AN ABSTRACT OF THE THESIS OF

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(Name) (Degree)

in FISHERIES AND WILDLIFE presented on May 4, 1972
(Major) (Date)

Title: YOLK-SAC MALFORMATION IN PACIFIC SALMON

Abstract approved: Redacted for Privacy
Dr. W. J. McNeil

Yolk-sac malformation has been observed to cause mortality of alevins of Pacific salmon raised in hatcheries. This study had two broad objectives:

1) to determine if there was a difference in incidence of malformation among salmon species and salmon hybrids, and
2) to identify environmental factors which cause malformed yolks.

Five Pacific salmon species (chum, pink, Chinook, sockeye, and coho) and two salmon hybrids (pink ♂ X Chinook ♀ and Chinook ♂ X pink ♀) were used in the experiments. Pink, chum, and sockeye salmon alevins exhibited a much higher incidence of malformed yolks than Chinook and coho salmon or salmon hybrids under standardized environmental conditions.

Environmental factors tested included temperature (8, 10, 12, and 14°C), water velocity (25, 100, 200, and 400 cm/hr), substrate (gravel and screen), and light.

Substrate had a profound effect on incidence of yolk-sac malformation. The incidence of malformed yolk appeared to be related to general level of activity (movement) in test groups. Little activity was observed in any of the test groups of alevins on gravel. On the screen substrate, chum, pink, and sockeye alevins exhibited considerable activity; whereas, Chinook, coho, and hybrid alevins exhibited little activity.

Temperature and velocity had a modifying effect on the incidence of malformed yolks, but screen substrate was found to be the principal cause of yolk-sac malformation. Where alevins are active, frequent rubbing of the yolk sac on the screen causes abrasions and coagulation of yolk material. The posterior part of the yolk sac typically elongates. Yolk coagulation appears to prevent the normal circulation of blood and contributes to high mortality of the affected alevins.

Yolk-sac Malformation in Pacific Salmon

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1972

APPROVED:

Redacted for Privacy

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Date thesis is presented May 1, 1972

Typed by Mary Jo Stratton for Hossein Emadi

ACKNOWLEDGEMENTS

I am deeply indebted to many people who contributed in so many ways to the completion of this study. I would like to express my sincere appreciation to my major professor, Dr. McNeil, for his counsel and suggestions throughout the study, and for his excellent help in criticizing and correcting the manuscript.

I am very grateful to Mr. Derek Poon, Dr. Anthony Novotny, and Mr. Donn Steven who provided the fish eggs for these experiments.

Thanks are also due to my close colleague Mr. Bernard Kepshire, for his help and companionship. Acknowledgements also are given to Messrs. Willie Breese, Robert Courtright, and Oliver Stephenson for their help. Thanks to Becky Knutson who typed the first draft copy of this paper.

I would also like to thank Mr. and Mrs. Litchfield, my host family for the time that I was preparing the manuscript in Newport.

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YOLK-SAC MALFORMATION IN PACIFIC SALMON

INTRODUCTION

Yolk represents the main portion (80 percent or more by weight) of a newly hatched salmon. The absence of well developed fins and the presence of a heavy yolk make it difficult for an alevin to swim during the first two or three weeks after hatching.

In natural spawning streams, eggs are buried in the gravel and the alevins remain buried in the gravel until the yolk is nearly or fully absorbed. In hatcheries, where alevins are usually held in screen trays or tanks with smooth bottoms, alevins tend to be active. The amount of activity may be influenced by factors such as light, water velocity, and movement of hatchery workmen. Premature swimming activity may contribute to the development of malformed yolks in salmon alevins.

The objective of this study was to determine the cause or causes of yolk-sac malformation, and to determine the role of environmental and genetic factors in the occurrence of the malformation. The malformation usually appears on the posterior portion of the yolk. It is associated with coagulation of yolk and formation of a fluid-filled vesicle posterior to the coagulated yolk. Scar tissue frequently forms over the surface of the affected area.

The malformation has been described for land-locked Atlantic salmon, Salmo salar, by Dumas (1966). According to Dumas, yolk-sac malformation is triggered by water temperature. Larvae raised in 53 and 47°F water showed the malformation, while larvae raised in colder water with fluctuating temperatures showed no evidence of malformation unless they were exposed to warmer water.

Chinook salmon, Oncorhynchus tshawytscha, exposed to constant rolling and crowding in barrel-type incubators exhibited a high incidence of yolk-sac malformation and a mortality of over 30 percent according to Jochimsen and Bedell (1968). These authors reported that the malformation was not pathogenic since no bacteria were found in the coagulated yolk. Wood (1968) reported that treatment of eggs and yolk sac fry of salmonid fishes with excess malachite green contributed to a coagulated yolk condition.

Although yolk-sac malformation is known to cause high mortality in salmon under certain circumstances, knowledge on factors contributing to its occurrence is limited. This study involved five species of Pacific salmon and two salmon hybrids to evaluate if there were genetic differences in susceptibility to the malformation. Effect of environmental factors such as temperature, light, velocity, and substrate on the occurrence of yolk-sac malformation were also evaluated during the study.

This study was supported by funds from the National Oceanic and Atmospheric Administration Sea Grant program (Contract No. GH97) and National Marine Fisheries Service (Project No. AFC-55).

GENERAL METHODS

Experimental groups of alevins were held in circular, plastic buckets (test cells) immersed in a water bath. Water entered from near the top of each test cell through a ring of 40 small holes positioned 1.5" below the water surface and was drained from near the bottom.

Each test cell was partitioned with a horizontal circular plywood frame covered with a fine screen. The screen was positioned 3.5" beneath the water surface. Water passed downward through the screen, but alevins were not able to penetrate the screen and were restricted to the surface layer of water. A gravel substrate was placed in some cells, while other cells had only bare screen as a substrate.

Water was drained from each test cell into a reservoir after it had passed through a bed of crushed rock and oyster shell (Figures 1 and 2). Water was pumped from the reservoir through a heat exchanger to adjust temperature before it was returned to the water bath and recycled past alevins.

Water velocity was controlled in each test cell by regulating the rate the water was allowed to drain from a cell. Lids were placed on some test cells to reduce light.

Heat exchange systems were constructed of 12 mm O. D. glass tubing immersed in a water bath of the desired temperature. Temperature was equalized in most cases after passing the test water

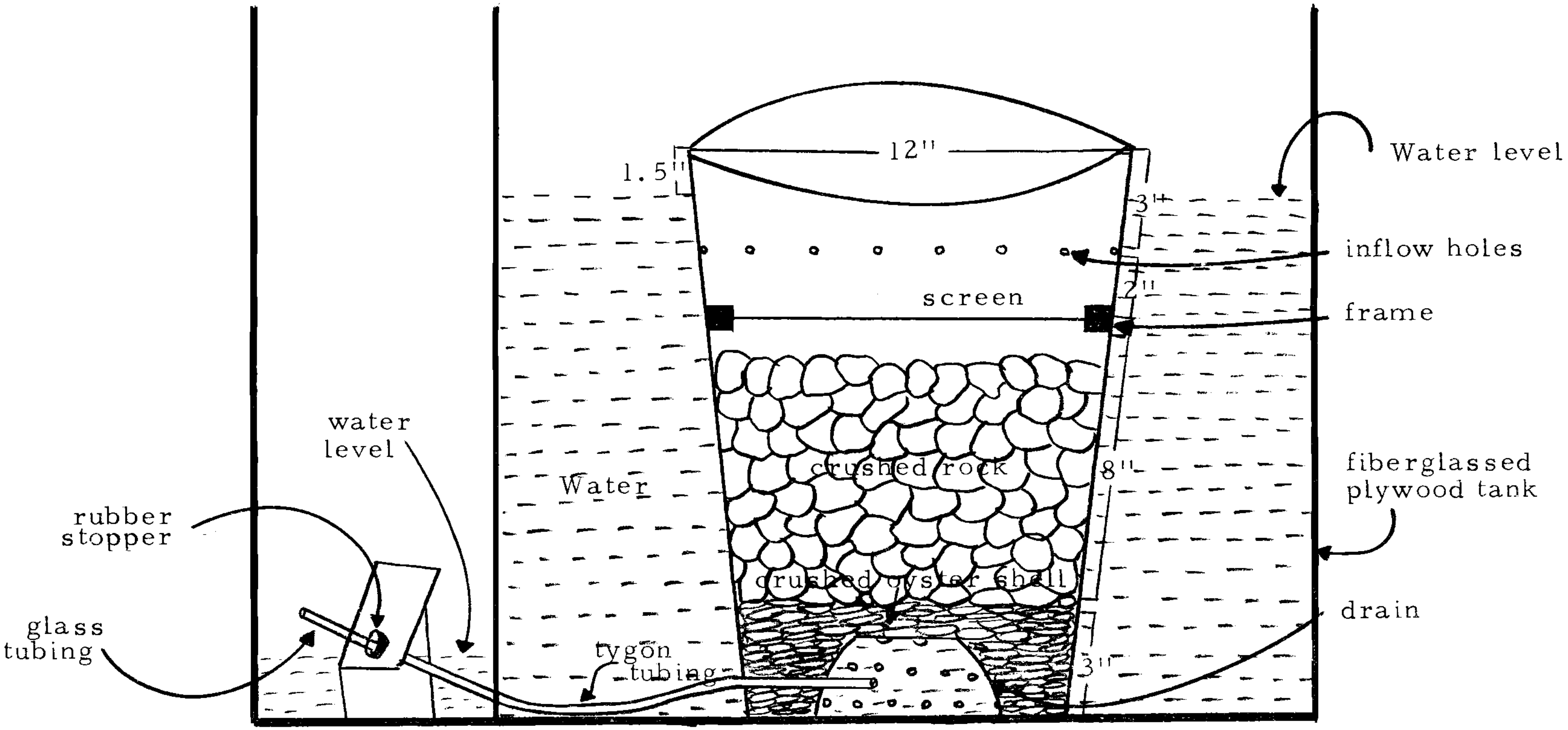

Figure 1. Schematic diagram showing the cross section of an experimental cell.

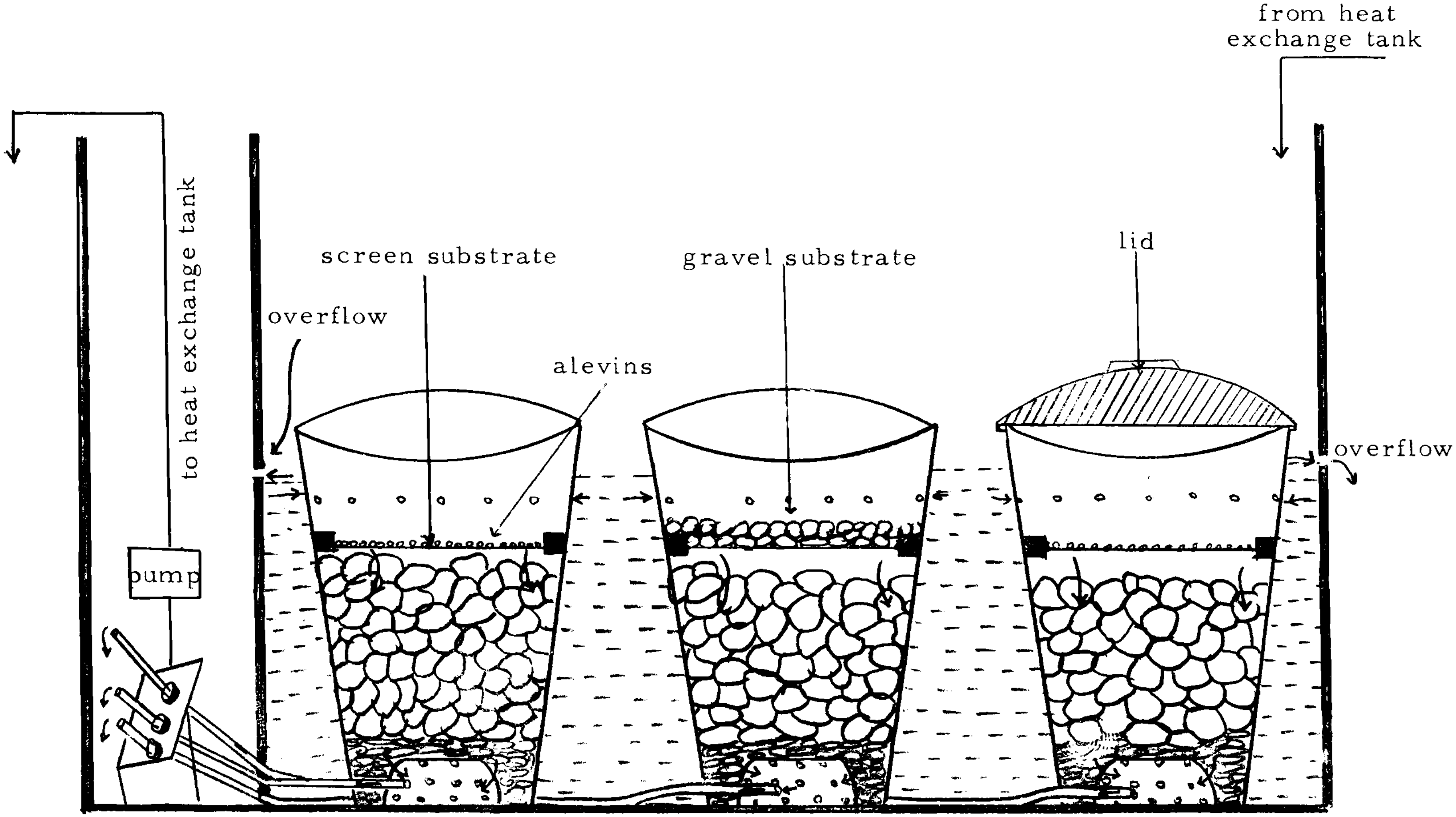

Figure 2. Schematic diagram showing the flow of water into and through the test cells.

through 250 feet of glass tubing before returning to the water bath and the test cells.

Water velocity was controlled in each test cell by the difference in head between the level of water in the water bath and the level of the discharge tube. Velocity was determined by the formula:

$$V = \frac{Q}{A}$$

where V = velocity in cm/hr,

Q = quantity of water in cm^3/hr, and

A = cross-sectional area of the test cell in cm^2.

Although lids were placed on certain test cells to reduce light, some light penetrated through the small holes which allowed water to enter the test cells. Extra precautions were taken with certain test cells to exclude light completely in experiments involving sockeye and coho salmon.

Substrate used in these experiments consisted of 1.0 to 1.5" diameter rocks and smooth nylon screen.

Although most water was recycled, enough water was added to each reservoir to renew the total volume about three times daily to avoid a build-up of toxic metabolites.

Seven stocks of fish were used in these experiments. They are listed in Table 1, which also identifies the source of fish and the cooperating agencies.

Table 1. Salmon used in these experiments along with the origin of stocks and cooperating agencies.

Species	Origin of stocks	Cooperating agencies
1. Chum salmon, *Oncorhynchus keta*	Olson Bay, Ak.	FCO, NMFS
2. Chinook salmon, *O. tshawytcha*	White River, Wn.	NMFS, FWS
3. Pink salmon, *O. gorbuscha*	Little Port Walter, Ak.	NMFS
4. Sockeye salmon, *O. nerka*	Columbia River, Wn.	FWS, EPA
5. Coho salmon, *O. kisutch*	Trask River, Ore.	FCO
6. Pink ♀ X Chinook ♂	Alaska & Washington	NMFS, FWS
7. Chinook ♀ X Pink ♂	Washington & Alaska	FWS, NMFS

Chum salmon eggs were shipped from the National Marine Fisheries Service (NMFS) Field Laboratory at Olson Bay, Alaska, and were fertilized and eyed at the Fish Commission of Oregon (FCO) Big Creek Hatchery before being shipped to Port Orford. Eyed eggs of Chinook, pink, and two hybrid salmon (pink ♂ X Chinook ♀ and Chinook ♂ X pink ♀) were supplied by the National Marine Fisheries Service field station at Manchester, Washington. The Chinook salmon parents came originally from the U.S. Fish and Wildlife Service (FWS) hatchery at White River, Washington. The pink salmon parents came originally from the National Marine Fisheries Service Field Station at Little Port Walter, Alaska. Sockeye salmon originated from the Columbia River, but the eyed eggs were supplied to us by the Corvallis

Fish Toxicology Laboratory of the U.S. Environmental Protection Agency (EPA). The eyed eggs of coho salmon were supplied by the Fish Commission of Oregon, Trask River Hatchery.

Upon arrival at Port Orford, eyed eggs were kept in $13^{o}C$ water to accelerate development. A few days before hatching (during hatching for coho) eggs were divided into test groups and were placed in the cells.

All three water baths were held initially at about $13^{o}C$, but the temperature was reduced gradually in at least two water baths by activating the heat exchange systems. Dead eggs and alevins were removed at least twice a week, and the mortalities were recorded. Mortality of eggs and some mortality of alevins resulting from factors other than yolk-sac malformation were excluded from analysis of malformed yolks.

Alevins were removed from test cells after some showed early signs of swimming. They were anaesthetized, and each alevin was examined for evidence of yolk-sac malformation. Selected groups of alevins exhibiting the malformation and not exhibiting the malformation were held in tanks and fed to evaluate possible delayed mortality after yolk absorption.

YOLK-SAC MALFORMATION IN CHUM SALMON

Eyed chum salmon eggs were received at Port Orford on August 31, 1971. They were divided among 23 test cells and held at elevated temperatures (13.0 to 16.5°C) until hatching commenced on September 12.

Incidence of Malformation

Four environmental variables were tested to determine their effect on incidence of yolk-sac malformation in chum salmon:

1) Substrate,

2) Water temperature,

3) Water velocity, and

4) Exposure to light.

Two types of substrate were evaluated--"screen" and "gravel." I first used a plastic netting with a 1/8" mesh opening for my screen substrate, but the first alevins to hatch were able to penetrate through the 1/8" opening into the underlying rock and gravel. Before hatching had progressed very far, I replaced the coarse screen with a fine nylon of the type used in Heath incubator trays. The few early hatching alevins escaping through the coarse screen were not considered in the analysis of the results.

My gravel substrate consisted of flat pebbles. They ranged in diameter from about 1.0 to 1.5". The pebbles formed a bed about 1.5

to 2.0'' deep overlying the fine screen substrate which prevented the alevins from penetrating more deeply into the test cells. Of the 23 test cells, nine had screen substrates and 14 had gravel substrates.

The experiments involved three temperature ranges (high, medium, and low). Fourteen test cells were held in the high range (12.5 to 13.5°C); six were held in the medium range (10.0 to 12.0°C); and three were held in the low range (8.5 to 12.5°C). The three temperature ranges are shown in Figure 3 for the 30-day experimental period September 15 to October 15.

The experiments with chum salmon involved four velocities: 25, 100, 200, and 400 cm/hr. Water velocity was held at 100 cm/hr in 17 test cells. Velocities of 25, 200, and 400 cm/hr each occurred in two cells.

Light was reduced in 17 test cells by fitting opaque lids over the top opening. Nevertheless, some incidental light was admitted through the small holes which served as entrance ports for water. There was no attempt to reduce light in six test cells, and these had no lids.

The incidence of yolk-sac malformation is given for each test cell in Table 2. Table 2 also gives for each test cell the range of water temperature, whether or not the cell was covered, water velocity, substrate, and number of alevins.

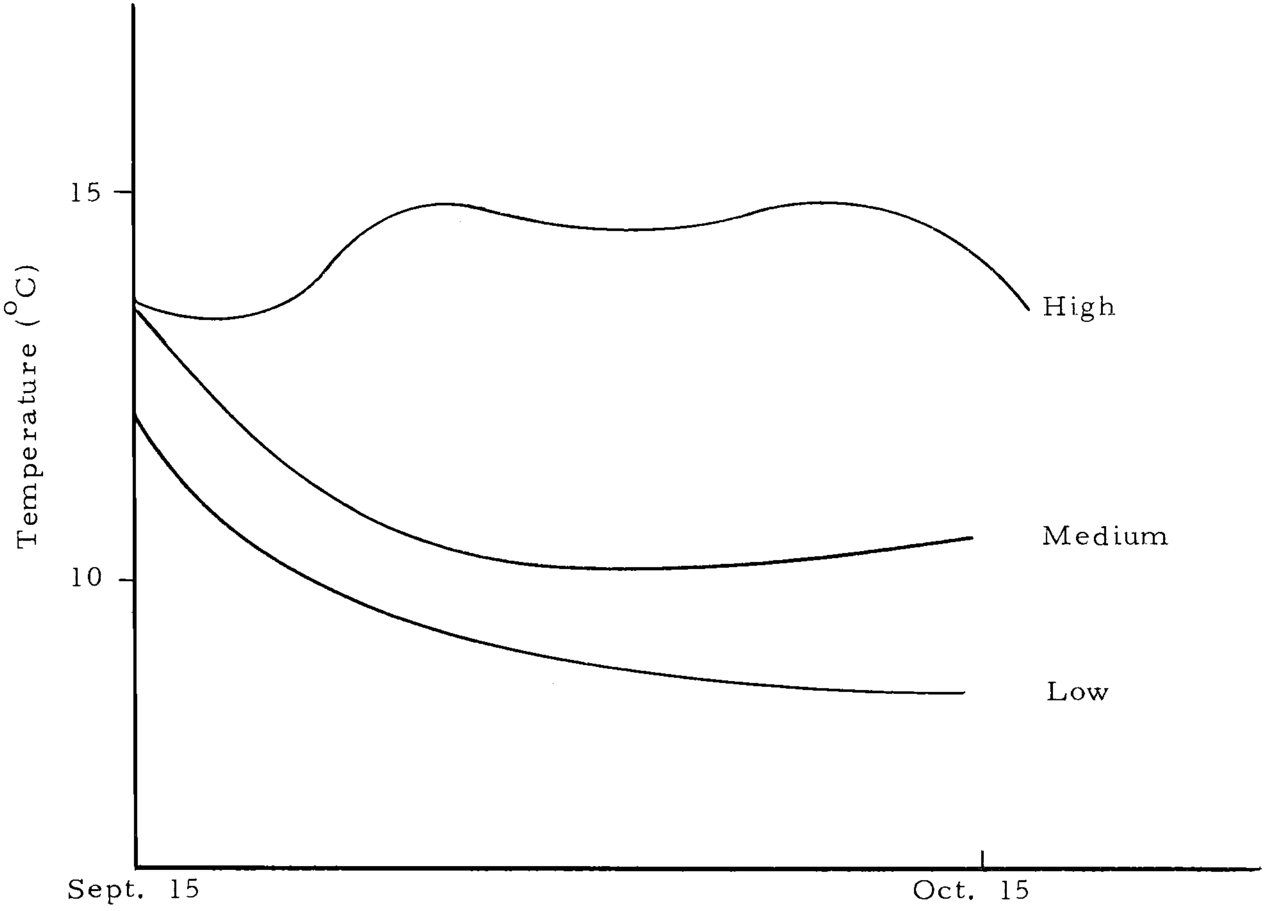

Figure 3. Three temperature ranges for experiments with chum salmon alevins.

Table 2. Incidence of yolk-sac malformation in chum salmon.

Cell no.	Temperature	Lid removed	Velocity (cm/hr)	Substrate	No. of alevins	No. of alevins with malformation	No. of alevins without malformation	% of Alevins with malformation
1	Low	No	100	gravel	80	21	59	26.30
2	Low	No	100	gravel	111	1	110	0.9
3	Low	No	100	screen	88	59	29	67.0
4	Medium	Yes	100	gravel	146	0	146	0
5	Medium	Yes	100	gravel	81	0	81	0
6	Medium	Yes	100	screen	165	55	110	33.3
7	Medium	No	100	gravel	147	0	147	0
8	Medium	No	100	gravel	126	1	125	0.8
9	Medium	No	100	screen	160	48	112	30.0
10	High	No	400	screen	122	25	97	20.5
11	High	No	400	gravel	80	0	80	0
12	High	No	200	screen	130	46	84	35.4
13	High	No	200	gravel	100	0	100	0
14	High	No	25	screen	139	47	92	33.8
15	High	No	25	gravel	92	0	92	0
16	High	No	100	screen	270	164	106	60.7
17	High	No	100	gravel	126	1	125	0.8
18	High	No	100	gravel	125	0	125	0
19	High	Yes	100	screen	102	48	54	47.0
20	High	Yes	100	gravel	80	0	80	0
21	High	Yes	100	gravel	140	0	140	0
22	High	No	100	screen	110	55	55	50.0
23	High	No	100	gravel	50	0	50	0

Effect of Substrate

There was high incidence of yolk-sac malformation in every cell where a screen substrate was used. With only one exception (cell no. 1, Table 2), the incidence of malformed yolks was low where a gravel substrate was used. The number of alevins exhibiting yolk-sac malformation in 14 cells with gravel totalled only 24. Of these, 21 were from cell no. 1.

Because the effect of substrate is obvious from inspection, no analysis is presented to test for significant effects of substrate on yolk-sac malformation. Rather, I have combined the incidence of malformation in all cells containing a gravel substrate and all cells containing a screen substrate for purposes of comparison (Table 3).

Table 3. Effect of gravel and screen substrate on incidence of yolk-sac malformation among chum salmon.

Substrate	No. of test cells	Total no. of alevins	No. of alevins with malformation	% of Alevins with malformation
gravel	14	1484	24	1.5
screen	7	1286	547	42.5

Effect of Temperature

To test for the effect of temperature on incidence of yolk-sac malformation, I used cells no. 3, 9, and 22 which had a screen substrate, an average water velocity of 100 cm/hr, and lids to reduce light. (The gravel substrate yielded mortalities which were too low to be useful for testing for effects of other factors.) Cell no. 3 had a low temperature range; cell no. 9 a medium temperature range; and cell no. 22 a high temperature range. χ^2 was used to test the null hypothesis that temperature had no effect on incidence of yolk-sac malformation, where other environmental factors were similar. The results of the χ^2 test are given in Table 4.

The null hypothesis was rejected, and the large χ^2 value supports the conclusion that temperature influenced the incidence of yolk-sac malformation. The incidence was lowest at the medium temperature range. No trend was apparent in the incidence of malformation with increasing or decreasing temperature in this experiment.

Effect of Water Velocity

To test for the effect of velocities of 25, 100, 200, and 400 cm/hr on incidence of yolk-sac malformation, I again used results from cells with screen substrate. This test involved only the high temperature range and cells with lids to reduce light. Data from cell nos. 10, 12, 14, and 22 were used to test with χ^2 the null hypothesis that velocity had no effect on incidence of malformation. The χ^2 test is summarized in Table 5.

The value of χ^2 (3 d.f.) = 23.34 (prob. < .001) leads to rejection

Table 4. χ^2 test of null hypothesis that temperature has no effect on incidence of yolk-sac malformation in chum salmon, where other environmental factors[1] are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
Low temperature (cell no. 3)	(48.17) 29	(39.80) 59	88
Medium temperature (cell no. 9)	(87.60) 112	(72.40) 48	160
High temperature (cell no. 22)	(60.20) 55	(49.80) 55	110
Total	196	162	358

χ^2 (2 d.f.) = 32.89^{***} (The null hypothesis is rejected.)

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[1] Substrate was screen, water velocity was 100 cm/hr, and lids reduced light.

Table 5. χ^2 test of null hypothesis that water velocity has no effect on incidence of yolk-sac malformation in chum salmon where other environmental factors[1] are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
25 cm/hr (cell no. 14)	(91.0) 92	(48.0) 47	139
100 cm/hr (cell no. 22)	(72.0) 55	(38.0) 55	110
200 cm/hr (cell no. 12)	(85.1) 84	(44.9) 46	130
400 cm/hr (cell no. 10)	(79.9) 97	(42.1) 25	122
Total	328	173	501

χ^2 (3 d.f.) = 22.34*** (Prob. < .001) (The null hypothesis is rejected.)

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[1] Substrate was screen, temperature was in the high range, and lids reduced light.

of the null hypothesis. However, the data do not give definition to a trend showing a dependence of incidence of malformation to increasing or decreasing water velocity.

Effect of Light

Some test cells were left open without lids, while others were covered to reduce light. χ^2 was used to test the hypothesis that open exposure to light (i. e., cells with lids removed) had no effect on incidence of malformation. Again cells with screen substrate were used, and two separate tests were made.

The first test was in the high temperature range with a water velocity of 100 cm/hr (cell nos. 19 and 22). The second test was in the medium temperature range with a water velocity of 100 cm/hr (cell nos. 6 and 9). The results are given in Table 6.

Since neither χ^2 value is significant, it is concluded that the removal of lids has no effect on incidence of malformation.

Form of Malformation

The yolk sac of alevins raised in gravel substrate (all species) is rather rounded, while it is more elongated in alevins which do not exhibit yolk-sac malformation, but are raised on a screen substrate. Figure 4 illustrates the shape of a normal yolk sac in gravel, and Figure 5 shows the elongate yolk sac in chum salmon alevins from a screen substrate.

Table 6. χ^2 test of null hypothesis that removal of lids has no effect on incidence of yolk-sac malformation in chum salmon where other environmental factors are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
A. First test in high temperature range and 100 cm/hr water velocity			
No lid (cell no. 19)	(52.4) 54	(49.6) 48	 102
Lid present (cell no. 22)	(56.6) 55	(53.4) 55	 110
Total	109	103	212
χ^2 (1 d.f.) = 0.20 (Not significant)			
B. Second test in medium temperature range and 100 cm/hr water velocity			
No lid (cell no. 6)	(112.7) 110	(52.3) 55	 165
Lid present (cell no. 9)	(109.3) 112	(50.7) 48	 160
Total	222	103	325
χ^2 (1 d.f.) = 0.415 (Not significant)			

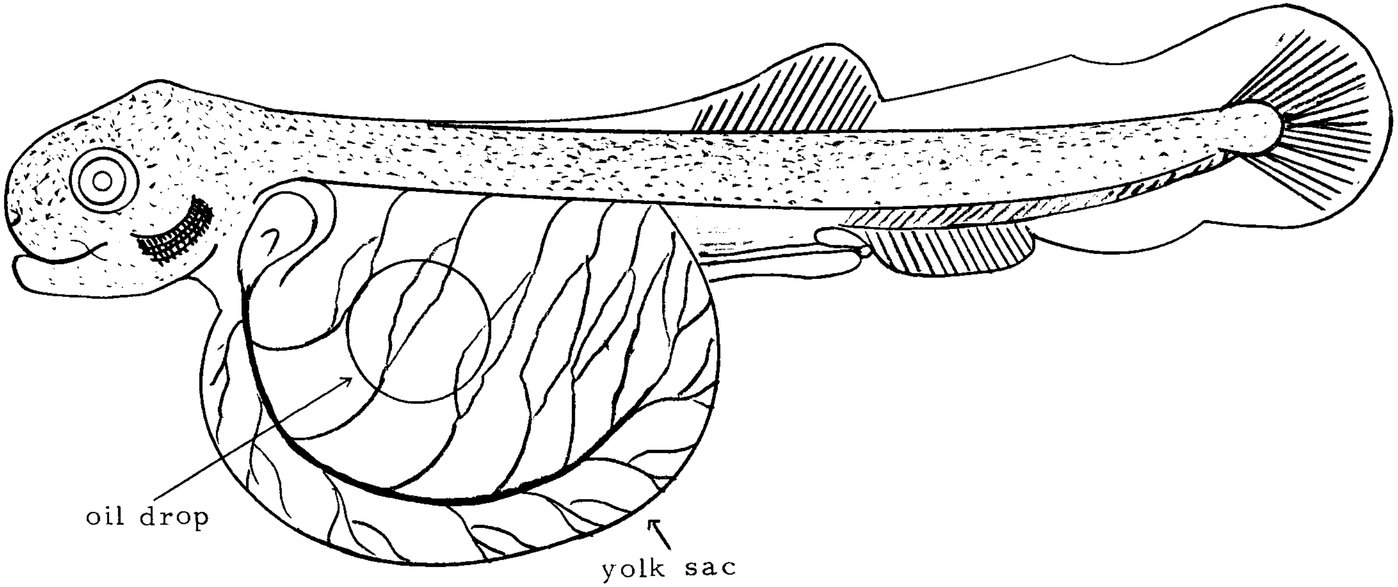

Figure 4. Normal shape of the yolk sac in chum salmon alevins raised in gravel.

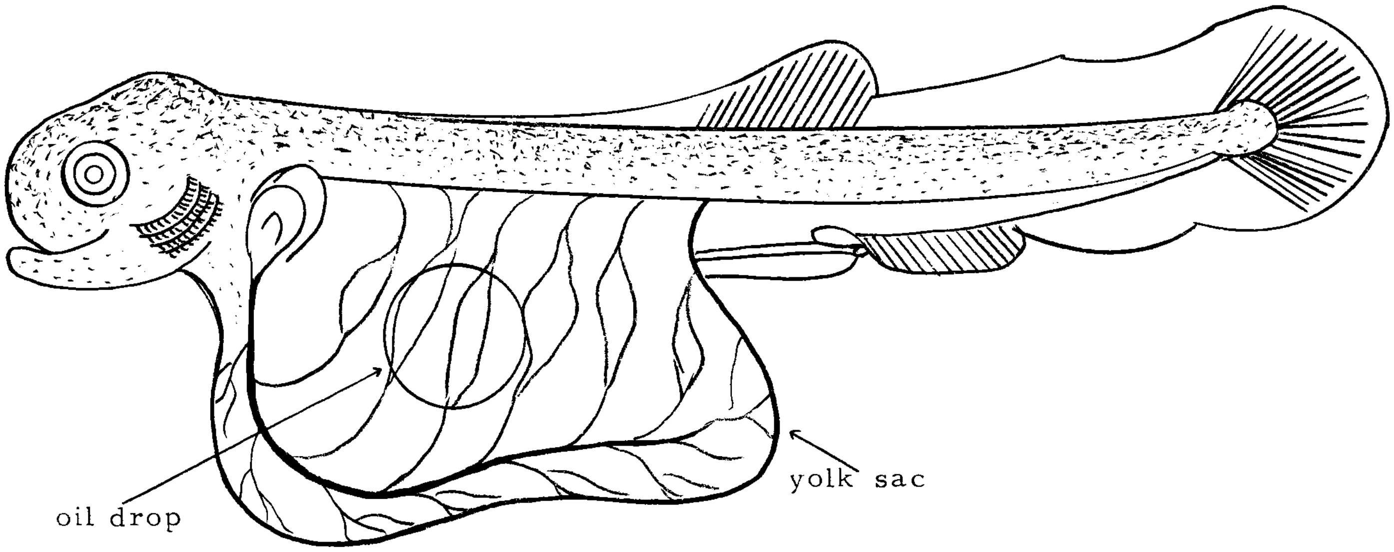

Figure 5. Common shape of the yolk sac in chum salmon alevins raised on a screen substrate.

The first visual evidence of yolk-sac malformation in chum salmon occurred 12 days after hatching in the high temperature range. Figures 6 and 7 illustrate the form of malformation as it is frequently observed in chum salmon.

The malformation is thought to result from mechanical injury (rupture) of the yolk sac. Such injury may facilitate the penetration of water into the yolk and probably ruptures the blood vessels which form a network on the surface of the yolk. The malformation is characterized by coagulation of yolk often accompanied by formation of a liquid-filled vesicle posterior to the coagulated region.

Early development of yolk-sac malformation commonly leads to death of alevins. Mortality appears to be reduced before absorption of yolk is completed where the malformation develops late during the alevin stage. Other unpublished research at our laboratory (Derek Poon, personal communication) suggests that fry resulting from alevins with malformed yolks experience high delayed mortality.

Observation on Behavior of Alevins

Chum salmon alevins are much more active on a screen substrate than on a gravel substrate. The activity continues until the alevins display evidence of a weakened condition, possibly resulting from a malformed yolk or other injuries.

Alevins commonly display a "head down" attitude on a screen

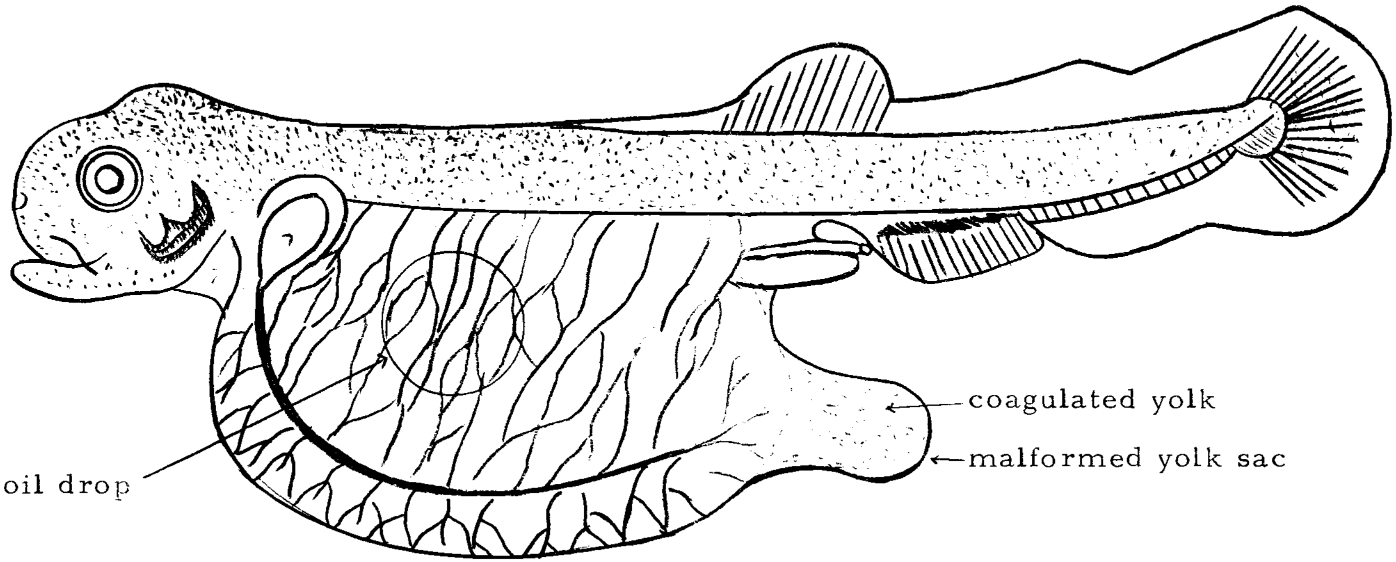

Figure 6. Yolk-sac malformation in chum salmon. This illustrates a case of malformation where the alevin will survive to commence feeding after absorption of yolk sac.

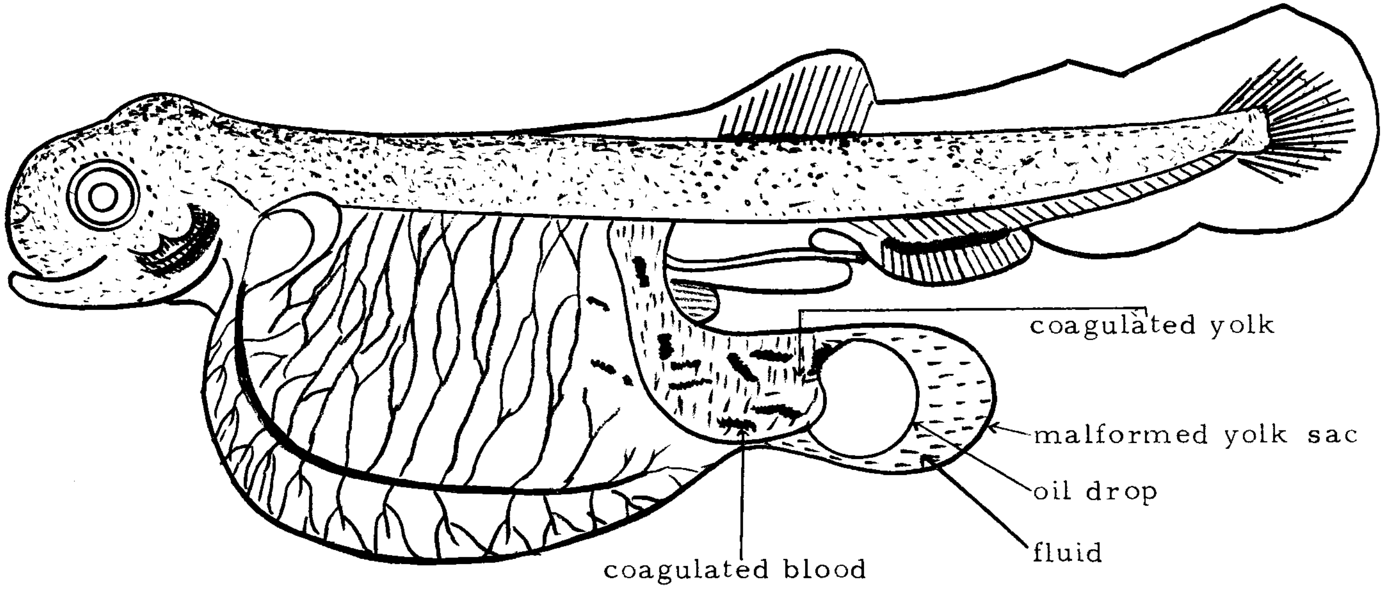

Figure 7. Yolk-sac malformation in chum salmon. This illustrates an extreme case of malformation which commonly leads to mortality in the alevin stage.

substrate, probably in an attempt to "burrow" into the substrate. I have observed abrasion around the mouth, particularly the lower jaw, on many chum salmon alevins. Mouth injury was often accompanied by fungus infection and death.

Another problem that can result from abnormal behavior (activity) of alevins on a screen substrate is dislocation of the oil drop. The oil drop normally is in the anterior or the middle part of the yolk. Disler (1953) was the first to describe dislocation of the oil drop and its effect on the fish. He states:

> ". . . unlike the embryo in natural condition, the embryo in nurseries (hatcheries) usually lie head downward. This position causes the oil globule to shift posterial in the yolk-sac. It is often squeezed between the chorda and the basis of the pelvic fins. The abnormal position of the oil globule often results in deformation of the internal organs."

YOLK-SAC MALFORMATION IN PINK SALMON

Eyed pink salmon eggs were received at Port Orford on October 9. They began to hatch in the test cells on October 18.

Incidence of Malformation

As with chum salmon, I studied the effects of four environmental variables on incidence of yolk-sac malformation (substrate, temperature, velocity, and light). The experiments were reduced, however, from 23 test cells in the case of chum salmon to 11 test cells for pink salmon.

The experiments with chum salmon revealed very low incidence of yolk-sac malformation on a gravel substrate, and the relatively large number of test cells where gravel substrate was used provided no basis to evaluate the effect of other environmental factors. With pink salmon, only three test cells had a gravel substrate and eight had a screen substrate. The substrates were the same as described previously for chum salmon.

As with chum salmon, three temperature ranges (warm, intermediate, and cool) were used. However, the temperatures remained relatively uniform during the experimental period (Oct. 18-Nov. 23) because of better control achieved with the heat exchange systems. The high temperature fluctuated from 11.0 to 12.5°C, the intermediate

Table 7. Incidence of yolk-sac malformation in pink salmon.

Cell no.	Temperature	Lid removed	Velocity (cm/hr)	Substrate	No. of alevins	No. of alevins with malformation	No. of alevins without malformation	% of alevins with malformation
1	Low	No	100	screen	91	28	63	30.7
2	Medium	Yes	100	screen	77	17	60	22.1
3	Medium	No	100	screen	90	8	82	8.8
4	High	No	400	screen	179	51	128	22.5
5	High	No	200	screen	142	25	117	17.6
6	High	No	25	screen	198	37	161	18.7
7	High	No	100	gravel	163	0	163	0
8	High	No	100	gravel	180	3	177	1.7
9	High	No	100	screen	198	52	146	26.3
10	High	Yes	100	screen	179	53	126	29.6
11	High	Yes	100	gravel	167	1	166	0.6

temperature from 9.5 to 10.5^{o}C, and the low temperature from 7.5 to 8.5^{o}C. Figure 8 shows the three temperature ranges.

The same velocities (25, 100, 200, and 400 cm/hr) were used for pink salmon as with chum salmon. Eight test cells had a velocity of 100 cm/hr and one cell was held at each of the other velocities.

Light was reduced in eight test cells by covering them with lids. Three cells had no lids.

The incidence of yolk-sac malformation is given for each test cell in Table 7. Table 7 also gives for each test cell the range of water temperature, whether or not the cell was covered, water velocity, substrate, and number of alevins.

Effect of Substrate

Presence of gravel reduced the incidence of yolk-sac malformation from 23.4 percent to 0.8 percent. This compared to a reduction from 42.5 percent to 1.5 percent for chum salmon alevins. Data on incidence of malformation among pink salmon alevins from the gravel and screen substrates are summarized in Table 8.

Table 8. Effect of gravel and screen substrate on incidence of yolk-sac malformation in pink salmon alevins.

Substrate	No. of test cells	Total no. of alevins	No. of alevins with malformation	% of Alevins with malformation
gravel	3	510	4	0.8
screen	8	1154	271	23.4

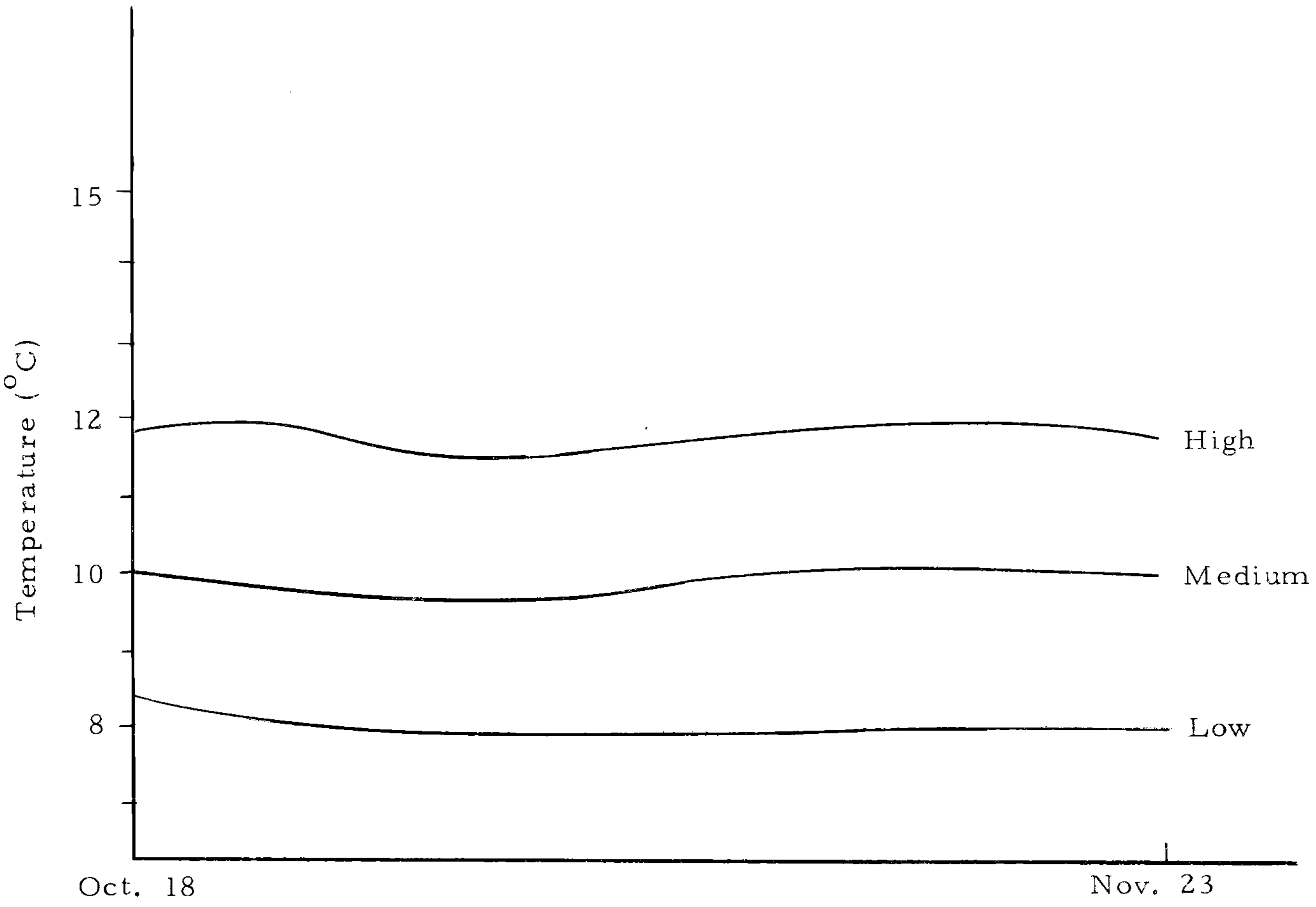

Figure 8. Three temperature ranges for the experiment with pink and Chinook alevins.

Effect of Temperature

Effect of temperature on yolk-sac malformation was evaluated in test cells with a screen substrate, water velocity of 100 cm/hr, and with lids to reduce light. Data from cell nos. 1, 3, and 9 were used to test the null hypothesis that temperature had no effect on incidence of yolk-sac malformation where other environmental factors were similar. The results of the χ^2 test are given in Table 9.

The test results were very similar to those obtained with chum salmon (Table 4). The null hypothesis is rejected, and the large χ^2 value supports the conclusion that temperature affects the incidence of yolk-sac malformation. As with chum salmon, the incidence was lowest at the medium temperature. No consistent trend was apparent in the incidence of malformation in pink salmon with increasing or decreasing temperature.

Effect of Water Velocity

The experiment on water velocity utilized the high temperature range, screen substrate, and cells with lids. Data from cell nos. 4, 5, 6, and 9 were used to test with χ^2 the null hypothesis that velocity had no effect on incidence of malformation. The χ^2 test is summarized in Table 10.

The value of χ^2 (3 d.f.) = 8.63 (prob. $\approx$ .04) caused me to reject

Table 9. χ^2 test of null hypothesis that temperature has no effect on incidence of yolk-sac malformation in pink salmon where other environmental factors[1] are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
Low temperature (cell no. 1)	(69.9) 63	(21.1) 28	91
Medium temperature (cell no. 3)	(69.0) 82	(20.9) 8	90
High temperature (cell no. 9)	(152.0) 146	(46.0) 52	198
Total	291	88	379

χ^2 (2 d.f.) 14.30^{***} (The null hypothesis is rejected.)

---

[1] Substrate was screen, water velocity was 100 cm/hr, and lids reduced light.

Table 10. χ^2 test of null hypothesis that water velocity has no effect on incidence of yolk-sac malformation in pink salmon where other environmental factors[1] are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
25 cm/hr (cell no. 6)	(152.4) 161	(45.6) 37	198
100 cm/hr (cell no. 9)	(152.4) 146	(45.6) 52	198
200 cm/hr (cell no. 5)	(109.3) 117	(32.7) 25	142
400 cm/hr (cell no. 4)	(137.8) 128	(41.2) 51	179
Total	552	165	717

χ^2 (3 d.f.) 8.63* (The null hypothesis is rejected.)

---

[1] Substrate was screen, temperature was in the high range, and lids reduced light.

the null hypothesis, since a similar test with chum salmon produced a somewhat higher χ^2 value of 12.84 (prob. $\approx$.01). As with chum salmon, there was no obvious linear or curvilinear relation between velocity and incidence of malformation.

Effect of Light

There were two tests to evaluate the effect of light on incidence of yolk-sac malformation in pink salmon alevins. The first test was in the high temperature range with a water velocity of 100 cm/hr. It included cell nos. 9 and 10. The second test was in the medium temperature range with a water velocity of 100 cm/hr. It included cell nos. 2 and 3. The results of the χ^2 tests are given in Table 11.

The χ^2 value for the first test was not significant, but the χ^2 value for the second test was significant at the 5 percent level. Similar tests with chum salmon gave χ^2 values which were not significant (Table 6). Removal of the lid to allow more light on alevins may have contributed to some increase in the incidence of yolk-sac malformation in pink salmon, but the additional light was not a major factor when tested against cells with lids. However, cells with lids admit some light through holes for water passage; hence, the darkened cells are not totally blacked out.

Table 11. χ^2 test of null hypothesis that removal of lids has no effect on incidence of yolk-sac malformation in pink salmon where other environmental factors are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
A. First test in high temperature range and 100 cm/hr water velocity			
No lid (cell no. 10)	(129.1) 126	(49.9) 53	179
Lid present (cell no. 9)	(142.9) 146	(55.1) 52	198
Total	272	105	377
χ^2 (1 d.f.) = 0.51 (Not significant)			
B. Second test in medium temperature range and 100 cm/hr water velocity			
No lid (cell no. 2)	(65.5) 60	(11.5) 17	77
Lid present (cell no. 3)	(76.5) 82	(13.5) 8	90
Total	142	25	167
χ^2 (1 d.f.) = 5.70* (Significant at Prob. $\approx$ 0.02)			

Form of Malformation

Yolk-sac malformation was first observed in pink salmon 21 days after hatching. The extreme form of yolk-sac malformation, as illustrated in Figure 5 for chum salmon, was not common in pink salmon. The typical form of the malformation observed in pink salmon is illustrated in Figure 9.

Observations on Behavior of Alevins

On gravel, pink salmon alevins exhibited somewhat less activity than chum salmon. For example, penetration into the substrate was delayed until about two days after hatching. In the case of chum salmon, penetration occurred immediately after hatching.

Alevins on screen substrate exhibited almost constant activity. Any disturbance heightened the level of activity. These observations were similar to those for chum salmon.

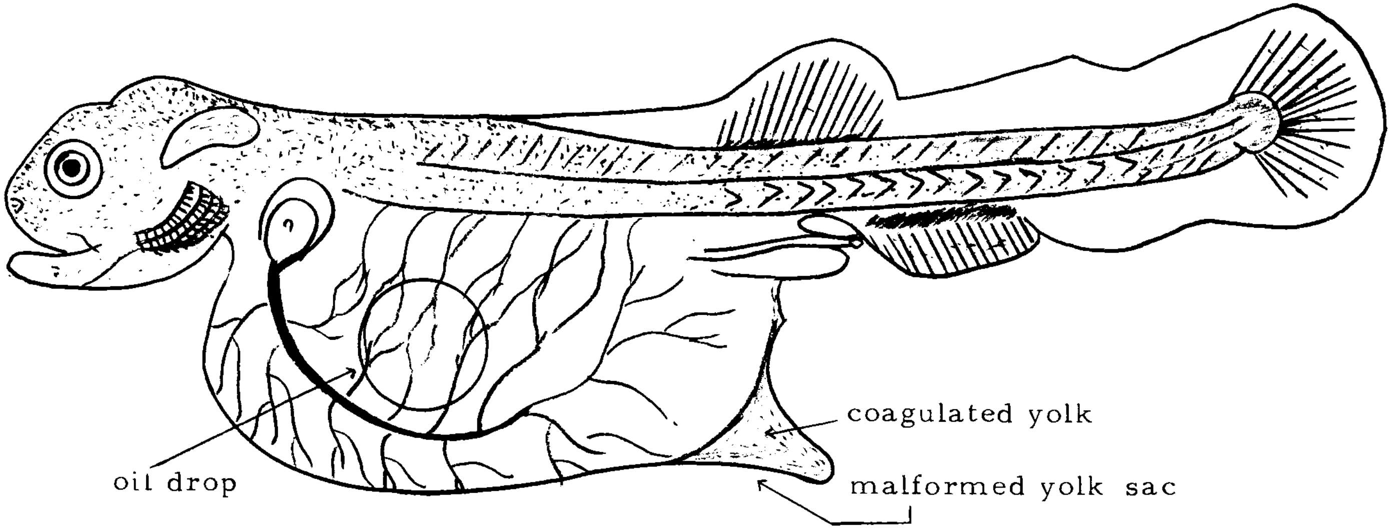

Figure 9. Yolk-sac malformation in pink salmon. This illustrates the most common form of malformation observed in this species.

YOLK-SAC MALFORMATION IN CHINOOK SALMON

Eyed Chinook salmon eggs were received at Port Orford on the same date as pink salmon eggs (October 9). Hatching commenced in the test cells on October 16.

Incidence of Malformation

The experiments were very similar to those with chum and pink salmon. The variables tested were substrate, temperature, velocity, and light. Thirteen test cells were used. Five cells had a gravel substrate and eight had a screen substrate. The temperature ranges were identical to those described for pink salmon (Figure 7).

The incidence of yolk-sac malformation was very low under all test conditions. The experimental conditions and test results are given in Table 12.

For Chinook salmon, the screen substrate had little or no effect on incidence of yolk-sac malformation. This result was quite different than for chum and pink salmon. Table 13 gives the incidence of malformation for alevins from the two substrates. No evaluations were possible with Chinook salmon on effect of temperature, water velocity, or light on incidence of yolk-sac malformation under all test conditions.

Table 12. Incidence of yolk-sac malformation in Chinook salmon.

Cell no.	Temperature	Lid removed	Velocity (cm/hr)	Substrate	No. of alevins	No. of alevins with malformation	No. of alevins without malformation	% of alevins with malformation
1	Low	No	100	gravel	75	0	75	0
2	Low	No	100	gravel	75	0	75	0
3	Low	No	100	screen	100	0	100	0
4	Medium	Yes	100	screen	82	0	82	0
5	Mdeium	No	100	screen	52	0	52	0
6	High	No	400	screen	171	0	171	0
7	High	No	200	screen	195	0	195	0
8	High	No	25	screen	190	0	190	0
9	High	No	25	gravel	193	0	193	0
10	High	No	100	gravel	191	0	191	0
11	High	No	100	screen	174	0	174	0
12	High	Yes	100	screen	190	2	188	1.05
13	High	Yes	100	gravel	188	0	188	0

Table 13. Effect of gravel and screen substrate on incidence of yolk-sac malformation in Chinook salmon alevins.

Substrate	No. of test cells	Total no. of alevins	No. of alevins with malformation	% of Alevins with malformation
gravel	5	722	0	0
screen	8	1154	2	0.2

Form of Malformation

In Chinook salmon, the malformation did not exhibit an extreme form as illustrated in Figure 5 for chum salmon. Figure 10 illustrates the observed form of yolk-sac malformation in Chinook salmon alevins.

Observations on Behavior of Alevins

Chinook salmon alevins did not exhibit high levels of activity on the screen substrate, which was so typical for chum and pink salmon. This lack of activity probably explains why the incidence of malformation was low.

Chinook salmon alevins raised on a gravel substrate exhibited little evidence of penetration into the gravel even where the lids were removed from the test cells.

Some alevins were transferred from low and intermediate

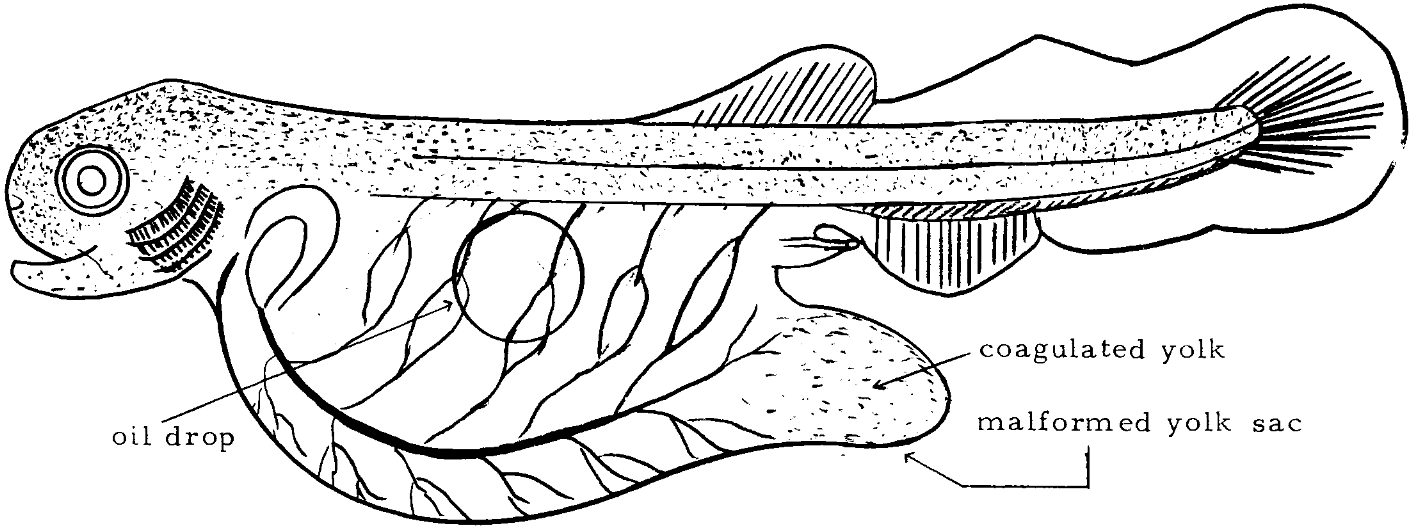

Figure 10. The observed form of yolk-sac malformation in Chinook salmon alevins.

temperatures to 14°C in an attempt to increase their activity. But even this failed to cause any significant increase of activity or incidence of yolk-sac malformation.

YOLK-SAC MALFORMATION IN SALMON HYBRIDS

When the experiments were planned, it was hypothesized that incidence of yolk-sac malformation would be higher in pink salmon than in Chinook salmon. The results reported in the previous two sections on pink and Chinook salmon have confirmed this hypothesis.

Two series of experiments were run on hybrid crosses of Chinook and pink salmon to gain further insight into the role of genetic control of the incidence of yolk-sac malformation in Pacific salmon. The first series of experiments involved pink male X Chinook female hybrids, and the second series involved Chinook male X pink female hybrids.

Eyed eggs of salmon hybrids were received at Port Orford on October 9. They began to hatch in the test cells on October 16.

Incidence of Malformation

Five test cells were used for each of the hybrids. Three cells contained a screen substrate and two cells contained a gravel substrate. Only two temperature ranges were used in these experiments (Figure 11). The experimental conditions and test results are given in Table 14.

The incidence of yolk-sac malformation was the same (about three percent) for both hybrids raised on a screen substrate (Table 15).

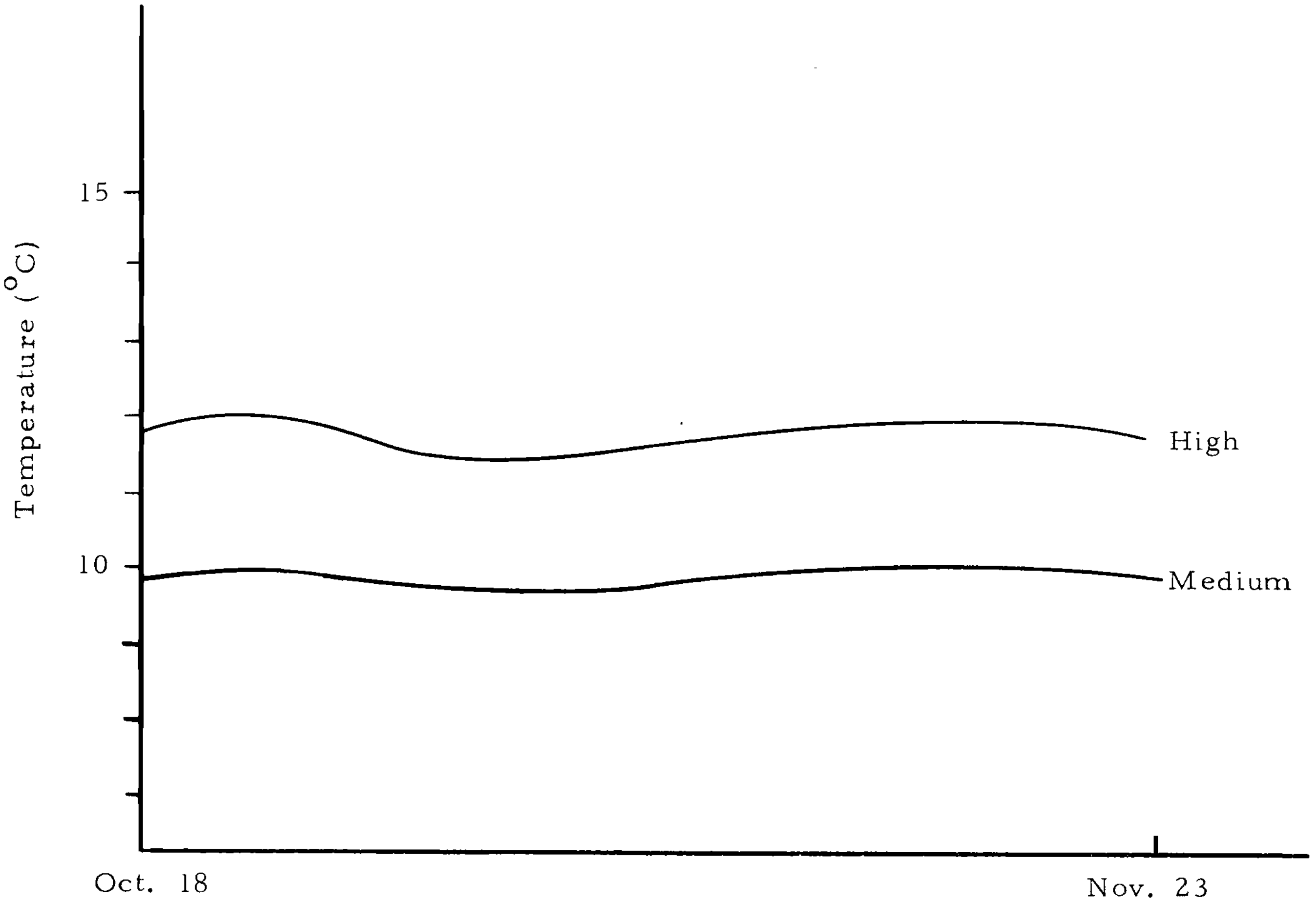

Figure 11. Temperature ranges for experiments with hybrid salmon alevins.

Table 14. Incidence of yolk-sac malformation in salmon hybrids.

Cell no.	Hybrid (♂X ♀)	Temperature	Lid removed	Velocity (cm/hr)	Substrate	No. of alevins	No. of alevins with malformation	No. of alevins without malformation	% of Alevins with malformation
1	Pink X Chinook	Medium	No	100	screen	80	2	78	2.5
2	"	High	No	100	screen	101	5	96	4.95
3	"	High	No	100	gravel	100	0	100	0
4	"	High	Yes	100	screen	110	2	108	1.82
5	"	High	Yes	100	gravel	58	0	58	0
6	Chinook X Pink	Medium	No	100	screen	61	0	61	0
7	"	High	No	100	screen	60	2	58	3.3
8	"	High	No	100	gravel	87	0	87	0
9	"	High	Yes	100	screen	104	4	100	3.85
10	"	High	Yes	100	gravel	81	0	81	0

Table 15. Effect of gravel and screen substrate on incidence of yolk-sac malformation in salmon hybrids.

Substrate	No. of test cells	Total no. of alevins	No. of alevins with malformation	% of Alevins with malformation
Pink ♂ X Chinook ♀ Hybrid				
gravel	2	158	0	0
screen	3	291	9	3.1
Chinook ♂ X Pink ♀ Hybrid				
gravel	2	168	0	0
screen	3	225	6	2.6

The observed percentage of malformation was much lower for the hybrids than for the pink salmon parental stock (about 23 percent) and considerably higher than for the Chinook salmon parental stock (only 0.2 percent).

Form of Malformation

Yolk-sac malformation in hybrid salmon was very similar in appearance to that already described for pink and Chinook salmon alevins (see Figures 7 and 8).

Observations on Behavior of Alevins

The pink ♂ X Chinook ♀ hybrid alevins mostly behaved as Chinook alevins. In the first two weeks of their life they did not exhibit much activity. They started to feed much earlier than Chinook, pink, or Chinook ♂ X pink ♀ alevins.

Mortality was rather high in the Chinook ♂ X pink ♀ hybrid eggs and alevins. Truncated heads and malformed eyes were common. These malformations were unrelated to incidence of malformed yolks. The alevins were active, but not as active as pink salmon alevins.

YOLK-SAC MALFORMATION IN SOCKEYE SALMON

Eyed sockeye salmon eggs were received at Port Orford on November 14. They began to hatch in the test cells on December 4.

Incidence of Malformation

The experiment with sockeye salmon paralleled that already reported for chum, pink, and Chinook salmon. The same four environmental variables (substrate, temperature, velocity, and light) were evaluated. A total of 23 test cells was used (eight with a gravel substrate and 15 with a screen substrate).

Water temperature was held near 8, 10, 12, and 14°C in individual cells during the experimental period (December 4-January 22). Figure 12 shows the four temperatures used in tests with sockeye salmon.

Test water velocities were 25, 100, 200, and 400 cm/hr.

Light was reduced in certain test cells with lids. One test cell was surrounded with black plastic to achieve complete darkness.

The incidence of yolk-sac malformation is given in each test cell in Table 16. Table 16 also gives for each test cell the water temperature, degree of protection from light, water velocity, substrate, and number of alevins.

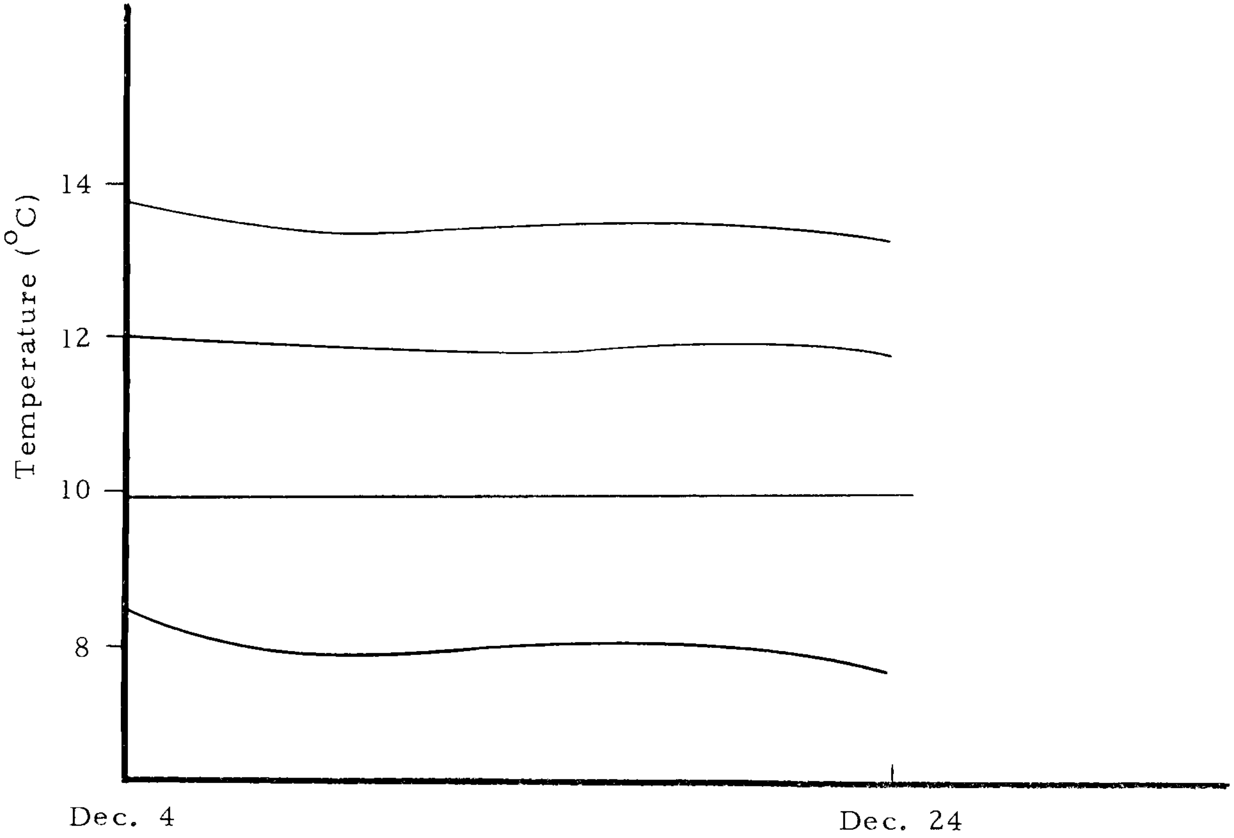

Figure 12. Temperature ranges for experiments with sockeye salmon alevins.

Table 16. Incidence of yolk-sac malformation in sockeye salmon.

Cell no.	Temperature (°C)	Lid removed	Velocity (cm/hr)	Substrate	No. of alevins	No. of alevins with malformation	No. of alevins without malformation	% of Alevins with malformation
1	8	No	100	screen	93	4	89	4. 30
2	8	No	100	gravel	94	0	94	0
3	10	Yes	100	screen	97	6	91	6. 20
4	10	Yes	100	gravel	84	0	84	0
5	10	No	100	screen	97	4	93	4. 10
6	10	No	100	gravel	89	0	89	0
7	12	No	100	screen	91	5	86	5. 50
8	12	No + black plastic	100	screen	20	0	20	0
9	12	No	400	screen	75	10	65	13. 30
10	12	No	200	screen	86	6	80	7. 00
11	12	No	200	gravel	66	0	66	0
12	12	No	25	screen	64	4	60	6. 30
13	12	No	100	nylon screen	81	8	73	9. 90
14	12	No	100	gravel	70	0	70	0
15	12	No	100	gravel	76	1	75	1. 30
16	14	No	400	screen	77	21	56	27. 20
17	14	No	200	screen	85	12	73	14. 10
18	14	No	25	screen	68	7	61	10. 30
19	14	No	100	screen	158	26	132	16. 50
20	14	No	100	screen	70	13	57	18. 50
21	14	No	100	gravel	76	0	76	0
22	14	Yes	100	screen	100	18	82	18. 0
23	14	Yes	100	gravel	65	0	65	0

Effect of Substrate

Presence of gravel greatly reduced the incidence of yolk-sac malformation in sockeye salmon. This result agreed with results already reported for chum and pink salmon. Data from gravel and screen substrates are summarized in Table 17.

Table 17. Effect of gravel and screen substrate on incidence of yolk-sac malformation in sockeye salmon alevins.

Substrate	No. of test cells	Total no. of alevins	No. of alevins with malformation	% of Alevins with malformation
gravel	8	620	1	0.2
screen	15	1262	144	11.4

Effect of Temperature

Tests with sockeye salmon revealed an increasing trend in incidence of yolk-sac malformation with increased temperature (Figure 13). Similar tests with chum and pink salmon indicated that temperature had an effect on incidence of malformed yolks, but these earlier experiments were not adequately designed to reveal a consistent trend relating the incidence of malformed yolks to increasing temperature.

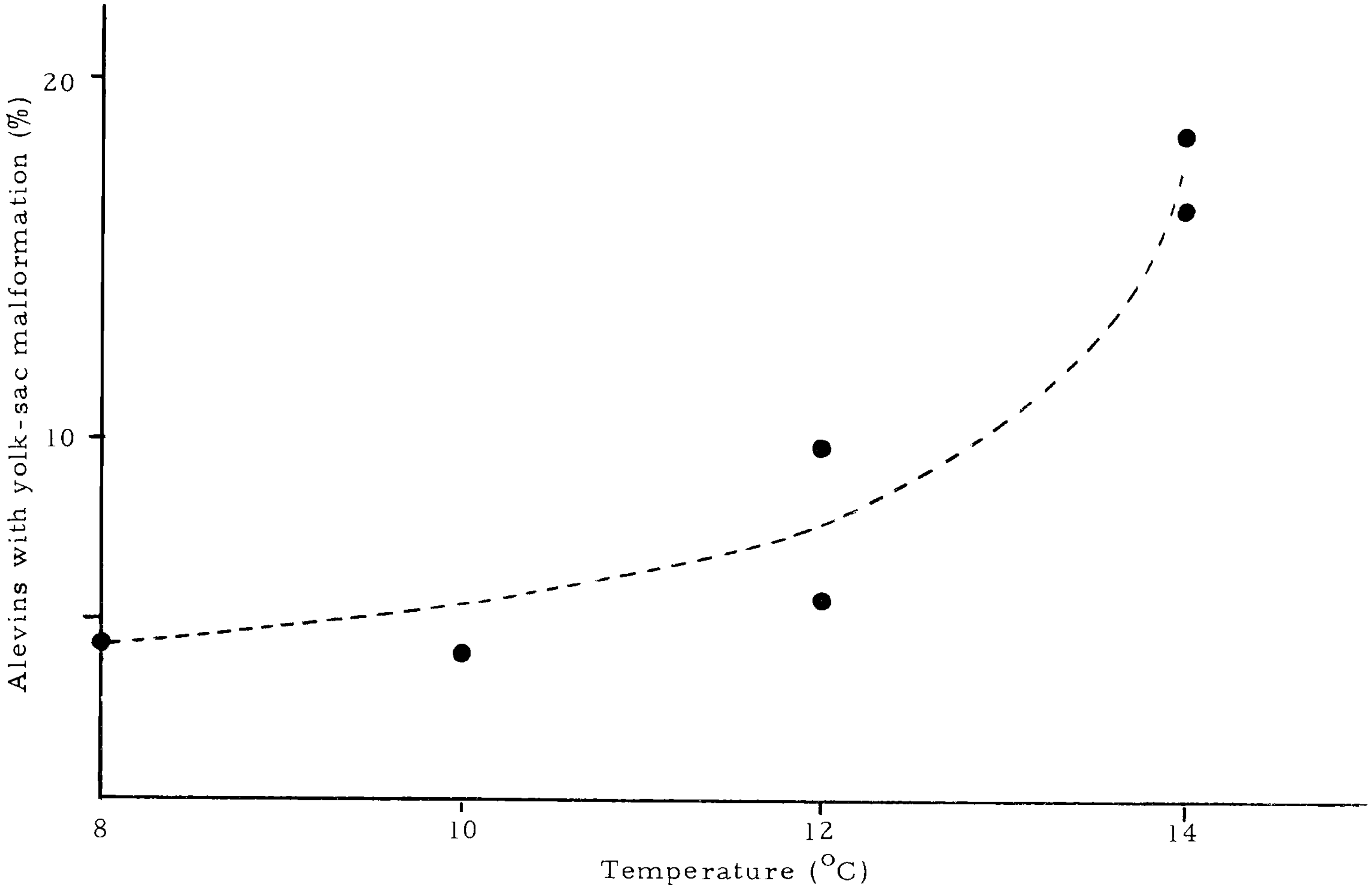

Figure 13. Incidence of yolk-sac malformation in sockeye salmon alevins in relation to water temperature.

Effect of Water Velocity

There were two experiments on effect of water velocity on incidence of yolk-sac malformation in sockeye salmon. One experiment was at 12°C and the other at 14°C. The percentage of alevins with malformed yolks is plotted against velocity in Figure 14.

Both experiments revealed a trend of increasing incidence of malformation with increased velocity. Furthermore, the incidence of malformation at 14°C was consistently higher than at 12°C for all velocities tested. This reinforces the conclusion that incidence of malformed yolks increases with temperature.

Effect of Light

There were two tests to evaluate the effect of light on incidence of yolk-sac malformation. The first test was at 10°C and a water velocity of 100 cm/hr. The second test was at 14°C and a water velocity of 100 cm/hr. Results of the χ^2 tests are given in Table 18.

Neither χ^2 value was significant. These results generally agreed with results of similar tests with chum and pink salmon alevins.

Form of Malformation

In sockeye salmon, the malformation did not exhibit an extreme form as illustrated in Figure 5 for chum salmon. Figure 15 shows the most common form of yolk-sac malformation in sockeye salmon.

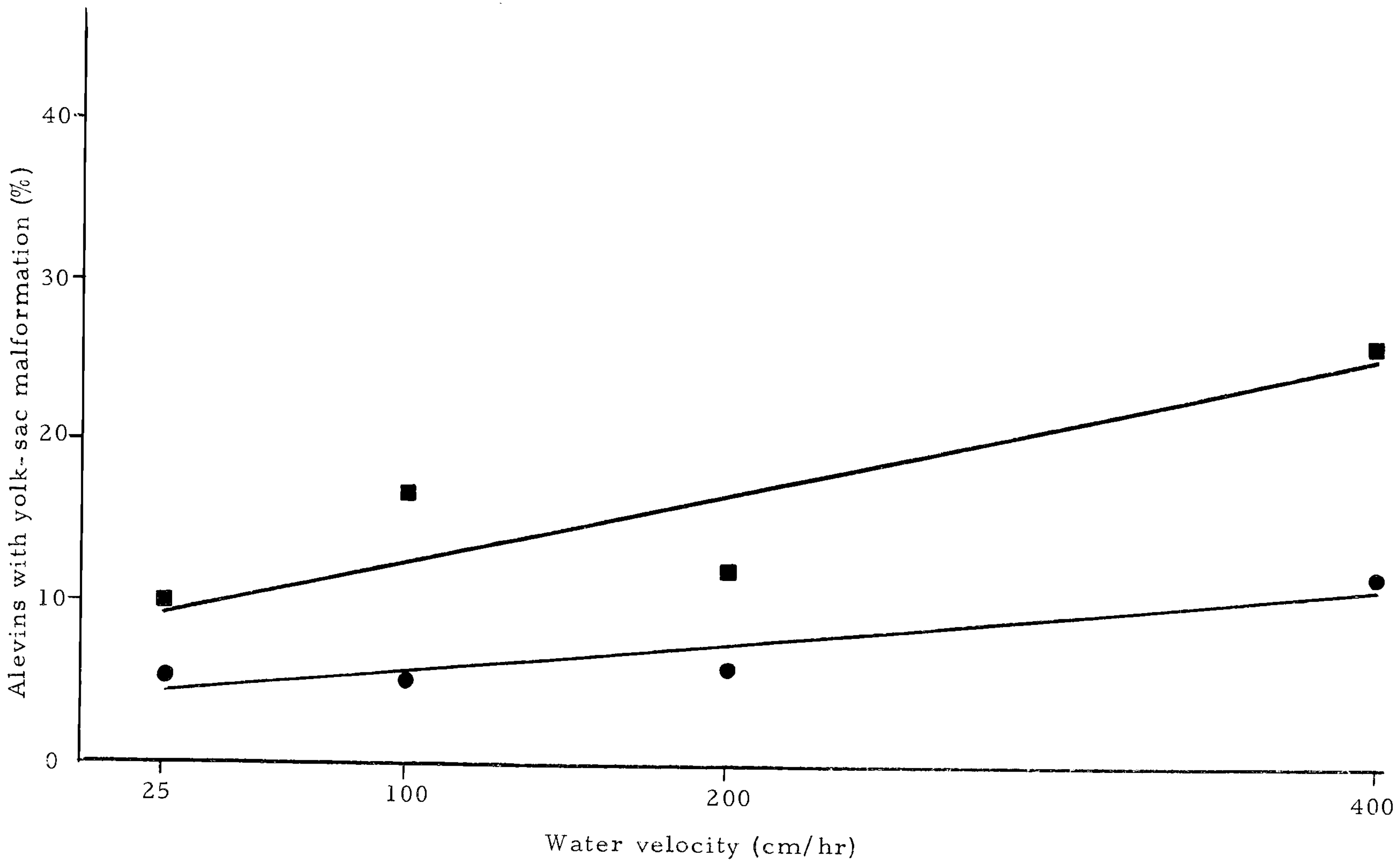

Figure 14. Incidence of yolk-sac malformation in sockeye salmon in relation to water velocity and temperature.

Table 18. χ^2 test of null hypothesis that removal of lids has no effect on incidence of yolk-sac malformation in sockeye salmon, where other environmental factors are similar.

	No. of alevins without malformation	No. of alevins with malformation	Total
A. First test at 10°C and 100 cm/hr water velocity			
No lid (cell no. 3)	(92.0) 91	(5.0) 6	97
Lid present (cell no. 5)	(92.0) 93	(5.0) 4	97
Total	184	10	194
χ^2 (1 d.f.) = 0.43 (Not significant)			
B. Second test at 14°C and 100 cm/hr water velocity			
No lid (cell no. 22)	(82.9) 82	(17.1) 18	100
Lid present (cell no. 19)	(131.1) 132	(26.9) 26	158
Total	214	44	258
χ^2 (1 d.f.) = 0.10 (Not significant)			

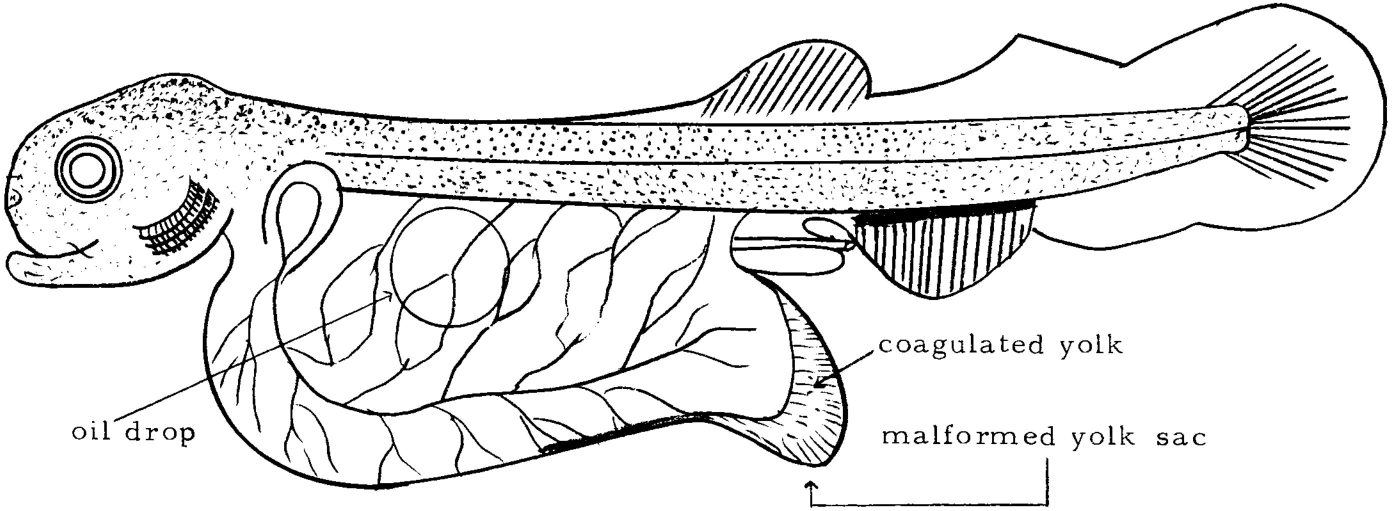

Figure 15. The most common form of yolk-sac malformation in sockeye salmon.

Observations on Behavior of Alevins

Sockeye salmon alevins are relatively active. In gravel, most of them penetrated into the substrate within two hours after hatching. On a screen substrate, they exhibit constant movement similar to chum and pink salmon alevins.

Sockeye salmon alevins began to leave the substrate and swim much earlier in development than the other species. As early as ten days after hatching, alevins in the warmest water were observed swimming. Within three weeks after hatching, sockeye salmon alevins were free swimming under all test conditions.

YOLK-SAC MALFORMATION IN COHO SALMON

Eyed coho salmon eggs were received at Port Orford on December 15. Hatching commenced on December 25.

Incidence of Malformation

An extensive series of experiments was set up with coho salmon, involving 29 test cells. I had intended to gather more detailed information on effects of temperature and water velocity on incidence of malformation, and some preliminary information on effects of crowding. However, the incidence of malformation was too low under all test conditions to complete a meaningful evaluation of the variables under study. The experimental conditions and test results are given in Table 19. The temperature ranges are given in Figure 16.

For coho salmon, as with Chinook salmon, the screen substrate appeared to have little or no effect on incidence of yolk-sac malformation up to the time the alevins began to leave the bottom and swim in the surface water. Only alevins from four test cells, all with screen substrate, showed evidence of malformation. These were cell nos. 19 (1.0 percent), 21 (1.0 percent), 26 (2.1 percent), and 27 (10.1 percent). Table 20 summarizes the incidence of malformed yolks observed to the swim-up stage in gravel and screen substrates.

After evaluating the incidence of yolk-sac malformation among

Table 19. Incidence of yolk-sac malformation in coho salmon.

Cell no.	Temperature (°C)	Lid removed	Velocity (cm/hr)	Substrate	No. of alevins	No. of alevins with malformation	No. of alevins without malformation	% of Alevins with malformation
1	8	No	100	screen	99	0	99	0
2	8	No	100	gravel	100	0	100	0
3	8	Yes	100	screen	97	0	97	0
4	10	Yes	100	screen	100	0	100	0
5	10	Yes	100	gravel	100	0	100	0
6	10	No	100	screen	1013	0	1013	0
7	10	No	100	screen	103	0	103	0
8	10	No	100	gravel	98	0	98	0
9	12	No	100	screen	99	0	99	0
10	12	No	400	screen	96	0	96	0
11	12	No	400	gravel	100	0	100	0
12	12	No	200	screen	103	0	103	0
13	12	No	200	gravel	100	0	100	0
14	12	No	100	gravel	100	0	100	0
15	12	No	100	screen	102	0	102	0
16	12	No	25	screen	101	0	101	0
17	12	No	25	gravel	100	0	100	0
18	14	No	400	screen	96	0	96	0
19	14	No	200	screen	100	1	99	1.0
20	14	No	25	screen	96	0	96	0
21	14	No	100	screen	196	2	194	1.00
22	14	No	100	screen	98	0	98	0
23	14	No	100	gravel	97	0	97	0
24	14	Yes	100	screen	96	0	96	0
25	14	Yes	100	gravel	97	0	97	0
26	14	No	100	screen	1012	21	991	2.10
27	14	No	100	screen	524	53	471	10.1
28	14	No	100	screen	150	0	150	0
29	14	No	100	screen	120	0	120	0

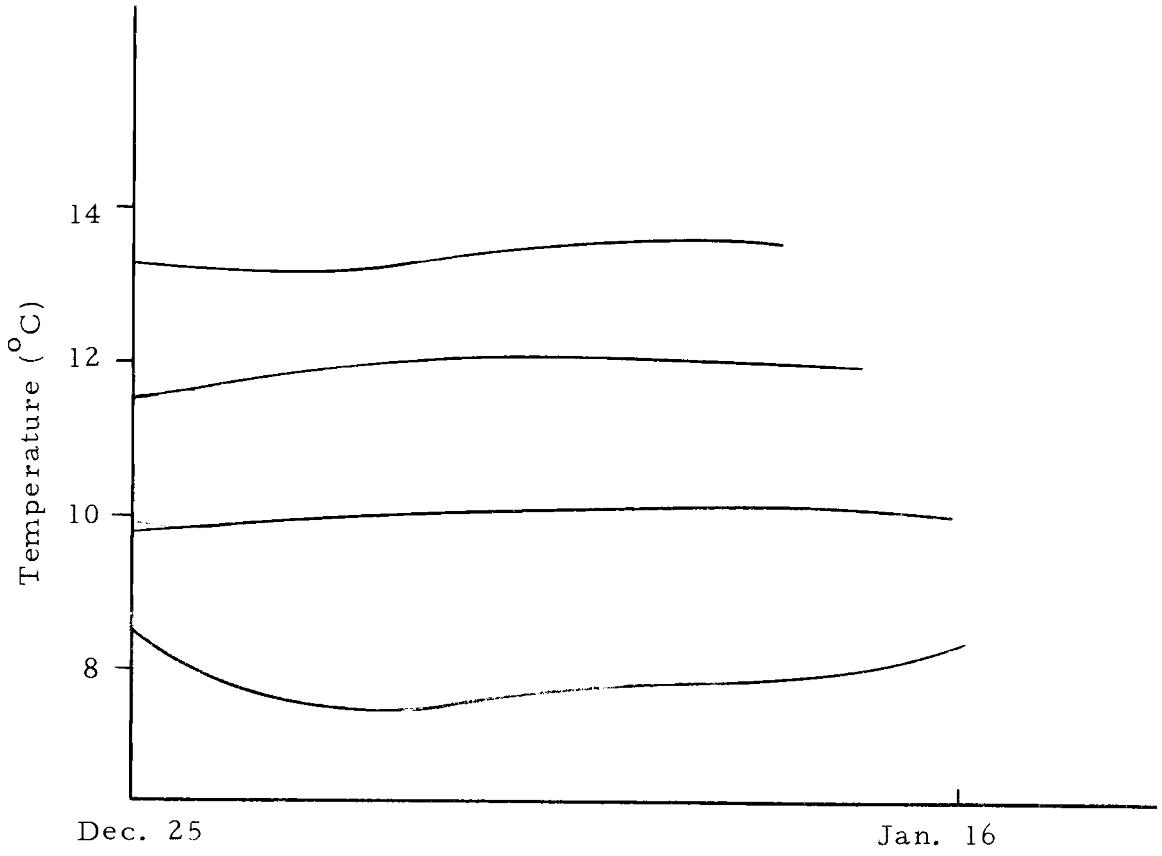

Figure 16. Temperature ranges for experiments with coho salmon alevins.

Table 20. Effect of gravel and screen substrate on incidence of yolk-sac malformation in coho salmon alevins.

Substrate	No. of test cells	Total no. of alevins	No. of alevins with malformation	% of Alevins with malformation
gravel	9	793	0	0
screen	20	4500	77	1.7

alevins held in the test cells, I transferred them to 100-gallon holding tanks, where the water temperature ranged between 9 and 10°C. Alevins from certain cells were combined to form four groups.

Group I consisted of all the alevins from cells 26 and 27. These two cells were characterized by having relatively high stocking densities (1012 and 524 alevins), screen substrate, and high temperature (14°C). They also had highest incidence of malformed yolks.

Group II alevins were from cell no. 6 which was characterized by high stocking density (1013 alevins), screen substrate, and a 10°C temperature.

Group III alevins were from the remaining 17 test cells with a screen substrate.

Group IV alevins were from nine test cells with a gravel substrate.

Observations were continued on the four groups of alevins for 14 days until they completed absorption of their yolk. Alevins in groups II, III, and IV appeared to develop normally, and no additional malformed yolks or mortalities were observed in any of these three groups. However, alevins in Group I showed substantial additional malformed yolks. Of the 1462 apparently normal alevins transferred to the holding tank from cell nos. 26 and 27 to form group I, 330 (22.6 percent) developed malformed yolks. All of these fish died. I suspect that these alevins already had damaged yolks which I failed to detect prior to their being transferred. There was no way to determine if these later malformations were predominantly from cell 26 or 27.

Form of Malformation

The malformation was less obvious from inspection in coho salmon alevins than in any of the other species of hybrids. The most common form of the malformation is shown in Figure 17.

Observations on Behavior of Alevins

Coho salmon alevins exhibited little activity, even when disturbed. Some alevins penetrated into the gravel substrate, but some remained exposed on the surface of the gravel.

Some alevins exhibited free swimming as early as 12 days after hatching at the warmest temperature. Early free swimming tended to parallel that observed with sockeye salmon alevins, but the percentage of the alevins exhibiting early free swimming was lower than for sockeye.

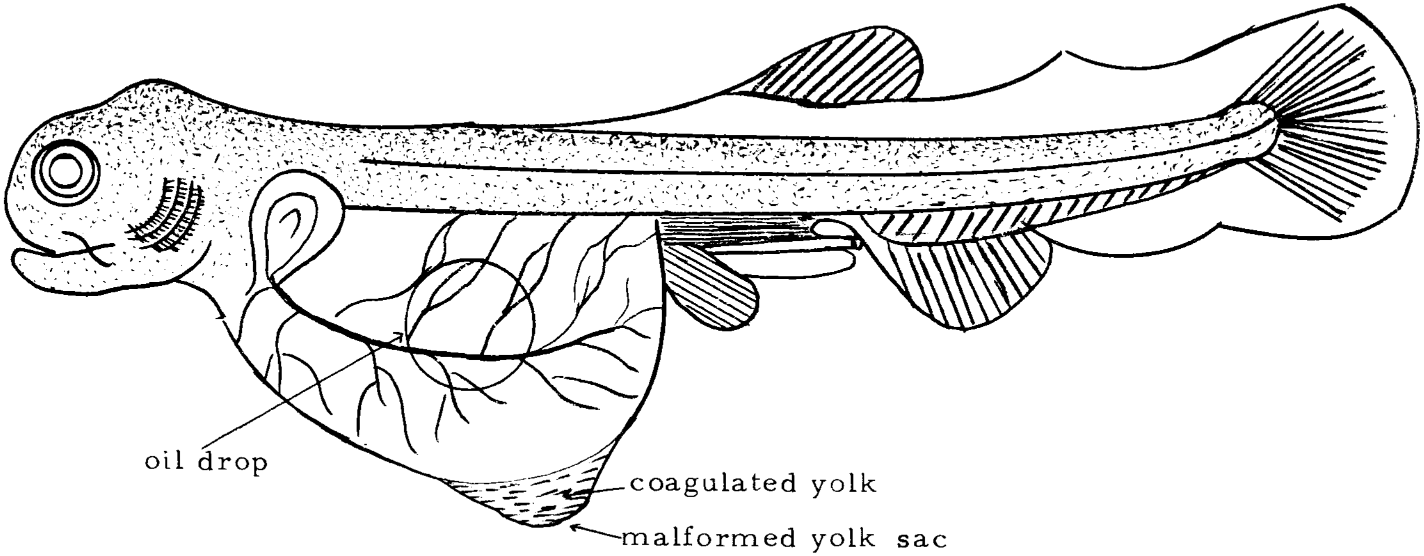

Figure 17. The most common form of yolk-sac malformation in coho salmon.

Yolk absorption progressed more rapidly for coho salmon than for the other species and hybrids.

CONCLUDING DISCUSSION

Pacific salmon have evolved a spawning behavior which requires, under natural conditions, that their eggs be buried in a gravel substrate. The behavioral response of alevins to their substrate has evolved over many generations. Hatcheries have significantly altered the natural environment by substituting an artificial flat surface (screen or tank) for a bed of gravel. The effect of this artificial substrate on survival of hatchery salmon is an important question requiring further investigation.

Recent studies have focused on the effect of the hatchery environment on efficiency of conversion of yolk to body tissue (Brannon, 1965; Bams, 1967; Poon, 1970). The general conclusion to be derived from these studies is that salmon alevins are more active in the hatchery environment than in the natural environment. This heightened activity results in increased metabolic cost for maintenance and decreased conversion of yolk to body tissue, i. e., growth is reduced to the fry stage and hatchery fry are smaller than wild fry.

Another problem associated with the hatchery environment is the possible development of yolk-sac malformation in alevins. The literature on this subject is sparse, but the problem has been identified by Mr. Poon of OSU (Personal communication) as being of potential significance to hatchery propagation of pink and chum salmon.

The incidence of yolk-sac malformation appears to be related to the activity of alevins. The more active alevins are more susceptible to the malformation than the less active alevins.

Description of Yolk-sac Malformation

Yolk-sac malformation resulted from the frequent rubbing of the yolk-sac on the substrate. In most cases this will result in an abrasion on the yolk membrane, particularly in the posterior part of the yolk-sac. The abrasion will increase the permeability of the yolk membrane in the injured areas and allow water to penetrate into the yolk (in some cases yolk will be released from the yolk-sac). Yolk coagulation and scar tissue are the primary visual symptoms of the malformation. If abrasion has occurred in the posterior part of the yolk (the most common condition), the coagulated yolk gives a triangular appearance to the posterior part of the yolk sac. If an abrasion occurs on the side of the yolk, one or two points of constriction will separate a part of yolk in the posterior part of the yolk sac. In all different forms of yolk-sac malformation, the coagulation and constriction will prevent the normal circulation of blood in the affected areas as well as areas of yolk adjacent to the injured portion. Such injury can result in mortality of alevins in extreme cases.

Since coagulated blood was found around the coagulated yolk sac in some instances it was thought that the malformation might result from blood discharged from broken capillaries into the yolk. This idea was rejected after injecting blood into the yolk of a group of Chinook salmon alevins and comparing them to another group that had superficial injuries of the yolk membrane. No malformation or coagulation was noticed in those injected with blood, and the injected blood could be seen in the yolk a few days after injection. Typical coagulation and malformation was observed in the other group, however, which had their yolk membrane injured.

Posterior displacement of the oil drop, which also is caused by the activity of alevins, was found to be associated with yolk-sac malformation in some instances, especially in chum salmon. Displacement of the oil drop may result in the breakdown in the structure of the yolk, as well as a rupturing of capillaries. It is accompanied by the collection of a fluid in the posterior part of the yolk sac behind the oil drop.

Differences Among Species and Hybrids

Chum salmon appeared to be the most active alevins among the species and hybrids that I tested. Chinook salmon alevins appeared to be the least active. Pink and sockeye salmon alevins were also very active. Coho and hybrid salmon alevins (Chinook X pink) did not exhibit high levels of activity. The salmon species and hybrids generally ranked as follows with respect to incidence of yolk-sac malformation in my tests:

Chum > Pink > Sockeye > Hybrids > Coho > Chinook

My experiments involved wild stocks of pink and chum salmon; hatchery stocks of sockeye, Chinook, and coho salmon; and crossbred wild and hatchery stocks of hybrid salmon. I did not test for possible differences in sensitivity of different races within a species to yolk-sac malformation.

However, it is possible that a wild race may be more susceptible to yolk-sac malformation than a hatchery race. Selection for alevins which exhibit little activity under hatchery conditions may create a race of hatchery salmon adapted to the artificial hatchery environment. Such selection could arise if a high percentage of alevins with yolk-sac malformation die; whereas, those which do not exhibit yolk-sac malformation will have an improved chance to survive. If activity is to some degree a heritable trait, the new hatchery race may be less sensitive to the hatchery environment and less susceptible to the occurrence of yolk-sac malformation than the ancestral stock.

Another genetic adaptation which may reduce yolk-sac malformation in hatchery races is early swimming activity as observed in sockeye and coho salmon alevins in my experiments. Such early swimming would reduce contact with the artificial substrate of the hatchery and perhaps reduce the chance of injury to the yolk. Contact between the yolk sac of active alevins and screen substrate is the major factor contributing to injury of the yolk and occurrence of yolk-sac malformation.

My experiments on Chinook salmon revealed a very low incidence of malformed yolks, but this result was different from that reported by Jochimsen and Bedell (1968) who studied yolk-sac malformation in Chinook salmon held in barrel-type incubators. The barrel-type incubator keeps alevins in constant motion from upwelling water. Thus, the upwelling action of incoming water may have contributed to the high incidence of malformed yolks reported for Chinook salmon alevins by Jochimsen and Bedell. Although Smith (1916) found that Chinook salmon alevins held in light exhibit a restlessness and activity not shown by those kept in darkness, no pronounced activity was observed in Chinook salmon alevins used in my experiments, even where lids were removed from the test cells. According to Richard Bole of the Oregon Fish Commission Trask River Hatchery (personal communication), both spring and fall Chinook salmon alevins do not show much activity before the free-swimming stage under hatchery conditions.

The observed activity of pink X Chinook hybrid alevins leads me to conclude that the Chinook salmon parental stock had a dominant influence, regardless of the sex of the parents. The percentage of yolk-sac malformation was 2.6 and 3.1 in the two hybrid crosses, while it was 23.4 in the pink and only 0.2 in Chinook salmon alevins.

Early mortality up to yolk absorption varied considerably among groups of alevins exhibiting yolk-sac malformation. Table 21 summarizes mortalities for the various species and hybrids.

Table 21. Mortality rate of alevins with yolk-sac malformation in different species up to button-off stage.

Species	% Mortality within alevins with yolk-sac malformation
Chum	40.6
Chinook	0
Pink	31.7
Pink X Chinook	0
Chinook X Pink	100
Sockeye	54.6
Coho	100

Role of Substrate

Presence of a gravel substrate prevented the occurrence of malformed yolks under most of my test conditions. In my experiments, the overall incidence of yolk-sac malformation among alevins of all species and hybrids under all test conditions was only 0.7 percent on the gravel substrate and 10.7 percent on the screen substrate. The difference was even more pronounced for the more active chum, pink and sockeye salmon alevins. Contact with gravel prevents alevins from becoming active even when subjected to other environmental factors such as temperature and water velocity which can alter activity and affect their vulnerability to injury and malformed yolks.

Alevins on a screen substrate not only lack shelter, but the screen can be harmful to species of salmon with active alevins. The screen can cause abrasions on both the anterior and posterior parts of the body. Such abrasions can result in fungus infection and yolk-sac malformation in alevins.

Modifying Effect of Other Environmental Factors

Water temperature and velocity have a modifying influence on the occurrence of yolk-sac malformation. Light (under my test conditions) was not an important factor. However, there is other evidence that light plays an important role. As it has been mentioned before, light was not completely eliminated from my test cells by lids placed over the cells. Some light penetrated through the small holes used as entry ports for water. I can only conclude that different intensities of light in my tests exerted similar influences on the activity of alevins and the incidence of yolk-sac malformation. Marr (1965) has described the importance of light as a factor which increases the locomotor activity of salmon alevins. The premature swimming of chum salmon, in hatcheries, has also been ascribed to light (Kolgaev, 1963).

The percentage of malformed yolks varied with temperature. The incidence of yolk-sac malformation increased in sockeye salmon and salmon hybrids with the increased temperature. Although the incidence of malformed yolks was low for coho salmon alevins, there was some evidence of a temperature effect. Coho salmon alevins held on a screen substrate at relatively high density in 10°C water did not show any evidence of yolk-sac malformation, while those held at 14°C experienced 2.1 and 10.1 percent yolk-sac malformation.

Although activity of salmon alevins would be expected to increase with increased temperature, the incidence of yolk-sac malformation in chum and pink salmon alevins was lower at the medium than either the low or high temperature ranges. If increased temperature increases the activity of alevins, and if yolk-sac malformation is related to the activity, one wonders how the higher incidence of malformation could occur at the low temperature range? In the case of chum salmon, there was poor control over temperature, especially during the early stages of the experiment. However, this was not the case for pink salmon. The problem warrants further study.

The incidence of yolk-sac malformation was affected by water velocity as well as temperature in several experimental groups. For pink and chum salmon, the incidence of malformed yolks showed no consistent relationship with increasing or decreasing water velocity. However, there was a clear relationship between increased incidence of malformed yolks and increased water velocity for sockeye salmon alevins.

The velocity and turbulence of water potentially can stimulate activity in salmon alevins. Water turbulence may vary in different regions of a test cell and cause the alevins to gather in the areas of low turbulence. I would expect the higher velocities to stimulate activity of alevins more than the lower velocities.

My test velocities of 25, 100, 200, and 400 cm/hr were less than velocities normally found in standard hatchery incubators. The concentration of dissolved oxygen in my experimental cells, regardless of the water velocity, was always above 6 mg/l. Therefore, activity was not attributed to oxygen privation under my test conditions.

SUMMARY

1. Yolk-sac malformation was found to be caused by frequent rubbing of the yolk sac on the substrate.
2. The incidence of yolk-sac malformation varies in different species of Pacific salmon, and has direct relationship with the activity of alevins. Chum, pink, and sockeye salmon alevins appear to be highly susceptible to yolk-sac malformation. The susceptibility of Chinook and coho salmon alevins is much reduced over the other species.
3. Gravel substrate will alleviate or prevent the occurrence of yolk-sac malformation, regardless of other environmental stresses.
4. Water temperature and water velocity have a modifying effect on incidence of yolk-sac malformation where alevins are raised on a screen substrate.
5. Although light had no effect on incidence of yolk-sac malformation under my test conditions, other studies suggest that absolute darkness will reduce the incidence of malformation.

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