

FISH DIVISION Oregon Department of Fish and Wildlife

Chinook salmon at-sea maturity studies 1976-1978, including a summary of Oregon Fish Commission ocean salmon maturity sampling 1948-1975

CHINOOK SALMON AT-SEA MATURITY STUDIES 1976-1978, including a SUMMARY OF OREGON FISH COMMISSION OCEAN SALMON MATURITY SAMPLING 1948-1975

Malcolm H. Zirges

INFORMATION REPORT SERIES, FISHERIES NUMBER 84-5

Oregon Department of Fish and Wildlife Marine Region Newport, Oregon 97365

June 1984

Funds Supplied in Part by the aaanadromous Fisheries Act, PL 89-304 Segment 3 of Federal Grant-in-Aid Project AFC-92 Agreement Number 80-ABD-PM1B Administered through the Pacific Marine Fisheries Commission

TABLE OF CONTENTS

	Page
INTRODUCTION	1
PREVIOUS WORK	3
Observation of Returning Fish	4 4 4
Studies of Physical Changes in Gonad Tissue	4
Gross Physical Changes	
Cell-Level Changes	10
Studies of Chemical Changes in Gonad Tissue	10
METHODS	12
Selection of Method for Determining Maturity	12
Sampling	15
At-Sea Sampling	
Sport Fishery Sampling	16
Development of Mature/Immature Separation Criteria	
Treatment of Differences by Sex	
Females	
Males	
Developing Histological Separation Criteria	19
RESULTS AND DISCUSSION	23
The 1976-1978 Maturity Sampling	
Historical Sampling	23
Methodology	25
Percent Maturing by Length	
Sex and Age Composition	
The 1952-1956 Data	33
ACKNOWLEDGMENTS	39
REFERENCES	40
APPENDIX J - Sample Data	42
Coho Sex and Length Frequencies	
Chinook Age, Sex, and Length Frequencies	
APPENDIX II	54
MELLIDIA 11	-

INTRODUCTION

Chinook salmon are exploited in the ocean beginning with their second year of life. Although male chinook mature as early as age-2 and females as early as age-3, many individuals, particularly females, mature as late as age-6. The ocean troll fishery historically has exerted the greatest pressure on three year old chinook, a significant portion of which are still immature.

Troll and sport chinook salmon fisheries have historically been regulated primarily through minimum size and season length regulations. These regulations are designed to protect immature chinook salmon which still have significant potential growth remaining. This objective is balanced, however, by a desire to fully harvest maturing fish not needed for spawning escapement before they leave the ocean.

Maturity information is needed to assist in the management of ocean salmon fisheries. For example, chinook jacks (early-maturing age-2 male fish), are less likely to be harvested after they leave the ocean and are rarely needed on the spawning grounds. A high percentage of jacks in a given ocean fishery might warrant adjusting minimum size limits to increase harvest of these smaller fish. A stock needing greater protection might be conserved effectively by closing an area or time period when a large percentage of maturing females are available thus providing increased spawning escapement.

A recent controversial example is the chinook fishery off the southern Oregon-northern California area which includes both depressed and early-maturing runs with a high percentage of jacks. Maturation rates of chinook salmon available to ocean fisheries in this area are poorly understood, particularly for those fish caught early in the season near the legal minimum commercial size limit of 26 inches. Various management problems in recent years have caused the Pacific Fisheries Management Council, which is responsible for salmon management in the 3 to 200 mile zone offshore, to alter chinook harvest regulations including size limits. For example, the minimum size limit for commerciallycaught chinook has changed from 26 to 28 inches north of Cape Falcon, Oregon. The sport-caught chinook limit has changed from 20 to 24 inches north of Cape Falcon, and ranged from zero to 22 inches south of that point. These changes have generated considerable pressure to either retract these new limits or enact them uniformly coastwide.

The need for maturity information reaches almost every aspect of chinook management. The "ideal" size limit is a function of balancing gains from additional growth by undersize (and immature) fish released and re-caught later against losses from shaker and natural mortality plus emigration of maturing fish. Protecting potential spawners or optimizing yield by taking advantage of growth potential also requires evaluating percentage of maturing fish.

In 1976, ODFW Ocean Salmon Program personnel began analysis of file data and implemented at-sea sampling of chinook salmon length-frequencies and other maturity parameters. Although this work initially addressed chinook along the entire Oregon coast, the size limit issue resulted in a change of focus in 1977 to small fish off the south coast. An effort was made to: (1) determine the most suitable method of detecting maturity in ocean-caught chinook salmon, particularly in the 20-28 inch size range, and (2) to utilize this method to assess the relative abundance of mature and immature chinook in the mixed stock ocean fisheries off southern Oregon.

The study was expanded in 1978 to a joint program with the California Department of Fish and Game under the Anadromous Fisheries Act, PL 89-304,

-2-

Grant-in-Aid project AFC-92 (administered through the Pacific Marine Fisheries Commission). This included development of an improved method for determining maturity in small male fish with the assistance of personnel of the Oregon Cooperative Fishery Unit at Oregon State University.

This report contains a summarization of chinook salmon maturity sampling data collected during the past by ODFW (earlier the Fish Commission of Oregon or FCO), and data collected by ODFW during the 1976-78 project. It presents maturity information derived from that data, and includes methodologies for a promising histological technique developed with the particular help of the Coop Fish Unit for determining state of maturity in male chinook salmon.

PREVIOUS WORK

A number of previous studies have examined stage of maturity in chinook salmon. Although numerous methods to differentiate maturity were tried, these studies met with limited success. This is because the maturation process proceeds over a relatively long period of time, months at least, but evidence is only physically obvious and measurable late in the process.

With salmon, which are all eventually caught or return to freshwater to spawn, one obvious approach is to discover percent maturing at each age in a stock by direct observation on the spawning grounds. This method provides information which is very stock specific. Where maturity information is desired for fish caught in a specific offshore fishery, approaches tried have included (1) tagging fish in the ocean and then observing how soon they return to freshwater, (2) various methods of measuring physical changes in gonadal tissue of the fish in question, and (3) testing for chemical changes in gonad or other glandular tissue.

-3-

Observation of Returning Fish

Direct observation of mature returning fish is a difficult way to obtain enough information to determine maturity rates in offshore fisheries. Managers need to know which fish being caught are maturing--in the mixed lot of fish being harvested in the fishery being managed. This requires tagging fish at sea and then sorting tags recovered on the spawning grounds between those recovered the year of tagging (tagged fish was maturing) from those recovered in later years (tagged fish was immature). Not only is this method slow, because it may take years to be sure all potential tag recoveries are in, but sample sizes are usually very small due to the costs of tagging and low tag recovery rates.

ODFW has conducted a number of ocean tagging programs, but low recovery rates have generally limited the usefulness of the information obtained. An interesting aspect obvious in this type of data, however, is the indication that the larger, faster growing individuals in a year-class are also the early maturing individuals (Figure 1). As is noted above, tag recovery rates from these studies were generally low and provided limited information for maturity determinations.

Studies of Physical Changes in Gonad Tissue

GROSS PHYSICAL CHANGES. A number of methods of measuring and comparing gross changes in organ (particularly gonad) sizes associated with maturation have been tried including (1) establishing some fixed minimum gonad size determined to be maturing by visual examination of a large number of samples, and (2) comparison of the ratio of gonad to body-size development (growth), either directly or using some sort of maturity index (for examaple gonadweight/body-length), to identify divergent maturing gonads.

-4-

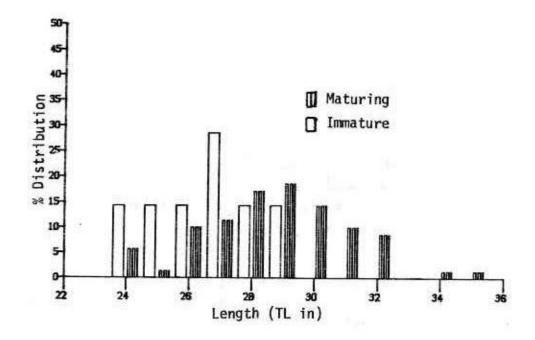


Figure 1. Maturity by length category of Age-3 chinook salmon tagged at sea 1948-1961 by FCO and returning to freshwater in the same year (maturing) and in following years (immature □).

Gonad tissue is a relatively small percentage of fish body weight before maturity. Physical methods are based on the expectation that immature gonad tissue increases in size linearly with fish growth, while maturing gonads enlarge disproportionately to fish growth. Robertson (1958) found that "growth of infantile testis parallels growth of the other components of the trout until the process of gonadal maturation begins to occur". This appears to be true in chinook salmon also as is shown in a plot of dressed weight to gonad weight for immature female chinook salmon (Figure 2). Robertson also determined that gonad size is most closely related to fish length, an easier measurement to obtain than fish weight.

-5-

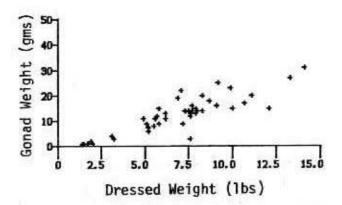


Figure 2. Example of the relationship of gonad size to fish size (parallel growth) in female chinook salmon sampled off the Columbia River during 1953.

Gonad weight and volume are almost perfectly proportional (Figure 3). This is useful since volume measurements are easier to obtain on the rolling deck of a boat while weight is usually easier to sample on shore. The close relationship (within measurement error) allows either method of gonad size characterization to be used interchangeably.

VanHyning (1973) sampled chinook gonads in the Columbia River area and plotted gonad weight against fish length and also gonad weight-frequency distributions. He visually separated larger (maturing) gonads from smaller (immature) ones in his figures, and extrapolated results from relatively easy to categorize large fish back into the data points from small fish where immature and maturing gonads were similar in size and difficult to separate.

-6-

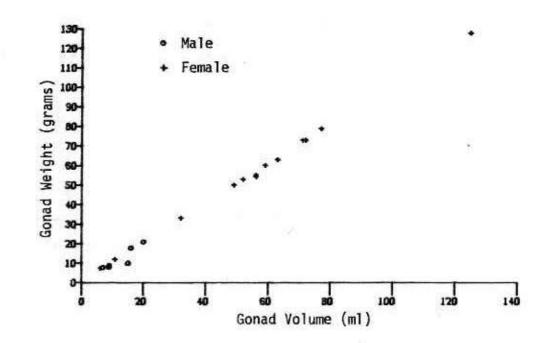


Figure 3. Plot of gonad weight against gonad volume for chinook salmon sampled at sea June 12, 1953 off Newport, OR. ESTIMATED GONAD WEIGHT in gms = 1.0195 (GONAD VOLUME in m1) - .2841

Wright and Bernhardt (1972) examined maturity rates of chinook salmon caught off Washington and the Columbia River in June to September of the 1970 and 1971 ocean fisheries. They measured the volume of chinook gonads, plotted volume histograms, and identified modes which they felt identified immature versus maturing fish. Although they noted that there was some overlap early in the fishing season, making separation suspect at that time, they concluded that fixed gonad volumes could be selected to categorize maturing fish over all fish sizes. They decided that all age-3 and older female fish with gonads over 25 ml in volume, and male fish with gonads over 5 ml in volume, were maturing.

-7-

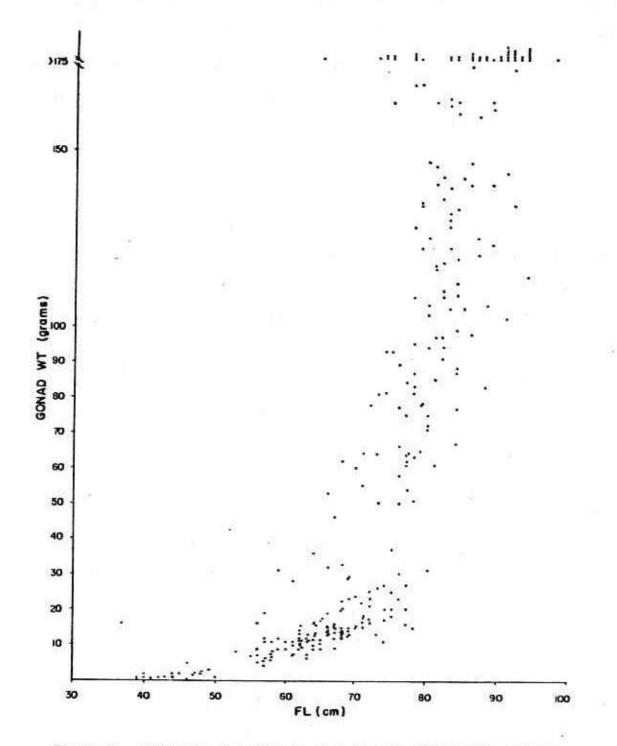
Other workers have also utilized physical examination of gonads to determine maturity in chinook or other salmon species with generally good results (Godfrey, 1961; Fiscus, 1969; Ishida and Miyaguchi, 1958). This technique appears to be sufficiently robust for female chinook salmon where gonad size increases dramatically during maturation, but it is not definitive for male fish.

Determination of the critical size separating mature from immature gonads has been inconsistent among studies, however, with no clearly superior method that might be applied over the full size spectrum of fish caught by the commercial fisheries. This is particularly true with respect to the need, and difficulty, of verifying state of maturity in small male fish. For example, 25 ml was used in early FCO studies as the separation point between mature and immature male chinook, and this is a workable value in early fall months. Wright and Bernhardt (1972) used 5 ml which is reasonably appropriate in mid-summer. But during the important management period, in late spring and early summer when the fish are small, no reliable single value or method has been proposed.

A plot of 293 female chinook sampled by ODFW personnel between 1952 and 1956 shows clear separation of maturing gonads that are enlarging exponentially from non-maturing gonads that are only enlarging steadily in parallel with fish growth (Figure 4). Note, however, that it also demonstrates the relative simplicity of determining female fish maturity since they mature at large sizes (older ages) and their gonads increase greatly in size near maturity.

The same technique is less clearcut for male fish. Male gonads are proportionally smaller and increase in size at a much slower relative rate as they approach maturity, and measurement error may be near the differences

-8-



1

Figure 4. Plot of gonad size to fish size in 293 female chinook salmon collected off Oregon between 1952 and 1956.

observed--especially early in the fishing season. Thus the divergence of maturing from non-maturing is less obvious in plots of male gonad weights. Figure 5 is a plot of 241 male chinook sampled by the FCO during July and August between 1952 and 1956. It clearly shows the clustering effect caused by relatively small gonad sizes, and wide gonad size dispersion caused by fish maturing at all sizes (and ages).

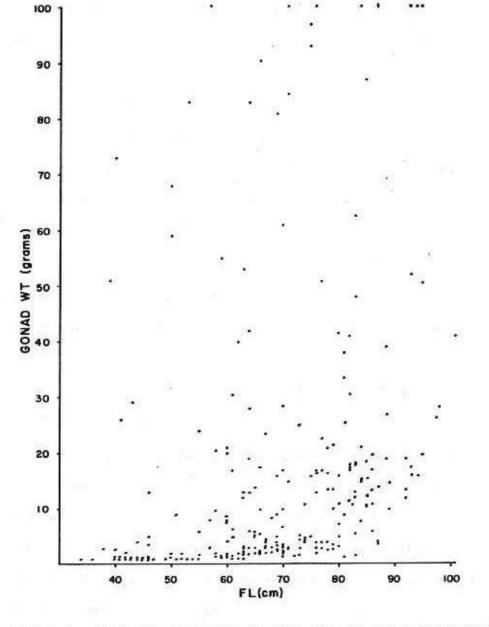
CELL-LEVEL CHANGES. The cell structure within the gonads changes as maturity nears. From a resting state, gonadal tissue cells must evolve through a number of stages before becoming the final sex products. In salmonids, resting spermatogonia evolve into primary and then secondary spermatocysts, then spermatids, and finally into spermatozoa. Presence of any stage other than the resting state provides de facto evidence that the maturation process has begun.

An in-depth study of gonad tissue development in rainbow trout (Salmo gairdneri) by Robertson (1958) showed that gonad tissue cell development could be detected in male fish prior to any external evidence of the maturation process. Chestnut (1970) identified similar changes in coho salmon gonad tissues and used them to study the effect of pituitary hormones on maturation. These studies suggested that histological examination of gonad tissue might be a workable method of detecting maturation in small male salmon.

Studies of Chemical Changes in Gonad Tissue

Some studies have also been conducted to detect chemical developments associated with maturation within gonadal tissue. These methods involve testing for changes in the production of some chemical, typically a hormone. Techniques employed have focused primarily on serological methods to identify blood serum factors associated with maturity.

-10-



L.

1.5

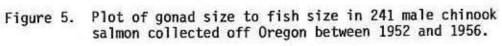
r-

8

ſ

E1

3



-11-

Serological techiques have been used widely in humans and other mammals to detect pregnancy. The method has been less applied to fishes, but Utter, Ridgway, and Hodgins (1964) and Utter and Ridgway (1965, 1967) reported on the application of this method to several fish species. They found the method workable for detecting maturing female fish, but that it is unsuitable for males.

FCO personnel conducted preliminary field testing of this technique on chinook salmon in 1962. Although maturing females were apparently detected dependably, the need for fresh (at-sea) blood samples and inapplicability to male fish limited the usefulness of the technique and it was not pursued.

METHODS

Methods involving a modification of previous gonad-volume to fish-length techniques plus a histological technique were developed to identify maturing chinook salmon.

Selection of Method for Determining Maturity

After examination of previous maturity studies conducted by ODFW and FCO personnel, and similar studies reported in the literature, the method of plotting gonad-volume to fish-length was felt to provide the clearest maturity determination over the widest range of fish sizes. This method is also very straightforward to implement. It requires measuring only fish length and gonad weight (or volume), and these parameters are readily converted, if necessary, between fork and total length or between volume in milliliters and weight in grams.

However, the subjective nature of this method did not allow reliable detection of maturation in male gonads because they are relatively too small and the relative growth differential between maturing and immature gonads too

-12-

little at early stages of maturation. The gonad-volume to fish-length method had to be supplemented to improve its accuracy with male chinook--particularly small males early in the fishing season. A histological technique was developed with the help of personnel of the Oregon Cooperative Fishery Unit at Oregon State University which provided a means of identifying the early stages of spermatogenesis. This method was also tested on female gonad tissue but was not satisfactory due to problems with the high lipid content of female sex cells.

Large numbers of gonad and fish size samples have been taken by ODFW personnel since 1948. Unfortunately, the maturity determination criteria originally used for much of these data are unknown, but some of the original samples were usable as a baseline data set and were summarized to help establish mature/immature separation criteria. Previous studies generally did involve some measurement of gonad weight or volume, and fish length or weight. Necessary conversions between different methods of measuring fish length were made using the formula:

TOTAL LENGTH in inches = 1.05625 (FORK LENGTH in centimeters) + 1.71728 (Van Hyning, 1951)

It was evident from data plots produced using previous methods that any separation line between plotted mature and immature gonad sizes must be sloped to reflect the growth of the gonad as the fish itself grows. Also, an arbitrary fixed separation criteria, such as the 5 ml criteria used by Wright and Bernhardt (1972), would cause some small maturing fish to be mis-classified as immature and some large immature fish to be mis-called as mature as shown in Figure 6.

-13-

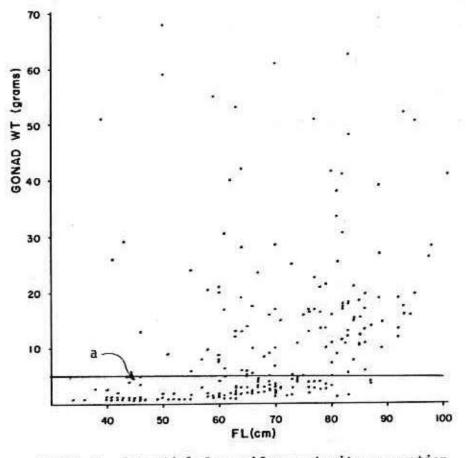


Figure 6. Potential for uniform maturity separation line set at a gonad weight of 5 grams (≅5ml) causing some maturing fish (a) to be categorized as immature. (data from Figure 5)

The basic method selected for this study involved plotting a large number of samples of gonad volume against fish length. Immature gonads were then separated from maturing gonads by their relative position on the graph--immature gonads around a nearly straight line sloping upward with increasing fish size, and maturing gonads departing almost exponentially from this line. This process is very straightforward for female chinook which mostly mature at older ages (large sizes), but this basic method was refined by also examining histological samples of gonad tissue from each male fish to verify state of maturity and clarify correct separation line placement.

Sampling

AT-SEA SAMPLING. Past maturity sampling was typically conducted during at-sea tagging or other studies aboard chartered vessels. Many of these early programs were large in scope with considerable sea time. They resulted in large sample sizes which, unfortunately, have not been obtainable in recent years due to limited charter funding.

Sampling during the 1976 and 1977 fishing seasons was conducted by ODFW personnel primarily during ride-along trips aboard commercial salmon troll vessels. This initial sampling effort was focused at sea to provide access to fish being caught offshore from the Klamath River, California, to the Coquille River, Oregon.

Samplers aboard trollers (1) removed scale samples for aging, (2) recorded sex, (3) obtained the volume of both complete gonads (by water displacement in a graduated cylinder), and (4) measured fork length in centimeters of each chinook caught and retained. Graduated cylinder sizes used were 25 ml for male gonads and 100 ml for female gonads.

Although samplers did obtain 65 and 296 usable samples in 1976 and 1977, respectively, with many from off northern California, relatively low numbers of small chinook were sampled. Since trollers could not legally retain chinook under 26 inches in length, and an individual boat usually saw relatively few fish, overall at-sea sampling proved to be an inefficient technique for obtaining small fish.

-15-

SPORT FISHERY SAMPLING. Minimum size limits continued to be an issue before the Pacific Fishery Management Council so in 1978 an effort was made to increase the number of small chinook sampled by also examining the catch of sport charterboats as they returned to the Port of Coos Bay. We also had determined by then that gonad tissues might be examined histologically to help verify maturity in small male chinook, and samplers were instructed to begin removing and preserving a one-inch section from the anterior portion of each gonad sampled for later study.

We obtained 297 samples during the 1978 season, including 226 gonad tissue sections, and because sport minimum size limits were then 22 inches (versus a commercial minimum of 26 inches), sport fishery sampling permitted many gonads to be collected from small fish. Most of these tissue samples were fixed in Bouin's solution and stored in 70% ethyl alcohol for later histological preparation (we originally used a 10% buffered formalin solution as a fixative but encountered problems with tissue brittleness) (Appendix II). Development of Mature/Immature Separation Criteria

Sample analyses were designed to determine only whether a given fish was maturing, i.e. would return to freshwater in the year caught (and sampled), or was immature.

Since the gonad-volume to fish-length method requires plotting as many data points as possible to provide a clear picture of where mature gonads diverge from immature ones, the large data set available from the 1952-1956 period was used to generate mature/immature baseline plots. Gonad volume and fish length data from this period were plotted separately for each sex to produce the scatter diagrams shown in Figures 4 and 5. The divergence of exponentially-growing maturing fish from steadily-growing immature fish can be seen in these figures.

-16-

Treatment Differences by Sex

FEMALES. For female chinook, which mature only at larger (older) sizes, and whose gonads increase in size at a high rate in proportion to body size during maturation, the gonad-volume to fish-length plot provides enough information to determine a mature-immature separation baseline.

Immature female gonads were subjectively identified as those gonad-volume/fish-length data points at small fish sizes defining a line sloping upward with increasing fish sizes, and those data points remaining near that line after maturing (faster growing) gonads have diverged at larger fish sizes. Data points in the center of the total data point distribution, where diverging points overlapped the linearly distributed points, were ignored.

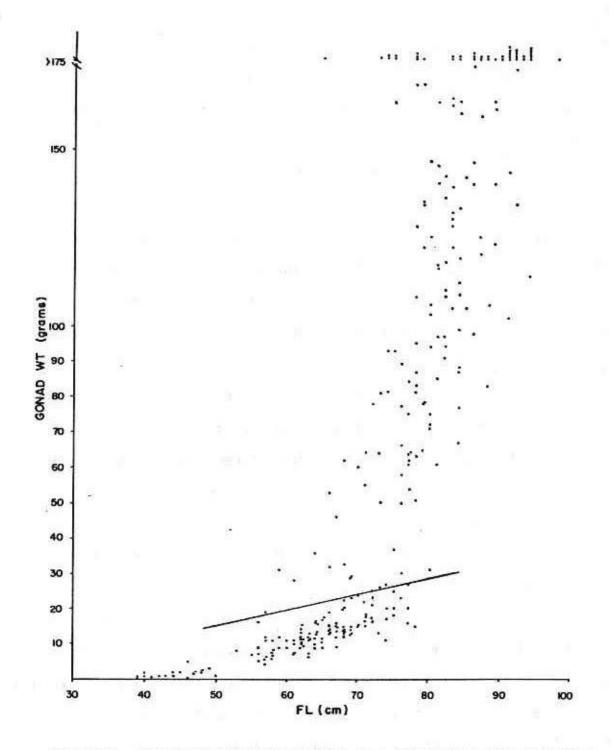
A least-squares linear regression line was fitted through data points identified as immature gonads, and an upper confidence band line (P=.95) determined and used as the separation line between immature and maturing female fish samples. The equation for this separation line is:

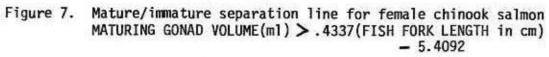
GONAD VOLUME in ml = .4337 (FISH FORK LENGTH in cm) - 5.4092 and any female gonad-volume/fish-length data point falling above this line is declared maturing (Figure 7).

MALES. The basic gonad-volume to fish-length plotting method was also used to determine maturity of male gonads. However, since male chinook mature at all sizes (ages), and male gonads are relatively small and do not exhibit a clear divergence of maturing from immature ones, plots of gonad-volume to fish-length alone did not provide enough information for subjective identification of immature and maturing gonads (refer again to Figure 5).

Male gonad tissue samples collected in 1978 were examined histologically to identify immature and maturing gonads. These known data points were then

-17-





-18-

overlaid onto the 1952-1956 male gonad-volume/fish-length data plot to provide a clearer indication of where immature and maturing gonads diverged. This allowed the variance inherent in immature gonad samples discovered histologically to define the upper limit of gonad data points called immature for regression line determination.

Developing Histological Separation Criteria

Histological preparation of gonadal tissue requires fixing, dyeing, and slide preparation of gonad samples using standard microtechniques moddified to suit the material under study. The preparation process used was developed by personnel of the Oregon Cooperative Fisheries Unit at Oregon State University, and the technique methodology is listed in Appendix II. Additional descriptions of appropriate histological methodologies are also available in Yasutake and Wales (1983).

Histologically prepared gonad tissue samples were evaluated for state of maturity by first examining the entire sample collection once to develop a feel for salmond cell structures and development stages present. Then guidelines developed by Robertson (1958) to follow maturation in rainbow trout were used to develop criteria to identify visual cues of maturation in chinook gonad cells. Photographs and explanations of gonad tissue cell structures provided by Chestnut (1970) and Yasutake and Wales (1983) were also useful in establishing reference criteria.

Spermatogenesis, the development of spermatozoa from gonad tissue cells, proceeds through several visually distinct stages. Figures 8a to 8d illustrate the most distinct stages of spermatogenesis observed in male chinook gonad tissue samples taken during 1978.

The extremes of cell development shown in photographs (a) versus (d) of Figure 8 clearly illustrate immature versus maturing gonad cells,

-19-

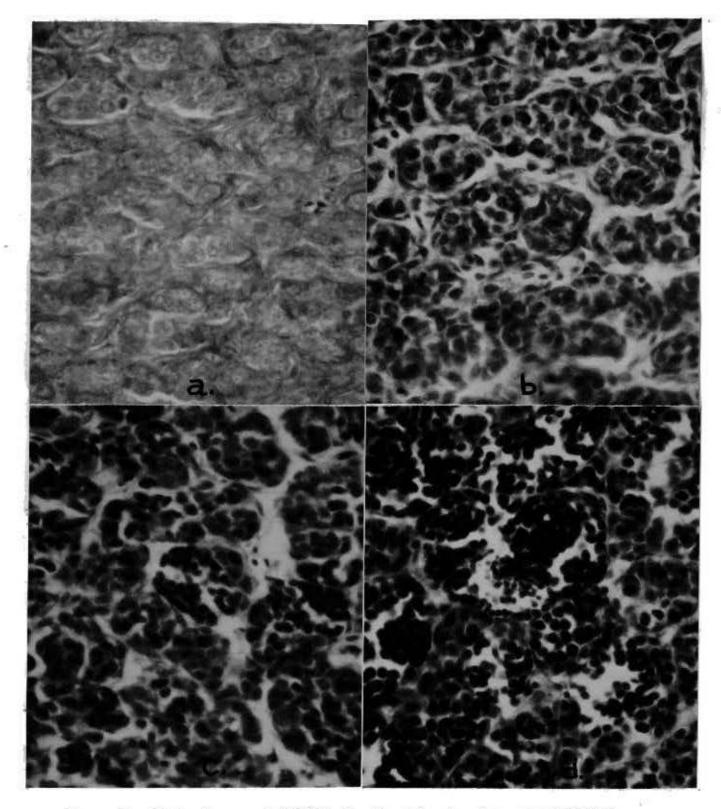


Figure 8. Photomicrograph (X400) of male chinook salmon gonad tissue showing stages of spermatogenesis. (a) resting, no cell development; (b) probably immature, but primary spermatocytes are grouped into lobules with distinct septa; (c) maturing, lobules enlarged and primary spermatocytes have developed into secondary spermatocytes; (d) maturing, some secondary spermatocytes have divided into clusters of spermatids. respectively. The presence of spermatids, the immediate precursor to spermatozoa, in (d) leave no doubt that this gonad was maturing. The gonads shown in photographs (b) and (c), however, show stages which should be evaluated further. Increased staining density apparent in cell nuclei of (c) were interpreted as evidence of increased miotic activity and the transition of primary spermatocytes to secondary spermatocytes--and maturation the year collected. In stage (b), however, greater variability in staining densities made interpretation less clear and gonads at this stage were called immature but with some question. Proper interpretation of this early stage may require from very careful attention to uniformity in staining and other tissue preparation procedures.

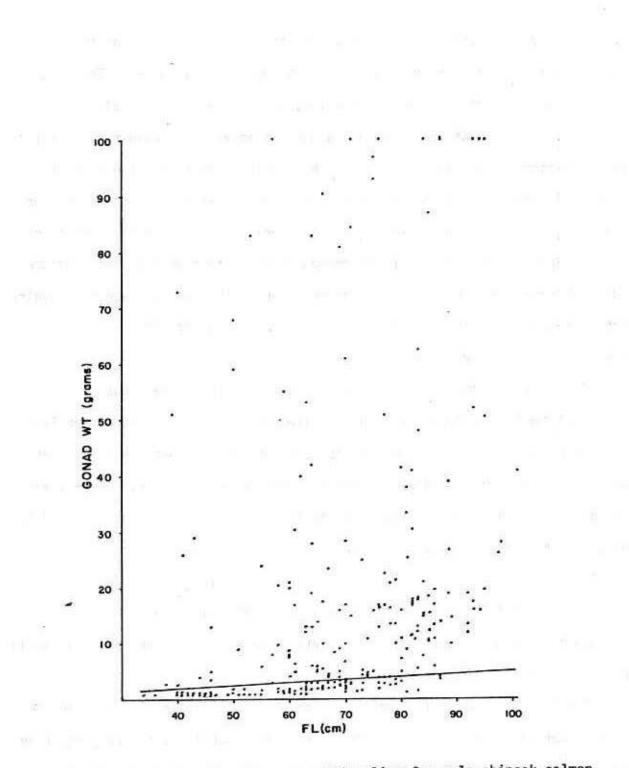
Once the location of immature gonads included in Figure 5 had been confirmed by histological means, a least-squares linear regression line was fitted to those data points as was done for female gonads, and an upper confidence band (P=.95) also determined. This regression line, however, was forced through zero (fish length = 0) because maturing male gonads can and do occur at small fish sizes. The equation for the male gonad separation line is:

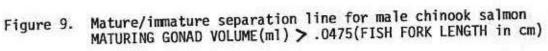
GONAD VOLUME in ml = .0475(FISH FORK LENGTH in cm)

and any male gonad volume/fish length data point falling above this line would be called maturing (Figure 9).

Finally, each maturity sample data point, from both the 1952-56 and the 1976-78 periods, was compared to the upper confidence limit boundary equation determined for each sex, and each fish sampled was designated as mature or immature.

-21-





-22-

RESULTS AND DISCUSSION

The 1976-1978 Maturity Sampling

A total of 676 fish were sampled during the 1976-1978 study of which 567 (285 males and 282 females) were usable for maturity determination. Histological samples were taken from 226 of these fish to verify stage of maturity. These samples were obtained over the entire south coast area from Coos Bay to Crescent City, Ca, and from May to October (Table 1).

While the focus of this report is maturity information, at-sea sampling also provided information on length frequency, species composition, and sex composition of whatever came aboard fishing vessels. This information provides a useful picture of salmon catch composition during those times off the south coast area. Listings of this information, for both chinook and coho, are presented in Appendix I.

Historical Sampling

A considerable number of chinook salmon maturity samples were collected by ODFW and FCO over the past 30+ years, but methodologies used to determine maturity were inconsistent. Even when a similar basic method such as the gonad-size fish-size relationship was used in more than one study, different criteria for separating between mature and immature fish were chosen by different investigators. Several investigators also did not measure gonad weights or volumes to the degree of accuracy necessary to identify maturation, for example, gonad volume sample measurements taken in 1962 were recorded to the nearest 5 ml which does not permit detection of maturity in male samples.

A limited amount of historical data could be evaluated using the present techniques. Sampling and measurement methods were found to be acceptable for 1176 gonad-volume to fish-size samples collected from off Astoria to Newport between March and October of the years 1952 through 1956. All data from these

-23-

	Length Month											
Increment		117. -	May	ay June		July				September		Combined
(TL)	.)	N	Percent	N	Percent	N	Percent	N	Percent	N	Percent	Percent
17 i	inches	8				1	0					0
18								1	100			100
19						3 3 4 9 11 5	59	1	100	1	100	60
20						3	66.6	1	0	4	50	50
21						2	50	5	60	4 2 5	100	66.
22						4	50	6	50	5	80	60
23						9	22.2	6	66.6			37.
20 21 22 23 24 25 26 27						11	18.2		60	4	50	40
25		1	100	2	50	5	20	10 12 12 13 8	50	1	0	42.
26		1 4	100 25	2 20	60	* 17	11.8	12	58.3	1	100	42.
27		14	71.4	24	37.5	58	32.8	13	38.5	1	100	40
28 29 30		11	72.7	22	54.6	58 43	37.2	8	100		- 1946	52.
29		1	100	12	66.7	20	55	7	57.1			60
30		7	85.7	18	66.7	20	85	3	100			79.
31		5	80	10	90	15	86.7	2	50			84.
31 32		4	100	10 8	87.5	15 25	92	1	100			92.
33		1	100	6	100	11	81.8	1	100			89.
34		2 2 1	100	4	100	3	100		10001005			100
35		2	100	5	80	9	100	2	100			94.
36		1	0	1	100	11 3 9 5 2	100					85.
37				1	100	2	100	1	100			100
38						1	100					100
39						1	100					100
40						1	100					100
41												
42		1	100									100

11

Table 1. Percent maturing chinook salmon by one-inch length increment by month of catch off southern Oregon (Coos Bay to Crescent City, CA) 1976-1978.

-24-

five years were combined and treated as one large sample. Although this data set represents north coast chinook only and may not be comparable to south coast or even the same stocks today, it does provide some comparison to present study data.

Methodology

The method used to identify maturing individuals developed during this study is felt to represent a substantial improvement over techniques used in previous studies. Some methodology problems were encountered with samples taken from male fish caught early in the 1976-1978 fishing seasons, but this was mostly associated with development of the histological technique.

The gonad-volume/fish-length method alone is felt to provide accurate identification of maturing female chinook. For males, the best method was a combination of (1) graphing gonad-volume/fish-length to identify rapidly growing maturing gonads, and (2) histological examination of gonad tissue to confirm maturation in small male fish. Both methods, however, require samples of at least 10 fish.

Although the histological technique used to verify maturation in small male fish proved to be useful and necessary, difficulty was encountered in classifying some male gonads at very stages of spermatogenesis, and some maturing males examined in this study may have been classified as immature. It is felt that this problem would be overcome by placing particular attention would be overcome by placing attention on uniform application of the histological preparation techniques, particularly staining procedures.

Percent Maturing by Length

Methods used to identify maturing male fish in previous studies probably resulted in mis-classification of many maturing fish as immature--particularly in the case of jacks. Although some mis-identification may also have occurred

-25-

among the 1976 to 1978 samples due to problems mentioned above, it is felt that most of the 567 gonads collected between 1976 and 1978 were accurately identified to state of maturity. The samples were collected over the entire salmon fishing season periods, and show a generally coherent picture of rates of maturity of chinook salmon caught in the sport and commercial fisheries between Coos Bay and Crescent City, CA.

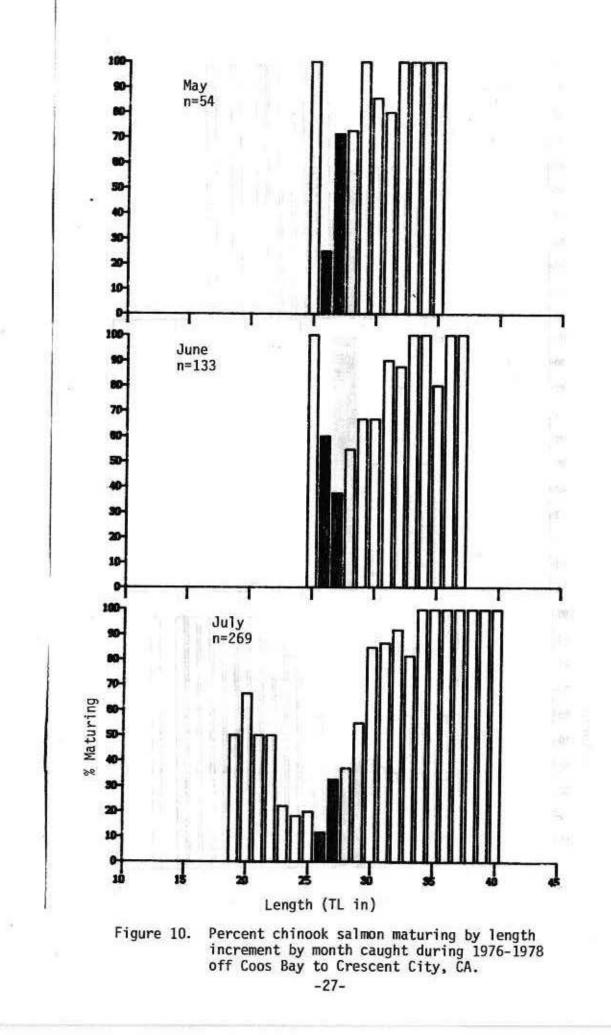
Subarea samples within this fairly large area were, unfortunately, too small to permit identification of any localized differences in maturity rates--as might be found in unique stocks fished at the mouths of specific rivers. However, no differences were apparent within the data collected.

Figure 10 shows the percent maturing within each one-inch length increment, by month, found in the 1976-1978 samples, and this data is also listed in Table 1. It is readily apparent that the present commercial minimum size limits (26 and 28 inches) very nearly bracket the least-mature fish of the broad size range being caught.

Table 2 shows that the percentage of chinook salmon maturing between the 26 inch north coast limit drops from just over one-half maturing in May to a low of just over one-quarter in July, and then increases again to almost one-half maturing in August and all maturing in September (although the 100% September value may be an artifact of small sample size). But in all cases except September the lowest percent maturing is between 26 and 28 inches.

This pattern is true generally for both sexes although they do exhibit different maturation patterns. Figure 11 shows that both in males, which can mature at all ages exposed to the ocean fishery, and in females, which mature only at older ages, the present commercial legal minimum size limits are very near the sizes with lowest percent maturing. But note that female fish did not enter the catches until at least 25 inches while shorter male fish were

-26-



1...

[

1...

1.5

1.1

14

1.1

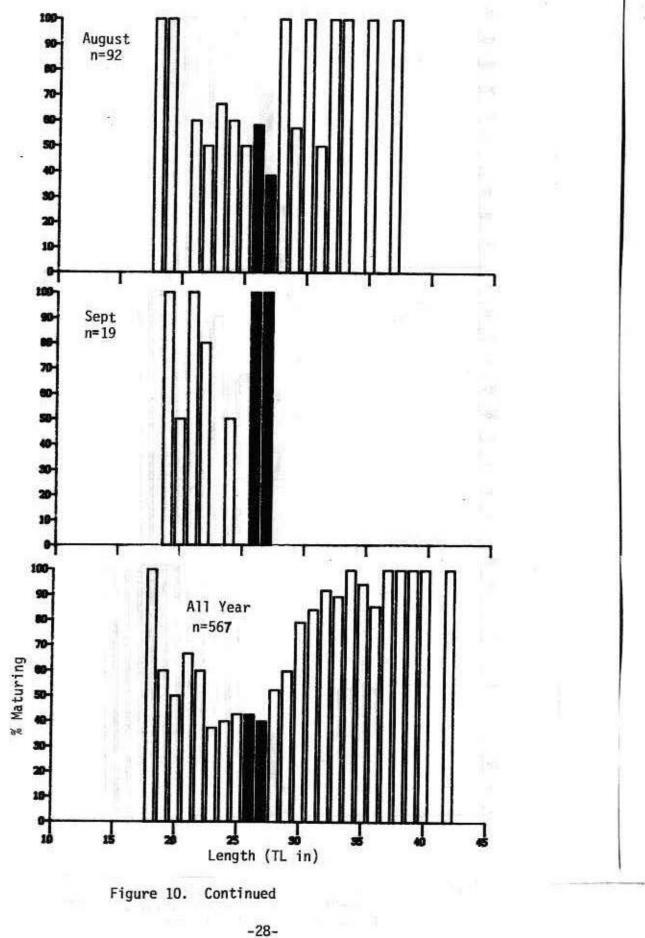
177

13

1.1

1

(D



not only caught but showed an increased percentage maturing in the smaller sizes. This suggests that most of the small chinook caught in the sport fishery below the commercial minimum size limit are males and are maturing as jacks.

	Length Increments							
Month	26 inches	27 inches	26 to 28					
May	25.0%	71.4%	61.1% (n=18)					
June	60.0%	37.5%	47.7% (n=44)					
July	11.8%	32.8%	28.0% (n=75)					
August	58.3%	38.5%	48.0% (n=25)					
September	100%	100%	100% (n=2)					
SEASON	42.6%	40.0%	40.9% (n=164)					

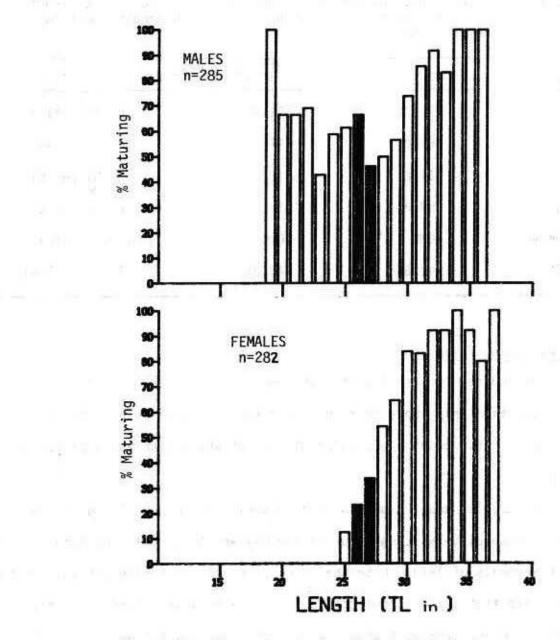
Table 2. Percent maturing chinook salmon in the 26 and 27 inch length increments by month of catch off southern Oregon (Coos Bay to Crescent city, CA) 1976-1978.

Sex and Age Composition

The sex and age composition of fish sampled in the smaller size categories, particularly those near the under the 26 and 28 inch commercial minimum size limits, provide a clearer picture of which fish are affected by those limits.

Figures 12, 13, and 14 show the size distribution of age-2, age-3, and age-4 fish, respectively, caught during the May and June, July and August, and September segments of 1978. Note that virtually all age-2 fish are smaller than 26 inches, and that present commercial minimum size limits impact primarily age-3, but also some age-4, fish. The present south coast sport limit (20 inches) allows retention of all ages of fish caught.

-29-



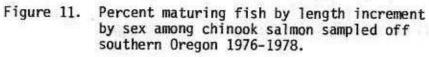
LI

U

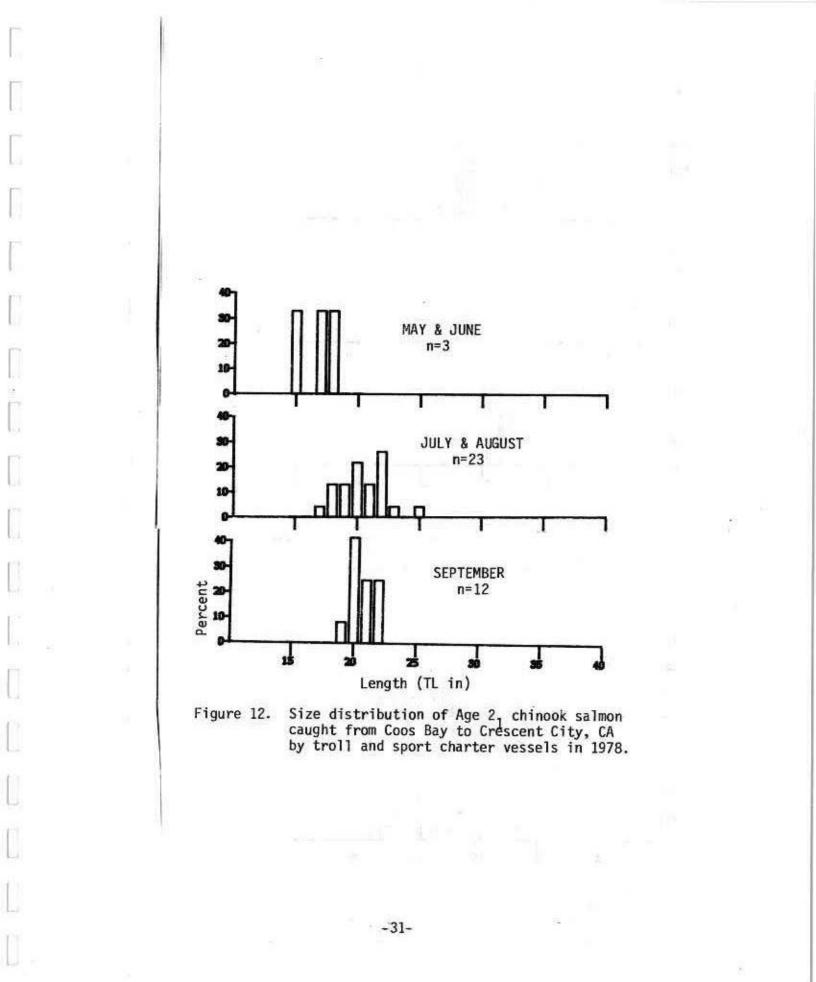
Į

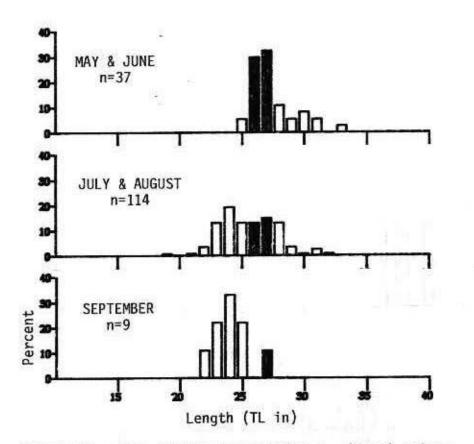
0

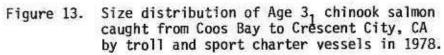
L

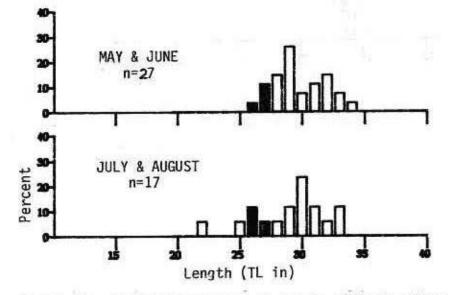


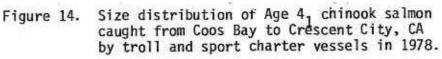
-30-











Small fish, those between 20 and 26 inches caught by the sport fishery are mostly Age-2, and are mostly jacks. Figure 15 shows that almost 80% of the age-2 fish caught are males, and Figure 16 shows that most about 80% of these males were maturing and would have returned as jacks.

Small female fish in the catch are mostly immature. Most fish just over the commercial minimum size limits are Age-3. Almost 40% of this age group are female, and only about 55% of these female chinook are maturing (Figures 15 and 16). Large female chinook in the catch are mostly maturing.

The implication of these data is that the south coast commercial troll fishery is catching significant numbers of immature chinook of both sexes in the 26 to 28 inch size bracket. In order to reduce the percent immature fish in the commercial catch to below 50%, the minimum size limit would have to be increased to 28 or even 29 inches. This assessment would also hold true for the sport fishery except that the harvest of jack chinook salmon, which are under 26 inches, by this fishery is beneficial.

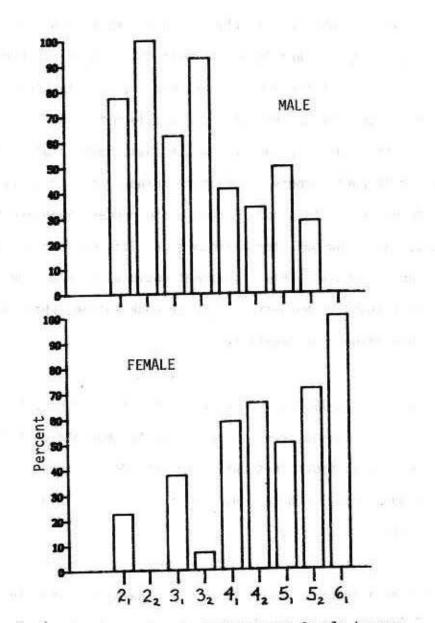
The 1952-1956 Data

Sample sizes from the 1952-1956 at-sea work, particularly during the important months of July and August, were large enough to generate useful maturity schedules. Figure 17 shows this data, and provides information closely comparable to the 1976-1978 data presented in Figure 10. This information is also listed in Table 3.

Note that the percent maturing among smaller-range fish is significantly lower in the 1952-1956 data (central and northern area sampling) than in the 1976-1978 data. The percentage of maturing fish 26-28 inch fish sampled from May to October was only 25.7% in 1952-1956 compared to 40.9% in 1976-1978.

These maturity rates generally agree with those reported in unpublished analyses of the 1952-1956 and other.past maturity sampling off Oregon. Some

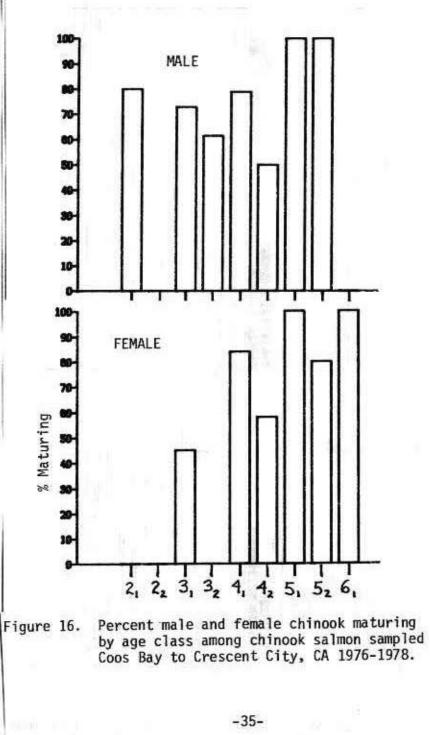
-33-





Percent male and percent female by age class among chinook salmon sampled Coos Bay to Crescent City, CA 1976-1978.

-34-



L I

1 L

1

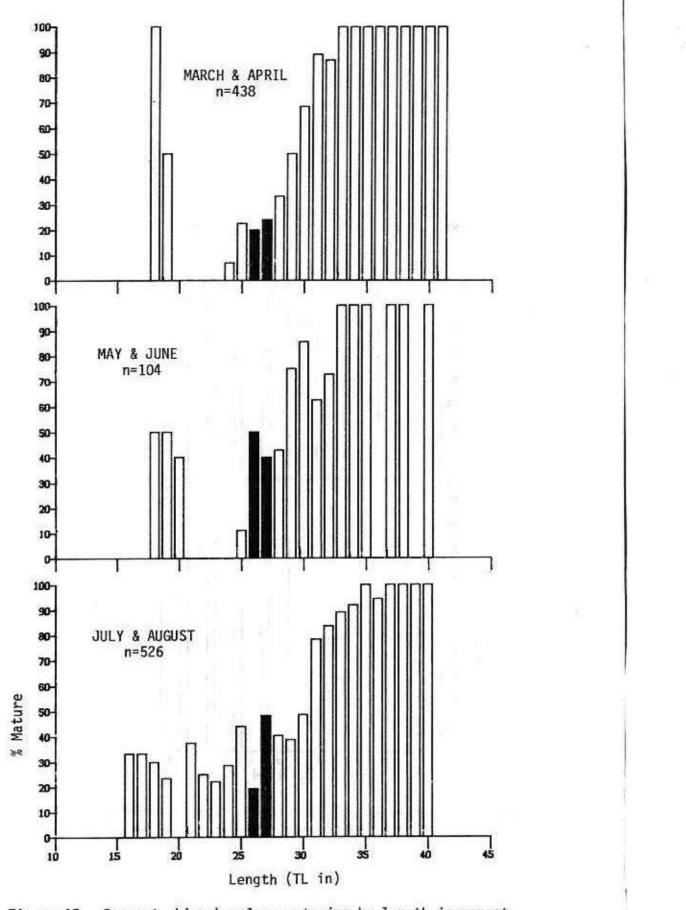
10

1 U.

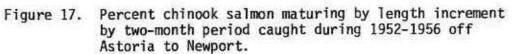
1.1

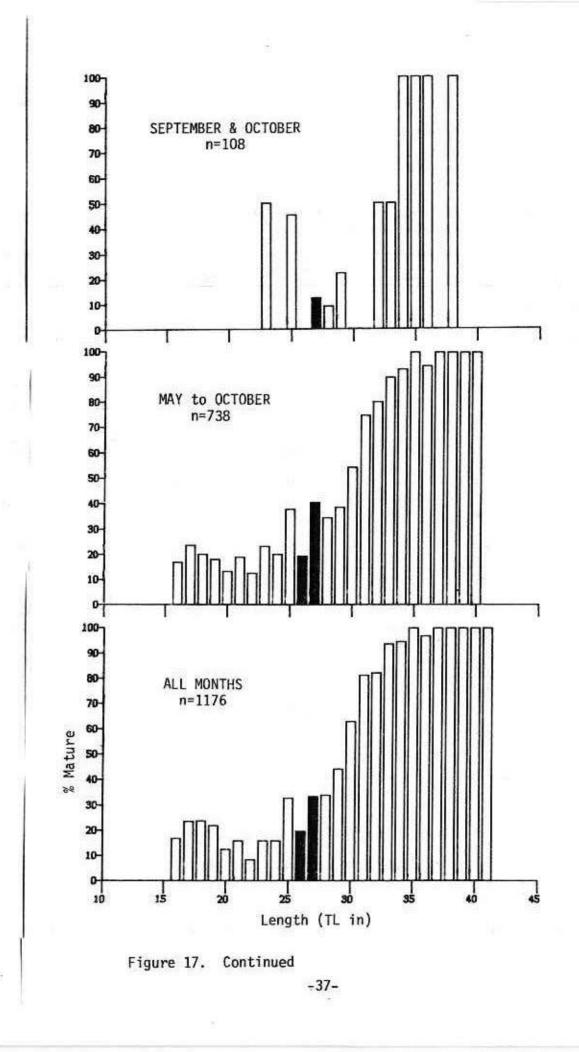
U

L



Li





L. L., ET. L. 1 ŧ. 1 L 6. ET. 14 1 C 1.1 L

[

۱.,

of the early data, however, appears suspect since past methods often under- or over-estimated the percent maturing--particularly whenever large numbers of Age-2 fish (including jacks) were present in samples.

Increment		Months Mar-Apr May-June July-Aug Sept-Oct May-Oct								
(TL)	Mar-Apr	May-Oct	Combined							
14 inches				0	0	0				
15	0		0	0	Ō	Ō				
16	112	0	33.3	0	16.7	16.7				
17		\$5.0) 	33.3	0	23.5	23.5				
18	100	50	30	0	20	23.8				
19	50	50	23.5	0	17.9	21.9				
20	0	40	0	0	13.3	12.5				
21	0	0	37.5	0	18.9	15.8				
22	0	0	25	0	12.5	8.3				
23	0	0	22.2	50	23.1	15.8				
24	7.1	0	28.6	0	20	15.9				
25	22.7	11.1	44	45.4	37.8	32.8				
26	20	50	19.4	0	19.2	19.6				
27	24.2	40	48.3	12.5	40.5	33.3				
28	33.3	42.9	40.5	9.1	34.5	33.9				
29	50	75	38.7	22.2	38.6	44.2				
30	68.6	85.7	48.7	100121214	54.3	62.9				
31	89.3	62.5	78.6		75	81.3				
32	87	72.7	83.7	50	80.4	82.3				
33	100	100	89.3	50	90.2	93.8				
34	100	100	92.1	100	93.3	94.7				
35	100	100	100	100	100	100				
36	100		94.4	100	94.7	96.9				
37	100	100	100		100	100				
38	100	100	100	100	100	100				
39 -	100		100		100	100				
40	100	100	100		100	100				
41	100					100				

Table 3. Percent maturing chinook salmon by one-inch length increment by month of catch off central and northern Oregon 1952-1956.

Differences between Figures 17 and 10 suggest maturation characteristics of chinook caught off northern versus southern Oregon are dissimilar--assuming the data has not been otherwise confounded over time. Since the size range of chinook caught off both areas are comparable, the overall age (size) compositions are also probably similar. This suggestion that a higher percentage of stocks caught off the south coast mature at younger ages than do those caught off northern Oregon may suggest that some management measures such as minimum size limits should differ between the two areas.

ACKNOWLEDGMENTS

Personnel of the Oregon Cooperative Fishery Unit at Oregon State University provided considerable advice and assistance on histological techniques used in this study, and prepared the histological mounts of gonad tissues. Mary Scherer of the unit, in particular, refined the staining and mounting techniques and prepared most of the gonad tissue mounts. Amy Stuart and Betsy Hunt of the Oregon Department of Fish and Wildlife drew or computer-generated graphics presented in the report.

-39-

REFERENCES

- Chestnut, Charles W. 1970. The pituitary gland of coho salmon (Oncorhynchus kisutch) and its function in gonad maturation and thyroid activity. PhD Thesis, Simon Fraser University, Burnaby, B.C. 130 p.
- Cleaver, Frederick C. 1969. Effects of ocean fishing on 1961-brood fall chinook salmon from Columbia River hatcheries. Fish Comm. Ore., Res. Repts. 1(1) 76 p.
- Fiscus, Hugh. 1965. Biological observation on the 1964 Columbia River (ocean) sport salmon catch. Olympia, Wash. Dept. Fish., 13 p. (processed).
 - . 1966. The 1965 Columbia River (ocean) sport fishery. Part II. Biological observation. Olympia, Wash. Dept. Fish., 13 p. (processed).

. 1969. Biological observations on the sport salmon catch at the mouth of the Columbia River in 1966 and 1967. Olympia, Wash. Dept. Fish., 35 p. (processed).

- Fraser, C. McLean. 1917. On scales of the spring salmon. Contrib. Can. Biol. studies from Biol. Sta. Can., 1915-1916:21-32.
- Godfrey, Harold. 1961. Method used to distinguish between immature and maturing sockeye and chum salmon taken by Canadian exploratory fishing vessels in the Gulf of Alaska. Int. N. Pac. Fish. Comm., Bull. 5:17-26.
- Haw, Frank, Henry O. Wendler, and Gene Deschamps. 1967. Development of Washington state salmon sport fishery through 1964. Wash. Dept. Fish., Res. Bull. 7, 192 p.
- Henry, Kenneth A. 1971. Estimates of maturation and ocean mortality for Columbia River hatchery fall chinook salmon and the effect of no ocean fishing on yield. Fish Comm. Ore., Res. Repts. 3:13-27.
- Informal Committee on Chinook and Coho. 1969. Reports by the U.S. and Canada on the status, ocean migration and exploitation of northeast Pacific stocks of chinook and coho salmon, to 1964. Vol. 1, Report by the United States Section. 107 p.
- Ishida, T., and K. Miyaguchi. 1958. On the maturity of Pacific salmon (Oncorhynchus nerka, O. keta, and O. gorbuscha) in offshore waters with reference to the seasonal variation in gonad weight. Bull. Hokkaido Reg. Fish. Res. Lab., 18:11-22.
- Lesh, Ernest W. 1978. Maturity and size of chinook salmon (Oncorhynchus tschawytscha) caught off northern California during 1977. Ms. Ocean Salmon Proj., Anad. Fish. Branch, Calif. Dept. Fish and Game. 23 p.
- Parker, Robert R., and Walter Kirkness. 1956. King salmon and the ocean troll fishery of Southeastern Alaska. Alaska Dept. Fish., Res. Rept. 1, 64 p.

- Rich, Willis H. 1925. Growth and degree of maturity of chinook salmon in the ocean. Bull. Bur. Fish. 41:15-90.
- Ridgway, G.J., G.W. Klontz, and C. Matsumoto. 1962. Intraspecific differences in serum antigens of red salmon demonstrated by immunochemical methods. Int. N. Pac. Fish. Comm., Bull. 8:1-14.
- Robertson, O.H. 1958. Accelerated development of testis after unilateral gonadectomy, with observations on normal testis of rainbow trout. U.S. Fish & Wildl. Serv., Fish. Bull. 127:9-30.
- Utter, Fred M., George J. Ridgway, and Harold O. Hodgins. 1964. Use of plant extracts in serological studies of fish. U.S. Fish & Wildl. Serv., Spec. Sci. Rept. Fish. No. 472. 18 p.
- Utter, Fred M., and George J. Ridgway. 1965. A serologically detected serum factor associated with maturity in english sole, Parophrys vetulus, and Pacific halibut, Hippoglossus stenolepis. U.S. Fish & Wildl. Serv., Fish. Bull. 66(1):47-58.
 - . 1967. Cross-reactive properties of antisera prepared in rabbits by stimulations with teleost vitellins. U.S. Fish & Wildl. Serv., Fish. Bull. 66(2):203-207.
- VanHyning, Jack M. 1951. The ocean salmon troll fishery off Oregon. Pac. Mar. Fish. Comm., Bull. 2:43-76.

. 1973. Factors affecting the abundance of fall chinook salmon in the Columbia River. Fish Comm. Ore., Res. Repts. 4(1) 87 p.

Wright, Sam, and John Bernhardt. 1972. Maturity rates of ocean-caught chinook salmon. Pac. Mar. fish. Comm., Bull. 8:49-59.

Yasutake, William T., and Joseph H. Wales. 1983. Microscopic anatomy of salmonids: An atlas. U.s. Fish & Wildl. Serv., Res. Publ. 150, 190 p. Ū.

Ľ

[

[]

[]

[]

0

Q

Catch Area	Date	Length (FLam)		Catch Area	Date	Length (FLom)	
Off Brookings	2 Aug 1976	64.0	F	Off Brookings	23 Aug 1976	66.0	F
Crescent City to	4 Aug 1976	57.5	F	45		61.0	M
Brookings		66.0	M			63.0	F
Off Brookings	4 Aug 1976	61.0	F		24 Aug 1976	76.0	MFFFF
	5 Aug 1976	68.0	F		a. , kig 13/0	64.5	E
		65.0	Μ			64.0	F
	11 Aug 1976	64.5	М			04.0	
		62.0	F	Crescent City to	20 May 1978	46.5	
		68.0	F	Brookings	24 May 1978	49.5	
		66.5	M F	of contings	21149 15/0	50.5	
		66.0	F			56.0	E
		70.5	F			52.5	F
		66.0	M				F
		62.5	M		25 May 1978	57.5	F
		68.0	M		23 may 1970	<52	
		69.0	F			<52	
		60.0	F			<52	
		61.5	M			<52	
		62.0	M			49.5	
		65.0	F			52.0	-
		66.0	F		27 Mar. 1070	56.0	F
		71.5	F		27 May 1978	<52	
		63.5	F			<52	
		64.5	M			<52	
		65.0	M			<52	
		67.0	M F			<52	
		68.5	F			51.3	
		66.0	F			59.5	M
	12 Aug 1976	60.0	M			54.9	м
	12 109 15/0	73.5	F			54.3	F
		65.0	F			53.4	F
		68.0	M			58.6	M
		65.0				52.7	F
	13 Aug 1976	62.5	M			58.5	F
	15 Aug 1970	65.0	M			55.3	F
rescent City to	23 Aug 1976	58.0	M			56.0	F
Brookings	CJ MUY 15/0		F F F F		3 June 1978	<52	
Di Con nigs		63.5 71.0	2			<52	
			F			<52	
f Brookings	23 Aug 1976	67.0	5			<52	
, prookings	CJ HUY 19/0	60.0	5			50.5	
		60.0	M			50.7	
		55.5	F			49.3	
		66.5	M			50.5	
		61.0	F			56.5	F
		61.5	M			59.8	F
		66.5	M			54.0	M

COHO SALMON

COHO	SALMON

Catch		Length		Catch	Dete	Length	5
Area	Date	(FLan)	Sex	Area	Date	(FLam)	263
Crescent City to	3 June 1978	53.6	М	Crescent City to	10 June 1978	59.4	F
Brookings		52.2	F	Brookings		57.5	F F F M F
bi oon mgs		55.0	F			55.2	F
		56.1	F			59.0	M
		64.3	M			53.6	F
		56.5	F			54.7	M
		53.8	F			53.0	M
		59.0	F			57.3	F
		56.5	F	Off Cape Arago	16 June 1978	58.3	M
		58.3	F	orr cape in ago	10 0010 15/0	56.3	MFFFFF
	4 June 1978	<52	.42			57.6	F
	4 June 1970	<52				59.2	F
		<52				58.5	F
		<52				59.6	F
		<52				65.8	
				-		59.7	F
		50.3	r.			57.5	
		56.2	F			58.9	M F F M
		53.1	M			59.3	F
		55.9	M				F
		57.0	M	000 0 111 01	10 1 1070	57.3	M
		55.4	F	Off Coquille River	16 June 1978	62.5	M
	1.	56.9	F			61.3	M
	6 June 1978	<52			17 1 1070	49.6	М
		<52		Off Cape Arago	17 June 1978	<52	
		48.4				35.1	
		43.6				59.3	M
		55.4	F F F			58.5	M
		56.5	F		1 1 1070	55.1	F
		57.5	F	Off Coquille River	17 June 1978	53.3	M
		55.9	F	Just N. Cape Blanco	17 June 1978	<52	-
		56.5	F M			54.7	F
		60.7	М			55.4	F
		54.9	F F M			52.8	F
		54.4	F			47.5	M
		54.2	F	A REAL PROPERTY AND A REAL PROPERTY AND A		46.9	Μ
		54.8	М	Just S. Cape Blanco	17 June 1978	60.0	М
		54.3	M	- i Him	18 June 1978	62.4	F
	7 June 1978	53.1	M F			62.2	M
		57.9	M			55.1	M
		55.6	M			59.6	F
		56.0	M			54.2	WFMMFMF
		55.1	F			58.4	F
	10 June 1978	<52				56.5	М
	8 10 10 18 18 19 19	<52				58.5	М
		<52				57.9	M F F
		59.8	F	1		55.0	F

-43-

COHO SALMON

L

1.1

1.4

-1

ind.

1

Catch Area	Date	Length (FLom)	Sex	Catch Area	Date	Length (FLcm)	Sex
Just S. Cape Blanco	18 June 1978	49.9	М	Off Coos Bay	20 June 1978	64.0	F
		59.4	M			61.8	м
		49.6	M			53.7	Μ
		59.3	F			67.5	M
		53.0	F F M	8		64.0	F
		58.8	Μ			57.4	F
		>52	F			59.7	F
		47.8	Μ			63.5	WFFFM
		60.5	M			59.7	М
		58.9	M			59.0	F
		60.0	F			56.5	M F F F
		57.9	F			59.5	F
	19 June 1978	61.3	Μ		21 June 1978	60.3	F
		60.0	Μ			61.8	M
		63.8	F			54.0	F
Off Coos Bay	20 June 1978	60.5	M			57.5	F
1990)		60.4	Μ	2010 - P		55.4	M F F F M F
		66.1	F	Off Cape Arago	21 June 1978	47.0	M
		58.8	F F	and solve to elle		64.0	F
		53.5	F			51.9	M
		61.1	F			55.3	M
		60.6	M			58.5	F
		59.5	F			63.9	M
		59.2	M			57.5	M F
		55.5	F			58.4	M
		55.3	F F F			60.5	M F F M
		57.2	F			59.5	F
		57.3	М			60.5	M
		67.0	Μ	3.8	25 June 1978	59.4	F
		51.5	F			58.4	M
		59.5	M			57.1	F
		54.4	M			60.1	M
		60.0	F			60.4	F
		52.6	F			58.5	F F
		59.4	F M F			62.9	M
		57.3	F			54.0	M
		46.0	M			55.6	M
		53.0	M			54.5	М
		60.9	F			52.8	M
		59.6	M F F M			59.5	F
		55.7	M			62.0	F
		61.0	M			61.6	M F F F F F F
		53.0	M			53.5	F
		55.3	M			59.0	F
		52.9	M F			57.1	F
		59.3	M			59.5	F
		0010	22 U		1.8	00.0	103

COHO SALMON

Catch Area	Date	Length (FLam)	Sex	Catch Area	Date	Length (FLam)	Sex
OFF Cases Aware	25 June 1978	52.0	м				
Off Cape Arago	25 Julie 1970	60.1		(J)			
		55.0	F				
		59.3	F				
		58.7	F				
		55.8	M F F F F				
		52.4	M				
		53.7	F				
		61.6	M				
		57.4	F				
		64.0	M				
		48.7	M				
		62.5	M				
		54.0	M				
		58.7	M				
		62.5	F				
		59.9	F	8			
		57.0	F				
		59.6	M	0			
		45.4	M				
		56.7	M				
	26 June 1978	62.2	M				
		52.6	м				
		46.0	Μ				
		58.4	Μ	6			
		53.0	F				
		59.0	F F	1			
		66.8	м				
Off Coos Bay	28 June 1978	56.6	м				
orr coost may	2000-2000-0000-0020-000-000-000-000-000	58.0	Μ				
		56.5	М				
		57.5	M				
		57.6	Μ				
		55.2	М	1			
		59.7	F	1			
		58.7	F				
		54.7	F				
		62.0	м				
		48.9	м	1			

Note: <52 are shakers under 22 in (TL) min size limit, others under 52 on tagged and released.

1976 Coho Sex Ratio - All adults so implies 32% of male coho matured as jacks. 21 male

31 female

1978 Coho Sex Ratio - Suggests at least 12% of male coho matured as jacks (probably significantly 104 male more because some shakers were themselves jacks).

117 female

CHINOOK	SALMON

Catch Area	Date	Length (FLom)	Sov	Aco	Catch Area	Date	Length	Ser	har
A CO	Date	(r.c.ii)	JEX	nge	Area	Date	(FLan)	Sex	Ag
Off Coos Bay	29 June 1976	61.5	F	31	Off Coos Bay	2 Aug 1976	60.5	м	3
	2 July 1976	77.0	M	31 41			61.5	M	3333
		85.5	M	31			62.5	F	3
		78.5	F	31 31	Crescent City				
		96.0	M	41	to Brookings	5 Aug 1976	83.5	M	4
		80.0	F	42	us brookings	5 / Ly 15/ 5	68.0	F	3
		77.5	M	42 Unk.			64.0	F	3
Crescent City			1.255		Off Coos Bay	8 Aug 1976	70.0	F	3
to Brookings	9 July 1976	65.0	М	31	011 0005 Day	0.09 25/0	70.0	M	4] 3] 3] 3] 3] 4]
and a state of the state of the		61.0	M	31		9 Aug 1976	60.5	-	
		77.0	F	41	Crescent City	5 109 15/0	00.0		
		81.0	M	41	to Brookings	12 Aug 1976	71.5	F	31
	11 July 1976	79.0	M	41	Off Coos Bay	18 Aug 1976	69.5	M	31 31 31 41
	11 0019 1010	67.0	F	31	on was buy	10 /10 10/0	64.0	F	31
		67.0	F	31		19 Aug 1976	79.5	F	1
		63.0	F	31 31	Crescent City	15 Aug 15/0	13.5		41
		72.0		31	to Brookings	23 Aug 1976	65.0	E.	2.
		75.0	F	31	w brookings	23 Huy 1970	66.5	F F	21
		69.5	Ē	41			76.0	F	21
		75.0	Ē	21		24 Aug 1076		F	41
		76.5	F	21		24 Aug 1976	68.5	M	31
		76.0	MFFFFFMFFFF	๚๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛๛	OFF Coor Dave	OC Aug 1070	63.5	F	31
			F	31	Off Coos Bay	26 Aug 1976	68.0	M	31314131313141
		69.0	Pi F	32			88.0	F	41
	16 July 1976	64.5	F	42	OCC Case Day	17 1077	CT 0		-
	10 July 19/0	69.0	r	42	Off Coos Bay	17 May 1977	65.0	М	31
		72.0	F	31			73.0	M	41
		72.0		31			70.5	М	41
		70.0	M	31			81.5	M	41
		65.0	M	31			66.0	F	31
		73.5	r.	31			86.0	F	41
	10 1.1 1070	66.0	F F F	31			71.5	F	41 41 41 31 41 31 41 31 41 31 41
	19 July 1976	68.0	t.	31			82.0	Μ	41
		65.0	F	31		18 May 1977	65.5	F	41
		70.0	F F F F	31	Crescent City	000000000000000000000000000000000000000	12223	33	1
	00 1 1 1070	77.0	F.	41	to Brookings	22 May 1977	68.0	F	40
	20 July 1976	72.0	F	41 31 41 31 31 31			74.0	F	31
off Coos Bay	20 July 1976	84.5	F	41	Off Coos Bay	23 May 1977	64.0	F	42
29 - M		66.5	F	31			67.5	М	31
	24 July 1976	63.5	М	31			101.5	F	61
rescent City				1000		24 May 1977	64.5	FFFMFFF	31
to Brookings	31 July 1976	92.0	М	51			84.5	F	4031423161314131
		64.0	F	42			64.0	Μ	31
	a second day	68.5	M F F	41	Crescent City				5
	1 Aug 1976	84.5	F	41	to Brookings	25 May 1977	76.5	F	41
		62.5	F	51 42 41 31 31 31			67.5	F F F	41 31 31 31 31
	2 Aug 1976	60.5	F	31		30 May 1977	66.0		31
		62.5	M	31			62.5	M	31

-46-

CHINOOK	SALMON
CUTINOUV	JUTIN

Catch	Dete	Length	Court	8	Catch	Dete	Length	C	
Area	Date	(FLcm)	Sex	Age	Area	Date	(FLan)	Sex	Pige
Crescent City					Off Coos Bay	17 June 1977	71.0	м	3
to Brookings	30 May 1977	72.5	M	31 41	Contrast score of		79.5 79.0	F	4j 4j
	31 May 1977	70.5	F	31	Just North of				
	2 June 1977	73.5 63.5	F	31 31	Brookings	17 June 1977	72.0 78.0	M	343443433344133441344133445434134425533314423
		68.5 66.5	M	31 31 31 31 31 31 31 31 31 50			66.0 75.0	F M	3
	3 June 1977	75.5	M	31			63.5	F	4
		72.0	M F	31			67.5 71.0	M	3
		74.5	F	41			65.0	F	3
		71.0 63.0	F	42 31	· · ·		73.5 65.0	F M	3
		76.0 68.0	М	42 31 41 31	2 A 4		71.0 63.0	M F	4
Off Coos Bay	3 June 1977	73.5	F			565. V.N. 107.51 (1925)	63.5	M	3
		67.5 62.0	F M	- 31 31	Off Coos Bay	18 June 1977	84.0 86.5	M	4
		67.0	F	31			66.0	M	3
		69.0 62.0	F	$31 \\ 31$	1		82.5 70.5	F M	4
Crescent City	7 June 1977	83.5	F	41	Off Brookings	19 June 1977	71.0 66.0	M	3
to Brookings	7 June 1977	71.0	F	41 31			66.0	F	4
	10 June 1977	63.5 68.0	F	31 42			82.5 77.0	M F	52
		66.0	F	31		20 June 1977	65.0	F	3
		71.0 66.0	F F	42313131313131			65.0 66.0	M	41
		65.0	F	31			68.0 68.0	F	4
		65.0 67.5	F	31			68.0	M	5
		70.0 72.5	F	42	Off Coos Bay	21 June 1977	68.5 63.5	FM	52
		65.0	F	41 31 31	on allos day		63.5	M F	3]
		63.5 78.0	M F	31 42		24 June 1977	64.5 83.5	F	3] 41
		67.5	FMFFMFM	- 2.			72.0 62.5	M	42
	11 June 1977	75.5 74.5	F	31 41	Crescent City				
	14 June 1977	67.0 62.5	M	31	to Brookings	1 July 1977	83.0 76.0	M	41
	14 UURC 13/7	69.0	M	42 - 314131314241			81.0	м	4] 4] 4] 4] 4] 4] 4] 4] 4] 4]
Off Coos Bay	17 June 1977	74.5 81.5	M	41			80.0 82.0	M	31
on was buy		73.5	F	42			79.0	M	41

-47-

1

CHINOOK	CALIMAN
CUTINOOV.	JUN

Catch Area	Date	Length (FLcm)	Sex	Age	Catch Area	Date	Length (FLcm)	Sex	Age
Crescent City					Off Brookings	11 July 1977	67.0	F	31
to Brookings	1 July 1977	77.0	F	31	orr brookings	in only is??	75.5	M	41
w brookings	1 outj 15/7	94.0	F	41			73.5	м	3
		76.0	F	31			71.5	F	3
	2 July 1977	74.5	F F	11			75.0	F	1
	2 JULY 19/7	75.5	M	41			64.0	M	7
		80.5	M M	51		17 1.1. 1077	85.0	E	2
		74.0	F	31 41 31 41 31 41 31 41 41		17 July 1977	73.0	M	*] 2.
	2 1.1., 1077		M	41			75.0	M F M F	2
	3 July 1977	71.0		- A-				M	2
		79.0	M	41			70.0	M	2]
		80.0	M	41	2		72.0	ra F	3]
		79.0	M	41			78.0	F M	41
		71.0	F	31			65.0		32
	4 1.1. 1077	83.0	F	41			67.5	M	2]
	4 July 1977	81.5	M	41			66.0	5	3]
	5 July 1977	73.5	F F	41			69.0	5	2]
		87.5	r.	41			76.0	p.	51
	C 1 1 1077	77.5	F	41			65.5	5	5]
	6 July 1977	71.0	М	41			64.0	5	21
	0.1.1.1077	65.5	M	31		10 1 1 1077	77.0	M	51
Off Brookings	8 July 1977	72.0	F	31		18 July 1977	66.0	WFFFFFMFFFF	21
		69.0	М	31			62.0	5	2]
		65.5	M	31			63.5	F	31
		63.0	F F	31			62.5	Ē	31
		83.0	1	41			82.5	r	41
		66.5	F	31			68.5		5]
	0 1 1 1077	64.5	F	31			80.0	M	21
	9 July 1977	63.0	М	31			63.0	M	31
		63.5	M	31			83.0	M	21
		65.0	F	31			73.0	M	21
		67.5	М	31			66.0	M F	21
		75.5	M	31			79.5	E.	21
		68.0	M	21			69.0	M	21
		64.5	F.	31			66.5	M	21
		64.0	M	21			63.5	m	21
		68.5	Ē.	21		10 1.1. 1077	65.5	F	21
		63.0	5	31	5	19 July 1977	63.5	M F M	21
		75.5	M	41	12		71.0		21
		67.5	E.	31			89.0	Ē	41
		71.0	FMFFMFFMFFM	᠂ᡧᠯᠯᠯᠭᠯᠯᠯᠯᠯᠯᠯᠯᠯᠯᡘ᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉᠉			65.5	M F F	31
		68.5	M	21			68.5	F	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
	10 1 1 1077	64.0	+	31	0		72.0	r	31
	10 July 1977	75.5	t	41	Crescent City	00 1 1 1077	02.0		4
	11 July 1977	68.0	M	31	to Brookings	20 July 1977	82.0	M	41
		67.5	M F	31			67.5	F	31
		65.0	F	31			66.0	F	41 31 31 41
		65.5	F	31			86.00	Μ	41

CHINOOK	SAL	MON
	-	

Catch Area	Date	Length (FLan)	Sex	Age	Catch Area	Date	Length (FLan)	Sex	Age
					a				
Crescent City				2	Crescent City	05 1 3 1077	70.0	-	
to Brookings	20 July 1977	87.5	F	51	to Brookings	25 July 1977	78.0	F	4
		66.0	М	31			64.0	Μ	3
		63.5	М	41			85.5	F	52
		65.0	M	41	624-cm 84		83.0	F	-
		66.0	M	31	Off Rogue River	26 July 1977	63.5	М	3
		67.0	F	41	1000 100000 00-1		82.0	F M	4
		66.5	F	31			76.0	M	4
		67.5	M	31			64.0	F	3
		63.0	F	31			72.5	M	3
		64.0	F	31			76.5	М	31
		64.0	M	31			68.5	M	3
		77.5	M	41		27 July 1977	85.5	F	4
		81.5	F	41		27 0019 2377	68.5	M	3
	21 July 1977	71.0	M	41			68.5	м	1
	21 Outy 19/7	65.0	M	71			71.0	F	2
		78.0		21	1		68.0	F	1
			M	41			67.5	M	2
		71.0	M	5131414131413131313131414141314142523131			70 5	E I	5
		84.0	F	52			79.5	F	4
		65.5	M	31			77.5	F	4
		63.5	M	31			77.5	M	4]
		65.0	F	42			67.5	F	3]
		63.0	F	31			88.0	Μ	5]
		65.5	F	31			96.0	M F F	4]
		63.0	F	31			83.5	F	41
		64.0	Μ	42			77.0	F	3
		65.5	F	31			67.5	М	31
		63.5	M	31			84.0	F	4
		65.0	F	31			80.0	M F	41
	22 July 1977	77.5	F	41		28 July 1977	68.0	F	3
		64.0	F	31			66.5	F	3
		63.5	M	31			83.0	M	41
		65.0 72.5	F	31			85.5	F	4
		72.5	M	42			67.5	F F F M	4
		63.5	M F	31			71.0	F	31
		66.0	M	31			84.0	M	31
		66.0	M	31	1 1 5		81.5	F	31
		63.5	F	42			83.0	M	41
		65.0	F	31			71.0	M	31
		65.0 66.0 67.5 63.5	M F F M M F F M F F	፞ ጞ፝፝፝፝፝፝፝ኯ፝ጞጞጞጞጞ፟፟፟፟፟ጞጞጞጞጞጞጞጞጞጞጞጞጞ፟፟፟ጞጞ፟፟፟፟			65.5	M	4 3 5 - 3 4 4 3 3 3 4 3 4 3 4 3 4 4 4 3 5 4 4 3 5 4 4 3 3 4 4 4 3 5 4 4 3 3 4 4 4 3 5 4 4 3 3 4 4 4 3 5 5 4 4 3 5 4 4 3 5 4 4 3 5 4 4 3 5 4 4 3 5 5 4 4 5 5 5 4 4 5 5 5 4 4 5 5 5 7 5 4 4 5 5 5 7 4 4 5 5 5 7 5 7
		67.5	M	41			71.0	M	31
		63.5	F	31			63.5	м	3
		76.0	F	40			68.0		3
		62.0	M	22			63.5	F	2
	25 3.1. 1077	62.0	E	41	5		63.0	F	2
	25 July 1977	63.0 63.0 75.5	F	41			75.5	M F F F F	2
		13.5	F	41			06 E	Ē	1
		68.5	F	41			86.5	1. C	4

CHINOOK SALMON

Catch Area	Date	Length (FLcm)	Sex	Age	Catch Area	Date	Length (FLom)	Sex	Age
Off Roque River	28 July 1977	73.0	м	31	Crescent City				
on hogue kiver	20 Outy 1977	/5.0		-1	to Brookings	7 June 1978	61.7	F	31
Crescent City					to thookings		77.4	F	31 41 31 41 41 42 41 52 R
to Brookings	20 May 1978	72.5	м	4.			64.3	F	31
w brookings	20149 15/0	65.0	M	31			62.6	E	31
		65.0	M	41 31 31 31 31		10 June 1978	70.5	M	4
		63.0	F	31		10 0010 10,0	69.2	F	4
		66.0	F	31			61.6	M	4
		62.5	M	-1	Off Cape Arago	16 June 1978	79.9	M	4
		64.0	M	- 31 42 42 31 42	orr cape hrago	10 0010 15/0	81.0	F	5
		65.0	E	An			80.1	F	R
		75.5	M F F F	42	Just South of		00.1	50	8
		60.5	÷	22	Cape Blanco	17 June 1978	76.8	M	4]
			M	51	Cape Diano	1/ 0016 15/0	61.7	M	A
	24 Mar. 1070	68.0	E E				62.7	M	1
	24 May 1978	64.0	E.	-			72.1	M	42 42 41 41 41
		64.5	E.	31			70.3	F	4
		64.0	F F F	31		18 June 1978	69.4	M	
		67.5	m	51		10 Julie 19/0	77.0	E	7.
		78.5	F	31				F	4
		75.0	Μ	51	and the second		80.3	F F	4
		72.0	М	31			62.6	F	42
		69.9	М	31	1.53		62.3	M	3]
	25 May 1978	77.5	F F F F F	41			61.0	M	- 414142333333333333541333413341
		74.5	F	41	2 C		61.7	F	3]
	27 May 1978	65.5	F	41	1		60.6	M	3]
		65.6	F	41			72.8	F	2
		64.9	F	31			65.4	M	3]
		62.1	Μ	31	1		76.7	F	52
		72.0	F F M	41			64.4	F	41
		65.9	F	41			62.6	F	3]
		66.9	М	41		18.7	68.1	M	3]
		75.6	F	51		19 June 1978	74.0	F	41
	3 June 1978	68.2	М	41	Bulling and Barry		61.9	М	3]
		68.9	F	41	Off Coos Bay	21 June 1978	69.0	M	41
	4 June 1978	61.9 61.5	F	31	20 20 CZ 88	22 June 1978	59.4	М	-
	6 June 1978	61.5	М	41		23 June 1978	51.0	F	
		78.4	F	41		25 June 1978	72.1	М	31
		65.7	F	31			64.9	М	31
		65.2	F	42			73.5	М	41
		66.1	FFMFFFFF	41			42.5	М	2]
		71.0	F	42			64.8	М	- 3]3]4]2]3]3]2]423]2]
		63.6	F	31			71.3	F	3
	7 June 1978	67.8	M	31			36.6	M F	2
		63.2	M	41			72.0	F	4
		61.7	M	31313131313141414131314141514141314141314241423131413131	6		74.5	F	3
		63.5	M	31		30 June 1978	40.3	м	21

-50-

CHINOOK SALMON

Catch Area	Date	Length (FLam)	Sex	Age	Catch Area	Date	Length (FLam)	Sex	Age
			522				Non-Sec.	1.65	÷.
Off Coos Bay	1 July 1978	56.9	M	31	Off Coos Bay	16 July 1978	59.6	F F	3
		59.2	M	31			56.9	F	-
	4 1 1 1070	47.0	F	21 31			62.4	F F F	3
	4 July 1978	57.4	F	31		21 July 1978	66.6	F	4
	0 1 1 1070	62.5	M	41			75.9		3
	8 July 1978	55.3	F	31			75.6	Μ	4]
		70.4	F	41 31			67.8	Μ	3]
		56.9	М	31			75.0	м	3]
	11 1 1 1070	47.4	M	R			66.7	F F F	31
	11 July 1978	40.2	M	-			63.4	F	3]
	12 July 1978	67.0	M	31		22 July 1978	62.2	F	31
		66.4	F	31			65.0	М	31
		62.8	M	31 31 31 31			69.0	М	41
	13 July 1978	60.3	F	31			78.8	F	41
		71.9	F M	41		and the second	72.5	F	41
	14 July 1978	57.3	M	31		23 July 1978	58.3	F F F	31
		47.6	Μ	4323333322333333223		10000000000000000000000000000000000000	61.7	F	31
		55.4	Μ	31		27 July 1978	55.9	M F F	31
		52.5	M	31			58.2	F	31
		50.8	М	31			45.9	F	31
		55.0	M	31			42.8	Μ	21
		49.2	М	21		Station English	53.9	Μ	31
		54.9	М	31		28 July 1978	66.4	Μ	31
	15 July 1978	64.7	м	31			66.1	M	31
		58.1	F	31			55.5	Μ	31
		58.7	М	31		29 July 1978	55.0	F	31
		30.8	М	22			53.5	F F M	31
		56.7	M	31			53.7	M	31
		54.0	F F			30 July 1978	57.0	-	32
		65.0	F	41			55.2	Μ	31
		53.6	M M	41			56.0	M	31
		52.6	М	31			55.8	F	31
		74.3	Μ	41			46.0	Μ	21
		78.2	M F	41			59.6	F	31
		39.8	F	21			46.9	FMFMFFF	21
		64.2	M	31			44.3	F	21
		67.4	F	42			64.2	M	31
		67.8	М	31		4 Aug 1978	71.5	F	41
	16 July 1978	64.0	Μ	31			62.0	F	41
		64.4	M	31			51.3	F	21
		73.7	M F F	- 4141314141213142313131314131313131312142		5 Aug 1978	64.2	М	42
		55.8	F	31		9 Aug 1978	59.2	М	42
		57.1	F	31		10 Aug 1978	49.4	M	32
		58.9	M	31		11 Aug 1978	67.0	F	31
		56.8	M	31		12 Aug 1978	51.8	F	21
		51.2	M	21		1777, 1877-946-75, 1777-144, 1747-0	55.5	M	343433333334444333333233333333333333333
		68.1	M	42					-

-51-

CHINOOK SALMON

Catch		Length			Catch	Data	Length (FLan)	Sex	Ane
Anea	Date	(FLan)	Sex	Age	Area	Date	(FLOII)	JEX	Age
Off Coos Bay	12 Aug 1978	59.6	м	31	Off Coos Bay	19 Aug 1978	62.3	F	R
		59.5	M F F	31			64.0	F	3
		63.6	F	31			53.3	F	2
		43.3	M	21			49.8	М	3
		70.0	M M	31			62.0	F	3
	68.6	F	31		22	74.5	Μ	3	
		50.4	M	21			56.0	М	3
		69.5	M F	41			43.0	F F M F F F	2
	13 Aug 1978	47.5	F	21			60.0	F	3
	10 / 10 20/0	56.0	M	31		20 Aug 1978	58.2	M	3
		63.9	M	31		22 Aug 1978	57.0	F	-
		63.5		31		1 THE 1 YO M. THEY CO.	60.7	F	4
		67.8	F	31			62.3	F	3
		57.5	F	31		25 Aug 1978	56.4	-	3
		57.4	M	31		26 Aug 1978	59.4	Μ	3
		56.1	F	31			62.0	Μ	5
	14 Aug 1978	70.0	F	42	21 24 1		61.2	м	3
	14 huy 15/0	67.0	F	31			54.0	M	3
	69.6	WFFWFFF	31			67.6	M F F	3	
	60.3	F	31			61.9	F	3	
	52.4	M	21			66.8	M	3	
	60.0	M	30			52.8	M	3	
		63.4	M	32		27 Aug 1978	57.7	M	3
	16 Aug 1070	61.5	M F F F F	31		ci ing soro	53.8	M	3
	15 Aug 1978	64.5	E.	31			56.0	M F	3
		58.5	Ē	21			55.9	M	2
			Ē	21			45.3	M	2
		67.5	5	21			51.0	M M F	2
		60.1	M	21			67.1	F	2
		64.2	M F F M	31		28 Aug 1978	59.3	M	2
		71.0	r.	31		20 AUG 1970	73.0	M	5
		48.3		41			68.2	M M	2
		59.2	M	31			61.7	M	2
		62.8	F	31		29 Aug 1978	59.9		2
	10 1 1070	67.3	5	41		29 Aug 19/0	61.0	F F F	3
	16 Aug 1978	51.5	M	31			62.0	E	2
		61.2		31			49.4	M	2
		64.8	m	31			56.3	M	2
	10 1 1070	57.5	r.	42			66.4	M	2
	19 Aug 1978	56.1	M	32					5
		54.0	M	31		1 See 1079	53.0	M F M	4
		64.2	F.	31		1 Sep 1978	53.3	M	2
		61.1	M	32		2 Sep 1978	58.1		3
		54.8	M	31			53.4	M	3
		63.6	M	31			53.1		4
		62.0	F M F M F M M F F M	ຠຠຠຆຠຠຆຆຆຆຠຠຠຠຠຠຆຆຠຠຆຆຠຠຆຆຠຠຆຆຠຠຆຆຠຠຆຆຠຠຆຆຠຠຆ			51.9 65.1	M F M F	4
		50.4	F	R			47.0	M	32333233-433353333333332222333333333332-332242
		57.8	M	42			47.0	M	4

-52-

I

CHINOOK	SALMON	
GILLINON	JUL MI	

Catch Area	Date	Length (FLcm)	Sex	Age	Catch Area	-	Date	Length (FLam)	Sex	Age
Off Coos Bay	3 Sep 1978	48.2 55.9	F F F F F F F	፝፝ጟጟ፝፟፝፝፝፝፝ጟጟጟ _፟ ቘ፟፟ጟ፟፝ጟ፠፠ፚፚጟጟ፟፟፝፠ፚፚፚፚፚ						
		47.0 57.1	F	32						
		59.5	F	31						
		54.5 52.0	F	31 R						
	4 Sep 1978	59.1	M F	31						
	5 Sep 1978	45.8 57.0	M M	21 32						
	19 Salara	56.5 47.3	M F	32						
	10 Sep 1978	47.3	M M	21 21						
		63.8 56.3	M F F	31						
	11 Sep 1978	57.4	F	32						
		47.4 51.8	M	21 21						
	12 Sep 1978	53.0	M M F	32						
		48.6 51.0	M	21						
	16 6 1070	49.5	M M	21						
	16 Sep 1978	61.1	n	32						
					P					
					1.1					
					1 C					
					1					
R = Regenerate										

-53-

APPENDIX II - HISTOLOGICAL METHODOLOGY

The procedures used for preparing histological mounts of chinook salmon gonad tissues included (1) FIXING the newly-collected tissue, (2) WASHING out the fixative and STORING the tissue in a preservative, (3) IMBEDDING the tissue in paraffin, (4) taking a SLICE of tissue and MOUNTING the tissue slice on a microscope slide, and (5) STAINING the tissue mount.

- 1. FIXING Fresh Gonad Tissue Samples
 - Remove samples from fish and place in BOUINS' or BUFFERED FORMALIN solution (refer to recipes).
 - 2. Allow samples to remain in fixative solutions 24 hours if using Bouin's solution or 48 hours if using Buffered Formalin.

Recipe for BOUIN'S SOLUTION

75 ml formalin

25 ml picric acid

5 ml glacial acetic acid

Mix regents under fan hood; avoid breathing the fumes. Wash immediately if contact with skin.

NOTE: Picric acid is considered explosive; exercise care in storage of pure material.

Recipe for BUFFERED FORMALIN

40 ml formaldehyde 60 ml distilled water CaCO crystals

Mix regents under fan hood; avoid breathing the fumes. Wash immediately if contact with skin. Shake solution with CaCO crystals everday for 10 days then decant off clear liquid.

NOTE: All ethanol solutions can be used in the raw form; they do not need to be redistilled.

- II. WASHING and STORING Samples
 - A. If BOUIN'S solution used:
 - Rinse samples with water to remove excess bouins solution from samples.
 - Place samples in 50% ethanol until ready to proceed with the following.
 - 3. After 24 hours in 50% ethanol transfer to 70% ethanol.

- B. If Buffered Formalin used:
 - Soak samples in water overnight to remove formalin from tissue.
 - 2. Place samples in 70% ethanol.

III. IMBEDDING in Paraffin

- A. Gonad tissues were imbedded in paraffin using an AUTOTECHNICON machine and the following instructions:
 - 1. Turn on switch located on right side of machine.
 - Black switch on left corner of machine lifts up cover of machine. Push up to start; push down to stop.
 - Lift up cover of machine and fill baskets with appropriate solutions. (refer to handout on solutions needed).
 - Add paraffin if needed to wax containers; make sure these containers are plugged in.
 - 5. Set timer
 - a. Timer is located inside the front plastic cover. It is the large dial on the right hand side.
 - b. The metal pin on the left (inside the front plastic cover) should be pushed down.
 - c. Release the lock on the timer dial and place the metal projection on the dial at the last notch (the 7 1/2 mark). One complete cycle takes approximately 10 hours, so allow additional onal time so that the samples do not sit in the wax for an excessive amount of time. A good time to start the cycle is in the afternoon; the cycle will run through the night and the remaining part of the procedure can be started the following morning.
 - 6. Attach the sample basket to the cover of the machine.

1 sample basket: start over the water solution

2 sample baskets: start one over wax, one over the water solution (Note: When the machine starts, the cover will turn so that (for 1 sample basket) the sample basket will start the cycle in ethanol; (for 2 sample baskets) one sample basket will start in the water and one will start in ethanol.

- 7. Close cover of machine.
- Push up the metal pin located inside the front plastic cover on left.
- Make sure the metal switch located under the black switch on left corner of machine is set for 24 hours. The cycle of the autotechnicon has started.
- B. Once the AUTOTECHNICON cycle is completed, the samples should be removed from the wax as soon as possible. (This prevents excessive heating of the samples)

1. Plug in the wax sample warmer and turn on the tissue-tek machine (both switches up). Add additional paraffin if needed. Allow time for the wax to melt. Put metal sample trays in the side shelf of the sample warmer. Once the wax has melted in these machines, proceed with the following.

- Open the cover of the AUTOTECHNICON, remove the sample basket, close the cover, and turn the AUTOTECHNICON off.
- Put samples in sample warmer. Remove wax from sample basket and watch glass by soaking them in xylol.
- Remove sample from wax in sample warmer and place it on front shelf of sample warmer. Label tissue embedding ring with sample number.
- 5. Fill metal sample tray with wax.
- Set sample in bottom of metal sample tray; set tray on cold spot of tissue-tek.
- Set tissue embedding ring on metal tray so that flaps of metal tray are exposed.
- Add additional wax to fill tissue embedding ring.
- Set metal tray on cold portion of tissue-tek for approximately 30 minutes.
- When wax has cooled sufficiently, remove metal tray. Return metal tray so side shelf of sample warmer.
- Remove wax from sample molds by dipping them in xylol. Do not leave them in xylol for any length of time, as the xylol will break down the plastic.
- Turn the sample warmer and tissue-tek off; clean off any wax that spilled on the machines.
- C. Recipes for the AUTOTECHNICON

Starting from the two wax containers, towards the left, the solutions are as follows;

- 1. distilled water
- 2. 30% ethanol
- 50% ethanol with LiCO₃
- 4. 50% ethanol with LiCO-
- 5. 70% ethanol
- 6. 70% ethanol
- 7. 95% ethanol
- 8. 95% ethanol
- 9. 100% ethanol
- 10. Xylol

The 50% ethanol with $LiCO_3$ is a saturated solution. Mix ethanol and $LiCO_3$ together in a separate bottle, then pout the solution into the autotechnicon baskets after the excess $LiCO_3$ has settled to the bottom of the bottle.

Always change solutions if they have been in the AUTOTECHNICON baskets for a long period of time, as the ethanol solutions will absorb water from the air.

Fill the AUTOTECHNICON baskets up to about 15 cm from the top of the basket. Periodically change the paraffin in the wax containers.

IV. SLICING and PREPARING the slides of the samples

Allow the waxed samples to set for 24 hours before slicing them.

- Turn on the slide warmer, set the temperature at approximately 30°C. Fill the water bath with water, turn it on, and set the temperature at approximately 47°C.
- 2. Instructions for using the microtome and for slicing samples
 - Attach knife to microtome. Be very careful with the knife; it it extermely sharp.
 - b. Label slides with sample number (2 slides/sample). Place a very small drop of Mayers albumin on the slide and rub it over the surface of the slide. Set slide on slide warmer.
 - c. Place the waxed samaple in the vise of the microtome (white case in with plastic flaps diseways). Screw down vise until the sample is secured.
 - d. Gently move the knife towards the samaple until the blade just barely touches the wax. Tighten the vise on knife.
 - e. Turn the wheel on the right hand side of microtome (moves sample up and down, and closer to the knife).
 - f. Proceed to cut into was until the sample is reached. When an adequate portion of the samaple is appearing in the slices, take 3 adhering slices and gently place them in the water bath. (The water bath warms the wax so that the slices lay smoothly on the slide). Repeat this procedure a second time so that 2 slides/sample are made.
 - g. Take the appropriately labeled slide from the slide warmer, place it in the water under the wax slice, bring the slide up under the slice and remove the slice with the slide from the water. Place the slide on the slide warmer and leave there for 24 hours.
 - h. When finished with knife (or if it becomes dull), sharpen it in the microtome knife sharpener using the fine sharpening compound.
 - i. After the slides have remained on the slife warmer for 24 hours, they are ready for the staining process.
 - j. Turn off machines and clean them up.
- V. STAINING the tissues prepared on slides.
 - A. Procedures
 - 1. Xylol I: 2-3 minutes. Removes paraffin from sections.
 - Xylol II: 2 minutes. Removes any residual paraffin.

3. 100% ethanol: flush 5-8 times. Do not let tissue dry.

- removes xylol from sections.
- 95% ethanol: 1-2 minutes.
- 5. 50% ethanol saturated with Li2CO3: 1-2 minutes or until yellow color
- disappears from sections.
- 6. Distilled water: 2-3 minutes.
- 7. Harris hematoxylin: at least 3 minutes; longer if stain is old.
- Tap water: flush several times. Removes excess stain.
- 9. Tap water: flush until all excess stain is removed.

10. Acid alcohol: 10-15 seconds. Dye is removed from cytoplasm and remains only in nuclei. 11. Alkaline water: 1-2 minutes until sections turn dark blue. 50% ethanol: 30 seconds - 1 minute. Dehydrates sections. 95% ethanol: 30 seconds - 1 minute. Dehydrates sections. 14. Eosin: 2 minutes; cannot overstain. Stain longer for formalin fixed tissues. 95% ethanol: flush 2-3 times. Removes excess dye. 15. 16. 100% ethanol: flush 2-3 times. Removes excess dye. 17. Clearing Xylol: flush 2-3 times; do not leave slides in excessively long, as xylol is acid and tends to destain the tissue. 18. Mount in neutral Canada balasm or synthetic resin (Paramount). B. RESULTS Nuclei-dark blue Cytoplasm-red Collagen-red Cartilage-gradation from light to dark blue C. RECIPES FOR STAINING Harris hematoxylin: 1 g hematoxylin in 10 cc 100% ethanol 20 g alumnium sulfate (Ammonium alum) in 200 cc warm water bring the above to a boil and add the following: 0.5 g mercuric oxide boil mixture 1 minute and plunge flask into cold water and cool rapidly under running water and add the following; 4 cc glacial acetic acid 2. Eosin Y: 0.5% in 95% ethanol; 5.5 g eosin/liter pH=5.4-5.6 (adjust with .1 N HC1) Acid alcohol: 1 liter 35% ethanol 3 cc concentrated HC1 4. Xylol: full strength Alkaline water

- water to which NaHCO3 to NH4 OH has been added to give solution a slightly alkalinity.
- 50% ethanol saturated with Li₂CO₃
- Alcohol series: 100%, 95%, ethanol
- NOTE: All ethanol solutions can be used in the raw form; they do not need to be redistilled.