center longline survey on legs 3–7 in cross-shelf gullies or on the continental slope in the Gulf of Alaska. North Pacific Fishery Management Council Sablefish management areas are delineated: East Yakutat = EYAK, West Yakutat = WYAK, Central Gulf of Alaska = CGOA, and Western Gulf of Alaska = WGOA.

age 4 because by this age the great majority have migrated to the study area (Rutecki et al. 2016).

Sablefish are collected annually for aging using a random, systematic sampling design so that samples are taken from all depths at 100–200-m intervals at each station. Leg 3 begins on July 5 at the southeastern portion of the Gulf of Alaska in the East Yakutat management area (Table 1; Figure 1). The survey continues west along the continental slope and ends on August 26 on the west side of the Central Gulf of Alaska. Dates of sampling for each leg are in Table 1. Different scientists are deployed on each leg of the survey, and the personnel on each leg varies each year.

Sablefish sampling.—All Sablefish were measured (fork length, mm) and weighed (g) on a motion-compensating scale. Sagittal otoliths were extracted and stored dry in vials. Otoliths were aged by the AFSC Age and Growth

Program using standard, validated methods (Fargo and Chilton 1987; Kimura and Anderl 2005; Kimura et al. 2007). For ovary removal and inspection, the abdomen was opened behind the pectoral fins with a slit across the width of the fish. This procedure is done instead of opening the entire body cavity lengthwise because the fish on the survey are processed on-board and commercially sold after the survey. The gonads were viewed from the opening in the body cavity and the maturity classified. Biologists took photographs of the ovaries inside and outside the body cavity before putting them into individual cloth bags and submerging them in a 19-L bucket containing ExCell Plus tissue fixative.

Maturity classification.—Ovaries were classified macroscopically at sea using five ovarian development phases used on the surveys since 1996: immature, mature, spawning, spent, and resting. However, only immature, mature,

and resting were identified in 2015, and these stages are described in Table 2. Different scientists are deployed on each leg and so annual training is used to standardize macroscopic maturity classifications. However, the interpretation is subjective because the survey takes place approximately 6–8 months prior to the spawning period in Alaska. During this time period fish that will spawn do not all have ovaries that have obvious, macroscopic signs of oocyte development (Figure 2). There is no category for skip spawning because this reproductive strategy in Sablefish was first documented in 2011, and it is difficult to identify without histology.

There was a second category of macroscopic maturity classification termed “standardized maturity.” For this method, maturity was classified after the survey from photographs by a single scientist with experience in Sablefish maturity classification (comparing histological analyses of oocytes and ovarian structures to photographs of fresh ovaries).

TABLE 1. Start and end dates and the sampling area of each leg of the annual Alaska Fisheries Science Center longline survey in the Gulf of Alaska (GOA). The dates remain the same each year.

Leg	Start date	End date	Management area
3	July 5	July 19	East Yakutat
4	July 21	July 22	West Yakutat
5	July 24	August 2	West Yakutat
6	August 5	August 15	Central GOA (east side)
7	August 17	August 26	Central GOA (west side)

Histological slides were prepared from sections from the middle of the ovary for gauging maturation and skip spawning. Each sample included a portion of the ovarian wall. The thickness of the ovarian wall has been used in several fish taxa, including Sablefish (Rideout and Tomkiewicz 2011; Rodgveller et al. 2016), to determine if a fish has previously spawned and if it is skip spawning. A “thin” ovarian wall is easy to discern from a “thick” ovarian wall for Sablefish: in Rodgveller et al. (2016) an immature mean wall width was 35 μm (95% CI = 30–41 μm) and the mean wall width for a skip spawning fish was 318 μm (95% CI = 267–370 μm). Previously, Rodgveller et al. (2016) found that oocyte development did not vary among locations in both ovaries. This indicated that a single sample was adequate for gauging maturation.

Ovarian tissues were embedded in paraffin, sectioned at 5–6 μm , stained with hematoxylin, and counterstained with eosin. Histological slides were examined microscopically and the stages of oocyte development were recorded (Table 3). The maturity classification was based on the most advanced oocyte stage present in the ovary as well as other characteristics (Table 2); ovaries with perinucleolar oocytes (primary growth) as the most advanced stage were classified as immature, and ovaries containing oocytes in late-stage cortical alveoli or vitellogenic stages were categorized as mature (i.e., will spawn in the coming spawning season) (Tables 2, 3; Figure 3). Although the maximum oocyte size for Sablefish was not present this time of year (~1.5 mm; Hunter et al. 1989), there were vitellogenic oocytes present on all legs of the survey (Figure 3). Vitellogenic oocytes were categorized into

TABLE 2. Sablefish ovarian maturity classification and accompanying oocyte development stages (see Table 3) identified either macroscopically or histologically during July and August in the Gulf of Alaska. Oocyte stages under “macroscopic maturity classification” are the stages that should be present if the ovary is classified accurately. Histologically, ovaries were classified based on the most advanced oocyte stage present. Only maturity classifications documented in this study are included.

Structures defining maturity	Maturity	Oocyte stage
Macroscopic maturity classification		
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 discernible.	Mature	One or more of stages 4–6
Ovaries large, not flaccid; no oocytes are discernable.	Resting	1 and possibly 2
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.	Mature	3
Yolk accumulated within eosinophilic spheres (vitellogenesis) (broken down into three stages for summer).	Mature	One or more of stages 4–6



FIGURE 2. Examples of Sablefish ovaries classified using histology slides as mature or immature. These images illustrate that classifying maturity macroscopically at sea would sometimes be difficult because of a lack of distinguishing macroscopic features during the summer months in the Gulf of Alaska.

TABLE 3. Sablefish oocyte development stages identified with histology during July and August in the Gulf of Alaska.

Oocyte development stage	Oocyte stage	Mature
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

three stages, based on the relative proportion of the cytoplasm filled with vitellogenic spheres, to track fine-scale oocyte development throughout the summer survey (Figure 3). If ovaries had perinucleolar and/or early cortical alveolar oocytes accompanied by evidence of previous spawning they were classified as skip spawning (Table 2). The signs of previous spawning 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. Images of histological slides from skip-spawning Sablefish ovaries can be found in Rodgveller et al. (2016).

Length- and age-at-maturity analysis.—The age and length at 50% maturity were compared by survey leg and by maturity classification method (macroscopic and microscopic) using the following logistic regression formula:

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

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

$$p_a = \frac{m_a}{n_a}, \quad (2)$$

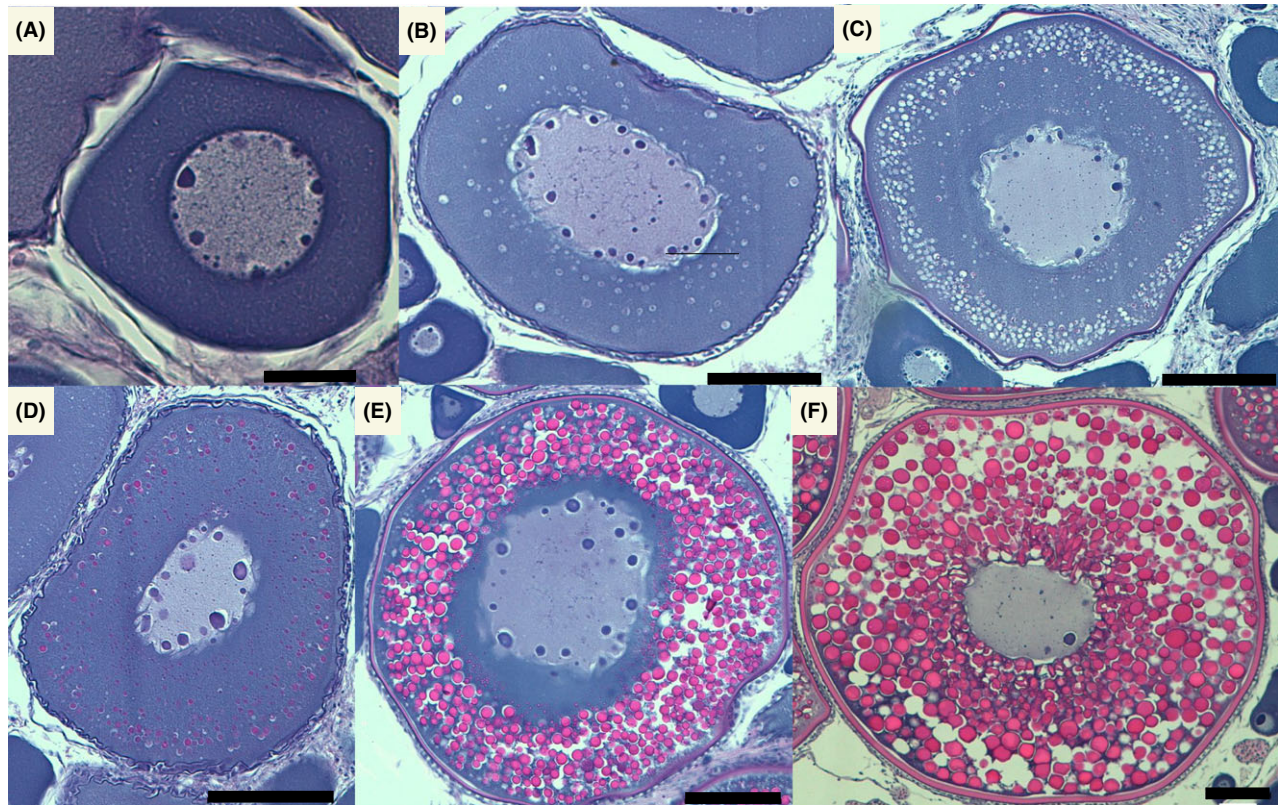


FIGURE 3. Oocyte development stages observed in histological slides of Sablefish ovaries in July and August: (A) perinucleolar, (B) early cortical alveoli, (C) late cortical alveoli, (D) vitellogenesis stage 1, (E) vitellogenesis stage 2, and (F) vitellogenesis stage 3. In all panels, the black scale bar is 100 μ m.

where m_a was the number of mature fish observed at age a or length a , and n_a was the total number of fish at age a or length a . We used the binomial likelihood to fit the observed proportion mature at age or length 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 in two ways: (1) fish determined to be skip spawning were classified as mature, or (2) they were classified as immature. Method 1 categorizes skip-spawning fish and fish that will spawn as functionally mature, where maturity is defined as those fish that are capable of spawning. Method 2 utilizes a definition of maturity that includes only those that are physiologically capable of spawning in this cycle.

The nonparametric 95% confidence intervals for each estimated logistic curve parameter ($a_{50\%}$ and δ) were calculated using 1,000 bootstraps. Within each survey leg, the logistic parameters for each maturity classification method were compared with one another for statistical significance. If there was no overlap between the 95% confidence intervals, there was a significant difference between the parameters at an alpha (α) of 0.05 (Davidson and Hinkley 1997).

RESULTS

Sample

A total of 624 female Sablefish were sampled for maturity on survey legs 3–7 (Table 4). All fish were aged, with the exception of the 37 fish collected on leg 4. These samples were used for comparing classification methods and for length at maturity but not for age-at-maturity analyses. Data were pooled for legs 4 and 5 because there were only 37 fish on leg 4 and because the two stations sampled on leg 4 overlapped with the area sampled on leg 5 (Figure 1). The age range of fish collected during the survey was 2–62 years (Figure 4). Immature fish, defined using histology, ranged in age from only 1 year to 14 years and in length from 41 to 72 cm; mature fish ranged in age from 3 to 62 years and in length from 51 to 96 cm; skip-spawning fish ranged in age from 5 to 46 years and in length from 55 to 95 cm (Figure 4).

Oocyte Development

The transitional cortical alveoli stages (stages 2 and 3) between perinucleolar and vitellogenic stages were uncommon, particularly stage 3 (Figure 5). The proportion of fish with early stage vitellogenic oocytes (stage 4)

TABLE 4. The number of female Sablefish sampled for maturity per survey leg in the Gulf of Alaska. The matrix of values shows where at-sea macroscopic and microscopic methods were in agreement. There was no attempt to identify skip spawning using macroscopic methods. The cells in gray denote that there was disagreement, and the “% dissimilar” represents the proportion of samples where there was disagreement. The sum of all values for each leg is equal to the total number of fish sampled. Also included is the number of fish per leg that were classified microscopically as skip spawning, and whether the skip-spawning fish were classified macroscopically as mature (will spawn) or immature.

Leg	Macroscopic	Microscopic		% Dissimilar	% Dissimilar for leg	Skip spawn
		Immature	Will spawn			
3	Immature	22	4	15	11	6
	Will spawn	20	169	11		
4	Immature	2	0	0	0	1
	Will spawn	0	34	0		
5	Immature	38	2	5	5	1
	Will spawn	5	100	5		
6	Immature	20	2	9	29	1
	Will spawn	33	64	34		
7	Immature	13	0	0	28	2
	Will spawn	27	58	32		

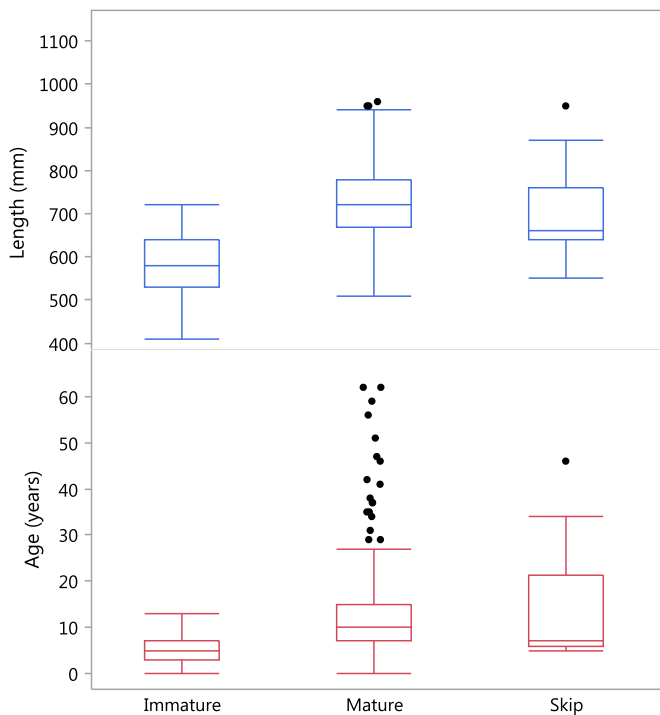


FIGURE 4. Box plot of the number of female Sablefish sampled in the Gulf of Alaska by maturity category and either length or age. Maturity was classified by analyzing histology slides. The box encompasses the 1st and 3rd quartiles, with the median represented by the horizontal line within the box. The whiskers are 1.5 times the interquartile range. In cases where the furthest data point was closer than the computed whisker range, the furthest data point was used for the whisker. Black circles are points outside this range.

decreased throughout the survey and the proportion of fish with the most developed oocytes (stage 6) increased at the end of the survey on leg 7 (Figure 5).

Maturity Classification: Macroscopic and Microscopic

The only macroscopic maturity stages identified were immature, mature, and resting. Agreement between microscopic and at-sea macroscopic classification of maturity varied by leg (Table 4). The highest disagreement was on legs 6 and 7 (both in the central Gulf of Alaska and both sampled in August). The source of disagreement on legs 6 and 7 was that fish were classified as mature (will spawn) macroscopically and as immature microscopically. There was low disagreement on leg 5 and none on leg 4 (2-d leg). The disagreement on leg 3 was moderate, compared with the relatively high disagreement on legs 6 and 7. The disagreement on leg 3 occurred for both categories (immature and will spawn).

Skip-Spawning and Previous Spawning

A total of 11 fish were classified as skip spawning using microscopic methods, which constituted 2% of mature fish (i.e., skip-spawning fish + those maturing towards spawning) (Table 4). Skip-spawning fish were found on all legs except for leg 6. In nine cases the fish were classified macroscopically as mature, one was classified as immature, and one as resting. Leg 3 had the most fish that would skip spawning ($N = 6$). The majority of fish that would skip spawning were under 18 years old (72%) (Figure 6).

There were sparse postovulatory follicles or residual eggs observed in some ovaries on all survey legs: 19 (9% of samples) on leg 3, 5 (14%) on leg 4, 12 (8%) on leg 5, 8 (7%) on leg 6, and 3 (3%) on leg 7. These residual structures from previous spawning were not present in all fish with other indications of past spawning (such as thick stroma, blood vessels within the ovary, and a loose oocyte structure).

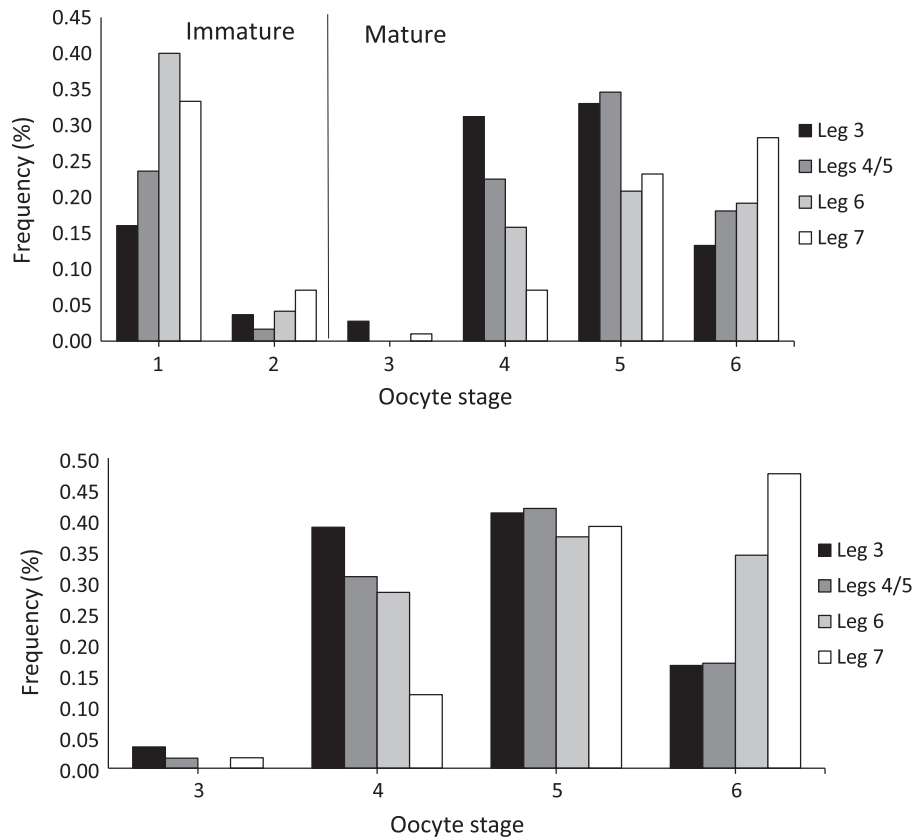


FIGURE 5. Frequency of each oocyte developmental stage (Table 3) by leg of the Alaska Fisheries Science Center Gulf of Alaska longline survey in July and August, where the developmental stage is the most advanced oocyte stage in the ovary. The top panel includes ovaries with oocytes in all stages of development. The bottom panel is the frequency of Sablefish with ovaries with maturing oocytes only (only stages 3–6 included in percentages).

Skip-Spawning Categorization for Maturity Models

When microscopic methods were used, fish that were predicted to skip spawning were either categorized as immature or mature in logistic age- and length-at-maturity models. The classification of skip-spawning fish as immature or mature had minimal effects on the curve parameters because there were only one to six skip-spawning fish on each leg. Parameters for all models are in Table 5.

Age and Length at Maturity: A Comparison of Classification Methods

Using the macroscopic at-sea method resulted in significantly younger and smaller estimates of age and length at 50% maturity on legs 6 and 7, and for all data pooled, compared with the other maturity classification methods, i.e., there was no overlap between the bootstrapped, non-parametric confidence intervals (Table 5; Figures 7, 8). The at-sea macroscopic method resulted in an $a_{50\%}$ that was 2.6 years younger and 7.9 cm smaller than the other classification methods on leg 6 and resulted in an $a_{50\%}$ that was 2.3 to 2.5 years younger and 6.2 to 7.7 cm smaller than the other methods on leg 7 (Table 5). When data

from all legs were pooled, the $a_{50\%}$ for at-sea macroscopic data was 1.2 to 1.4 years younger or 3.2 to 4.3 cm smaller than when other methods were used. The macroscopic at-sea data also yielded steeper slopes for the age-at-maturity curve for legs 6 and 7 and for all data pooled and also a steeper slope for length at maturity on leg 7 (Table 5). The standardized macroscopic method yielded very similar results to the microscopic methods on all legs, and there were no significant differences between age or length-at-maturity curve parameters (Table 5; Figures 7, 8).

Age and Length at Maturity: A Comparison of Survey Legs

Using the macroscopic at-sea method to classify maturity, the youngest ages at maturity and the smallest lengths at maturity were on legs 6 and 7. Conversely, when using the standardized macroscopic or microscopic methods, the youngest and smallest ages and lengths at maturity were on legs 3 and 5. The standardized macroscopic and microscopic methods yielded very similar results; when using these methods, length and age at maturity increased from leg 3 to leg 6 and then decreased

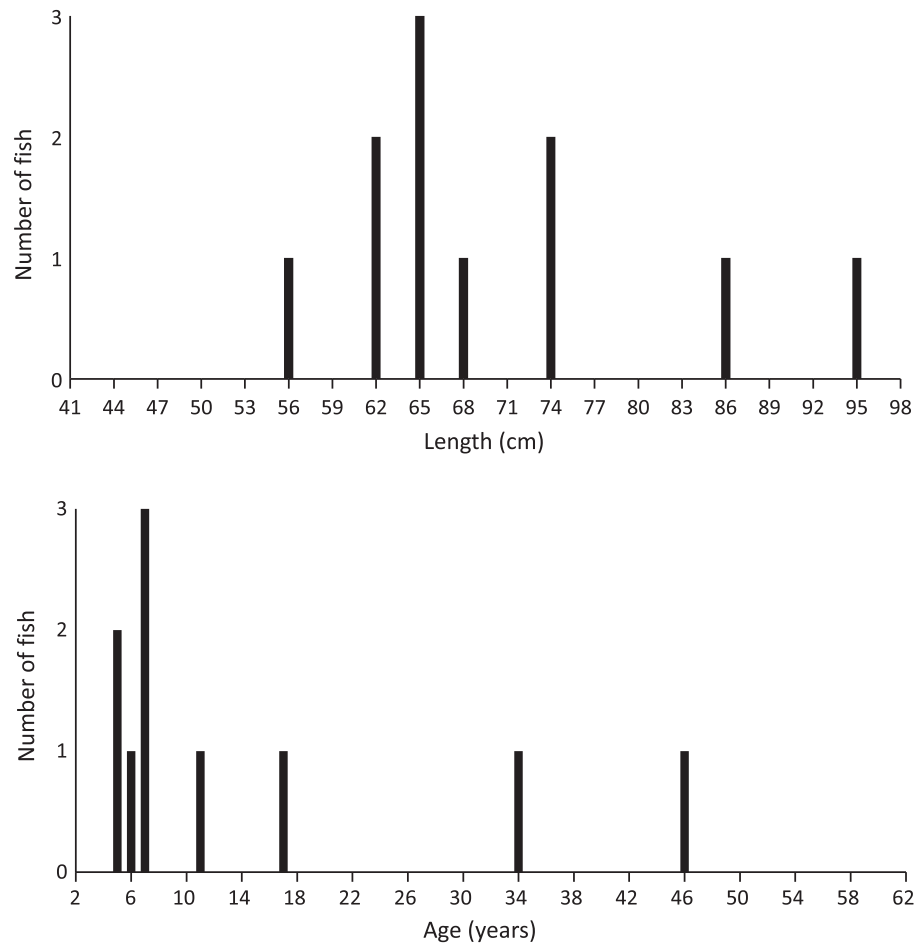


FIGURE 6. Number of female Sablefish sampled in the Gulf of Alaska predicted to skip spawning by length and age.

on leg 7, but leg 7 had the second highest estimates after leg 6 (Table 5).

DISCUSSION

Classification Methods

The at-sea macroscopic method resulted in earlier or smaller estimates of age or length at maturity for legs 6 and 7 than the microscopic or the standardized macroscopic methods; however, there were no differences on legs 3 and 4–5. This discrepancy could be related to the sampling date, differences in the time of the onset of maturation by geographic area, or differences in the population structure by area. For example, there could be more first-time spawning fish in one area, which are sometimes confused with immature or resting fish at a higher rate than fish that have spawned before (McBride et al. 2013). The differences between methods could also be due to an observer effect introduced because there were different staff on each leg. An observer effect has not been quantified

because this study was only carried out in 1 year and different scientists were on each leg. Although 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 examining microscopic structures in histological slides in combination with photographs of fresh ovaries. Scientists on the survey seldom have experience with fish reproduction studies, particularly with Sablefish, and some staff may not be deployed on the survey every year. Pictures of a variety of ovaries that are immature and mature are used for training, but because there are not obvious, discrete differences in ovary sizes between fish that are immature and maturing, the training does not appear to always be effective. Although the differences between model results using different classification methods were large on legs 6 and 7, it is possible that the differences will fluctuate annually. Maturity classifications using all three methods over several years would be required to determine if the differences we saw between methods on each leg are a regular occurrence.

TABLE 5. Age-at-maturity (years) and length-at-maturity (cm) logistic regression parameters for Sablefish collected on legs 3–7 of the Alaska Fisheries Science Center longline survey in the Gulf of Alaska. At-sea macroscopic (Macro), standardized macroscopic (Standardized macro), or microscopic (Micro) methods were used for classifying maturity. When using microscopic methods, fish that were predicted to skip spawning (SS) were treated as either immature (SSImm) or mature (SSMat) in logistic models. There were no skip-spawning fish on leg 6. The 95% confidence intervals calculated using bootstraps are given in parentheses. A lack of overlap in the confidence intervals for parameters for each leg (data within each row are compared to one another) is denoted by a different letter to the right of the confidence intervals (z or y). If there are no letters in a row then there was overlap in the confidence intervals for all parameter estimates.

Leg	Age or length	Micro SSImm	Micro SSMat	Standardized macro	Macro
50% maturity					
Pooled	Age	6.1 (5.8–6.3) z	6.0 (5.7–6.2) z	6.1 (5.9–6.4) z	4.8 (4.5–5.0) y
3	Age	5.7 (5.2–6.1)	5.6 (5.3–6.0)	5.6 (5.2–6.0)	5.1 (4.7–5.4)
5	Age	5.8 (5.5–6.7)	5.6 (4.9–6.6)	5.9 (5.3–6.8)	5.6 (5.0–6.2)
6	Age	6.9 (6.3–7.5) z		6.8 (6.3–7.5) z	4.8 (4.3–5.3) y
7	Age	6.2 (5.6–7.1) z	6.0 (5.5–6.7) z	6.1 (5.5–7.2) z	3.8 (3.0–4.3) y
Pooled	Length	62.6 (61.6–63.4) z	62.2 (61.4–63.2) z	61.5 (60.6–62.3) z	58.3 (57.5–59.4) y
3	Length	58.1 (55.4–61.1)	58.3 (55.7–60.6)	59.5 (57.2–60.8)	56.8 (54.0–58.8)
4 + 5	Length	60.3 (58.7–61.6)	59.7 (57.9–61.4)	60.5 (59.3–61.8)	60.0 (59.2–61.7)
6	Length	64.2 (62.4–65.3) z		64.2 (62.8–65.6) z	56.3 (54.4–58.7) y
7	Length	63.0 (61.6–64.9) z	62.5 (61.1–64.1) z	62.7 (61.1–64.1) z	55.3 (53.4–56.3) y
Slope					
Pooled	Age	0.86 (0.76–0.97) z	0.89 (0.80–1.02) z	0.80 (0.72–0.87) z	1.22 (1.18–1.37) y
3	Age	1.05 (0.94–1.37)	1.08 (0.93–1.28)	1.16 (1.00–1.42)	1.31 (1.16–1.58)
5	Age	1.30 (0.95–1.99)	1.35 (0.89–1.84)	1.14 (0.80–1.73)	1.42 (1.13–2.29)
6	Age	0.92 (0.80–1.15) z		0.95 (0.82–1.11) z	1.35 (1.16–1.72) y
7	Age	0.97 (0.80–1.21) z	1.06 (0.90–1.34) z	0.98 (0.77–1.33) z	1.99 (1.70–92.65) y
Pooled	Length	0.29 (0.24–0.36)	0.32 (0.29–0.39)	0.34 (0.28–0.41)	0.42 (0.36–0.54)
3	Length	0.18 (0.14–0.31)	0.25 (0.19–0.36)	0.35 (0.26–0.58)	0.38 (0.26–0.74)
4 + 5	Length	0.38 (0.27–0.74)	0.40 (0.32–0.74)	0.44 (0.30–0.88)	0.56 (0.42–4.55)
6	Length	0.32 (0.22–0.53)		0.31 (0.23–0.45)	0.34 (0.26–0.52)
7	Length	0.34 (0.26–0.51) z	0.34 (0.26–0.51) z	0.35 (0.26–0.49) z	1.01 (0.69–7.67) y

Although histology provides the most specific information on ovarian structures and development, it is more time consuming and more expensive than macroscopic methods. Histology requires time to (1) extract, label, and store ovaries at sea, (2) prepare tissues for a laboratory to create slides (or for a biologist to create their own slides), and (3) collect information on the ovarian structures from each slide and enter the data. The standardized macroscopic method requires the at-sea scientists to take extra time to take photographs of the ovary and for the experienced scientist to look at the photographs from the entire survey. However, the time it takes to review photographs and enter data is significantly less than the time required to analyze histology slides and enter the more detailed data on ovarian development. The at-sea macroscopic method takes the least amount of time because a photograph is not required. Histology is also more expensive than both macroscopic methods: the microscopic method requires fixatives and other supplies, staff time to prepare tissues, shipping of supplies and samples, slide preparation, and staff time to analyze slides and enter data. The standardized macroscopic methods requires a camera and

staff time to analyze the photographs. In terms of data quality, there are misclassification issues with the at-sea macroscopic method, which we documented on legs 6 and 7. Currently, neither the at-sea macroscopic nor the standardized macroscopic methods can be used to identify skip spawning.

Considering data quality and costs, the standardized macroscopic method may be practical to use when there are time and fiscal constraints. However, in a species where skip spawning has been documented and the rate has the potential to affect age and length at maturity, histology may still be required to identify skip-spawning fish. If this is the case, a combination of standardized macroscopic and microscopic methods could be used to minimize costs but maintain accuracy. One potential method could entail the collection of all ovaries at sea and subsequently histological slides are produced only for fish that have moderately sized ovaries or where there was some uncertainty in the standardized macroscopic classification. Alternatively, a random subsampling procedure could be used for monitoring the accuracy of the standardized macroscopic methods and for estimating skip-spawning rates.

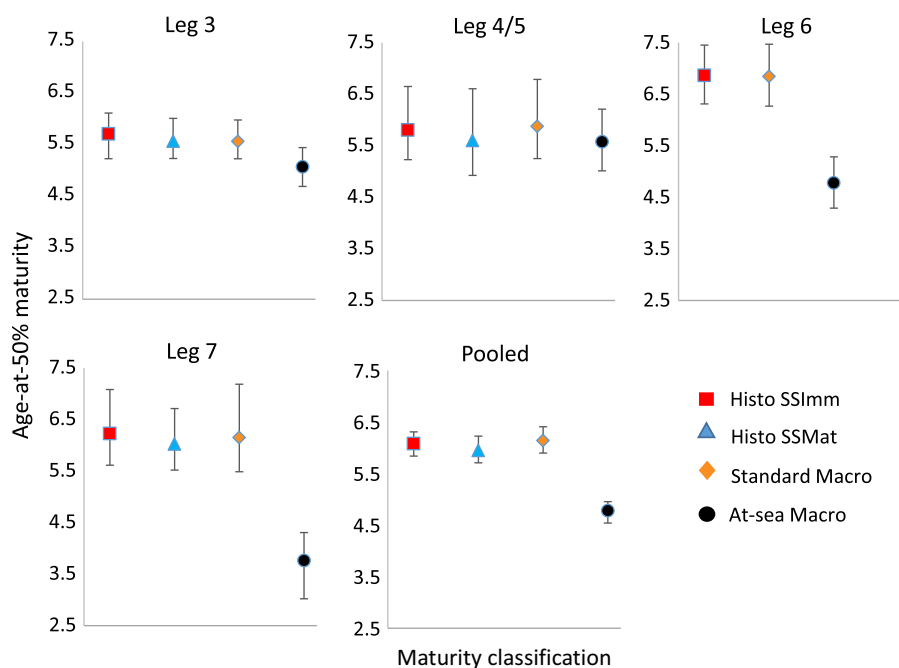


FIGURE 7. Parameters for age at 50% maturity for Sablefish collected on legs 3–7 of the longline survey in the Gulf of Alaska. Estimated values and 95% confidence intervals, calculated using bootstraps, are presented for four different maturity classification methods: at-sea macroscopic (At-sea Macro), standardized macroscopic (Standard Macro), microscopic where skip spawners were classified as immature (Histo SSImm), or microscopic where skip spawners were classified as mature (Histo SSMat).

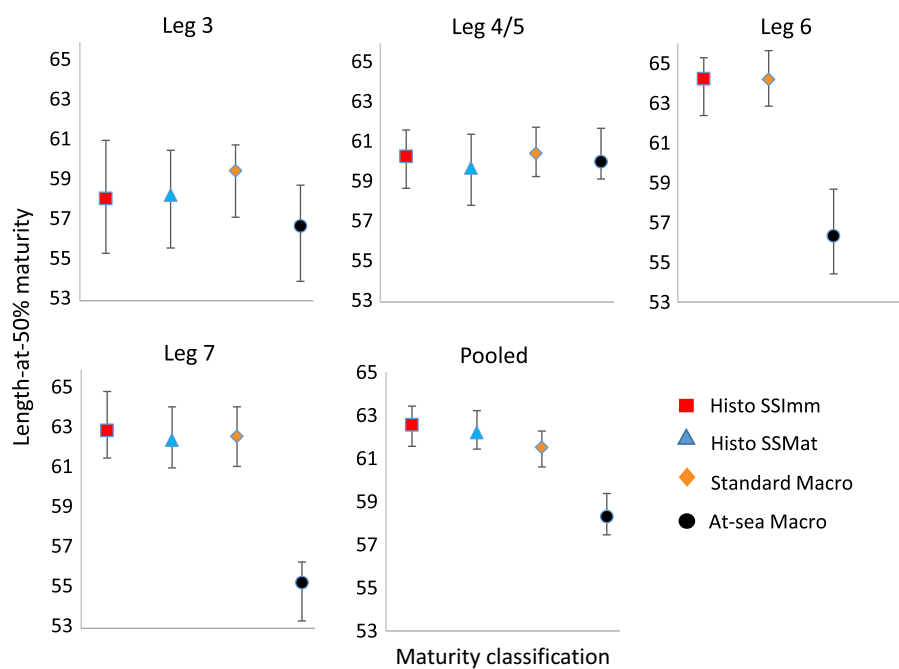


FIGURE 8. Parameters for length at 50% maturity for Sablefish collected on legs 3–7 of the longline survey in the Gulf of Alaska. Estimated values and 95% confidence intervals, calculated using bootstraps, are presented for four different maturity classification methods: at-sea macroscopic (At-sea Macro), standardized macroscopic (Standard Macro), microscopic where skip spawners were classified as immature (Histo SSImm), or microscopic where skip spawners were classified as mature (Histo SSMat).

The skip-spawning rate in the summer of 2015 (2%) was lower than in previous studies of Sablefish in Alaska: 21% in December 2011 (Rodgveller et al. 2016) and 6% in December 2015 (Rodgveller et al. 2018). The greater prevalence of skip spawning in 2011 had a larger impact on maturity-at-age estimates than in 2015. In 2011, the $a_{50\%}$ when skip spawners were considered immature (i.e., the physiological definition of maturity was used) was 3.1 years older than when they were considered mature (Rodgveller et al. 2018). In December 2015 it differed by only 0.6 years, and in summer 2015 it only changed the $a_{50\%}$ by 0.1–0.2 years, depending on the leg. A latitudinal range in age-at-maturity estimates was found in female Sablefish off the west coast of the United States (waters south of British Columbia, Canada) ($a_{50\%}$ = 4.9–11.0 years; Head et al. 2014). These data also indicate that Sablefish maturity can be variable and may be related to environmental conditions.

The North Pacific Ocean was in a cool phase during the 2011 Sablefish collection and was in a warm, positive Pacific Decadal Oscillation during 2015 (Zador 2015; North Pacific Marine Science Organization 2016a). Although the warm water in 2015 negatively affected many taxa in shallow water, such as crab, salmonids, birds, and mammals (North Pacific Marine Science Organization 2016b), our results from 2015 show that skip spawning was less prevalent during this warm period. It is unknown how changes in temperature and productivity closer to the surface may affect animals that reside in deeper water. However, it is possible that the colder surface water was associated with the higher skip-spawning rate in 2011 and the warmer water with the lower skip-spawning rate in 2015. More years of data are required to link environmental fluctuations to energy reserves and skip-spawning rates for Sablefish.

Skip spawning is plastic in other species as well (Atlantic Cod *Gadus morhua*, Rideout and Rose 2006; Argentine Hake *Merluccius hubbsi*, Macchi et al. 2016) and can be related to the energetic condition of the female and restricted rations (Burton and Idler 1987; Rideout and Rose 2006; Skjæraasen et al. 2009; Rideout and Tomkiewicz 2011). For Sablefish, the energy storage prior to when vitellogenesis has been initiated will dictate whether a fish will skip spawning. Oocytes were in various stages of vitellogenesis in July and so the time when this energetic threshold occurs is likely before July. Lab studies may provide information on the condition threshold for skip spawning and the time of year when adequate energy storage is required.

There are potential methods for adapting the standard, two-parameter logistic model, which asymptotes at 100%, to incorporate skip spawning. Adaptations depend on how skip spawning is related to size or age, the longevity of the species of interest, and the annual or spatial variability

(e.g., Secor 2008; Brooks 2013). In the current study and the winter 2011 and 2015 collections, fish at younger ages were skip spawning, while older fish were not (Rodgveller et al. 2018). This indicates that adding parameters to add flexibility to the model so that the curve can asymptote at values lower than 100%, or so that the maturity curve can take on a new shape (such as a dome), are not required. If there was evidence that older fish were skip spawning, exploration of alternative maturity models would be warranted.

The age-at-maturity data currently used in the Alaska Sablefish stock assessment was collected in the early 1980s using ovaries sampled in the summer and classified using macroscopic methods; in addition, only lengths were collected and were later converted to ages using an age-length matrix (Hanselman et al. 2007). Although the methods and timing are not preferable, the parameters for the age-at-maturity curve used in the stock assessment population model match closely to the age-at-maturity parameters from legs 6 and 7. The slopes from legs 6 and 7, when using microscopic or standardized macroscopic methods, range from 0.92 to 1.06, depending on the leg and whether skip-spawning fish are classified as mature or immature; the slope used in the assessment is 0.87. The $a_{50\%}$ on legs 6 and 7 range from 6.0 to 6.9 years, and the $a_{50\%}$ currently used in the assessment is 6.6 years. Using only the macroscopic assignment of maturity from at-sea personnel on legs 6 and 7 of the survey from 1996 to 2012, there was a wide range in the annual estimates of $a_{50\%}$ (5.5 to 8.6 years) (Rodgveller et al. 2016); therefore, the similarity between what is used in the assessment and what we found on legs 6 and 7 may be from random chance. Because the leg 6 and 7 results are so close to what is currently being used in the stock assessment, there may not be meaningful changes to management if the new data from this study are used in the Alaska Sablefish population model. However, data from the winter of 2011 and 2015 were not as similar to what is being used in the assessment model and the implications of these differences on management may be more significant.

Sampling Timing

In Alaska, the time frame of the summer longline survey encompasses the period in the reproductive cycle when Sablefish are either in a resting stage or have initiated oocyte maturation. For Sablefish, there are several issues with this summer sampling. Maturing oocytes on all legs were at various developmental stages, ranging from late cortical alveoli to later stages of vitellogenesis. This indicates that all Sablefish do not initiate maturation at the same time. Because there were ovaries in very early stages of maturation on every leg, it is possible that ovaries showing no signs of development will progress into vitellogenesis after the summer sampling

time frame. These fish could either appear to be immature or skip spawning during the summer. This may be one reason why the most fish predicted to skip spawn were on leg 3. Classification of a fish as immature or skip spawning, when it will in fact spawn, was least likely on leg 7, when more fish had progressed into later stages of vitellogenesis. Therefore, classifications on leg 7 will provide the most accurate predictions of which fish will spawn in the coming season. In addition, sampling occurred over a broad time frame and so the timing of development in each geographic area is still unknown. To test this, collections in each area would be required within the same time frame. This is not likely to occur on a fishery-independent sampling platform.

It is important to consider the potential for seasonal migrations and annual variation in migration patterns when sampling at any time of year. In December 2011, the majority of skip-spawning fish were in cross-shelf gullies (Rodgveller et al. 2016), and in summer of 2015 73% were on the slope. It may be that skip-spawning fish congregate in gullies only in the winter. In addition, during the winter sampling that was done with trawl gear, the sex ratio was heavily skewed towards males (K. Echave, National Oceanic and Atmospheric Administration, Alaska Fisheries Science Center, personal communication), whereas they are near 1:1 on the summer longline survey (Hanselman et al. 2017). This may indicate that there was some vertical migration or aggregation associated with the sex of the fish close to the spawning season. The survey design in the summer provides comprehensive sampling because the longline survey samples a wide range of depths at each station. For special surveys, gear configurations should be flexible enough to sample a wide range of depths in order to sample the depth range where both males and females are concentrated.

December sampling has proven to be preferred to summer months in Alaska because it is closer to the spawning season (Rodgveller et al. 2016). However, sampling after the summer survey but prior to spawning can be difficult to accomplish because of inclement weather and because there are no regular bottom trawl or longline surveys after August in Alaska. Some periods in the fall would provide more accurate maturity classifications than much of the summer because fish that will spawn will have oocytes in vitellogenesis. Unfortunately, there is very little commercial fishing effort between September and mid-November, the end of the federal individual fishing quota season in Alaska. However, fishery samples could be useful for evaluating if there is geographic or annual variation in the timing of development in the summer. There are potential sampling bias issues with fishery samples because the

industry typically targets areas and depths with older, larger fish and fewer young, small fish. If development timing is earlier in older or larger fish, as documented in other taxa (e.g., *Sebastes* spp.; Nichol and Pikitch 1994; Rodgveller et al. 2012), then fishery samples will not be adequate.

Recommendations

In this study the macroscopic at-sea method was not successfully validated for all legs of the survey. If using samples from the summer survey, the microscopic results indicate that the most reliable maturity classifications will come from the last leg of the survey, using either the standardized macroscopic or microscopic methods or a combination of the two. This is because oocytes will be in later stages of development at the end of the survey and because there may be misclassifications when using the at-sea macroscopic method. A caveat to this plan is that the geographic sampling area would be reduced to a portion of the central Gulf of Alaska. However, this is near the center of the distribution of Sablefish in Alaska, Sablefish are highly migratory (they are not likely to have discrete stocks with differencing life history) (Hanselman et al. 2014), and it is still a broad geographic area. Because of its size, it is not possible to sample all of the Gulf of Alaska during months of the year when maturity can be accurately classified, especially on a regular basis.

For many species, sampling platforms are only available during a time of year that is not ideal for maturity classification. It is important to study the time course of oogenesis to define the time period when spawning or skip spawning in the coming season can be predicted with accuracy and what methods are required. For example, the macroscopic at-sea method may be useful when spawning is imminent but is not useful during earlier months. These results demonstrate that accurate maturity classifications may be collected during months that are not preferable when histology or a single scientist with experience is used for classifying all samples, even if classifications are made from photographs.

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