# Evaluation of the Macroscopic Staging Method for Determining Maturity of Female Walleye Pollock *Theragra chalcogramma* in Shelikof Strait, Alaska

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Abstract: Macroscopic or visual staging is the primary method for determining maturity status of walleye pollock *Theragra chalcogramma* stocks in the Gulf of Alaska and the Bering Sea, although its accuracy has not been established. To address this, maturity data and samples were taken during several annual spawning surveys of pollock conducted in Shelikof Strait, Gulf of Alaska. Comparison of histological and macroscopic staging methods on 126 ovary samples resulted in a 25% misclassification rate, with 75% of these misclassifications reclassified into the adjacent stages. Misclassifications were most common among developing stages 2 and 3, and the prespawning stage 4. Paired readings of female pollock maturities were made at sea during the surveys, with readers disagreeing on classification stage in 36% of 411 observations. Reader disagreements were most common between stages 3 and 4 (11.4%), stages 4 and 5 (5.8%) and stages 7 and 8 (4.8%). Errors made across the boundaries of stages 3 and 4 as well as stages 2 and 8 can impact the estimates of length at 50% maturity and consequently the spawning stock biomass. These types of errors were observed in the histological validation process, but due to the very low incidence of stages 3 and 8 in the survey population (1.8% and 0.4%, respectively), the contribution of these potential errors to maturity assessments can be considered small.

# INTRODUCTION

Maturity estimates are an important component of fisheries assessments, allowing biologists to determine the reproductive potential of fish populations and monitor changes in biological characteristics of exploited fish stocks. In many fisheries, harvest recommendations rely on the spawning potential of the population, which is based on assessments of reproductive condition collected during biological surveys and from sampling commercial catches.

Alaska populations of walleye pollock *Theragra chalcogramma* (hereafter pollock) support one of the largest single-species fisheries in the world. Currently, the eastern Bering Sea fishery accounts for the great majority of the harvest, with the Gulf of Alaska fishery substantially reduced below peak landings in the mid-1980s. Pollock in Shelikof Strait spawn in late March and April, in a distinct spawning event thought to represent the primary reproductive output for the year. Scientists aboard National Oceanic and Atmospheric Administration (NOAA) research vessels have monitored this spawning aggregation annually since 1981 (except 1982 and 1999) using acoustic methods

combined with opportunistic trawls for identifying fish seen acoustically. Trawl catches provide biological data for pollock abundance estimation, including maturity data and ageing structure samples. Acoustic or echo integration-trawl (EIT) surveys provide a component of the pollock abundance estimates used in stock management. In maturity estimation, female gonad development is of primary concern, as stock assessment scientists are monitoring spawning potential, which is considered a proxy for annual egg production. Recent reductions in pollock spawning biomass observed in Shelikof Strait have prompted an evaluation of pollock maturity data.

Estimates of length and age at 50% maturity derived from survey data vary from year to year, prompting scrutiny of the maturity staging process on which these indices are based. It is important to discern between potential errors in maturity data and true biological shifts in maturity characteristics.

In general, fisheries surveys rely on macroscopic staging of maturity due to the large numbers of samples that can be collected and the lack of required instruments and specialized procedures. However,

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in many cases the accuracy of macroscopic staging is unknown (West 1990). Reducing the continuous process of ovarian development to discrete categorical classification based on external examination and a basic written description of the stage may result in individual interpretations of staging characteristics, even by experienced sampling personnel. Additionally, certain reproductive states, such as those not exhibiting active spawning characteristics cannot be reliably identified by macroscopic methods (Hunter and Macewicz 2001).

The definitive classification of maturity status is obtained by examining histological preparations of ovarian tissue. Histological sections reveal the detail of oocyte development, which presents less ambiguity in assigning maturity status. Several studies concerning histology of pollock ovarian development have been made (Tanino et al. 1959; Hinckley 1987; Merati 1993; Stah 2004), although a histological validation of maturity classification for this species has not been directly addressed. Macroscopic staging has been validated with histological methods for the Atlantic cod *Gadus morhua* (Sorokin 1957; Morrison 1990; Tomkiewicz et al. 2003), which undergoes comparable ovarian development.

Other objective methods for assessing maturity are available to fisheries biologists, such as measurements of egg diameter (Ramsay and Witthames 1996), monitoring of hormone and vitellogenin levels (Merati 1993), and the use of morphometrics and the gonadosomatic index (GSI; Demartini and Lau 1999). Most of these approaches suffer from technological complexity and increased sample processing time requirements, and result in much smaller sample sizes.

Maturity data collected during pollock surveys in Alaska from 1979 to 1996 was based on a 5-stage scale developed specifically for pollock, which was expanded into an 8-stage scale in 1996 that remains in use. The 8-stage scale was modeled after Maier's (1908) general teleost maturity classification, and was refined through an international collaboration of scientists working with pollock, allowing comparisons of maturity data to be made among research groups.

The principal limitation of macroscopic staging is its apparent subjectivity. The process of macroscopic staging consists of readers applying qualitative and quantitative criteria outlined in written descriptions of the stages to an ovarian sample without the aid of magnification. The written stage descriptions may not be referenced continually, which often results in readers relying on individual search images, a process that highlights the potential for subjectivity to compromise the accuracy of the data. Categorical criteria are more

definite, as they require the presence of a character, whereas quantitative criteria require the reader to estimate relative sizes of the ovary or eggs. Quantitative criteria are also changing gradually through ovarian development, requiring readers to establish hard boundaries between stages. In separating these stages, we rely on the readers to make a quick estimate of these values without measurement, a decision that may be influenced by surrounding observations.

Maturity stage data are collapsed into a binomial value of mature and immature, which are then modeled to determine length and age at 50% maturity ( $L_{50}$  and  $A_{50}$ ). The decision on which maturity stages should be considered potential contributors to the spawning population is often difficult to determine due to the lack of information on the duration of maturity stages, specifically for those fish that are not actively spawning at the time of observation.

The working definition of mature fish has also varied among researchers, with the term encompassing all non-virgin fish (Rideout et al. 2005), or alternatively only fish that will spawn in a given spawning season (Hunter et al. 1992). Evidence of previous season spawning is generally not visually discernible in pollock, as in related gadoids (Bromley and Casey 2001). Thus the latter seasonal definition of maturity is used during the Shelikof Strait spawning survey, which is timed to capture the peak spawning activity.

In separating those fish that are likely to spawn in the Shelikof Strait spring spawning event from those that will not, the maturity assessment process relies on two boundaries in the maturity cycle. One boundary separates immature and developing fish from those in prespawning condition (prespawning boundary) between stages 3 and 4. The other boundary separates spawned-out fish from those that are unlikely to have spawned in the current spawning event (post-spawning boundary) in stages 7, 8 and 2.

The objectives of this study are to evaluate the effectiveness of the macroscopic staging method in separating female pollock across these two stage boundaries. Additionally, the accuracy of the 8-stage scale is examined and recommendations for its improvement are suggested.

# **METHODS**

## **Biological Data Collection**

Standard biological data, consisting of length, weight, sex, maturity, and otoliths for ageing are routinely collected during the annual Shelikof Strait EIT (SSEIT) survey conducted aboard the NOAA ship *Miller Free*-

man. For this study, pollock ovary samples and weights for histological analyses were taken from Shelikof Strait during the 2004 and 2005 winter survey seasons. and augmented with samples of spawned out fish from Sanak Trough in February 2005 (Figure 1). Additional samples were also taken during transit through Shelikof Strait in February 2005 to sample earlier developing stages of the spawning aggregation. All weights were measured at sea using a motion-compensated electronic platform scale with a resolution of 2 g. For estimating reader agreement, individual female pollock were read by several readers. Paired readings of 411 fish were made during SSEIT surveys in 2003, 2004 and in Sanak Trough and Shelikof surveys in 2005. Written stage descriptions and photographs of the maturity stages were available to the personnel in the sample processing area for reference.

# Histology

Histological sections were made from preserved ovary samples collected at sea. A cross section of one of the posterior lobes of the ovary nearest to the vent was removed, embedded in paraplast, and sectioned at 6–10

μm. Sections were stained using a standard hematoxylin and eosin procedure (Luna 1968). Mounted slides were digitized using a microscope-mounted camera, and analyzed using image analysis software Image J1. A portion of the scanned section was selected and the individual oocytes within the selected area were staged into development classes using appearance and size criteria. Established oocyte development criteria for teleosts were used in assigning oocyte classes (Morrison 1990; Tyler and Sumpter 1996), with several oocyte stages grouped into development classes (Table 1). Histological samples were classified by identifying the most advanced oocyte stage present in the ovary and referencing previous pollock histological studies (Hinkley 1987; Merati 1993). Ovarian development was classified into 9 stages, with the first 8 stages equivalent to the macroscopic maturity classification, and stage 9 representing female pollock with abnormal ovaries or abandoned spawning. Histological stage criteria were based on corresponding macroscopic descriptions in the 8-point pollock maturity scale (Table 2). Stages 1 and 6 were regarded as sufficiently unique in appearance and were not addressed by comparisons and histological verification.

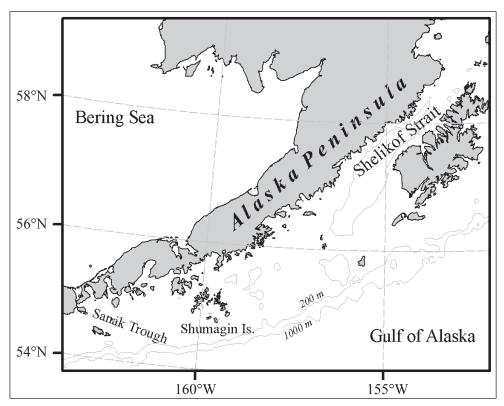


Figure 1. Walleye pollock spawning survey locations sampled for maturity data and ovary tissue samples. Maturity data was collected from Shelikof Strait from 2003 to 2005. Additional samples of spawned out fish were collected in the Sanak trough in 2005.

Table 1. Histological characteristics of primary oocyte development classes observed in ovaries of walleye pollock used for histological classification of ovarian maturity.

Oocyte Class	Description
UY	Perinucleous stage oocytes, have no chorion. Round centered nucleus clearly distinct from heavily stained basophilic cytoplasm, in later perinucleus stages zoning of cytoplasm is observed.
DV	Oocytes undergoing vitellogenesis, increasing in size. Encompasses the yolk-vessicle stage, primary and secondary yolk stages. Yolk granules appear in cytoplasm starting at the oocyte perimeter. Chorion is visible.
MA	Uniform darker staining yolk globules occupy the whole cytoplasm. Nucleus become sickle shaped and migrates toward the cell edge (nuclear migration)
HY	Oocytes has smooth lightly staining cytoplasm, and an irregular shape due to dehydration during fixation. Some oocytes have ovulated and are free inside the ovary.
POF	The empty follicle appears as an irregular ribbon shape with an empty center. Tissue appears granular
ATR	Atretic oocyes (a type) appear irregular in appearance, with non-uniform granular cytoplasm. Weakly staining cytoplasm represents re-absorbing yolk material.

Table 2. A comparison of macroscopic and histological characteristics of walleye pollock ovary development across maturity stages.

Maturity stage	Macroscopic stage description	Histological stage description	Most advanced oocyte class	Frequency of occurence <sup>a</sup>
1 - Immature	ovary transparent colorless to gray eggs invisible to eye	-	-	31.5
2 - Developing I	length is less than half of ventral cavity single eggs can be seen with magnifying glass occasionally small orange ovaries	ovary contains unyolked oocyte(UY) embedded in lamellar tissue occasional presence of atretic oocytes(ATR) in final stages of re-absorption	UY	21.6
3 - Developing II	ovaries opaque with blood capillaries occupy about half the length of ventral cavity eggs visible to eye as whitish, granular	developing oocytes begin vitellogenesis (DV), increase in size, reserve unyolked oocytes remain	DV	1.8
4 - Prespawning I	ovaries orange, reddish occupy about 2/3 of ventral cavity eggs clearly discernible, opaque	ovary filled with large, mature, fully yolked oocytes (MA) in final stages of maturation	MA	21.4
5 - Prespawning II	ovaries fill ventral cavity some eggs translucent (hydrated)	most oocytes are in final stages of maturation, some hydrated oocytes (HY) free from follicles	НҮ	19
6 - Spawning	roe runs with slight pressure most eggs hydrated (translucent) with few opaque eggs left in ovary	-	-	1.7
7 - Spent I	ovaries not yet fully empty few opaque eggs left in ovary	some unspawned hydrated oocytes seen among post-ovulatory follicles (POF) and few arthretic oocytes	POF	2.6
8 - Spent II (resting)	ovaries empty, red a few eggs in the state of reabsorption	ovary containing reabsorbing postovulatory follicles and atretic oocytes thick ovary wall	POF	0.4
Abandoned Spawning <sup>b</sup>	-	ovary containing large percentage of arthretic oocytes, without post- ovulatory follicles	ATR	-

<sup>&</sup>lt;sup>a</sup> Represents average percentage of macroscopic stage occurrence in Shelikof Strait, 1996–2004.

<sup>&</sup>lt;sup>b</sup>Observed only by histological methods.

# **Maturity Indices and Error Modeling**

The 8-point scale macroscopic classification data is used as a basis for determining the proportion mature at length by assigning stages 4 through 8 as mature or spawning, and stages 1 through 3 as immature, which includes virgin fish and non-virgin fish that will not spawn during the Shelikof Strait spawning event. Maturity was modeled using a logistic regression in the general form

$$p_i = \frac{1}{1 + e^{-(\alpha + \beta E)}}$$

and the logit transformation

$$h \frac{p_i}{1 - p_i} = \alpha + \beta E,$$

where  $p_i$  equals the proportion mature at length i,  $\alpha$  and  $\beta$  are the fitted parameters, and FL is the fork length. Parameter values were estimated using general linear model fitting in Matlab software (v. 7.0.1; Mathworks Inc, 2004). The length at 50% maturity was estimated as the negative ratio of the parameters (- $\alpha/\beta$ ).

Female spawning stock biomass (SSB) was estimated as

$$SSB = \sum_{i=1}^{n} p_i b_i ,$$

where  $p_i$  is the proportion mature at length i and  $b_i$  is the biomass at length i. The proportion mature is estimated from the logistic model.

The potential effects of maturity misclassification on  $L_{50}$  and SSB were modeled by applying classification error to a select size group. Aggregate maturity data from survey years 2000 to 2005 showed that female fish in the size range of 31 to 55 cm represented the great majority of the variation in maturity at length (Figure 2). Observations below this size range were considered juvenile or immature; observations above this size range were considered adult or mature. This simulation evaluated the effect of introducing random classification errors in the transitional range of observed survey data collected for SSEIT survey years from 2003 to 2005. A thousand simulation runs were made at each error rate from 0 to 0.3 in increments of 0.05. This was accomplished by estimating a random probability of misclassification equal to the error rate (0-0.3) being tested for each observation using Matlab software (Version 7.01, Mathworks, Inc.).

# **RESULTS**

Gonad development in pollock proceeds similarly to other gadoid species, with spawners entering an annual cycle of maturation, spawning, and recovery periods (Figure 3). The majority of females encountered dur-

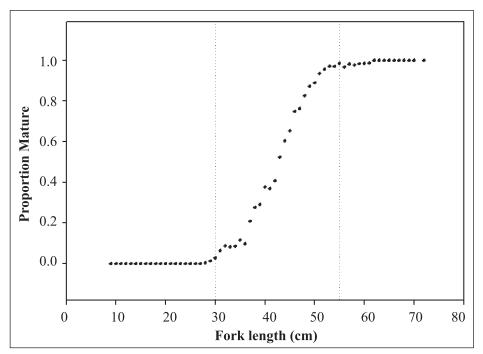


Figure 2. Female walleye pollock maturity data from Shelikof Strait echo integration-trawl surveys conducted in winter from 2000 thru 2005. Three length strata represent the general maturity pattern in pollock.

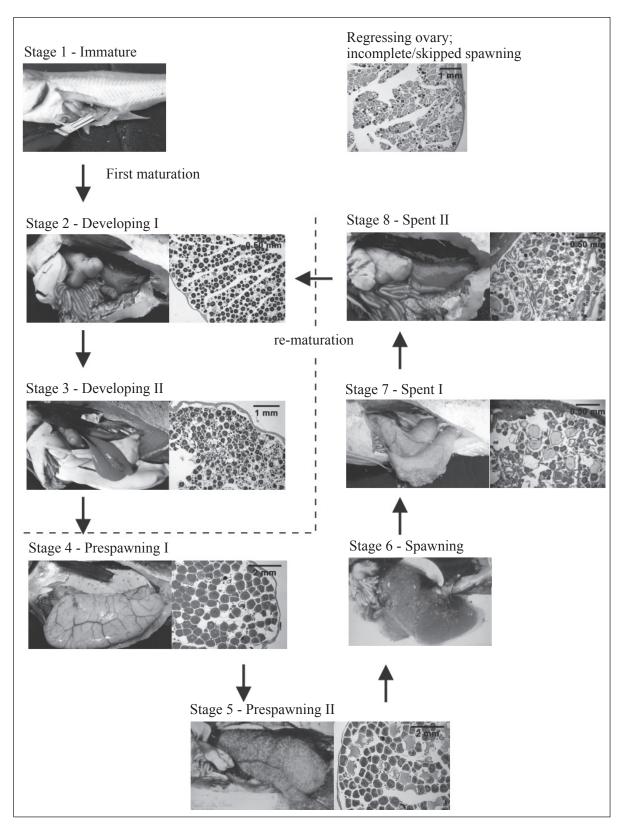


Figure 3. Maturity cycle of female walleye pollock. The typical appearance of the ovary is shown next to a histological section. The dotted line separates immature and mature stages. An example is shown of a regressing ovary, possibly constituting a separate stage. Stages 1 and 6 were not examined histologically.

ing SSEIT surveys from 1996 to 2005 were in early maturation stages 1 and 2 (47.6%), and in prespawning stages 4 and 5 (44.4%), although all stages were observed.

# **Histological Verification of Macroscopic Staging**

The distribution of oocyte classes in each stage observed in pollock typifies group synchronous spawning, as groups of oocytes mature and reserve cells are seen during all stages (Figure 4). Histological verification of 126 ovary samples resulted in an overall correct classification rate of 75% (Table 3). Of the 32 misclassified observations, 24 (75%) were reclassified into an adjacent stage, with stage 8 considered adjacent to stage 2 in the maturity cycle. The direction of reclassified stages suggests an underestimate of maturity, as 11 reclassified observations were changed from immature to mature fish, and none of the originally mature fish were reclassified as immature. This finding may represent a personal bias of the individual reader responsible for the macroscopic staging. It may also be influenced by the sample composition, as collections were stratified by maturity and therefore overrepresented uncommon and potentially problematic stages. The transitional stage 3 was proportionally the most commonly misclassified stage, apparently sharing macroscopic appearance with reabsorbing fish, as well as early prespawning fish (Figure 3). Due to the importance of these stages in the prespawning boundary, the written description of stage 3 appears inadequate to resolve it from similar appearing but developmentally distinct individuals, specifically postspawners and those with regressing ovaries.

A large number of macroscopic classification errors were also seen between the commonly observed stages 4 and 5, which are separated by the presence of hydrated eggs. Many of these samples contained hydrated eggs near the lumen of the ovary, which could not be seen externally but were present in the histological sections. In the histological classification, stage 9 was added to define those ovarian samples characterized by large levels of atresia, or obvious abnormalities (Figure 3). The exact reproductive status of these individuals is not known, although the high level atresia may be indicative of individuals resorbing yolk material prior to complete maturation. These individuals were encountered in low numbers, generally outside of the spawning aggregation.

# **Paired Reader Comparison**

Paired reader comparisons showed an overall reader disagreement of 36% in all observations across all stages with the highest rates observed between stages 3 and 4 (11.4%), and stages 7 and 8 (4.9%; Table 4). In only 8 of the 411 (1.9%) observations did the reader disagreement involve nonadjacent stages. Stage 7 classifications showed low conformity (10%), indicating that the current written descriptors of this stage may not be detailed enough for accurate macroscopic classification. When comparing individual reader data on a binomial mature/immature basis, the disagreement rate decreased to 12%. The primary contributor to this value is lower reader agreement involving stages 3 and 4, at the prespawning boundary.

# **Modeling Reader Error**

The effect of random misclassification of the transitional individuals had differing effects, with the direction and magnitude of change in  $L_{50}$  and SSB varying by survey year (Figure 5, top). The sensitivity of  $L_{50}$ and SSB to random misclassifications appears to be regulated by the sizes of the dominant age class. In the survey years examined, the strong 1999 year class is mostly immature in 2003, progressing toward mostly mature in 2005 (Figure 5, bottom). Specifically, in 2003 survey data, increasing misclassification rates resulted in a greater proportion of the dominant biomass appearing mature, and due to the smaller size distribution of the biomass in this year, the  $L_{50}$  estimate was lower. In the following years, size distribution had shifted toward larger, mature fish and the misclassification error effect is reversed, with more mature individuals being randomly reclassified as immature.

# **DISCUSSION**

Errors in field-collected maturity data can have multiple sources, such as incomplete or inaccurate stage descriptions, or reader inexperience. Estimating this error requires the development of validation techniques based on histology and inter-reader comparisons. Comparing histological and macroscopic maturity classification methods relies on an accurate histological interpretation of the written stage description, and an understanding of the stage evolution in the context of the maturity cycle. It is assumed that maturity stages represent distinct developmental states that can be defined by macroscopic and histological characteristics. Finding equivalent histological descriptors of the macroscopic stages is not an objective process in

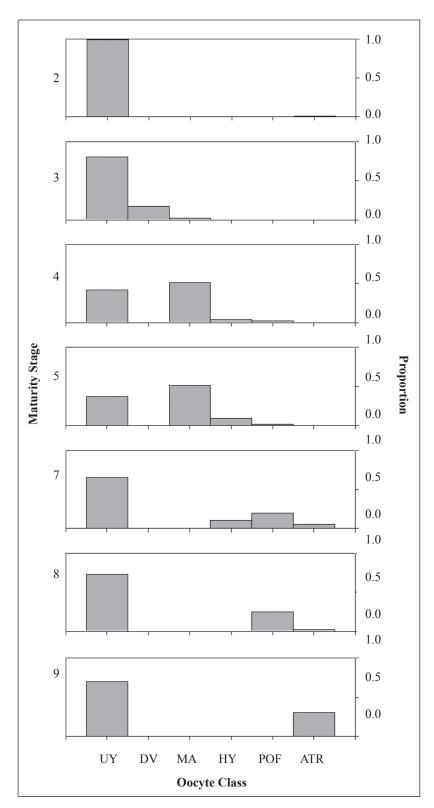


Figure 4. Oocyte class composition of pollock maturity stages. Observations were made from ovary sections of walleye pollock collected in Shelikof Strait, Alaska. Oocyte class definitions: OY=unyolked oocytes, DV=developing oocytes (start of vitellogenesis), MA=mature fully yolked oocytes, HY=hydrated oocytes, POF=post-ovulatory follicles, ATR=atretic (reabsorbing) oocytes.

itself. The most advanced oocyte stage present is the primary indicator for histological staging. Only some macroscopic staging criteria rely on oocyte characteristics, and only on a basic level observable with the naked eye.

The historical basis of the current pollock macroscopic staging is based on external anatomical appearance, not on histological classification of oocyte development. Consequently, the researcher must determine the oocyte development levels that can define the macroscopic stages, primarily by equating the functional definitions of the maturity stages. For example, the onset of oocyte hydration would indicate the late prespawning macroscopic stage 5, and a high level of postovulatory follicles and atretic oocytes would indicate an early postspawning condition or stage 7. There is room for some subjectivity in this decision, although certainly less than is inherent in macroscopic staging. Therefore, histological methods are assumed to provide an adequate methodology for evaluating the quality of macroscopic stage data, given the appropriate histological interpretation of stage development.

Classification errors that influence maturity assessments are primarily those that involve the boundary

stages. The designation of stages 3 and 4 as the prespawning boundary separating spawning fish from the immature fish and nonspawning adults was influenced by the need to integrate the historical 5-point scale with the currently used 8-point scale. Stage 3 is considered a late development stage, contained within the "developing" stage 2 in the 5-point system, although it contains oocytes that have progressed into the cortical alveoli stage, which is indicative of spawning later in the current season in Atlantic cod (Morrison 1990). Merati (1993) commented that the 5-point scale in use at the time was not adequate to resolve vitellogenic individuals that may spawn later in the season from those spawning next year.

Reader errors commonly occur across the postspawning boundary during macroscopic staging (Tomkiewicz et al., 2003) due to the similarity in ovary size and appearance between these adjacent stages. In histological sections, the thickness of the ovary wall appeared to be greater in postspawning fish (stages 7 and 8) relative to prespawning fish (stages 2 and 3). However, this characteristic is difficult to resolve with macroscopic inspection, and cannot be used as a criterion for distinguishing across the postspawning boundary.

Table 3. Histological verification of walleye pollock macroscopic stage data collected in Shelikof Strait. Dotted lines represent the division between immature and mature stages.

	Macroscopic stage									
		1	2	3	4	5	6	7	8	Total
	1									
92	2		22							22
stage	3		1	7						8
	4			5	31	3				39
Histological	5				10	22		1		33
olog	6				l I					
stc	7		3					2	2	7
$\Xi$	8		3						10	13
	9			4	 			'		4
	Total		29	16	41	25		3	12	126

Table 4. Paired reader comparison of walleye pollock macroscopic stage data collected in Shelikof Strait. Dotted lines represent the division between immature and mature stages.

	Reader 2									
		1	2	3	4	5	6	7	8	Total
	1	4	3		l					7
	2	1	58		! !					59
	3		1	38	35					74
<u>ler</u>	4			12	116	16	1	2		147
ead	5				6	66		5		77
$\aleph$	6				i i	1	3			4
	7				i			2	8	10
	8		1		l I			12	20	33
	Total	5	63	50	157	83	4	21	28	411

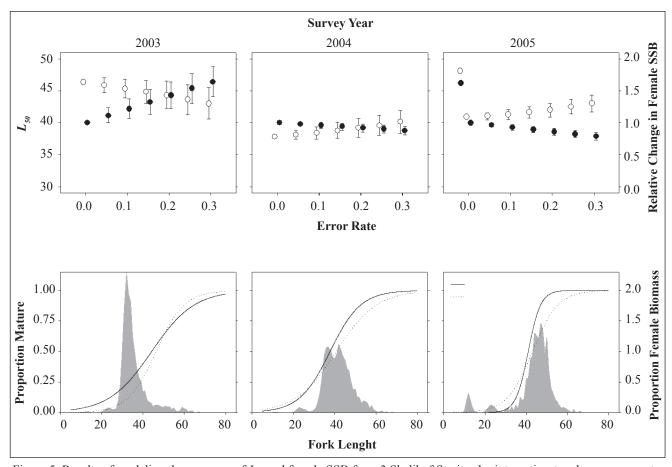


Figure 5. Results of modeling the response of  $L_{50}$  and female SSB from 3 Shelikof Strait echo integration-trawl survey years to increasing random error. Logistic regressions of unaltered data and data with 30% random misclassification error introduced are shown for each survey year with the total female biomass by length shown shaded in gray. Error bars show the standard deviation of the values over 1,000 runs.

The temporal component of the pollock spawning event in Shelikof Strait also plays a role in the allocation of potential spawners because the spawning population estimates for the area are derived in context of a single spawning event. However, some fish that were classed as immature could potentially mature and spawn at a later time after the primary spawning event has ended. In fact, demersal trawl surveys conducted by Alaska Fisheries Science Center scientists encounter pollock in various stages of development when conducting summer surveys in the Gulf of Alaska. The spring spawning event in Shelikof Strait represents a relative index of the level of spawning activity in the Gulf of Alaska, limiting the definition of mature individuals to those spawning within this event. In the Bering Sea, the more protracted pollock spawning season and the lack of a definitive, dominant single spawning event make the maturity boundary more difficult to ascertain due to the larger period of time available for maturation of "developing" individuals (Stahl 2004).

Classification of stage 3 was a common problem with both comparing readers and histological validation. Macroscopic distinction between the prespawning boundary stages 3 and 4 appears to rely heavily on subjectively estimated quantitative criteria—namely ovary and egg size—whereas other stages may be defined by coloration or the presence of hydration. Pollock classified into maturity stage 3 are generally rare in SSEIT surveys, comprising approximately 1.8% of all fish observed in survey years from 2000 to 2005—making their allocation to the non-spawning stock as well as their potential misclassification less significant to the maturity assessments.

Histologically verified macroscopic stage misclassifications and reader disagreements display a generally expected pattern of misclassifying adjacent stages. This suggests that at least some of the observed samples may have been in transition between stages, exhibiting some characteristics of both bounding stages. In some cases, macroscopic examination does not reveal

characteristics of the maturity stage which are apparent in histological sections. This is the case with stages 4 and 5, where the primary separating character is oocyte hydration. Despite their high misclassification rate, both these frequently observed stages designate mature pollock, making their misclassification of low overall importance to the population maturity assessment. Likewise, high disagreement among readers was observed between stages 7 and 8. The high incidence of this type of misclassification indicates that these stages should probably be combined, as the macroscopic resolution between these stages is not very high. If the validation data collected with an 8-point scale relation were collapsed to the 5-point scale, following the accepted stage overlap, the total misclassification rate would be reduced from 25% to 17%.

Modeling the effects of reader misclassifications provides insight into the sensitivity of maturity assessments to reader errors. The directional trends of spawning biomass and  $L_{50}$  estimates in response to random error were somewhat unexpected (Figure 5). The effect of low maturity data accuracy appears to be dependent on the underlying population structure. Substantial changes in SSB and  $L_{50}$  seen at high error rates, although 30% misclassification rates are unrealistic based on misclassification rates between mature and immature fish observed in this study. Histological validation resulted in an estimated 8.7% error rate in mature/immature designations. In the histological samples, fish macroscopically staged 3 represented 12.7%, much higher than their occurrence in the survey data (1.8%). A more reasonable estimate of the overall

error rate in SSEIT surveys can be made by weighing the stage-specific error rates by their frequency of occurrence. This can be done by generating a classification error matrix from Table 3, where columns are proportioned to reflect proportions misclassified by stage. The observed macroscopic stage frequencies are then multiplied by this matrix, analogous to the use of length-age keys used to reconstruct age distribution from length data. This approximation yields an error rate of 5.5% in mature/immature designations over the survey years from 2000 to 2005.

When we look at the maturity stage frequency of the SSEIT surveys, it is clear that the spawning component of the population can be separated using macroscopic observations, primarily because at least one side of the bounding stages between the spawners and nonspawners is observed in low frequency. If we consider the central task of maturity staging to be the separation of spawners and nonspawners, macroscopic maturity data is adequate for estimating the SSB and  $L_{\rm so}$ .

Compared to histological methods, the pragmatic advantage of macroscopic classification is difficult to overcome. However, its accuracy needs to be established and periodically monitored. This analysis showed that the macroscopic 8-point maturity scale has a significant misclassification rate, which the users of this data should be aware of. If a higher level of accuracy is mandated for the stage-specific data, certain stage descriptions should be revised and the readers made aware of more detailed criteria, or alternatively, the number of stages should be reduced if there are few justifications for its complexity.

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