

FACTORS AFFECTING GROWTH AND SURVIVAL
OF COHO SALMON (*Oncorhynchus kisutch*)
AND CHINOOK SALMON (*O. tshawytscha*)
IN SALTWATER NET-PENS IN PUGET SOUND

Conrad V. W. Mahnken and F. William Waknitz
Northwest and Alaska Fisheries Center
National Marine Fisheries Service
National Oceanic and Atmospheric Administration
2725 Montlake Boulevard East
Seattle, WA 98119

ABSTRACT

Experimental marine net-pen systems for the culture of Pacific salmon have been developed in the United States and Canada and have resulted in the establishment of fish farms in Washington, Alaska, Maine, and British Columbia. Fish farmers have encountered biological problems that constrain production through decreased growth and survival. These factors include: feed amounts (ration), dissolved oxygen levels and rearing densities, saltwater adaptation, physiological stage of smoltification, and disease. This paper discusses the relative severity of these problems and current practices for solving them.

INTRODUCTION

The continued decline of freshwater habitat for natural and hatchery stocks of salmonids and rising capital costs for new hatchery construction have mandated the need for new and economical methods of salmonid culture in the Northwest region of the United States. In the past decade, commercial rearing of salmonids to "pan-size" in saltwater net-pens has developed as one of the best new techniques to produce traditional species for the consumer markets.

Marine culture of salmonids is not unique to the Pacific Northwest. Rainbow trout (*Salmo gairdneri*) and Atlantic salmon (*S. salar*) are cultured in salt water in many North Sea countries (Novotny, 1975). Rainbow and Pacific salmon (*Oncorhynchus* spp.) are raised in salt water in Japan (Tomiyama, 1972). Several methods are used; however, various types of floating cages (net-pens) are the predominant culture containers.

Since 1969, the National Marine Fisheries Service (NMFS) has conducted research on the marine culture of salmonids at its station on Clam Bay in Puget Sound near Manchester, Washington. This research has

led to a small but growing industry (Mahnken, 1972). Although other methods of marine culture have been evaluated, the bulk of our effort has concentrated on describing and solving problems associated with floating net-pen culture of Pacific salmon, primarily coho (*O. kisutch*) and chinook (*O. tshawytscha*).

We have also cultured and evaluated the three other species of Pacific salmon indigenous to the northeastern Pacific rim: sockeye (*O. nerka*), chum (*O. keta*), and pink (*O. gorbuscha*) in addition to one species (*O. masu*), native to the western Pacific Ocean. Several anadromous species of the genus *Salmo* (i.e., Atlantic salmon [*S. salar*]), steelhead trout (*S. gairdneri*), and cutthroat trout (*S. clarki*) have been similarly cultured. Coho and chinook salmon have emerged as the best two species for use in commercial net-pen culture in the Pacific Northwest due primarily to their relative disease resistance and the availability of eggs from state hatcheries. Therefore, this paper will address some of the more pronounced biological problems associated with net-pen culture of these two species.

Topics discussed in this paper include diseases and their prevention, smoltification and saltwater adaptation, feeding strategies, effect of body size on growth, and loading density.

EFFECT OF DISEASE ON SURVIVAL

Infectious disease is the most important single cause of mortality associated with marine culture of Pacific salmon and trout in Puget Sound. The three most serious diseases are bacterial in origin: vibriosis, furunculosis, and kidney disease. Outbreaks of disease occur whenever fish are subjected to a combination of stress and proper environmental conditions for the growth of pathogenic organisms. Physiological stress caused by direct transfer to salt water, handling, high population densities, high summer temperatures, or low dissolved oxygen in the pens (as a result of marine organisms fouling the net) may result in outbreaks of disease.

The most common and devastating pathogen is *Vibrio anguillarum*, the etiologic agent of vibriosis, which has been frequently documented as the cause of mortality in marine cultured salmonids (Cisar and Fryer, 1969; Fryer et al., 1972; Rucker, 1959; Antipa, 1976; Novotny, 1975, 1978). At least two pathogenic serotypes of *V. anguillarum* have been identified (Harrell et al., 1976). This organism is the cause of a serious disease when saltwater temperatures exceed 12°C (July through mid-October in Puget Sound), but outbreaks may occur whenever cultured populations of fish are subjected to stress. Vibriosis can reach epidemic proportions during these periods in the close confines of the net-pens (Novotny, 1978). The threat of disease abates in October as saltwater temperatures drop but some chronic loss generally continues into the winter.

Another bacterial disease, furunculosis, caused by *Aeromonas salmonicida*, is also responsible for high mortalities in salt water. Fish contract the disease in fresh water, and it exacerbates when smolts are transferred to salt water. Handling and osmoregulatory stress are the probable major causes of furunculosis outbreaks. Simultaneous infections of both vibriosis and furunculosis can occur (Novotny, 1978).

Treatment with antibiotics and avoidance of stress constitute the only method for control of furunculosis as no effective vaccines have been developed.

Kidney disease (KD) is a chronic bacterial infection that becomes evident in net-pens during the winter. The causative agent is a bacterium of the genus *Corynebacterium*. Although a less acute disease than either vibriosis or furunculosis, KD can nevertheless cause significant economic loss to the salmon grower. Infection occurs in fresh water and is carried to salt water, but the disease does not usually become evident until the first winter in salt water when the fish have grown to about 100 g. The organism continues to cause death and retard growth to maturity. Kidney disease is, therefore, a serious disease in brood stocks. Antibiotic therapy has shown limited success as a treatment for kidney disease, but at this time no effective vaccines have been developed.

Recently, attention has been focused on the prevention of vibriosis by vaccination, and bacterins have been developed and successfully used in the laboratory and in field tests. Bivalent bacterins that include both pathogenic serotypes of *V. anguillarum* are now available commercially and are being used on a regular basis by net-pen owners in Puget Sound.

The perfection of suitable vaccination systems has proven to be a difficult task (Fryer, 1977). A number of methods of antigen delivery have been tried with varying degrees of success. They include: injection, oral administration, vacuum infiltration, hyperosmotic infiltration, direct addition to the water, and the spray method. Injection, oral administration, and hyperosmotic infiltration techniques are the most commonly used. Rohovec et al. (1975) demonstrated that oral immunization is effective while others (Antipa, 1976; Harrell et al., 1975; Antipa and Amend, 1977) showed injected bacterins to work well. The more recent and novel techniques of hyperosmotic infiltration (Amend and Fender, 1976) and spraying (Gould, 1977) show great promise because of the ease of rapidly administering bacterin to large numbers of fish.

Immunity imparted by vibrio vaccines is neither totally efficacious nor permanent. The protection provided by the vaccine is relative and can be overwhelmed by stress, such as from handling, or by adverse environmental conditions, such as high water temperatures or low dissolved oxygen. Furthermore, field data for any given year are dependent on year-to-year environmental variations, husbandry techniques, presence and serotype of the pathogen, potency of the vaccine, and a multitude of other qualifying factors. Such data should only be used in relative comparisons between vaccinated and unvaccinated groups of salmonids held in saltwater net-pens in 1977 and 1978. They should not be interpreted as either the "average" or the "potential" protection afforded by vaccines. It is, nevertheless, useful to present such data as illustrations of the efficacy of immunization against vibriosis during a 2-year period when strong natural challenges did occur.

METHODS AND MATERIALS

The data used in this presentation are from studies of smoltification in Columbia River hatchery stocks, which were transferred to saltwater net-pens at the NMFS station near Manchester, Washington, from the hatcheries between 10 March and 2 May 1977 and between 3 March and 10

July 1978. After arrival at the station, one-half of each population was intraperitoneally injected with a vaccine-antibiotic solution, and the remaining noninjected half were marked by removal of the adipose fin to distinguish them from the injected fish. The bacterin-antibacterial preparation is used to avoid the normal 2-week, post-immunization delay before introducing the fish to salt water. All test groups were then transferred to full strength salt water (29 ppt) and maintained in small nylon net-pen enclosures. Casualties were removed daily and necropsies performed to determine cause of death. Every 30 to 60 days all test groups were anesthetized with Tricaine Methanesulfonate, individually measured to the nearest millimeter, and weighed to the nearest 0.1 g.

RESULTS

Results are presented as the average difference in survival between injected and noninjected fish for each species and are given in Figure 1. All stocks seemed to retain the immunity imparted by the vaccine-antibiotic solution for 60 to 90 days. Mortality after 60 to 90 days was about the same in injected and noninjected populations. Spring chinook and coho salmon were the only stocks tested for more than 180 days; after that time it appeared that protection in the injected fish had diminished. It should be noted that after 180 days, mortality was slightly higher in injected spring chinook and coho salmon than in their non-injected cohorts.

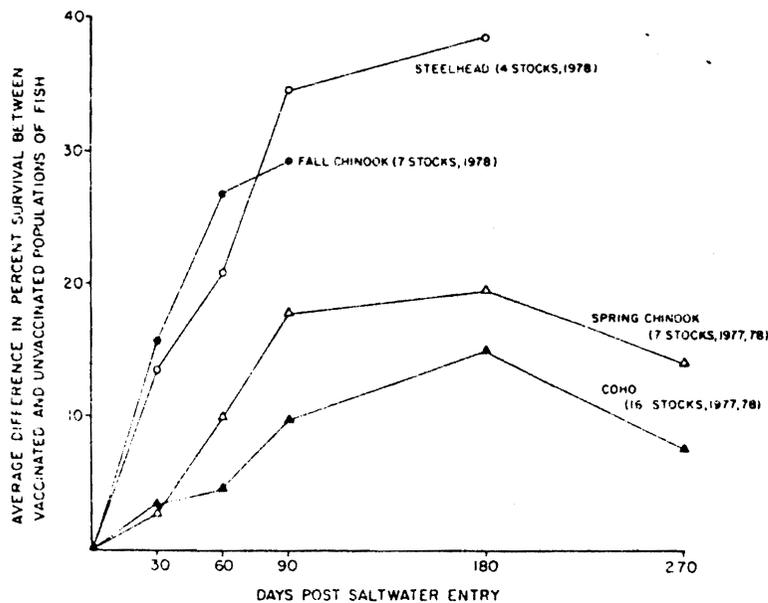


Figure 1. Improved survival of intraperitoneally vaccinated salmonids held in saltwater net-pens in Clam Bay, at the NMFS station near Manchester, Washington (1977-78).

The vaccine-antibiotic solution was least efficacious for spring chinook and coho salmon with injected fish demonstrating approximately 10 and 15% improved survival, respectively, after 90 days of exposure to salt water.

In contrast, injection improved survival approximately 30 and 35%, respectively, for fall chinook salmon and steelhead trout during the first 90 days of saltwater exposure. Necropsies performed on casualties from the 1977 experiments indicated that most of the loss was due to vibriosis (Table 1). These findings suggest that either spring chinook and coho salmon are inherently more resistant to *V. anguillarum* than fall chinook salmon and steelhead trout or that the inclusion of the antibiotic in the solution compensates for the possible effects of other bacterial pathogens normally affecting these species. For example, several of the steelhead trout stocks had an extensive history of bacterial infection during freshwater rearing that may have been suppressed by the antibiotic during the critical period of saltwater entry. Other factors such as size dependent immunocompetence (fall chinook populations averaged only 3 to 5 g at saltwater entry) may also dictate survival rates.

TABLE 1. Cause of Mortality in Six Test Groups of Marine Cultured Pacific Salmon, Clam Bay, Washington, 1977

| Species and test group | Mortality (%) | | | |
|------------------------|-------------------------|--------------------------|------------------|------------------|
| | Vibrio 775 ^a | Vibrio 1669 ^b | BKD ^c | All other causes |
| Coho | | | | |
| Toutle | 75.0 | 6.3 | 2.0 | 16.7 |
| Eagle Creek | 82.6 | 4.4 | 6.5 | 6.5 |
| Bonneville | 67.4 | 2.2 | 2.2 | 28.3 |
| Kalama Falls | 68.9 | 3.3 | 3.3 | 24.6 |
| Spring chinook | | | | |
| Carson | 61.9 | 9.5 | 4.7 | 23.8 |
| Eagle Creek | 60.0 | 20.0 | 0.0 | 20.0 |
| Mean | 69.3 | 7.6 | 3.1 | 20.0 |

^a*Vibrio anguillarum* strain 775. ^b*Vibrio anguillarum* strain 1669.

^cBacterial kidney disease.

It should also be noted that our vaccination technique varied from those used by commercial growers who usually vaccinate several weeks before saltwater entry. Although the injected vaccine-antibiotic mixture decreases mortality caused by the organism, the ability of the vaccine alone to impart immediate protection is unknown in this case.

EFFECT OF BODY SIZE AND RATION ON GROWTH
OF MARINE CULTURED COHO

One of the fundamental laws of growth for all animals is that growth rate is greatest in young animals and decreases with increasing size and age (Needham, 1964). Numerous investigators have shown salmonids to exhibit similar characteristics of rapidly decreasing growth rate as they grow larger (Brett et al., 1969; Brett and Shelbourn, 1975; Cooper, 1961; Brett, 1974; Shelbourn et al., 1973).

Brett et al. (1969) found temperature to be a major factor limiting growth of young sockeye salmon in fresh water. Optimum growth occurred at approximately 15°C in fish fed excess rations. Temperature affected specific growth rate to a much greater extent in small fish (0.5-1.5 g) than in large fish (4.2-30.1 g) and is, therefore, an important consideration during freshwater rearing when fish are small (Shelbourn et al., 1973). Thus temperature, although limiting growth in small salmonids, can be expected to have somewhat less effect on growth of larger salmon grown in the relatively moderate thermal climate of Puget Sound. Size is the major factor affecting growth in healthy populations.

METHODS AND MATERIALS

Experiments were conducted at the NMFS station near Manchester, Washington, from July 1974 to May 1975 to determine the scope for growth of coho salmon fed commercially prepared Oregon Moist Pellets (OMP). Replicated groups were established in net-pens and fed varying feed amounts (rations) for the purpose of establishing the relative degree to which ration and body weight determine growth rate.

A coho salmon population was divided into six replicated test groups of 150 fish each and fed commercial OMP II rations of 1.0, 2.0, 3.0, 4.0, and 5.0% of body weight per day, respectively, with the sixth group fed until food was refused (demand ration). All other procedures were as previously described in the disease section. Weekly average saltwater temperatures ranged from a high of 14°C in August to a low of 7.1°C in March.

RESULTS

Our data show the major effect that fish weight has on specific growth rate (Fig. 2). Specific growth rate in fish fed to excess, as marked by the solid line, range from a high of about 1.75% body weight/day in small 25-g fish to a low of about 0.40% body weight/day in fish larger than 200 g, a four-fold decrease over an 11-month marine culture period. Changes in specific growth rate attributable to temperature are seen as part of the variance of excess ration points above the line and are of second order in importance, effecting changes no greater than about 0.50% body weight/day. Although the precise effect of temperature on growth is not known, it is interesting to note that growth rate in the very large fish did not recover even though saltwater temperatures increased in the spring months. We conclude that the effect of size on growth of coho salmon is at least three times as important as the effect of temperature under conditions of excess food and within the range of temperature normally experienced in Puget Sound.

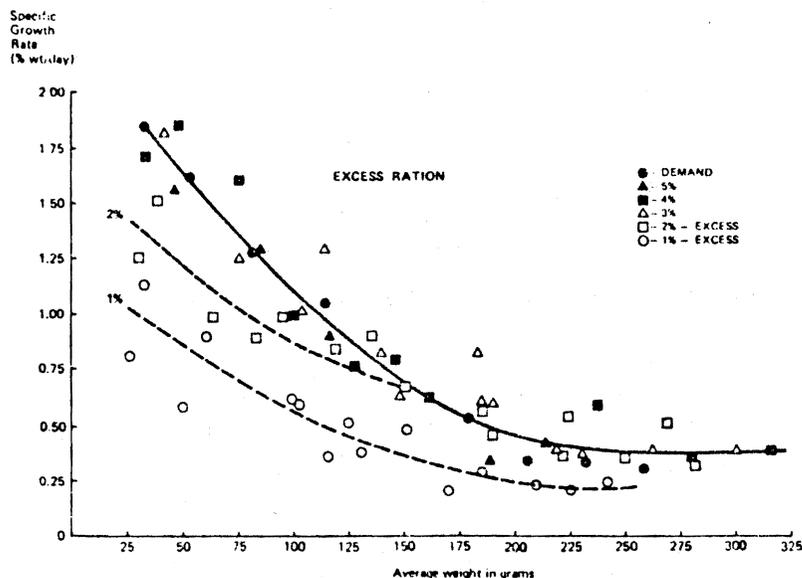


Figure 2. Specific growth rates for marine cultured coho salmon fed excess and restricted rations of Oregon Moist Pellet. Solid line represents growth rates of fish fed on excess rations and broken lines, growth rates of fish fed on restricted rations. A 3-point moving average was used and the lines were fitted by eye.

A major factor affecting growth of young salmon under culture is the amount of food fed (ration). Brett and co-workers found ration to be a major factor limiting growth of young sockeye salmon grown at 15°C (Brett et al., 1969; Brett and Shelbourn, 1975). In these studies, specific growth rate¹ remained steady when fish were fed a fully consumed fixed ration. When a size was reached where the ration became excessive or size-limited, specific growth rate declined. The smallest fish (4 g) consumed the greatest percentage of fixed ration per day (10-12%) while the largest fish (50 g) consumed the smallest fixed ration per day (4-6%).

Our results, although conducted in water averaging colder than 15°C, show similar results (Fig. 2). Rations of 3% of body weight per day became excessive at 60 g while 2% ration did not become excessive until fish reached 150 g. One percent ration was never excessive even in fish weighing 250 g. These results indicate that salmon growers who frequently feed pelleted foods on demand (4-6% body weight per day) several

¹ Specific growth rate is defined as: $G = \frac{\ln_e W_1 - \ln_e W_0}{t_1 - t_0} \times 100$, where W is wet weight and t is time in days (Ricker, 1958).

times daily are feeding excessive and wasteful amounts. It is probable that feed amount is seldom limiting. But more attention should be focused on the relationship between ration, body weight, and water temperature if the grower is to achieve either optimum conversion or maximum growth rate.

DISSOLVED OXYGEN IN NET-PENS

Net-pens afford the opportunity to study stocking density effects independent of the buildup of metabolic waste normally associated with rearing containers like raceways. We have found net-pens to be relatively free of feed and fecal wastes while remaining high in dissolved oxygen (DO) even at high fish stocking densities. Commercial farms in Puget Sound have occasionally experienced low concentrations of DO in large net-pens during slack tides but only when flow through the net is restricted by marine fouling organisms or if the nets are inordinately large.

The problem of oxygen depletion in large net-pens is worthy of consideration--not only during the summer when nets are most likely to foul but also during the fall when there is a natural decline of dissolved oxygen. Summer values for DO in Puget Sound generally exceed 10 ppm while fall values may reach a low of 4 ppm in October. It is during this latter period that low DO is most likely to occur in net-pens and frequently coincides with the fall outbreak of vibriosis.

METHODS AND MATERIALS

In the summer of 1972, we monitored dissolved oxygen and flow in a 22 m³ hexagonal net-pen (3.7 m deep) that contained up to 2,270 kg of juvenile chinook salmon. Eight series of measurements were conducted that ranged in time from 24 to 72 hours during which continuous DO, temperature, and tidal current measurements were recorded. These measurements allowed us to determine the effect of tidal advection and fish respiration on the oxygen concentrations inside and outside the pen. A typical portion of one such series is presented in Figure 3. At one point, no measurable tidal current could be detected outside the cage (0213-0505 hours on 11 August as indicated by arrows). The time expected for total depletion of DO inside the pen from fish respiration is estimated at 3.5 hours, yet despite the lack of tidal current, dissolved oxygen in the cage remained at an average of 7.75 ppm during the 30-hour period, only 0.25 ppm lower than ambient water. These data, typical of the other experiments conducted that summer, have led us to believe that some process other than tidal advection is causing water exchange through the nets. The swimming activity of the fish may cause flushing of the cage providing the net is relatively free of fouling. We have frequently noticed that the circular, uni-directional swimming of schooled fish in the pens can create visible currents evidenced by whorls of small surface waves radiating from the center of the pen to the outer perimeter. The swimming behavior of salmon is probably an important factor in flushing the pens.

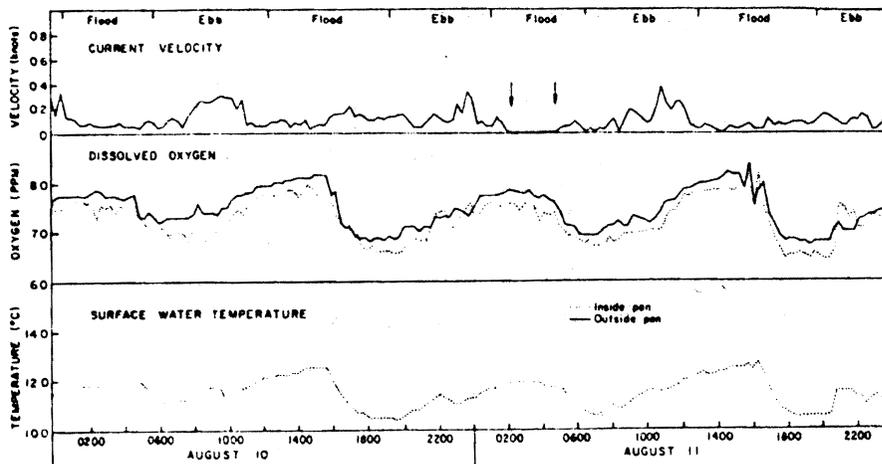


Figure 3. Current velocity, dissolved oxygen, and sea surface temperature in a 220 m³ hexagonal net-pen containing an estimated 2,000 kg of chinook salmon (9 kg/m³).

EFFECT OF STOCKING DENSITY AND PEN SIZE ON GROWTH

In 1975, we undertook to determine the effect of static loading density (kg fish/m³ of water) and net-pen volume on growth and survival of coho salmon at much higher densities than are normally employed by growers. Commercial growers had aimed for a harvest density of approximately 16 kg of fish/m³ of water as suggested by Mahnken et al. (1970). This figure had not generally been exceeded on a production scale for lack of information on the effect of higher loading densities.

METHODS AND MATERIALS

In early July, approximately 70,000 underyearling (zero-age) coho salmon (average weight 20.0 g) were transferred from freshwater raceways to a saltwater holding pen at the NMFS station near Manchester, Washington. All fish were vaccinated intraperitoneally against *Vibrio anguillarum*, fed on a dry diet for 2 weeks, and redistributed to 18 experimental pens.

All tests were conducted in one of 3 different size pens (5, 10, and 108 m³) and at 5 stocking densities (Table 2). Final stocking densities of 8, 16, 32, 48, and 64 kg/m³ were achieved in December 1975. Fish in each pen were monitored monthly for growth and mortality either by direct counts or by subsampling, depending on pen size and population density. Estimates of total biomass and number of fish were made from

counts, subsamples, bulk weights, and daily mortality records. At termination in December 1975, the entire population of each pen was bulk weighed and counted, except in the 108 m³ pen where all fish were bulk weighed and subsampled to determine the number of remaining fish.

TABLE 2. Experimental Design for Coho Stocking Density Experiment

| Stocking density (kg/m ³) | | Net-pen volume (m ³) | | |
|---------------------------------------|----------------------|----------------------------------|----|-----|
| Initial | Final (projected) | 5 | 10 | 108 |
| | | Number of pens | | |
| 1.6 | 8.0 | 2 | 2 | - |
| 3.2 | 16.0 | 2 | 2 | - |
| 4.8 | 32.0 | 2 | 2 | 1 |
| 8.0 | 48.0 | 1 | 1 | - |
| 11.2 | 64.0 | 1 | 1 | - |

RESULTS

Results of the study showed growth was comparable at all densities and pen sizes tested until the end of the third month when the fish in the 48.0 and 64.0 kg/m³ density pens began to show reduced growth (Fig. 4). By termination of the experiment, the fish in these higher density pens lagged in growth by about a month behind the 8.0, 16.0, and 32.0 kg/m³ density pens. But the most surprising results were that the fish in the largest and smallest pens showed better growth than those in the intermediate size pens (Fig. 4). At the end of the 6-month period, the 8 m³ pens lagged in growth by approximately 15 days behind the smaller 5 m³ pens at the 8.0 kg/m³ loading density. But at the higher densities, the relative differences in growth between the 8 and 5 m³ pens lessened and at the highest density (64.0 kg/m³) differences were not significant.

Survival was comparable among all densities and pen volumes and no major disease outbreaks occurred. But slightly more parred fish were seen in the higher density pens, a natural result of reduced growth and subsequent parr reversion (see smoltification section).

We are at a loss to explain why growth was better in the small and large pens than in the intermediate size pens. However, we feel confident that the effect is real and not an experimental artifact because the relationship held at all densities tested. It is possible that territorial behavior plays a role. Territorial behavior develops at an early age in coho salmon, and we have seen size dominant individuals establish territory under certain conditions in net-pens. The development of size hierarchies in marine cultured coho salmon is demonstrated by increasing dispersion in size between the largest and smallest individuals as the population grows. The size hierarchy effect on growth is probably related to an established order of dominance in salmonids with the large, dominant fish growing fastest (Brown, 1957). It is possible that in the smaller and larger saltwater pens, it is difficult for dominant fish to establish territory, i.e., either too small a pen to estab-

lish a suitable territory or too large a territory to defend when food is delivered to the interior of a large net-pen.

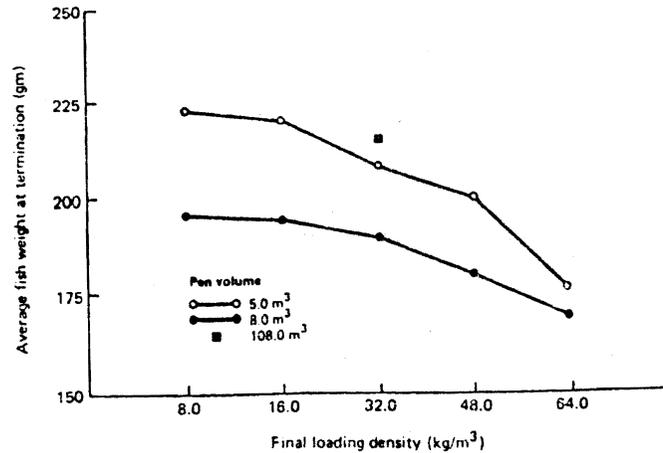


Figure 4. Effect of stocking density on growth of coho salmon reared in saltwater net-pens, Clam Bay, Washington.

Using the 5 m³ pen as a model and extrapolating to the larger commercial size pen, the commercial salmon grower can expect to significantly increase yield from net-pens by increasing rearing density from 16.0 to 32.0 kg/m³ while experiencing less than a 7% reduction in growth and no increase in mortality. Even at a density of 48.0 kg/m³ a grower will realize less than a 10% reduction in growth, but care must be exercised in using higher densities because of greater risk of disease transmission.

SMOLTIFICATION AND SEAWATER ADAPTATION

The failure of coho and chinook salmon to adapt to salt water is a major cause of mortality and reduced growth in saltwater net-pens. The cause of mortality is osmoregulatory dysfunction while the cause of reduced growth is reversion to the freshwater parr state (coho salmon only). A variety of factors influence the ability of Pacific salmon to adapt to salt water, not the least being size and time of saltwater entry, disease, and the physiological state of smoltification.

Many salmonid species undergo physiological, morphological, and behavioral changes coinciding with their migration to salt water. This progressive developmental transformation from the freshwater parr form to a saltwater-tolerant form is termed smoltification.

Determining the optimum time for transfer from the freshwater hatchery to saltwater net-pens is not a simple matter. Transfers traditionally are made when certain behavioral characteristics are noted or

When defined physical changes are observed (silvering, fin color, size, etc.). However, these criteria are not always accurate, do not always coincide with the actual peak of smoltification, and cannot be used to quantitate the smoltification process as they do not necessarily correspond to the time when the fish are fully prepared physiologically for salt water. The result has been that growers often transfer to salt water stocks of salmon that contain some parr or incompletely smolted fish that succumb to osmoregulatory dysfunction or redevelop many structural characteristics of the parr. These fish do not grow in salt water and mortality is high. We have seen commercial populations that have contained up to 80% of these stunted fish several months after transfer to salt water.

Stunted growth of coho salmon in salt water has been documented by Mahnken (1973) and by Clarke and Nagahama (1977): Two forms of stunted coho salmon may develop in sea water. The first form is the undersized fish that is incompletely smolted and although appearing silvery upon saltwater entry becomes progressively darker sometime after transfer. These fish look like freshwater parr and maintain a high condition factor. The second form is the fish that enters sea water as a silvery smolt, grows, and remains as an apparent smolt for a period of weeks or months, then gradually assumes some characteristics of the parr (parr marks, darkening of skin). Fish of this type cease growing and condition factor drops. Whether reversion to the parr stage has occurred in this second form of stunting is not known.

It has been suggested by Clarke and Nagahama (1977) that stunting is not a function of size at saltwater entry but rather a function of slow growth. They also point out another vexing aspect of coho salmon stunting: their ability to acclimate to salt water and maintain plasma sodium concentrations only slightly above the normal level. The ability of the premigratory form of coho salmon to adapt and survive in salt water for an extended period of time seems to be unique among the congeneric species of Pacific salmon, although we have observed a few parred chinook salmon surviving in sea pens some weeks following saltwater entry (Waknitz and Mahnken, unpublished data).

In most local stocks, smolting of yearling coho salmon occurs during April and May at a size of about 15 g. At ambient temperatures in rivers and streams of the Pacific Northwest, growth to smolt size requires 12 to 14 months of stream residence or hatchery rearing after fertilization. For economic reasons, commercial growers accelerate the growth of coho salmon using warm ground water or heated stream water of 12-15°C to achieve smoltification by the first summer after hatching. Under ideal conditions, smolting can occur as early as the first week in June but generally does not occur until late June or July (Fig. 5). Growers program freshwater growth of 0-age smolts to coincide with warm summer temperatures in the grow-out pens in salt water.

But the critical timing of saltwater entry is often jeopardized by the inability of the 0-age coho salmon to reach smolt size. Furthermore, smoltification is cyclic and photoperiod-dependent in anadromous salmonids; reversion to a nonmigratory parr form in fresh water begins shortly after the summer solstice in June (Fessler and Wagner, 1969). If a grower is unable to transfer fish by late spring for lack of growth or incomplete smolting, he must choose between holding the fish over for an additional year, or risk serious loss by transfer of poorly smolted fish to salt water.

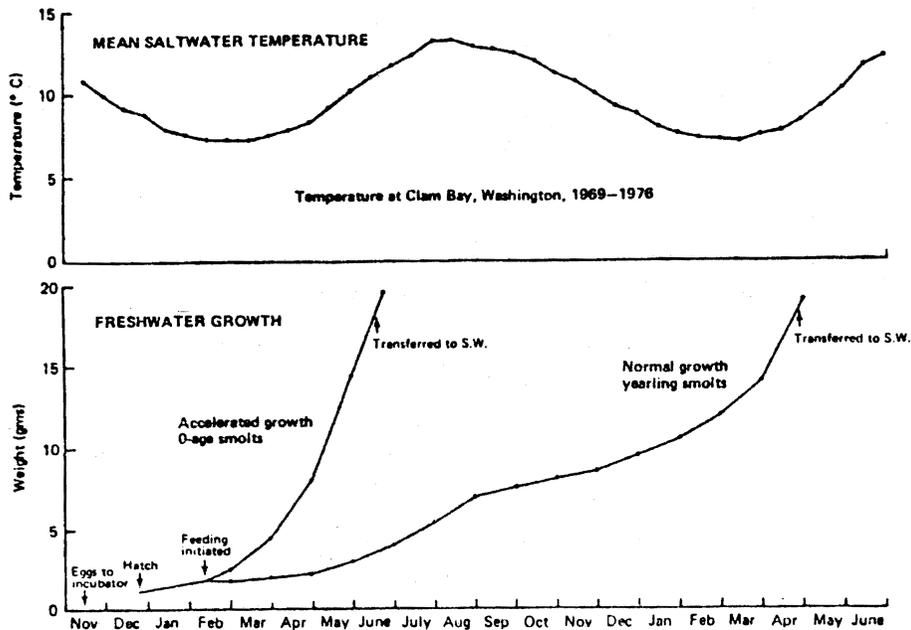


Figure 5. Diagrammatic representation of freshwater growth of coho salmon reared on accelerated and normal temperature regimes (after Brannon et al., 1976). Saltwater temperatures in Clam Bay, Washington, are presented as an example of net-pen temperatures at time of transfer to salt water (S.W.).

Accelerated chinook salmon attain saltwater entry size (7 g) by late May and can be successfully adapted to salt water providing they have smolted. Hence the period of acceleration is not as critical as with coho salmon.

The following discussion is based on data from two experiments conducted at the Manchester field station in 1973 and 1977 and sheds some light on the chronology of events and the consequences of introducing poorly smolted coho and chinook salmon into saltwater net-pens.

METHODS AND MATERIALS

From May to November 1973, experiments were conducted to determine what lasting effects early conversion to salt water would have on 0-age coho salmon. Serial entry experiments were carried out where coho salmon from a single hatchery stock were divided into four experimental groups and transferred to salt water at various times and sizes.

Experimental fish were obtained from the Skykomish Hatchery (Washington Department of Fisheries) as eggs. The growth of these fish was accelerated so that they were nearly ready to smolt by early May 1973. At that time, they were placed in fresh water in 8 circular tanks, 100 fish to a container. At approximately 3-week intervals, starting on 9

May, two replicate tanks were switched to full strength salt water (29 ppt). No attempt was made to acclimate the populations to salt water. By 10 July all fish had been converted to salt water and were transferred to net-pens at dockside. The presence or absence of parr marks was noted at all weighing periods thereafter. Weighing, feeding, and vaccination procedures were as described in the disease section. A majority of the first 200 fish transferred to salt water (average 10.3 g) had smolted by the time of their saltwater entry on 10 July.

The second body of data is from 1977 experiments conducted to determine the status of smoltification in hatchery stocks. Both coho and chinook salmon were transferred to saltwater pens from Columbia River hatcheries in various stages of smoltification, and their growth and survival were monitored over a period of 150-210 days in salt water. The fish were weighed, measured, vaccinated, and fed as described in the previous section on disease. The studies, carried out under production conditions, were supplemented with biochemical assays for Na⁺-K⁺ activated gill ATPase and serum thyroxine at the hatcheries.

RESULTS

Results from the 1973 serial entry experiment with coho salmon show growth, mortality, and reversion to a pre-smolt state to be related to the season and mean size of the fish at time of conversion to full-strength salt water. By winter 1973, more than 60% of the fish in the first saltwater entry group were visually judged to be parr. The percentage of parr was decreased in a treatment-related manner to a low value of 15% for the last group introduced to salt water (Fig. 6).

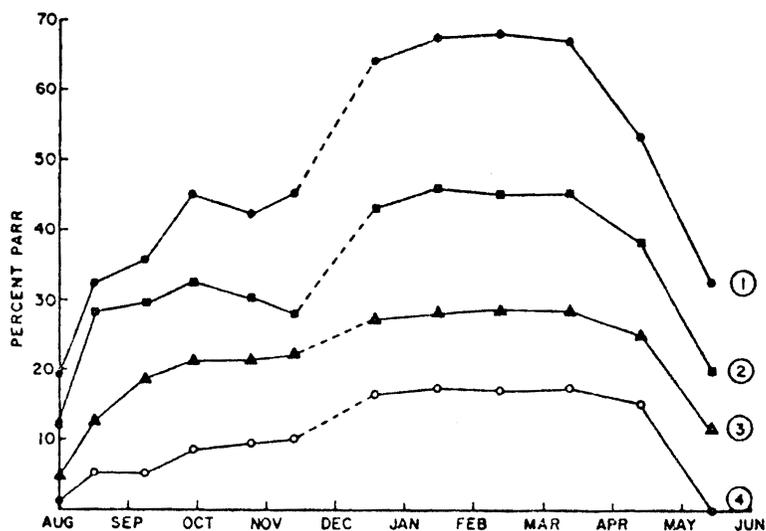


Figure 6. Percentage of parred fish in four populations of coho salmon grown in saltwater net-pens. Circled numbers indicate serial entry groups--see text for explanation.

Similarly, mortality also paralleled treatment; the first group to enter salt water suffered greater than 25% mortality by the end of the experiment while the last group to enter experienced less than 10% mortality (Fig. 7). The second and third serial entry groups performed intermediately.

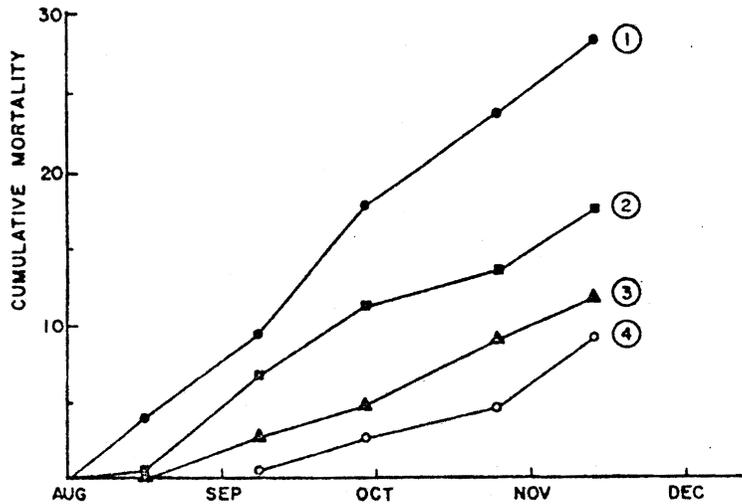


Figure 7. Cumulative mortality in four populations of coho salmon grown in saltwater net-pens. Circled numbers indicate serial entry groups--see text for explanation.

Examination of Figure 8 reveals that the growth rate of smolt and parr within the four test groups was the same and not affected by time of entry to salt water. However, mean population growth (smolt and parr combined) over time is greatly affected by season and mean fish weight at time of conversion to salt water (Fig. 9). The conclusion is obvious: the greater the percentage of parrred fish in the population at time of saltwater entry, the poorer the growth and survival of the population. Furthermore, it appears that a critical growth path must be maintained even in salt water to avoid reversion to parr (Fig. 10). In this supporting figure, compiled from the 1978 experiment, the apparent increase in size of parr and transitional animals is the result of reversion from smolt + transitional + parr rather than true growth. Thus, poorly growing smolts, unable to attain a critical size during a period of declining photoperiod, become transitionals and transitionals become parr, creating the false impression that growth is taking place.

This presents serious implications for the commercial grower. Coho salmon placed in a marine net-pen system at the wrong size or time of year or both would eventually present severe problems. Mortality would increase and more importantly, growth would be greatly reduced, possibly to the extent that the time required to produce a marketable product becomes economically limiting.

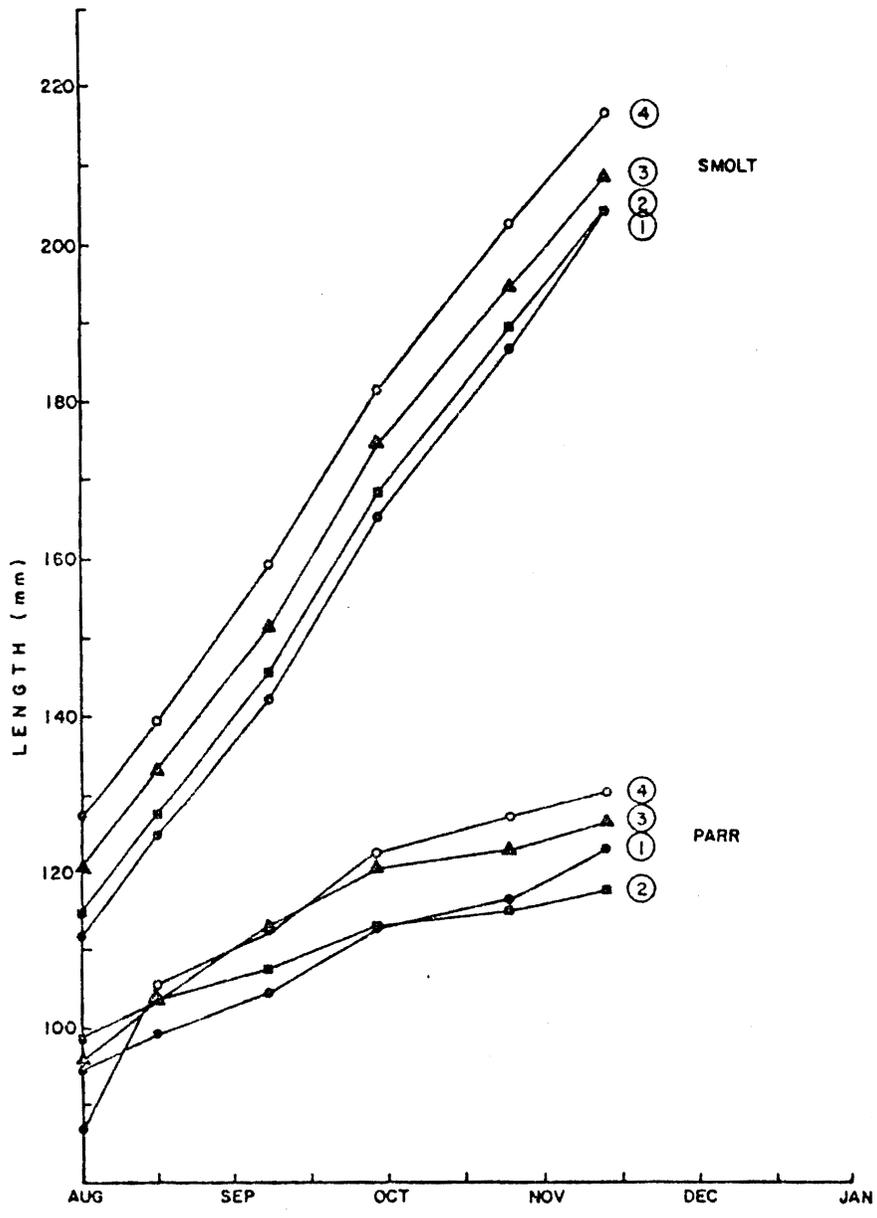


Figure 8. Mean size for parr and smolted fish in four populations of coho salmon grown in saltwater net-pens. Circled numbers indicate serial entry groups--see text for explanation.

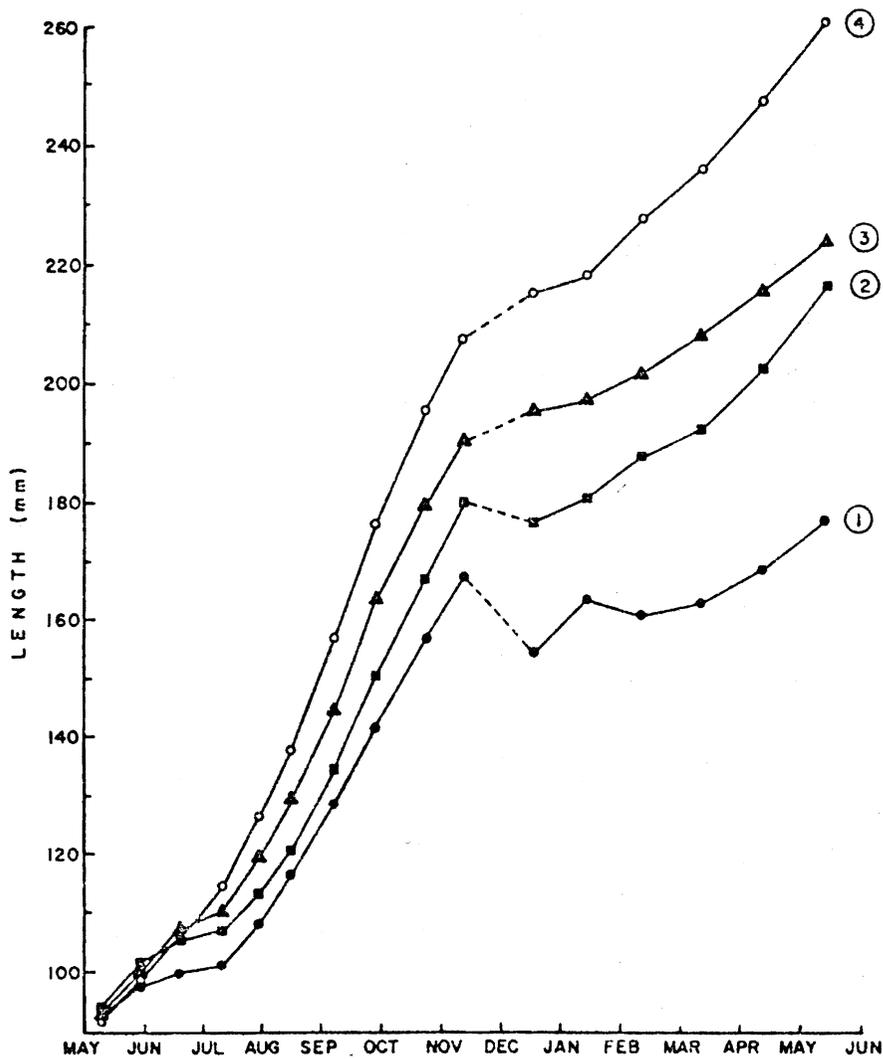


Figure 9. Mean size for four populations of coho salmon grown in salt-water net-pens: smolted and parred fish combined for each lot. Circled numbers indicate serial entry groups--see text for explanation. Broken line represents a period of losses of experimental fish due to vibriosis.

Unlike poorly smolted coho salmon that can survive for extended periods in sea water, poorly smolted chinook salmon die within the first week due to osmoregulatory dysfunction. Obvious osmoregulatory loss, as evidenced by dehydration in chinook salmon, is not frequently seen in coho salmon; instead, long-term chronic losses occur as small incom-

pletely smolted fish either remain in the parr stage or revert from smolt to transitional and parred stages.

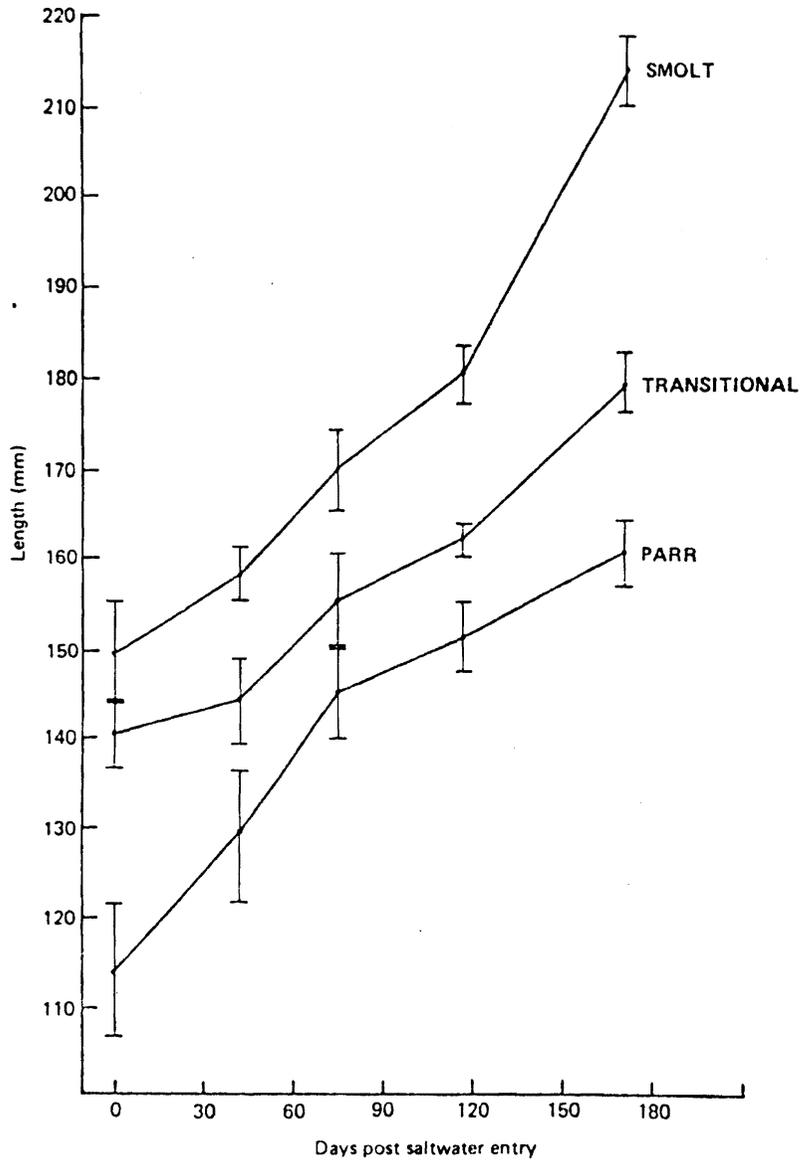


Figure 10. Average length of smolt, parr, and transitional fish in 7 stocks of marine culture coho (1978). Bars indicate 1 standard deviation.

ENZYME AND HORMONE INDICATORS OF SMOLTIFICATION DURING FRESHWATER REARING

While in the freshwater hypotonic environment, fish absorb water through mucous membranes and gills, excreting copious amounts of dilute urine. In the hypertonic saltwater environment, dehydration is prevented by the drinking of salt water and the partial shutdown of urine excretion by the kidney. The salt water consumed replaces water lost to the environment by osmotic diffusion through the body surfaces. Excess salt accumulates from drinking salt water and from diffusion, primarily through the epithelial cells of the gill lamellae. These salts, primarily sodium ion, are excreted by an active transport system located in the gill (Parry, 1966; Potts, 1968) and the hindgut.

The active transport system is a major energy-requiring process activated by hormonal and enzymic changes during smoltification. Among the most useful indicators of the active transport system's functional level in smolting salmonids is an increase in the sodium-potassium stimulated enzyme, adenosine triphosphatase ($\text{Na}^+\text{-K}^+$ ATPase). Studies have shown that salmon and trout undergoing the parr-smolt transformation will show a rise in $\text{Na}^+\text{-K}^+$ ATPase activity in the microsomal fraction of gill tissue (Zaugg and McClain, 1969, 1970; Zaugg and Wagner, 1973). This rise occurs prior to entry into salt water and is followed by a further increase once the fish is in salt water (Dickhoff and Folmar, 1978; Giles and Vanstone, 1976). Presumably, the higher $\text{Na}^+\text{-K}^+$ ATPase activities in hypertonic salt water are related to osmo-ionoregulatory functions.

Studies conducted at the NMFS station near Manchester, Washington, in 1977 and 1978 have shown a strong relationship between the spring surge of $\text{Na}^+\text{-K}^+$ ATPase in hatchery stocks of chinook salmon and their subsequent survival in saltwater net-pens. High $\text{Na}^+\text{-K}^+$ ATPase activity at the hatcheries resulted in good adaptability to salt water. Low $\text{Na}^+\text{-K}^+$ ATPase activity resulted in immediate losses from osmoregulatory dysfunction, as judged by dehydration in mortalities. Two examples, one of a well smolted stock (Eagle Creek) and one of a poorly smolted stock are given in Figure 11. Both stocks were moved to saltwater pens at approximately the same average weight, but the stock with high $\text{Na}^+\text{-K}^+$ ATPase (Eagle Creek) experienced much less mortality when introduced to the pens (Fig. 12). In coho salmon stocks, the relationship is not as clearly defined. Low enzyme activity at the hatchery (Fig. 13) did not result in high initial mortality upon saltwater entry (Fig. 14), but it appeared to be related to stunting and parr reversion in the later summer and fall months.

Recent work by Dickhoff et al. (1977) and Dickhoff and Folmar (1978) have shown smoltification in coho salmon to be regulated by endocrine control mechanisms and that stunting in salt water is probably due to endocrine dysfunction. These workers have shown a distinct thyroid hormonal surge during smoltification that is related to a variety of developmental and physiological phenomena, including $\text{Na}^+\text{-K}^+$ ATPase activity (Folmar, Zaugg, and Dickhoff, unpublished). The magnitude, duration, and time of onset of the thyroxine (T-4) surge varied in the stocks investigated. Their information shows a close relationship between the peaks of thyroid hormone levels and $\text{Na}^+\text{-K}^+$ ATPase. Furthermore, these important studies show that thyroid gland activity is modified while the fish is in fresh water, long before the morphological changes of stunting in salt water can be observed, and that there is a depressed thyroid

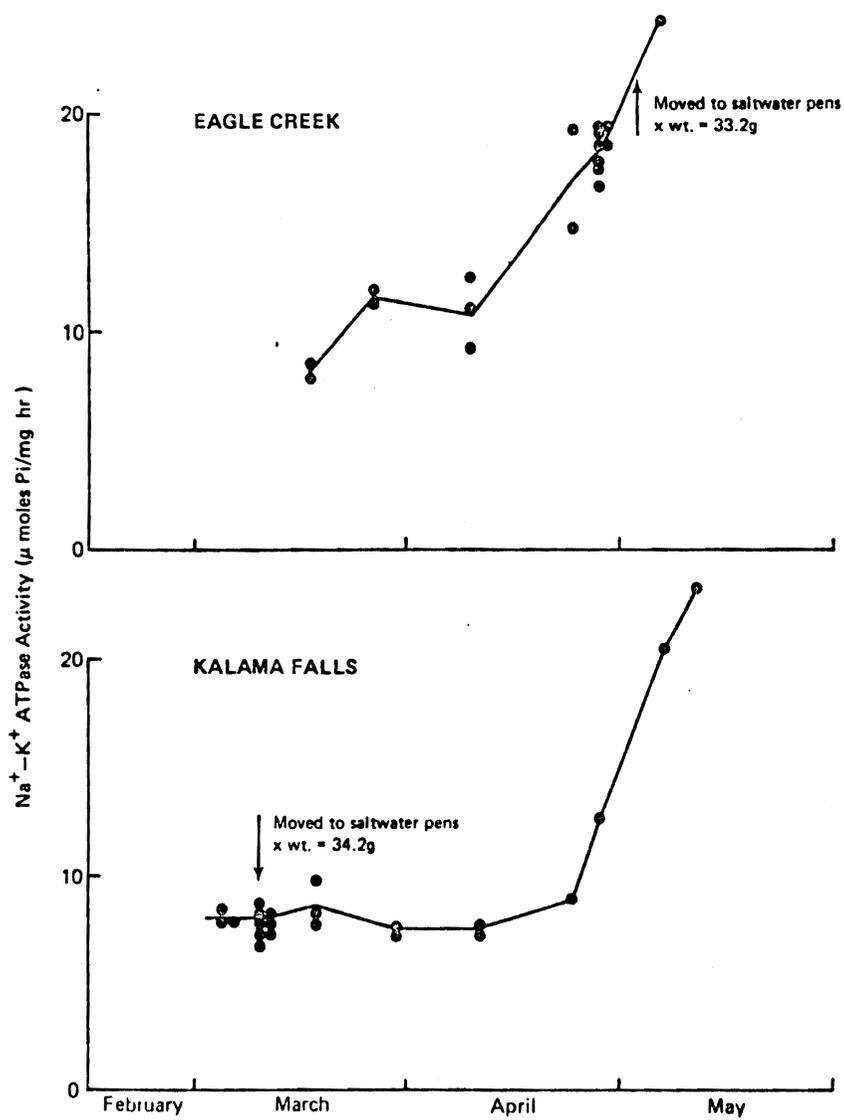


Figure 11. Na⁺-K⁺ ATPase activity in the gills of two stocks of spring chinook salmon at Columbia River hatcheries. Saltwater testing, as indicated by arrows, coincided with release time from the hatchery. Small populations were maintained in fresh water beyond time of release from hatchery to determine scope of enzyme activity.

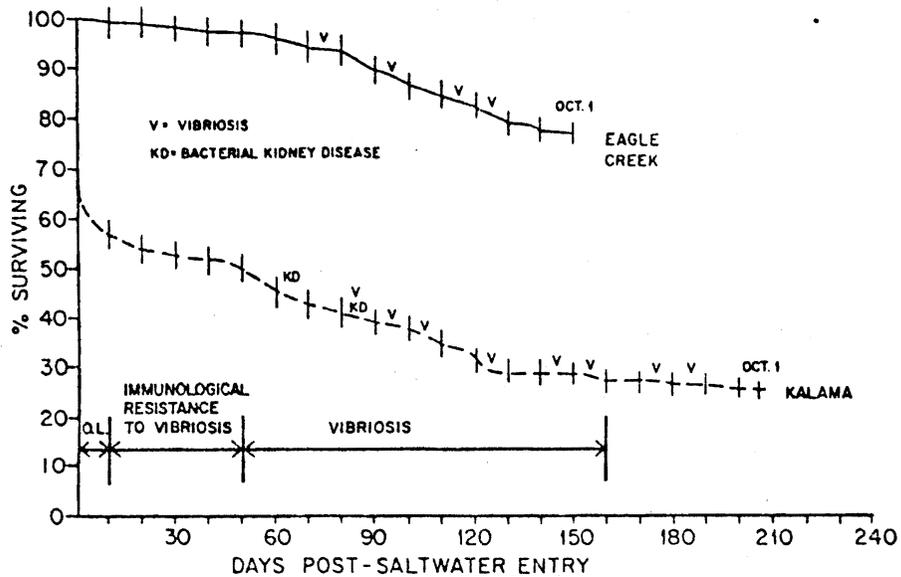


Figure 12. Survival of 2 stocks of spring chinook salmon in saltwater net-pens. Disease mortalities occurring during 10-day intervals are indicated on curve. Death resulting from osmoregulatory dysfunction is indicated by O.L.

release of T-4 in the plasma in stunted fish in sea water. Clarke and Nagahama (1977) offered a plausible hypothesis for saltwater stunting based on histological studies of coho salmon pituitaries. They suggested that the stunting process occurs as a result of decreased secretion of thyroxine. Since thyroxine is known to affect growth hormone (GH) secretion in coho salmon (Higgs et al., 1976), it is plausible that depressed GH is the cause of stunting. The use of sensitive radioimmune assays for thyroxine in smolting salmonids may afford a rapid a priori index of the physiological extent of smoltification in hatchery populations, thus reducing the chance of premature transfer to saltwater pens.

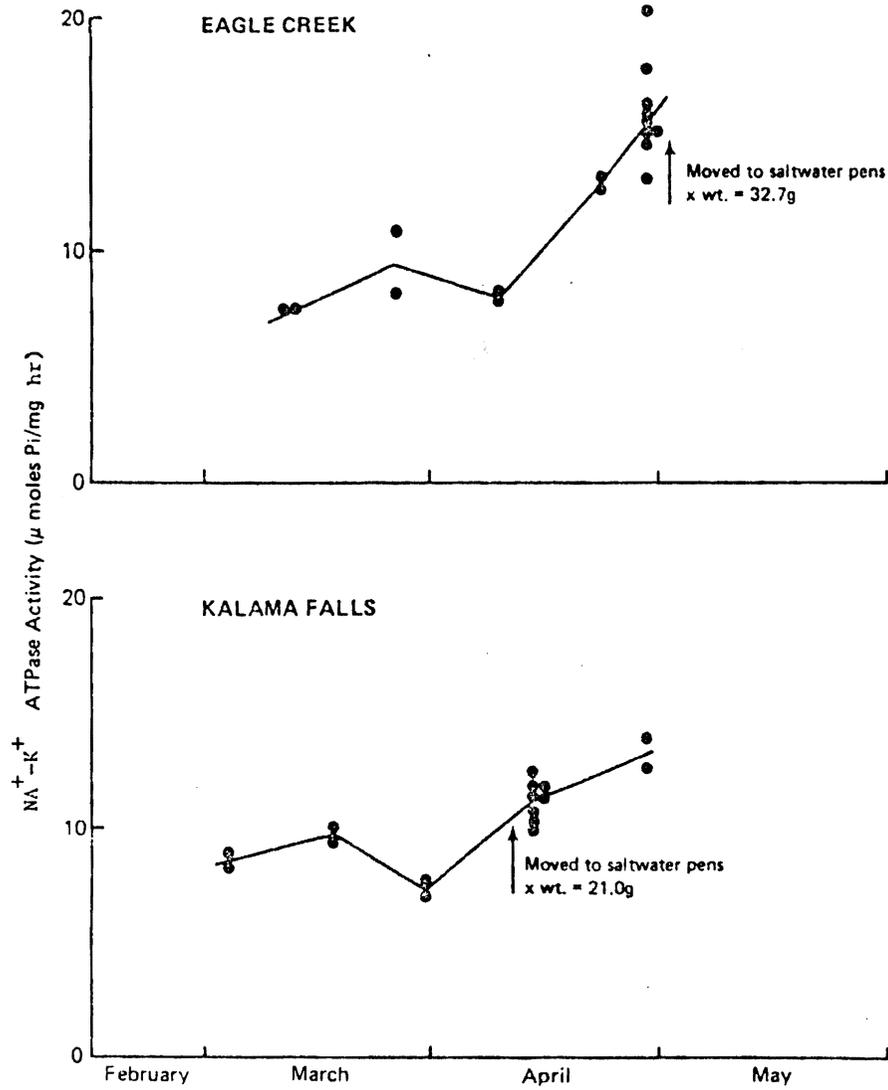


Figure 13. $\text{Na}^+ - \text{K}^+$ ATPase activity in the gills of coho salmon at Columbia River hatcheries. Saltwater testing, as indicated by arrows, coincided with release time from the hatchery. Small populations were maintained in fresh water beyond time of release from the hatchery to determine scope of enzyme activity.

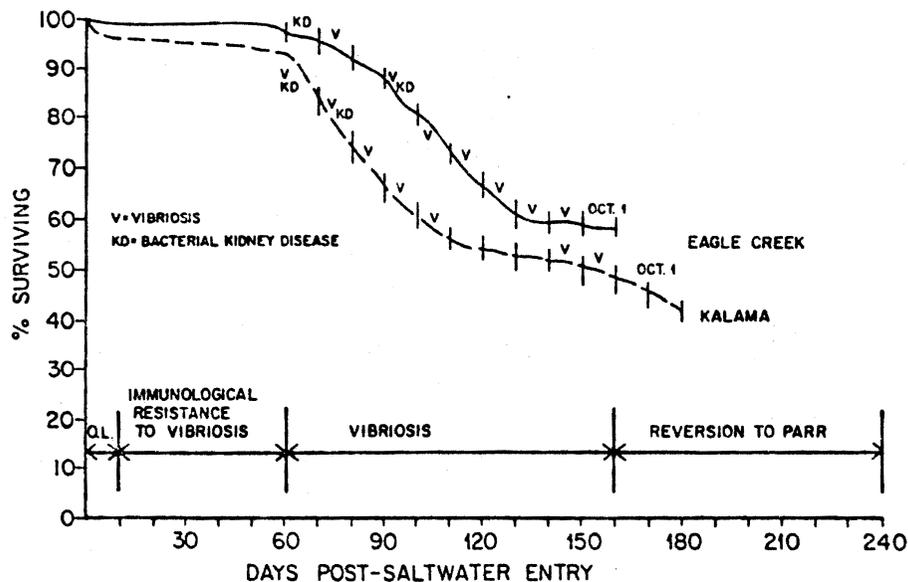


Figure 14. Survival of 2 stocks of coho salmon in saltwater net-pens. Disease mortalities occurring during 10-day intervals are noted on curve. Death resulting from osmoregulatory dysfunction is indicated by O.L.

LITERATURE CITED

- Amend, D. F., and F. C. Fender. 1976. Uptake of bovine serum albumin by rainbow trout from hyperosmotic solutions: a model for vaccinating fish. *Science* 192(4241):793-794.
- Antipa, R. 1976. Field testing of injected *Vibrio anguillarum* bacterins in pen-reared Pacific salmon. *Journal of the Fisheries Research Board of Canada* 33:1291-1296.
- Antipa, R., and D. F. Amend. 1977. Immunization of Pacific salmon: comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins. *Journal of the Fisheries Research Board of Canada* 34:203-208.
- Brannon, E. L., R. E. Nakatani, and L. R. Donaldson. 1976. Waste heat employment for accelerated rearing of coho salmon. Sea Grant Reprint WSG-TA 77-4, University of Miami Conference of Waste Heat Management and Utilization.
- Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. *Journal of the Fisheries Research Board of Canada* 26:2363-2394.

- Brett, J. R. 1974. Tank experiments on the culture of pan-size sockeye salmon (*Oncorhynchus nerka*) and pink salmon (*O. gorbuscha*) using environmental control. *Aquaculture* 4:341-352.
- Brett, J. R., and J. E. Shelbourn. 1975. Growth rate of young sockeye salmon, *Oncorhynchus nerka*, in relation to fish size and ration level. *Journal of the Fisheries Research Board of Canada* 32:2103-2110.
- Brown, M. E. 1957. Experimental studies of growth. Pages 361-400 in M. E. Brown (ed.), *Physiology of Fishes*. Academic Press, New York.
- Cisar, J. O., and J. L. Fryer. 1969. An epizootic of vibriosis in chinook salmon. *Bulletin of the Wildlife Disease Association* 5:73-76.
- Clarke, W. C., and Y. Nagahama. 1977. The effect of premature transfer to seawater on growth and morphology of the pituitary, thyroid, pancreas and interrenal in juvenile coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Zoology* 55:1620-1630.
- Cooper, E. L. 1961. Growth of wild and hatchery strains of brook trout. *Transactions of the American Fisheries Society* 90:424-438.
- Dickhoff, W. W., L. Folmar, and A. Gorbman. 1977. Relationship of thyroxine, and gill $\text{Na}^+\text{-K}^+$ adenosinetriphosphatase in coho salmon, *Oncorhynchus kisutch*. *American Zoologist* 17:857 (abstract).
- Dickhoff, W. W., and L. C. Folmar. 1978. Relationship of thyroxine and gill adenosinetriphosphatase (ATPase) in coho salmon (*Oncorhynchus kisutch*). *Journal of Experimental Zoology* (in preparation).
- Fessler, J. L., and H. W. Wagner. 1969. Some morphological and biochemical changes in steelhead trout during parr-smolt transformation. *Journal of the Fisheries Research Board of Canada* 26:2823-2841.
- Fryer, J. L., J. S. Nelson, and R. L. Garrison. 1972. Vibriosis in fish. Pages 129-133 in R. W. Moore (ed.), *Progress in Fishery and Food Science*, Vol. 5, University of Washington Publications in Fisheries, Seattle, Wash.
- Fryer, J. L. 1977. Development of bacterins and vaccines for control of infectious diseases in fish. Oregon State University, Sea Grant College Program Publications, ORESU-T-77-012. OSU, Corvallis, Ore.
- Giles, M. A., and W. E. Vanstone. 1976. Changes in ouabain sensitive adenosinetriphosphatase activity in gills of coho salmon (*Oncorhynchus kisutch*) during parr-smolt transformation. *Journal of the Fisheries Research Board of Canada* 33:54-62.
- Gould, R. W. 1977. Development of a new vaccine delivery system for immunizing fish and investigation of the protective antigens in *Vibrio anguillarum*. Ph.D. Thesis, Oregon State University, Corvallis.
- Harrell, L. W., H. M. Etlinger, and H. O. Hodgins. 1975. Humoral factors important in resistance of salmonid fish to bacterial disease. I. Serum antibody protection of rainbow trout (*Salmo gairdneri*) against vibriosis. *Aquaculture* 6:211-219.

- Harrell, L. W., A. J. Novotny, M. H. Schiewe, H. O. Hodgins. 1976. Isolation and description of two vibrios pathogenic to Pacific salmon in Puget Sound. U.S. Department of Commerce, National Marine Fisheries Service, Fisheries Bulletin 74:447-449.
- Higgs, D. A., E. M. Donaldson, H. M. Dye, and J. R. McBride. 1976. Influence of bovine growth hormone and L-thyroxine on growth, muscle composition and histological structure of the gonads, thyroid, pancreas and pituitary of coho salmon (*Oncorhynchus kisutch*). Journal of the Fisheries Research Board of Canada 33:175-200.
- Mahnken, C. V. W., A. J. Novotny, and T. Joyner. 1970. Salmon mariculture potential assessed. American Fish Farmer 2(1):12-15, 27.
- Mahnken, C. 1972. The future of mariculture in Puget Sound. In First Annual Marine Technology Society, Pacific Rim Conference, Seattle, Washington.
- Mahnken, C. V. W. 1973. The size of coho salmon and time of entry into seawater: Part I. Effects on growth and condition index. Pages 30-31 in Proceedings of the 24th Annual Northwest Fish Culture Conference.
- Mahnken, C. V. W. 1976. Status of commercial net-pen farming of Pacific salmon in Puget Sound. Proceedings World Mariculture Society 6:285-298.
- Needham, A. E. 1964. The Growth Process in Animals. Sir Isaac Pitman and Sons Ltd., London.
- Novotny, A. J. 1975. Net-pen culture of Pacific salmon in marine waters. Marine Fisheries Review 37(1):36-47.
- Novotny, A. J. 1978. Vibriosis and furunculosis in marine cultured salmon in Puget Sound, Washington. Marine Fisheries Review 40(3):52-55.
- Parry, G. 1966. Osmotic adaptation in fishes. Biological Review 41:392-444.
- Potts, W. T. W. 1968. Osmotic and ionic regulation. Annual Review of Physiology 30:73-104.
- Ricker, W. E. 1958. Handbook of Computations for Biological Statistics of Fish Populations. Fisheries Research Board of Canada, Bulletin 119.
- Rohovec, J. S., R. L. Garrison, and J. L. Fryer. 1975. Immunization of fish for control of vibriosis. Pages 105-112 in Proceedings of 3rd U.S.-Japanese Meeting on Aquaculture. Special Publication of Japan Fisheries Agencies and Sea Regional Fisheries Research Laboratory, Niigata, Japan.
- Rucker, R. R. 1959. *Vibrio* infections among marine and freshwater fish. Progressive Fish Culturist 21:22-25.
- Shelbourn, J. E., J. R. Brett, and S. Shirahata. 1973. Effect of temperature and feeding regime on the specific growth rate of sockeye salmon fry (*Oncorhynchus nerka*) with a consideration of size effect. Journal of the Fisheries Research Board of Canada 30:1191-1194.

- Tomiyama, T. 1972. Fisheries in Japan: Salmonidae. Japanese Marine Products Photo Material Association, Michi Nada, Tokyo.
- Zaugg, W. S., and L. R. McLain. 1969. Inorganic salt effects on growth, seawater adaptation and gill ATPase of Pacific salmon. Pages 293-306 in O. W. Neuhaus and J. E. Halver (eds.), Fish in Research. Academic Press, New York.
- Zaugg, W. S., and L. R. McLain. 1970. Adenosinetriphosphatase activity in gills of salmonids: seasonal variations and saltwater influence in coho salmon (*Oncorhynchus kisutch*). Comparative Biochemistry and Physiology 35:587-596.
- Zaugg, W. S., and H. H. Wagner. 1973. Gill ATPase activity related to parr-smolt transformation and migration in steelhead trout (*Salmo gairdneri*): influence of photoperiod and temperature. Comparative Biochemistry and Physiology 45:955-965.