Using Cell-Cycle Analysis to Measure Growth of Walleye Pollock (*Gadus chalcogrammus)* Larvae

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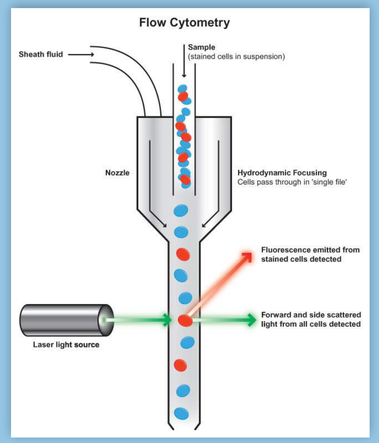
Preliminary results of a new method for measuring the growth rate (mm/day) of Walleye Pollock (*Gadus chalcogrammus)* larvae are presented here. This method uses cell-cycle analysis of muscle cell nuclei measured with flow cytometry. An advantage of using flow cytometry is that it is faster than counting otolith daily increments to determine growth, particularly when increments are difficult to discern due to slow growth.

Why Growth Rate Is Important

Hypotheses used to explain the survival of fish larvae are all based on fast growth being advantageous, thus growth rate is a critical factor affecting their survival. Field studies have shown that survival is greater among faster growing larvae within a population. Accurate measurement of growth will lead to better understanding the relationship between environmental variability and Walleye Pollock larvae survival in Alaskan waters.

Flow Cytometry

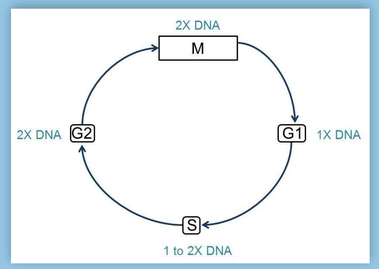
Flow Cytometry is a technique by which individual cells or nuclei are stained with target-specific dyes, passed through laser beams, and their fluorescence and light-scattering properties are measured. DNA-specific and RNA-specific dyes were used in this study to simultaneously stain those nucleic acids and measure the fluorescence of each.



source: flowcytometry.med.ualberta.ca

Cell Cycle

The cell cycle is divided into two basic parts, interphase and mitosis (M). Interphase consists of three phases: gap 1 (G1), DNA synthesis (S), and gap 2 (G2). Cell growth occurs during the G1 phase. For cells to divide they must first replicate their DNA (S phase), and then grow and produce the structures necessary for mitosis (G2 phase). The amount of DNA within the nucleus increases during the S phase, and G2 and M have twice the amount of DNA that is present in G1.

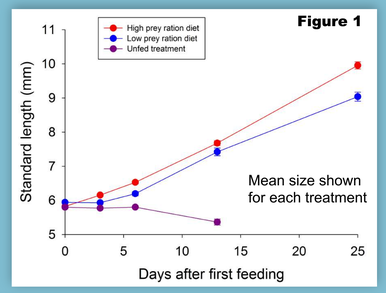


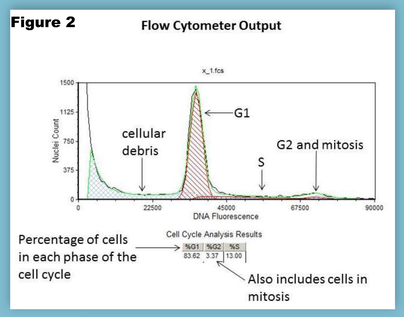
Methods

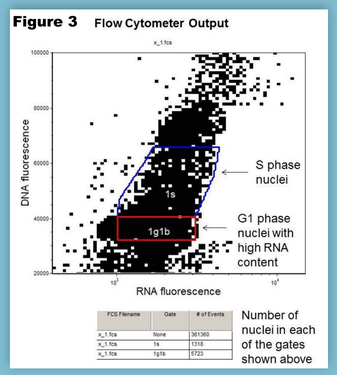
Walleye Pollock larvae were reared in the laboratory at 6°C using a high prey ration diet, a low prey ration diet, and an unfed treatment (Fig. 1). Larvae were frozen at -80°C for later analysis.

For each larva the percentage of cells in S, G2 and M was calculated based on DNA fluorescence (Fig. 2). Those phases indicate cells that may divide and therefore are related to growth. The ratio of the number of S phase nuclei to the number G1 phase nuclei with high RNA content (RSG1) was used as another indicator of cell growth that was based on the progression of cells from G1 to S (Fig. 3).

A generalized additive model (GAM) to predict growth rate was formulated beginning at first feeding to 25 days thereafter. Covariates tested were standard length (SL), body depth at anus, proportion of cells in the S phase, proportion of cells in the G2 phase and M, and RSG1.







Results

The best fit model had SL, proportion of cells in the S phase, and proportion of cells in the G2 phase and M for covariates, and $R^2 = 0.84$. An independent group of larvae was used to test model accuracy. Predicted growth was more accurate for larvae > 7.5 mm SL, than for smaller larvae where accuracy was much more variable.

Standard length (mm)	Actual growth rate (mm/d)	Predicted growth rate (mm/d)	SE	% error
5.61	0.00	0.02	0.01	> 100
5.85	0.01	0.03	0.01	> 100
6.05	0.08	0.06	0.01	34
6.08	0.04	0.06	0.01	70
6.55	0.11	0.10	0.01	9
6.56	0.05	0.09	0.01	71
7.44	0.06	0.11	0.01	81
7.76	0.14	0.14	0.01	0
9.35	0.14	0.14	0.01	0
9.45	0.14	0.14	0.01	0
9.93	0.16	0.18	0.01	12

Future Work

1. Larvae reared at 1.5° and 3°C will be included to cover a range of temperatures that typically occurs in the Bering Sea and Gulf of Alaska.
2. The model will be field tested using Gulf of Alaska larvae and those results will be compared to growth rates calculated using the otolith technique.

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