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Title: In Search of the Optimum Stocking Density for Chum
Salmon (Oncorhynchus keta) Eggs in Shallow Matrix Substrate
Incubators

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Abstract approved:

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Within the tested range of 1000 - 7000 eyed eggs/ft.² gravel substrate (1.08 - 7.53 eggs/cm.²), the optimum stocking density for chum salmon (Oncorhynchus keta) eggs in shallow matrix substrate incubators occurred at 3000 - 4000 eggs/ft.². Premature fry, which predominated early emergence, showed greater variability in lipid content than fry at peak emergence. Cumulative premature fry emergence was lowest at 3000 - 4000 eggs/ft.² and highest at 5000 - 7000 eggs/ft.². Early emerging fry are considered to be of lesser quality than peak emerging fry because the observed increase in variation in lipid content (i.e. primary energy reserve) may lead to increased variation in survival during seaward migration.

Neither survival (from egg stocking to emergence) nor the gross body composition (determined by proximate

analysis) of emerging fry were affected by egg stocking density. Development indices decreased in a linear fashion throughout emergence but the slope and intercept of the line did not differ among egg stocking densities. The frequency of yolk sac abnormalities was negligible in all treatments. The water content of fry increased from early to late emergence while dry weights remained relatively constant. The implications of this observation are discussed.

In Search of the Optimum Stocking Density
For Chum Salmon (Oncorhynchus keta) Eggs
in Shallow Matrix Substrate Incubators

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TABLE OF CONTENTS

INTRODUCTION.....	1
MATERIALS AND METHODS.....	6
RESULTS AND DISCUSSION.....	12
Fry Survival.....	12
Emergence Timing.....	14
Development Index.....	17
Proximate Analysis.....	20
Yolk Sac Abnormalities.....	28
SUMMARY AND CONCLUSION.....	29
BIBLIOGRAPHY.....	33

LIST OF ILLUSTRATIONS

<u>Figure</u>		<u>Page</u>
1	Arrangement of incubation cells with water inlets connected in parallel to assure uniform water quality among them	7
2	Cross-section of an incubation box showing pattern of water flow	7
3	Fry survival from egg stocking to emergence	13
4	Emergence timing in densities stocked with eyed eggs	15
5	Comparison among initial stocking densities of emerging fry development indices as a function of time	18

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1a	Correlations between proximate analysis components for three phases of fry emergence period	22
1b	Summary statistics for proximate analysis components compared in table 1a	22
2	Correlations between development indices (K.D.) and standardized proximate analysis components for three phases of fry emergence period	24

In Search of the Optimum Stocking Density for Chum
Salmon (Oncorhynchus keta) Eggs in Shallow Matrix
Substrate Incubators

INTRODUCTION

The optimum stocking density for chum salmon (Oncorhynchus keta) eggs in shallow matrix substrate incubators has never been clearly defined. While the present operating levels are considered conservative, they need to be compared with a wide range of densities to determine if fry production can be increased without lowering egg to fry survival or fry quality. Because the ultimate goal of a chum salmon hatchery is to increase returns of adults from the sea, it is most important to estimate the quality (fry to adult survival potential) of fry emerging from various egg stocking density treatments.

The design of the Netarts shallow matrix substrate incubator accomodates the following requirements for successful incubation of chum salmon eggs and alevins (McNeil,1968; Poon,1970; Lannan,1975; Bams and Simpson, 1977):

1. a nontoxic, clean water supply of sufficient flow to supply adequate levels of dissolved oxygen and remove nitrogenous wastes;
2. separation of alevins into small clusters to

reduce energy expending interactions and maximize growth;

3. a rugose substrate to cushion the delicate yolk sacs of alevins;
4. exclusion of light from the incubators;
5. an appropriate temperature regime to support eggs and alevins.

The present operation of these incubators involves spreading fertilized eggs in a monolayer on screens located above the gravel substrate, resulting in a density of 2000 - 2500 eggs/ft.² of substrate (2.15 - 2.69 eggs/cm.²). Although increasing the egg stocking density could potentially increase the efficiency of these incubators, it is not known what effect this would have on the first three incubation requirements and thus on the survival and quality of chum fry.

In fact, the effects of egg stocking density on fry survival and quality are poorly understood for all Pacific salmon species. Dill(1967) noted that egg burial density in natural redds could affect emergence timing, and that alevins of coho salmon (O. kisutch) in laboratory aquaria responded to higher densities by net movement towards the water inlet. No significant effects of greater egg density were reported for eyed chum salmon eggs (Dill,1967; Dill and Northcote,1970) or eyed eggs of chinook salmon

(O. tshawytscha) (Thomas,1975) in gravel incubators. A very narrow range of densities were tested in all cases, suggesting that critical levels were never reached. A maximum stocking density was measured above which survival decreased for sockeye salmon (O. nerka) eggs in a deep gravel incubator; none of the tested densities affected emergence timing or fry quality (Ginetz,1976).

The objective of this study was to determine the stocking density of chum salmon eggs in shallow matrix substrate incubators that would produce the largest possible number of high quality fry. Quality in this context refers to the capacity of salmon fry at emergence to survive the transition from the freshwater to oceanic environment, and ultimately to survive until they return to freshwater as mature adults (Koski,1975). Because survival in the wild cannot be measured directly until after fry are released from a hatchery, indicators have been developed to predict the quality of emerging fry. Four indicators of fry quality were used in this study: emergence timing, development index (K.D.), proximate tissue analysis, and the frequency of yolk sac abnormalities.

Emergence timing is often used to predict the viability of hatchery fry immediately after release because wild fry are known to synchronize their migrations with the

greatest availability of estuarine food (Bams,1969; Kron,1976). Before migrating downstream, wild fry become more streamlined (by increasing length faster than weight) and thus better adapted to swimming. The readiness of emerging fry to swim downstream has been ascertained by relating weight and length measurements in a development index (Bams,1970). The value of proximate analysis is that it may reveal qualitative differences among fry that have equal weights and/or development indices. For example, the magnitude of a fish's energy reserves may influence its survival after release from a hatchery. Adult returns from chinook fingerlings containing 7.9% lipid were significantly greater than from those containing 4.1% lipid (Burrows,1969). A plentiful source of energy is also crucial for seaward migrating fry considering that they must avoid predation and survive periods of fasting, salinity increases, and changes in water velocity and diet. It appears that stored lipid is the main energy source utilized by chum and pink salmon (O. gorbuscha) fry during migration and early seawater growth. Large depletions in lipid content occur when chum fry are experimentally transferred from fresh to salt water (Saddler et al.,1971), when they are starved in the laboratory (Parker and Vanstone,1966), and when they migrate from nearshore to the open sea (Parker and Vanstone,1966; Saddler et al.,1971;

Vanstone et al.,1970). The frequency of yolk sac abnormalities is another good measure of chum fry quality since some abnormalities have been shown to cause death before yolk absorption is completed (Emadi,1973).

A wide density range (limited only by the availability of eggs) was tested in this study to increase the chances of identifying the optimum density level. All densities were tried with green or newly fertilized eggs and with eyed eggs just about to hatch in order to determine whether the biological requirements of embryos or alevins limit the optimum density level.

MATERIALS AND METHODS

The densities tested in this experiment were: 1000, 2000, 3000, 4000, 5000, 6000, and 7000 eggs per square foot (1.08 - 7.53 eggs/cm²) of one inch graded smooth gravel substrate. Three replicates of each density were stocked with green eggs and three with eyed eggs, using forty-two separate incubation cells. Each cell was a scale model of a Netarts shallow substrate matrix incubator (Lannan, 1975) which provided one square foot (929 cm²) of substrate area. Figure 1 shows the arrangement of the incubation cells: two rows with twenty-one cells per row. Water intakes of the cells in each row are connected in parallel by a central water distribution channel located between the rows. The entire block of cells was outdoor, alongside the production incubators at the Netarts chum salmon hatchery. The incubators were covered throughout the experiment.

Water diverted from the head box of the production incubators flowed into the central distribution channel via a four inch (10.16 cm) I.D. PVC pipe, supplying each cell with 1.6 - 1.8 gpm (0.10 - 0.11 L/s) through its own water inlet, as depicted in figure 2. Figure 2 also shows the water flow pattern through a given incubation cell.

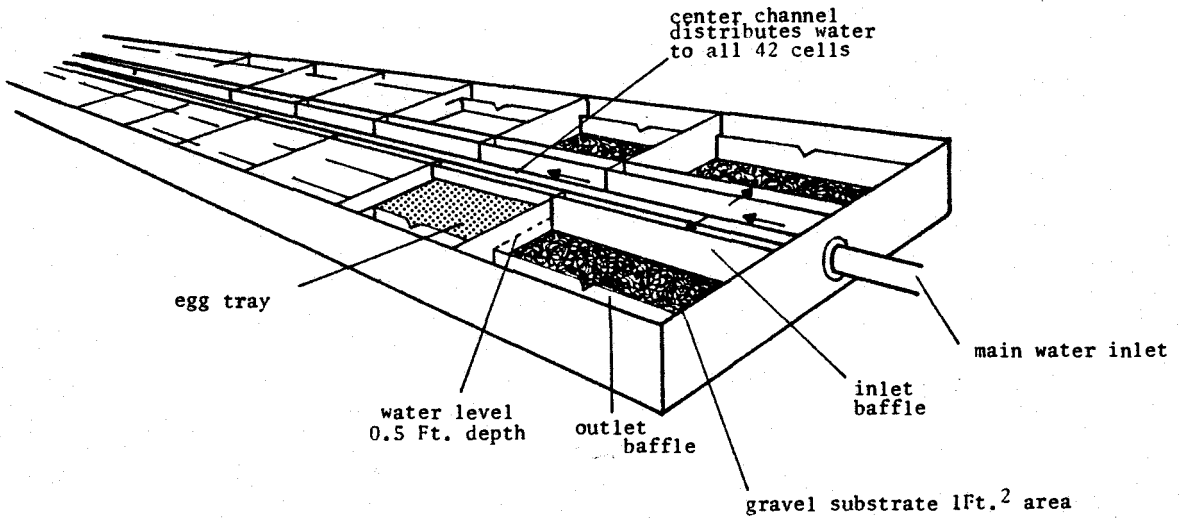


Figure 1. Arrangement of incubation cells with water inlets connected in parallel to assure uniform water quality among them

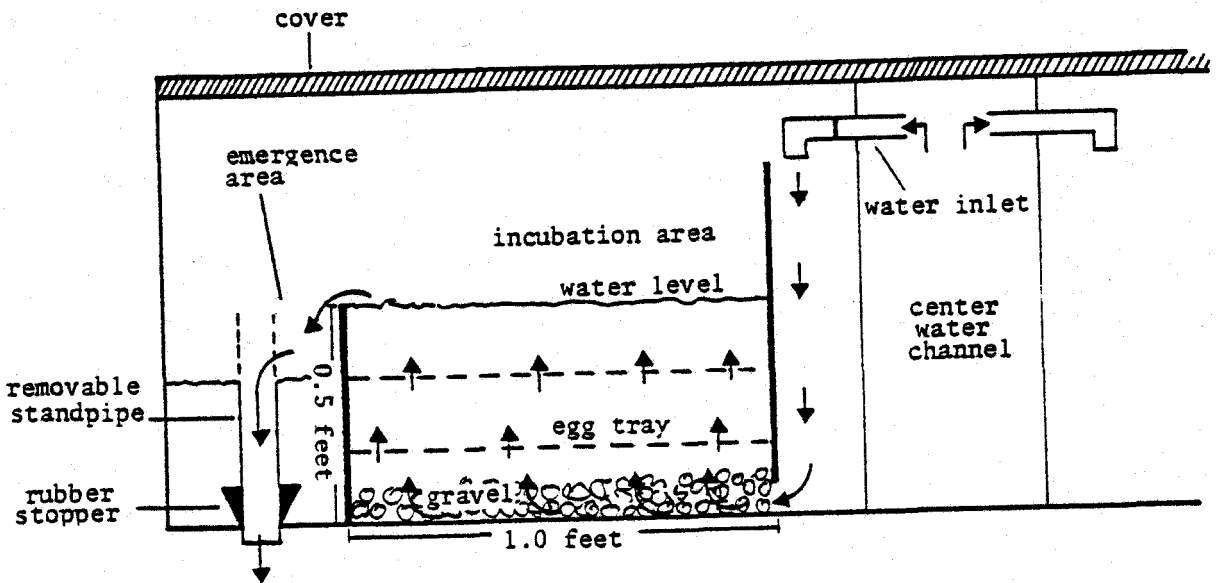


Figure 2. Cross section of an incubation box showing pattern of water flow

The water enters the incubation area under an upstream baffle, moves through the gravel monolayer, then up through the eggs sitting in even layers on one-quarter inch (6.35 mm) Vexar screen trays, and finally cascades over the downstream baffle into the emergence area. Hatched alevins pass through the holes in the egg tray screen to settle on the gravel. The egg trays are removed when hatching is completed. A notch in the top of the downstream baffle facilitates volitional emergence of fry. A standpipe maintains the water level in the emergence area. Narrow slots near the top of the standpipe allow water to exit while precluding escapement of fry. The standpipe is inserted through a rubber stopper in the floor of the incubator, making it easily removable for collection of emerged fry.

A random numbers table was used to assign egg stage and density treatments to the forty-two cells. Green egg treatments (G-treatments) were stocked using females and males spawned on the same day. It was assumed that selecting eggs from only one part of the adult run would not bias the results of the experiment. Fertilized eggs from four females were mixed well in one bucket and a 150 egg sample was withdrawn to determine the quantity of water displaced per egg. This volumetric relationship was then used to count the eggs from the bucket into the individual cells.

This procedure was repeated until all treatments were stocked. Live eyed eggs were taken from the production incubators to stock the eyed egg treatments (E-treatment). These eggs were selected on the basis of having been fertilized on the closest possible date to that of the G-treatments. Not enough ripe females were available on the day the G-treatments were stocked to set aside eggs for the E-treatments. Bi-weekly monitoring during the experiment included checking flow rates and levels of dissolved oxygen (with a YSI model 57 meter and #5739 oxygen-temperature probe) and ammonium nitrogen (with a Hach model NI-8 test kit) of the water in the emerging areas.

Eggs in the G-treatments were addled (shocked) and the dead eggs removed at the eyed stage. The dead eggs from each cell were counted by the volumetric method and then subtracted from the number of eggs stocked to determine survival from stocking to the eyed stage.

Emerging fry from each cell were counted daily with notes taken on their predominant visual appearance, using the six stage classification system described by Koski (1975). These data were used to graph emergence timing. The equation used for logistic transformation of the dependent variable in this graph is: $p' = \ln(p/1-p)$ where p = cumulative % of total emergence/100 (Neter and Wasserman, 1974). The daily counts of emerging fry were totalled to calculate survival in each cell from stocking

to emergence as follows:

$$\text{percent survival} = \frac{\text{no. emerged fry}}{\text{no. eggs stocked}} \times 100.$$

Samples for fry quality determinations were taken three times during emergence (see abscissa of figure 4), i.e. at early, peak, and late emergence. Two percent, but not less than three fish, were drawn from each cell's daily emergers by counting the fry into a bucket of water, vigorously stirring the water, and removing the required number with a dip net. Since each fry in the bucket had an equal and independent chance of being removed, the sample was considered to be random. Each fry was anesthetized in a 0.01% solution of tricaine methane sulfonate (MS-222), blotted dry, weighed, measured for forklength, examined for visual appearance and yolk sac abnormalities, wrapped in aluminum foil with an identification number, and quick frozen in liquid nitrogen. A development index was calculated for each sampled fry using the equation

$$\text{K.D.} = \frac{10 \sqrt[3]{\text{wet wt. in mg}}}{\text{length in mm}} \quad (\text{Bams, 1970}).$$

Three frozen fish per cell per sampling date (early, peak, and late emergence) were randomly selected for proximate analyses. One fish was used for dry weight, water, and ash determinations, the second for total lipid, and the third for total nitrogen. Dry weights were determined by heating fish on tared aluminum pans to constant weight at 80°C. Water content was calculated by

subtracting dry weights from wet weights. Each dried fish was then incinerated to constant weight in a muffle furnace at 450°C to yield ash weight. The ammonia released after modified micro-Kjeldahl digestion (Dennis Gordon, Oregon Seafoods Laboratory, Astoria, Oregon, personal communication) was measured with an Orion #95-10 ammonia-sensing gas electrode connected to an Orion model 701 pH meter to estimate total nitrogen (Farmer, Ritter, and Ashfield, 1978). A standard solution of bovine serum albumin was used to determine recovery rates of the digestions. Ammonium chloride dissolved in a solution identical to that of the fish digests was used to check the accuracy of the ammonia probe. Since the amount of non-protein nitrogen is negligible (Brett et al., 1969), total nitrogen may be taken as an indicator of protein content. Lipid was determined by the method of Folch, Lees, and Stanley (1957) with tripalmitin used as a standard. Carbohydrate content was not determined because it is not more than 0.5% of wet weight in salmon (Brett et al., 1969).

Arcsine transformations, analyses of variance, two sample t tests, Student-Newman-Kuels (SNK) multiple range tests, and correlation coefficients were calculated according to Zar (1974).

RESULTS AND DISCUSSION

Fry Survival

Fry survival from egg stocking to emergence for seven densities is illustrated in figure 3. Arcsine transformations were performed on the data to calculate means and standard deviations, followed by retransformation of the statistics for graphing purposes. Conclusions based on analysis of variance of these data are: 1. there is no significant stocking density effect on survival ($\alpha = .05, P > .25$); 2. there is a significant effect of egg stage (at stocking) on survival, i.e. E-treatments show higher survivals than G-treatments ($\alpha = .05, .0005 < P < .001$). This egg stage effect may be due to a bias introduced when only live eyed eggs were used in stocking the E-treatments. This procedure selected for survival to the eyed stage in the E-treatments, without doing the same in the G-treatments.

Average survival for all G-treatments up to the eyed stage was $60.22 \pm 2.81\%$. Such low survival confounds the evaluation of the remainder of the experiment on G-treatments. Thus, in all subsequent sections the results for E-treatments only will be presented.

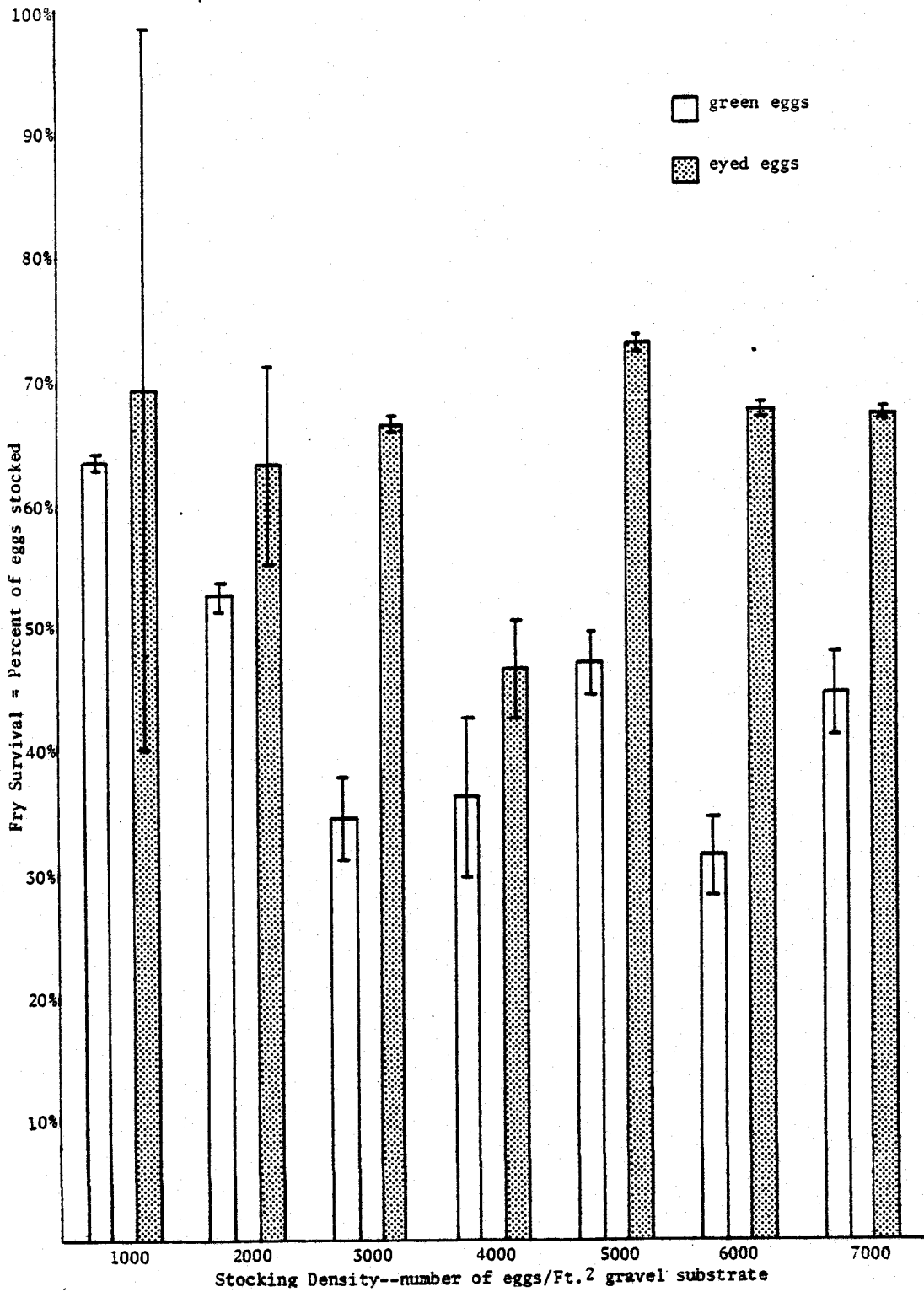


Figure 3. Fry Survival--From egg stocking to emergence

Emergence Timing

The influence of density on emergence timing is illustrated in figure 4. Emergence dates and corresponding notes on the visual appearance of emerging fry are on the abscissa. The cumulative percent of total emergence is on the ordinate. Within the entire emergence period of fifty-nine days there is a peak period coincident with the steepest slopes of the curves. This peak lasts approximately eighteen days (3/18/79 to 4/4/79). Premature yolk sac fry predominate before peak emergence. "Buttoned up" fry (i.e. fry that have fully absorbed their yolk sacs) do not appear until late emergence.

There are density related differences among the relative positions of the emergence curves from ten to fifty percent emergence, resulting from differences among slopes and locations of inflection points. Displacement of the curves to the left of the graph (i.e. towards the early emergence of premature fry) is greatest in the high densities (5000 - 7000 eggs/ft.²), least in the intermediate densities (3000 - 4000 eggs/ft.²), and intermediate in the lowest densities (1000 - 2000 eggs/ft.²). Premature fry emergence is greatest at the highest densities, presumably as a response to overcrowding in the gravel substrate. The reason for the intermediate densities having lesser premature emergence than the lowest ones

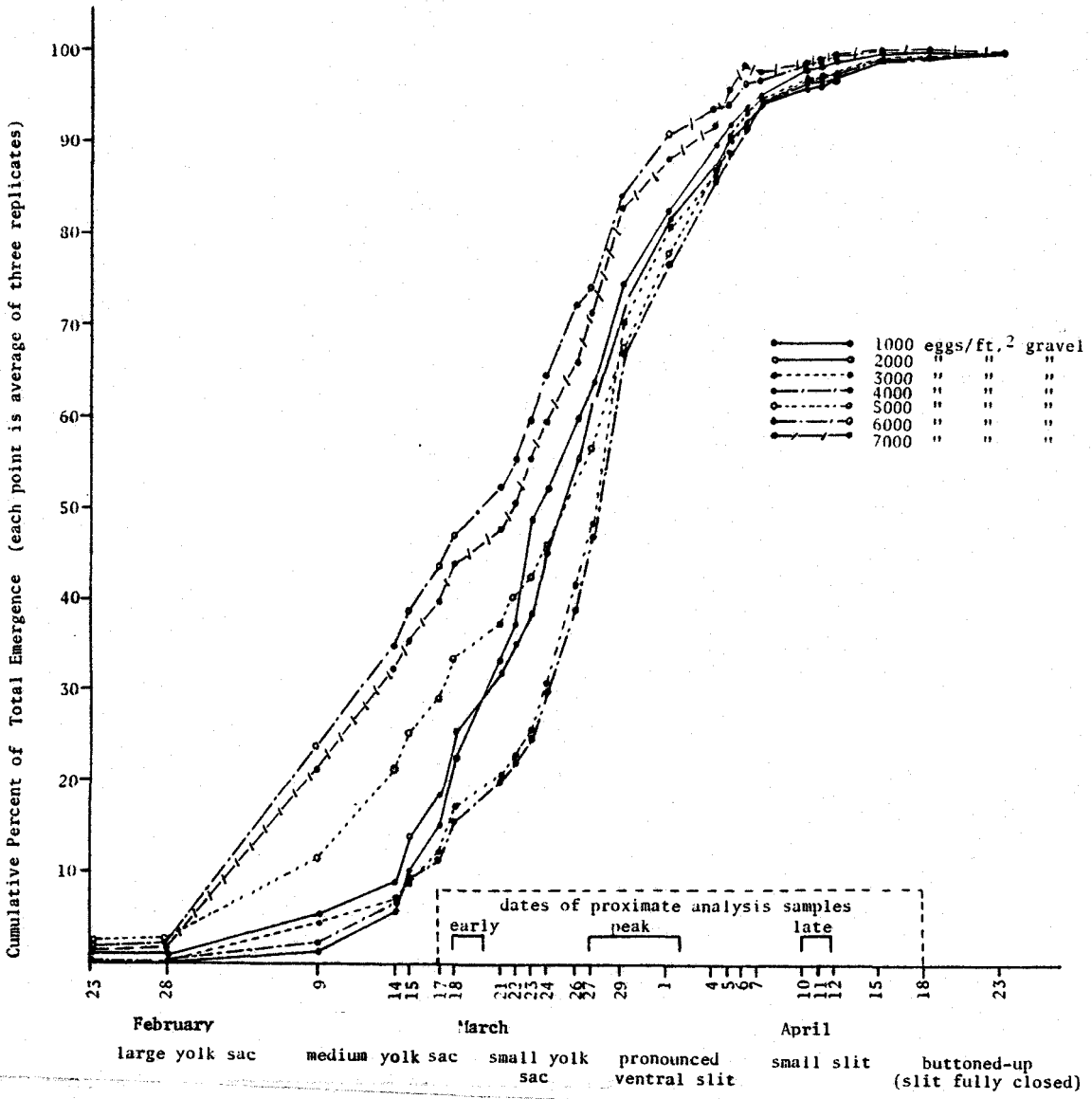


Figure 4. Emergence timing in densities stocked with eyed eggs

may be related to the gregariousness of chum alevins and fry. This behavior of developing chum salmon was observed during this experiment and in past years of incubation at Netarts. Unfortunately, these data do not suggest a mechanism for the influence of gregariousness on premature emergence.

Differences in the slopes of the curves, i.e. the rates of emergence, reveal a second density related trend illustrated by comparing the time intervals between twenty and fifty percent cumulative emergence. High densities exhibit the slowest emergence rate (12 - 14 days), lowest densities a faster emergence rate (10 days), and intermediate densities the fastest rate (6 days). The aforementioned gregarious behavior of chum fry may partially explain why the intermediate densities, not the low ones, exhibit the most rapid emergence rate.

To test whether these density related differences among the emergence curves were due to chance alone, the curves in figure 4 were first made linear by plotting logistic transformations of the dependent variable against time. The resulting slopes and intercepts were computed and compared in a series of t tests, where data from the 2000/ft.² density (the current operating density in Netarts boxes) were compared to each higher density.

Conclusions from these SNK tests ($\alpha = .05$) are:

1. fry from the 6000/ft.² treatment emerge at a slower rate than those from the 2000/ft.² treatment ($P < .001$); but emergence rates from the 5000/ft.² and 7000/ft.² treatments are not significantly different from that of the 2000/ft.² treatment ($0.2 < P < 0.5$);
2. fry from the 3000/ft.² and 4000/ft.² treatments emerge at a faster rate than those from the 2000/ft.² treatment ($P < .001$);
3. cumulative premature fry emergence from the 5000 - 7000/ft.² densities is greater than from the 2000/ft.² density ($P < .001$);
4. cumulative premature fry emergence from the 3000 and 4000/ft.² densities is lower than from the 2000/ft.² treatment ($P < .001$).

Development Index

Notes on the visual appearance of emerging fry (bottom of figure 4) indicate that fry emerge at progressively more mature stages of development over time. The relationship between initial stocking density and this change in emerging fry maturity is addressed in figure 5. Development indices (K.D.) are used as a quantitative measure of development stage (Bams, 1970). K.D. is

Stocking Density--Eggs/Ft.² gravel substrate

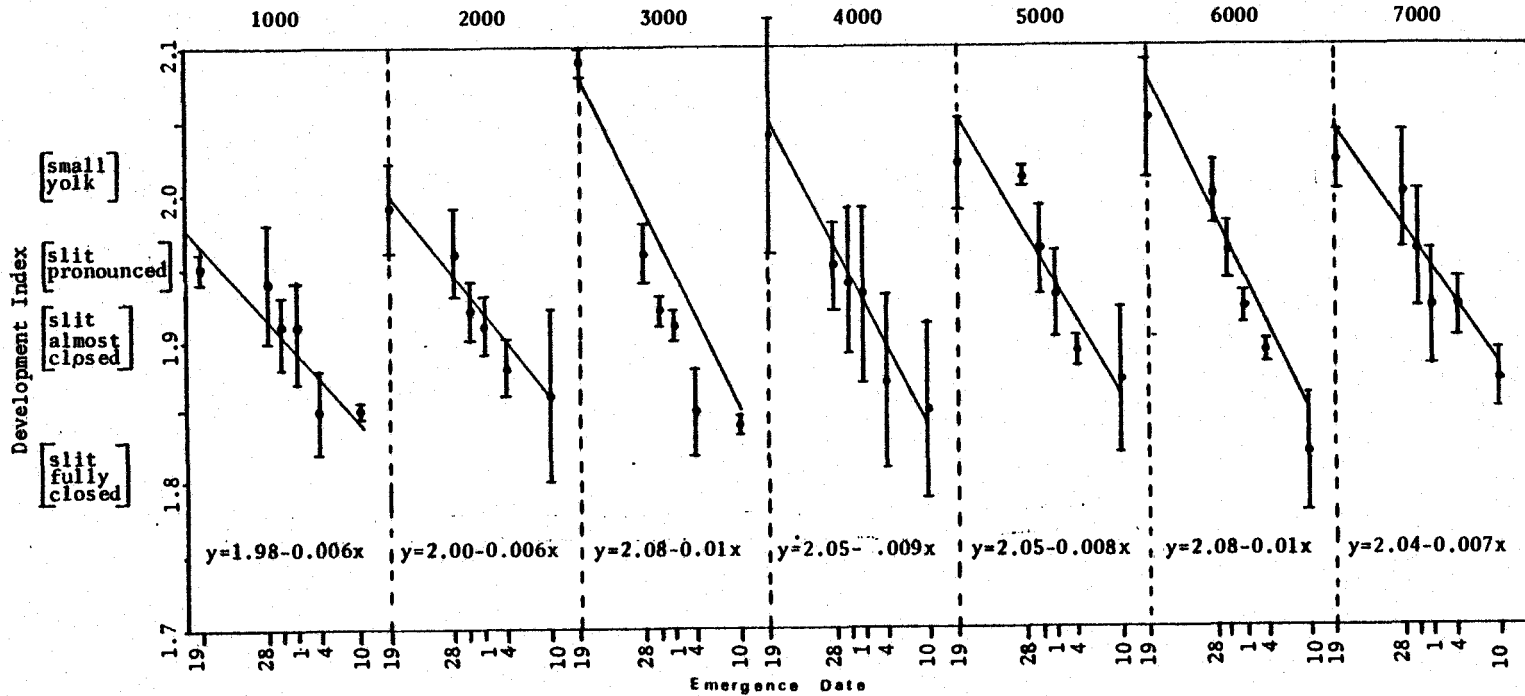


Figure 5. Comparison among initial stocking densities of emerging Fry Development Indices as a function of time

graphed as a function of emergence time, with a separate regression line plotted for each density. Notice that all the lines have negative slopes: K.D. decreases over time in all densities. This observation corroborates those of Bams (1970) and Poon (1977).

If all graphs are compared for a given day, the considerable overlap among standard deviations becomes apparent. This suggests that initial differences in stocking densities did not lead to differences among the development indices of concurrently emerging fry, nor among the rates of change in the indices over time. Comparison of the slopes of the regression lines using SNK tests ($\alpha = .05$) confirmed that the rate of decrease in K.D. values or the rate of fry maturation during the emergence period is independent of egg stocking density. The only exception to this pattern is the difference between the slopes of 1000/ft.² and 6000/ft.² treatments which is significant at $\alpha = .05$. The Y - intercepts of the seven linear regressions were also compared using SNK tests ($\alpha = .05$) to identify significant density related differences in K.D. values at the start of emergence. Conclusions from these tests are:

1. fry from 1000/ft.² densities started emerging at a more developed stage than fry from the 2000, 3000, 5000, 6000, and 7000/ft.² densities ($P < .001$);

2. the difference between 1000/ft.² and 4000/ft.² values is significant at $\alpha = .1$.
3. fry from 2000/ft.² treatments initiated emergence at a more developed stage than fry from the 7000/ft.² treatments ($.01 < P < .025$);
4. there are no significant differences in K.D. values of first emergers among the 2000 - 6000/ft.² densities.

It is concluded that the egg stocking density effect on the development stage of first emergers is significant only when extremes of the density range are included in the comparison. Specifically, the most mature fry at the start of emergence were produced by the lowest density, and first emergers from the second lowest density were more mature than those from the highest density, while K.D. did not differ significantly among fry at intermediate densities.

Proximate Analysis

No significant effect of egg stocking density on the dry weight, water, lipid, and total nitrogen content of emerging fry was apparent when the data were evaluated by analysis of variance. Although the levels of these components varied with time of emergence (early, peak, or late), the variations in ash content of all the samples

were not significantly different regardless of egg stocking density, emergence date, or the stage of eggs stocked. The implication of these results is that the proximate composition of fry did not differ among the seven densities. However, the presence of density related biochemical differences too subtle to be detected by proximate analysis cannot be ruled out.

The data of the seven densities were pooled and examined for relationships between emergence time and proximate analysis. The correlations between dry weight and other proximate components are presented in table 1a. The outcomes of statistical tests are also reported. Summary statistics for the data are listed in table 1b. A correlation coefficient measures the intensity of association between two variables, neither of which is dependent on the other. For example, an "r" value computed from correlating lipid content with dry weight may be considered to measure the amount of variability in lipid content accounted for by variation in dry weight. Zero correlation means that a change in magnitude of one variable does not infer a change in magnitude of the other.

Lipid is significantly correlated with dry weight only in peak emergence fry. Consequently, an increase in the magnitude of dry weight at peak emergence implies an increase in the magnitude of lipid content. The same

Table 1a.. CORRELATIONS BETWEEN PROXIMATE ANALYSIS COMPONENTS FOR THREE PHASES OF FRY EMERGENCE PERIOD

<u>Emergence Period</u>	<u>Correlated Variables</u>	<u>r</u>	<u>Outcome of testing $H_0: \rho = 0$ at $\alpha = 0.05$</u>
Early	dry wt. and lipid	0.165	retain H_0
Peak	" " " "	0.604*	reject H_0 (.002 < P < .005)
Late	" " " "	0.142	retain H_0
Early	dry wt. and water	-0.014	retain H_0
Peak	" " " "	0.524*	reject H_0 (.01 < P < .02)
Late	" " " "	0.488*	reject H_0 (.02 < P < .05)
Early	dry wt. and total N	0.275	retain H_0
Peak	" " " " "	0.239	retain H_0
Late	" " " " "	0.104	retain H_0

*denotes statistical significance at $\alpha = 0.05$

Table 1b. SUMMARY STATISTICS FOR PROXIMATE ANALYSIS COMPONENTS COMPARED IN TABLE 1a.

<u>Emergence Period</u>	<u>Mean \pm Standard Deviation (n = 21)</u>			
	<u>mg lipid</u>	<u>mg total N</u>	<u>mg H₂O</u>	<u>mg dry wt.</u>
Early	24.97 \pm 7.29	9.71 \pm 1.73	245.23 \pm 35.51	84 \pm 14.0
Peak	25.24 \pm 3.83	10.83 \pm 2.89	284.01 \pm 46.21	90 \pm 9.0
Late	23.64 \pm 5.93	10.86 \pm 1.91	315.5 \pm 42.53	87 \pm 3.0

correlations for early and late emergence fry are very low. Furthermore, the lipid contents of early and late emergers are more variable than those of peak emergers as seen in the standard deviations reported in table 1b. Apparently, correlation with dry weight accounts for little of these relatively high variations.

The correlation between water content and dry weight goes from very low in early emerging fry to a significant value at peak emergence. This trend is similar to that seen in the lipid-dry weight correlations. The water - dry weight correlation is also significant in late emerging fry. In contrast, correlations between total nitrogen and dry weight are insignificant. Means and standard deviations are similar throughout emergence (table 1b) indicating that the total nitrogen content of emerging fry varies little as dry weight and/or emergence timing vary.

Correlations of proximate analyses with development indices were used to test for qualitative differences among early, peak, and late emerging fry. The results are reported in table 2. Sample values for lipid, total nitrogen, and water content were standardized, i.e. divided by their respective dry weights, before correlating them with K.D. values.

Table 2. CORRELATIONS BETWEEN DEVELOPMENT INDICES (K.D.)
AND STANDARDIZED PROXIMATE ANALYSIS COMPONENTS FOR
THREE PHASES OF FRY EMERGENCE PERIOD

<u>Emergence Time</u>	<u>Mean ± S.D.(n = 21)</u>	<u>Variables</u>	<u>r</u>	<u>Outcome of testing</u> <u>H₀: ρ=0 at α =.05</u>
		<u>lipid/dry wt.</u>		
Early	0.3069 ± 0.1264	K.D.	-0.26	retain H ₀
Peak	0.2797 ± 0.0412	and	0.41	retain H ₀
Late	0.2740 ± 0.0677	lipid/dry wt.	0.39	retain H ₀
		<u>N/dry wt.</u>		
Early	0.1198 ± 0.0347	K.D.	-0.35	retain H ₀
Peak	0.1206 ± 0.0343	and	0.29	retain H ₀
Late	0.1267 ± 0.0285	N/dry wt.	-0.14	retain H ₀
		<u>water/dry wt.</u>		
Early	3.06 ± 1.05	K.D.	-0.22	retain H ₀
Peak	3.15 ± 0.54	and	-0.355	retain H ₀
Late	3.64 ± 0.49	water/dry wt.	-0.219	retain H ₀

Although the correlations are insignificant throughout the emergence period, there are some interesting trends:

1. correlation coefficients are generally greatest at peak emergence;
2. means of the standardized components differ only slightly in magnitude throughout emergence;
3. the magnitude of standard deviations around the mean decreases 33-50% from early to peak emergence (except for nearly equal nitrogen/dry wt. values).

Based on these trends, it appears that while the development indices of fry change in magnitude throughout the emergence period, their average gross body composition, on a dry weight basis, remains relatively constant. Variation around this average (due to individual fry differences) is influenced by emergence timing, reaching a minimum at peak emergence; yet it is always poorly accounted for by correlation with corresponding K.D. values regardless of the emergence date.

Another apparent trend in table 2 deserves attention. The water/dry weight ratio is always negatively correlated with K.D. suggesting that fry gain water as they mature (i.e. as K.D. decreases). Indeed, late emergence (mature) fry have a greater average water content than early emergence (premature) fry, whereas the increase in average dry weight from early to late emergence is much less (table 1b).

Finally, notice that the means for the ratio of water to dry wt. (table 2) increase from early to late emergence. These results indicate that fry maturation is accompanied by increased water gain and/or retention.

The mechanisms involved and the adaptive value of this hydration cannot be determined from these data although some reasonable hypotheses can be formulated. Atlantic salmon (Salmo salar) alevins show a similar increase in water content during development concurrent with a decrease in specific gravity (Peterson and Metcalf, 1977). Peterson and Metcalf were able to account for these changes by measuring the changes in relative amounts of dense, dehydrated yolk (55-60% water) and less dense, hydrated embryonic tissue (84-85% water) associated with the alevin's development. Thus, the different water contents of early and late emerging fry in this study may be related to different yolk contents. According to Weisbart (1967), Hayes and Armstrong (1942) showed that the increased hydration of Salmo salar alevins in fresh-water was due to a net uptake of water from the environment, although the alevins were also obtaining water from their yolks. Weisbart believes that such an ability to regulate water, as well as ions, explains the regulation of plasma osmolality, plasma Na^+ , and plasma Cl^- concentrations he observed in chum and pink salmon alevins

exposed to seawater. He also observed improvement of osmoregulatory capabilities in fry of these species. Given that net water gain and retention are often part of a teleost's osmoregulatory strategy in saltwater (Prosser, 1969), the increased water content of late emerging fry in this study may reflect changes in their physiology in anticipation of the transition from maintaining hydro-mineral balance in a hypotonic (freshwater) to hypertonic (saline) environment. Black (1951) associates such osmoregulatory changes with the increased water content seen in chum fry held in freshwater past the time of migration.

A secondary result of increased water content may be increased buoyancy in the water column which facilitates passive drifting with the current, a behavior frequently observed in the downstream migration of chum fry (McDonald, 1960; Bakkala, 1970). Fry are known to fill their air bladders at the termination of yolk absorption (Bakkala, 1970; Peterson and Metcalf, 1977) and this is probably their primary means of increasing buoyancy.

The K.D. relationship is frequently called a "condition factor" (Dill and Northcote, 1970; Bams and Simpson, 1977; Farmer et al., 1978; Donaldson et al., 1979), implying that K.D. values are quantitative measures of condition or quality. This meaning must be applied with caution. Fry with equal wet weights and lengths (the

terms in the K.D. equation) may have subtle differences in water or lipid content, which may influence their survival in seawater. Putting them on the same quantitative scale (such as K.D.) for comparison may be misleading.

Yolk Sac Abnormalities

The total sample frequency of yolk sac abnormalities was observed to increase from low to high egg stocking densities. Yet, all frequencies, ranging from 0.0002% at 3000 eggs/ft.² to 0.0029% at 7000 eggs/ft.² are considered negligible.

SUMMARY AND CONCLUSION

Results from testing the effects of initial egg stocking density (eggs/ft.² gravel substrate) and the stage of eggs stocked, using chum salmon eggs in shallow-matrix substrate incubators, are summarized below.

1. Initial stocking density did not affect fry survival.
2. Survival was significantly higher in cells stocked with eyed eggs than in those stocked with green eggs; thus only observations on eyed eggs are reported.
3. Egg stocking density affected emergence timing by altering the rate of fry emergence and the magnitude of early or premature fry emergence; intermediate densities (3000-4000 eggs/ft.²) displayed the fastest emergence rate and the lowest frequency of premature fry emergence.
4. Development indices were not significantly different among density treatments, except when the comparison included K.D. values of first emergers from densities at extreme ends of the tested range.
5. K.D. values decreased in a linear fashion throughout the emergence period. The rate of decrease was not affected by egg stocking density.

6. Initial egg stocking density had no significant effect on the proximate composition of emerging fry. Therefore, data for the densities were pooled to examine the effects of emergence timing on proximate analyses of fry.
7. The presence of density related differences among the body compositions of fry too subtle to be detected by proximate analyses, but important enough to affect fry quality, cannot be excluded.
8. Two interesting trends were found when the proximate composition of fry were compared by time of emergence. The variations in lipid, lipid/dry wt., and water/dry wt. values were greater in early emerging fry than during peak emergence. The water content of fry increased from early to late emergence, while dry weights remained relatively constant. Since this hydration of late emerging fry may indicate a readiness for osmoregulation in a hypertonic medium, it is postulated that late emergence is beneficial to the seawater survival of fry.
9. The frequency of yolk sac abnormalities was negligible in all egg stocking densities.

The emphasis placed on biological versus economic considerations will influence how these results are

interpreted to determine an optimum egg stocking density for a particular chum salmon hatchery. For example, the biological optimum (i.e. the density that yields the highest quality fry) would be the best application for stock enhancement and elimination of egg shortages. In the case of a private ocean ranching business concerned with profitability or a public hatchery faced with funding constraints, economic optimization may be more important.

Results with the eyed egg stockings showed that survival was not lowered by density increases. Since the cost of incubating each egg will decrease as density increases via economies of scale, the economic optimum for the range of densities tested in this study occurs at 7000 eggs/ft.². In contrast, the biological optimum is most influenced by the effects of stocking density on fry quality. The two observations having greatest implications for biological optimization are that the emergence of premature fry was minimized in the intermediate densities (3000 - 4000 eggs/ft.²) and that premature, early emerging fry show greater variability in lipid content than fry at peak emergence. Increased variation in the magnitude of this lipid energy reserve can be considered an undesirable aspect of early emergence since it may lead to increased variation in survival during seaward migration.

The biological optimum for egg stocking density may be

defined as the one which minimizes the incidence of premature fry. For the incubator design and the density range tested in this experiment, the optimum occurs at 3000 to 4000 eyed eggs per square foot of gravel substrate.

BIBLIOGRAPHY

- Bakkala, Richard G. 1970. Synopsis of biological data on the chum salmon, Oncorhynchus keta (Walbaum) 1972. FAO Fish. Synopsis No. 41, Circ. 315. 76 p.
- Bams, R. A. 1969. Adaptations of sockeye salmon associated with incubation in stream gravels, p. 71-87. In T. G. Northcote (ed.) Symposium on salmon and trout in streams, 1968. H. R. MacMillan Lectures in Fisheries, Univ. of British Columbia, Vancouver, B.C.
- Bams, R. A. 1970. Evaluation of a revised hatchery method tested on pink and chum fry. J. Fish. Res. Bd. Canada 27: 1429-1452.
- Bams, R. A., and K. S. Simpson. 1977. Substrate incubators workshop - 1976. Report on current state-of-the-art. Fish. Mar. Serv. Res. Dev. Tech. Rep. 689: 68 p.
- Black, V. S. 1951. Changes in body chloride, density, and water content of chum (Oncorhynchus keta) and coho (O. kisutch) salmon fry when transferred from fresh water to sea water. J. Fish. Res. Bd. Canada 8: 164-177.
- Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. J. Fish. Res. Bd. Canada 26: 2463-2394.
- Burrows, R. E. 1969. The influence of fingerling quality on adult salmon survivals. Trans. Am. Fish. Soc. 4: 777-784.
- Dill, L. M. 1967. Behavioral ecology of chum salmon (O. keta) and coho salmon (O. kisutch) alevins in the gravel. M. S. Thesis, Univ. of British Columbia, Vancouver, B. C. 83 p.
- Dill, L. M. and T. G. Northcote. 1970. Effects of environmental factors on the survival, condition, and timing of fry. J. Fish. Res. Bd. Canada 27: 196-201.

- Donaldson, E. M., U. H. M. Fagerlund, D. A. Higgs, and J. R. McBride, 1969. Hormonal enhancement of growth, p. 456-597. In W. S. Hoar, D. J. Randall and J. R. Brett (ed.) *Fish Physiology*, Vol. VIII. Bioenergetics and Growth. Academic Press, New York.
- Emadi, H. 1973. Yolk-sac malformation in Pacific salmon in relation to substrate, temperature, and water velocity. *J. Fish. Res. Bd. Canada* 30: 1249-1250.
- Farmer, G. J., J. A. Ritter, and D. Ashfield. 1978. Seawater adaptation and parr-smolt transformation of juvenile Atlantic salmon, salmo salar. *J. Fish. Res. Bd. Canada*. 35: 93-100.
- Folch, J., M. Lee, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226: 497-509.
- Ginetz, R. M. J. 1976. Fulton River upwelling gravel incubator for sockeye salmon. *Fish. Mar. Serv. Tech. Rep. PAC/T-76-10*: 45 p.
- Hayes, F. R. and F. H. Armstrong. 1942. Physical changes in the constituent parts of developing eggs. *Can. J. Res. D.* 20: 99-114.
- Koski, K. V. 1975. The survival and fitness of two stocks of chum salmon (Oncorhynchus keta) from egg deposition to emergence in a controlled-stream environment at Big Beef Creek. Ph. D. Thesis, University of Washington, Seattle, 212 p.
- Kron, T. 1976. Pink salmon (O. gorbuscha) time of release in relation to their estuarine food supply - some preliminary observations, p. 210-211. In G. K. Gunstrom (ed.) 1976 Northeast Pacific pink and chum salmon workshop. Alaska Dept. of Fish and Game. 264 p.
- Lannan, J. E. 1975. Netarts chum salmon hatchery, an experiment in ocean ranching. Oregon State Univ., Sea Grant College Program, Publ. No. ORESU-H-75-001. 28 p.
- McNeil, 1968. Development of a streamside incubator for culture of Pacific salmon. Dept. Fish. Wildl., Oregon State Univ., Corvallis, Oreg., Prog. Rep., June 27, 1968. 14 p.

- McDonald, J. 1960. The behaviour of Pacific salmon fry during their downstream migration to freshwater and saltwater nursery areas. J. Fish. Res. Bd. Canada 17: 655-676.
- Neter, J. and W. Wasserman. 1974. Logistic response function, p. 329-335. In J. Neter and W. Wasserman. Applied Linear Statistical Models. Richard D. Irwin, Inc. Illinois. 842 p.
- Parker, R. R. and W. E. Vanstone. 1966. Changes in chemical composition of central British Columbia pink salmon during early sea life. J. Fish. Res. Bd. Canada 23: 1353-1384.
- Peterson, R. H. and J. L. Metcalfe. 1977. Changes in specific gravity of Atlantic salmon (Salmo salar) alevins. J. Fish. Res. Bd. Canada 34: 2388-2395.
- Poon, D. C. 1970. Development of a streamside incubator for culture of Pacific salmon. M. S. Thesis, Oregon State Univ., Corvallis, Ore. 84 p.
- Poon, D. C. 1977. Quality of salmon fry from gravel incubators. Northwest and Alaska Fisheries Center Processed Report. 253 p. Available from: NMFS, Northwest Fisheries Center, Seattle, Wa.
- Prosser, C. L. 1969. Water: Osmotic Balance, p. 6-47. In C. L. Prosser and F. A. Brown, Jr. Comparative Animal Physiology. W. B. Saunders Co., Philadelphia, Pa.
- Saddler, J. B., K. V. Koski, and R. D. Cardwell. 1972. Fatty acid alterations during migration and early sea water growth of chum salmon (Oncorhynchus keta). Lipids 7: 90-95.
- Thomas, A. E. 1975. Effect of egg concentration in an incubation channel on survival of chinook salmon fry. Trans. Am. Fish. Soc. 2: 335-337.
- Vanstone, W. E., J. R. Markert, D. B. Lister, and M. A. Giles. 1970. Growth and chemical composition of chum (Oncorhynchus keta) and sockeye (O. nerka) salmon fry produced in spawning channel and natural environments. J. Fish. Res. Bd. Canada 27: 371-382.

- Weisbart, M.. 1968. Osmotic and ionic regulation in embryos, alevins, and fry of the five species of Pacific salmon. Can. J. Zool. 46: 385-397.
- Zar, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc. Englewood Cliffs, N. J. 620 p.