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# The effect of whole body lipid on early sexual maturation of 1 + age male chinook salmon (*Oncorhynchus tshawytscha*)

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## Abstract

Early sexual maturation of male chinook salmon (maturation 1 to 4 years prior to females in the same age class) results in reduced effectiveness of stock enhancement programs and a financial loss to the salmon farming industry. Previous studies in Atlantic salmon have shown that the age of maturity in males is affected by growth and/or body energy stores, but the relative roles of these two factors are not well understood. Therefore, an experiment was designed to determine when spermatogenesis was initiated, to characterize the endocrine changes during the onset of puberty in male salmon, and to determine if the level of whole-body lipid affects the incidence of early male maturation in a wild stock (Yakima River) of 1 + spring chinook salmon. Fry were fed a commercial diet from February until August and were then divided into groups of 320 fish (mean weight, 5.6 g) and fed one of five experimental diets (two replicate groups/diet) containing 4%, 9%, 14%, 18% or 22% lipid and 82%, 77%, 73%, 69%, or 65% protein for 13 months. Fish were reared on natural photoperiod and ambient temperature (6°C to 16°C), and pair-fed to a level based on the tank with the lowest feed consumption. Fish were weighed monthly and sampled to determine body composition, pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, plasma insulin-like growth factor I (IGF-I) levels, and stage of gonadal development.

Throughout the experimental period the mean fish weight was similar among treatment groups. However, from December through the end of the experiment in the following September, maturing males were significantly larger than nonmaturing fish. Initial lipid levels in 0-age experimental fish were near 6%, which is similar to wild fish of the same stock and age captured in the Yakima

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River during August. Fish fed diets containing more than 4% lipid increased in whole-body lipid content during the first 2 months of feeding and then maintained at relatively constant levels during the course of the experiment. Whole-body lipid levels for the dietary treatment groups averaged 5.6%, 7.1%, 8.2%, 9.4%, and 9.6% from October through the following September.

Based on histological examination of the testes of experimental fish, type B spermatogonia and primary spermatocytes were first observed in some of the yearling males during November. These were designated maturing males. Pituitary FSH levels were significantly higher in maturing than nonmaturing males at this time and for the remainder of the study. Pituitary FSH levels increased as spermatogenesis proceeded in maturing fish, whereas pituitary LH levels increased in maturing 1 + males only during July and August, when testes were in late stages of spermatogenesis and in September during spermiation. Plasma IGF-I levels were significantly higher in maturing males than nonmaturing fish from December through the end of experiment. Since maturing males were significantly larger than nonmaturing fish of both sexes from December through September, the difference in IGF-I levels could be due to differences in growth or due to maturation.

The percentage of maturing males was significantly influenced by whole-body lipid, increasing from 34% in fish fed the 4% lipid diet to 45% in fish fed the 22% lipid diet. These data suggest that whole-body lipid levels influenced the incidence of maturation of male spring chinook salmon. In addition, both endocrine and histological indicators suggest that maturation was initiated in males approximately a full year prior to the time the fish will spawn. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Chinook salmon (*Oncorhynchus tshawytscha*); Early male maturation; Spermatogenesis; Gonadotropin; Insulin like growth factor-I

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## 1. Introduction

The salmonids have been described as phenotypically plastic, opportunistic generalists because of the variation observed in the length of their life cycle stages (Thorpe, 1989). Spring chinook salmon spend 1 or more years in fresh water before migrating to sea and return after 1 to 4 years in spring or early summer prior to spawning in the fall (Healey, 1991). Early male maturation (sexual maturation at a younger age than that of females) occurs in wild spring chinook salmon but appears to be much more common in fish reared in hatcheries (Foote et al., 1991). Rates of early male maturation as high as 80% have been reported in captively reared fish (Unwin and Glova, 1997).

Jonsson and Jonsson (1993) and Thorpe (1994) have discussed the ecological advantages of early male maturation and have, along with others, discussed possible factors that influence this life cycle strategy. Evidence has been presented which demonstrates that genetic (Thorpe and Morgan, 1980; Gjerde, 1984a,b; Hard et al., 1985; Heath, 1992; Silverstein and Hershberger, 1992), abiotic (Saunders et al., 1982; Taylor, 1989) and biotic (Thorpe, 1986) factors play a part in life history strategy. Rowe and Thorpe (1990a,b) have shown that growth rate or body size during specific periods of the year influence the rate of early male maturation. Several researchers have recently suggested that fish may assess body energy stores and that critical thresholds for size and energy storage must be met at a specific time of year or maturation is inhibited (Rowe et al., 1991; Simpson, 1992; Thorpe, 1994; Kadri et al., 1995; Silverstein et al., 1997, 1998; Hopkins and Unwin, 1997).

The relative importance of body energy stores, growth rate, and size in permitting maturation to occur and the endocrine mechanisms whereby metabolic hormones influence the reproductive system are not well understood. In addition, the period when spermatogenesis is initiated and likely to be influenced by nutritional status has not been defined through direct histological assessment of the testis and endocrine measurements which would indicate the activation of the reproductive axis. Therefore, we designed a study to assess the effect of whole-body lipid stores on the incidence of maturation in 1 + male spring chinook salmon that had similar rates of body growth. The testes were examined histologically and reproductive hormones were measured at monthly intervals to establish the period when spermatogenesis was initiated in this stock of fish under our experimental conditions.

Both pituitary gonadotropins and androgens are involved in regulating spermatogenesis (Miura et al., 1991, 1992) in fish. In salmon, follicle-stimulating hormone (FSH) increases in the pituitary and plasma during spermatogenesis; whereas, luteinizing hormone (LH) increases during the time of spermiation (Swanson, 1991; Prat et al., 1996). Growth is regulated primarily by growth hormone and insulin-like growth factor I (IGF-I) (Duan, 1998) and plasma levels of IGF-I are correlated with both growth rate and size (Beckman et al., 1998). We hypothesized that growth may influence maturation via an interaction between these two endocrine systems. Therefore, we measured gonadotropins and IGF-I during the course of the study.

## 2. Materials and methods

### 2.1. Fish husbandry

Spring chinook salmon (*Oncorhynchus tshawytscha*) gametes were obtained from wild adults returning to the Yakima River near Cle Elum, WA in December 1992. Fertilized eggs were obtained from a pooled sample at the hatchery and incubated in Heath trays and maintained on dechlorinated municipal water at 8°C until hatching in February 1993. At the swim-up stage, fry were ponded into circular fiberglass tanks (1.5-m diameter) supplied with flow-through dechlorinated municipal water (8°C–15°C) and were fed a commercial diet (Biodiet, BioOregon, Warrenton, OR) according to the manufacturer's specifications from first feeding until the start of the experiment in August 1993.

Examination of a number of fish prior to the start of the experiment revealed that some fish were infected with bacterial kidney disease (BKD). Therefore, fish were fed a commercial diet containing erythromycin (100 mg/kg fish/day) for 3 weeks prior to the initiation of the experiment. In August 1993, fish were graded to remove the largest and smallest fish, and were then randomly distributed into 10 fiberglass tanks (130 l; 200 fish per tank) supplied with dechlorinated municipal water (4 l/min). Initial mean ( $\pm$  S.D.) fish weight was  $5.6 \pm 1.2$  g. During the experimental period, fish were reared on natural photoperiod and ambient water temperature, with the exception of summer months when water was chilled so that the maximum temperature would not exceed 16°C. Water temperature varied seasonally and ranged from a maximum of 16°C (July and August) to a minimum of 8°C (January). A failure in the chilling system in August

1994 caused the temperature to briefly rise to 20°C, which necessitated moving the fish to a new location and larger tanks (720 l). During September 1994, the experiment was terminated and all fish were sacrificed.

## 2.2. Diets and feeding

The objective of this study was to determine whether whole-body lipid levels, not size, affected the percentage of male spring chinook salmon which sexually matured at 2 years of age. To create fish with graded levels of whole-body lipid, fish were reared on diets containing excess protein (more than required for maximum growth, > 55%, Delong et al., 1958) and five different levels of lipid. Ration was controlled to maintain similar size among the various dietary treatments. Samples were collected at monthly intervals to monitor seasonal changes in body size, whole-body lipid levels, gonad development and levels of pituitary gonadotropins as indices of sexual maturation, and plasma levels of insulin-like growth factor I (IGF-I) as an index of growth.

Five experimental diets (Table 1) were prepared every 4 months and were stored at –20°C. The composition of experimental diets is shown in Table 2. The diets differed in the levels of protein (82%, 77%, 73%, 69%, 65%) and lipid (4%, 9%, 14%, 18%, 22%). The amount of feed fed to each tank of fish each day was determined using the method described by Shearer et al. (1997a). On day 1 of the experiment, the average amount of feed consumed by the fish in each tank was noted. For the remainder of the first month, fish in each tank were fed 90% of the amount of feed consumed by the group that ate the least feed on day one. Fish were fed slightly below satiation to ensure that all the feed would be consumed. The day after each monthly sampling all groups

Table 1  
Formulation of the experimental diets

Ingredient	Diet				
	1	2	3	4	5
<i>g / kg diet</i>					
Dried fish muscle <sup>a</sup>	680	640	606	576	548
Gelatin	120	114	108	102	97
Wheat gluten	60	57	54	51	49
Ascorbic acid	10	10	10	10	10
Arginine	6	6	5	5	5
Herring oil	36	91	140	184	223
Choline Cl	12	11	11	10	10
Trace mineral mix <sup>b</sup>	1	1	1	1	1
Carboxymethyl cellulose	24	23	22	20	19
Binder <sup>c</sup>	24	23	22	20	19
CaH <sub>2</sub> PO <sub>4</sub>	18	17	16	15	15
Vitamin mix <sup>d</sup>	18	17	16	15	15

<sup>a</sup>Supplied by NMFS, Kodiak, AK 94% protein, 2% lipid, 4% ash.

<sup>b</sup>USFWS, No. 3 (Hardy 1989).

<sup>c</sup>Algea Produkter, Lier, Norway.

<sup>d</sup>USFWS, Abernathy (Hardy, 1989).

Table 2

Composition of experimental diets

Protein and lipid determined by analysis. Energy calculations based on: protein, 23.6; lipid, 36.2 and carbohydrate, 17.2 kJ/g (Brafield, 1985).

Component	Diet				
	1	2	3	4	5
Protein (%)	82	77	73	69	65
Lipid (%)	4	9	14	18	22
Protein/Energy (mg/kJ)	40	36	33	30	28
Energy kJ/g	20.8	21.5	22.3	22.8	23.3
Energy (% from protein)	93	84	77	71	66

were fed to satiation. The amount of feed to be fed to each tank of fish for the remainder of the month was then calculated as follows:

$$(C \times 0.9 \times N) + \frac{\left( \frac{(W_L - W) \times N}{F} \right)}{d}$$

where:  $C$  = the average amount of feed consumed/fish in the tank that ate the least feed the first day after sampling; 0.9 = a factor to help insure that all feed fed was consumed;  $N$  = the number of fish in the tank;  $W_L$  = the largest mean fish weight of all the groups;  $W$  = the mean weight of the fish in the tank;  $F$  = the feed efficiency in the tank the previous month;  $d$  = the number of feeding days before the next sampling.

The fish were normally fed 5 days per week, but if they failed to consume their allotted ration in 5 days, they were fed on days 6 and 7. Feed was withheld for 2 days prior to sampling. During weeks 1–4, 9–12, and 17–20, erythromycin phosphate was added at the rate of 100 mg/kg fish/day to all diets to control BKD. No antibiotics were fed after week 20. Feed consumption and mortality were recorded daily.

### 2.3. Sampling

Fish were bulk weighed and counted at approximately monthly intervals. Prior to each monthly weighing, 10 fish from each tank were randomly removed and sacrificed for determination of length, weight, sex, state of gonadal development, and whole-body lipid levels. From December 1993 through September 1994, pituitary glands, blood and gonads were collected from the sacrificed fish. Fish were anesthetized in 0.05% bicarbonate-buffered tricainemethanesulphonate (MS-222; Argent Chemical Laboratories, Redmond, WA) and blood was collected from the tail vein in heparinized hematocrit tubes after severing the caudal peduncle. Blood was centrifuged at  $1000 \times g$  and plasma was stored at  $-70^\circ\text{C}$ . Pituitaries were removed and frozen in liquid nitrogen, then stored at  $-70^\circ\text{C}$ . Gonads were removed and fixed in Bouin's fixative for 24 h, then stored in 70% ethanol. Carcasses were frozen at  $-20^\circ\text{C}$  for whole-body lipid determination.

At the end of the experiment, September 1994, all fish (approx. 100/tank) were sacrificed and measured (fork length and weight). Additional sampling of blood, pituitaries, gonads and carcasses was performed on the first five females, five immature males and five maturing males collected randomly from each tank. In addition, gonad and liver weights were recorded. All remaining fish were sexed and each fish was classified as nonmaturing or maturing based on gross morphology of the gonad and gonadosomatic index (GSI).

#### *2.4. Whole-body lipid analysis*

Whole-body lipid levels were determined on pooled samples of 10 fish from each tank throughout the experiment and on individual fish from the sampling at the end of the experiment (September 1994). Fish were ground and dried to constant weight at 105°C and a subsample was extracted with dimethyl chloride using the soxhlet method. Dietary lipid was determined for each new batch of feed, and percent lipid between batches of any particular diet varied less than 1%. Condition factor (CF) was calculated as  $CF = (w/l^3) \times 100$ , where  $w$  = weight in g and  $l$  = length in cm. Data from wild chinook salmon of the same stock and age as those used in the experiment but sampled from the native habitat (Yakima River) were kindly provided by Brian Beckman (Beckman et al., 2000).

#### *2.5. Hormone analyses*

Total plasma IGF-I levels were determined following acid-ethanol extraction (Daughaday et al., 1980) using the method of Moriyama et al. (1994) with recombinant salmon IGF-I and anti-barramundi IGF-I serum purchased from Gro-Pep (Adelaide, Australia). A complete validation of this assay using commercial components is described in Shimizu et al. (2000). Pituitary levels of follicle-stimulating hormone (FSH, previously called GTH I) and luteinizing hormone (LH, previously called GTH II) were analyzed by radioimmunoassay (Swanson et al., 1989). Pituitaries were briefly sonicated in 0.5-ml barbital buffer (0.75 M sodium barbital, 10 mM EDTA, 1 mM PMSF, pH 8.6) and centrifuged at  $10,000 \times g$  for 10 min. Pituitary extracts were diluted in assay buffer (0.75 M sodium barbital, 1.0% bovine serum albumin, pH 8.6) prior to assay.

#### *2.6. Gonad histology*

Testes were removed and fixed in Bouin's fixative for 24 h, then stored in 70% ethanol until processed for histology. Tissue was dehydrated through a series of increasing concentrations of ethanol, imbedded in Paraplast, sectioned (6  $\mu$ m) and stained with hematoxylin and eosin. Stage of spermatogenesis were determined by light microscopic observation according to Schulz (1984) and is briefly described in Table 3.

#### *2.7. Statistics*

Statistical analyses were performed using Statview™ (Abacus Concepts, Berkeley, CA, 1992), and multiple mean comparisons were made using the Fisher PLSD test.

Table 3  
Stages of spermatogenesis observed during experimental period<sup>a</sup>

Date	Nonmaturing	Maturing
September 27, 1993	I	
October 25, 1993	I	
November 29, 1993	I	II
December 27, 1993	I	II
January 31, 1994	I	II
February 28, 1994	I	II
March 25, 1994	I	II
May 2, 1994	I	II, III, IV
May 31, 1994	I	III, IV
July 5, 1994	I	IV
August 4, 1994	I	IV, V
September 12, 1994	I	IV, V, Va

<sup>a</sup>Criteria for stage of spermatogenesis according to Schulz (1984). I = spermatogonia, type A and Sertoli cells as intralobular types; II = spermatogonia types A and B, primary spermatocytes; III = spermatogonia, primary and secondary spermatocytes, spermatids; IV = spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoa; many spermatogenic cysts; no spermiation; V = no or few spermatogenic cysts; lobules filled with spermatozoa; Va = no spermatogenic cysts; lobules filled with spermatozoa; spermiation.

Percentage data were arcsine transformed prior to analysis. Analyses were performed using mean tank values as the unit of observation and  $P < 0.05$  was accepted as significant unless otherwise stated.

### 3. Results

#### 3.1. Gonad development

From September through December 1993, no gross morphological signs of male maturation such as enlarged testes were observed. Histological analyses of testes collected from fish during this period indicated that the majority of males remained in Stage I of spermatogenesis, and these fish were classified as nonmaturing. However, testes from some individuals collected during November and December 1993 were in Stage II of spermatogenesis (presence of type B spermatogonia and in some cases primary spermatocytes) and these fish were classified as maturing.

The first gross morphological signs of maturation of male fish were observed during January 1994, when a slight enlargement of the anterior portion of the testes was seen in 14% of the males fed more than 4% lipid. Histological examination confirmed that these testes were in Stage II of spermatogenesis. From that point on, males with testicular enlargement were designated as maturing males.

A random sample (20/month) of males classified as maturing was collected from January through March, and analyses revealed that the testes were in Stage II of spermatogenesis. By April, individuals were found in Stages II to IV. From May through July, Stages IV and V were observed. Finally, during September the majority of

maturing males were in Stage V or Va, with a few fish expressing milt with gentle abdominal pressure. Nonmaturing fish throughout the year remained in Stage I. Stages of spermatogenesis observed at each sampling point are summarized in Table 3. Since

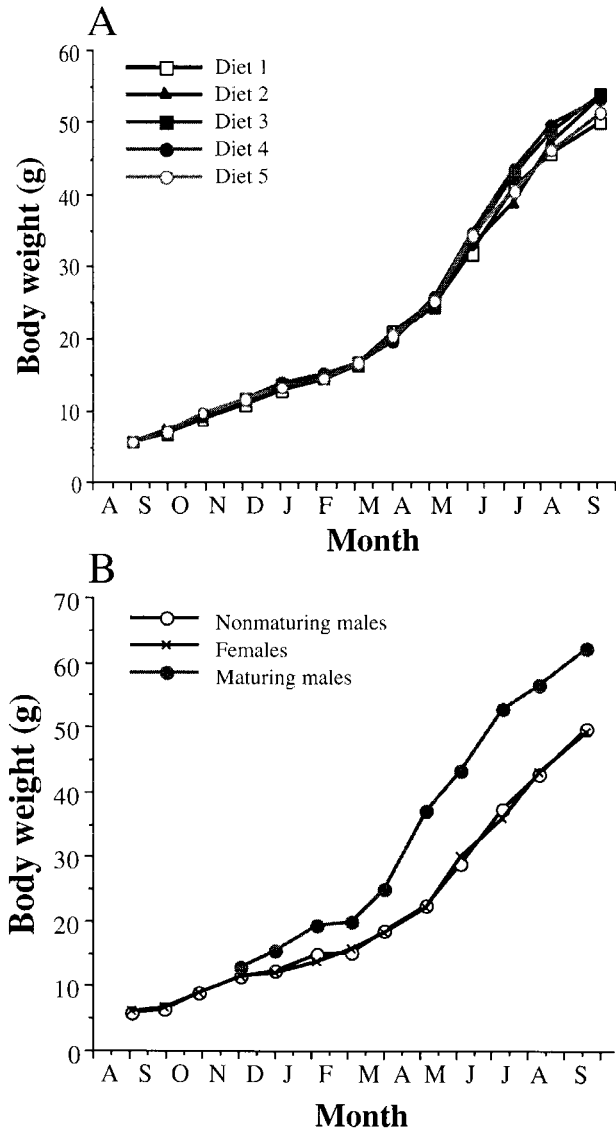


Fig. 1. Mean weight ( $n = 2$  tanks/treatment) of 1+ spring chinook salmon fed experimental diets containing varying levels of protein and lipid (A). Data are calculated by bulk weight of fish in tank/number of fish per tank. Mean weight of female, nonmaturing and maturing male fish sampled at monthly intervals during the experimental period (B). Maturing males were significantly ( $P < 0.05$ ) larger than nonmaturing fish from December through the end of the experiment.



we did not analyze all testes collected, we could not quantify the proportion of fish at each stage for each sampling period.

### 3.2. Growth and whole body lipid

Growth of fish in terms of body weight was similar among the five treatment groups, with body weight gradually increasing from August 1993 to March 1994, at which time

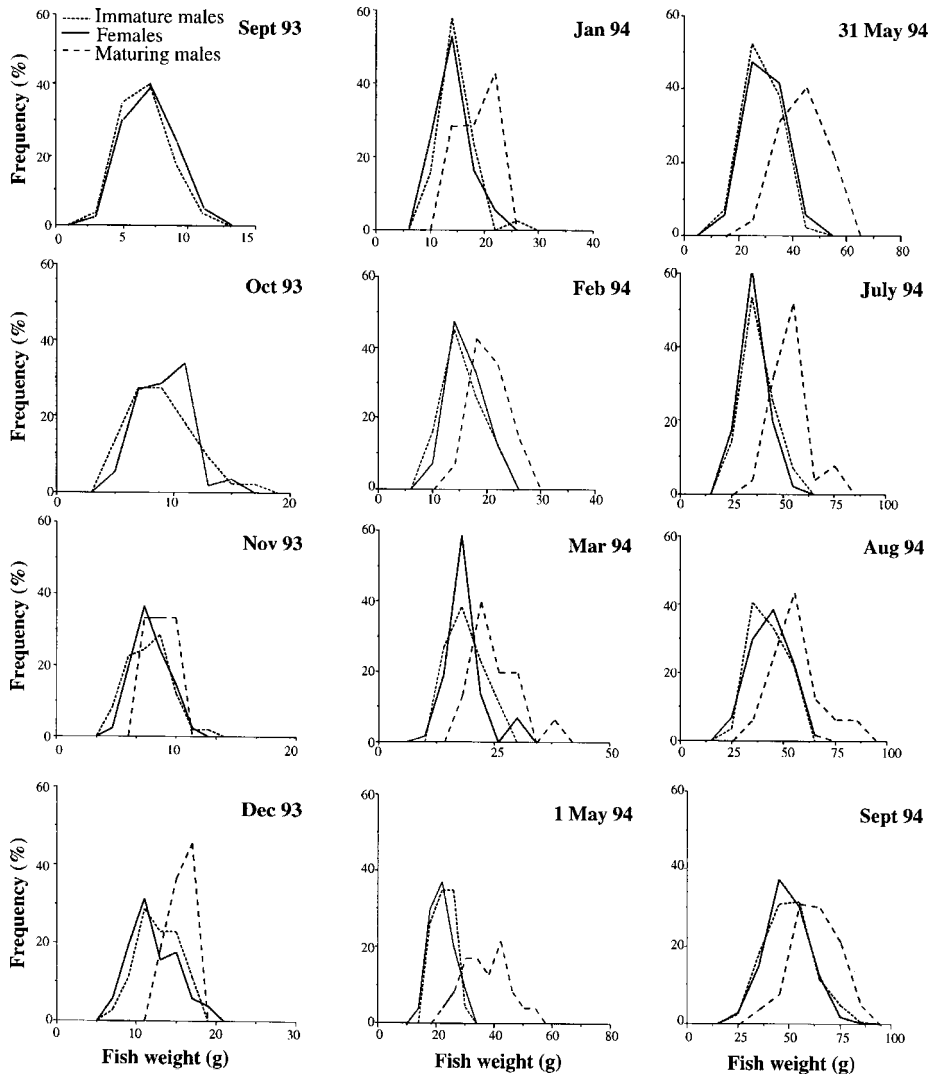


Fig. 2. Weight frequency distribution of immature males and females, and maturing male chinook salmon at each sampling;  $n=100$  fish per sampling period. The sex ratio and number of maturing males varied at each sampling.

Table 4

Final weight<sup>a</sup>, feed efficiency<sup>b</sup>, percent maturation<sup>c</sup>, and whole-body lipid<sup>d</sup> of fish at the end of the experiment (September 1994)

Treatment	Final weight (g)	Feed fed (g)	Feed efficiency	Mortality (%)	Male maturation (%)	Whole-body lipid (%), mature males	Whole-body lipid (%), immature males	Whole-body lipid (%), females
1	48.8 ± 0.4	46.0	0.94	4.5	33.5 ± 1.5	6.3 ± 0.2	5.2 ± 0.4	6.1 ± 0.1
2	52.5 ± 1.0	46.4	1.01	1.3	31.5 ± 3.5	6.8 ± 0.2	7.2 ± 0.3	7.5 ± 0.3
3	53.3 ± 0.1	47.2	1.01	2.8	40.0 ± 8.0	8.0 ± 0.3	9.0 ± 0.1	8.6 ± 0.7
4	52.9 ± 1.6	46.4	1.02	3.8	43.5 ± 11.5	8.5 ± 0.5	10.5 ± 0.5	10.5 ± 0.5
5	50.6 ± 2.8	46.4	0.97	10.4	45.5 ± 4.5	8.5 ± 0.6	10.1 ± 0.4	11.2 ± 0.5

<sup>a</sup>Based on mean tank weight, mean ± S.E.<sup>b</sup>Wet weight gain/dry feed fed.<sup>c</sup>Mean ± S.E., *n* = 2.<sup>d</sup>Based on five males, five females and five mature males per tank, *n* = 2 tanks per treatment, mean ± S.E.

the rate of growth increased in all treatments. Fish fed the diet containing the highest level of lipid ate the least and thereby drove the levels of feed offered to the other treatments. However, mean fish weights did not differ significantly among the treatment groups except at the final sampling (Fig. 1a), when fish fed the lowest fat diet were

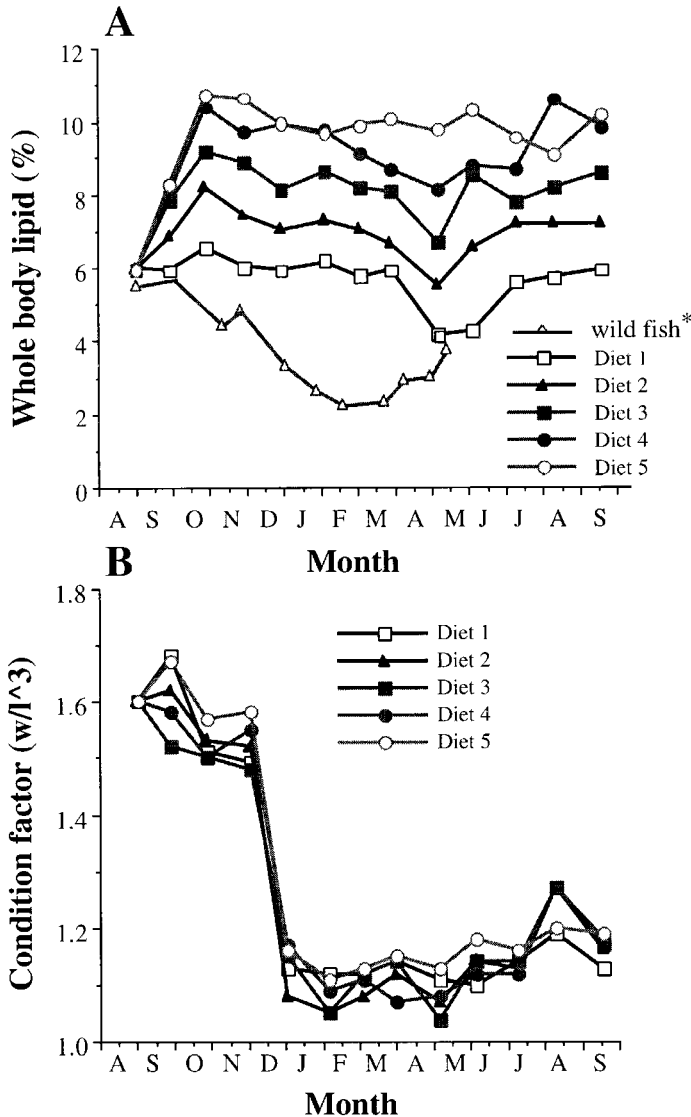


Fig. 3. Whole-body lipid levels (A) and condition factor (B) of 1+ chinook salmon fed experimental diets containing varying levels of dietary lipid and protein. See Table 1 for description of diets. Data are mean of replicate tanks. Whole-body lipid levels were determined on pooled carcasses of 10 fish per tank. \* Data from wild fish of the same stock collected during the same year are shown with permission from authors (Beckman et al., 2000).

significantly smaller than all other fish. Comparison of the weights of fish sampled from all treatment groups indicated that nonmaturing males and females grew at equivalent rates (Fig. 1b). However, males that were classified as maturing were significantly larger in body weight than nonmaturing males or females. The larger size of maturing males was also evident in weight frequency distributions of all fish sampled monthly (Fig. 2).

Overall mortality (Table 4) was 4.6% and the majority of this occurred in one replicate of treatment 5 (18.5%). Postmortem examination revealed the presence of bacterial kidney disease. Feed efficiency for all treatments averaged  $99 \pm 4.8\%$  (weight gain/feed fed) and did not differ between treatments (Table 4).

In all groups, whole-body lipid increased during the first 2 months of feeding experimental diets (September and October 1993) and then decreased slightly throughout the winter and early spring, reaching their lowest level in April 1994 (Fig. 3a). There was a significant effect of diet on whole-body lipid levels, and relative differences in whole-body lipid among the groups were generally maintained throughout the study. Although slight fluctuations in whole-body lipid levels were observed in experimental fish, levels remained relatively constant compared to wild fish (Fig. 3a), which declined in whole-body lipid from 6% in August to 2% in February and increased again in the

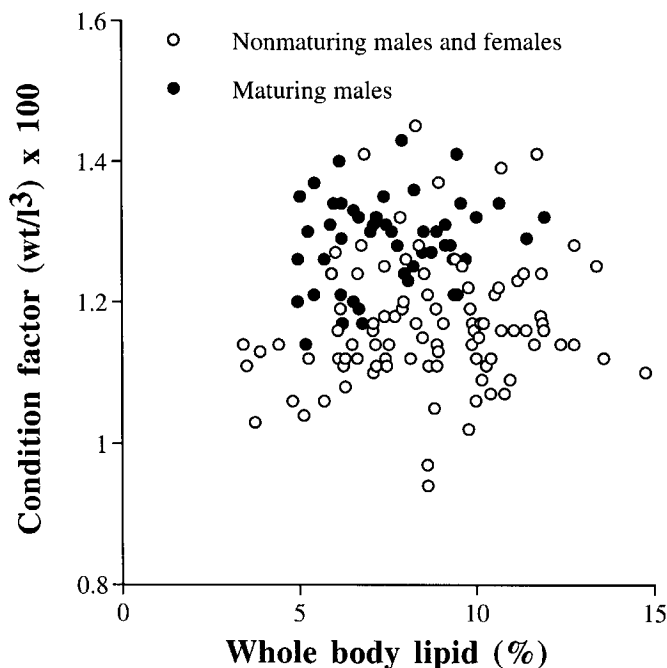


Fig. 4. Relationship between individual whole-body lipid level and condition factor in September 1994. Measurements were made on five immature males, five females and five maturing males from each replicate tank ( $n = 150$ ). No differences in the relationship between condition factor and whole-body lipid due to sex or maturity were found.

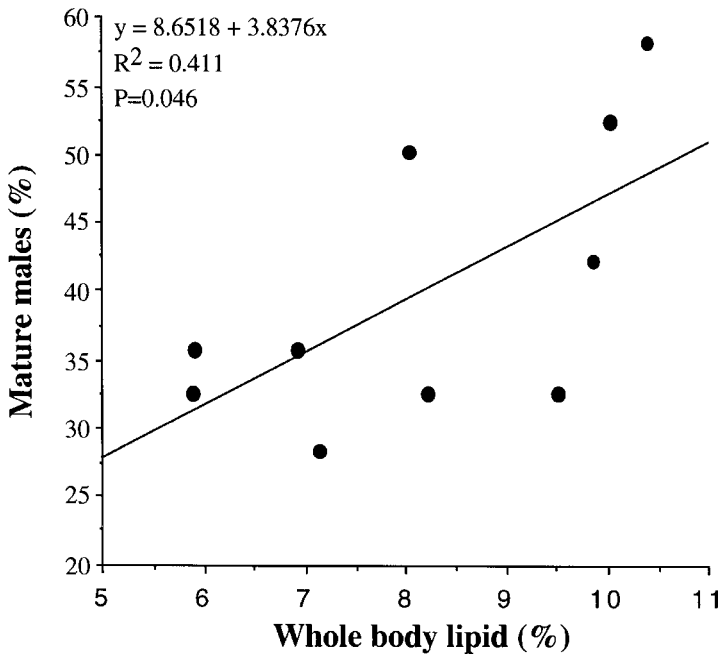


Fig. 5. Percent maturing 1<sup>+</sup> age male spring chinook salmon in each tank at the end of the experiment (September 1994) in relation to mean whole-body lipid levels in December (1993) when we found a significant correlation between these two parameters (Table 5).

spring months to almost 4%. Condition factor did not differ among treatment groups at any sampling (Fig. 3b). Condition factors remained constant from August to November 1993 (approximately 1.5) but declined markedly, to approximately 1.1, between the

Table 5

Correlation of percent whole-body lipid<sup>a</sup> at each month and percent maturation of males in September 1994

Date	Correlation coefficient	P value
October 25, 1993	0.604	0.0645
November 29, 1993	0.590	0.0732
December 27, 1993	0.642	0.0439
January 31, 1994	0.633	0.0481
February 28, 1994	0.486	0.1599
March 25, 1994	0.573	0.0843
May 2, 1994	0.533	0.1158
May 31, 1994	0.635	0.0474
July 5, 1994	0.519	0.1282
August 4, 1994	0.717	0.0172
September 12, 1994	0.610	0.0605

<sup>a</sup>From a pooled sample of 10 fish per tank,  $n = 10$  tanks.

November and December 1993 samplings (Fig. 3b). There appeared to be no relationship between condition factor and body lipid levels at the end of the experiment (Fig. 4).

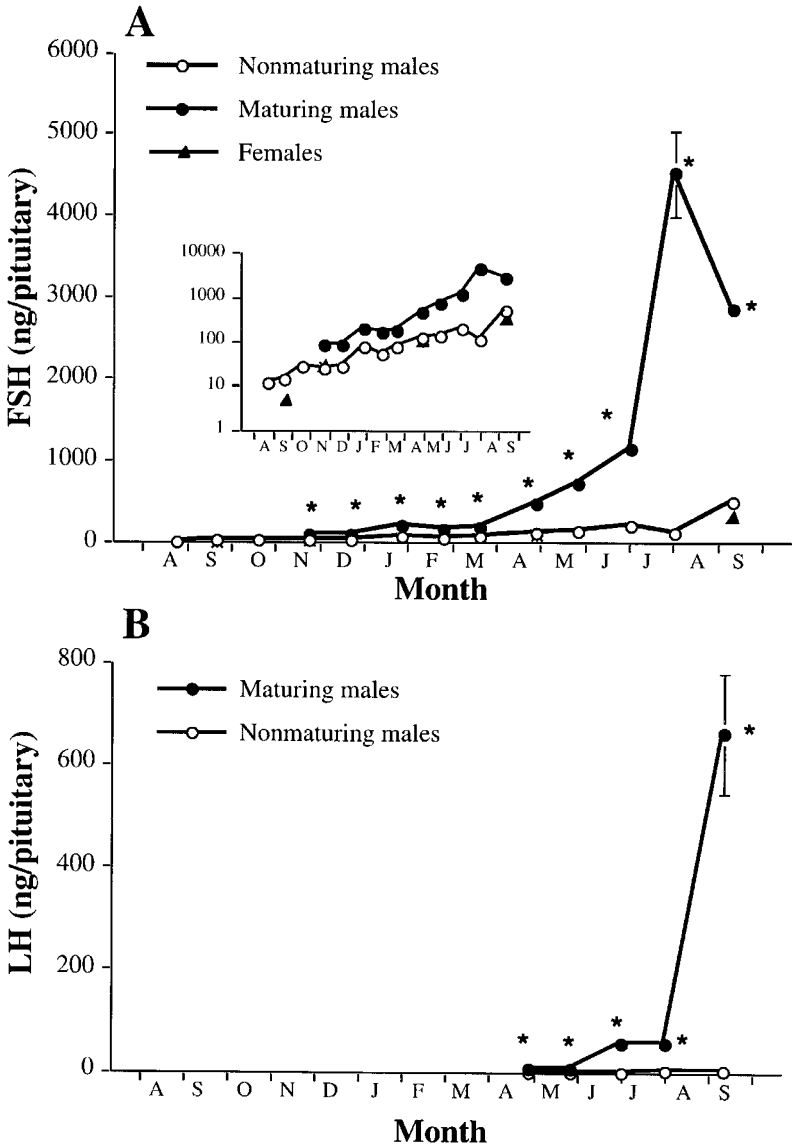


Fig. 6. Pituitary levels of FSH (A) and LH (B) in nonmaturing male and female, and maturing male 1+ spring chinook salmon sampled during the experimental period. Insert in (A) represents log-transformed data. Data are means  $\pm$  standard errors of 10–60 replicates. There was no effect of diet on pituitary FSH or LH levels, but significant differences ( $P \leq 0.05$ ) due to maturity were observed as indicated by asterisk.

Since gonadal histology indicated that the maturation process appeared to begin between the November and December 1993 samplings, regression analysis was performed to determine if there was a relationship between the mean levels of body lipid in each tank of fish in December 1993 and the incidence of maturation in September 1994 (Fig. 5). The percentage of males maturing at 2 years of age in September 1994 was significantly positively correlated with average whole-body lipid levels during December 1993, January, May and August 1994 ( $P < 0.05$ ). While maturation rate appeared to be positively correlated with whole-body lipid during October and November 1993, it was not statistically significant (Table 5).

### 3.3. Hormone levels

Pituitary FSH and LH levels were measured in all male fish sampled during the experimental period, and from females collected during September 1993 and 1994. Pituitary FSH levels were significantly higher in maturing males than nonmaturing fish of either sex (Fig. 6a). Levels of pituitary FSH in maturing males increased during the year, but most dramatically from July to August 1994 when the transition to later stages of spermatogenesis was observed in the testes samples (Table 3). Pituitary LH levels were not detectable in nonmaturing fish. In maturing males, pituitary LH (Fig. 6b) levels increased slightly from May to July 1994, and increased more than a hundred-fold from August to September 1994. It was not possible to measure plasma levels of either FSH or LH due to insufficient plasma from individual fish for the analyses. However, increases in pituitary gonadotropins generally precede increases in plasma levels of these hormones and are indicative that the reproductive endocrine axis is activated.

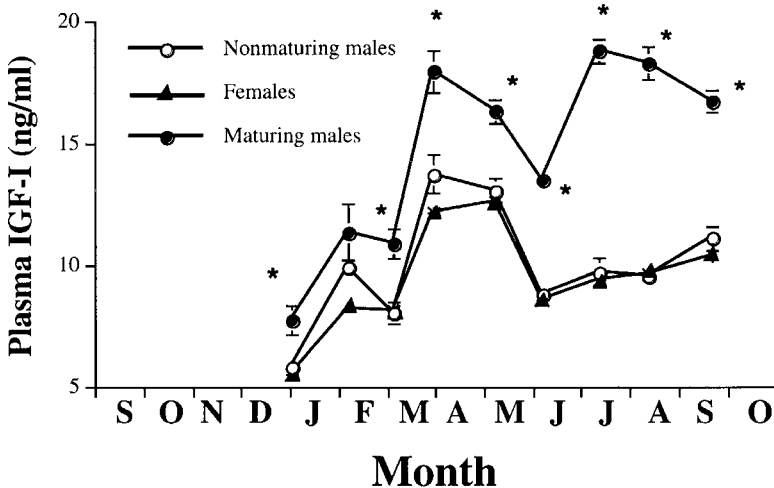


Fig. 7. Plasma IGF-I levels in nonmaturing male and female, and maturing male 1+ spring chinook salmon sampled during the experimental period. Data are means  $\pm$  standard errors of 10–60 replicates. There was no treatment effect on plasma IGF-I levels except the last sampling point (see Fig. 8); however, significant ( $P \leq 0.05$ ) differences between maturing and nonmaturing fish are indicated by asterisk.

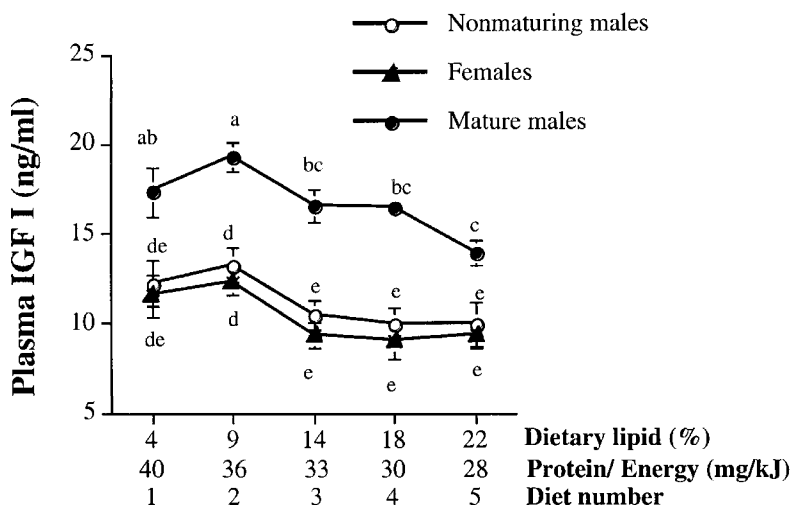


Fig. 8. Plasma IGF-I levels in immature male and female, and mature male 1+ spring chinook salmon sampled during September 1994 in relation to dietary protein/energy ratio and dietary fat. Data are mean  $\pm$  standard errors,  $n = 10$  per data point. Significant differences ( $P \leq 0.05$ ) between treatment groups are indicated in the figure. No differences were found between immature male and female fish; however, for all treatments mature males had significantly higher plasma IGF-I levels than immature fish of either sex.

There was no significant dietary treatment effect on plasma IGF-I levels except at the final sampling date. However, there was a significant effect of sampling date and maturity status. At all samplings, plasma levels of IGF-I (Fig. 7) were significantly higher in maturing males than nonmaturing fish of both sexes. In all fish, there was a seasonal increase in IGF-I levels from December to the following spring when IGF-I levels peaked during March and April, and then declined by the end of May. In maturing males, levels of IGF-I increased again in July and remained elevated through the end of the experiment. At the last sampling, September 1994, there was a significant treatment effect on IGF-I levels, with both maturing and nonmaturing fish fed Diet 2 having significantly higher IGF-I levels than those fed Diets 3, 4, and 5 (Fig. 8). Levels of IGF-I in fish fed Diet 1 were similar to those fed Diet 2, but because of high variance they did not differ from IGF-I levels in fish fed Diets 3, 4, or 5. Among the diets fed in this experiment, Diets 1 and 2 were the lower in fat and higher in protein:energy ratio (Table 2) than the other three diets. Thus, there was a tendency for IGF-I levels to be higher in fish fed diets higher in protein:energy.

#### 4. Discussion

There were four major findings that resulted from this study. First, we were able to successfully manipulate whole-body lipid levels and control size of juvenile spring chinook salmon with diet and feeding regime. Second, we confirmed that a critical period when maturation is initiated in male spring chinook salmon is a full year prior to



spawning. Third, the rate of maturation of 1<sup>+</sup> age male chinook salmon was correlated with whole-body lipid levels during autumn, when maturation was initiated. And fourth, pituitary FSH levels increased as spermatogenesis progressed and plasma IGF-I levels were higher in maturing male fish than nonmaturing fish; however, these fish were also larger than nonmaturing males. Therefore, the effect of size or maturity on IGF-I levels could not be distinguished.

The diets and feeding regime used in the present study successfully produced groups of fish with similar growth rates but different levels of body fat. Daily growth was approximately 0.6%, which is less than that normally observed under hatchery conditions (about 2.0%; Hardy, 1991). This may be due to the relatively low food consumption of fish fed the diet containing the highest level of lipid. Since the food consumption of this group was used to calculate the ration for all of the other groups, ration was consequently reduced across all treatments.

In another study, we found that feed intake was lower in fat compared to lean fish (Shearer et al., 1997b). Therefore, it is likely that the low feed intake observed in fish fed the highest level of dietary lipid was due to the high energy density of this diet or the high level of body lipid in these fish (Shearer et al., 1997b). The maximum body fat level observed at the end of the experiment was 10%, which is higher than that observed in hatchery fish of similar size (8%) by Higgs et al. (1985). This may be due to the high energy densities (23.3 to 28.0 kJ/g) of the diets used in our study, which were higher than diets normally fed (18.1 to 20.8 kJ/g) to juvenile chinook salmon (Silver et al., 1993). Despite the relatively low growth, we were able to maintain graded levels of body fat among the treatment groups throughout the duration of the experiment.

In November 1993, the males classified as maturing were similar in weight to the nonmaturing males and females, but by December, the mature males were significantly larger than nonmaturing fish. Males showing testicular development were larger than nonmaturing males and females for the remainder of the experiment. A weight increase associated with male sexual maturation has previously been reported (Aksnes et al., 1986; Taylor, 1989; Kadri et al., 1996; Tveiten et al., 1996; Stead et al., 1999).

Since our results showed no difference in size between maturing and nonmaturing males during November 1993, our results support the suggestion by Foote et al. (1991) that maturation is initiated prior to the increase in growth that is observed in maturing fish. However, it should be noted that although no statistical difference in size was observed during November, it was apparent from weight frequency distributions that there was a trend of higher body weights in maturing males than nonmaturing fish of either sex. Therefore, our data are insufficient to determine whether the physiological commitment to initiate maturation occurs first, and that enhanced growth occurs as a result of this process (i.e. anabolic reproductive hormones), or whether higher growth causes the fish to initiate maturation. Recent studies by Silverstein et al. (1998) have shown that both growth and body adiposity affect the percentage of male chinook salmon that mature at 2 years of age. It would be difficult to conclude that growth does not affect the onset of maturation, since maturation rates were clearly altered by growth. High growth rate or large body size may influence the onset of maturation, and anabolic steroids such as androgens that increase during maturation further stimulate growth in maturing fish.

Condition factors were similar in all groups throughout the study, irrespective of body-fat levels. Condition factor is an index of body shape, and the decrease observed between November and December 1993 could be due to a morphological change associated with smoltification (Folmar and Dickhoff, 1980). Juvenile spring chinook salmon show physiological and morphological changes associated with smoltification during the fall as underyearling fish and again in the subsequent spring (Beckman and Dickhoff, 1998). Based on the whole-body lipid determinations made at the end of the experiment, there appears to be no relationship between whole-body lipid and condition factor. These results are in contrast with those of Herbing and Friars (1991), but agree with those of Kadri et al. (1996). It is possible that condition factor may be an appropriate index of body fat levels in some species during part of the life-cycle (Chellappa et al., 1995), however, it should not be used universally for this purpose.

The main objectives of the experiment were to determine when maturation was initiated and if there was a relationship between whole-body lipid level and the incidence of sexual maturation. In our study, regression analysis of mean tank whole-body lipid levels in December, and the incidence of male maturation in the following September, indicated that a higher incidence of maturation was associated with higher whole-body lipid levels in the previous winter. From the start of the experiment in August 1993 until August 1994 only pooled lipid samples were analyzed. It was therefore impossible to determine if the males that initiated maturation had higher whole-body lipid levels than nonmaturing males. Thus, we could not confirm the hypothesis put forth by Thorpe (1994) and Silverstein et al. (1997, 1998) that males making the physiological commitment to mature were those that exceeded a threshold lipid level the fall before final maturation occurred. However, we were able to confirm with both endocrine and histological data that maturation was initiated in some fish during the autumn, a full year in advance of the spawning period.

At the end of the experiment, when lipid analyses were performed on individual fish, whole-body lipid levels of maturing males were lower than nonmaturing males. Whole-body lipid analyses were performed on all nonmaturing males and females fed diet 3 ( $n = 119$ ) and the lipid levels were found to have a mean of 7.9%, a range from 5.5% to 11.9%, and to be normally distributed. The lipid levels of nonmaturing fish analyzed in the other treatments ( $n = 20$  fish/treatment; data not presented) also appeared to be normally distributed. The lower lipid level in mature males is most likely due to mobilization of stored energy for gonadal growth (Ware 1980; Roffe, 1983).

Numerous studies have implicated the role of the pituitary in controlling sexual maturation (Billard et al., 1990; Billard, 1993). The formation of spermatogonia does not appear to be under the control of the pituitary gland; however, both pituitary gonadotropins and androgens are involved in regulating spermatogenesis (Miura et al., 1991, 1992). In this study, the differentiation of spermatogonia and formation of primary spermatocytes was correlated with elevations in pituitary FSH levels, suggesting an activation of the pituitary–gonad axis. We first observed this transition during November and December, 10 months before fish produced mature sperm. We concluded from these data that a critical period for initiation of spermatogenesis is a full year prior to spawning, as has been previously suggested by others (Thorpe, 1991; Thorpe et al., 1992; Mangel, 1994; Berglund, 1995; Silverstein et al., 1998). The level of FSH in the

pituitary of maturing fish increased drastically during the spring as spermatogenesis proceeded, reaching a peak level in August. The spring rise in pituitary FSH occurred when more advanced stages of spermatogenesis were observed in cysts within the tubules in the testis. This also corresponded to a time when LH could be detected in the pituitary of maturing males. LH levels in the pituitary increased most dramatically during the period when mature spermatozoa were present in the testis and sperm duct.

In previous studies, we have shown that plasma levels of FSH increase in adult coho salmon during early stages of spermatogenesis and then decline at spawning, whereas LH levels increase at the time of spermiation (Swanson, 1991). Similar results have been obtained in recent studies of rainbow trout (Prat et al., 1996; Gomez et al., 1999). Pituitary levels of FSH are also elevated in precocious masu salmon (Amano et al., 1993, 1994). These results, combined with studies of steroidogenic activity of FSH (Planas and Swanson, 1994), strongly indicate that FSH, not LH, plays a critical role in the initiation of spermatogenesis in fish.

At the outset of this study, we were interested in measuring levels of a metabolic hormone that regulates growth and to determine whether levels of this hormone could be used to distinguish growth differences among individual fish during a period when maturation was initiated. Thorpe (1994) and others have suggested that fish perceive their “growth opportunity,” which in turn influences the “decision” or physiological commitment to mature. The endocrine mechanism by which this occurs is not known. We speculated that a metabolic hormone may be a critical signal to the reproductive system that there are sufficient energy reserves or that growth rate is sufficient to initiate maturation for the subsequent year.

In fish, as well as other vertebrates, growth is regulated by a complex suite of hormones including pituitary growth hormone (GH), thyroid hormones, pancreatic hormones and a variety of growth factors. GH stimulates hepatic production of IGF-I, which in turn acts peripherally to stimulate tissue growth. The GH-IGF-I axis is influenced by photoperiod, temperature (Bjornsson et al., 1995; McCormick et al., 1995), and nutrition (Perez-Sanchez et al., 1995).

Beckman and Dickhoff (1998) have suggested that the GH-IGF-I axis provides the animal with an integrated signal for season, temperature, and food supply. Therefore, hormones from the growth axis may influence reproduction via direct effects on the brain-pituitary-gonad axis. Recent studies have also shown that IGF-I is produced in the testis (Le Gac et al., 1996), stimulates spermatogonial cell division (Loir and Le Gac, 1994) and IGF-I receptors have been localized in various cell types within the testis (Le Gac et al., 1996). In addition, IGF-I has been shown to elevate pituitary FSH content and GnRH-induced FSH release in coho salmon (Baker et al., 2000), GnRH-induced FSH and LH release in rainbow trout (Weil et al., 1999), and LH release in the eel (Huang et al., 1998, 1999). Whether these effects are endocrine, or occur via a paracrine/autocrine mechanism is not known. Because of the role of IGF-I in both growth and reproduction, we measured IGF-I during the critical period when growth may be influencing the physiological commitment to maturation.

In our study, we found that maturing fish had higher IGF-I levels than nonmaturing fish from December through the end of the experiment in the following September. However, because the maturing fish were also larger we cannot determine whether the

increase in IGF-I was related to growth and/or maturation. IGF-I levels were elevated when significant size and growth differences were noted between maturing and nonmaturing males from December through September. This is consistent with studies by Beckman et al. (1998) who demonstrated a significant correlation between plasma levels of IGF-I and growth rate. Therefore, the combined observations of elevated total IGF-I levels in the peripheral circulation of larger maturing fish, and direct effects of IGF I on pituitary FSH content are consistent with IGF-I acting as a metabolic trigger for puberty in fish.

At the last sampling (September 1994), we found that IGF-I levels were higher in fish fed diets containing the lowest fat content but highest in protein/energy ratios. This result is consistent with the observations of Perez-Sanchez et al. (1995) who found that increases in protein intake influence the GH–IGF-I axis in a marine fish by increasing plasma IGF-I and liver GH receptors. At the end of the experiment, we analyzed individual body-fat levels and plasma IGF-I and did not find a significant positive correlation of IGF-I with body-fat levels (data not shown). Additionally, 1 year prior to maturation there were no significant differences in IGF-I levels due to the treatments which created groups of fish with graded levels of whole-body lipid. This is in contrast to Silverstein et al. (1998) who reported a correlation between plasma IGF I levels and whole-body lipid in chinook salmon 1 year prior to maturation.

The spring increases in IGF-I levels that we observed in both maturing and nonmaturing fish were similar to those reported by Beckman et al. (1998). Previous studies have also shown smoltification-associated increases in IGF-I levels (Lindahl et al., 1985; Duguay et al., 1994). The autumn increases in IGF-I observed in the maturing fish in our study were not associated with smoltification because the fish would have smolted during the previous autumn or spring. This autumn increase in IGF-I is similar to that reported by Moriyama et al. (1997) in amago salmon; however, we did not observe the decline in plasma IGF-I in fully mature fish that occurred in the amago salmon. This may be associated with the feeding status of the fish at the time of the sampling since fasting will cause a reduction in plasma IGF-I levels (Duan, 1998). In the present study, fully mature male fish were still feeding.

Previous studies have suggested that there are two critical periods during the process of maturation (Thorpe, 1991; Mangel, 1994; Berglund, 1995). First, there is a critical period during autumn, 1 year prior to maturity, when sexual maturation (puberty) is initiated. Second, there is a period in the spring, when maturation is permitted to continue if environmental conditions and threshold size or energy status are sufficient to support gonadal development. The important distinction between these two periods is that the second is “permissive.” In other words, the maturation process starts in the autumn, and can either be permitted to continue in the spring or inhibited. There is considerable evidence that size, growth rate, and energy storage are important factors that influence the physiological commitment to sexual maturation (Alm, 1959; Rowe and Thorpe, 1990b; Rowe et al., 1991; Berglund, 1995). In most studies to date, the relative importance of these factors could not be discerned.

In our study, where growth in terms of body size was controlled and body fat levels were altered, we found that higher body-fat levels during December were associated with higher rates of male maturity in the following year. In studies conducted subse-

quently, we have found an interactive effect of body size and whole-body adiposity on maturation of male spring chinook salmon (Shearer et al., 1997a,b; Silverstein et al., 1998). Together, these studies indicate that the rate of maturation of male salmonids can be manipulated by controlling growth and adiposity. Since we have confirmed in our present study that the autumn is a critical period for the initiation of spermatogenesis, it is clear that manipulations of growth and adiposity during this season are critical to affect maturation rates of males in the subsequent year.

These data have important implications for controlling age of maturity in captively reared male salmonids. It may be desirable to reduce the number of males maturing at 1 and 2 years of age to avoid asynchronous age of maturity of male and female fish. Data from the present study and others indicate that it may be possible to reduce the number of males maturing at 2 years of age by reducing growth and fat levels during the autumn, a full year prior to maturation. Because reduced ration 8 to 12 months prior to spawning decreases maturation rates and reduced ration 4 to 8 months prior to spawning reduces fecundity of female rainbow trout (Bromage et al., 1992), the effects of manipulations of both ration and fat levels that we have studied in male chinook salmon need to be examined in females.

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