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## NORTHWEST FISHERIES CENTER

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# PROGRESS REPORT ON ESTUARINE HUSBANDRY RESEARCH OF $197 \uparrow$ ² AND 1973 BROOD SALMONIDS 

 AT LITTLE PORT WALTER, ALASKA, JANUARY 1-DECEMBER 31, 1974 byWilliam R. Heard, Roy M. Martin, and Alex C. Wertheimer



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PROGRESS REPORT ON ESTUARINE HUSBANDRY RESEARCH OF 1972 AND 1973 BROOD SALMONIDS AT LITTLE PORT WALTER, ALASKA, JANUARY 1-DECEMBER 31, 1974 l/

## BACKGROUND AND OBJECTIVES

During the spring of 1972 the Alaska Department of Fish and Game (ADFGG) and the National Marine Fisheries Service (NMFS) entered into a cooperative research effort to test and develop the concept of fry-to-smolt salmonid husbandry in estuarine pens at the Little Port Walter (LPW) field station on south Baranof Island. This concept, based in part on preliminary pen-rearing studies at LPW and elsewhere, may represent a viable method of artificially producing smolts in Alaska without the expense associated with standard hatchery rearing facilities. If the pen-reared smolt concept proves to be biologically and economically feasible, ADF\&G can utilize it in fishery enhancement and developmental programs. In addition to the cooperative program at LPW, ADF\&G is simultaneously testing three additional pilot estuarine pen-rearing facilities, one in the Kachemak Bay area of Cook Inlet, one at Starrigavan Bay near Sitka, and one in Auke Bay at Fish Creek near Juneau.

Overall objectives of estuarine husbandry research at LPW are to continue feasibility studies on (1) fry-to-smolt culture of those species that normally grow to smolt stage in freshwater nursery areas (coho, chinook, and sockeye) and (2) short-term culture of those species that normally go directly to sea as fry (pink and chum). The principal emphasis will continue to be on fry-to-smolt culture of coho. An integral part of these broad objectives is the development, testing, and refinement of procedures, equipment, and materials--in essence the methodology and technology--required for this type of husbandry.

Although most estuarine husbandry research at LPW has occurred in floating pens with nylon mesh nets, we are also testing various types of floating raceways (both horizontal and vertical in configuration) for use either independently or in concert with nets to achieve the overall program objectives.

This progress report is organized in three separate parts to cover the estuarine husbandry activity at LPW during the period January 1 through December 31, 1974. The three parts are: Part 1--Overwinter and Spring Fry-to-Smolt Culture of 1972 Brood Coho Salmon; Part 2--Short-Term PenRearing of 1973 Brood Pink and Chum Salmon; and Part 3--Initial Fry-toSmolt Husbandry of 1973 Brood Sockeye Salmon.

PART 1--OVERWINTER AND SPRING FRY-TO-SMOLT CULTURE OF 1972 BROOD COHO SALMON
Fry-to-smolt husbandry of coho in estuarine pens at LPW falls naturally into three basic temporal sequences. These sequences or phases relate to natural biological events (such as fry emergence, initial feeding and primary growth, fry-to-parr development, parr-smolt transformation, and

1/Wertheimer, Fisheries Technician, was also temporarily employed by the Alaska Department of Fish and Game during part of the period covered by this report.
smolt emigration) that occur in a step-by-step yearly pattern. The three husbandry phases and their salient events are Phase 1: late spring, summer, and early fall (initial husbandry with primary growth and fry to advanced parr development); Phase 2: late fall, winter, and early spring (overwinter husbandry with no growth and initial parr-smolt transformation); and Phase 3: late spring and early summer (terminal husbandry with secondary growth, final parr-smolt transformation, and smolt release).

This general husbandry pattern would also apply to other species such as chinook or sockeye salmon where the end result was an age 1 smolt coinciding roughly with the downstream migration and initial ocean 1ife of wild smolts from freshwater nursery areas.

The specific time intervals in the three husbandry phases depend upon the seasonal climatic events of a given calendar period. Of particular significance in the estuarine pen-rearing program at LPW is the necessity to achieve sufficient growth at low and intermediate salinities during the Phase 1 time period to enable juvenile coho to successfully osmoregulate and maintain good health at high salinities during the Phase 2 time period. Winter husbandry conditions at LPW are frequently characterized by heavy snowfall and severe surface icing conditions in the estuary. This necessitates that Phase 2 husbandry occur in deep nets that deemphasize surface water layers. A1so, even when the estuary is not ice covered during the winter, surface water salinities are higher than at other times due to reduced freshwater runoff. The general temporal relations of the three fry-to-smolt husbandry periods are illustrated (Figure 1) in a schematic diagram. The areas of potential overlap between Phase 1 and 2 and between Phase 2 and 3 husbandry depends on the relative mildness of weather during fall and spring months.
Significant growth does not occur during most Phase 2 husbandry due to cold water temperatures. However, two important biological events do occur during this period--husbandry in fall seawater salinities and initial parr-smolt transformation. Neither event can occur successfully unless fish are of sufficient size. Overall success in the LPW estuarine rearing program therefore hinges on growth achieved in the 120- to 150-day primary growth period of Phase 1 husbandry. Based on several lines of evidence, mean lengths and weights of coho should equal or exceed 75 mm (fork length) and 5 g ( 80 fish per pound) at the end of this period. Husbandry activity associated with 1972 brood coho at LPW during Phase 1 (summer and early fall of 1973) was covered in an earlier progress report.

Winter (Phase 2) Husbandry
Approximately 280,0001972 brood coho salmon were arranged in 12 separate estuarine pens at LPW between September and November 1973 for overwinter husbandry. Each pen was maintained as a discrete population unit


Figure 1.--Generalized schematic diagram showing three temporal phases of coho salmon fry-tosmolt husbandry in estuarine pens at Little Port Walter over a hypothetical 12- to 15 -month period. Shaded areas between phases indicate periods of possible overlap depending on specific weather. Labeled horizontal arrows denote growth and physiological sequences. Vertical arrows on growth trend line identify key time-size events expressed in mean fork length. Single vertical arrow defines threshold size for successful acclimation to full seawater salinities and overwinter parr-smolt transformation. Double vertical arrows identify target time-size sequence for age I smolts. Dashed beginnings of Phase 1 and trend line indicate variable starting time for initial fry husbandry.
throughout the winter period. The usual procedure at LPW is to maintain individual population identities until that particular population (pen) is changed by subdividing or combining it with others. Daily or cumulative dead fish, increments in mean weight, length frequencies, and other pertinent biological data are maintained on the population unit basis. The population units, overall dimensions of the nets, mesh size of net webbing, and the inclusive dates between population assessments for each of the 12 overwinter pens are summarized in Table 1. Population assessments are made by determining the total weight of all fish in the population and the average weights of individual fish.

Husbandry nets were cleaned at periodic intervals throughout the winter period. This was done by crowding fish to one portion of the net, pulling part of the webbing to the surface, and washing it with a high pressure spray of water. The procedure was repeated until all or most of the webbing in the net was cleaned. The 12 nets were cleaned in this manner during the winter at least monthly. By late February, as lengthening photoperiod and increased light during a period of clear weather began to increase biofouling growths on webbing, the nets were cleaned at about 2 -week intervals. This net-cleaning procedure was not entirely satisfactory due to crowding fish into small areas that had loose quantities of biofouling growths, uneaten food, and fecal material. In March we revised the net-cleaning procedure by attaching a flexible 2 -inch hose to the suction intake of a gasoline-operated centrifugal pump and "vacuuming" loose material from the bottom of the net before fish were crowded and the net webbing washed. This vacuuming procedure was accomplished by divers working the suction hose along the bottom panel of webbing from outside the net. This revised procedure became especially helpful in reducing handing stress when fish were crowded into a small part of the net for any purpose.

In addition to cleaning nets, other routing activities during the winter husbandry period associated with estuarine pen-rearing included feeding fish, removing snow from walkways around nets, operating a compressed air-perforated hose system to control ice formation in specific areas, removing dead and dying fish from nets, and collecting temperature and salinity data. Feeding rates during the winter period varied from 2 to 6 times per day depending on water temperatures, snow and ice conditions, and feeding responses of the fish. Walkway snow removal and surface water ice control was intermittent depending on conditions. Dead and acutely distressed fish accessible with long-handled dip nets were usually removed daily from each pen. A diver in a neoprene wet suit generally removed other accumulated carcasses from the bottom of husbandry nets at about 10-day intervals with a hand-held collecting net. A two-pen recording thermograph with thermocouple probes located at the surface and 2 -meter depth was maintained on the husbandry float. Salinities were determined intermittently with an Findico refractometer from water samples collected at various depths and locations.

Table 1.--Population units, dimensions, and mesh sizes of nets and dates of consecutive population assessments for each of twelve 1972 brood coho salmon populations maintained overwinter during 1973-74 in estuarine husbandry at Little Port Walter

| Population unit (pen number) | Dimensions of net (feet) |  |  | Square mesh size (inch) | Dates of consecutive population assessments |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  |  |  | 1973 | 1974 |
| 25 | 12 | 12 | 12 | 1/4 | Oct. 31 | March 28 |
| 28 | 12 | 12 | 12 | 1/4 | Nov. 2 | March 28 |
| 32 | 12 | 25 | 12 | 1/4 | Oct. 2 | April 1 |
| 33 | 12 | 12 | 12 | 1/4 | Nov. 5 | Apri1 1 |
| 34 | 12 | 12 | 12 | 1/4 | Oct. 1 | March 29 |
| 35 | 12 | 25 | 12 | 1/4 | Sept. 17 | Apri1 1 |
| 36 | 12 | 25 | 12 | 1/4 | Sent. 18 | Anril 1 |
| 37 | 13 | 26 | 8 | 1/4 | Sept. 27 | March 26 |
| 38 | 12 | 12 | 12 | 1/4 | Oct. 2 | March 29 |
| 39 | 10 | 20 | 8 | 1/4 | Sept. 20 | March 26 |
| 40 | 12 | 12 | 10 | 3/8 | Oct. 26 | March 24 |
| 41 | 12 | 25 | 10 | 3/8 | Oct. 30 | Apri1 12 |

$1 /$ Dates when total weight of population determined and the number of fish in the population estimated. This estimate is based on total weight of all fish and average weights of individual fish--expressed as number of fish per pound or kilogram.

An 18- to 20-inch-high barrier fence was positioned around the periphery of each of the 12 overwinter pens to prevent land otter access into the nets. Surface net covers were not practical due to the rapid buildup of snow on the covers. Two types of fences were used. On four pens $(32,35,36$, and 40$)$ the rigid tubular aluminum frames that husbandry nets were secured to were raised so that the top part of the net webbing continuing above the waterline formed the fence. On the remaining pens a temporary wire mesh fence was secured to the flotation collar around the pens. No known otter predation occurred in any pens during the winter, although otter were frequently seen in the vicinity.

## Overwinter Coho Populations

It is convenient to evaluate overwinter status of 1972 brood coho in estuarine pens at LPW on the basis of several parameters for individual population units. The numbers of fish in each population and mean fish weights (expressed as fish per pound) for each of the 12 overwinter pens, based on consecutive population assessments in fall and spring, are given in Table 2. Also given in Table 2 are data on overwinter survival, observed and actual losses for each pen. Overwinter survival is based on changes in numbers of fish in each population. Observed losses are the numbers of dead or dying fish removed from the population, and actual losses are the numerical differences in numbers of fish in the populations between fall and spring.

Overwinter survival ranged from 56.0 percent in Pen 40 to 81.4 percent in Pen 37 (Tab1e 2). Overall survival throughout the winter period, based on the estimated total number of 279,300 juvenile coho salmon in the fall and 186,400 in the spring, was 66.7 percent. Most of the observed loss during this period was disease related; it was primarily associated with continued chronic effects of the presumed vibriosis epidemic that occurred during the late summer of 1973 and of a confirmed vibriosis outbreak that occurred during mid-winter.

A1though the average weight of fish in all pens increased during the winter period (Table 2), it is not possible to distinguish between real growth and biased loss of smaller fish in the populations. Some real growth probably took place especially in the last half of March and early April as water temperatures began rising and feeding responses and food intake increased. No population data, however, were collected that would separate this growth from earlier selective loss of smaller fish during the winter. Two comparative length frequencies from Pen 32 (one based on a random sample October 2, 1973, when the population was established, the other from dead and dying fish on January 8, 1974, during the vibriosis outbreak) clearly show (Figure 2) a disease loss bias of smaller fish. This selective loss is probably related to salinity stress (osmoregulation difficulty) and greater vulnerability to disease pathogens.
Table 2.--Comparisons in 1972 brood coho salmon populations in 12 estuarine pens at Little Port Walter between
fall 1973 and spring 1974 ${ }^{1 /}$ showing number and mean weight of fish, overwinter survival, fish lost (observed and acutal ${ }^{2 /}$ ) and percentage of lost fish observed.

| Population unit (pen number) | Fish in population (thousands) |  | Percentage overwinter survival | Mean weight of fish (fish/1b.) |  | Fish losses (thousands) |  | Percentage of lost fish observed |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Fall | Spring |  | Initial | Final | Observed | Actual |  |
| 25 | 14.2 | 11.1 | 78.2 | 80 | 77 | 1.5 | 3.1 | 48 |
| 28 | 14.2 | 10.8 | 76.1 | 80 | 77 | 2.0 | 3.4 | 58 |
| 32 | 37.1 | 21.4 | 57.7 | 76 | 65 | 12.9 | 15.7 | 82 |
| 33 | 20.5 | 15.1 | 73.6 | 78 | 76 | 4.3 | 5.4 | 80 |
| 34 | 19.4 | 12.0 | 61.9 | 79 | 70 | 5.8 | 7.4 | 78 |
| 35 | 37.9 | 25.3 | 66.8 | 78 | 65 | 10.1 | 12.6 | 80 |
| 36 | 39.8 | 25.0 | 62.8 | 74 | 66 | 13.0 | 14.8 | 88 |
| 37 | 28.0 | 22.8 | 81.4 | 67 | 64 | 5.2 | 5.2 | 100 |
| 38 | 16.9 | 10.8 | 63.9 | 77 | 76 | 4.2 | 6.1 | 69 |
| 39 | 17.4 | 10.0 | 57.5 | 92 | 80 | 5.3 | 7.4 | 72 |
| 40 | 10.0 | 5.6 | 56.0 | 73 | 70 | 0.8 | 4.4 | 18 |
| 41 | 23.9 | 16.5 | 69.0 | 60 | 57 | 1.0 | 7.4 | 14 |
| Total | 279.3 | 186.4 | -- | -- | -- | -- | -- | -- |
| Mean | -- | -- | 66.7 | -- | -- | 66.1 | 92.9 | 71 |

$\underline{1}$ Dates of consecutive population assessments in fall and spring are given in Table 1 .
$\underline{2 /}$ Includes all observed losses and cumulative undetected losses of fish.


Figure 2.--Length frequencies (grouped by $5-\mathrm{nm}$ intervals) of coho salmon in an estuarine husbandry unit (pen 32) at Little Port Walter from a random sample of the population on October 2, 1973, and from dead fish in the population on January 8, 1974, during a mid-winter vibriosis epidemic.

Mean weights were determined from random samples from Pens 34, 35, and 38 in mid-December. A comparison of these data with similar data from the same pens when the populations were established in early October fails to indicate if any real growth was occurring during the early part of the winter. Mean weight of fish from the three pens, expressed as numbers of fish per pound, remained the same in Pen 34 (79 in October versus 78 in December), increased slightly in Pen 35 (73 in October versus 68 in December), and decreased in Pen 38 ( 77 in October versus 86 in December). The lot weights from which the December data were derived were 7.6 pounds from Pen $34,11.4$ pounds from Pen 35, and 7.1 pounds from Pen 38.

The portion of actual loss in the 12 overwinter pens that was observed and recorded varied from 100 percent in Pen 37 to only 14 percent in Pen 41 (Table 2). Actual losses include all observed and cumulative undetected losses of fish. Factors possibly contributing to undetected losses in the pens could include cannibalism, predation by birds and land otters, escapes through webbing (due both to holes and to mesh sizes too large for smaller fish), and to disappearance of dead fish in the pens before they are removed due to invertebrate scavengers (primarily amphipods).

We believe that amphipod scavenging on carcasses was the most important cause of undetected loss of coho in estuarine pens during the 1973-1974 winter period. Amphipod populations in Little Port Walter Bay appear to reach peak numbers during the winter and they are readily observed in the estuarine husbandry pens, especially at night. Two tests were conducted in February 1974 to determine the disappearance rates of juvenile coho carcasses due to amphipod (and possibly other invertebrate) scavenging.

On February 8, 100 coho carcasses were placed in a cylindrical container approximately 2 feet in diameter and 1-foot deep that was covered with $1 / 4$-inch-mesh webbing. The container was suspended in the estuary at the 10 - to 12 -foot depth equivalent to the bottom of the husbandry nets. Ten days later the container was retrieved and no remnants including skeletal features of any carcasses remained. A second test began February 18 when 25 carcasses were placed in the container. The fate of this group was checked daily. After 1 day, fleshy tissue on all but one of the 25 carcasses was gone. Skeletons were essentially intact and could be easily counted. Skeletons from all 25 carcasses were still sufficiently intact to be counted on the second and third days. On the fourth day only 19 individual skeletal components were discernible; by the fifth day, February 23, only 11 carcass remains (mostly craniums) could be counted. A few bones remained on the sixth day, but individual carcasses could not be counted. On the basis of these two tests it appears the 10 -day diver collections of cumulative carcasses (accumulated carcasses that are inaccessible to daily dip net collections) may only account for about 50 percent of the fish that die in the husbandry nets that are not recovered daily.

The accountability of actual losses among the 12 population units was lowest in Pens 40 and 41 where only 18 and 14 percent of the
overwinter loss was recorded (Tab1e 2). Two factors believed to account for this low rate are: (1) these pens had the only $3 / 8$-inch-mesh nets in use; and (2) no 10-day cumulative carcass collections by divers were made in them. Although the populations estab1ished in Pens 40 and 41 in late October were among the largest juvenile coho at LPW, smaller individual fish in these populations could escape through $3 / 8$-inch meshes, especially when fish were crowded into a small area or frightened into the webbing. The $3 / 8$-inch-mesh nets were used only because additional $1 / 4$-inch-mesh nets were not available. We suspect that a significant part of the combined unaccounted overwinter loss of 10,000 fish from these two nets was small fish that escaped through the $3 / 8$-inch-mesh webbing.

## Midwinter Vibriosis Epidemic

Disease losses of 1972 brood coho at LPW reached epidemic proportions during late summer in 1973. A1though a diagnostic culture of the causative pathogen was not made, all symptoms were similar to the confirmed vibriosis epidemic among 1971 brood juvenile coho at LPW during the summer of 1972 . A variety of treatments, including oral drug therapy and environmental modification of the husbandry medium, were initiated to control the pathogen; results of those treatments were covered in an earlier progress report. Roughly one-third of the coho on hand died in a 6 -week period during late summer 1973. Chronic losses of 100 to 200 fish per day (approximately 0.04 to 0.08 percent per day) continued throughout most of the fall and early winter period.
In late December 1973 and early January 1974, disease losses began to increase rapidly. By mid-January losses reached and exceeded 1.0 percent per day ( 2,500 to 3,000 fish per day). This high rate lasted only 4 or 5 days before slackening. Mortality then declined almost to the early December levels before a second increase occurred in late February. This second increase in winter mortality peaked in mid-March at about 0.25 percent per day ( 500 fish per day). The observed overwinter mortality for cumulative 10-day intervals from late November 1973 through late May 1974 illustrates (Figure 3) the relative severity of the two peaks of winter disease losses.

Logistics to and from LPW during the winter are at best very difficult. In an attempt to diagnose the causative pathogen of this winter epidemic a group of terminally ill diseased coho were sent by mail plane to Sitka on January 20 for examination by the ADF\&G fish pathologist. A tentative diagnosis based on microscopic examination of the distribution and morphology of bacteria on several fish implicated both bacterial kidney disease and vibriosis as possible causes of the epidemic.

In mid-February (with the assistance of Adam Moles of the Auke Bay Fisheries Laboratory) several trypticase soy agar (TSA) bacterial culture plates were sent to LPW by mail. The resident technician at LPW innoculated the plates with streaks and smears from dying fish and returned them by subsequent mail flight to Auke Bay for incubation.


Figure 3.--Observed coho salmon mortality in 12 estuarine pens at Little Port Walter summed by approximate 10 -day intervals between November 26, 1973, and May 27, 1974, to show the effects of a winter vibriosis epidemic. The combined populations totaled 279,000 fish in November and 186,000 fish in April.

Isolation and subsequent culture of the principal bacteria provided material for presumptive diagnostic tests. The primary pathogen from the LPW coho was a gram negative salt and oxidase positive bacterium, eliminating Corynibacterium (the causative pathogen of bacterial kidney disease) and providing a reasonably strong presumptive diagnosis for Vibrio spp.

Surface water temperatures varied from $32^{\circ}$ to $42^{\circ} \mathrm{F}$ and averaged about $38^{\circ} \mathrm{F}$ during the period heavy disease losses were occurring (Figure 4). Although temperatures at 2 meters frequently were $3^{\circ}$ to $4^{\circ} \mathrm{F}$ warmer than surface temperatures, the occurrence of a vibriosis epidemic at these low temperatures, to our knowledge, has not been previously reported.

The mean monthly salinities, and ranges, encountered from surface and 2 -meter depths adjacent to the overwinter pens from January through May 1974 are given in Figure 5.

The total loss of 1972 brood coho during winter husbandry was 93,000 fish, about one-third of the number present at the beginning of the winter period. Oral drug therapy using Oregon Moist Pellets (OMP) treated with oxytetracycline (OTC) was initiated on January 20 (just after the peak losses had occurred) and continued until February 23. A total of 290 pounds of medicated OMP was fed at LPW during this interval. Food intake and metabolism were limited because of low water temperatures, and we do not know if any beneficial effect resulted from feeding medicated food.

Vibrio Control with Fresh Water
Vibrio is primarily a marine bacterium. There were indications during the summer of 1973 that transferring Vibrio-infected fish to fresh or low-salinity water reduced the virulence of the pathogen in the fish population. A second test was conducted during the winter vibriosis epidemic to further evaluate the possible beneficial effect of moving infected fish into fresh water.

On January 23, 1974, a random sample of 1,240 juvenile coho was collected from Pen 32 and divided into a freshwater lot of 350 fish and an estuarine lot of 890 fish for further husbandry. The mortality rate in the Pen 32 population at this time was about 200 fish per day (out of an estimated total population of 28,000 fish), and it was assumed that infected fish in the sample were proportional to the overall population. Daily vibriosis losses in Pen 32 had peaked 6 days earlier at 1,163 fish. The coho population in Pen 32 in late. January averaged about 70 fish per pound in weight.

The 350 freshwater fish were put into two aquaria in the LPW wet lab. The aquaria ( 200 fish in a 100-gallon unit; 150 fish in an 80 -gallon unit) each had a 5 gpm single-pass freshwater flow. The 890 estuarine

Figure 4.--Surface water temperature at Little Port Walter estuarine husbandry float, October 1973 to September 1974. Smoothed by a moving average of 2 days.


Figure 5.--Monthly salinities measured from January through May 1974 at irregular 1to 3-day intervals from surface and $2-\mathrm{m}$ depths at Little Port Walter Bay adjacent to the estuarine husbandry pens. Vertical bars are the range of values observed; horizontal bars are mean monthly values. Numbers in parentheses are monthly sample sizes. Other salinities measured at the same depths in husbandry nets approximated these values.
fish were placed in a 4- by 8-by 4 -foot net of $1 / 8$-inch-mesh suspended from the main husbandry float.

Both the freshwater and estuarine lots were fed regularly. Uneaten food, fecal material, and other debris were siphoned from the aquaria twice weekly. To prevent poor tidal flow through the estuarine net because of biofouling growths on the webbing, the estuarine fish were transferred to clean nets on February 9 and 26 and March 10, 17, and 25. To avoid undue handling stress on these fish, replacement nets were installed around the net in use and the fish were gently herded into the clean net through a lowered panel of the soiled net. Dead fish were removed and recorded daily from each lot. The experiment was terminated on March 31. Freshwater temperatures varied from $35^{\circ}$ to $36^{\circ} \mathrm{F}$ during this study and surface water estuarine temperatures from $33^{\circ}$ to $41^{\circ} \mathrm{F}$.

Overall survival of the Vibrio-infected fish during the 67-day period was 89.4 percent in fresh water and 78.2 percent in estuarine water. Of particular interest was the pattern of mortality in the two lots. Although mortality in the estuarine lot occurred at a fairly steady rate throughout the study, in fresh water there was initially a high mortality that quickly subsided to almost zero after 2 weeks (Figure 6). This initial loss in the freshwater lot may have involved terminally diseased fish that were unable to withstand the osmoregulatory shock of moving from salt water back into fresh water. The leveling off of mortality in the freshwater fish suggests the epidemiology and virulence of Vibrio can be at least partially controlled through management of the husbandry environment. The increase in mortality in the estuarine fish in early March (Figure 6) was concurrent with the March increase in mortality among all the estuarine pens (Figure 3).

Spring (Phase 3) Husbandry
Population assessments ending the winter husbandry period were completed in late March and early April. All population units were weighed, subsampled for length frequencies, and transferred to clean nets. Some groups were combined to form new populations, and others were continued as the same population established the previous fall. Eight populations were maintained for varying lengths of time during the spring husbandry period. Most populations were released in late May or early June, coinciding roughly with the timing of peak emigration of wild coho smolts from Sashin Creek.

The beginning and final numbers and mean weights of fish, overall survival, and inclusive dates for each population unit (pen number) maintained during the spring husbandry period are summarized in Table 3. Survival of 1972 brood coho during this period ranged from 81 to 100 percent (average, 93 percent). The lowest survival ( 81 percent in Pen 37) was again associated with the use of a $3 / 8$-inch-mesh net and an unknown


Figure 6.--Mortality of coho salmon taken from a vibriosis-infected estuarine pen population (pen 32) on January 23, 1974, and cultured separately in freshwater and estuarine environments until March 31, 1974.
number of smaller fish escaping from the population. Except for a 2-day period when a $3 / 8$-inch-mesh net was used to temporarily hold a new composite population, all other husbandry nets used during the spring period were $1 / 4$-inch mesh.

Growth in the various populations during the spring period depended in part on how long each population was cultured and when it was released. Except for four lots from the composite population (Pen 44, Table 3) that were released in early April or retained after the principal late May-early June release period, the mean weight of fish in the population at the time of release varied from 37 to 50 fish per pound. In terms of increases in mean weight during the spring, the best growth performance was in Pen 43 where fish increased from 77 to 44 per pound (a 75 percent gain) between March 28 and June 1. Other populations showed similar but slightly less gain over a similar time period. The composite population (Pen 44) averaged 38 fish per pound on May 31. One lot of 10,000 fish from this population averaged 22 fish per pound on June 30 while another lot of 2,000 fish averaged 7 fish per pound on September 6 .

An empirical relationship between mean fork length and number of fish per pound (Figure 7) was derived from a series of 100 observations on 1972 brood coho salmon in estuarine husbandry at LPW. In each observation mean fork lengths (derived from a length frequency histogram) and average numbers of fish per pound were determined from fish from the same population at the same time. The numbers of fish per pound were determined from counts of fish in 1- to 12 -pound samples weighed in water in tare-balanced plastic containers. Numbers of fish in length frequency histograms used to derive a mean length for each observation varied from 80 to 340 fish per histogram.

To define the length-weight relation of individual coho in estuarine husbandry at LPW, we made paired length and weight measurements on 485 fish in 1974. With one exception, the fish were selected at random, usually in conjunction with other population assessment activities; the nonrandom sample was on September 8 when 19 larger fish were selected from the final lot of Population 44. To avoid handling stress and biased weights, fish were not fed on days when length-weight data or any other population assessment data were collected. Fish were anesthetized, measured to the nearest millimeter, blotted once on each side with an absorbent paper towel to remove excess moisture, and weighed to the nearest 0.1 g on an Ohas tri-beam balance.

The dates, population sources, numbers of fish processed, and mean lengths and weights (and ranges) of the five series of individual length-weight observations are summarized in Table 4. These data were averaged to arrive at a mean weight for each length recorded. An empirical length-weight curve was derived from these mean values (Figure 8).
 FORK LENG TH (MILLIME TERS)
Figure 7.--Relation between mean fork length and numbers of fish per pound for 1972 brood coho salmon in the estuarine husbandry program at Little Port Walter. See text for explanation of how values were determined. Curve drawn by inspection.

Tab1e 3.--Comparisons in 1972 brood coho salmon in eight estuarine populations at Little Port Walter during the spring (primarily) of 1974, showing initial and final numbers, mean, weights, survival, and inclusive dates of husbandry. unit (pen number) lation (thousands)


Totals
186.4173 .0

Mean
92.8

1/ The later are dates of release for each population; terminal population assessments including total weight; length frequency and fish per pound data were usuallv collected 3 to 5 days before release.

2/ Includes population units 39 and 40 in Tables 1 and 2.
3/ Includes population units 25 and 28 in Tables 1 and 2.
4/ Includes population units 32,35 , and 36 in Tables 1 and 2. This population was developed as a composite group from which seven different lots of fish were marked and released with coded wire tags between April and September. After population 44 was developed, portions of it were held for varying lengths of time in other nets associated with marking and release of separate lots.

Table 4.--Summary of five series of paired length-weight observations on 1972 brood estuarine pen-reared coho salmon at Little Port Walter during 1974.

| Date | Population unit (pen number) | Number of paired observations | $\begin{aligned} & \text { Fork length (mm) } \\ & \text { Mean } \quad \text { Range } \\ & \hline \end{aligned}$ |  | $\begin{aligned} & \text { Weight (g) } \\ & \hline \text { Mean Range } \end{aligned}$ |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| May 29 | 44 | 103 | 103.3 | 66-139 | 11.2 | 2.3-25.9 |
| May 30 | 38 | 141 | 97.6 | 64-127 | 11.1 | 2.6-19.9 |
| June 29 | 44 | 106 | 127.4 | 96-159 | 20.4 | 8.6-42.9 |
| August 23 | 44 | 116 | 158.1 | 79-215 | 48.9 | 4.0-111.1 |
| September 8 | 44 | 19 | 222.1 | 193-242 | 122.0 | 78.6-146.8 |

Coded Wire Tagging
Eight lots of 1972 brood coho ranging from 2,276 to 10,806 fish per lot were marked with adipose fin marks and coded wire tags before release. The primary purposes of coded wire tagging were to measure (1) the effects of variations in size and time of smolt releases on ocean survival, (2) the effects of variations in husbandry history on ocean survival, and (3) the effects of 1 -day and 11-day exposures of two lots of smolts released in a non-natal area on imprinting and subsequent homing or straying. The source (Population unit), binary code identification, date, size, number released, and primary treatment designations are summarized in Table 5 for the eight lots of coded wire tagged coho.

A composite population (Pen 44, Table 3) was developed at the beginning of the spring husbandry period to provide a randomized pool of estuarine pen-reared coho with similar husbandry histories for most of the marked release lots. Theoretically, this procedure should randomize any population differences in the groups mixed together. Seven of the eight marked lots were from this composite pool. Fish from the one marked lot not from the composite population (data $1 / 4$ from Pen 41) were selected for marking because they may have had a significantly different husbandry history. Coho in Pen 41 were cultured throughout their first summer of life in fresh water without any saline experience. They were transferred to full seawater pens (from freshwater rearing pools on the LPW warehouse dock) without any gradual salinity acclimation at a mean weight of 60 fish per pound in October 1973.

The composite population was initially mixed together in a 12 - by 25 - by 12 -foot net with $3 / 8$-inch mesh and then randomly subdivided temporarily into three 12 - by 12- by 12 -foot nets with $1 / 4$-inch mesh. As individual lots were tagged and arranged for releasing throughout the spring, the remaining portions of the composite population were consolidated into a single 12 - by 25 - by 12 -foot net with $1 / 4$-inch mesh.

During the initial mixing, a significant number of smaller fish were inadvertently graded out of the composite population by escaping through meshes of the $3 / 8$-inch mixing net. A total of 71,700 coho were put into the mixing net on March 31 and April 1. Only 58,500 coho were subsequently removed when the population was subdivided into random groups on April 1, leaving an estimated 13,200 fish that escaped during the mixing and randomizing procedure. Surprisingly, only five fish (all between 80 and 85 mm fork length) were actually gilled in meshes of the mixing net. Although this inadvertent grading of smaller fish from the composite population may limit the validity of direct comparisons between the composite population (Pen 44) and the other unmarked groups released (Table 3), it should not affect direct comparisons between the various lots of marked fish from the composite population (Table 5).
$\underline{3}$ This unit had a varied husbandry history; see text for exnlanation.

After the escape of smaller fish from the composite population a school of several thousand juvenile coho was observed around the husbandry floats and nets. Because no intentional releases had been made and this school was not evident before April 1, there was no doubt that most of the fish were "escapes" from the composite population. Two samples of these fish were collected by suspending a 12-by 12- by 12-foot net with $1 / 4$-inch mesh from a husbandry float so that the net, with the assistance of a diver underwater to herd fish, could be used as a lift net. A sample of 328 of the escaped coho caught on April 7 averaged 73.6 mm fork length (range 52 to 89 mm ). On April 8, 2, 272 were caught with the lift net and marked with coded wire tags (data 1/2, Tab1e 5); they were released on April 9. A sample of 262 of these fish averaged 77.4 mm fork length (range 59 to 97 mm ), while the weight of all 2,272 averaged 101.6 fish per pound.

Fin marking and coded wire tagging operations were done under an open-sided shed on the LPW husbandry float. The wire tags all had Agency 3 (NMFS) binary codes. Agency codes and data codes were verified on samples of wire from each of the eight lots marked.

On the basis of coded wire tagging activities at LPW in 1973 and 1974, a reasonably effective system of processing fish through this marking procedure has evolved. Certain key elements in the procedure, such as a recirculating, filtered, and cooled anesthetic water system, were patterned after successful juvenile salmon-marking programs e1sewhere. Two important features of the LPW system are: (1) considerable flexibility in the manpower requirements and (2) the ability to maximize coded wire tag output with only one tag injector at each level of available manpower. The system can function with as few as two or as many as six people--four is probably the most efficient number.

The coded wire tag marking procedure for estuarine pen-reared fish at LPW begins by crowding fish to be marked to one end or a corner of the husbandry net. This is done with a "crowder bar," starting at the opposite end of the net and working the net webbing over the bar while moving the bar toward the designated corner of net to hold the confined fish. Depending on the size of fish to be marked, 1- to 12 -pound lots were dipped from the husbandry net, weighed (in water) in a tare-balanced plastic container (Figure 9), and stored temporarily (5 to 20 minutes) in covered shallow fiber glass tubs. From these tubs small samples of 20 to 40 fish (depending upon the number of workers available and the prevailing rate of marking) were transferred with dip nets to a compartmentalized anesthetic tray (Figure 10).

The anesthetic tray was located on one end of the main worktable adjacent to the tag injector in such a way that workers moved freely around three sides of the tray. Compartments in the tray were formed with perforated polypropylene plastic dividers so that water in the tray flowed freely between compartments. Inlet and outlet fittings were plumbed into opposite ends of the tray, and anesthetic water flowed from
the outlet fitting through a small 110-volt AC fluid transfer pump (mounted beneath the top shelf of the worktable). Anesthetic water passed from the transfer pump through a 100 -foot-long 1-inch-diameter coiled polyethylene plastic pipe suspended 20 to 30 feet beneath the work shed. This served as a cooling coil to keep anesthetic water tempered to roughly the same ambient water temperatures in the husbandry nets. From the cooling coil, water returned to the inlet side of the anesthetic tray after passing through an improvised cartridge-type glass wool filter to remove particulate material (especially the fleshy lobes of clipped adipose fins).

As fish were anesthetized, the adipose fins were excised with corneal surgical scissors and passed by hand to the anesthetic tray compartment closest to the tag injector (Figures 11 and 12). From this compartment, the tag injector operator moved the fish to the headmold of the tag injector (Figure 13).

To maximize actual operation of the injector and to avoid unnecessary hand motion by the tag injector operator, especially when a backlog of anesthetized fin-marked fish accumulate, an optional step of handing fish to the operator was used. This was done by a designated person placing fish (properly oriented) into the left hand of the operator while he completed the tag injection cycle with the preceding fish in his right hand (Figures 12 and 13). With minimal movement the operator transferred the fish from his left to his right hand in such a manner that the fish (now in the right hand) was close to and in a position for activating the next tag injection cycle. After a few hours experience, a high degree of dexterity in this procedure can be developed, and tag injection rates of 1,000 to 1,200 fish per hour can be achieved. Handing fish to the operator, however, is an optional step and depends on available manpower and other circumstances. At LPW we used this step intermittently for about 60 percent of the actual tagging. Most of our tagging rates averaged around 700 fish per hour for a four-man crew.

The tag injection cycle is activated with a foot switch by the operator when the head of the fish is properly positioned in the headmold of the tag injector (Figure 14). Once the cycle is activated a "critical" 250 -millisecond interval passes when the fish should not move. It is during this interval that the injector needle and wire tag are inserted into the rostrum of the fish.

After the fish is tagged it is placed by the operator in the funnel of the quality control device (QCD). This funnel is visible in the lower left part of Figure 12 and the upper right part of Figure 14. Once in this funnel the fish passes through the QCD which (1) magnetizes the tag in the fish, (2) detects and confirms that the fish contains a microwire tag and that the tag is magnetized, and (3) directs fish that do not pass these steps into a separate recovery container at the lower end of the QCD (Figure 15). After all fish from a weighed sample have been through the tagging procedure, those rejected as not being properly
tagged are again passed through the QCD. If their nontagged status is confirmed, they are retagged with the tag injector. With an experienced, conscientious crew the retagging rate at LPW was usually less than 1 percent of the fish handled.

When tagged fish in the anesthetic recovery tub regained equilibrium, they were transferred to a husbandry net designated for holding the particular lot of marked fish until scheduled release. Random subsamples from coded wire tagged lots were held in separate nets or aquaria (Figure 16) to test the extent of initial shedding of the wire tags. Three test groups (totaling 947 fish held from 1 to 14 days) had a tag retention factor of 0.9989 .

## Smolt Releases

A total of 173,0001972 brood estuarine pen-reared coho were released in the LPW vicinity between early April and early September 1974. This number includes the 13,200 known escapes from the composite population on April 1. Of this total, 65,000 ( 38 percent) were coded wire tagged before release. Excluding two marked lots released at Toledo Harbor (data $1 / 5$ and $1 / 6$, Table 5) all other marked and unmarked lots of 1972 brood estuarine pen-reared coho were released at the floating husbandry facility in LPW Bay. A11 fish were released between 2100 and 0100 hours (depending on the length of daylight) by dropping three sides of the husbandry net. This release procedure prevented any terminal handling stress.

As releases were made throughout the spring and especially after the principal release in late May-early June, large schools of coho smolts were evident in the inner and outer portions of LPW Bay. Schools of coho smolts were also observed during midsummer in several parts of Big Port Walter Bay.

Release of the two lots of coho smolts at Toledo Harbor was designed to measure the effects of short (1-day) and long (11-day) exposure of estuarine pen-reared coho smolts from LPW to non-natal rearing area water on imprinting and subsequent homing and straying. Toledo Harbor (about 20 surface acres in size) is a protected area somewhat circular in shape along the Chatham Strait shoreline about 2 miles south of the entrance to LPW. A narrow entrance to the harbor (Figure 17) provides protection from heavy seas. Toledo Harbor Creek, a stream with observed flows from an estimated 5 to 200 cfs , enters the southwest portion of Toledo Harbor. The stream has no fish fauna, either endemic or introduced, because of a barrier falls at the head of tide (Figure 18).

Two estuarine husbandry pens were towed by skiff to Toledo Harbor from LPW in mid-May and anchored off the mouth of Toledo Harbor Creek. The 11-day exposure lot of coho (data $1 / 5$, Table 5) was coded wire tagged from May 17-19 and transported to one of the husbandry pens at Toledo Harbor on May 20. The 1-day exposure lot for Toledo Harbor (data 1/6)


Figure 9.--Weighing estuarine pen-reared coho smolts in tare-balanced plastic container of water. This is first step in LPW coded wire tagging procedure. After weight of a particular lot of fish is determined, the fish are held temporarily in fiber glass tubs seen in background


Figure 11.--As fish become anesthetized, adipose fins are excised and the fish is passed by hand to the compartment nearest the tag injector (upper left in this photograph).


Figure 10.--Small quantities of fish are transferred by dip net from the fiber glass storage tub to a compartmentalized anesthetic tray. An exact count of fish in each weighed lot is determined.


Figure 12.--Overhead view showing relationship of anesthetic tray to tag injector. Person standing in middle is passing fin-clipped fish by hand to the injector operator, an optional step (see text for explanation).


Figure 13.--Tag injection sequence while using optional step of passing fish by hand to injector operator. As fish in operator's right hand is placed in headmold and the injector is activated by foot switch, a second fish is placed into left hand of operator. When injector cycle is finished, operator places fish in his right hand into funnel of the quality control device about 10 inches to the right of operator's hand. The fish in left hand is now transferred to the right hand with minimal movement. During this transfer from the 1 eft to the right hand, the fish is gently rolled $180^{\circ}$ so that its head is in proper alignment and close to the headmold of the injector.


Figure 15.--As fish pass through the quality control device, tags are magnetized and verified. Improperly marked fish are sorted into separate containers. Note in this sequence that no one is passing fish to the tag injector operator and he is retrieving the next fish to be tagged from anesthetic with his left hand.


Figure 16.--Subsamples of coded wire tagged lots of fish are held in aquaria or nets to determine the initial postmarking shedding rate of tags.
during injection sequence.


Figure 17.--Two lots of EPR coho were released in Toledo Harbor as part of a imprinting-homing study. In this view the mountains of Kuiu Island across Chatham Strait can be seen beyond the narrow entrance to Toledo Harbor.


Figure 18.--Toledo Harbor has no anadromous fish stocks because of a barrier falls just above high tide elevation. Two lots of coho were held and released off the mouth of this stream after 1-day and 11-day exposure periods.
and the control lot (data 1/7) were tagged at LPW from May 20 to 29. Data $1 / 6$ coho were transported to the second husbandry pen at Toledo Harbor on May 30. Both groups of coho taken to the Toledo Harbor pens were transported in fiber glass tubs in an outboard motor powered skiff. As each tub was loaded at LPW, light-proof covers were placed over the tubs and not removed until the coho were transferred into nets at Toledo Harbor. Oxygen stress in the hauling tubs was avoided by limiting the number of fish. With moderately calm seas in Chatham Strait, the time required for the coho smolt hauling trips from LPW to Toledo Harbor (from initial loading to final fish into Toledo Harbor nets) varied from 10 to 12 minutes. The 11-day exposure lot (data $1 / 5$ ) was fed to satiation four times daily at Toledo Harbor from May 21 through May 31.

A coordinated release of both Toledo Harbor lots (data $1 / 5$ and $1 / 6$ ), the marked LPW control lot (data 1/7), and three unmarked populations at LPW (Pens 33, 37, and 42--Table 3) was synchronized at 2300 hours on May 31.

## PART 2--SHORT-TERM PEN REARING OF 1973 BROOD PINK AND CHUM SALMON

Pink and chum salmon fry normally migrate directly into estuarine nurseries upon emerging from natal spawning gravels. An exception to this is that some chum salmon fry in certain circumstances may feed and grow for a short period in freshwater before entering the estuary. Short-term husbandry, primarily of chum salmon fry in estuarine and fresh water ponds, has been conducted in an attempt to improve ocean survival.

Short-term husbandry of pink or chum salmon fry is based on a rationale involving one or more of the following factors: (1) the greatest part of the total marine mortality occurs shortly after fry migrate to their initial estuarine nursery area; (2) theoretically at least, ocean survival can be significantly inproved by producing and releasing a fry larger than normal migrant size; and (3) fry emergence in artificial incubation systems tends to be earlier than emergence of wild fry and may not coincide with suitable environmental conditions in the estuary. Interim husbandry in these situations may provide an overall marine survival advantage.

Pink salmon, which have a 2-year life cycle, probably follow precisely fixed seasonal schedules on the timing of migrations of fry from fresh water to estuaries and from estuarine to oceanic nurseries. Timing may therefore be the single most important biological consideration in any short-term rearing of pink (or chum) salmon fry. During the spring and early summer of 1974 we reared and released a group of 1973 brood Sashin Creek pink and chum salmon in estuarine pens in LPW Bay to begin developing expertise in this area for testing the rationale of some of the above points.

## Adults, Eggs, and Incubation

Adult pink and chum salmon were collected at Sashin Creek weir and held for ripening either in the freshwater "weir pond" immediately downstream from the weir or in holding pens in LPW Bay. A total of 3,606 pink salmon and 67 chum salmon passed through Sashin Creek weir in 1973. of these totals, 335 pinks ( 192 females and 143 males) and 11 chums ( 5 females and 6 males) were retained for gametes, killed for fecundity counts, or otherwise prevented from entering Sashin Creek to spawn naturally. These adults were collected from throughout the run to avoid a genetic or timing bias from one portion of the rm.

In late August and early September, approximately 255,000 pink and 12,000 chum eggs were spawned and placed in Heath incubators. These eggs were picked and reseeded in October after reaching the eyed stage. Survival from spawning to the eyed egg stage was about 97 percent. Eggs were seeded for eyed egg-to-fry stage incubation as follows: (1) pink salmon- $-173,000$ eggs were seeded equally in 38 Heath trays and 75,000 eggs were seeded in a $1-\mathrm{m}^{2}$ circular gravel incubator; (2) chum salmon-12,000 eggs were seeded equally in four Heath trays.

During the spring of 1974, 71,858 pink fry emerged volitionally from the one gravel incubator between March 29 and April 30. These averaged 196 mg per fry (2,328 per pound). A total of 171,000 pink fry were "emerged" from the 38 Heath trays between March 27 and April 3, 1974; they averaged 178 mg per fry (2,550 per pound). An estimated 11,000 chum salmon fry were removed from the four Heath trays on April 3 and 4, 1974; they averaged 400 mg per fry (1,134 per pound).

Estuarine Husbandry
Pink salmon incubated in gravel and Heath units were maintained as separate husbandry entities to determine (1) if differences in fry weight at emergence remained throughout the short-term pen-rearing period and (2) if survival during the husbandry period was the same for both groups. Unfortunately, because of material and equipment limitations and other activities associated with the terminal coho penrearing, we were unable to maintain the two groups of pink fry at the same densities in the same types of husbandry nets. A1though feeding schedules and other husbandry activities were standardized as much as possible, differences in the numbers and sizes of nets and densities of fry in nets negate precise factoral comparisons in postemergent rearing of Heath and gravel pink fry. Heath pink fry were placed in two 12 - by 12 - by 6 -foot nets with $1 / 8$-inch mesh on April 2 (85,000 fry per net). Grave1 pink fry were placed in four 4-by 8-by 4 -foot. nets with $1 / 8$-inch mesh as they emerged between late March and late April (18,000 fry per net).

Overall survival of Heath and gravel pink fry from initial husbandry in nets through June 4 was 67 and 74 percent respectively. Near anoxic water due to clogged meshes in the two Heath fry nets in late May accounted for most of the differential mortality in the two groups. On June 4, Heath and gravel pink fry averaged 695 mg ( 653 per pound) and 643 mg (706 per pound) respectively.

Pink fry were transferred into six 12 - by 12 - by 4 -feet nets with $3 / 16$-inch mesh on June 4 (four Heath fry and two gravel fry nets) and reared until July 3 when all but about 10,000 fish were released. Heath and gravel pink fry averaged 1.58 g (287 per pound) and 1.75 g ( 260 per pound) in weight on July 3. Comparative growth of Heath and gravel pink fry in estuarine pens at LPW between early April and early July is shown in Figure 19. Survival of Heath and gravel pink fry in husbandry nets between June 4 and July 3 was 97 and 91 percent. Overall survival of Heath and gravel pink fry in estuarine husbandry nets from initial numbers of fry through the principal release on July 3 was almost identical, 66.3 and 66.8 percent respectively. The weighted average of 151,285 pink fry released on July 3 was 1.65 g per fry ( 275 per pound) (Table 6).

A combined group of about 10,000 Heath and grave1 pink fry was retained from July 3 until September 6, 1974, in a 12- by 24 - by 12 -foot net with 1/4-inch mesh. Survival of these pinks was excellent throughout a period (July-August) when vibriosis outbreaks occurred at LPW in 1972 and 1973. Estimates of the numbers of fish in the combined Heath-grave1 population were 10,162 on July 3, and 10,467 on August 14. Terminal processing and marking provided an individual count of 10,063 fish on September 4-6. Growth of these fish showed (Figure 20) a twelvefold increase in mean weight from 1.62 g ( 280 per pound) on July 3 to 19.89 g ( 22.8 per pound) on September 5. A total of 9,905 fish were released from this group in early September.
Chum salmon fry were reared in a 4 - by 8 - by 4 -foot net with $1 / 8$-inch mesh from April 4 until June 29. Mean weight of these fish (Figure 19) increased from 0.4 g to 3.2 g ( 140 per pound) during this period. Survival of the 11,000 chum fry from April 4 through June 5 was 88.7 percent. Survival continued high throughout most of June, however, on June 25 land otters entered the chum salmon net and ate approximately 4,200 fry. On June 29, 5,627 chum fry were released. Overall estuarine husbandry survival was 51.2 percent.

Pink and chum salmon fry were fed Oregon Moist Pellets throughout the estuarine husbandry period. They were fed to satiation 8 to 12 times daily. Estimated feeding rates varied from about 4 to 10 percent of body weight per day.

Table 6.--Summary of 1973 brood Sashin Creek pink and chum salmon reared in estuarine pens and released in Little Port Walter Bay in 1974 including numbers, mean sizes, and dates of release.


Pink salmon

| Heath 1A | 24,427 | July 3 | 287 | 1.58 | 58.2 |
| :--- | :---: | :---: | :---: | :---: | :---: |
| Heath 1B | 24,699 | July 3 | 270 | 1.68 | -- |
| Heath 2A | 27,981 | July 3 | 293 | 1.55 | 58.6 |
| Heath 2B | 28,508 | July 3 | 297 | 1.53 | -- |
| Fravel 1 | 25,454 | July 3 | 232 | 1.96 | 57.7 |
| Gravel 2 | 20,216 | July 3 | 287 | 1.58 | 60.4 |

Subtotal for July 3 releases $1 /$

Heath/Grave1
(combination)

| 9,905 | Sent. $6 \stackrel{2}{2}$ | 23 | 19.7 | 132.1 |
| :--- | :--- | :--- | :--- | :--- | :--- |

Total, pink salmon
161,190
Chum salmon

| Heath 1 | 5,627 | June 29 | 140 | 3.24 | 71.2 |
| :--- | :--- | :--- | :--- | :--- | :--- |

1 Chums released on June 29 and pinks released on Tuly 3 were not individually marked but were fed OPM from June 22-27 medicated with oxytetracycline (OTC). A subsample indicated 98.9 nercent of the fry had detectable OTC marks when released.
$\underline{2 /}$ Pinks released September 6 were individually marked with adinose fin marks and a binary coded wire tag (Agency 3; data 1/10). A sample of 339 fish held 12 days indicated an initial tag retention factor of 0.9941 . Applying this factor to the number released 9,846 ninks were released with wire tags.


Figure 19.--Growth of 1973 brood pink and chum salmon fry in estuarine pens at Little Port Walter from early April until early July 1974. Some pink salmon fry originated from a gravel substrate incubator, while other pink and all chum salmon fry originated from Heath tray incubators. Points are dates when mean weight was measured. The initial point for pink salmon gravel fry is date $50 \%$ had volitionally emerged. Horizontal line through this point defines the emergence period.


Figure 20.--Growth of 1973 brood pink salmon fry in estuarine pen at Little Port Walter during July, August, and early September, 1974. Population was a combination of gravel- and Heath-incubated fry shown in Figure 19.

## Marking and Release

A summary of the 1973 brood pink and chum salmon reared for varying lengths of time in estuarine pens and released at LPW in 1974 is reviewed in Table 6. The pink salmon released on July 3 and the chum salmon released on June 29 were 'marked' for differentiation from others by feeding oxytetracycline (OTC) treated food from June 22-27. A subsample of 93 pink fry collected just prior to release and examined in February 1975 indicated that 98.9 percent of the fry had detectable OTC marks on the distal ends of rib bones and on vertebral centrums.

The pink salmon "smolts" released on September 6 were individually marked with excised adipose fins and binary coded wire tags (Agency 3; data $1 / 10$ ). Scales on these fish were very deciduous and many fish lost scales when processed through the same coded wire tagging procedure described previously for coho. Initial post-tagging loss was not high (only 2 of 339 fish died in a 12-day post-tagging holding period), but subsequent deaths among these fish due to scale loss during tagging could be substantial. Heavy scale loss also occurred with this same group of pink salmon earlier on August 14 at an average weight of 12.6 g when they were transferred into a clean net and subsampled for growth data. No scale losses were noted in handling smaller 1973 brood pink and chum fry at LPW earlier during the 1974 estuarine husbandry rearing period.

Survivors of the 161,190 estuarine pen-reared 1973 brood pink salmon released at LPW in 1974 will return as adults in 1975. Returning adults will be monitored for marked fish to separate reared fish from wild fry. The 3,271 pink salmon that spawned in Sashin Creek in 1973 had a potential complement of $3,022,904$ eggs ( 1,516 females $\times 1,994$ mean fecundity). Freshwater survival (potential egg-to-fry) of 1973 brood spawners in Sashin Creek was only 0.22 percent, the second lowest in 34 years (in 1946 an escapement of 933 pink salmon had a freshwater survival of 0.16 percent). The estimated production of 6,508 wild pink fry from 1973 brood spawners was made from hydraulic streambed sampling in Sashin Creek in late March 1974. The future outcome of the odd-year line of Sashin Creek pink salmon hinges essentially on the ocean survival of the 161,190 hatchery-incubated and pen-reared fry released in 1974.

In this study we reared pink salmon fry from early April until early July ( 90 days) and early September ( 150 days). Chum salmon fry were reared for 87 days before release. Each of these groups (certainly the 150-day pinks) were probably reared long enough to adversely influence timing of migration patterns. As previously discussed, delay in timing of normal migratory patterns may be an important factor in postemergent rearing of pink and chum fry. The combined Heath-gravel pink fry lot was reared until September for reasons precluding an earlier release including: (1) the planned husbandry of pink salmon through the high vibriosis-prone period of July and August and (2) evaluation of coded wire tagging on pink salmon. The first releases of pink and chum fry in late June and
early July were not made sooner in order to attain sufficient growth of fry to (1) insure successful OTC bone marks and (2) minimize possible predation from estuarine pen-reared coho smolts released (principally in late May and early June (Tables 3 and 5).

PART 3--INITIAL FRY-TO-SMOLT HUSBANDRY OF 1973 BROOD SOCKEYE SALMON
The generalized time-growth schedule for husbandry of age 1 coho smolts in the estuarine pen-rearing (EPR) program at LPW (Figure 1) should also relate closely to age 1 smolt husbandry for sockeye salmon. It remains, however, to be seen if sockeye can physiologically follow the same time-size salinity acclimation pattern as coho salmon. We began research in 1974 with progeny of the 1973 brood Port Herbert sockeye stock to gain insight into the suitability of using sockeye in a fry-to-smolt EPR program.

Port Herbert, located approximately 4 miles north of Port Walter, is a 3 -mile long fiord. A short stream, Nakvassin Creek, (about 0.5 mile long) enters the back end of Port Herbert from 74-acre Nakvassin Lake. Nakvassin Lake is the freshwater nursery area for a sockeye stock that apparently spawns in an inlet stream to the lake or perhaps on the lake beaches. We have no information on the biology of the Nakvassin Lake sockeye other than general timing on the occurrence of adults in the estuary off the mouth of Nakvassin Creek. Bright sea-run adults usually appear off the stream mouth in mid-July, and by late August all have apparently migrated into the lake. Escapements during the past 8 years probably have ranged from 2,000 to 4,000 fish annually.

## Adults, Eggs, and Incubation

One of the initial purposes of working with the 1973 brood Nakvassin Lake sockeye stock was to evaluate the possibility of ripening adult sockeye salmon in saltwater holding pens. In recent years we have routinely held bright coho salmon for 4 to 6 weeks until ripe in estuarine pens at LPW. To test the feasibility of this procedure on sockeye we seined 96 bright sockeye from the estuary at the mouth of Nakvassin Creek on July 28-30, 1973. Seining was done at low tide and the captured adults were placed in a 13 - by 26 - by 8 -foot net with $3 / 8$-inch mesh anchored nearby.

The holding pen containing the adult sockeye was towed from Port Herbert to the inner part of LPW Bay on August 1, 1973. Here the pen was anchored at the head end of the bay just out of the main discharge pattern from Sashin Creek.

By September 24, 40 of the adult sockeye had died without fully ripening. Live fish remaining in the pen included 28 males and 28 females. Some of the dead fish showed initial signs of ripening secondary sexual development (male kype and reddish body color). Other, nonripe fish, especially males, died in late September and early October although some males did become ripe during this period. The first ripe female was spawned on October 7. Between October 7 and 26, eggs were taken from 14 females. A full egg complement was not available from all

14 fish; some apparently extruded part of their eggs in the holding net and in others, all the eggs never became fully ripe. Other females died during this interval without any eggs rupturing free of the ovaries. The ripe eggs were mixed "dry" with fresh milt, although some difficulties occurred with the simultaneous availability of eggs and good quality milt. We attempted to fertilize some eggs with bloody, water milt that could have had low gamete viability.

By October 26 all but five adult sockeye (four females, one male) had died or had been killed for spawning. The last male either escaped from the holding pen or was eaten by an otter on October 27 and the remaining four females (now ripe) were killed without spawning.

Approximately 40,000 sockeye eggs were spawned at LPW in September and October, 1973. After picking off dead eggs on January 19, 1974, 37,800 live eyed eggs were available for eyed egg-to-fry stage incubation. This is about 22 percent of the 175,000 eggs potentially available in the adults held for ripening in the estuarine holding pen. In holding adult coho salmon in estuarine pens for ripening and spawning, we usually successfully spawn over 90 percent of the eggs potentially available, and rear them at least through fertilization and the initial zygote cleavage stages.

The sockeye eggs were incubated overwinter in Heath tray incubators at LPW. The first hatching was noted on March 24, 1974.

## Husbandry of Sockeye Fry

On June 22, 1974, sockeye fry were removed from the incubator trays and placed in a 12-foot-diameter by 3 -foot-deep plastic-1ined pool on the LPW warehouse dock. This pool had a sing1e-pass freshwater flow of 20 gpm . The number of fry was estimated at 23,600 and the mean weight was 0.148 g per fry ( 3,070 per pound). Fry were fed 10 to 14 times daily. Uneaten food, fecal matter, and debris were siphoned regularly from the bottom of the pool.

Losses of sockeye fry in the pool were almost nonexistent until August 9, when 2,700 fish died overnight, apparently from gas bubble disease. The occurrence of supersaturated air in the husbandry water was associated with waterline construction activity which permitted free air to enter the water source in Sashin Creek and become compressed into solution under pressure. This error was detected and corrected on August 10.

Mean weight of the sockeye fry increased from 0.148 g on June 22 to 0.308 g ( 1,471 per pound) on July 16 to 1.560 g (291 per pound on August 24 (Figure 21).

On August 24, the sockeye averaging 52.9 mm fork length ( 38 to 63 mm range), were divided into two lots and placed in floating vertical raceways. The experimental 12- by 12 - by 8 -foot raceways were made from nylon reinforced plastic fabric (impervious to water) with $3 / 16$-inch-mesh webbing sewn into the bottom. The raceways were arranged initially so that one received a
single-pass flow of 10 to 30 gpm fresh water, the other a sing1e-pass flow of 5 to 15 gpm saline water at about $15 \%$ oo salinity. Variable flows in both units were due to changes in hydraulic head caused by tidal fluctuations.

Sockeye were retained in the raceway husbandry units until late October; however, the venturi hydraulic injector device that mixed fresh water and seawater in the estuarine raceway became inoperative sometime in early October. Throughout most of October both raceways received freshwater flows, although the one unit still received a flow of only 5 to 15 gpm .

The mean weight of sockeye in the two raceways on September 18 (based on counts of one 5-pound sample from each) was: freshwater umit, 3.69 g (123 fish per pound) ; and estuarine unit, 3.58 g (127 fish per pound). On October 31 the mean weight of the sockeye (based on counts of 8.0- and 6.1- pound freshwater unit samples and 7.6and 7.0 -pound estuarine unit samples) was: freshwater unit, 6.85 g (66.3 per pound) ; and estuarine unit, 6.55 g ( 69.3 per pound).

We do not know if the slightly greater growth indicated in the freshwater raceway unit was due to osmoregulatory difficulties from husbandry at 15 \%/oo salinity from August 24 until early October, lower flows through the estuarine raceway, or possibly sampling bias. If serious osmoregulatory problems were involved it seems the growth difference would have been greater. Mortalities were nil in both units throughout the raceway husbandry period. The mean weights of sockeye from both raceway units were combined for the September 18 and October 31 data points shown in Figure 21.

On September 18, 2,500 sockeye were removed from each raceway ( 5,000 total) and flown to Sitka for use by ADF\&G as test fish in a selective fish toxicant study.

A11 sockeye were removed from both raceways on October 31, 1974, and placed in a 12 - by 25 - by 12 -foot net with $1 / 4$-inch mesh for overwinter husbandry (Phase 2--see Figure 1) in the LPW estuary. An estimated 16,800 sockeye ( 8,500 from estuarine raceway unit and 8,300 from freshwater raceway unit) were placed in the net. This recombined sockeye population averaged 84.2 mm fork length and 6.68 g weight (67.9 per pound) ; and they apparently adapted readily to the salinity conditions in the net. Surface salinities to 0.5 -meter depth generally ranged between 4 and $10 \% 00$. Below 0.8 -meter depth salinities ranged from 28 to $32 \%$. Sockeye normally remained in the lower half of the net except when rushing to the surface in response to food. From November 1 to December 18 fewer than 25 dead fish were removed from the sockeye population.


Figure 21.--Growth of 1973 brood Nakvassin Lake sockeye salmon in freshwater pool and floating raceways at Little Port Walter during 1974.
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