Biochemical Relationship Between Otolith and Somatic Growth in the Rainbow Trout *Oncorhynchus mykiss*: Consequence of Starvation, Resumed Feeding, and Diel Variations

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Teleost otoliths are calcium carbonate concretions which have increments consisting of a bipartite structure of light and dark rings. These structures are known to be formed on a daily basis (Pannella 1971). Since otolith and fish size are highly correlated for a variety of marine and freshwater species (Campana and Neilson 1985), it is possible to estimate growth-rate histories of individual fish by measuring the width of otolith increments. Volk et al. (1984) averaged otolith increment widths over each week and found a linear regression of mean increment width with somatic growth rate in chum salmon *Oncorhynchus keta* under controlled feeding. Nishimura and Yamada (1988) successfully back-calculated the growth rate of walleye pollock *Theragra chalcogramma* by using mean otolith width measured every 10 increments. However, it remains to be shown whether the rate of otolith growth reflects somatic growth rates in terms of daily or subdaily trends.

Recently several workers (Secor and Dean 1989, Reznick et al. 1989, Wright et al. 1990) reported an uncoupling of the relationship between otolith and somatic growth in fish populations experiencing slow and fast somatic growth. Similar uncoupling was exaggeratedly induced in hypophysectomized goldfish *Carassius auratus*, in which somatic growth in length was completely inhibited but otolith continued to grow at a reduced rate (Mugiya 1990).

Since the otolith (sagitta) occurs within the sacculus which is anatomically closed in rainbow trout *Oncorhynchus mykiss*, it is feasible to take out the otolith-containing sacculus without any leakage of the endolymph (Mugiya 1984). An *in vitro* preparation of the isolated sacculi was used for indicating the current growth rate of otoliths, which reflected the *in vivo* physiological state at the time when the fish were sampled (Mugiya 1984, 1987). Somatic growth is a balance between catabolic and anabolic components in protein metabolism (Miglavs and Jobling 1989). Since accretive growth should be directly associated with protein synthetic capacity, RNA-DNA ratios in muscle are widely used as an indicator of the short-term or current somatic growth rate (Bulow 1987).

The aim of the present study is to clarify the biochemical relationship between otolith and somatic growth on a daily basis, using starved and then fed rainbow trout. The otolith growth–fish growth relationship was also examined at 6-hour intervals.
over a 24-hour period. Otolith and somatic growth were defined by the rate of in vitro calcium deposition on otoliths and RNA-DNA ratios in white trunk muscle, respectively. Serum calcium concentrations were examined for diel variations based on previous work on the relationship between serum calcium and otolith growth (Mugiya 1984).

**Materials and methods**

We performed two experiments with food and diel effects on somatic and otolith growth. Immature rainbow trout *Oncorhynchus mykiss* weighing 100-130g were obtained from a trout farm and acclimated to experimental conditions for at least 4 weeks before use. Throughout the acclimation and experimental periods, they were maintained in a pair of outside concrete ponds (3.8m² x 0.7m in depth) which were supplied with running water at 14 ± 0.5°C, and fed commercial trout pellets once a day at around 1500 hours, unless otherwise stated. The ration fed was about 1.5% of body weight.

**Starvation and refeeding**

This experiment was carried out in October 1988. Dusk occurred at 1700 hours and dawn at 0545 hours. Sixty fish were randomly divided into two groups (30 fish per group), and separately assigned to one compartment of the paired ponds. One group was starved for 5 days and then fed. During these periods, they were sampled on days 1 (45 hours from last feeding), 2, 3, and 5 after starvation, and on days 1 (19 hours from the first refeeding), 2, 3, and 4 after refeeding. The other group was fed throughout the period and sampled as the control on the third day of starvation and on the second day of refeeding when only the experimental group was sampled. Sampling was conducted alternately between experimental and control groups at 0945-1045 hours every day. At each sampling, 4-6 fish were gently netted one at a time, and immediately bled by cutting the tail of the fish. After bleeding, a pair of sacculi containing otoliths were isolated, placed in an incubation medium, and then the next fish was netted. The remaining part of the body was stored at -40°C and analyzed within 5 days for RNA-DNA ratios. Data from the control group were pooled for statistical analyses, as little difference was found among the sampling days.

**Diel variation**

This experiment was carried out in December 1988. Dusk occurred in 1600 hours and dawn at 0700 hours. To examine the diel relationship between otolith and somatic growth, 25 fish were stocked in each compartment of the paired ponds, and 7 fish each were randomly sampled at 6-hour intervals of 1000, 1600, 2200, and 0400 hours over a 24-hour period. Sampling was conducted alternately from one compartment at 1000 and 2200 hours, and from the other compartment at 1600 and 0400 hours. This sampling regime is recommended for minimizing handling effects. After fish were netted one at a time, blood was immediately collected from the caudal vessels by cutting the tail of the fish and draining it into test tubes. After centrifugation, the separated sera were stored at -40°C for 6-24 hours and analyzed for total calcium concentrations by flame photometry using an atomic absorption spectrophotometer (Hitachi #518). After the blood collection, sacculi were isolated for incubation, and the remaining part of the body was stored for RNA and DNA analyses. In this experiment, the fish were starved throughout the sampling day.

**Otolith incubation**

Otolith-containing sacculi were isolated according to a previously described technique (Mugiya 1987). Isolated sacculi were incubated in 50mL of a Ringer solution (Mugiya 1986) containing 46Ca (New England Nuclear) at a concentration of 1 x 10⁴ Bq/mL. Incubation was carried out with oxygenation at 14°C for 2 hours.

After incubation, sacculi were rinsed several times in 46Ca-free Ringer solution and the otoliths were separated under a binocular microscope. The separated otoliths were lightly rinsed in water, placed in each counting vial, dried overnight at 80°C, and then weighed. They were solubilized in a mixture of 0.2mL perchloric acid and 0.2mL hydrogen peroxide (Mugiya 1987), and added to Scintisol EX-H (Wako Chem.) for radioactive counting (liquid scintillation spectrometer, Aloka LSC-673). The rate of otolith growth was evaluated in terms of microgrammes of calcium deposited per unit otolith weight.

**RNA and DNA determinations**

RNA-DNA ratios were estimated in the white muscle collected from an area of the dorsoanterior trunk, using a modification of the Schmidt and Thannhauser method (Buckley and Bulow 1987). Briefly, white muscle (1.00g) was homogenized with cold distilled water and adjusted to 10.0mL. A 1.4-mL aliquot of the homogenate was used for the estimation of nucleic acids. RNA and DNA were purified with 0.6N cold HClO₄, and then extracted with 0.3N KOH and 0.6N hot HClO₄, respectively. Both acids were quantified from the absorbance of the extracts at 260nm. RNA and DNA
standard solutions were prepared using RNA from yeast and DNA from salmon sperm (Wako Chem.).

Statistics
Student's *t*-test for unpaired observations was applied to assess statistical significance of differences between mean values. Significance was accepted at *P*<0.05.

Results

Starvation and refeeding

The rate of *in vitro* calcium deposition on otoliths ranged from 0.06 to 0.08 μg/mg·hour in the control group. These data are pooled and presented as the mean level with standard errors (Fig. 1). Effects of starvation and refeeding on the rate are expressed as a percentage against the control level. Starvation induced an inhibitory effect (*P*<0.05) on the rate of calcium deposition on otoliths by day 2, decreasing to approximately half of the control. This level remained almost unchanged until day 5, the last day of starvation. On refeeding, the starvation-induced decrease recovered to the control level as early as day 1. However, this recovery was transient, followed by a significantly (*P*<0.05) reduced calcium deposition on day 2. This reduced calcium deposition recovered to the control level on day 4 after refeeding (Fig. 1).

Muscle RNA and DNA concentrations and their ratios are presented in Table 1 and Figure 2, respectively. Starvation and refeeding affected RNA concentrations, but DNA concentrations remained constant during the experiment (Table 1). Therefore, changes in RNA-DNA ratios are primarily attributable to changes in RNA concentrations.
Table 2  
Diel variations in muscle RNA and DNA concentrations (mg/g) in rainbow trout *Oncorhynchus mykiss*. Each plotted value represents mean ± SE of 7 fish for serum calcium and of 12-14 otoliths. *P<0.05 for 1600 hours; **P<0.001 for 1600 hours.

<table>
<thead>
<tr>
<th>Time of day (h)</th>
<th>RNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1.26 ± 0.08</td>
<td>0.66 ± 0.04</td>
</tr>
<tr>
<td>1600</td>
<td>1.30 ± 0.08</td>
<td>0.72 ± 0.02</td>
</tr>
<tr>
<td>2200</td>
<td>0.66 ± 0.04*</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td>0400</td>
<td>0.80 ± 0.04</td>
<td>0.68 ± 0.04</td>
</tr>
</tbody>
</table>

Values are mean ± SE of 7 fish.  
*P<0.01 for 1600 hours.

RNA-DNA ratios ranged from 1.91 to 2.73, with a mean value of 2.44 in the control group. One day’s starvation had no effect on the ratio (Fig. 2). The first significant (P<0.05) effect occurred on day 2 after starvation, followed by further decrease to approximately half of the control on day 3. This reduced level apparently remained essentially unchanged through day 5. In contrast to the response observed for otoliths, refeeding had no effect on the recovery in RNA-DNA ratios the next day (Fig. 2). Ratios increased gradually with feeding and recovered to the control level on day 4 after refeeding.

Diel variation

Serum calcium concentrations varied dielly by approximately 4%, and this variation was statistically significant at P = 0.05 (Fig. 3). Calcium concentrations were high during the daytime, but a nadir occurred at 2200 hours.

The profile of diel variations in calcium deposition on otoliths was essentially the same as that of serum calcium concentrations (Fig. 3). The high rate of calcium deposition at 1600 hours rapidly decreased by 70% (P<0.001) to a nadir at 2200 hours, followed by an increase toward 0400 hours.

Diel variations in muscle RNA and DNA concentrations and their ratios are presented in Table 2 and Figure 4, respectively. Diel variations were significant in RNA concentrations and RNA-DNA ratios. The profile of variations in the ratios was similar to that of calcium deposition on otoliths: high ratios during the daytime were followed by a steep decrease between 1600 and 2200 hours. The lowest ratio occurred at 2200 hours, and this ratio was highly significant (P<0.001) compared with the ratio at 1600 hours. Differences between 1000 and 1600 hours were not statistically significant in either otolith or muscle, showing that the criterion of similar growth profiles was fulfilled.

A comparison of variations in calcium deposition on otoliths and RNA-DNA ratios in muscle showed positive relationships through several days after starvation and during the diel experiment. However, uncoupling was found in the recovery processes with refeeding: calcium deposition on otoliths transiently recovered on day 1 after refeeding, while RNA-DNA ratios did not (compare Figures 1 and 2).
Discussion

RNA-DNA ratios in muscle have been widely accepted as an index of current somatic growth rates in various species of marine and freshwater fish (Bulow 1987). These ratios are affected by various factors such as season (Bulow et al. 1981), ration size (Bulow 1970, Buckley 1979, Wilder and Stanley 1983, Jürss et al. 1986), temperature, salinity (Jürss et al. 1987), and lunar cycles (Farbridge and Leatherland 1987). Various toxicants also reduce the ratios (Barron and Adelman 1984). The present study presents an additional finding with regard to variations of RNA-DNA ratios: the ratios had distinct diel variations, showing higher values during daytime than nighttime. It is desirable to confirm such a profile of variations by further examinations at shorter and longer time-intervals.

Endocrinologically, RNA-DNA ratios are under the control of growth-regulating hormones. Hypophysectomy reduced the ratios, and replacement therapy with beef growth hormone restored the ratios to a normal level in bullheads Ictalurus melas (Kayes 1979). Circadian periodicities in the surge of growth-regulating hormones are well documented in higher vertebrates (Kato et al. 1982). Although few comparable references are available in fish, cyclic variations in growth hormone have been reported in plasma and pituitary levels in salmonids (Leatherland et al. 1974, Leatherland and Nuti 1982, Bates et al. 1989). Therefore, the cyclic surge of this hormone in combination with other hormones is probably a cause for diel variations in RNA-DNA ratios in the present fish.

Effects of starvation or restricted food on RNA-DNA ratios have been repeatedly reported. A 4-week starvation induced an approximately 40% decrease in the ratios in rainbow trout Oncorhynchus mykiss (Jürss et al. 1986). Brook trout Salvelinus fontinalis also had the ratio reduced by 44% after 22 days of restricted feeding (Wilder and Stanley 1983). Bulow (1970) showed that RNA-DNA ratios directly reflected different somatic growth rates induced by nutritional manipulation. However, it is not clear how fast food deprivation affects this ratio. Although the time course of the effect will depend on various factors such as temperature, fish sizes, sexual status, etc., the present study revealed that the first effect of starvation on RNA-DNA ratios in muscle appeared on day 2 (67 hours from last feeding) in adult rainbow trout Oncorhynchus mykiss. This inhibitory effect was exaggerated with time, but the ratios never decreased below half the control level even on day 15 after starvation (data are not presented here). This level (55% of the control) is similar to trout values in the literature mentioned above, and therefore it appears to be a basal level in RNA-DNA ratios in white muscle of rainbow trout.

Starvation-induced decrease in RNA-DNA ratios recovered with the resumption of daily feeding at a level of 1.5% of body weight. The recovery processes were rather steady, taking 4 days in the present study. Bulow (1970) showed a more rapid recovery in golden shiners Notemigonus crysoleucas: 2 days feeding at 6% body weight seemed to be enough for the recovery of a starvation-induced decrease in ratios to the normal level. This disparity may be explained by the amount of food used and/or species difference. Lied et al. (1983) reported that a starvation-induced decrease in RNA-DNA ratios recovered to the normal level within 8 hours after refeeding ad libitum in cod Gadus morhua. However, it is uncertain whether this recovery was due to the result of food assimilation or to diel variations in the ratios, because control values were not available at each determination time in the study.

Recently Miglavs and Jobling (1989) reported that a growth spurt (compensatory growth) transiently occurred following realimentation after a period of food restriction in juvenile Arctic char Salvelinus alpinus. Nevertheless, no corresponding increase occurred in tissue RNA-DNA ratios. This led them to present a limit to using the ratios as a growth index. However, since their somatic growth rate was calculated from a cumulated increase in body weight for 28 days, it is uncertain whether or not the rate remained high on the last day, when the RNA-DNA ratios were determined.

When somatic and otolith growth rates were biochemically compared at 6-hour intervals over a 24-hour period, or after food deprivation, they were coupled at a qualitative level. In spite of these positive relationships, evidence of an allometric relationship between otolith and somatic growth has been accumulated, especially under suboptimal conditions. For example, Mostegaard et al. (1988) examined the effect of temperature, fish size, and somatic growth rate on otolith growth rate in Arctic char Salvelinus alpinus and found an uncoupling between somatic and otolith growth rates at hyperoptimal temperatures. Based on these results, they suggested that metabolic activity, not necessarily somatic growth rate, governs otolith growth rate. Somatic growth rate results mainly from the balance between protein synthesis and degradation, and hyperoptimal temperatures would accelerate both components, especially degradation, resulting in no somatic growth (Houlihan et al. 1988). However, since somatic growth is comparable with components from metabolic rates within the range of optimal temperatures (Webb 1978), the muscle RNA-DNA ratio, an index for protein synthesis, will be a reflection of metabolic components at an appropriate temperature. Therefore, it appears reasonable to use this ratio for examining the relationship between somatic and otolith growth rates, even if otolith growth is a function of metabolic rate.
In the present study, the transient recovery of otolith growth occurred on the first day following refeeding. Although the definite reason for this remains unexplained, an accelerated recovery process after growth suppression is well known as “repletion” in mammalian skeletal tissue formation (Linkhart et al. 1988). Mechanisms for repletion would differ depending on tissues. Since the otolith is an acellular product secreted by otolith-forming cells (Saitoh and Yamada 1989), a possible explanation for otolith repletion is as follows: Starvation may interrupt the releasing activity partly due to the reduced processing of the secretory product. This would inversely result in some accumulation of otolith precursor materials in the cells (Anderson and Capen 1976). Refeeding stimulates release of the accumulated precursors through some factors (e.g., calcium-calmodulin interaction; Mugiya 1986) regulating the secretory activity. A steady-state recovery in synthesis and processing of the secretory product is probably a time-consuming process, running parallel to the recovery of RNA-DNA ratios in muscle. These possibilities await further examination.

In the present study, we have demonstrated corresponding variations in otolith calcification and RNA-DNA ratios in muscle, but a causal relationship between specific growth rate and otolith calcification remains to be studied.

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**Citations**


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