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Title:	THE EFFECTS OF X-IRRADIATION, DIETHYL-
	STILBESTROL, AND SIZE AT TIME OF RELEASE
	ON THE EARLY SEXUAL MATURATION OF COHO
	SALMON (ONCORHYNCHUS KISUTCH)
Abstra	Redacted for privacy
	Dr. C. F. Bond

Precocious male coho are of limited economic value and control of their numbers returning to the hatchery or the increased survival of full-sized adults would be beneficial. Factors influencing early sexual maturation and attempts to suppress this maturation of males through the incorporation of a hormone in the diet, treatment of parr with X-irradiation and control of the size at time of release by manipulation of the food were investigated.

The investigation was carried out at the Fish Commission of Oregon's Big Creek Hatchery from June 1964 to January 1967 using the 1963 and 1964 broods of coho. A portion of the treated animals of the 1963 brood was retained through adult maturation in fresh water at Big Creek and a similar group was held in salt water at Bowmans Bay, Washington.

Fish in one production pond received diethylstilbestrol incorporated into the Oregon Moist Pellet diet at 200 µg/g from November 1964 through April 1965. Juvenile coho that received the various exposures of X-irradiation were taken to Corvallis for treatment and then returned to Big Creek. An experiment involving manipulation of diet to achieve two distinct sizes of smolts was initiated in April 1965 and carried through to March 1966, at which time the smolts were released.

Juvenile coho salmon that received the hormone in their diet produced only half as many precocious males as the control when reared in fresh and saltwater ponds and only one quarter as many precocious males when released to the ocean. The adult survival for the two groups was equal in the ponds although only 30% of the hormone group was recovered at the hatchery as compared to the control. No long term effects on the viability of the gametes was noted but the mean length of fish in the hormone group was significantly smaller than that of the control fish.

X-irradiation of parr did not inhibit early sexual maturation.

No long term effects on the viability of gametes was noted. X-irradiation is not a practical tool for suppression of gonad development because juvenile coho have a low tolerance.

Early sexual maturity is positively related to size of smolt at time of release. The release of larger smolts resulted in a

12-fold greater return of precocious male coho, and almost a two-fold greater return of adults.

From the results future suggestions for investigation are outlined. They include: further investigation of chemical suppression of the pituitary; various feeding regimes in an attempt to control growth; manipulation of hatchery procedures to produce smolts that would be migrants in their first year of life and combinations of the above.

The Effects of X-irradiation, Diethylstilbestrol, and Size at Time of Release on the Early Sexual Maturation of Coho Salmon Oncorhynchus kisutch

bу

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THE EFFECT OF X-IRRADIATION, DIETHYLSTILBESTROL, AND SIZE AT TIME OF RELEASE ON EARLY SEXUAL MATURATION OF COHO SALMON ONCORHYNCHUS KISUTCH

INTRODUCTION

In recent years salmon hatcheries along the Pacific Coast have been plagued by the return of large numbers of precocious male coho salmon (Oncorhynchus kisutch). These small fish are of limited value to the commercial and sport fisheries and create handling and disposal problems at hatcheries. Greater benefit could be realized if fewer precocious males and more adult coho returned. To the species the precocious male may be of important biological value, for it can act as a safety factor ensuring fertilization of deposited eggs, and can provide exchange of genetic material between two brood years. Nevertheless, the above economic problems indicate the need for a better understanding of early sexual development. This study involved attempts to suppress precocious maturation of males through feeding a diet containing a hormone, X-irradiation of the juvenile, and manipulation of food to control the size of smolt at time of release.

Almost all coho salmon rear for one or two years in fresh water before migrating to the sea. Also, most spend two growing seasons in the ocean, returning on their spawning migration late in the year following that in which they entered the sea (Gribanov,

1948; Marr, 1943; Pritchard, 1940; and Shapovalov and Taft, 1954). Various other minor age groups have been observed in coho, including fry that made the seaward migration during the same season as emergence. In some areas significant numbers of precocious males return late in the same year they went to sea.

Early maturation occurs in wild fish as well as those of hatchery origin and is not restricted to coho salmon. Precociously developed males are found in spawning migrations of sockeye salmon (Oncorhynchus nerka), chinook salmon (Oncorhynchus tshawytscha), and steelhead trout (Salmo gairdneri) in the Pacific Northwest. As a rule there are fewer precocious males in the northern than in the southern portions of the geographical range of these fishes. Precocious males may constitute a considerable portion of the normal escapement; for example, the coho spawning run to the Tenmile Lake system in southern Oregon was composed of 30 to 70% precocious males in some years. The spawning run entering Waddell Creek, California, averaged 17% early maturing males during the years 1931 to 1939 (Shapovalov and Taft, 1954).

There is no published evidence that coho salmon mature sexually as parr as in chinook salmon O. tshawytscha (Robertson, 1957; Gebhards, 1960) and Atlantic salmon Salmo salar (Dahl, 1910; Orton, Jones, and King, 1938; Jones and King, 1952). However, Dr. C. E. Bond (personal communication) noted that a student, Joe Wetherbee,

Lake, near Florence, Oregon. R. Mc Queen (Oregon Fish Commission, personal communication) found some of the smolts leaving Big Creek, a lower Columbia River tributary, maturing sexually.

Makeeva and Nikol'skii (1965) indicated that small, precocious males are fairly common in Eurasian waters among species of Salmo,

Oncorhynchus and Salvelinus. Males of Oncorhynchus masou are the most variable of all salmonids in their life history. Some are sexually mature before their first winter in fresh water, other males (yamame) become mature in fresh water and never migrate to the ocean whereas others after one or two years in fresh water spend one winter in the ocean before returning to fresh water to reproduce (Tanaka, 1965).

Alm (1959) from his experiments and literature review concluded that early maturity is a result of growth rate or genetic influence or both. In a detailed survival experiment using chinook salmon, Robertson (1957) fertilized eggs with milt from precocious male fingerlings and found "normal" development of eggs and fry. Offspring were examined periodically to the end of their first year and no precociously mature males were found. Neave and Pritchard (1947) fertilized normal coho eggs with sperm from precocious and normal adult males and released the fingerlings after marking. There was no significant difference in the number of returning

precocious males and adults. The authors concluded that precocious males do not tend to reproduce their own kind. However, Mr. J. Graybill (personal communication) used sperm from normal and precocious males to fertilize eggs from normal coho females and noted that early sexual maturation is predominantly a heritability trait of the female. In his experiments some female's progeny produced 15 times as many precocious males as did eggs from other females fertilized by the same male.

The hypothesis tested in this investigation was that control of precocious sexual maturation could be achieved by suppression of gonad development. The suppression was attempted by: (1) feeding a diet incorporating a synthetic hormone, diethylstilbestrol; (2) treatment of parr with X-irradiation; and (3) controlling size at time of release by manipulating the amount of food fed the juvenile salmon.

Activation of the pituitary gland and subsequent release of gonadotropic hormone induces maturation of the gonad. Schmidt et al., (1965) showed that immature male S. gairdneri increased their gonad weight when injected with pituitary extract of adult O. tshawytscha. Yamamoto (1953, 1955, 1962), Berkowitz (1941), and others fed female gonadotropic hormones to tropical fish and noted a change in the sex. Whereas, Ashby (1956) noted that estradiol and testosterone added to the culture medium of alevin brown trout, Salmo trutta, only retarded gonadal development and produced

hypertrophy of the somatopleure. Svardson (1943) reported that oestrone arrested the growth of young Lebistes and caused testes to remain in an undeveloped state. Jaap and Thayer (1944) noted a pseudo-caponizing effect in male chickens fed diethylstilbestrol; it tended to eliminate "cockiness" in early maturing broilers. I found no report of the effects of prolonged treatment of salmon fingerlings with hormones. But, I postulated that a female gonadotropic hormone in the food of salmon parr, prior to smoltification, might suppress development of the testes and result in a reduction of the number of precocious males. A synthetic estrogen (diethylstilbestrol) could provide "false information" to the pituitary thus inhibiting the release of further gonadotropins responsible for the development of the testes.

Another postulate was that development of rapidly dividing cells, such as cells of gonadal tissue of young salmon, could be suppressed by X-irradiation. Solberg (1938) noted an inhibitory effect on the formation of spermatocytes from spermatogonia in Oryzias latipes, X-irradiated with 1980R. Konno and Egami (1966) also noted that testes of fish Oryzias latipes when irradiated with 1/10 - 8kR were reduced in weight within two weeks. In fish irradiated with smaller doses, the testicular weight began to increase about one month after irradiation and at two months became approximately normal. Fish irradiated with 8kR of X-rays showed severe damage of spermatogonia, spermatocytes and spermatids within 24 hours.

A relationship between the size of smolts at time of release and the percentage of early maturing adults has also been noted (Jeffries, 1959; Mastin, 1955; Noble, 1958; Salo and Bayloff, 1958; Wallis, 1968). These investigators also found a relationship between the size of smolts at release and survival to adulthood. Schmidt et al., (1965) noted that greater stimulation of the testes of juvenile S. gairdneri occurred in larger fish when injected with adult salmon pituitary extract. The last postulate I examined was whether large body size at time of smoltification is related to a higher incidence of precocious males.

METHODOLOGY

Description of Facilities

Big Creek, Oregon

Experimental fish were reared at the Fish Commission of Oregon's Big Creek Hatchery, located in Clatsop County, east of Astoria. The hatchery is located on Big Creek, a tributary to the lower Columbia River about 18 miles from the mouth.

Following incubation and hatching, the alevins were held inside the hatchery until "swim-up," at which time the fry were placed in concrete recirculating ponds, 80 feet long, 20 feet wide, and 4 feet deep. The fry were originally stocked at 500,000 per pond and as they grew were subdivided to other ponds, until a density of 80,000 per pond was reached. The experimental fish were held in similar facilities.

Bowmans Bay, Washington

Bowmans Bay Marine Biological Laboratory is situated at sea level on Fidalgo Island near Deception Pass, Washington. At the time of the experiments it was operated by the Washington Department of Fisheries.

Experimental coho smolts from Big Creek Hatchery were transported via tank truck to Bowmans Bay on April 15, 1965, and placed in two 40 by 16 foot concrete raceways provided with a water supply of 20 to 30 gallons per minute. Water depth was maintained in each raceway at approximately 3 feet.

Fresh water from Pass Lake and sea water (up to 31.5% salinity) from an intake in Bowmans Bay were mixed to provide a range of salinities from 15 to 30%. The smolts were introduced into the raceways that contained water of 15% and were subject to 5% daily increase until acclimated to full strength sea water.

Temperatures of the saltwater supply ranged from 6.7 C in early April to about 11.7 C in late June 1965; in 1966 temperatures were less variable and ranged from 8.3 C to 10.6 C during a comparable period.

Experimental Design

Experimental Fish

Fish used in the study were of the 1963 and 1964 Big Creek brood stock (Table 1). Adults were spawned in November 1963 and 1964 and the fertilized eggs were incubated in standard egg

Table 1. Experimental groups of 1963 and 1964 brood coho salmon.

		1963		<u>19</u> 64	4
Groups	A	В	C (144)	D	E
Diet (Oregon Moist Pellet)	Hormone Incorporated November 1964 to April 1965	Regular	Regular	Regular, High Ration April 1965 to March 1966	Regular, Low Ration April 1965 to March 1966
Dispos ition of Smolts	1000 to be reared to maturity in fresh water	1000 to be reared to maturity in fresh water	Four groups of 1500‡ coho to be irradiated at various dosages	Released to ocean March 1966	Released to ocean March 1966
	1000 to be reared to maturity in salt water	1000 to be reared to maturity in salt water	One group of 1300 [±] coho to be sham irradi-ated		
	40, 000 ⁺ to be released for ocean maturation	40, 000 ^{,†} to be released for ocean maturation	Half of each irradiated group and control to be reared to maturity in fresh and salt-water ponds		
			400 selected large smolts subdivided in half and each group reared to maturity in fresh and saltwater ponds		

baskets provided with water from a constant temperature spring (8.9 C).

The fry (1963 brood) were placed in the concrete ponds in February and March 1964. The fish were graded in July 1964 to reduce the size variation within the 21 ponds of fingerlings. Small fingerlings (< 80 per pound) were transferred to separate ponds for more intensive feeding.

In late October and early November 1964, approximately 115,000 coho fingerlings were removed from general hatchery production and placed in three ponds for some of the studies described in this report (Appendix I). All fish were fed Oregon Moist Pellet (Hublou et al., 1959) from the first feeding until their release as smolts.

Hormone Treatment

A study was conducted at the Fish Commission of Oregon's Clackamas Laboratory in September 1964 to determine the palatability to salmon of a diet containing diethylstilbestrol. Oregon Moist Pellet containing 100 and 200 µg of diethylstilbestrol per gram of food was fed to young coho fingerlings for approximately one month. The fingerlings ate pellets containing either level of diethylstilbestrol

equally well and suffered no apparent adverse effects. The higher level of diethylstilbestol was chosen for the study.

Coho fingerlings held in pond 28 were fed diet containing the hormone from November 6, 1964 to April 13, 1965. The daily ration was divided into two or three feedings and was broadcast to the fish by hand.

The original plan was to give equal amounts of food to the experimental fish and to those of general hatchery production (1.6% of body weight per day). In early January 1965 the fish on the hormone diet had frayed fins and minor secondary infections of two parasites (Epistylus and Trichodina). Following formalin treatment for the parasites the amount of food being fed was increased for the experimental fish and their designated controls. By the end of February, the fish receiving the hormone diet again appeared healthy although a small percentage had apparently been unilaterally blinded, possibly by the severe nipping that had occurred earlier.

Hormone-fed coho and marked control coho were released

April 13, 1965 into Big Creek (Table 2). Prior to release 2,000

fish from each of the diet groups were set aside for rearing in fresh
and saltwater ponds. One-half of each group was transported to

Bowmans Bay to be reared in salt water. The remainder were to
be reared at Big Creek in fresh water.

Table 2. Data on release of 1963 brood coho salmon at Big Creek Hatchery

	<u>.</u>	-	Average size					
Group	Date Released	Number	Fork length (cm)	(No. per pound)	Fin mark			
Hormone	April 13, 1965 April 15, 1965	43,969 (ocean rearing) 1,000 (Bowmans Bay) 1,000 (Big Creek)	13.5	(16.8)	Left ventral-right maxillary (LV-RM)			
Control	April 13, 1965 April 15, 1965	41, 250 ¹ (ocean rearing) 1, 000 (Bowmans Bay) 1, 000 (Big Creek)	14.4	(12, 8)	Right ventral-left maxillary (RV-LM)			
Production	November March April	889, 908 ² 836, 878 15, 920 ²	No data	(18.8) (15.7) (14.2)	None			

¹ Actual number released.

^{2.} Estimated number released.

X-irradiation Treatment

X-irradiation of coho yearlings to arrest development of the gonadal tissue was carried out in February and March prior to smoltification. Because treatment of sufficient numbers of presmolts for release in the ocean as originally intended was physically impossible, all treated fish were retained. Irradiation was carried out under the direction of Dr. F. P. Conte of the Radiation Center, Oregon State University.

Fish were held at the Oregon Game Commission Laboratory, Corvallis, for approximately one week prior to X-irradiation, and three to four days before treatment were marked by fin excision to indicate the dosage.

The source of radiation was a General Electric Maxitron 300 X-ray therapy unit. The machine was operated under the following conditions: 300 kVp, 20 mA, HVL selector at 2.0 mm Cu, and beam monitor registering .35 x 25. Approximately 110 fish were placed in 15 cm of water in a 20 gallon plastic container, which was placed under the beam for the desired exposure time. The water was aerated and the fish were allowed free movement within the container. Coho fingerlings that were designated as controls received the same treatment as the exposed group including sham irradiation. The fish were held at the OGC Laboratory for one week prior to their

return to Big Creek. Approximately one-half of each irradiated group (0 to 800 R) were reared in salt water at Bowmans Bay and the remainder in fresh water at Big Creek. At the time of irradiation the coho fingerlings ranged from 10.5 to 14.0 cm. In mid-April the fingerlings taken to Bowmans Bay averaged 14.5 cm in length.

All fish reared in fresh and salt water were fed Oregon Moist

Pellet. This pellet was developed for production of juvenile fish and

did not appear to provide adequate nutrition for coho reared to maturity in the fresh and saltwater ponds.

Size at Time of Release

Coho remaining in pond 30 (after removal of irradiates and their controls) were graded and approximately 400 of the largest smolts (> 14.0 cm) were retained for artificial rearing to test the hypothesis that larger smolts produce a higher percentage of precocious males. This group of large smolts was divided into subgroups of 200 each; one group was reared in fresh water at Big Creek and the other reared in salt water at Bowmans Bay.

Two groups of coho salmon were chosen at random from the 1964 brood Big Creek production at an average size of 206 fish per pound. They were separated in April 1965 and fed Oregon Moist Pellet at two predetermined levels to provide extremes in average size at time of release. The daily ration was determined on the basis of

temperature, rate of food conversion, and desired size of smolt. 1

All experimental fish were marked by fin excision in January 1966 and released with production fish in mid-March 1966. The group receiving the high ration was marked anal-right ventral (An-RV) and released at an average size of 15.1 cm (10.7 fish per pound). The group receiving the low ration was marked anal-left ventral (An-LV) and released at an average size of 11.4 cm (27.3 fish per pound). Data on release statistics of the 1964 brood experimental and production groups are shown in Table 3.

Sampling Procedures

Following the release of the 1963 brood fish for migration to the ocean, the coho (hormone-treated and control; X-irradiates and control) retained in fresh and salt water were sampled monthly from June 1965 to December 1966, when the experiment was terminated. At each sampling period seven to ten coho from each treatment group were randomly taken, killed, and preserved for later study. The preserved fish were measured to the nearest millimeter, weighed with a Mettler K-7 balance, sex noted, and the gonads removed and weighed. The ratio of gonad weight to body weight multiplied by 100 is known as the gonadosomatic index (GSI). This relationship gave

¹D. A. Leith, Fish Commission of Oregon designed feeding schedule.

Table 3. Data on release of 1964 brood coho salmon at Big Creek Hatchery.

	.					Averag	e size	
Group	Date released	Numb er	Pounds	Gain in weight (pounds)	Pounds of food fed	Fork length(cm)	(No. per pound)	Fin mark
High Ration	March 16, 1966	59, 984	5, 632	5, 339	10, 746	15,1	(10,7)	Anal-right ventral
Low Ration	March 16, 1966	83, 649 ¹	3,062	2, 652	4, 689	11,4	(27.3)	(An-RV) Anal-left ventral (An-LV)
Production	March 14 to 18, 1966	1, 236, 180 ²	6, 784			12.9	(18,0)	None

Actual number released.

² Estimated number released. In addition 321, 100 unmarked fingerling released into Big Creek, July 1965.

an indication of the male fish that would likely mature later in the year. Various tissues and organs were removed from irradiated and control fish and prepared for histological sectioning.

All dead fish were removed from the ponds daily and frozen. Dead fish were measured and sex and general condition noted each month. A 5 to 10% loss occurred from mid-April to the end of November 1965. Although few fish died in all treatment groups through May 1966, almost 50% died during the summer and fall months prior to termination in December 1966 (Appendices II and III). Approximately 20 to 30% of the fish dying in the salt and freshwater ponds had major lesions of the kidney, liver, and spleen. Dr. J. Fryer, Department of Microbiology, Oregon State University, identified the problem as kidney disease (Corynebacterium).

The coho salmon held at Bowmans Bay and at Big Creek were individually inspected in November 1965 for signs of sexual maturity. Those fish from which sperm could be freely expressed were killed and preserved for further study. All dead fish and samples taken prior to this time were examined also for sexual maturity.

Coho salmon returning from the ocean entered Big Creek in September; the majority did not migrate upstream until mid-October and November. Upstream migrants were captured at a weir 3 miles from the mouth.

In 1965 fish taken at the weir were usually checked daily,

marks recorded, and precocious males killed. A sample of marked fish was obtained and frozen for later study.

In the fall of 1966, adults from the control group and the group that had been fed the hormone diet as fingerlings returned from the ocean to the Big Creek weir. A random sample of marked fish was measured to compare the growth of the various treatment groups. Similarly, a number of marked fish was killed and spawn taken to determine if treatment as fingerlings had long term effects on viability of eggs and sperm. Fish Commission of Oregon personnel collected data from adults originating from the 1964 brood in the fall of 1967.

Following nearly three years of rearing in the ponds, the experimental fish remaining in the fresh and salt water were sacrificed in December 1966. All survivors were measured and examined for marks (identifying the treatment received as fingerlings), sex, sexual maturity, and general condition. A random sample was preserved for further study.

A number of fish from each treatment group was artificially spawned to determine the viability of the eggs and sperm. The eggs obtained were taken to the laboratory of the Oregon Game Commission in Corvallis or were reared at Big Creek Hatchery.

Spawning and Incubation

Ova were removed by incision of recently killed females and placed in dry spawning pans. Each female's eggs, of the fish reared in salt and freshwater ponds, were divided into three groups to be fertilized with sperm from three separate males. Eggs from adult coho, returning from the ocean, were divided into four groups to be fertilized with sperm from four different males. Milt was expressed by hand from recently killed males. The "dry" method of fertilization was used, i.e., milt and ova were combined before adding water. After fertilization and washing, the eggs were set aside in gallon jars for one hour to water-harden. The jars were transported to Corvallis and the eggs, following disinfection with zonite, were placed in a Robertson incubator with each tray divided into fourths. The position of each experimental group of eggs within the incubator was selected at random. There was a 5 hour delay before eggs taken at Big Creek were placed in the incubator and a 9 hour delay for eggs from Bowmans Bay.

To test further the viability of eggs and sperm from the various treatment groups, 10 to 15 females from each freshwater reared group were spawned and fertilized with milt from four to seven males. Following water-hardening the eggs were placed in baskets in troughs at Big Creek Hatchery and reared to "swim-up" fry. Spawn from

ocean adults originating from the control and hormone groups was handled in a similar manner.

The eggs were checked regularly, unfertilized and dead eggs were counted and removed. The percentage of "swim-up" fry for each series was calculated.

RESULTS

Effect of Hormone Diet on Early Maturation

Pond Reared Coho

The coho salmon reared at Bowmans Bay and Big Creek Hatchery were individually inspected for sexual maturity in November 1965.

No females were mature, but the incidence of precocious maturation in the control males was twice that found in those receiving the hormone, diethylstilbestrol, as fingerlings (Table 4). More than twice as many coho matured in fresh water as in salt water. Increased occurrence of early maturation in fresh water is probably due to the differences in growth between the two environments for the last seven-month rearing period (Figure 1). The fish held in fresh water were considerably larger and more uniform in size than their counterparts reared in salt water. The lesser growth in the salt-water pond is not understood but might be nutritional.

In both the fresh and saltwater ponds a significant reduction in the number of males that matured precociously occurred in those coho that received the hormone, diethylstilbestrol, as fingerlings $(P \leqslant .001 \text{ and } P \leqslant .05, \text{ respectively}).$

The mean fork length of precocious males was less for salmon reared in salt water than for salmon reared in fresh water

Table 4. Biological statistics of hormone-fed coho maturing precociously in fresh and salt water in November 1965.

		water	Fresh	water
	Hormone	Control	Hormone	Control
Number of Smolts	1000	1000	1000	1 000
Length (cm)	13. 5	1.4.4	13. 5	14.4
Precocious Males, November 1965	13	26	32	72
Precocious Males in Samples and Deaths in Ponds	3	4	7	12
Total Precocious Males (%)	16	30	39	84
	(1, 6)	(3, 0)	(3.9)	(8.4)
Percentage Males of Smolts Retained	55.9	51.3	51.8	49.8
Percentage Precocious of Male Smolts	3,1	6, 0	8.1	17.4
ength (cm)	25.6	26, 1	28.1	28,9
Probability Level (P)	.0	5	.00:	I

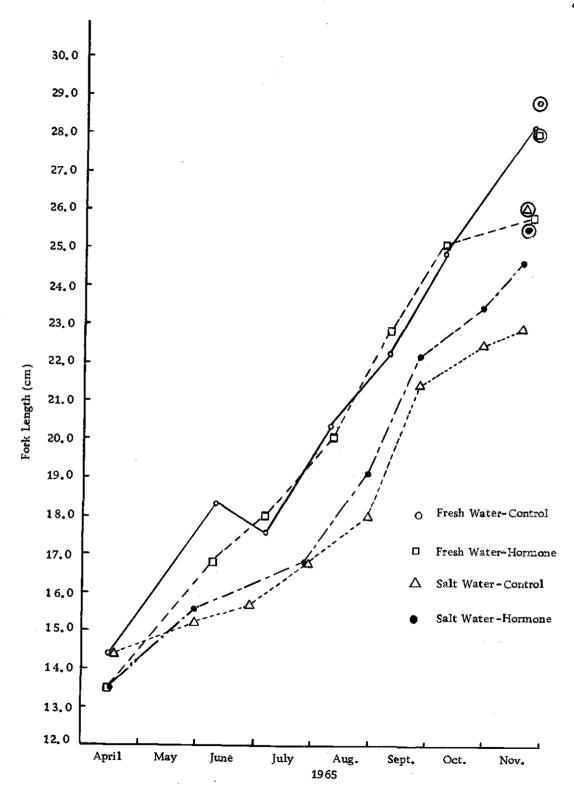


Figure 1. Growth rate of male coho fed a hormone as juveniles and reared in fresh and salt water. (Circled values represent mean length of precocious males, November 1965.)

(Table 4, Figure 2). In both salt and fresh water the mean fork length of control fish was greater than treated fish. This difference approximated the magnitude of the initial differences in the two groups as smolts. However, the rate of growth of fish reared in salt water was considerably less than the growth rate of fish reared in fresh water. Possibly in the saltwater environment, only those fish in which the pituitary had already activated development of the testes continued to develop, whereas in fresh water, because of better growth, others were stimulated and matured later.

At maturity, coho in fresh water had larger testes than those reared in salt water (Table 5). There was no significant difference in gonad size between the control or those that received the hormone diet as fingerlings.

Ocean Reared Coho

Precocious coho males first appeared at the Big Creek weir on September 8, 1965; the last was recorded on December 9. Although large numbers of coho were observed in lower Big Creek in September, the main upstream migration of precocious males and adults did not occur until mid-October to mid-November and appeared related to stream flow. No stream flow data were recorded at Big Creek during 1965, but on days immediately following freshets the number of fish entering the weir increased markedly. There was no

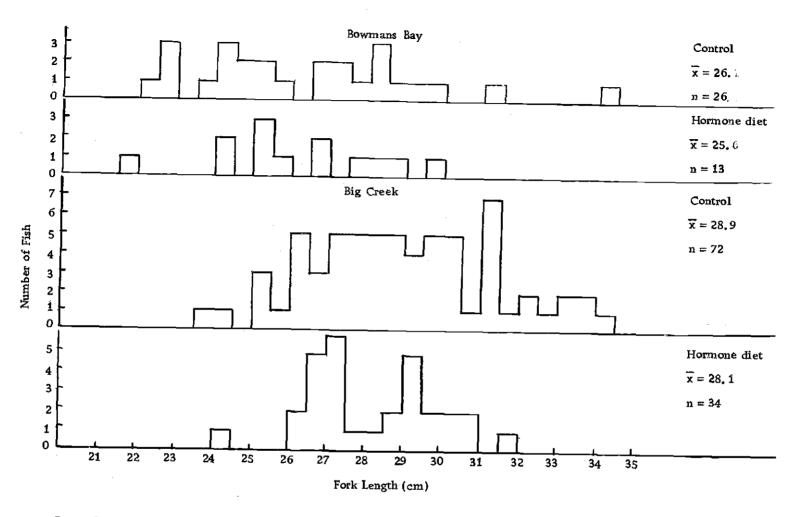


Figure 2. Length frequency distribution of precocious male coho of different smolt origin reared in fresh and saltwater ponds, 1965.

Table 5. The mean length, weight and GSI of precocious males in fresh and salt water, 1965.

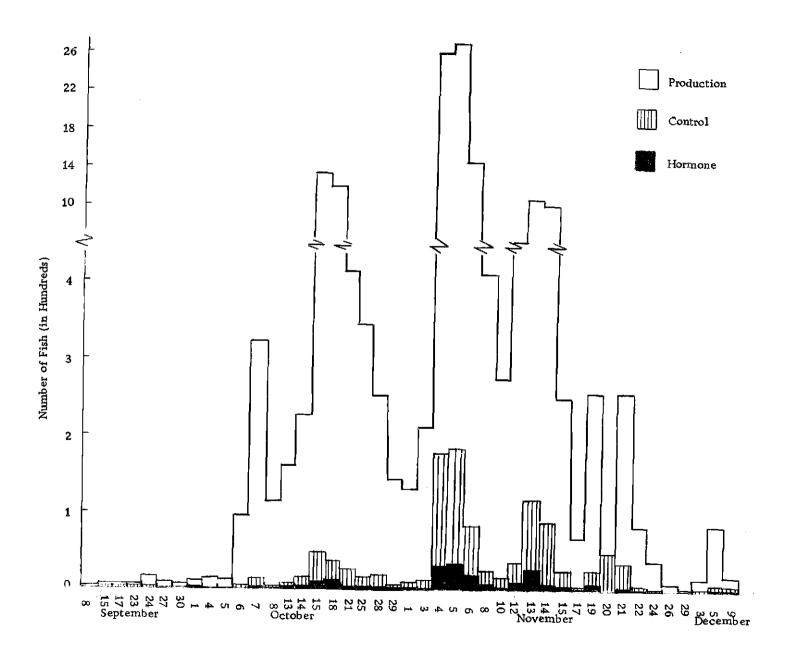
	Fre sh v	vater	Salt :	Salt water		
	Control	Hormone	Control	Hormone		
ength (cm)	28. 9	28.1	26.1	25.6		
Weight (g)	305. 2	276.9	220.7	199.0		
SI	6, 56	6.47	5.71	5, 36		
(range)	(4.92-8.66)	(4.19~8.59)	(3.07-7.88)	(4, 29-6, 74)		
1	65	30	23	11		

apparent difference in rates of migration of marked precocious males (control and hormone groups) and those from general hatchery production. The pattern of daily arrival at the weir is shown in Figure 3.

A total of 16,690 precocious males was checked at the Big Creek weir. Of this total, 1,372 were experimental fish that had been released as smolts in April 1965. Of the two groups of experimental fish released, a return of 1,117 control fish or 2.54% of the smolts released to 255 hormone fish or 0.62% of the smolts released was recorded. These numbers are minimal because numbers of fish taken by sport fishermen in the Columbia River, Big Creek Slough, and Big Creek below the weir were not included although an extensive fishery existed in these areas. Calculation of the minimal percentage of return of hatchery production as precocious males for the 1963 brood year is complicated because 0.89 million smolts were released in November 1964 and another 0.85 million smolts were released in March and April 1965. Considering that 15, 300 unmarked precocious males were handled at the weir, the percentage return of precocious males was somewhere between 0.85% based on the total release of 1.7 million fish and 1.7% based on the 0.85 million smolts released in March and April.

Part of the difference between the control group and the hormone group in return of precocious males may be related to the larger size of the control smolts at time of release. At that time the hormone

Figure 3. The migration pattern of precocious male coho of different smolt origin returning from the ocean, 1965.



group appeared to be inhibited in growth, even though the daily rations being fed were equal in terms of percentage of body weight. The control fish were all quite silvery and exhibited external characteristics of smolts at time of release. However, those coho that had been on the hormone diet were quite dark and had prominent parr marks. Although the fish fed the hormone appeared similar to parr, their physiological condition was such that they had no apparent difficulty adapting to sea water when transferred to Bowmans Bay, Washington. Conte et al. (1966) presented data showing that, under accelerated growth conditions, seawater adaptation by coho occurred approximately 5 months prior to smoltification and the normal period of downstream movement.

Two females from the control group were found to be sexually mature in 1965. Because of the method of handling precocious males at the weir, these fish were not discovered until they had been dead for several hours. They were 42.0 and 44.5 cm in length.

Lengths of precocious males collected at the Big Creek weir were recorded on a number of days throughout the spawning migration. No significant differences in length within the three groups were noted over the spawning season, although differences in lengths among groups were recorded (Figure 4). Snout-to-fork length was used rather than eye-to-fork length as the morphological alteration associated with secondary sexual development had not occurred at the time

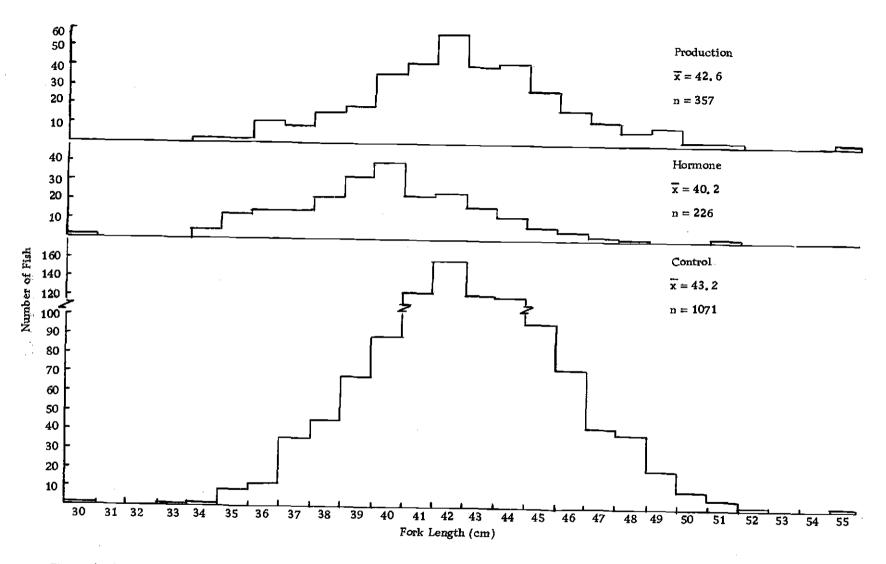


Figure 4. Length frequency distribution of precocious male coho of different smolt origin returning from the ocean, 1965.

of trapping. The mean length of control fish (43.2 cm) was very similar to that of general hatchery production (42.6 cm) but both were considerably larger than precocious males originating from hormone-fed smolts (40.2 cm).

Hormone treated fish were considerably smaller than the controls, but the gonadosomatic index of the testes for the two groups was similar (Table 6).

Table 6. The mean length, weight and GSI of precocious males returning from the ocean, 1965.

	Hormone	Control
Length (cm)	40, 3	43.1
Weight (g)	789 . 7	981. 2
GSI (range)	6, 27 (3, 23-8, 31)	6,90 (4,16-10,04)
n	13	49

Effects of X-irradiation on Early Maturation

Growth of the irradiated groups and their control reared in fresh water was fairly uniform until November 1965 (Figure 5). During their last year of life (1966) the retained coho showed considerable variation in growth.

At the time of precocious maturity in November 1965, many of the fish reared in salt water were considerably smaller than those

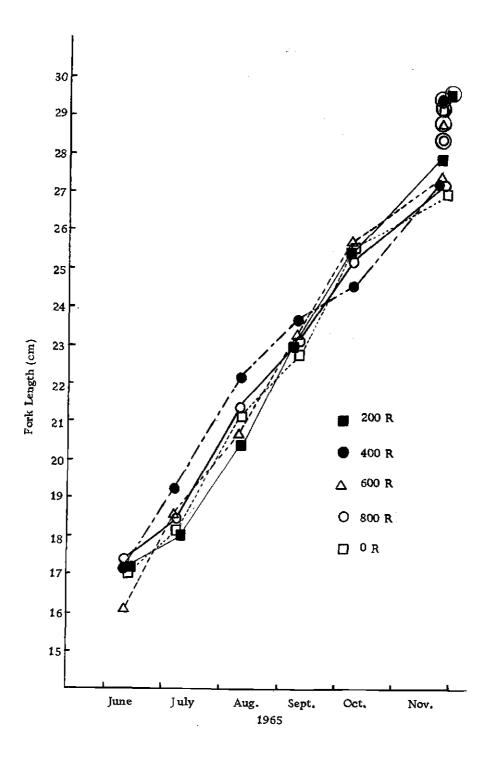


Figure 5. Growth rate of X-irradiated male coho in fresh water. (Circled values represent mean length of precocious males, November 1965.)

reared in fresh water (Figure 6). As noted in the coho fed the hormone diet and their control, numbers of X-irradiated fish and their control had apparently not fed, as evidenced by their emaciated condition. Others that appeared healthy and robust were not much larger than the smolts originally placed into the system.

In both ponds many fish died between May 1966 and the termination of the experiment in December 1966. Many factors were probably involved in this excessive mortality during their final year of life.

Water temperatures in the two environments averaged about 14 C during the summer. This is higher than ocean temperatures and probably had some adverse effects. The fish were fed Oregon Moist Pellet (developed for production of juvenile salmonids) which was probably inadequate for fish of this size. A number of the dead fish showed signs of having a severe kidney infection.

No differences in the number of precocious males were found between irradiated fish and the control fish examined in November 1965 (Tables 7 and 8). From 4.6 to 8.7% of the X-irradiated smolts and their controls matured in fresh water (Table 7), as compared with 2.9 to 4.3% of the smolts in salt water (Table 8). In addition, there is no apparent relationship between the number of mature precocious males produced and the level of X-irradiation to which they were exposed. The only difference noted was that twice as many coho matured precociously in fresh water as in salt water.

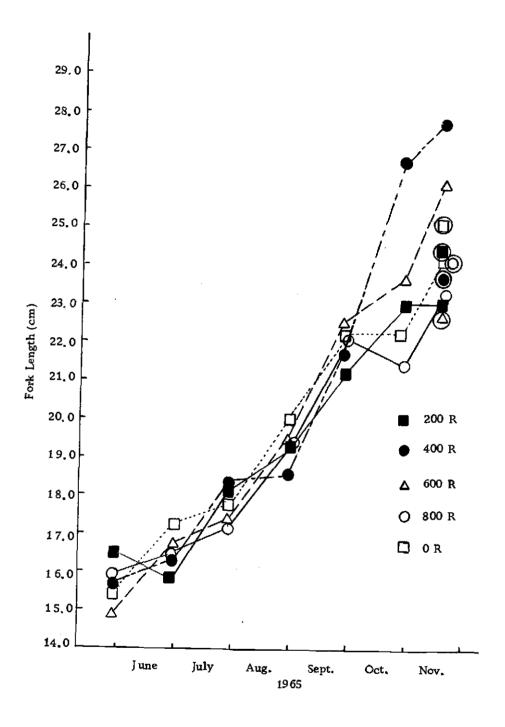


Figure 6. Growth rate of X-irradiated male coho in salt water. (Circled values represent mean length of precocious males, November 1965.)

Table 7. Biological statistics of X-irradiated coho maturing precociously in fresh water in November 1965.

	200	400	Total Exposure (R) 600	800	0
Number of Smolts	833	815	788	794	676
Precocious Males, November 1965	49	52	35	56	32
Precocious Males in Samples and Deaths in Pond	7	9	1 ·	9	7
Total Precocious Males (%)	56 (6,7)	61 (7.5)	36 (4. 6)	65 (8 . 2)	39 (5.8)
Percentage Males in Smolts Retained	48.5	52.3	53, 2	50.3	48.7
Percentage Precocious of Male Smolts	14.1	14.6	8.7	16.6	11,9
Length (cm)	29,6	29.3	28.8	28.4	29.0

Table 8. Biological statistics of X-irradiated coho maturing precociously in salt water in November 1965.

					
	200	400	Total Exposure (R)		
		400	600	800	0
Number of Smolts	750	750	777	761	650
Precocious Males,	16	26	23	21	26
November 1965	i i			:	
Precocious Males in Samples and Deaths in Pond	6	1	3	4	2
Total Precocious	22	27	26	2 5	2 8
Males (%)	(2,9)	(3, 6)	(3.3)	(3, 3)	(4, 3)
Percentage Males in Smolts Retained	49. 8	52.5	53.4	53.4	56,9
Percentage Precocious of Male Smolts	6.0	7.1	6, 6	6.3	7.8
Length (cm)	24.4	23.8	23.0	24,2	25. 2

The mean length of irradiated precocious males and their control was similar in both fresh water (Figure 7) and salt water (Figure 8). However, the mean length of fish maturing in fresh water was 4.0 to 5.0 cm larger than those from saltwater rearing.

Effect of Size at Release on Early Maturation

Pond Reared Coho - 1963 Brood

The state of maturity of the 200 large coho fingerlings that were selected for pond rearing was also inspected in November 1965.

Thirty-two of these fish matured precociously in fresh water and 29 in salt water (Table 9). These fish represented 30 and 24%, respectively of the males present in the two environments. Selection of larger smolts resulted in a higher percentage of males than females in the sample, suggesting that males have a greater mean growth rate. Males that matured in fresh water were slightly larger than the males that matured in salt water (Figure 9).

The percentage of smolts maturing precociously was two to four times greater in the groups of selected large smolts than in the two unselected control groups reared under similar conditions (Table 10). The large smolts had a greater mean size as precocious males than the other control groups (Table 10).

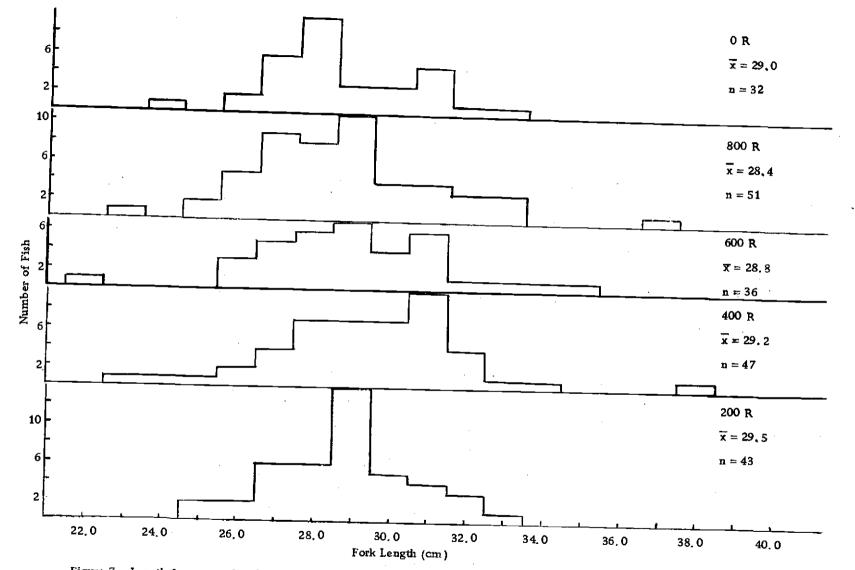


Figure 7. Length frequency distribution of precocious male coho X-irradiated as juveniles and reared in fresh water, 1965.

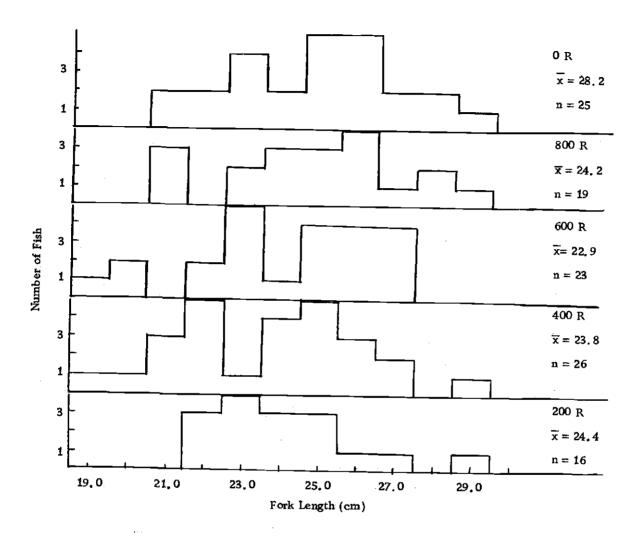


Figure 8. Length frequency distribution of precocious male coho X-irradiated as juveniles and reared in salt water, 1965.

Table 9. Biological statistics of selected large coho maturing precociously in fresh and salt water in November 1965.

	Big Creek	Bowmans Bay
Number of Smolts	200	200
Precocious Males November 1965	29	27
Precocious Males - Deaths in Pond	3	2
Total Precocious Males (%)	32 (16,0)	29 (14.5)
Percentage Males of Smolts Retained	54.9	61.8
Percentage Precocious of Male Smolts	29 , 9	23,6
Length (cm)	29.7	27.3

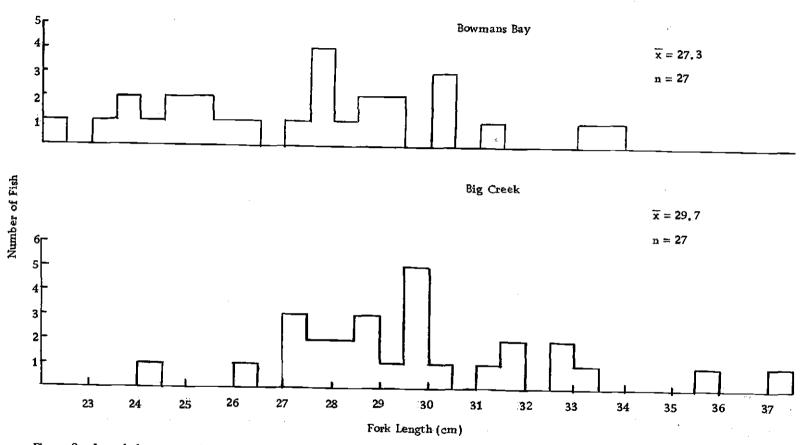


Figure 9. Length frequency distribution of precocious male coho selected as large smolts and reared in fresh and salt water, 1965.

Table 10. Biological statistics of experimental controls and selected large coho maturing precociously in fresh and salt water in November 1965.

		Sa lt water				
	Control (Hormone)	Control (Irr adiation)	Selected Large Smolts	Control (Hormone)	Control (Irradiation)	Selected Large Smolts
Number of Smolts	1000	650	500	1000	676	200
Mean Length (cm)	14.4	14.5	> 14.0	14.4	14.5	> 14.0
Number of Precocious Males (%)	30 (3.0)	28 (4,3)	29 (14. 5)	84 (8.4)	·39 (5.8)	32 (16.0)
Percentage Males of Smolts Retained	51.3	56 .9	61.8	49.8	48,7	54.8
Percentage Precocious of Male Smolts	6,0	7.8	23,6	17.4	11,9	30.0
Length (cm)	26.1	25.2	27.3	28.9	29.0	29.7

Ocean Reared Coho - 1964 Brood

In the fall of 1966, all precocious males returning to Big Creek Hatchery were examined for fin marks. A total of 11,668 early maturing males was recovered. A total of 150 precocious males originating from smolts receiving a low ration and 1,590 precocious males from a high ration returned or 0.18 and 2.65%, respectively of the numbers released (Table 11). These values are minimal as many precocious males were restricted from the hatchery trap during periods of peak migration.

Fork lengths were obtained from a random sample of the finmarked and non-marked precocious males (Figure 10). The group fed the high ration showed the largest average length (45.0 cm); those fed the low ration the smallest average length (41.6 cm). The hatchery production was intermediate with a mean length of 43.0 cm.

An estimate of the number of precocious males that returned from hatchery production is not available as no tetracycline mark (a hatchery mark) examination was performed. I assume the majority of non-marked precocious males were from hatchery production rather than from natural production in Big Creek.

A return of 2.54% of the smolts released (1963 brood, control group) were recovered as precocious males at the Big Creek weir whereas 2.65% of the 1964 brood smolts (high ration) were recovered

Table 11. Number and mean length of precocious male coho of the 1964 brood of different smolt origin returning from the ocean, 1966.

	High Ration	Hatchery Production	Low Ration
Number of Smolts	59,984	1, 236, 180	83, 649
Mark	An-Rv	none	An-IV
Length (cm) (range)	15, 1 (11, 2-23, 0)	12.9 (9.5-17.3)	11.4 (7.8-15.3)
Number of Precocious Males Recorded at Hatchery Weir	1590	9,921 1,2	150 ¹
Percentage Precocious Males	2, 65	.80	0, 18
Length (cm)	45.4	43.0	41.6

¹ Minimal returns as not all fish ascending the stream were allowed entry into the trap because of crowded conditions.

²Also includes some stream reared early maturing males.

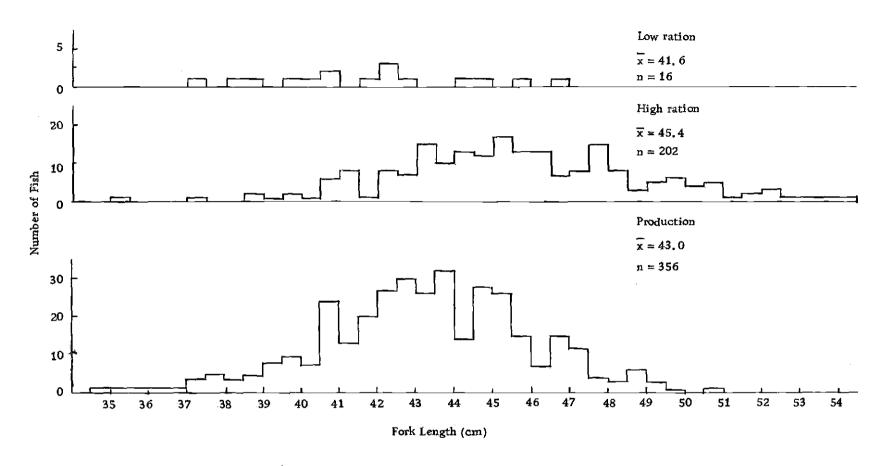


Figure 10. Length frequency distribution of precocious male coho of different smolt origin returning from the ocean, 1966.

as precocious males. That fish of the 1964 brood were of larger mean size than the 1963 brood as smolts (\overline{x} = 15.1 cm: 14.4 cm) is probably responsible for the increased return of precocious males.

Gonad Size in Precocious and Maturing Adults

Examination of the gonadosomatic index (GSI) indicated that as early as June 1965 those males that probably would have matured precociously could be distinguished in fresh water (Figure 11, Appendix IV). The GSI (0.19) for two males sampled at the end of May in the salt water indicate they probably also would have matured precociously (Appendix V). In June some males exhibited a GSI (0.12-0.74) at least three to four times that of the normal male (0.03-0.04) population sampled. By August the GSI of maturing fish was 100 times that of the normal male population. The GSI reached a peak in September and October and then declined as maturation was completed (Figure 11, Appendix IV and V).

Following the removal of precocious males, the population of coho that remained was sampled at regular intervals. The GSI increase over the months was the same as that witnessed for those fish that matured precociously (Figure 11). A 100-fold difference in GSI was noted between maturing males in July 1966 and immature males (Figure 11). At the time the experiment was terminated a few males in fresh water were still immature and this was readily

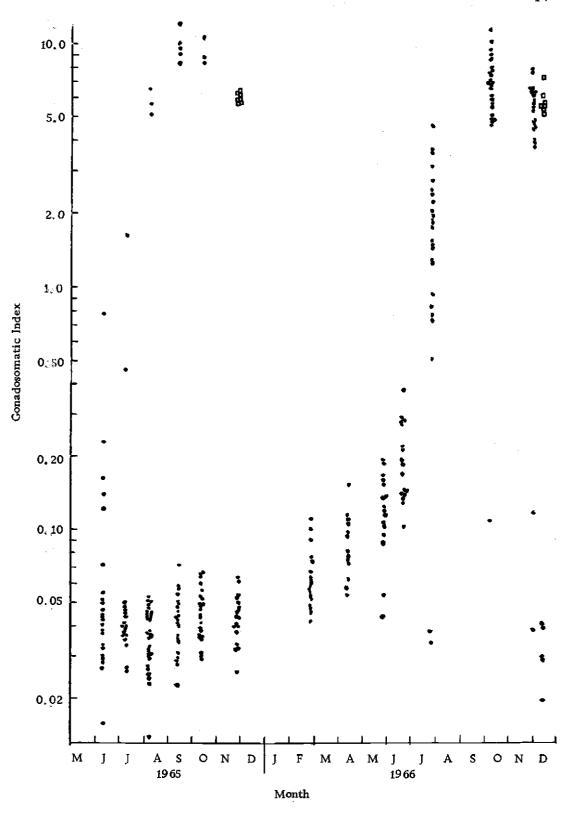


Figure 11. Gonadosomatic index of male coho reared in fresh water. (

(

- Sexually mature.)

borne out by their GSI (Figure 11). In salt water a greater number of males failed to mature following 20 months of rearing (total for all groups: 19 immature males in fresh water; 246 immature males in salt water).

Maturation of Adults in Salt and Freshwater Ponds

Hormone Diet

Following 35 months of rearing there was comparable survival in the hormone and control groups (Table 12). Although equal survival allof the two groups in fresh and saltwater ponds was noted, significant differences in the state of sexual maturity occurred (Table 12). More males matured in fresh water than salt water, and only half as many females matured in salt water as in fresh water. Very few three year old males remained sexually immature in fresh water but in the saltwater pond almost one-third of the males were still sexually immature after 20 months. Twenty to 30% of the females were sexually immature in fresh water whereas more than 55% were immature in salt water. Coho reared in fresh water were slightly smaller than their salt water counterparts but did not show as much variability in size (Appendix VI). The sexually immature fish were approximately 20 cm smaller than the mature fish.

The gonad weight comprised 16 to 19% of the total body weight in mature females but in males it comprised only 6 to 7%. There

Table 12. Maturity statistics of hormone-fed coho in salt and fresh water in December 1966. (Percentage of total).

		Fres	h water		Salt water				
	Hor	mone	Co	ntrol	Horn	none		ontrol	
	No.		No.		No.		No.		
	(%) 		(%)		(%)		(%) —————		
Mature Males	1 29	(42,9)	159	(50, 2)	118	(37.7)	128	(38, 0)	
Mature Females	106	(35.2)	127	(40. 1)	57	(18, 2)	74	(22, 0)	
Immature Males	10	(3.3)	1	(0,3)	51	(16.3)	45	(13.3)	
Immature Females	56	(18. 6)	30	(9.5)	87	(27.8)	90	(26,7)	
Total	301		317		313		337		

appeared to be no important differences in gonad weights between those that received the hormone diet as juveniles and the controls.

X-irradiation

No substantial difference was noted in survival to the "normal" adult state of the various irradiated and control groups. Similarly there is little difference between the total survivors in fresh and salt water although differences in state of sexual maturity occurred (Table 13). At least 85% of the coho matured in fresh water within each treatment group and control whereas only 55 to 65% matured in salt water (Table 13). Approximately 2% of the males in fresh water failed to mature whereas up to 16% of the males in salt water did not mature (Table 13).

Fish maturing in fresh water had a smaller mean size (FL = 36.9 cm) than those maturing in salt water (FL = 40.8 cm) Appendices VII and VIII].

Gonad weight expressed as a percentage of the total body weight in mature females ranged from 11 to 28% whereas in males it comprised only 3 to 8%.

Selected Large Smolts

Approximately equal numbers of large smolts survived the rearing in the salt and freshwater ponds. A total of 90% of the

Table 13. Maturity statistics of X irradiated coho in salt and fresh water in December 1966. (Percentage of total).

Sex	200 R		400 R		600 R		800]	R	0 R	
					Fresh wat	er				
Mature Males	1 03	(45,4)	1 07	(47.3)	101	(44. 1)	91	(45, 2)	100	(49.8)
Mature Females	101	(44.5)	101	(44.7)	114	(49.8)	80	(39.8)	76	(37.8)
Immature Males	1	(0,4)	4	(1,9)	-		2	(1.0)	-	
Immature Females	22	(9.7)	14	(6, 2)	14	(6.1)	28	(13.9)	2 5	(12, 4)
Total	227		226		229		201		201	
					<u>Salt wa</u>	ter				
Mature Males	70	(30. 6)	88	(38.3)	84	(40,8)	75	(36, 2)	73	(37.8)
Mature Females	61	(26, 6)	64	(27.8)	47	(22.8)	46	(19,3)	51	(26.4)
Immature Males	27	(11.8)	28	(12, 2)	26	(12. 6)	33	(15,9)	31	(16, 1)
Immature Females	71	(31.0)	50	(21.7)	49	(23, 8)	53	(25.6)	3 8	(19.7)
Total	229		230		206		207		193	

freshwater reared group matured compared to 78% of those reared in salt water (Table 14). Again, the freshwater group had a smaller mean size but showed less variability in size than the saltwater group (\$\overline{x}\$ FL= 39.4 cm:40.5).

Table 14. Maturity statistics of selected large coho maturing in salt and fresh water in December 1966. (Percentage of total)

	Fresh	water	Salt	water	
	Number	(%)	Number	(%)	
Mature Males	23	(40, 4)	31	(46.3)	
Mature Females	2 8	(49.1)	21	(31.3)	
Immature Males	1	(1.8)	5	(7/5)	
Immature Fema les	5	(8.8)	10	(14.92)	
Total	57		67		

Maturation of Adults of Ocean Release

Hormone Diet

Adult coho salmon of the 1963 brood were first checked on September 19, 1966 at the Big Creek weir. Adult male coho generally arrived first at the weir. At the end of October 1966, 50% of the males in the run had been recorded. The females were only a few days behind. By mid-November more than 60% of the adult females had been recorded (Appendix IX). The cumulative percentage return of the marked and unmarked coho recorded at the weir is presented in Appendix IX.

The control fish returning from the ocean were larger than those that received the synthetic estrogen as fingerlings (Figure 12). The control males were slightly larger than females, whereas males were slightly smaller than the females in the hormone group (Figure 12).

The marked adults (experimental fish) examined at the weir in 1966 showed approximately a 1:1 sex ratio for both treated and control groups (Table 15). This was significantly different from the return of hatchery production where the ratio of males to females was 1:1.7 (Table 15).

The 1966 adult escapement of 12,390 resulted in the recovery of 733 marked and 11,657 unmarked coho. Totals of 215 hormone and 518 control adults were recovered, representing returns of 0.49% and 1.25%, respectively, of the numbers released (Table 15).

The coho that received the hormone, diethylstilbestrol as juveniles produced less than one-half as many adults as the control group (0.49:1.25). But there is no significant difference in the adult male and female ratio between the two groups. These returns were minimal as they do not account for marked fish caught by sport fishermen. Also, during the peak of the adult migration the weir had to be closed for a number of hours on two days in order to clear the trap of the accumulated fish. Because of this closure, considerable numbers of adult and precocious males dropped back downstream,

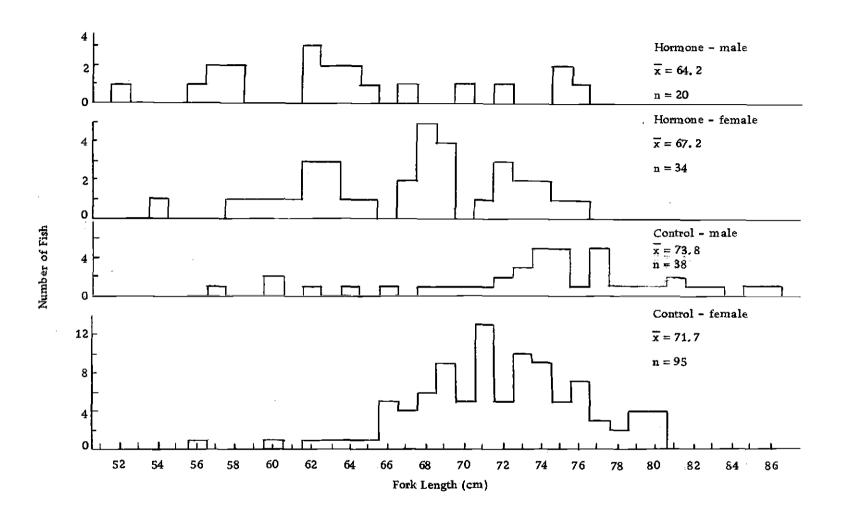


Figure 12. Length frequency distribution of ocean returning adults of the 1963 brood of different smolt origin.

Table 15. Sex composition and mean length of precocious and adult coho of the 1963 brood returning from the ocean.

Group	Age Class	5ex	No. Recov ered	Sex Composition (%)	Percentage of No. Released	Range	Fork Length (cm) Mean
Hormone 2	Precocious Males	2 55	100.0	0, 62	30. 5 -51. 5	40, 2	
	3	Adult Males	1 08	50. 2	0. 245	52,0-76,5	64.2
		Adult Females	1 07	49.8	0.243	54 .0- 76 . 0	67 . 2
Control 2	Precocious Males	1, 117	100.0	2,54	30.0-55.0	43.2	
	3	Adult Males	247	47.7	0, 60	57.0-86.0	73.8
		Adult Females	271	5 2, 3	0, 66	56.0-80.0	71.7
Hatchery 1 Production ¹	1	Precocious Males	15, 300	100,0	0.89 ² -1.8 ³	34.0-55.5	42,6
	• 3	Adult Males Adult Females	4, 51 2 7, 145		0.26^{2} - 0.52^{3} 0.41^{2} - 0.84^{3}	No data	

¹ A Release Split Between November, March and April.

 $^{^{\}mbox{2}}$ Return calculated on release of 1.74 million smolts.

 $^{^{3}}$ Return calculated on release of .84 million smolts.

where some spawning occurred. No estimates were made of the number of fish that spawned below the weir.

The percentage return of marked fish in Table 15 does not include those fish taken in the Columbia River commercial gill-net fishery, which was sub-sampled in late September and early October. During the sampling period 41 fish bearing Big Creek marks were noted among the 43,000 fish sampled. The Columbia River gill-net fishery was responsible for taking 409,500 coho salmon in 1966. Utilizing direct proportions this estimate would indicate that approximately another 390 marked fish would have returned to the hatchery if no fishery existed (95 hormone group: 295 control). Similarly another 54 hormones and 159 control coho were recorded in the Oregon and California commercial troll and sport fisheries. These numbers are generally expanded by a factor of 3 to approach the actual catch. However there was no reported sampling of the Alaska, British Columbia and Washington commercial troll and sport fisheries that would allow compilation of an estimate of the total number of adult coho produced by these experimental groups.

Size at Time of Release

The Fish Commission of Oregon gathered the data on the adult escapement of the 1964 brood coho and the material has been analyzed and published by A. K. Johnson (1970). In summary:

"The 1967 total adult escapement (11, 444) resulted in the recovery of 780 fin-marked and 10, 664 non-fin marked fish. A total of 424 (high ration) and 356 (low ration) adults was recovered, representing a return of 0.71% and 0.43% respectively of the numbers released.

Based on total numbers released there was no significant difference in percentage return of adult males between high ration (0.20) and the low ration (0.18); however, the return of the females from the high ration (0.51) was over twice that of the low ration (0.25); adult male to female sex ratios were 1:2.6 and 1:1.4 respectively (Appendix X). Analysis of variance indicated that adult males were significantly larger than adult females in both groups (P < 0.01), while the high ration precocious males, adult males, and adult females were each significantly larger than corresponding low ration fish (P < 0.01). (Appendix X).

Viability of Gametes and Offspring of Experimentally Treated Adults

Pond Reared

No apparent difference in survival of gametes and offspring to the "swim-up" fry stage was noted between the control and the experimental fish in the following paired groups of pond reared coho:

- group fed the diet containing hormone and reared in fresh water (Tables 16, 17, Appendix XI);
- group fed the diet containing hormone and reared in salt water
 (Appendix XII);
- group X-irradiated as juveniles and reared in fresh water
 (Tables 16, 17, Appendix XI);
- 4) group X-irradiated as juveniles and reared in salt water (Appendix XII).

Table 16. Viability in percent of offspring from coho treated with 800 R X-invadiation or fed a hormone in the diet as juveniles and reared to maturity in fresh water.

		Female	
	1	2	3
Hormone			
Male			
1	60,9	79.2	3.2
2	56,4	69. 1	9,6
3	38.5	72, 2	11.1
800 X-irradiation			
•	1	2	."3
1	26.3	46.5	66, 5
2	34.1	54.8	57.3
3	30. 6	50.8	57.2
Control			
	1	2	3
1	71,4	27.5	53,6
2	77,6	50.3	61.7
3	62,0	17.1	61, 4

Table 17. Viability of offspring from coho treated with X-irradiation or fed a hormone in the diet as juveniles and reared to maturity in fresh water.

Treatment	Number of Eggs	Number of Unfertilized Fggs ¹ (%)	Number of "Swim-up" Fry	Percentage Survival to "Swim-up" Fry
200 R	9647	635 (6. 6)	8730	90.5
400 R	7573	554 (7.3)	6727	88.8
600 R	7582	424 (5. 6)	6 9 85	92. 1
800 R	4865	3 33 (6.8)	4319	88.8
Hormone	8526	753 (8.8)	7510	88.1
Control	7769	765 (9.8)	6545	84.2

¹ Includes all non-eyed eggs.

The eggs from 10 to 15 females from each of the hormone group, four X-irradiated groups, and control group incubated at Big Creek showed equal survival to the "swim-up" fry stage (Table 17). All groups survived better than those eggs brought to Corvallis for incubation.

Eggs obtained from the fish reared in salt water showed extremely poor survival (0 to 17%) to the "swim-up" fry stage in the hormone, X-irradiated and the control groups (Appendix XII). This low survival is similar to results obtained by A. Novotny (personal communication) in saltwater ponds at Manchester, Washington. He obtained approximately 3% survival to the "swim-up" fry stage from coho reared to maturity in salt water.

Ocean Reared

Eggs from adults that received the hormone, diethylstilbestrol, as juveniles showed a higher survival to the "swim-up" fry stage than the control group of eggs (Table 18, Appendix XIII). However, poor survival was experienced in the top trays of the incubator where one-quarter of the control eggs were located, probably due to leakage of light or supersaturation of nitrogen. Eggs incubated at Big Creek (hormone and control group) showed no difference in survival (> 90%) to the "swim-up" fry stage.

Table 18. Viability in percent of offspring from ocean returned coho fed a hormone in the diet as juveniles.

		F	emale	
	1	2 	3	4
Hormone				
Male				
1	25.9	9 2. 6	76,7	63.5
2	87.8	67.7	86.3	63.2
3	86,5	92.2	86.0	73.3
4	90.0	90.5	87.3	74, 2
Control	•			
Male				
1	9,9	92.3	81.4	80, 6
2	72.5	90.6	28.9	4.5
3	49.8	83.5	27.1	44.8
4	46.3	74.4	63.1	7.7

DISCUSSION

Hormonal Suppression of Maturation

Juvenile coho salmon that received the hormone, diethylstilbestrol, in their diet and were released for ocean rearing returned only one-quarter as many precocious males as the control. Those retained and reared in fresh and saltwater ponds produced half as many precocious males as the control. The coho fed the hormone were smaller and looked similar to parr at release time raising the thought that the smaller size influenced the number of coho maturing precociously. But, fewer precocious males resulted from the group fed the diet containing the hormone than hatchery production smolts which were released at a smaller size, as well as a month earlier. Coho fed the hormone appeared smolt-like within 10-14 days following removal from the hormone diet even though the presence of the hormone was still detectable in the tissues (Hisaw, 1959).

Depression of gonadal activity in terms of tissue growth and development is postulated for the reduction in early maturation of coho salmon fed the hormone diethylstilbestrol as juveniles. No sex reversals or histological changes in the gonad were noted in sampled fish. My results support the findings of Ashby (1956, 1965) and Svardson (1943) who both noted that steroid hormones retarded gonad

development and arrested growth. Neither author found evidence of sexual inversion. Yamamoto (1969) however, ascribes Ashby's results to the fact that administration of the hormone to the trout alevins was not started until gonadal differentiation had already been established.

McShan and Meyer (1946) showed that diethylstilbestrol is an effective inhibitor of the succinoxidase system of liver and pituitary tissue. This might explain why I found lowered precocious development in those males that received the hormone diet as well as reduced growth. The pituitary was probably inhibited from its normal endocrine role. Hishida (1965) administered radioactive diethylstilbestrol (monoethyl-1-¹⁴C) to medaka (Oryzias latipes) and showed that it was selectively incorporated into the juvenile gonad. Since this study, Hoar et al., (1967) used Methallibure as a chemical blocking agent of gonadotropic functions. Their conclusions follow:

"it acts in a manner comparable to that which follows surgical hypophysectomy. Spermatogenesis is either completely suspended or greatly depressed. The pituitary-gonadal relations were investigated and in general the findings were consistent; the pituitary regulates both gametogensis and steroidogensis."

Pandey (1970) also found that Methallibure completely blocked production and release of gonadotropins in juvenile guppies, <u>Poecilia</u> reticulata Peters, but did not completely block production and/or release of pituitary gonadotropins in the adult guppy.

Diethylstilbestrol did not appear to depress survival of coho reared to maturity in salt and freshwater ponds, but an increased mortality of coho fed the hormone was noted for fish returning from the ocean. Adult escapement to the hatchery of the hormone group was only 30% of the control. Had the diethylstilbestrol treatment functioned in the desired manner a greater return of adult fish would have been anticipated.

Increased ocean predation is only one of the probable causes of lower survival of ocean released fish. In the saltwater rearing pond approximately 15 to 20% of all groups of coho failed to adjust. Some became very emaciated; others looked healthy but did not grow. In the ocean similar fish would readily succumb to predators or other factors of natural mortality. Because a number of fish exhibited poor physical condition and comparable growth was not attained in the fresh and saltwater ponds research into the diet of fish being reared past the normal release time is necessary. The Oregon Moist Pellet is excellent for production of juvenile fish but does not appear to meet the nutritional requirements of fish being reared to adulthood in fresh and saltwater ponds.

There was no significant difference in the gonadosomatic index of coho from the hormone and control groups; however, the mean length of control fish was significantly greater than the hormone group. The hormone appeared to inhibit the coho from attaining

Eggs obtained from the diethylstilbestrol treated fish showed no adverse effects to the "swim-up" fry stage but, the females that received the hormone as juveniles were significantly smaller than the control fish and had fewer and smaller eggs. This fact along with reduced size and ocean survival could conceivably eliminate any advantage from the hormone due to gonad retardation.

Effect of X-irradiation

X-irradiation of presmolt coho salmon (200 to 800R) did not inhibit early sexual maturation. Equal numbers of irradiated and control fish matured. Irradiated males that were sacrificed for histological examination of the testes showed no damage to spermatogonia or disruption of spermatogensis. The first samples however, were not collected until three to four months following X-irradiation and regeneration of affected cells may have taken place. No long term effects of X-irradiation were found in coho reared in fresh and salt water since equal numbers of experimental groups were recovered after 35 months of rearing. Survival of the "swim-up" fry stage of eggs taken from irradiated adults was similar to that of the control.

Solberg (1938), however, noted an inhibitory effect on the formation of spermatocytes. Follenius (1952a, b) X-irradiated young

Lebistes reticulatus with 6000R and found complete sterilization of the testes, but sterilization did not hinder differentiation of the secondary sexual characteristics. Ulrikson (1969) utilizing cobalt-60, irradiated bluegills (Lepomis macrochirus) with 1000 to 10,000R and noted temporary sterility in male fish. He also noted that survival time decreased with increased exposure. By 120 days the males started to produce sperm. Ulrikson further noted that 1000R was a sublethal dose but did not greatly decrease the number of sexual products produced.

In subsequent short term studies carried out at Oregon State University, coho salmon juveniles were irradiated with 800, 1100, 1400, and 1700R of X-rays. Nearly all coho died after being treated with 1400 and 1700R and held in fresh or saltwater tanks. Histopathological changes were noted in intestine and gill with severe damage to gill lamellae. Testes of these fish (1400 and 1700R) showed severe damage to primary and secondary spermatogonia, and often complete degeneration of all cells. Testes of coho irradiated with 800R showed no deleterious effect and their post-treatment condition approximated that of the controls. Conte (1965) noted that 1000R was about the LD₅₀ for irradiated coho transferred to salt water. Thus, X-irradiation is not a successful means of controlling precocious maturation in coho.

Size at Time of Release

Natural and hatchery populations of coho with rapid freshwater growth often contain substantial numbers of precocious males. Willis (1963) reported that release of six broods of coho in Gnat Creek (Oregon) produced returns of 52 to 74% precocious males. Morgan and Henry (1959) reported that 45% of the returning coho to the Tenmile Lake system (Oregon) were precocious males during the 1955 to 1956 spawning season. Their observations indicated that smolts from the lake system were substantially larger than smolts from adjacent stream systems.

Selected large smolts removed from hatchery production showed a preponderance of males within the group. Fish Commission of Oregon (1957) personnel also showed that in a given group of graded coho fingerlings, 60 to 80% of the larger individuals were males. It is well established that the number of scale circuli is related to length of salmonids, with larger fish having greater number of circuli. Fish Commission biologists examined scales of coho from several broods returning to Klaskanine hatchery and noted that precocious males resulted from fingerlings with the greater number of freshwater circuli than normal adults (Fish Commission of Oregon, 1958). This suggests that precocious males were produced by the larger juveniles.

Early sexual maturation appears to be a combination of genetic make-up and potential for rapid growth. Alm (1959) noted that fish that showed the most rapid growth often also matured at an earlier age. Schmidt et al., (1965) noted that a greater response was achieved in large immature male Salmo gairdneri for a given dose of adult salmon pituitary extract. Mastin (1955) found that those female coho returning to Bandon hatchery after only one summer in the ocean were 8 inches or longer when released as smolts. Fish smaller than 7 inches spent two summers in the ocean whereas the intermediate smolts (7 to 8 inches in length) returned following one or two summers of ocean residence. Graybill (personal communication) has shown the importance of the female's contribution to early maturing males. Further analysis of his data will indicate whether progeny of females that produced the majority of precocious males also have high overall survival of their progeny and thus make a significant contribution to the coho population.

The 1964 brood coho on the high ration had a greater escapement of precociously mature males (1:0.08) and adults (1:0.61) than the low ration groups. This supports the reports of earlier workers who noted that a relationship appeared to exist between the size of smolt, at time of release, and the percentage of precociously mature males as well as survival to adulthood (Jeffries, 1959; Kelez, 1937; Noble, 1958; Salo and Bayliff, 1958; Wallis, 1968). It has also been

noted that the best adult returns have been from smolts released during the normal downstream migration period (March to June).

November releases have generally failed to return many adult fish (Salo and Bayliff, 1958; Wallis, 1968). The percentage return of adult males from the two groups was approximately equal but twice as many females of the high ration group returned as adults as did those of the low ration group. As many males of the high ration group matured precociously, possibly a comparably survival and escapement of adults, as witnessed in the females, would have been observed had fewer matured precociously.

Wallis (1968), summarizing five Fish Commission of Oregon experiments, noted that larger fingerlings produced greater returns of precocious males than comparable smaller-size groups. In four of the five experiments the larger-sized fingerlings (15 to 20 per pound compared to 18 to 75 per pound) also produced more adult fish; the exception was the Big Creek stock of the 1953 brood that involved graded fish with a much larger size differential than those in the other experiments (15 per pound and 7 per pound). Wallis believes that this grading selected a greater proportion of males and because larger sized fish tend to mature earlier a greater proportion of this group returned as précocious males.

Equal numbers of selected large smolts matured precociously when held in the fresh and saltwater ponds. In other control groups

only half as many matured in salt water as in fresh water. Early maturation is apparently largely determined prior to downstream migration. Early sexual maturation appears to be expressed if the rate of growth has been adequate or some critical size reached. Of the selected large smolts, because of genetic make-up and exceptional early freshwater growth, all those predestined to become precocious males did so even when placed in saltwater ponds. In the other control groups only those predestined males that had attained the necessary releasing factors upon being placed in salt water matured precociously, whereas in fresh water all males with the genetic potential probably matured precociously because of the better growth and lack of physiological stress.

Coho that matured precociously in all experimental groups, in the salt and freshwater ponds November 1965, were larger than the immature fish sampled at the same time. Similarly, those males that indicated they would have probably matured precociously (high GSI values) in the monthly samples were generally always larger than "immature" males sampled at a comparable time. In both the 1963 and 1964 brood years the highest precocious return and adult escapement resulted from the largest smolts. Smolts released from the 1964 brood were larger, returned a greater percentage of precocious males, but had a smaller adult escapement than the control group of the 1963 brood.

Suggested Experiments for Future Investigation

If further work on control of maturation is considered it should include chemical inhibitors of the pituitary as one of the areas of research. Hoar and co-workers found that Methallibure greatly depressed or completely suspended spermatogenesis in a number of fish. Similarly, diethylstilbestrol used in this study appeared to control development of the testes although it also retarded growth. Reduced amounts and earlier incorporation into the diet, as well as use of other estrogenic substances could be investigated and prove beneficial.

Precocious maturation appears to be genetic as well as size dependent. Graybill (personal communication) has data indicating that the heritability contributions from certain female coho and also precocious male coho are important factors in early maturation. In this study I noted that selected large smolts were predominantly males and that a large segment (30%) of these larger smolts matured precociously. I found also that many of the male fish showing the most rapid rate of growth matured precociously. When the fish reaches a critical size the pituitary apparently responds by releasing gonadotropins that initiates the maturation process. We do not know whether size per se or rate of growth initiates this activity of the pituitary.

Early maturation is most prevalent in those areas where there are hatcheries with favorable water temperatures for growth or a productive stream or lake system, capable of producing a rapidly growing large smolt. In hatchery operations early maturation could possibly be controlled by grading in the first summer and removing the upper 10 or 15% of the largest fish, which are often predominantly males - the individuals that contribute most to early maturation.

Size can also be controlled through feeding. With the diets presently in use many hatcheries find it possible to produce large juveniles (30 to 60 per pound) by early summer and then feed little more than a maintenance diet until the following spring.

Hatchery smolts must be released at a larger size than natural stream produced smolts to achieve a good return of adults. Presently hatcheries often feed at an intermediate or a high ration to obtain an initial rapid growth and release smolts averaging 15 to 20 per pound. This practice results in a large number of precocious males but also a fair adult escapement. Until a demand can be created for these precociously maturing fish, hatcheries will remain confronted with the problem especially since precocious males have a higher survival because of shorter ocean residence than normal adults. Therefore, it would probably be advantageous to: 1) Adjust the feeding schedule so the initial growth of juveniles would be less and smolts would reach release size slowly. Thus if rapid initial growth

activated the endocrine system that produced precocious males the problem would be abolished. 2) Manipulate the spawning time and incubation temperature so that "swim-up" fry would be obtained as early as possible. Then through intensive feeding and use of artificial light, smolts could be produced that would migrate to the ocean in their first year of life. If these smolts returned as "normal" adults after two years in the ocean the life-cycle would have been shortened and three rather than two broods every six years could be achieved.

Wagner (1971) found that photoperiod was the main environmental factor controlling the parr-smolt metamorphosis in steelhead trout. He also presented data that suggests that more male fish (steelhead, S. gairdneri) matured in the reverse (30%) and accelerated (35%) photoperiod than in the natural or the decelerated photoperiod (0 to 1.5%) causing him to suggest (personal communication) that a period of decreasing photoperiod prior to smoltification may be one of the stimuli to the pituitary that can bring about the release of gonadotropins that eventually results in precocious maturation.

Garrison (unpublished data) and Donaldson (1969) both released 0-aged coho smolts in June at a size comparable to normal stream smolts and neither reports the return of precocious males although fair numbers of adults were recovered. These smolts were only subjected to natural increasing photoperiod before release.

In conclusion, with the modern facilities and diets available

today various hypotheses could be readily tested. Potential areas of research are: 1) chemical inhibition of the pituitary or use of steroid hormones; 2) manipulation of the diet to reduce rapid initial growth; 3) removal of the largest parr (upper 10-15% of size group) during first summer of growth; 4) identification of the females responsible for most precocious males in the population and exclusion of them from spawning if their progeny do not make significant contributions to adult escapement; 5) manipulation of the time of spawning and incubation temperature to reduce the time required to obtain "swim-up" fry, so that smolts could be produced through intensive feeding in their first year of life. These potential areas of research could be investigated individually or in combination.

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Appendix I. Size composition of juvenile coho salmon of the 1963 brood in October 1964 and their planned experimental use.

		Pond		
	28	29	30	
Number of pounds of coho fingerlings	2474	2320	1509	
Fish per pound	18.9	19.0	19.2	
Number of coho fingerlings (estimate)	46, 699	44, 191	28, 974	
Treatment	Group to receive hormone incorporated diet	Group to receive normal diet-control	Group to provide irriates, control and selected large smolts	

Appendix II. Statistics for 1963 broad coho reared to maturity in fresh water.

	_	_				Hormone		Selected large	
	200 R	400 R	600 R	800 R	Control	diet	Control	<u>smolts</u>	Total
Number of smolts	833	815	788	794	676	1,000	1,000	200	6, 106
Deaths to November 22, 1965	26	16	32	35	12	37	30	8	196
Samples to	53	45	5 1	48	47	50	47		341
November 1965			÷	e e e	V		:		
Total precocious males	56	61	36	65	39	39	84	32	412
Precocious males of smolts retained (%)	6.72	7.48	4,57	8.18	5.77	3,90	8.40	16.0	
Percentage precocious of male smolts	14.1	14.6	8.6	16.6	11.9	8.1	17.4	29,9	
Deaths from November 22, 1965 to November 23, 1966	401	395	377	381	325	447	448	96	2 _s .870
Samples to December 1966	65	60	62	60	52	76	63	2	440
Survivors								-4	
Mature	204	208	215	171	176	235	286	51	1, 544
Immature	23	18	14	30	2 5	66	31	6	226
Questionable marks									56
Number of fish accounted for	82 8	803	787	<i>7</i> 90	676	950	989	195	
Inadvertantly discarded at hatchery									23
Unaccounted loss									9

Appendix III. Statistics for 1963 brood coho reared to maturity in salt water.

	200 R	400 R	600 R	800 R	Control	Hormone diet	Control	Selected large smolt	Totals_
Number of s molts	750	750	777	761	650	1, 000	1,000	200	5, 888
Deaths to November 19, 1965	37	3 8	56	63	38	85	63	11	391
Samples to November 1965	57	58	63	64	55	63	57		417
Total precocious males	22	27	26	25	28	16	30	29	203
Precocious males of smolts retained (%)	2,93	3,60	3, 34	3.28	4.31	1, 60	3,00	14.5	
Percentage precocious of male smolts	6, 0	7.1	6.6	6, 3	7.8	3, 1	6.0	23, 6	
Number of survivors	632	62 5	628	607	5 2 5	830	847	157	4,851
Deaths from November 22, 1965 to November 30, 1966	332	318	335	320	267	386	430	92	
Samples to December 1966	56	59	58	62	54	61	59		
Survivors Mature	131	152	131	121	124	175	202	50	
Immature	98	78	75	86	69	138	135	15	
Number of fish accounted for	733	730	7 44	741	<i>6</i> 35	924	976	197	5, 860

Unaccounted loss (probably due largely to cannibalism)

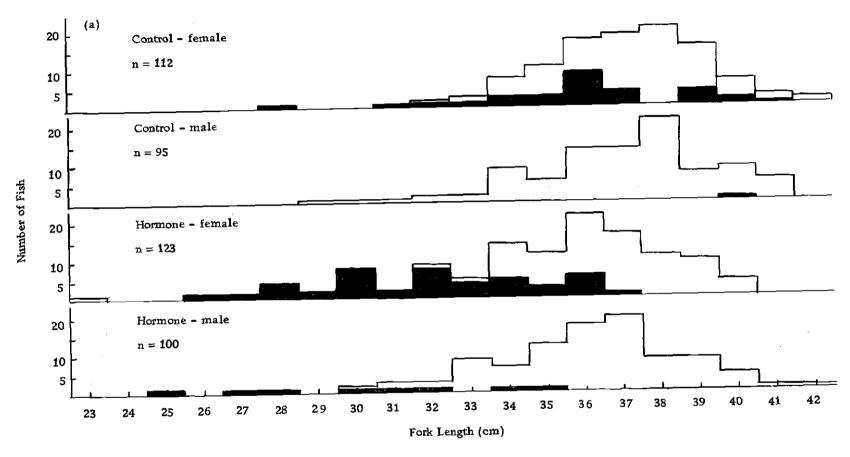
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Appendix IV. Gonadosomatic index of male coho reared in fresh water from June through November 1965.

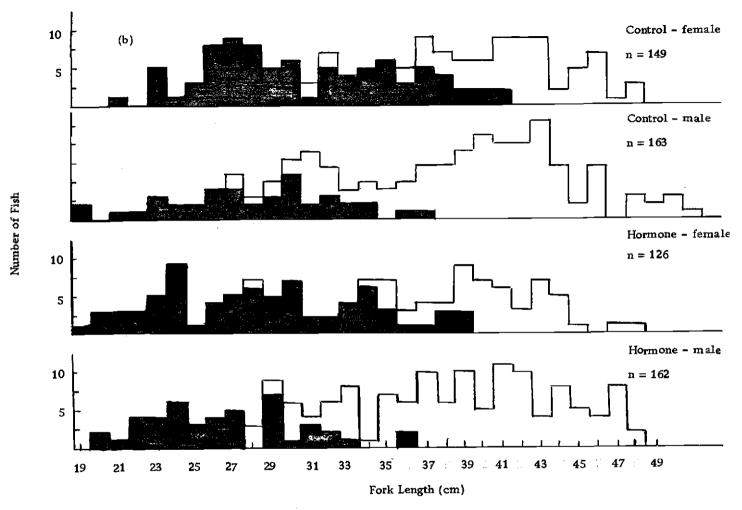
	Experi-	Length	Weight	Gonad	GSI	X fork	X GSI
	mental	(cm)	(g)	Weight		length	
	Group			(g)		of im-	
						matures	
June	400 R	19.3	76.9	0.57	0,74	16.6	0,03
	400 R	18.6	73.5	0.17	0, 23	10.0	0,00
	control	18.0	64.9	0.09	0, 14	8	
	control	19.8	77.7	0.13	0. 17	17.5	0.041
	contr ol	20, 5	90.4	0, 12	0,12		
July	400 R	21.2	107.7	1,72	1.59	18.9	0.03
-	control	21.8	123. 8	0, 57	0,46	17. 6	0.04
August	200 R	24.6	196.4	11. 19	5,70	19.6	0,04
Ŭ	400 R	24.2	201.1	10. 17	5.06	21.7	0.04
	800 R	25.5	258.8	16.94	6, 62	20.4	0, 04
September	400 R	24.3	257, 1	31.52	12, 26	23.5	0.05
•	800 R	23.1	208.5	20, 24	9.71	22,8	0.04
	800 R	23.9	203.5	17. 19	8,45	22.0	0.04
	Hormone	2 5.5	286.5	25.96	9.06	22.3	0,04
	control	23.8	199.2	20.00	10.04	22.6	0,04
October	400 R	26.1	298.5	32, 27	10.81	24.1	0,04
	800 R	26.3	267.2	22, 19	8,3	25.0	0.04
	control	27.5	309.0	27, 23	8.81	24.3	0, 05
November	(means of	sacrificed p	recocious mal	es)			
	200 R	29.5	324.3		5.74	27.9	0, 04
	400 R	29.5	327.2		5.96	27.3	0.04
	800 R	28.7	302.6		5.85	27, 2	0.04
	control	28.9	306.0		5 . 79	25.0	0,05
	hormone	28.1	285.6		6, 49	25.9	0.04
	control	28.5	299.1		6, 24	28.7	0,04

Appendix V. Gonadosomatic index of male coho reared in salt water from May through November 1965.

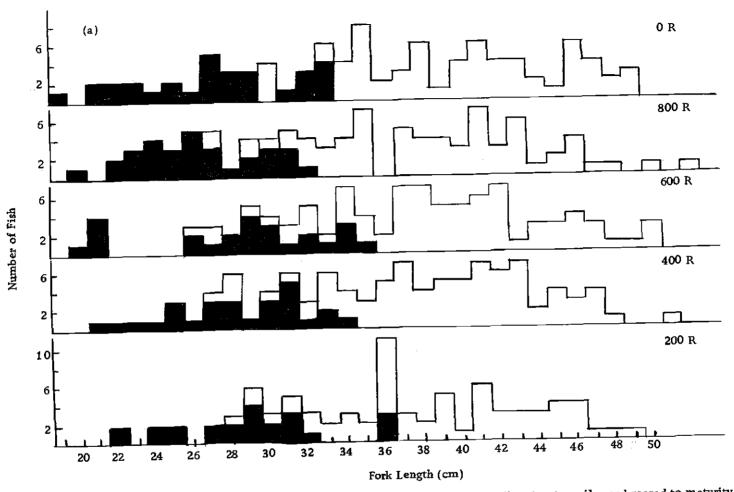
	Experi- mental Group	Length (cm)	Weight (g)	Gonad Weight (g)	GSI	X Fork Length of im- matures	X GSI
May						_	
•	200 R	16.9	48.9	0.095	0. 1 9	15.9	0,04
	200 R	17.8	58.7	0.11	0, 19	20.7	
June							
	800 R	19,1	74. 2	0.16	0, 22	16.1	0.06
	control	18.5	60,5	0, 27	0,45	16.9	0.05
j uly							
	200 R	1 9. 6	83.3	0, 17	0. 20	18.0	0.05
	400 R	20, 2	90.5	0, 23	0, 25	17.4	0, 04
August							
	200 R	22.0	135.6	8, 18	6.03	18.9	0, 06
	800 R	20, 0	100, 6	4.34	4.31	19.3	0.06
September							
	800 R	24.2	197.6	16.71	8.45	21,7	0, 05
	control	24.8	220, 2	22, 23	10.09	20.7	0, 05
November	(Means of	sacrificed p	recocious mal	es)			
	200 R	24.4	178		5.45	23, 0	0.08
	400 R	23.8	168.9		5, 20	22,8	0.06
	800 R	24.2	173.1		5,26	23, 2	0.04
	control	25.2	192.3		5. 58	24.2	0.05
	control	26, 1	220.7		5.71	23.0	0, 05



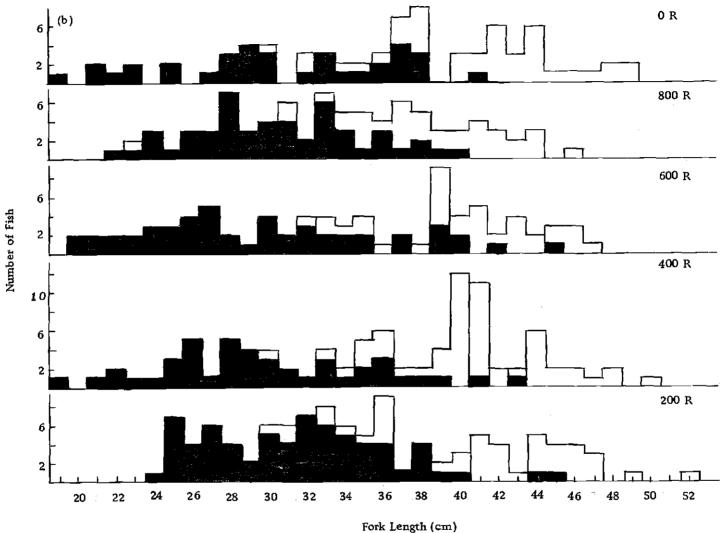
Appendix VI. Length distribution of mature and immature "adult" coho, fed a hormone diet as juveniles, and reared to maturity in: a) fresh water; b) salt water. (Solid area denotes sexually immature fish.)



Append ix VI. Continued

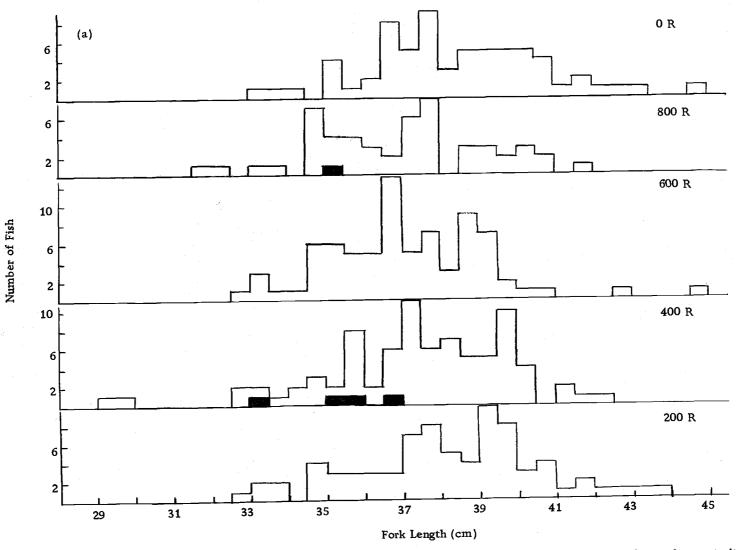


Appendix VII. Length distribution of mature and immature "adult" coho X-irradiated as juveniles and reared to maturity in salt water: a) males; b) females. (Solid area denotes sexually immature fish.)

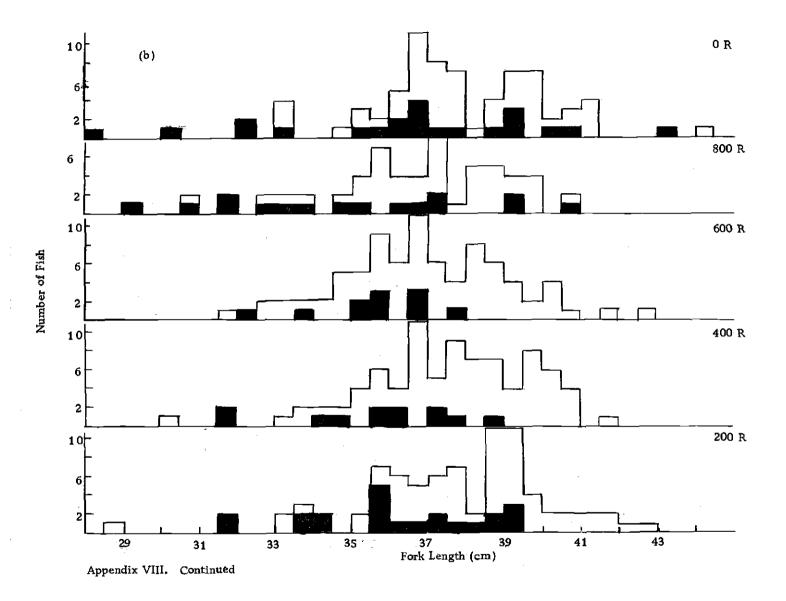


Appendix VII. Continued

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Appendix VIII. Length distribution of mature and immature "adult" coho X-irradiated as juveniles and reared to maturity in freshwater: a) males; b) females. (Solid area denotes sexually immature.)



Appendix IX. Number of adult male and female coho of the 1963 brood returning from the ocean in 1966.

	ocean in 1966	·				
			Male		_	
Date	Total	Accumu-	Total	Ac cumu-	Total	Ac cumu-
1966		lative (%)	Control	lative (%)	Hormone-	lative (%)
		<u> </u>			fed	
9-17	3	.06				
9-18	5	.10				
9-20	7	. 15				
9-21	10	.21				
9-22	13	.27				
9-23	17	.36				
9-24	21	.44				
9∺25	24	.51				
9-27	32	. 68				
9-30	35	.74				
10-3	36	.76				
10-6	38	.80	1	.4		
10-10	46	.97				
10-20	467	9,9	3	1.2	1	.9
10-21	1034	21.9	37	15.0	12	11.1
10-22	1229	26.0	49	19.8	1 5	13,9
10-23	1301	27.5	50	20, 2		
10-24	1426	∍30, 26	53	21.5		
10-25	1577	33,4	71	28.7	23	21.3
10-26	2016	42.6	89	36.0	30	27.8
10-27	2141	45.3	103	41.7	34	31.5
10-28	2241	48.4				
10-31	2418	5 1. 2				
11-1	2475	5 2. 4	105	43.5		
11-2	2480	52.5	109	44.1	36	33.3
11-4			113	45.7		
11-9	2487	52.6				
11-10	· 2583	54.6	114	46.2	37	34.3
11-12	3039	64.3	138	55.9	42	38.9
11-14	3501	74.1	158	64.0	54	50.0
11-15	3931	83. 2	193	78, 1	74	68.5
11-16	4056	85.8	198	80.2	79	73.2
11-17	4095	86.6	201	81.4	80	74.1
11-18	4116	87.1			81	75.0
11-20	4259	90.1	202	81.8	82	76.0
11-21	426 8	90.3				
11-23	4280	90.5			_	
11-28	4439	93.9	217	87.9	91	84.3
12-1	4630	97. 9	237	97.0	102	94.4
12-2	4663	98.6	242	98.0	103	95.4
12- 5	4700	99.4	244	100	105	97.2
12-8	4711	99.7	246	e -	106	98.1
12-12	4721	99.9	247	100	107	99.1
12-15	4726	100			108 108	100 100
12-16		100	247			

Appendix IX. Continued

			<u>Fer</u>	nales		
Date	Total	Accumu-	Total	Ac cumu=	Total	Ac cumu-
1966	e .	lative (%)	Control	lative (%)	Hormone- fed	lative (%)
9-17						
9-18						
9-20						
9-21						
9-22	2	.03				
9-23	3	.04			1	.9
9-24	4	.05				
9-25	5	.07				
9-27	23	.30	1	.4	2	1.9
9-30	24	.31				
10-3	26	.34			3	2.8
10-6	27	.35				
10-10	33	.43			4	3.7
10-20	2 85	3.7	3	1.1	5	4.7
10-21	883	11.5	22	8.1	15	14.0
10-22	1 108	14.5	31	11.4	18	16.8
10-23	1193	15.6	32	11.8		
LO-24	1318	17.2	38	14.0	19	17.8
10-25	1489	19.4	44	16.2	31	29.0
10-25 10-26	2252	29.4	65	24.0	35	32.7
10-27	2402	31.3	82	30.3	39	36.4
10-28	2502	32.7	02	50,5		- •
10-23	2897	37.8	86	31.7		
10-31 11-1	2912	38.0	95	36.1	41	38.3
11-2	3270	42.7	105	38.7	42	39.3
11-2 11-4	3274	42.7	100	55.		•
11 - 4 11 - 9	3332	43.5				
	3654	47.7	114	42.1	45	42.1
11-10	4816	62.8	145	53.5	60	56 . 1
11-12	5663	73.9	183	67.5	71	66.4
11-14 1 1-1 5		84. 1	217	80.1	86	80.4
11-15 11-16	6442 6704		217 237	87.5	87	81.3
	6704	87.5	237 241	88.9	88	82.2
11-17	6 76 6	88.3	241	90.7	88	82.2
11-18	6814	88.9	254	92.6	92	86.0
11-20	6999	91.3	251	93.4	32	00.0
11-21	7133	93.1	253	95.4	93	87.0
11-23	7135	93.1	864	07.4		93.4
11-28	7353	9610	264	97.4	100	100
12-1	755 1	98.5	271	100	107	100
12-2	7585	99.0			107	
12-5	7635	99.6				
12-8	7656	99.9				
12-12	7 659	99.9				
12-15	7662	100	_	4.0-	40=	400
12-16	7 663	100	271	100	107	100

Appendix X. Sex composition and mean length of precocious and adult coho of the 1964 broad that originated from large and small smolts achieved by feeding two levels of food. 1

Age Class	=	Number recovered	Percentage of number recovered	Percentage of number rele ased	Fork length (cm)				
					Range	Mean	Standard error		
2	Precocious Males	1, 590	1 00. 0	2, 65	35.0-54.5	45.4	0.23		
3	Males	117	27.59	0, 20	59.0-85.0	74.1	0,58		
	Females	307	72.41	0.51	54.0-82.0	70,7	0.32		
2	Precocious Males	150	100.0	0. 18	37.0-46.5	41,6	0.67		
3	Males	151	42, 42	0. 18	57.0-85.0	72.5	0.52		
	Females	205	57.58	0, 25	50.0-79.0	68.9	0.37		
	Class 2 3	Class Sex 2 Precocious Males 3 Males Females 2 Precocious Males 3 Males	Class Sex recovered 2 Precocious Males 1,590 3 Males 117 Females 307 2 Precocious Males 150 3 Males 151	Age Class Number recovered of number recovered 2 Precocious Males 1,590 100.0 3 Males 117 27.59 Females 307 72.41 2 Precocious Males 150 100.0 3 Males 151 42.42	Age Class Number recovered of number released of number released 2 Precocious Males 1,590 1 00.0 2.65 3 Males 117 27.59 0.20 Females 307 72.41 0.51 2 Precocious Males 150 1 00.0 0.18 3 Males 151 42.42 0.18	Age Class Number recovered of number recovered of number rele ased Range 2 Precocious Males 1,590 1 00.0 2.65 35.0-54.5 3 Males 117 27.59 0.20 59.0-85.0 Females 307 72.41 0.51 54.0-82.0 2 Precocious Males 150 1 00.0 0.18 37.0-46.5 3 Males 151 42.42 0.18 57.0-85.0	Age Class Number recovered of number released of number released Range Mean 2 Precocious Males 1,590 100.0 2.65 35.0-54.5 45.4 3 Males 117 27.59 0.20 59.0-85.0 74.1 Females 307 72.41 0.51 54.0-82.0 70.7 2 Precocious Males 150 100.0 0.18 37.0-46.5 41.6 3 Males 151 42.42 0.18 57.0-85.0 72.5		

¹Data on adults from A. K. Johnson, Fish Commission of Oregon.

Appendix XI. Viability of gametes and offspring from coho treated with 800 RX-irradiation, or fed a hormone in the diet as juveniles and reared to maturity in fresh water.

						Female				
ale	Treatment	Number of Eggs	1 Number Unfertilized Eggs. ¹	Number "Swim-up" Fry (%)	Number of Eggs	Number Unfertilized Eggs ¹	Number "Swim-up" Fry (%)	Number of Eggs	3 Number Unfertilized Eggs ¹	Number "Swim-up" Fry (%)
1	Hormone Diet	271	89	155 (60,9)	318	57	252 (79, 2)	154	137	5 (3.2)
2		250	97	124 (56, 4)	291	60	201 (69.1)	230	144	22 (9,6)
3		117	67	44 (38. 5)	216	46	156 (72, 2)	225	175	25 (11.1)
1	800R X-irradiation	110	65	29 (26, 3)	299	30	139 (46.5)	236	71	157 (66.5)
2		132	82	45 (34.1)	206	32	113 (54.8)	211	87	121 (57.3)
3		186	120	57 (30. 6)	181	53	92 (50 _. 8)	264	111	15 1 (57.2)
1	Control	269	35	192 (71.4)	302	81	83 (27.5)	209	74	112 (53,6)
2	v.	179	29	139 (77.6)	314	89	158 (50. 3)	201	72	124 (61.7)
3		166	22	103 (62.0)	316	111	54 (17. 1)	189	46	116 (61.4)

¹ Includes all non-eyed eggs.

Appendix XII. Viability of gametes and offspring from coho treated with 800R X-irradiation or fed a hormone in the diet as juveniles and reared to maturity in salt water.

			1			Female 2	3				
Wale	Treatment	Number of Eggs	Number Unfertilized Eggs ¹	Number "Swim-up" Fry (%)	Number of Eggs	Number Unfertilized Eggs 1	Number "Swim-up" Fry (%)	Number of Eggs	Number Unferti liz ed Eggs ¹	Number "Swim-up" Fry (%)	
1	Hormone Diet	413	408	0 (0)	365	354	0 (0)	30 6	205	44 (14.4)	
2		390	374	3 (0,8)	320	279	2 (0, 6)	336	2 23	59 (17. 5)	
3		317	317	0 (0)	274	264	:3 (1.1)	392	338	39 (9,9)	
1	800R X-irradiation	2 46	242	3 (1.2)	242	242	0 (0)	153	140	2 (1,3)	
2		22 6	223	0	374	374	0 (0)	203	200	1 (0,5)	
3		427	417	3 (0,7)	393	393	0 (0)	320	318	2 (0, 6)	
1	Control	280	270	3 ((1, 1)	411	387	4 (1.0)	333	316	2 (0, 6 ₎	
2		518	494	7 (1,35)	534	347	61 (11, 4)	352	315	6 (1, 7)	
. 3		436	435	1 (0, 2)	601	531	25 (4,2)	370	337	17 (4. 6)	

Appendix XIII. Viability of gametes and offspring from ocean returned coho fed a hormone in the diet as juveniles.

Male	Treatment	Number of Eggs	1 Number Unfertilized Eggs ¹	Number "Swim-up" Fry (%)	Number of Eggs	2 Number Unfertilized Eggs ¹	Female Number "Swim-up" Fry (%)	Number of Eggs	3 Number Unfertilized Eggs ¹	Number "Swim-up" Fry (%)	Number of Eggs	4 Numb <i>e</i> r Unfertilized Eggs ¹	Number "Swim-up" Fry (%)
i	Hormone Diet	618	80	160 (25,9)	798	35	739 (92.6)	767	44	581 (75,7)	535	42	340 (63, 5)
2 .		735	8	646 (87.8)	873	100	591 (67,7)	1019	21	880 (86, 3)	729	107	461 (63.2)
3	·	650	31	562 (86 <u>.</u> 5)	938	32	865 (92 , 2)	893	29	768 (86 ₄ 0)	904	50	663 (73.3)
4		780	16	702 (90, 0)	991	75	897 (9 0, 5)	859	g _.	750 (87, 3)	768	128	570 (74.2)
1 .	Control	515	190	51 (9.9)	1048	38	967 (92,3)	718	93	\$85 {81, 4}	799	63	644 (80, 6)
2		821	150	595 (72.5)	1137	51	1030 (90, 6)	709	272	205 (28,9)	669	233	30 (4,5)
3		650	145	324 (49.8)	1078	70	9 00 (83,5)	995	258	270 (27.1)	897	244	402 (44.8)
4		1230	165	569 (46.3)	991	43	737 (74.4)	944	125	596 (63,1)	728	122	56 (7,7)

¹ Includes all non-eyed eggs.