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Evaluation of Endocrine Indices of Growth in Individual Postsmolt Coho Salmon

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Abstract.—Plasma levels of the hormones insulin and insulin-like growth factor-I (IGF-I) were assessed as indices of growth for individual juvenile coho salmon *Oncorhynchus kisutch*. Smolts were tagged in April, introduced to seawater in May, and reared at two different feeding levels. Fish lengths and weights were obtained in June, August, September, and November. Plasma samples were obtained in September and November. There was a consistent, robust relation (over both feeding rates and sampling dates) between plasma IGF-I and the instantaneous growth in length of individual fish when growth was measured over a 6-week period. There was no consistent association between plasma insulin level and growth, size, or condition factor. Plasma IGF-I levels were also measured in postsmolt coho salmon captured in September from the Strait of Georgia and Puget Sound and were found to be similar to those in laboratory fish.

Several investigators have suggested that the growth rate of juvenile salmon in the first few weeks or months after ocean entry is correlated with overall survival to the adult stage (Holtby et al. 1990; Friedland et al. 2000). However, Fisher and Pearcy (1988) found similar growth rates for juvenile coho salmon *Oncorhynchus kisutch* during years of high and low survival. In addition, it has been postulated that coho salmon growth or condition in late summer and fall influences the survival rate (Beamish and Mahnken 2001). Overall, there appears to be some correlation between ocean conditions and salmon survival (Ryding and Skalski 1999; Cole 2000; Hobday and Boehlert 2001), yet the mechanism(s) by which ocean conditions set or modulate salmon survival are still unknown.

Resolution of questions about the relation between the oceanic growth rate of juvenile salmon and their survival to the adult stage depends on developing accurate methods of assessing growth of fish in the ocean. Several of the studies mentioned above used the spacing or number of circuli on scales to index growth. A primary disadvantage of this method is that rope trawls, which are widely used for salmonid research on the Pacific coast (Beamish et al. 2000), may rub most of the scales off fish during capture. In addition, there may be some difficulties associated with assessing the time interval over which successive circuli are formed (Fisher and Pearcy 1990). Thus, alternative indices of growth could be useful.

A number of biochemical methods have been used to assess growth or condition of fish captured in the ocean, including various enzyme assays and RNA: DNA ratio, protein concentration, and lipid assessments (Mathers et al. 1992; Guderley et al. 1996; Couture et al. 1998; Dutil et al. 1998; Majed et al. 2002). There does not appear to be any consensus on the best method. Indeed, many studies of the growth of fish in the ocean use a suite of measures. Some of these methods have limited utility for trawl-caught fish aboard a fishing vessel owing to fish's dying in the net or on deck, prob-

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lems with capture stress, and logistical problems associated with sample collection at sea.

These considerations led us to examine the hormones insulin and insulin-like growth factor-I (IGF-I) as growth indices. Both hormones play important roles in stimulating and supporting cellular growth (Mommsen 1998). Thus, these hormone levels could be related to growth rates. Indeed, several studies have reported associations among plasma insulin or IGF-I levels and feeding or growth (Storebakken et al. 1991; Sundby et al. 1991; Pérez-Sánchez et al. 1995; Beckman et al. 1998, 2001; Larsen et al. 2001; Pierce et al. 2001; Mingarro et al. 2002). Finally, the ability to process samples quickly (e.g., more than 300 samples/week) makes endocrine assays an attractive alternative to conventional techniques involving scale or otolith analysis.

Couture et al. (1998) made several suggestions as to the properties that biochemical indicators of growth should possess. First, such indicators of growth should be required for growth and not simply reflect it. Second, the indicators should be proportional to the growth rate and independent of recent growth history or condition. Third, since growth in length is physiologically different from growth in weight, biochemical indicators of growth may show different relations between growth and length and growth and weight. Plasma hormone levels have not previously been evaluated as growth indicators based on all of these criteria.

This work focuses on coho salmon *Oncorhynchus kisutch*, both in the laboratory and at sea. Coho salmon have one of the simpler life histories among Pacific salmon species (Groot and Margolis 1991). Smolts generally enter the ocean after 1–2 years of freshwater residence during a fairly narrow temporal window (April through June) (Weitkamp et al. 1995). In addition, coho salmon generally mature after 18 months in the ocean. Thus, it is relatively easy to infer the age and residence time of coho salmon captured in the ocean.

Our objective in this study was to assess two endocrine mediators of growth, insulin and IGF-I, as indices of growth based on the criteria of Couture et al. (1998). Two approaches were used: (1) we assessed the relations between growth and hormones in controlled laboratory experiments with individually marked fish, and (2) we compared blood hormone levels of juvenile coho salmon captured in the ocean with those from fish in laboratory experiments to assess the feasibility of using blood hormone levels from trawl-caught fish as growth indices.

Methods

Fish rearing and sampling.—Yearling coho salmon (1997 brood) were obtained from the Minter Creek Hatchery, Washington, and transferred to the National Marine Fisheries Service's Manchester Field Station, Manchester, Washington, by truck. Fish were weighed and measured, uniquely tagged with passive integrated transponder (PIT) tags, and held in freshwater for 2 weeks prior to transfer to seawater on 28 April 1999. Fish were reared in outdoor 3.7-m-diameter fiberglass tanks supplied with filtered and UV-treated seawater (salinity, 28‰). Temperature ranged from 9°C in May to a seasonal peak of 14°C in July and August, decreasing to 10°C at the last sampling in November. Fish (n = 1,300) were divided into two groups, low- and high-feed, there being two tanks of fish for each feeding treatment. By means of the bioenergetics model of Cho (1992), a daily ration was calculated to produce a mean size of 100 g for the low-feed group and 200 g for the high-feed group by October 1999. These targets resulted in feeding rates of 2% of body weight/d for the high-feed group in June, decreasing to 1.2% of body weight/d in November. Similarly, the low-feed group received 1% of body weight/d in June, and the ration decreased to 0.6% of body weight/d by November. Fish were fed a commercially available diet (BroodSelect; Moore Clark). Between 21 July and 10 August, approximately 40% of the experimental fish died from an infection of *Flexibacter maritimus*. This disease was manifested by serious necrosis of the tail (tail rot). Fish that developed early signs of the disease (eroding tail fins) inevitably died. Surviving fish grew according to projections. The incidence of disease decreased to zero by September (when sampling was initiated) and no diseased fish were sampled.

One tank (observation) of fish from each feeding treatment was used solely to determine a size-frequency distribution to guide sampling in the second tank (physiology). Destructive sampling was conducted on fish from this second tank. On each sampling date, PIT tags were read and lengths and weights were recorded for fish from the observation tanks. A size-frequency distribution was determined from these measurements for each feeding treatment. This size frequency was then divided into quartiles: small fish, medium-small fish, medium-large fish, and large fish. Fish from the physiology tank were evenly selected for sampling from these quartiles as they were weighed and measured. Thus, sampled fish were not ran-

TABLE 1.—Mean values and SEs of plasma insulin-like growth factor-I (IGF-I) and insulin, size, instantaneous growth rate (%/d), and condition factor (100,000·weight/length^3) of postsmolt coho salmon. Fish were fed at either low or high levels and sampled in September and November. The low feeding level consisted of feed equivalent to 1% of body weight per day in June, decreasing to 0.6% by November; the high feeding level consisted of feed equivalent to 2% of body weight in June, decreasing to 1.2% by November. Mean values with identical lowercase letters are not significantly different (ANOVA; $P < 0.05$); n = number of fish sampled for a given date and feeding treatment.

	September				November			
	Low feed		High feed		Low feed		High feed	
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE
IGF-I (ng/mL)	39.9 z	1.6	45.2 z	1.9	27.4 y	1.5	27.9 y	1.4
Insulin (ng/mL)	1.6 x	0.06	3.8 z	0.25	1.8 x	0.1	3.1 y	0.2
Length (mm)	199.6 x	3.2	211 xy	4.4	220.3 yz	3.5	237 z	5
Weight (g)	102.5 x	4.7	134 y	9.9	131.9 xy	6.3	178 z	12
Growth rate								
Length	0.26 y	0.01	0.35 z	0.02	0.17 x	0.01	0.26 y	0.01
Weight	0.7 y	0.05	1 z	0.07	0.34 x	0.04	0.55 xy	0.1
Condition factor	1.24 xy	0.01	1.29 z	0.01	1.2 x	0.02	1.26 yz	0.01
n	59		58		42		42	

domly obtained from the physiology tank as a whole; rather, fish were evenly chosen from the size range of fish in the tank. The fish collected for destructive sampling were sequestered in a 2-m-diameter tank until all fish in the tank were measured. Blood samples (see below) from fish from the high-feed tank were obtained in the afternoon of the first day of sampling. Fish from the low-feed group were sampled for blood the next morning. These differences in sampling time were necessitated by logistical difficulties associated with traveling from Seattle to the Manchester Field Station. Fish were not fed on either day of sampling. Lengths and weights of all fish were obtained in April (at tagging), June, August, September, and November. Blood samples were collected only in September and November.

Fish were killed singly in a lethal concentration (0.2 g/L) of tricaine methanesulfonate. The tail was severed and blood collected in heparinized glass tubes. Whole blood was stored on ice and then centrifuged at 3,000 × gravity for 3 min. Plasma was separated from red blood cells and stored at −80°C until hormone analyses were performed. Plasma insulin levels were measured by

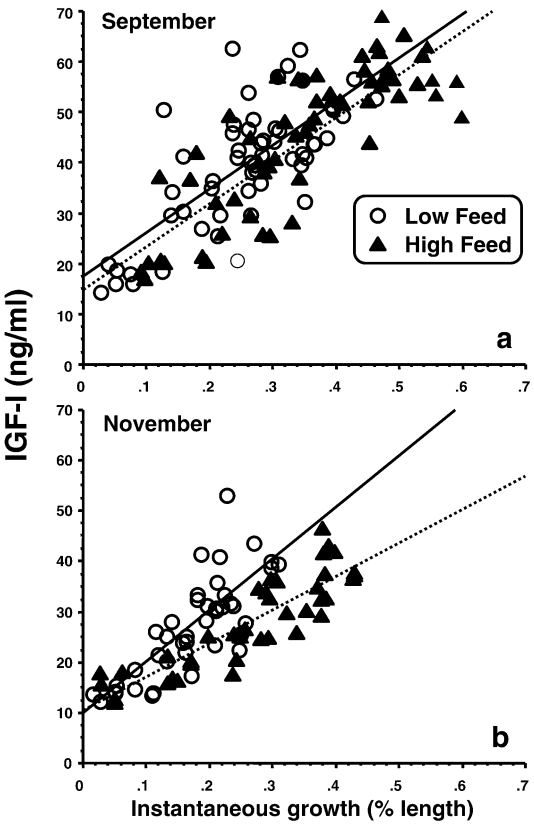

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FIGURE 1.—Scatterplots showing the relation between instantaneous growth rate per day and plasma insulin-like growth factor-I (IGF-I) level for individual postsmolt coho salmon reared at two feeding rates and sampled in **(a)** September and **(b)** November 1999. The low-feed group received feed equivalent to 1% of body weight per day in June, decreasing to 0.6% by November; the high-feed group received feed equivalent to 2% of body weight per day in June, decreasing to 1.2% by November. Significant regression relations are indicated by solid (low-feed group) or dashed lines (high-feed group).

TABLE 2.—Results of regression analysis for plasma insulin-like growth factor-I (IGF-I) and insulin levels vis-à-vis size, growth rate (%/d), and condition of postsmolt coho salmon raised at two different feeding levels and sampled in September and November. Regression coefficients, slopes, and intercepts (Int.) are not reported for nonsignificant relations ($P > 0.05$). See Table 1 for additional information.

	September									
	Low feed					High feed				
Variable	*F*	*P*	r^2	Slope	Int.	*F*	*P*	r^2	Slope	Int.
IGF-I										
Length (mm)	33.9	<0.0001	0.38	0.3	−22.7	61.6	<0.0001	0.53	0.33	−24.6
Growth rate (length)	63.8	<0.0001	0.54	86.5	17.5	123.6	<0.0001	0.69	85	14.9
Weight (g)	23.3	<0.0001	0.30	0.15	20.5	43.5	<0.0001	0.44	0.16	23.9
Growth rate (weight)	45.1	<0.0001	0.45	24.5	22.6	42.8	<0.0001	0.44	17.8	26.8
Condition factor	0.1	0.71				2.9	0.09			
Insulin										
Length (mm)	2.8	0.1				7.0	0.01	0.11	0.02	−0.42
Growth rate (length)	7.3	0.01	0.12	1.5	1.2	0.3	0.59			
Weight (g)	2.9	0.09				7.6	0.008	0.12	0.11	2.4
Growth rate (weight)	11.0	0.002	0.17	0.55	1.2	0.1	0.8			
Condition factor	1.2	0.29				29.2	0.003	0.15	7.4	−5.8

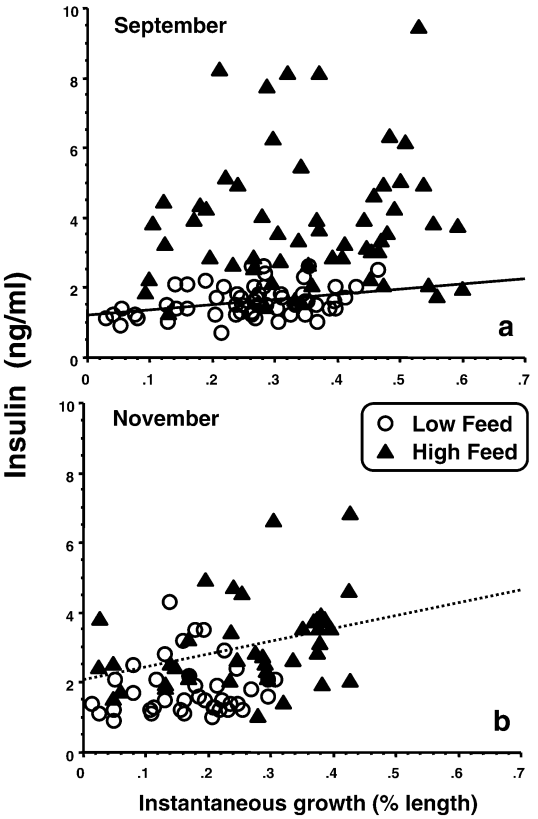

FIGURE 2.—Scatterplots showing the relation between instantaneous growth rate per day and plasma insulin level for individual postsmolt coho salmon reared at two feeding rates and sampled in **(a)** September and **(b)** November 1999. See the caption to Figure 1 for additional information.

radioimmunoassay (RIA; Plisetskaya et al. 1986). The plasma IGF-I levels of extracted plasma were determined by RIA using components obtained from GroPep, Ltd. (Adelaide, Australia), as described by Shimizu et al. (2000).

Ocean sampling.—Coho salmon were captured in the central Strait of Georgia off of Nanaimo, British Columbia, and in the northern Puget Sound off of Port Townsend, Washington, during standard 30-min surface trawls (Beamish et al. 2000) in September 1998. Fish were generally alive in the net and died as they were brought on deck and sorted. Postsmolt coho salmon (based on size) were randomly selected and placed on crushed ice. Blood was collected from fish (as described above) within 45 min of their being brought on deck. Plasma samples were kept at −20°C on the ship and transferred within 96 h to a −80°C freezer, where they were stored until analyzed.

Data analysis.—Length and weight data were used to calculate instantaneous growth rates and condition factors. The equation for instantaneous growth rates (for both length and weight) was

$$\text{Growth}\,(\%/\text{d}) = 100 \times [\log_e(s_2) - \log_e(s_1)]/(d_2 - d_1),$$

where s_2 is length or weight on day 2, s_1 is length or weight on day 1 and $d_2 - d_1$ is the number of days between measurements. Condition factor was computed from the equation

$$\text{Condition factor} = 100{,}000 \times \text{weight (g)}/\text{length (mm)}^3.$$

Differences among mean values were detected

TABLE 2.—Extended.

Variable	November									
	Low feed					High feed				
	F	*P*	r^2	Slope	Int.	*F*	*P*	r^2	Slope	Int.
IGF-I										
Length (mm)	42.2	<0.0001	0.52	0.3	−40.0	58.8	<0.0001	0.61	0.18	−25.8
Growth rate (length)	62.2	<0.0001	0.62	101	10.0	123.8	<0.0001	0.77	66.4	10.4
Weight (g)	34.9	<0.0001	0.48	0.2	5.5	33.9	<0.0001	0.47	0.06	13.6
Growth rate (weight)	99.0	<0.0001	0.72	33.9	16.1	68.8	<0.0001	0.64	17.7	18
Condition factor	0.5	0.49				1.4	0.24			
Insulin										
Length (mm)	0.7	0.4				13.0	0.009	0.26	0.02	−1.8
Growth rate (length)	0.1	0.71				5.2	0.03	0.12	3.7	2.1
Weight (g)	1.3	0.26				20.2	<0.0001	0.35	0.01	1.4
Growth rate (weight)	0.6	0.44				9.3	0.004	0.20	1.4	2.3
Condition factor	4.2	0.05	0.10	3.5	−2.4	8.9	0.005	0.19	5.4	−3.7

using analysis of variance (ANOVA) followed by Scheffé's multiple-range test ($P < 0.05$). Simple linear regression was used to assess the relations between hormone levels and fish size, growth rate, and condition factor for each feeding treatment and each date when plasma samples were obtained. Differences among regression slopes were determined using analysis of covariance (ANCOVA). Stepwise multiple regression analysis was performed with hormone levels and growth stanzas (June–August, August–September, and September–November) to determine the contribution of each growth stanza to the overall regression. The residuals of the regression of IGF-I on growth rate (length) were calculated and regressed on length measures to assess whether length was related to any of the residual variation. Statistical analyses were carried out in Statview (SAS Institute 1998).

Results

Mean size, condition factor, growth rate, and plasma IGF-I level varied significantly among fish from the low-feed and high-feed treatments in September and November (Table 1). Fish in the high-feed group were larger in both length and weight in September and November than fish in the low-feed group as a result of the higher growth rates they obtained in the August–September and September–November periods. Fish from the high-feed group also had significantly higher condition factor in both September and November. Average plasma IGF-I values did not vary significantly between the high- and low-feed treatments but decreased from September to November in both groups, reflecting the lower growth rates from August–September to September–November in both

TABLE 3.—Analysis of covariance table showing the significance of rearing tank treatment (month and feeding level; [main effect]) and growth rate in terms of length and weight (covariates) on plasma insulin-like growth factor-I levels.

Effect	df	Sum of squares	Mean square	*F*	*P*
Length					
Tank-treatment	3	142.7	47.6	1.4	0.23
Growth rate	1	7,565.3	7,565.3	228.4	<0.0001
Interaction	3	111.4	37.1	1.1	0.34
Residual	189	6,260.2	33.1		
Weight					
Tank treatment	3	520.6	173.5	4	0.008
Growth rate	1	6,729.1	6,729.1	155.9	<0.0001
Interaction	3	376.9	125.6	2.9	0.04
Residual	189	8,157.2	43.2		

TABLE 4.—Results of regresson analysis for the relation between (1) the residuals of the relationship between insulin-like growth factor-I level and growth rate in terms of length and (2) length of postsmolt coho salmon raised at two different feeding levels (see Table 1) and sampled in September and November. Regression coefficients are not reported for nonsignificant relations ($P > 0.05$).

	Low feed			High feed		
Month	F	P	r^2	F	P	r^2
Sep	2.4	0.12		6.8	0.01	0.11
Nov	1.9	0.18		0.5	0.47	

TABLE 5.—Stepwise regression analysis of the relation between plasma insulin-like growth factor-I level and the growth stanzas preceding sampling for postsmolt coho salmon in September or November.

Step	Growth interval	F to remove	r^2
	September		
1	Aug–Sep	147.4	0.604
2	Jun–Aug	14.6	0.650
	November		
1	Sep–Nov	27.8	0.512
2	Aug–Sep	12.9	0.578
3	Jun–Aug	4.9	0.598

treatments. Unlike IGF-I, plasma insulin levels varied significantly among feeding treatments in both September and November, the levels of high-feed fish being significantly greater than those of low-feed fish in both cases.

Plasma IGF-I was significantly related to the rate of growth in length and weight under both feeding conditions in September and November (Figure 1; Table 2). The regression coefficients for instantaneous growth in length ($r^2 = 0.54$–0.77) were generally higher than for instantaneous growth in weight ($r^2 = 0.44$–0.72). Similar relations were found when growth rates were calculated as grams per day or millimeters per day rather than as percent per day for both feeding treatment in both months (data not shown).

Plasma IGF-I was also significantly related to length and weight under all conditions. However, the regression coefficients for the relations between plasma IGF-I and size were almost always lower (seven of eight comparisons) than the corresponding regression coefficients for plasma IGF-I and growth rate ($r^2 = 0.72$ for plasma IGF-I and instantaneous growth [weight] in November for the low-feed group versus 0.48 for plasma IGF-I and weight for the same group in November). Plasma IGF-I was not significantly related to condition factor.

Plasma insulin was not consistently related to growth rate (Figure 2; Table 2). When a significant association was found (four of eight tests), the regression coefficient was low ($r^2 = 0.12$–0.20). Similarly, there was no consistent association between plasma insulin and size. While plasma insulin was significantly related to condition factor in three of the four groups tested, regression coefficients were low ($r^2 = 0.10$–0.19). Based on these results, insulin did not appear to be a good predictor of growth and was not analyzed further.

The relations between plasma IGF-I and growth were compared for each feeding treatment for both September and November to determine whether there was a constant slope. No significant differences in the slopes of the IGF-I–instantaneous growth (length) equations were found, but the slopes of the IGF-I–instantaneous growth (weight) equations differed (ANCOVA; Table 3). Further analysis revealed that the IGF-I and instantaneous growth (weight) data in September had a binomial relation ($y = 17 + 47x - 15.7x^2$; $F = 67.5$, $P < 0.0001$, $r^2 = 0.55$). Plasma IGF-I and instantaneous growth (length) data for both treatments and for both September and November were pooled to examine the overall relation of IGF-I to growth. These data resulted in a highly significant linear equation ($y = 13.2 + 85.7x$; $F = 341.1$, $P < 0.0001$, $r^2 = 0.64$). A binomial relation was not supported ($P > 0.05$ for the second-order polynomial).

The residuals of the IGF-I–growth rate (length) regression were not significantly related to length in three of the four cases tested (Table 4). This suggests that little of the variability found in IGF-I–growth rate (length) relations is explained by fish size. We examined whether the growth of an individual fish in an earlier period influenced IGF-I levels in a subsequent period with multiple regression. Plasma IGF-I level was best related to the growth rate of fish in the interval in which the plasma sample was obtained (Table 5). For example, for fish sampled in November, the September–November growth interval contributed the most to the overall growth–plasma IGF-I relation ($r^2 = 0.51$), the August–September and June–August growth rate contributions to the overall relation being small (August–September incremental $r^2 = 0.07$; June–August incremental $r^2 = 0.02$).

Mean plasma IGF-I values for fish from the laboratory experiment, pooled in growth increments of 0.10%/d (length), varied significantly (Figure

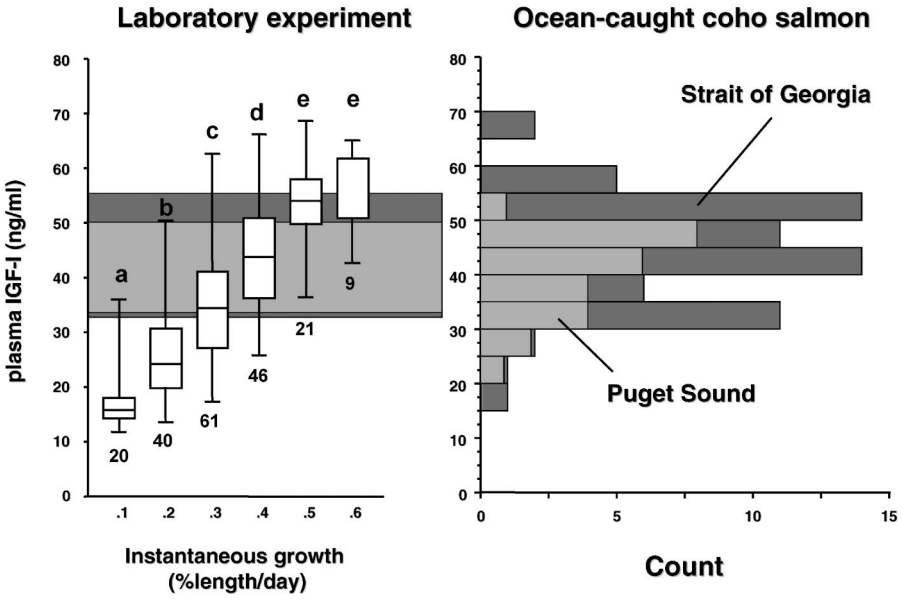

FIGURE 3.—The box-and-whisker plots in the left-hand panel show (1) the median (horizontal lines), (2) the 25th and 75th percentiles (bottoms and tops of the boxes), and (3) the full range of values (whiskers) for insulin-like growth factor-I (IGF-I) in postsmolt coho salmon reared in a laboratory setting at either a high or a low feeding rate (see the caption to Figure 1) that were sampled in September and November 1999, as grouped by growth increment. The number of fish in each growth increment is shown below the box; boxes with different lowercase letters differ significantly ($P < 0.05$) with respect to median value. The shaded areas show the distribution of the means (±SDs) of plasma IGF-I values from postsmolt coho salmon captured in either the Strait of Georgia or Puget Sound in September 1999 in relation to measured growth rates and the IGF-I values of laboratory fish. The right-hand panel shows a frequency distribution of plasma IGF-I values in the ocean-caught fish.

3). The range of IGF-I values within each growth increment was relatively high. However, there was a stair step increase in both the minimum and maximum IGF-I values with increases in growth increment.

Postsmolt coho salmon captured in the Strait of Georgia in September averaged 241 mm in length and 170 g in weight (Table 6). They were larger than fish sampled from either high- or low-feed treatments. Coho salmon from Puget Sound were significantly smaller than Strait of Georgia fish and similar in size to the high-feed fish. Mean plasma IGF-I levels of ocean-caught coho salmon were similar to those found in the low- and high-feed treatment groups sampled in September and did not differ significantly from each other. Plasma IGF-I was significantly related to length, weight, and condition factor for coho salmon captured in the Strait of Georgia (Table 7). None of these associations were significant for coho salmon caught in Puget Sound.

Individual plasma IGF-I values of fish captured in the Strait of Georgia and Puget Sound ranged from roughly 15 to 70 ng/mL (Figure 3). The majority of fish captured had values ranging from 30 to 60 ng/mL. A similar range of IGF-I values was found in the low- and high-feed treatment fish. Thus, individual plasma IGF-I levels in ocean-caught fish were within the range observed in fish from the laboratory experiment.

The plasma IGF-I values of fish from the low- and high-feed treatments corresponded to growth

TABLE 6.—Mean values and SEs of insulin-like growth factor-I level, size, and condition factor (100,000·weight/length^3) of postsmolt coho salmon captured in either the Strait of Georgia or Puget Sound in September 1998. Means with identical lowercase letters are not significantly different ($P < 0.05$); n = the number of fish sampled for a given date.

	Strait of Georgia		Puget Sound	
Variable	Mean	SE	Mean	SE
IGF-I (ng/mL)	43.9 z	1.2	40.2 z	1.6
Length (mm)	241 z	3.3	216.1 y	5.4
Weight (g)	169.4 z	7.2	133.3 y	11.8
Condition factor	1.17 y	0.01	1.25 z	0.02
n	69		26	

TABLE 7.—Results of regresson analysis for relations between plasma insulin-like growth factor-I level and size and condition factor (100,000·weight/length^3) of postsmolt coho salmon captured in the Strait of Georgia and Puget Sound. Regression coefficients, slopes, and intercepts (Int.) are not reported for nonsignificant relations ($P > 0.05$).

	Strait of Georgia					Puget Sound				
Variable	F	P	r^2	Slope	Int.	F	P	r^2	Slope	Int.
Length (mm)	12.3	0.0008	0.16	0.16	5.2	0.5	0.47			
Weight (g)	16.1	0.0002	0.20	0.08	29.7	1.1	0.32			
Condition factor	9.0	0.0040	0.13	40.30	−4.0	0.2	0.36			

rates (length) ranging from less than 0.1%/d to almost 0.6%/d (Figure 3). Very few ocean-caught fish had plasma IGF-I values corresponding to those of experimental fish with growth rates of less than 0.1%/d. In contrast, there were significant numbers of ocean-caught fish that had plasma IGF-I values corresponding to those of experimental fish with growth rates exceeding 0.5%/d.

Discussion

Organismal growth is undeniably mediated by the endocrine system. In particular, the growth hormone–IGF-I axis is essential for stimulating growth in vertebrates (Lupu et al. 2001). Both cartilage and muscle cell growth are dependent on the actions of growth hormone and IGF-I (Nicoll et al. 1999). In fish, IGF-I treatment increases whole-body growth and has been shown to increase muscle and cartilage growth (McCormick et al. 1992; Negatu and Meier 1995; Chen et al. 2000). Several studies have also suggested that plasma IGF-I levels are correlated with either feeding level or growth rate (Pérez-Sánchez et al. 1995; Beckman et al. 1998, 2001; Larsen et al. 2001; Pierce et al. 2001; Mingarro et al 2002). Together, these data indicate that IGF-I meets the first criterion of Couture et al. (1998) for a biochemical growth index: IGF-I directly stimulates growth.

Our results show that plasma IGF-I is more strongly related to growth rate than to either size or condition factor. The coefficient for the growth relation (instantaneous growth in length or weight) was consistently higher than that for the size relation (length or weight) in both September and November. In addition, length had little relation to the residuals from the IGF-I–instantaneous growth (length) regressions. No significant association was found between IGF-I and condition factor. Moreover, Beckman et al. (2001) found no link between body adiposity and plasma IGF-I level. Insulin-like growth factor-I thus meets the second criterion of Couture et al. (1998): it has a stronger relation with growth than with size or condition factor.

A multiple-regression model suggests that IGF-I levels predominantly reflect current growth rate within the 6-week growth stanzas encompassed in this experiment. Previous growth stanzas added relatively little (<10%) to the multiple-regression relation. Pierce et al. (2001) found that IGF-I levels in individual coho salmon could be related to growth over 4-week periods. Recent results (Beckman et al. 2004) demonstrated that the plasma IGF-I levels of juvenile coho salmon reflect growth rates over approximately 2-week periods. This fulfills an additional requirement of the second criterion of Couture et al. (1998): IGF-I reflects the current growth rate, not previous growth history.

The regression coefficients for the IGF-I–instantaneous growth (length) equations were higher than those for the IGF-I–instantaneous growth (weight) equations, particularly in September. The shape of these relations also varied. The relation involving length was linear, while that involving weight was best approximated by a binomial equation. This meets the third criterion of Couture et al. (1998): the relations between plasma IGF-I level and growth in length and weight are not identical.

We are not suggesting that IGF-I levels can provide a precise estimate of growth rate. In neither September nor November did mean IGF-I values differ between the low-feed and high-feed treatments. Although significant differences in growth rate were found in both cases, the magnitudes of the differences were not large. In individual fish we evaluated IGF-I–growth rate (length) associations over the growth rate range of 0.02–0.56%/d (a 25-fold difference). Mean growth rates varied from 0.26%/d to 0.35%/d (a less than 0.5-fold difference) in September and from 0.17%/d to 0.26%/d (a 0.5-fold difference) in November. While it would certainly be desirable to develop an assay that can accurately distinguish between groups of

fish growing at rates that differ by relatively small amounts, an assay that can distinguish between relatively large differences in growth rates may still prove useful.

The novelty of this work does not lie in suggesting that there is a link between feeding level, growth rate, and plasma IGF-I level. This has been accomplished in previous work (Pérez-Sánchez et al. 1995; Beckman et al. 1998, 2001; Larsen et al. 2001; Pierce et al. 2001; Mingarro et al. 2002). However, it should be noted that several studies have failed to find an association between IGF-I and growth rate (Silverstein et al. 1998; Plisetskaya 1998; Nankervis et al. 2000). In addition, one might criticize the experimental design used in this study: the treatment tanks were not replicated and plasma samples were obtained from fish from the different treatments at different times. However, our study was designed to address two questions: (1) whether hormone levels are directly related to growth rate (i.e., does a significant regression equation exist?) and (2) whether different feeding levels alter the relation between hormone levels and growth rate (i.e., do regression equations differ between feeding treatments and sampling dates?). Attaining these objectives was not compromised by the experimental design. In essence, we conducted four independent assessments of the association between individual hormone levels and growth rate. In each case, IGF-I levels were related to growth rate and the slopes of the IGF-I–growth rate (length) equations did not differ. To our knowledge, only Pierce et al. (2001) has shown a similar association between individual plasma IGF-I values and growth rates in a fish (coho salmon). The present results constitute the most rigorous evaluation to date of plasma IGF-I level as a growth indicator in fish.

The differences in mean plasma insulin value between high- and low-feed fish may be due to the differences in sampling time. The low-feed fish were fasted at least 16 h more than the high-feed fish. Plasma insulin levels in salmonids clearly respond to feeding and fasting (Navarro et al. 1992). However, regardless of sampling time, insulin values never displayed a strong link to growth rate. Several studies have indicated that insulin levels are related to either size or growth rates (Hilton et al. 1987; Sundby et al. 1991; Storebakken et al. 1991; Baños et al. 1998; Plisetskaya 1998; Silverstein et al. 1998; Rungruangsak-Torrissen et al. 1999). Our results suggest that these associations are not strong enough to encourage the use of plasma insulin levels as a growth indicator.

This study does not report on an extensive oceanic sampling program for postsmolt coho salmon. Instead, plasma IGF-I values for ocean-caught postsmolts were included as a first step in validating the use of such values as a growth index for ocean-caught fish. The values we obtained from the ocean-caught fish seem reasonable; although they were on the high side of the range found in the laboratory fish, the ocean-caught fish were bigger than the laboratory fish and had a robust condition factor. Certainly, these fish had been growing over the course of the summer. The IGF-I values that we obtained from these fish would support the thesis that the fish were growing at relatively high rates when they were caught. There was a weak association between plasma IGF-I level and both size and condition factor in Strait of Georgia fish. The slopes of the regression equations were roughly similar to those found in the laboratory experiments, though the regression coefficients were lower. This might be expected, as the range of IGF-I values found in the ocean-caught fish was smaller than that found in the laboratory experiments. No association was found between size or condition factor and plasma IGF-I level within the Puget Sound fish, perhaps because fewer fish were examined. Overall, the results from the ocean-caught fish are consistent with a relationship between IGF-I and growth rate in these samples.

We provide no proof that there was no ''stress'' effect on plasma IGF-I values for the ocean-caught fish, yet there is no evidence that stress effects did occur. Indeed, we did not obtain our laboratory samples in a manner that would alleviate stress. Fish were netted, anesthetized, weighed, measured, and transferred to a holding tank for a period of minutes to hours, then netted a second time, placed in a bucket, and killed. If the stress of handling and sampling does affect plasma IGF-I values, it does so in a manner proportional to growth rate. In addition, recent work by Kajimura et al. (2003) demonstrated that injections of cortisol (one of the major stress hormones) did not change plasma IGF-I levels in Mozambique tilapia *Oreochromis mossambicus* until more than 24 h postinjection. Finally, no short-term (<24-h) effect of handling stress on plasma IGF-I levels in California sheephead *Semicossyphus pulcher*, jack mackerel *Trachurus symmetricus*, or longjaw mudsucker *Gillichthys mirabilis* has been found (Kevin Kelley, California State University, Long Beach, personal communication). Together, these results support further investigation on plasma IGF-I values as a growth index for ocean-caught fish.

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