Length and age at maturity of female petrale sole (*Eopsetta jordani*) determined from samples collected prior to spawning aggregation

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Abstract—The problem of bias in female petrale sole age and length-at-maturity relationships caused by sampling from spawning aggregations was investigated. Samples were collected prior to aggregation, and histological methods were used to determine maturity status. Mature and immature fish were classified by inspecting oocytes for the presence of yolk in September, when substantial divergence in yolked and unyolked oocyte diameters had been observed. Comparison of macroscopic and microscopic assessment of maturity showed that maturity status cannot be determined accurately by using macroscopic inspection during the summer. Female petrale sole from the central Oregon coast were 50% mature at 33 cm and 5 years of age. Comparison of data from our study with data used in recent petrale sole stock assessments showed that both sampling bias and the use of samples from seasons when status cannot be accurately determined have likely caused errors in fitted maturity relationships.

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Petrale sole (*Eopsetta jordani*) have been the target of a valuable commercial trawl fishery off the U.S. West Coast since before World War II (Sampson and Lee[1]). Management and assessment of petrale sole is complicated by the fact that modern fishing activity targets winter deepwater aggregations of spawning fish (Fig. 1). Because most of the available female maturity data are derived from the winter commercial fishery, they are potentially biased with respect to age and length at maturity (e.g. small mature fish are more likely to be sampled than small immature fish). Some summer samples are available for analysis, including those from the National Marine Fisheries Service triennial shelf survey (e.g. Zimmermann et al., 1994). However, in the summer, when mature and immature fish are well mixed, maturity status is not easily determined visually because of the similarity in appearance between ovaries of immature fish and those of fish in the "resting" state (Ketchen and Forrester, 1966).

The age and length at maturity for female fish is an important parameter in many stock assessment models. Clark (1991), Lunsford (1999) and Al-Jufaily (1996) have demonstrated that variation in the age at 50% maturity, especially in relation to the median age of recruitment to the fishery, can have a major influence on the calculation of a target fishing rate. Target fishing rates are currently used by the Pacific Fishery Management Council (PFMC) to manage most groundfish stocks (Clark, 1991; PFMC[2]). An example of a target fishing rate would be $F_{40\%}$, the fishing mortality rate that would reduce spawning stock biomass per recruit to 40% of the unexploited level. The potential influence of errors in estimating the median age of female maturity can perhaps best be illustrated with an example. In Lunsford's (1999) study of maturity of Pacific ocean perch

[1] Sampson, D. B., and Y. W. Lee. 1999. An assessment of the stocks of petrale sole off Washington, Oregon and Northern California in 1998. *In* Pacific Fishery Management Council. 1999. Appendix to the status of the Pacific coast groundfish fishery through 1999 and recommended acceptable catches for 2000: stock assessment and fishery evaluation. Pacific Fishery Management Council, 2130 SW Fifth Avenue, Suite 224, Portland, Oregon, 97201.

[2] Pacific Fishery Management Council. 2000. Status of the Pacific Coast groundfish fishery through 2000 and recommended biological catches for 2001: stock assessment and fishery evaluation. (Document prepared for the council and its advisory entities.) Pacific Fishery Management Council 2130 SW Fifth Avenue, Suite 224, Portland, Oregon 97201.

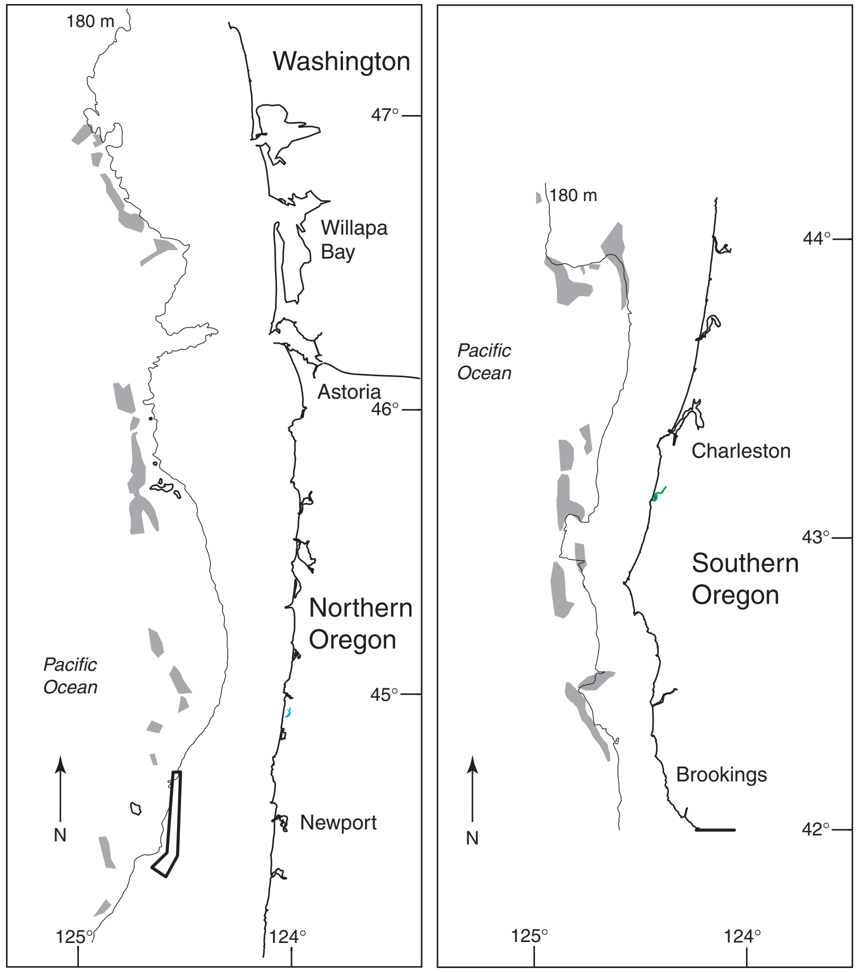

Figure 1

Sampling area for petrale sole (bold-outline polygon) off Newport, Oregon, and areas where winter trawl fishing for petrale sole is concentrated (gray polygons). Also shown is the 180-m depth contour.

(*Sebastes alutus*), a shift in the estimated median age of maturity for female fish from 7.5 to 10.5 years decreased the $F_{40\%}$ value from 0.110 to 0.076, a decrease of 31%. Accordingly, the use of potentially biased maturity data from the winter commercial fishery for petrale sole adds to the uncertainty in stock assessments. The accuracy of maturity information used in recent petrale sole stock assessments is also uncertain because of two additional factors: outmoded data and the blending of samples across space and time (Sampson and Lee.[1]; Turnock et al.[3]). Maturity samples are obtained from various locations and times, and are often combined to produce a general relationship for stock assessment purposes. The combined relationship may poorly account for geographical differences, long-term changes in maturity, or for inadequacies in some samples (e.g. lack of immature fish) (Sampson and Al-Jufaily, 1999) and can result in poorly determined curves that bias and degrade the fit of the combined maturity relationship. The principal objective of our study was to collect maturity data for female petrale sole from the late summer to early fall period and compare them with the maturity data used in recent stock assessments (Sampson and Lee[1]; Turnock et al.[3]). Although sampling in late summer and early fall is probably optimal for assessing female maturity in the petrale sole population, some ovaries may be difficult to classify accurately as mature or immature by macroscopic inspection. A second objective of our study was to evaluate female petrale sole maturity by using microscopic examination of stained thin sections and compare these results with those obtained from simple visual inspection.

The petrale sole maturity data used in recent stock assessments have been obtained from the Oregon Department of Fish and Wildlife's commercial fishery sampling program (Sampson and Lee[1]; Turnock et al.[3]). Potential

[3] Turnock, J., M. Wilkins, M. Saelens, and C. Wood. 1993. Status of West Coast petrale sole in 1993. Appendix G. *In* Status of the Pacific Coast groundfish fishery through 1993 and recommended biological catches for 1994: stock assessment and fishery evaluation. Pacific Fishery Managemenmt Council, 2130 SW Fifth Ave., Suite 224, Portland, OR 97201.

bias in some of these data, arising from spawning aggregation, argues that a different sampling approach might be useful for trying to generate unbiased maturity data from samples of the commercial catch. A third objective of our study was to evaluate problems with available maturity data from fishery samples in light of histological evaluations of maturity stages and to recommend an alternative approach for sampling the commercial catch for age and length at maturity.

Materials and methods

Petrale sole were collected by trawl during August and September 2000 by using a chartered commercial fishing vessel from Newport, Oregon. Sampling was conducted over a very limited geographic range to minimize any spatial variation in the maturity ogive. Individual sampling sites were selected to attempt to sample across the available size range of petrale sole, based on the skipper's experience and expertise. Fish were sorted at-sea according to total length (±0.5 cm), and fifteen fish from each 1-cm size interval were retained for maturity sampling. Upon returning to port, the fish were measured again and sex was determined by dissection. An ovary was removed and assigned a macroscopic maturity stage following the criteria of Hagerman (1952). Only experienced samplers were used to assign macroscopic maturity stages to minimize any errors in visual staging. Otoliths were removed from all female fish for subsequent age determination, and one ovary was preserved for histological examination to determine maturity. Ages were determined by using the break-and-burn technique for sagittal otoliths (Chilton and Beamish, 1982). Consistency of age determination was evaluated by using a blind double reading.

Ovaries were preserved in 10% buffered formalin and later transferred to 70% ethanol for storage. Tissue samples from the midsection of the ovary were then embedded in paraffin, sectioned at 5 µm, and stained with Harris's hematoxylin and eosin *Y*. The samples were examined and classified as mature or immature based on the presence or absence of dark-staining yolk globules (vitellogenin).

The diameter (µm) of the largest spherical nonatretic oocyte in the most advanced oocyte stage from each of five microscope fields was also measured and used to calculate a mean maximum oocyte diameter (MMOD) for each sectioned ovary (West, 1990; Nichol and Pikitch, 1994). MMOD was compared for mature and immature fish. A divergence in MMOD between mature and immature fish was taken as evidence that the presence or absence of dark-staining yolk globules was an accurate indicator of maturity status. Samples from August and September were compared by using this approach. The accuracy of visual examination of ovaries was then evaluated by comparing the maturity status from visual examination with status determined after sectioning, staining, and microscopic examination.

Logistic regression was used to fit sigmoid length-maturity and age-maturity curves. The model fitted had the general form

$$P_{x_1} = e^{(b_0 + b_1 x_1)}/(1 + e^{(b_0 + b_1 x_1)}),$$

where p = the probability that a fish is mature in a given length (cm) or age category x_1; and
b_0 and b_1 are parameters that define the shape and location of the fitted curve.

The predicted length or age at 50% maturity was calculated as

$$L \text{ (or } A)_{50} = -b_0/b_1.$$

Maturity data collected by the Oregon Department of Fish and Wildlife (ODFW) from the 1986–2000 commercial trawl fisheries were also analyzed and compared with the data collected in our study. The most recent petrale sole stock assessment (Sampson and Lee[1]) relied on maturity data used for the 1993 assessment (Turnock et al.[3]), which in turn were derived from combined ODFW port sampling data for the years 1986–91. We fitted a maturity-length relationship to these data for comparison with the maturity data from our study.

We also summarized the ODFW data from 1986–91 and 1992–2000, by month and maturity status, to compare with our data and assess their adequacy for determining length at maturity for female petrale sole. All of these samples were classified by a simple visual evaluation of maturity stage. To examine the effect that a lack of immature fish may have had on estimated maturity curves from these two time periods, we split the data into groups, according to season. Samples from the months of November through February were classified as "fall–winter" samples. These samples represented the seasonal time period when the maturity status of petrale sole was most readily determined visually, but also the time period when samples were most likely to be from spawning aggregations. Samples from April through September were classified as "spring–summer" and represented the seasons in which fish were not aggregated for spawning, but the time when maturity status was most difficult to determine by inspection of the ovaries.

Results

Comparison of visual maturity determinations with results obtained from microscopic evaluation showed that maturity of petrale sole cannot be reliably determined by simple visual inspection in August or September (Tables 1 and 2). For ovaries classified as immature by inspection in August, 16.7% proved to be mature upon microscopic evaluation. Of those classified initially as mature, 6.5% showed no microscopic evidence of vitellogenesis. By September, all of the ovaries classified by inspection as mature, did prove to be vitellogenic. However, 27.9% of those classified as immature, proved to be vitellogenic after viewing the stained sections with a microscope.

MMOD increased markedly in vitellogenic ovaries between August and September, but in nonvitellogenic ovaries the increase was minimal (Fig. 2). The resulting divergence in MMOD and the absence of fish with MMOD

Table 1

Details of petrale sole collected by trawl aboard the FV *Olympic*.

Date	Location	Females	Males	Size range (cm)
8 Aug 2000	4428°N 12434°W	140	103	24–51
23 Aug 2000	4446°N 12432°W	24	46	27–49
26 Sep 2000	4428°N 12435°W	117	123	26–50
Total		281	272	24–51

Table 2

Comparison of macroscopic and microscopic determination of maturity in female petrale sole collected by trawl.

	Macroscopic classification		Microscopic classification	
Sample month	Condition	Number of fish	Number immature (%)	Number mature (%)
August	immature	102	85 (83.3)	17 (16.7)
	mature	62	4 (6.5)	58 (93.5)
September	immature	61	44 (72.1)	17 (27.9)
	mature	56	0 (0.0)	56 (100)
Total		281		

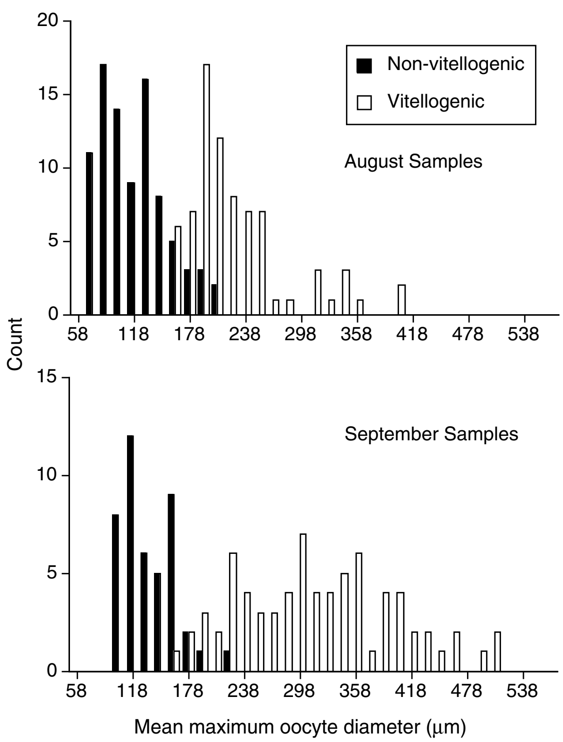

Figure 2

Comparison of mean maximum oocyte diameters (µm, see text) for petrale sole with vitellogenic and nonvitellogenic oocytes, for samples collected in August and September 2000.

around 180 µm suggested that by September, the microscopic classification of ovaries provided an accurate identification of maturity status of individual fish. Immature fish were well represented in the September collections, indicating the fish were still well mixed on the feeding grounds (Table 2). Therefore, the September maturity data generated from microscopic inspection were used to fit age- and length-at-maturity relationships for female petrale sole (Fig. 3). The resulting curves fitted the data well and chi-square tests on the residuals indicated no problems with lack of fit caused by overdispersion (Pearson chi-square of 5.714 [with 20 degrees of freedom] for length, and 0.643 [with 11 degrees of freedom] for age, $P>0.999$). The fitted relationships indicated that female petrale sole off the central Oregon coast were 50% mature at about 33 cm and at about 5 years of age. Complete maturity was obtained at about 40 cm and about 9 years of age (Table 3).

Examination of the monthly samples that, as an aggregate, were used to fit the 1986–91 maturity curve showed that numerous samples were included from the March through August period, especially from the port of Charleston, Oregon (Table 4). The inclusion of these summer samples explains some of the flatness in the fitted maturity curve from Turnock et al.[3] (Fig. 4, L_{50}=30.6, slope=0.29). A maturity curve fitted to these data, but excluding spring and summer samples, was much steeper (Fig. 4, L_{50}=33.56, slope=0.50).

Inspection of the 1986–91 and 1992–2000 maturity data from the winter months, when errors in determining maturity should be minimized (Tables 4 and 5, Fig. 5), showed some evidence of the type of bias that can result from sampling spawning aggregations. First, very few im-

Table 3

Results of logistic regression analysis of histologically determined maturity status of petrale sole versus length (cm) and age (September samples only).

Independent variable		Coefficients	Standard error	Chi-square	P-value	L_{50} or A_{50}	95% confidence
Length						33.10 cm	32.13–33.93
	constant	–24.593	4.572	28.936	0.0001		
	length	0.743	0.136	29.759	0.0001		
Age						5.15 yr	4.68–5.54
	constant	–6.358	1.417	20.122	0.0001		
	age	1.234	0.261	22.349	0.0001		

mature fish were sampled, even though the commercial fishery samples showed a similar lower size range to the fish collected in our study (Fig. 6). The lack of immature fish was most evident in the 1986–91 November–February samples from Charleston, Oregon, where 170 fish were sampled for maturity, and only one fish was reported as immature. The 1992–2000 Charleston data also showed low numbers of immature fish. These samples also showed groups of very small fish, and most or all of them were classified as mature (Fig. 5). This kind of bias, where small fish encountered are more likely to be reported as mature than similar-size fish sampled from a well-mixed population, would also act to flatten the resultant maturity curve. The Astoria winter data included more immature fish, and accordingly produced steeper maturity curves. However it was unclear to what degree bias from sampling spawning aggregations may have influenced these curves.

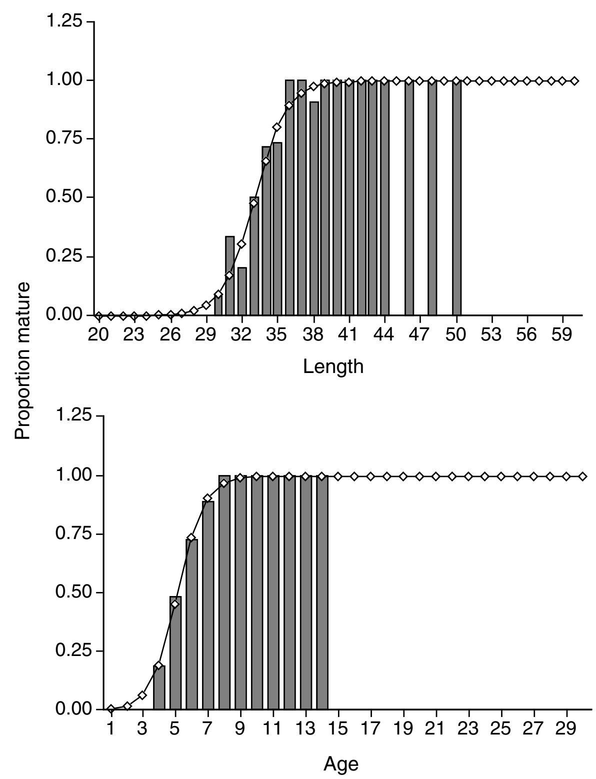

Figure 3

Proportion mature for female petrale sole, by age and length (FL,cm), from September samples. Maturity status was determined by histological examination by using presence and absence of vitellogenesis to determine maturity. Fitted curve is also shown (Table 3).

Discussion

Although it is known that late fall and winter is a better time for visually assessing female maturity status for petrale sole, our data showed clearly that late summer and early fall maturity samples, for which there was a visual determination of maturity, should not be used for estimating maturity in this species. Although our study did not specifically examine ovaries from spring collections, the general timing of ovarian development in this species suggests that this caution can be extended to all late spring and summer samples. Our results are similar to the findings of Ramsay and Witthames (1996), who suggested that visual maturity evaluations of the common sole (*Solea solea*) are generally only reliable during a limited portion of the year.

The maturity data developed in our study (Figs. 3 and 4) suggest a larger size at 50% maturity (33.1 cm vs. 30.7 cm) and a much steeper maturity curve for petrale sole than indicated by the 1986–91 sample data used in the last two petrale sole stock assessments (Turnock et al.[3]). Although the curves appeared to be quite different, the length at 50% maturity increased by only 2.4 cm. The most recent stock assessment model indicates an annual fishing mortality rate, for fully exploited size groups, of 0.285 (Sampson and Lee[1]). Incorporating a revised maturity ogive from our study decreased the equilibrium fishing rate to 0.268, a change of about 6% (Sampson[4]). This modest effect on harvest rate seems odd, given the large apparent difference between the curves in Figure 3. A possible explanation for this discrepancy is that the results depend heav-

[4] Sampson, D. B. 2000. Personal commun. Coastal Oregon Marine Experiment Station and Department of Fisheries and Wildlife, Oregon State University, Hatfield Marine Science Center, Newport, OR 97365.

Table 4

Summary of the numbers of mature and immature petrale sole sampled, from the Oregon bottom trawl fishery, 1986–91.

Port	Month	Immature	Mature	Total
Astoria	November	20	130	150
Astoria	December	0	167	167
Astoria	January	2	135	137
Astoria	February	32	10	42
Winter total		54	442	496
Astoria	March	18	61	79
Astoria	October	7	26	33
Astoria total		79	529	608
Charleston	November	0	37	37
Charleston	December	1	68	69
Charleston	January	0	24	24
Charleston	February	0	40	40
Winter total		1	169	170
Charleston	March	16	64	80
Charleston	April	0	37	37
Charleston	June	14	115	129
Charleston	July	27	123	150
Charleston	August	71	162	233
Charleston	October	2	78	80
Charleston total		131	748	879
Total (all ports)		210	1277	1487

Table 5

Summary of numbers of mature and immature petrale sole sampled by season and port from the Oregon bottom trawl fishery, 1992–2000.

Port	Season	Immature	Mature	Total
Astoria	Winter (Nov–Feb)	19	242	261
	Spring–fall (Mar–Oct)	4	89	93
Charleston	Winter (Nov–Feb)	20	347	367
	Spring–fall (Mar–Oct)	31	179	210
Brookings	Winter (Nov–Feb)	0	56	56
	Spring–fall (Mar–Oct)	0	142	142
Total		84	995	1079

ily on assumptions about fishery selectivity and discard used in the latest assessment model–parameters that are poorly known for most West Coast fish stocks. The modest effect on harvest rate found in our study also does not preclude larger effects for other, later maturing stocks. In the example given earlier from Lunsford's (1999) simulation work with Pacific ocean perch, the drop in target harvest rate was much larger. In that instance, a shift of 3 years in the median age of female maturity resulted in a 31% drop in the target harvest rate. In that example, spawning stock biomass per recruit also fell to 31%, well under the target of $F_{40\%}$.

The maturity data developed in our study differ from those developed by Turnock et al.[3] primarily in the steepness of the maturity curve. It could be argued that the earlier curve is flatter simply because it incorporates data from a wider geographic range. A latitudinal cline in length at 50% maturity, as has been postulated for many flatfish stocks (Ketchen and Forrester, 1966; Castillo, 1995; Brodziak and Mikus, 2000), could cause flattening

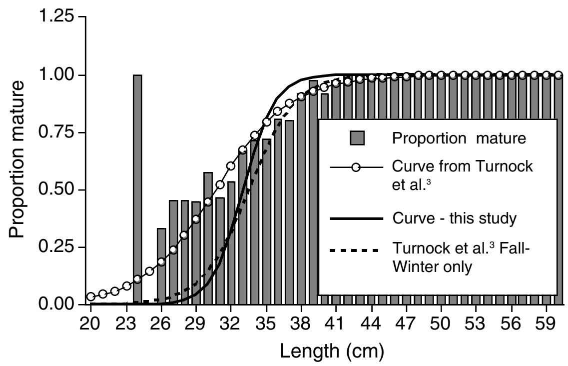

Figure 4

Comparison of fitted petrale sole female maturity curves from September samples (our study) and 1986–91 commercial fishery data used in the last two stock assessments (Turnock et al.[3]). Bars show 1986–91 maturity data used to fit the curve from Turnock et al.[3] Dashed line shows the maturity curve fitted to the 1986–91 samples by using only data from the fall–winter period.

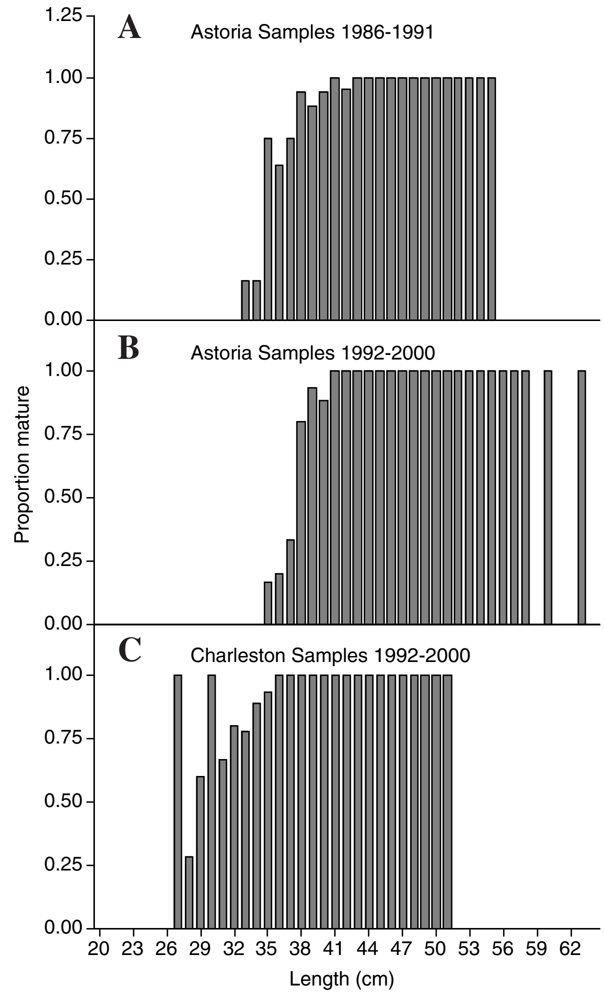

Figure 5

Proportion of female petrale sole identified as mature from November–February samples of trawl landings in Astoria and Charleston, Oregon, 1986–1991 and 1992–2000.

in a combined maturity curve. However, errors in determining maturity status, caused by including samples from time periods when maturity status cannot be accurately assessed visually, would have the same effect. This can be seen in a comparison of the maturity data generated in our study from macroscopic and microscopic evaluations. The macroscopic curve (bars and solid line in Figure 7) is flattened in relation to the curve according to microscopic evaluation (dashed line). The curve is also shifted to the right because, in this case, more large fish were incorrectly classified than small fish. At other times of the year, errors could be more common with smaller fish and the curve would shift the other way. In either case, however, the curve should, in theory, be flatter than the true maturity curve. This flattening of the maturity curve is caused by the way the most likely errors change along a maturity ogive. In small or young fish, which are mostly immature, most of the errors encountered will be immature fish mistakenly considered mature. At larger sizes or older ages, the reverse will be true. Near the inflection point, the two types of errors offset each other and have little impact on the slope because the population is split roughly 50/50 with respect to maturity. These effects, in combination, tend to flatten the resulting curve.

Compared to a length at 50% maturity of 33 cm for the central Oregon coast (our study), the Astoria data from the commercial fishery are consistent with an increase in the length at 50% maturity with latitude, as suggested by Ketchen and Forrester (1966) and Castillo (1995). The methods developed in our study could be applied to petrale sole samples collected over a wider geographic range to shed more light on how length at maturity varies with latitude. Best (1961) reported a length at 50% maturity of 35.5 cm for female petrale sole from California waters, and Harry[5] reported 40.0 cm for the Columbia River area. In comparison with the recent data from Astoria fishery samples (Fig. 5) and from the central Oregon coast (Fig. 3), the data from Harry[5] suggest a very large decrease in the length at 50% maturity for female petrale sole since the 1960s. If this decrease is a biological reponse to exploitation, it could explain how petrale sole stocks have held up quite well under heavy commercial harvest (Sampson and Lee[1]).

The data presented in our study suggest that the collection of maturity data by sampling on spawning aggregations does cause bias in estimates of female age and length at maturity for petrale sole. Use of spring and summer maturity samples in data sets used to estimate age and length at maturity has also caused errors. Our analysis shows that the overall effect on estimates of age and length

[5] Harry, G. Y. 1959. Time of spawning, length at maturity, and fecundity of the English, petrale and Dover soles (*Parophrys vetulus, Eopsetta jordani,* and *Microstomus pacificus*, respectively). Fish. Comm. Oregon, Research Briefs 7(1):5–13.

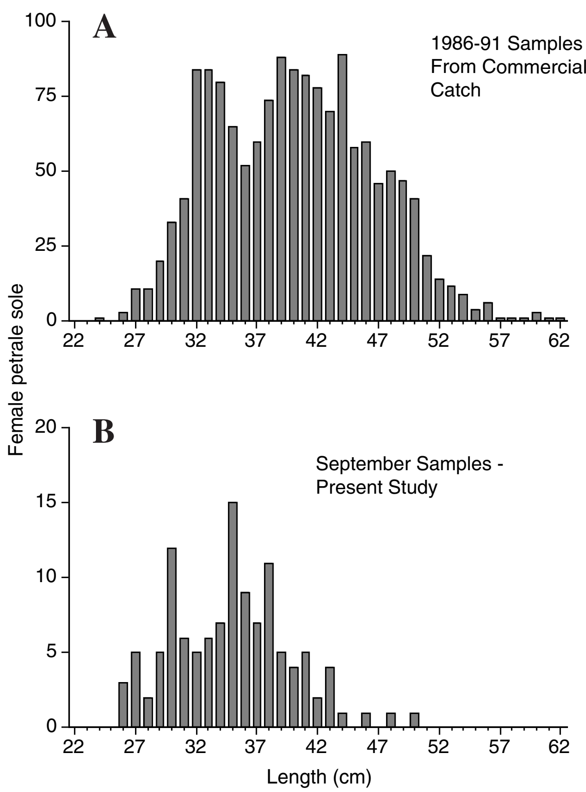

Figure 6

Comparison of female petrale sole length frequency from the 1986–91 commercial fishery samples and the September samples collected in our study. The 1986–91 samples were also used to fit the maturity curve from Turnock et al.[3]

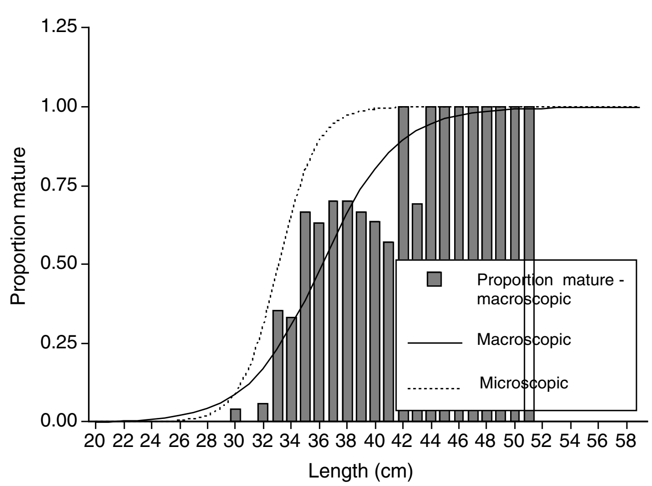

Figure 7

Comparison of maturity curves fitted to the data from our study. Bars show proportion of fish mature by length from the macroscopic evaluations of the August and September samples. The solid line is the fitted curve for those data. The dashed line is the fitted curve from microscopic evaluations of the September samples only.

at 50% maturity has been small. Collection of early fall samples from the commercial catch, followed by histological preparation and classification based on presence or absence of vitellogenin, should yield more accurate maturity information for female petrale sole than winter sampling on spawning aggregations or maturity sampling where only a visual assessment of ovary condition is made.

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