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EFFECTS OF SUBSTRATE DEPTH, SEEDING DENSITY, AND FLOW RATE ON PRODUCTION OF PINK SALMON (ONCORHYNCHUS GORBUSCHA) FRY FROM INCUBATORS WITH PLASTIC SUBSTITUTES FOR GRAVEL SUBSTRATE

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EFFECTS OF SUBSTRATE DEPTH, SEEDING DENSITY, AND FLOW RATE ON PRODUCTION OF PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) FRY FROM INCUBATORS WITH PLASTIC SUBSTITUTES FOR GRAVEL SUBSTRATE

by

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Abstract

Eyed eggs of pink salmon, Oncorhynchus gorbuscha, were incubated in 40 experimental incubators to assess responses to controllable conditions of incubation. The fry were collected after volitional emergence and evaluated for fork length, wet weight, incubation period, and survival. Two plastic materials, AstroTurf* and Intalox* saddles, were tested as substrates at five depths ranging from 5.1 to 45.7 cm, five seeding densities from 0.28 to 0.94 $eggs \cdot cm^{-3}$, and five apparent velocities (discharge per unit cross-sectional area of the incubator) of waterflow from 255 to 1,309 $cm^3 \cdot h^{-1} \cdot cm^{-2}$. However, these ranges were reduced (new ranges: 13.3-45.7 cm, 0.414-0.940 $eggs \cdot cm^{-3}$, and 499-1,103 $cm^3 \cdot h^{-1} \cdot cm^{-2}$) when 20% of the experimental units were rejected because of suspected technical errors in execution of the experiments. Fry from the remaining experimental incubators were compared with each other and with fry from an incubator having gravel substrate 103 cm deep, seeding density of 0.098 $eggs \cdot cm^{-3}$ of gravel, and apparent water velocity of 303 $cm^3 \cdot h^{-1} \cdot cm^{-2}$. AstroTurf was inferior to saddles by all four measures of fry response regardless of the values of the control variables. Fry from saddle substrates that are 13.3-29.5 cm deep are predicted to be longer and heavier, emerge nearer the time that alevins emerge from natural redds in Auke Creek, and survive to emergence better than fry from a gravel incubator. This prediction holds even at the highest seeding density tested in saddle incubators, 10-fold that in the gravel incubator.

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* Reference to trade name does not imply endorsement by National Marine Fisheries Service.

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INTRODUCTION

Plastic substrates are potential substitutes for gravel in incubators for salmon (Oncorhynchus spp.). The low density of plastics, compared with gravel, offers savings in transportation and handling costs at remote hatchery sites. High porosity of plastic substrates, often double that of gravel, may allow increased seeding densities and more economical use of hatchery space and water supply.

Recently, two plastic materials, AstroTurf[1] and Intalox saddles, have been used as substrates in salmon incubators. Plastic turf was first used as an incubation substrate at the Auke Creek Hatchery, Alaska (Bailey and Taylor 1974a); plastic saddles were first used at the Craig Brook National Fish Hatchery, Maine (Leon 1975). Both experiments succeeded in producing fry; however, these plastic substrates have not been compared. Furthermore, the fry in these studies were not compared with fry from an acceptable standard substrate. Gravel is such a standard: pilot hatcheries using gravel have successfully enhanced salmon runs (Bams 1972, 1974; Bailey et al. 1976). Therefore, fry from incubators with plastic substrates should be compared with fry from incubators with gravel substrate for intelligent evaluation of plastic substrates.

There are no published definitions for the operating limits of incubators with plastic substrates--limits, such as substrate depth, seeding density, and flow rate, that produce satisfactory fry. Incubators have only been operated conservatively with unknown excess water and space for alevins. Because substrate types differ in porosity and size and shape of interstices, their operating limits probably differ and require individual evaluation.

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[1] Reference to trade name does not imply endorsement by the National Marine Fisheries Service.

In our experiment, we compared turf and saddle substrates as substitutes for gravel in salmon incubators and delimited, if possible, combinations of substrate depth, egg seeding density, and flow rate for these plastics that produce fry comparable or superior to those from a gravel incubator. The gravel incubator was operated under conditions known to have augmented the run of pink salmon (Oncorhynchus gorbuscha) to Auke Creek (see Bailey et al. 1976). Fry quality at time of volitional emergence was measured in terms of length, weight, incubation period, and survival.

EXPERIMENT

For approximately 7 months, pink salmon eggs were incubated in 40 individual incubators that had plastic substrates, either turf or saddles. Fifteen selected combinations of substrate depth, egg seeding density, and waterflow were tested (Table 1). We evaluated the effects of these variables on length and weight of volitionally emerged fry, length of incubation period, and survival to emergence. For comparison, eggs were incubated in a gravel incubator, and the fry used as a standard of fry quality. In a previous experiment, 2% of 43,307 fin-marked fry from this gravel incubator survived in the ocean and returned to spawn in Auke Creek (ocean survival). Ocean survival at Auke Creek has averaged 3.6% (range, 0.2-10.0%) based on eleven annual estimates.[2]

Collection and Eyeing of Eggs

The test incubators were seeded with eggs from pink salmon that returned to Auke Creek between 7 and 13 September 1976. Eggs were collected from 307 females (see Bailey and Taylor 1974b for method). Seven females were spawned into a dry plastic pail, the eggs were then mixed

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[2] Unpublished data on file at Northwest and Alaska Fisheries Center Auke Bay Laboratory, NMFS, NOAA, P.O. Box 155, Auke Bay, AK 99821.

Table 1.--Experimental conditions in the incubators and the observed responses. T = turf; S = saddles.

	Experimental conditions							Responses			
Treatment number	Substrate depth (cm)	No. of eggs	Substrate vol. (cm^3)	Egg density (eggs cm^{-3})	Apparent velocity (cm^{3}h^{-1} cm^{-2})	Flow rate (cm^{3}min^{-1})	Substrate type	Incubation period (days)	% survival	Weighted mean length (mm)	Weighted mean weight (mg)
1	13.3	2,877	6,950.3	0.414	499	4,344	T	212*	98*	32.34*	272.0*
	13.3	2,877	6,950.3	0.414	499	4,344	S	220*	88*	32.63*	287.4*
2	37.5	8,113	19,596.7	0.414	499	4,344	T	207	86	32.15	273.3
	37.5	8,113	19,596.7	0.414	499	4,344	S	219	89	32.53	278.4
3	13.3	5,602	6,950.3	0.806	499	4,344	T	211	87	32.03	274.2
	13.3	5,602	6,950.3	0.806	499	4,344	S	220	93	32.62	291.5
4	37.5	15,795	19,596.7	0.806	499	4,344	T	203	91	31.94	268.9
	37.5	15,795	19,596.7	0.806	499	4,344	S	218	82	32.53	278.8
5	13.3	2,877	6,950.3	0.414	1,103	9,608	T	214	101	32.05	261.8
	13.3	2,877	6,950.3	0.414	1,103	9,608	S	220	107	32.38	283.1
6	37.5	8,113	19,596.7	0.414	1,103	9,608	T	207	87	32.07	266.9
	37.5	8,113	19,596.7	0.414	1,103	9,608	S	219	95	32.40	277.1
7	13.3	5,602	6,950.3	0.806	1,103	9,608	T	211	106	32.12	275.5
	13.3	5,602	6,950.3	0.806	1,103	9,608	S	219	97	32.88	293.1
8	37.5	15,795	19,596.7	0.806	1,103	9,608	T	203	88	31.86	266.5
	37.5	15,795	19,596.7	0.806	1,103	9,608	S	217	92	32.55	285.4
9	5.1	1,626	2,665.2	0.610	801	6,976	T	212*	77*	32.08*	282.9*
	5.1	1,626	2,665.2	0.610	801	6,976	S	219*	120*	32.58*	296.0*
10	45.7	14,568	23,881.9	0.610	801	6,976	T	202	87	31.70	266.1
	45.7	14,568	23,881.9	0.610	801	6,976	S	218	94	32.47	278.6
11	25.4	3,717	13,273.5	0.280	801	6,976	T	213*	66*	32.27*	278.1*
	25.4	3,717	13,273.5	0.280	801	6,976	S	220*	125*	32.64*	299.2*
12	25.4	12,477	13,273.5	0.940	801	6,976	T	206	91	32.09	270.4
	25.4	12,477	13,273.5	0.940	801	6,976	S	219	98	32.40	288.1
13	25.4	8,097	13,273.5	0.610	293	2,549	T	209	80	32.07	268.0
	25.4	8,097	13,273.5	0.610	293	2,549	S	219	88	32.99	298.4
14	25.4	8,097	13,273.5	0.610	1,309	11,403	T	208	84	32.08	275.5
	25.4	8,097	13,273.5	0.610	1,309	11,403	S	220	91	32.46	284.1
15	25.4	8,097	13,273.5	0.610	801	6,976	T	208	84	32.08	273.1
	25.4	8,097	13,273.5	0.610	801	6,976	T	207	91	32.08	267.6
	25.4	8,097	13,273.5	0.610	801	6,976	T	208	87	32.14	271.1
15	25.4	8,097	13,273.5	0.610	801	6,976	S	219	97	32.54	288.9
	25.4	8,097	13,273.5	0.610	801	6,976	S	218	100	32.92	288.9
	25.4	8,097	13,273.5	0.610	801	6,976	S	219	101	32.64	291.2
16	25.4	8,097	13,273.5	0.610	255	2,223	T	208	83	31.96	264.3
	25.4	8,097	13,273.5	0.610	255	2,223	T	206	88	32.00	264.9
	25.4	8,097	13,273.5	0.610	255	2,223	T	206*	73*	31.87*	262.3*
16	25.4	8,097	13,273.5	0.610	255	2,223	S	219	89	33.10	293.5
	25.4	8,097	13,273.5	0.610	255	2,223	S	219*	98*	32.50*	287.2*
	25.4	8,097	13,273.5	0.610	255	2,223	S	218	88	32.71	291.9

*--suspect observations omitted from analyses.

and divided into seven aliquots, and each aliquot was fertilized with sperm from a different male. Each male was used only once. The eggs and sperm were gently mixed, washed with a 50:50 mixture of creek water (temperature 13°C) and hatchery water (temperature 8°C), and immediately placed in Heath tray incubators where the eggs were kept until eyed. Each tray was loaded with eggs from 14 females, and flow rate was set at 19 l·minute^{-1}. Malachite green treatments, 5 ppm for 1 hour at 10-day intervals between 11 September and 21 October, inhibited fungus growth. When the eggs were eyed, dead eggs (4.8% of the total eggs collected) were removed, and all of the live eggs were placed in one container where they were mixed thoroughly. The eggs were then placed in a gravel incubator and the 40 test incubators. Burrows' (1951) volume displacement method was used to approximate the number of eggs in each incubator.

Water Temperatures

Water for the Auke Creek Hatchery comes from nearby Auke Lake. We measured water temperature at the hatchery daily with an electronic thermometer (to the nearest 0.1°C). While pink salmon eggs were being collected, the water cooled from 8° to 7°C. Thereafter, it gradually cooled to 3.2°C on 17 December and remained between 2.9° and 3.2°C until 27 March 1977. The water warmed from 3.2°C on 27 March to 5.2°C on 11 May when the last fry left the incubators.

Incubators

The incubation chambers of the 40 test incubators were square in a horizontal cross section with an area of 523 cm^2. The height of the incubators varied so that regardless of substrate depth, about 8 cm of water was between the top surface of the substrate and the outlet pipe at the top of the chamber. Unfiltered water entered the bottom of the incubator and passed into the incubation chamber through a plate perforated with 2.38-mm holes. Waterflow to each incubator was checked daily and controlled by a gate valve. The effluent from each incubator was diverted into a screened bucket that spilled excess water but retained fry.

The incubation substrates were FH-01 AstroTurf and 2.54-cm Intalox plastic saddles. Turf was cut to fill the incubation chamber to the desired depth and loaded vertically (blades horizontal). Vertical loading is superior to horizontal loading (blades vertical).[3] We loaded saddles into the incubators and leveled them by hand to the desired depth. The saddles were not arranged in the incubator to either increase or decrease the number loaded into each incubator. Eggs were placed directly on top of the substrates and covered with a loosely fitted screen to reduce the flushing of alevins from the incubators. When the eggs hatched, most of the alevins worked their way down into the substrate. From 21 December 1976 through 13 March 1977, a few alevins (<5%) found a way past the screens. These alevins were caught in screened buckets and returned to their respective incubators.

The gravel incubator was a fiber-glass box similar to the one used by Bailey and Taylor (1974b). It measured 1.2 m in each dimension and contained about 1.5 m^3 of gravel. The box was seeded with 150,000 eggs (0.098 eggs$\cdot cm^{-3}$ of gravel), and the flow was set at 75 l$\cdot$minute^{-1} (303 $cm^3 \cdot h^{-1} \cdot cm^{-2}$). Gravel incubators operating at these values for depth, seeding density, and waterflow have successfully augmented natural production of pink salmon at Auke Creek (Bailey et al. 1976). Fry from the gravel incubator were not included in the statistical design to be discussed next; however, fry produced in the gravel were used to establish acceptable performance for fry from the plastic substrates.

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[3] Unpublished data on file at Northwest and Alaska Fisheries Center Auke Bay Laboratory, NMFS, NOAA, P.O. Box 155, Auke Bay, Ak 99821.

Statistical Design of Experiment

The statistical design that we chose to predict effects of substrate type, substrate depth, seeding density, and waterflow through incubators was based on a central composite rotatable (CCR) design from response surface analysis (see Cochran and Cox 1964). In our modification of the CCR design, the six repeated observations at the center point of the original design were replaced by two groups of three repeated observations each. The two groups of repeated observations were centered with respect to seeding density and depth of substrate, but waterflow differed between the groups. The 16 treatment points of the three-factor space included in the design--substrate depth, seeding density, and waterflow--were evaluated once by experiment for each of the two plastic substrates (Table 1).

Each incubator provided one observation from each of the four responses--incubation period, survival, length, and weight (Table 1). Each of the four responses was analyzed separately. The general quadratic response surface appropriate to the experimental design and assumed to describe the observations from a substrate is

$$y_u = \mu + \sum_{i=1}^{3} \beta_i \, x_{iu} + \sum_{i=1}^{3} \beta_{ii} \, x_{iu}^2 + \sum_{i<j}^{3} \sum^{3} \beta_{ij} \, x_{iu} \, x_{ju} + e_u \; ;$$

where,

$\underline{y_u}$ is the response being considered at the u-th point in the experiment for the substrate;

μ is a constant;

β_1, β_2, and β_3 are the coefficients corresponding to linear effects of depth, density, and flow;

$\underline{x_{iu}}$ is the value of the i-th controlled variable, depth, density, or flow, at the u-th point;

β_{11}, β_{22}, β_{33}, β_{12}, β_{13}, and β_{23} are the coefficients corresponding to quadratic effects of depth, density, and flow and to interactions between these variables; and

$\underline{e}_u$ is the discrepancy between the observed response, y_u, and the response expected from the quadratic response surface.

The quadratic surface for each response was fitted using BMDP1R (Dixon et al. 1977). We analyzed each response separately for turf and saddles for a total of eight analyses for the four responses and two substrates. The repeated observations at two of the treatments in our modification of the CCR design provided an estimate of experimental error. This estimate was used to construct a test for lack of fit of the quadratic surface (e.g., Draper and Smith 1981).

Substrate depths ranged from 5.1 to 45.7 cm. The minimum depth was twice the depth that induced by its shallowness an excessively high rate of premature migration from turf and gravel incubators in earlier experiments at Auke Creek.[4] The maximum depth was nearly nine times deeper, equal to one-half the width of a roll of turf as purchased.

Seeding densities ranged from 0.280 to 0.940 eggs$\cdot$cm^{-3}. The lowest density was slightly less than the highest density previously found (see footnote 4) not to produce adverse effects. The highest density selected for this experiment was almost six times that used in gravel incubators at Auke Creek (Bailey et al. 1976) and seven times the maximum recommended by Bams and Simpson (1977).

In the reported regression analysis of fry response to substrate depth, seeding density, and apparent velocity, we expressed density in eggs per cubic centimeter of substrate. However, we subsequently observed that

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[4] Unpublished data on file at Northwest and Alaska Fisheries Center Auke Bay Laboratory, NMFS, NOAA, P.O. Box 155, Auke Bay, AK 99821.

alevins in a transparent incubator with 0.7-m deep gravel descend and repose in a dense layer at the bottom. Therefore, density of eggs per unit of cross-sectional area of the incubator may be more appropriate. The regression model contains a cross-product term of depth of substrate (cm) with density ($eggs \cdot cm^{-3}$) equal to density per unit area ($eggs \cdot cm^{-2}$). Consequently, important linear effects due to density on an area basis were included.

Flow rates ranged from 2,223 to 11,403 $cm^3 \cdot minute^{-1}$. Oxygen requirements of the alevins dictated the lower limit of the flow rate, and oxygen content of the of incubator effluents was maintained at or above 6.3 $mg \cdot l^{-1}$. Based on past experience at Auke Creek, we assumed that oxygen content of the water supply would decline to 8 $mg \cdot l^{-1}$ near the end of the incubation period when the oxygen requirement of alevins approaches a maximum, 0.028 $mg \cdot alevin^{-1} \cdot hour^{-1}$ (Bailey et al. 1980). The lower limit was to satisfy the oxygen needs within even the most densely seeded incubators.

Our assumption that the oxygen content of the water supply would decline to 8 $mg \cdot l^{-1}$ proved erroneous. The winter of 1976-77 was exceptionally mild in southeastern Alaska, and Auke Lake was ice covered for only 6 weeks in December and January. Consequently, oxygen content of the water dropped only to 13.3 $mg \cdot l^{-1}$, almost 100% saturated, at the end of February when the oxygen content was minimum (effluents had >10.8 $mg \cdot l^{-1}$ of oxygen at this time).

Physical effects of water currents on eggs and alevins dictated the upper limit for waterflow. We set the upper limit of flow well below 18,289 $cm^3 \cdot min^{-1}$. In preliminary tests made before substrate was added, this flow was the lowest observed to physically disturb eggs lying on or next to the perforations that allowed water to enter in the bottom of the incubation chambers.

We expressed waterflow as apparent velocity, $cm^3 \cdot h^{-1} \cdot cm^{-2}$ (discharge per unit cross-sectional area of incubator) because it is commonly used in describing waterflow in salmon incubators. Apparent velocity was calculated by dividing the flow of water supplied to an incubator by the horizontal cross-sectional area of the incubator.

Observations

The responses of fry to the controlled environmental conditions in each incubator were determined by comparing length, weight, incubation period, and survival of fry from the different incubators. Volitionally emerged fry were counted each morning. Fry from turf and saddle incubators were counted by hand when they were removed from the screened buckets at the outlet of each incubator. The number of fry from gravel incubators was estimated by subsampling the emerging fry with a cone-shaped sampling device[5] that retains an estimated 41 fry per 1000 fry (Bailey et al. 1976). Twice weekly, samples of 30 fry from each incubator were preserved in 5% Formalin. Only samples of fry collected on the dates of approximately 25%, 50%, and 75% of the cumulative total emergence were measured. Preserved fry were measured after 6 weeks when changes in length and weight were considered negligible. Lengths were measured to the nearest millimeter and wet weights to the nearest milligram. We defined the incubation period as the number of days from fertilization of the eggs to the date when 50% of the fry had emerged from the incubator. Survival was estimated as the ratio of number of fry emerging to the estimated number of eyed eggs seeded in an incubator. When survival was good, underestimates of the number of eggs

[5] A blueprint for the cone-shaped fish sampler was supplied by the Washington Department of Fisheries.

seeded resulted in survival estimates >100%. Weighted means of pooled lengths and pooled weights of fry from each incubator were computed from the samples of emergent fry that were measured.

RESULTS

Survival

Observed survival rates provide evidence of technical or recording errors of which we had been unaware and which caused us to modify analysis of all four variables. Survival estimates ranged from 66% to 106% in turf and from 82% to 125% in saddles (Table 1). Treatments 9 and 11 draw attention immediately: treatments with the highest estimates of survival in saddles correspond to the lowest estimates of survival in turf. An examination of residuals from the fitted quadratic surfaces for survival of fry in incubators with turf or saddles convinced us that not only were observations under Treatments 9 and 11 suspect but also those of Treatment 1 and a pair under Treatment 16. These treatments, ignoring sign, had the four largest residuals for turf incubators and the four largest residuals for saddles. Further, under each treatment with suspect observations, the large residuals for survival in turf and saddle incubators were of opposite sign. Such residuals would occur, for example, if we counted fry from one substrate as fry from the other substrate under the four treatments. Residuals under turf are statistically independent of those under saddles if neither technical nor recording errors occurred, but the remarkable coincidence of these residuals strongly indicates such errors probably occurred. An exhaustive search of records and reexamination of procedures, including the physical location of incubators in the hatchery, yielded no clue to the source of the alleged errors. Regardless, in further analyses, we omitted observations on any of the four response variables from the suspect incubators.

Survival from fry to egg in the remaining incubators was greater for saddle incubators than for turf incubators in 11 of the 13 remaining treatments. Survival estimates in turf incubators ranged from 80% to 106% and in saddle incubators from 82% to 107%. Previous experience in variation of volumetric estimation of the number of eggs seeded indicates unfeasible estimates slightly greater than 100% are not suspicious when survival is good. Average survivals for all included treatments were 89.3% and 93.4% for fry from turf incubators and saddle incubators, respectively.

Survival in the turf incubators was fitted satisfactorily by the quadratic surface ($\underline{P}$ = 0.21) (Table 2); however, the regression was not significant ($\underline{P}$ = 0.15). Therefore, average survival, 89.3%, was used to predict survival in the factor space of control variables.

Table 2.--Analysis of variance of survival: turf and saddle substrates.

		Turf		Saddles	
Source	df	SS	F	SS	F
regression	9	499.348	2.39	499.892	4.86
lack of fit	3	101.923	2.74	59.378	6.48
pure error	3	37.167		9.167	

Survival in the saddle incubators may not be satisfactorily fitted by the quadratic surface because the significance level is suspiciously small ($\underline{P}$ = 0.08) (Table 2). Estimated survival of fry in saddle incubators at the repeated points of the design (i.e., Treatments 15 and 16) were remarkably consistent compared with those from turf incubators (Table 2; compare sums of squares of pure error). An underestimate of pure error for saddle incubators may have caused the suspicious test for lack of fit. The regression is significant ($\underline{P}$ = 0.03), if the model is adequate, and explains 88% of variation in survival.

If we use the regression equation (Table 3) to predict survival, survival in saddle incubators decreases with increased seeding density and substrate depth (Figure 1). Water velocity does not greatly affect the relation of survival to seeding density and substrate depth but appears to improve survival slightly at velocities greater than the lowest velocities included in our study. Survival is overestimated at lowest seeding densities and substrate depths.

Fry Length

Weighted means of lengths for fry from saddle incubators were greater than those for fry from turf incubators in each of the 13 treatments retained. Weighted mean lengths of fry from turf incubators ranged from 31.70 to 32.15 mm and for fry from saddles ranged from 32.38 to 33.10 mm (Table 1). Simple averages of weighted mean lengths for the experiment were 32.03 and 32.63 mm for fry from turf and saddle incubators, respectively.

Weighted means of lengths for fry from the turf incubators seem adequately described by the quadratic surface ($\underline{P}$ = 0.14) (Table 4). If the fit is all right, the regression is significant ($\underline{P}$ = 0.009) and explains 93% of variation in length. The prediction surface for fry length in turf incubators (Table 3) indicates little change of length at experimental substrate depths <29.5 cm, but length decreases sharply with further increases in depth (Figure 2). Effect of seeding density is also negative in deeper turf. Velocity has only slight effect.

Weighted means of length for fry from the saddle incubators probably are adequately described by the quadratic surface ($\underline{P}$ = 0.67) (Table 4). However, the regression is not significant ($\underline{P}$ = 0.35) so the simple average of the weighted means for the experiment, 32.63 mm, was used to predict length of fry in the saddle incubators.

Table 3. Estimates of the coefficients of the general quadratic prediction model[a] when model fit is significant.

Parameter	Survival in saddles (%)	Length in turf (mm)	Weight in saddles (mg)	Incubation period in turf (days)
μ	99.34204	32.10144	289.81180	207.72314
β_1	-5.67537	-0.02112	-5.38972	-3.77128
β_2	-4.19111	-0.03866	1.86757	-1.77881
β_3	0.97156	0.01554	-2.12724	0.08030
β_{11}	0.27654	-0.11479	-1.28866	0.31552
β_{22}	0.81933	0.03362	-2.25006	0.54572
β_{33}	-3.33641	-0.01718	-0.07509	0.03740
β_{12}	2.39884	-0.07508	0.62729	-0.28073
β_{13}	2.14693	-0.04755	2.27921	-0.03084
β_{23}	1.65196	-0.00513	3.02593	-0.03092

[a] $$\hat{y}_u = \hat{\mu} + \sum_{i=1}^{3} \hat{\beta}_i x_{iu} + \sum_{i=1}^{3} \hat{\beta}_{ii} x_{iu}^2 + \sum_{i<j}^{3} \sum^{3} \hat{\beta}_{ij} x_{iu} x_{ju} ;$$

where,

$$x_{1u} = \frac{\text{depth (cm) at u-th point } -25.4}{12.081},$$

$$x_{2u} = \frac{\text{density (eggs cm}^{-3}\text{) at u-th point } -0.610}{0.1962},$$

$$x_{3u} = \frac{\text{apparent velocity (cm}^3\text{h}^{-1}\text{cm}^{-2}\text{) at u-th point } -801}{302.021}.$$

Table 4. Analysis of variance of length: turf and saddle substrates.

Source	df	Turf SS	Turf F	Saddles SS	Saddles F
regression	9	0.186	8.259	0.517	1.42
lack of fit	3	0.012	4.00	0.089	0.58
pure error	3	0.003		0.154	

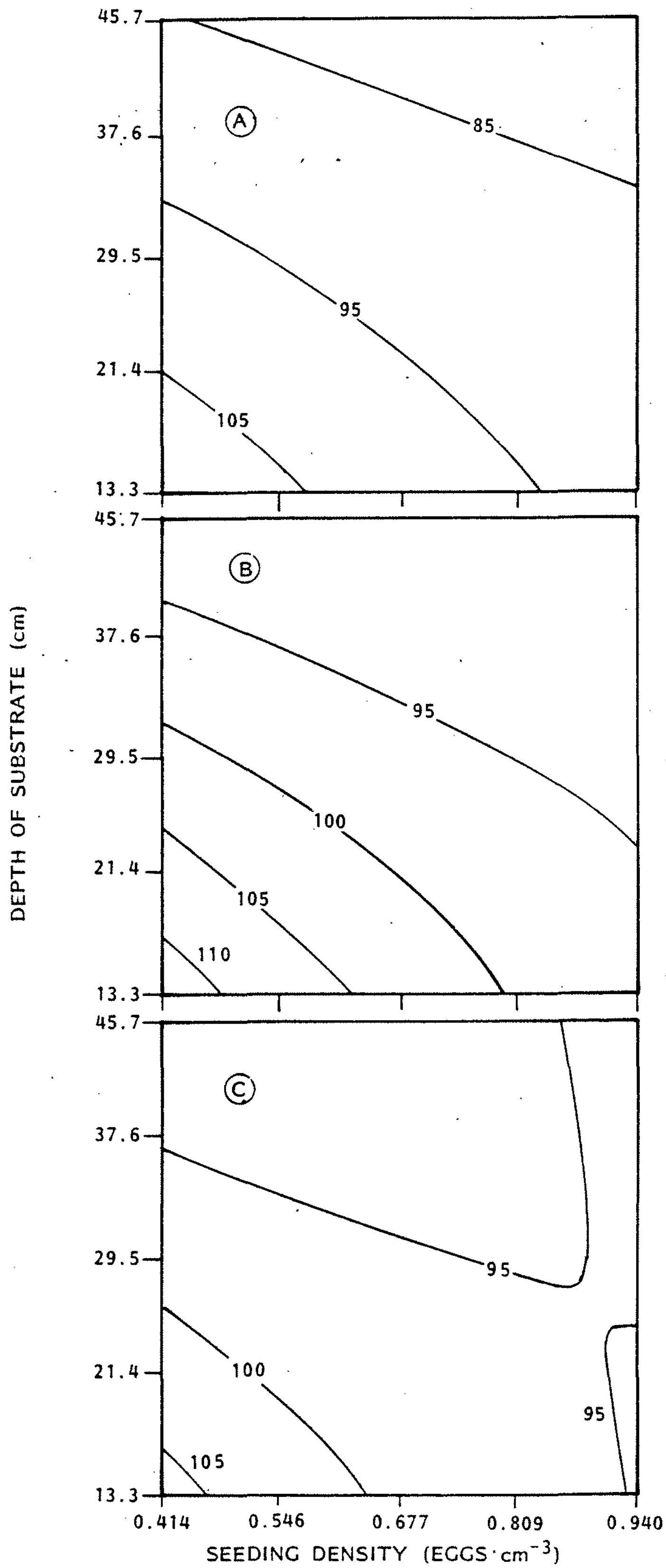

Figure 1.--Contours of predicted survival (%) in saddles as related to substrate depth and seeding density at apparent velocity of (A) 499 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, (B) 801 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, and (C) 1,103 $cm^3 \cdot h^{-1} \cdot cm^{-2}$.

Fry Weight

Weighted means of weight for fry from saddle incubators were greater than those for fry from turf incubators in each of the 13 treatments retained (Table 1). Weighted means of fry from turf incubators ranged from 261.8 to 275.5 mg and for fry from saddle incubators ranged from 277.1 to 298.4 mg. Simple averages of weighted mean weights for the experiment were 269.3 and 286.9 mg from turf and saddles, respectively.

Adequacy of the quadratic surface in describing fry weight is questionable in saddle incubators (*P* = 0.03) but not in turf incubators (*P* = 0.15) (Table 5). Again, the weights of fry in saddle incubators at repeated Treatments 15 and 16 (Table 1) were much less variable than the weights of fry in turf incubators (Table 5; compare sums of squares of pure error for turf and saddle incubators). Possibly, the mean square for pure error is underestimated for fry in saddle incubators.

Table 5. Analysis of variance of weight: turf and saddle substrates.

		Turf		Saddles	
Source	df	SS	F	SS	F
regression	9	187.828	1.63	546.630	5.58
lack of fit	3	60.771	3.80	60.382	12.33
pure error	3	15.980		4.899	

The regression of weighted mean weights for fry in turf incubators is not significant (*P* = 0.28), so the simple average of weighted mean weights for the experiment, 269.3 mg, was used to predict weight. The regression for weights of fry from saddles explains 89% of variation in weight and is

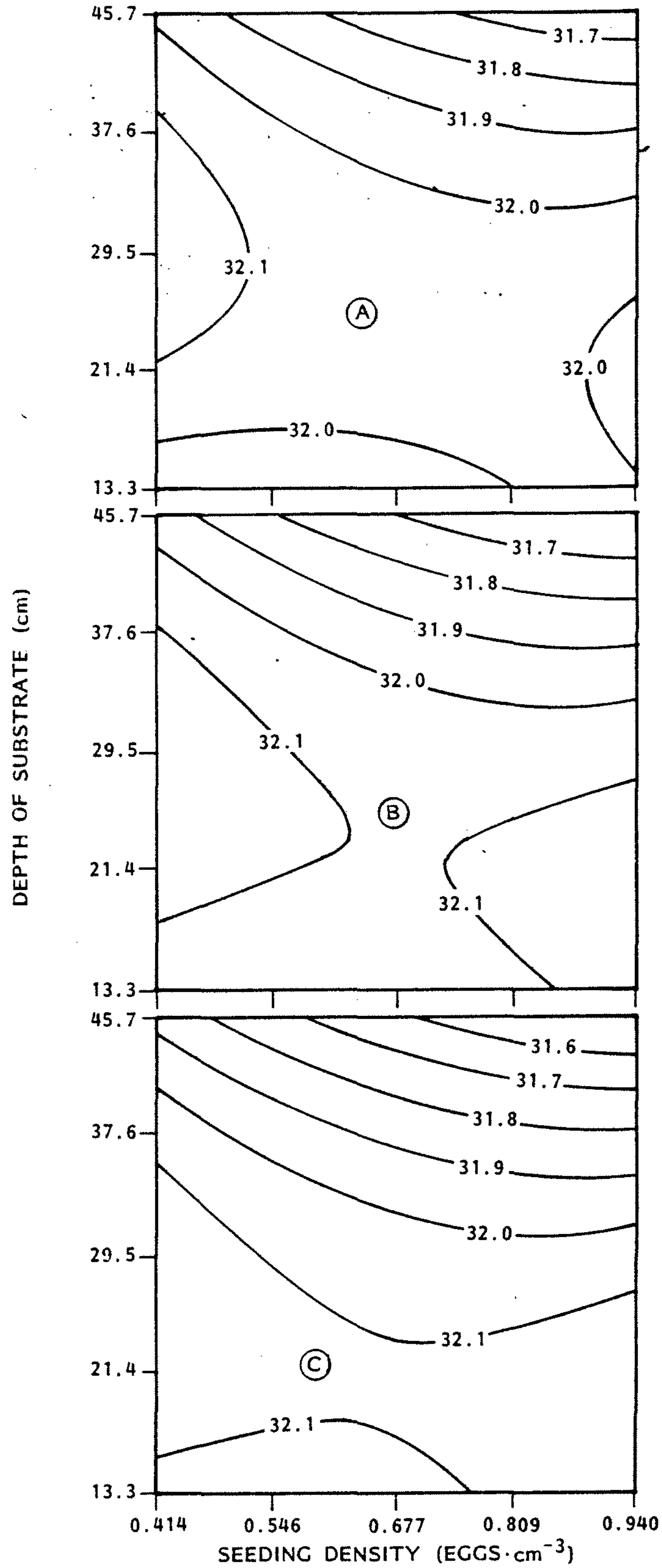

Figure 2.--Contours of predicted length (mm) in turf as related to substrate depth and seeding density at apparent velocity of (A) 499 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, (B) 801 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, and (C) 1,103 $cm^3 \cdot h^{-1} \cdot cm^{-2}$.

significant ($\underline{P}$ = 0.02) if lack of fit is overlooked. With only the mean for the experiment as an alternative, we choose to tentatively accept the quadratic surface to predict weight in saddles (Table 3) in view of its apparent explanatory power. These predictions in the lower portion of the experimental range of velocity are perfectly plausible.

Weight, at low velocities, is predicted to decrease with increase in depth of saddle substrate as well as with increase in seeding density (Figure 3A). At higher velocities, weight predictions continue to decrease with increased substrate depth, but the predictions for effect of seeding density become increasingly implausible with higher velocity (Figures 3B, 3C). At the higher velocities, an increase in seeding density is predicted to increase fry weight. The main inference to draw from these predictions is the negative effect of depth of substrate. Supportive of this inference are three direct comparisons for effect of depth with seeding density and apparent velocity held fixed: Treatments 3 and 4, 5 and 6, and 7 and 8 (Table 1). In each case, increase in depth caused reduction in weight.

Incubation Period

In each of the 13 treatments retained, incubation periods for fry from turf incubators were shorter than those from corresponding treatments in saddle incubators. Incubation periods for fry in turf incubators ranged widely from 202 to 214 days and for fry in saddles only from 217 to 220 days. Averages of incubation periods for the experiment were 207.4 and 218.8 days in turf and saddle incubators, respectively.

Incubation periods for fry from the turf incubators were fitted satisfactorily by the quadratic surface ($\underline{P}$ = 0.46) (Table 6). The regression was significant ($\underline{P}$ = 0.001) and explained 96% of variation in incubation period. The surface (Table 3) predicts that incubation period will decrease with increases in depth of turf or with increases in density (Figure 4). Effect of velocity is negligible.

Table 6. Analysis of variance of incubation period: turf and saddle substrates.

		Turf		Saddles	
Source	df	SS	F	SS	F
regression	9	140.077	16.46	7.574	1.76
lack of fit	3	3.006	1.13	1.196	0.72
pure error	3	2.667		1.667	

Incubation periods of fry in the saddle incubators were fitted satisfactorily by the response surface ($\underline{P}$ = 0.60) (Table 6). However, the regression is not significant ($\underline{P}$ = 0.25). The little variation in incubation period and lack of significance of the regression justify using the mean incubation period (218.8 days) for experiments with saddle incubators for predicting incubation period.

Gravel Incubator

Survival of fry from the gravel incubator was 88.9%, within the range of survival of fry from either turf or saddle incubators. The weighted mean of fry lengths from the gravel incubator was 32.15 mm ($\underline{s}^2$ = 0.0058), which lies at the upper end of the range of mean fry lengths from turf incubator but is less than any of the mean lengths of fry from saddles. The weighted mean of fry weights from the gravel incubator was 281.1 mg ($\underline{s}^2$ = 3.43), greater than

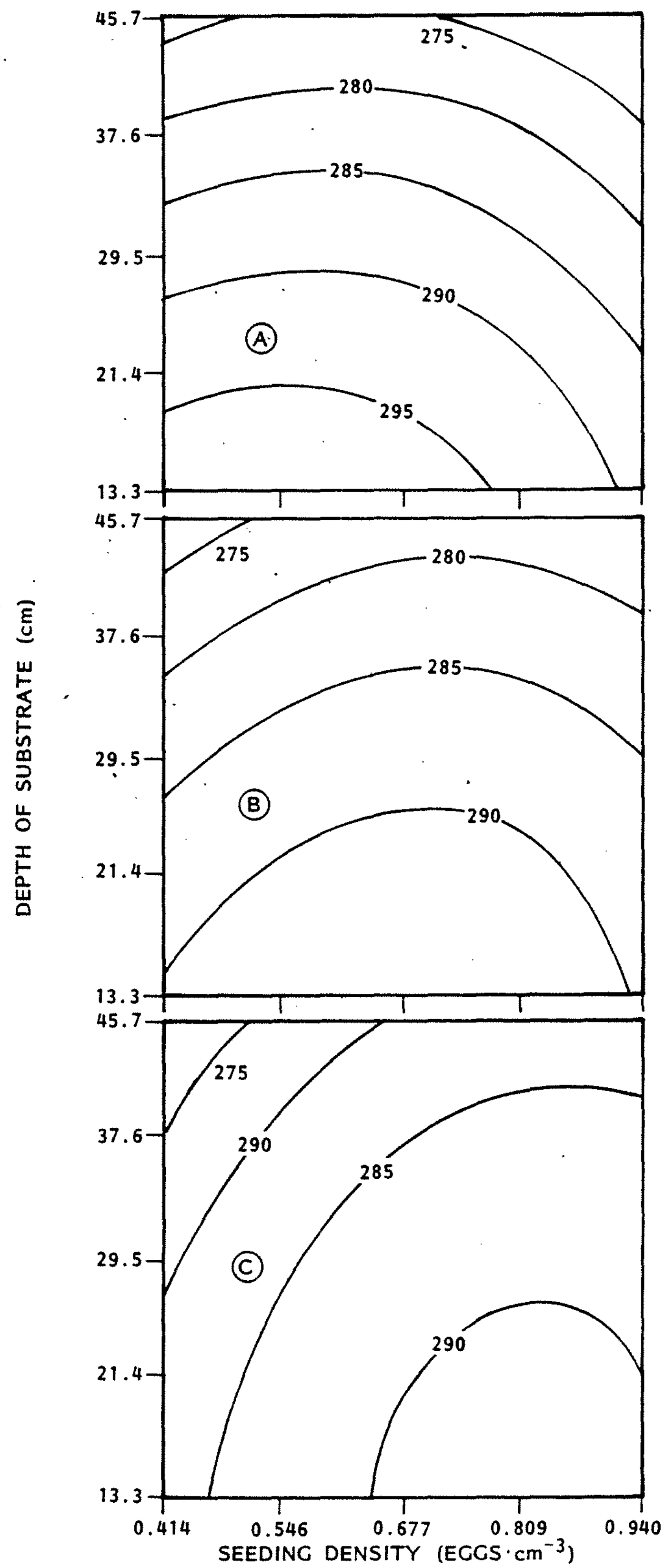

Figure 3.--Contours of predicted weight (mg) in saddles as related to depth of substrate and seeding density at apparent velocity of (A) 499 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, (B) 801 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, and (C) 1,103 $cm^3 \cdot h^{-1} \cdot cm^{-2}$.

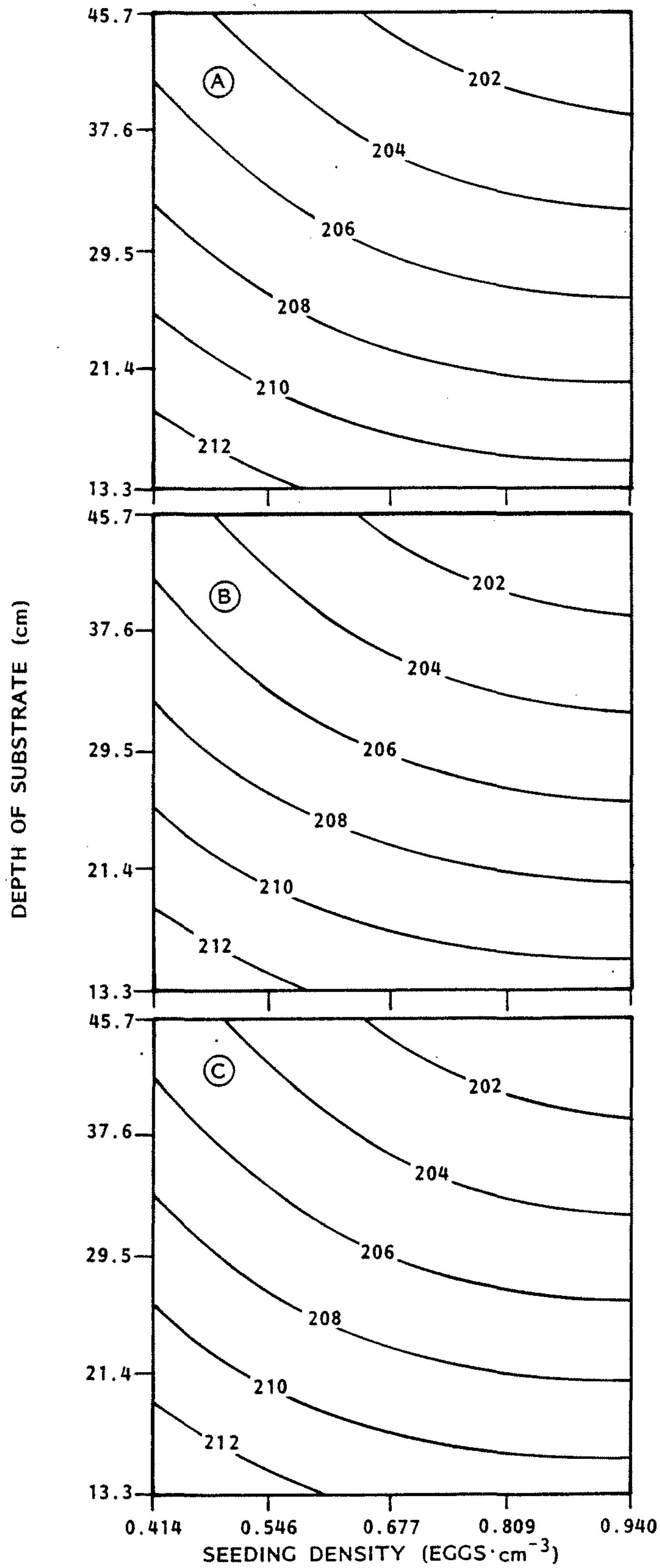

Figure 4. Contours of predicted incubation periods (days) in turf as related to depth of substrate and seeding density at apparent velocity of (A) 499 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, (B) 801 $cm^3 \cdot h^{-1} \cdot cm^{-2}$, and (C)1,103 $cm^3 \cdot h^{-1} \cdot cm^{-2}$.

any of the mean weights of fry from turf incubators but within the range of mean weights of fry from saddle incubators. The incubation period for fry from the gravel incubator was 216 days, greater than any of the incubation periods for fry from turf incubators and less than any incubation periods for fry from saddle incubators.

DISCUSSION

The type of plastic material used as substrate for incubating salmon eggs affects survival, time elapsing between fertilization and volitional emergence, and physical size of alevins. Shape of the substrate directly affected survival: no trapped fry were seen among the smooth, rounded saddles; however, fry were trapped among the blades of AstroTurf and died because they could not escape from the incubators. We could not accurately count alevins trapped in the turf because a brown organic precipitate filled spaces in substrates at the Auke Creek Hatchery (Bailey et al. 1976) and obscured the alevins. Estimated survival of alevins from fertilization to emergence from saddles was greater than from turf incubators in 11 of the 13 treatment combinations presumed valid. Mean survivals for this experiment were 89.3% and 93.4% for turf and saddles, respectively. Because trapping reduced survival in turf incubators, we expected survival to decrease detectably with increases in depth of the substrate if trapping caused substantial mortality. Such decrease, however, was not apparent from regression analysis: the effect is hidden by other uncontrolled factors affecting survival.

Alevins from saddle incubators were longer and heavier than those from turf incubators under every combination of the controlled variables in our experimental design. Our presumption is that large size is beneficial, provided time of emergence is correct.

Early emergence of alevins has been a recurring problem at Auke Creek Hatchery. At hatcheries such as the one at Auke Creek, where unfed fry are released to go to sea immediately after they emerge, shorter incubation periods mean earlier release and probably reduced marine survival. For example, fry released 33 days after another group had eight times greater survival to adult return, even though the fry were of comparable size when released (Taylor 1980). We observed a 40% higher marine survival for marked fry with a midpoint release date of 19 April 1977 as compared with fry released only 6 days earlier with a midpoint release date of 13 April.[6]

Alevins from saddle incubators had longer incubation periods than those from turf under every treatment combination attempted. The differences were substantial and ranged from 6 to 15 days, depending on the particular treatment combination of controlled variables (Table 1). The advantage again goes to saddle incubators as later emergence should result in higher ocean survival, provided the delay is not too great. We believe the emergence from saddles nearly coincided with emergence of fry in Auke Creek for reasons to be presented shortly. Therefore, we feel the time of emergence from saddle incubators was not too late.

Fry from the saddle incubators were clearly better than fry from the turf incubators if fry quality is judged by survival to emergence, size of alevins produced, and time to emergence. Fry from saddle incubators also were superior to fry from the gravel incubator.

Seeding density in the gravel incubator was only 0.098 eggs$\cdot$cm^{-3}, less than one-half the minimum seeding density and only about one-tenth the maximum seeding densities attempted in the plastic substrates. Yet, fry from

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[6] Unpublished data on file at Northwest and Alaska Fisheries Center Auke Bay Laboratory, NMFS, NOAA, P.O. Box 155, Auke Bay, AK 99821.

saddles were longer than those from the gravel incubator under any of the 13 treatment combinations of controlled variables retained for analysis. Fry from saddle incubators were also heavier in 9 of 13 treatment combinations. Only when the plastic substrate was 37.5 or 45.7 cm deep, the deepest levels, did fry weigh less than those from the gravel incubator.

We believe later emergence from the hatchery is beneficial to ocean survival of fry at Auke Creek. With our present lack of better information, we consider simultaneous emergence of hatchery alevins with naturally produced alevins to be ideal. Midpoint of emergence from the saddle incubators was 217-220 days after fertilization, compared with 216 days for the gravel incubator. At this hatchery, alevins tend to emerge about 3 days earlier from gravel incubators than from natural redds in Auke Creek. The longer time spent in the saddle incubators moves the emergence nearly coincident to that for naturally produced fish.

Although the predictions of survival in saddles were too high at low seeding densities and shallow depths, we do not regard the estimates as lacking merit in comparing survival in the saddle substrate with that in the gravel substrate. Estimates of survival in both substrates were determined by the same techniques; the larger gravel incubator simply had more eggs. Unless an unexplained bias has favored saddle incubators, the comparison is valid. In 10 of 13 treatment combinations retained for analysis, estimated survival from saddles exceeded that for the gravel incubator. Even if a bias were to favor saddles, survival must generally have been very high in the saddle incubators.

The prediction surface for survival in the saddle incubators is consistent with intuition: survival is expected to decline with increased seeding density (Figure 1). Therefore, the general shape of the surface has probably been correctly determined. Although survival predicted by the surface is too high over at least portions of the factor space, the important feature of the surface is that highest survival occurs at shallow depths for any seeding density. Optimal conditions for growth in weight and for survival also occur in shallow depth of saddle substrate, and these variables suffer only slight reduction at even the highest seeding densities attempted. Growth in length and time of emergence were not detectably affected by the controlled variables. At shallow depths, saddle incubators produced longer and heavier fry than the gravel incubator, time of emergence was closer to the time of emergence of naturally produced alevins, and survival to emergence in the saddle incubators was probably higher than survival to emergence in the gravel incubator. We predict these advantages hold even when seeding densities in saddle incubators are tenfold those in the gravel incubator.

Obviously, our study does not adequately compare saddles and gravel as incubation substrates because we had no replications of the gravel incubator and did not explore the effects of the controlled variables on alevins in gravel. However, plastic saddles did produce fry equal or superior to fry from a gravel incubator. The fry produced by this gravel incubator survived well in the ocean and, therefore, constituted an appropriate standard for comparison.

CONCLUSIONS

If we define acceptable fry from this experiment as fry whose quality must equal or exceed the quality of fry from the gravel incubator and stipulate that all measures of fry quality--length, weight, incubation period, and survival from the eyed-egg stage--must be satisfied, then we can draw several conclusions about the merits of plastic substrates. These conclusions are valid even though a portion (20%) of the observations was discarded because of suspected technical errors in execution of the experiment. The quality of fry from the turf incubators, which were operated in the range of conditions tested in this experiment, was unacceptable. For fry from the turf incubators, the range of mean weights that were retained for analysis was below the mean weight of fry from the gravel incubator. Only one treatment in the turf incubators produced fry that had a mean length equal to that of fry from gravel. All other treatments in the turf incubators produced smaller fry. Although survival to emergence of fry from turf incubators did not appear impaired compared with survival to emergence of fry from the gravel incubator, incubation period of fry in the turf incubators was severely reduced by 2 days to 2 weeks depending on conditions. Plastic turf, as used in this experiment, was not an acceptable substitute for gravel if volitional emergence coincident with natural emergence of wild fry is desired. But even more clearly, plastic turf is not acceptable because it is inferior to plastic saddles by all our criteria.

Acceptable fry were produced in the saddle incubators over a wide range of experimental conditions of the control variables. Only depth of saddle substrate appeared limiting for recommendations among levels of the three control variables (substrate depth, seeding density, and apparent velocity) within the region explored. Fry length and incubation period were acceptable anywhere over the region of control variables explored: depths from 13.3 to 45.7 cm, seeding densities from 0.414 to 0.940 eggs$\cdot$cm^{-3}, and apparent velocities from 499 to 1,103 cm$^3\cdot$h$^{-1}\cdot$cm^{-2}. Survival and weight of fry in the saddle incubators were acceptable unless depth of saddles exceeded about 29.5 cm, the midpoint of the range explored.

Strictly speaking, the substrate depth at which survival and weight were acceptable is predicted to depend on the other control variables, but the value of 29.5 cm in depth of saddles can be recommended regardless of values of other variables within the region explored. Depth of saddles might be reduced below 13.3 cm and still maintain the quality of the fry, but such depths were not included in the analysis.

Saddles have superior performance at shallow depths, an advantage when used in stacked-tray incubators. Stacked tray incubators with saddle substrates should have several advantages over deep-box incubators. First, water is continually aerated during its passage through the stacked-tray incubator. The result is a potentially greater and more uniform oxygen supply for the eggs and alevins. Second, production of fry per unit volume of substrate is greater in relatively shallow trays than in deep boxes such as the gravel incubator. Alevins tend to reside in the bottom 5-10 cm of substrate (Bams and Crabtree 1976), and added layers of substrate are an unnecessary expense. Third, and most important, production of fry per unit of space in the hatchery is greater for stacked, shallow trays than for a single, deep box. In conclusion, plastic saddles can be used as an efficient substitute for gravel in the production of high-quality pink salmon fry.

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