EFFECT OF FEEDING VERSUS STARVATION ON DNA SYNTHESIS IN RAINBOW TROUT FRY

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ABSTRACT

The DNA synthetic activities of 73-day old rainbow trout, *Oncorhynchus mykiss*, fed and starved for 29 days were quantified in individual blood cells using flow cytometry. The fraction of cells at the G2 and S stages during the cell division cycle, representing potential for cell proliferation, growth and condition parameters (condition factor and specific growth rate) were always greater in fed fry than in starved fry.

INTRODUCTION

The RNA/DNA ratio has been used as an indicator of the physiological condition of aquatic organisms, particularly the growth and nutritional status of fish larvae (Blow, 1987; Buckley and Lough, 1987; Clemmesen, 1993, 94; Westerman and Holt, 1988, 1994; Canino, 1994). Poor nutritional condition contributes to low protein synthesis, slow growth, and thus, results in a low RNA/DNA ratio (Chung et al., 1993). The analytical techniques for determining RNA and DNA concentrations have been well established for pooled fish larvae and also sensitive assays have been developed for a single fish larvae (Karstein and Wallenburger, 1972, 1977; Calderon and Buckley, 1993; Clemmesen, 1994; Canino and Calderon, 1995). Individual fish tissues may respond differentially to physiological condition or nutritional status (Chung et al., 1988, 1993). Thus, measurement of the RNA/DNA ratio of the cells of individual tissues may provide a more accurate index of physiological condition than whole fish homogenate. The growth rate of
different organs may vary, and therefore, interpretation of the RNA/DNA ratio in whole fish homogenate as an index of condition may be difficult (Theilacker and Shen, 1993). Most studies carried out to date have quantified nucleic acid concentrations of individual tissues, a single whole fish or pooled fish larvae. Few studies have been performed to determine nucleic acid concentrations in single cell types from fish larvae (Theilacker and Shen, 1993).

To refine nucleic acid analysis of a single cell types, we utilized flow cytometry (Theilacker and Shen, 1993) to quantify DNA in the erythrocytes of rainbow trout, Oncorhynchus mykiss, larvae. These cells were chosen because they are present in quantity, free of contamination from other tissues and already exist in a suspension of single cells suitable for flow cytometry (Darzynkiewicz, 1991). The objective of this study was to determine condition factor, specific growth rate, and DNA synthetic activities during the G2 and S stages of the cell division cycle, and to use these values, as indicators of the physiological condition of fed or starved rainbow trout.

MATERIAL AND METHODS

Rainbow trout fry were purchased in October 1994 from Spring Valley Trout Farm, Langley, British Columbia and were acclimated to laboratory conditions. Water temperature was ±10°C. The 79-day old fry were cultured during 29 days in a flow through system at the West Vancouver Laboratory. The fry were fed ad libitum with trout chow #3 or fasted during the experimental period. The total wet weight and total length of 20 fry were measured at 4, 9, 14, 20, and 29-day intervals in each experiment. Fry blood sampled with glass capillary by heart puncture, about 1-3 μL, was transferred into a 1.5-ml centrifuge tube filled with 200 μL cryoprotectant on ice. The cryoprotectant was prepared with 1 mL fetal bovine serum, 1 mL dimethyl sulfoxide (DMSO), and 4 mL Eagle Minimum Essential Solution Medium (MEM). We dissociated blood cells by pipetting about 10 times in cryoprotectant, and then, immediately added an equal volume of 0.08N HCl on ice to ensure nucleic acid stability in the cell preparation (Theilacker and Shen, 1993). The fry were frozen on dry ice and the blood samples treated with cryoprotectant were kept at -20°C until nucleic acid concentrations were determined by flow cytometry using the Coulter Epics XL.

The fluorochrome, acridine orange solution was added to the thawed sample prior to flow cytometry (Protocols 21, 1978).

Condition factor (K) was calculated by K = (W x 10,000) / L3 (Busacker et al., 1990), where L is standard length in mm and W is wet weight in mg. Specific growth rate (SGR) was obtained by SGR = (InW2 - InW1) x 100 / (t2 - t1), where W1 and t1 were total wet weight and time at the beginning of experiment and W2 and t2 final wet weight and time (Ricker, 1979).

RESULTS AND DISCUSSION

Average total wet weight of the fry increased from 612.4 to 1558.6 mg for those fed ad
*libitum*, and decreased to 385.8 mg for those fasted during 29 days (Fig. 1). Average body length increments were from 39.7 mm to 52.3 mm for those fed continuously and almost similar values without much change for those fasted (Fig. 2). Therefore, condition factor (K) was maintained at approximately 10 for those which were fed and dropped to less than 7 for those maintained without any food for 29 days (Fig. 3). Specific growth rate (SGR) varied from about 6 to 9 mg g⁻¹ day⁻¹ for those fed *ad libitum*, however, negative growth occurred in those fasted 9 days to 29 days (Fig. 4). The percentage of blood cells

Figure 1. Total wet weight change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.

Figure 2. Total length change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.

In the G2 and S stages of the cell division cycle were 1.52-3.13% for those fed continuously and ranged from 0.28 to 2.1% for those fasted for 29 days (Fig. 5). However, percentage of cells undergoing division was always higher in those fed than in those fasted during entire experimental period. The Student t-test results indicated that all
parameters tested were significantly different \( (p < 0.05) \) between those fed and starved, except for total body length.

The percentages of cells in the G2 and S stages of the cell division cycle are considered to be independent from external factors, and dependent upon intracellular activities (Hartwell and Weinert, 1989; Murray and Kirschner, 1989). Therefore the fraction of

![Figure 3. Condition factor change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.](image)

![Figure 4. Specific growth rate change of rainbow trout fry, *Oncorhynchus mykiss*, fed and starved for 29 days.](image)

cells in the G2 and S stages of cell division is an indicator of the numbers of dividing cells under varying nutritional or physiological conditions. The percentages of dividing blood cells showed that the growth and nutritional conditions of the rainbow trout fry tested are well correlated with the G2 and S stages of cell proliferation.
CONCLUSION

The total body wet weight and total length changes, condition factor, specific growth rate, and percentage of blood cells in the S2 and G stages of the cell cycle indicated that nutritional status is an important factor which determines the physiological condition of rainbow trout fry. In this study we compared fry exposed to extreme conditions i.e. satiation versus starvation. It remains to be determined whether measurement of DNA synthesis can detect differences in condition after measurable moderate changes in nutritional status.

Figure 5. Percentages of the DNA synthesis activities during the G2 and S stages of the cell division cycle in rainbow trout, Oncorhynchus mykiss, fed and starved for 29 days.

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