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Evaluation of Two Methods to Determine Maturity of Pacific Halibut

by

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ABSTRACT

Two methods to determine maturity of Pacific halibut (*Hippoglossus stenolepis*) are evaluated and compared. A visual method is based on an examination of ovaries whereas a serological method involves an immunodiffusion analysis of blood or tissue fluid samples. Less than 3% of the estimates from summer and winter samples disagreed, an indication that both methods provide similar results during most of the year. An important implication for halibut management is that female maturity data collected in the past using the visual method are correct and useful. The variation in age of 50% maturity between seasons probably results from the seasonal migration of mature halibut, and is not an artifact resulting from difficulties in visually determining maturity in summer. In addition, results showed that the serological method may have wider application than the visual method. Although each method has advantages and limitations, they rely on different approaches and have complementary applications.

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INTRODUCTION

Management of Pacific halibut *(Hippoglossus stenolepis)* depends in part on a good understanding of its reproductive biology. Maturity studies have been conducted as part of biological investigations by the IPHC (Thompson 1915, 1916, and 1917; Schmitt and Skud 1978; St-Pierre 1984; and Clark and Parma 1995). Results suggest that size or age at maturity has changed over time and therefore needs to be monitored periodically to assess reproductive changes in the population.

Maturity of halibut varies with sex, age, and size of the fish. St-Pierre (1984) examined setline catch data from research cruises conducted between 1924 and 1982 by the IPHC during the November-March period. The data show that the coastwide average age at which 50% of the individuals became mature was 8 and 12 years for males and females, respectively. Males, especially in Area 2B, may mature as young as 5 years (28%) while 2.1% were reported still immature at 20 years or older. Females may mature as young as 7 years (2.0%) while 1.4% appeared to be still immature at 20 years or older. No indication of senescence was mentioned concerning these older immature fish.

Estimates of age at 50% maturity vary greatly between geographical locations and seasons, making differences in age of 50% maturity difficult to interpret. Schmitt and Skud (1978) suggested that the difference in maturity between areas reflected variation in growth rate. Likewise, they reported that the estimated age of 50% maturity within the same area was generally lower in winter samples than in summer samples, often by as much as three years. They explained that this trend may reflect differences in the geographic distribution of mature and immature fish during the winter (spawning). If immature fish do not participate in migrations to spawning grounds, only immature fish resident on the spawning grounds are fully represented in winter samples and the age of 50% maturity is biased low as it represents the spawning component of the stock rather than the population in general. St-Pierre (1984) reached the same conclusion and the winter tagging data indicate that mature halibut migrate actively to and from the spawning grounds. The spawning grounds in Area 2C and Area 3A east of Cape St. Elias appear to acquire a net gain from the immigration of mature fish from other regions of the coast located to the west and south, some fish arriving from considerable distances. This implies that the age of 50% maturity from summer samples is more representative of the population, if mature and immature females are randomly intermingled during the summer. Therefore, observations of seasonal differences in age of 50% maturity can be explained in a large measure by the movement of mature fish.

The traditional method used to determine maturity in halibut involves a vi-

sual examination of gonads. Schmitt and Skud (1978) acknowledged that the distinction between mature and immature females is sometimes unclear during the summer, and this uncertainty also contributes to the seasonal differences in estimated age of maturity. Thompson (unpub.)[1] and other IPHC biologists (pers. comm.)[2] expressed concern that they may erroneously designate some mature females as immature during the summer. Halibut with maturing ovaries, i.e. passing from the immature to the mature stage, are especially hard to differentiate from immature at times because of the protracted spawning period (November through March). The question of maturity requires distinguishing if the ovaries are sufficiently developed for that fish to participate in the first upcoming spawning without knowing the specific spawning time (5 month period) of that particular fish. Incorrectly labeling maturing ovaries as mature would result in lower estimates of the age of 50% maturity in summer.

A serological technique to detect the antigenic component in maturing female pleuronectids was used by Utter and Ridgway (1967) to determine maturity in halibut. They tested this technique on female English sole (*Parophrys vetulus*) and Pacific halibut. Their results for halibut sampled during the winter spawning season compared favorably with those from the visual examination of ovaries. They found that the presence of the maturity component in serum samples varied seasonally in English sole, and such qualitative variation in mature halibut was not found in winter and spring samples. However, annual qualitative variation could not be studied as samples were not available for the summer or autumn. They also suggested that the separation of mature females from HM+ immature females appears possible by quantitative means during the spawning season. At that time, the HM serum levels of mature females had titers above 200 compared to less than 2 found in some immature females. In addition, Utter (1964) and Utter and Ridgway (1967) reported that most kidney samples from mature female halibut, which were handled and eviscerated similarly to commercially-landed fish, contained detectable amounts of the maturity component. On this basis, they suggested that the serological technique might be successfully applied to eviscerated halibut, at least during the spawning season. They also indicated that this technique may not endanger living halibut; repeated bleedings of four starry flounder (*Platichthys stellatus*) held in captivity apparently did not endanger them. Thus, the serological method did show promise for successful application to tagged halibut, with the potential to overcome some of the shortcomings of the visual method.

The first objective of this study was to compare the two methods for maturity determination in halibut, evaluate the reliability of the visual method used commonly by the IPHC, and identify the probable cause of the seasonal differences in the age of 50% maturity. The second objective of the study was to determine if the maturity component can be detected in serum taken from mature females at any time of the year, and thus establish the seasonal accuracy of the serological method. The third objective of this study was to evaluate the potential for application of the serological method to commercially landed halibut.

This report describes the application of the visual and serological methods to determine the maturity of female halibut and documents the procedure followed

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[1] William F. Thompson diary. IPHC. P.O. Box 95009, Seattle, WA 98145-2009.

[2] IPHC. P.O. Box 95009, Seattle, WA 98145-2009.

when using the serological method. The serological results are used to compare the accuracy and confirm the validity of the visual method. The assumption is that the serological method is accurate and therefore, the agreement indicates that the visual method is also accurate. We also investigate if the serological method has complementary or wider applications than the visual method. Finally, we evaluate the advantages and weaknesses of each method.

MATERIALS AND METHODS

Data Collection

Data on fork length, age, sex, maturity (visual method), and samples for serological tests were collected on IPHC research cruises off Kodiak Island, Alaska, during late August and early September 1979, and outside southeastern Alaska during late January to mid-February 1980 (Figure 1). The primary objective of these research cruises was to tag and release as many halibut as possible. Age, sex, and maturity data were obtained only from those fish unsuitable for tagging. The sex of each of these fish and maturity of females were determined by a visual examination of gonads, and the left otolith was collected for subsequent aging. The criteria for identifying the maturity of halibut are described below in the visual method section. All halibut were measured to the nearest centimeter. The ages of fish were estimated by counting the growth zones on the otoliths after the otoliths had cleared in a 50% glycerin solution.

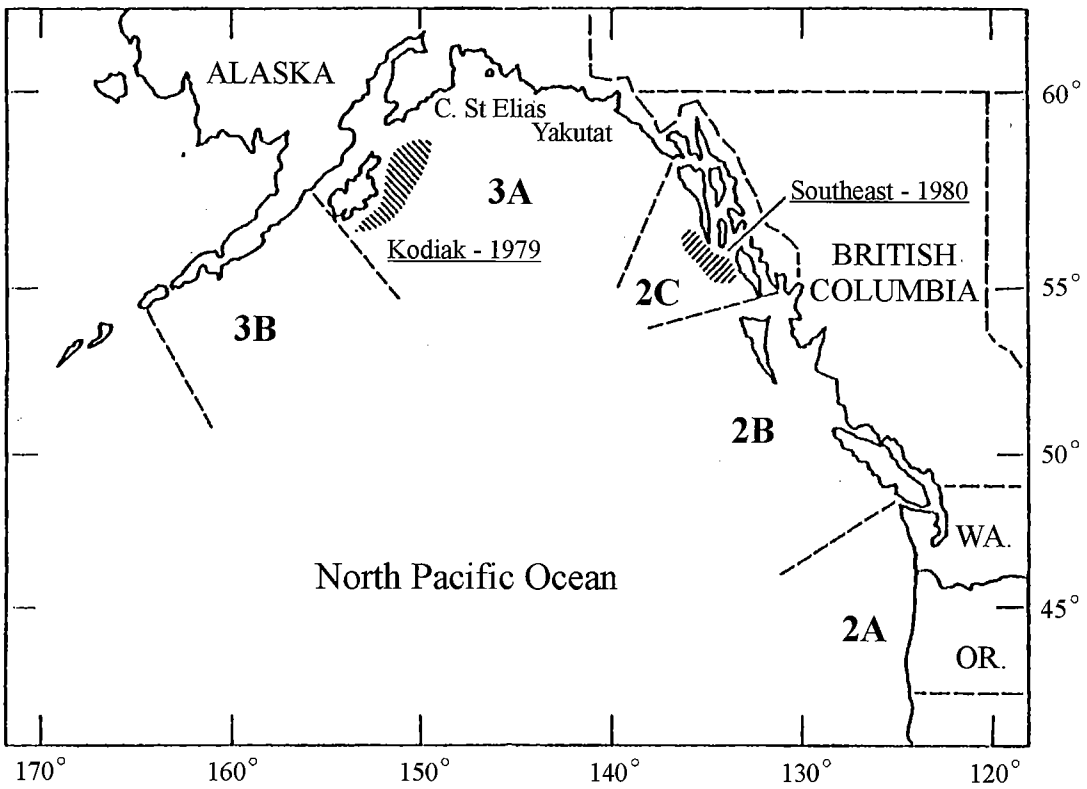

Figure 1. Regions sampled during the IPHC research cruises in August-September, 1979 and January-February, 1980.

Visual Method

No secondary sexual characteristics had been identified for halibut prior to 1988. Therefore, sexual differentiation between halibut was usually through physical appearance of the gonads after cutting open the fish and its maturity customarily determined by visual examination of the testis or ovary. Males are classified as immature when the testes are very small, firm-textured, and pink colored. In mature males, the testes are especially soft and plump, pink to whitish in color, swollen in appearance, and the outer face near the center of the testes is usually marked by a deep crease. The determination of maturity in recently mature, small-sized males is difficult as considerable shrinkage of the testes take place during the resting period (summer). However, the maturity determination of males of similar size is easier and more precise during the fall-winter-spring period, at which time the maturity is determined either by the extrusion of milt or by the texture or swollen condition of the testes (St-Pierre 1984). Males may ripen as early as late August and many are still extruding milt as late as June of the following year, a few months prior to or after the ripening phase in females.

The maturity determination of females is made by cutting open the ovary and inspecting it for any indication of oocyte development. Immature, maturing, and mature ovary stages are shown from right to left in Figure 2. Females are classified as immature when the ovaries are small and firm, pink, reddish, or translucent in color, and the oocytes are not visible macroscopically. A maturing female has gonads still firm and small but slightly larger than those in the immature stage, the development of blood capillaries is progressing, the ovaries are whitish, yellow, or reddish in color, and the oocytes are barely visible to the naked eye. Maturing females are considered insufficiently developed to participate in the first upcoming spawning season. A mature female has large gonads with a well-developed vascular system and depending on the time of the year, opaque, translucent, or clear eggs are present in the ovaries. A spent female has slack, flabby, or bloodshot ovaries with large deflated blood vessels, few or no remaining translucent eggs, and developing oocytes are usually visible. Most mature females are found in the ripe phase between October through March.

Serological Method

The serological method was described thoroughly by Utter (1964) and only a summary is given here. This method detects the presence of a serum vitellin component in mature females, called the HM factor by Utter and Ridgway (1967). They reported that the mechanism for vitellin synthesis and its presence in the blood serum was...

> "under the control of the pituitary, estrogen produced in the ovary stimulates production by the liver of proteins that are passed through the blood to the ovary and there utilized in yolk formation."

The serological method involves a double-diffusion precipitin analysis in which the antiserum, containing antibodies specific to the HM factor, and the test solution diffuse toward one another in a semisolid medium. If the test solution contains the antigenic substance, the HM factor, a precipitate line is formed in the zone where antigen molecules meet specific antibodies in optimal proportions.

Sample Collection

Of the fish unsuitable for tagging, only those between 80 cm and 130 cm were sampled for serological testing because immature and mature females are generally well represented in this size range (Schmitt and Skud 1978). Whole blood samples were taken from 77 halibut (54 females, 21 males, and 2 tagged fish) during the summer cruises and from 68 halibut (53 females, 13 males, 1 tagged, and 1 unknown) during the winter cruises. Of the blood and tissue samples collected from 28 halibut during early September, tissue samples from most immature fish (24) were not tested because of time constraints and the high correlation between blood and tissue samples for the 3 fish tested. Blood samples from three tagged halibut (their age, sex, and maturity unknown) were taken for serological maturity determination and the immediate effect on their survival was noted.

Procedures for taking blood and tissue samples were as follows. Shortly after a suitable fish was brought aboard the vessel, whole blood was withdrawn from the caudal artery into a 4 ml evacuated glass tube equipped with a 1-inch, 22-gauge, holder-needle assembly. The blood sample was frozen in the tube shortly after collection. As the fish was eviscerated, about 1 cm^3 fragments from the gonads, gill, kidney, liver, and tongue and about 3 ml of eye fluids were placed in separate plastic vials, labeled, and frozen.

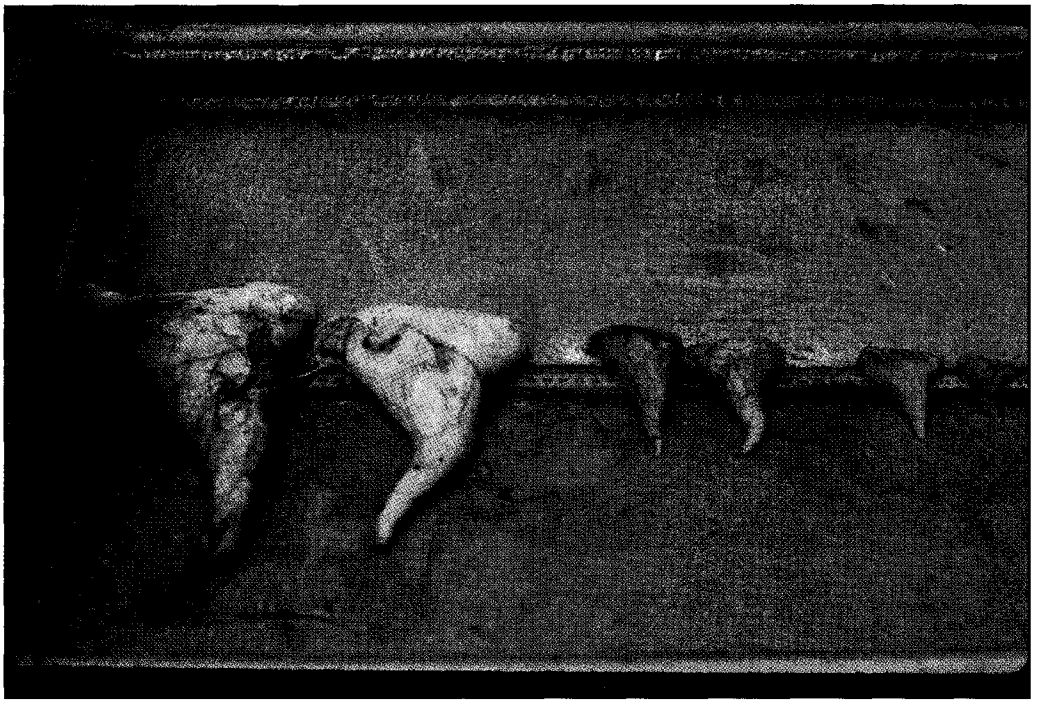

Figure 2. The immature, maturing, and mature stages are shown from right to left, respectively.

Test Procedure

The antiserum used in this study was produced by intraperitoneal injections of two female rabbits with homogenates of 0.5 ml whole-egg extract from starry flounder and 1.0 ml Freund's Complete Adjuvant, which acts as a booster. The starry flounder egg extract was used because it had produced satisfactory results with halibut serum (Utter 1964; Utter and Rigway 1967) and because ripe ovaries from starry flounder were more easily obtained than from halibut. Each rabbit was injected three times at intervals of about two weeks. A week after the last injection, a 25-ml blood sample was taken by heart puncture. Serum from each blood sample was tested for the presence of adequate concentrations of antibodies by immunodiffusion with starry flounder whole-egg extract. The concentration of antibodies in one rabbit was too low for precipitin analysis and this rabbit was given one more injection, which proved sufficient. Three 25-50 ml blood samples extracted from each rabbit at intervals of about three days were pooled, and the serum containing antibodies to the HM factor was decanted and frozen for use in all tests.

Tests for the presence of the HM factor were made on microscope slides covered with a blue-stained medium. The composition of the medium (from Ridgway et al. 1962; Utter and Ridgway 1967) was 1.50% Difco agar, 0.72% sodium chloride, 0.60% tri-sodium citrate dihydrate (used as buffer to prevent precipitation of calcium salts), 0.01% merthiolate Lilly, 0.01% trypan blue, and distilled water. The pH was adjusted to 6.7 with hydrochloric acid. When not in use, the medium was refrigerated. In preparation for use, the medium was liquefied in a boiling water bath so that it could be spread easily in a layer about 1 mm thick over the microscope slide. The medium quickly solidified on the slide and 14 wells were suctioned from it in two hexagonal patterns (Figure 3). The wells provided test positions for eight samples. A drop of antiserum (A) was placed in each center well and a drop of starry flounder egg extract (C) was placed in four positions so that each unknown was adjacent to a control for easy comparison. A drop of whole blood from halibut was placed in each of the remaining eight wells. The slides were then stored in a moistened, plastic box and heated in a 50°C oven for eight to twelve hours. Subsequently, the slides were examined on a dark-field illuminator for the presence of precipitate lines. For example, precipitate lines (Figure 3) formed between the antiserum A and the blood samples in positions 1, 7, and 8 indicate that the corresponding halibut were mature females (HM+) and the remainder were immature females or males (HM-). Tissue samples were treated in a similar manner, but they usually were ground and centrifuged at 3,400 rpm for 15 seconds to yield sufficient fluid for testing.

RESULTS

Comparison of Methods

The comparison of visual and serological determinations of maturity showed nearly perfect agreement, regardless of season (Table 1). Maturity determinations by these methods differed for only 3 of 107 females (2.8%). One female judged to be immature in the August-September sample showed an HM+ reaction, and two females judged to be mature in the January-February sample showed HM- results. In addition, all 34 males tested gave HM- results, as expected, and serum samples

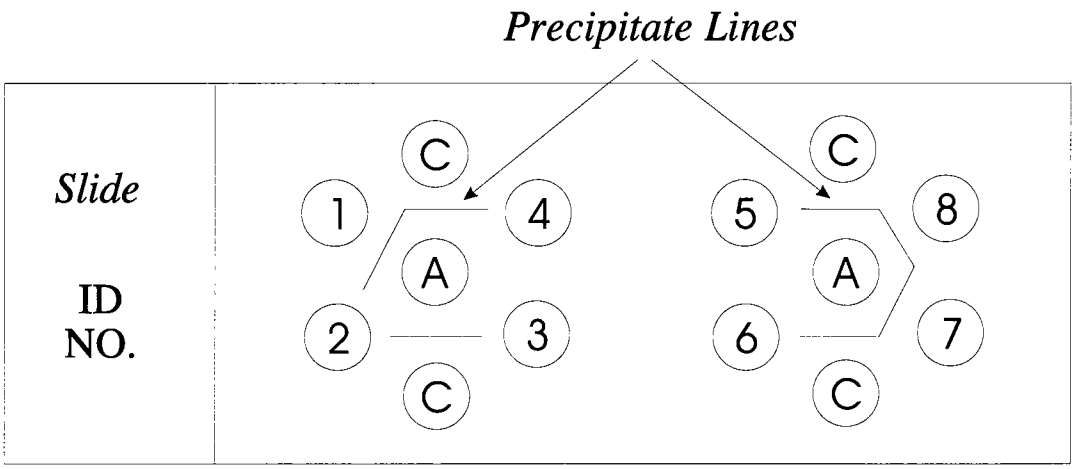

Figure 3. Schematic diagram of microscopic slide showing HM+ (precipitate lines) and HM- reactions.

from the three fish tagged indicated that none were mature females. The immediate effect of taking a blood sample from a live halibut was not lethal, although recoveries of the three fish that were sampled, tagged and released, have not been reported.

Of the four sets of tissue samples tested, only one was from a mature female. Fluids from its ovary, kidney, liver, gill, and tongue samples showed HM+ reaction, although the reaction with tongue fluid was relatively weak. Eye fluid from the mature female showed no reaction (HM-). Three sets of tissues from immature females gave HM- results.

Catch Composition

The length of every fish caught was recorded and the totality of fish unsuitable for tagging were sampled for sex and age composition (Table 2). The sex composition of the total catch for each location sampled was determined from the proportion of known males and females in each 5 cm length interval. Similarly, the total number of mature and immature females in each length interval was projected from their known proportion, as determined from the visual method. Each sex and maturity stage was treated separately during the projection of the dead fish sample to the total number of fish caught at each age and length interval using the method described by Hardman and Southward (1965). Hoag et al. 1979 indicated that the likelihood of bias should be small in such circumstances because the age and sex compositions were estimated for each length interval and then projected to the total number of fish in the interval.

The estimated length and age compositions of mature and immature females are presented in Tables 3 and 4, respectively. The estimated age and length compositions of mature and immature females showed that all females were immature at ages less than 8 years or lengths less than 95 cm, and all were mature by age 17 or at sizes greater than 139 cm in length. The percentage of mature females in the August-September sample was greater for the age range (except age 8) in which both immature and mature females were represented than in the January-February sample. However, the percentage of mature females by length interval generally showed the opposite trend. Females in the winter sample were mature at smaller sizes than those in the summer sample, a logical consequence of migratory move-

Table 1. Maturity of female halibut collected during the Kodiak Island and southeastern Alaska surveys from the visual and serological methods.

Length Interval (cm)	Kodiak Island Survey				Southeastern Alaska Survey			
	Visual Method		Serological Method		Visual Method		Serological Method	
	Mature	Immature	Positive (mature)	Negative (Immature)	Mature	Immature	Positive (Mature)	Negative (Immature)
80-84	-	3	-	3	-	-	-	-
85-89	-	4	-	4	-	-	-	-
90-94	-	5	-	5	-	2	-	2
95-99	-	8	-	8	-	7	-	7
100-104	-	5	-	5	-	7	-	7
105-109	-	4	-	4	-	8	-	8
110-114	-	7	1*	6	-	4	-	4
115-119	1	4	1	4	-	6	-	6
120-124	4	6	4	6	6	1	6	1
125-129	2	-	2	-	5	-	3	2*
130-134	-	-	-	-	4	1	4	1
135-139	1	-	1	-	1	-	1	-
140-144	-	-	-	-	1	-	1	-
Total	8	46	9	45	17	36	15	38

*Disagreement between methods.

ments by mature females to spawning sites as previously indicated by Skud (1977) and St-Pierre (1984).

The age and length of 50% maturity were estimated through fitting a functional (logistic regression) relationship using the statistical package GLIM (Generalized Linear Interactive Modelling) documented by Parma[3]. This analysis has the advantage of using the full data sets to interpolate the maturity points instead of only the information in the two adjacent age increments. The number of observations for which we have associated age and length data are 474 and 652, respectively for 1979 and 1980.

The estimated age and length of 50% maturity are shown in Table 5. The age of 50% maturity was higher in the winter sample while the length of 50% maturity was lower when compared to the summer sample.

[3] Parma, A. M. 1993. Estimation of halibut maturity as a function of length. Report of Assessment and Research Activities 1992. Int. Pac. Halibut Comm. 113-120.

Table 2. Number of fish tagged and sex compositions of the dead fish in number of males, immature and mature females by 5 cm length interval from the tagging surveys off Kodiak Island in 1979 and southeastern Alaska in 1980. Maturity determination based on the visual method.

Length Interval	Kodiak Island Survey (Aug.-Sept., 1979)					Southeastern Alaska Survey (Jan.-Feb., 1980)				
	No. of Fish Tagged	Maturity of Females			No. of Males	No. of fish Tagged	Maturity of Females			No. of Males
		Immature	Mature	Unknown			Immature	Mature	Unknown	
40-44	1	1	0	0	2	1	0	0	0	0
45-49	6	3	0	0	1	1	0	0	0	0
50-54	27	3	0	0	14	0	0	0	0	9
55-59	49	11	0	0	21	6	3	0	1	10
60-64	81	16	0	0	31	17	3	0	0	21
65-69	76	18	0	0	44	58	15	0	1	32
70-74	98	18	0	0	27	103	30	0	0	70
75-79	75	19	0	1	25	122	27	0	1	74
80-84	66	20	0	0	25	120	29	0	3	60
85-89	40	22	0	1	36	100	31	0	3	49
90-94	39	26	0	0	22	89	23	0	4	27
95-99	40	30	0	0	24	67	37	2	3	31
100-104	20	30	0	0	29	79	44	1	4	30
105-109	27	30	2	0	25	83	31	4	4	20
110-114	30	22	0	0	21	83	22	8	1	17
115-119	21	22	6	2	11	75	25	8	1	6
120-124	22	14	14	0	6	86	12	18	3	6
125-129	29	7	9	1	9	69	1	24	0	4
130-134	13	0	18	0	2	55	4	27	3	3
135-139	13	0	24	1	1	60	2	26	1	4
140-144	17	0	20	0	3	70	0	25	1	0
145-149	6	0	11	0	1	60	0	29	3	0
150-154	6	0	8	1	0	49	0	19	2	1
155-159	7	0	18	0	0	36	0	21	3	0
160-164	1	0	8	0	0	24	0	18	2	0
165-169	8	0	14	0	1	17	0	22	0	0
170-174	2	0	10	0	0	7	0	22	0	0
175-179	1	0	1	0	0	3	0	21	0	0
180-184	1	0	5	0	0	0	0	8	0	0
185-189	0	0	2	0	0	0	0	6	0	0
190-194	1	0	1	0	0	0	0	4	0	0
195-199	-	-	-	-	-	0	0	8	0	0
200-204	-	-	-	-	-	0	0	1	0	0
205-209	-	0	2	0	0	0	0	3	0	0
210-214	0	0	1	0	0	0	0	1	0	0
215-219	-	-	-	-	-	0	0	1	0	0
Total	823	312	174	7	381	1,540	339	327	44	474

Table 3. Estimated number of immature and mature females based on the visual method for maturity determination, and percent mature by 5 cm length interval from the tagging surveys off Kodiak Island in 1979 and southeastern Alaska in 1980.

Length Interval	Kodiak Island Survey (Aug.-Sept., 1979)				Southeastern Alaska Survey (Jan.-Feb., 1980)			
	Number of Immature	Number of Mature	Total Number	Percent Mature	Number of Immature	Number of Mature	Total Number	Percent Mature
40-44	1	0	1	0.0	-	-	-	-
45-49	8	0	8	0.0	-	-	-	-
50-54	8	0	8	0.0	-	-	-	-
55-59	27	0	27	0.0	6	0	6	0.0
60-64	45	0	45	0.0	5	0	5	0.0
65-69	41	0	41	0.0	37	0	37	0.0
70-74	57	0	57	0.0	62	0	62	0.0
75-79	54	0	54	0.0	65	0	65	0.0
80-84	49	0	49	0.0	74	0	74	0.0
85-89	39	0	39	0.0	76	0	76	0.0
90-94	47	0	47	0.0	74	0	74	0.0
95-99	52	0	52	0.0	77	4	81	4.9
100-104	40	0	40	0.0	98	2	100	2.0
105-109	44	3	47	6.4	83	11	94	11.7
110-114	37	0	37	0.0	62	22	84	26.2
115-119	36	9	45	20.0	74	24	98	24.5
120-124	23	23	46	50.0	42	63	105	60.0
125-129	16	21	37	56.8	3	81	84	96.4
130-134	0	30	30	100.0	11	74	85	87.1
135-139	0	38	38	100.0	6	76	82	92.7
140-144	0	35	35	100.0	0	96	96	100.0
145-149	0	17	17	100.0	0	92	92	100.0
150-154	0	15	15	100.0	0	68	68	100.0
155-159	0	25	25	100.0	0	60	60	100.0
160-164	0	9	9	100.0	0	44	44	100.0
165-169	0	21	21	100.0	0	39	39	100.0
170-174	0	12	12	100.0	0	29	29	100.0
175-179	0	2	2	100.0	0	24	24	100.0
180-184	0	6	6	100.0	0	8	8	100.0
185-189	0	2	2	100.0	0	6	6	100.0
190-194	0	1	1	100.0	0	4	4	100.0
195-199	-	-	-	-	0	8	8	100.0
200-204	-	-	-	-	0	1	1	100.0
205-209	0	2	2	100.0	0	3	3	100.0
210-214	0	1	1	100.0	0	1	1	100.0
215-219	-	-	-	-	0	1	1	100.0
Total	624	272	896	30.4	855	841	1,696	49.6

DISCUSSION

Validation of the Methods

The almost complete agreement between the visual and serological determinations of maturity suggests that both methods provide the same measure of maturity, not only during the winter spawning season but also during the late summer. Utter (1964) reported similar agreement for samples collected during February, April, and May. Thus, both methods probably are valid year-round although samples collected during early summer are needed to confirm this fully. Also, the visual determinations of maturity were a composite of observations by several biologists so the good agreement was not the result of an exceptional ability by any single biologist.

Interpretation of Discrepancies

One of the discrepancies between visual and serological determinations of maturity was a female in the August-September sample whose ovaries appeared to be immature but whose serum showed an HM+ reaction. This female was 10-years-old and 111 cm in length, well below the size at which most females in this sample were mature, and may have been in transition from the immature to the mature stage. The most probable biological explanation is that this particular female had developing oocytes in an early stage of formation which were not detected during the visual examination. It is conceivable that this female was tardy in maturing her eggs when compared to the others and was indeed going to participate in the upcoming spawning, likely toward the end of the period in late March or the beginning of April. Under such a scenario, that female still had ample time (7 months) to bring her eggs to maturity. Such possible outcomes exemplify why fish approaching the maturing stage are at times difficult to classify as immature or mature.

An alternative biological explanation is that this female may have produced egg vitellin in relatively small amounts so that her ovaries lacked visible oocytes, yet her serum showed an HM+ reaction. Thompson (1915) reported that several generations of eggs are present in halibut ovaries simultaneously and the smallest group, invisible to the naked eye, provide eggs to be spawned a year or more later. Perhaps this female was producing egg vitellin for these oocytes a year or more in advance of her first spawning, which may explain the HM+ reaction and lack of visible eggs. Similarly, Utter (1964) reported that six out of 44 comparisons of halibut maturity determined by visual examination showed differences, all of which were immature females that gave HM+ results. His quantitative analysis indicated that the concentration of the HM factor in the serum of these immature females was very low in comparison to that of mature females (Utter 1964; Utter and Ridgway 1967). Therefore, a quantitative evaluation of the HM factor, wherein low concentrations indicate maturing but not yet mature females, may provide a more definitive measure of maturity. Maturity determination estimates based on such a quantitative criterion are likely to be in agreement with the visual determination. It also may be more biologically meaningful because a mature fish is considered to be capable of spawning during the forthcoming spawning season, whereas a maturing fish is usually not.

Table 4. Estimated number of immature and mature females based on the visual method of maturity determination, and percent mature by age from the tagging surveys off Kodiak Island in 1979 and southeastern Alaska in 1980.

Age	Kodiak Island Survey (Aug.-Sept., 1979)				Southeastern Alaska Survey (Jan.-Feb., 1980)			
	Number of Immature	Number of Mature	Total Number	Percent Mature	Number of Immature	Number of Mature	Total Number	Percent Mature
4	6	0	6	0.0	-	-	-	-
5	24	0	24	0.0	-	-	-	-
6	110	0	110	0.0	14	0	14	0.0
7	148	0	148	0.0	64	0	64	0.0
8	111	1	112	0.9	202	3	205	1.5
9	113	11	124	8.9	189	6	195	3.1
10	57	17	74	23.0	166	42	208	20.2
11	32	50	82	61.0	117	53	170	31.2
12	20	61	81	75.3	67	111	178	62.4
13	3	30	33	90.9	17	134	151	88.7
14	0	31	31	100.0	11	163	174	93.7
15	0	19	19	100.0	3	113	116	97.4
16	0	14	14	100.0	5	105	110	95.5
17	0	17	17	100.0	0	62	62	100.0
18	0	9	9	100.0	0	14	14	100.0
19	0	3	3	100.0	0	13	13	100.0
20	0	2	2	100.0	0	12	12	100.0
21	-	-	-	-	0	9	9	100.0
22	0	3	3	100.0	0	1	1	100.0
23	0	2	2	100.0	-	-	-	-
24	0	1	1	100.0	-	-	-	-
25	0	1	1	100.0	-	-	-	-
Total	624	272	896	30.4	855	841	1,696	49.6

The two remaining discrepancies in this study, one in which females with visibly mature ovaries gave HM- results, had no apparent biological basis. Several factors suggest that the visual and serological determinations were correct, but a mix-up of samples occurred. Errors in labeling the samples either at the time of collection or at the laboratory seem likely because the serological tests for both females were made on the same microscope slide, and the controls gave HM+ reactions, an indication that the serological tests were performed properly. Also, these discrepancies occurred in the winter sample when the visual method to determine maturity is most easily and accurately applied. The females were 12 and 13 years and both measured 127 cm. Thus, both were older than the estimated age of 50% maturity and at a size where most females were mature, so the visual determination was probably correct.

Table 5. Estimated age and length of 50% mature using the logistic regression method.

	1979 (Summer)			1980 (Winter)		
Predictor	a*	b*	50% Mature	a*	b*	50% Mature
Age	-13.28	1.2206	10.9	-13.27	1.1377	11.7
Length	-25.61	0.2094	122.3	-24.35	0.2035	119.7

a* and b* are the parameters of the logistic regression.

Cause of Variation in Age of 50% Maturity

The relationship of halibut among the regions is not well understood. Skud (1977) suggested that many immature females may not participate in the spawning migration with the mature females while Schmitt and Skud (1978) proposed that different geographic distributions of mature and immature females during the spawning season is the probable explanation for the differences in age of 50% maturity. St-Pierre (1984) attributed the difference in the age of 50% maturity observed between regions during the winter to spawning migration of mature fish. Schmitt and Skud (1978) reported that within the same region, estimates of ages of 50% maturity obtained from winter samples were generally lower than from summer samples, suggesting that immature halibut are not fully represented on the spawning grounds. Their data also show that the age of 50% maturity varies monthly within the same season and has changed over the years, although by not more than one year.

The results of the current study show a higher age of 50% maturity (11.7 years) for the winter sample from southeastern Alaska compared to 10.9 years for the late summer sample off Kodiak Island (Table 5). The length of 50% maturity for the winter sample is 119.7 cm compared to 122.3 for the late summer sample. Although the age of 50% maturity is usually lower during the winter that what is observed during the summer, the reverse happened in this study. However, the samples in this study were from different regions and the relationship among halibut in these regions is not well understood. For example, results of tagged fish released on Area 2C (southeastern Alaska) spawning grounds indicate that 98.3% were recovered primarily on the summer feeding grounds of Areas 2C, 2B, and 2A (St-Pierre 1984). Conversely, 95.2 % of those released between Cape St. Elias and Kodiak Island were recovered on the summer feeding grounds in or west of that area. Fish from those two areas apparently have different seasonal distributions,

and therefore the results of 50% maturity from those two samples are hardly comparable.

Seasonal migrations or different geographic distributions of mature females probably explain the variation in age of 50% maturity between regions and seasons. The alternative explanation of unreliable maturity determinations appears unlikely in the light of the results of this study. In the current study, nearly all mature females caught during the January-February cruise off southeastern Alaska were spent and possibly some of the spawning fish may already have started their migration back to their respective summer feeding grounds. Consequently, these females may not truly reflect spawning aggregations. The late summer sample off Kodiak Island may not be entirely representative of the summer distribution either, as the schooling of migrating mature halibut at the edge of the continental shelf may already have begun. Such schooling in preparation for spawning has been documented by St-Pierre (1984) and is readily noticeable during October and November.

Applications and Limitations

The potential for application of the serological method to determine maturity of either tagged or commercially-landed halibut cannot be established until more data are available, although preliminary results are encouraging. The three tagged halibut were not noticeably affected at the time blood samples were taken (Williams pers. comm.)[4], but the effect on their survival after release is unknown. Utter and Ridgway (1967) reported that repeated bleedings of captive starry flounder apparently did not affect their survival in captivity. Although this potential problem must be studied thoroughly before the serological method is used widely for live halibut, the technique shows promise. In addition, preliminary results from the tissue samples showed that fluid from a variety of tissues may be used to determine maturity, thereby increasing the probability that most mature females, although eviscerated, can be identified in commercial landings. For example, kidney and gill fragments are often found in eviscerated halibut, and the presence of either will suffice. Utter and Ridgway (1967) reported that 38 out of 41 halibut kidney samples collected four days after evisceration gave accurate results although disagreements occurred with three individuals with extremely low serum concentration. However, the tissue samples in the present study were obtained at the time the fish was eviscerated, and further study is needed to establish the length of time that samples may be taken after evisceration and still provide accurate results.

The present study was undertaken in 1979 and subsequent changes in fish holding methods aboard commercial vessels have reduced the potential value of the serological technique using kidney or gill fragments for sampling commercial landings. Although storing fish in crushed ice continues to be the most common method aboard commercial halibut vessels, an increasing number of vessels have converted to either refrigerated seawater or slush ice (seawater chilled with ice) to

[4] Williams, Gregg H. IPHC. P.O. Box 95009, Seattle, WA 98145-2009.

store fish. Such a practice, even if commercial halibut fishing was permitted during the spawning season, would likely dilute the amounts of the maturity component (HM) in samples to undetectable levels and/or result in mixing of fluids from more than one fish. Therefore, the use of different halibut storage techniques by vessels complicates the random sampling of commercial catches for maturity determination via the serological method.

A potential alternative to these techniques was developed in the late 1980s and involved an accurate visual procedure to determine the sex in live Pacific halibut from the shape and appearance of the urogenital vent (St-Pierre 1992). Furthermore, the maturity of halibut having never spawned and those having participated in several spawnings can accurately be determined from the appearance of the urogenital vent. However, the procedure is not applicable to halibut at the stage preceding initial spawning as the urogenital vent still retains the shape of an immature fish. Also, the procedure, has proved difficult to apply to some females which have obviously participated in their first spawning as indicated by visual inspection of the ovaries. First time spawners may have lower fecundity and their release of eggs may be insufficient in some cases to alter noticeably and permanently the size of the urogenital opening. Unfortunately, this sex determination procedure is not applicable to halibut landed commercially as the urogenital vent is usually cut through or scraped off during evisceration of the fish.

Advantages and Shortcomings of Each Method

The principal advantages of the visual method are that males and females are readily separated and that a trained observer can determine the sexual maturity quickly, accurately, and easily under shipboard conditions. The visual method of maturity determination has several shortcomings including possible unreliability with some maturing fish. The method is limited to halibut caught on research cruises where a qualified observer can examine the gonads because commercial catches are eviscerated at sea. Also, it requires that the fish be sacrificed if maturity data are needed, thereby precluding their release as tagged fish for migration and mortality studies.

The serological technique is a valuable investigating tool which could be used to validate other methods of maturity determination. The method appears to inflict no apparent harm to live halibut. Therefore, the serological method shows promise for successful application to tagged fish and to commercially-landed halibut packed in ice. However, the serological method in itself is not without shortcomings. The predominant limitation is that the method does not discriminate between males and immature females as the serological test gives an HM- result for both. In addition, the test procedure in the laboratory is rather lengthy, conditions at sea are not always favorable to the collection of samples, and special attention aboard vessels is needed in the storage and labelling to avoid mixing samples.

The serological method could be used to obtain maturity data of female fish where sex is determined by the shape of the urogenital pore. Thus, the method of visual determination of sex in halibut will segregate males from females and the serological method could separate immature from mature females.

SUMMARY AND CONCLUSIONS

The visual and serological methods for determining the sexual maturity of female halibut were evaluated and compared. An important implication of the agreement between visual and serological methods is that the maturity data collected formerly by IPHC biologists are correct. The visual and serological methods apparently can be successfully applied year-round to determine maturity of female halibut. The serological and visual methods may have wider application when used jointly to overcome the shortcomings inherent to a specific method. This supports the theory that seasonal differences in maturity schedules observed in past studies using visual methods are real and probably related to spawning migrations. Age and length estimates of 50% maturity are more representative of the halibut population in an area when they are based on summer rather than winter sampling.

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LITERATURE CITED

Clark, W. G. and A.M. Parma. 1995. Re-evaluation of the 32-inch Commercial Size Limit. Int. Pac. Halibut Comm. Tech. Rep. No. 33: 34 p.

Hardman, W. H. and G. M. Southward. 1965. Sampling the commercial catch and use of calculated lengths in stock composition studies of Pacific halibut. Int. Pac. Halibut Comm. Rep. No. 37: 32 p.

Hoag, S.H., C.C. Schmitt, and W.H. Hardman. 1979. Size, age, and frequency of male and female halibut: setline research catches, 1925-1977. Int. Pac. Halibut Comm. Tech. Rep. 17: 112 p.

International Pacific Halibut Commission. 1991. Int. Pac. Halibut Comm. Annual Report 1990: 52 p.

Ridgway, G. J., G. W. Klontz, and C. Matsumoto. 1962. Intraspecific differences in serum antigens of red salmon demonstrated by immunochemical methods. Int. North Pac. Fish. Comm. Bull. No. 8: 1-13

Schmitt, C. C. and B. E. Skud. 1978. Relation of fecundity to long-term changes in growth, abundance and recruitment. Int. Pac. Halibut Comm. Sci. Rep. 66: 31 p.

Skud, B. E. 1977. Drift, migration, and intermingling of Pacific halibut stocks. Int. Pac. Halibut Comm. Sci. Rep. No. 63: 42 p.

St-Pierre, G. 1984. Spawning locations and season for Pacific halibut. Int. Pac. Halibut Comm. Sci. Rep. No. 70: 46 p.

St-Pierre, G. 1992. Visual determination of sex in live Pacific halibut. ICES J. mar. Sci., 49: 373-376.

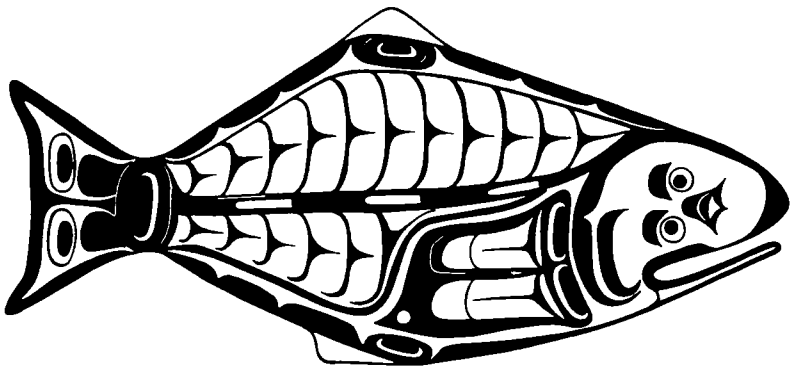
Thompson, W. F. 1915. A preliminary report on the life-history of the halibut. Province of British Columbia. Report of the Commissioner of Fisheries: 76-99.

Thompson, W. F. 1916. The problem of the halibut. Province of British Columbia. Report of the Commissioner of Fisheries: 130-140.

Thompson, W. F. 1917. The egg production of the halibut of the Pacific. Province of British Columbia. Report of the Commissioner of Fisheries: 35-38.

Utter, F. M. 1964. Biological and immunological aspects of an antigenic factor associated with maturity in female flatfish. M.S. Thesis, University of Washington, Seattle: 66 p.

Utter, F. M. and G. J. Ridgway. 1967. A serologically detected serum factor associated with maturity in English sole, *Parophrys vetulus*, and Pacific halibut, *Hippoglossus stenolepis*. Fishery Bulletin, Vol. 66, No. 1: 47-58.



HALIBUT CREST – *adapted from designs used by Tlingit, Tsimshian and Haida Indians.*