Delayed Photoperiod Induces Increased Seapen Survival, Increased Growth Rate and Increased Uniformity of Size of Coho Salmon (*Oncorhynchus kisutch*) Transferred Into Seawater at Age 0+, Under Intensive Culture Conditions

by

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ABSTRACT

The effect of delayed photoperiod on coho salmon fry first fed as late as March and April and transferred to seapens in their first summer, was measured by survival and growth rates. In 1987, three lots of fry, progeny of wild broodstock, were first fed as follows: lots 1 and 2 from Robertson Creek (49°20'N, 124°59'W) on 3 and 17 March respectively, and lot 3 from Kitimat (54°7'N, 128°38'W) on 12 April. For each lot, 2 batches of 200 were held in 200L barrels at 48°56'N, 124°59'W under 3 photoperiod regimes: 1) natural daylength (control); 2) 9.5h constant light for the first 60 days and then natural photoperiod; and 3) photoperiod cycle delayed to start at the winter solstice.

In August, 50 liquid nitrogen branded fish from each of the 18 batches were transferred to one of two 6x6x6m seapens. After 6 months, both photoperiod manipulations increased seawater survival, final mean weights, and uniformity of weight, for all lots. Seawater survival of 9.5h constant (64%) and delayed groups (69%) were significantly different ($p<0.001$) from controls (21%). Mean roundweights (±1SD) of 9.5h constant (401±194g) and delayed (412±135g) groups differed significantly ($p<0.0001$) from controls (74±129g).

Hence, when egg-take times and incubation temperatures result in first feeding as late as March or April (previous studies have used February or earlier), photoperiod manipulation can be used to increase survival and growth rates of coho transferred to sea in their first summer. The total final biomasses achieved were 4.3 (control), 73.4 (9.5h) and 83.6kg (delayed). Simple farm based photoperiod manipulation offers the salmon aquaculture industry the possibility of early transfer of young coho salmon to seawater grow out pens together with improved growth and survival in comparison to the normal life history and the resultant economic benefits of a condensed production cycle [redacted]

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INTRODUCTION

Most coho salmon (Oncorhynchus kisutch) in British Columbia spawn in October and November, with the eggs hatching early the next spring. The exact timing of hatching is strongly dependent on the spawning time and incubation temperatures. After the eggs hatch, the alevins remain in the gravel until most of the yolk sac is consumed usually in March or April, at which time they swim up out of the gravel and remain in freshwater streams or lakes usually for one year (Scott and Crossman 1979; Carl et al. 1977). In the spring of their second year, coho salmon, typically about 20-50g, undergo a coordinated set of changes, smoltification, that enables them to migrate downstream into seawater without experiencing high mortalities from the hypertonic medium in which they will live for about 18+ months before returning to their natal stream to spawn and die (Scott and Crossman 1979).

Smoltification is the coordinated physiological and behavioral adaptations of a juvenile salmonid that allows for normal growth and development in the ocean. Visual changes associated with smoltification include silvering of the body and blackening of fin margins along with an overall streamlining of body form. Physiological changes include 1) an increase in hypoosmoregulatory ability (decrease in plasma and tissue chloride, decrease in glomerular filtration rate, dramatic increase in gill Na+, K+ -ATPase activity and increased salinity preference and tolerance)(Hoar 1988; Wedemeyer et al. 1980), 2) an increase in growth rate (Vanstone and Markert 1968), 3) changes to lipid and carbohydrate stores (reduction in total body lipids, qualitative change of lipids to fats that are more highly unsaturated, reduced liver glycogen and elevated blood glucose)(Hoar 1988; Sweeting et al. 1985), and 4) changes in the endocrine system including activation of thyroid, interrenal, and growth hormone cells (Folmar and Dickhoff 1980; Sweeting et al. 1987; Wedemeyer et al. 1980). Behavioral changes include a decrease in territoriality, an increase in schooling and out-migration to the ocean (Hoar 1976).

For the profitable culture of coho salmon, it is advantageous to manipulate their life history with two prime objectives of 1) inducing early smoltification to permit early transfer into seawater and 2) maximizing survival in seawater and growing fish quickly to a marketable size. These objectives can be accomplished if coho can be induced to smoltify in time for transfer to seawater during their first summer. Also, there are several problems associated in keeping salmon parr in freshwater for more than one year. These include: 1) the temperature of freshwater becoming too high in late summer which often leads to increased fish health problems; 2) production often limited by the amount of freshwater available for rearing in the late summer; 3) when fry require ponding the next year, year old salmon parr are still occupying rearing containers. Thus, there is a strong interest in the intensive salmon farming industry of B.C. in being able to induce the parr-smolt transformation during the first summer and thus being able to transfer coho into seawater as 0+ coho salmon.

Induction of smoltification in the first summer can be accomplished by induction of early hatching of eggs, by induction of early spawning (Zaugg et al. 1986) or by increasing incubation temperature, or by inducing rapid smoltification of juveniles. Three methods used to induce rapid smoltification during the first summer are to increase the temperature of the rearing water, hormonal methods, and photoperiod manipulations. Temperature increase is limited by an undesirable increase in size variability (Clarke and Shelbourn 1986, Clarke 1989, Clarke and Shelbourn 1989), fish health problems and a decrease in seawater adaptability above certain optimal temperature parameters. Also heating rearing water is limited by the expense and technical difficulties inherent in heating the millions of liters of flow through water used each day by many coho culture facilities. While hormonal treatments show promise to increase early smoltification and increase growth rates, the resulting product may be hard to sell to health conscious consumers.

Under laboratory conditions it has been shown that photoperiod manipulations can be used to increase the seawater adaptability and early seawater growth of coho in their first year (Clarke et al. 1989; Thorarensen and Clarke 1989; Clarke and Shelbourn 1986). Coho reared under delayed photoperiod (delayed increase in daylengths) when compared to those reared under natural photoperiod conditions demonstrated (Brauer 1982; Clarke and Shelbourn 1986): 1) more uniform growth (decreased coefficients of variation for weight); 2) greater seawater adaptability; 3) earlier seawater adaptability; 4) greater food conversion efficiency; 5) seawater growth rate five times faster.

Clarke and Shelbourn (1986) achieved these results with a photoperiod phase delay of 1 or 2 months used on swim-up fry that emerged around February 1st from eggs that were incubated in ground water with a reasonably uniform temperature of 10.5°C. Clarke and Shelbourn (1986) found that groups of coho exposed to a 1 month delayed photoperiod had greater and earlier seawater adaptability and more uniform growth than fish exposed to natural photoperiod. Although fish exposed to a one month phase shift had greater (significantly different) mean weight by August 15 of their first year and greater (significantly different) mean growth rate (% * day^{-1} measured between February 28 and August 15) than fish exposed to natural photoperiod, fish exposed to a 2 month delayed photoperiod did not (Clarke and Shelbourn 1986). This allowed for the possibility that a 1 month delay was optimal and that 2 month or greater phase delays would not increase freshwater growth rates. As many salmon culture facilities use surface water from lakes and streams with temperatures ranging between 1° and 7°C, with these lower temperatures swim-up fry do not emerge until March or April, 3-4 months after the winter solstice. Thus, the effects of phase delays greater than 1 month are of interest to the aquaculture industry of B.C.

The objectives of the present study are to investigate the following, under conditions found in the aquaculture industry of B.C.:

1. Can coho salmon that emerge as swim up fry as late as March or April (3-4 months after the winter solstice) be induced to smoltify in their first summer using delayed photoperiod manipulations?
   - A. Is it possible using a 3-4 month phase shifted photoperiods on fish ponded in March and April to induce smoltification during the first summer?
   - B. Is it possible using a 9.5 hour constant photoperiod for sixty days and then natural photoperiod on fish ponded in March and April to induce smoltification during the first summer?
   - C. To compare a 9.5 hr constant photoperiod for sixty days starting at first feeding with a phase delayed photoperiod.
2. Will the two experimental photoperiods increase uniformity of size?
   - A. By time of transfer into seawater?
   - B. After at least 4 months in seawater?
3. Will the two experimental photoperiods increase seawater survival rates?
4. Will the two experimental photoperiods influence an increase in mean whole body wet weight?
   - A. By time of transfer into seawater?
   - B. After at least 4 months in seawater?

MATERIALS AND METHODS

SITE LOCATION

To obtain rearing conditions and fish husbandry practices as close as possible to intensive culture conditions, the present study was carried out at an intensive culture facility. The San Mateo Bay intensive salmon culturing facility is located in San Mateo Bay (48°56'N, 124°59'W), a protected inlet of Alberni Inlet on the West coast of Vancouver Island, British Columbia. The facility has been operational since the mid-nineteen seventies. It was operated primarily as a family operation up until the mid-nineteen eighties when it was purchased by International Aquaculture Capital Corporation. The hatchery facility, capable of incubating at least 2 million salmon eggs, had just been completed in the fall of 1986. Thus, the eggs used in the present experiment were the first set of eggs incubated in the new hatchery facility. During the spring of 1987, fresh water rearing facilities were being constructed for the first year of the expanded production operation. Over 1.2 million smolts (coho and chinook, Oncorhynchus tshawytscha) were produced in 1987.

The shore line rises steeply from San Mateo Bay for about six hundred feet to a lake which has been dammed to increase its water storage capacity. The San Mateo facility is the sole user of water from the lake. During peak use, the salmon rearing facility uses approximately 20 million liters of water per day. No salmonids are known to reside in the lake and the water is thought to be free of salmonid infectious diseases. Water is drawn out of the lake via two outlet pipes. By varying the depth of the inlet pipes and mixing of water from the two pipes some control of water temperature to the hatchery and fresh water rearing facilities is afforded.

The site had traditionally been a seapen grow-out site for at least 10 years. However, due to high incidence of Bacterial Kidney Disease (B.K.D.) the seapen grow out function was being phased out in preference to seapen rearing in other locations with better flushing characteristics. A small intermittent creek which flows into San Mateo Bay has a freshwater influence during parts of the year on the seawater netpens located in the bay.

Eggs were incubated in freshwater and fish reared in freshwater and seawater at the San Mateo Bay Intensive Salmon Culture Facility.

EXPERIMENTAL ANIMALS

Swimup coho salmon fry (Oncorhynchus kisutch) were obtained from two allotments of eggs originally collected from Robertson Creek wild broodstock at the Robertson Creek Hatchery (49°20'N, 124°59'W) on November 12 and November 26, 1986 and one allotment of coho eggs collected from Kitimat River wild broodstock at the Kitimat Hatchery (54°7'N, 128°38'W) approximately one month later. All eggs were incubated in water from the lake as part of the salmon farm production operation. All eggs were incubated under similar conditions although water temperature varied seasonally (Figure 1).

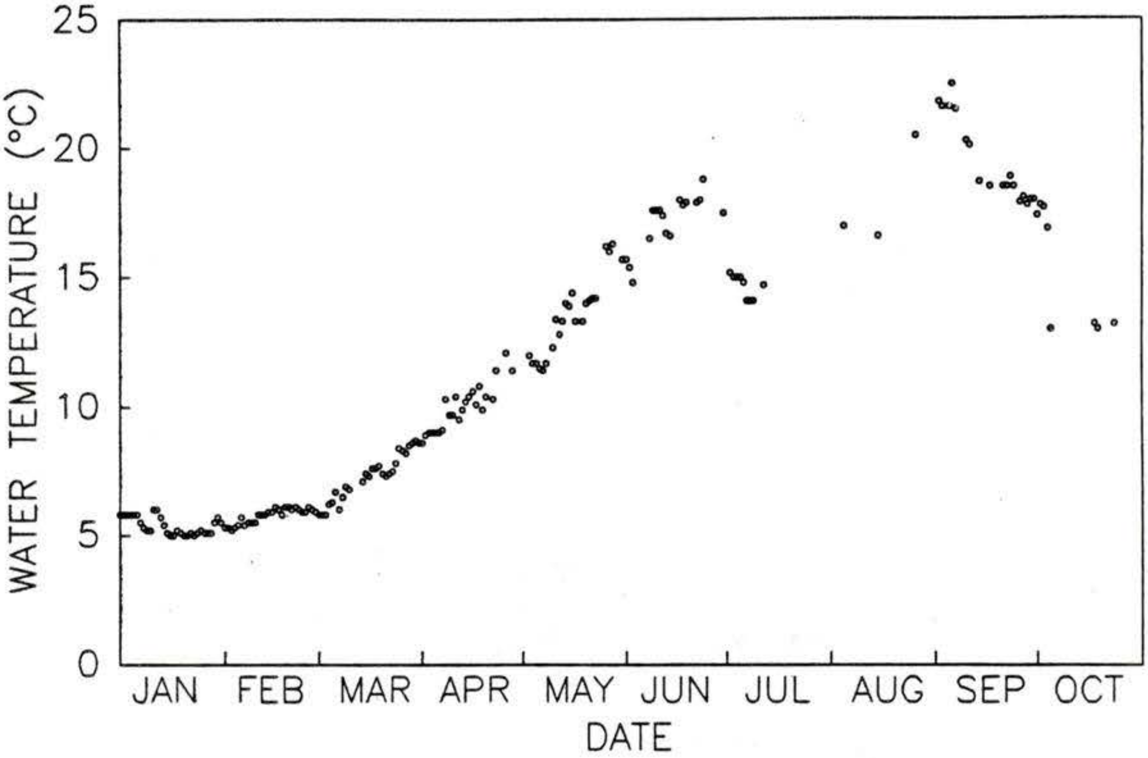


Figure 1: Daily average temperatures of water supplying incubation trays and freshwater rearing barrels during 1987. Intake moved to deeper and cooler water on June 30 and October 5. Maximum daily fluctuation about 2°C.

FRESHWATER REARING

PONDING DATE LOTS

In 1987, three lots of fry were ponded from incubation trays into freshwater rearing barrels and first fed as follows: Robertson Creek early (R1) and Robertson Creek late (R2) on the 3 and 17 March respectively, and Kitimat (K) on 12 April, 1987. To enable investigation of the usefulness of photoperiod manipulation as a technique to induce early smoltification over a range of ponding dates, ponding dates were chosen to give the largest spread of ponding dates possible from the fish available at the San Mateo Bay Hatchery.

To decrease the chance of any subsequent differences that might be observed between fish of different ponding dates being attributable mainly to specific parental crosses, progeny from a variety of parental crosses within a common stock were used for each of the three ponding dates. The early and late ponded Robertson fry are expected to be genetically similar to each other. However, it has been found under certain conditions that progeny of Kitimat River coho exhibit faster seawater growth rates than progeny of coho spawning further South in B.C. (Pers. comm. Dr. Ruth Withler, Pacific Biological Station, Nanaimo, B.C.). Thus, the progeny of Kitimat River coho are expected to be less genetically similar to the early and late Robertson Creek progeny than the early and late Robertson Creek progeny are to each other. Thus it is expected that genetic differences may confound some potential ponding date effects of this study.

Swimup fry were ponded when yolk was no longer visible in the yolk sacs of the majority of fish in an incubation tray. For each ponding date lots, the swimup coho fry were randomly allotted to each of 6 barrels (3 photoperiod treatments each with 2 batches): water was drawn down in the bucket and fish quickly scooped, counted and placed into barrels. Fish were weighed by adding all 200 fish from each barrel into a tared quantity of water. To minimize stress on fish at such an early life stage, transport and allocation to barrels was done as quickly as possible. Aeration stones were used to facilitate gas exchange in the water while the fish were counted into the barrels.

PHOTOPERIOD MANIPULATION

Egg and swim-up fry while in incubation trays were covered with black plastic to exclude light, and were exposed to low incandescent lighting only while attended.

For each of the three ponding date lots, starting at the time of ponding, 2 batches of 200 fish were exposed to the three following photoperiods: 1) natural daylength (control); 2) 9.5h constant light for the first 60 days and then natural photoperiod (Figure 2); and 3) photoperiod cycle delayed to start at the daylength of winter solstice (Figure 3). The extent of photoperiod delay thus equals the number of days after December 22 that the fish were ponded. Ponding dates of 3 and 17 March and 12 April correspond to 72-, 86- and 112-day delays, respectively.

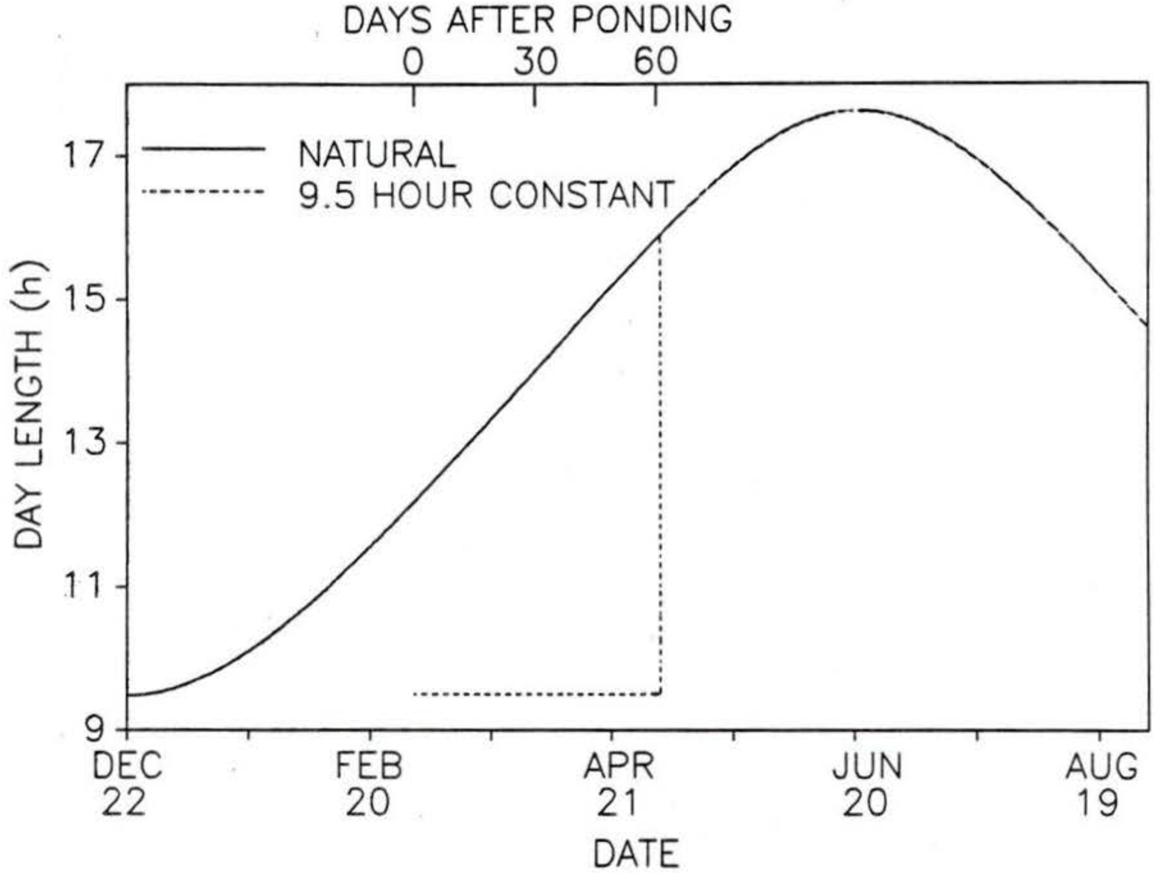


Figure 2: Daylengths of 1) natural photoperiod and 2) 9.5 hour constant for first 60 days and then natural photoperiod for fish ponded on March 3.

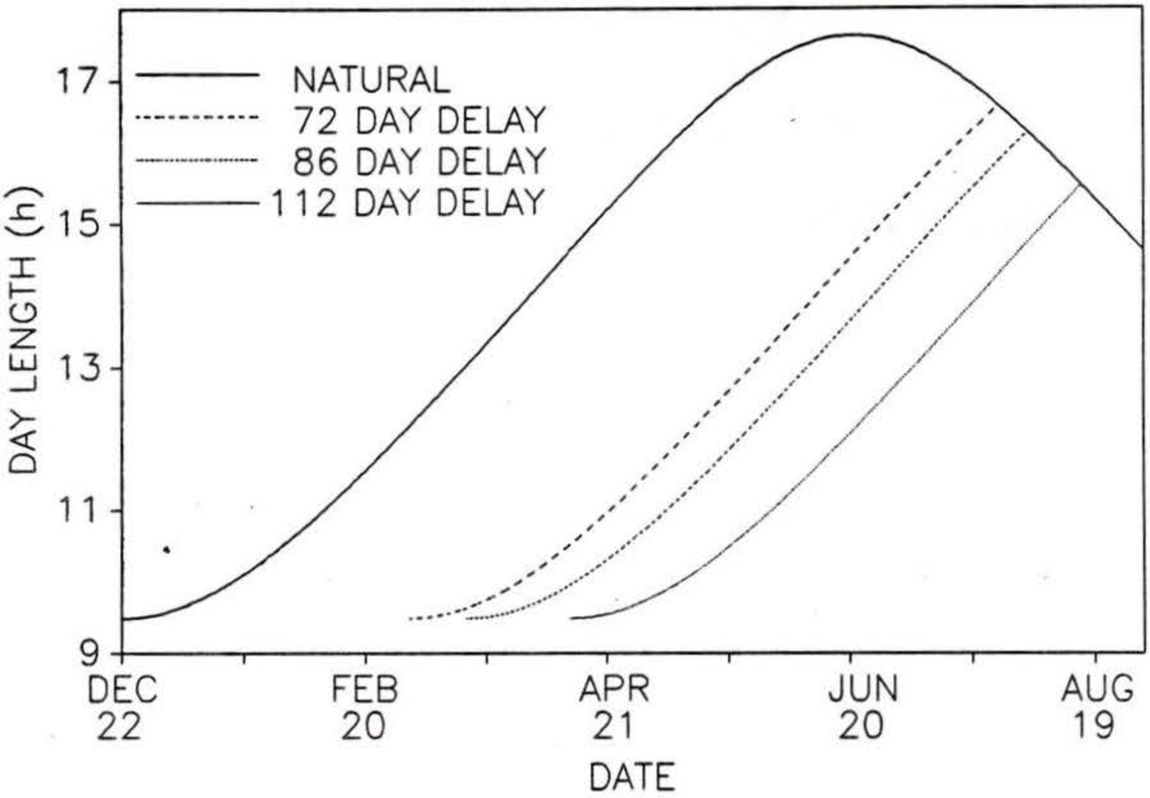


Figure 3: Seasonal daylength cycles for normal, 72, 86, and 112 day delayed photoperiod phase shifts which correspond to ponding dates of 3 and 17 March and 12 April, respectively.

The barrels containing coho subjected to natural photoperiod treatment were left uncovered and the fish were exposed to the ambient light cycles at San Mateo Bay. Production staff often kept a flood light on all night which illuminated the experimental barrels sufficiently for people to work at night in an emergency.

The barrels containing coho subjected to the 9.5 hour constant photoperiod treatment, had covers taken off in the morning at 07:00 PST (Pacific Standard Time) and replaced 9.5 hours later. After sixty days the covers were left off, except for Kitimat barrels which had covers left off after 69 days.

The barrels containing coho subjected to the phase delayed photoperiod treatment, had the start of morning civil twilight (centre of sun's disk is 6° below the horizon) used as the beginning of daylength. The covers were then replaced in the evening after the required daylength. The covers were subsequently taken off after evening nautical twilight (centre of sun's disk is 12° below the horizon) and before morning nautical twilight, so that daylength could begin the next day with the start of the ambient civil twilight. The time the covers were removed during the night was randomized to prevent this being perceived by the fish as a regular chronological event to which they might have cued. The daylength (number of hours of daylight plus the number of hours of civil twilight summed for morning and evening) required for each day of the delayed phase shifted photoperiods, was calculated from sunrise-sunset tables calculated for the coordinates of San Mateo Bay by the Dominion Astrophysical Observatory.

Figure 3 shows that after a certain date in July each of the delayed photoperiods would have called for a longer daylength than the ambient (natural) photoperiod. Instead, once the daylength of the simulated photoperiods equalled the ambient photoperiod, the covers were left off the barrels and thus the simulated photoperiods declined with the natural photoperiod.

EXPERIMENTAL DESIGN AND PHYSICAL LAYOUT OF TREATMENTS

Treatments consisting of combinations of three ponding dates and three photoperiods were arranged in a factorial design with two batches within each of the nine combinations. Spatial arrangement of the treatments was a matter of introducing randomness within certain constraints. The single row of barrels in the background

Figure 4: Photograph of barrel layout.

PHOTOPERIOD TREATMENTS					
NATURAL		9.5h CONSTANT		DELAYED	
K-B	K-A	K-B	K-A	K-B	K-A
R2-B	R1-A	R2-B	R1-A	R1-B	R1-A
R1-B	R2-A	R1-B	R2-A	R2-B	R2-A

Figure 5: Diagram of spatial distribution of treatments. Symbols for ponding groups are: R1 = Robertson 3 March; R2 = Robertson 17 March; K = Kitimat 12 April. Letter following hyphen indicates batches A and B.

of Figures 4 & 5 was not constructed when the Robertson Creek progeny required ponding. For convenience, each of the three photoperiod treatments was assigned to two adjacent columns of barrels (Figures 4 & 5). For each barrel of the bottom row, a designation of Robertson early or Robertson late was determined from a random numbers table. The second row was then matched to the bottom row with early next to late within all six columns (Figure 5). After the Robertson fish were ponded, the third row (top row in Figures 4 & 5) of barrels was constructed into which Kitimat River progeny were ponded on April 12. For each pair of barrels with the same ponding date and photoperiod combination, barrels to the right of Figures 4 and 5 were designated as position A and ones to the left as position B. This non-random position designation allowed for analysis to be performed to determine if a gradient existed along the rows of barrels that might have affected the results. If a particular variable is found to be either greater or lesser at position A than at position B, this could lead to a suspicion that a gradient existed along the rows of barrels. Although no gradient was expected to be found, it was felt prudent to check for one given the layout of photoperiod treatments.

To decrease the chance of differences observed being attributable to the effects of particular barrels, the locations of the individual barrels were rotated: fish were removed from one barrel and placed into a second barrel which was then put back into the place the former barrel had occupied. Rotation of barrels with batches of fish maintaining their spatial distribution was done at least ten times.

BARRELS

The barrels had previously contained fruit juice concentrate. Their tops were cut off and the barrels were thoroughly cleaned with Ovadine, a hatchery disinfectant. The sides of all barrels were covered in black plastic to prevent light passing through the sides of barrels. Thick black plastic covers were fashioned to exclude light from barrels during the simulated nights. These covers were tied around the barrel and had short sections of water pipe fastened into the tops of them using neoprene seals to allow water to enter while excluding light. Removable screens were placed over the top of each barrel to keep predators out.

WATER FLOWS AND QUALITY

Water was initially supplied by two water pipes to each barrel. The dual supply was precautionary in case one ceased to function. Both lines were taken from the main hatchery supply and had independent valves and pressure gauges. Thus the water pressure via both lines could be regulated and monitored. Vertical pipes supplying each pair of barrels (Figure 4) were equipped with a tap to regulate flows. Duality of the water supply was lost after approximately one month due to need of the plumbing fixtures by the production side of the San Mateo Bay operation. When the fish were first ponded, flows were set at 15 LPM (Litres per Minute) and gradually increased to 30 LPM by early August, 1987.

Oxygen readings were taken periodically with a YSI (Yellowstone Instruments) model 51-B temperature and oxygen meter. The lowest oxygen reading recorded at a barrel outflow was 8.0 $mg*L^{-1}$ on 11 September, 1987 when the water temperature was 20°C. pH readings were taken periodically in all barrels at the surface near the inflow, halfway down the barrel and in the outflow standpipe, with a Western Digital model PHD10-2PK pH/mV meter. Readings ranged from pH 6.0 to 6.5. Conductivity of water in the rearing barrels as determined with a YSI model 33 S-C-T (Salinity-Conductivity-Temperature) meter on 31 May 1987 ranged from 135 to 140 $\mu mhos*cm^{-1}$.

At the bottom of each barrel was an outlet screen that attached via a bulkhead fitting to a stand pipe which was exterior to the barrel and could be rotated to adjust water height. To prevent loss of fish from the barrels when the bottom screens became blocked, a small overflow screen was placed in the top edge of each barrel.

All eighteen barrels were cleaned daily. Fish were removed from the barrels on an approximately weekly basis during the warmer summer months and less frequently during the spring and fall when algal growth was slower, to allow the barrels to be thoroughly scrubbed and disinfected with Ovadine. Barrel rotation was done at the same time.

Two notable fish kills resulted from inadequate water quality. The first occurred on 19-20 May 1987 when the hatchery manager opened a valve to admit air into a collapsed water line thus supersaturating the water with air; within 24 hours fish were found dead in the experimental barrels, presumably from gas bubble disease. The air valve was then immediately closed. At least 172 experimental fish were killed with a even distribution of sizes. The second fish kill occurred on 13 September 1987 when the sole remaining water intake to one of the delayed Kitimat barrels was knocked off and remained undetected for an unknown period of time. Twenty-two delayed Kitimat died, presumably of hypoxia.

DISEASE AND VACCINATION

No disease outbreaks were noted during the freshwater rearing stage and no medication was administered to any of the experimental fish at any time during the course of either freshwater or seawater rearing. All fish were vaccinated against Vibriosis, a disease caused by bacteria of the genus Vibrio, prior to transferring fish to seawater netpens. All fish including those not transferred to seawater were vaccinated. Two treatments were administered, separated from each other, from anesthetization and from transfer to seawater, by approximately two weeks (Appendix 1). Robertson coho were vaccinated on May 31 and about two weeks later and Kitimat coho were vaccinated on July 18 and August 15, 1987. Fish were vaccinated by dipping for twenty seconds into a mixture containing 10 parts water to 1 part inactivated whole cell culture. The first dip was done with autogenous V. anguillarum (product of Biomed Research Laboratories, Seattle, Wash.) and the second using a mixture of autogenous V. ordalii and autogenous V. anguillarum (products of Microtek Research & Development Ltd., Sidney, B.C.).

FEEDING

Experimental fish were fed by hand in a similar manner to production fish. When first ponded, fish were fed Oregon Moist Pellets Starter Food approximately every ten minutes, often from 07:00h, PST or Pacific Daylight Savings Time, whichever was in effect at the time, until dusk.

Because fish were only fed when the barrels were uncovered, fish exposed to natural photoperiod fish received more hours of feeding than fish exposed to either of the manipulated photoperiods. As fish grew, pellets size was increased and dry pellets manufactured by White Crest were added to the diet according to the manufacturer's recommendations. A mixture of pellet sizes were fed to the experimental fish in excess of satiation. Ratê of feeding had decreased to approximately once every half hour by July, 1987.

DURATION OF FRESHWATER REARING

Freshwater rearing was terminated during the third week of October 1987.

SAMPLING OF FRESHWATER COHO

Sampling for whole body wet weight and fork length of a random sample of fifty fish per batch was performed 5 or 6 times for each batch during freshwater rearing (Table 1).

Table 1: Dates of freshwater sampling of coho.

PONDING GROUPS		
MARCH 3 EARLY ROBERTSON	MARCH 17 LATE ROBERTSON	APRIL 12 KITIMAT
Apr 23-24	Apr 23-24	
May 28-29	May 29	May 29-30
Jun 26-Jul 5	Jul 9-11	Jun 26
Jul 22-25	Jul 25-27	Aug 4-5
Sep 20	Sep 22	Sep 18
Oct 23-25	Oct 23-25	Oct 19-22

Fish were anesthetized using a 50 $mg \cdot l^{-1}$ solution of MS-222 (Ethyl m-aminobenzoate methanesulfonate) buffered with sodium bicarbonate, measured for forklength to the nearest mm, rolled gently in a paper towel to remove excess moisture and weighed to the nearest 0.1 g on an electronic balance. Balances used included a Yamoto LabTop-Ace 60B, a K-tron 600g balance and a Sartorius balance. All balances read within 0.05g of an Ohaus calibration weight of fifty grams. A silvering index based on visual inspection of the fish was then recorded on a scale of one to five:

1 = distinct bar-like parr marks that extend above and below lateral line

2 = parr marks not as deep and more oval than above but still cross lateral line

3 = parr marks just touch or do no cross lateral line

4 = parr marks almost invisible, edges of anal fin starting to darken, some silvering of sides

5 = no trace of parr marks; silvery sides; slender body; darkened edges of anal fin margin.

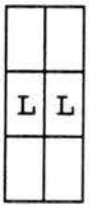
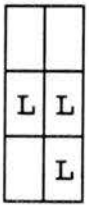
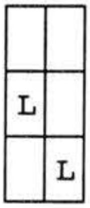
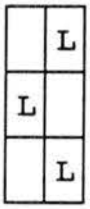
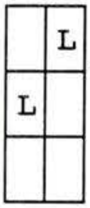
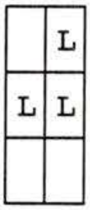
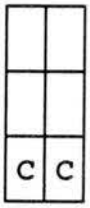
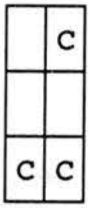
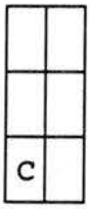
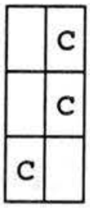
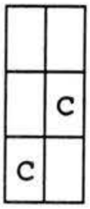
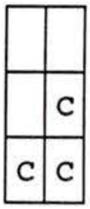
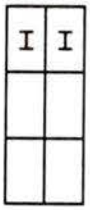
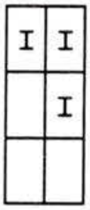
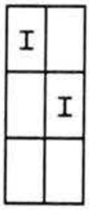
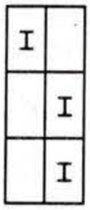
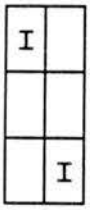
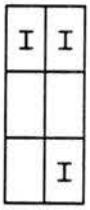
A silvering index of 1, indicated a fish exhibiting visual characteristics associated with being a parr, and 5 with being a smolt.

During sampling, coho were temporarily kept in small buckets which were aerated using airstones supplied with air from scuba tanks and/or a small electric air pump. Aeration was used at all times when fish were removed from the larger 200L barrels and kept in smaller containers. A Western Digital model PHD10-2PK pH/mV meter was used to monitor pH of anesthetizing water and sodium bicarbonate was added when required to maintain a pH between 6 and 7. Tru-touch plastic gloves were used when handling fish.

LIQUID NITROGEN FREEZE BRANDING

Prior to transfer to seawater, 55 fish from each batch were nitrogen freeze branded (Busack 1985; Mighell 1969). Three 12 pt. letter brands were borrowed from Ruth Withler (Pacific Biological Station, Nanaimo, B.C.). Since it was important to be able to distinguish coho from 18 separate batches by brands for at least 6 months, it was decided not to rely solely on a single letter brand but to use a combination of brand locations as follows. Three positions on each side above the lateral line of the fish were allotted for branding: caudal to the dorsal fin, under the dorsal fin and rostral to the dorsal fin. A throw of a dart with eyes closed, decided that batches A would receive two brands (one per side) while batches B would receive three brands (one on the left side and two on the right). As the position of the brand (i.e., close to gills versus close to tail) might affect fish health, it was decided that all fish of a given ponding date would have a left brand in the same location. This was done in this study because differences between photoperiod treatments are more important than differences between ponding dates. Location of the left and right brands was done using the pseudo-random numbers generator on a Hewlett Packard 11C calculator (Table 2).

Table 2: Location of brands on fish. Brands shown from dorsal view with head of fish toward top of page.

PONDING DATE	PHOTOPERIOD TREATMENTS					
	NATURAL		9.5H CONSTANT		DELAY	
	Batches A	B	Batches A	B	Batches A	B
MARCH 3						
MARCH 17						
MARCH 12						

Robertson coho were anesthetized, measured for length and weight and freeze branded on July 25 and 26, 1987. Kitimat coho were done on August 4 and 5, 1987. Fish were branded for 7 seconds at each location with care taken not to freeze the lateral line.

SEAWATER REARING

Fifty branded fish from each of the eighteen batches were transferred to seawater netpens during August, 1987 (Appendix 2). Robertson Lot 1 and Lot 2 and Kitimat on August 4, 15 and 28, respectively. Seawater temperatures were higher than optimal (Table 3). Previous studies have shown that 10 to 15 °C are preferable temperatures to transfer into, whereas poor growth and survival have been associated with transfer into 17 °C seawater (Harache et al. 1980).

Table 3: Freshwater rearing temperatures, and seawater rearing temperatures and salinities at time of transfer into seawater netpens. Temperature and salinity at surface (0.1m) and near bottom of netpens (4.0m) are shown. * = salinity meter inoperative.

PONDING DATE	DATE OF TRANSFER	FRESHWATER TEMP. (°C)	SEAWATER			
			TEMPERATURE (°C)		SALINITY (^{o}/oo)	
			0.1m	4.0m	0.1m	4.0m
MARCH 3	AUG 4	17.0	20.0	15.7	*	*
MARCH 17	AUG 15	16.6	17.5	16.5	27.3	*
APRIL 12	AUG 28	21.0	16.9	15.5	27.4	29.0

Batches A and B (Figure 5) went into seapens A and B, respectively. Netpens were located 1m apart and were each 4m x 4m x 4m, with the bottom of the net sagging down to 4.5m. A single predator net was set around both seapens. Feeding was initially done at approximately 8-10 times per day and gradually decreased to 2 times per day by November

1987. A variety of pellet sizes appropriate for the range in size of fish in the netpens were fed in excess of satiation at each meal. Temperature and salinity of the water in the netpens were measured daily at 0.1, 1.0, 2.0, 3.0 and 4.0m using a YSI Temperature-Conductivity-Salinity meter (Figures 6 and 7). Salinity as determined by the YSI meter was periodically checked against a Beckman Induction Salinometer model RS7-C standardized with Copenhagen standard seawater. Daily secchi disc depth readings were also taken as indicators of algal blooms that might adversely affect fish health. The shortest secchi disk depth of 2m was recorded on 11 September 1987 during an algae bloom. An oxygen profile taken at 1m intervals from the surface to 5 m with a YSI oxygen meter revealed oxygen readings in the range of 7.3 to 8.7 $mg*L^{-1}$. At 4m depth $7.3mg*L^{-1}$ of oxygen was recorded inside the netpen and $8.6mg*L^{-1}$ outside the netpen, indicating reasonable flow through the net at that time.

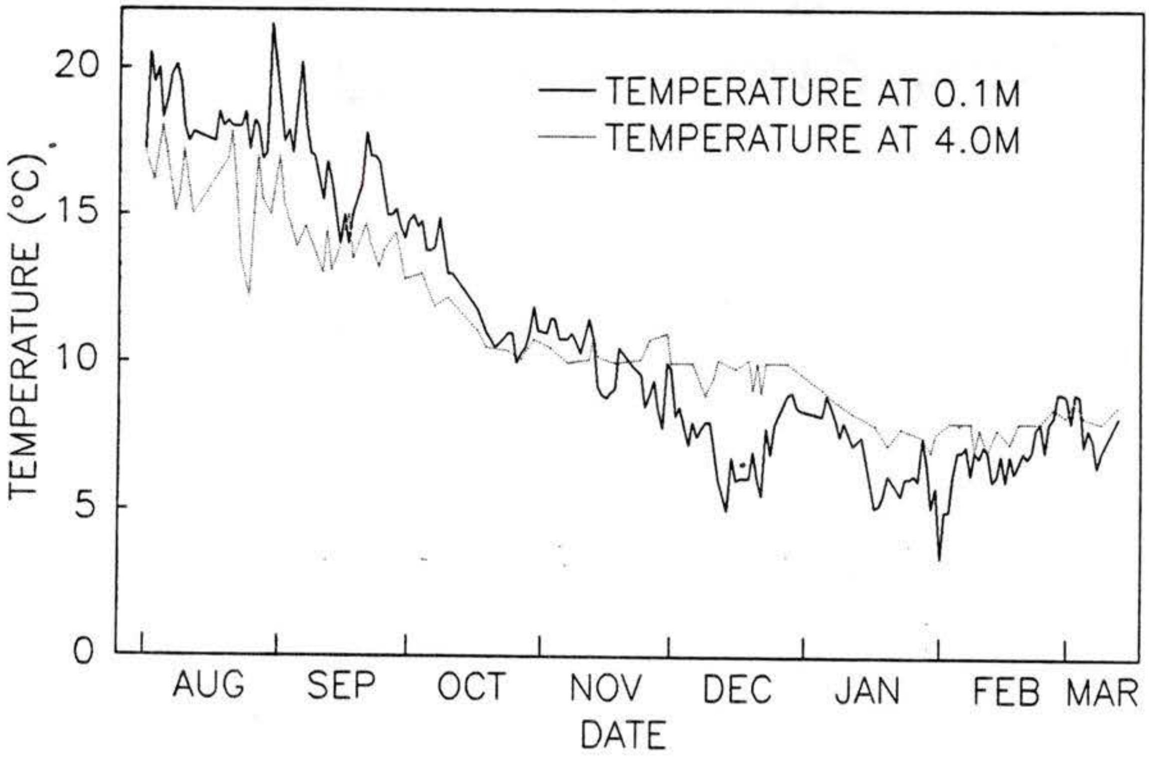


Figure 6: Temperature at surface and bottom of seapens.

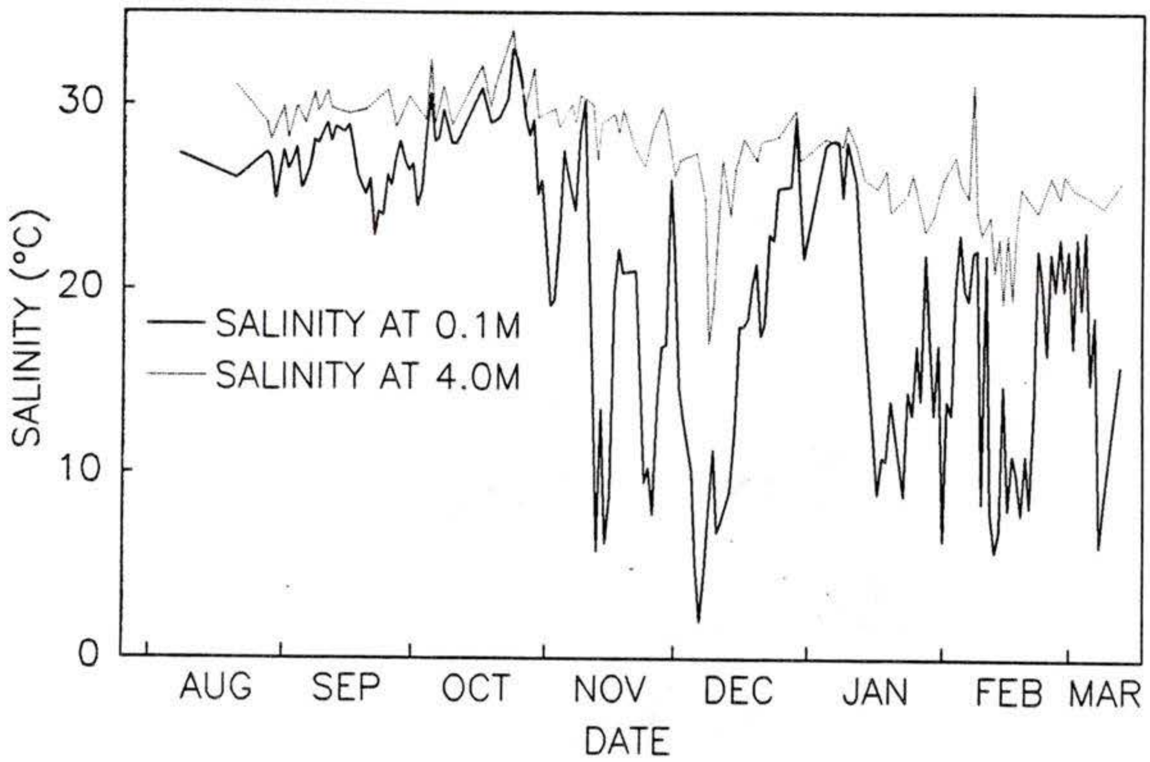


Figure 7: Salinity at surface and bottom of seapens.

The netpens were checked for holes and dead carcasses removed on approximately a weekly basis by S.C.U.B.A. diving. During late August and September, as many as 10-15 parasitic copepods were noticed on a few fish. These were identified as Caligus clemensi by staff of the Pacific Biological Station (PBS), Nanaimo, B.C. The wounds caused by these parasites can leave fish susceptible to secondary infection and as few as 5 lice per fish are reported to have killed smolts (Case History Report #870483, PBS). Heavy parasitic infections will affect growth of fish because fish stop feeding and use up energy in flashing to get rid of parasites (Case History Report #870483, PBS).

Coho in both netpens were turned over to production operations of the salmon farm on 15 March 1988 and harvested for the restaurant trade a few months later.

SAMPLING OF SEAWATER COHO

On 20-23 November 1987 and 11-12 March 1988 the following parameters were measured for each fish in both netpens: parr reversion (yes/no), fork length and weight. The same method for anesthetizing and measuring fish for length and weight were used as in freshwater except that no buffer was required due to the buffering capacity of seawater. A Sartorius balance was used for the November sampling and a Western Scale (indicator DF2000NS with a load cell model No. WSB2015) was used for the March sampling. Fish that appeared emaciated with a thin body, loose skin and parr markings were classified as parr revertants: including stunts and parr revertants as described by Hoar (1988) and Folmar et al. (1982). Figure 8 shows a healthy post-smolt and Figure 9 shows two parr revertants.

TERMINOLOGY USED

The following terminology is used throughout the thesis:

Lots: 3 lots (lot = fish ponded on a particular date)
Photoperiod treatments: 3 photoperiod treatments
Groups: 9 groups (batches combined)
Batches: 18 batches (2 batches within each of the 9 ponding date and photoperiod combinations)

CONDITION FACTOR

Condition factor, the ratio of the weight of a fish divided by the cube of its length, has been used as an indicator of fish health and smoltification. A reduction in condition factor has been noted as part of smoltification, especially in Atlantic salmon (Salmo salar), (Bjornsson et al. 1989).

Figure 8: Photograph of post smolt sampled from seapen on March 3, 1988. Note brand on left side in front of dorsal fin.

Figure 9: Photograph of two parr revertants sampled from seapen on March 3, 1988. Note loose skin especially along ventral surface.

Condition factor (K) was determined as:

$$K=((Wt)*(Fl^{-3}))*10^{6}$$

where: Wt = whole body wet weight in grams

Fl = fork length in millimeters.

STATISTICAL ANALYSIS

SAS (Release 5.18) general linear modeling (GLM) programs, applicable to use on unbalanced data sets, were the main programs used for statistical analysis.

The SAS procedure GLM determines four sums of squares which are determined from the following, with-interaction, overparameterized model for 2-way crossed classification (Searle 1987):

$$E_{(y_{ijk})} = \mu + \alpha_i + \beta_j + \phi_{ij}$$

where:

$E(y_{ijk})$ = expected value of the kth element in the ith level of factor A and the jth level of Factor B

μ = general mean

α_i = effect due to the ith level of Factor A

β_j = effect due to the jth level of Factor B

ϕ_{ij} = effect due to the interaction of level i of Factor A with level j of Factor B

SAS GLM calculates four sums of squares: Type I (Sequential), Type II (Each-after-all-others), Type III (Σ-restricted) and Type IV (Hypotheses). Type III sum of squares are considered in analyses used in this thesis. The Type III sum of squares for Factor A indicates the additional reduction in sum of squares due to fitting Factor A to the model over and above that achieved by fitting the general mean, Factor B, and the interaction of Factors A and B, to the model. The Type III sum of squares for Factor A, and the interaction of Factors A and B, are also determined as the additional reduction in sums of squares after fitting the other three components to the model. Tests of hypotheses were done using the mean square for batches of ponding lots and photoperiod treatment as an error term in the F-ratio test.

The underlying assumptions for the analysis of variance are: 1) that the effects of the different factors are additive and, 2) the residuals from the model are normally and independently distributed with the same variance (Snedecor and Cochran 1980).

Although equality of variance can be tested with Bartlett's test, this test was not used as it is not very efficient and is highly sensitive to nonnormality (Zar 1984). Analysis of variance is robust enough to operate well even with considerable heterogeneity of variance (Zar 1974). In regard to testing for homogeneity of variance before making an analysis of variance test for homogeneity of means in which homogeneity of variance is assumed, Box (1953) offers the following comment: "To make the preliminary test on variances is rather like putting out to sea in a rowing boat to find out whether conditions are sufficiently calm for an ocean liner to leave port!". However, due to the importance of the analysis of variance results for variables determined after 7 months of seawater growth, variances for each of the batches within each of the ponding date x photoperiod treatment combinations for the variables of interest are presented in Appendix 5. Testing the assumption of homoscedasticity for the variables of interest is considered in Appendix 5, using the tabulated variances and the Fmax-test (Sokal and Rohlf 1969; David 1952; Rohlf and Sokal 1969).

As the validity of analysis of variance is affected only slightly by modest deviations from normality (Zar 1974), no tests of normality were made.

Multiple comparisons with an experiment-wise $\alpha=0.05$, were performed using Scheffé's method (Scheffé 1953) as it is applicable to unbalanced data (Devore 1982). Scheffé's method however is less powerful than both Tukey's method and the Student-Newman-Keuls test (Zar 1984).

Analysis of survival data was done using contingency table analysis. Testing for goodness-of-fit was done using the log-likelihood ratio test. This test is preferred over chi-square testing for goodness-of-fit based on theoretical grounds (Sokal and Rohlf 1969). Sokal and Rohlf (1969) recommend that "the G-test should be uniformly employed for goodness-of-fit tests, and if the computation is done by computer we recommend its use exclusively." When significant differences in survival between all ponding groups and/or all photoperiod treatments were found from contingency table analysis, contingency tables were subdivided and further goodness-of-fit testing performed.

RESULTS

GROWTH IN FRESHWATER

The late-July/early-August freshwater morphometric sampling was done on those fish branded and subsequently transferred to seawater in August 1987. This sampling was the last morphometric sampling before seawater entry and is therefore the best data to indicate the morphometrics of fish just prior to transfer into seawater. These samplings occurred 10 to 24 days, depending on ponding lot, prior to the transfer of 50 fish from each of the 18 batches into seawater. Fish were not measured at the time of transfer to seawater as anesthetizing and handling stress might have adversely affected seawater adaptability.

Freshwater data from the September and October samplings are presented primarily to indicate the growth characteristics of the 18 batches of experimental coho when not transferred to seawater in their first summer. September freshwater data was also used to interpolate the mean weight at time of transfer to seawater of each of the 18 batches.

SURVIVAL

Survival in freshwater was not recorded as an experimental variable. However, no disease outbreaks were noted and less than 30 dead fish, which usually had signs of physical trauma, were found in the freshwater rearing containers throughout the experimental period, apart from those killed during the previously mentioned supersaturation and low flow incidents. Occasionally fish were drawn against the end of the siphon used for daily cleaning of each of the barrels. Over a dozen fish were found throughout the period of the experiment with rings on their bodies that matched the diameter of the vacuum pipe; many of these fish subsequently died.

There were sufficient fish in all batches to allow for fifty to be transferred to seawater from each batch in August 1987 and still keep some fish in freshwater for future measurements. By September and October 1987, some early Robertson batches remaining in freshwater contained fewer than the 50 fish desirable for sampling. Otherwise, sufficient numbers of fish survived in freshwater in all batches to allow for fish morphometric sampling until the end of freshwater rearing in October 1987.

MEAN WEIGHTS

Swimup coho in all batches of all three ponding lots were approximately 0.3g when ponded.

Sampling of the March 3 and 17 ponded lots in late May (Table 1), revealed little difference in mean fish weight amongst the 6 batches within ponding lots (Figures 10 and 11). Mean weights in the March 3 ponding lot ranged from 4.09 to 4.64g, and in the March 17 ponding lot from 3.80 to 4.59g.

Sampling of the April 12 ponded groups on May 30, 1987, revealed little difference between mean weights of batches within photoperiod treatments (Table 4). For weights recorded on May 30, GLM revealed a significant ($P=0.0004$) effect of photoperiod treatment and Scheffé's multiple comparison revealed a significant (experiment-wise $\alpha=0.05$) difference between all pairwise comparisons of photoperiods. The fish exposed to natural photoperiod had the greatest mean weight, followed by fish exposed to delayed and 9.5h constant photoperiods (Table 4).

By the late-July early-August sampling session, for all ponding dates, fish that had been subjected to either the 9.5 hour constant photoperiod or the phase delayed photoperiod had greater (significantly different with

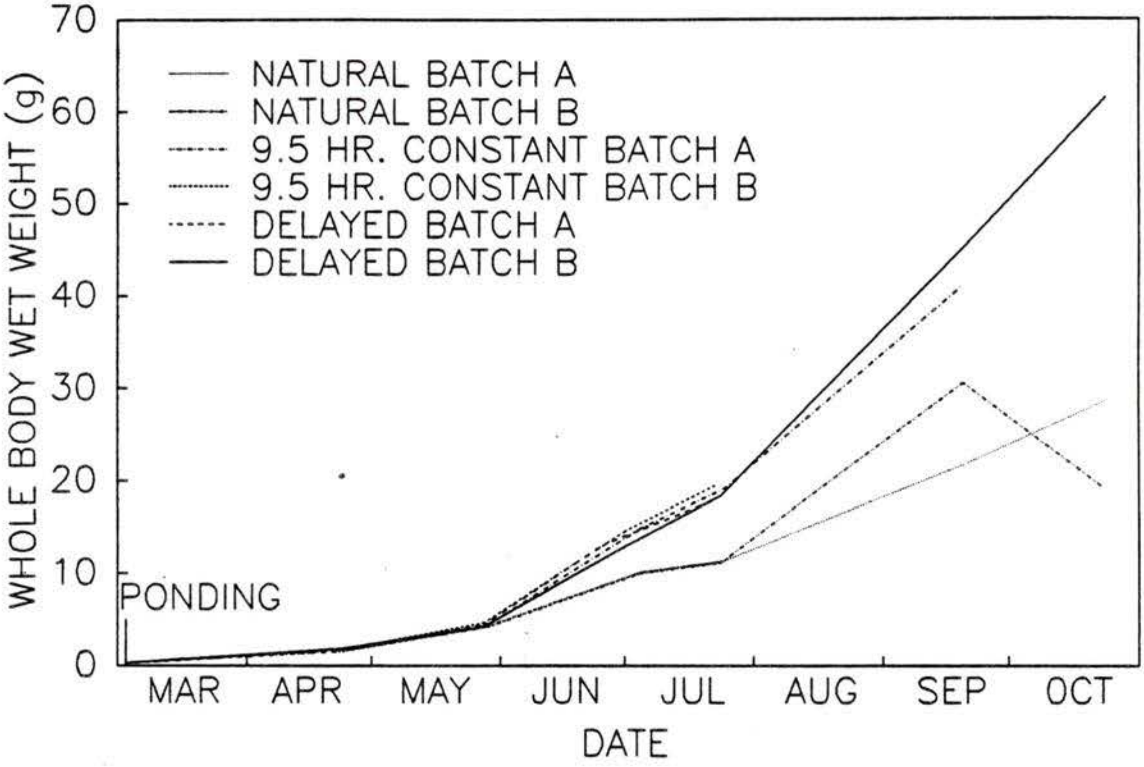


Figure 10: Mean weights in freshwater of coho ponded on March 3, 1987 (Robertson lot 1) as a function of time of year for the 2 batches within each of the 3 photoperiod treatments. See Table 1 for sampling dates.

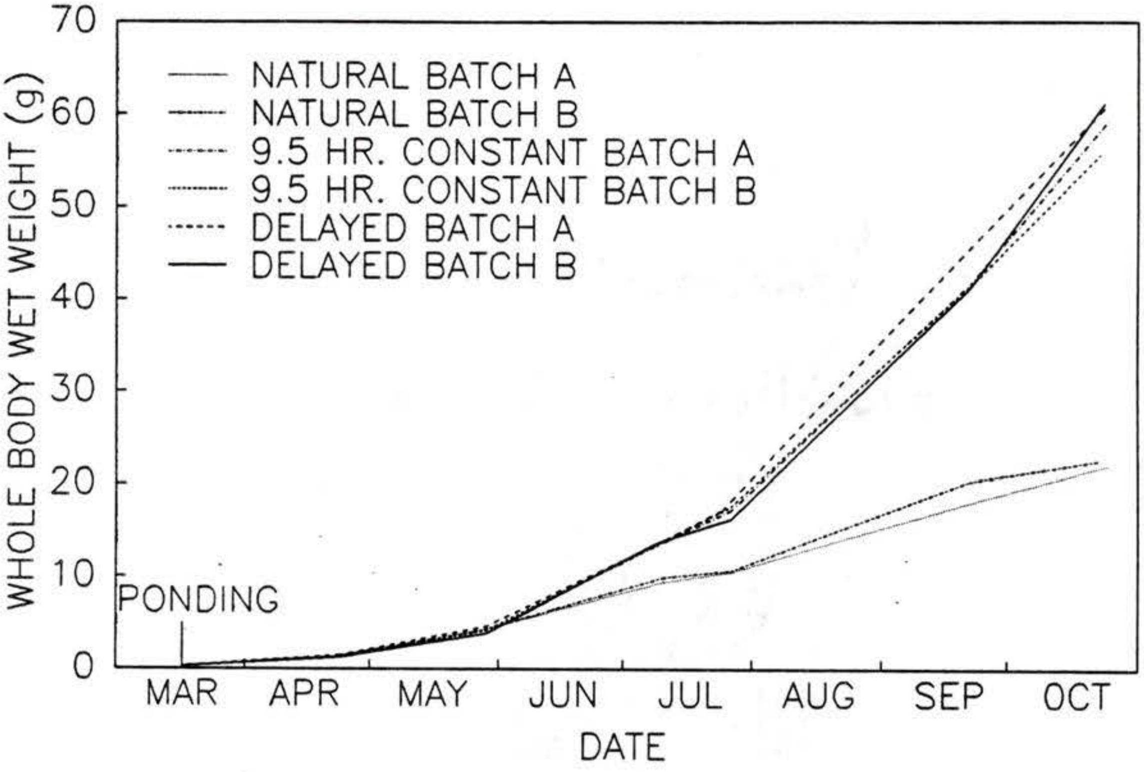


Figure 11: Mean weights in freshwater of coho ponded on March 17, 1987 (Robertson lot 2) as a function of time of year for the 2 batches within each of the 3 photoperiod treatments.

Table 4: Mean weight, standard error, standard deviation, coefficient of variation and number of coho ponded April 12 and sampled May 29-30, 1987. Bat.=Batch.

PHOTOPERIOD TREATMENT	BAT. (#)	mean WT (g)	1 S.E. (g)	1 S.D. (g)	C.V. (%)	n
NATURAL	A	2.80	0.10	0.72	26	51
	B	2.80	0.10	0.74	26	50
9.5 H	A	2.37	0.07	0.51	22	50
	B	2.39	0.08	0.55	23	50
DELAY	A	2.52	0.07	0.52	21	50
	B	2.54	0.07	0.51	20	50

experiment-wise $\alpha=0.05$) mean weights than those exposed to ambient light since ponding (Table 5). Within all three ponding lots, a significant effect ($P=0.0153$, 0.0023, and 0.0095 for March 3, March 17, and April 12 ponding groups, respectively) of photoperiod treatment on mean weight was found. Scheffé comparisons with an experiment-wise $\alpha=0.05$, revealed that for the March 3 and 17 ponded fish, the mean weights of photoperiod manipulated fish were significantly greater than those of non-photoperiod manipulated fish and that no significant difference in mean weights existed between batches exposed to the delayed and to the 9.5h photoperiods. For the April 12 ponded groups, the groups exposed to a 9.5h constant photoperiod had a significantly greater mean weight than both non-photoperiod manipulated groups and delayed photoperiod groups.

Table 5: Mean weight, standard error, standard deviation, coefficient of variation and number of coho sampled late-July/early-August 1987. Bat.=Batch R1=Robertson lot 1 ponded on March 3, R2=Robertson lot 2 ponded on March 17, K=Kitimat lot ponded on April 12, Nat=Natural, 9.5H=9.5 hour, Delay=Delayed.

POND-ING DATE	PHOTO-PERIOD TREAT-MENT	BAT. (#)	MEAN WT (g)	1 S.E. (g)	1 S.D. (g)	C.V. (%)	n (#)
R1	NAT.	A	11.37	0.51	3.65	32	51
		B	11.14	0.54	3.84	34	51
	9.5H	A	19.14	0.82	5.75	30	49
		B	19.51	0.88	6.21	32	50
	DELAY	A	17.81	0.82	5.78	32	50
		B	18.38	0.68	4.84	26	51
R2	NAT.	A	10.52	0.35	2.49	24	52
		B	10.61	0.37	2.69	25	52
	9.5H	A	17.18	0.66	4.73	28	52
		B	17.59	0.77	5.53	31	52
	DELAY	A	17.29	0.95	6.82	39	52
		B	16.29	0.63	4.61	28	53
K	NAT.	A	8.29	0.26	1.87	23	53
		B	8.36	0.31	2.26	27	54
	9.5H	A	11.84	0.32	2.32	20	53
		B	11.87	0.37	2.67	22	52
	DELAY	A	9.06	0.32	2.34	26	52
		B	9.89	0.33	2.41	24	53

By the end of freshwater rearing in October 1987, the number of fish remaining in each of the March 3 ponding batches was fewer than 50. Three batches had been terminated (Figure 10) because of insufficient fish for sampling. Periodic seawater challenge testing, which required terminal sampling, was a major contributor to the decline in the number of fish in all batches. The October sampling mean weights ± 1S.E.(n) of natural batch A, natural batch B and delayed batch B were 10.30 ± 5.8g(25), 19.45g ± 1.8(7), and 61.55g ± 4.29(41), respectively. Thus the trend established in July of the photoperiod manipulated fish being larger, appears to have continued until October (Figure 10). This was not tested statistically due to the vastly unequal sample sizes. Also, the decrease in mean weight of natural batch B from September to October seen in Figure 10, reflects the large variation of the September sampling (1 S.D. = 26.8g), and that five large fish that were present in the September sampling, were not present in the October sampling.

For fish ponded on March 17, the trend of the photoperiod manipulated fish being larger than fish exposed to ambient light, persisted from July until the end of freshwater rearing in October 1987 (Figure 11). No significant difference was found between the two photoperiod manipulative treatments for freshwater mean weights measured in the third week of October 1987.

For fish ponded on April 12, a distinct separation in mean weights between all photoperiod manipulated batches and natural photoperiod batches was not evident until the mid-October sampling (Figure 12). In the other two ponding lots separation in mean weights between natural photoperiod batches and photoperiod manipulated batches was evident by the July sampling. In October, all 4 batches of photoperiod manipulated Kitimat coho had greater mean weights than the natural photoperiod fish (Figure 12).

Comparison of mean weights at time of transfer into seawater among ponding dates are discussed in the seawater results section.

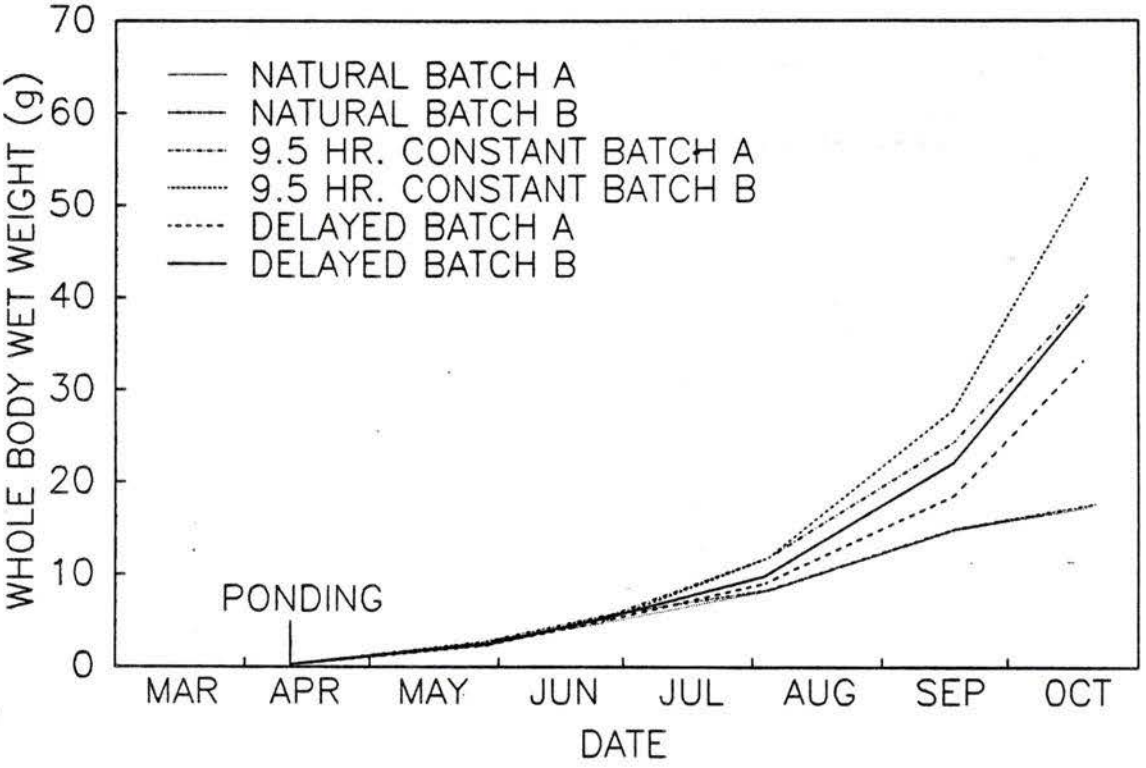


Figure 12: Mean weights in freshwater of coho ponded on April 12, 1987 (Kitimat) as a function of time of year for the 2 batches within each of the 3 photoperiod treatments.

UNIFORMITY OF SIZE

At the time of the late-July/early-August sampling (Table 5), and at the time of the October freshwater sampling (Table 6), within all three ponding lots, the standard deviations and coefficients of variations either overlap between the different photoperiod treatments or were larger for photoperiod manipulated batches. Thus, photoperiod manipulation does not appear to have increased uniformity of growth by the time of the last sampling session before transfer to seawater nor by the time of the October freshwater sampling.

Table 6: Mean weight, standard error, standard deviation, coefficient of variation and number of coho sampled October 19 to 25, 1987. Bat.=Batch R1=Robertson lot 1 ponded on March 3, R2=Robertson lot 2 ponded on March 17, K=Kitimat lot ponded on April 12, Nat=Natural, 9.5H=9.5 hour, Delay=Delayed. Note: Natural batch A experienced the low flow (hypoxia) incident in September 1987, in which at least 22 fish died.

POND-ING DATE	PHOTO-PERIOD TREAT-MENT	BAT. (#)	MEAN WT (g)	1 S.E. (g)	1 S.D. (g)	C.V. (%)	n (#)
R1	NAT.	A	28.92	6.25	31.26	108	25
		B	19.45	1.83	4.85	25	7
	9.5H	A	--	--	--	--	0
		B	--	--	--	--	0
	DELAY	A	--	--	--	--	0
		B	61.55	4.35	27.53	45	40
R2	NAT.	A	22.07	1.68	11.00	50	43
		B	22.56	0.98	7.69	34	62
	9.5H	A	59.35	4.53	34.55	58	58
		B	55.68	3.74	32.43	58	75
	DELAY	A	60.72	3.64	22.71	37	39
		B	61.23	2.40	19.83	32	68
K	NAT.	A	17.29	0.89	4.63	27	27
		B	17.67	1.50	12.53	71	70
	9.5H	A	40.34	3.50	30.11	75	74
		B	53.09	4.70	35.78	67	58
	DELAY	A	33.25	2.12	17.16	52	65
		B	39.12	2.14	19.99	51	87

CONDITION FACTOR

Within all three ponding lots, all 6 batches showed a decrease in mean condition factor between the late-June/early-July sampling and the late-July/early-August sampling (Figures 13, 14, and 15). A significant difference (P=0.0004, 0.0096, and 0.0005, respectively, for

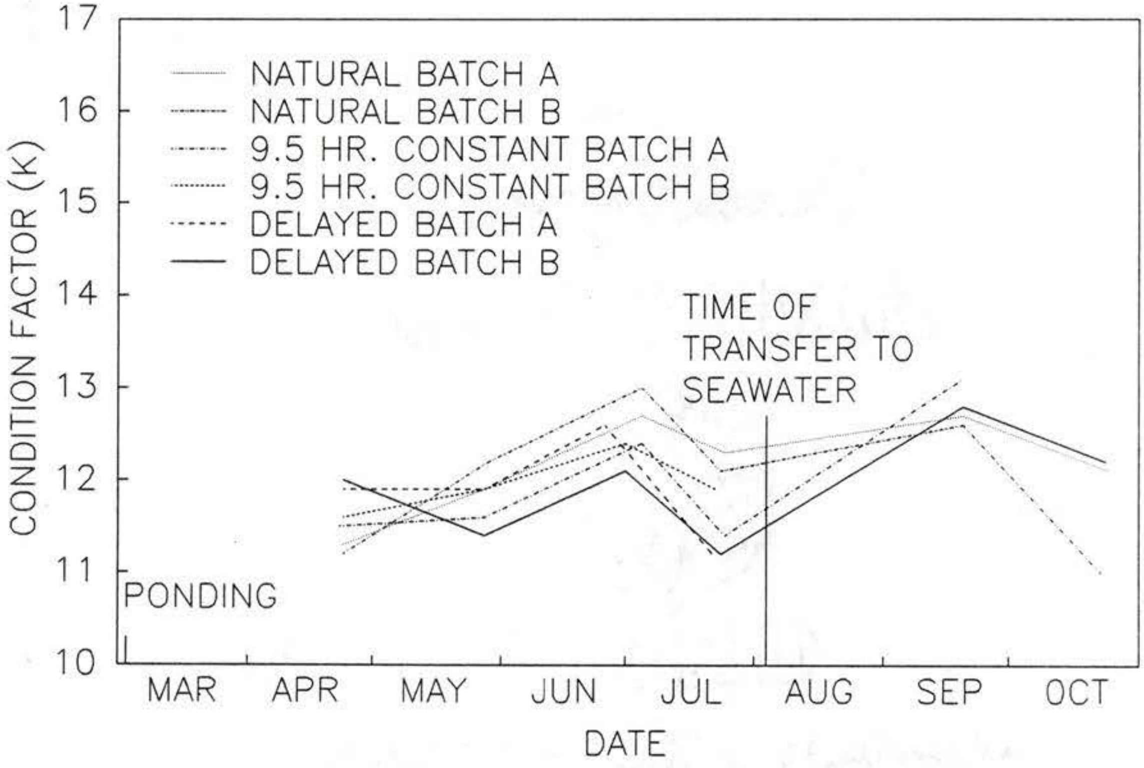


Figure 13: Freshwater condition factor of coho ponded on March 3, 1987.

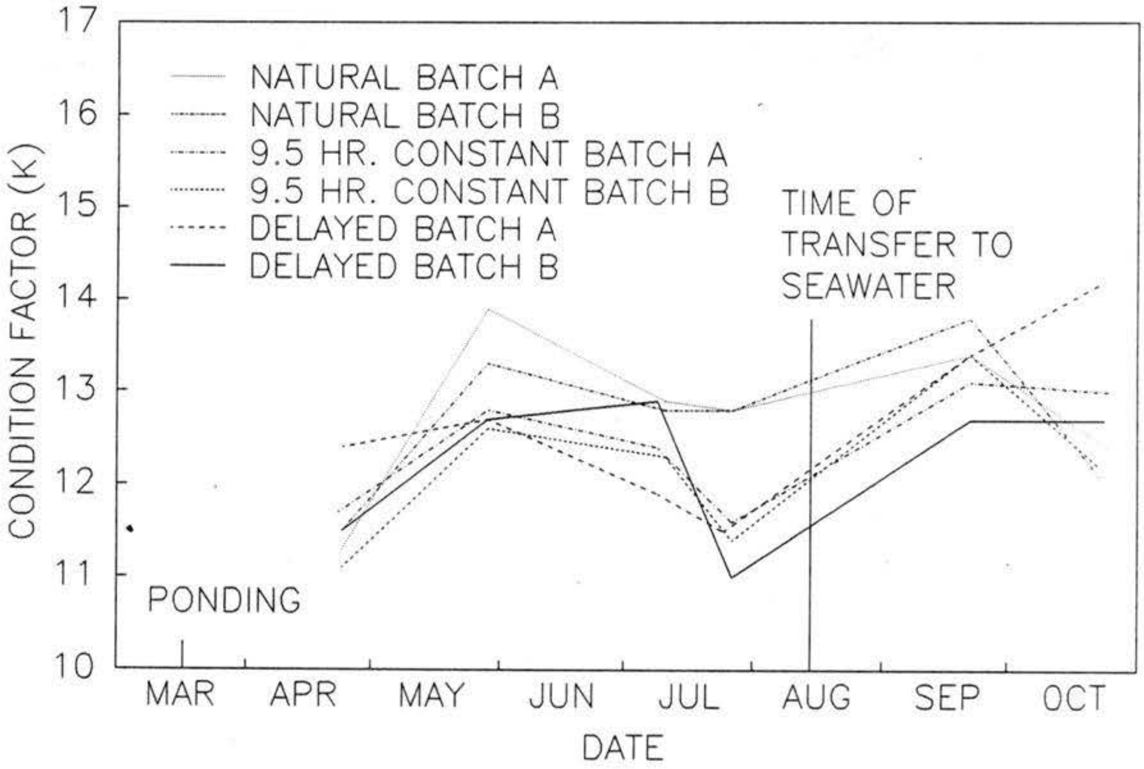


Figure 14: Freshwater condition factor of coho ponded on March 17, 1987.

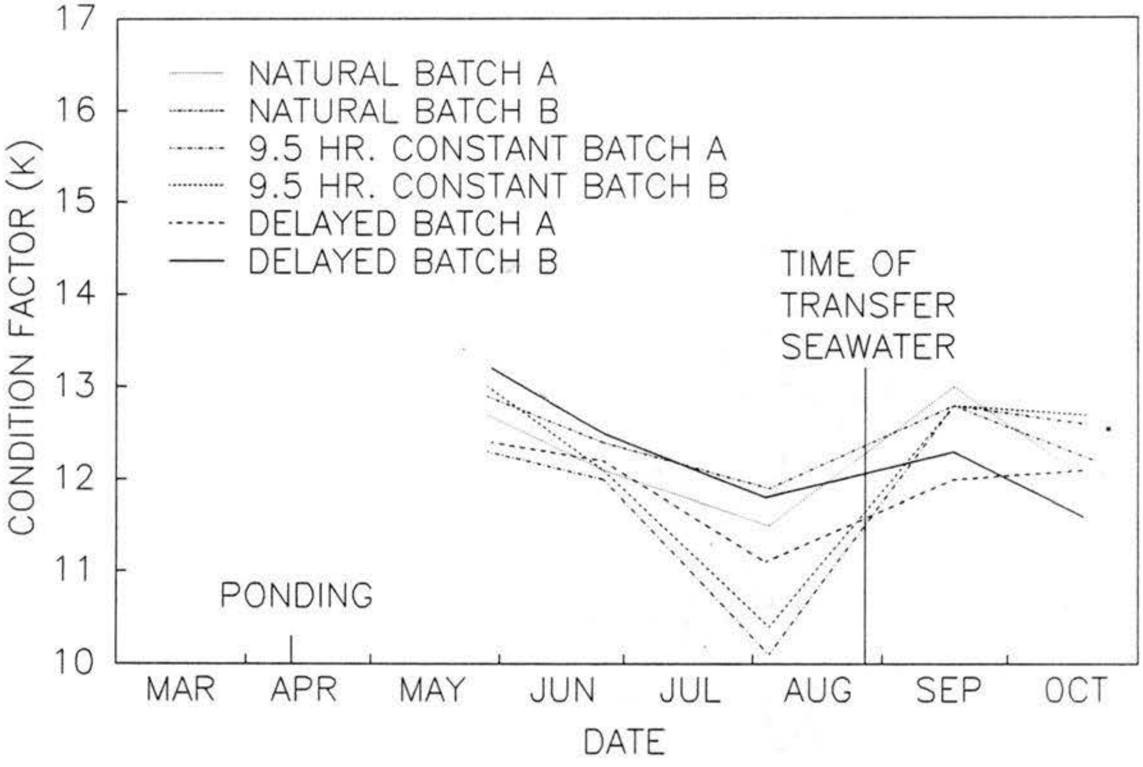


Figure 15: Freshwater condition factor of coho ponded on April 12, 1987.

the three ponding dates in chronological order) was found between the condition factors determined during the June 26-July 5 sampling and those determined during the July 22-25 sampling.

Due to the time between samplings, it is not possible to determine if the lowest mean condition factor of the various batches occurred before or after the late July sampling (Figures 13, 14, and 15).

For fish ponded on March 3 and 17, all 4 photoperiod manipulated batches, when measured during the late-July sampling, had lower mean condition factors than the 2 batches exposed to ambient photoperiod (Figure 13, 14). For fish ponded on April 12, when measured during the early-August sampling, the two 9.5h constant photoperiod batches had lower mean condition factors than did the natural and delayed batches, (Figure 15).

For the late-July/early-August sampling, a nearly significant difference (P=0.0823) was found for condition factors amongst photoperiod treatments for fish ponded on March 3. For the same sampling session a significant difference in condition factor was found amongst photoperiod treatments for fish ponded on March 17

(P=0.0175) and April 12 (P=0.0102). In the March 17 ponding lot, fish exposed to photoperiod manipulation had significantly different condition factors than those exposed to natural photoperiod and the photoperiod manipulated groups were not significantly different from each other. For the April 12 ponded groups, the groups exposed to 9.5h photoperiod had significantly different condition factors from both the natural and the delayed photoperiod groups. The delayed groups did not have significantly different condition factors from the natural photoperiod groups.

SILVERING INDEX

Silvering index was determined for coho during the late-July/early-August sampling, the last freshwater sampling before transfer into seawater. Within all three ponding lots, contingency table analysis demonstrated a significant difference ($P<0.0005$) in frequency of silvering indices amongst the three photoperiod treatments.

Within all ponding lots, over 85% of fish in each of the 6 natural photoperiod batches had silvering indices of 3 or less whereas over 60% of fish in each of the 12 photoperiod manipulated batches had silvering indices of 4 or greater (Figures 16, 17, and 18). This indicates a positive association between both of the photoperiod manipulated treatments and an increased percentage of fish that are smolt-like in appearance.

At the time of the last freshwater sampling before transfer to seawater, a significant ($P<0.0005$) difference was found from contingency table analysis amongst ponding dates for frequency of silvering indices. This same result was found when comparing ponding dates within all three photoperiod treatments.

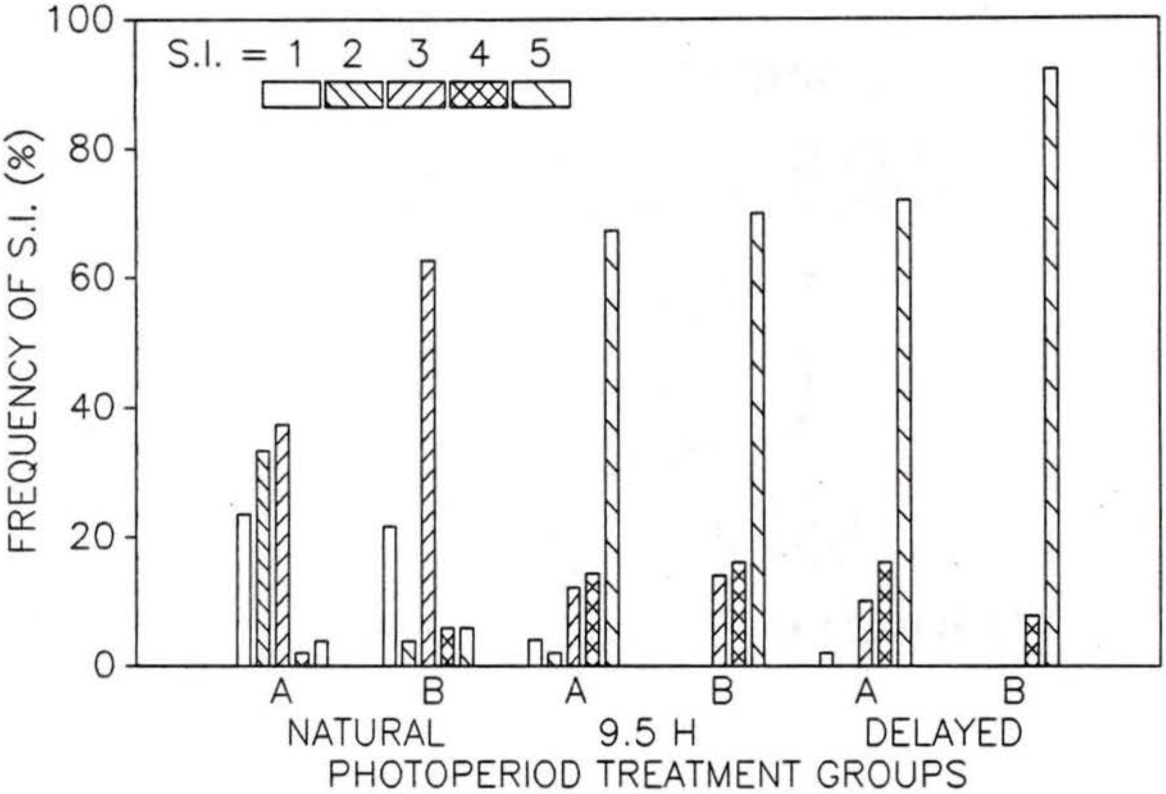


Figure 16: Silvering index frequency determined during July 22 to 25 sampling for each of the 6 batches ponded on March 3, 1987.

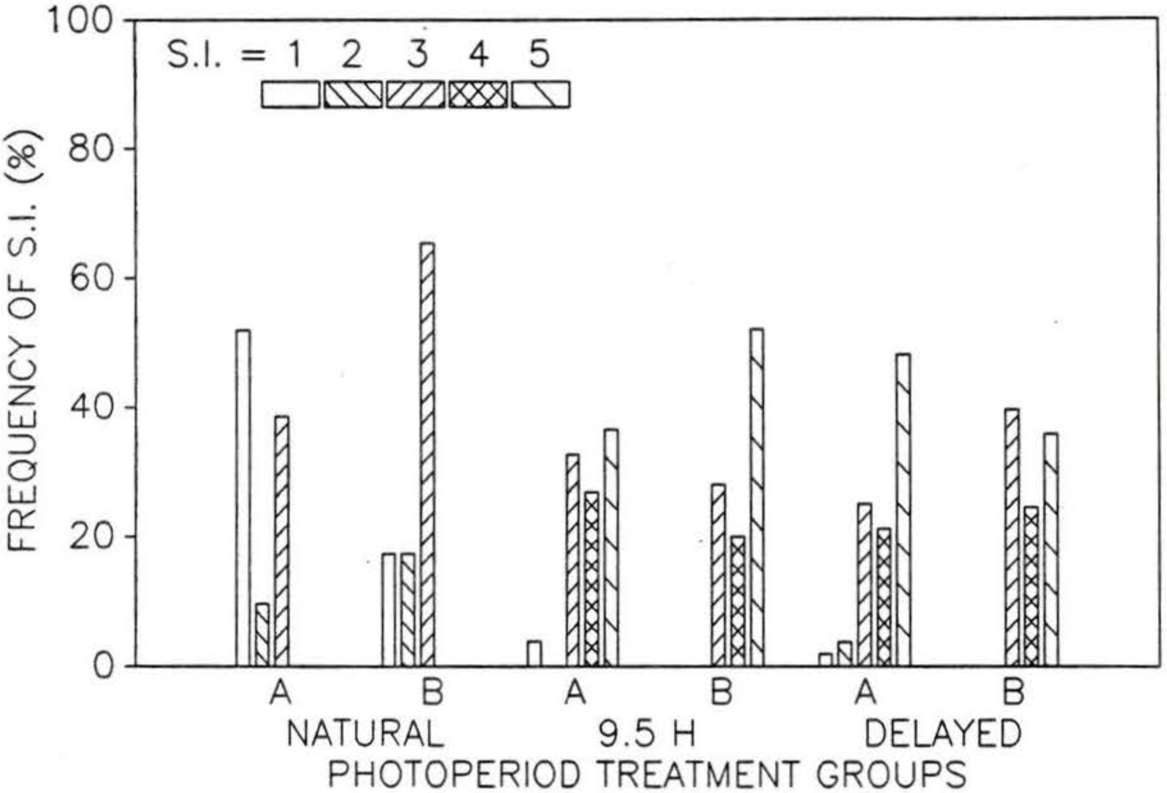


Figure 17: Silvering index frequency determined during July 25 to 27 sampling for each of the 6 batches ponded on March 17, 1987.

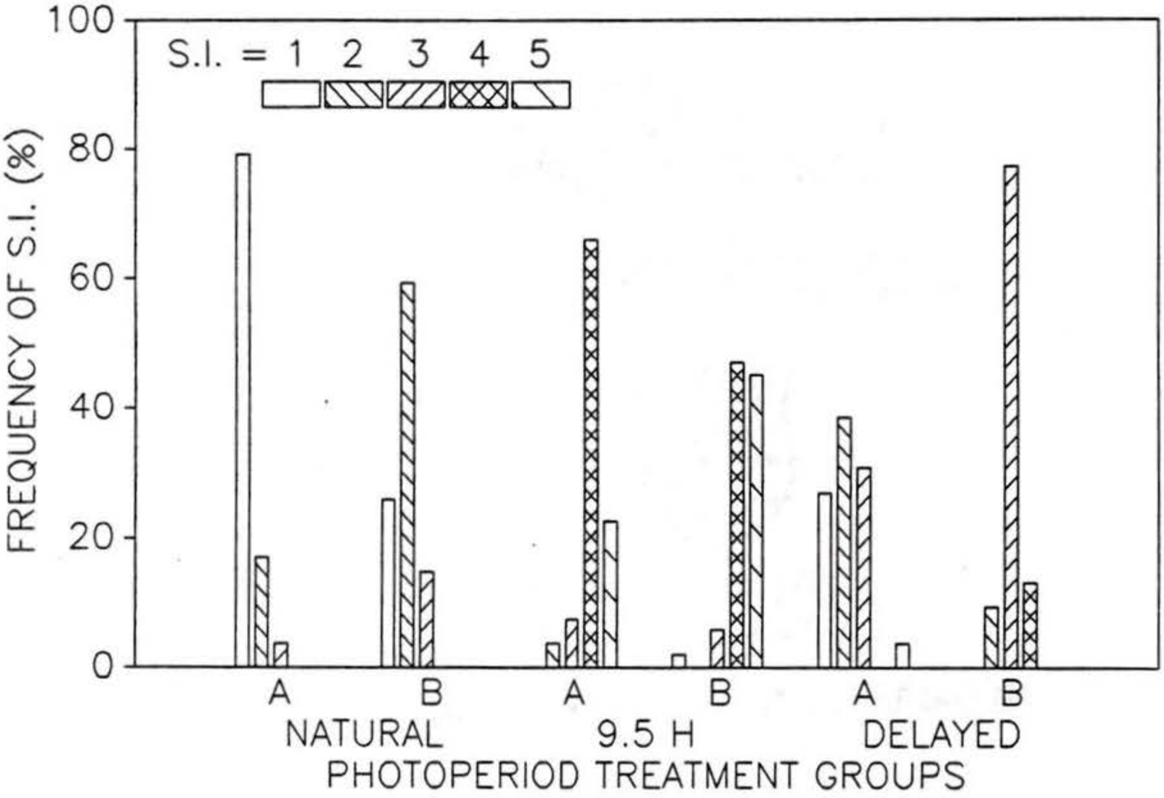


Figure 18: Silvering index frequency determined during August 4 to 5 sampling for each of the 6 batches ponded on April 12, 1987.

Means silvering indices for each of the photoperiod and ponding dated combinations (batches combined) were calculated (Table 7). Within all ponding lots, groups exposed to manipulated photoperiod had greater mean silvering indices than groups exposed to natural photoperiods. Within all photoperiod treatment, March 3 ponded groups had the largest mean silvering indices, and, except for the 9.5h photoperiod group, April 12 ponded fish had the lowest mean silvering index.

Table 7: Means silvering indices at time of last freshwater samplings before transfer into seawater.

PHOTOPERIOD TREATMENT	PONDING DATE	MEAN S.I.
NATURAL	MARCH 3	2.500
	MARCH 17	2.173
	APRIL 12	1.570
9.5 H	MARCH 3	4.475
	MARCH 17	4.078
	APRIL 12	4.202
DELAYED	MARCH 3	4.743
	MARCH 17	4.029
	APRIL 12	2.600

GROWTH IN SEAWATER

Because the data from the March 1988 sampling represents the last seawater sampling done, it will be emphasized in the presentation of seawater results. The November sampling data will be used when time series data is presented. The November 1987 and March 1988 samplings occurred after the fish had been in seawater for approximately 3 and 7 months, respectively.

In presenting some of the analyses, parr revertants have been excluded. Such analyses are referred to as being for post-smolts.

Most brands were of sufficient clarity for identification purposes in both the November and March samplings: 12 fish in November and 3 fish in March, could not be identified by their brands. Generally, brands were more easily discernable on larger fish, and brands were easier to read during the March sampling than during the November sampling.

SURVIVAL

Survival was recorded for both the total number of fish surviving and the number of post-smolts surviving out of the 50 fish transferred into seawater for each of the 18 batches (Table 8).

Table 8: Percent of coho surviving in seawater at time of November 1987 (number before slash) and March 1988 (number after slash) samplings for the 2 batches within each of the 9 photoperiod treatment X ponding date combinations. Total=Parr revertants included; P.S.=Post-smolts only; R1=Robertson lot 1 ponded on March 3; R2=Robertson lot 2 ponded on March 17; K=Kitimat ponded on April 12; Bat=Batch.

GROUP	PHOTOPERIOD TREATMENT					
	Nat		9.5		Delayed	
	Total.	P.S.	Total	P.S.	Total	P.S.
R1 Bat=A	28/32	28/32	56/54	50/54	66/62	64/62
Bat=B	32/24	28/20	62/58	56/58	82/76	80/76
R2 Bat=A	12/06	08/06	70/60	54/56	52/54	42/52
Bat=B	24/16	20/16	86/70	72/60	68/68	66/68
K Bat=A	30/26	28/24	80/78	80/78	80/82	80/82
Bat=B	22/20	18/20	68/64	68/64	70/70	70/70

Percent total survival (includes parr revertants and post-smolts) for each of the 18 batches, from the time of seawater transfer until the November 1987 sampling, ranged from 80% to 12% (Table 8). Percentage total survival for batches, from the November 1987 sampling until the March 1988 sampling, ranged from 114% (R1 Nat Bat=A: 14 fish in November to 16 fish in March) to 50% (R2 Nat Bat=A: 6 fish in November to 3 in March)(Table 8). Two possible explanations for the increase in total number of fish counted in 3 batches (R1 Nat Bat=A: 2 fish; R2 Delayed Bat=A: 1 fish; K Delayed Bat=A: 1 fish) from the November sampling to the March samplings are: 1) some fish not identifiable by brands in the November 1987 sampling had grown and their brands therefore had become larger and these fish could be identified by their brands during the March 1988 sampling; 2) the section of the net separating measured and unmeasured fish was accidentally dropped into the water during the November sampling and although not observed, as many as four fish may have swum from the unmeasured side to the measured side before the partition was again raised above the water line.

In all 18 batches, higher mortality occurred in the 3 months from seawater entry in August 1988 until the November 1987 sampling than occurred during the 4 months from November 1987 sampling until March 1988 sampling (Table 8).

For the March 1988 sampling, log-likelihood ratio goodness-of-fit analysis, showed no significant difference in survival between batches (P=0.689 with parr revertants included and P=0.841 with parr revertants excluded). Therefore, for graphical presentation of survival data, batches were combined (Figure 19). Survival was found to

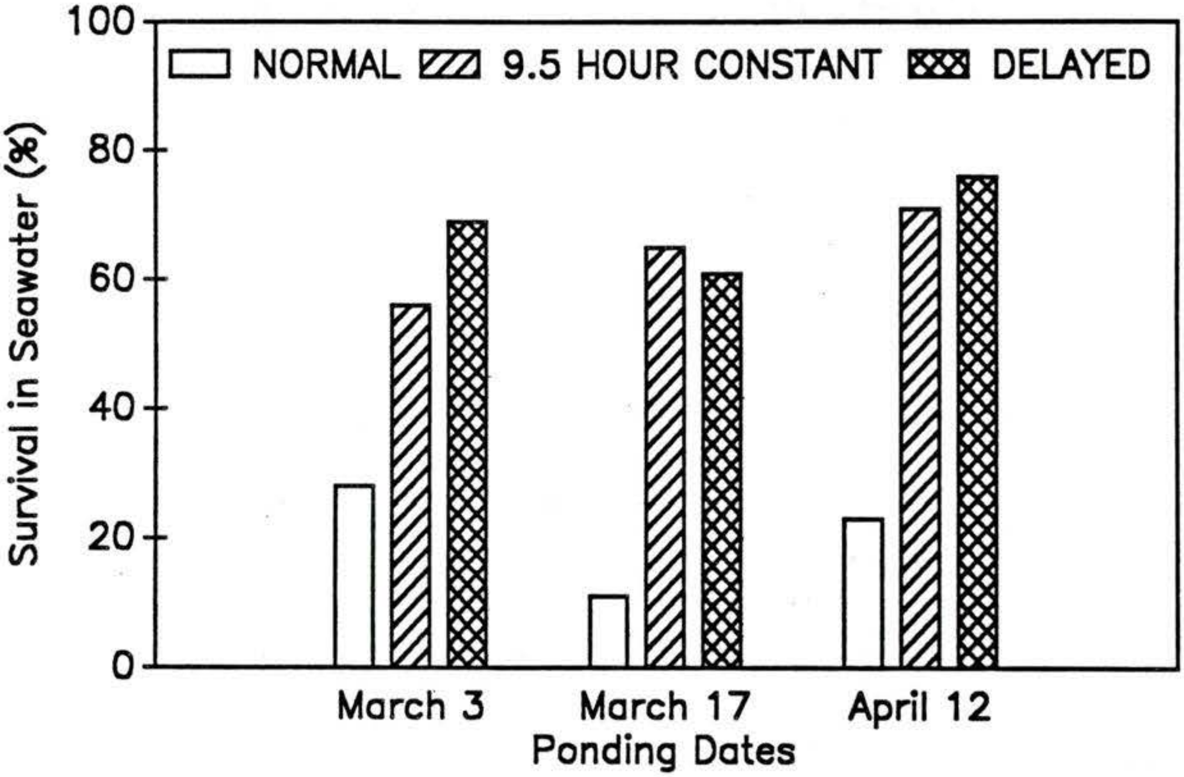


Figure 19: Percent of coho salmon surviving (excluding parr revertants) 7 months after transfer into seawater for each photoperiod x ponding date combination.

be significantly different amongst photoperiods ($P<0.0005$) with groups of fish that had been exposed to 9.5h constant and delayed photoperiods having greater seawater survival than those groups exposed to the ambient photoperiod at San Mateo Bay (Figure 19). Among Robertson progeny exposed to natural photoperiods, the earlier ponded group exhibited significantly better seawater survival ($P=0.006$ for post-smolts). However, the April 12 ponding group also had better seawater survival than did the March 17 ponding group ($P=0.035$ for post-smolts). Within ponding dates, photoperiod manipulated groups had better survival than did non-photoperiod manipulated groups ($P<0.0005$ for all ponding groups) and all three groups exposed to delayed photoperiod had slightly, but not significantly, better survival than those groups exposed to the 9.5h constant photoperiod (Figure 19). For the March 3 ponding lot, there was a borderline significant difference ($P=0.057$) in survival between the two photoperiod manipulated groups for survival (Appendix 3). For the other two ponding lots, no significant difference in survival between the two photoperiod manipulated groups was found (Appendix 3).

PARR REVERTANTS

Thirty nine parr revertants were identified in the November sampling and 11 in the March sampling (Table 9). One of the parr revertants in the November sampling, could not be identified by its brands. The total number of parr revertant within each of the 3 photoperiod treatments was greater in November 1987 than in March 1988 (Table 9).

Table 9: Number of parr revertants at time of November 1987 and March 1988 samplings, for the 2 batches within each of the 9 ponding date X photoperiod combinations. Bat=Batch.

GROUP		PHOTOPERIOD TREATMENT					
		Nat		9.5		Delayed	
		Nov.	Mar.	Nov.	Mar.	Nov.	Mar.
R1	Bat=A	0	0	3	0	1	0
	Bat=B	2	2	3	0	1	0
R2	Bat=A	2	0	8	2	5	1
	Bat=B	2	0	7	5	1	0
K	Bat=A	1	1	0	0	0	0
	Bat=B	2	0	0	0	0	0
Total		9	3	21	7	8	1

In both the March and the November samplings the groups exhibiting the least number of parr revertants were the Kitimat for ponding date and delayed for photoperiod treatment group (Table 9 and Figure 20).

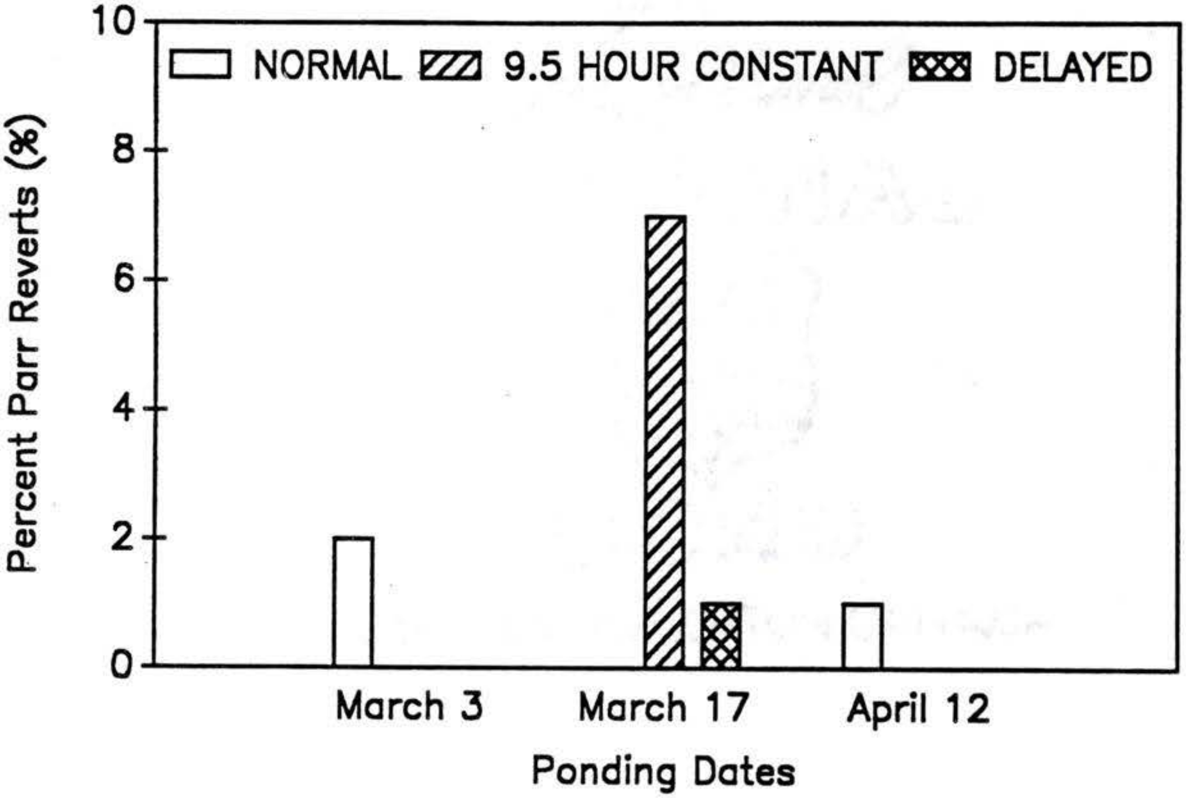


Figure 20: Percent parr revertants (#p.r./#transferred) 7 months after being transferred into seawater for each photoperiod treatment and ponding date combination.

The same general trends discussed above also hold true when percentage of parr revertants are considered as a percentage of those fish surviving (Figure 21).

Due to the sparsity of parr reversion in the data obtained (Table 9), no statistical analysis was performed to determine if ponding date or photoperiod treatment affected the occurrence of parr reversion.

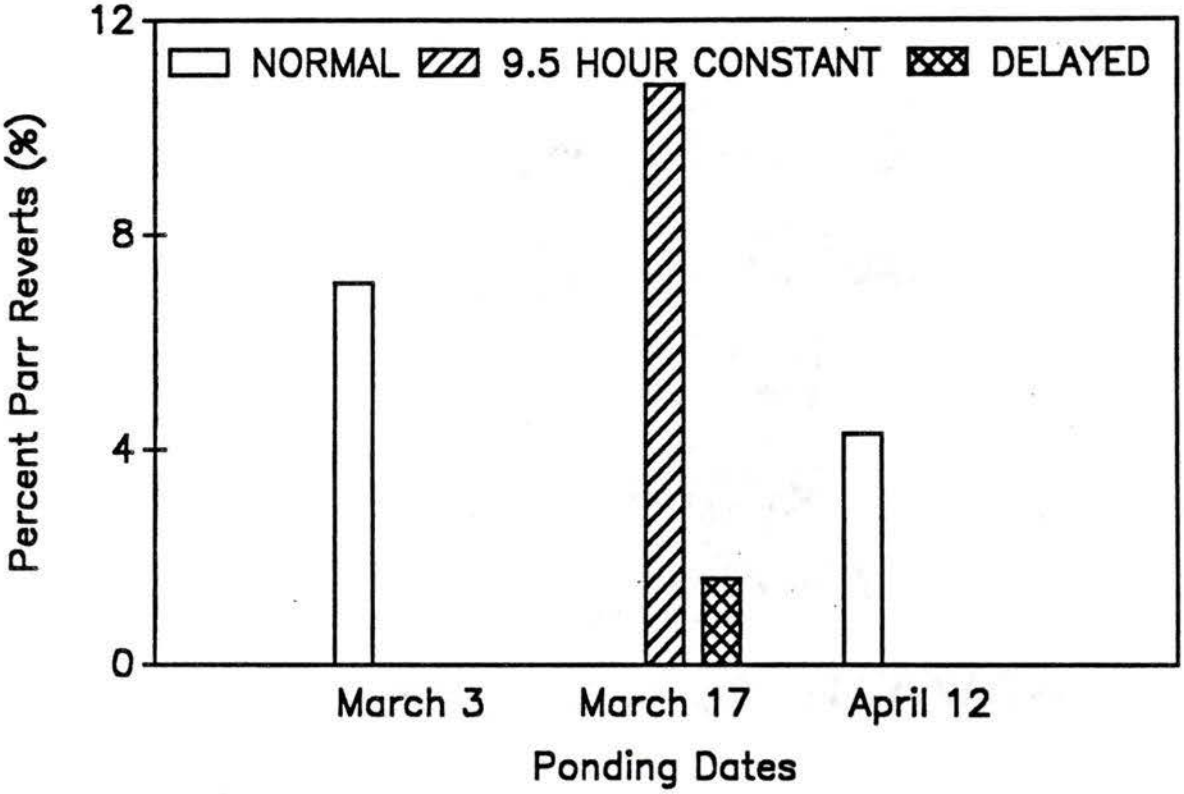


Figure 21: Percent parr revertant (#p.r./#surviving) 7 months after being transferred into seawater for each photoperiod treatment and ponding date combination.

MEAN WEIGHT

To determine if photoperiod treatment or ponding date affected size of coho at time of transfer into seawater and seven months after transfer into seawater, analysis of variance was performed on whole body wet weight and fork length data. Photoperiod treatment and ponding date were the two factors in the two way cross classification fixed effects model. Each factor had three levels with two experimental units per cell. These two experimental units have previously been called batches.

Due to the difference in survival amongst cells for data collected after approximately seven months in seawater, with all survivors being measured for the variable of interest, the March 11-12 1988 sampling data used in the analysis of variance is unbalanced. To balance the data would required discarding a large amount of information. Therefore, analysis of variance was performed on the unbalanced data using the SAS procedure GLM. Parr revertants were omitted from the analysis, due to their abnormal development and limited life expectancy.

Mean Weights at Time of Transfer into Seawater

Mean weights at time of transfer into seawater were determined by interpolation using mean weights of the last freshwater sampling before transfer into seawater (late-

July/early-August sampling) and the first freshwater sampling after 50 fish/batch had been transferred into seawater (September sampling) (Table 10). No mean weights at time of transfer could be calculated for the 9.5h constant batch B and the delayed batch A for early Robertson fish, because insufficient freshwater fish were present at the time of the September 20 sampling to determine mean weights for these two batches. As no variances could be determined for the 9.5h constant and the delayed group, it was not possible to formally test for differences in mean weights at time of transfer into seawater amongst the three photoperiod treatments. However, it does appear that both photoperiod manipulated batches had greater mean weights at time of transfer into seawater than did the natural photoperiod batches (Table 10).

Table 10: Mean weights (g) at time of transfer into seawater, determined by interpolation, for the 18 batches. BAT.=Batch.

PHOTOPERIOD TREATMENT	BAT. (#)	PONDING LOTS R1	PONDING LOTS R2	PONDING LOTS K
NATURAL	A	13.19	13.03	11.72
	B	14.82	13.88	11.78
9.5 H	A	22.99	25.35	18.36
	B		25.55	20.24
DELAY	A		27.35	14.11
	B	23.46	24.61	16.44

GLM analysis of mean transfer weight data, excluding the early-Robertson lot, showed highly significant effects due to photoperiod treatments, ponding dates and, the interaction of photoperiod treatments with ponding dates (Table 11). The significant interaction effect does not invalidate conclusions about the main effects as the model used was sigma-restricted (Searle 1987). However, it does preclude performing multiple comparisons analysis.

Table 11: Anova table of interpolated mean weights at time of transfer into seawater. Tests of hypothesis using the type III MS for bat(lgt*grp) as an error term.

Source of variation	df	type III SS	f value	P-value
Main effects				
Photoperiod treatment	2	217.275	75.63	0.0001
Ponding date	1	114.824	79.94	0.0001
Interaction	2	40.502	14.10	0.0054

Within the late-Robertson and Kitimat lots, photoperiod treatments were found to have significant (P=0.0029 and 0.0197, respectively) effects on mean weight at time of transfer into seawater. Within both of these lots, by visual inspection of Table 10, it appears that photoperiod manipulated batches had greater mean weights at time of transfer into seawater than did the natural photoperiod batches, and little if any difference in mean transfer weight existed between the two manipulated photoperiods.

Growth Rate Increase at time of Transfer into Seawater

All of the photoperiod manipulated batches exhibited a marked increase in growth rate when transferred into seawater over that experienced in freshwater (Figures 22, 23, and 24). In Figure 22, 23, and 24 lines extending until October 19-25 1987 indicate freshwater growth and lines extending until March 11-12 1988, indicate seawater growth. Growth rates were not calculated, as the size at time of transfer was interpolated, and fish were only sampled for weight on two occasions after being transferred into seawater.

Effect of Barrel Position on Subsequent Seawater Mean Weight, Fork Length and Biomass

To determine if a gradient existed along the rows of barrels (Figures 4 and 5) that might have affected the results, the position effect of barrel within light treatments was investigated. Hypothesis testing of position within light treatment was done using lot x position within light treatment as the error term. Position effect was highly non-significant (P=0.9937, 0.9259, and 0.9545, respectively) for mean weight, mean forklength and biomass measured on March 11-12, 1988. Thus, the null hypothesis that physical position of barrels did not affect these three variables is accepted. Furthermore by visual inspection of the means (Appendix 4),

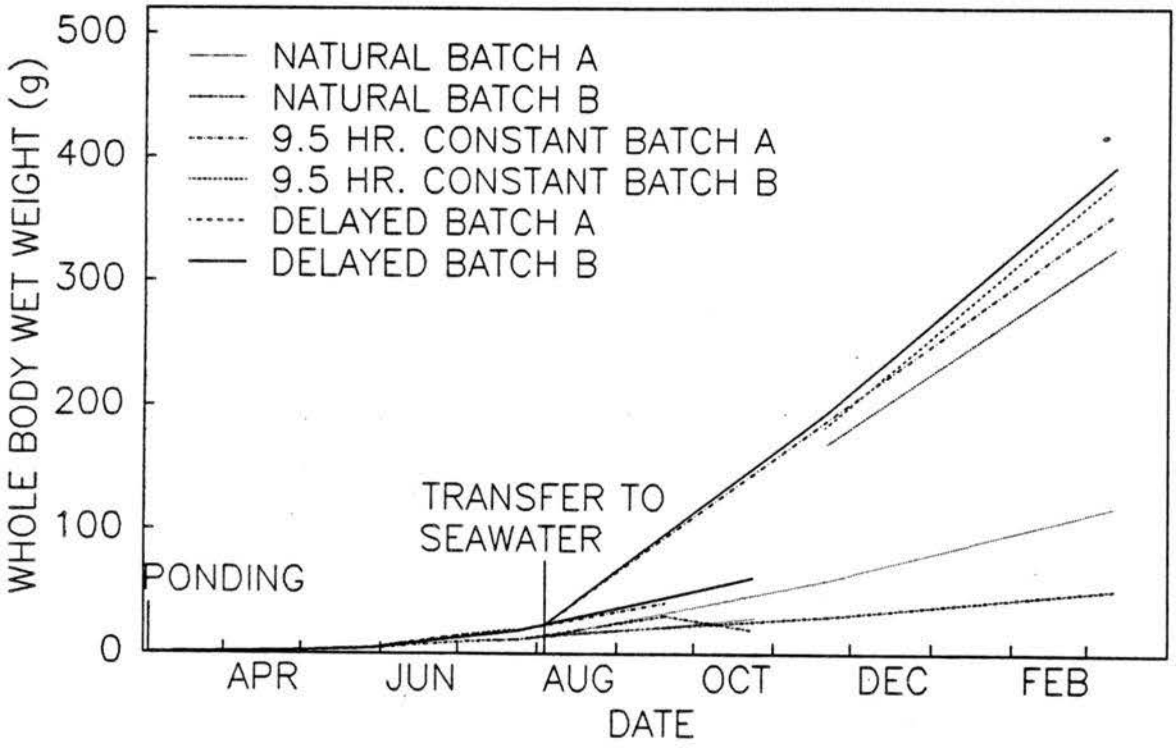


Figure 22: Mean whole body wet weights of the 6 Robertson March 3 ponded batches.

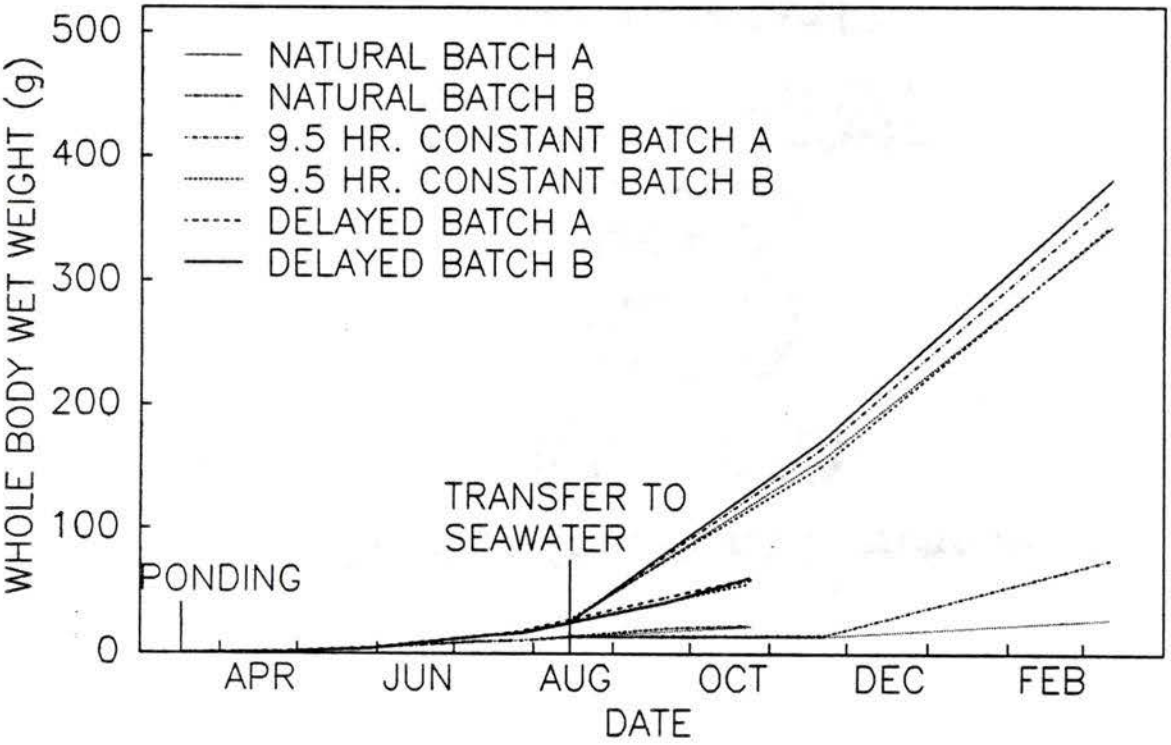


Figure 23: Mean whole body wet weights of the 6 Robertson March 17 ponded batches.

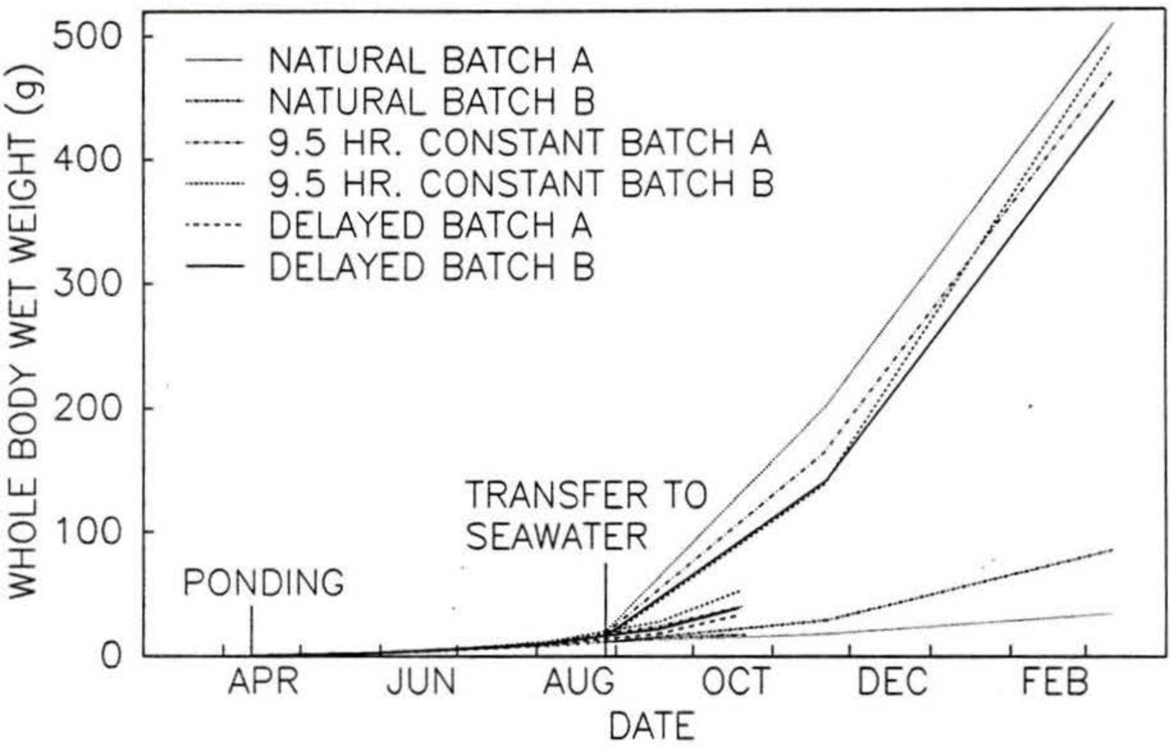


Figure 24: Mean whole body wet weights of the 6 Kitimat April 12 ponded batches.

it can be seen that positions within light treatments toward one end of the rows of barrels were not always greater than means toward the other end.

Analysis of March 11-12 Weight and Fork Length Data

For whole body wet weights determined on March 11-12, 1988, photoperiod treatment (P=0.0001) and ponding date (P=0.0033) were found to have significant effects on mean weight (Table 12). However, for forklengths determined on March 11-12, 1988, photoperiod treatment was found to significantly (P=0.0001) affect mean forklength but ponding date had no significant (P=0.1304) affect on mean forklength (Table 13). Based on the tentative assumption that fork length varies approximately with the cubic root of whole body wet weight, an analysis was run using the cubic roots of fish weight (Table 14). The resulting analysis gave similar results to those found for the variable forklength (cf. Tables 13 and 14).

Although a significant (P=0.0415) interaction effect was found for the variable weight, this was not considered to invalidate the analysis due to the extremely low P-values for the main effects (Table 12).

Table 12: Anova table of whole body weights determined seven months after transfer to seawater. Tests of hypothesis using the type III MS for bat(lgt*grp) as an error term.

Source of variation	df	type III SS	f value	P-value
Main effects				
Photoperiod treatment	2	4,506,149.0	130.22	0.0001
Ponding date	2	400,179.1	11.56	0.0033
Interaction	4	270,512.2	3.91	0.0415

Table 13: Anova table of fork lengths determined seven months after transfer to seawater. Tests of hypothesis using the type III MS for bat(lgt*grp) as an error term.

Source of variation	df	type III SS	f value	P-value
Main effects				
Photoperiod treatment	2	1,055,323.0	128.85	0.0001
Ponding date	2	21,101.3	2.58	0.1304
Interaction	4	19,830.1	1.21	0.3709

Table 14: Anova table of cubic roots of whole body wet weights determined seven months after transfer to seawater. Tests of hypothesis using the type III MS for bat(lgt*grp) as an error term.

Source of variation	df	type III SS	f value	P-value
Main effects				
Photoperiod treatment	2	546.125	135.81	0.0001
Ponding date	2	13.329	3.31	0.0834
Interaction	4	12.594	1.57	0.2642

Using Scheffé's method, multiple comparisons were performed with an experiment-wise $\alpha=0.05$ to find between which photoperiod treatments and between which ponding dates significant differences existed. In comparing photoperiod treatments, the results were consistent for all three variables (Table 15). Both photoperiod manipulated groups were significantly different from the natural photoperiod groups and no significant difference was found between the two photoperiod manipulated groups.

Table 15: Scheffé's Multiple comparisons of photoperiod treatments for the variables of whole body wet weight, forklength and the cubic root of whole body wet weight as determined during the March 11-12, 1988 sampling. Means with the same letter are not significantly different.

VARIABLE	PHOTO- PERIOD TREATMENT	SCHEFFE'S GROUPING	Mean	n
WT (g)	NATURAL	A	74.0	59
	9.5H	B	401.1	183
		B		
	DELAY	B	411.8	203
FL (mm)	NATURAL	A	160.6	59
	9.5H	B	312.7	183
		B		
	DELAY	B	322.7	203
WT ($g^{1/3}$)	NATURAL	A	3.584	59
	9.5H	B	7.028	183
		B		
	DELAY	B	7.277	203

In comparing ponding dates, the results were reasonably consistent for all three variables (Table 16). For the variables weight and cubic root of weight, both March ponding lots were significantly different from the April ponding lot and no significant difference was found between the two March lots. However, for forklength, the March 17 and April 12 ponding lots were not significantly different from each other. For all of the variables a significant difference was found between the earliest and latest ponding dates (Table 16).

Table 16: Scheffé's Multiple comparisons of ponding dates for the variables of whole body wet weight, forklength and the cubic root of whole body wet weight as determined during the March 11-12, 1988 sampling. Means with the same letter are not significantly different.

VARIABLE	PONDING LOTS	SCHEFFE'S GROUPING	Mean	n
WT (g)	MARCH 3	A	317.8	149
		A		
	MARCH 17	A	334.9	128
	APRIL 12	B	423.5	168
FL (mm)	MARCH 3	A	287.6	149
		A		
	MARCH 17	A B	292.1	128
		B		
	APRIL 12	B	309.3	168
WT ($g^{1/3}$)	NATURAL	A	6.430	149
		A		
	9.5H	A	6.561	128
	DELAY	B	7.006	168

For the March 11-12, 1988 sampling, all photoperiod treatments had similar standard deviations despite the larger mean weights of photoperiod manipulated fish (Figure 25).

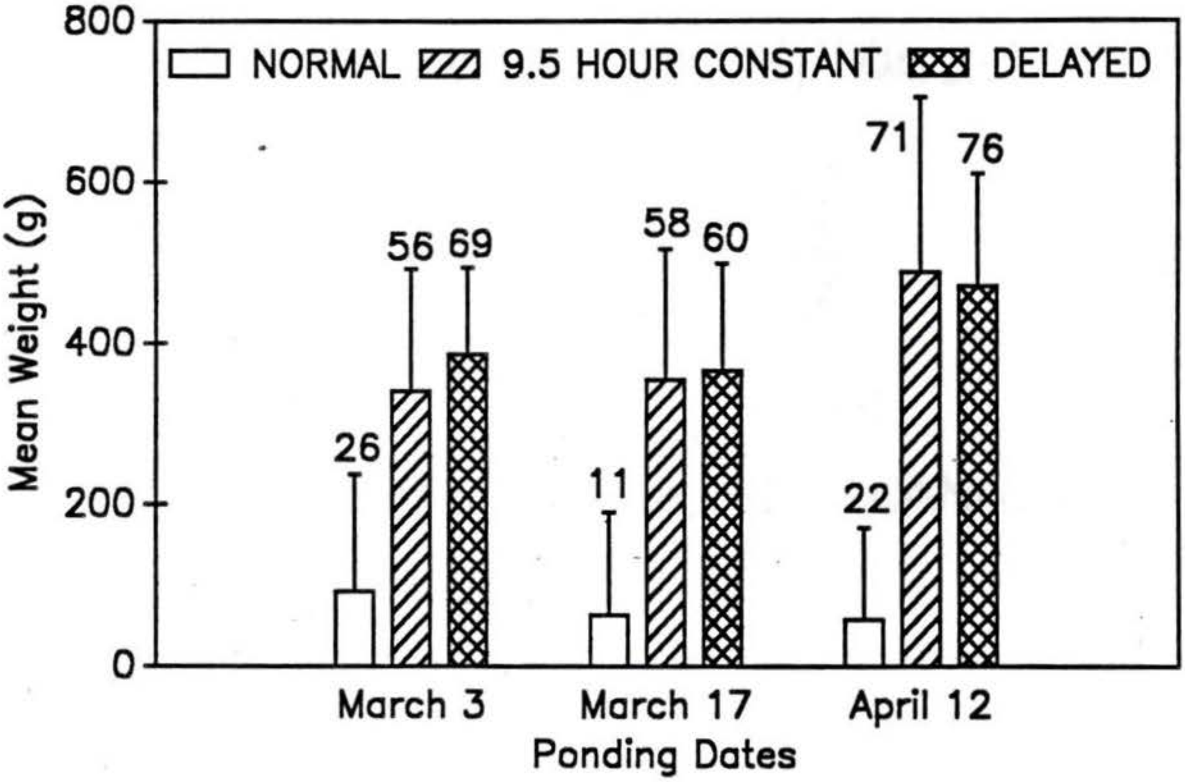


Figure 25: Mean wet weight (± 1 S.D.) of coho salmon for each ponding date and photoperiod treatment combination when measured on March 11-12, 1988. Numbers above bars indicate numbers of post-smolts surviving.

For weights measured in March 1988, within each of the three ponding dates, mean weights of batches within phototoperiod treatments were not significantly different (P=0.5459, 0.6787 and 0.4366, respectively in chronological order of ponding) and significant differences (P=0.0036, 0.0001, and 0.0038, respectively in chronological order of ponding) were found amongst photoperiod treatments. Multiple range testing using Scheffé's method within each of the ponding lots determined that for the variable weight, that natural photoperiod fish were significantly different from both 9.5h constant and delayed photoperiod fish and that the two manipulated photoperiods were not significantly different from each other.

In all ponding lots, groups that had been exposed to photoperiod manipulation had significantly heavier mean weights than did fish exposed to natural photoperiod (Table 17 and Figures 22, 23, and 24).

Table 17: Mean weight, standard error, standard deviation, coefficient of variation and number of fish in sample for all 18 batches sampled March 11 and 12, 1988.
R1 = Robertson lot 1 ponded on March 3; R2 = Robertson lot 2 ponded on March 17; K = Kitimat ponded on April 12.

POND-DATE	PHOTO-PERIOD TREAT-MENT	BAT. (#)	mean WT (g)	1 S.E. (g)	1 S.D. (g)	C.V. (%)	n (#)
R1	NATURAL	A	118	44	175	148	16
		B	52	19	61	117	10
	9.5 H	A	354	30	155	44	27
		B	327	28	150	46	29
	DELAY	A	379	22	122	32	31
		B	393	16	94	24	36
R2	NATURAL	A	28	17	29	104	3
		B	76	53	149	196	8
	9.5 H	A	365	30	157	43	28
		B	344	31	167	49	29
	DELAY	A	344	34	173	50	26
		B	382	15	89	23	34
K	NATURAL	A	34	4	14	41	12
		B	86	53	169	197	10
	9.5 H	A	469	41	253	54	38
		B	509	30	168	33	32
	DELAY	A	492	21	133	27	41
		B	446	25	145	33	35

BIOMASS

Biomass is a useful descriptive statistic as it combines survival and mean weights for all ponding date and photoperiod treatments. However analysis is limited due to lack of measures of variability.

For biomass determined on March 11-12, 1988, photoperiod treatment (P=0.0001) and ponding date (P=0.0015) were found to have significant effects on biomass (Table 18). Although a significant (P=0.0339) interaction effect was found for the variable biomass, this was not considered to invalidate the analysis due to the extremely low P-values for the main effects(Table 18).

Table 18: Anova table of biomass determined seven months after transfer to seawater. Tests of hypothesis using the MS for bat(lgt*grp) as an error term.

Source of variation	df	Sum of Squares	f value	P-value
Main effects				
Photoperiod treatment	2	619204419	117.27	0.0001
Ponding date	2	76965662	314.58	0.0015
Interaction	4	44600797	4.22	0.0339

Scheffé's method revealed that a significant difference existed between fish ponded in March and those ponded in April. However, no significant difference was found between the two March ponding dates.

By Scheffé's method, biomass of natural photoperiod fish was significantly different from both the manipulated photoperiods and the latter were not significantly different from each other. To further compare the biomass of the two photoperiod manipulated treatments, analysis of variance was performed on the two manipulated photoperiod treatments, with the natural photoperiod treatment excluded from the analysis. No significant difference (P=0.1801) was found between the biomasses of fish exposed to the two manipulated photoperiod treatments. However, a one sided t-test, revealed that the biomass of fish exposed to delayed photoperiod treatments was moderately significantly greater (P=0.0901) than the biomass of fish exposed to the 9.5h constant photoperiod treatment.

Analysis of variance excluding fish exposed to natural photoperiod was also run by ponding lots. In all cases, biomass was not significantly different between the two photoperiod manipulated groups. However, the earliest ponding group had an associated P-value of 0.1052 whereas later ponding groups had associated P-values of 0.7100 and 0.7630 for the March 17 and April 12 ponding groups. The associated P-values of the one sided t-test that the biomass of fish exposed to the delayed photoperiod treatment was greater than the biomass of fish exposed to

9.5h constant photoperiod treatment are 0.0526, 0.3550, and 0.3680 for the March 3 and 17, and April 12 ponding dates, respectively. This suggests that the delayed photoperiod is more effective than the 9.5h photoperiod for fish ponded in early March. It is possible that a difference does exist between the two manipulated photoperiods, but that with only two batches per ponding date and photoperiod combination there was insufficient evidence to show a significant difference.

For all ponding dates, biomass of the two photoperiod manipulated groups were greater (significantly different) from natural photoperiod (Table 18). It can also be seen that for all ponding groups, that delayed photoperiod resulted in greater biomass, although not significantly so, at the time of the March 11-12 sampling. The Kitimat lot that was ponded on April 12 had greater biomass than either of the two Robertson lots ponded earlier in the year (Figure 26).

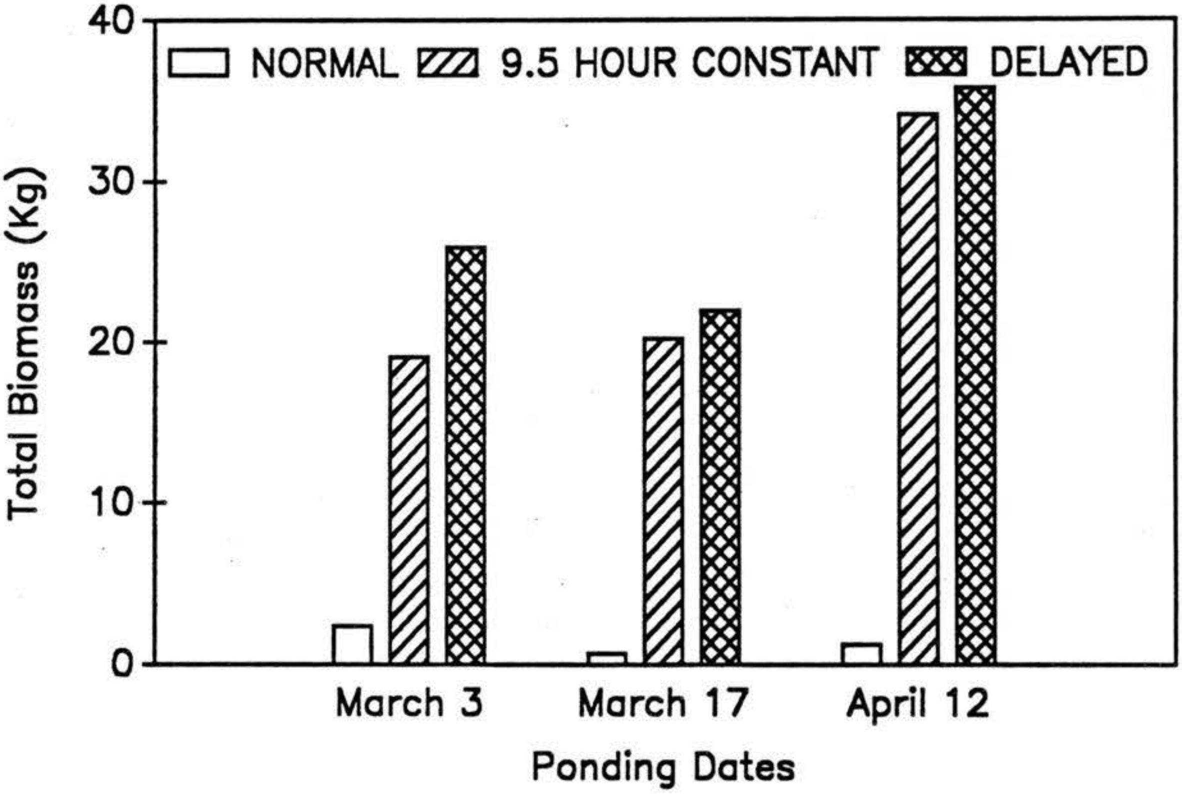


Figure 26: Total biomass of coho salmon 7 months after being transferred into seawater for each ponding date and photoperiod treatment combination.

CORRELATION BETWEEN FRESHWATER SILVERING INDEX AND SURVIVAL AFTER SEVEN MONTHS IN SEAWATER, AND TO MEAN WEIGHT AFTER SEVEN MONTHS IN SEAWATER

The correlation of the nine means for the silvering indices determined in the last freshwater sampling before transfer into seawater (Table 7) with subsequent survival of post-smolts and with subsequent mean weights of post-smolts after approximately seven months of seawater growth was determined (Figures 27 and 28). The resulting correlation coefficients for subsequent seawater survival ($r=0.7243$) and for subsequent mean weight ($r=0.7319$) are both positive and significantly ($0.02<P<0.05$) different from zero. Thus, silvering index in freshwater appears to be linearly associated with subsequent seawater survival. If the correlations are obtained excluding those groups exposed to natural photoperiod, non-significant ($0.20<P<0.50$) negative correlations result.

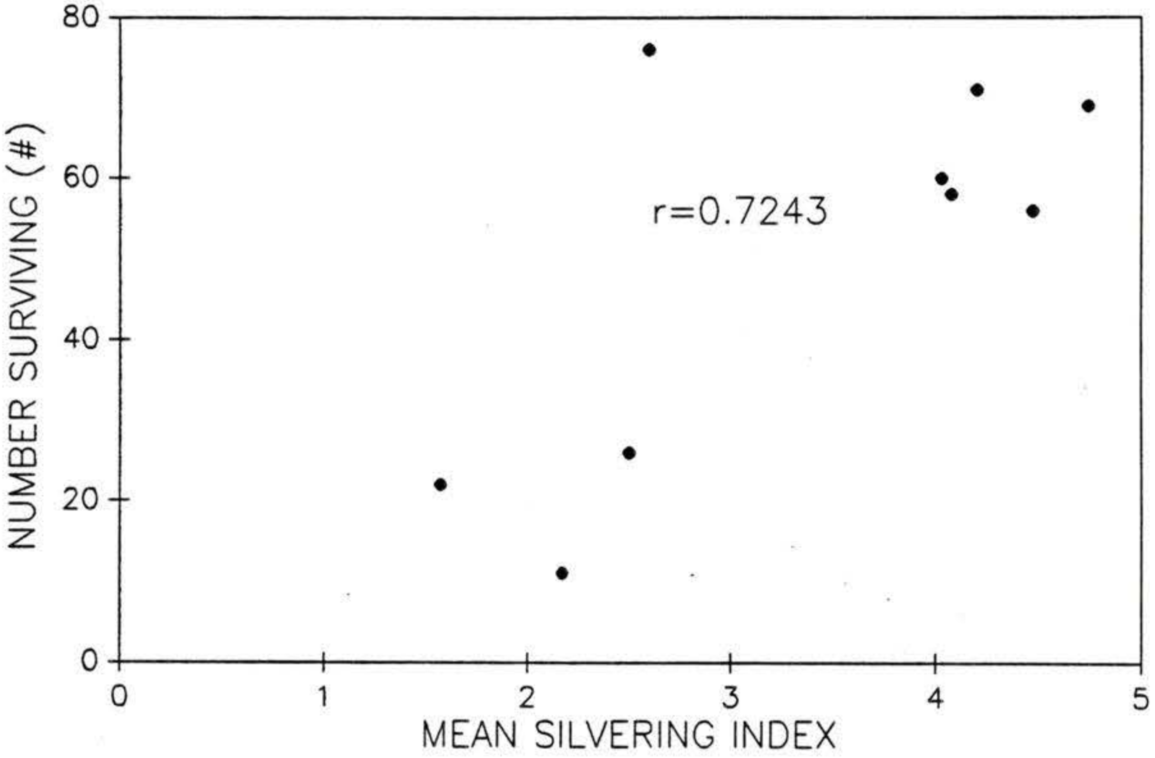


Figure 27: Scatter plot for each ponding date and photoperiod combination of mean silvering index at last freshwater sampling and subsequent survival after approximately seven months in seawater.

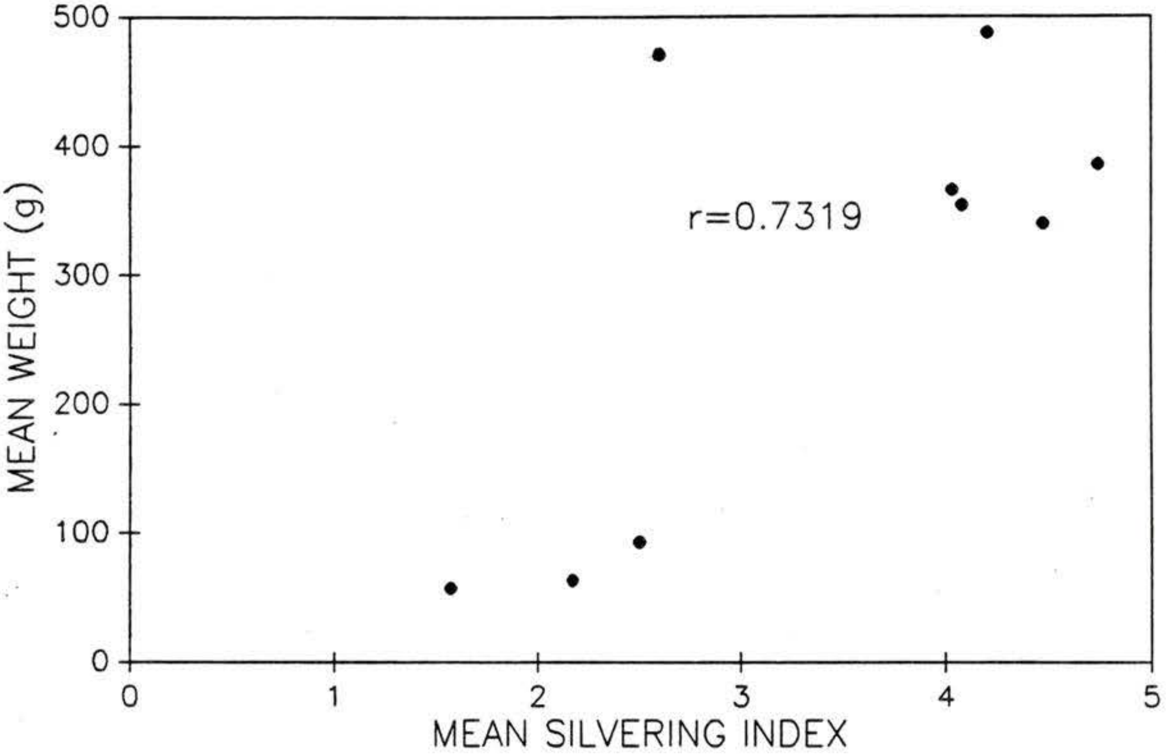


Figure 28: Scatter plot for each ponding date and photoperiod combination of mean silvering index at last freshwater sampling and mean fish weight after approximately seven months of seawater growth.

CORRELATION BETWEEN MEAN WEIGHT AT TIME OF TRANSFER INTO SEAWATER TO SURVIVAL AFTER SEVEN MONTHS IN SEAWATER, AND TO MEAN WEIGHT AFTER SEVEN MONTHS IN SEAWATER, AND TO TOTAL BIOMASS AFTER SEVEN MONTHS IN SEAWATER.

Due to the small number of fish surviving in some batches after seven months in seawater, batches are combined for presentation of the data (Table 19). Mean weights at time of transfer were determined by interpolation using means from the last freshwater sampling before seawater transfer and the means of the first freshwater sampling after 50 fish per batch had been transferred into seawater.

Table 19: Mean weights at time of transfer determined by interpolation, survival of post-smolts after seven months in seawater, and mean weights of post-smolts after seven months in seawater, and total biomass after seven months in seawater.

PHOTOPERIOD TREATMENT	PONDING DATE	TRANSFER MEANS WT (g)	SURVIVAL (#)	SEAWATER MEANS WT (g)	TOTAL BIOMASS WT (kg)
NATURAL	MARCH 3	14.01	26	92.77	2.412
	MARCH 17	13.46	11	63.18	0.695
	APRIL 12	11.75	22	57.32	1.261
9.5 H	MARCH 3	22.99	56	340.45	19.065
	MARCH 17	25.45	58	354.74	20.220
	APRIL 12	19.30	71	487.33	34.113
DELAYED	MARCH 3	23.46	69	386.12	25.870
	MARCH 17	25.98	60	365.93	21.956
	APRIL 12	15.28	76	470.62	35.767

Positive non-significant correlations were obtained for mean weight at time of transfer with subsequent seawater survival ($r=0.638$; $0.05<P<0.10$), with subsequent mean seawater weight ($r=0.637$; $0.05<P<0.10$), and with subsequent biomass after approximately seven months in seawater ($0.10<P<0.20$) (Figures 29, 30, and 31). If the above correlations are obtained excluding those fish exposed to natural photoperiod, significant negative correlations result ($0.02<P<0.05$ for correlation with subsequent survival and mean weight and $0.01<P<0.02$ for correlation with biomass).

The groups with the largest mean weights at time of transfer did not have the best survival when sampled on March 11-12, 1988 (Figure 29). It can be seen in Figure 29 and Table 19 that two groups with similar mean transfer weights 13.46g and 15.28g, had become the groups with the worst and best survivals, respectively, when counted on March 11-12, 1988.

The groups with the largest mean weights at time of transfer did not have the largest mean weights when sampled on March 11-12, 1988 (Figure 30). It can be seen in Figure 30 and Table 19 that two groups with similar mean transfer weights 13.46g and 15.28g, had became the groups with the second smallest and second largest means weights, respectively, when measured on March 11-12, 1988.

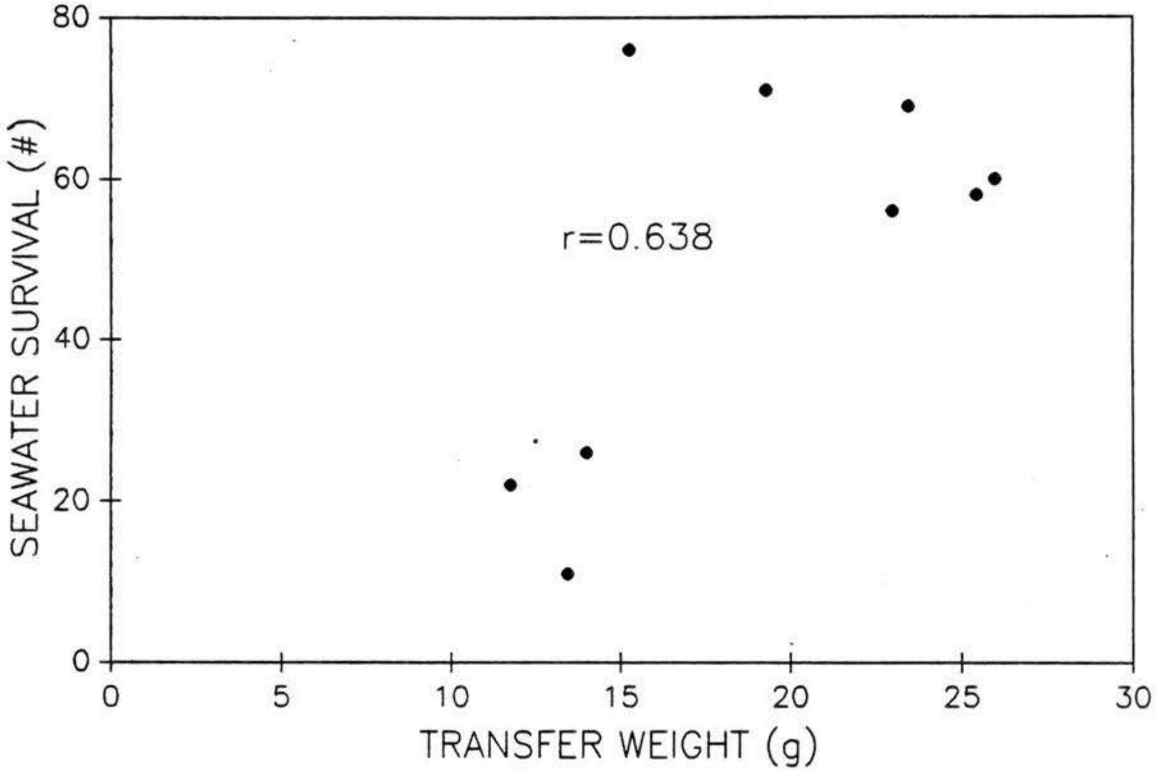


Figure 29: Scatter plot for each ponding date and photoperiod combination of mean interpolated weight at time of transfer into seawater, and survival after seven months in seawater.

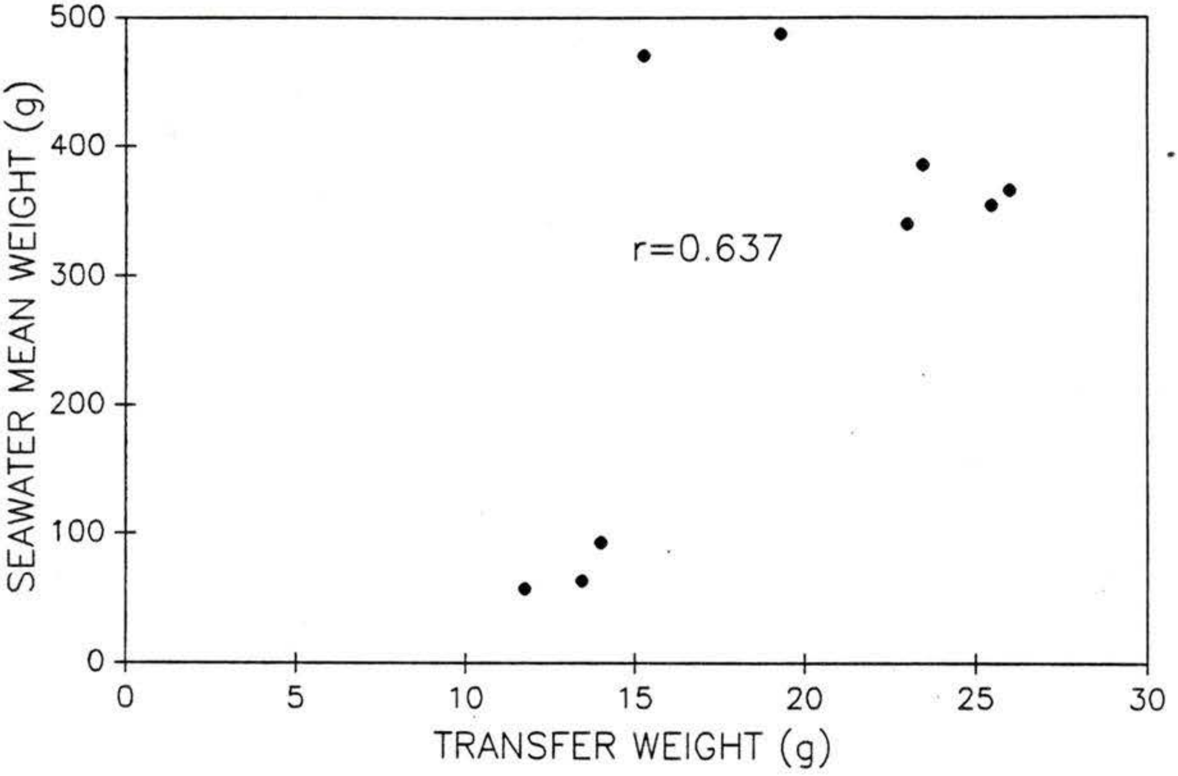


Figure 30: Scatter plot for each ponding date and photoperiod combination of mean interpolated weight at time of transfer into seawater and mean weight after seven months in seawater.

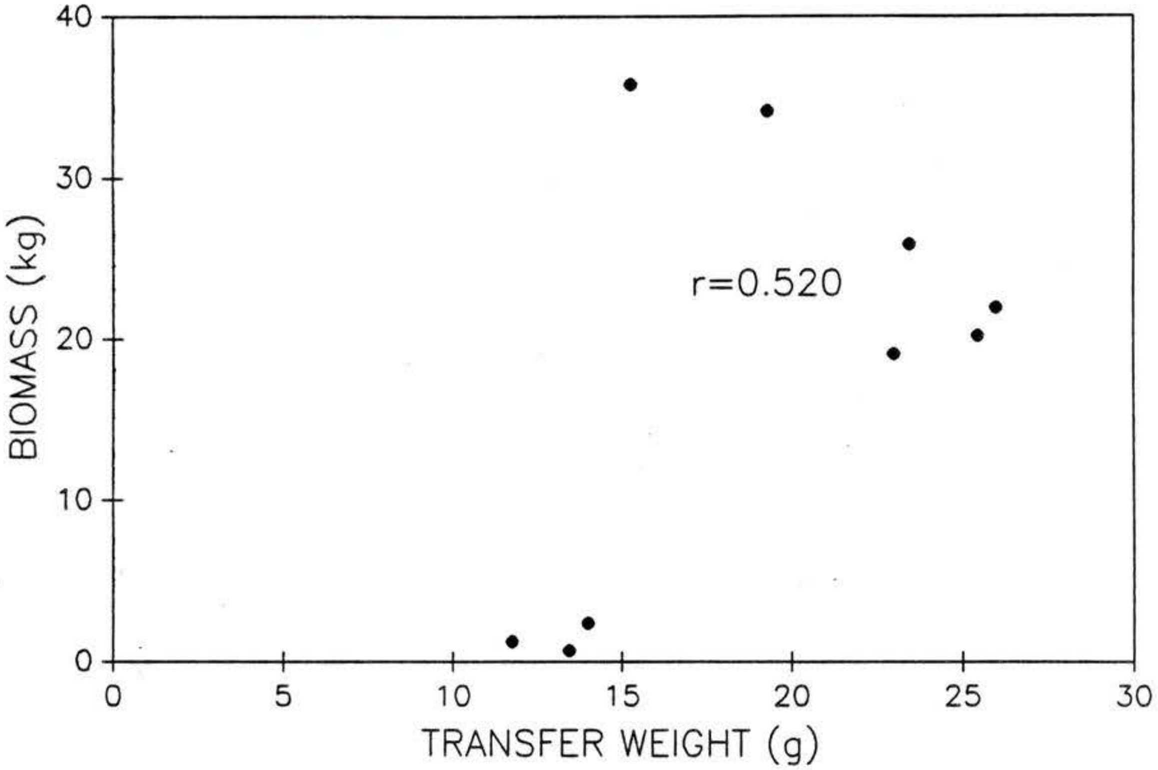


Figure 31: Scatter plot for each ponding date and photoperiod combination of mean interpolated weight at time of transfer into seawater and total biomass after seven months in seawater.

DISCUSSION

The present study has demonstrated that exposure to a three or four month delayed photoperiod increased growth in freshwater and increased seawater survival and growth of coho transferred into seawater in their first summer. Clarke and Shelbourn (1986) demonstrated that exposure of coho salmon to one or two month delayed photoperiod from the time of first feeding, had a profound effect on the growth of coho in fresh water, their capacity to hypoosmoregulate following a 24-h seawater challenge and their ability to grow in seawater. However, until the present study confirmed the increase in seawater survival and growth using a three and four month phase delay, it was not known if increased seawater growth and survival would result when phase delays of greater than two months were used.

Likewise, it was not known until the present study if photoperiod manipulations could be used to induce coho ponded as late as March and April to smoltify sufficiently to allow them to survive and grow well when transferred into seawater in their first summer. The present study demonstrated good survival and growth rates using photoperiod manipulation on coho ponded as late as April 12.

In the present study, both manipulated photoperiods increased the mean weight of fish by July 1987, but no increase in uniformity of weight was found. Clarke and Shelbourn (1986) found a two month delayed photoperiod increased growth rate and uniformity of size of coho after 5.6 months rearing in freshwater at 8, 11, and 14°C. They also found that increasing freshwater rearing temperature from 8°C to 11°C and 14°C was associated both with an increase in growth rate and variability of growth. This increase in variability with increasing rearing temperature may help to explain why Clarke and Shelbourn (1986) found after 5.6 months of freshwater rearing, an increase in uniformity of fish weight associated with photoperiod manipulation whereas none was found in the present study after 4 and 8 months of freshwater rearing in water temperatures ranging as high as 18.8°C.

Photoperiod manipulation to induce early smolting involves two steps; priming and induction. The priming phase, of about two months duration starting at time of ponding, requires exposure to a short-day photoperiod which sensitizes coho fry to subsequent long-day photoperiod (Clarke 1989). The induction phase, starting about two months after time of ponding and continuing until transfer into seawater, requires exposure to long-day photoperiod (Clarke 1989). If coho are exposed to long-day photoperiod during the first two months, the fry become unresponsive to subsequent long-day photoperiods, which inhibits growth and smolting (Clarke 1989). Various levels of night illumination during the first two months, have also been found to inhibit smolting (Thorarensen et al. 1989). Thorarensen et al. (1989) found the threshold for the inhibition may be close to 0.0001 lux. In the present study, the light levels received by those fish exposed to the delayed photoperiods which were uncovered for most of the night and exposed to a floodlight, were greater than this threshold of 0.0001 lux. Thus, the 9.5h constant photoperiod batches which were covered all night might be expected to exhibit better subsequent seawater survival and mean weights than those batches exposed to floodlighting at night. However, the delayed photoperiod groups, which were exposed to flood lighting at night, had non-significantly better survival (69% versus 64%), and non-significantly larger mean weights (412 versus 401g) after seven months in seawater, than did than those coho exposed to 9.5h constant photoperiod which were kept covered by heavy gauge black plastic for the full duration of the simulated night. Therefore, it may be that the fish exposed to delayed photoperiod responded to an abrupt end to daylight or that they were in complete, or almost complete darkness, for a critical period of the day. Perhaps it is not so much light at night that is inhibitory to smolting but light during the first few hours after dark, which was the only part of the night that the delayed photoperiod groups were in complete darkness. This corresponds with the idea of critical times of the day when illumination and darkness are important in triggering smolting as has been demonstrated using skeleton photoperiods by Thorarensen and Clarke (1989). It is interesting to note that in Atlantic salmon (Salmo salar), night illumination has been found to have a stimulatory effect on growth (Bjornsson et al. 1989).

Relating condition factor as determined 3-4 weeks before transfer to seawater, to subsequent seawater survival and growth was considered invalid as sampling was not frequent enough to determine if condition factor had increased or decreased between the last freshwater sampling date before seawater transfer and the time when fish were transferred into seawater. If a reduced condition factor is assumed to be an indicator of smoltification (see below), an increasing condition factor at time of transfer might have indicated that the fish were being transferred too late, and an equal but increasing condition factor at time of transfer might indicate the fish were being transferred too early.

A significant linear association was found between silvering indices determined 3-4 weeks before seawater transfer and subsequent seawater survival and mean weights after seven months in seawater. This association does not mean that silvering indices determined 3-4 weeks before time of transfer into seawater are necessarily a reliable predictive index of smoltification. From Figures 27 and 28, it can be seen that groups of fish with similar mean condition had drastically different seawater survival and mean weights. After seven months in seawater, two groups that had similar mean silvering indices of about 2.5 as measured 3-4 weeks before transfer into seawater, subsequently became one group with a total biomass of 2.3 kg and another with a total biomass of 35.8 kg. This demonstrates the lack of reliability of the mean silvering index, as determined 3-4 weeks before seawater transfer, has for predicting subsequent seawater survival and growth.

The findings in the present study that superficial features such as silvering and low condition factor were not reliable predictive indices are confirmed by other studies. Bjornsson et al. (1989) found that for Atlantic salmon (Salmo salar) there is no general correlation between changes in condition factor and the chain of light-induced changes in growth hormone, growth and hypoosmoregulatory ability. Hoar (1988) in his review article, states that silvering indices and condition factor are not reliable predictive indices, since these features may persist for a time after the readiness to migrate to seawater has passed.

Another index which has been used to predict readiness for seawater transfer is mean weight. In the present study, mean weight at time of transfer into seawater was not significantly correlated, to seawater survival, to mean weight after seven months in seawater, or to total biomass after seven months in seawater. Based on the data, it is possible to speculate, but with little certainty due to the very small number of data points, that 15 to 20g was the optimal transfer weight and groups with either larger or smaller mean weights would experience reduced survival and growth rates (Figures 29 and 30). It can also be speculated that some groups were transferred at their prime and others too late. However, for groups exposed to photoperiod manipulation, the March 17 ponded groups transferred on August 15 had similar biomasses after approximately 7 months in seawater to the March 3 ponded groups transferred on August 4, 1987 (Table 19 and Figure

26). This reduces the plausibility of the last speculation that some groups were transferred too late. A third possibility is that the analysis is being biased by the use of genetically dissimilar lots of fish. If Kitimat fish are excluded from the analysis a positive correlation exists (Table 19 and Figures 29, 30, and 31). Likewise if all lots exposed to natural light are omitted from the analysis, a significant correlation exists, however this time it is a negative correlation. This emphasizes the danger of basing firm conclusions on such a small number of data points. Perhaps the safest speculation is that exposure to photoperiod and genetic inheritance combined are more important to smoltification than mean weight at time of transfer is alone. This is in no way meant to imply that a critical minimum size does not exist.

The most conclusive test of how complete smoltification is when coho are transferred into seawater, is how well they survive and grow after transfer into seawater. Some salmon farms have had coho exposed to a 9.5 h constant photoperiod that grew well for the first 6 weeks after transfer into seawater and then growth tapered off with no appreciable growth noticed after 8 weeks in seawater (pers. comm. Clayton Brenton, General Manager, Dalmar Sea Farms Ltd.). For about 4 months in the fall of

1986, the coho at Dalmar Sea Farms on the east coast of Vancouver Island remained at about 150g (pers. comm. Clayton Brenton, General Manager, Dalmar Sea Farms Ltd.). In the published papers reviewed, growth up to 6 weeks after transfer into seawater of 9.5 hour constant photoperiod coho had been reported but with no follow up on longer term seawater growth being reported (Clarke and Shelbourn 1986). Such photoperiod manipulation would have been of little economic benefit to salmon farming of marketable coho if coho stopped growing after 6 weeks in seawater. In the present study, photoperiod manipulated coho were monitored for seven months in seawater. Although growth rate was not monitored, the mean size of fish in all groups was substantially larger when measured in March 11-12 1988 than it had been when measured the previous November. About 2 months after the March 11-12 sampling the coho were harvested and sold to the restaurant market, thus confirming the applicability of photoperiod manipulation in rearing coho to a marketable size very quickly. By March 11-12, 1988 the total biomass in each of the 3 groups exposed to natural photoperiod was less than 2.5 kg. In comparison, the total biomass in each of the 6 groups exposed to photoperiod manipulation was over 20 kg with mean weight over 340g.

Based on time considerations, the earlier ponded coho would have been growing for a longer period of time and therefore would be expected to be the largest in all samplings performed. This was found for the first few freshwater samplings, however after seven months in seawater the last ponded fish had the largest mean weights. Two plausible explanations for the groups ponded last becoming the largest after approximately seven months in seawater are 1) time of transfer to seawater (see above), and 2) genetic difference (see below). A third possibility is that environmental conditions, including photoperiod, that adults experience during development of their sex products may affect priming or inhibition of smolting of their progeny. Thus Kitimat adults that spawn later in the year, under conditions of shorter days and lower temperatures as compared to Robertson adults, may predispose the Kitimat progeny to quicker growth under the experimental photoperiod and water temperatures they experienced at San Mateo Bay. Genetic versus environmental effects could be separated by holding Robertson creek adults in the Kitimat River under ambient photoperiod during maturation of the sex products and subsequently transporting Robertson Stock milt and eggs and Kitimat milt and eggs to San Mateo Bay where various progeny could be raised under photoperiod manipulated conditions. If progeny of Kitimat stock still performed better in seawater than progeny of Robertson Creek stock, that would indicate that the differences in mean weight and survival in seawater was attributable to genetic variation between the two stocks.

Parr reversion data was non-conclusive in the present study. Although insufficient data exist for statistical analysis, in both the March and the November samplings the groups exhibiting the least number of parr revertants were the Kitimat for ponding date and delayed for photoperiod treatment (Table 9 and Figure 20). Parr reversion data is difficult to analyze due to its dependence on time of sampling. Parr revertants are susceptible to high mortalities, thus their frequency of occurrence often changes substantially between samplings. Although trends have been commented on above, it should be emphasized that these observations may not reflect real differences in number of parr revertants amongst photoperiod treatments and ponding dates.

When measured after approximately seven months in seawater, prior exposure of coho to manipulated photoperiods had resulted in a significant increase in survival, mean weight, mean forklength, and total biomass for all three ponding dates. The total biomass resulting from the exposure of coho to phase delayed photoperiod was 12 % greater (significantly greater P=0.0901) than that resulting from exposure of coho to the 9.5h constant photoperiod. This moderately significant result might reflect that there is actually no difference between the two treatments or that the experimental design did not permit the sensitivity needed to determine if a real difference existed. In hypothesis testing the error term used was the batch (experimental unit) within each photoperiod and ponding date combination. Thus, with a measure of variability within groups being determined from only two experimental units, only 6 degrees of freedom were available for testing the main effects. Therefore, hypothesis testing is not likely to determine small but real differences between levels of the effects. To determine if a difference in the effectiveness of the two manipulated photoperiods actually exists would require performing another experiment with a larger number of experimental units within each photoperiod treatment and ponding date combination. The main conclusion that can be reached comparing the two photoperiods in the present study is that a difference may exist but the results of the present study did not give enough information to confirm a significant difference at the 5% significance level. The present study only showed a significant that groups of coho exposed to delayed photoperiod had greater biomass after seven months than did groups exposed to 9.5h constant photoperiod at the 9% significance level.

Another unmeasured aspect of freshwater rearing, was stress on the fish which might have affected their health and subsequent seawater adaptability and growth. According to many guide books on hatchery management the stress of constant handling, especially when coupled with repeated anesthetizing and measuring of fish should have strongly adversely affected fish health (Anonymous 1971). The extent to which handling stress affected subsequent seawater survival and growth in the present study is not known. In the absence of repeated handling, seawater survival and growth would have perhaps been even better than they were. Efforts were taken throughout the experiment to ensure that all batches received an equal amount of handling. Despite handling and anesthetizing stress many of the fish survived and grew well in seawater.

In the present study, the value and applicability of two similar photoperiod manipulations have been demonstrated under conditions as they existed at at least one intensive salmon culture facility in British Columbia. Furthermore it has been shown that progeny from Kitimat stock ponded as late as April 12, had better seawater survival and mean weights after seven months in seawater, than did Robertson Creek stock ponded a month to a month and a half earlier. Further study is need to determine if the 12 % increase in total biomass resulting from using 3-4 month delayed photoperiod versus a 9.5h constant photoperiod on fish ponded in March and April is a real increase or merely an experimental artifact. If real, a 12 % increase in biomass after seven months in seawater, especially from the group that had received fewer hours of feeding while in freshwater and had greater uniformity of weight after seven months in seawater, should be a favorable prospect for commercial salmon farmers in British Columbia.

SUMMARY

The results of the present study have provided the following information in response to the objectives of the study.

1. Can coho salmon that emerge as swim up fry as late as March or April (3-4 months after the winter solstice) be induced to smoltify in their first summer using delayed photoperiod manipulations? It was found that photoperiod manipulation could induce smoltification in fish ponded in March and April and transferred to seawater during their first summer.

    A. Is it possible using a 3-4 month phase shifted photoperiods on fish ponded in March and April to induce smoltification during the first summer? Both the three and four month phase delayed photoperiods were successful in inducing smoltification in fish transferred to seawater in their first summer, with over 60% of the fish transferred in each ponding lot surviving as post-smolts approximately seven months after transfer into seawater (Figure 19 and Table 20).

    B. Is it possible using a 9.5 hour constant photoperiod for sixty days and then natural photoperiod on fish ponded in March and April to induce smoltification during the first summer? Both the three and four month phase delayed photoperiods were successful in inducing smoltification in fish transferred to seawater in their first summer, with over 55% of the fish transferred in each ponding lot surviving as post-smolt approximately seven months after transfer into seawater (Figure 19 and Table 20).

    C. To compare a 9.5 hr constant photoperiod for sixty days starting at first feeding with a phase delayed photoperiod. Groups of fish exposed to the delayed photoperiod had non-significantly better survival and non-significantly larger mean weights than groups exposed to the 9.5h constant photoperiod (Table 20. This resulted in a moderately significant (P=0.090) 12% increase in biomass (Table 20 and Figure 32). The groups exposed to delayed photoperiod, on average, had greater uniformity of size as determined by visually comparing coefficients of variation and also had fewer parr revertants than those groups exposed to the 9.5 h photoperiod (Table 20).

2. Will the two experimental photoperiods increase uniformity of size?
    A. By time of transfer into seawater? No increase in uniformity of size was found after approximately 4 and 8 months of freshwater rearing.
    B. After at least 4 months in seawater? After approximately seven months in seawater, all groups of fish exposed to manipulated photoperiods had small coefficients of variation in comparison to those fish exposed to the ambient photoperiod at San Mateo Bay (Table 20).
3. Will the two experimental photoperiods increase seawater survival rates? Yes, both manipulated photoperiods increased seawater survival (Table 20).
4. Will the two experimental photoperiods influence an increase in mean whole body wet weight?
    A. By time of transfer into seawater? Within ponding lots, batches that had prior exposure to manipulated photoperiod treatments had greater mean weights (Table 10), although this could not be tested statistically due a highly significant interaction effect (Table 11).
    B. After at least 4 months in seawater? Significantly larger mean weights were recorded in all groups exposed to manipulated photoperiod in comparison to those groups exposed to the ambient photoperiod at San Mateo Bay (Table 20).

Table 20: Seawater survival, mean whole body wet weight, percent coefficient of variation, total biomass, and percent of fish surviving that are parr revertants for control, 9.5 hr constant and delayed photoperiods as measured on March 11-12, 1988.

Photo-Period Treatment	Seawater Survival (%)	Mean Weight ± 1S.D. (g)	Coefficient of Variation (%)	Total Biomass (kg)	Parr Reverts (%)
Control	21	74 ± 129	174	4	5.6
9.5 hr	64	401 ± 194	48	73	2.6
Delayed	69	412 ± 135	33	84	0.5

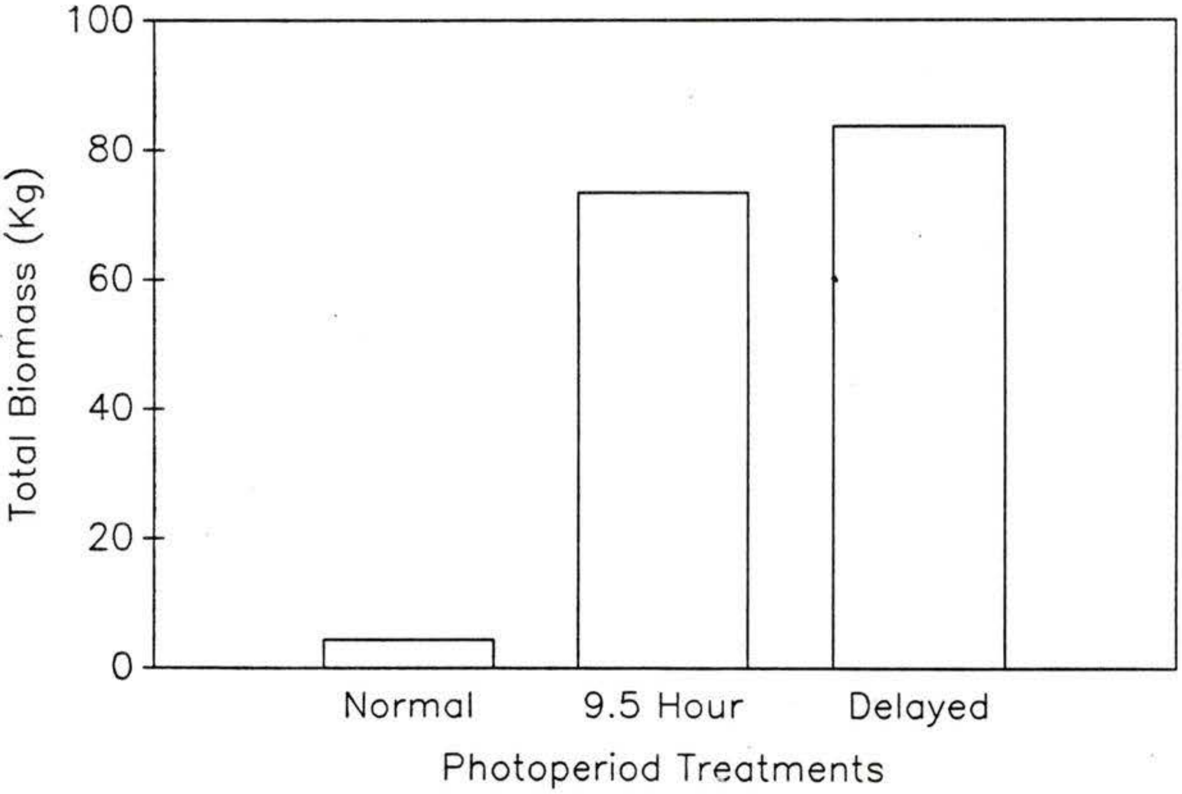


Figure 32: Total biomass of coho salmon after approximately 7 months in seawater for each photoperiod treatment.

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APPENDICES

APPENDIX 1: EVENT TIMING FOR FRESHWATER REARING

note: date of 2nd vaccination of Robertson lots 1 and 2 still missing

Robertson Lot 1

March 3: Ponded and began feeding + wet weight of 200 fish.
April 23/24: Wt/Fl.
May 10: S.W. Challange testing.
May 19-20: Supersaturation of water supply.
May 29: Wt/Fl
May 31: Vaccination
June 30: Deeper water intake 19.5 to 15°C and 9mg D.O.$^*L^{-1}$
June 31: S.W. Challange testing.
July 1-5: Wt/Fl
July 1-5: SW Challange testing
July 5: S.W. Challange testing (3 barrels)
July 22-25: Branding
August 4: Transfer into seawater.
Sept 18-20: S.W. Challange testing.
Sept 20: Wt/Fl
Oct 22: S.W. Challange testing
Oct 23-25: Fl/Wt

Robertson Lot 2

March 17: Ponded and begin feeding + wet weight of 200 fish.
April 23/24: Wt/Fl.
May 12: S.W. Challange testing.
May 19-20: Supersaturation of water supply.
May 29: Wt/Fl.
May 31: Vaccination
June 30: Deeper water intake 19.5 to 15°C and 9mg D.O.$^*L^{-1}$
July 7-11: S.W. Challange testing
July 9-11: Wt/Fl
July 25-27: Branding
August 15: Transfer into seapens
Aug 28-Sept 2: SW Challange testing
Sept 20-22: SW Challange testing
Sept 22: Fl/Wt
Oct 23-25: Wt/Fl

APPENDIX 1: EVENT TIMING FOR FRESHWATER REARING - continued

Kitimat

April 12: Ponded and begin feeding + wet weight of 200 fish.
May 19-20: Supersaturation of water supply.
May 30: Wt/Fl
June 20: Covers taken off 9.5 hour constant:(69 days)
June 26: Wt/Fl
June 27: S.W. Challange testing.
June 30: Deeper water intake 19.5 to 15°C and 9mg D.O.*L^{-1}
July 18: Vaccination
Aug 4-5: Branding
Aug 15: Vaccination
Aug 28: Transfer into seapens
Sept 13: 22 delayed coho died of hypoxia (crow on pipe).
Sept 18: Wt/Fl
Oct 18-20: SW Challange testing
Oct 19-22: Wt/Fl

APPENDIX 2: EVENT TIMING FOR SEAPEN REARING

Aug 4: Transfer of 50 Robertson Lot 1 coho to seapens.
Aug 15: Transfer of 50 Robertson Lot 2 coho to seapens.
Aug 28: Transfer of 50 Kitimat coho to seapens.
Sept 11: Dissolved oxygen measurements
Nov 20-23: Fl/Wt
Nov 23: Storm
Dec 28: Dissolved oxygen
Mar 11-12: Fl/Wt and Branding

APPENDIX 3: SURVIVAL OF COHO APPROXIMATELY SEVEN MONTHS AFTER TRANSFER INTO SEAWATER, INCLUDING AND EXCLUDING PARR REVERTANTS

Table 21: Survival of coho seven months after transfer to seawater, including and excluding parr revertants. Log-likelihood ration goodness-of-fit analysis and subdivision by ponding date and photoperiod treatments are presented. P.R. = Parr revertants included; P.S. = Post smolts only (parr revertants excluded); LGT = photoperiod treatment (1 = natural, 2 = 9.5h constant, 3 = delayed); LOT = ponding date (1 = March 3, 2 = March 17, 3 = April 12); BAT = batch; STRIP = strip of barrels; SURV = number surviving.

DATA SET USED	Comparison of	Controlling for		Likelihood Ratio Chi-Square	
				With P.R. P-value	P.S. P-value
ALL PONDING DATES AND PHOTO-PERIOD TREATMENTS	LGT*SURV			0.000	0.000
	LOT*SURV			0.026	0.005
	BAT*SURV			0.689	0.841
	STRIP*SURV			0.000	0.000
	BARR*SURV			0.000	0.000
	LOT*SURV	LGT=1		0.007	0.017
	LOT*SURV	LGT=2		0.084	0.057
	LOT*SURV	LGT=3		0.072	0.051
	LGT*SURV	LOT=1		0.000	0.000
	LGT*SURV	LOT=2		0.000	0.000
	LGT*SURV	LOT=3		0.000	0.000
	BAT*SURV	LOT=1	LGT=1	0.372	0.170
	BAT*SURV	LOT=1	LGT=2	0.687	0.687
	BAT*SURV	LOT=1	LGT=3	0.129	0.129
	BAT*SURV	LOT=2	LGT=1	0.104	0.104
	BAT*SURV	LOT=2	LGT=2	0.294	0.685
	BAT*SURV	LOT=2	LGT=3	0.150	0.102
	BAT*SURV	LOT=3	LGT=1	0.475	0.629
	BAT*SURV	LOT=3	LGT=2	0.122	0.122
	BAT*SURV	LOT=3	LGT=3	0.158	0.158

Table 21 continued: Survival of coho seven months after transfer to seawater, including and excluding parr revertants.

DATA SET USED	Comparison of	Controlling for		Likelihood Ratio Chi-Square	
				With P.R. P-value	P.S. P-value
MARCH 3 AND MARCH 17 PONDING LOTS	LGT*SURV			0.000	0.000
	LOT*SURV			0.191	0.072
	BAT*SURV			0.072	0.141
	STRIP*SURV			0.000	0.000
	BARR*SURV			0.000	0.000
	LOT*SURV	LGT=1		0.002	0.006
	LOT*SURV	LGT=2		0.193	0.775
	LOT*SURV	LGT=3		0.235	0.183
	LGT*SURV	LOT=1		0.000	0.000
	LGT*SURV	LOT=2		0.000	0.000
	BAT*SURV	LOT=1	LGT=1	0.372	0.170
	BAT*SURV	LOT=1	LGT=2	0.687	0.687
	BAT*SURV	LOT=1	LGT=3	0.129	0.129
	BAT*SURV	LOT=2	LGT=1	0.104	0.104
	BAT*SURV	LOT=2	LGT=2	0.294	0.685
	BAT*SURV	LOT=2	LGT=3	0.150	0.102
MARCH 17 AND APRIL 12 PONDING LOTS	LGT*SURV			0.000	0.000
	LOT*SURV			0.007	0.001
	BAT*SURV			0.935	1.000
	STRIP*SURV			0.000	0.000
	BARR*SURV			0.000	0.000
	LOT*SURV	LGT=1		0.023	0.035
	LOT*SURV	LGT=2		0.363	0.054
	LOT*SURV	LGT=3		0.022	0.015
	LGT*SURV	LOT=2		0.000	0.000
	LGT*SURV	LOT=3		0.000	0.000
	BAT*SURV	LOT=2	LGT=1	0.104	0.104
	BAT*SURV	LOT=2	LGT=2	0.294	0.685
	BAT*SURV	LOT=2	LGT=3	0.150	0.102
	BAT*SURV	LOT=3	LGT=1	0.475	0.629
	BAT*SURV	LOT=3	LGT=2	0.122	0.122
	BAT*SURV	LOT=3	LGT=3	0.158	0.158

Table 21 continued: Survival of coho seven months after transfer to seawater, including and excluding parr revertants.

DATA SET USED	Comparison of	Controlling for		Likelihood Ratio Chi-Square With P.R. P-value	Likelihood Ratio Chi-Square P.S. P-value
MARCH 3 AND APRIL 12 PONDING LOTS	LGT*SURV			0.000	0.000
	LOT*SURV			0.164	0.141
	BAT*SURV			0.368	0.326
	STRIP*SURV			0.000	0.000
	BARR*SURV			0.000	0.000
	LOT*SURV	LGT=1		0.417	0.508
	LOT*SURV	LGT=2		0.027	0.027
	LOT*SURV	LGT=3		0.267	0.267
	LGT*SURV	LOT=1		0.000	0.000
	LGT*SURV	LOT=3		0.000	0.000
	BAT*SURV	LOT=1	LGT=1	0.372	0.170
	BAT*SURV	LOT=1	LGT=2	0.687	0.687
	BAT*SURV	LOT=1	LGT=3	0.129	0.129
	BAT*SURV	LOT=3	LGT=1	0.475	0.629
	BAT*SURV	LOT=3	LGT=2	0.122	0.122
	BAT*SURV	LOT=3	LGT=3	0.158	0.158
NATURAL AND 9.5H CONSTANT PHOTO PERIOD-TREATMENTS	LGT*SURV			0.000	0.000
	LOT*SURV			0.189	0.050
	BAT*SURV			0.869	0.618
	STRIP*SURV			0.000	0.000
	BARR*SURV			0.000	0.000
	LOT*SURV	LGT=1		0.007	0.017
	LOT*SURV	LGT=2		0.084	0.057
	LGT*SURV	LOT=1		0.000	0.000
	LGT*SURV	LOT=2		0.000	0.000
	LGT*SURV	LOT=3		0.000	0.000
	BAT*SURV	LOT=1	LGT=1	0.372	0.170
	BAT*SURV	LOT=1	LGT=2	0.687	0.687
	BAT*SURV	LOT=2	LGT=1	0.104	0.104
	BAT*SURV	LOT=2	LGT=2	0.294	0.685
	BAT*SURV	LOT=3	LGT=1	0.475	0.629
	BAT*SURV	LOT=3	LGT=2	0.122	0.122

Table 21 continued: Survival of coho seven months after transfer to seawater, including and excluding parr revertants.

DATA SET USED	Comparison of	Controlling for	Likelihood Ratio Chi-Square	
			With P.R. P-value	P.S. P-value
9.5H CONSTANT AND DELAYED PHOTO-PERIOD TREATMENTS	LGT*SURV		0.226	0.087
	LOT*SURV		0.029	0.006
	BAT*SURV		0.489	0.608
	STRIP*SURV		0.483	0.230
	BARR*SURV		0.027	0.011
	LOT*SURV	LGT=2	0.084	0.057
	LOT*SURV	LGT=3	0.072	0.051
	LGT*SURV	LOT=1	0.057	0.057
	LGT*SURV	LOT=2	0.558	0.774
	LGT*SURV	LOT=3	0.423	0.423
	BAT*SURV	LOT=1 LGT=2	0.687	0.687
	BAT*SURV	LOT=1 LGT=3	0.129	0.129
	BAT*SURV	LOT=2 LGT=2	0.294	0.685
	BAT*SURV	LOT=2 LGT=3	0.150	0.102
	BAT*SURV	LOT=3 LGT=2	0.122	0.122
	BAT*SURV	LOT=3 LGT=3	0.158	0.158
NATURAL AND DELAYED PHOTO-PERIOD TREATMENTS	LGT*SURV		0.000	0.000
	LOT*SURV		0.010	0.011
	BAT*SURV		0.622	0.622
	STRIP*SURV		0.000	0.000
	BARR*SURV		0.000	0.000
	LOT*SURV	LGT=1	0.007	0.017
	LOT*SURV	LGT=3	0.072	0.051
	LGT*SURV	LOT=1	0.000	0.000
	LGT*SURV	LOT=2	0.000	0.000
	LGT*SURV	LOT=3	0.000	0.000
	BAT*SURV	LOT=1 LGT=1	0.372	0.170
	BAT*SURV	LOT=1 LGT=3	0.129	0.129
	BAT*SURV	LOT=2 LGT=1	0.104	0.104
	BAT*SURV	LOT=2 LGT=3	0.150	0.102
	BAT*SURV	LOT=3 LGT=1	0.475	0.629
	BAT*SURV	LOT=3 LGT=3	0.158	0.158

APPENDIX 4: EFFECT OF FRESHWATER BATCH POSITION ON SUBSEQUENT SEAWATER MEAN WEIGHTS, FORKLENGTHS AND BIOMASSES, OF THE MARCH 11-12, 1988 SAMPLING

Table 22: Mean weight, mean forklength and mean biomasses for March 11-12, 1988 sampling sorted for comparison by position. NAT=Natural, 9.5H=9.5 hour constant, Delay= Delayed, R1=Robertson lot 1, R2=Robertson lot 2, K=Kitimat.

PHOTO PERIOD TREAT-	POND- ING DATE MENT	POSITION[1]	N[2] (#)	MEAN WT (g)	MEAN FL (mm)	N[3] (#)	MEAN BIOMASS (g)
ALL	ALL	A	222	364.1	295.7	9	8982
ALL	ALL	B	223	361.1	298.5	9	8947
NAT	ALL	A	31	76.6	161.5	3	791
NAT	ALL	B	28	71.2	159.6	3	665
9.5H	ALL	A	93	404.4	313.2	3	12538
9.5H	ALL	B	90	397.6	312.2	3	11928
DELAY	ALL	A	98	416.9	321.6	3	13617
DELAY	ALL	B	105	407.1	323.7	3	14248
NAT	R1	A	16	118.0	184.5	1	1888
NAT	R1	B	10	52.4	156.9	1	524
NAT	R2	A	3	28.0	125.7	1	84
NAT	R2	B	8	76.4	156.3	1	611
NAT	K	A	12	33.5	139.9	1	402
NAT	K	B	10	85.9	164.9	1	859
9.5H	R1	A	27	354.4	306.8	1	9569
9.5H	R1	B	29	327.4	297.0	1	9496
9.5H	R2	A	28	365.4	307.2	1	10232
9.5H	R2	B	29	344.4	295.4	1	9988
9.5H	K	A	38	468.7	322.4	1	17812
9.5H	K	B	32	509.4	341.3	1	16301
DELAY	R1	A	31	378.5	315.9	1	11735
DELAY	R1	B	36	392.6	323.3	1	14135
DELAY	R2	A	26	344.5	294.6	1	8956
DELAY	R2	B	34	382.4	321.6	1	13000
DELAY	K	A	41	491.7	343.0	1	20159
DELAY	K	B	35	445.9	326.1	1	15608

[1] Position and batch are often interchangeable terms. Position is used to describe the physical locations of the experimental units during freshwater rearing (Figures 4 and 5) whereas, batch refers to an experimental unit within each of the photoperiod treatment and ponding date combinations. Batch A=Position A and Batch B=Position B

[2] Survival of post smolts and sample size for mean weights and forklengths

[3] Sample size for mean biomasses

APPENDIX 5: VARIANCES OF WHOLE BODY WET WEIGHT, FORKLENGTH AND CUBIC ROOT OF WHOLE BODY WET WEIGHT

The Fmax-test relies on the tabulated cumulative probability distribution of a statistic which is the variance ratio of the largest to the smallest of several sample variances (Sokal and Rohlf 1969). The cumulative probability distribution of Fmax $\alpha[a,n-1]$ assumes that the df of all variances are equal, but an approximate test can be made using the lesser of the degrees of freedom of the two variances (Sokal and Rohlf 1969). Fmax tables in Rohlf and Sokal (1969) were used.

In the following tables, the highest variance, and a considerable contributor to the heterogeneity of variance found is in batch A of 9.5h photoperiod treated coho that were ponded on April 12, 1987. Removing this batch would in most cases reduce the Fmax value found to less than the critical value at $P=0.05$, especially when considering combined batches.

Table 23: Variances of each of the 2 batches within each of the 9 ponding date X photoperiod combinations for whole body weight. Variances when batches combined are also shown.

Ponding Date	Photo-period	Variance		
		Bat=A	Bat=B	Combined
March 3	Nat	30643	3704	20779
	9.5	23988	22486	22972
	Delay	14997	8874	11573
March 17	Nat	819	22239	16242
	9.5	24772	27779	25945
	Delay	30083	7849	17495
April 12	Nat	186	28489	13020
	9.5	64325	28069	47472
	Delay	17719	20991	19492

For batches separate, largest ratio is: 64325/186 = 346
For batches combined, largest ratio is: 47472/11573 = 4
For batches combined, Fmax 0.05[9,66] = 2.26
therefore, variance ratio considered significant at $P<0.01$.

Table 24: Variances of each of the 2 batches within each of the 9 ponding date X photoperiod combinations for cubic root of whole body weight. Variances when batches combined are also shown.

Ponding Date	Photo-period	Variance		
		Bat=A	Bat=B	Combined
March 3	Nat	3.2692	1.0087	2.4429
	9.5	2.2732	2.1843	2.1952
	Delay	1.1853	0.4676	0.7941
March 17	Nat	0.9545	2.9638	2.3511
	9.5	2.0922	3.1582	2.6062
	Delay	2.8584	0.3438	1.4801
April 12	Nat	0.2223	2.8182	1.3866
	9.5	4.3614	2.2040	3.3870
	Delay	1.2062	2.2831	1.7112

For batches separate, largest ratio is: 4.3614/0.2223 = 20
For batches combined, largest ratio is: 3.3870/0.7941 = 4
For batches combined, Fmax 0.05[9,66] = 2.26
therefore, variance ratio considered significant at $P<0.01$.

Table 25: Variances of each of the 2 batches within each of the 9 ponding date X photoperiod combinations for fork length. Variances when batches combined are also shown.

Ponding Date	Photo-period	Variance		
		Bat=A	Bat=B	Combined
March 3	Nat	6623.1	2103.2	4918.5
	9.5	4537.4	4303.0	4360.1
	Delay	2597.6	851.7	1646.2
March 17	Nat	1545.3	5346.2	4255.5
	9.5	4041.7	5840.4	4904.3
	Delay	5337.9	651.8	2808.6
April 12	Nat	262.8	5623.9	2710.0
	9.5	8663.9	4083.1	6572.0
	Delay	2268.9	4238.5	3203.5

For batches separate, largest ratio is: 8663.9/262.8 = 33
For batches combined, largest ratio is: 6572.0/1646.2 = 4
For batches combined, Fmax 0.05[9,66] = 2.26
therefore, variance ratio considered significant at $P<0.01$.

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