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LUNAR CYCLES OF COHO SALMON, *ONCORHYNCHUS KISUTCH*

II. SCALE AMINO ACID UPTAKE, NUCLEIC ACIDS, METABOLIC RESERVES AND PLASMA THYROID HORMONES

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SUMMARY

[^{14}C]glycine uptake by scales *in vitro* was measured in coho salmon (*Oncorhynchus kisutch* Walbaum) smolts at different times during several semi-lunar cycles. There was a clear cyclical pattern of glycine uptake during the semi-lunar period.

Evidence for semi-lunar cycles of liver and muscle RNA:DNA ratios, carcass water content, haematocrit, and plasma triglyceride, glucose and cholesterol levels was also found in coho salmon parrs.

Plasma L-thyroxine (T4) levels exhibited a cyclical pattern during the semi-lunar cycle in parrs sampled in March when plasma T4 levels tended to be low, but no such pattern was seen in parrs sampled in January when the plasma T4 levels were relatively high (1·39–1·88 $\mu g\,dl^{-1}$ in January compared with 0·38–0·83 $\mu g\,dl^{-1}$ in March).

There were no apparent semi-lunar cycles in liver mass:body mass ratios and plasma triiodo-L-thyronine (T3) levels.

Changes in growth parameters (nucleic acid levels and glycine uptake by scales) and the content of nutrient reserves are discussed in relation to the semi-lunar patterns of growth in length, growth in mass, and food intake in this species.

INTRODUCTION

Coho salmon (*Oncorhynchus kisutch*) parr and smolts exhibit short-term rhythmical patterns of changes in body mass, body length and food intake (Farbridge & Leatherland, 1987), the periodicity of which has been proposed to be correlated with the semi-lunar cycle.

Increases in body mass or length are relatively crude estimates of growth, which is generally considered to be the accretion of protein and cell proliferation (Love, 1980; Calow, 1985), because changes in body mass can be brought about by accumulation of other materials such as lipid or by alterations in physical activity (Randall, Holeton & Stevens, 1967; Stevens, 1972). Less crude methods of assessing short-term growth

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in fishes have been used, including amino acid uptake by scales and changes in tissue nucleic acid content. Both parameters are considered to reflect tissue growth. The *in vitro* uptake of [^{14}C]glycine by scales is correlated with growth in length and mass for several fish species (Ottaway & Simkiss, 1977; Ottaway, 1978; Adelman, 1980; Goolish & Adelman, 1983*a*,*b*; Smagula & Adelman, 1983) and appears to be sensitive to changes in growth occurring within hourly periods (Ottaway, 1978). Tissue RNA content is highly correlated with protein synthesis in most vertebrates that have been studied (Munro & Fleck, 1969; Munro & Gray, 1969); rapidly growing organisms synthesize and accumulate the RNA needed for protein synthesis.

In the present study, scale amino acid uptake and tissue RNA:DNA ratios were examined in coho salmon smolts and parrs, respectively, at different times during several semi-lunar cycles to evaluate whether the semi-lunar rhythms in growth in mass reported previously (Farbridge & Leatherland, 1987) are reflected in similar rhythms in growth measures at the tissue level.

The process of growth is associated with complex processes of nutrient acquisition, storage, mobilization and catabolism. Consequently, cycles of growth in mass and food intake are likely to be associated with cycles of body reserves of various metabolites. Changes in the tissue or plasma content of several carbohydrate and lipid reserves during the lunar cycle were measured in the present study.

The thyroid hormones have been implicated in growth processes in teleost fishes (Eales, 1979; Leatherland, 1982), and earlier reports have correlated changes in thyroid activity in salmonid fishes with lunar events (e.g. Grau *et al.* 1981; Grau, Specker, Nishioka & Bern, 1982). Plasma L-thyroxine (T4) and triiodo-L-thyronine (T3) levels were measured in the present study to evaluate whether there is evidence of a semi-lunar phasing of thyroid function correlated with the semi-lunar growth cycles.

MATERIALS AND METHODS

General experimental conditions

The coho salmon, *Oncorhynchus kisutch*, used in the study were hatched and raised in the laboratory (Farbridge & Leatherland, 1987).

During the course of several studies investigating the lunar periodicity of growth cycles in coho salmon (Farbridge, 1985), various tissues were sampled (approximately 10 fish/sample). The source of fish, stage of development, mass, dates of tissue sampling and the tissue constituents examined in each trial are shown in Table 1.

In all experiments, the fish were maintained in constantly running and aerated well water (seasonal temperature range of 9–11°C) under a 12 h:12 h L:D artificial photoperiod. They were fed to excess three times a day, 7 days a week, with a commercial salmonid pellet (Martin Feed Mill, Elmira, Ontario).

Table 1. *Source of fish, stage of development, dates of sampling and tissue components measured in coho salmon*

Trial	Source of gametes	Stage of development (mass, in g, ± S.D.)	Dates of sampling	Tissue components measured
A	Michigan	smolt (117·8 ± 22·20)	May 3, 7, 11 (1984)	glycine uptake by scales
B	Michigan	smolt (126·7 ± 47·97)	June 5, 9, 13, 17 (1984)	glycine uptake by scales
C	Ontario	smolt (50·4 ± 20·85)	May 9, 11, 13, 15, 17 (1984)	glycine uptake by scales
D	Ontario	parr (22·2 ± 10·37)	January 18, 20, 22, 24, 26 (1985)	carcass water carcass lipid liver lipid plasma triglyceride plasma T4 plasma T3 muscle RNA:DNA haematocrit
E	Ontario	parr (25·2 ± 8·35)	March 4, 6, 8, 10, 12, 14 (1985)	carcass water plasma cholesterol plasma glucose plasma T4 plasma T3 muscle RNA:DNA liver RNA:DNA haematocrit
F	Ontario	parr (29·23 ± 9·17)	March 18, 20, 22, 24, 26, 28 (1984)	carcass water plasma T4 plasma T3 liver RNA:DNA haematocrit

Methods of analysis

Amino acid uptake by scales

Scales were removed from a small area posterior to the dorsal fin and just above the lateral line, using fine forceps. They were incubated in fish saline (Wolf, 1963) containing 0·4 μCi ml^{-1} [^{14}C]glycine (>100 mCi mmol^{-1}; Amersham Corporation, Oakville, Ontario) (3 scales/400 μl saline) for 2 h at ambient water temperature (Goolish & Adelman, 1983*a*,*b*), rinsed in saline containing no isotope for 15 min, dried at 40°C, weighed (±0·05 mg), and dissolved with Soluene (Packard, Dorval, Quebec). The digested scales were counted in an LKB Wallac Beta counter using Instagel (Packard). Quench was estimated by sample channels ratio. For each sample, 10 scales were incubated in saline to determine background, and 10 scales, killed by microwaves, were incubated in saline containing [^{14}C]glycine to determine non-specific absorption of [^{14}C]glycine. Uptake of [^{14}C]glycine was expressed as d.p.m. mg^{-1} corrected for background and non-specific absorption. Each sample represented scales from nine fish (10, 10 and 5 scales/fish for trials A, B and C,

respectively). This number of scales/replicate was necessary in order to obtain precise measurement of tissue mass.

Plasma analysis

Blood was collected, by caudal severance, in heparinized microvette blood collection tubes (Sarstedt Canada Inc., St Laurent, Quebec), and plasma was stored in plastic screw-cap vials at −20°C. Haematocrit was recorded. Plasma T4 and T3 levels were measured using a highly specific radioimmunoassay (RIA) (Canadian Bioclinical Inc., Scarborough, Ontario). Plasma glucose and triglyceride content were measured colorimetrically using appropriate assay kits (Sigma Chemical Company, St Louis, MS). Plasma cholesterol levels were measured using the method of Bhandaru, Srinjvasan, Pargaonkar & Berenson (1977).

Carcass water content

Carcasses were stored in sealed plastic bags at −20°C until the total water content was measured by freeze-drying.

Carcass and liver lipid content

Carcass (dry) and liver total lipid content were measured in duplicate samples using a modification of the Bligh & Dyer (1959) extraction method (Herbes & Allen, 1983).

Muscle and liver nucleic acid content

Liver and muscle (approximately 0·5 g from the dorsal body wall anterior to the dorsal fin) samples were rapidly removed and stored in plastic screw-cap vials at −196°C, before DNA and RNA levels were measured fluorometrically by the method of Karsten & Wollenberg (1972, 1977). Nucleic acid content was expressed as an RNA:DNA ratio.

DNA and RNA [25 μg RNA (type IV from calf liver) ml^{-1} in PBS; Sigma Chemical Company] added to liver and muscle homogenates were completely recovered. Treatment of RNA with RNAase A eliminated fluorescence. Fluorescence of RNAase A solution was negligible. The RNAase A resistant fluorescence of liver and muscle homogenates was sensitive to deoxyribonuclease I [100 μg DNAase I (type III from bovine pancreas) ml^{-1} in 125 mmol l^{-1} $MgCl_2$ neutralized with NaOH; Sigma Chemical Company, St Louis, MS]. The fluorescence due to DNA and RNA increased linearly in intensity with the amount of tissue homogenate added. The fluorescence of the ethidium bromide was stable for more than 1 h after addition of the reaction mixtures. The fluorescence of the heparin solution (125 μg heparin ml^{-1} in PBS) was negligible.

Reproducibilities of liver DNA and RNA were 2674·3 ± 228·4 (±S.D., $N = 10$) and 4029·5 ± 271·8 ($N = 10$) $\mu g\, mg^{-1}$, respectively, and reproducibilities of muscle DNA and RNA were 379·4 ± 41·9 ($N = 10$) and 401 ± 54·5 ($N = 10$) $\mu g\, mg^{-1}$, respectively. A single freezing and thawing did not affect liver or muscle DNA or RNA levels.

Liver: body mass ratios

Liver: body mass ratios (LW: BW) were calculated from the following equation:

$$\text{LW:BW} = \frac{\text{LW}}{\text{BW}} \times 100,$$

where LW and BW represent liver and body mass, respectively.

Statistical analysis

If there is a lunar influence on the physiology of these fish, then a cyclical pattern should be evident when measurements from several studies are plotted on a common semi-lunar scale. Consequently all measurements were plotted in this way and means were compared using one-way analysis of variance. Where significance is indicated ($P < 0{\cdot}05$), differences between all peak and low values were compared with least significant means procedures (Steele & Torrie, 1960); the critical level of significance for testing hypotheses was $P \leqslant 0{\cdot}05$.

RESULTS

Amino acid uptake by scales

The scales obtained from salmon parr were too small for accurate estimates of amino acid uptake. Consequently, measurements were made on smolts only. Moreover, the size of scales from smolts necessitated that scales from several fish be pooled in order to be weighed with acceptable precision ($\pm 0{\cdot}05$ mg).

The uptake of [^{14}C]glycine by scales showed a cyclical pattern over the semi-lunar cycle with a significant peak and trough occurring approximately 6 days after and 3 days before the occurrence of new and full moons, respectively (Fig. 1A).

Tissue nucleic acid content

Liver

Liver RNA: DNA ratios showed a cyclical pattern over the semi-lunar cycle with a significant trough approximately 1 day before the occurrence of new and full moons (Fig. 1B).

Muscle

Muscle RNA: DNA ratios showed a cyclical pattern over the semi-lunar cycle with a significant peak approximately 1 day after the occurrence of new and full moons (Fig. 1C).

Carcass water and total lipid content

Carcass water content showed a cyclical pattern over the semi-lunar cycle with a significant trough occurring approximately 4 days before the occurrence of new and

full moons (Fig. 1D). The change from 1 to 3 days after the occurrence of new and full moons was significant, but the following changes from 3 to 5 and 7 days after the occurrence of new and full moons were not.

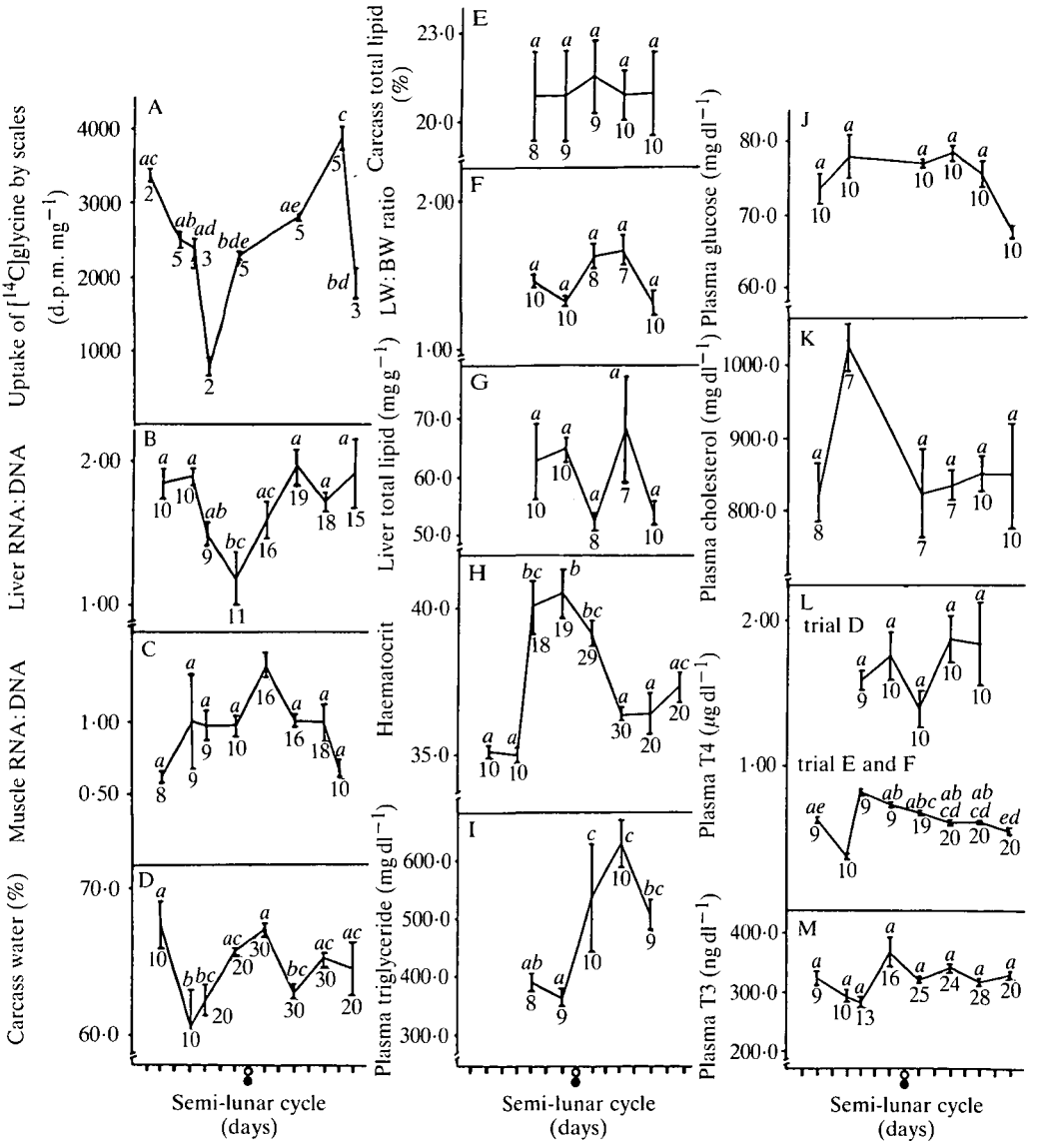

Fig. 1. (A) Mean uptake of [^{14}C]glycine by scales; (B) liver RNA:DNA ratios; (C) muscle RNA:DNA ratios; (D) carcass water content; (E) total lipid content; (F) liver:body mass ratios; (G) liver total lipid content; (H) haematocrit; (I) plasma triglyceride levels; (J) plasma glucose levels; (K) plasma cholesterol levels; (L) plasma L-thyroxine (T4) levels and (M) plasma triiodo-L-thyronine (T3) levels (±S.E.M., N), in coho salmon during the semi-lunar cycle (new and full moons are indicated as filled and open circles, respectively). Same lower-case letter indicates no significant difference between means.

There was no apparent cycling in the total lipid content of the carcass during the part of the semi-lunar cycle studied (Fig. 1E).

Liver: body mass ratio and liver total lipid content

There was no apparent cycling in liver: body mass ratios or liver total lipid content during the part of the semi-lunar cycle studied (Fig. 1F,G).

Haematocrit and plasma metabolite and thyroid hormone levels

Haematocrit showed a cyclical pattern over the semi-lunar cycle with a significant peak occurring approximately 1 day before the occurrence of new and full moons (Fig. 1H).

Plasma triglyceride levels showed evidence of a cyclical pattern over the part of the semi-lunar cycle studied; they were significantly lower before the occurrence of new and full moons than after (Fig. 1I).

Plasma glucose levels were significantly lower 7 days after the occurrence of new and full moons (Fig. 1J).

Plasma cholesterol levels showed a cyclical pattern over the part of the semi-lunar cycle studied, with a significant peak approximately 4 days before the occurrence of new and full moons (Fig. 1K).

Plasma T4 levels were significantly higher in trial D (collections made in January) than in trials E and F (collections made in March) and consequently were not pooled. There was no apparent cycling of plasma T4 levels in trial D during the part of the semi-lunar cycle studied (Fig. 1L). Plasma T4 levels for trials E and F showed a cyclical pattern, with a significant trough and peak approximately 4 days and 3 days, respectively, before the occurrence of new and full moons (Fig. 1L).

There was no apparent cycling of plasma T3 levels during the semi-lunar cycle (Fig. 1M).

DISCUSSION

This study provides evidence of a semi-lunar cycle of [^{14}C]glycine uptake by coho salmon scales *in vitro*. The observations suggest that the cyclical changes in body mass observed in coho salmon (Farbridge & Leatherland, 1987) may reflect changes in the rate of protein synthesis and are not necessarily the result of fluctuations in fat deposition or water content, although this last possibility cannot be excluded.

Growth in mass of coho salmon smolts tended to be higher before new and full moons rather than after (Farbridge & Leatherland, 1987), while the uptake of amino acids by scales was at its highest approximately 6 days after new and full moons. Growth in length has been shown to be out of phase with growth in mass in brown trout (Brown, 1946) and possibly in coho salmon (Farbridge & Leatherland, 1987). Since it is likely that scale growth is associated with growth in length rather than mass (Pannella, 1980), it may be that the observed cycle of amino acid uptake reflects a cycle of growth in length rather than a cycle of growth in mass *per se*.

Tissue RNA:DNA ratios have been successfully correlated with annual changes of growth in fish (Haines, 1973, 1980; Bulow, Zeman, Winningham & Hudson, 1981; Thorpe, Talbot & Villarreal, 1982), and growth changes due to starvation (Bulow, 1970), growth hormone replacement after hypophysectomy (Kayes, 1978, 1979), and exposure to toxic substances (Barron & Adelman, 1984). However, it is important to note that, according to Dunn & Schotman (1981), messenger RNA can be stored in the cell and thus new proteins can be induced cytoplasmically at the translational level.

Liver RNA:DNA ratios in this study were lowest at about the time of new and full moons and highest midway between new and full moons. Growth in mass and food consumption of coho salmon parr showed a similar relationship (Farbridge & Leatherland, 1987), suggesting that protein synthesis by the liver is most active during periods of rapid growth in mass and feeding.

Muscle RNA:DNA ratios were at their highest about the time of new and full moons, when growth in mass tended to be low (Farbridge & Leatherland, 1987). Indirectly, this supports the idea that growth in mass and length are out of phase in this species and that protein synthesis by muscle is most active during periods of rapid growth in length.

Since feeding and sampling occurred at the same time each day in each trial, the differences observed in certain tissue metabolites and thyroid hormones during the semi-lunar cycle support the hypothesis that the fish are experiencing changes in growth and feeding patterns. Changes in plasma metabolites may reflect different levels of nutrient intake or changes in metabolism. Unfortunately, although there is a considerable amount of information about the relationship between appetite and changes in plasma nutrient levels in higher vertebrates, the results from the small number of studies on fish are often contradictory (Fletcher, 1984).

The rhythm of food intake (Farbridge & Leatherland, 1987) suggests that there may be changes in lipogenesis. Dietary protein, and perhaps carbohydrate (Cowey & Sargent, 1979), must be stored as lipid during the period of high food intake in order to meet the energy demands of the fish during the period of low food intake when the fish may convert the assimilated resource into length (Farbridge & Leatherland, 1987). Insulin may be important in regulating this process (Ince & Thorpe, 1978). However, liver and carcass total lipid reserves remained apparently unchanged, as did LW:BW ratios, although plasma triglyceride and cholesterol levels did show evidence of a cyclical pattern. It is interesting that neither of these liver parameters changed in the light of the cyclical pattern observed in liver RNA:DNA ratios. There may have been changes in the dynamics of tissue metabolites which were not reflected in absolute levels. Moreover, the fish possessed large amounts of abdominal lipid stores which might have masked small changes in carcass lipid content.

Carcass water content was highest at the time of new and full moons, which is correlated with times of decreased growth in mass (Farbridge & Leatherland, 1987). One would think that the fish might be less active at this time and thus might be expected to show decreased carcass water content, the result of decreased permeability of the gills to oxygen and, consequently, to water because of reduced

oxygen demands (Randall *et al.* 1967; Stevens, 1972). Carcass water content was most variable in trial E (pooled data are shown) just before the spring equinox, indirectly suggesting that these fish are more active at this time for the reasons stated above. Rainbow trout were also observed to be more active at this time (Farbridge, 1985), suggesting that the spring equinox represents an important event for these fish.

Haematocrit values exhibited a marked cyclical pattern which was similar to that observed for carcass water content.

Thyroid hormones are considered to play a permissive role in the growth of fish, potentiating the anabolic effects of other hormones, particularly growth hormone (McBride, Higgs, Fagerlund & Buckley, 1982). Evidence from studies on fish suggests that the conversion of T4 to T3 may be a prerequisite for thyroid hormone action and that, as in mammals, T3 appears to be the most biologically active thyroid hormone (Eales, 1979; Leatherland, 1982). It is likely, therefore, that absolute plasma T3 levels are regulated. Consequently, it is not surprising that there was no obvious cycling pattern in T3. Most thyroid hormones are bound to proteins in the blood; only free hormone is thought to have biological activity (Leatherland, 1986). The assays for T4 and T3 measure total concentrations, both bound and unbound, and thus provide no information on the ratio of unbound to bound. The ratio may be more informative than the absolute values for the thyroid hormones. Moreover, the rate of secretion of T4 and T3 from the thyroid follicles, the rate of conversion of T4 to T3, and the rate of catabolism and excretion of T4 and T3, as well as removal by receptor binding, will determine the amount of biologically active thyroid hormone available at a particular time.

Plasma T4 levels were low just after the period of rapid growth and increased during the period of low growth in mass and perhaps high growth in length. The administration of T4 has been found to increase the rate of growth in fish (Donaldson, Fagerlund, Higgs & McBride, 1979). More amino acids are channelled into protein synthetic pathways after administration of thyroid hormones (Plisetskaya, Woo & Murat, 1983). However, Higgs *et al.* (1977) observed that the administration of bovine growth hormone and 17-methyltestosterone resulted in greater growth in length, whereas the administration of T4 resulted in greater growth in mass. This would appear to contradict the pattern observed in this study, although it may be more complicated than this since T4 has been observed to stimulate the release of growth hormone from pituitary somatotrops (Sage, 1967; Leatherland & Hyder, 1975; Higgs, Donaldson, Dye & McBride, 1976).

Plasma thyroid hormones are elevated during the process of smoltification, which for coho salmon normally occurs during the spring of their second year (Hoar, 1939). However, plasma thyroid hormone levels were higher in January (trial D) than in March (trials E and F). Grau *et al.* (1981, 1982) reported 'surges' of plasma T4 at the new moon closest to the spring equinox in salmonids. However, no such surge in plasma T4 was evident in the study reported here, although measurements were taken about the time of the full moon and not of the new moon in trial E.

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