Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins in coho salmon fasted for varying periods

Munetaka Shimizu,1,2 Kathleen A. Cooper,2 Walton W. Dickhoff,1,2 and Brian R. Beckman1

1Northwest Fisheries Science Center, National Oceanographic and Atmospheric Administration Fisheries, Seattle, Washington; and 2School of Aquatic and Fishery Sciences, University of Washington, Seattle, Washington

Submitted 20 November 2008; accepted in final form 22 May 2009

Shimizu M, Cooper KA, Dickhoff WW, Beckman BR. Postprandial changes in plasma growth hormone, insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins in coho salmon fasted for varying periods. Am J Physiol Regul Integr Comp Physiol 297: R352–R361, 2009.—We examined postprandial changes in circulating growth hormone (GH), insulin, insulin-like growth factor (IGF)-I, and IGF-binding proteins (IGFBPs) in yearling coho salmon under different feeding regimes. Fish were initially fasted for 1 day, 1 wk, or 3 wk. Fasted fish were then fed, and blood was collected at 4-h intervals over 26 h. After the various periods of fasting, basal levels of insulin were relatively constant, whereas those of IGF-I, IGFBPs, and GH changed in proportion to the duration of the fast. A single meal caused a rapid, large increase in the circulating insulin levels, but the degree of the increase was influenced by the fasting period. IGF-I showed a moderate increase 2 h after the meal but only in the regularly fed fish. Plasma levels of 41-kDa IGFBP were increased in all groups within 6 h after the single meal. The fasting period did not influence the response of 41-kDa IGFBP to the meal. IGFBP-1 and GH decreased after the meal to the same extent among groups regardless of the fasting period. The present study shows that insulin and IGF-I respond differently to long (weeks)- and short (hours)-term nutritional changes in salmon; insulin maintains its basal level but changes acutely in response to food intake, whereas IGF-I adjusts its basal levels to the long-term nutritional status and is less responsive to acute nutritional input. IGFBPs maintain their sensitivity to food intake, even after prolonged fasting, suggesting their critical role in the nutritional regulation of salmon growth.

GROWTH OCCURS WHEN THERE IS A net positive difference between the anabolic and catabolic processes of metabolism. Metabolism rapidly shifts between anabolic and catabolic processes on the order of minutes to hours, depending on current energy intake and expenditures. Growth varies at longer time scales of days to weeks and even months, depending on net energy intake averaged over these same days and weeks. Metabolism directly responds to current stimuli, while changes in growth rate are due not only to current intake but also to nutrient reserves stored over time. Thus growth is integrally connected to metabolism.

Regulation of growth and metabolism is among the primary functions of the endocrine system. Both growth and metabolism are mediated and modulated through a myriad of differing factors that include integration and stimulation through the hypothalamic-pituitary axis; however, some primary mediators have been identified in the peripheral circulation (23). Pancreatic hormones such as insulin and glucagon are important regulators of anabolic and catabolic processes, respectively (24, 33). Growth is mediated through the actions of growth hormone (GH)-insulin-like growth factor (IGF)-I system. Interestingly, insulin and IGF-I are structurally related peptides, which arose from gene duplications before the emergence of the Chondrichthyes (8). Endocrine mechanisms have developed such that these structurally similar hormones modulate differing yet linked aspects of the physiology of metabolism and growth.

The kinetics of insulin and total IGF-I in the circulation are markedly different. Circulating insulin shows a rapid change in response to a meal and its half-life in the circulation is less than 5 min (6). In contrast, total IGF-I level is relatively stable, and its half-life is ~12 h in humans and fish (9, 15). This difference is attributed, at least partly, to the presence of a family of six IGF-binding proteins (IGFBPs). Among the six IGFBPs, IGFBP-3 carries more than 80% of circulating IGF-I (49). IGFBP-3 prolongs the half-life of IGF-I by forming a ternary complex with IGF-I and an acid-labile subunit (ALS) in mammals (1). Approximately 20% of IGF-1 binds to other IGFBPs, and less than 1% exists in the free form (13, 49). The levels of free IGF-I are presumably regulated in part by short-term changes in IGFBP-1 (13, 22). IGFBP-1 is the only form that shows a marked change following food intake in mammals (22, 35). Changes in IGFBP-1 level are primarily due to a suppressive effect of insulin at the level of transcription, and IGFBP-1 and insulin levels are generally inversely related (22). One of the primary effects of the IGFBPs is to limit interactions between IGF-I and the insulin receptor by the reduction in free IGF-I levels, as IGF-I is capable of activating the insulin receptor (10).

In fish, at least five IGFBP sequences can be found in the fugu and zebrafish genome databases, and cDNA sequences of six IGFBPs have recently been reported for rainbow trout (Oncorhynchus mykiss) (17). Multiple IGFBPs are also present in the circulation of fish species. Western ligand blotting of fish plasma/serum typically reveals three IGFBP bands at 20–25, 25–30, and 40–50 kDa (18). The 20–30-kDa and 40–50-kDa IGFBPs are believed to be fish homologs of mammalian IGFBP-1 and -3, respectively. This assumption is based on the similarities of their molecular size and physiological regulation, although the exact identity of the circulating fish IGFBPs is still not clear. In salmon, the 22-kDa IGFBP has been identified as IGFBP-1 (38). Salmon 41-kDa IGFBP appears to be a functional homolog of IGFBP-3 (39), while its amino acid...
sequence is most similar to IGFBP-2 (36, 42). These IGFBPs are under gross nutritional control (37, 39). However, their overall physiological role and response to graded nutritional stimuli have not been fully examined in fishes.

A common paradigm for investigating factors involved in metabolism and growth is a fasting time course. Animals on a positive nutritional plane are taken off feed, and the change in the levels of various factors is monitored. Those involved in anabolic processes and growth generally decrease and those involved in catabolic processes increase (12, 31). We have performed an inverse of this experiment, by fasting animals and then following a time course after feeding. In particular, we fasted coho salmon for differing durations to place the animals in differing degrees of catabolic stress. This helped us to differentiate between the upregulation of anabolic and growth processes after ending the fast, as we assumed that the initiation of growth processes would be diminished in fasted fish. Thus, we compared changes in circulating plasma level of insulin, (total) IGF-I, GH, IGFBP-1 and 41-kDa IGFBP of fasted and fed coho salmon after 1-day, 1-wk, and 3-wk fasts.

MATERIALS AND METHODS

Fish. One-year-old coho salmon (Oncorhynchus kisutch) [fork length 16.1 ± 0.8 cm (means ± SE), body weight 48.7 ± 2.6 g, n = 363] were reared in fresh water at the Northwest Fisheries Science Center in Seattle, WA. They were maintained in recirculated fresh water in circular fiberglass tanks under incandescent light; flow rate was 25 l/min; temperature ranged from 10.5°C to 13.0°C; photoperiod was adjusted to that of Seattle, WA, weekly (48°N). Fish were fed standard rations (0.6–1.0% body wt/day) of a commercial diet (Biodiet Grower; Bioproducts, Warrenton, OR) prior to the start of the experiment.

Experimental design and sampling procedure. Prior to the initiation of sampling, fish in each of two tanks were fasted for 1 day (18 h), 1 wk or 3 wk (six tanks total). On August 1, 2003, fish in one tank of each treatment were fed to satiation at 1400. Fish in the other tank received no meal. These treatments resulted in six groups with each treatment were fed to satiation at 1400. Fish in the other tank of sampling, fish in each of two tanks were fasted for 1 day (18 h), 1 wk or 3 wk, no meal. Fish were sampled before and after the single meal at day 1

RESULTS

significant effects of treatment and length were found in an ANCOVA analysis of IGF-I levels (fast: F = 3.9, P = 0.02; length: F = 93.6, P < 0.0001; interaction F = 1.5, P = 0.24; Fig. 1A). Individual IGF-I levels were standardized from each fasting treatment utilizing the following regression relations (Fast0: IGF-I = −3.9 + 0.11-length, P < 0.0001, r2 = 0.15; Fast1: IGF-I = −11.0 + 0.13-length P < 0.0001, r2 = 0.40; Fast3: IGF-I = −4.0 + 0.07-length, P < 0.0001, r2 = 0.32). Standardization resulted in removing the size relation with IGF-I values (Fig. 1B). Similarly, significant effects were found with an ANCOVA analysis of 41-kDa IGFBP levels in relation to fasting period with length as a covariate (fast: F = 29.9, P < 0.0001; length: F = 202.9, P < 0.0001; interaction F = 23.8, P < 0.0001). The following regression equations were used to length standardize the 41-kDa IGFBP data (Fast0: 41-kDa IGFBP = −40.1 + 1.22-length, P < 0.0001, r2 = 0.13; Fast1: 41-kDa IGFBP = −162.3 + 1.8-length P < 0.0001, r2 = 0.28, Fast3: 41-kDa IGFBP = −141.3 + 1.52-length, P < 0.0001, r2 = 0.22). No significant effects were found when the insulin, GH, or IGFBP-1 data were analyzed similarly.

Basal plasma insulin levels were similar among the three groups (fasted for 1 day, 1 wk, or 3 wk) at the beginning of the experiment (i.e., 0800–1200 on day 1) (Fig. 2). Plasma insulin levels in fish receiving no meal showed no sign of diel rhythm in any group. When fish received a meal at 1400, insulin increased within 2 h in all groups and remained elevated for more than 24 h. The magnitude of the insulin response was influenced by feeding history, with the highest postprandial
elevation (17.7-fold increase compared with no meal control) occurring in fish fasted for the shortest period, while lowest in fish fasted for 3 wk (9.6-fold increase) (Figs. 2 and 7A).

Fasting fish for 3 wk significantly decreased basal circulating IGF-I levels compared with both 1-day and 1-wk fasted groups (Fig. 3). A clear increase in plasma IGF-I was seen after a meal for the regularly fed fish (1-day fasted group) after 2 h (Fig. 3A). However, this increase was lower than found in insulin and diminished within 14 h. Fish fasted for more than 1 wk showed no clear IGF-I response to the single meal (Figs. 3, B and C, and 7B).

Fasting significantly decreased basal plasma 41-kDa IGFBP levels (Fig. 4). Fish without a meal showed no indication of a diel rhythm in any group. Postprandial plasma 41-kDa IGFBP levels became elevated in all groups by 6 h and remained high for over 24 h (Fig. 4). The single meal caused similar increases in 41-kDa IGFBP levels among groups despite differences in preprandial levels (Figs. 4 and 7C).

Fig. 1. Relationship between body length and IGF-I before (A) and after (B) length standardization (see METHODS) of coho salmon fasted for 1 day, 1 wk or 3 wk. Because IGF-I values were positively correlated with body length, random size variation of fish could mask treatment effects. IGF-I values were, therefore, standardized to body length to remove the size influence.

Fig. 2. Circulating insulin levels in 1-yr-old coho salmon fasted for 1 day (A), 1 wk (B), or 3 wk (C) prior to the sampling and either fed a meal (closed symbols) or not (open symbols). Values are expressed as means ± SE of six or seven fish. *Significant difference between meal and no meal groups for a given time point.
Fig. 3. Circulating IGF-I levels in 1-yr-old coho salmon fasted for 1 day (A), 1 wk (B), or 3 wk (C) prior to the sampling and either fed a meal (closed symbols) or not (open symbols). IGF-I values were standardized with body length and shown as means ± SE of six or seven fish. *Significant difference between meal and no meal groups for a given time point.

Fig. 4. Circulating 41-kDa IGFBP levels in 1-yr-old coho salmon fasted for 1 day (A), 1 wk (B), or 3 wk (C) prior to the sampling and either fed a meal (closed symbols) or not (open symbols). 41-kDa IGFBP values were standardized with body length and shown as means ± SE of six or seven fish. *Significant difference between meal and no meal group for a given time point.
IGFBP-1 levels increased in relation to the length of the fasting period (Fig. 5). There was some fluctuation in IGFBP-1 in the unfed groups, but these did not follow a consistent pattern. When fish received a single meal, plasma IGFBP-1 began declining within 6 h and became significantly lower than in unfed fish in all groups by 14 h (Fig. 5). The effect of the single meal was similar among the three groups (Figs. 5 and 7D).

Plasma GH levels varied directly with fasting; significantly higher levels were found in fish fasted for longer periods (Fig. 6). Feeding a meal resulted in rapid and significant decreases in plasma GH, with significant differences between fed and fasted groups found 2 h postfeeding in all treatments. These differences between fed and fasted fish were sustained throughout the duration of the time course in the 1 day fasted fish, whereas differences between fed and fasted fish were transitory and disappeared after 6 h in the 3-wk fasted groups.

**DISCUSSION**

Circulating levels of insulin and (total) IGF-I present distinctly different patterns of response to short- and long-term nutritional changes in salmon. Basal levels of insulin were relatively constant during fasting (weeks) and rapidly increased after feeding (hours). In contrast, basal IGF-I levels directly reflected fasting status, as these levels directly decreased with the extent of the fast. However, acute response of IGF-I levels to food intake was minimal. In short, insulin and IGF-I levels adjusted to nutritional status differently by changing magnitude of acute response and basal level, respectively. These differing patterns lend insight into both the differing physiological roles of IGF-I and insulin and mechanistic differences in the regulation of plasma IGF-I and insulin levels.

Insulin appears to respond to short-term changes in nutritional status while IGF-I response is related to longer-term integrated trends in nutritional status. After a meal, nutrients are absorbed from the gut into the bloodstream. One of the primary roles of insulin is to direct nutrients from the blood into cells and tissues (23). Thus plasma insulin levels increase rapidly after a meal in response to increased glucose and amino acid loading of the bloodstream (28). In this experiment, plasma insulin levels remained high for an extended period after feeding. This is likely due to the fact that fish were fed to satiation, and in many cases, these same fish still had full stomachs 24 h after the meal (data not shown). Regardless of the fishes’ long-term nutritional status (fasting for 1 day or 3 wk), it is advantageous to rapidly remove and store nutrients from the blood. Thus one sees a similarly rapid increase in insulin after a meal across fasting state.

The rapid postprandial increase in fish insulin was similar to what has been found in previous studies (27, 32, 44). However, we found that the degree of the insulin increase depends on the feeding history of fish; fish that had been fed daily showed the maximum postprandial insulin response after a meal, whereas the insulin rise was progressively lower in fish fasted for longer periods. This suggests that insulin synthesis decreased, insulin sensitivity increased, or the stimulatory signals for insulin release were somehow reduced in fasted fish (34, 51).

Previous studies have demonstrated that fasting reduces plasma IGF-I levels in salmon (26, 31). These data suggest that one of the ways that growth processes are fine-tuned is to...
modulate the response of plasma IGF-I levels to a meal based upon longer-term nutritional state. Thus, we found little response of plasma IGF-I level to a meal after the fish had been fasted for a week. In addition, we found depleted basal IGF-I levels in these fish, suggesting that growth processes had been down-regulated by the fasting condition. These data suggest that it takes more than one meal to recondition fasting salmon to resume growth processes, as there was no IGF-I response to a meal in fasted fish. The present study was undertaken as part of a validation of the use of circulating IGF-I as a growth index in fish. Increasing evidence in teleosts suggests that circulating IGF-I levels positively correlate with growth rates of individuals and could be used to evaluate growth performance of fish for aquaculture and stock assessment (3, 30). The present study expands our knowledge of the efficacy of IGF-I as a growth indicator, as the effect of recent feeding history was examined.

The differing patterns of insulin and IGF-I in response to fasting and refeeding may also be reflections of differences in production, storage, release, and clearance of these hormones. Insulin peptide is stored in secretory granules in the endocrine pancreas. Large and rapid increases in plasma insulin levels are consistent with the release of hormone from storage (24). In contrast, IGF-I is secreted constitutively, as it is produced in hepatic cells (47). Plasma IGF-I levels do not demonstrate the dramatic increase (release) and decrease (clearance) of insulin following a meal. Instead, plasma IGF-I levels are relatively stable as clearance from the blood is retarded by IGF-I binding to several different IGFBPs. In mammals, more than 95% of circulating IGF-I is bound to IGFBPs, whereas a small fraction (<1–5%) is unbound (free IGF-I) (13). This is also the case for salmon (40). Under a wide range of physiological conditions, free IGF-I level is a reflection of equilibrium between total IGFs and IGFBPs, especially IGFBP-1, and change in free IGF-I is relatively rapid comparable to insulin (11). Thus, the pattern of postprandial changes in free IGF-I might be different than that of total IGF-I. Given that free IGF-I plays roles in glucose homeostasis and feedback regulation of GH release (11), it might also be important to assess free IGF-I levels in future studies.

Length standardization is a common data transformation in ecological and evolutionary studies (19) but has not to our knowledge been conducted in endocrinology. We chose to take this approach given a relatively small sample size per time point (\(n = 7\)) and the relation we found between size and both IGF-I and 41-kDa IGFBP. The transformation resulted in reducing the variance associated with mean IGF-I or 41-kDa IGFBP level induced by random variation among the size of fish sampled at any one time point and thus allowed us to determine differences in IGF-I or 41-kDa IGFBP between treatments with greater precision. There were large differences in the \(y\)-intercept of the regression lines of size and both IGF-I and 41-kDa IGFBP. The transformation resulted in reducing the variance associated with mean IGF-I or 41-kDa IGFBP level induced by random variation among the size of fish sampled at any one time point and thus allowed us to determine differences in IGF-I or 41-kDa IGFBP between treatments with greater precision. There were large differences in the \(y\)-intercept of the regression lines of size and both IGF-I and 41-kDa IGFBP between treatments, demonstrating that nutritional condition plays a large role in determining these levels (lower IGF-I or 41-kDa IGFBP levels in fasted fish). However, the analysis also suggests that there is an underlying relation between basal IGF-I and 41-kDa IGFBP levels and fish size. The mechanistic source(s) of the relations between size and IGF-I or 41-kDa IGFBP are unknown; however, size may also serve as a proxy for energy reserves such as lipid that were unlikely to be greatly depleted in a 3-wk fast (21). The fact that no relations were found among length and insulin, GH, or

![Fig. 6. Circulating growth hormone levels in 1-yr-old coho salmon fasted for 1 day (A), 1 wk (B), or 3 wk (C) prior to the sampling and either fed a meal (closed symbols) or not (open symbols). Values are expressed as means ± SE of six or seven fish. *Significant difference between meal and no meal group for a given time point.](http://ajpregu.physiology.org/)
IGFBP-1 suggest that metabolic conditions (such as feeding and fasting) have a primary and overriding role in regulating levels of these factors.

The responses of IGFBPs (41-kDa IGFBP and IGFBP-1) add another aspect of response by salmon to nutritional condition; basal levels of salmon IGFBPs changed during fasting, as well as responding quickly to food intake, regardless of the fasting period. These data suggest that one of the main components of the regulation of plasma IGF-I levels and the growth process is variation in the production and circulating levels of IGFBPs.

![Graphs showing changes in IGF-I, IGFBP-1, GH, and insulin levels](image-url)

Fig. 7. Insulin (A), IGF-I (B) and 41-kDa IGFBP (C), IGFBP-1 (D) and GH (E) levels averaged for the 24-h period after a meal. Columns are means ± SE of seven time points. Symbols sharing the same letters are not significantly different from each other (two-way ANOVA followed by Fisher’s paired least significant difference test, *P* < 0.05).

The 41-kDa IGFBP is a main carrier of circulating IGF-I and thus physiologically equivalent to mammalian IGFBP-3 (39), although perhaps structurally most similar to mammalian IGFBP-2 (17, 36, 42). The basal levels of circulating 41-kDa IGFBP were reduced by fasting. However, unlike insulin and IGF-I, the response of 41-kDa IGFBP to a single meal was independent of feeding history, and there was a rapid increase in 41-kDa IGFBP after a meal. This result suggests that the pathway by which nutrition regulates the production of 41-kDa IGFBP was inactive but not unresponsive after a fast. In addition, the different patterns of response of IGF-I and 41-
kDa IGFBP to a meal suggest that differential regulation of IGF-I and 41-kDa IGFBP is possible. In general, IGF-I and 41-kDa IGFBP are well correlated in fish (5, 31, 39), and GH stimulates plasma levels of both of these factors (25, 39, 41). The decline in plasma IGF-I levels during fasting, despite high levels of plasma GH, is thought to be due to GH resistance by the liver (14). Fasting is known to impair the GH receptor signaling pathway (2). Given the significant decrease in GH in fasted fish after a meal, the increase in 41-kDa IGFBP and the lack of response in IGF-I in these fasted fish, there is more to learn about 41-kDa IGFBP regulation in fish. Specifically, in mammals, it has been shown that IGFBP-3 is not produced in hepatocytes but rather in Kupffer cells that are also found in the liver (52). The production of IGF-I and IGFBP-3 by different cell types in mammals(i.e., IGF-I by hepatocytes and IGFBP-3 by Kupffer cells) would certainly allow for differential regulation of production if the same arrangement occurred in fishes.

In mammals, postprandial IGFBP-3 levels are relatively stable over 24 h (48), while salmon 41-kDa IGFBP showed a relatively rapid response to a meal in 6 h. There appears to be a significant mechanistic difference between the mammalian and piscine IGFBP systems. No study of piscine systems has been able to demonstrate the presence of a ternary complex of IGF-I, 41-kDa IGFBP, and ALS (9, 40, 46). ALS is a relatively large protein that greatly retards clearance of IGF-I and IGFBP-3 in mammals. An apparent lack of the ternary complex may explain why the response of the 41-kDa IGFBP in this study was relatively rapid compared with mammalian IGFBP-3 (7).

IGFBP-1 mainly acts as an inhibitor of IGF-I actions presumably through sequestering free GH-I from the circulation (22). Its levels increase when animals are in catabolic states, such as fasting and stress. Consistent with the previous finding in salmon (37), plasma IGFBP-1 levels increased during fasting for 1 to 3 wk. When fish were fed, plasma levels started to decline by 14 h. The relatively slow response of IGFBP-1 to food intake is partly due to the fluctuation of IGFBP-1 levels in the unfed group. When IGFBP-1 levels were compared within a group over time, IGFBP-1 levels significantly declined 6 h after a meal (data not shown). There was no significant effect of feeding history on the responsiveness of IGFBP-1 to a meal, despite a trend of a greater decrease in fasted fish (data not shown). Taken together, IGFBP-1 and 41-kDa IGFBP respond to food intake differently but maintain their sensitivity even after prolonged fasting, which should allow them to fine-tune activity/availability of circulating IGF-I under a wide range of nutritional status in salmon.

As expected, plasma GH levels increased with fasting (29, 31, 43). A novel finding of this study is the rapid and significant decrease found in GH levels after a meal. Decreased GH levels were sustained through the time course in the 1-day fasted fish, while the depression was transitory in the 3-wk fasted fish, lasting only through a few hours. GH secretion is generally thought to be influenced by negative feedback from circulating IGF-I, and it has been suggested that IGFBP-1 plays a role in this regulation by influencing free IGF-I levels (11). We did not see a clear correlation between decreases in GH and either increases in IGF-I or decreases in IGFBP-1 in our data; however, there were a number of components of the IGF system and GH regulators that we did not measure (including IGF-II other IGFBPs, ghrelin, and smatostatin) (16, 20), leaving our view of cause-and-effect relation incomplete.

We integrated and condensed the results of our time course by averaging over all postprandial time points and then reported mean plasma values of the endocrine factors that we measured (Fig. 7). There are two clear patterns: one shows a decrease in response to a fast and an increase in response to a meal (insulin, IGF-I, 41-kDa IGFBP), while the other pattern shows an increase with fasting and a decrease in response to a meal (IGFBP-1, GH). These patterns reflect the short-term metabolic role of insulin, with almost all regulation of its abundance relating to its response to a meal and the longer-term growth role of IGF-I, with little response to a meal and greater alterations in basal abundance. Moreover, these patterns suggest the fundamental role that the IGFBPs play in regulating IGF-I; the level of both binding proteins varies in response to a meal with little response by IGF-I itself. Finally, there is a striking repetition of pattern found in the GH and IGFBP-1 levels, both with regard to fasting and in response to a meal. The functional consequences of the similarity of these patterns await further study.

Perspectives and Significance

We measured a time course of postprandial endocrine response in groups of coho salmon under varying degrees of catabolic distress. The variation in patterns of response helps us distinguish between the metabolic and growth-regulating roles of these endocrine factors and represents one of the most complete characterizations of changes in circulating levels of components of the IGF system in any nonmammalian vertebrate. These patterns show a basic commonality of regulation of the endocrine growth axis across taxa, suggesting that the discrimination of metabolic and growth regulation function by insulin family peptides and IGFBPs has a long evolutionary history (8).

ACKNOWLEDGMENTS

We thank Brad A. Gadberry and Paul J. Parkins, School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, for the maintenance of fish. We also thank Dr. Erika Plisetskaya, School of Aquatic and Fishery Sciences, University of Washington, and Dr. Penny Swanson, Northwest Fisheries Science Center, National Oceanographic and Atmospheric Administration Fisheries, Seattle, WA, for providing insulin antisemur and help in insulin assay, respectively.

GRANTS

This project was supported by an internal grant from the Northwest Fisheries Science Center, National Oceanographic and Atmospheric Administration Fisheries; National Research Initiative Competitive Grant no. 2003-35206-13631 from the U.S. Department of Agriculture Cooperative State Research, Education, and Extension Service; and a grant-in-aid for Scientific Research from Japan Society for the Promotion of Science.

REFERENCES


AJP-Regul Integr Comp Physiol • VOL 297 • AUGUST 2009 • www.ajpregu.org


