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Title: QUALITY OF SALMON FRY FROM GRAVEL INCUBATORS

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The performances of newly-established gravel incubation systems designed to use unfiltered water for production of unfed salmon fry were evaluated at Netarts Bay, Oregon, and at Little Port Walter, Alaska.

Research was divided into two phases. The first consisted of laboratory studies to develop fry quality testing procedures and to concurrently investigate effects of substrate, stocking density, water velocity, exposure to light, and incubator design on fry quality. The second consisted of field comparisons of gravel incubator fry to parent stock wild fry at the two sites.

Fry quality criteria included egg and alevin mortality, frequency of physical abnormalities, migration pattern and timing, post-emergence growth, size and stage of development, and performance in stress tests; size was the primary criterion.

Chum salmon incubated without substrate support and exposed periodically to light experienced intense photonegative reaction leading to yolk-sac malformation, high mortality, and loss of fry size;

provision of a shallow gravel substrate negated these adverse effects. By comparison, chinook salmon treated similarly experienced milder photonegative reaction and insignificant mortalities and yolk-sac malformation; fry size and post-emergence growth appeared to be unaffected by substrate rugosity.

Apparent water velocities of 50 to 100 cm/hr and stocking densities of 1.29 to 2.58 pink salmon eggs/cm^2 produced equivalent fry size without accelerated developmental rates. Increasing velocity to 300 cm/hr or density to 5.16 pink salmon eggs/cm^2 accelerated developmental rates without affecting fry size. The influence was more significant for velocity ($p<0.025$) than for density ($p<0.10$).

Incubator design or substrate rugosity and depth had little influence on relative fry size of chum salmon given favorable conditions of low stocking density and darkness; gravel incubator fry, however, performed better in stress tests than fry incubated on a smooth substrate.

Deep and shallow matrix gravel incubator designs at Little Port Walter and two types of shallow matrix gravel incubator designs at Netarts Bay produced fry of at least equivalent size to wild fry; the deep gravel incubator tested at Little Port Walter was stocked at 0.23 eggs/cm^3, which is up to nine times higher than stocking densities used in similar systems elsewhere. At Little Port Walter, hatchery fry migrated at a stage of development similar to wild fry,

but at Netarts Bay, hatchery fry migrated mostly at less mature developmental stages than wild fry.

Although no difference in size was detected between fry incubated in shallow matrix or deep matrix gravel incubators in laboratory or field studies, judgements on the preferred system must await evaluation at the adult stage.

Quality of Salmon Fry from Gravel Incubators

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Derek Clinton Poon

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I. INTRODUCTION

Increased demands and price increases have maintained fishing pressure for Pacific salmon despite their continuing population declines from historic levels (e. g. Fulton 1970; Bakkala 1970). The causes for the decline are multiple and complex, but overfishing and the loss or degradation of freshwater habitats are primary factors. Artificial propagation has been cogent in replacing and supplementing natural propagation.

Of the five species of salmon[1] in the United States, the three species which require freshwater feeding--coho (Oncorhynchus kisutch), chinook (O. tshawytscha), and sockeye (O. nerka)--have received the most propagation emphasis due to their higher economic values. The commercial species of pink (O. gorbuscha) and chum (O. keta) salmon, both of which migrate to sea as unfed fry, have received relatively little attention--despite being, in theory, the simplest to produce since no feeding is required. The heavy reliance by the Japanese and the Russians on successful pink and chum salmon

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[1] In this study, egg or embryo refers to prehatching stage; alevin to post hatching and fry to the stage at or near complete yolk absorption. Larval period is the alevin stage up to the fry stage. Post-emergence period is the period beyond the fry stage.

hatchery program is testimony to propagation potentials of these two species (Atkinson 1976; McNeil, personal communication, 1976; Kanid'yev et al. 1970).

Early North American hatcheries, however, fared poorly. Since the first hatchery was built on the Sacramento River in 1876, millions of salmon fry have been released by private and public hatcheries from California to Alaska. But by 1930, it became obvious that adult returns were generally poor. Foerster (1938) concluded after intensive studies that artificial propagation provided no advantage over natural spawning, and because of his report, all salmon hatcheries were closed in British Columbia in 1937. The basic reasons for failures of early hatchery efforts were nutrition deficiencies, diseases, and the lack of understanding of the biological and environmental incubation requirements.

Recently, technological developments in diets and disease control have helped establish financially sound coho and chinook programs in the Pacific Northwest (Wahle et al. 1974; Worland et al. 1969), and applications of the "simulation concept" have resulted in improved systems for production of unfed fry, primarily for sockeye, pink and chum salmon.

The simulation concept, or gravel incubation, stemmed from a recognition that traditional hatcheries and the natural spawning bed have widely different environmental conditions, that these differences

may be responsible for the production of poor quality hatchery fry with inferior survival potentials, and that remedial measures consist of provision of simulated natural conditions in hatcheries. Although the concept is not new (e. g., Babcock 1911; Robertson 1919; Shapovalov 1937; Shapovalov and Berrian 1940; Carl 1940; Foerster 1946; Wickett 1952; Shelton 1955), its application in production scale is a recent development in North America. The Japanese and Russians have apparently recognized the same concept and are now using gravel for incubation in their hatcheries (Atkinson 1976; Mathews and Senn 1975; Kolgaev 1963; Kolgaev and Zhirnova 1966; McNeil, personal communication, 1976).

Under natural conditions, eggs and alevins repose in darkness under a protective layer of gravel through which water flow is laminar. There is little physical activity until salmon begin to emerge as free-swimming fry. Survival from egg to fry in the wild is typically low (e. g., Ellis 1969; Hunter 1959; Olson and McNeil 1967; McNeil 1962, 1966; Pritchard 1948; Parker 1962), averaging around 10 percent for salmonids (Royce 1959). For pink salmon, the range can be 0. 8-21. 7 percent (Ellis 1969). According to Koski (1975) factors generally responsible for the heavy population attrition in fresh water include: 1) the composition and stability of the gravel bed; 2) the quality and quantity of intragravel water (i. e., water occupying the interstices within the streambed); and 3) the density

of spawners and redd superimposition. Changes in the climatic conditions (droughts, freezing, etc.) produce an additive effect. Limiting factors on natural freshwater survival are well studied and documented (e. g., McNeil 1962, 1966; Neave 1953; Bams 1969; Foerster 1968; Wickett 1958).

In conventional hatchery operations, eggs are concentrated in trays stacked vertically or placed in horizontal troughs. The water flow through the trays is turbulent and re-aeration is usually provided between trays. Alevins may be held in flat incubation trays until after their yolk has been absorbed, or they may be transferred to open troughs or ponds to complete their development. The fish are often exposed to artificial or natural light prematurely. Since freshwater survival in hatcheries can regularly exceed 90 percent, a gain of 5- to 10-fold in fry production can be expected when compared to natural production.

Despite freshwater survival advantages, hatchery fry have many observable differences from wild fry. Brannon (1965) reported that the high water velocity through a hatchery incubator forces sockeye alevins to exercise prematurely at the expense of growth. He also found that exposure to light retards development, increases mortality, and slows yolk absorption. Other physiological differences observed in hatchery fry include: yolk-sac malformation (Emadi 1973), deformation of gut (Disler 1953), translocation of liver

(Nishida and Kobayashi 1971), and fat distrophy (Kolgaev and Zhirnova 1966).

Concerns about the apparent inferior status of hatchery fry led to experimentation with gravel incubation. One early test (Vibert 1956) with Atlantic salmon and three species of trout demonstrated that a gravel substrate produced fry which were better able to withstand temperature extremes, swift currents, and predation than fry from a smooth hatchery trough. More recently, Bams (1967) found that relative to standard hatchery methods, natural incubation and conditions which simulated natural conditions produced the largest sockeye fry which were also better able to perform in swimming and predation tests. Apparently, a rugose substrate maintained alevins in a preferred and quiescent upright position, thus reducing yolk energy used for non-growth activities and affecting better yolk conversion efficiency (Marr 1963, 1965; Bams 1969). The cumulative adverse effects of hatchery conditions probably reduced survival potential of hatchery fry when compared to wild fry (e. g., Miller 1954; Salo and Bayliff 1958; Vincent 1960).

Fry quality would not be as critical if fry are to be fed to a larger size before release. But when they are to be released as unfed fry, any initial disadvantages may have dire consequences. In nature, fry to adult survival (including fishing mortality) typically varies between one and five percent for pink and chum salmon (e. g. Ellis

1969; Parker 1962, 1964; Hunter 1959; Atkinson 1976; McNeil and Bailey 1975). For the initial period of around 40 days when fry are aggregated at the inshore coastal areas, the rate of mortality is highest and has been measured at 55 to 70 percent for the entering Bella Coola, British Columbia pink salmon population (Parker 1968). This mortality occurs concurrently with stresses associated with adaptation to the salt water environment, and the majority of the early losses were attributed by Parker (1968) to predation which was apparently non-random and directed at the smaller individuals of the population (Parker 1968; Walker 1974). The rigors of the early marine environment may be inimical to the hatchery fry and may explain failures of the early propagation of pink, chum, and sockeye salmon (Foerster 1938; Noble 1963).

In hopes of producing propagated fry with better survival potentials, applications of the simulation concept led to development of spawning channels in the 1950's and gravel incubators in the 1960's.

Spawning channels are usually water diversion canals with silt-free gravel, water flow regulation, and controlled spawner density. Early success of channels constructed in Canada (Cooper 1972; Fraser 1972) led to development of production channels which now produce adult returns comparable to natural production (Paine 1974; Fred Fraser, personal communication, 1976). In Washington, experimental channels which can produce fry survival of 75 percent or more

have been demonstrated at Big Beef Creek (Shroeder 1974; Koski 1975). A variation of the spawning channel, the egg incubation channel (where fertilized eggs are planted into the channel gravel) has apparently been quite successful (Finn 1974; Thomas and Shelton 1968).

Relative to spawning channels, gravel incubators provide savings in cost, space, and water requirements. These incubators basically incubate eggs and alevins above or under a gravel substrate within the incubator box. The stocking densities are much higher per unit incubation area than the channels. Incubator boxes are compact and can generally be operated both outdoors and indoors.

Work on gravel incubators in North America started in the 1960's with three groups of workers: 1) Bams (Fisheries Research Board) and Wilson (Fisheries Service) of Environment Canada; 2) Bailey and Heard of the National Marine Fisheries Service (NMFS), Alaska Region; and 3) McNeil of Oregon State University (OSU). The designs of these incubators differed primarily in the depth of the gravel substrate--Environment Canada and NMFS both use a deep gravel matrix where incubation is within the substrate whereas OSU uses a shallow gravel matrix where incubation is above a single layer of gravel. Secondary differences between these designs include: 1) water filtration, 2) water source, 3) species and stock incubated, 4) egg stocking methods, 5) box design and flow pattern, 6) stocking

density, 7) apparent water velocity, and 8) gravel type and size range. In addition, post-emergence feeding was used by some workers prior to fry release, but this operational phase will not be considered within this report which is limited to evaluation of unfed fry. A summary of the design of each incubator and publications describing the technical details are presented in Table 1.

Information on the performance of these gravel incubators is incomplete, but available data have proven that the application in some form can produce the desired results in closing the quality gap between cultured and wild fry. Bams was the first worker to demonstrate with three generations of pink salmon fry released at Headquarters Creek that cultured fry were equivalent to wild fry in efficiency of yolk conversion to body tissue and that returns of marked hatchery adults approached to within nine percent of marked natural adults (Bams 1972, 1974, 1976, and personal communication, 1976). Results on other systems, however, have generally been inconclusive. Bailey et al. (in press) recently documented that pink salmon fry to adult survival of hatchery fish was 59 percent that of wild fish (0.79% versus 1.35%) at Auke Creek for the 1972 brood year. Evaluation of fry quality was based on size at migration and migration timing, but relative yolk conversion efficiency was not assessed. Wilson's system, and several variations of it, have tested out successfully on chum salmon; the design can apparently produce high survival, although relative conversion

Table 1. Design and operational parameters for gravel incubators tested in North America in the late 1960's.

Location	Species	Incubator design		Gravel substrate			Egg stocking	Water flow		References
		Unit size[1]	Flow pattern	Approx. depth	Type	Size range		Vel. /egg den.	Appar.[2] vel.	
		m		cm		cm	Eggs/cm^3 [3]	1/min/1000 eggs	cm/hr	
Whiskey Creek, Oregon	Chum	1.2x8.5x1.2	Lateral	2.5	Crushed rock	0.6 to 1.9		0.20 to 0.27	800[4] to 1000	McNeil 1969; Poon 1970; Lannan 1975
Sashin Creek, Alaska	Pink	1.1 diameter x 0.3 depth	Upwelling	30	Beach gravel	1.3 to 3.2	0.23 to 0.28	0.27 to 0.22	116	Bailey and Heard 1973[5]
Auke Creek, Alaska	Pink	1.2x0.9x0.9	Upwelling	76	River gravel	1.9 to 3.2	0.18	0.50	300	Bailey and Taylor 1974; Bailey et al. 1975, 1976
Hook Nose Creek and Headquarters Creek, B. C.	Pink and[6] Chum	1.2x2.4x1.2	Upwelling	89	Crushed rock	1.9 to 3.2	0.03	0.50	71	Bams 1970, 1972, 1974, 1976; Bams and Crabtree 1976
Blaney Creek, B. C.[7]	Chum	1.5x2.7x1.0	Upwelling	91	River gravel	1.9 to 3.8	0.12	0.57	407	Wilson 1974

1/ Unit size is size of incubator fed by a single pass of water flow.
2/ Apparent velocity = cm^3/hr of water passing perpendicular to each cm^2 of substrate surface.
3/ 3 eggs per cm^2 of substrate.
4/ Calculated as lateral flow through vertical cross-section of water column.
5/ Report on small scale prototype.
6/ Density and flow data given for pink salmon only.
7/ Design parameters derived from field tests at Chehalis River, Big Qualicum River, and Inches Creek, British Columbia.

efficiency and ocean survival are yet inconclusive (Wilson 1974; Fred Fraser, personal communication, 1976). Physical operation of the Netarts Bay shallow gravel matrix chum salmon system (McNeil 1968; Poon 1970; Lannan 1975) and the deep gravel matrix pink salmon system at Little Port Walter (Bailey and Heard 1973) has been successful, but no attempt was made to mark and release comparable unfed hatchery and wild fry at either location; adult returns, therefore, can not be conclusively evaluated. There is encouraging evidence that the Netarts system is generating hatchery returns--the chum salmon run returning to Netarts Bay has increased from a few to several hundred adults prior to the operation of the hatchery to over 3000 adults in 1974 and 800 adults in 1975. Lannan (personal communication, 1976) estimated that the return of adults to the Netarts hatchery at 0.5%. No estimate has been made on returns of unfed hatchery fry at Little Port Walter.

In order to critically assess the merits of the Netarts and Little Port Walter system, research on performance of gravel incubators was initiated jointly by OSU, NMFS (Alaska Region), and the Oregon Department of Fish and Wildlife in 1969 as a follow-up to a previous cooperative program on the development of the prototype streamside incubator at Netarts Bay (Poon 1970). The general objective of the research was to evaluate the performance of gravel incubators at Netarts Bay and at Little Port Walter by measuring quality of the

hatchery fry compared with wild fry. To attain this objective, however, two prerequisites have to be met: 1) development of standardized methods of measuring quality of unfed fry, and 2) knowledge of how variations of existing design criteria (e. g., stocking density, water velocity, substrate rugosity and depth) might affect fry quality. The former is needed as a measurement tool, the latter is needed for interpretation of the performance data and for recommendations for design improvements.

Efforts at measuring fry quality have been far from standard primarily because few workers define quality in concrete terms. The most commonly accepted definition of fry quality is "the capacity for survival" (cf. Vibert 1956; Bams 1969; Koski 1975), which is in turn divided into subcapacities specific for given periods of the animal's life history. Egg to adult survival is considered as the ultimate criterion of fry quality, but to be useful to technological development, a working definition covering all accessible (manage able) portions of the life cycle is needed. For this work, the following defintion based on increased understanding of the incubational and the early marine environment of pink and chum salmon is adopted.

Quality unfed migrant fry have the following characteristics:

1. High degree of genetic variability adapted to the incubation site (can be assumed if stocks are local and genetically sound spawner selection and egg fertilization techniques are

practiced).

2. Egg-to-fry survival at 75 percent or higher.
3. Negligible frequency of physical abnormalities.
4. Yolk conversion efficiency and larval behavior equivalent to wild fry.
5. Stage of development and timing at migration equivalent to wild fry.
6. Fast initial growth rate, plus good stamina and resistance to stress during early marine residence.
7. Fry-to-adult survival equivalent to wild fry and repeatable from generation to generation.

This working definition is an optimum one since data on all characteristics are usually not possible; it is useful, however, because speculations about success of a propagation program can be reduced by satisfying characterististics one through six, and success can be demonstrated by characteristic seven.

Past attempts at measuring fry quality have approached the above format; where the program was successful, most of the characteristics were satisfied (Bams 1970, 1972, 1974, 1976). Table 2 presents recent work on testing or measuring fry quality. For this research, methodology was investigated on measurement of the characteristics listed above, except for adult survival of marked hatchery fish, which was not financially feasible.

Table 2. Some recent research on quality testing of unfed salmon fry.[1/]

	Fry Quality Index	Reference
1.	Morphometrics	routinely measured in most studies, e.g., Bams 1970, 1972, 1974, 1976; Bailey et al. 1975 1976; Blackett 1974; Mead and Woodall 1968; Dill 1970; Koski 1975; Wells and McNeil 1970.
2.	Survival	
3.	Stage of development at migration	
4.	Timing of migration	
5.	Swimming ability	Bams 1967; Vibert 1958; Thomas et al. 1969; Dill 1970
6.	Predator avoidance	Bams 1967; Vibert 1958; Mead and Woodall 1968; Dill 1970; Beall 1972
7.	Resistance to stress	Vibert 1958
8.	Post-emergence growth	Vanstone et al. 1970; Leon 1975; Kepshire and McNeil 1972
9.	Response to light	Brannon 1965; Mead and Woodall 1968
10.	State of nutrition	Vanstone et al. 1970; Mead and Woodall 1968; Koski 1975
11.	Resistance to starvation	Bilton and Robbins 1973; Ivlev 1961; Koski 1975

1/ For quality testing on fingerling salmonid, consult Burrows (1969).

Definition of fry quality criteria provides the basis for testing and developing incubation systems. Successful operation of gravel incubators is based upon manipulation of a number of parameters, and the quality of fry produced from a given system represents a specific combination of those parameters (c. f. Table 1). The complexity of factors which must be considered for deriving design criteria have been discussed recently by McNeil and Bailey (1975) for salmon ranching (simulation systems), Bams (1969, 1970, 1974, 1976 and Bams and Crabtree (1976) for gravel incubators, and Koski (1975) for spawning channels.

Although information is available on most design parameters, application of the data is often difficult because: 1) variables interact and effects of single variables may be masked; 2) information derived may be specific for particular species and stocks; and 3) existing design criteria (Table 1) are generally site specific and often formulated as safe levels with little information on acceptable latitudes.

A comprehensive study of all possible design parameters would be desirable but impractical. This research, therefore, focused on six parameters judged to be the most pertinent for operation of systems at Netarts Bay and at Little Port Walter: 1) substrate depth, 2) stocking density, 3) water flow, 4) exposure to light, 5) stocking of eyed and newly fertilized eggs, and 6) incubator design.

The research had three specific objectives: 1) to develop

procedures for quantification of fry quality, 2) to determine the effects of key incubator design variables on fry quality, and 3) to evaluate the quality of pink and chum fry produced from production gravel incubators at Netarts Bay and at Little Port Walter. These objectives were achieved in two phases. Phase one covered the objectives one and two simultaneously in laboratory studies conducted during the brood years 1969 through 1971. Phase two addressed objective three in field studies conducted during the 1971 brood year using natural stocks of pink and chum salmon.

II. DESCRIPTION OF PRINCIPAL RESEARCH LOCATIONS

Five research facilities and four salmon streams were referenced in this study.

The five research facilities consisted of four locations on the Oregon coast and one in Alaska. The Oregon facilities were the OSU Swanson Aquaculture Laboratory at Netarts Bay, the OSU Marine Science Center at Newport, the OSU Marine Laboratory at Port Orford, and the Oregon Fish Commission Elk River Hatchery near Port Orford (Figure 1). The Alaskan facility was the NMFS Little Port Walter Research Station on Baranof Island in Southeast Alaska (Figure 2).

The four salmon streams consisted of two Oregon streams and two Alaska streams. The Oregon streams were Whiskey Creek of Netarts Bay and Edson Creek near Port Orford. The Alaska streams were Sashin Creek of Little Port Walter Bay and Lover's Cove Creek of Big Port Walter Bay.

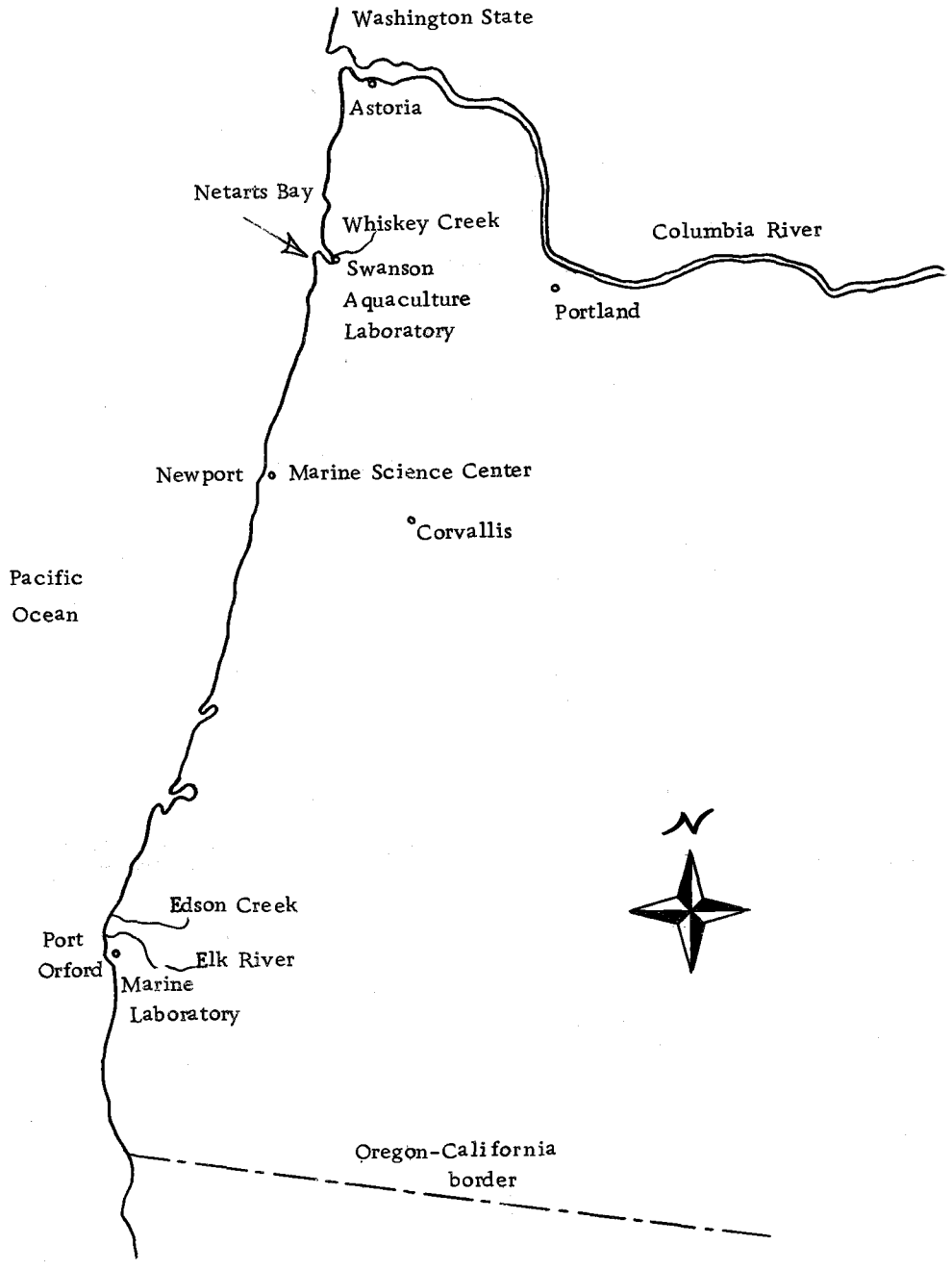

Figure 1. Location of research facilities on the Oregon coast.

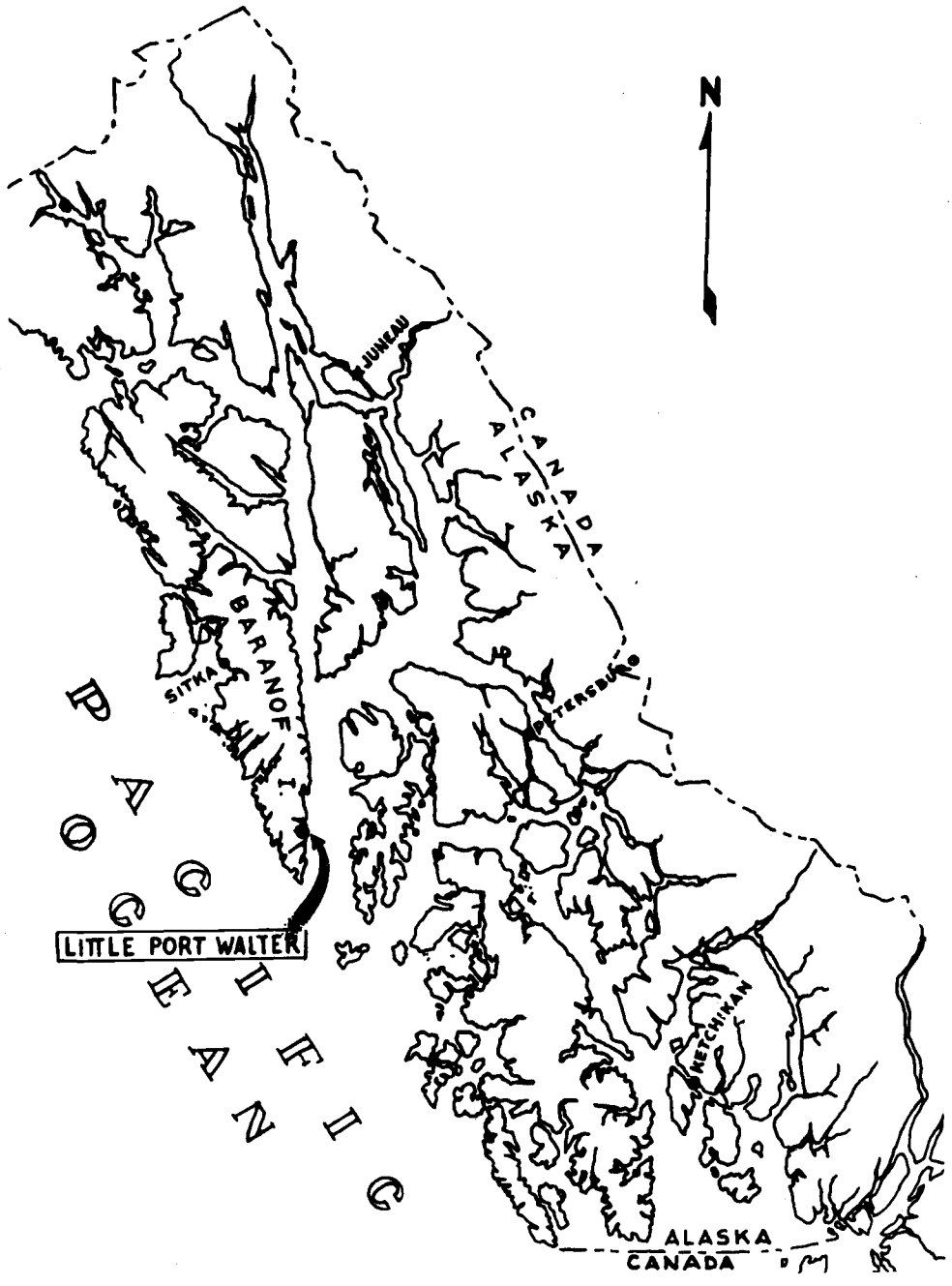

Figure 2. Location of Little Port Walter research facility in Southeast Alaska (taken from Merrell, 1962).

III. GENERAL TESTING PROCEDURES

Development of procedures to test fry quality proceeded with increasing sophistication and comprehensiveness. Hatchery fry were compared with wild fry to evaluate relative quality. Laboratory studies involved comparisons among groups of fry exposed to different treatments. In all, six quality criteria were used, and they could be classified as discrete or continuous criteria.

Discrete criteria, or enumerative criteria, included: 1) egg and alevin mortality, 2) frequency of physical abnormalities, and 3) migration pattern and timing. Continuous criteria, or criteria measured over a range of values, consisted of 4) size, 5) stage of development at migration, and 6) performance in stress tests.

A. Egg and Alevin Mortality

High survival is the essence of any propagation program. Mortality, however, is meaningful not only because of production assessment, but also as an index of unfavorable conditions within the incubation environment.

Mortality assessment was made by actual count whenever feasible, and by volumetric estimates otherwise. The accuracy of estimates was generally within 3-5% of the true value, except when the number stocked was estimated, or if mortality was estimated

from a difference between an estimated stocked number and an estimated survival number. Under these conditions accuracy of an estimate was thought to be within 10% of the true value.

B. Frequency of Physical Abnormalities

Two basic types of abnormalities were observed--monstrosities and yolk-sac malformation. Of the two, monstrosities were negligible and were therefore ignored.

Yolk-sac malformation and some causative factors have been documented by Emadi (1972, 1973). The abnormality ranges from a simple protuberance at the end of the yolk sac, to an elongated yolk sac, to constriction and rupture at the end of the elongation. The malformation is apparently caused by swimming activity, which often leads to abrasion of the epithelial tissue from contact with the substrate. Extreme cases often led to death; sublethal effects, however, are not well known.

When the malformation was observed in this study, activity during incubation and a subsequent loss of size were also observed. Frequency of yolk-sac malformation was assessed as a percentage of the test population affected.

C. Migration Pattern and Timing

It is important for fry to migrate when conditions in the estuary

or lake are favorable, and when fry are at an optimum stage of development (near total yolk absorption) for survival. In either case, substantial deviations from the optimum may have dire consequences. For example, if the fry migrate at a time when food is not abundant in the estuary, their survival potential is reduced. Likewise, if fry emerge either with too much yolk or somewhat undernourished (assuming no extraneous feeding), they may not exhibit optimum swimming performance, thus increasing their vulnerability to predation.

In this study, time to emergence and emergence pattern were assessed only for the 1971 brood year where test populations were capable of volitional migration. The data were recorded as number of days from fertilization to 50% cumulative emergence.

D. Post-emergence Growth

The relative ability of different groups of fry to feed and grow is used to index physiological well-being after incubation. Fast initial estuarine growth should allow migrants to reach a size less susceptible to predators (Parker 1971).

Growth experiments were conducted by one of two basic methods: comparison of test groups in separate rearing tanks or comparison of test groups differentially marked and reared in common tanks. Experimental periods were kept short. Studies of long duration, while more meaningful in defining growth trends, were rejected due to time

constraints and potential disease outbreaks.

An effort was made to establish criteria for post-emergence growth, but results were sparse, due primarily to the inability to standardize and control rearing conditions under field situations. Only the 1969 brood year experiments produced acceptable data; other studies conducted in 1970 and 1971 were either limited or inconclusive.

E. Size and Stage of Development

The amount of yolk material available for larval growth is fixed, and amount of body tissue is a measurement of conversion efficiency from yolk to body tissue without extraneous feeding. Thus, size is an index of the capacity of a given incubational environment to promote larval growth. Other factors being equal, larger fry will likely have a higher capacity for survival since larger fry are better able to swim and to avoid predators (Bams 1969), two capacities crucial to survival during the initial high mortality period after entry into the estuary.

Stage of development, however, is as crucial as size since the advantage of size can only be realized if fry migrate at a stage of development which allows optimum swimming performance. Quantification of stage of development at a common time reference is used to index relative larval growth rates, which are influential in

determining the length of the incubation period and the time of migration.

Size during the larval period changes as a function of time. Changes in length, wet weight, and total dry weight for a hypothetical population, but based on empirical data from this study, are shown in Figure 3. Length and wet weight both reach a maximum as dry weight declines. Any instantaneous assessment of size, therefore, must be referenced by the stage of development. To evaluate migrant fry which are known to migrate at a wide range of developmental stages (Bams 1970, 1972, 1974, 1976; Bailey et al. 1975, In Press; Blackett 1975; Dill 1970), size should be compared at a common stage of development, otherwise interpretations may be confounded (cf. Bams 1970, p. 1451 for examples of caveats in data interpretation).

In taking size measurements, fish were measured either live and anesthesized or after being preserved in 5 or 10 percent formalin for at least six weeks. Fork lengths of individual fish were read to 1.0 or 0.5 mm, and wet weight and dry weight were read to the nearest 1.0 or 0.1 mg respectively on individual fish or a group of 5-20 fish. Dry weights were taken after 24 hours of oven drying at 100°C.

The stage of development was quantified by either a visual or a calculated method.

The visual method is simply a rating assigned to a group of fish for their degree of yolk absorption, e.g., 1/2 mm yolk gap, fully

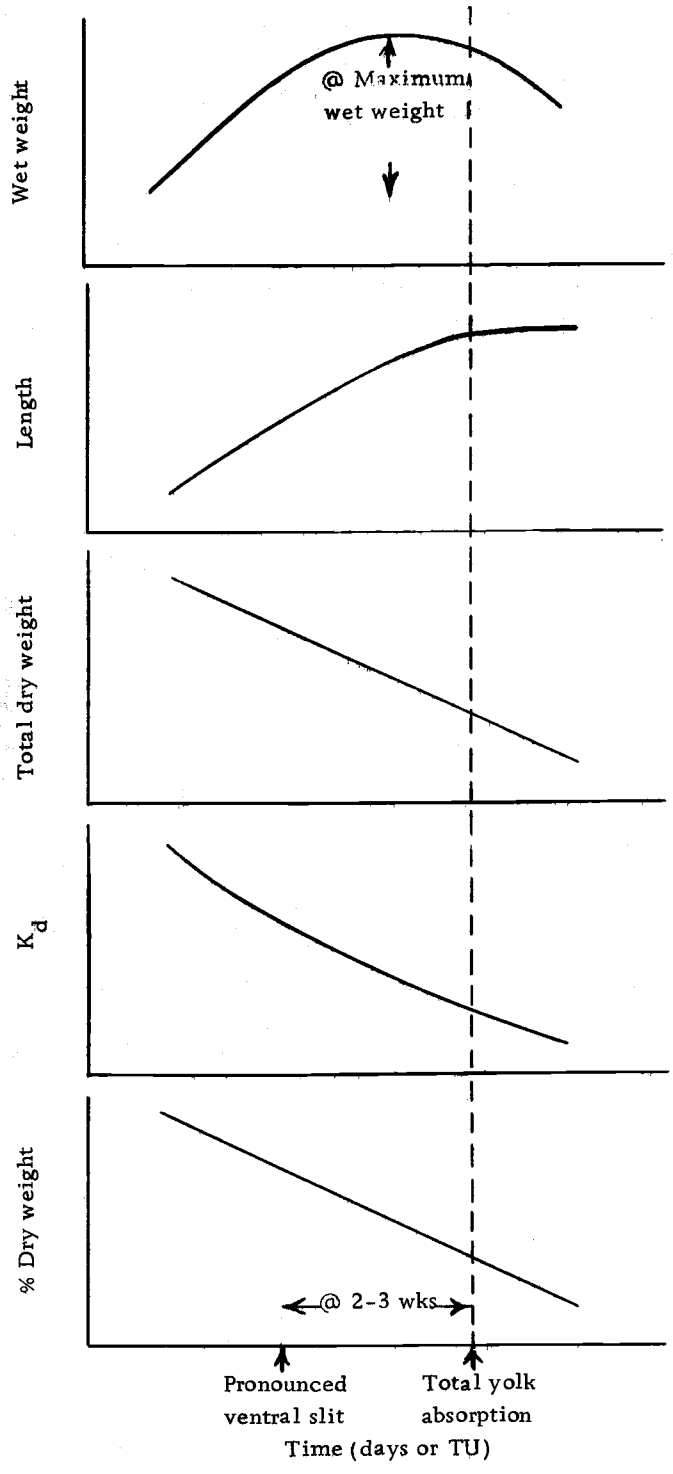

Figure 3. Relationship of wet weight, length, total dry weight, K_d, and % dry weight to time over the terminal yolk absorption period for a hypothetical population of salmon alevins.

buttoned, etc. A more quantitative visual method which assigned to each fish a rating of 0 to 4, based on the degree of yolk absorption, was abandoned because precision became questionable near and after total yolk absorption.

Stage of development was sensitively quantified by the calculated indices--a condition factor developed by Bams (1970), and an index based on weights developed empirically for this study.

The Bams index (Kd) is based on the relative changes in larval length and wet weight, the ratio of which are largely independent of absolute fish size:

$$Kd = \frac{10 \times \sqrt[3]{\text{wet weight in mg}}}{\text{length in mm}}$$

This index exhibits a linear decrease during the latter part of the developmental period up to about 1 week after attainment of maximum wet weight, roughly a period of two weeks (Figure 3).

A second index (% dry weight) developed in this study utilizes the relative proportion of total dry weight to total wet weight and is descriptive of the rate at which the dense yolk material is converted to the less dense body tissue.

$$\% \text{ dry weight} = \frac{\text{total dry weight in mg}}{\text{total wet weight in mg}} \times 100$$

This index is negatively linear with time over the same period of larval development as Kd (Figure 3) and is also largely independent

of absolute fish size. Its use, however, was limited to early laboratory experiments because of procedural difficulties with dry weight determinations (discussed later).

In order to compare size of fish at a common stage of development, two methods were used. The first compared treatments at the stage of development of maximum length or wet weight and was applied when populations were accessible for random sampling through the terminal larval period. The second used a regression model of size (length or dry weight) regressed on stage of development (Kd or % dry weight). It was used for populations not accessible for random sampling, and for which maximum fry size was not determined, e. g., deep gravel incubator fry with volitional migration.

Procedures for the regression method are as follows:

1) From indigenous samples, determine rate of growth over the terminal larval period, i. e., size unit/time unit, developmental index unit/time unit, and size unit/developmental index unit.

2) Determine the median of the developmental index distribution and use this as the common developmental index (CDI).

3) Convert size measurement to a common stage of development using the formula:

$$CS = OS - [(ODI-CDI)(SCS)]$$

where CS = Converted size or size measurement converted to the common developmental index

OS = observed or unconverted size measurement

ODI = observed developmental index

CDI = common developmental index

SCS = size conversion slope, i. e., size unit/developmental index unit

4) Compare converted size measurement (CS) of treatment populations.

Application of regression methods was successful in most evaluations except when gravel incubators were compared against Heath incubators operated at moderate to high stocking densities. The developmental rate of alevins in Heath incubators sometimes were much higher than for alevins in other types of incubators, and calculated regression equations were not always comparable. Fortunately, large size differences between fry from Heath incubators and fry from other gravel incubation treatments generally made precise evaluation techniques unnecessary.

F. Performance in Stress Tests

Performance in stress tests reflected how a given test population might perform under similar stresses in nature. Two stress tests were used in this study; the swimming stamina test and the anoxia test.

The swimming stamina test required test fish to swim at a

sustained speed in a swimming tube under a step acceleration flow schedule. Performance in this test theoretically reflected the fish's swimming stamina necessary for foraging and predator avoidance.

The anoxia test subjected test fish to a period of time in the atmosphere and measured their ability to survive hypoxial stress.

Test procedures for evaluation of performance were established and used during the 1971 brood year laboratory studies. Applications of the methods, however, were not extended to evaluation of production-scale gravel incubator fry because large differences in migration timing between propagated and wild fry precluded testing at a common time frame.

IV. LABORATORY STUDIES ON INCUBATOR DESIGN

The purposes of the laboratory experiments on incubator design variables were (1) to define the optimum incubator conditions which will produce high quality fry, and (2) to develop procedures for comparative fry quality testing. Experiments were conducted at the OSU Port Orford Marine Laboratory during the 1969 brood year and at the Netarts Swanson Aquaculture Laboratory during the 1970 and 1971 brood years. A brief description of these experiments is as follows.

In 1969, the most basic design variable--substrate rugosity (screen versus shallow gravel matrix)--was tested to derive an expected quality difference which could be measured by comparative fry quality tests. Two species, chum and chinook, were used in this study.

In 1970, stocking density and water flow were tested for the shallow gravel matrix design using pink and chum salmon. These two variables are the most crucial design criteria for the operation of the Netarts system, and the findings were to be used for the design of other types of gravel incubators.

In 1971, comprehensive experiments were designed to test substrate rugosity and depth and incubator design. A test was also set up to evaluate the singular and synergestic effects of substrate and light. Pink and chum salmon were used in these studies.

In addition to the 1971 laboratory studies at Netarts Bay, hatchery operation at Little Port Walter in the same year provided the opportunity to use wild fry as a quality standard in tests of seeding with eyed versus green eggs, stocking of deep gravel matrix incubators at two densities, and a comparison of shallow gravel matrix, deep gravel matrix, and Heath incubators. These experiments are presented under the section on comparison of hatchery versus wild fry (cf. Field Studies, p. 149).

A. Effects of Substrate on Chum and Chinook Salmon

Previous research on trout and Atlantic salmon (Marr 1963, 1965) and on sockeye salmon (Bams 1969) has demonstrated the need of a rugose substrate during incubation to maintain alevins at a preferred upright position and thus minimize the loss of size due to unnecessary larval activities. This concept has been central to gravel incubation technology; little work, however, has been done to evaluate the need for rugosity in other species of Pacific salmon.

To determine this need for chum salmon, the target species of the OSU gravel incubation program, and to test fry quality evaluation procedures concurrently, the 1969 brood year laboratory experiments were designed to evaluate the effects of substrate rugosity on fry quality in a one-way classification of treatments experiment with two treatments: shallow gravel matrix and flat screen. Parallel

experiments were conducted at the OSU Port Orford Marine Laboratory with wild stocks of chum salmon from Netarts Bay and chinook salmon from the Port Orford area to provide a replicate for the experimental design and to measure possible species differences. Primary fry quality criteria tested were maximum fry weight and post-emergence growth.

The chum salmon study consisted of a single comparison of fish incubated on the two substrates. Fry quality evaluation was not extended to post-emergence growth due to a 50% mortality experienced by fish raised on a screen substrate.

The chinook study consisted of two parallel experiments which subjected each of two egg sizes of chinook salmon to three substrate treatments: 1) gravel, 2) screen, and 3) the Heath incubator tray. The experiment was carried through successfully for both the incubation and post-emergence phases with the two egg sizes serving to replicate the experiment. No major mortality problems were encountered throughout most of the study. Some mortalities occurring near the end of the experiment did negate one experimental feeding tank, but the overall findings were not affected. This experiment was conducted in cooperation with the Oregon Department of Fish and Wildlife Elk River Hatchery located near Port Orford. Dr. Paul Reimers of the Department conducted simultaneous incubation experiments in deep matrix gravel boxes and Heath incubators using

the same stock of chinook eggs used in this experiment. Dr. Reimers' data will be referred to under discussion.

1. General Procedures

a. Chum Salmon Study

Fish were held in 378.5 liter fiberglass-lined plywood tanks with upwelling flow. A fine-mesh wire screen provided a false bottom. This screen served as the non-gravel substrate or it supported a single layer of crushed rock with size range of 0.6 to 1.9 cm. A black plastic cover excluded light from the tanks.

Approximately 2300 eggs from a single day of spawing at the OSU Netarts Bay Streamside Incubator were eyed at the Newport Marine Science Center and transported to the Port Orford Marine Lab on January 20, 1970.

On January 22, 1970, 700 eyed chum salmon eggs were placed in two tanks (#1 and #2) with a screen and gravel substrate respectively and at an egg density of 0.123 eggs per cm^2. Water velocities[2/] were set at 50.7 cm/hr. The experimental apparatus was checked daily and water flows adjusted when necessary. Records were kept on dead alevins removed, water temperature, and water velocity. Water temperature range was recorded to the nearest degree Fahrenheit with a Taylor Maxi-mini thermometer and water velocity were measured in ml/15 seconds with a graduate cylinder and stopwatch. The experimental cells were exposed to light for a short

2/ All water velocities in this research are apparent water velocities calculated as flow volume per unit time per unit area.

period while being inspected for dead fish.

The temperatures observed during the experiment ranged from 9.5°C to 12.2°C with a mean of 11.1°C. The average flow rate in each tank was--tank #1: 47.40 cm/hr with a range of 16.90 to 84.51 cm/hr; tank #2: 47.68 cm/hr with a range of 16.90 to 88.73 cm/hr.

Periodic growth measurements were suspended early in the experiment due to high mortality of test fish on the screen substrate. Instead, samples were taken after completion of hatching on February 9 and at button-up on March 14. In each sampling, 20 fish were selected from several concentrations of alevins or fry on the substrate to minimize sample bias. Sampled fish were anesthesized with MS-222, wiped dry with a moist soft tissue, and weighed 5 or 10 at a time on a top-loading Mettler electric balance accurate to ± 10 mg.

b. Chinook Salmon Study

Heath incubation trays at the Elk River hatchery and the experimental tanks identical to those used in the chum salmon study were utilized for the chinook salmon study. Heath incubators at Elk River were exposed to subdued natural and artificial light. The Heath incubator is constructed of fiberglass. Egg trays have a surface area of 1280 cm^2 and a fine screen substrate.

Experimental tanks at Port Orford were set up as in the chum study except that a baffle system created two experimental cells in the front half of the false bottom screen; each cell encompassed one quarter of the original false-bottom area. Water flow through each

tank was controlled by the valve on the water line, and flow through the two cells of each tank was controlled by adjusting the height of the overflow tube from each cell. While tanks #1 and #2 were being used for the chum study, tanks #3 and #4 were converted into cells #3 through #6 with the following treatment design:

Tank #	Cell #	Substrate	Size of eggs
3	3	gravel	small eggs
3	4	gravel	large eggs
4	5	screen	small eggs
4	6	screen	large eggs

For the post-emergence growth experiment, false bottoms of each tank were simply removed to convert the tanks into feeding tanks, and black plastic was placed over the front half of the tanks to provide cover for the fish.

Two chinook females from Edson Creek near Elk River were spawned on December 10, 1969, and their eggs eyed separately in Heath incubator trays at Elk River Hatchery. Egg sizes 25 hours after fertilization were: large eggs--9.10 mm; small eggs--8.23 mm. One thousand large eggs and 1,000 small eggs were transferred to the Port Orford Marine Lab on February 2 for initiation of substrate experiments. Remaining eggs (about 5500 large eggs and 4500 small eggs) were kept in Heath incubator trays at Elk River as the third treatment for the experiment. Fry from Heath incubators were later

transferred to Port Orford for the post-emergence growth experiments.

On February 4, 500 eggs were stocked in each of the four experimental cells, giving an egg density of 0.35 eggs/cm^2. Flow rate for each cell was set at 135 cm/hr. By comparison, the Heath incubator was stocked at 4.30 eggs/cm^2 for large eggs and at 3.50 eggs/cm^2 for small eggs with an apparent velocity of 710 cm/hr (4 gpm).

During the incubation phase, the flow control system proved to be inadequate for maintaining the prescribed flow rate, particularly in equalizing flows for the two cells in each tank. A modification was made which maintained the compatibility between the gravel and screen cells of each egg size but changed the designated flow rates experienced by each egg size. For each tank, the flow rate was adjusted first for the total flow (combined flow of the two overflows); then cells #4 and #6 with large chinook eggs were adjusted to the desired flow, and cells #3 and #5 with small chinook eggs received the left-over flow. In practice, one cell in each tank always received more flow, and changes of flow in one tank affected flow capacities of other tanks on the same water line. Consequently, the flow rate was changed to 115 cm/hr for the large chinook and 150 cm/hr for the small chinook eggs. Observed average flow rates and their respective ranges were as follows:

Cell #	Egg Size	Substrate	Flow Rate in cm/hr	
			Average	Range
3	small	gravel	140.90	67.61-253.52
4	large	gravel	116.37	84.51-194.37
5	small	screen	151.39	76.06-236.62
6	large	screen	113.71	67.71-267.04

On March 29, a 43-day feeding experiment was set up in two tanks, one for each egg size. About two hundred Heath incubator fry of each egg size were transported from Elk River to Port Orford on March 19 for the feeding experiment. A 3% mortality (12 large chinook fry) was experienced during this transport due to handling. No mortalities were observed after these fish were placed in tanks.

Two hundred fish from each of the three treatments were differentially marked and placed into one tank. Fry from large chinook eggs were placed in tank #1 and fry from small chinook eggs in tank #2. An upper caudal clip was given to the Heath fish, a lower caudal clip to the gravel fish, and no marks were placed on the screen fish. The differential clipping favored the screen fish purposely; thus if the gravel fish should show a favorable growth rate the difference cannot be attributed to an advantage gained from marking.

Unlike the incubation phase, flow control in the feeding tanks was not a problem and the exchange rate was maintained at 3.8 to 4.7 liters per minute. Temperatures ranged from 10.0°C to 15.0°C and

averaged 11.7°C during the study.

Two rations of Oregon Moist Pellet diet (from a single batch) were used to feed at a 6% and a 2% wet body weight. A 6% ration is sufficient to maintain good growth at the given temperature. The 2% ration is less than sufficient and will accentuate any competition existing between fish within a tank. The 6% ration was used for the first 23 days. After the 23rd day, each treatment group was divided randomly and one half of the test fish was transferred to new tanks (fry from large chinook eggs to tank #5, fry from small chinook eggs to tank #6). The 6% ration was fed to one group (tanks #5 and #6), and the 2% ration was fed to the other group (tanks #1 and #2) over the last 20 days of the experiment.

In feeding the fish, a small amount of food was sprinkled at a time over a small surface area to give the faster and more alert fish an advantage in cropping off food pellets dropping through the water column. The tanks were cleaned at approximately weekly intervals with a vacuum hose to remove feces and uncropped food off the bottom. Daily temperatures, mortalities, and flow rates were monitored. Flow through each tank was maintained betwen 3.8 to 4.7 liters per minute.

To establish growth during incubation, seven samples were taken--beginning at hatching and ending when an obvious loss in wet weight was detected. The Heath incubator fish at Elk River were

sampled on the same sampling dates, transferred to Port Orford, and processed at the same time as the samples from the other two treatments. Processing methods were identical to those used in the chum study.

For post emergence growth determination, samples of n=30 fry were taken from each tank for wet weight measurements at about weekly intervals. The last sample of the incubation phase served as the starting measurement for this phase. Sampled fish were weighed individually or up to 10 fish at a time using previously described methods.

2. Results

a. Chum Salmon Study

Egg to fry survival was 88 times higher for fish raised on gravel than for fish raised on a screen substrate. Cumulative mortality experi- by the two treatments were: screen fish 50%; gravel fish 0.57%.

The cause of the observed mortality difference can be attributed primarily to the relative degree of photonegative reactions. For the screen fish, alevins assumed a head down position and tended to aggregate for mutual physical support, particularly bunching in corners. When exposed to light during the brief daily inspection, intense avoidance reaction of scurrying and "digging" took place, resulting in scrapping of the ventral yolk-sac and the lower jaw.

Fungus infected the eroded tissues. In the earlier alevin stages, photonegative reactions were at the highest, and there was more "digging" than scurrying. But as the alevins matured, they would do more dashing in addition to "digging." In contrast to the screen alevins, gravel alevins took on an upright position wedged between the interstices of the gravel. They exhibited a more subdued photonegative reaction when exposed to light, aggregated to a lesser degree, dug less intensely, and were less likely to dash long distances over the substrate. As with the screen fish, photonegative reaction was more intense in the earlier alevin stages and decreased with maturation. Both groups were swimming off the substrate well before total yolk absorption.

Yolk-sac malformation and high mortality occurred exclusively among the screen fish. Within the first 5 days after conclusion of hatching (hatching spread out over 16 days), coagulated yolk condition was observed on most dead alevins. This condition was characterized by a plug of coagulated yolk near the surface of the upper mid-ventral portion of the yolk-sac. Red lines often radiated from the coagulated yolk and a red spot was generally seen under the eye. The yolk-sac, however, appeared round. Twelve days after total hatching, yolk-sac malformation was observed on live and on dead alevins. The malformation varied from a slight coagulated tit on the posterior ventral tip of a round yolk-sac to an elongation of the

yolk-sac to a sausage shape. Sometimes coagulated yolk protruded from the tip of the elongated sac; other times the elongation had a small appendix. Physical tissue erosion and fungus were observed on the lower jaw and on the yolk-sac of these malformed alevins. Mortality of the alevins reached 21% by the conclusion of hatching, and increased to 50% at button-up.

Observations on the newly hatched alevins and the button-up fry for both treatments are summarized in Table 3. Fry from gravel fish were 5.0% heavier than the surviving fry from the screen substrate.

b. Chinook Salmon Study

Chinook alevins exhibited a milder photonegative response than chum salmon, with gravel alevins being somewhat less active than their counterparts on the screen substrate. The difference in behavior between the gravel and the screen alevins was quite apparent but was not as extreme as the difference observed with chum salmon. Because of the mild photonegative reaction, physical abnormalities and mortalities were few; their occurrence in the four experimental cells are as follows:

Cell #	Egg Size	Substrate	Total mort. + abnorm.	Abnormalities
3	small	gravel	3 (0.6%)	1
4	large	gravel	13 (2.6%)	2
5	small	screen	2 (0.4%)	1
6	large	screen	10 (2.0%)	1

Table 3. Comparisons between gravel and screen substrate chum salmon.

	Observation	Substrate	
		Screen	Gravel
1.	Hatching	From January 25 to February 9	
2.	Cumulative Mortality		
	February 9	21%	--
	22	31%	--
	March 3	48%	--
	14	50%	0. 57%
3.	Alevin position	Head down	Upright, supported by substrate
4.	Photonegative reaction	Intense	Subdued
5.	Coagulation in normally-shaped yolk sac	Observed prior to February 21, 12 days after total hatching	Not observed
6.	Yolk-sac Malformation	Observed after February 21 in over 95% of mortalities	Not observed
7.	Wet weight measurements		
	Alevins:		
	February 2	$\overline{X}$ = 281 mg n=20 weighed one at a time	
	Feburary 9	$\overline{X}$ = 303 mg n = 20 weighed 5 at a time	$\overline{X}$ = 295 mg n = 20 weighed 5 at a time
	Fry:		
	March 14	$\overline{X}$ = 399 mg n = 20 weighed 10 at a time	$\overline{X}$ = 419 mg n = 20 weighed 10 at a time

All abnormalities were monstrosities and not yolk sac malformation. Some white spots were observed on a number of alevins but no apparent stress or mortality resulted from this condition. Other biological parameters of (1) body position as affected by substrate type, (2) time of swim-up, and (3) reduction of photonegative reaction with maturation were similar to observations made for chum salmon.

Differences in larval growth between treatments were small (Appendix 1, Figure 4). The maximum wet weight observed for each treatment of each egg size are ranked as follows:

Rank	Large chinook treatment and average wet weight in mg	Small chinook treatment and average wet weight in mg
1	Heath 581	gravel 480
2	gravel 575	Heath 469
3	screen 567	screen 462

Rank number one was 2.5% (14 mg) and 3.9% (18 mg) heavier than rank number three for the large and small chinooks respectively; these differences were not significant at the 99% level ($F_{2,7}=1.849$ for large chinook and $F_{2,5}=9.699$* for small chinook), although the difference of the small chinook experiment was significant at the 95% level.

Developmental rate of the Heath incubator fish was slower than that of the gravel and screen fish due to colder water temperatures at the Elk River Hatchery. Developmental differences between

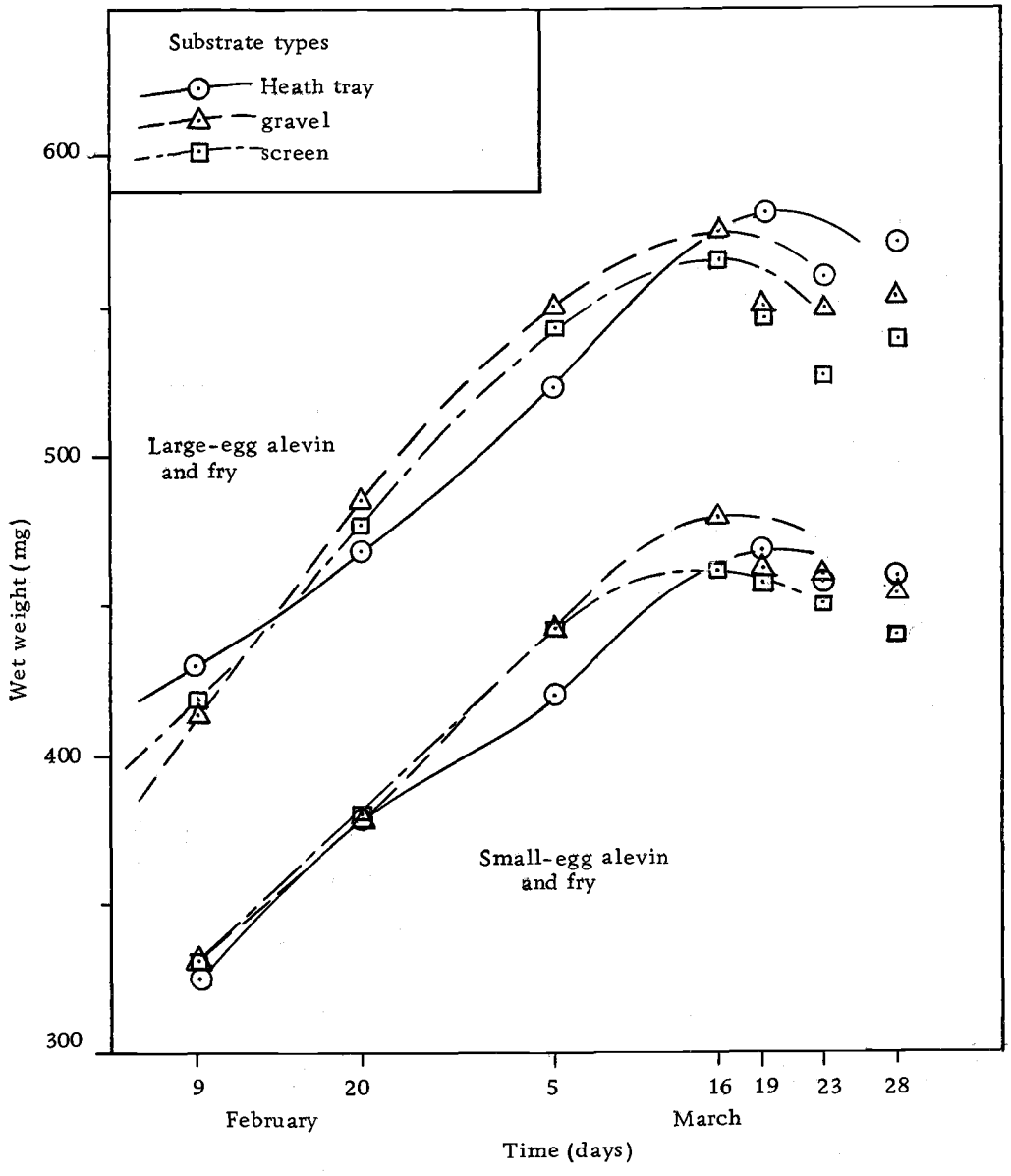

Figure 4. Changes in mean wet weight during incubation for chinook salmon from large and small eggs incubated on three substrates.

treatments were not obvious from visual inspection of the fish during sampling, but on March 19, the date Heath fry were transferred from Elk River to Port Orford, the cumulative temperature units at Port Orford were estimated to be 86 TU (^{o}C) or about 8 days ahead of Elk River. Based on growth curves (Figure 4), however, the Heath fish reached maximum wet weight at about 3 days after the gravel and screen fish. Since the temperature recorders at the two sites were not calibrated against one another, the empirical data from the growth curves were considered a more accurate determination of the developmental difference.[2a]

The general feeding behavior of fry resulting from the various experimental groups of alevins was vigorous. Competition existed within each tank as some fish swam faster than others and were better able to crop off the food pellets. The lower ration treatment apparently enhanced this competition.

Mortalities observed throughout this phase are as follows:

Tank #1	5	
#2	0	
#5	158	All occurred on April 22, 1970
#6	16	All occurred on April 22, 1970

[2a]Based on information generated in subsequent laboratory studies, the higher water flow rate in the Heath incubator may have increased the rate of development thus decreasing the developmental difference estimated by temperature units.

No explanations were found for the selectiveness and suddenness of this kill. Evidence of stress was not observed in any of the fish prior to mortality, suggesting that the causative agent was not of a chronic nature.

The growth experiment was continued for 43 days. During this period Heath fish had a slower growth rate than either the gravel or screen fish, which exhibited nearly identical growth rates (Figure 5). This trend was established during the first 23 days of 6% ration and was accentuated during the last 20 days under the 2% ration (Appendix 2). Table 4 summarizes growth rates of test groups of chinook salmon. Due to mortalities encountered, the 6% ration treatment for large chinooks was terminated after April 22, but the 2% ration treatment was sufficient to establish an accentuated growth difference consistent with the trend observed for the small chinooks (Figure 5).

3. Discussion

Species difference in photonegative reactions made provision of a rugose substrate crucial for the chum salmon but not for chinook salmon.

For the chum salmon study, the intense photonegative reaction of the screen fish was primarily a function of exposure to light and the lack of a gravel substrate, but several other factors contributed. While the gravel substrate provided hiding places and served as a

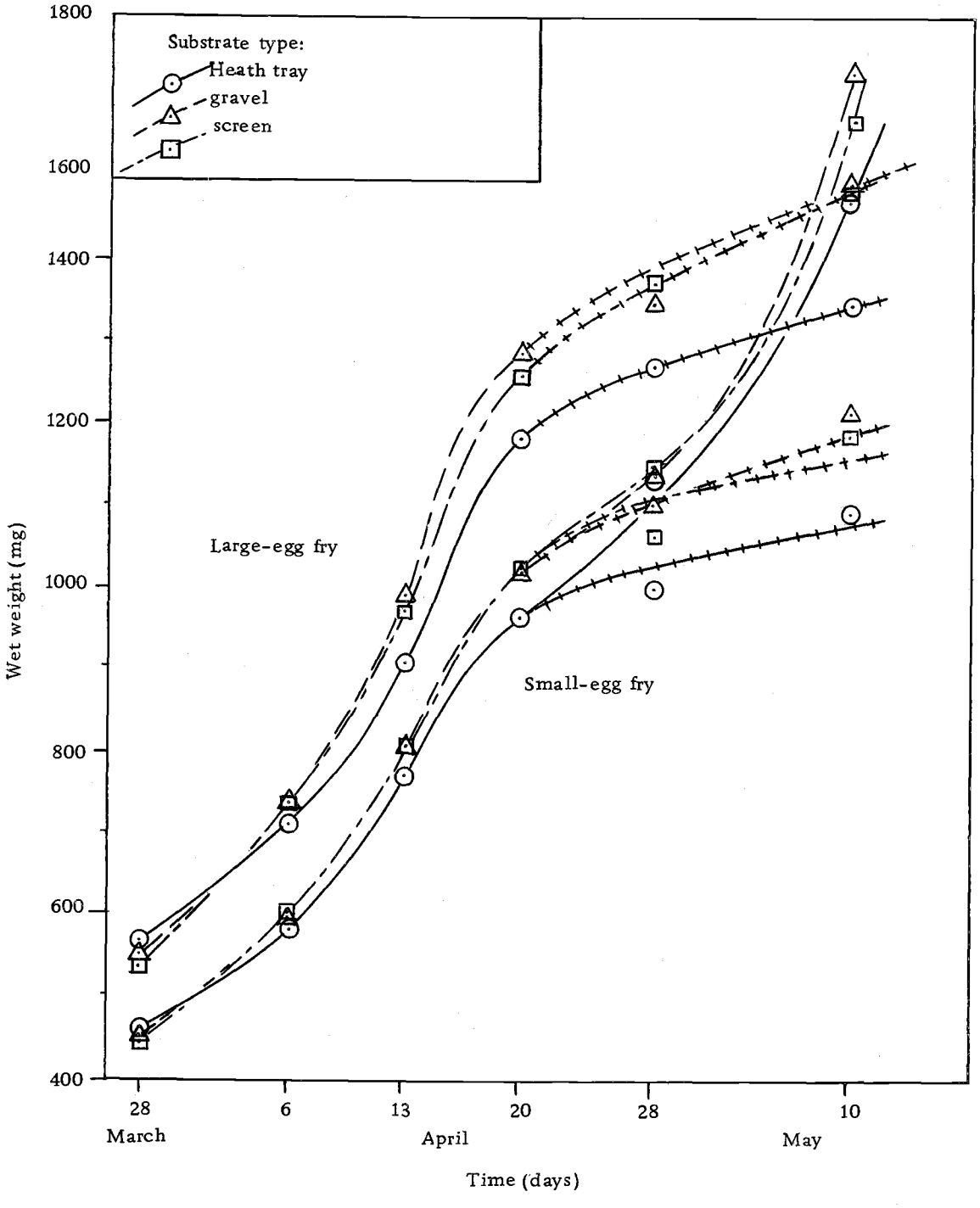

Figure 5. Changes in mean wet weight during post-emergence feeding studies conducted with chinook salmon fry from large and small eggs incubated on three substrates. Fish were fed a 6% ration for the first 23 days, and a 6% and 2% ration for the last 20 days. Cross hatched lines represent 2% ration; non cross hatched lines represent 6% ration.

Table 4. Average wet weight increase (mg/day) for two groups of chinook salmon fry fed under two rations for 43 days.

Feeding period and ration	Fry from large eggs			Fry from small eggs		
	Heath	Gravel	Screen	Heath	Gravel	Screen
First 23 days at 6% ration	26.48	31.91	31.30	21.96	24.57	25.39
Last 20 days at 6% ration	--- No data taken due to mortalities---			25.55	30.35	27.20
Last 20 days at 2% ration	8.05	10.30	11.25	6.45	9.75	8.05

camouflage, the white screen offered no protection. Further, the large screen area and its coarse texture contributed to the occurrence of yolk-sac malformation and high mortality. The observed low survival of the screen fish was supported by concurrent experiments conducted at Netarts Bay using the same stock of eggs. In a gravel versus non-gravel substrate experiment where test cells were periodically exposed to daylight for inspections, mortality was close to 100% for all non-gravel substrate treatments whereas negligible mortalities were sustained for gravel substrate cells.[3] Results of the Netarts Bay experiment and this experiment are generally in agreement with results of other studies where salmonid alevins without substrate support were exposed to a variety of light sources (e.g., Brannon 1965; Eisler 1957; Smith 1916).

For the chinook salmon study, however, a substrate apparently provided little advantage during incubation even though experimental conditions were similar to the chum salmon study. Based on the righting response concept (Marr 1963, 1965; Bams 1969), the experiment should have produced a fry size hierarchy of: 1) gravel fish (with a substrate support), 2) screen fish (without substrate support), and 3) Heath fish (without substrate support and with higher flow and stocking density). The experimental data, however, did not indicate a clear size difference among the three treatments.

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[3]Mr. Lou Bowen, 1970. OSU Swanson Aquaculture Laboratory. Personal communication.

Experimental conditions were the most uniform and comparable in the Port Orford study. The results showed that gravel fry were significantly larger than screen fry for the small "egg-size" experiment but not for the large "egg-size" experiment; thus the advantage of substrate support was not conclusively demonstrated for chinook salmon. Why results of the two "egg-size" experiments differed is not known. The proportionally greater difference observed in the small "egg-size" experiment may have been caused by the 23% higher water flow (150 versus 115 cm/hr) for these experimental cells. The higher flow may have increased the fry size difference between treatments by 1) increasing the size of the gravel fish through increased delivery of oxygen and removal of waste products, 2) decreasing the size of the screen fish through increased stimulation of non-growth activities, or 3) combining the effects of the above two factors.

In assessing the Port Orford reared gravel and screen fry against the Elk River reared Heath incubator fry, the lack of a reduced fry size for the Heath incubator fish would suggest that substrate provided little advantage during incubation. The difference in incubation site for the Heath fry, however, introduced differences in the experimental conditions which may have influenced the experimental results. Heath fish at Elk River experienced a higher flow rate and stocking density, were exposed to chronic but lower levels of light (whereas Port Orford

fish were exposed to periodic but higher levels of light), and experienced slightly colder water temperatures than fish at Port Orford. The effects of these differences on fry size are difficult to assess. Differences in flow and density probably had a neutral effect since advantages gained from the higher water flow would be negated by increased activities enhanced by crowding. Light exposure differences may be influential, but the effects of chronic versus periodic exposure to light are not known. Finally, the water temperature difference was probably too small to cause a difference in fry size.

Because of the many unanswered questions, results from this study were inconclusive. The suggestion that substrate had little effect on incubation of chinook salmon, however, received strong support from independent experiments conducted by Dr. Paul Reimers at the Elk River Hatchery. With the same eggs used in this experiment, Dr. Reimers compared the Heath incubator fish with fish which were incubated within deep matrix gravel incubators, a treatment which excluded all light and provided physical support at the same time. While Dr. Reimer's work can not be directly compared to this work due to differences in weighing and sampling techniques, our independent conclusions can be compared. Dr. Reimers observed for both large and small egg fry no significant difference between the maximum size of Heath fry and deep gravel box migrants; the gravel fry migrated at about maximum or slightly below maximum weight

(at or past total buttoning), thus no further weight gains were expected and the maximum weights of the two treatments can be compared. Based on this evidence, light and substrate appeared to have a minimal influence on growth.

Whether the effects of incubation will show up after emergence was not considered in Dr. Reimers' experiment, but post-emergence feeding of this study suggested that incubation history may have an influence on the ability of chinook fry to feed and grow. After the incubation phase, the small size difference between the Heath fish and the other two treatments developed into a significant difference with feeding, viz., Heath fish grew at a significantly slower rate than the other two groups. Possible explanations for this observation are the following: 1) the observed trend is transient--a longer feeding period would have allowed the Heath fish to catch up; 2) differential fin-clipping biased the results against the Heath fish; 3) some factor in the incubation phase caused the observed difference.

In evaluating these options, #1 is unlikely because the difference between the growth rates was basically maintained throughout the experiment, and was increasing under the 2% ration. Number 2 is a possibility, but both the gravel and Heath incubator fish were fin-clipped and the gravel fish were growing better than the Heath fish. A more likely explanation is the disparity in the incubation environment.

Differences in the incubation environment can be defined by four factors: 1) The Heath fish, because of a colder temperature regime, were about 3 days behind in development at the beginning of feeding. 2) During the transport of Heath fish from Elk River to Port Orford, the handling stress had a long term effect. 3) Heath fish were incubated without substrate support. 4) Heath fish were exposed to chronic level of light whereas the gravel and screen fish were exposed for short durations only during the daily inspection.

Developmental stage may have possible influence, but it is more likely to be an advantage or a neutral factor since it is the fish which start feeding too late, rather than too early, which experience feeding difficulties (Palmer et al. 1951; Hurley and Brannon 1969). Handling may be influential, but handling mortality affected only large chinooks, and both large and small chinooks exhibited the same growth patterns; moreover, no further delayed mortality was observed after the fish recovered from the immediate effects of handling. Lack of substrate is a possible causative factor but the screen fish, which also were without substrate support, exhibited a higher growth rate than the Heath fish; the screen fish, however, were not fin-clipped whereas the Heath fish were. Chronic exposure to light may be a significant factor as it has been suggested as a cause of faulty sight formation (Disler 1953) and of decreased alertness (e. g., Eisler 1957; Brannon 1965) both of which would influence the ability to feed. Smith (1916)

demonstrated that chinook salmon reared under exposure to continuous lighting were not able to feed and grow as well as fish reared under total darkness. This evidence, however, cannot be used directly since no information exists on the comparison of chronic versus periodic exposure to light.

In contrast to the Heath fish, gravel and screen fish maintained virtually identical growth rates, suggesting that the effects of substrate treatments had no influence on their ability to feed. The differential marking, however, was biased in favor of the screen fish. The suggestion, therefore, is that the gravel fish might have grown better given equal marking advantage to all treatments.

Results of this experiment left a basic question unanswered. If no light exposure was experienced by the experimental fish, or if observations were made under red light, would results differ?

Based on evidence from this study, the chinook salmon will probably demonstrate no size difference due to substrate rugosity. For the chum salmon, no postulation can be made; the severe degree of larval activities and occurrence of yolk-sac malformation, however, are not generally observed with domesticated chum salmon stocks. Data from other studies suggest that a single answer is not probable as species and stock-specific responses to substrate incubation are likely.

Research on Atlantic salmon and some trout species has yielded

consistent results. Marr (1965) incubated Atlantic salmon under darkness and demonstrated that alevins reared on a rugose substrate had higher yolk conversion efficiency than alevins reared on a smooth substrate. Other Scottish workers (Anonymous 1969) showed that when

> ...taking the wet weight of alevins from the smooth-floored trough, which had been exposed to daylight, as 100%, the wet weights of alevins from covered troughs were (a) with a smooth floor, 104%; (b) with a grooved floor, 114%; and (c) with the floor covered with stones, 119%.

Leon (1975) found that Atlantic salmon reared on a plastic substrate exhibited better growth and feeding behavior than those salmon reared on a smooth substrate.

Other research on trout and Pacific salmon, however, suggested different results. Emadi (1972, 1973) reared all five species of Pacific salmon under various incubation environments and found that chum, pink, and sockeye salmon exhibited higher levels of larval activity on a gravel substrate than coho and chinook salmon. Eisler (1957) found that light increased larval activity and reduced subsequent fry size for chinook salmon, but found no size or activity difference for coho salmon treated similarly. Haempel and Lechler (1931) found distinctly different photonegative reactions between three species of trout.

The above differences in larval behavior pointed out the need to

determine genetic as well as environmental components in the development of incubation technology, and that a given incubational system may not be equally adaptable to all species and stocks.

B. Effects of Stocking Density and Water Velocity on Pink and Chum Salmon

Previous research on levels of water velocity and egg density for incubation systems have had limited applications to gravel incubators for pink and chum salmon due to differences in species used, fry quality evaluation techniques, and experimental conditions. Investigations on steelhead trout (McNeil 1968) and on coho and chinook salmon (Poon 1970) provided preliminary but limited fry quality data. Only unpublished mortality data on chum salmon reared in deep gravel small scale incubators were cited by Bams (1970). Brannon (1965) studied the range of velocity, light, and oxygen under standard hatchery conditions; the lack of a supporting substrate and the high range of velocity in his study, however, precluded direct applications to gravel systems. Other studies on effects of water velocity evaluated the embryonic period up to hatching only (Shumway et al. 1964; Silver et al. 1963).

Given limited research data, levels of velocity and density used in present gravel systems were established as safe levels primarily through operational experience (Table 1). In general, flow levels are above optimum and stocking density is low, thus use of water and

building space is not maximized. In order to allow more efficient operations, information will be needed on acceptable ranges of velocity and egg density, particularly the lower limits of water flows and the upper limits of stocking density.

Principal objective of the 1970 brood year studies, therefore, was to determine the combination of stocking density and water velocity which will produce the best quality pink and chum salmon fry. While shallow gravel matrix test cells were used to tailor data for the Netarts system, generated information may also be useful as a reference to design other gravel incubators.

All experiments were conducted at the OSU Netarts Bay Swanson Aquaculture Laboratory using wild stocks of pink salmon from Lover's Cove Creek near Little Port Walter in southeastern Alaska, and chum salmon from Whiskey Creek, Oregon. A similar experimental design was common to both studies--a factorial design of three levels each of stocking density and water velocity. A non-substrate control was run in the Heath incubator for comparison. Fry size was compared by regression models.

The general objective of the study was met by the pink salmon study, but the chum salmon study was compromised by premature migration during incubation and also by a 9-hour water stoppage at the end of the incubation phase.

1. General Procedures

a. Pink Salmon Study

Two basic incubators were used--small scale shallow gravel matrix test cells and the Heath incubator.

A test cell consisted of a wooden box made from plywood and measured 30. 5 cm by 30. 5 cm by 41. 3 cm. Water upwelled through a false bottom made from fine mesh hardware cloth on a wooden frame. The false bottom was lined with a single layer of crushed rocks (0.64 cm to 1. 9 cm in diameter) to provide the shallow gravel matrix substrate. The outlet was located near the top of the cell through a 2. 5 cm (i. d.) outflow blocked with a fine-mesh plastic to prevent premature migration. Water flow was controlled by a screw clamp placed on the flexible inflow line. Light was excluded from the cell by an opaque lid, but some light entered cells via the overflow holes. The Heath incubator was also kept in subdued light, but the amount of exposure was higher than for the test cells due to small gaps in the cover.

Pink salmon eggs from 149 females were spawned on September 8 at Lover's Cove Creek (near Little Port Walter) and transported to the Oregon Fish Commission Big Creek Hatchery where they were fertilized after 12 hours in transit using methods described by Poon and Johnson (1970). These eggs were eyed and then taken on October 14 to the Netarts Laboratory. Survival at the eyed stage was 94% (216, 800 eggs) and the trip from Big Creek to Netarts

resulted in no additional egg mortality. Average egg diameter was 0.64 cm at the eyed stage. The eggs reached 50% hatching on November 14 and the alevins reached button-up by January 16, 1971.

Nine gravel incubation cells were set up with water velocities of 50, 100, and 300 cm/hr, and stocking densities of 1200, 2400, and 4800 eggs per cell (1.29, 2.58, 5.16 eggs/cm^2 respectively) on October 27. One Heath incubator tray was stocked with 4100 eggs (3.20 eggs/cm^2). Daily records were kept of water velocities and mortalities. Water temperature during the study ranged from 3.4°C to 13.4°C with an average of 8.9°C.

Maintenance of the designated water velocities was hampered by clogging due to high silt content of the water during periodic freshets. Silt accumulated in flexible tubing leading to the experimental cells, and later in the experiment, with algal growth. This clogging slowed the water flow and necessitated some flushing, particularly with the higher velocity cells. The overall effect of the clogging was to lower the observed average flow from their designated levels (Table 5).

The siltation problem also hampered the operation of the Heath incubator. Because the Heath tray and its cover were made from fine mesh screen, accumulated silt, and for the cover, algae and silt, had to be washed off periodically. Water flow to the Heath incubator was kept at a constant 18.9 lpm through the experiment.

Table 5. Water velocities, stocking densities, and mortalities of the 1970 brood year pink and chum salmon velocity and density experiments.

Species	Experimental cell number	Velocity in cm/hr			Stocking density[1]	Mortality in %		
		Designated	Observed[2]			Total	Egg	Alevin
			average	S.D.	Eggs/cm^2			
Pinks	1	50	48.5	8.0	1.29	0.42	0.42	0.00
	2	50	50.3	6.8	2.58	0.29	0.29	0.00
	3	50	49.6	7.6	5.16	0.19	0.19	0.00
	4	100	96.7	14.5	1.29	0.42	0.42	0.00
	5	100	99.5	13.9	2.58	0.21	0.21	0.00
	6	100	98.7	23.1[4]	5.16	0.19	0.15	0.04
	7	300	278.9	30.5	1.29	0.08	0.08	0.00
	8	300	280.5	26.1	2.58	0.08	0.08	0.00
	9	300	276.2	37.9	5.16	0.23	0.21	0.02
						$\bar{X}$ 0.23	0.23	0.01
	Heath Tray	887 (18.9 lpm)	887		3.20	10.32	0.78	9.54
Chums	1	25	24.3	4.7	0.96	4.50	1.50	3.00
	2	25	23.9	6.4	1.92	7.25	3.63	3.63
	3	25	24.4	6.2	3.85	5.83	2.83	3.00
	4	75	72.8	14.2	0.96	3.67	1.25	2.42
	5	75	71.7	14.4	1.92	4.58	1.96	2.63
	6	75	71.0	16.7	3.85	6.38	3.40	2.98
	7	225	213.7	25.5	0.96	2.83	1.25	1.58
	8	225	196.2	35.8	1.92	4.42	2.33	2.08
	9	225	219.5	29.2	3.85	4.04	2.06	1.98
						$\bar{X}$ 4.83	2.25	2.59
	Heath Tray 1	887	887		0.94	19.09	11.67	7.42
	2	(18.9 lpm)			1.88	8.34	3.46	4.88
	3				3.75	10.02	4.08	5.94
						$\bar{X}$ 12.48	6.40	6.08
					$\bar{X}$ without 1st tray	9.18	3.77	5.41

1/ Pink egg average diameter: 0.643 cm. Chum egg average diameter: 0.744 cm.

2/ N = 159 for pinks; N = 128 for chums.

3/ Excluding mortalities and sampled fish.

4/ Bending of incoming tygon tube necessitated periodic adjustments.

Four random samples of n=20 fry per sample were taken from each test cell over the 9 days prior to complete closure of the abdominal slit. The fry were anesthetized and processed live for wet and dry weights using bulk weighing methods. To generate regression data for the size conversion procedure, Heath incubator fish were sampled for 16 days prior to closure of the ventral slit.

The sample size of 20 fish was justified by comparing average wet and dry weights of three groups of 20 fish sampled from the same population; no significant difference was found at the 5% level for wet weights ($F_{2,60}=2.27$) or for dry weights ($F_{2,60}=1.49$).

Fry quality was assessed by the criteria of size, stage of development, and post-emergence growth. Fry size was evaluated by the regression method using dry weight as the size index and % dry weight as the stage of development index (cf. p. 26). Because of the lack of rearing facilities, post-emergence growth was compared only between Heath fish and gravel fish from one test cell. This test compared incubator design and provision of a substrate rather than density and velocity. Growth was measured to evaluate relative ability of gravel and Heath fry to feed and grow; size measurements were taken only at the beginning (January 16, the date of the last incubation sample) and at the end of the experiment 73 days later. Fry from gravel test cell #3 were selected for comparison with fry from the Heath incubator because this cell represented the least

favorable gravel incubation treatment (highest density and lowest flow), and their performance can be taken as the expected minimum performande of the gravel fish. Approximately 3700 fry from the Heath and 4800 fry from gravel cell #3 were placed into two 568 liter plastic-lined indoor rearing tanks with water exchange rates of approximately 7.6 lpm. Test fish were fed on a diet of dry Abernathy pellets several times a day until satiation. At the end of the feeding period, water level of the tanks was lowered to concentrate the fish, and a random sample of approximately 100 to 150 fish was taken from each tank for live bulk weight and individual length measurements. Water temperature ranged from 4.5°C to 11.1°C with an average of 7.8°C during this growth phase.

b. Chum Salmon Study

As in the pink study, two basic incubators were used--small scale shallow gravel matrix test cells and the Heath incubator. Timing overlap of the two studies precluded the use of the same cells twice. Cells used in this study were non-toxic plastic and measured 40.6 x 30.6 x 32.4 cm deep. Water upwelled through a plexi-glass false bottom drilled with 0.32cm holes. A single layer of crushed rocks provided the shallow gravel matrix substrate. Flow was controlled with a screw clamp on the flexible inflow tubing, and an opaque lid excluded light, although a little light could penetrate through the outflow hole.

Unlike the pink salmon test cells, these test cells were

shallower, with a water depth above the substrate of about 7.6 cm. The outflow was a small hole (10 mm ID), to which a flexible tubing was attached to lead effluent to a trough. The outflow was not blocked by a plastic mesh, as in the pink salmon study, due to an erroneous assumption that alevins would not attempt to escape through a small opening.

Chum eggs for the experiments were taken on November 13-14. Approximately 80,000 eggs eyed in the production hatchery tanks at Netarts Bay were stocked in the incubator cells on January 17. Average egg diameter at the eyed stage was 0.744 cm. Fifty percent hatching occurred on January 27 and fry were buttoned-up by March 26. Eggs were water-hardened for approximately one hour before being spread on the egg screens in the production tanks. Some delayed mortality was encountered over most of the incubation period in all experimental treatments.

Nine gravel incubator cells were set up with water velocities of 25, 75, and 225 cm/hr, and with stocking densities of 1200, 2400, and 4800 eggs per cell (0.96, 1.92, and 3.85 eggs/cm^2 respectively) on January 17. Three Heath incubator trays were stocked with the same number of eggs per tray for egg densities of 0.94, 1.88, and 3.75 eggs/cm^2 respectively. Daily records of flow rates and mortalities were kept as in the pink experiment (Table 5). Water temperature ranged from 3.4°C to 11.7°C with an average of 7.5°C.

The siltation and clogging problems observed in the pink experiment were also observed in the chum experiment. In addition, the unscreened outflows allowed alevin and fry escapements. As early as February 19, about 23 days after 50% hatching, some escaped alevins were observed in the common outflow trough; but since the trough received effluent from all test cells, it was impossible to determine the source of the escaped fry. Subsequent efforts to stop these escapes were ineffective. Because of this escapement problem, valid data were derived only for assessment of hatching success under the specified velocity and density conditions, and for the derivation of the fry size regression model using only Heath incubator fish. The three Heath trays were combined during sampling to derive one set of data for regression analysis.

Seven samples of n=20 fry each were taken from the three Heath incubator trays between March 7 through March 26, the period immediately before total buttoning. Sample processing proceeded as described for the pink experiment, except length was measured in addition to wet and dry weight.

Fry quality testing procedures described for the pink salmon study were used for this study under the limitations specified previously. Unlike the pink study, the inclusion of length data allowed calculation of the Kd stage of development index; and, comparative size evaluation models were extended to all combinations of length

or dry weight regressed on Kd or percent dry weight (cf. p. 26).

The post-emergence phase of the chum experiment was eliminated when on the final sampling day a nine hour electricity outage and water stoppage killed over 90% of the Heath incubator fry but caused negligible mortalities in the gravel cells.

2. Results

For both pink and chum salmon, alevins in the gravel test cells were distributed evenly over the substrate area, and their activities were observed to be lower than alevins on the flat Heath trays. Activities in the Heath, however, may have been stimulated further by somewhat higher exposure to light.

a. Egg and Alevin Mortality

For both the pink and chum experiments, mortality was higher for the Heath fish than for the gravel fish; differences between gravel cells, however, were smaller (Table 5).

1. Pink Salmon Study

For the pink experiment, the total (egg to fry) mortality in the Heath incubator was 45 times higher than the average total mortalities experienced in the gravel cells (10.32% versus 0.23%). Mortality in the gravel cells was negligible and consisted almost exclusively of eggs (0.23%), whereas in the Heath trays, alevin mortality (9.54%) dominated.

2. Chum Salmon Study

Mortality of chum salmon was higher than for pink salmon. For unknown reason, egg mortality in the Heath trays averaged 2.8 times higher than the average from the gravel cells (6.40% versus 2.25%). The mortality in tray #1 was unusually high for unknown reasons. Excluding tray #1 from analysis, the average egg mortality was 3.77%, which is reasonably close to the average for the gravel cells (2.25%). Within the Heath incubator, the lower density tray #2 had slightly lower mortality than tray #3 (3.46% versus 4.08%).

Among the gravel cells, egg mortality appeared to be lowest at the lowest density, regardless of water velocity. To test this correlation statistically, a preliminary test for interaction between velocity and density was run by plotting egg mortality on stocking density for each of the three velocities (Figure 6). On this plot, lines for 25 cm/hr and 225 cm/hr were parallel, but the 75 cm/hr line was not due to one aberrant point; this single point was considered insufficient evidence to reject an interpretation of no interaction. Accordingly, the effects of density on mortality was tested by a chi-square test at each of the three velocities. Test results showed that density at 25 and 75 cm/hr was significant at the 1% level in producing a difference in egg mortality ($\chi^2_{2df} = 12.966^{**}$ and 23.598^{**}, respectively); at 225 cm/hr, the test was significant at the 10% level

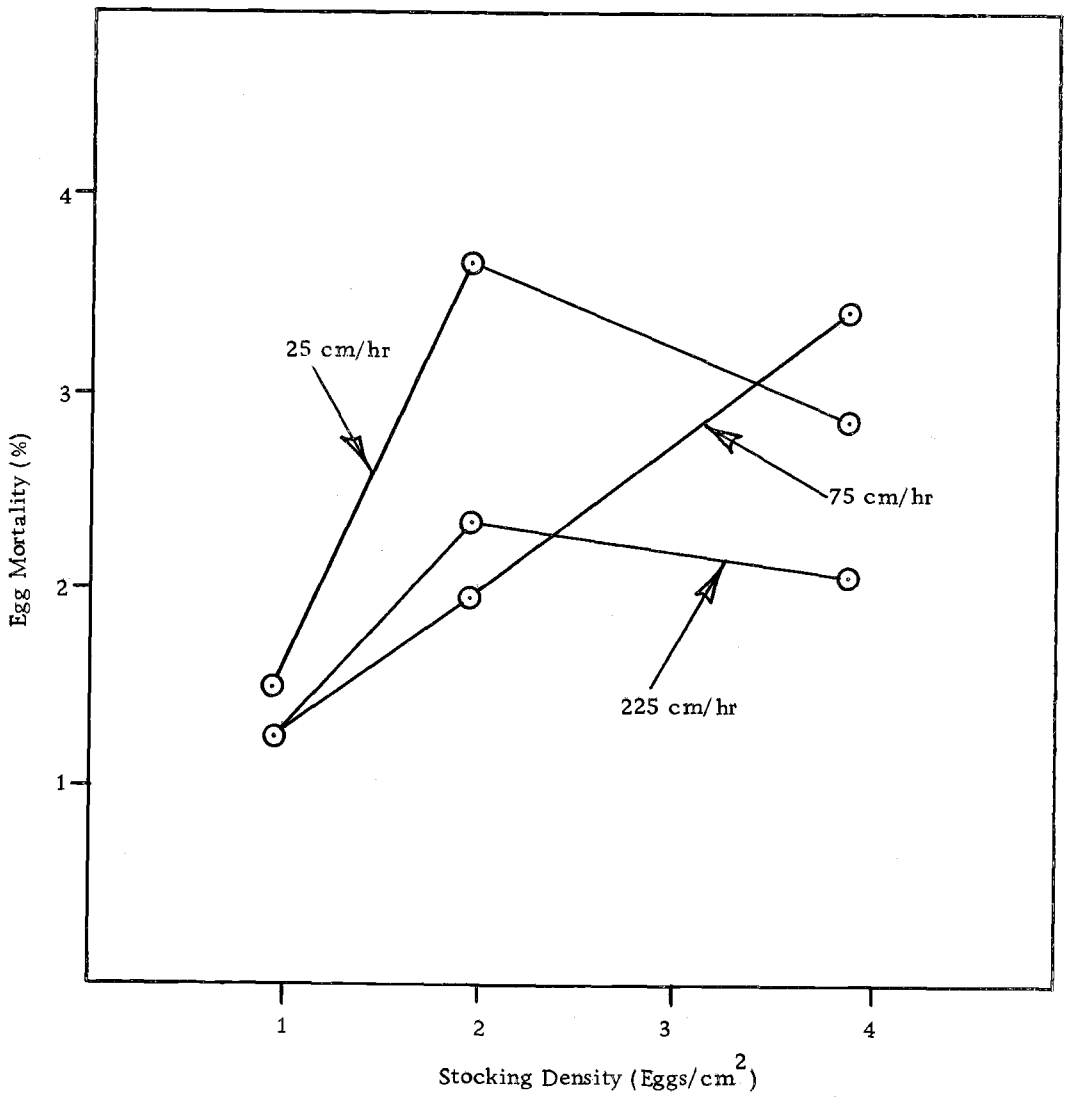

Figure 6. Relationship of egg mortality to stocking density for three water velocities tested in the 1970 brood year chum salmon velocity and density laboratory experiment.

($\chi^2_{2\,df}$=4.821). At all water velocities, the lowest density (0.96 eggs/cm^2) apparently produced a lower mortality than the two higher densities (1.92 and 3.85 eggs/cm^2).

Chum alevin mortality in the Heath incubator averaged about two times higher than the average of the gravel cells (5.41% vs. 2.59%). Within the Heath trays, the higher density tray #3 again had a slightly higher mortality than the lower density tray #2 (5.94% versus 4.88%).

The chum mortality assessment for the incubation phase stopped on the day of the last fry sample, March 26, which was also the day of the 9 hour electricity outage. After the outage, and the accompanying water stoppage, the mortalities which were suffered as a direct consequence of this incident were assessed as follows:

Incubator:	#1	#2	#3	#4	#5	#6	#7	#8	#9	Heath
# of Mortalities:	1	22	21	4	2	7	2	3	5	over 90% of population

b. Frequency of Physical Abnormalities

Mild to severe cases of yolk-sac malformation were observed exclusively and at virtually 100% frequency on the Heath incubator fish in both the pink and chum experiments.

c. Fry Size and Stage of Development

1. Pink Salmon Study

Three regression equations were developed for the Heath and gravel incubator fish: dry weight on time, % dry weight on time, and dry weight on % dry weight (Appendix 3). Changes of dry weight and percent dry weight over time were more pronounced for the Heath fish than for the gravel fish (Figures 7 and 8); but when these two parameters were regressed against each other, i.e., dry weight against percent dry weight, the difference in growth rates decreased (Figure 9). Given the rate differences, both calculated sets of regression data were used in the size conversion for the respective incubation treatment. Size and stage of development comparisons in this study, however, were facilitated because Heath fish were visibly smaller and were more advanced than most of the gravel fish throughout the sampling period (Appendix 4). Statistical treatments therefore were limited to the gravel fish only.

Statistical comparisons of relative size were made of grand means of average dry weights for each treatment on each sample date (Appendix 4, Figure 10). Grand means were converted to a common stage of development by the formula:[4]

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[4]See p. 26 for discussion of formula parameters.

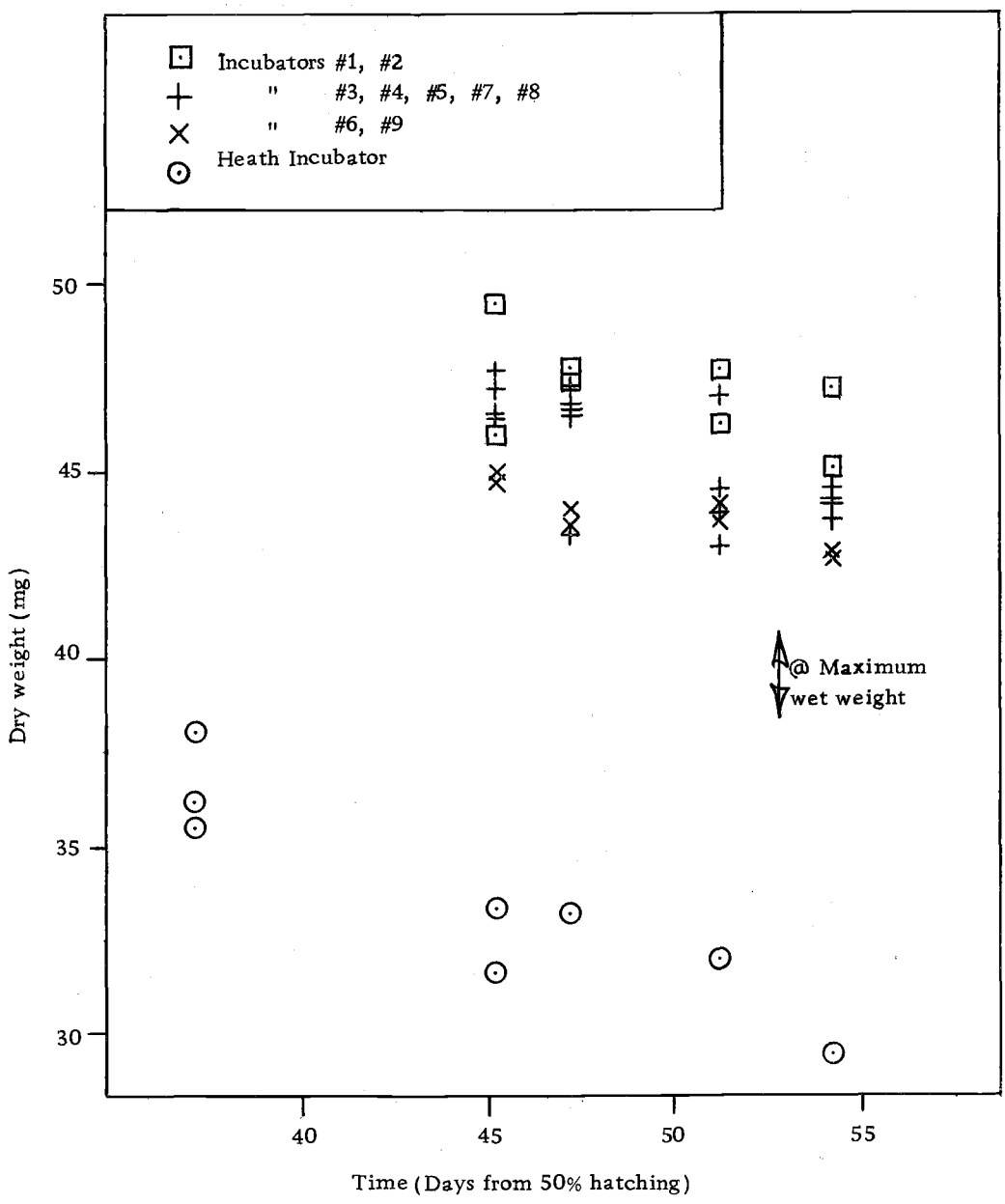

Figure 7. Relationship between dry weight and time during the terminal yolk absorption period for the 1970 brood year pink salmon density and velocity experiment. Each point is an average of 20 fry.

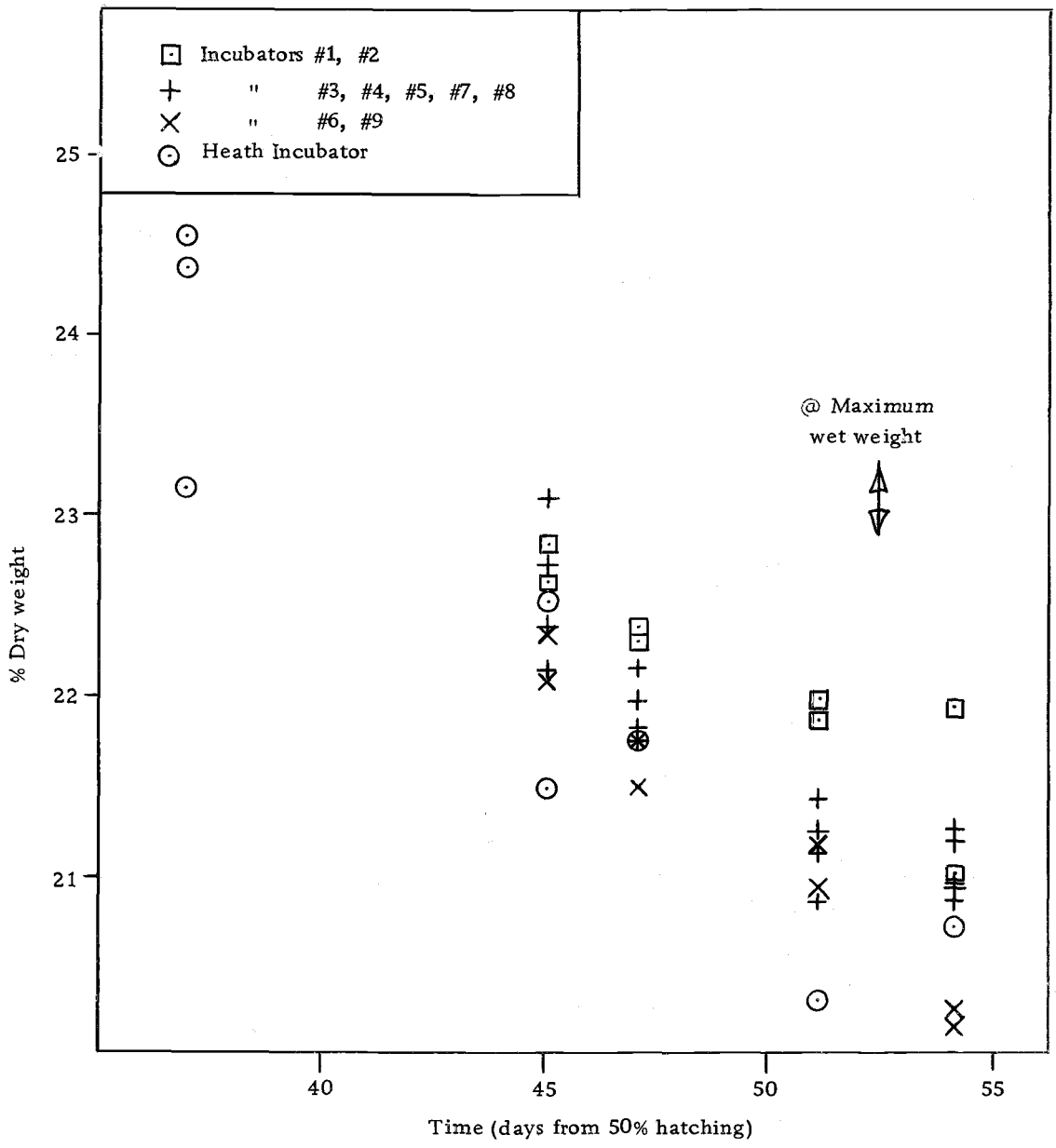

Figure 8. Relationship between % dry weight and time during the terminal yolk absorption period for the 1970 brood year pink salmon density and velocity experiment. Each point is an average of 20 fry.

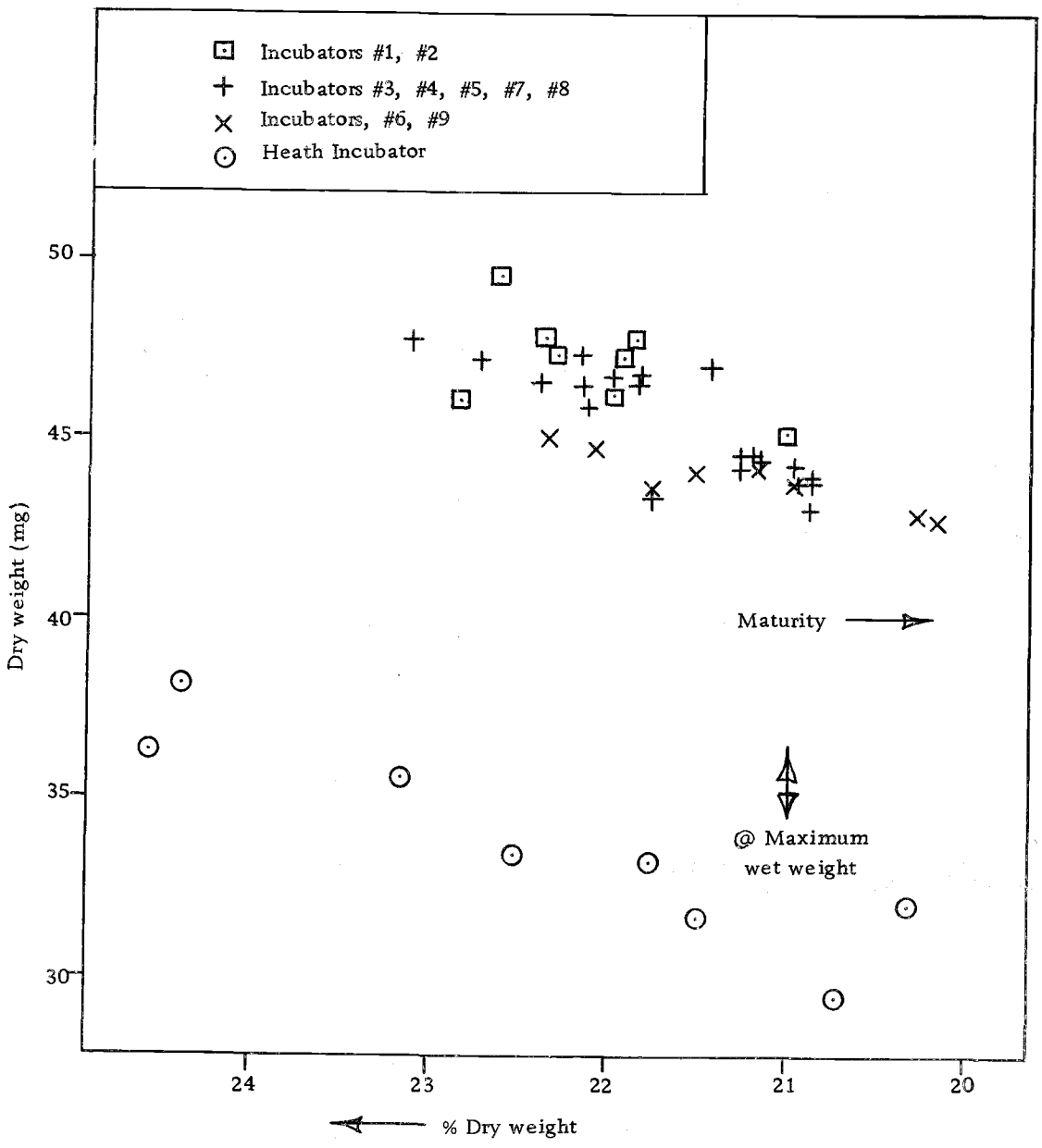

Figure 9. Relationship between dry weight and % dry weight of chronological samples taken during the terminal yolk absorption period for the 1970 brood year pink salmon density and velocity experiment. Each point is an average of 20 fry.

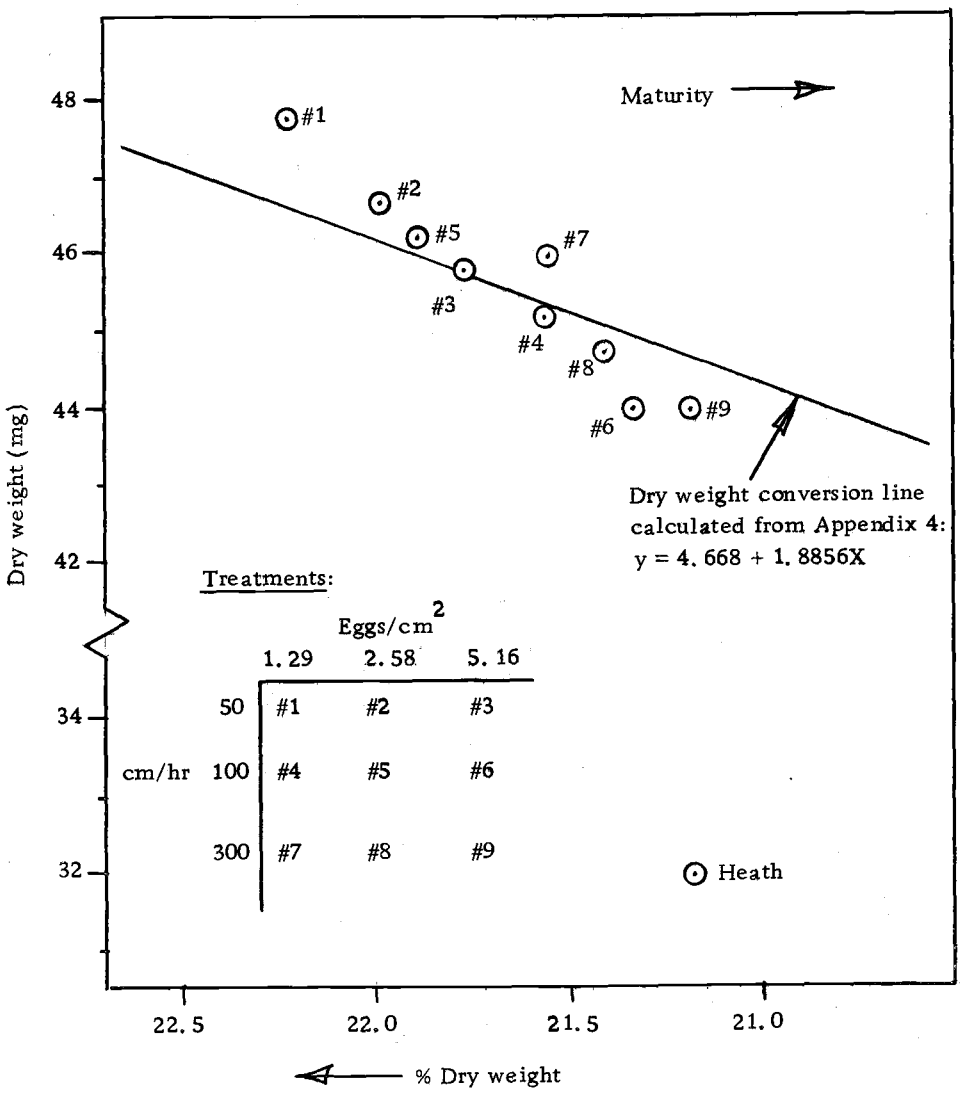

Figure 10. Relationship between dry weight and % dry weight for all treatments of the 1970 brood year pink salmon density and velocity experiment. Each point is a grand mean of four samples (n=20 fry per sample) taken over the terminal yolk absorption period from 1/7/71 through 1/16/71 (cf. Appendices 3 and 4).

$$\text{converted dry weight} = \text{observed dry weight} - [(\text{observed \% dry weight} - 21.59)(\text{conversion slope}^{*})]$$

* 1.8856 for gravel treatments; 1.6200 for Heath treatment

and tabulated by ranks (Table 6). Effects of stocking density and water velocity were then analyzed by a modified procedure of the analysis of variance for a two-way classification of treatments with single observations per cell. Prior to the analysis of variance, possible interaction between the two treatments of stocking density and water velocity was tested by plotting dry weight against stocking density for each of the three velocities and determining if the lines were parallel (Figure 11). The results showed that except for one aberrant point in the 100 cm/hr line, the three lines would have been approximately parallel. This was considered to be sufficient evidence to assume no interaction effects. The analysis of variance was modified, therefore, to assign to main effects all variance due to interaction; thus calculation of error terms was possible despite the lack of true replicates in the experiment. The analysis resulted in no significant difference for either water velocity ($F_{2,4} = 5.56$) or stocking density ($F_{2,4} = 6.21$) on size at the 5% level, although significant differences existed for both treatments at the 10% level (Appendix 5). In contrast to the small size differences among gravel fish, Heath fish were smaller than gravel fish by 28.41% in average wet weight and by 28.39% in average dry weight.

Table 6. Ranking of average size[1/] (dry weight) and stage of development[1/] (% dry weight) for all treatments of the 1970 brood year pink salmon density and velocity study.

Size ranking (heaviest to lightest)					Stage of development ranking (most to least developed)			
Rank	Incubator	Density/velocity	Dry weight[2/] after conversion	Dry weight before conversion	Rank	Incubator	Density/velocity	% Dry weight
			mg	mg				%
1	#1	low/low	46.52	47.71	1	Heath	med/high	21.19
2	#7	low/high	45.94	45.86	2	#9	high/high	21.19
3	#2	med/low	45.87	46.60	3	#6	high/med	21.33
4	#5	med/med	45.51	46.08	4	#8	med/high	21.41
5	#3	high/low	45.44	45.76	5	#4	low/med	21.54
6	#4	low/med	45.18	45.09	6	#7	low/high	21.55
7	#8	med/high	44.90	44.56	7	#3	high/low	21.76
8	#9	high/high	44.67	43.92	8	#5	med/med	21.89
9	#6	high/med	44.47	43.98	9	#2	med/low	21.98
10	Heath	med/high	32.52	31.87	10	#1	low/low	22.22

1/ Each mean is the grand mean of four samples of n = 20 fry each taken over the terminal yolk absorption period from 1/7/71 through 1/16/71.

2/ Conversion formula for incubators #1 to #9:

Converted dry wt.= observed dry wt. - [(observed % dry wt. - 21.59)(1.8856)]

Conversion formula for Heath incubator:

Converted dry wt.= observed dry wt. - [(observed % dry wt. -21.59)(1.6200)]

see p. 26 for discussion of formula parameters.

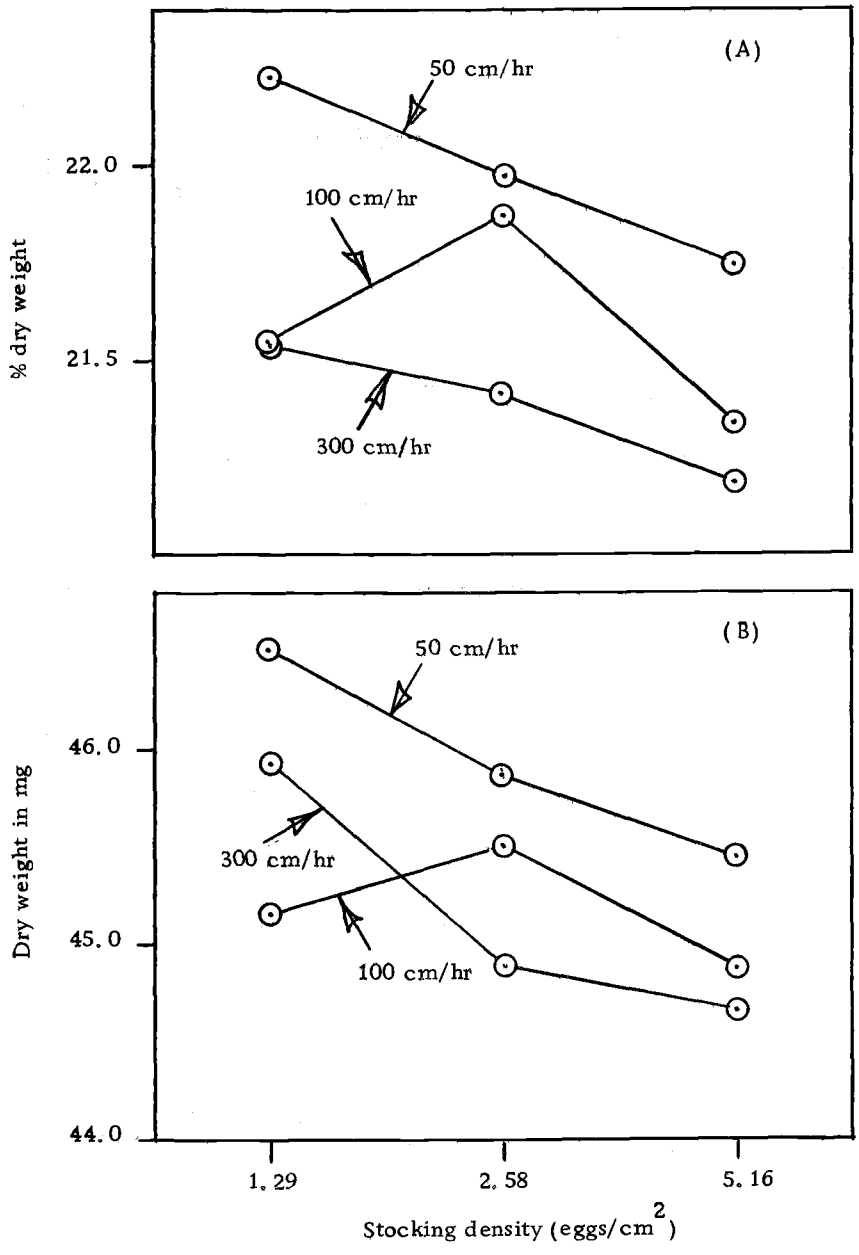

Figure 11. Relationship of (A) % dry weight and (B) dry weight to stocking density at three water velocities for the 1970 brood year pink salmon density and velocity experiment.

Procedures for evaluating effects of density and water velocity on relative stage of development (Table 6 and Figure 11) were similar as for evaluating their effects on size. There was no evidence of interacting effects between the variables, and the analysis of variance test assumed no interaction. There was a significant difference for velocity at the 2.5% level ($F_{2,4}$=11.01*) but no significant difference for density at the 5% level ($F_{2,4}$=4.46), although the latter was significant at the 10% level (Appendix 6).

To identify the velocity at which significant differences in stage of development occurred, the nine treatment means were pooled across densities (yielding three means), ranked, and all possible paired means compared with the Least Significant Difference (LSD) method at the 5% and at the 1% level (Appendix 7). At the 5% level, fry from the 300 cm/hr treatment were significantly more advanced than fry from the 50 and 100 cm/hr treatments, and no difference existed between the latter two treatments. At the 1% level, fry from the 300 cm/hr treatment were significantly more advanced than fry from the 50 cm/hr treatment only, and again, no difference existed between fry from the 50 and 100 cm/hr treatments. It appears that a significant increase in developmental rate probably occurred between 100 and 300 cm/hr. When the Heath fish were compared to gravel fish (Appendix 6, Figure 10) they were as advanced in development as the most advanced gravel treatment--cell #9, the cell with

the highest stocking density and water velocity.

While effect of density was not highly significant, inspection of pooled means across velocities showed that an increase in developmental rate was obvious at the highest density of 5.16 eggs/cm^2 whereas little difference existed between the lower densities of 1.29 and 2.59 eggs/cm^2 (Appendix 6).

2. Chum Salmon Study

Size and stage of development data generated over 19 days (7 sample dates) prior to button-up from the combined Heath incubator fish exceeded the stages of development at which fry generally migrate (Appendix 8), but plots of these data illustrated linear changes of each parameter with time and their applicability to derivation of regression models (Figures 12 and 13). For size parameters[5] dry weight had a better linear fit than length (Figure 12); for stage of development parameters, percent dry weight had a better linear fit than Kd (Figure 13).

To derive regression data for comparative size evaluation with chum salmon, calculations were limited to the last five sample dates over 2 weeks prior to button-up, the period of expected fry migration (Appendix 9). All parameters showed high correlation coefficients

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[5]Wet weight was not used as a size parameter.

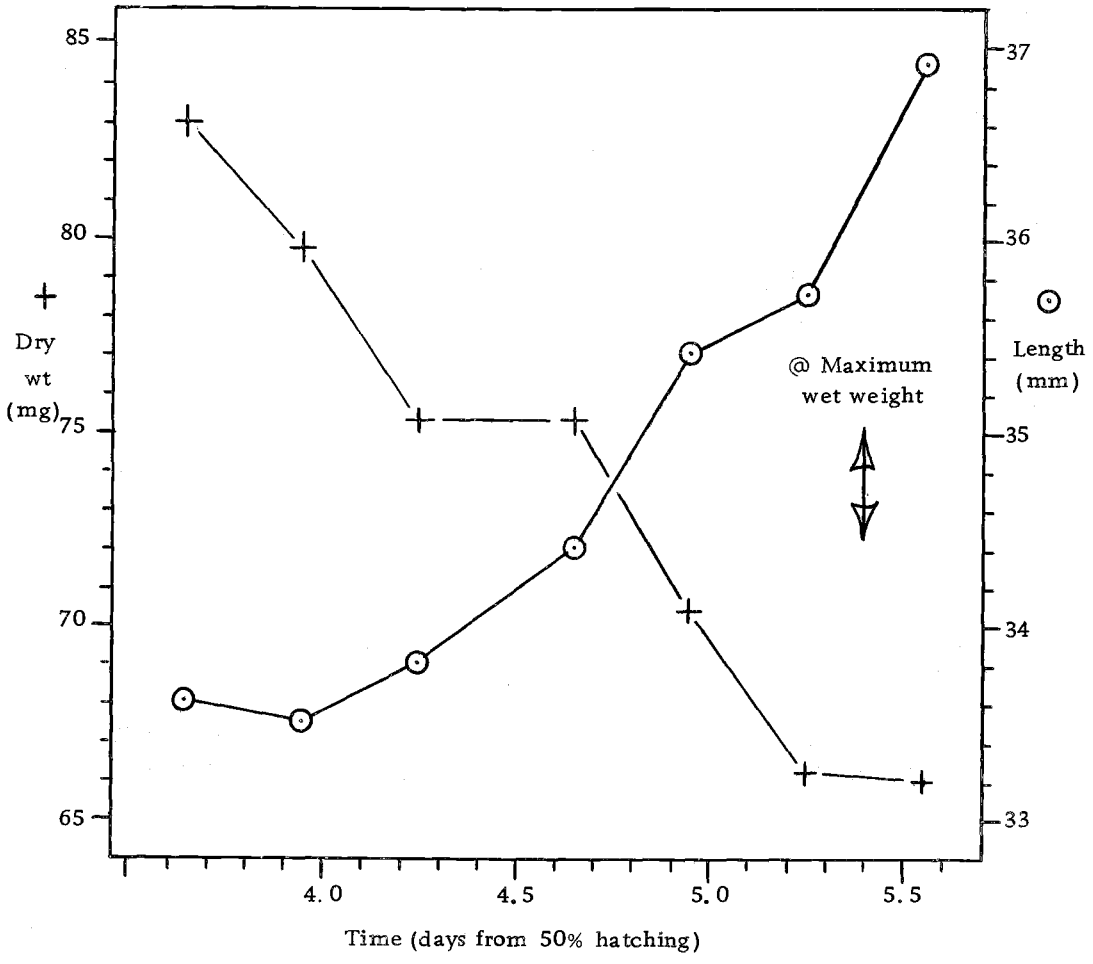

Figure 12. Relationship between dry weight and time and length and time during the terminal yolk absorption period for the 1970 brood year Heath incubator chum salmon fry. Each point is an average of n=20 fry except for the first two dates for which n=10 fry.

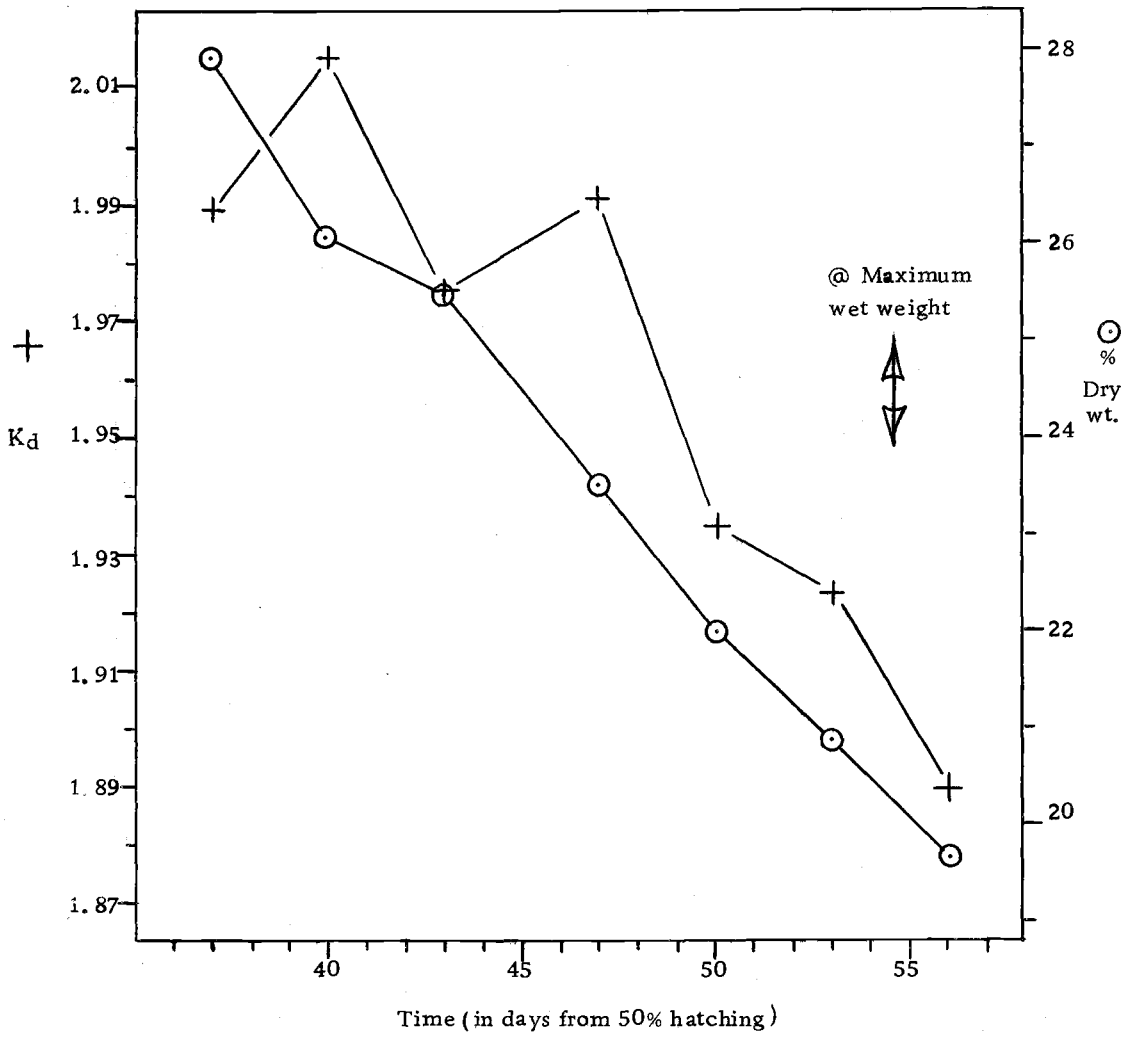

Figure 13. Relationship between % dry weight and time and Kd and time during the terminal yolk absorption period for the 1970 brood year Heath incubator chum salmon fry. Each point is an average of n=20 fry except for the first two dates for which n= 10 fry.

of approximately 0.9 or higher. The four size conversion regression models, i.e., length on Kd, length on percent dry weight, dry weight on Kd, and dry weight on percent dry weight, all demonstrated sufficient goodness of fit (Figures 14 and 15).

Since sample size and numbers are limited, the derived regression trends should be considered as general trends which can be better defined under more exhaustive sampling.

d. Post-emergence Growth

Gravel fish were 52% heavier and 14% longer than the Heath incubator fish at the end of the feeding period which lasted 73 days. The mortalities observed for both treatment groups were mostly non-feeding "pin-heads."

Mortality and the single growth measurement on the pink salmon experiment are tabulated below:

	Initial Measurement (1/16/71)		Final Measurement (3/30/71)		Mortality
	length mm	wet weight mg	length mm±95% CI	wet weight mg	%
gravel cell #3	--	208.0	42.6±1.0	633.5	12
Heath	--	142.5	37.3±0.9	417.7	51

The outstanding difference between the two populations was the dominance of small fish (Figure 16) and high occurrence of "pin-head" fish among Heath incubator fish.

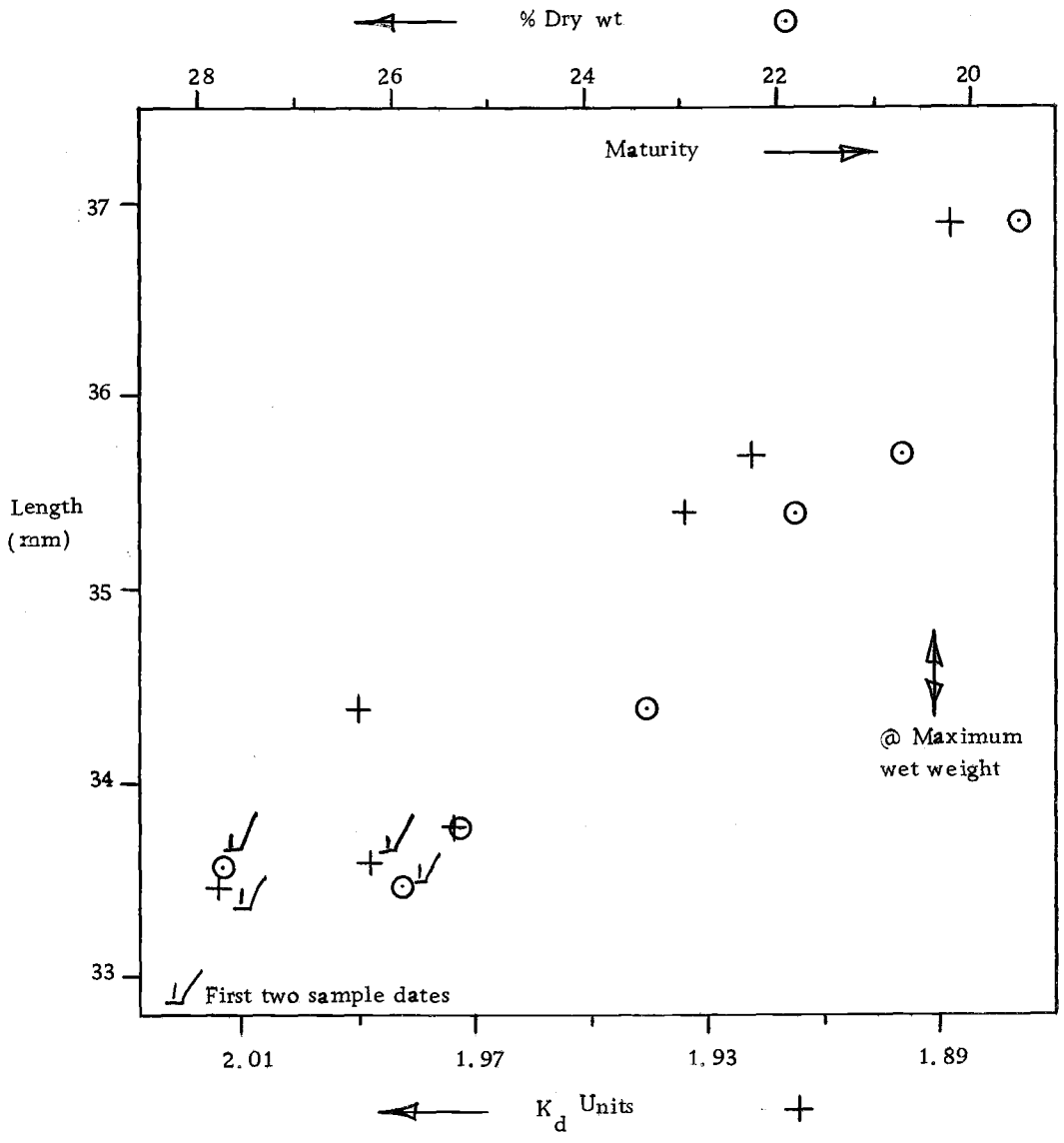

Figure 14. Relationship between length and K_d and length and % dry weight of chronological samples taken during the terminal yolk absorption period for the 1970 brood year Heath incubator chum salmon fry. Each point is an average of n=20 fry except for the first two sample dates for which n= 10 fry.

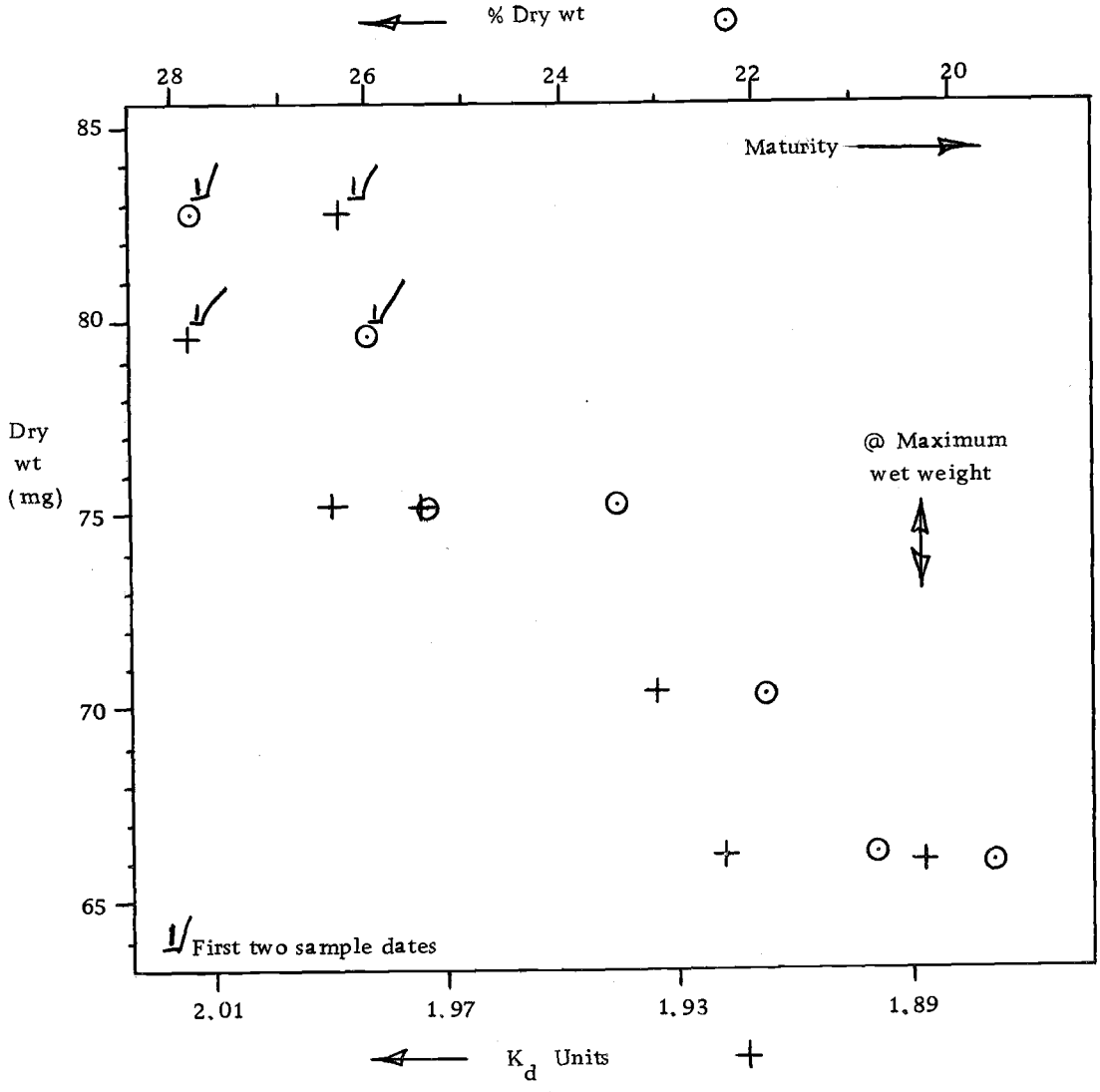

Figure 15. Relationship between dry weight and K d and dry weight and % dry weight of chronological samples taken during the terminal yolk absorption period for the 1970 brood year Heath incubator chum salmon fry. Each point is an average of n=20 fry except for the first two sample dates for which n=10 fry.

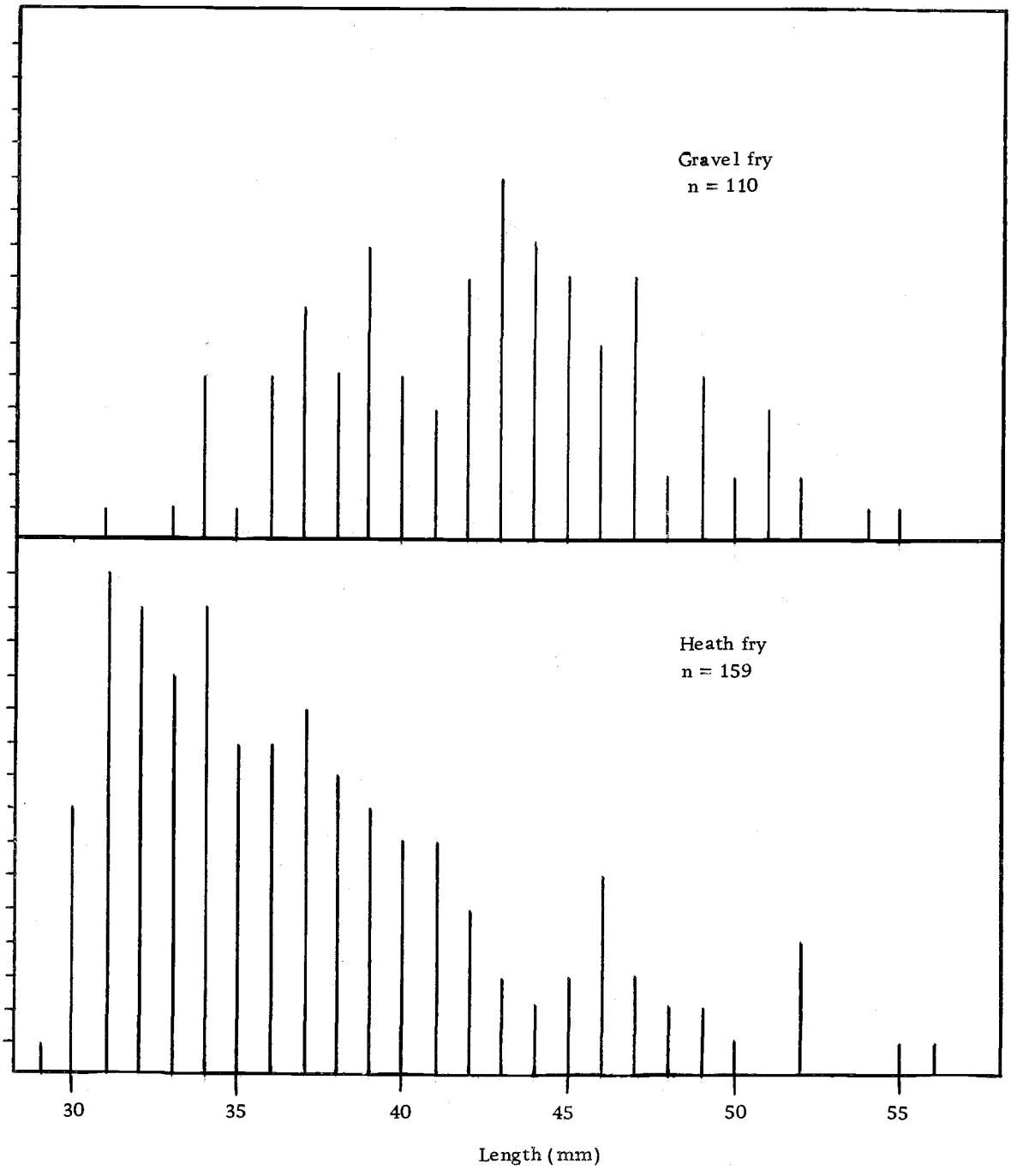

Figure 16. Length distributions for random samples of the 1970 brood year Heath and gravel pink salmon fry after 73 days of feeding.

3. Discussion

Since the chum salmon data were limited to demonstration of regression models and egg mortality assessment, the following discussion will be addressed primarily to pink salmon data. The purpose was to determine the combined levels of water velocity and stocking density which produce good quality fry. Fry size was expected to be the primary quality criterion since unfavorable environmental conditions from increased crowding and accumulation of waste metabolites would increase non-growth energy expenditures, leading to decreased yolk to tissue conversion efficiency.

Under the range of velocity and density tested, however, the experimental results produced no highly significant fry size difference, although differences in mortality and stage of development among test cells were observed.

The high egg-to-fry survival experienced in the pink salmon experiment are not infrequent in hatchery operations, however. For example, Bams (unpublished chum salmon data cited in 1970 paper) in a similar velocity and density experiment with deep matrix gravel incubators reported average eyed egg-to-fry survival of 97. 7% and green egg to eyed egg survival of not less than 95%. It is significant that high survival in this experiment occurred under less than optimum water quality conditions with the shallow gravel matrix design.

The lack of a size difference was contrary to expectation and suggested that larval activities were approximately equal in all test cells. This apparent aberrancy might be explained by several factors: 1) Removal of mortalities meant no waste metabolite accumulation from presence of dead eggs or alevins. 2) Waste metabolite build-up was mitigated by distribution of alevins over the entire substrate area during most of the incubation period. 3) Alevins were able to self-aerate and could withstand lower circulations. 4) The shallow gravel matrix offered little resistance to the water flow so stagnant flow areas were limited. These conditions are similar to the velocity experiment conducted by Brannon (1965) on sockeye salmon. Brannon also demonstrated no fry size difference under true velocities of 180 to 5400 cm/hr at egg density of 1.9 eggs/cm^2. Work with steelhead trout (McNeil 1968) and coho and chinook salmon (Poon 1970), however, resulted in the establishment of a lower velocity threshold of 25 to 50 cm/hr and 54 cm/hr respectively (with a single layer of eggs), below which fry size was affected. But because size assessments were not accompanied by calculations of developmental rates, interpretation of the latter data was made difficult; nevertheless, a lower limit on velocity certainly should exist. Mortality data from the present chum salmon study supported the existence of a lower velocity limit since fry produced at the lowest velocity of 25 cm/hr and stocking densities of 1.92 and 3.85 eggs/cm^2 were more

vulnerable to hypoxic conditions. Thus, given the conditions of this experiment, a lower limit for flow rate should probably be at 50 cm/hr with maximum stocking density of 5.16 pink salmon eggs per cm^2. Operation at this low flow, however, may be easily hampered by egg mortalities, flow channelization and water siltation. Higher velocities will generally be preferred even though increasing water velocity may lead to accelerated development.

Based on this study, velocity above 100 cm/hr and density above 2.58 pink eggs/cm^2 accelerated developmental rate; the influence was apparently more significant for velocity ($p<0.025$) than for density ($p<0.10$). Rate of yolk utilization is primarily controlled by temperature and the amount of available yolk, and under a given temperature regime, yolk absorption rate is considered constant even if metabolic demands vary (Bams 1969). Brannon (1965) showed that developmental rates were not affected by changes in true water velocity (from 180-27,000 cm/hr) even when fry size was reduced from excessive larval activities. Differences in developmental rates, however, do exist in other cases even when temperature exposures were equivalent. In laboratory tests, large differences of up to several weeks have been observed by Bailey et al. (1975) between deep gravel incubators and Heath incubator fry reared under the same water supply. In this experiment and in my subsequent studies, similar developmental rate differences were also observed under

constant temperature regimes. Reports of hatchery fry reaching button-up ahead of natural fry are also not uncommon.

Factors associated with fish density and flow velocity which cause developmental rate differences are not clear, although some environmental parameters are known to be responsible. Lowering dissolved oxygen levels will decrease developmental rates for salmon embryos and alevins (e. g., Alderice et al. 1958; Garside 1959, 1966; Silver et al. 1963; Brannon 1965). Exposure to various light sources can change hatching time and time to total yolk absorption, although the effects may be to decrease or increase the developmental rate, depending on the species and the wavelengths of the light (e. g., Eisler 1957; Bell and Hoar 1950; Smith 1916; Brannon 1965).

Factors which might have altered developmental rates among test cells in this study could not have been light since the limited exposure was similar in each test cell. The effects of dissolved oxygen levels, however, can not be ruled out; although considered as nonlimiting based on observations of high survival and low alevin activities, differences may have occurred among test cells, particularly at the microenvironmental level surrounding each alevin. If delivery of dissolved oxygen at the substrate level were the cause of the observed developmental rate difference, that would explain increased developmental rates from increased flow rates; increased densities, however, also increased developmental rates even though

decreased dissolved oxygen availability might be expected. This aberrancy might be explained by the positive correlation between density and true velocity--when egg density is increased, interstices in the gravel matrix are decreased, and true velocity is increased. Since density varied by a factor of four fold, increase in velocity and in dissolved oxygen delivery might be substantial.

Potential developmental rate differences between hatchery and wild fry as caused by velocity and density during incubation, are quite conceivable since water flow and dissolved oxygen levels are typically low in nature. Apparent velocity through spawning gravel generally range from 2.4-126 cm/hr (e.g., Wickett 1958; McNeil and Ahnell 1964; Vaux 1967) and dissolve oxygen level can range from saturation to close to depletion depending on the characteristic of the streambed and the distribution and survival of the buried eggs (e.g., Phillips and Campbell 1961; Koski 1975; McNeil 1966; Wickett 1954; Coble 1961). In general, intragravel dissolved oxygen levels in spawning bed can be assumed to be below saturation and lower than in the hatchery water supplies.

A more cogent consequence of developmental rate differences is the potential calculation error with size conversion procedures. In this study, size on stage of development regression models have a built-in compensatory effect for rate differences, i.e., a fast growth rate regressed on a fast development rate may be

proportional to a slow growth rate regressed on a slow development rate. Thus, even if developmental rates of two treatments differed, calculated size conversion factors (size unit per developmental index unit) may be approximately equal. Using data from this study as an example, developmental rate of the Heath fish was significantly faster than the gravel fish, but very similar size conversion factors of 1.62 and 1.89 were calculated for Heath and for gravel fish respectively (Appendix 3). Size conversion for the gravel test cell fry using either one of the two calculated slopes would have produced very similar converted sizes, thus the conclusion of no size difference between test cells would be the same and the differences in developmental rates were compensated successfully by the regression model. If, however, equal developmental rates were assumed for all test cells, and no size conversions were implemented before size comparison, the analysis would have yielded a significant size difference, which would have been erroneous. This illustrates the potential errors when size is evaluated without proper consideration of developmental rates and the relative stage of development of sampled fry.

When the gravel test cells were compared with the Heath incubator, fry quality differences were large and succinct, i.e., 28% reduction in dry and wet weight, occurrence of yolk sac malformation and higher alevin mortality, accelerated developmental rate, and

depressed post-emergence growth. The reason for these differences can not be ascertained since conditions in the Heath incubator are different in many respects from the gravel test cells. The higher mortality rate of the Heath also precluded direct comparison of the Heath and gravel incubator populations. It can be postulated, however, that unfavorable conditions within the Heath may include higher flow rate, higher siltation in the water, higher exposure to subdued light, and lack of supporting substrate. The significance of each factor can only be defined by crucial experiments. Based on the 1969 experiments, light and substrate may be the key determining factors in the observed quality differences.

In the post-emergence growth comparison, differences between Heath and gravel fish in growth rate and in frequency of stunted and pinheaded fish may be caused not only by factors during incubation, but also by possible retardation of initial feeding. Palmer et al. (1951) found that pink and chinook fry fed when their ventral yolk slits were closed or were almost closed experienced a lower growth rate, higher frequency of stunted fish, but no difference in mortality when compared to fish fed at an earlier stage of development. Hurley and Brannon (1965), however, found that initial feeding of sockeye salmon can be withheld for up to 7 days after total yolk absorption before significant reduction of growth is observed. Both studies concurred that given differences in developmental rate within any population,

waiting until all fry were buttoned will mean that some fry will experience a period of starvation. In this study, Heath fry were approximately 3 days more matured than gravel fry as estimated from the difference in developmental index of 0.46% dry weight. From visual inspection, 75% of the fry from both groups were buttoned, and the more mature status of the Heath fry was not obvious. Nevertheless, it is possible that a larger proportion of the Heath fry experienced starvation thus leading to the feeding difficulties. Ultimately, the poor growth exhibited by the Heath fry was probably caused by an accumulation of many unfavorable incubation treatments and can be more appropriately classified as another index of generally poor fry quality.

In summary, under the experimental conditions of this study, water velocities of 50 to 100 cm/hr and stocking densities of 1.29 to 2.58 pink salmon eggs per cm^2 produced large fry without accelerated developmental rates. Increasing velocity to 300 cm/hr or density to 5.16 pink salmon eggs per cm^2 acclereated developmental rates without affecting fry size; some increase short of these upper levels may produce no developmental rate increase, however. In comparison, a Heath incubator operated at 5 gpm and at 3.2 eggs/cm^2, but without a gravel substrate and with exposure to subdued light, produced fry of inferior quality by all criteria.

Finally, application of these experimental results from small

gravel cells to production scale Netarts Bay incubators may not be direct because of two considerations. First, expansion of small scale experiments to large scale production invariably encounters scale transition problems which must be identified and resolved. Second, the upwelling flow of the test cells differs from the lateral flow of the Netarts production tanks, and effects of flow pattern variations must also be identified and considered.

C. Effects of Substrate, Light, and Incubator Design on Pink and Chum Salmon

Further laboratory experiments on effects of some gravel incubation design parameters were conducted with 1971 brood year fish. The previous two years of experiments yielded data on the need for a gravel substrate, comparisons between incubator designs, effects of stocking density and water velocity, and development of comparative fry quality testing procedures. The final year addressed remaining parameters defined in the stated objectives and explored cogent questions which developed during the course of the previous experiments.

To formulate the objectives and design of the present experiments, the following points were considered:

(1) Data from the substrate and incubator design experiments had been complicated by exposure to some degree of light.

(2) The effects of gravel depth, i. e., subsurface or surface incubation--a key design parameter--had not been investigated for pink and chum salmon. A previous study using steelhead eggs found no difference between the two methods (McNeil 1968); but the lack of correlation of size with stage of development and the existence of species difference in salmonid incubation requirements found in this study, and in work such as by Emadi (1972) necessitated a re-evaluation.

(3) Incubator design was tested in previous experiments, but a simultaneous comparison of the major incubator designs, specifically, the trough, Heath, and upwelling box designs, had not been done. The trough and Heath incubators are commonly used by conventional hatcheries. If provision of a substrate improved the quality of fry produced, savings could result should gravel incubation technology become a standard approach.

(4) More comprehensive information was needed to follow up on the comparative quality testing procedures already developed.

Considering the above points, the objective of this experiment was to investigate the effects of the following gravel incubator design variables on fry quality: substrate (smooth or screen, shallow gravel matrix, deep gravel matrix), incubator design (trough, Heath, upwelling box), and exposure to light (none, exposed). Stocking density was kept low--at one loose layer or less--to eliminate

influence on the other variables.

Two studies were completed at the Netarts Bay Swanson Aquaculture Laboratory, one using pink salmon eggs from Little Port Walter, and one using the native chum salmon eggs from Netarts Bay.

For the pink salmon study, light was excluded totally from all incubators. Two experiments--A and B--were conducted with the following design.

Experiment A investigated the variables of substrate and incubator design with a stocking density of 1.72 eggs/cm^2. The treatments were:

SUBSTRATE	INCUBATOR DESIGN	
	Heath	Trough
Screen	3 cells	3 cells
Shallow gravel matrix	---	3 cells
Deep gravel matrix	---	3 cells

Experiment B was a subsidiary experiment to investigate the feasibility of providing a gravel substrate in the Heath incubator tray. Surplus eggs from experiment A were divided into four lots of 2900 eggs each (2.27 eggs/cm^2)--two lots were placed into two trays without gravel and two lots into two trays lined with a single layer of gravel. Because of the subsidiary nature of this experiment, replicates were treated as single test cells during sampling. This procedure minimized sample numbers at the expense of variability

assessment. The intent was to use the triplicates of experiment A to provide information on expected variations within treatments.

For the chum salmon study, a single comprehensive experiment was conducted to test the variables of incubator design, substrate, and light in the following design:

Substrate	Incubator design and exposure to light			
	Upwelling Box	Heath		Trough
	Exposed[6]	Exposed	Not Exposed	Not Exposed
Smooth or screen	--	1 cell	2 cells	2 cells
Shallow gravel matrix	---	1 cell	2 cells	2 cells
Deep gravel matrix	2 cells	---	---	---

The stocking density was kept constant at 1.2 eggs/cm^2 for all test cells.

Both the pink and chum study extended previous efforts in developing fry quality testing procedures by conducting performance tests for the first time.

In the pink salmon study, incubator clogging and alevin movements negated the trough treatments of experiment A. The cause of the problems was corrected for the subsequent chum study.

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[6]Since alevins incubated beneath the surface of the substrate, they were not exposed to light striking the substrate surface.

1. General Procedures

a. Pink Salmon Study

Two types of incubators were used in this experiment: the previously described Heath incubator and a new trough incubator. The trough incubator, which resembled a conventional hatchery trough, consisted of two 305 cm X 81.3 cm X 30.5 cm troughs, each separated into two channels for a total of four channels. Each channel was constructed from 5.1 cm X 30.5 cm lumber and divided by baffles into an inflow compartment, three 50.8 cm X 33.0 cm X 30.5 cm test cells, and an outflow compartment. These compartments were screened off from each other. Lateral and upwelling flow patterns were created through each of the test cells by baffles. Water flowed from the head tank into each trough via individual lines equipped with control valves. Surface area of each test cell (1355 cm^2) was designed to be about the same as the Heath tray.

To create the substrate treatments, 2.5 cm wooden-framed egg trays lined with fine mesh hardware cloth were placed in each test cell but elevated 1.3 cm off the bottom to allow water to upwell through each tray. An unlined tray provided the screen treatment; a tray lined with a single layer of 0.64 cm to 1.9 cm crushed rocks provided the shallow gravel matrix treatment; two single layers of crushed rocks with the eggs stocked in between constituted the deep

gravel treatment or subsurface gravel incubation. Light was totally excluded from all test cells by a black plastic cover.

Eggs were obtained from Sashin Creek at Little Port Walter. On September 9, about 52, 500 eggs (30 females, 20 males; about 1750 eggs/female) were transported to Netarts Bay and fertilized using the delayed fertilization method (cf. Poon and Johnson 1970). Eyed eggs were used to stock the experimental incubators on October 20, 1971.

Nine of the 12 test cells available and the Heath incubator were used for two experiments on substrate and incubator design. The non-random allocation of the three substrate treatments for the test cells was:

In Flow Compartments	Test Cells			Out Flow Compartments
→	#10 not used	#11 not used	#12 not used	→
→	#7 Deep gravel substrate	#8 Shallow gravel substrate	#9 Screen substrate	→
→	#4 Shallow gravel substrate	#5 Screen substrate	#6 Deep gravel substrate	→
→	#1 Screen substrate	#2 Deep gravel substrate	#3 Shallow gravel substrate	→

Diagrammatic top view of troughs

Water flow to both the trough and Heath incubator was set at about 15 liters per minute and was adjusted every several days as necessary. Water temperature ranged from 1.1°C to 13.4°C with an average of 7.2°C (cf. Appendix 11).

There were problems with clogging of the fine mesh screen of the egg trays and the escape of alevins from test cells. Alevins were able to squeeze through small crevices despite screens between compartments. The stocked eggs reached 50% hatching on about November 6. By November 15, a small number of alevins were observed to have escaped. Substantial escapement of alevins was confirmed by November 7, invalidating the substrate comparisons between the treatments in each trough.

Many deep gravel fish (estimated at 10% of the treatment populations), previously buried, had moved out onto the surface of the substrate; these fish along with many fish from the shallow gravel treatment penetrated beneath the trays and onto the smooth trough bottom.

Concurrent with the alevin escapement, heavy silt in the hatchery water began to clog the fine mesh screen of the test cells; clogging was accentuated in gravel-lined trays. As a result, the water flow fluctuated widely between troughs, varying between 9.5 to 15 lpm.

Because of clogging and alevin escapement, modification in the design of Experiment A was made on December 3, 1971. All screen

trays of the non-gravel treatment were removed from the troughs and all alevins not on or in a gravel substrate were siphoned out of the trough and excluded from the experiment. Left in the trough were the shallow or deep gravel alevins. Since accounting of the number of alevin remaining in each gravel cell could not be done, the integrity of the original stocking density was lost; the remaining fish were now treated as "gravel" fish (without differentiation for gravel depth) incubated under a variable flow velocity and stocking density.

The effects of the heavy mineral silt load on the behavior of the incubating alevins were not assessed. It was assumed that adaptive mechanisms such as reverse coughing and mucous cleaning of the gills were used by the fry to cope with the problem (cf. Bams 1969; Stuart 1953).

Within the limitations of the experiments, fry sampling was conducted for the following treatments:

Experiment A: Heath tray No. 1, Heath tray No. 2, Heath tray No. 3, Trough No. 1, Trough No. 2, and Trough No. 3.

Experiment B: Heath trays No. 4 and No. 5 (treated as one cell) Gravel lined Heath trays No. 6 and No. 7.

During the period of terminal yolk absorption, December 14, 1971 through January 14, 1971, five random samples of 30 fry each were taken at approximate weekly intervals from each of the above

treatments.

The number of valid treatments available for evaluation was limited by partial negation of trough incubator treatments in Experiment A. In hindsight, the rationale for sampling trough "gravel" fish was determined to be unjustifiable because the test populations were under suspect after the non-random removal of non-gravel fish. This judgement, unfortunately, means that replicates in Experiment B should have been sampled as replicates rather than as (combined) single cells.

In reorganizing the data for fry quality evaluation, all trough treatments were eliminated from consideration and Heath incubator treatments of experiments A and B were combined into three treatments as follows:

Density (eggs/cm^2)	Screen Substrate	Shallow Gravel Substrate
1.72	3 cells	
2.27	2 cells (combined as one)	2 cells (combined as one)

The minor density difference was not expected to generate observable quality differences; rather, the triplicate cells were used as a measure of variability within treatments.

Regression data needed for fry size and stage of development evaluation was taken from length, wet weight, and kd data generated from all Heath treatments. Since treatments differed in provision of

a gravel substrate, possible developmental rate differences were investigated but no discernible differences were found. Consequently, all treatment data were combined to generate one set of regression models.

Regression models did not include dry weight and % dry weight during the 1971 brood year due to a problem with oven drying of the samples which invalidated the results. Size and development evaluation, therefore, was limited to length and kd data.

Comparative fry quality testing procedures used in this study did not include swimming stamina test nor the post-emergence growth test, but anoxia tests were conducted for the first time.

Two anoxia tests were conducted 7 days apart--one in freshwater at the Netarts Swanson Aquaculture Laboratory, and one in salt water at the Newport Marine Science Center. Based on pilot testing, procedures were adopted which tested triplicate samples of 50 fry from each treatment. Test fry were exposed to air for 30 minutes in paper-towel lined, perforated, gallon-size plastic buckets. After the exposure period, buckets and fry were placed into aerated water and survivals assessed after a 24 hour recovery period. A control was run without exposure to anoxia.

In order to test the effects of substrate only, the three treatments were reduced to two--Heath, screen substrate (at 1.72 and 2.27 eggs/cm^2) and Heath, shallow gravel substrate (at 2.27

eggs/cm^2). Fry used for the first test on December 29 had not reached total buttoning and some differences were detected between treatments in the stage of development. By the time of the second test on January 5, however, the difference in development had diminished to a visually undetectable level. Unfortunately, the second test was conducted after the fry had been converted to salt water only three hours earlier--the stress from anoxia combined with osmotic stress produced nearly 100% mortality for all three test groups. Therefore, data for the second test had to be deleted.

b. Chum Salmon Study

Incubation systems included the previously described Heath and trough incubators, plus a new upwelling basket. Use of the trough incubator was modified in response to the siltation problems encountered during the pink salmon study. Instead of providing three substrate treatments within the trough, only two were provided--the smooth substrate and the shallow gravel substrate. The deep gravel substrate was provided in upwelling baskets rather than within the trough.

All four channels of the trough incubator were utilized in this study, but only the front test cell was used in each channel. The substrate treatments were affected on the trough bottom itself, without the use of trays. The smooth substrate was simply the

unlined bottom, whereas the shallow gravel substrate was a single layer of crushed rocks. The deep gravel substrate was provided in a plastic upwelling basket with a false bottom 32.4 cm in diameter (826 cm^2 surface area) and lined with 5-inches of crushed rock. Flow to the baskets was regulated by a hose clamp on the incoming water line.

To set up the light X substrate treatments in the Heath incubator, another Heath incubator body was constructed of wood to provide the light exposed housing for the two substrate treatments of screen and shallow gravel matrix. The original Heath housing was blacked out totally for other Heath incubator treatments. The amount of light which penetrated the "exposed" Heath housing was primarily indirect natural light coming in at a nearby window, but artificial light was also present whenever the hatchery lighting was on. During the course of this experiment, use of artificial lighting in the hatchery room was minimized.

Eggs were obtained from one day's spawning at Netarts Bay on November 16, 1971. These eggs were eyed in general hatchery boxes and then stocked eyed in the test incubators. The November 16 egg take consisted of 21 females and 21 males for a total of about 52,500 eggs, of which 18,000 eyed eggs were used for this study.

Experimental cells were stocked on January 18, 1972, based on the previously established design. Since substrate area of the test cells differed--the Heath and trough cells being larger than the

upwelling box cell by 1.64 to 1.00--the stocking density and water flow were adjusted to equivalents as follows:

Incubator	Density		Flow Rates	
	# eggs stocked per cell	eggs/cm^2	Volume lpm	Velocity $cm^3/hr/cm^2$
Heath	1600	1.18	18.9	838
Trough	1600	1.18	1.0	44
Upwelling Box	1000	1.21	0.60	44

Flow to the trough and upwelling box was lowered to discourage alevin movement experienced in the pink experiment. The Heath incubator, however, was maintained at its recommended 18.9 lpm (5 gpm) flow.

To maintain the experimental conditions, flow rates were periodically checked and adjusted when necessary. Mortalities were checked by a very brief look under the test cell covers, but since dead alevins were very rare no picking was necessary except with the Heath, screen substrate, exposed treatment. Temperature records were kept as in the pink study (cf. Appendix 11).

The clogging and alevin movement problems which occurred in the pink salmon study did not occur in this study; flow fluctuation was, therefore, minimal. As in the last study, however, many alevins in the deep gravel treatment moved to the surface of the gravel during incubation, partially negating the sub-surface deep

gravel incubation treatment. As early as February 7, 1972, about 100 alevins (10% of the population) were observed above the substrate. These alevins did not move into the water column and out of the incubator, but when they migrated out of the cell near the fry stage, they were trapped, enumerated, and accumulated for quality testing.

Fry were sampled from two types of migration patterns--volitional and non-volitional. Except the deep gravel cells, which had volitional migration, all test incubators fall in the non-volitional category.

To evaluate size and stage of development, 30 fry were taken from each test cell on two dates: March 14, and March 18, 36 and 40 days after hatching. For the two deep gravel test cells, samples were taken from cumulations of the migrated population of about 68% and 70% respectively--the last 30% of the deep gravel population was not represented in the size and stage of development evaluation, but these fry were represented in subsequent performance testing.

Fry quality evaluation in this study was the most thorough of the entire study. Data on every fry quality index was gathered except for post-emergence growth. Due to time constraints, however, performance testing was conducted without the Heath, shallow gravel, exposed treatment.

Conversion data for size and stage of development evaluation was generated from chronological samples taken from two

treatments--(1) the Heath, shallow gravel, unexposed treatment and (2) the Heath, screen, exposed treatment--over 18 days surrounding the time of maximum fry weight (March 10 to March 28). Regression lines were calculated using March 14 and March 18 (two sample dates) data points as the pivot to put the maximum precision on length conversion (cf. p. 26). As discussed under the Pink Salmon Study, complications with oven drying procedures precluded the use of dry weight and % dry weight data.

Both anoxia and swimming performance tests were conducted successfully at the Newport Marine Science Center using random samples of fry transported from Netarts Bay to Newport on March 18. At the time of testing, all treatment groups were at a similar stage of development as judged by visual observations.

The anoxia test was run in freshwater on March 26, using the apparatus and methods previously employed for the pink salmon study. Based on pilot testing with chum salmon fry, procedures were established which would test replicate 30 fry samples from each treatment. The fry were exposed to air for seven minutes, and survivals were assessed after a 24 hour recovery period.

The swimming stamina test was conducted in a swimming tube apparatus described by Butler and Millemann (1971). The fish were confined in a transparent plexiglass tube 108 cm long and 10 cm inside diameter. Two circular plastic screens and one wire screen, each

with a different mesh size, were attached inside the tube at the front end to smooth out the flow pattern. A movable plastic screen was placed near the downstream end to allow introduction and removal of the fish. Water flow was generated by a cast iron centrifugal pump and was controlled precisely up to a maximum flow of 87 cm per second with a diaphragm valve. A black plastic cover was placed over the central section of the tube and bright lights were directed at both ends to help confine test fish within the middle section (which had the most uniform flow pattern), eliminate influence from outside movements, and discourage premature dropout of fatigued fish.

Pilot testing was conducted from March 19 to March 25 to determine the ideal step acceleration schedule, the number of fry per test, and the best method of handling and rating stamina. It was recognized that procedures established must accommodate the maximum number of test runs within the limited time frame when unfed test fry were at their peak swimming performance, i. e., near total closure of the ventral slit.

The established procedures tested a sample of 50 fry according to a step acceleration water velocity schedule (Appendix 10) which allowed an initial 30 minutes of acclimation at a low velocity (7. 71 cm/sec) before the velocity was increased at an increment of 4% maximum flow rate every five minutes. Test fry were placed into the swimming tube using a funnel and large diameter plastic tubing,

and the velocity schedule was initiated immediately. As exhausted fish were pinned against the back screen and could not swim away with agitation of the screen, they were considered fatigued and removed from the tube; the time interval was recorded and the fish were preserved in 10% formalin in one of three bottles which separated the first 10, the middle 30, and the last 10 fish. The separation was not perfect as more than one fish may drop out at a time. After the fortieth fatigued fish was processed the test was terminated by flushing the remaining fish from the tube with a burst of high velocity. The fry in the middle 30 group were later measured for length, weight, and Kd developmental index as support data with which to evaluate the swimming stamina as well as another evaluation of fry size between treatments. The first 10 and the last 10 fish were excluded to minimize the expected variations in test populations.

Swimming stamina was rated in minutes of endurance and was derived as an average of two ratings from each test. Since "time" was recorded at five minute intervals, rating calculations were based on the midpoint of the given interval, e. g., midpoint of the 85-90 minute interval is 87. 50 minutes. Accordingly, the two ratings of each test consisted of (1) the time of the 25th fatigued fish and (2) the median between the "time" of the 11th and 40th fatigued fish. The average of these two ratings balanced a rating based on a single fish (25th fish) with a rating assigned to all 30 fish.

Actual testing took place over a four day period from March 26 to March 29. Eight separate tests were run on six treatment groups, with duplicate tests on two groups to assess expected variability within treatments. Water temperature, dissolved oxygen level, and water exchange rate of the apparatus were maintained as constant as possible.

2. Results

a. Pink Salmon Study

The total elimination of light from the Heath incubator trays reduced the mortalities to a negligible amount, estimated at less than 5% for all trays.

From inspection of random samples of 69 to 117 alevins taken from each tray 19 to 30 days after hatching, no yolk-sac malformation was found (Table 7) General observations during the course of the study also failed to detect any malformations. However, after some alevins were held for several days in a bucket exposed to light and devoid of a gravel substrate, up to 5% of the alevins developed mild malformations.

No significant difference in size and stage of development was detected between fry from gravel and screen substrates. Regression calculations used for data analysis are tabulated on Appendices 12

Table 7. Occurrence of yolk-sac malformation (YSM) among random samples of treatment groups of the 1971 brood year pink salmon laboratory study, experiments A and B.

Date of sample	Incubator	Substrate[1/]	N	# alevins[2/] with YSM	% of alevins with YSM
11/25/71	Heath	gravel	125	0	0
11/25/71	Heath	screen	140	0	0
12/05/71	Heath	gravel	69	0	0
12/05/71	Heath	screen	77	0	0
12/03/71	Trough	screen	189	0	0
12/03/71	Trough	screen	408	14	3.43[3/]
12/05/71	Trough	gravel	132	2	1.51[3/]
12/08/71	Trough	gravel	60	1	1.66[3/]

1/ gravel substrate is single layer of crushed rock.

2/ malformation was very mild.

3/ alevins had been removed from test cells and placed for several days in flat bottom holding tanks exposed to subdued natural light.

and 13. The size conversion model of average length plotted on average Kd value (Figure 17) showed no discernible developmental rate difference between treatments, thus all data were combined to derive one set of regression models for all size conversion procedures.

Based on the expected within treatment variation determined from the triplicate screen substrate treatment at 1.72 eggs/cm^2, assessment of the pooled treatment means resulted in no difference in size or stage of development between fry from the gravel and screen substrate treatments at 2.14 eggs/cm^2 (Table 8). Difference in converted mean lengths between the higher density gravel and screen treatment was 0.288 mm compared to the within treatment variation of 0.532 mm between the triplicate lower density screen treatments. Moreover, ranking of the converted lengths placed the gravel treatment as rank #2 between the non-gravel treatments (Table 8).

Unconverted mean stage of development (Kd value) of the gravel treatment was about 7 days (0.0230 Kd units) less advanced than the comparable screen treatment at 2.27 eggs/cm^2. The within treatment variations of the triplicate, however, were about 8 days (0.0256 Kd units), with the most to least developed of trays #3, #2, and #1 respectively. It is not known why the variation between and within treatments was large.

Results of the anoxia test showed that gravel fish consistently

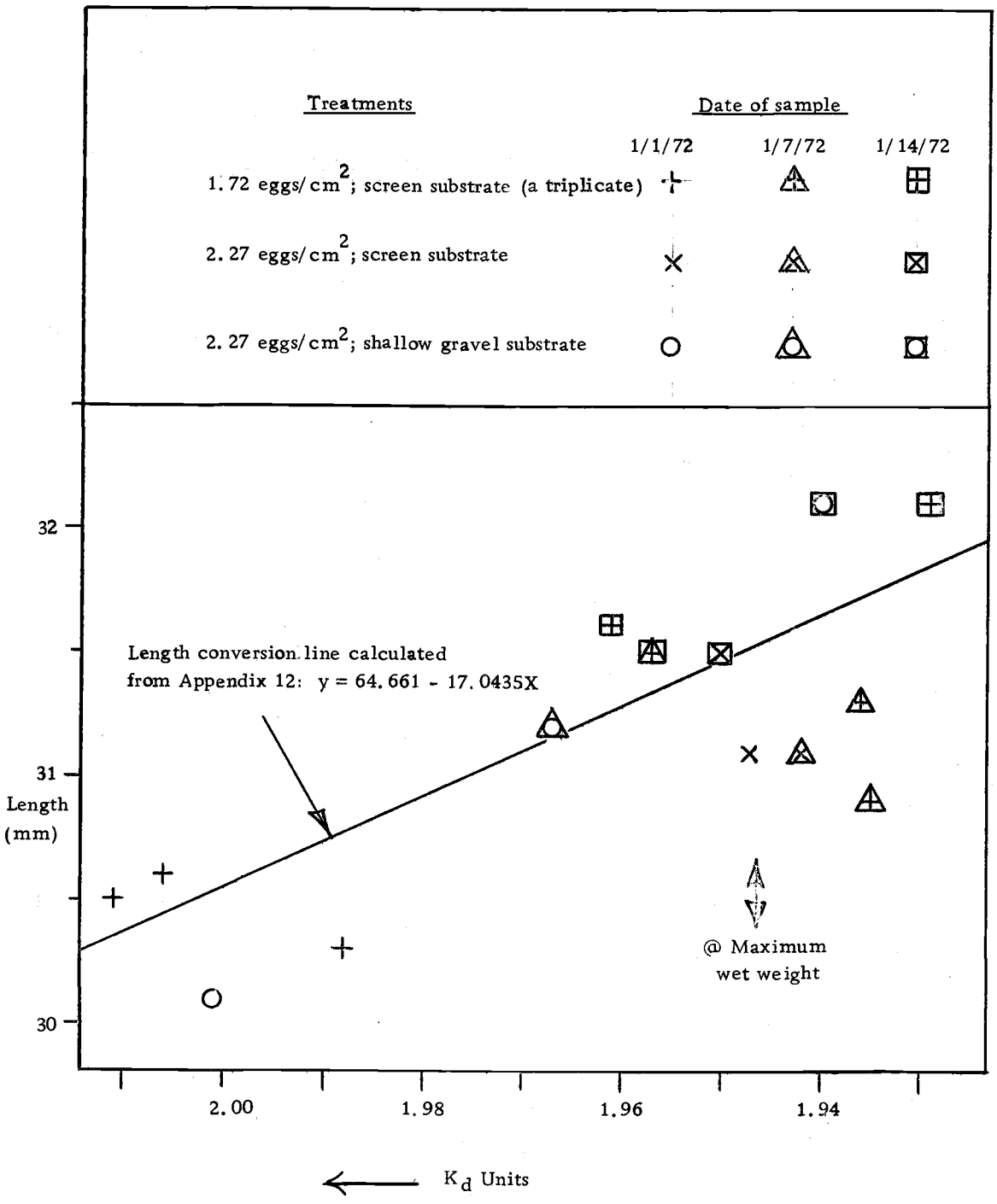

Figure 17. Relationship between length and K_d of fry sampled on three dates during the terminal yolk absorption period for three treatments of the 1971 brood year pink salmon laboratory study, experiments A and B. Each point is an average of 30 fry.

Table 8. Ranking of average[1/] size (length) and stage of development (Kd) for three treatments of the 1971 brood year pink salmon laboratory study, experiments A and B.

Size ranking (longest to shortest)					Stage of development ranking (most to least developed)			
		Length						
Rank	Treatment[2/]	After[3/] conversion	Before conversion		Rank	Treatment	Kd units	
		$\bar{X}$	$\bar{X}$	$S^2_{\bar{X}}$[3/]			$\bar{X}$	$S^2_{\bar{X}}$[4/]
		mm	mm	$\times 10^{-3}$ mm				$\times 10^{-5}$
1	1.62 eggs/cm^2, screen substrate (#1)	31.453	31.20	12.0	1	2.14 eggs/cm^2 screen substrate	1.9463	2.22
2	2.14 eggs/cm^2, gravel substrate	31.265	31.13	10.1	2	1.62 eggs/cm^2, screen substrate (#3)	1.9507	2.00
3	1.62 eggs/cm^2, screen substrate (#2)	31.214	31.13	10.0	3	1.62 eggs/cm^2, screen substrate (#2)	1.9663	2.67
4	2.14 eggs/cm^2, screen substrate	30.977	31.23	14.3	4	2.14 eggs/cm^2, gravel substrate	1.9693	2.44
5	1.62 eggs/cm^2, screen substrate (#3)	30.921	31.10	9.9	5	1.62 eggs/cm^2, screen substrate (#1)	1.9763	1.78

1/ Each mean is pooled from three samples of n=30 fry each taken over the terminal yolk absorption period from 1/1/72 to 1/14/72.

2/ One treatment; 1.62 eggs/cm^2, screen substrate, is a triplicate.

3/ Conversion equation: Converted length = Unconverted length - [(observed Kd - 1.9613) (-16.86107)] See page 26 for discussion of formula parameters.

4/ $S^2_{\bar{X}} = V\left(1/3 \sum_{i=1}^{3} \bar{X}_i\right) = (1/3)^2 [V(\bar{X}_i)]$ where $V(\bar{X}_i) = \frac{S^2_{X_i}}{30}$

had higher survival than screen fish (average of 88.7% versus 71.3%), but the ability to survive was decidedly affected by the stage of development (Table 9). Twenty-eight percent of the gravel fish had closed ventral slits versus 47.3% for the screen fish. Moreover, inspection of the live and dead fry after the experiment showed that a higher proportion of the dead fish were buttoned. Thus, the less advanced fish were apparently less likely to succumb to hypoxic conditions than the more advanced fish, and the higher survival of the gravel fish may be caused by their less developed status or by their higher capacity to withstand test conditions.

b. Chum Salmon Study

Of all experimental treatments, only one treatment--the Heath, screen substrate, exposed treatment--experienced significant mortality. Approximately 500 alevins were removed from the tray of 1600 eggs for a mortality rate of 31%. All other cells had less than 1% mortality.

Alevins from the Heath tray exposed to light and lacking a gravel substrate exhibited 91.1% yolk-sac malformation; whereas, the remaining cells had 0 to 5.1% yolk-sac malformation (Table 10). The severity of malformation varied: the screen substrate, exposed Heath fish exhibited mild to extreme cases with a large proprotion of extreme cases; in contrast, malformation in all other treatment groups was very mild, i.e., only a slight elongation of the yolk sac. Deep gravel fish were not evaluated for malformation

Table 9. Results of anoxia test conducted on 12/29/71 for two combined Heath incubator treatments of the 1971 brood year pink salmon laboratory study, experiments A and B. Each treatment tested with triplicates of 50 fry each.

Substrate	Percent surviving test		Stage of development as indexed by % with closed ventral slits		
	Overall	Per triplicate	Mortalities	Survivors	All fish
	%	%	%	%	%
Gravel	88.7	90	40	20	22
		88	67	27	32
		88	67	27	30
Screen	71.3	66	76	42	54
		72	71	39	48
		76	67	32	40

Table 10. Occurrence of yolk-sac malformation (YSM) among random samples of treatment groups of the 1971 brood year chum salmon laboratory study. Samples taken on 2/21/72.

Incubator	Substrate[1/]	Exposure to light	N	# Alevins with YSM	% of alevins with YSM
					%
Heath	screen	yes	45	41	91.1
Heath	gravel	yes	48	0	0
Heath	screen	no	36	0	0
Heath	gravel	no	32	0	0
Trough	smooth	no	39	2	5.1[2/]
Trough	gravel	no	40	1	2.5[2/]

1/ Gravel substrate is single layer of crushed rock.

2/ Malformation very mild.

since most alevins were within the substrate and were not accessible for sampling.

The chum salmon study provided the most comprehensive data to evaluate the effect of environmental parameters on size and stage of development. Exposure to light with screen substrate caused a reduction in fry size, and simultaneous exposure to light and high velocity were associated with accelerated development on both gravel and screen substrates. Length, wet weight, and Kd data (Appendix 14) generated for regression calculations were representative of the expected changes with time of these parameters during the terminal larval stage. The approach of wet weight to a maximum is not precise and often difficult to pinpoint (Figure 18). Length and Kd, however, both approximate a linear function, particularly near the period of maximum wet weight (Figures 19 and 20). The size conversion model, length regressed on Kd, was also slightly curvilinear (Figure 21) but quite suitable for a least square fit. To maximize precision on size conversions, all least square lines were determined using the two sample dates (36 and 40 days after fertilization) as the midpoint of the lines (Appendix 15).

Of the two treatments selected for regression calculations, the Heath, screen substrate, exposed group, developed at a slightly faster rate than the Heath, shallow gravel substrate, unexposed group (Appendix 15, Figure 20). This developmental rate difference,

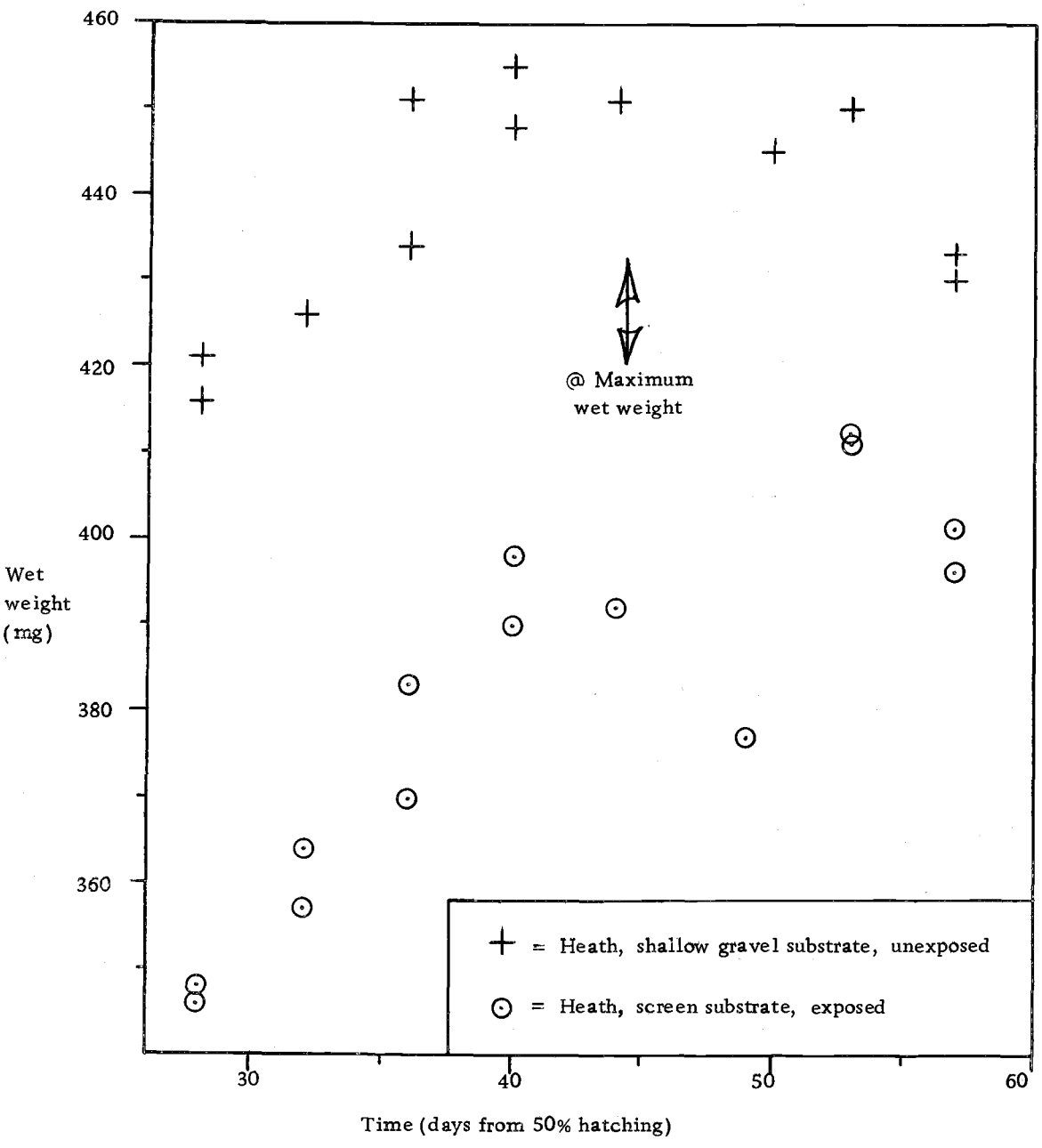

Figure 18. Relationship between wet weight and time during the terminal yolk absorption period for two treatments of the 1971 brood year chum salmon laboratory study. Each point is an average of 30 fry.

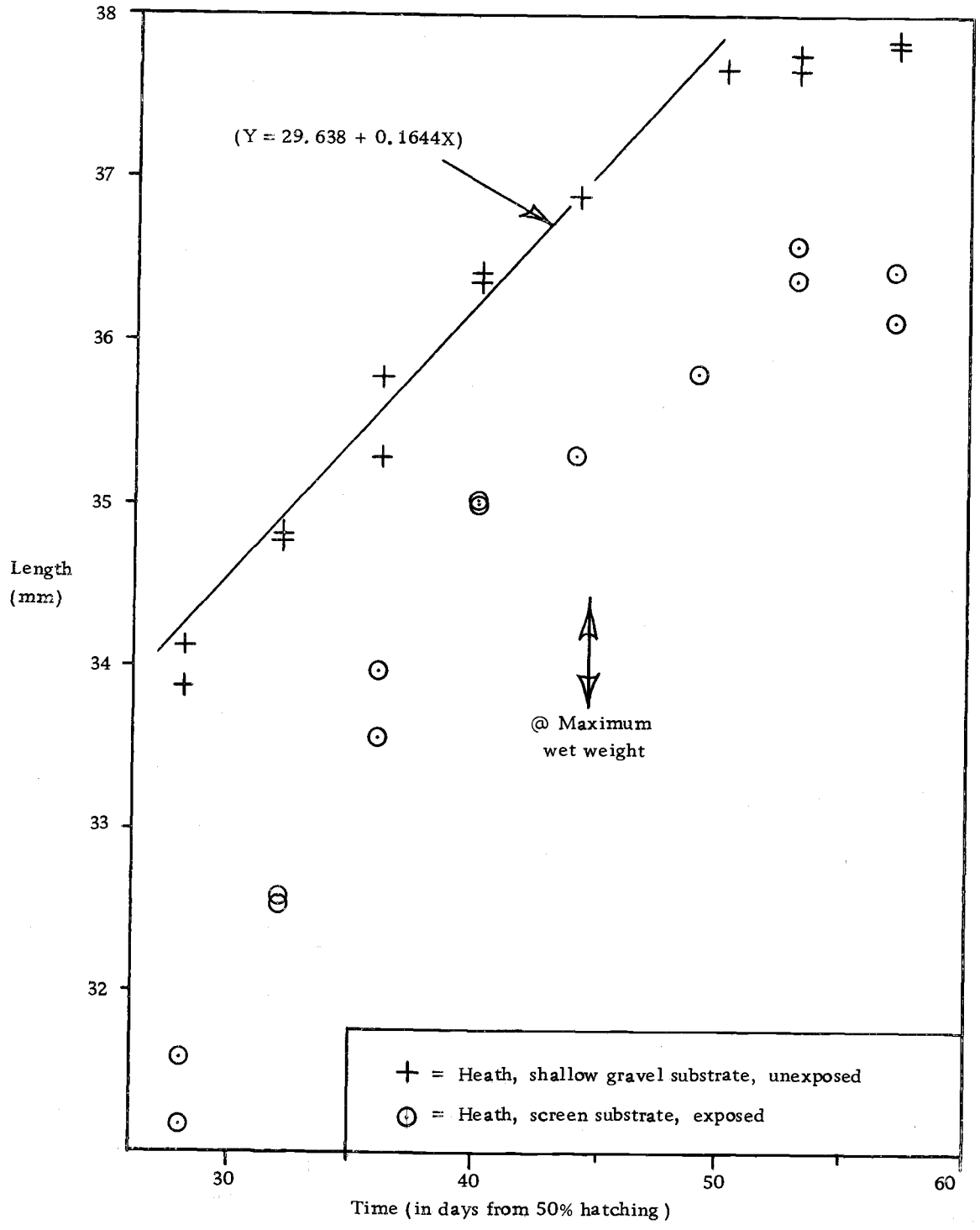

Figure 19. Relationship between length and time during the terminal yolk absorption period for two treatments of the 1971 brood year chum salmon laboratory study. (Each point is an average of 30 fry.

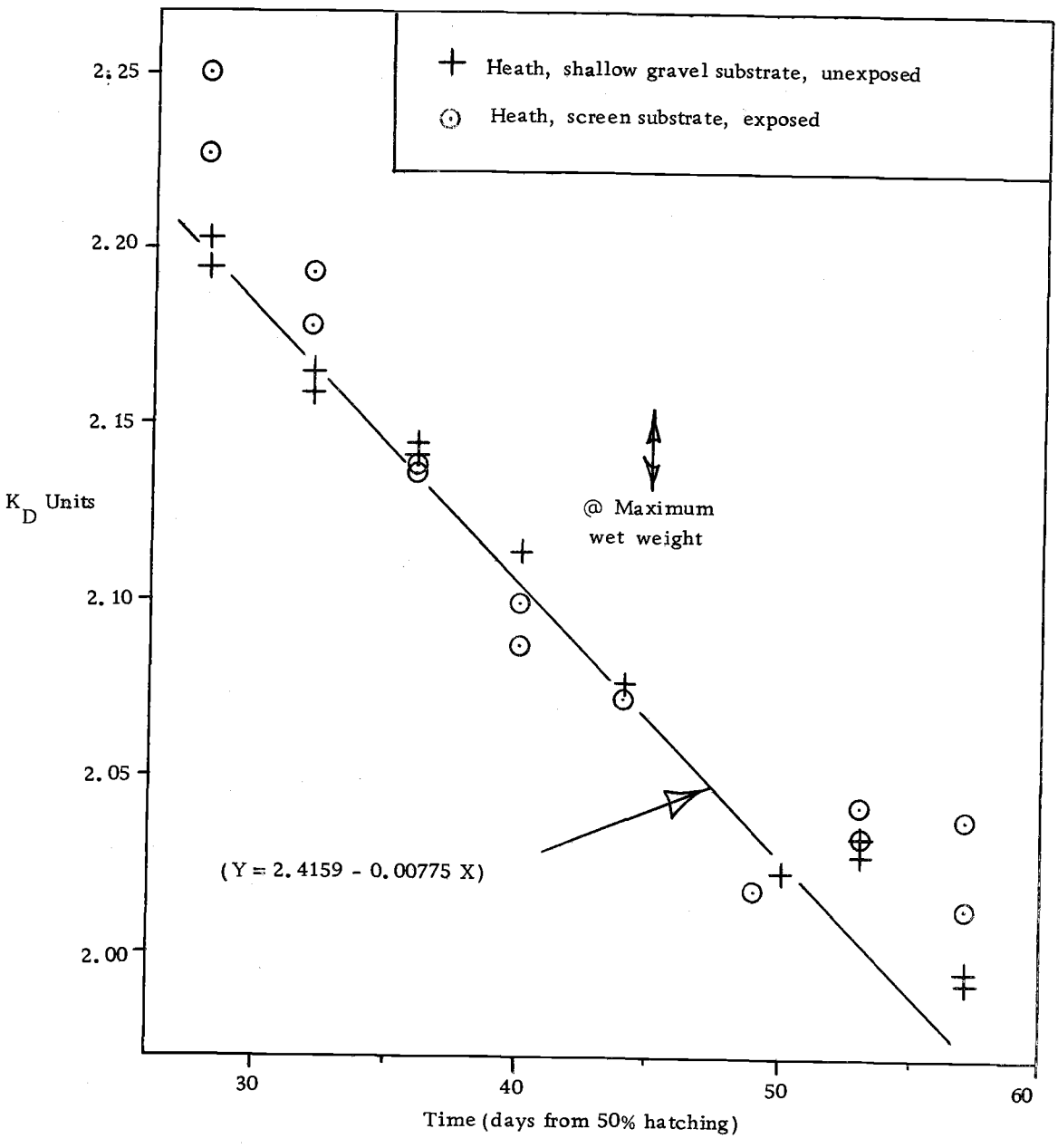

Figure 20. Relationship between K_d and time during the terminal yolk absorption period for two treatments of the 1971 brood year chum salmon laboratory study. Each point is an average of 30 fry.

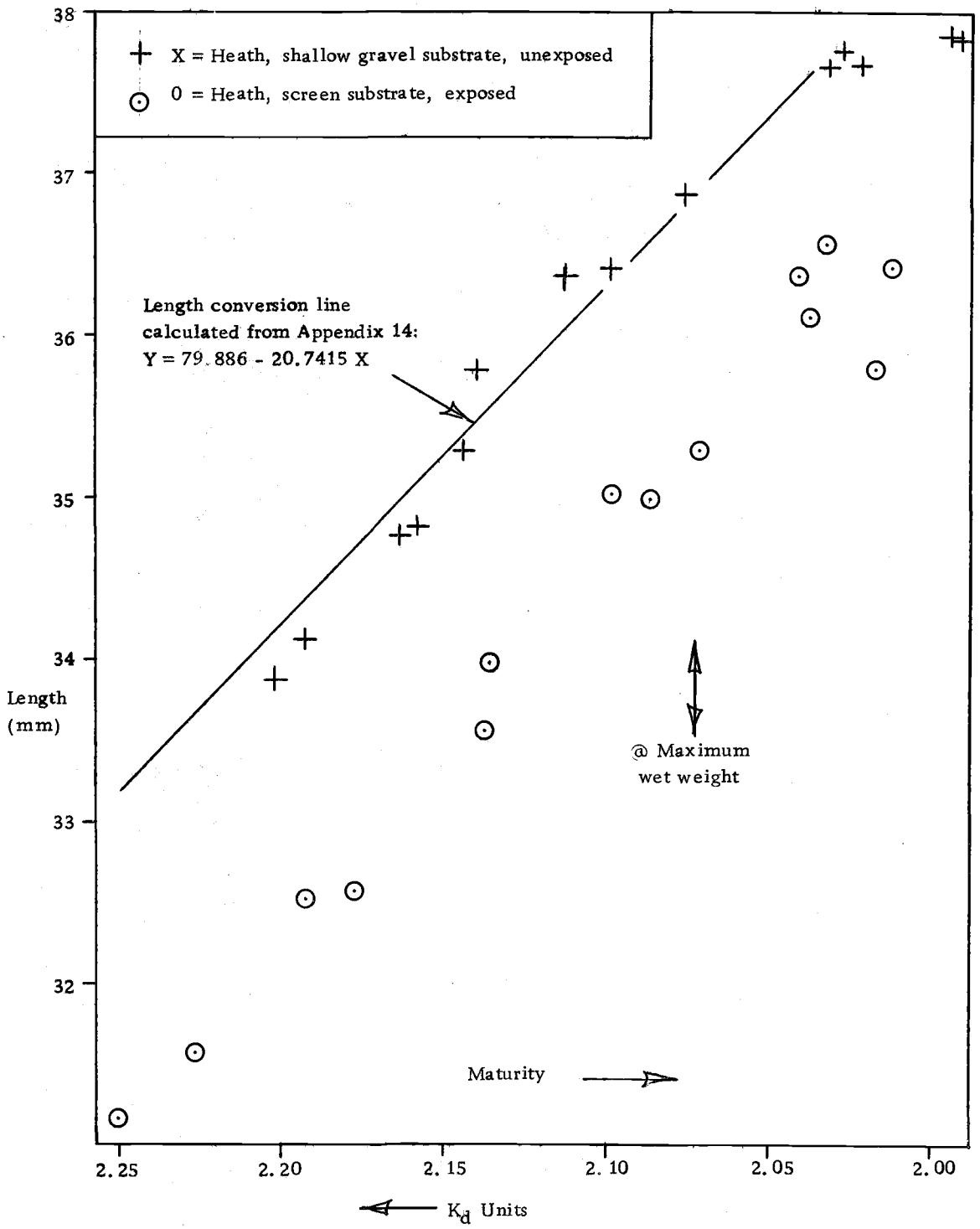

Figure 21. Relationship between length and K_d of chronological samples taken during the terminal yolk absorption period (3/6/72 to 4/4/72) for two treatments of the 1971 brood year chum salmon laboratory study. Each point is an average of 30 fry.

however, can be attributed mostly to the early data points (at 28 to 36 days after fertilization) since by the time both groups approached maximum wet weight (36 to 45 days after fertilization), their developmental stage was about equivalent (Figures 20 and 21). When length was regressed on Kd, the difference in rate was reduced by the compensatory effect of this model (cf. discussion on p. 88), and the small difference between the two groups in their length unit per Kd unit slope would mean that size conversion with either slope would result in very similar converted lengths at a common Kd value.

Of the two calculated regression lines, size conversion slopes were similar but the line calculated for the Heath, shallow gravel substrate, unexposed group transected the majority of data points plotted on the length versus Kd graph (Figure 22). Thus, the slope of this line was used for size conversion for all treatments.

Prior to assessment of relative length and Kd for treatment groups, data format was simplified by eliminating the distinction of true replicates versus repeated samples from one treatment since no difference can be discerned between the two[7] (Figure 22).

In assessing relative lengths, the ranking of all converted mean lengths showed that fry from the Heath, screen substrate, exposed treatment were clearly shorter than fry from the other treatments by

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[7]The deep gravel baskets, because of their volitional migration were excluded from this comparison.

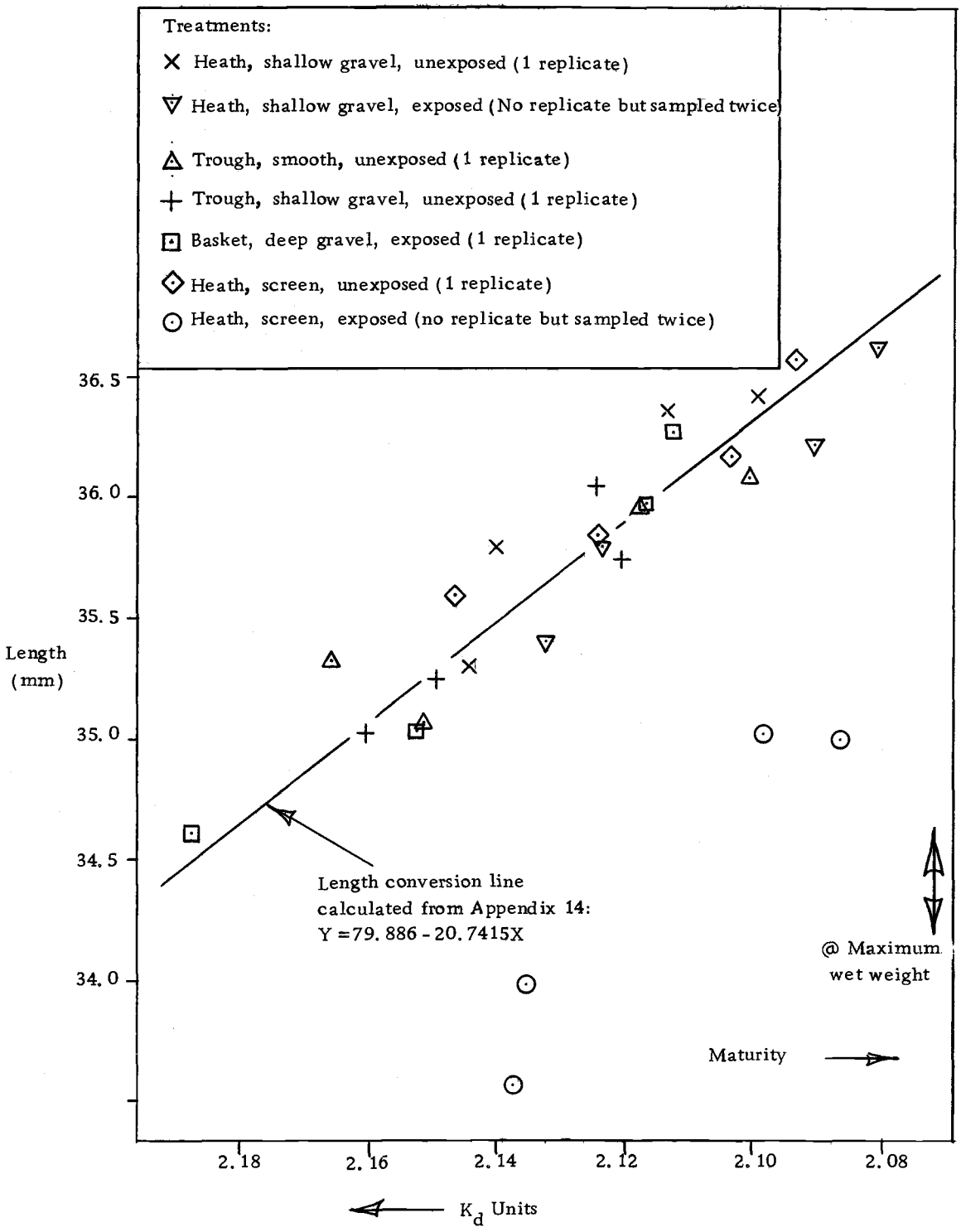

Figure 22. The relationship of length and K_d of fry sampled on two dates (3/14/72, 3/18/72) during the terminal yolk absorption period for all treatments of the 1971 brood year chum salmon laboratory study. Each point is an average of 30 fry.

about 1. 5 mm; whereas, all other treatments were separated by only 0. 356 mm (Table 11), a negligible difference relative to the size of their 2 X SE intervals (Figure 23).

For analysis of stage of development, two trends were clear from inspection of the plot of length on Kd (Figure 22): 1) the higher velocity Heath incubator treatments were more advanced than the slower velocity trough and basket incubator treatments, and 2) the two groups exposed to light--the Heath, shallow gravel substrate, exposed treatment, and the Heath, screen substrate exposed treatment--were the most advanced groups. The existence of both trends were clear on the first sample date, but the distinction was dissipating by the time of the second sample date. And, despite initial differences in development, the groups reached maximum wet weight at about the same time. This catching-up trend can be demonstrated by comparing Kd values over time for the two regression lines derived from the Heath, shallow gravel substrate, unexposed treatment versus the Heath, screen substrate, exposed treatment (Figure 20).

The basket, deep gravel substrate, exposed group, the only group with volitional migration and sampling of the most advanced fry, was the least advanced group on the first sample; if the entire population had been available for sampling, Kd measurements might have been even less advanced.

To determine whether observed developmental rate differences

Table 11. Ranking of average size[1/] (length) and stage of development[1/] (Kd) for all treatments of the 1971 brood year chum salmon laboratory study.

Size ranking (longest to shortest)					Stage of development ranking (most to least developed)			
Rank	Treatment	Converted[2/] length mean	Unconverted length mean	Unconverted length variance[3/]	Rank	Treatment	Kd mean	Kd variance[3/]
		mm	mm	X 10^{-3} mm				X 10^{-5}
1	Heath, shallow gravel, unexposed	35.973	35.975	7.790	1	Heath, shallow gravel, exposed	2.1073	1.44
2	Heath, screen, unexposed	35.895	36.053	6.503	2	Heath, screen, exposed	2.1150	1.70
3	Basket, deep gravel, exposed	35.835	35.468	7.757	3	Heath, screen, unexposed	2.1175	1.57
4	Trough, shallow gravel, unexposed	35.815	35.520	6.493	4	Heath, shallow gravel, unexposed	2.1250	1.82
5	Trough, smooth, unexposed	35.801	35.610	7.370	5	Trough, smooth, unexposed	2.1343	1.94
6	Heath, shallow gravel, exposed	35.619	35.988	7.880	6	Trough, shallow gravel, unexposed	2.1393	1.34
7	Heath, screen, exposed	34.186	34.395	10.92	7	Basket, deep gravel, exposed	2.1428	1.69

1/ Means pooled from four samples (30 fry per sample): two samples on 3/14/72, two samples on 3/18/72; cf. Appendix 14.

2/ Converted length = Unconverted length - [(observed K_d - 2.1251)(-20.7415)]. See p. 26 for discussion of formula parameters.

3/ $V(1/4 \quad \overline{X}_i) = (1/4)^2 \quad [V(\overline{X}_i)]$, where $V(\overline{X}_i) = \frac{S^2_{Xi}}{30}$

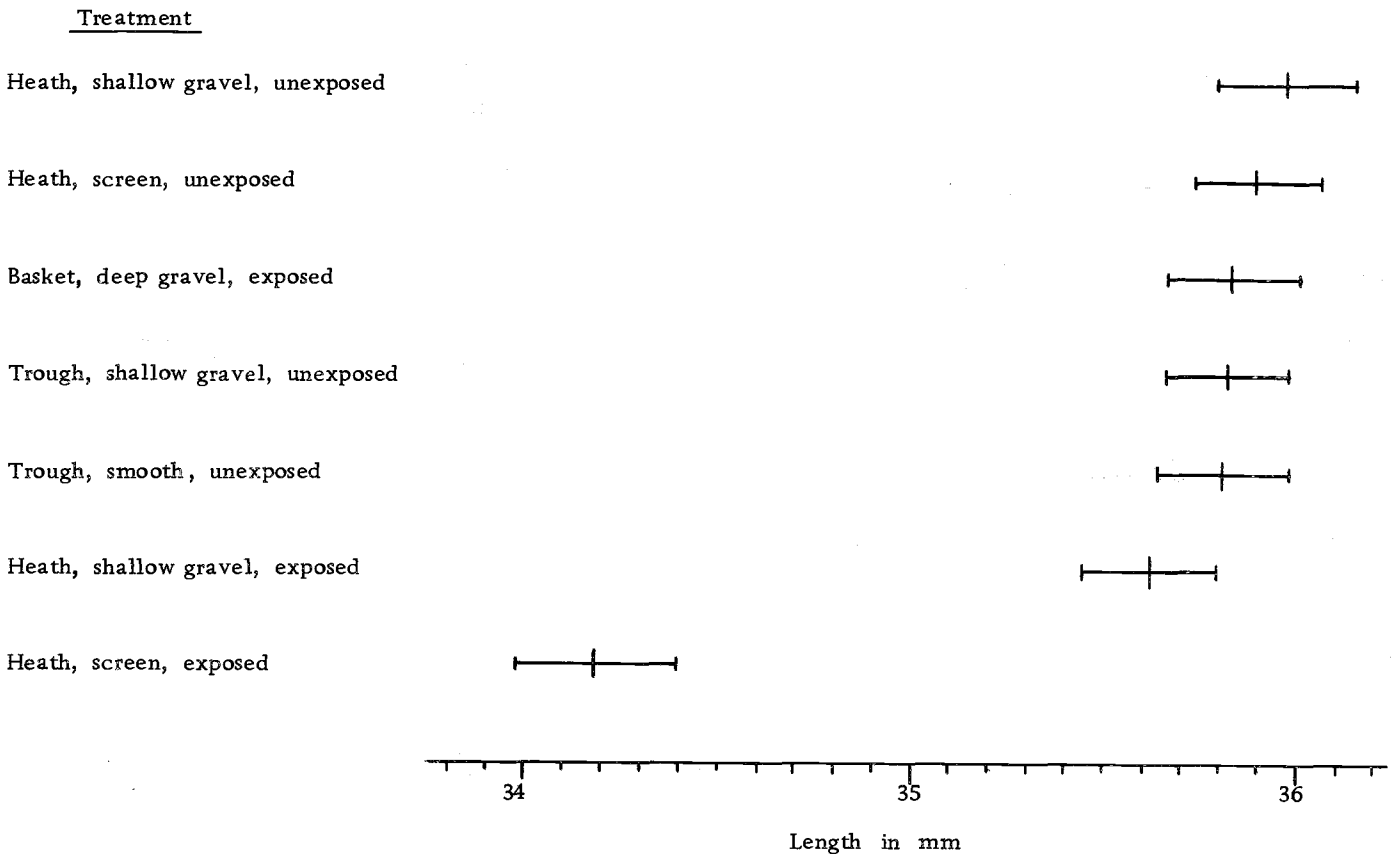

Figure 23. Length (corrected to common K_d of 2.1251) of chum salmon fry of the 1971 brood year laboratory study. Each line represents two times the standard error of the pooled mean from four samples of 30 fry per sample.

were significant, the pooled mean Kd with their 2 X SE intervals were plotted according to ranking to examine confidence interval overlaps (Figure 24). The results supported the two trends established previously; but since confidence intervals overlapped in the middle ranks, only the extreme ranks were considered different from each other, i. e., the two exposed Heath groups were more advanced than the two trough treatments and the basket treatment.

In anoxia tests, gravel substrate fish generally out-performed screen and smooth substrate fish. When data from the anoxia test were ranked according to percentage survivals, results fell into two distinct categories: four high and two low survival groups (Table 12). The two screen substrate Heath treatments had average survival percentages of 23. 3 and 28. 3, whereas the rest of the treatments had average percentages which ranged from 56. 6 to 80. 0. Given the variations exhibited by the duplicate treatments, no further breakdown of these categories seemed justified, although rank #4, the trough, shallow gravel substrate, unexposed treatment, had slightly lower survivals than the first three ranks.

In the two incubator designs in which substrate was tested--the Heath and trough--substrate enhanced performance for the Heath fish but not for the trough fish. The reason for this difference is not known, but it seems reasonable to suggest that the higher velocity in the Heath incubator, which was the primary difference between these

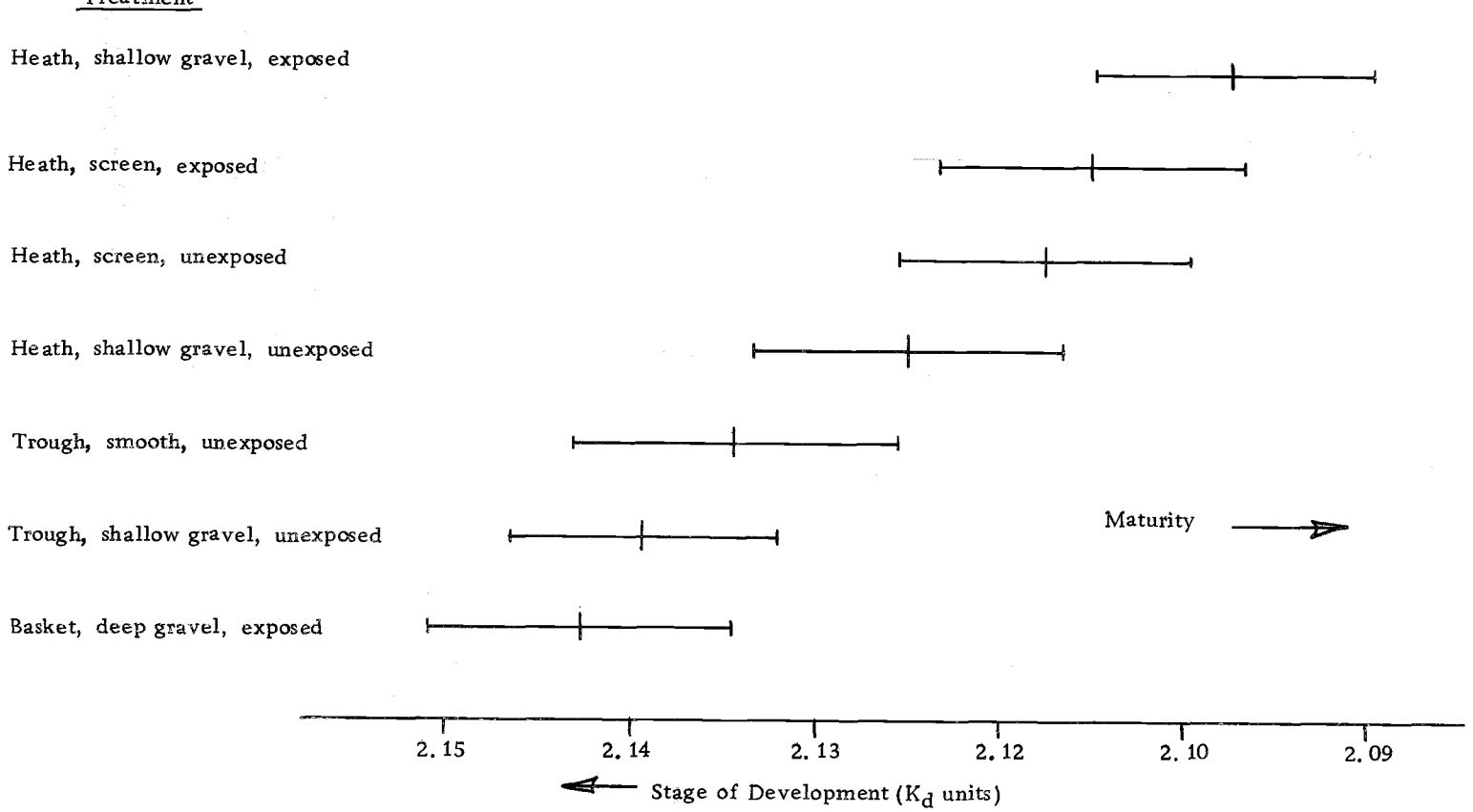

Figure 24. Uncorrected development index (K_d) of chum salmon fry of the 1971 brood year laboratory study. Each line represents two times the standard error of the pooled mean from four samples of 30 fry per sample.

Table 12. Results (ranked by survival) of the anoxia test conducted on 3/26/72[1/] for the 1971 brood year chum salmon laboratory study. Each treatment tested with replicate of n = 30 fry each.

Rank	Treatment			% of fry surviving test	
	Incubator	Substrate	Exposure to light	Overall	Per replicate
				%	%
1	Basket	deep gravel	yes	80.0	86.7 73.3
2	Heath	shallow gravel	no	71.7	66.6 76.7
3	Trough	smooth	no	67.8	62.1 73.3
4	Trough	shallow gravel	no	56.6	63.3 50.0
5	Heath	screen	no	28.3	33.3 23.3
6	Heath	screen	yes	23.3	20.0 26.7
		Control		100.0	100.0

1/ All fry were near closure of ventral slit.

two incubator designs, would be the likely responsible factor.

Execution of the swimming stamina test was successful with test conditions maintained close to constant. During the test period, water temperature ranged from 14.8 to 15.5°C, dissolved oxygen ranged from 9.0 to 9.3 mg/liter, and the water exchange rate ranged from 8.4 to 8.6 liters per minute. Behaviorally, test fish maintained their positions within the center covered section of the tube and dropped back only when nearing exhaustion. They apparently avoided impingement on the back screen until total exhaustion, since fatigued fish recovered from the tube exhibited little physical movements during handling. Variations in thresholds of positive rheotactic responses were apparently minimal.

Although not as succinct as the anoxia test, results of the swimming stamina tests (Table 13) supported the existence of two quality categories. The estimated within treatment variations were 2.50 and 1.25 minutes as indexed by the two true replicates ran with the trough, smooth substrate, unexposed treatment and the Heath, screen substrate, unexposed treatment (Table 13). Thus, probable stamina differences were subjectively assigned to rating differences of 5 minutes or more. When ratings were tabulated by ranks, gravel treatments ranked #1 through #3 and screen and smooth substrate treatments ranked #4 through #8 (Table 13). Ranks #1 and #8 were separated by a rating difference of 13.75 minutes, within which two

Table 13. Ranking of swimming stamina (endurance time) of treatments of the 1971 brood year chum salmon laboratory study. Each test conducted with 50 fry.

Rank	Date of test in 1972	Treatment[1]	Endurance time[2]				Morphometrics of test populations[3]						
			At 25th drop-out		median[4] time for 10th to 30th drop-out	stamina[5] rating	N	Length		Wet weight		Kd[6]	
			interval	mid-point of interval				mean	variance	mean	variance	mean	variance
			min.	min.	min.	min.		mm	mm	mg	mg		
1	3/26	Trough, s. gravel, unexposed	105-110	107.5	110.0	108.75	31	37.31	1.128	431.5	1244.7	2.025	.00180
2	3/28	Heath, s. gravel, unexposed	105-110	107.5	107.5	107.50	32	37.38	1.065	435.0	1984.7	2.025	.00157
3	3/28	Basket, d. gravel, exposed	105-110	107.5	105.0	106.25	29	37.12	1.440	426.6	1892.0	2.026	.00193
4	3/29	Trough, smooth, unexposed	100-105	102.5	107.5	105.00	27	37.15	0.958	412.9	1313.7	2.003	.00136
5	3/28	Trough, smooth, unexposed	100-105	102.5	102.5	102.50	31	37.18	1.176	410.5	830.0	1.999	.00156
6	3/27	Heath, screen, unexposed	100-105	102.5	102.5	102.50	29	37.09	1.019	422.3	1045.1	2.022	.00129
7	3/26	Heath, screen, unexposed	100-105	102.5	100.0	101.25	30	37.02	1.215	428.3	1465.1	2.035	.00250
8	3/27	Heath, screen, exposed	90-95	92.5	97.5	95.00	30	35.88	0.839	376.6	1038.4	2.011	.00138

1/ S. gravel = shallow gravel; d. gravel = deep gravel.

2/ Refer to step acceleration schedule on Appendix 10 for reference to velocity in cm/sec.

3/ While test population was N = 50, only the middle 30 (±3) fry were processed for comparison.

4/ Median time = midpoint between interval of 10th drop-out and interval of 30th drop-out.

5/ Rating derived by averaging mid-point of interval of 25th drop-out with median time for 10th to 30th drop-out.

6/ All test fry were closed to total buttoning; any differences in stage of development between treatments were undetectable by visual inspection.

natural breaks can be detected between ranks #4 and #5 and between #7 and #8. Using the minimum difference of 5 minutes as the criterion, three categories can be distinguished: the 1st category of ranks #1 and #2 (108.75 and 107.50 minutes) was probably different from the 3rd category of ranks #5 through #8 (102.50 through 95.00 minutes), and may be different from the 2nd category of ranks #3 and #4 (106.25 and 105.00 minutes). But since rank #5 (102.50 minutes) is the lower rating of its replicate rank #4 (107.50 minutes), it was removed from the lowest category of #5 through #8 and placed into the middle category with ranks #3 and #4. To present these rankings under treatment names, the trough and Heath, shallow gravel substrate, unexposed treatments (ranks #1 and #2) probably possessed more swimming stamina than the Heath, screen substrate, exposed and unexposed treatments (ranks #6 through #8) and may have possessed more stamina than the basket, deep gravel substrate, exposed treatment and the trough, smooth substrate, unexposed treatment (ranks #3 through #5). These data are consistent with results of the anoxia test; again, where substrate was tested in the Heath and the trough incubator, provision of a gravel substrate enhanced the performance of the Heath fish more so than the performance of the trough fish.

Swimming stamina data evaluations were qualified by some differences in length, wet weight, and stage of development. Only

fish from one treatment, rank #8 (Heath, screen substrate, exposed treatment) were distinctly shorter in length than the higher ranks by 1.1 to 1.5 mm and lower in weight by 34 to 58 mg. The first seven ranks were separated by a length difference of 0.29 mm and a wet weight difference of 24.5 mg., both insignificant relative to their 95% confidence intervals (Figure 25 and 26). The replicate trough, smooth substrate, unexposed treatments, however, were slightly lower in weight than other treatments.

The Kd values of all treatments were separated by 0.036 units, or approximately four to five days in development. This difference was expected since the tests were conducted over a four day period and the Kd values of each test day generally decreased (became more developed) with advancing days (Table 13). However, the trough, smooth substrate, unexposed replicate (Ranks #4 and #5) appeared to consist of slightly more developed fish than other treatments (Figure 27), which would account for their slight drop in wet weight (Figure 26). It is probable that this small difference would not affect their performance rating.

Finally, in distribution of fatigued fish over the time intervals, non-homogeneity in stamina was detected in three out of the eight test groups (Figure 28); these three treatments exhibited distinct bimodal distributions whereas the other five treatments exhibited roughly normal distributions. In two of the three bimodal distributions--ranks #6

Treatment	Stamina rating by rank	minutes
Trough, shallow gravel, unexposed	108.75	
Heath, shallow gravel, unexposed	107.50	
Basket, deep gravel, exposed	106.25	
Trough, smooth, unexposed	105.00	
Trough, smooth, unexposed	102.50	
Heath, screen, unexposed	102.50	
Heath, screen, unexposed	101.25	
Heath, screen, exposed	95.00	

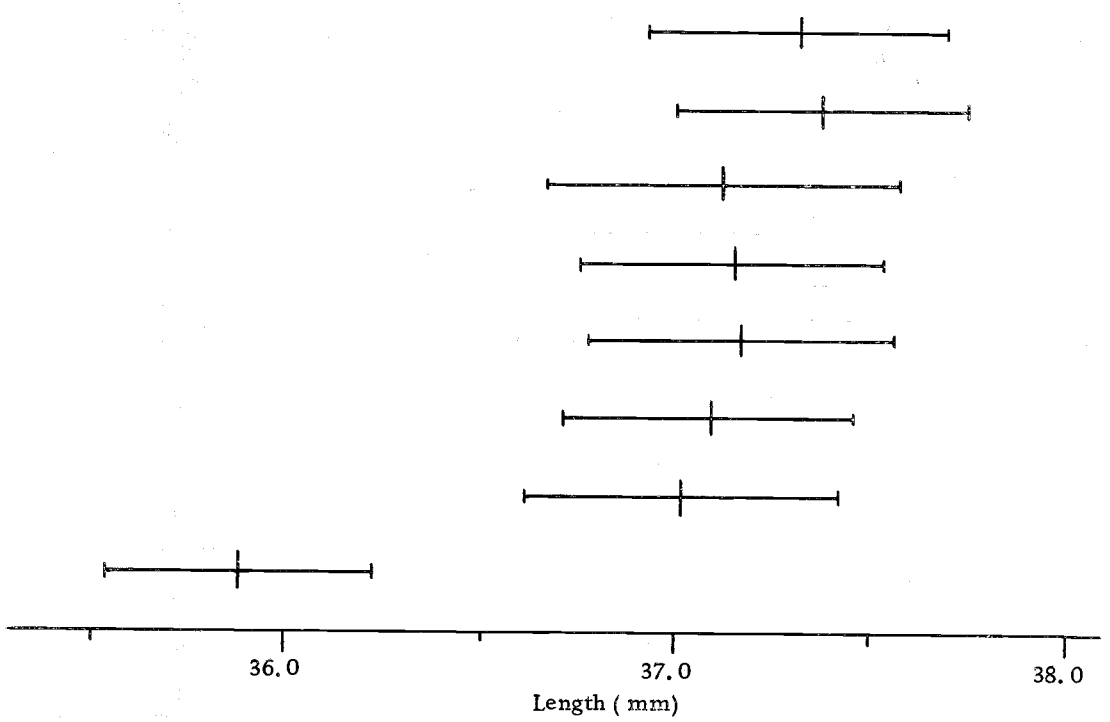

Figure 25. Average lengths (in mm) of eight groups of unfed chum salmon fry tested for swimming stamina. Each line represents a mean and 95% confidence interval for approximately 30 fry.

Treatment	Stamina rating by rank	minutes
Trough, shallow gravel, unexposed	108.75	
Heath, shallow gravel, unexposed	107.50	
Basket, deep gravel, exposed	106.25	
Trough, smooth, unexposed	105.00	
Trough, smooth, unexposed	102.50	
Heath, screen, unexposed	102.50	
Heath, screen, unexposed	101.25	
Heath, screen, exposed	95.00	

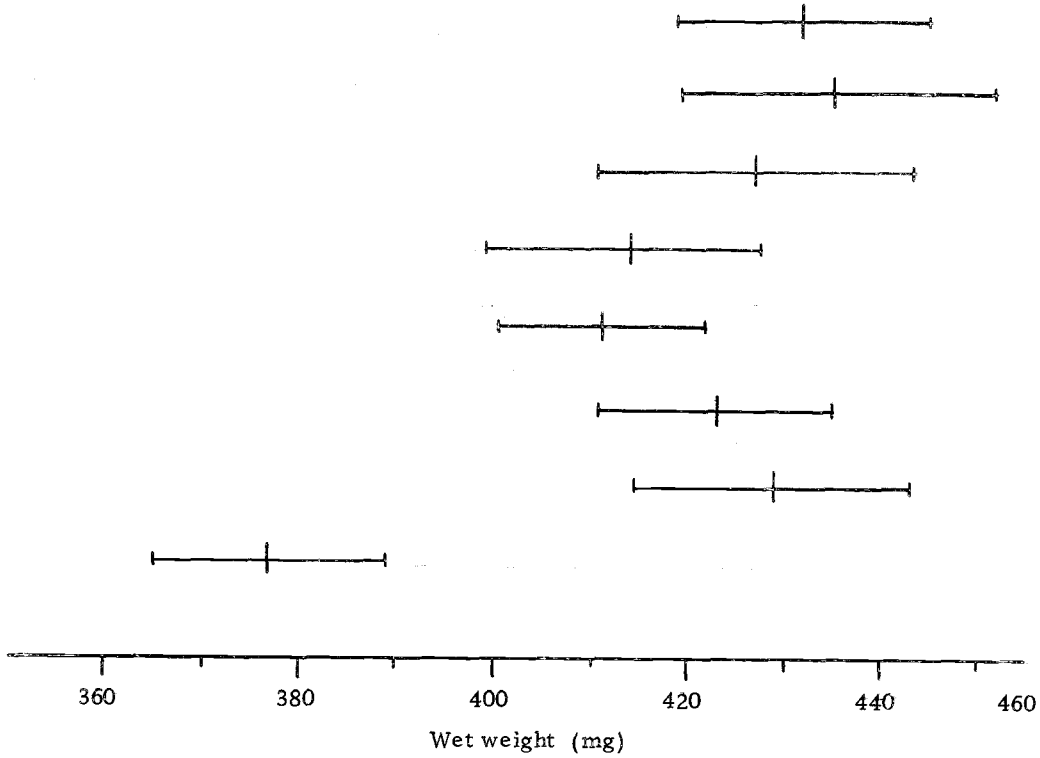

Figure 26. Average wet weight (in mg) of eight groups of unfed chum salmon fry tested for swimming stamina. Each line represents a mean and 95% confidence interval for approximately 30 fry.

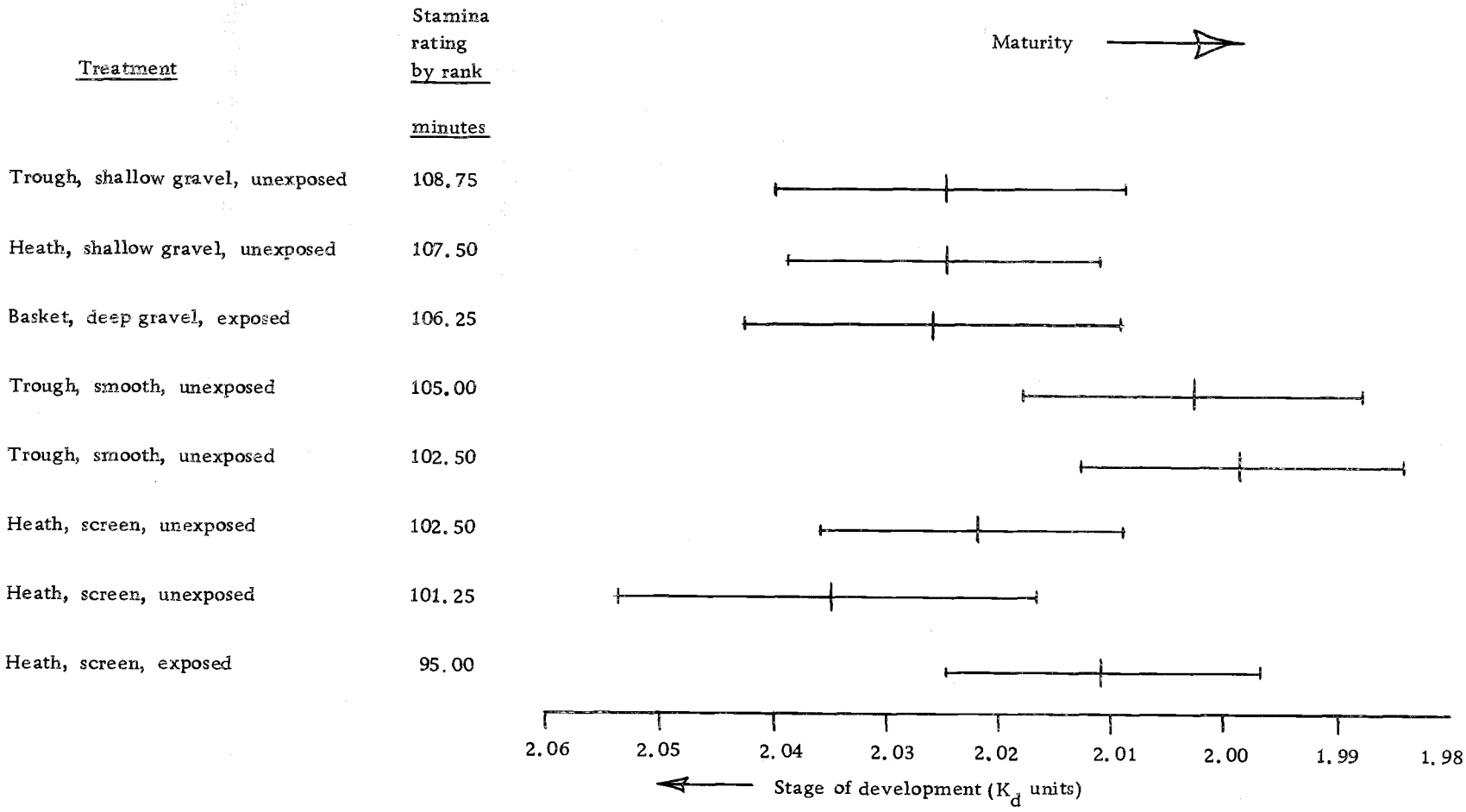

Figure 27. Average stage of development (K_d) of eight groups of unfed chum salmon fry tested for swimming stamina. Each line represents a mean and 95% confidence interval for approximately 30 fry.

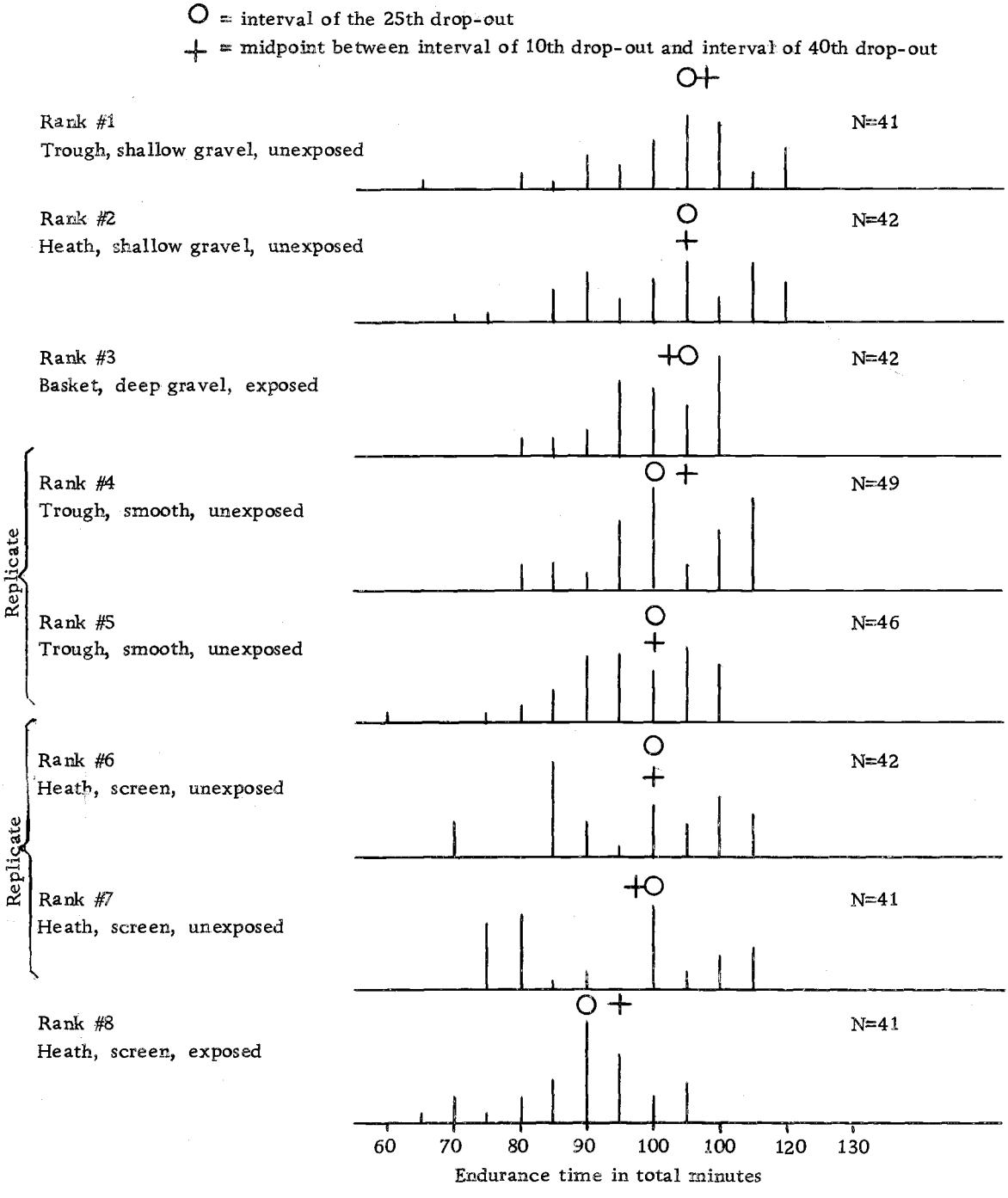

Figure 28. Distribution of drop-outs during step acceleration swimming stamina tests for eight groups of chum salmon fry of the 1971 brood year laboratory study. Each test was run with 50 fry and was terminated when the 40th fry dropped out.

and #7, the two replicates for the Heath, screen substrate, unexposed treatment--the first mode fell below the stamina rating suggesting an unproportionally high frequency of lower stamina fish. The other bimodal distribution was exhibited by rank #4, the replicate of rank #5, the trough, smooth substrate, unexposed treatment. Unlike the other bimodal distributions, both modes of this distribution occurred above the stamina rating, suggesting an unproportionally high frequency of higher stamina fish. For practical interpretations of survival potential, the consistent occurrence of lower performance modes with the two Heath, screen substrate, unexposed treatments may indicate that a higher proportion of the population may possess lower stamina and thus may have lower survival potentials.

Since bimodal distribution occurred in ranks #6 and #7, it should have occurred in the lower stamina rank #8 also. But rank #8--the Heath, screen substrate, exposed treatment--had already experienced a 31% mortality which undoubtedly eliminated the lower performance fish from the population.

Data generated from the swimming stamina tests were more comprehensive and included calculated Kd values. By comparison, the anoxia test assumed equivalent stage of development based on visual observation; Kd values could not be calculated since morphometric measurements from dead and live fish are not the same. Confidence on data from the anoxia test was therefore not as high.

Fortunately, results of both tests are in general agreement so it seems likely that the assumption of comparable stage of development in the anoxia test was generally valid.

3. Discussion

Results of these experiments on the relative effects of substrate, exposure to light, and incubator designs on fry quality provided information for evaluation and improvement of present gravel incubator designs. Given the low level of stocking densities in this study, it is recognized that increased density and the accompanying density-dependent factors may alter interpretations of test parameters. This discussion is based primarily on results from the chum salmon study although the results of the more limited pink salmon study were consistent with data from the chum salmon study.

The general conclusion from the pink salmon study was that a shallow gravel substrate had little influence on relative size of Heath incubator fry reared at lower density and in darkness.

For the chum salmon study, the inclusion of performance testing added greater dimension to fry quality testing. Results of the anoxia test and the swimming stamina test were in general agreement except for the effects of a gravel substrate on trough fry reared under darkness--the anoxia test resulted in no performance difference whereas the swimming stamina test detected a possible

improved performance for the gravel fry. Because the swimming stamina test data were deemed more reliable, they were used to formulate conclusions.

Interpretation of swimming performance of unfed salmon fry can often be confounded by a number of common problems, which include the variations in thresholds of positive rheotactic response, influence of length, and performance variations given comparable lengths. Of these, length has the dominating influence on swimming ability (e. g., Bainbridge 1958; Vincent 1960; Bams 1967; Dill 1970). For example, in an experiment which demonstrated that wild and gravel incubator fry had better swimming and predator avoidance capability than conventional hatchery fry, Bams (1967) concluded that the factor most responsible for the hierarchy of performance was length. Hence, to avoid the inevitable conclusion that longer fish swim better, fish of comparable lengths are generally tested together. Performance variations within length classifications, however, are high (cf. Bainbridge 1960; Vincent 1960, Vibert 1956; Bams 1967), and much of this variation can be accounted for by differences in stage of development, and the thresholds of positive rheotactic response, i. e., the fish dropping out prior to total exhaustion.

The influence of stage of development is a function of the degree of body streamline. Fry carrying external yolk are hydrodynamically inferior to a streamlined fry with less yolk (e. g., Thomas et al.

1969; Bams 1967). There is a stage of development when swimming performance is optimum for unfed fry; before or beyond this stage, performance drops drastically. For sockeye fry, Bams (1967) found this stage to be near total yolk absorption at calculated Kd values between 1.80 and 1.85 based on measurements of preserved fry.

The effect of behavioral variations is more complex. Fish in stamina tests often drop out prematurely due to fright response, response of other fish, and sometimes unknown reasons. Addressing the thresholds of positive rheotactic response, Bams (1967, p. 1139) stated that "It is this variation in response threshold which I consider to be the main source of variation in this type of experiment."

In this study, most of the above sources of variations were mitigated. The influence from stage of development was controlled by testing when all fry were near total yolk absorption. The average Kd values differed by 0.035 Kd units (Table 13) which is small compared to the range of optimum performance of 0.05 units calculated by Bams (1967). Length, however, was not controlled in selecting random samples of test fry, but fortunately, average lengths were comparable except for the single low-performing Heath, screen substrate, exposed treatment, the fry of which were definitely shorter. Thus, performance measured quality independent from the influence of length and stage of development. By measuring the median 30 fry from each sample of 50 fry, the wide variations

between individuals within a population was replaced with the smaller variations around a mean performance, which yielded proportionately smaller within treatment differences. Finally, the variations from differences in thresholds of positive rheotactic response was minimal because only the exhausted fish would tolerate impingement on the back screen.

In sum, the swimming stamina test procedures apparently avoided problems generally encountered by similar studies. Problems which remained for this study were the lack of a clear definition of the change of performance with stage of development, and the limited number of replicates for each treatment. Neither of these factors, however, should alter the test results significantly.

The swimming test was crucial to the evaluation of substrate depth, which was the most important difference between incubation systems tested. This variation from none, to shallow, to deep is a direct function of the degree of simulation of the natural streambed. The question is how far simulation should go to reap the advantages of improved fry quality. Operation of the deep gravel design is made difficult by the large volume and weight of substrate and the possible need for water filtration to control clogging. However, this design can accommodate higher stocking density per unit floor space than the shallow gravel matrix design. Operation of the shallow gravel matrix design is relatively simple due to the small volume

of substrate and capability to operate without water filtration, even with relatively high silt content in the water supply. By dispersing alevins over a two-dimensional plane, it provides poor efficiency in space utilization.

Results from this study on substrate supported the advantage of gravel substrate (either shallow or deep) over a flat substrate, given no exposure to light; this conclusion, surprisingly, was based not on relative yolk conversion efficiency, i. e., relative fry size, but primarily on the relative performance in the swimming stamina tests. The lack of a size difference is consistent with data from the chinook salmon experiment in the 1969 brood year of this research and again indicated that provision of a substrate provided no advantage to reduction of larval activities. This result would be contrary to the righting response concept demonstrated with sockeye salmon (Bams 1969) and with Atlantic salmon (Marr 1963, 1965; Anonymous 1969), and suggest that need for gravel support during incubation may be genetically controlled. The detection of performance differences between gravel incubator fry and non-gravel conventional incubator fry supported results of similar performance test run by Bams (1967) on sockeye salmon and by Vibert (1956) on Atlantic salmon; one key difference, however, is that size difference is not a factor in this study whereas the gravel fry were generally longer in these other studies.

The disproportional high number of low stamina fry in the Heath, screen substrate, unexposed treatment (Figure 28) may cause poor performance in other activities also, such as the ability to forage. If this postulation has merits, the high frequency of stunted and pin-headed fry observed in the Heath fry of the 1970 growth study may have been caused partly by a higher proportion of smaller, low-stamina fry.

While advantages of gravel incubation have been established, results in this study produced no size or performance difference between shallow and deep gravel matrix fry, and are thus consistent with work on steelhead trout by McNeil (1968). The comparison in this study was complicated by surface incubation of about 10% of the deep gravel matrix fry, whose upward movement was apparently caused by heavy siltation (Bams 1969). This complication is not expected to change the experimental results decisively, but it did weaken the power of the comparison.

The lack of a size difference between the gravel and screen or smooth substrate treatments might have been due in part to the elimination of light. According to results from the light X substrate comparison, screen substrate alevins exposed to light experienced distinctly reduced survival and fry size whereas exposed shallow gravel substrate alevins had equivalent survival and fry size as gravel and screen substrate treatments reared in the

dark. These results, therefore, indicated that provision of a substrate would negate adverse influences of light-induced activities. What is not known, however, is whether other documented adverse influences of light, such as loss of alertness and possible lowered capacity to feed extraneously (cf. discussion on p. 51) would still be manifested. The Heath, shallow gravel substrate, exposed treatment was unfortunately excluded from the stress tests so no data on performance can be inferred for gravel fry which were exposed to light. Until more information can be derived about the influence of light on fry quality and subsequent survival, the practical conclusion at this point is to exclude light and provide a gravel substrate. Given the possible effects of light, previous fry quality differences attributed to conventional incubators might have been caused partially by light-induced activities, and that introduction of a gravel substrate to these incubators may reduce some of the loss in fry size.

In comparing various incubators, design differences which may influence fry quality include flow rate, flow pattern, and substrate depth. The results indicated that fry size and performance are enhanced by a gravel substrate whereas developmental rate is accelerated by the higher flows of the Heath incubator, particularly the exposed Heath treatments. When a gravel substrate, either deep or shallow, was provided, all fry were equivalent in size and performance regardless of incubator origin. Only when substrate was

removed and when light was introduced was there detectable quality differences. Thus, provision of a gravel substrate and elimination of light seem to take precedence over incubator design.

Gravel substrate in the Heath incubator appeared to help enhance fry performance more than gravel substrate in the trough incubator, although this difference was not distinct (Table 13). It is not known why this difference should exist--intuitively, if the higher velocity in the Heath incubator adversely affected screen substrate fry, then a difference in fry size should have been detected. But there was no size difference.

The accelerated development experienced by Heath incubator fry did not influence the time to complete yolk absorption since the difference in stage of development dissipated prior to button-up. But early developmental rate differences will influence the validity of size evaluation procedures based on constant developmental rates (cf. discussion on p. 88).

In this study, the accelerated development in the Heath incubator fish was probably caused by the high velocity compared to the lower velocity in the trough and deep gravel basket; whether increased velocity would also accelerate development in the trough and the deep gravel basket is not known since no such tests were run.

Differences in the stage of development among treatments were eliminated at button-up in this study, but this difference persisted

in the 1970 density and velocity experiment with pink salmon and also in experiments conducted by Bailey et al. (1975) comparing different gravel and smooth or screen substrate incubators. Whether fry preserve their relative time to total yolk absorption may be dependent upon density in addition to velocity, the ranges of which were higher in studies where developmental differences persisted through button-up. It may be that the combined effects of density and velocity increased the developmental rate sufficiently such that compensatory mechanisms were either not enough or did not operate. Given accelerated development, early yolk absorption may necessitate holding and feeding the fry in order to release them under favorable estuarine conditions; therefore, producing fry which migrate at the proper time would be advantageous from a biological as well as from an economic perspective.

In this study, no control was maintained over the quality of the water supply and it is not known how the energy expenditure for physiological and behavioral mechanisms needed to combat siltation might influence experimental results and fry quality. Water filtration is a key difference between the proven Bams boxes and all other gravel incubator designs, but how much filtration is necessary to preserve high fry quality is still not identified but would be most pertinent to design of incubational facilities.

Finally, fry quality criteria of high survival and conversion

efficiency are good approximations of survival potential, and is often the only feasible measurement of quality. The occurrence of performance differences in this study, given equivalent survival and fry size, underscored that all fry quality criteria are only estimates of survival potentials. As a case in point, Bams (1976; personal communication, 1976) demonstrated that while egg to fry survival of his Headquarters Creek gravel system increased from 68.3 to 74.9 to 93.6 percent in three generations, and with fry size equivalent between propagated and wild fry on all three tests; corresponding fry to adult survival favored the wild fry by 1.5, 2.5, and 9.1 percent respectively. The causes of this difference will be addressed later, but the data suggest the preliminary nature of fry quality judgements based on survival and fry size alone.

V. FIELD STUDIES ON INCUBATOR DESIGN

The 1971-1972 field season was phase II of this research and addressed the evaluation of production gravel incubators at Sashin Creek, Little Port Walter, Alaska, and at Whiskey Creek, Netarts Bay, Oregon. This effort represented the final analysis of the fundamental hypothesis of this research, i. e., if environments of these gravel incubators simulated optimum conditions of the natural streambed, then propagated fry should be equivalent to wild fry in yolk conversion efficiency (relative size) and in other quantifiable quality criteria. If this hypothesis is rejected, then information accumulated in Phase I laboratory studies should provide some basis on which to evaluate results and to make recommendations for improved operations. The primary assumption is that the natural streambed at both locations will produce fry which can serve as quality standards.

The critical experiment subjected wild stocks of pink salmon at Little Port Walter and chum salmon at Netarts Bay to artificial and natural propagation and compared the quality of migrant fry from the two sources.

A. Description of Study Areas

Gravel incubation research began at Sashin Creek and at Netarts Bay in the late 1960's; preliminary efforts have been documented (cf. Table 1).

At Little Port Walter (Figure 1), where adult escapement and migrant fry have been enumerated since 1934, stream production and ecology are well studied (e. g. Merrell 1962; Olson and McNeil 1967; Ellis 1969). Under the direction of Mr. William Heard, prototype deep-gravel matrix incubator testing began in 1969 (Bailey and Heard 1973). During the 1971 brood year, propagation was centered in a small experimental hatchery located on Little Port Walter Bay; water to the hatchery was delivered by gravity from the top of a waterfall (approximately 1707 m from the creek mouth) via a 5. 08 cm line, the terminal portion of which passed 275 m under the bay. The hatchery water supply, therefore, is warmer than the creek water during winter time due to the warming effect of salt water overlying the terminal portion of the water line.

At Netarts Bay (Figure 2), development of the shallow gravel matrix "streamside incubator" was designed and initiated by Dr. William McNeil in 1968 (McNeil 1968; Poon 1970). Creek water for the hatchery was delivered from a settling pond immediately upstream from the hatchery. In 1971, Dr. McNeil replaced the streamside incubator with an advanced model, called the "Netarts Gravel Incubation Hatchery," which has become the standard production model (McNeil 1972). Descriptions of Whiskey Creek and the Native Chum salmon run, cumulative hatchery production records, and hatchery operation and improvements since 1972 were recently documented by

Dr. James Lannan (1975).

One key difference between the two study areas which was cogent to subsequent experimental designs was the quality of the hatchery water supply. The Sashin Creek watershed is unlogged, and the amount of settleable solids in the water supply is very low, even during freshets. At Netarts Bay, however, the Whiskey Creek watershed was clearcut, and high amounts of mineral silt enter the hatchery water supply during every freshet. Water filtration was not undertaken at either location, and the types of incubators tested were those models which can be operated under the respective water conditions.

B. General Procedures

Both deep and shallow gravel matrix designs were tested at Little Port Walter, but the deep gravel design was the production model. Only the shallow gravel matrix design was tested at Netarts Bay.

Experimental designs at both sites maximized information output by incorporating design variables testing wherever appropriate. At Little Port Walter, stocking density, method of egg stocking (eyed versus newly fertilized), and incubator design were tested. At Netarts Bay, the feasibility of a more efficient multiple layer design was tested; in addition, concurrent Laboratory studies on other design variables have already been described.

1. Pink Salmon Study at Little Port Walter

a. Incubation Systems

The following six incubation treatments were tested:

Incubator Number	Incubation Treatment
1	Deep gravel matrix; stocked with 70,000 green eggs
2	Deep gravel matrix, stocked with 70,000 eyed eggs
3	Deep gravel matrix, stocked with 85,000 eyed eggs
4	Replicate of #3
5	Shallow gravel matrix; multiple layer design; stocked with 20,000 eyed eggs
6	Heath incubator stocked with 20,000 eyed eggs

Beach gravel of 1.3 cm to 3.2 cm in diameter was used as the substrate material.

The deep gravel matrix incubator, an enlarged version of the prototype model (cf. Bailey and Heard 1973), is a circular fiberglass tank with a diameter of 1.1176 m, a depth of 38.7 cm, an area of 0.981 m^2, and a volume of 0.380 m^3. The incubator is filled to a

height of approximately 30. 5 cm for an effective gravel volume of about 0. 3 m^3. Water upwells through a finely perforated plexiglass false bottom 3. 2 cm off the tank bottom. Flow is controlled through a 1. 9 cm valve on the inflow line. The eggs are stocked into the incubator in four even layers buffered by gravel between layers.

The multiple layer, shallow matrix incubator, a modified version of the conventional "deep trough incubator," was designed within a hatchery trough measuring 325. 1 x 33. 0 x 28. 6 cm and baffled to create an upwelling flow through each of three subsections. Each subsection has inner surface dimensions of 87. 6 x 23. 5 cm. Wooden screen trays made with eight mesh per 2. 5 cm hardware cloth and with a surface area of 1652 cm^2 (20. 3 cm x 81. 3 cm) were lined with a single layer of gravel and stacked five deep in each subsection. For this study, only the first subsection (next to the inflow) was used; the remaining two sections were filled with coho eggs in another unrelated experiment. Eyed eggs were stocked directly onto the gravel on each tray. Flow to the incubator was controlled by a 1. 9 cm valve on the inflow line. Wooden lids were placed snugly over each subsection to exclude all light. Fry migration from the trays was made possible by removing a wooden rectangular plug at the center of the baffle to create a free flow area.

The Heath incubator was operated under total darkness by provision of a black plastic cover. Only the top six trays were used

for this study.

b. Securing Egg Population

A random sample of approximately 2% of the Sashin Creek pink run was taken at the weir trap from August 15 to September 6. Sampling generally took place whenever approximately 1000 females had entered the creek since the last sample period. Since sampling hampered continuous fish movement, it was carried out only when the fish were not pressing to enter the weir. Therefore, the actual number of females passing the weir before a sampling ranged from 855 to 2373. During sampling, ratios of male to female for both the creek and sampled fish were the same at any given period.

A preselected number of males and females were dipnetted out of the weir trap and transferred to holding pens (separating males and females) in a small reservoir next to the weir. Care was taken to avoid selection for size when dipnetting. On September 9, the last day of sampling, a total of 842 fish (317 females, 525 males) had been taken from a cumulative creek escapement of 41,603 fish (15,372 females, 26,231 males) with a sex ratio of 1.7 males to 1.0 female. After this date, 1221 additional fish were allowed into the creek to represent the late stock; these fish were not represented in my sampling.

During the fish holding period, two problems occurred which

affected sampled population. On August 30, 1971, a water stoppage at the reservoir killed 97 out of 754 fish held at that time. On September 10, 1971, a hole was discovered in the female holding pen and 52 fish had apparently escaped into the reservoir. Attempts to recapture these fish were unsuccessful since the water level could not be lowered while fish were still being held. Because of these problems, and with small numbers of fish spawned unripe, 222 females (70% of sampled females) were spawned successfully during four spawnings on August 28, September 10, September 3, and September 15. The average fecundity was 1837 eggs per female (n=30). Correcting for the normal egg loss during spawning a fecundity of 1750 was used to estimate the egg take of 388, 500 eggs for 222 females spawned.

The second spawning on September 3, the heaviest spawning (38. 7% of total egg take) of the four attempts, coincided with peak spawning activity in Sashin Creek riffles. Thus, maturation of artificially selected spawners coincided with the peak natural spawning period.

The spawning procedures for each egg take were as follows: 1) spawn approximately one gallon of eggs (about 14 females) into a bucket; 2) pour the egg bucket into a bucket with milt from an equivalent number of males; 3) mix gametes thoroughly to affect dry fertilization; 4) take the dry fertilized eggs to the hatchery within one half

hour and pour into an incubator to complete fertilization. All stored gametes were kept cool until introduction into water.

In utilizing the males for fertilization, previously spawned males were tagged with a spaghetti tag for identification and were not used again except in a few instances when a ripe unspawned male could not be found.

A small sample of fertilized eggs from each spawning was examined for embryonic development after 24 hours; fertilization success was 90% or above in all examinations.

c. Stocking and Maintaining Incubation Treatments

Hatchery incubators were stocked on October 21 with a total of 350,000 eggs. Because of the egg mixing and stocking procedures, incubators No. 3 and No. 4, the two high density deep gravel matrix incubators, contained eggs from the first two spawnings, and the green eggs stocked in the deep matrix gravel incubator No. 1 contained eggs from the third spawning only (Figure 29). Incubators No. 2, No. 5, and the Heath incubator contained eggs from all spawnings. Stocking densities and water velocities are summarized for each treatment in Table 14.

Daily air and water temperatures were recorded continuously with a Taylor two-needle thermograph at the hatchery and at Sashin Creek. Over the incubation period from stocking through the end of

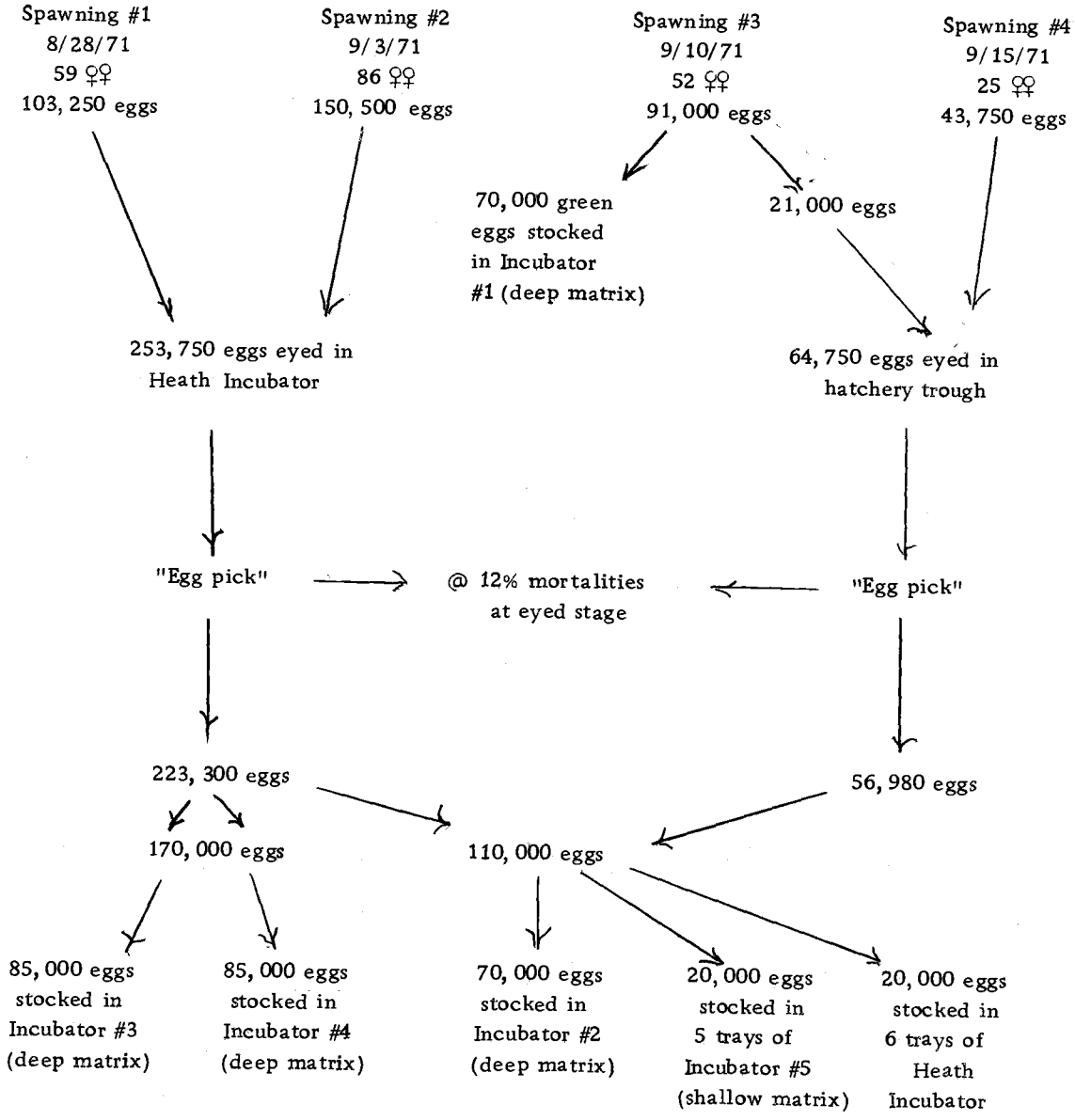

Figure 29. Distribution of pink salmon eggs for hatchery incubators of the 1971 brood year at Little Port Walter.

Table 14. Stocking densities and water velocities of the 1971 brood year pink salmon hatchery versus wild fry evaluation at Little Port Walter.

Incubator				Egg stocking				Designated water velocity[1]	
#	Design	Surface area[2]	Substrate volume	Date stocked[3]	Estimated # stocked	Egg density[4]			
		cm^2	cm^3			eggs/cm^2	eggs/cm^3	lpm	cm/hr
1	deep gravel matrix	9810	299,000	9/10/71	70,000	7.14	.230	18.9	116
2	deep gravel matrix	9810	299,000	10/22/71	70,000	7.14	.230	18.9	116
3	deep gravel matrix	9810	299,000	10/22/71	85,000	8.66	.280	18.9	116
4	deep gravel matrix	9810	299,000	10/22/71	85,000	8.66	.280	18.9	116
5	multiple layer shallow gravel matrix	1652	---	10/22/71	20,000 (5 trays @ 4000)	12.1 (2.42x5)	---	21.6[5]	784
Heath		1280	---	10/22/71	20,000 (6 trays @ 3300)	15.48 (2.58x6)	---	18.2	852
Sashin Creek		---	---	---	29,668,000[6]	---	---	---	---

1/ Water velocity = Apparent water velocity = Volume of water/unit time x $\frac{1}{\text{surface area}}$.
Except for incubator #5, all observed velocities were within 10% of designated velocities.

2/ Based on single top-view measurement.

3/ All incubators stocked with eyed eggs except #1 which was stocked with green eggs.

4/ Surface area based on single top view measurement for deep gravel incubator but included area of each tray for #5 and Heath incubator. Volume based on gravel substrate within incubator.

5/ Stepwise decrease to 15.9 lpm (677 cm/hr) on 12/13/71 and to 11.4 lpm (412 cm/hr) on 3/29/72 as necessitated by pre-mature flush-out of alevins.

6/ Calculated potential egg deposition.

migration, the hatchery temperature ranged from approximately[8] 0°C to 11°C with an average of 3.5°C; whereas, creek temperature ranged from approximately[8] 0°C to 13°C with an average of 1.9°C (Appendix 16). The large difference caused by the route of the hatchery water line under the estuary is apparent. From November to June, the creek was accumulating one-half or less of the temperature units accumultated by the hatchery. Creek water temperature went below 0°C[8] for extended periods during December through March. At the time of fry migration in April, the cumulative temperature units (to the end of April) of the hatchery was about twice the units of the creek (872 to 469 TU°C).

Water velocities of the incubators were checked periodically and were consistently within 10% of the designated levels; few adjustments were needed throughout incubation. Mortalities were picked at the eyed stage and were not assessed again until after migration was completed. With minimal mortality and cold water temperatures, fungus was not a problem.

Ten to 15% hatching was observed by November 18, and 50 to 70% by December 15. On the latter date, fry collection bags were installed on the outflows of each incubator to monitor premature emergence. A few alevins were caught in the collection bags as

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[8]Accuracy of Taylor thermograph was approximately ±1°C so temperature data are regarded as estimates and subfreezing temperatures of about -1°C were recorded.

early as December 28.

Some degree of premature emergence occurred in all incubators which allowed volitional migration. For the deep gravel matrix boxes (#1-#4), flush-outs of alevins prior to March were less than 1/2 of 1% of all migrants enumerated. For the shallow gravel matrix incubator (#5), however, premature flush-outs of alevins were 10.29% by March 1st.

The high amount of premature emergence in incubator No. 5 was apparently caused by the high water velocity and by movement of alevins and fry between trays and between compartments. The fish were able to squeeze through the small gaps on the periphery of the system and many ended up on the smooth substrate bottom of the trough, thus nullifying in part the substrate treatment for these fish. As early as December 13, 1971, inspection of the two empty sections netted 870 alevins. On December 13, an attempt to slow down the flush-outs by reducing the flow from 21.6 to 15.9 lpm was unsuccessful. A total of 14.07% had flushed-out by March 29, 1972, and these fry were discarded. On March 29, 1972, a further reduction in flow from 15.9 to 11.4 lpm did slow down the premature migration, but it also caused the fry to hold inside the trough, resulting in migration dates which were later than expected.

d. Trapping, Enumerating and Sampling Migrant Fry

Migrant fry from the hatchery and from the creek were trapped, counted, and subsampled for fry quality analysis. Because the hatchery water line passed under the bay, hatchery fish experienced a warmer temperature regime and migrated at an earlier date. Hatchery fry migrated from March 29 through May 20, whereas wild fry migrated from April 17 through June 28.

At the hatchery, migrants were trapped in perforated buckets placed at the outflow of each incubator. Enumeration was done by count up to approximately 300 fry; higher numbers of fry were estimated gravimetrically based on previous assessment of number of fry/unit-weight. Approximately 100 fry were subsampled weekly and preserved in 5% formaldehyde solution for morphological measurements. Extra samples were taken during peak migration periods.

Whenever the number of migrants fell below 100, daily catches were pooled to get the required sample size. This procedure was necessary only at the beginning and at the end of the migration period, and pooling was kept to within two to three days. All migrants not preserved or held for experimental purposes were released in the evening hours into the estuary.

For the Heath incubator, which has no volitional migration, sampling (taken from all trays and then combined) was done at

higher frequency from March 29 to May 19.

At the creek, trapping was done with a 30.5 X 92.7 cm (2826 cm^2) fyke net set at the weir between two pillars away from the main stream flow. The fishing area of the trap is maintained at the top layer of the water column by a large styrofoam float attached to the periphery of the net frame. A removable collection bag was snapped on to the end of the fyke net each evening and was removed for fry enumeration each morning. On June 3, a live box attachment replaced the catch bag which had a tendency to gather debris and cause clogging and fry mortality. The trap fished effectively throughout the migration period and its position was stationary except for seven days from April 30 to May 6 when it was moved to a position closer to the main flow. It was moved back because the water current was too swift in the more central position. Within the modifications of its operation, however, the trapping procedure provided an index of the relative number of migrants over the sampled period.

All enumeration of creek migrants was done by counts and sampling took place as with the hatchery fry. All sampled fry not preserved were released in the evening of the processing day. Sample pooling was necessary to obtain the proper sample size during the beginning and the end of migration. Pooling was extended for six days in the beginning and three days at the end.

e. Evaluating Fry Quality

Except for the deletion of performance testing and post-emergence growth, fry quality was evaluated per the methods described in the 1971 brood year laboratory studies. As in these studies, dry weight and percent dry weight data were invalidated by oven drying complications.

Performance testing was deleted because comparable test populations were not available at a common point in time, and because swimming stamina testing equipment was lacking. The post-emergence growth test was conducted at Little Port Walter Bay but escapement of significant numbers of fry during pen changing nullified this effort.

Mortality in the gravel incubators was estimated as the difference between the initial number stocked and the number of fry migrants and back checked with an estimation of the actual number of dead eggs and alevins remaining in the incubators. Dead eggs and alevins in the Heath incubator were hand counted. The creek estimate was based on pre-emergence egg pumping data coupled with the estimated potential egg deposition.

Fry size conversion data were taken from Sashin Creek eggs incubated at Netarts Bay for concurrent laboratory studies (Appendices 12 and 13). While the Netarts Bay experiment differed in

temperature regimes, incubation treatments, and strength of preservative, its conversion data were considered appropriate for use in this study because: 1) the eggs were from the same brood stock; 2) within these Netarts Bay data, there was no difference in developmental rates and fry size between the two treatments; 3) the data covered similar size and stage of development range as this experiment; 4) a common kd value (1. 945) was observed for maximum wet weight in Netarts and Little Port Walter fry, indicating that the difference in preservative strengths (5% versus 10% formalin) did not change the length-weight relationship substantially; and 5) in another experiment using the same brood of Sashin Creek eggs, but incubated at Auke Creek (Bailey and Taylor 1974), similar size conversion data were calculated.

In applying size and stage of development procedures, however, the difference in sampling methods precluded direct comparison of all incubation treatments. The Heath incubator samples were taken as random samples from a totally accessible population whereas samples from other incubation treatments were non-random samples of fry migrating at one point in time; consequently, the heterogenous Heath samples and the more homogenous samples from other treatments produced kd values which were not equivalent. Further, a maximum length can be observed for Heath fry whereas gravel fry generally migrate at sub-maximum lengths. Because of the above

factors, Heath fry were not evaluated directly against gravel incubator and wild fry.

Size evaluation for the Heath fry was implemented by comparing the observed maximum lengths (average of nine samples taken on and after April 14) of the Heath fry against the observed length and projected maximum length from other treatments. Projected maximum lengths were calculated with the expected rate of length increase per unit kd value increase (cf. Appendix 13) and an estimated kd value (approximately 1.920) for maximum length based on data from chronological samples of gravel fry.

In assessing the stage of development of Heath fry, there can be no reference to time of migration; thus, a qualitative comparison was made for the relative time required to reach button-up. This comparison was restricted to the hatchery incubators only since the wild fish experienced a different temperature regime.

In contrast to the Heath incubator evaluation, comparison of migrant fry from gravel incubators and the creek followed previously established procedures more closely. Again, the time required to reach button-up was not evaluated between the creek and hatchery fish due to the temperature regime difference.

2. Chum Salmon Study at Netarts Bay

a. Incubation Systems

At Netarts Bay, the incubation treatments consisted of the production hatchery incubators and an experimental multiple layer incubator.

The production incubators have been described in detail by McNeil (1972) and Lannan (1975); they resemble standard hatchery troughs but are larger, protected from light with wooden lids, and possess gravel-lined (0.64 to 1.91 cm crushed rocks) bottoms. The production hatchery consists of four banks of wooden incubator boxes, each bank having four 1.2 m by 2.4 m boxes bolted together end to end. Each box was divided by a baffle system into two 1.2 m by 1.2 m compartments with a capacity of 40,000 eggs per compartment. The total capacity of the four banks of boxes was 1.28 million eggs. Fertilized eggs were placed on four plastic screen trays stacked inside each 1.2 m by 1.2 m compartment and the hatched alevins dropped onto the gravel substrate to complete their development. Dead eggs were removed with the screen to be enumerated by volumetric methods.

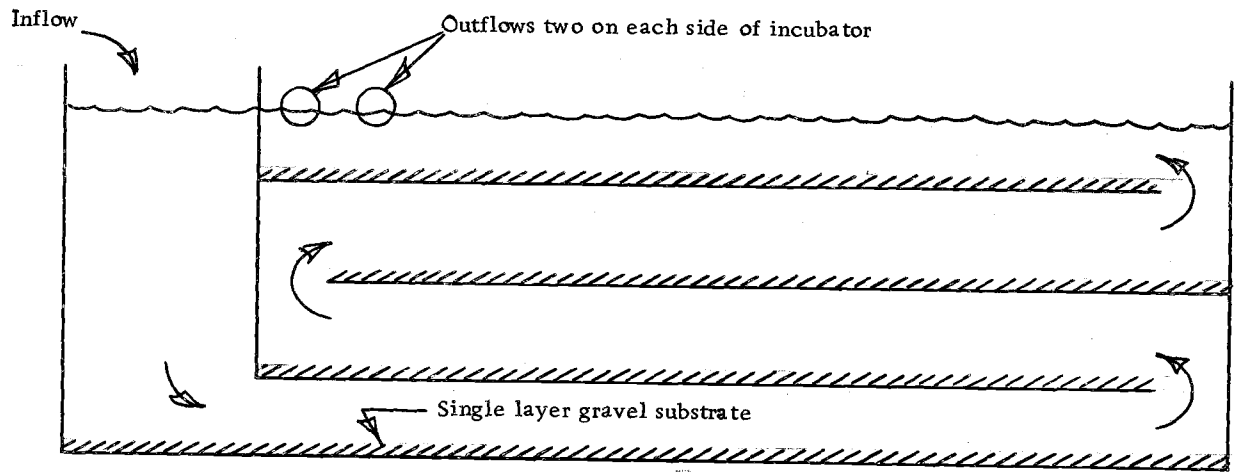
For this experiment, only one out of the four banks of boxes was used. The end compartment was left unstocked to allow a staging area for the migrating fry; to discourage movement of premature fry into this compartment, the lid was left slightly open to allow some

light penetration.

The multiple layer shallow matrix incubator was a gravel-lined four layer (three trays plus bottom) plywood box measuring 1.8 m by 0.6 m by 0.6 m (Figure 30). Water is introduced via the front baffle system to the bottom layer and upwells through each layer to the overflow holes near the top of the tank. A lid is provided to exclude light. Each wooden tray is weighted down by the substrate and can be removed for egg stocking. Eggs are placed on PVC framed plastic screens; unlike the single layer model, however, these screens are not removed after hatching since removal would necessitate the complete removal of each of the top trays.

b. Securing Egg Population

A random sampling of approximately 50% of the Whiskey Creek chum salmon run was the arrangement made with the Oregon Department of Fish and Wildlife for the operation of the Netarts Bay hatchery. Because a substantial amount of spawning goes on in an intertidal area below the adult collection trap near the high-tide line, and because some fish may get by the weir at high water, a 75/25 hatchery to creek sampling ratio was used. While spawners going over the weir at high water have not been observed, it is postulated that a few may get through. Final evaluation of spawner selection procedures revealed that the 50:50 formula was close to the actual



Diagrammatic side view: 1 inch = 1 foot

Figure 30. Multiple-layer shallow gravel matrix incubator tested in 1971 brood year at Netarts Bay. Drawn without lid, egg trays, eggs or alevins.

allocation.

Spawners were sampled from November 1 to November 27, with 50% escapement occurring on November 12, 1971. Using numbers from a random number table, 5 out of every 20 fish of each sex were selected, identified with a spaghetti tag between the fin rays of the dorsal fin, and released above the weir. The remaining 15 fish were separated by sex and held in holding pens in the reservoir next to the weir.

Hatchery spawning on a day-to-day basis extended from November 6 to December 1 using the same fertilization procedures previously outlined for the Little Port Walter study. However, since the number of females spawned each day was variable, the fish were not spawned to fill a gallon bucket at a time as at Little Port Walter, but were simply spawned on a one to one ratio of male to female. Fifty percent of the spawning was completed on November 18.

Spawner holding was hampered by some escapements. Between November 14 and November 23, 86 males and 8 females escaped from the pens into the settling ponds (204 males and 123 females were being held at this period). Although these escaped fish could go downstream and end up below the weir, or go upstream and be above the weir, surveillance of the downstream overflow and the area around the overflow indicated that the escaped fish most likely went upstream and spawned above the weir. After this escapement, allocation of males

for the creek was curtailed and only 3 more males (out of 20 processed) were released into the creek. The escapement, fortunately, occurred near the end of spawner sampling and consisted of many used males which had already been incorporated into the hatchery gene pool thus mitigating the effect of the escapement on the compatibility of the hatchery versus the wild population.

A large number of fish which arrived in the latter part of the run did not press at the weir but spawned below the weir. To enumerate these fish, unmarked spawned-out fish in this area were counted and removed daily. This count is a minimum count because fish which spawned and were washed away undetected were not enumerated. Any error, however, was probably higher for females than for males since females dominated the late portion of the run and were in higher numbers below the weir.

The final spawning record showed that of the 489 fish (277 males, 212 females) in the run, 233 fish (118 males and 115 females) were spawned for the hatchery, and 256 fish (159 males and 97 females) were allowed to spawn in the creek (Table 15). Of the 115 females spawned for the hatchery, approximately 110 female "equivalents"[9] were spawned successfully. With an adjusted (for wasted eggs during spawning) estimated fecundity of 2500 eggs per fish, 110 females

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[9]"equivalent" refers to the average fecundity of a female spawner.

Table 15. Sampling of Whiskey Creek chum salmon spawners for hatchery propagation during the 1971 brood year hatchery versus wild fry evaluation at Netarts Bay.

# fish processed									# fish spawned for hatchery[1]						# fish spawned in Whiskey Creek					
at weir			below weir[2]			Total			Total			above weir			below weir[2]			Total		
♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀	♂	♀	♂+♀
246	165	411	31	47	78	277	212	489	118	115[3]	233	128	50	178	31	47	78	159	97	256

1/ Actual number of fish held were 204 ♂♂ and 123 ♀♀, but 86 ♂♂ and 8 ♀♀ escaped between 11/14 and 11/23 and spawned in the creek.

2/ Based on counts of untagged spawned out fish removed from the creek.

3/ Actual egg content of the 115 ♀♀ was equal to the total fecundity of 110 ♀♀ because some females were partially spent.

yielded approximately 275,000 eggs for the hatchery.

The total number of fish processed for the creek showed an unusually high proportion of males to females (277:212), which can probably be explained by the low estimate on the number of unmarked females in the stream below the weir. There is a possibility that the male count was high due to the 86 escaped holding pen males which can be washed back down below the weir and be reprocessed. This was unlikely or was a low contribution because very few of the marked fish were ever observed again at the weir; they apparently were caught by debris in the creek above the weir.

Spawner distribution above the weir was impossible to assess due to extensive accumulation of logging slash and debris which provided hiding places for the fish. It was established, however, that a massive logjam approximately 650 m from the creek mouth restricted further spawner distribution upstream.[10] On a clear water day (November 17) when 70 marked fish were known to be above the weir, a survey over the entire length of the stream (about 5000 m) resulted in no observed spawners above the logjam. The possibility that spawners escaped detection in the upper creek was unlikely because most of this upper area was good quality spawning bed cleared from debris and also because other surveys also failed to detect

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[10]This observation was made again in 1972 and 1973 brood years by Dr. James Lannan of OSU. But since 1973, the logjam has been removed by the Oregon Department of Fish and Wildlife and access is apparently not a problem at this date.

spawners above the logjam.

c. Stocking and Maintaining Incubation Treatments

Hatchery eggs were separated into three categories: 1) 18,000 eggs taken on November 16 for laboratory experiments (cf. p. 92); 2) 240,000 eggs for incubation in the production hatchery boxes, and 3) 35,000 eggs (12.73% of total available eggs) accumulated from sub-samples taken from every spawned female (Figure 31).

The 35,000 eggs provided for the key experimental treatment of this study: the multiple layer box. The specific purposes of the box were to: 1) create an egg population which was as comparable as possible with the creek population--since 18,000 eggs were removed from the general hatchery for laboratory studies, the general hatchery did not represent the total sampled hatchery population; 2) test the multiple layer shallow gravel matrix box as a space saving alternative to the general hatchery design; 3) test shallow gravel matrix design under a favorable low density high flow condition; 4) create a situation where fry migration pattern can be determined accurately by trapping the entire experimental population. On January 22, 12,000 eyed eggs were distributed evenly between the layers of the box and the water velocity was set at 30 lpm; this gave a ten-fold lower stocking density and a two-fold higher water velocity than the production hatchery (Table 16). If these conditions were more favorable than the production hatchery, there should have been a detectable difference in fry

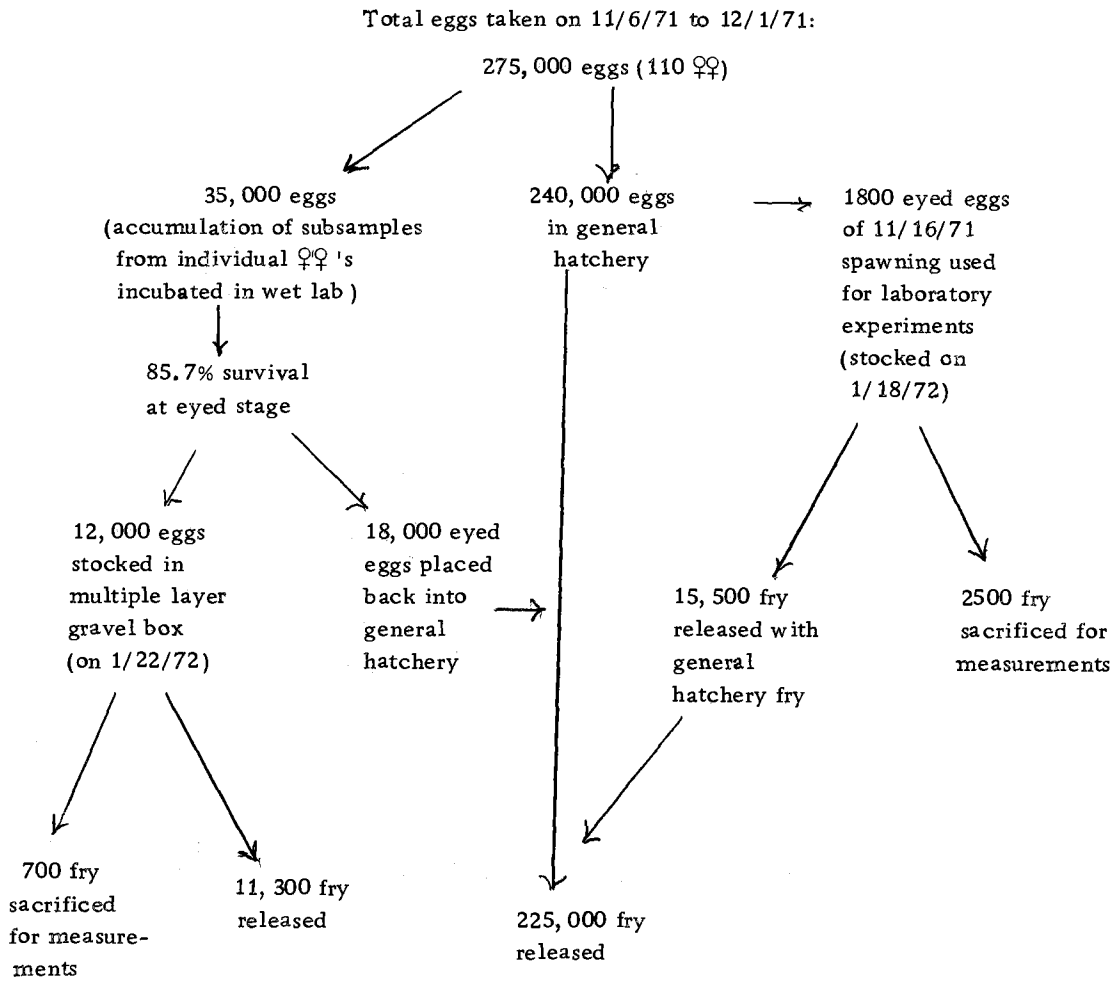

Figure 31. Distribution and survival of chum salmon eggs for hatchery incubators of the 1971 brood year hatchery versus wild fry evaluation at Netarts Bay.

Table 16. Substrate areas, stocking densities and water velocities for hatchery incubators of the 1971 brood year chum salmon hatchery versus wild fry evaluation at Netarts Bay.

Incubator	Total substrate area	Egg stocking		Water velocity	
		Estimated # stocked	Density		
	cm^2		$eggs/cm^2$	lpm	cm/hr[1/]
General Hatchery	93, 450[2/] (7 units @13350)	258, 000 (@ 40, 000/unit)	3.0	57-76	811-1081
Multiple layer box	36, 455 (4 layers)	12, 000	0.3	30	2345

1/ Assumes horizontal laminar flow. Actual flow approaches turbulent pattern with slowest velocity next to substrate.

2/ Each row has eight units, but last unit left empty for fry accumulation.

quality. Surplus eggs not used for this treatment were returned to the production hatchery boxes.

In maintaining and recording the experimental conditions, hatchery water temperature was recorded with a Taylor maximum-minimum thermometer. A temperature comparison was made daily between the creek water and the hatchery water. From the first spawning through the end of creek migration hatchery water temperature varied from 1°C to 13°C, with an average temperature of 7°C (Appendix 11). Water temperature in the creek and in the hatchery were virtually identical from December 6 through March 10. After this period, the hatchery water was about 0.5°C warmer on approximately one third of the daily spot readings up until April 21. Thus, the hatchery water appears to be slightly warmer over the terminal incubation period. Water velocities of the incubators were checked periodically and adjusted where necessary. Mortalities were assessed at the eyed stage and after migration for the multiple layer box; for the general hatchery boxes, mortalities were assessed after hatching from volumetric measurements of the dead eggs left on the egg screens.

No problem was experienced in maintaining designated incubator flow rates, and adjustments were infrequent. Since mortalities were minimal and eggs were well spread on egg screens, there was little fungus growth. No flush-out of alevins occurred in either incubation

treatment, but in the general hatchery, some downstream movement was evident with both alevins and fry. The movement intensified with advancing development, and neutral buoyancy was attained by most of the fry well before button-up. As early as February 21, an estimated 1,000 to 1,500 advanced alevins had accumulated in the end hatchery box, but they did not migrate out of the box. Movement of alevins and fry could not be observed easily in the multiple box, and it is not known if accumulation had occurred.

The heavy mineral silt in the water supply imposed no mechanical problems in hatchery operation. Biologically, the alevin's digging actions often removed settled silt from their immediate environment. It is not known if the presence of silt extracted an energy cost for silt-clearing physiological mechanisms (cf. Bams 1969; Stuart 1953).

Because of the access problem in Whiskey Creek, no attempt was made to assess incubation conditions or survival within the streambed. However, it was observed after freshets that scouring had occurred and some eggs and alevins were probably dislodged. The effects of siltation on intragravel flow and chemistry were not measured.

d. Trapping, Enumerating, and Sampling Migrant Fry

Migrants were trapped, enumerated and sampled from the

multiple layer box February 21 to April 20, from the general hatchery boxes February 24 to April 20, and from the creek March 6 to April 25. Sampled fry were preserved in 10% formalin.

All migrants from the multiple layer box were channeled into a large perforated bucket and all fry were hand counted daily and accumulated in the wet laboratory. Whenever 500 fry had been accumulated, or on every Monday--whichever came first--a sample of 60 fry was preserved, and the remaining fry released. Fifteen samples were taken over the migration period.

For the general hatchery, no attempt was made to count all the migrants, but the migration pattern was indexed by channeling a small portion of the outflow into a net placed inside a large bucket. Each day's catch was counted by hand and released into the holding pond; in total, an estimated 2.75% of the population was enumerated. In order to compare the size and stage of development of the general hatchery fry with those of the multiple layer box, two samples of 100 fry were preserved--one at the peak of the migration period and one 10 days later.

Fry migration in the creek was indexed by trapping a portion of the migrants. A winged migrant trap was placed at the high tide line, anchored into the streambed by steel fence posts, positioned in the middle of the mainflow (during low water periods). Another trap was placed at the overflow of the settling pond, the water of

which originated at a culvert connected to the upstream side of the weir. Both traps were checked each morning for trapped fry. The pond trap was not expected to net many fry, however, because: 1) the culvert opening is located near the bottom of the creek and fry generally migrate near the water surface; 2) the water drawn through the culvert is small relative to the water going over the weir, thus fry will likely migrate with the stronger flow.

The traps fished throughout the migration period except on two high water days for the creek trap and on seven high water days for the pond trap. As expected, 77% of the fry were trapped by the creek trap. The total migrant population cannot be extrapolated from the migration index since the number of fry which were missed on those high water days can not be estimated. Also, the stream flow at high water is no longer directed at the creek trap but is directed at the entire width of the creek. A total of 152 fry were trapped and all were preserved for quality analysis. Excluded from processing were two fry which had obvious freshwater growth and seven fry which were killed during holding within the trap.

e. Evaluating Fry Quality

Fry quality was evaluated in the same fashion as in the pink salmon study at Little Port Walter including the deletion of performance tests and post-emergence growth due to difference in migration

timing of hatchery and wild fry. Fry size conversion was implemented using data generated for the concurrent laboratory experiments (Appendix 15).

In contrast to the Little Port Walter study, a significant temperature difference did not exist between the hatchery and the creek, and Heath incubator fry were not involved, thus fry size and stage of development evaluation followed closely previously established procedures.

C. Results

1. Pink Salmon Study at Little Port Walter

The physical operation of the Little Port Walter hatchery went smoothly except for premature flush-outs of incubator No. 5. Dissolved oxygen apparently was not a limiting factor based on measurements of outflow water in some incubators. On April 13, outflow water of incubator No. 4 and the Heath incubator had dissolved oxygen levels of 10.8 and 12.6 mg/l respectively; the incoming water was at 12.4 mg/l and 2°C. Since this was during early migration when dissolved oxygen demand was at its highest in the incubation period, and since incubator No. 4 has the highest density and the lowest velocity, dissolved oxygen levels in other incubation units were probably non-limiting also.

a. Mortality

In all incubators where eyed eggs were stocked (all treatments except #1) survival was over 90% (Table 17); in two instances, survivals were 103% and 102% (Incubators 3 and 4) which were not outside of expected estimation errors of up to 6-10%. The only incubator with a lower survival (81%) was incubator #1 which was stocked with newly fertilized eggs. The relative numbers of mortalities left behind in the incubators supported the percentages estimated. As an illustration of possible high survivals only 182 dead eggs and 18 dead alevins were counted out of approximately 20,000 eyed eggs stocked in the Heath incubator; this was a 99% survival.

Most of the mortalities occurred before hatching. During the elimination of dead eggs in October, 12% mortality was estimated. For the entire hatchery, the total estimated migrant count of 337,253 fry represented 96% of the eyed and green eggs planted and 87% of the eggs spawned. This can be compared to the 5.38% estimated survival (based on egg pumping data) in Sashin Creek.

b. Frequency of Physical Abnormalities

Yolk sac malformation was not observed in any of the gravel incubator fish. In two samples of Heath incubator fish (March 29 and April 4) which had about 1 to 2 mm yolk gaps, yolk tits were observed

Table 17. Survival and migration timing for hatchery and wild pink salmon fry of the 1971 brood year at Little Port Walter.

Incubation site	Estimated[1] # eggs stocked	Estimated % survival	Dates (in 1972) of cumulative fry migration			Length of[2] incubation period
		%	5%	50%	95%	days
Incubator #1	70,000 (green)	81[3]	4/21	4/30	5/7	233
Incubator #2	70,000 (eyed)	99[4]	4/18	4/25	5/4	233
Incubator #3	85,000 (eyed)	103[4]	4/4	4/15	4/26	228
Incubator #4	85,000 (eyed)	102[4]	4/5	4/19	4/27	232
Incubator #5	20,000 (eyed)	91[4]	12/21[5]	5/2	5/14	240
Heath incubator	20,000 (eyed)	99[6]	----	----	----	---
Total Hatchery	350,000	96				
Sashin Creek	29,668,000[7] (green)	5.38[8]	5/3[9]	5/27[9]	6/14[9]	268[10]

1/ Accuracy of @ 5% per estimate.
2/ Period = # days from mean fertilization date to 50% cumulative fry migration.
3/ Large number of dead eggs found in gravel substrate after total emergence.
4/ Few dead eggs found in gravel substrate after total emergence.
5/ Early date is the result of premature flush-outs.
6/ Mortality was hand counted on 5/8/72: 182 eggs, 18 alevins.
7/ Calculated from: average fecundity X # female spawners.
8/ Estimated from pre-emergence egg pumping.
9/ Estimated from fyke-net index at weir.
10/ Mean fertilization date for creek was designated as 9/3/71, day of heaviest spawning.

on 22% and 14% of the sampled fish, respectively. The extent of the malformation can not be ascertained since no samples were taken prior to this stage of development. The incidence of malformation could not have been severe as mortality was estimated at 1%.

While YSM was not observed with gravel incubator fish, it was observed that normal alevins forced to become active in an environment without physical support did develop the condition. This observation suggests that activity may be a causative agent of yolk-sac malformation.

c. Migration Timing

Migration timing was vastly different between wild and hatchery fish but not between hatchery gravel incubators. All gravel incubator fry reached mid-migration at around 230 days after fertilization, whereas incubator No. 5 took 240 days and the wild fry took 268 days (Table 17). The delay in shallow gravel, low egg density incubator can probably be attributed to the slowing down of the water flow to control premature flush-outs and the subsequent holding of the fry inside the incubator. The difference in timing of the wild fry was caused by the large difference in temperature regime of the hatchery and the creek, although this difference was so large that timing was probably influenced by additional factors other than temperature; whether a timing difference will still exist if the temperature

difference were eliminated is not known.

The migration timing for the replicate deep gravel-high egg density treatments (incubators #3 and #4) demonstrated a low variability of about one to four days in the 5, 50, and 95% cumulative migration dates (Table 17). Given this expected variability, the number of days required to reach 50% migration was about the same for all gravel incubators except for the delayed shallow gravel-low egg density incubator.

d. Fry Size and Stage of Development

Heath fry reached an average maximum length of 31.5 mm, which was shorter than the weighted observed mean lengths (31.73 mm to 32.45 mm) of all other treatments (Appendix 17, Table 18, Figure 32). When the latter range was projected to their maximum lengths, the values were 32.1 mm to 33.0 mm; thus, the maximum observed lengths of the Heath fry were 0.6 mm (1.87%) to 1.5 mm (4.55%) shorter than the projected maximum lengths of other treatment fry.

Heath fry apparently possessed a higher rate of development and reached button-up before fry from other treatments. As early as April 14, when no other incubator had experienced a peak migration period, the Heath fry were already near total yolk absorption. By May 5, before the 50% cumulative fry migration of other incubators, all Heath fry had nearly closed yolk slits; migrants from other

Table 18. Ranking of the weighted means of size (length) and stage of development (Kd) of migrant pink salmon hatchery[1/] and wild fry of the 1971 brood year at Little Port Walter.

Size ranking (longest to shortest)								Stage of development ranking (most to least development)				
Rank	Incubator	N[2/]	Converted[3/] length $\bar{X}$	Observed length $\bar{X}$[4/]	Observed length $S^2_{\bar{X}}$[5/]	Observed wet weight $\bar{X}$[4/]	Observed wet weight $S^2_{\bar{X}}$[5/]	Rank	Incubator	N[2/]	Kd $\bar{X}$[4/]	Units $S^2_{\bar{X}}$[5/]
			mm	mm	$\times 10^{-3}$ mm	mg	mg					$\times 10^{-6}$
1	#5	8	32.44	32.45	5.5486	255.54	3.444	1	#3	7	1.9387	8.694
2	Creek	9	32.22	32.43	2.9974	249.91	1.456	2	Creek	9	1.9405	5.954
3	#2	9	32.18	31.94	3.2018	249.19	2.442	3	#4	9	1.9422	8.976
4	#1	8	31.95	31.73	3.9682	243.52	3.556	4	#5	8	1.9537	6.798
5	#4	9	31.65	31.84	3.5292	237.04	3.148	5	#1	8	1.9661	11.02
6	#3	7	31.51	31.75	5.0454	234.57	3.732	6	#2	9	1.9677	8.696

1/ Excluding Heath incubator fry

2/ Each sample within N is mean of 50 fry.

3/ Converted length = Observed length - [(Observed Kd - 1.9532)(-16.86107)] . See p. 26 for discussion of formula parameters.

4/ Weighted mean = $\Sigma W_i \bar{X}_i$, where W = weighing factor

5/ Weighted variance = $\Sigma W_i^2 V(\bar{X}_i)$, where $V(\bar{X}_i) = \frac{S^2_{X_i}}{50}$

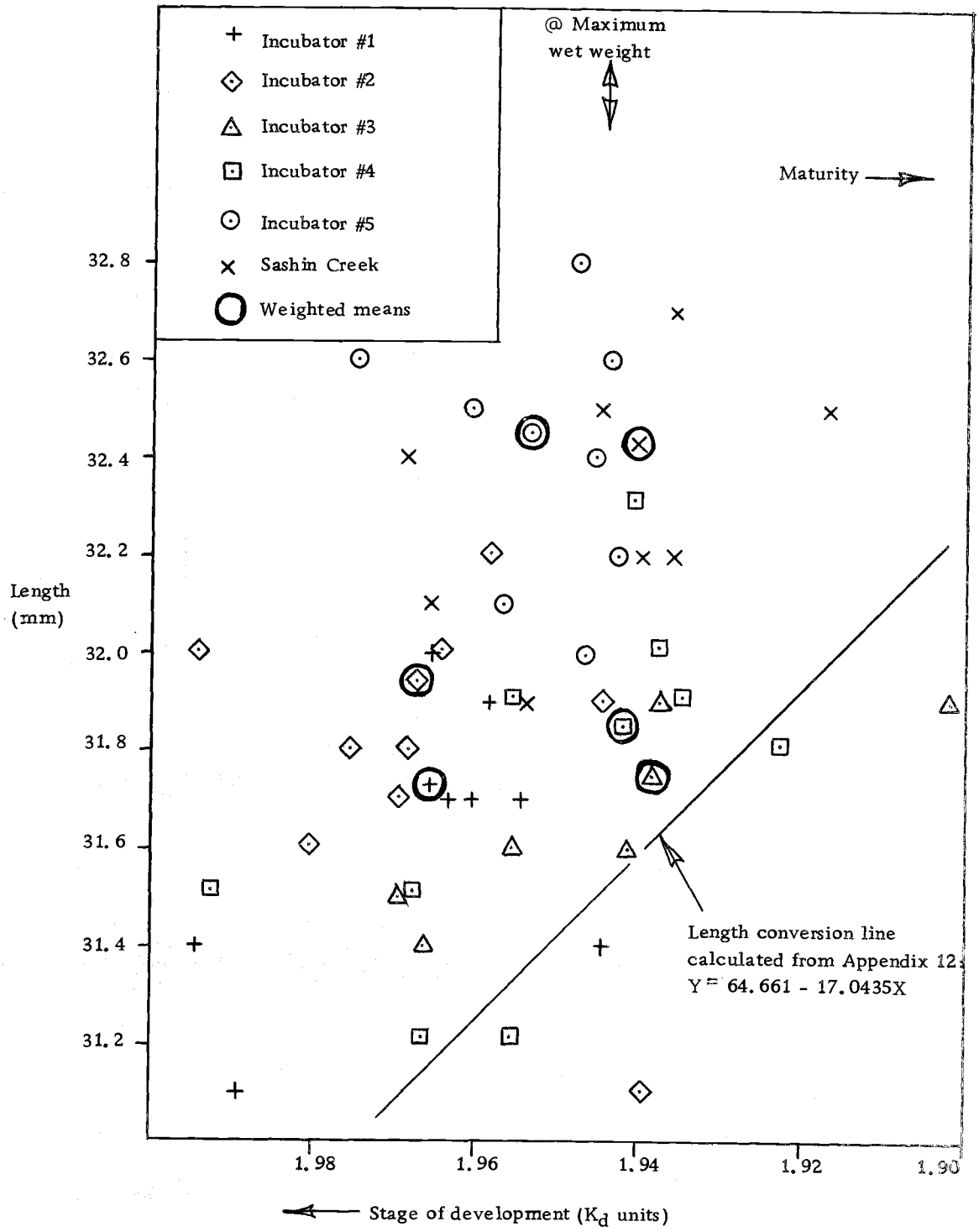

Figure 32. Relationship of length and K_d for pink salmon fry sampled at migration for all incubators during the 1971 brood year hatchery versus wild fry evaluation at Little Port Walter. Each point is an average of 50 fry.

incubators generally had yolk slits of 0. 5 mm to 1. 0 mm in width. The average stage of development was therefore more advanced for the Heath population than for other populations and this difference apparently persisted through button-up.

In assessing treatments other than the Heath incubator, converted weighted mean lengths and unconverted weighted mean kd values were ranked and their means plotted with their 2 x SE intervals for evaluation of differences between the means.

In assessing relative lengths, the derived size hierarchy was as follows:

Rank	Treatment
1	#5: multiple layer shallow gravel incubator
2	Sashin Creek
3	#2: deep gravel low egg density incubator
4	#1: deep gravel low egg density incubator (green eggs)
5	#4: deep gravel high egg density incubator
6	#3: deep gravel high egg density incubator

The difference in length between incubator #3 and #4, the replicate treatment, was 0. 23 mm; in contrast, the difference between incubator #5 and incubator #3 was 0. 93 mm (Table 18). Thus, a significant difference apparently existed between treatment means. The plot of mean lengths and thir 2 X SE intervals showed general interval overlaps between ranks but that a significant difference

probably exists between the first ranks 1, 2, and 3, and ranks 5 and 6 (Figure 33). These results yileded the following conclusions concerning relative fry size: 1) fry from the creek and from incubator #2 and #5 were equivalent in size and were longer than fry from other incubators; 2) the lower density deep gravel incubator produced longer fry than its higher density counterpart; 3) no size difference was observed between the use of green or eyed eggs for stocking deep gravel incubators; 4) no size difference was observed between shallow gravel-matrix fry and deep matrix fry.

In relating mean size to overall fry quality, small numbers of emaciated and deformed fry were observed in the terminal migration stages in hatchery incubators. These fry were apparently able to survive in the pampered environment of the hatchery but they are not expected to survive long after emergence in the wild, if they reach emergence at all.

The results of the stage of development assessment between gravel incubator and wild fry followed the same format described for relative size, except kd values are observed and not corrected values. The derived hierarchy of developmental stages from most to least advanced are as follows:

Rank	Treatment
1	#3 deep gravel high egg density incubator
2	Sashin Creek

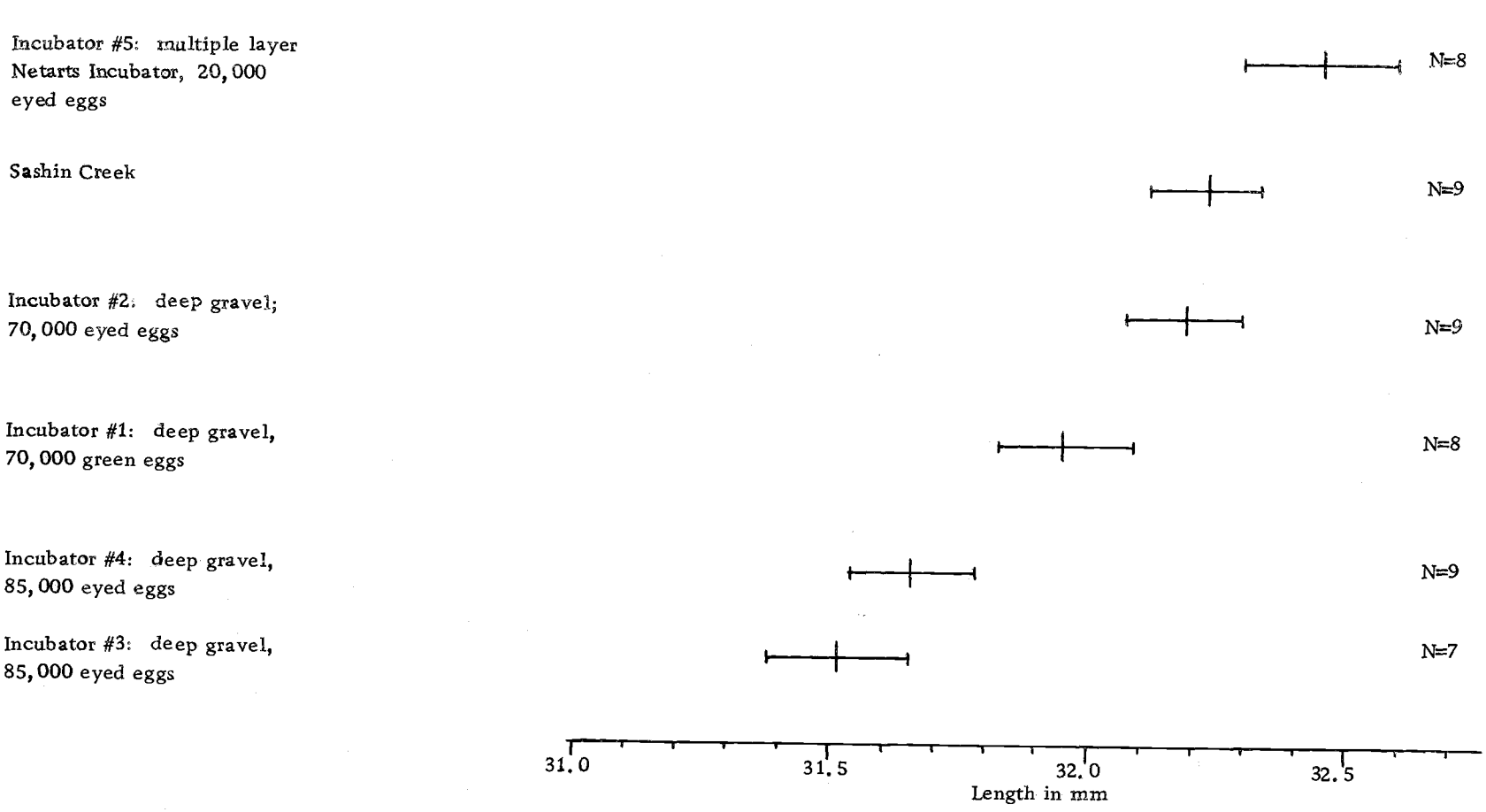

Figure 33. Length (corrected to common K_d of 1.9532) for migrant pink salmon fry produced from hatchery incubators and Sashin Creek during the 1971 brood year at Little Port Walter. Length of each line represents three times the standard error of the pooled means for N samples of 50 fry per sample.

3 #4 deep gravel high egg density incubator

4 #5 multiple layer shallow gravel incubator

5 #1 deep gravel low egg density incubator (green eggs)

6 #2 deep gravel low egg density incubator

The difference in kd between the replicate incubator #3 and #4 was 0.0035 kd units whereas the difference between #3 and #2 was 0.029 kd units (Table 18). Thus, a significant difference apparently existed between treatments. Plot of mean kd values of each treatment resembled closely the plot for mean lengths: general overlaps between ranks with #1, #2, and #3 being equivalent and significantly more advanced than ranks #5 and #6 (Figure 34). These results yielded the following conclusions regarding the relative stage of development at migration: 1) the two deep gravel high density gravel incubators and the wild fry were equivalent in stage of development and were more advanced than other treatment fry; 2) the higher density deep-gravel incubator produced more advanced fry than their lower density counterpart; 3) no difference was detected between use of green or eyed eggs for stocking deep gravel incubators; 4) the shallow gravel-matrix design (Incubator #5) was slightly less advanced than the deep gravel high density incubators (#3 and #4) and the wild fish, and was slightly more advanced than the low density deep gravel incubators (#1 and #2)--these differences may not be significant.

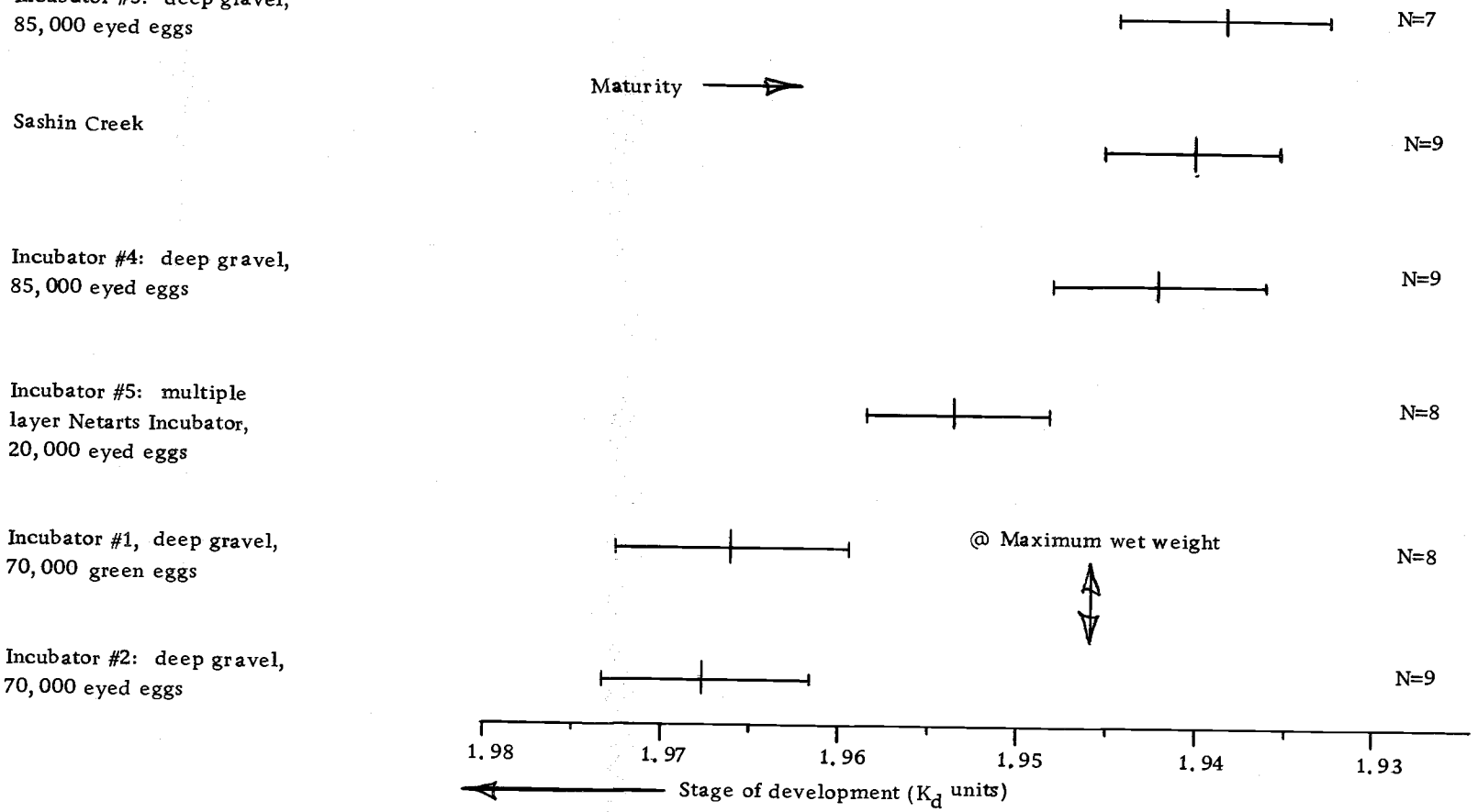

Figure 34. Uncorrected developmental index (K_d) of pink salmon migrant fry produced from hatchery incubators and Sashin Creek during the 1971 brood year at Little Port Walter. Length of each line represents two times the standard error of the pooled mean from N samples of 50 fry per sample.

Of significance to all above conclusions is that the number of days in incubation, i. e. from fertilization to 50% migration, was similar for all hatchery treatments except for the shallow gravel-matrix incubator (#5), the fry of which held within incubator for about 7 more days; thus, if holding had not occurred, the average kd value for #5 would have been higher (less mature) which would make its fry more equivalent to incubator #1 and #2 in average stage of development (Figure 34). Hence, if the length of incubation period can be assumed constant for all treatments, then the more advanced status of incubator #3 and #4 would reflect a higher rate of larval development relative to the other treatments.

2. Chum Salmon Study at Netarts Bay

The physical operation of the Netarts hatchery went smoothly throughout the 1971-1972 season. No biological problems were observed with the fish up to the migration stage.

a. Mortality

Of 240, 000 green eggs stocked in the general hatchery boxes, approximately 210, 000 fry migrated for estimated survival of 81. 4%. For the multiple layer box, the 12, 000 eyed eggs produced 11, 816 migrants for an estimated survival of 98. 5%. Since survival at the eyed stage was 85. 7% for the multiple layer box, the two hatchery

incubation treatments produced comparable survivals and mortalities. Accuracy of these estimates, given the errors of volumetric measurements, are projected to be within 5 to 10% (Table 19). Comparable mortality data from the creek are not available. Although the number of trapped fry (152) can not be used as an index of creek mortality, the low number trapped would suggest that overall survival was probably low.

b. Frequency of Physical Abnormalities

No yolk-sac malformation was observed in any of the hatchery or wild fish.

c. Migration Timing

The timing of the general hatchery appears to be about 5 days earlier than the multiple layer box and both hatchery incubators are earlier than the creek timing by about two to three weeks (Table 19). The effects of missing creek migration data on high water days are treated under discussion.

d. Fry Size and Stage of Development

Both timing and stage of development were different between creek and hatchery migrants. The later creek fry migrated when stage of development was close to total buttoning, wet weights were

Table 19. Survival and migration timing for hatchery and wild chum salmon fry of the 1971 brood year at Netarts Bay.

Incubation site	Estimated # eggs stocked	# migrants	Estimated % survival	Dates (in 1972) of cumulative fry migration[1/]		
				5%	50%	95%
General Hatchery	240,000 (green eggs)	210,000[2/] (estimated)	% 81.4	2/28	3/11	3/30
Multiple layer box	12,000[3/] (eyed eggs)	11,816 (hand-count)	98.5	3/4	3/16	4/5
Whiskey Creek	242,500[4/] (green eggs)	No data	No data	3/19	4/2	4/18

1/ Based on index population of general hatchery and Whiskey Creek, but on total population of multiple layer box.

2/ Estimation based on: (Estimated # eggs stocked - estimated # dead eggs). Alevin mortality is assumed to be negligible.

3/ Survival at eyed stage was 85.7%.

4/ Based on Potential egg deposited by 97 spawners.

on a decline, and lengths were near maximum. In contrast, hatchery fry migrated at an earlier stage of development which ranged from close to total buttoning to pronounced yolk gaps, and most migrants had not reached maximum wet weight or length (Appendix 18; Figure 35). For both creek and hatchery trapped migrants were increasing in length and stage of development with advancing time in the migration period. The observed range of development was much wider for the hatchery fish (2. 190-2. 050 Kd unit) than for the wild fish (2. 061-2. 016 Kd units) although the migration period was about the same (30 days).

The homogeneity of fry size could not be compared between the two populations since the number of wild fry samples was too small. Unlike the pink salmon migrants from deep gravel matrix incubators at Little Port Walter, no emaciated chum migrants were observed from any of the incubation sources at Netarts Bay.

The plot of length on Kd for the hatchery versus wild fry (Figure 35) not only revealed the clear linear relationship of length with advancing stage of development but also separated the three incubation treatments into a size hierarchy (from longest to shortest length) of: 1) multiple layer box, 2) general hatchery, and 3) wild fry. The weighted mean converted length and unconverted Kd for each incubation treatment can be ranked as follows:

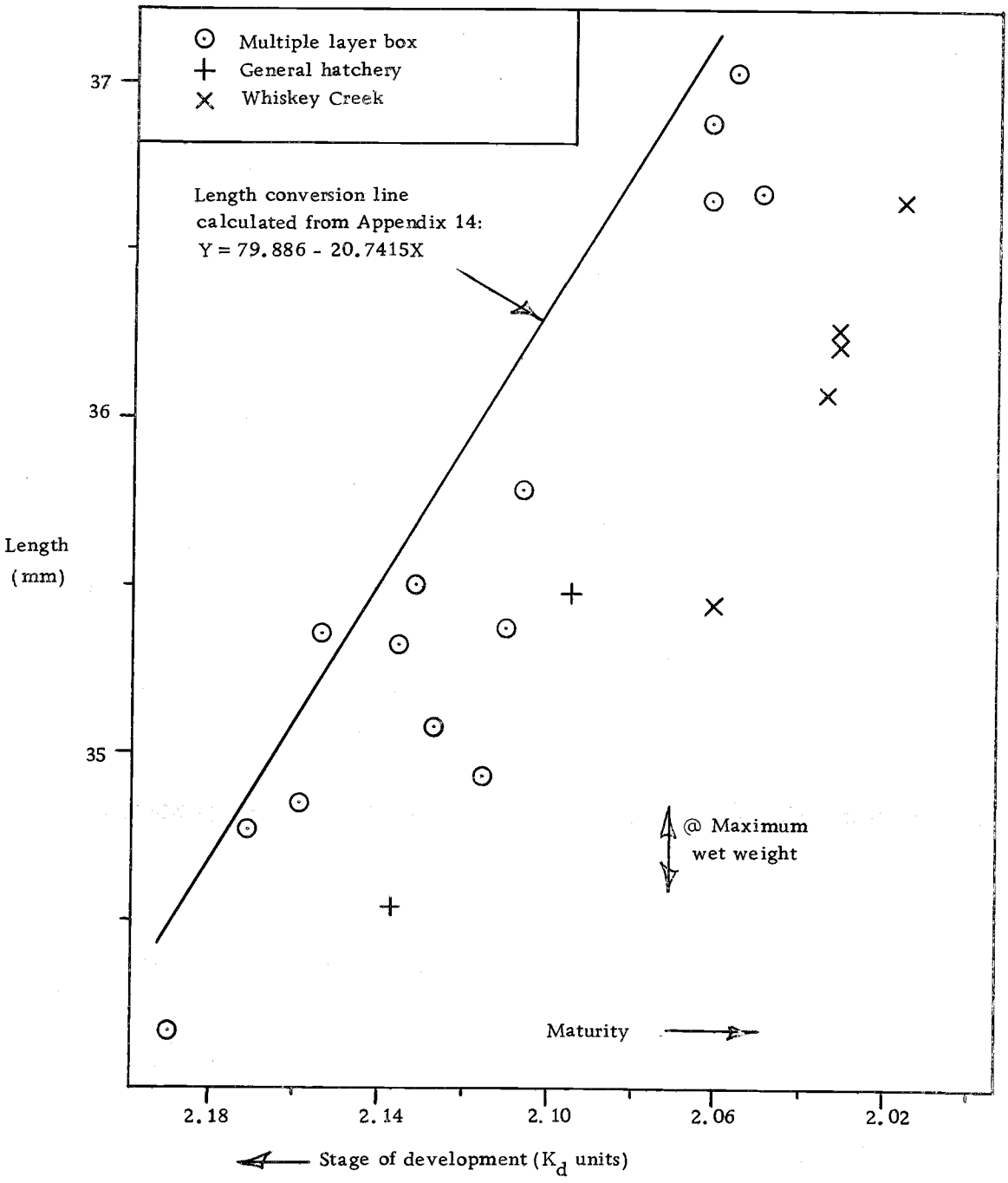

Figure 35. Relationship of length and K_d for chum salmon fry sampled at migration for all incubation treatments during the 1971 brood year hatchery versus wild fry evaluation at Netarts Bay. Each point is an average of 30 fry, except for general hatchery fish, for which each point is an average of about 100 fry.

Rank	Length (longest to shortest) Incubator	Converted length[11] mm	Stage of development (most to least advanced) Incubator	Observed Kd
1	Multiple layer box	36.524	Whiskey Creek	2.0346
2	General hatchery	35.892	Multiple layer box	2.1121
3	Whiskey Creek	35.330	General hatchery	2.1158

The creek fry were 1.194 mm (3.3%) shorter than hatchery (multiple layer box) fry. This difference, although small, is quite distinct and significant when considered with the clear linear trend.

The difference observed in stage of development (0.0775 Kd units or about 10 days) was less than expected based on the difference in migration timing of 16 and 21 days for multiple layer box and general hatchery, respectively, i.e., if the hatchery fry were held back until the time of creek migration, their stage of development would still have been 6 and 11 days more advanced. This difference could not be explained by a difference in water temperature, or by an inaccuracy of the creek migration index. The creek water, which warmed up slightly near the time of migration, would have caused an increase in developmental rate and a possible earlier migration timing, both of which would have decreased the timing difference.

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[11] Conversion based on equation on Table 11, with substitution for common developmental index entry appropriate for this set of data (cf. p. 26).

The accuracy of the creek migration index was influenced by two days of no data during high-flows for the creek trap (March 12, April 8) and for seven days for the pond trap (April 7-10, April 12-14). March 12, however, occurred very early in the migration period and few migrants were expected. All other dates fell after the calculated 50% cumulative migration day. Thus, any adjustments to the estimated migration pattern by inclusion of no data days would delay the 50% cumulation migration date which would increase rather than decrease the timing difference between the propagated and wild fry. The only conclusion, therefore, is that since hatchery fry were 6 to 11 days less developed than creek fry even with the adjustment for stage of development difference, they must have experienced a faster developmental rate.

The above analysis has not included the general hatchery fry, except for reference to timing difference. The limited sampling of these fry suggested, however, that they were longer than the wild fry but shorter than the multiple layer box fry. Their stage of development at migration also covered a range similar to the multiple layer box migrants--this assessment was based on visual inspection of the fry during daily migration checks.

D. Discussion

The null hypothesis of this study--that propagated fry should be

equivalent to wild fry in quality if environments of gravel incubators simulated the natural streambed--was satisfied by one or more incubators tested at each site by the criterion of relative yolk conversion efficiency, other parameters on fry quality were inconclusive. The combined results from laboratory and field studies on incubator design suggest that gravel incubators are basically sound and that their operation and design can be improved.

1. Pink Salmon Study at Little Port Walter

At Little Port Walter, conclusions about incubator performance were confounded by warmer water temperature leading to early migration of hatchery fry. Nevertheless, size and stage of development of fry from the low density, deep gravel incubator (#2), the multiple-layer, shallow gravel matrix incubator (#5), and Sashin Creek compared favorably. This is strong evidence that these incubators can produce high quality fry. Fry produced from other incubators, however, were judged to be of lower quality.

The Heath incubator contained 3300 eggs per tray (less than one-half full) and was held in darkness, procedures which should be optimum for this system; yet by comparison to other treatments, Heath fry were the smallest and reached button-up the earliest. In a similar experiment using the same brood of Sashin Creek eggs, Bailey and Taylor (1974) compared deep gravel incubator fry with

Heath fry incubated in darkness at 2000 alevins per tray. They found that the Heath fry were smaller and reached button-up earler than the gravel incubator fry. In the concurrent laboratory experiment at Netarts Bay, however, Heath incubator fry stocked at 2200 pink eggs (from Sashin Creek) per tray and 1600 chum eggs per tray produced no size or timing difference compared to gravel incubator fry. While the latter results appeared to be inconsistent with data from Little Port Walter and from Bailey and Taylor's work, significant differences in experimental treatments and conditions preclude a direct comparison among the three experiments, particularly since the data in question are based on relative measurements. Significantly, yolk sac malformation was found at Little Port Walter and not at Netarts Bay, suggesting that the higher stocking density at Little Port Walter might have produced a higher level of larval activity and lowered yolk conversion efficiency.

In addition to size and timing, the use of the Heath incubator for production of unfed fry may not be appropriate due to the lack of volitional migration. Release of Heath fry cannot be adjusted for inherent differences in stage of development between individuals within the population. In contrast, voluntary migration from the gravel incubators allows fry of advanced stages to migrate and less advanced fry can remain until they reach the proper stage of development.

Data from the deep gravel incubators provided some guidelines

for stocking density. Based on maximum yolk conversion, stocking density of 0.23 eggs/cm^3 produced fry equivalent in size to wild fry; whereas, a density of 0.28 eggs/cm^3 produced fry which were significantly smaller than wild fry. A density of 0.23 eggs/cm^3 is up to nine times higher than levels used by other workers (cf. Table 1). While the lower density fry were larger, they were also 0.03 Kd units less developed at emergence than the higher density fry; given the approximately equal number of incubation days the higher density apparently produced an increased rate of development. However, since both groups produced reasonably streamlined fish, the difference in stage of development was considered to be of secondary importance. Bams (1972, p. 1164) also observed that higher loading density at his Headquarters Creek hatchery relative to his Hooknose Creek hatchery may have caused a later stage of development at migration for the Headquarters Creek fry.

Comparisons between deep and shallow gravel matrix incubators did not produce evidence to favor either model. The key difference between them was not size or stage of development at migration, but rather the amount of alevin and fry movement, which appears to be resolvable by design refinements and reduced flow velocity. The lack of a stage of development difference was surprising since premature migration is commonly observed with shallow matrix systems (this point will be addressed later).

The relatively successful operation of the multiple layer shallow matrix gravel incubator may resolve the problem of low space utilization efficiency of the shallow matrix design. This approach is

basically similar to a stocking design developed by Salter (1975). The comparability of egg density per unit area of floor space between the deep and shallow models may not be a significant point of contention in the future.

Despite partial loss of substrate support for fry which had moved onto the smooth trough bottom, the shallow gravel matrix incubator produced robust fry; this suggested that the effects of substrate might not have been crucial given the favorable conditions of flow, density, and darkness. However, should density be increased, there will probably be a level when size would be reduced due to increased alevin activity, such as demonstrated with the Heath incubator fry.

As for the use of eyed or green eggs to stock the deep gravel model, the results from both methods were acceptable, based on overall survival, fry size, or stage of development at migration. Such favorable results, however, are predicated on relatively low egg mortality and favorable incubation conditions, the loss of either might adversely affect fry quality. The question, then, is one of safety versus cost: stocking eyed eggs is safer but costlier. Newly fertilized eggs are routinely used with success in British Columbia (Fred Fraser, personal communication, 1976), and elsewhere by this author. I, however, have also seen instances where use of green eggs produced total failures due to unexpected mortality. Thomas

(1975b), in assessing stage of egg development at time of planting on chinook salmon survival at the Abernathy egg incubation channel, obtained better survival from eyed than from green eggs. He also showed that success was more dependable with water-hardened green eggs than unwater-hardened green eggs. Acceptable results were produced in my studies with unwater-hardened green eggs, and the same techniques have also been employed at Netarts Bay with apparent success (Lannan 1975).

Finally, the generally favorable results at Little Port Walter were attained without the aid of water filtration, which has been a main feature of the Bams' system. The water at Little Port Walter is particularly free of fines, and fry quality may not have been affected for this reason. There is little doubt, however, that filtration will be necessary in order to operate deep gravel system with success at sites where water contains high levels of fines. Unfortunately, the degree of fines at which fry quality might be affected has not been determined.

2. Chum Salmon Study at Netarts Bay

The two shallow gravel matrix models tested at Netarts Bay produced fry of equivalent quality to wild fry based on the criterion of relative size. Unlike the systems at Little Port Walter, however, most propagated fry at Netarts Bay migrated well before complete

yolk absorption and at substantially earlier timing, therefore, overall survival potential of propagated fry may have been lowered despite the advantage in yolk conversion efficiency.

The premature migration at immature developmental stages was apparently characteristic of both shallow and deep gravel matrix models (cf. deep gravel matrix test cells in concurrent laboratory experiments) and the reason for it is not known. Premature migration has been correlated with increased levels of fines in the gravel substrate (Phillips et al. 1975; Koski 1966, 1975) and the cause has been attributed to stress from entrapment. Thomas (1975a) found that either increasing temperature 2.2°C or the level of turbidity will increase chinook fry migration from simulated incubation channels; his observations were supported by Coburn and McCart (1967) on pink salmon fry. Bams (1972, p. 1164) postulated that the extensive use of unfiltered water at his Hooknose Creek hatchery (Bams 1970) increased the amount of silt in the gravel interstices and may be one factor responsible for increased premature migration.

Turbidity and siltation did occur at Netarts Bay during high flows. The hatchery water supply was also slightly warmer by about 0.5°C near the terminal incubation period. Entrapment was also possible with the deep gravel test cells but not in the shallow matrix models. If early migration were limited to the shallow matrix systems only, then the obvious lack of restriction to fry movement in

the hatchery boxes may have been one causative factor. But since it occurred also with the deep gravel test cells, the variable of surface versus subsurface incubation seemed inconsequential. It is possible that entrapment stress, lack of restriction to fry movement, turbidity, siltation, and temperature increase operated together to cause the observed premature migration: entrapment stress and siltation would apply to the deep gravel test cells, the lack of confinement would act on the shallow gravel matrix systems, whereas turbidity and temperature increase operated on both. Further, whatever factors caused premature emergence in the hatchery, the observed accelerated development of the hatchery fry would serve to increase the timing difference.

If turbidity, siltation, and entrapment contributed to premature emergence and migration for hatchery fish, they did not seem to affect the wild Whiskey Creek fish. In contrast to hatchery fry, over 95% of the wild fry migrated when their ventral slits were almost closed. The few immature migrants were found predominantly in the earliest 10% of the migrant population. This observation is similar to Koski's observations on chum migrants previously subjected to high intragravel silt content at the Big Beef spawning channel (Koski 1975, p. 112 to 114). It is perhaps not unreasonable to expect behavioral differences between hatchery and wild fish since the intragravel environment of the stream bed differs from the hatchery in such

aspects as water velocity, flow pattern, and natural filtration; these differences may influence the migration mechanism of the fry.

As for a comparison of the two shallow gravel matrix models tested, data extrapolations are limited since the general hatchery was a production model and the multiple layer model was strictly a small-scale experimental model. There is some evidence that the higher flow and lower density of the multiple layer box produced slightly larger fry with a later timing. The differences, however, were not statistically significant. Possible application of the multiple layer box design on a larger scale must await further testing. Technically, there is no reason why this model might not serve as a space saving alternative as the multiple layer model at Little Port Walter, but the design will have to be modified to confine alevins and fry to their respective level within the box and to allow volitional migration at the proper time.

Combined observations from experiments at Little Port Walter and at Netarts, as well as from studies by Bailey and Taylor (1974) at the Auke Creek Hatchery in Alaska revealed a number of characteristics which seemed to be common to shallow matrix systems, whether tested with gravel or with an artificial substrate such as Astroturf®. Recognition of the pattern and design parameters which caused them may lead to changes which may improve fry quality. The common characteristics include: 1) uneven density distribution of alevins and

fry within incubator; 2) flush-out of alevins; 3) premature migration of immature fry; 4) early attainment of neutral buoyancy; 5) accelerated development which may lead to early button-up.

Design parameters which are responsible for the above characteristics appear to include flow rate, density, and confinement. The effects of flow and density on developmental rate has already been discussed. But in all shallow matrix systems in use today, flow rates have generally been higher than necessary based on dissolved oxygen requirements. The lack of confinement allows unhampered alevin and fry movement, whether random or nonrandom, leading to undesirable distribution and possibly flush-out. Early movements within an incubator sometimes allow alevins to swallow air and attain premature neutral buoyancy.

Many of the above problems might be mitigated or eliminated by decreasing flow to a level which will minimize movements and yet supply sufficient dissolved oxygen for proper development. Since established flow velocity and stocking density are a function of density distribution within the incubator, if the alevins and fry can be physically separated in discrete units, such as in stocking trays, not only might flow be decreased, but the unit density per unit floor space may also be increased.

The sum of the evidence would indicate that the shallow matrix system can apparently produce fry of comparable size as wild fry,

and that the common problems encountered are within the range of engineering refinements. Research and development should now test possible improvements, and follow with evaluation of ocean survival of marked fry.

Finally, evaluation of hatchery and natural production was based only on fry in this study, but occurrence of a significant size advantage for the hatchery fry necessarily imply that conditions within the streambed were less conducive to efficient yolk conversion. The latter possibility was surprising since fry produced from gravel incubators with optimum conditions were approximately equivalent but not significantly larger than wild fry (Bams 1970, 1972, 1974, 1976). Thus, the basic assumption that wild fry can serve as a quality standard must be questioned in this study. Given heavy sedimentation in the creek water due to logging activities in the watershed, it is possible that quality of wild Whiskey Creek fry was adversely affected.

The effects of logging and sedimentation on salmon and trout streams are well documented (e. g., Moring 1975; Hall and Lantz 1969; Cordone and Kelley 1961). Sedimentation can affect streambeds by reducing streambed permeability (McNeil and Ahnell 1964; Cooper 1965) and limit the delivery of dissolved oxygen to embryos and alevins. Exposure to low levels of dissolved oxygen can result in reduced alevin size (Shumway et al. 1964; Silver et al. 1963; Phillips

and Campbell 1961) and fry size (Mason 1969). Thus sedimentation may be responsible for the observed reduced wild-fry size in this study.

VI. GENERAL DISCUSSION

This discussion will address three perspectives pertinent to the overall research: 1) fry size as a quality criterion, 2) application of research results, and 3) areas for future research.

A. Fry Size as a Quality Criterion

In this research as well as in most gravel incubation research, fry size has been a basic quality criterion. Accurate size assessments are predicated upon the conversion of observed fry size to a common stage of development for comparison (cf. discussion on p. 22-27) and such conversions may be confounded if growth rates differed significantly between treatments or if sampling methods were not standardized (cf. p. 88; p. 164).

Fry size conversion made use of a regression method in this study, but its applications are contingent upon the satisfaction of the following assumptions.

1. All test populations are random samples from a common egg source.
2. Fry sampling (random or nonrandom) and processing procedures are the same for all treatments.
3. Conversion data are generated indigenously and are calculated for the range of developmental stages of the sampled fry.

4. The calculated size conversion factor (size unit per developmental index unit) is not significantly different among treatments.
5. The regression model applies only to the terminal larval phase and not to any other phase in the life history.
6. All growth is acquired from yolk and not from extraneous feeding.

Minor deviations from these assumptions may not alter experimental results but their occurrences should be noted.

Given accurate determinations of fry size and with other quality criteria being equal, predictions of survival potential might still be tentative. Data from the swimming stamina test in this research suggested that fry which experienced high survival and were of equivalent size may not perform equally, thus leading to possible variations in survival potentials (cf. discussion on p. 143 and 148). Bams (1972, 1974, personal communication, 1976) demonstrated that when hatchery egg-to-fry survival exceeded 75%, hatchery fry experienced higher ocean mortality than wild fry even though size of hatchery fry compared favorably with wild fry. These observations suggest that favorable incubation environments may optimize fry survival and size despite inherent viability differences, and that these differences may manifest themselves in performance testing and in subsequent ocean survivals. The implication, therefore, is that conclusions

based on fry size alone should be considered a first approximation to survival potential until survival itself can be measured at the adult stage, and that data on other fry quality criteria (cf. p. 11) be generated whenever possible to aid in predicting survival potentials.

B. Application of Research Results

This research generated information on how variations in some key design variables affect fry quality. The evaluation of production hatchery fry at Little Port Walter and at Netarts Bay determined relative merits of different gravel incubators. While experimental data served as useful bases on which to form preliminary judgements on approaches to gravel incubation technology, application of the results must be qualified by the following considerations: 1) this study represents one series of experimental results, the validity of which is subjected to confirmation by other independent studies; 2) results of both laboratory and field evaluations were probably specific for the site, species, and stock of salmon; 3) evaluation of the production hatcheries encompassed a single season at two sites--year to year variations in performance can exist and may modify present conclusions; 4) conclusions are based on fry quality criteria--final judgements should await conclusive proof from evaluation of adult returns.

C. Areas for Future Research

Given the useful but tentative nature of research results, the following general research areas can be identified to clarify, enhance, or perhaps negate my conclusions.

1. The question of shallow versus deep gravel matrix design for production of unfed fry remains unresolved by evaluation at the adult stage. The burden of the proof, however, is on the shallow gravel matrix design since one deep gravel matrix design has already been conclusively evaluated as a success by Bams (1970, 1972, 1974). Changes should be implemented in design and operation of the shallow matrix system to reduce premature migration at immature developmental stages and yet retain the favorable yolk conversion efficiency; marked fry can then be released to compare both designs with wild fry.
2. Design criteria experiments tested in this study should be repeated with different species and stocks and under varying conditions of water quality in order to broaden the application of established design guidelines. In addition to the parameters addressed in this study, the effects of stocking density, degrees of fines, dissolved oxygen, and waste metabolite levels (particularly at the micro-habitat level) should be investigated.
3. Since evaluation of adult returns is impractical for every new design idea, the method of unfed fry quality testing must be

improved for higher reliability. Emphasis might be placed on standardizing and streamlining testing procedures which should test diverse fry quality criteria and evaluate key questions with ocean survival. Much fruitful work can be directed at the statistical analysis of fry size evaluation models, particularly as affected by growth rate differences.

4. As already demonstrated by Bams (Bams and Crabtree 1976), gravel incubation technology must be evaluated over several generations to assess possible performance changes over a period of time. Continuous artificial propagation will invariably produce brood stocks which are selected for high freshwater survivals. Knowledge of the effects of this selection on ocean survivals and on genetically controlled components of the life cycle are cogent for application and improvement of the technology.

VII. RESEARCH SUMMARY

1. Performances of newly-established gravel incubation systems designed to use unfiltered water for production of unfed salmon fry were evaluated at Netarts Bay, Oregon, and at Little Port Walter, Alaska. The program was divided into two phases. The first consisted of laboratory studies to develop fry quality testing procedures and to concurrently investigate effects of key design variables on fry quality. The second consisted of field comparisons of gravel incubator fry to parent-stock wild fry at the two sites. Research covered three brood years of 1969, 1970, and 1971.
2. Fry quality criteria tested included egg and alevin mortality, frequency of physical abnormalities, migration pattern and timing, post-emergence growth, size and stage of development, and performance in stress tests; size was the primary criterion.

A. Laboratory Studies

3. Three brood years of phase one laboratory studies on design variables investigated the effects of substrate rugosity on chum and chinook salmon at the Port Orford Marine Laboratory in 1969, the effects of stocking density and water velocity on pink and chum salmon at Netarts Bay in 1970, and the effects of

substrate rugosity and depth, light, and incubator design on pink and chum salmon at Netarts Bay in 1971.

1. Effects of Substrate on Chum and Chinook Salmon

4. Exposure to light during periodic inspections and at other times stimulated photonegative reactions in all treatments.
5. Chum salmon without substrate support exhibited intense photonegative reactions leading to yolk-sac malformation, high alevin mortality, and a loss of fry size; provision of a gravel substrate mitigated photonegative reaction and negated all associated adverse effects.
6. Chinook salmon alevins exhibited mild photonegative reactions in comparison to chum salmon alevins; yolk-sac malformation and significant mortality were not experienced by any treatments, with or without substrate support.
7. No difference in fry size was demonstrated at the 99% confidence level between chinook salmon reared on a gravel, screen, or Heath tray substrate for either the small or large egg-size experiment; a significant difference, however, was observed for the small egg size experiment at the 95% confidence level.
8. Heath incubator chinook fry of both large and small egg size experiments exhibited slower post-emergence growth rates than their counterpart gravel and screen substrate fry.

2. Effects of Stocking Density and Water Velocity on Pink and Chum Salmon

9. Apparent water velocities of 50 to 100 cm/hr and stocking density of 1.29 to 2.58 pink salmon eggs per cm^2 produced equivalent fry size without accelerated developmental rates. Increasing velocity to 300 cm/hr or density to 5.16 pink salmon eggs/cm^2 accelerated developmental rates without affecting fry size; the influence was more significant for velocity ($p < 0.025$) than for density ($p < 0.10$).

10. In the chum experiment, egg mortality was significantly higher in the lower water velocity cells (25 to 75 cm/hr) and in the higher density cells (1.92 and 3.85 chum eggs/cm^2).

11. When compared to fry from gravel-lined test cells, Heath incubator pink salmon fry incubated at 3.2 eggs/cm^2 and exposed to subdued light exhibited loss in fry size, high mortality, yolk-sac malformation, accelerated developmental rate, and slow post-emergence growth with a high frequency of pinheaded and stunted fish.

3. Effects of Substrate, Light, and Incubator Design on Pink and Chum Salmon

12. Under darkness and at a stocking density of 1.7 to 2.3 eggs/cm^2, the provision of a shallow gravel substrate in the Heath incubator resulted in no improvement in fry size for pink salmon.

13. Under darkness and at a stocking density of 1.2 eggs/cm^2, chum salmon fry from deep gravel test cells, flat substrate trough and Heath incubators, and shallow gravel substrate trough and Heath incubators were all equivalent in size.

14. In a Heath incubator two x two factorial chum salmon experiment treating light exposure and substrate, only fry without substrate support and with exposure to light exhibited yolk-sac malformation, significant mortality, and loss in size; no difference was detected in any of these factors between the other treatments. Stocking density was 1.2 eggs/cm^2.

15. Swimming stamina and anoxia tests conducted with all experimental chum salmon treatments except fry from the Heath, exposed, shallow gravel substrate treatment resulted in gravel substrate fry performing better than flat substrate fry, regardless of incubator origin. There is strong evidence that performance differed given equal fry size.

16. In the swimming stamina test, Heath incubator chum salmon fry reared in darkness and without substrate support exhibited a bimodal performance distribution, suggesting a disproportionally high frequency of low performance fish.

17. There is some evidence that a gravel substrate in the Heath incubator enhanced the performance of the fry more so than a gravel substrate in the trough incubator.

18. About 10% of pink and chum salmon incubated in deep gravel test cells moved onto the substrate surface during incubation.

19. Fry from deep gravel test cells migrated well before complete yolk absorption.

B. Field Studies

1. Little Port Walter Study

20. Comparison of hatchery treatments versus wild fry at Little Port Walter was ranked from the largest to the smallest average fry size as follows:

Rank	Incubation Treatment (stocked with eyed eggs unless otherwise noted)
1	shallow gravel matrix
2	Sashin Creek (natural spawning)
3	deep gravel matrix, low egg density
4	deep gravel matrix, low egg density, stocked with green eggs
5	deep gravel matrix, high egg density
6	deep gravel matrix, high egg density
7	Heath incubator

Ranks #1, 2, and 3 were equivalent to each other and significantly larger than ranks #5, 6, and 7. Rank #7 was significantly smaller than ranks #1 to 6.

21. All gravel incubator fry migrated close to total yolk absorption with a maximum difference between weighted average developmental index of about 0. 03 Kd units.

22. Heath incubator fry incubated at 2. 6 eggs/cm^2 and in total darkness experienced accelerated development leading to a shorter time to button-up; a low frequency of yolk sac malformation was also observed.

23. The deep gravel incubator which produced fry of equivalent size to wild fry had a stocking density (0. 23 eggs/cm^3) which was higher by up to nine times than densities presently used by other workers.

2. Netarts Bay Study

24. Chum salmon fry produced from either the production hatchery or from the multiple layer box were significantly larger than wild fry; hatchery fry, however, migrated 16-21 days earlier at a wide range of relatively immature developmental stages. Over 95% of the wild fry migrated at or near total yolk absorption.

25. There is a suggestion that hatchery fry developed at a faster rate than the wild fry.

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APPENDIX

Appendix Table 1. Mean and range of wet weights (in mg) from hatching through button-up of chinook salmon from large and small eggs incubated on three substrates.

Date	Stage	N[1/]	Large eggs						Small eggs					
			Heath tray		Gravel		Screen		Heath tray		Gravel		Screen	
			mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
2/9/70	Newly hatched	20	430[2/3/]	380-480	413	406-420	418	414-422	325[2/]	310-350	330	322-336	332	324-336
2/20/70	Alevin	20	468	460-474	485	474-496	477	470-480	379	376-384	378	374-384	379	376-380
3/5/70	Alevin	20	523	516-526	550	540-558	544	524-554	420	406-430	443	440-448	441	436-448
3/16/70	Fry[4/]	20[5/] & 30	----		575	561-586	567	559-580	----		480	474-485	462	460-463
3/19/70	Fry	20[6/] & 40	581	577-589	550	549-551	548	546-550	469	466-472	463	457-469	459	458-459
3/23/70	Fry	20	560	557-563	550	540-559	527	520-533	458	453-463	460	458-462	451	444-457
3/28/70	Fry	20[5/] & 30	572	570-575	554	545-562	540	528-557	460	454-465	455	449-460	441	440-441

1/ From 2/9/70 to 3/5/70, fry processed 5 at a time. From 3/16/70 to 3/28/70, fry processed 10 at a time.
2/ Fish processed individually rather than 5 at a time.
3/ Measurements taken from fish killed during anesthesia.
4/ All fry have 1-2 mm. yolk gap.
5/ n= 30 for large chinooks; n= 20 for small chinooks.
6/ n=40 for large and small Heath chinooks, n=20 for all others.

Appendix Table 2. Post-emergence growth (in mg) at two rations for chinook salmon fry from large and small eggs incubated on three substrates.

Date	N[1/]	Ration	Large eggs						Small eggs					
			Heath tray		Gravel		Screen		Heath tray		Gravel		Screen	
		% wet wt.	mean	range	mean	range	mean	range	mean	range	mean	range	mean	range
3/28/70	20[2/] & 30	6	572	570-575	554	545-562	540	528-557	460	454-465	455	449-460	441	440-441
4/6/70	30	6	716	699-731	741	720-758	740	697-793	583	579-591	601	579-616	607	600-611
4/13/70	30	6	911	870-963	996	979-1018	979	975-984	773	769-779	808	800-812	811	797-825
			mean	95% C. I.	mean	95% C. I.	mean	95% C. I.	mean	95% C. I.	mean	95% C. I.	mean	95% C. I.
4/20/70	30	6	1181	1138-1223	1288	1249-1327	1260	1202-1320	965	936-993	1020	992-1047	1025	1001-1050
4/28/70	30	2	1271	1236-1306	1347	1294-1400	1378	1329-1426	1002	972-1032	1103	1072-1135	1063	1036-1089
		6	---[3/]---		---[3/]---		---[3/]---		1101	1060-1142	1139	1098-1180	1145	1115-1176
5/10/70	30	2	1342	1296-1388	1494	1439-1548	1485	1426-1544	1094	1059-1129	1215	1177-1254	1186	1147-1225
		6	---[3/]---		---[3/]---		---[3/]---		1476	1412-1539	1627	1559-1696	1569	1530-1609

1/ From 3/28/70 to 4/13/70, fry were processed 10 at a time; from 4/20/70 to 5/10/70, fry were processed individually.

2/ n=30 for large chinooks; n=20 for small chinooks.

3/ Discontinued due to heavy mortalities encountered on 4/22/70.

Appendix Table 3. Size conversion regression data calculated from Heath and gravel incubator fry for the 1970 brood year pink salmon laboratory study.

Axis		Heath fry[1/]			Gravel fry[2/]		
Y	X	Y-intercept	Slope	Corr. coef.	Y-intercept	Slope	Corr. coeff.
Dry weight	Days	50.996	-0.3903	-0.924	57.808	-0.2498	-0.516
% Dry weight	Days	32.179	-0.2226	-0.931	29.904	-0.1675	-0.831
Dry weight	% Dry weight	-2.441	1.6200	0.918	4.668	1.8856	0.785

1/ Based on eight regression points from samples taken on 12/30/70 to 1/16/71: each point is an average of 20 fry (cf. Appendix Table 4).

2/ Based on 36 regression points from samples taken on 1/7/71 to 1/16/71 from incubators #1 to #9. Each point is an average of 20 fry (cf. Appendix Table 4).

Appendix Table 4. Size and stage of development during terminal yolk absorption period[1/] of 1970 brood year pink salmon density and velocity experiment. Each measurement is an average of 20 fry processed 20 at a time.

Incubator	Density/velocity[2/]	7 January 1971			9 January 1971			13 January 1971			16 January 1971		
		Wet wt.	Dry wt.	% Dry wt.	Wet wt.	Dry wt.	% Dry wt.	Wet wt.	Dry wt.	% Dry wt.	Wet wt.	Dry wt.	% Dry wt.
		mg	mg	%	mg	mg	%	mg	mg	%	mg	mg	%
#1	low/low	218.9	49.52	22.62	213.8	47.80	22.36	210.6	46.25	21.96	215.7	47.28	21.92
#2	med/low	201.9	46.09	22.83	212.4	47.35	22.29	218.5	47.75	21.85	215.2	45.19	21.00
#3	high/low	208.1	47.28	22.72	215.0	46.89	21.81	210.2	44.64	21.24	208.0	44.21	21.25
#4	low/med	208.4	46.64	22.38	212.8	46.75	21.97	206.8	43.14	20.86	209.4	43.83	20.93
#5	med/med	206.9	47.77	23.09	214.1	47.43	22.15	210.4	44.47	21.14	210.8	44.64	21.18
#6	high/med	202.0	45.10	22.34	201.0	43.72	21.75	209.3	44.28	21.16	212.3	42.80	20.16
#7	low/high	207.6	45.90	22.11	213.4	46.56	21.82	219.8	47.09	21.42	210.3	43.87	20.86
#8	med/high	210.1	46.51	22.14	199.6	43.40	21.74	211.2	44.03	20.85	211.5	44.31	20.95
#9	high/high	202.9	44.81	22.08	205.1	44.10	21.50	209.1	43.81	20.95	212.0	42.97	20.27
Heath	high/med	148.2	32.61	22.00	153.2	33.32	21.75	157.8	32.05	20.31	142.5	29.51	20.71

1/ Period ranged from 45 to 54 days after 50% hatching. General visual index for each date is as follows. 1/7/71: about 1-2 mm ventral gap; 1/9/71: about 1-2 mm ventral gap; 1/13/71: 25% of population buttoned; 1/16/71 = 75% of population are buttoned.

2/ Cells #1-#9 have shallow gravel matrix; Heath incubator tray has no gravel substrate.

Appendix Table 5. Data input and output for the two way analysis of variance on effects of water velocity and stocking density on dry weight (in mg) of pink salmon fry, 1970 brood year laboratory study.

INPUT:

Velocity (cm/hr)	Density (Eggs/cm^2) 1.29	2.58	5.16	$\overline{X}$
50	1) 46.52	2) 45.87	3) 45.44	45.94
100	4) 45.18	5) 45.51	6) 44.47	45.05
300	7) 45.94	8) 44.90	9) 44.67	45.17
$\overline{X}$	45.88	45.43	44.86	

Heath: 32.52

OUTPUT:

Source	DF	SS	MS	F[1/]
Velocity	2	1.40376	0.70188	5.56
Density	2	1.56702	0.78351	6.21
Error	4	0.50491	0.12623	

1/ Table values at 2, 4 d.f. are α = .01: 18.00; α = .05: 6.94; α = .10: 4.32

Appendix Table 6. Data input and output for the two way analysis of variance on effects of water velocity and stocking density on stage of development (% dry weight) of pink salmon fry, 1970 brood year laboratory study.

INPUT:

Velocity (cm/hr)	Density (Eggs/cm^2) 1.29	2.58	5.16	$\bar{X}$
50	1) 22.22	2) 21.98	3) 21.76	21.99
100	4) 21.54	5) 21.89	6) 21.33	21.59
300	7) 21.55	8) 21.41	9) 21.19	21.38
$\bar{X}$	21.77	21.76	21.43	

Heath: 21.19

OUTPUT:

Source	DF	SS	MS	F[1/]
Velocity	2	0.56536	0.28268	11.01*
Density	2	0.22909	0.11454	4.46
Error	4	0.10271	0.02568	

1/ Table values at 2, 4 d.f. are α = .01: 18.00; α = .025 : 10.65; α = .05 : 6.94; α = .10 : 4.32

Appendix Table 7. Least Significant Difference (LSD) analysis on effects of water velocity on stage of development (% dry weight) at button-up for the 1970 brood year pink salmon laboratory study.

RANKING Rank (Most to least advanced)	Velocity treatment cm/hr	Mean stage of development (pooled) % dry weight
1	300	21.38
2	100	21.59
3	50	21.99

COMPARISON OF ALL PAIRED MEANS: $\bar{Y}_k - \bar{Y}_i$; where k and i are ranks

LSD at 5% level = 0.3632

LSD at 1% level = 0.6024

	k = 3	k = 2
i = 1	0.61**	0.21
i = 2	0.40*	--

Appendix Table 8. Size and stage of development during terminal yolk absorption period of the 1970 brood year Heath incubator chum salmon. Each measurement is a mean of 10[1/] or 20[1/] fry.

Date	Stage of development	N	Wet[1/] wt.	Dry[1/] wt.	Length		Kd	% Dry wt.
					mean	S.D.		
			mg	mg	mm	mm		%
3/7/71	pronounced ventral gap	10	298.18	82.89	33.6	1.43	1.988	27.80
3/10/71		10	306.71	79.67	33.5	1.43	2.014	25.98
3/13/71	1 to 2 mm ventral gap	20	296.52	75.24	33.8	1.39	1.974	25.38
3/17/71		20	321.41	75.28	34.4	1.35	1.990	23.43
3/20/71	1 mm ventral gap	20	321.24	70.35	35.4	1.69	1.934	21.90
3/23/71		20	323.48	67.24	35.7	2.32	1.922	20.79
3/26/71	close to buttoned	20	337.66	66.02	36.9	1.62	1.888	19.56

1/ processed in bulk.

Appendix Table 9. Size conversion regression data[1/] calculated from Heath incubator chum salmon fry for the 1970 brood year laboratory study.

Axis		Y-intercept	Slope	Corr. Coef.
Y	X			
dry weight	days	111.448	-0.8157	-0.952
length	days	23.643	0.2329	0.983
% dry weight	days	44.500	-0.4475	0.998
Kd	days	2.306	-0.00731	-0.899
wet weight	days	185.842	2.6951	0.921
dry weight	% dry weight	30.638	1.8093	0.947
dry weight	Kd	-126.553	101.6472	0.964
length	% dry weight	46.728	-0.5172	-0.979
length	Kd	88.653	-27.5071	-0.944

1/ Based on five regression points taken over the two weeks prior to button-up. Each point is an average of 20 fry (cf. Appendix 8).

Appendix Table 10. Step acceleration schedule for swimming stamina test.

Time interval	Flow through tube	
	Meter reading	Velocity
minutes	% max. flow	cm/sec.
0-30	8	7.71
30-35	12	11.57
35-40	16	15.43
40-45	17	16.39
45-50	18	17.35
50-55	19	18.32
55-60	20	19.28
60-65	21	20.25
65-70	22	21.21
70-75	23	22.17
75-80	24	23.14
80-85	25	24.10
85-90	26	25.06
90-95	27	26.03
95-100	28	26.99
100-105	29	27.96
105-110	30	28.92
110-115	31	29.89
115-120	32	30.85
120-125	33	31.82
125-130	34	32.78
130-135	35	33.74
135-140	36	34.71
140-145	37	35.67
145-150	38	36.64
150-155	39	37.60
155-160	40	38.56

Appendix Table 11. Netarts Bay hatchery water temperature (in °C) records for the 1971 brood year chum salmon study.

Date	Event	Cumulative Temperature units	Range	Average
11/06/71	First spawning	7.2		
11/16/71	Egg-take for Laboratory Study	91.9		
11/18/71	50% Spawning	107.2	1.1-11.1	6.9
01/28/72	50% Hatching	587.8		
03/30/72	Fry reached @ maximum wet weight	1053.1		
04/04/72	Last sample of Laboratory Study	1091.4		
04/16/72	End of migration-multiple layer box	1183.6	1.1-13.4	7.5
04/20/72	End of migration-general hatchery	1213.1		
			Overall: 1.1-13.4	7.2

Appendix Table 12. Size and stage of development during terminal yolk absorption period for the 1971 brood year pink salmon laboratory study, experiment A and B. Each mean derived from n=30 fry.

Experiment	Treatment: Stocking density	Treatment: Substrate	Date and Measurements[1]: 1/1/72[2] Length $\bar{X}$	1/1/72 Length s^2	1/1/72 Wet weight $\bar{X}$	1/1/72 Wet weight s^2	1/1/72 Kd $\bar{X}$	1/1/72 Kd s^2	1/7/72 Length $\bar{X}$	1/7/72 Length s^2	1/7/72 Wet weight $\bar{X}$	1/7/72 Wet weight s^2	1/7/72 Kd $\bar{X}$	1/7/72 Kd s^2	1/14/72[3] Length $\bar{X}$	1/14/72 Length s^2	1/14/72 Wet weight $\bar{X}$	1/14/72 Wet weight s^2	1/14/72 Kd $\bar{X}$	1/14/72 Kd s^2
	eggs/cm^2		mm	mm	mg	mg			mm	mm	mg	mg			mm	mm	mg	mg		
A	1.72	screen	30.5	0.672	231.3	416.82	2.011	0.0022	31.5	1.431	234.4	415.01	1.957	0.0018	31.6	1.145	238.5	596.33	1.961	0.0020
			30.6	0.731	231.6	308.46	2.006	0.0020	31.3	1.264	224.1	604.05	1.936	0.0016	31.5	0.740	235.5	496.95	1.957	0.0018
			30.3	0.782	220.1	615.84	1.988	0.0020	30.9	1.460	213.4	501.51	1.935	0.0019	32.1	0.464	238.8	626.76	1.929	0.0033
B	2.27	screen	31.1	1.651	222.0	599.62	1.947	0.0023	31.1	0.878	221.6	400.74	1.942	0.0011	31.5	1.328	231.3	619.86	1.950	0.0032
		gravel	30.1	1.030	218.5	577.50	2.001	0.0011	31.2	0.833	231.0	557.55	1.967	0.0025	32.1	0.809	242.8	387.29	1.940	0.0012

1/ On 2/2/72, one random sample taken from all treatments yielded the following data. Length (mm): $\bar{X} = 31.8$; $S^2 = 0.747$; Wet weight (mg): $\bar{X} = 198.8$; $S^2 = 548.64$; k_d: $\bar{X} = 1.834$; $S^2 = 0.0027$.

2/ All fry have narrow yolk gap.

3/ All fry close to total button-up.

Appendix Table 13. Size conversion regression data[1/] calculated from combined experiments A and B of the 1971 brood year pink salmon laboratory study.

Axis		Y-intercept	Slope	Correlation coefficient
Y	X			
Length	Days	25.146	0.09496	0.874
Kd	Days	2.1665	-0.00323	-0.653
Length	Kd	64.238	-16.8611	-0.766

1/ Based on 15 data points, each one a mean of 30 fry (cf. Appendix 12).

Appendix Table 14. Size and stage of development during terminal yolk absorption period for treatments of the 1971 brood year chum salmon laboratory study. Each mean derived from n=30 fry.

Date and days from 50% hatching	Treatment[1/]	Size				Stage of development		
		wet weight		length		kd units		visual index
		$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	
		mg	mg	mm	mm			
3/6/72	HGU-2	416.5	1567.2	33.88	1.667	2.203	.00460	
28 days	HGU-2	420.7	899.0	34.13	0.499	2.194	.00170	Large yolk protusion
	HE-1	348.4	1181.3	31.58	1.450	2.227	.00298	
	HE-1	346.2	1090.0	31.18	0.819	2.250	.00349	
3/10/72	HGU-2	426.9	1133.6	34.77	0.806	2.165	.00128	
32 days	HGU-2	425.6	1336.2	34.82	0.974	2.159	.00261	Medium yolk protrusion
	HE-1	357.4	822.5	32.58	1.639	2.178	.00486	
	HE-1	363.9	1340.8	32.53	1.051	2.193	.00309	
3/14/72	HGU-1	434.3	1437.3	35.30	1.234	2.145	.00282	
36 days	HGU-2	451.2	1719.2	35.80	0.976	2.141	.00153	Pronounced yolk slit
	HE-1	383.1	1290.2	33.98	1.388	2.136	.00156	
	HE-1	370.3	785.5	33.57	0.685	2.138	.00163	
	HGE-1	430.7	1381.9	35.38	1.150	2.133	.00186	
	HGE-1	438.8	1108.3	35.77	0.978	2.124	.00167	
	HU-1	442.6	1313.6	35.85	0.623	2.125	.00217	
	HU-2	448.0	1514.0	35.60	0.610	2.147	.00144	
	TU-1	430.6	1371.4	35.07	0.857	2.152	.00328	
	TU-2	448.0	1214.6	35.32	0.905	2.166	.00217	
	TGU-1	435.0	1209.2	35.03	0.585	2.161	.00136	
	TGU-2	436.2	1322.2	35.25	0.806	2.150	.00171	
	DGE-1	429.9	1162.7	35.03	0.930	2.153	.00261	
	DGE-2	434.5	1160.9	34.60	0.903	2.188	.00253	

Appendix Table 14. (Continued)

Date and days from 50% hatching	Treatment[1]	Size				Stage of development		
		wet weight		length		kd units		visual index
		$\bar{X}$	S^2	$\bar{X}$	S^2	$\bar{X}$	S^2	
		mg	mg	mm	mm			
3/18/72	HGU-1	448.1	1047.2	36.43	0.875	2.100	.00156	Medium yolk
40 days	HGU-2	455.1	1341.1	36.37	0.654	2.114	.00284	slit
	HE-1	390.4	1120.7	35.00	1.152	2.087	.00235	
	HE-1	397.9	1306.5	35.03	2.016	2.099	.00262	
	HGE-1	442.9	1426.9	36.60	0.662	2.081	.00220	
	HGE-1	434.3	1330.6	36.20	0.993	2.091	.00118	
	HU-1	442.2	1645.2	36.18	0.836	2.104	.00220	
	HU-2	450.5	1411.1	36.58	1.053	2.094	.00172	
	TU-1	442.1	1050.3	35.97	0.775	2.118	.00289	
	TU-2	436.6	1275.9	36.08	1.001	2.101	.00097	
	TGU-1	436.1	745.0	35.75	0.685	2.121	.00175	
	TGU-2	450.5	1572.2	36.05	1.041	2.125	.00162	
	DGE-1	451.0	1557.8	36.27	1.064	2.113	.00158	
	DGE-2	442.1	1019.1	35.97	0.826	2.117	.00139	
3/22/72	*HGU-1&2	451.2	1863.8	36.89	0.952	2.077	.00182	Narrow yolk
44 days	*HE-1	392.1	1046.5	35.30	1.041	2.072	.00251	slit
3/27/72	*HE-1	376.6	1139.8	35.81	0.836	2.015	.00157	Almost buttoned,
49 days								body streamlined

Appendix Table 14. (Continued)

Date and days from 50% hatching	Treatment[1/]	Size				Stage of development		
		wet weight		length		kd units		visual index
		$\bar{X}$	s^2	$\bar{X}$	s^2	$\bar{X}$	s^2	
		mg	mg	mm	mm			
3/28/72 50 days	*HGU-1&2	444.6	1953.0	37.68	1.232	2.023	.00131	
3/31/72 53 days	HGU-1	450.3	1616.0	37.67	0.644	2.033	.00231	
	HGU-2	450.1	1451.8	37.77	0.806	2.028	.00102	
	HE-1	412.0	1395.5	36.58	1.381	2.033	.00127	
	HE-1	411.4	1862.9	36.38	0.926	2.042	.00091	
4/4/72 57 days	HGU-1	432.5	2460.6	37.85	0.968	1.995	.00260	Almost buttoned
	HGU-2	429.6	2247.3	37.83	1.661	1.992	.00145	
	HE-1	395.8	1655.4	36.43	0.995	2.013	.00198	
	HE-1	400.8	1990.8	36.13	1.240	2.038	.00133	

1/ HGU = Heath, shallow gravel substrate, unexposed
HE = Heath, screen substrate, exposed
HGE = Heath, shallow gravel substrate, exposed
HU = Heath, screen substrate, unexposed
TU = Trough, smooth substrate, unexposed
TGU= Trough, shallow gravel substrate, unexposed
DGE = Basket, deep gravel substrate, exposed

1, 2, 1&2 stand for replicate numeral
* = random sample used for swimming stamina test, n = 50 fry

Appendix Table 15. Size conversion regression data[1/] calculated for two treatments of the 1971 brood year chum salmon laboratory study.

Axis		Heath	Shallow gravel substrate	Unexposed to light	Heath	Shallow gravel substrate	Exposed to light
Y	X	Y-intercept	Slope	Corr. Coef.	Y-intercept	Slope	Corr. Coef.
Length	Days	29.638	0.1644	0.983	26.4186	0.2021	0.948
Kd	Days	2.4159	-0.00775	-0.991	2.4900	-0.00971	-0.988
Length	Kd	79.886	-20.7415	-0.971	78.721	-21.0372	-0.969

1/ Based on eight regression points from samples taken over the terminal yolk absorption period (3/10/72 to 3/28/72; cf. Appendix 14). Each point is a mean of 30 fry.

Appendix Table 16. Hatchery and creek water temperature (^{o}C) records[1/] for the 1971 brood year at Little Port Walter.

Month	Cumulative # days	Hatchery		Creek	
		Cumulative TU	Temperature range	Cumulative TU	Temperature range
Sept.	30	265	7 to 11	248	5 to 13
Oct.	61	470	4 to 8	418	2 to 8
Nov.	91	596	3 to 6	489	0 to 4
Dec.	122	678	2 to 4	484	-1 to 2
Jan.	153	735	1 to 3	468	-1 to 0
Feb.	182	779	-1 to 3	456	-1 to 0
March	213	818	1 to 2	452	-1 to 2
April	243	872	1 to 3	469	-1 to 3
May	274	952	2 to 3	510	0 to 3
June	304			582	1 to 4
		range:	-1 to 11		-1 to 13
		average:	3.5		1.9

1/ Accuracy of thermograph was approximately $\pm 2^{o}$F so these data are regarded as estimates. Centigrade readings converted from Fahrenheit readings.

Appendix Table 17. Size and stage of development from hatchery and wild migrant pink salmon fry during the 1971 brood year hatchery versus wild fry evaluation at Little Port Walter. Each measurement is a mean of 50 fry.

Date (in 1972)	Incubator[1]	Length		Wet weight		Stage of development			
						kd		Visual index[2]	
		mean	variance	mean	variance	mean	variance		
		mm	mm	mg	mg			mm	yolk gap
3/29	4	31.5	0.784	249.3	618.1	1.993	.00138		
4/7	1	31.7	0.867	241.0	713.7	1.961	.00332		
	2	31.8	1.347	246.6	897.3	1.969	.00212		
	3	31.9	1.136	224.8	663.8	1.902	.00219		
	4	31.9	0.761	235.5	619.3	1.935	.00317		
	5	32.1	1.364	247.8	770.0	1.957	.00358		
	H(1-3)	31.6	2.211	224.5	1298.2	1.920	.00341		
	H(4-6)	30.8	2.151	203.2	1510.9	1.899	.00376		
4/10	4	31.9	1.087	243.4	790.6	1.956	.00169		
	H	31.2	1.796	210.7	1368.3	1.900	.00549		
4/14	1	31.4	0.602	245.9	758.0	1.995	.00336		
	2	31.8	1.451	248.6	967.3	1.976	.00177		
	3	31.9	1.031	237.7	913.8	1.938	.00161		
	4	31.2	0.665	228.4	506.7	1.956	.00174		
	5	32.0	1.061	242.5	690.8	1.947	.00278		
	H(1-3)	31.9	1.919	210.9	762.6	1.865	.00279	~0	
	H(4-6)	31.2	2.137	205.1	805.8	1.889	.00306		
4/17	3[3]	31.6	1.016	232.3	587.9	1.942	.00188		
4/19	4[3]	32.0	0.733	238.9	867.9	1.938	.00233		
4/21	1	31.1	1.096	238.0	932.2	1.990	.00262		
	2	32.0	0.978	248.5	684.9	1.965	.00302		
	3	31.6	1.174	237.7	604.5	1.956	.00220		
	4	31.8	1.061	229.6	608.9	1.923	.00182		
	5	32.2	0.880	246.7	707.1	1.943	.00202		
	H	31.7	2.042	225.2	1454.9	1.912	.00312	~0	
4/24	2[3]	31.6	0.575	245.7	664.9	1.981	.00220		
4/25	2	31.9	1.006	239.4	632.3	1.945	.00206		
	H	31.4	2.412	220.6	1309.0	1.917	.00317	0-1/2	
4/28	1	31.7	0.882	242.2	1223.6	1.964	.00345		
	2	32.2	0.939	253.1	729.0	1.959	.00249		
	3	31.5	1.151	241.1	974.7	1.970	.00267		
	4	32.3	1.217	246.1	715.0	1.941	.00219		
	5	32.6	1.024	254.7	546.1	1.944	.00107		
	H	31.4	2.245	198.7	1447.1	1.851	.00573		

Appendix Table 17. (Continued)

Date (in 1972)	Incubator[1]	Length		Wet weight		Stage of development		
						kd		Visual Index[2]
		mean	variance	mean	variance	mean	variance	
		mm	mm	mg	mg			mm yolk gap
4/24-29	SC	31.9	1.871	243.1	599.8	1.954	.00330	
5/1	1[3]	32.0	0.755	250.0	428.3	1.966	.00178	
	5[3]	32.5	0.826	259.5	773.4	1.961	.00188	
5/5	1	31.7	0.916	238.5	717.0	1.955	.00165	
	2	32.0	1.000	261.4	664.4	1.995	.00228	
	3	31.4	1.802	237.6	1026.4	1.967	.00555	
	4	31.5	1.479	238.4	666.1	1.968	.00268	
	5	32.4	2.159	252.1	1285.4	1.946	00212	
	H	31.1	1.527	192.1	1272.0	1.850	.00707	0
	SC	32.2	0.600	243.3	260.0	1.940	.00197	
5/8	H	31.6	1.959	193.0	1391.5	1.821	.00612	0
5/11	SC	32.5	1.030	252.5	545.4	1.945	.00230	
5/12	1	31.9	1.062	243.8	561.1	1.959	.00244	
	2	31.7	0.981	246.3	1334.4	1.970	.00668	
	5	32.6	0.823	266.3	521.5	1.975	.00148	
	H	31.7	1.576	196.0	1280.6	1.828	.00543	0
5/11-13	3	31.7	1.520	220.1	1368.4	1.898	.00805	some 0's
	4	31.2	2.064	232.4	695.4	1.967	.00516	some 0's
5/19	5	32.8	1.043	260.8	777.0	1.948	.00191	
	H	31.6	2.531	187.0	1284.5	1.802	.00559	0
5/19-20	1	31.4	0.961	229.4	501.6	1.945	.00431	
	2	31.1	1.706	224.1	2324.3	1.940	.01452	some 0's
5/20	SC[3]	32.5	0.908	242.2	411.3	1.917	.00175	
5/26	SC[3]	32.7	0.686	255.3	440.2	1.936	.00106	1/2
6/4	SC[3]	32.2	1.155	243.0	418.7	1.936	.00229	1/2
6/10	SC	32.4	0.619	261.4	551.4	1.969	.00179	1/2
6/16-17	SC	32.5	0.907	273.4	626.3	1.998	.00320	1/2
6/26-28	SC	32.1	1.062	252.7	580.8	1.966	.00221	1/2

1/ H = Heath incubator. Number within bracket indicates trays sampled. No bracket indicates a comprehensive sample from all trays. SC = Sashin Creek.

2/ General form: 95% or more of gravel migrants had between 1/2 to 1 mm (width) yolk slits; Heath incubator fish exhibited wide range of development. Entry made in this column only when developmental conditions are different from general form.

3/ Sample taken during a peak migration period.

Appendix Table 18. Size and stage of development from hatchery and wild migrant chum salmon fry during the 1971 brood year hatchery versus wild fry evaluation at Netarts Bay.

Incubation site	Sample		Size				Stage of development		
			Length		Wet weight		Kd units		
	Date (1972)	N	mean	variance	mean	variance	mean	variance	Visual index
			mm	mm	mg	mg			
General Hatchery Tanks	3/12	25	34.70	1.792	413.2	1143.6	2.147	.00361	Slight to medium yolk protrusion
		25	34.30	1.813	399.2	1803.9	2.146	.00562	
		25	34.52	2.052	393.2	1256.7	2.123	.00773	
		25	34.62	1.610	405.2	2758.2	2.134	.00448	
		20	34.60	1.411	402.8	1163.9	2.134	.00454	
	3/22	20	35.78	1.960	422.0	1199.6	2.097	.00561	Slight yolk protrusion
		20	35.70	1.563	406.3	1240.6	2.074	.00594	
		20	35.00	1.184	394.5	1553.2	2.094	.00549	
		20	35.80	1.905	428.9	2345.4	2.104	.00387	
		20	35.10	3.095	404.4	2258.4	2.105	.00474	
	Observed grand mean:		35.012		406.97		2.1158		
	Converted grand mean[1/]:		35.892						
Whiskey Creek	3/14-3/30	30	35.45	2.161	389.8	1152.4	2.061	.00572	Narrow yolk slit
	3/30-4/1	30	36.08	0.743	396.9	1976.4	2.034	.00195	almost buttoned
	4/1-4/4	30	36.22	0.719	399.1	1404.8	2.031	.00190	
	4/4-4/5	30	36.27	2.185	401.8	2771.2	2.031	.00304	
	4/5-4/19	23	36.65	1.214	404.6	1643.8	2.016	.00166	
	Observed grand mean:		36.134		398.44		2.0346		
	Converted grand mean[1/]:		35.330						

Appendix Table 18. (Continued)

Incubation site	Sample		Size				Stage of development		
			Length		Wet weight		Kd units		
	Date (1972)	N	mean	variance	mean	variance	mean	variance	Visual index
			mm	mm	mg	mg			
Multiple Layer Box	2/27	30	34.77	2.323	431.3	2297.7	2.171	.00231	Medium yolk protrusion
	2/28	30	34.17	2.851	421.1	3311.3	2.190	.00423	
	3/6	30	34.93	2.444	405.9	3078.8	2.116	.00200	
	3/9	30	35.32	1.974	431.1	2903.5	2.136	.00236	
	3/10	30	34.85	2.847	425.9	2042.1	2.159	.00513	
	3/11	30	35.50	2.845	434.2	24 4.9	2.132	.00414	
	3/13	30	35.08	2.519	416.1	2092.3	2.127	.00397	Slight to medium yolk protrusion
	3/14	30	35.35	1.778	441.7	1580.5	2.154	.00337	
	3/16	30	35.37	2.602	416.4	2377.8	2.110	.00362	
	3/18	30	35.78	1.684	428.8	1476.6	2.107	.00355	
	3/22	30	35.78	1.322	429.0	1520.8	2.106	.00261	
	3/28	30	36.65	0.985	433.0	2051.5	2.062	.00320	
	3/31	30	36.67	1.920	425.2	1719.2	2.050	.00284	
	4/3	30	36.88	1.253	441.4	2186.5	2.062	.00065	Almost buttoned
	4/5	30	37.03	1.654	443.0	2462.4	2.056	.00195	
Observed weighted mean:			35.720		430.090		2.1121		
Converted weighted mean[1/]:			36.524						

1/ Converted length = Observed length - [(Observed K_d-2.07335)(-20.7415)]. See p. 26 for discussion of formula parameters.