Final Report

to the

Bonneville Power Administration

Development of an Effective Transport Media for Juvenile Spring Chinook Salmon to Mitigate Stress and Improve Smolt Survival During Columbia River Fish Hauling Operations

Bonneville Power Administration

Contract No. DE-A1 79-82 BP-35460

Code No. 82-l 9

Kathleen J. Anderson, Project Officer

Submitted by:

U.S. Fish and Wildlife Service

National Fishery Research Center

Bldg. 204, Naval Station

Seattle, Washington 98115

Alfred C. Fox, Director

Gary A. Wedemeyer, Project Leader

Aldo N. Palmisano, Project Chemist

Louis E. Salsbury, Project Biologist

February 1985

Table of Contents

Abstract

Selected transport media consisting of mineral salt additions (Na^+, Cl^-, Ca^{++}, PO_4^{-3}, HCO_3^-, and Mg^{++}), mineral salts plus tranquilizing concentrations of tricaine methane sulfonate (MS-222), or MS-222 alone were tested for their ability to mitigate stress and increase smolt survival during single and mixed species hauling of Columbia River spring chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*). Successful stress mitigation was afforded by several formulations as indicated by protection against life-threatening osmoregulatory and other physiolqoical dysfunctions, and against immediate and delayed hauling mortality. Effects on the seawater survival and growth of smolts hauled in transport media were used as the overall criterion of success.

Of the fourteen chemical formulations tested, 10 ppm MS-222 emerged as top-rated in terms of ability to mitigate physiological stress during single and mixed species transport of juvenile spring chinook salmon at hauling densities of 0.5 or 1.0 lb/gallon. Immediate and delayed mortalities from hauling stress were also reduced, but benefits to early marine growth and survival were limited to about the first month in seawater. The two physical factors tested (reduced light intensity and water temperature) were generally less effective than mineral salt additions in mitigating hauling stress, but the degree of protection afforded by reduced light intensity was nevertheless judged to be physiologically beneficial.

List of Tables

List of Tables Continued:

List of Figures

Executive Summary

It is widely recognized that the anadromous fishery resources of the Columbia River and its tributaries have been seriously affected by the construction and operation of dams for hydroelectric power generation. Since the declining salmon and steelhead trout runs are partly due to fish passage problems encountered by downstream migrants, an experimental program was begun in 1965 by the National Marine Fisheries Service to collect migrating smolts and transport them past the dams. Currently, the transportation program is operated by the Corps of Engineers and utilizes both trucks and barges. Migrating juvenile salmonids collected at McNary Dam and hauled by truck or barge include not only spring chinook salmon (*Oncorhyncus tshawytscha*) and steelhead trout (*Salmo gairdneri*), but also wild and hatchery coho (*O. kisutch*), sockeye (*O. nerka*), and summer/fall chinook salmon (*O. tshawytscha*).

The transport system as now operated has been a qualified success; plainly beneficial to fall chinook and steelhead adult returns, and marginally beneficial to the spring chinook contribution. The reduced success rate with migrating spring chinook smolts has been attributed, at least in part, to hauling mortality resulting from the stress of the fish transport process.

The goal of the present project was to develop and test water chemistry modifications--transport media--for the single (chinook only) and mixed species (chinook with steelhead) hauling of juvenile spring chinook salmon that would mitigate stress, reduce immediate and delayed mortality, improve early marine survival, and have a low potential for causing hauling equipment corrosion problems.

Transport media consisting of mineral salt additions (Na^+, Cl-, Ca^{++}, PO_4^{-3}, HCO_3^-, and Mg++), mineral salts and tranquilizing concentrations of tricaine methane sulfonate MS-222, or MS-222 alone were tested. Successful stress mitigation was afforded by several formulations as indicated by protection against life-threatening osmoregulatory and other physiological dysfunctions, and against immediate and delayed hauling mortality. The two physical factors tested (reduced light intensity and water temperature) were generally less effective than mineral salt additions in mitigating hauling stress, but the degree of protection afforded by reduced light was nevertheless judged to be physiologically beneficial.

Of the fourteen chemical formulations tested, 10 ppm MS-222 emerged as top-rated for the single and mixed species hauling of juvenile spring chinook at leading densities of 0.5 and 1.0 lb/gal. Immediate and delayed mortalities from hauling stress and scale loss were reduced, but there were limited benefits to subsequent growth and survival in seawater. Transport media formulations containing MS-222 and $NaHCO_3$, CaC12; and CaC12 alone (50 ppm) were also promising, but the biological benefits of their regular use would probably be outweighed by the possibility of causing corrosion. As far as can be determined, MS-222 used alone will not contribute to equipment corrosion problems.

In conclusion, since a transport medium consisting simply of 10 ppm MS-222 reduced activity levels (potential for scale loss), did not stimulate oxygen consumption, and was effective in reducing stress during spring chinook hauling, it is recommended as the alternative of choice for Columbia River smolt transport whether or not equipment corrosion problems continue to be seen as a major constraint.

Introduction

It is widely recognized that the anadromous fishery resources of the Columbia River and its tributaries have been seriously affected by the construction and operation of dams for hydroelectric power generation. Because it was recognized that the declining Pacific salmon (Oncorhynchus sp.) and steelhead trout (Salmo gairdneri) runs -were partly due to fish passage problems encountered by downstream migrants, biologists began an experimental program in 1965 to collect migrating smolts and transport them past the dams (Park 1980). The transport program was put into practice by the U.S. Fish and Wildlife Service, Bureau of Commercial Fisheries (now the National Marine Fisheries Service) at Ice Harbor Dam in 1968 and was subsequently expanded to include Little Goose, Lower Granite, and McNary Dams. Transportation of smolts from 1968 to 1976 was limited to trucking from collection sites at the dams to release sites below Bonneville. From 1977 to the present, the transportation program, now run by the U.S. Army Corps of Engineers, has utilized both trucks and barges. Migrating juvenile salmonids collected at McNary Dam and hauled by truck or barge include not only spring chinook salmon (O. tshawytscha) and steelhead trout but also wild and hatchery-reared coho salmon (O. kisutch), sockeye salmon (O. nerka), and summer and fall chinook salmon (O. tshawytscha). Since 1977, the percentage of smolts transported has exceeded 49% of the available fish.

The transport system has been a qualified success; plainly beneficial to fall chinook salmon and steelhead adult returns, and marginally beneficial to the spring chinook salmon contribution (Park et al. 1984). Marking experiments show that many more transported steelhead and fall chinook salmon return to spawn. For example, adult steelhead returns to the Snake River arising from transported smolts is usually about 2%. However, the success ratio for spring chinook salmon is considerably less (Congleton et al. 1984). Before the 1975 brood year, the Columbia River chinook salmon contribution rates (Ice Harbor escapement plus Columbia River harvest) ranged from 0.1 to 2.18, but was usually above 0.5%. For the more recent brood years, however, the Columbia River contribution has been less than 0.23, even though the number of fish transported has increased (Raymond and Sims 1980). Even though transported fish contribute more returning adults than do untransported fish, the fact remains that transportation of chinook salmon smolts from Snake River dams to the Columbia River estuary has not reversed the downward trend in Idaho chinook salmon stocks (Congleton et al. 1984).

The difference in success among steelhead, and fall and spring chinook salmon suggests a basic biological impact or physiological difference between the species. A comparison of smolt survival to the lower Columbia River (Raymond and Sims 1980) with adult returns to the mid-Columbia and Snake River, lead Park and co-workers to suggest that the major mortality occurs in both stocks after release from the transport system or after fish pass the lower river dams, and that stress factors associated with smolt hauling operations might be involved (Park 1980, Park et al. 1984). Tests involving holding post-transport test groups at Bonneville Hatchery showed that the major delayed mortality (in some cases as high as 50%) occurred in spring chinook salmon rather than in steelhead. Thus, spring chinook appear to be physiologically less capable than steelhead of benefiting from tranportation, particularly in mixed species hauling of salmon with steelhead (Park et al. 1984).

It is not unexpected that survival after the stress associated with transportation systems is lower in spring chinook salmon than in the other anadromous species. Experience in salmonid fish culture has shown that spring chinook are among the least resistant to the stress of hatchery practices and to the stress-mediated fish diseases. As an example, spring chinook are probably the most susceptible of all the anadromous salmonids to bacterial kidney disease (D. Mulcahy, U.S. Fish and Wildlife Service, personal communication). Similarly, when smolt functionality has been impaired by adverse environmental factors, it is usually evident in chinook salmon after less than two weeks in sea water. In contrast, coho salmon may be able to survive for as long as several months before osmoregulatory capacity is finally overwhelmed or stress-mediated diseases are expressed (C. Mahnken, National Marine Fisheries Service, personal communication).

The physiologically detrimental effects associated with fish hauling operations and the use of transport media such as mineral salt additions or tricaine methane sulfonate (MS-222) to mitigate stress and improve survival have been under investigation for a number of years (McFarland and Norris 1958; Wedemeyer 1972; Long et al. 1977; Carmichael et al. 1983). For example, Hattingh et al. (1975) reported that the survival rates of several warm water species could be substantially increased by the addition of 10 ‰ NaCl to the hauling tank water. Other workers have shown that mineral salt additions can also mitigate stress and reduce the immediate and delayed mortality associated with the transport of salmonid fishes, especially in smolts hauled in water of low total hardness typical of Columbia River conditions (Wedemeyer 1972; Long et al. 1977; Haswell et al. 1982; Nikinmaa et al. 1983).

The physiological mechanism probably involves protection by the added electrolytes against the life-threatening osmoregulatory imbalances that result when tolerance limits to the stress of handling and crowding are exceeded. However, there is also reason to believe that the hyperglycemia and hypercortisolemia that Specker and Schreck (1980) have recently demonstrated in transported coho salmon may be amenable to mitigation by the use of mineral salts or other transport media (Wedemeyer 1972; Wedemeyer and McLeay 1981).

As judged by existing information, the transport media which appear to have the most potential for alleviating the life-threatening osmoregulatory and other physiological disturbances resulting from Columbia River fish hauling operations are salts of Ca^{++}, Mg^{++}, K^{+}, Na^{+}, Cl^{-}, or HCO_3^- and low (tranquilizing) levels of MS-222. Unfortunately, the addition of mineral salts to the water used to haul fish may also significantly increase the potential for equipment corrosion problems. Another potential problem which must be addressed in the development of transport media for smolts hauling is the fact that salt formulations containing Mg^{++} and K^{+} can actually decrease survival if significant scale loss has occurred (Wedemeyer and Casillas 1985). Thus, the addition of low concentrations of MS-222 to a transport medium may help in preventing scale loss (by reducing activity) as well as in reducing the severity of the physiological stress response itself. The use of such a transport medium would likely increase the present benefits of Columbia River spring chinook salmon hauling operations. Consequently, a research and development project on such stress mitigation procedures could be considered as a positive action toward increasing Columbia River chinook salmon returns.

A variety of physiological methods is available to evaluate the severity of stress and the time fish need for recovery (Pickering 1981; Schreck 1981). Those which were judged to be suitable for studies of hauling stress include the measurement of circulating levels of the corticosteroid stress hormones and secondary blood and tissue effects such as hyperglycemia, blood electrolyte imbalances, and depletion of liver glycogen and muscle adenylate energy charge (Wedemeyer and McLeay 1981). In Columbia River smolt hauling operations, the ability of transported fish to adapt to sea water, survive, and continue to grow and develop normally is also a necessary index of success in achieving stress mitigation. Thus, a seawater holding period was considered to be essential.

The goal of the present project was to develop and test transport media for the single and mixed species hauling of juvenile spring chinook salmon that would mitigate stress, reduce immediate and delayed mortality, improve early marine survival, and have a low potential for causing hauling equipment corrosion problems. Long-term tagging studies to assess contribution to the fishery and adult returns were beyond the scope of this project.

The following hypotheses were tested:

1. The use of transport media will mitigate acute stress and improve smolt survival in the single and mixed species hauling of juvenile spring chinook salmon.
2. A transport medium that mitigates acute stress and improves survival during single and mixed species hauling will also Improve smolt performance capacity as indicated by increased survival and growth in sea water.

Methods

The methods used to obtain the data needed to test the stated hypotheses were categorized under two tasks:

Task 1: Testing under controlled conditions. A hauling stress challenge test was developed and used in the laboratory, to evaluate the effectiveness of transport media formulations in protecting juvenile spring chinook against the life-threatening osmoregulatory and other physiological disturbances that are known to occur as a result of fish hauling operations.

Task 2: Field evaluations. The most promising of the transport media formulations identified in Task 1 were further evaluated by test-hauling hatchery spring chinook, Columbia River fall chinook and spring chinook salmon mixed with hatchery steelhead, to the marine field station of the Seattle National Fishery Research Center (SNFRC) located at Marrowstone Island, Washington. Protection against physiological dysfunctions, immediate and delayed hauling mortality, and benefits to long-term seawater growth and survival were used as the criteria of successful stress mitigation.

<u>Testing under controlled conditions</u>

Juvenile spring chinook salmon and steelhead trout were obtained from the Columbia River Eagle Creek or Wells Dam hatcheries and transported to the SNFRC Marrowstone Field Station (MFS). Roth species were acclimated to 4'-circular tanks at a loading density[1/] of 0.05 lbs/ft^3 or less, for at least

---

[1/] Fish loadings are given in English system units in this report to correspond with current usage in Columbia River fish transport operations.

four weeks prior to testing. These conditions have been previously shown to be minimally stressful (Wedemeyer 1972). During this period, the fish were fed the Terramycin medicated Oregon Moist Pellet diet (OMP) and given a prophylactic formalin treatment to control subclinical bacterial infections and external parasite infestations. Testing was not begun until feeding behavior was judged to be normal.

Hauling stress challenge tanks for the controlled laboratory testing required in Task 1 were constructed from six-gallon plastic containers (Fig. 1). They were marked to indicate 0.5-gallon increments, and overflow holes were drilled at the five-gallon level. Water lines with flowmeters and air lines with 6-inch aerators were plumbed to the bottom of each tank. Temperature control was achieved by partially submerging the challenge tanks in flowing water during tests. Translucent plastic tops were used to protect the experimental fish from visual disturbances during the challenge tests.

Challenge Test Protocol. Based on the average weight of the stock fish, the volume of water in the challenge tanks was adjusted to achieve the hauling density to be tested (0.5 lb/gal, 1.0 lb/gal, or 2.0 lbs/gal). The transport media formulation to he tested was then added at the pre-selected concentration, the water flow for temperature control was atarted, and aeration was begun. Initial water temperature and dissolved oxygen samples were taken.

Prior to each single or mixed species hauling stress challenge test, a lo-fish baseline (0-hr) sample was taken from the stock tank by netting groups of 2 or 3 spring chinook or steelhead into 8 L of 200-ppm MS-222[2/] neutralized with 300 ppm $NaHCO_3$. The anesthetized fish were weighed and measured (fork length), the caudal peduncle was severed, and the required amount

---

[2/] Concentrations are given as ppm (parts per million) instead of mg/L to correspond with current usage in Columbia River fish transport operations.

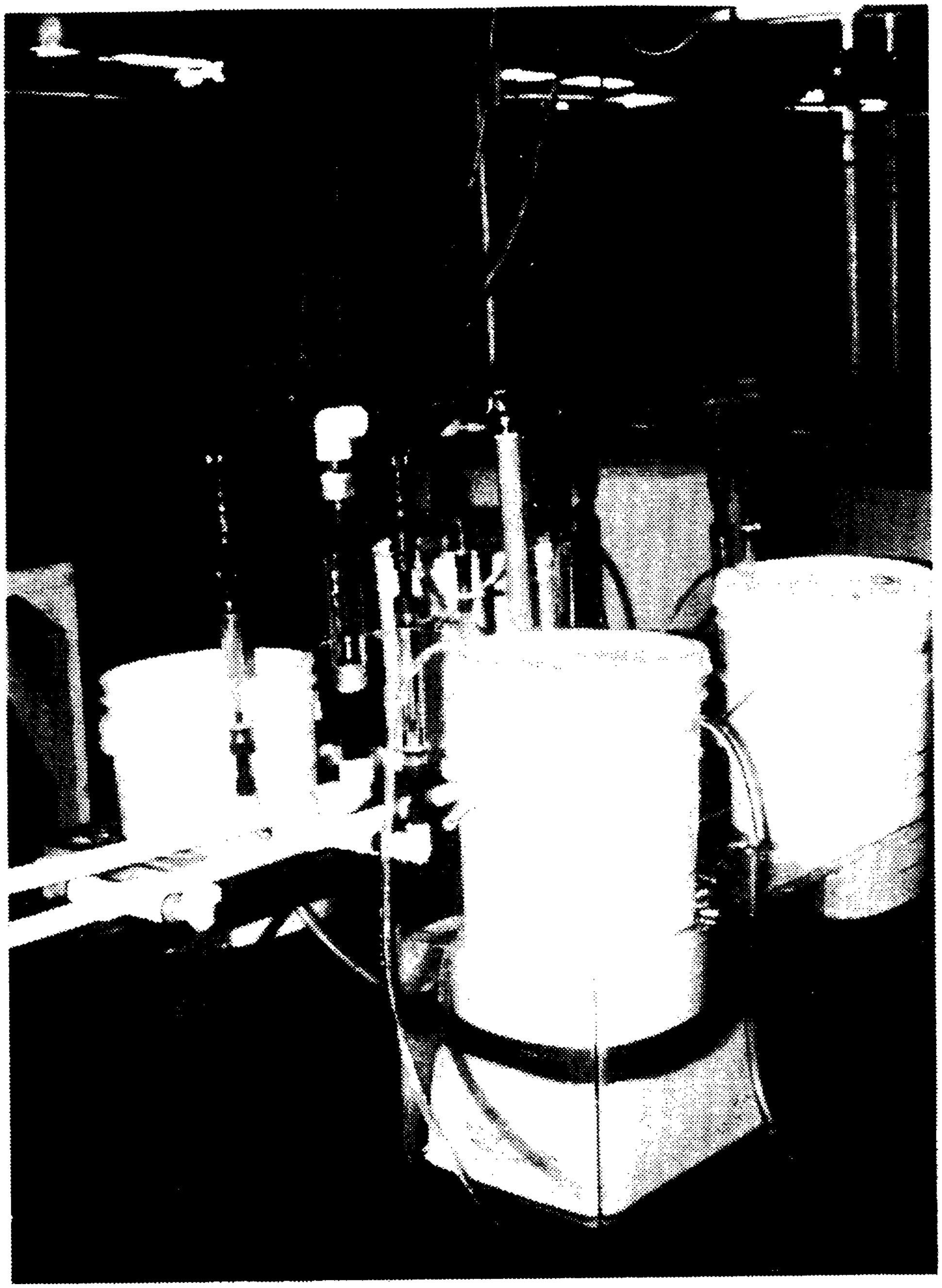

Figure 1. Hauling stress challenge tanks used to assess protection afforded by transport media. External water bath provided required temperature Control.

of blood was collected in heparinized Natelson tubes. Tissue samples were taken as appropriate. The water volume in the stock tank was then lowered sufficiently to increase the loadiny density of the remaining fish back to the initial conditions (usually 0.05 lb/ft^3, or less). This sampling protocol has been previously shown to minimize the influence of handling stress on physiological test results (Wedemeyer 1976).

To initiate a test, forty spring chinook or steelhead (as appropriate) were netted from the stock tanks directly into each test and control challenge tank, and a timer started. When pre-stressed fish were required,the technique of Schreck (1981) was used, in which groups of 4 or 5 fish were suspended above the water in a hand net for 30-60 sec before placing them in the challenge tanks. Blood samples from l0-fish subsamples were taken at 0, 1, 3, 6, and 24 hours during the test. To maintain a constant loading density in the challenqe tanks, the water level was lowered by the appropriate (pre-calculated) amount each time fish were removed. The dissolved oxyqen (DO) concentration was determined periodically in a water sample taken by siphon and the aeration rate adjusted as needed to maintain the DO at 1 O-12 ppm. After the six-hour sample was taken, the challenge tanks were quickly filled to the five-gallon overflow level to reduce the loading density to 0.05 lb/ft^3, or Less, and the remaining 10 fish were allowed an 18-hour recovery period. Aeration was discontinued and the water flow was adjustetl to about 0.2 gpm, resul ting in a flow index (lbs fish/gpm per inch of fish length) calculated to he equivalent to the flow index existing in the lightly loaded stock tanks. After 24 hours, final fish, DO, and water temperature samples were taken.

Mixed species protocol. For the mixed species hauling stress challenge tests, a protocol was developed which was judged to be physiologically repre sentative of conditions encountered by spring chinook salmon and steelhead during collection and holding operations at Columbia River dams (D. Park, National Marine Fisheries Service, personal communication).

About18 hours prior to each challenge test, 120 spring chinook and 120 steelhead from individual stock tanks loaded at about 0.05 lb/ft^3 were combined into a separate 4 foot circular tank and the water level adjusted to maintain the mixed species at the same (0.05 lb/ft^3) population density. Numbers of fish were chosen to maintain a 50:50 chinook/steelhead ratio. Because of natural size differences, the steelhead were generally larger than the salmon. For a few tests, steelhead of smaller or equal size were selected.

To initiate a mixed species hauling stress challenge test, fish were pre-stressed as before and transferred to the challenge tanks at the hauling density selected for testing: 0.5, 1.0, or 2.0 lbs/gal. Subsamples of 10 fish were taken at 0, 1, 3, 6, and 24 hours as usual. The 50:50 chinook/steelhead mixed species ratio in the challenge tanks was maintained in the lo-fish subsamples by taking sufficient numbers of fish at each time period to enable the collection of blood from at least five salmon and five steelhead.

The effectiveness of darkness vs. illumination in mitigating hauling stress was evaluated using mixed species challenge tests. Equal numbers of spring chinook salmon and steelhead smolts were removed from stock tanks, mixed, and held in two groups at 0.05 lb/ft^3 or less for 18 hours. One group was illuminated with (ambient) light at about 2 ft-candles, and the other was kept in darkness by covering the tank with black plastic. Prior to initiating the challenge tests, baseline blood samples were taken from 5 to 10 salmon removed from the single species stock tanks (controls) and from the light and dark mixed species test groups. Fish from the illuminated group were then transferred with as little stress as possible to a hauling stress challenge tank illuminated at a light intensity of 24 ft-candles, and fish from the darkened group were similarly transferred to a darkened stress challenge tank; both groups were tested at a hauling density of 1.0 lb/gal. Experimental conditions and fish sampling schedules were the same as previously described. When spring chinook smolts were removed for sampling, steelhead were also removed to maintain the 1:1 mixed species ratio.

Field Evaluations

Single and mixed species test hauls of spring chinook and steelhead smolts from Columbia River hatcheries to MFS were conducted to assess benefits of transport media to long-term seawater growth and survival. An insulated fish hauling tank with divided chambers for test and control groups was used. On arrival at each hatchery, an immediate 20-fish sample was taken from the raceway to establish pre-hauling baseline physiological values. Blood samples from ten fish were taken and the plasma frozen for later cortisol analyses. Blood and liver samples were taken from 10 additional fish for glucose, chloride, and glycogen determinations. Fish were handled and anesthetized as previously described to minimize sampling stress.

Prior to loading, the smolts were pre-stressed by crowding to approximate the physiological conditions experienced by migrating juveniles as a result of collection and holding procedures at dams. A wire mesh pen was constructed in a convenient hatchery raceway and smolts were netted into the pen to achieve a crowding density of 4 lbs/ft^3. They were then held for 18 hours--conditions previously shown to elicit a moderate stress response in salmonids (Wedemeyer 1976).

Shortly before departure, the hauling tanks were filled with untreated Columbia River water and the transport media formulation added to the test chamber. Aeration sufficient to maintain the DO at 10-12 ppm was provided. Approximately 5 lbs of fish at a time were alternately loaded into each test and control chamber to achieve a nominal hauling density of 0.5 lbs/gal, and blood and tissue samples were again taken. The water temperature was maintained at 10-11°C during each haul by adding chlorine-free ice as necessary.

After the 6-8 hour haul to MFS, 20 fish were again sampled from the test and control groups and the remainder transferred into 700-L freshwater holding tanks at a reduced loading density of 0.05 lb/ft^3. The smolts were then held in fresh water (at 10-11°C) for 10 days (the estimated time required for smolts trucked to the release site below Bonneville Dam to migrate to the sea). Periodic blood and liver samples were taken from the test and control groups during this freshwater holding period.

To initiate the seawater growth and survival evaluations, both the test and control groups were given a final stress challenge (by weighing and measuring) to determine if the smolts hauled in a transport medium tolerated subsequent stress after release better than the controls. Two-hundred fish from each group were netted into aerated tanks of 100 ppm MS-222 in groups of 5 to 10. After weighing and measuring, 100 smolts per group were randomly distributed to duplicate 700-L tanks and gradually acclimated to full-strength sea water by increasing the salinity to 10‰ for 24 hours, to 20‰ for an additional 24 hours, and then to 28‰ (full strength).

Effects of hauling on seawater growth and survival were assessed over a four-month period. Fish were fed the OMP diet at the rate of 2% of body weight per day. Test and control groups were weighed and measured, and the ration adjusted at monthly intervals. Results were expressed as monthly average weight, monthly weight gain, cumulative weight gain, and percent monthly and cumulative mortality.

For the test hauls of migrating Columbia River smolts, spring chinook salmon could not be obtained and we were required to substitute fall chinook collected at McNary Dam. To test haul fish of this smaller size, tanks were constructed of 20-gal plastic containers placed in a wooden frame inside a larger, water-filled, insulated steel tank. This arrangement allowed separate groups of the smaller fall chinook salmon to be test hauled at a loading of 0.5 lb/gal with adequate temperature control. Upon arrival at the McNary Dam fish facility, we normally collected baseline blood and tissue samples from fish in the raceways. In one case, a hauling barge was being filled and fish were removed from the raceways too quickly to allow us to collect samples. Thus, we were required to sample from the flume where smolts first enter the facility. As before, all fish were anestheized with 100 ppm MS-222 and blood or tissue samples were taken within 2 or 3 minutes after the fish were placed in the anesthetic. For adenylate determinations, white-muscle samples were taken posterior to the dorsal fin, quick-frozen between two stainless steel plates pre-cooled with liquid nitrogen (freeze-clamp technique), foil wrapped, and stored in liquid nitrogen for later analysis.

After the baseline samples were taken, additional fall chinook were netted into a bucket containing 5 gal of water until 2 to 3 lbs of fish were collected, resulting in a density of about 0.5 lb/gal. The fish were thus carried to the hauling truck at the loading density to be tested. After the control and test tanks were loaded, an additional lo-fish blood and tissue sample was taken from each group.

During the haul, the DO level was maintained at 10-12 mg/L by oxygen aeration. Since the Columbia River water temperature at McNary Dam was usually higher (19°C) than the water temperature at MFS (16°C), ice was placed in the outer tank to slowly cool the water in the inner (transport) tank to 16°C. After the 6-8 hour haul to MFS, lo-fish blood and tissue samples were again taken from the test and control groups and the remaining fish transferred to duplicate 700-L tanks at a loading density of 0.05 lb/ft^3 for the IO-day freshwater holding period. Periodic lo-fish blood and tissue samples were taken.

To initiate the seawater growth and survival evaluations of Columbia River fall chinook, fish from each test and control group were pre-stressed as before by being anesthetized, weighed, and measured, and then transferred in groups of 100 to separate circular tanks at a loading of 0.05 lb/ft^3 or less. Ten-fish blood and tissue samples were again taken from each group, and acclimation to seawater was begun by increasing the salinity to $10^{o}/oo$ for 24 hours, to $20^{o}/oo$ for an additional 24 hours, and then to $28^{o}/oo$ (full strength).

Test and control groups were maintained on the OMP diet at the 2% feeding rate and weighed and measured monthly. Mortalities were also weighed and measured. As before, results were expressed as average and cumulative monthly weight gain, and monthly and cumulative percent mortality.

Field evaluations of 10 ppm MS-222 as a transport medium were conducted with migrating fall chinook salmon collected at McNary Dam and hauled to the release site below Bonneville Dam as part of the routine fish transportation operstions by the Corps of Engineers (COE). Since it was not possible to compartmentalize the COE hauling trucks into test and control sections, two trucks were used.

Baseline blood and tissue samples were taken from fish in the McNary Dam raceways collected during the 12 to 24-hour period prior to COE trucking. The hauling trucks were then loaded, at a nominal density of 0.5 lbs/gal, and a second blood and tissue sample was taken from the test and control groups.

Because the fish were loaded in water from the raceways, the MS-222 transport medium under evaluation was introduced after loading by adding 114 g of MS-222 to the water at the pump intakes. This effectively circulated it through the entire system, giving a final concentration of 10 ppm.

After arrival at the release site below Bonneville Dam, a post-transport blood and tissue sample was taken from each control and test group and frozen for later cortisol and muscle adenylate analyses. The extent of mortality in the tank trucks and in the river at the release site could only be estimated visually, however, few dead fish were noted in either the test or control groups.

The benefits of transport media in mitigating stress during mixed species trucking was evaluated by test hauling pre-stressed spring chinook salmon mixed with steelhead (1:1 ratio) from the FWS Eagle Creek National Fish Hatchery to the MFS for seawater growth and survival evaluations.

As before, crowding was used to impose a pre-stress prior to hauling. Blood samples were taken from both species to determine baseline values and screened, 16 ft^3 compartments were constructed in two raceways. Steelhead were removed from an adjacent raceway, weighed, and accumulated in the two crowding compartments, until 250 fish (31.3 lbs) were present in each enclosure.

Then, 250 spring chinook (28.8 lbs) were loaded into each of the two compartments in a similar manner, yielding a final mixed species loading density of approximately 0.5 lb/gallon. The fish were held in the compartments for 18 hours to achieve the degree of pre-stress desired.

After the 18-hour holding period, each group was loaded into one of two compartments of the hauling truck to achieve the desired transport hauling density of 0.50 lb gallon. One compartment (treated) contained 10 ppm MS-222 ; the other (control) contained untreated water. We-transport blood samples were taken immediately after the fish were loaded, and the plasma was separated and frozen for later cortisol, glucose, and chloride analyses. The fish were then subjected to a 6-hour test haul to MFS. Oxygen aeration was used, and both water temperature and DO were monitored periodically throughout the trip.

Upon arrival at MFS, post-transport blood samples were taken and the fish distributed into 700-L freshwater tanks to reduce the loading density to 0.05 lb/ft^3 or less. Both treated and control groups were held in freshwater for 10 days befor seawater conversion, to allow for the time estimated to be required by migrating smolts to reach the sea after release from the COE hauling trucks below Bonneville Dam. Periodic blood samples were taken from the treated and control spring chinook salmon during the freshwater holding period and analyzed for cortisol, glucose, and chloride. To begin the four-month seawater survival and growth evaluations, test and control fish were pre-stressed by being anesthetized, weighed, and measured, and then acclimated to full-strength ($28^o/oo$) sea water over a 48-hour period, as before.

Physiological Methods and Data Analysis

Physiological methods. The protection against physiological stress afforded by the transport medium under test was assessed by evaluating the severity of the resulting stress response, the accompanying osmoregulatory disturbances, and the time needed for recovery. In most cases, hyperglycemia, hypercortisolemia, and liver qlycogen depletion were used as indices of the severity of the primary stress response, while hypochloremia was taken as an index of the secondary blood electrolyte disturbances that result from osmoregulatory dysfunction (Wedemeyer et al. 1984).

In certain tests, muscle adenylate concentrations were determined to provide additional information on physiological status. Sampling and analytical methods used for muscle adenylate were as follows: Fish were removed from the raceway or the hauling truck in groups of two or three and lightly anesthetized with 50 ppm MS-222 in 15 L of Columbia River water. The spinal cord was severed, and a small (0.5 to 1.0 g) sample of white muscle tissue was excised and frozen using the liquid nitrogen freeze clamp technique method (Faupel et al. 1972; MacFarlane 1981; Vetter and Hodson 1982). Total time between capture and freeze clamping was kept to less than three minutes. For analyses, duplicate 130-mg tissue samples were ground with liquid nitrogen to a fine powder, warmed to O°C for extraction with 6% perchloric acid, brought to pH 7 with 5N K_2CO_3, and diluted to final volume with tris buffer, pH 7.75, according to procedures described by Ivanovici (1980) and Vetter and Hodson (1982).

The analytical method used for adenylate analysis was the conventional luciferin-luciferase reaction, measured in an integrating biophotometer. Adenosine triphosphate (ATP) was measured directly: adenosine diphosphate (ADP) was converted to ATP with pyruvate kinase and determined by difference; and adenosine monophosphate (AMP) was converted to ATP with pyruvate kinase and adenylate kinase, giving a direct measure of total adenylates and AMP by difference (Holm-Hansen and Karl 1978). One of each pair of duplicate samples was analyzed immediately after extraction and the remaining sample kept frozen and analyzed one or two days later.

Data analyses. Statistical procedures for data analyses included a nonparametric t-test in the initial work-up of the data, followed by a standard analysis of variance (ANOVA), and Dunnett's multiple comparison test if significant differences between more than two means were needed. The overall effectiveness of the transport media formulations in reducing the severity of physiological stress was evaluated by measuring the areas under the hyperglycemic and hypochloremic response curves with a planimeter and comparing them with those from fish challenged or test-hauled in untreated water.

Results and Discussion

Laboratory

The single and mixed species hauling stress challenge tests were conducted at fish loading densities of 0.5, 1.0, and 2.0 lb/gal--conditions typical of or higher than those commonly used during Columbia River smolt hauling operations. The hyperglycemia and hypochloremia occurring as a result of challenge tests at these fish loadings were adequate for testing purposes and previous work had shown that the protection obtained against such osmomorequlatory and other physiological dysfunctions could be taken as an index of the degree of stress mitigation afforded by transport media formulations (Wedemeyer et al. 1984).

Columbia River water chemistry information (Appendices 1 and 2) was the basis for selecting the particular transport media formulations to be tested. Water chemistry at MFS, the site of the laboratory testing was comparable to Columbia River conditions (Appendices 3 and 4). The candidate formulations selected for testing are listed in Table 1. The basis for selection was the need to favorably affect the ionic composition of the water in the hauling truck, and thus minimize ionorequlatory dysfunction, while also minimizing the potential for equipment corrosion problems.

Initial evaluations of $NaHCO_3$ (0, 250, 500 ppm), $CaCl_2$ (0, 50, 100 ppm), and KC1 (0, 100, 200 ppm) in protecting juvenile spring chinook during single species hauling stress challenge tests showed that the highest concentrations were not necessarily the most effective in mitigating the osmoregulatory and other physiological disturbances resulting from the stress challenge tests. For example, sodium bicarbonate at 250 ppm afforded a moderate degree of protection against the hyperglycemia accompanying the primary

Table 1. Candidate transport media formulations and concentrations (ppm) tested.

Individual Compounds	Combinations
CaC12	CaC12 + MgS04
50	50 + 200
100	140 + 150
	140 + 40
KC1	
50	$CaCl_2$ + $NaHCO_3$
100	25 + 250
200	
	MS-222 + $CaCl_2$
$MgSO_4$	10 + 50
100	25 + 50
200	
	MS-222 + $NaHCO_3$
MS-222	10 + 250
5	25 + 250
10	
25	MS-222 + CaC12 + $MgSO_4$
	10 + 50 + 200
NaCl	
500	MS-222 + CaC12 + $NaHCO_3$
	10 + 50 + 250
$NaHCO_3$	25 + 50 + 250
100	
250	
500	
Phenyl ethyl alcohol	
25	
50	
100	

stress response, whereas 500 ppm, provided only minimal benefit. Similarly, the 530 ppm level was no more beneficial than 250 ppm in protecting against the hypochloremia resulting from secondary osmoregulatory disturbances. In the case of $CaCl_2$, 50 ppm during a stress challenge was as protective as 100 ppm. In contrast, tests with KC1 revealed that100 ppm afforded no stress mitigation and that a concentration of 200 ppm actually increased the severity of the physiological disturbances. For completeness, we retested KC1 and $NaHCO_3$ at lower concentrations, but benefits were again minimal. Several additional formulations designed to enhance environmental conditions during Columbia River smolt hauling operations were then tested: NaCl (500 ppm), $MgSO_4$ (100 and 200 ppm), MS-222 (5, 10, and 25 ppm), CaC12 + $NaHCO_3$ (50 + 250 ppm), MS-222 + CaC12 (10 + 50 ppm), MS-222 + CaC12 (25 + 50 ppm), MS-222 + $NaHCO_3$ (25 + 250 ppm), and MS-222 + $CaCl_2$ + $MgSO_4$ (10 + 50 + 200 ppm). Phenyl ethyl alcohol was included because of its potential as both a transport medium and bacteriostatic agent. Because of previous work with MS-222 that had revealed a potential for adverse stimulation of metabolic rates (Piper et al. 1982), we conducted oxygen consumption tests were to rule out any potential for problems. Results from both single and mixed species tests (spring chinook salmon alone, and spring chinook salmon and steelhead in equal numbers) conducted at loading densities of 0.5, 1.0, and 2.0 lb/gal, showed that the increase in oxygen consumption by fish in MS-222 at anesthetic concentrations does not occur at the lower, tranquilizing, concentrations used in the hauling stress challenge tests. At 10 ppm, MS-222 slightly reduced oxygen consumption of spring chinook tested singly or in the presence of steelhead. For chinook salmon tested singly, typical hourly consumption rates were: 210 mq O_2/kg body weight per hour for fish in untreated water (controls), and 190 for fish in 10 ppm MS-222. For chinook in the presence of steelhead, typical results were: 210 mg O_2/kg/hr for controls, and 160 for fish in 10 ppm MS-222.

The effectiveness of the other formulations tested in reducing the severity of the stress response,as judged by success in reducing the area under the hyperglycemic response curve, ranged from a 5% reduction (100 ppm KCl) to as much as 62% (25 ppm MS-222 + 250 ppm $NaHCO_3$). A summary of the protection against hyperglycemia (an indirect measure of adrenaline production) is given in Table 2. The rank-order effectiveness of these transport media in mitigating the secondary osmoregulatory disturbances resulting from the hauling stress challenge tests (as judged by success in alleviating hypochloremia) is given in Table 3. As seen, the formulations that were most effective in alleviating the primary stress response were not necessarily effective in protecting against secondary osmoregulatory dysfunctions. Phenyl ethyl alcohol was completely ineffective and was not tested further. To evaluate the overall effectiveness of the transport formulations, the results from Tables 2 and 3 were combined, and a revised rank-order effectiveness list of the single species hauling stress challenge test results was prepared (Table 4). Benefit/cost ratios were also calculated based on the cost of treating 100 L of water. Although the top-rated compound was MS-222 at 25 ppm, the fourth ranked compound, MS-222 at 10 ppm, was almost as effective and had a higher benefit cost ratio. The second and third ranked formulations provided only marginally better stress mitigation than 10 ppm MS-222 and had the disadvantage of potential for causing equipment corrosion problems because of their mineral salt content. Consequently we selected 10 ppm MS-222 for

Table 2. Rank order effectiveness of transport media formulations in reducing the severity of the primary stress response during hauling of juvenile spring chinook salmon, as judged by success in alleviating hyperglycemia. Results given as percent decrease in area under the blood glucose response curve relative to control fish tested in untreated water.

Transport media formulation	Concentration (ppm)	Decrease in hyperglycemia relative to control fish (%)
MS-222 + $NaHCO_3$	25 + 250	62
MS-222	10	56
MS-222 + CaC12	10 + 50	50
MS-222	25	46
MS-222 + $CaCl_2$	25 + 50	41
$NaHCO_3$	2 50	23
$CaCl_2$	100	23
MS-222 + $CaCl_2$ + MgS04	10 + 50 + 200	22
CaC12	50	22
NaCl	500	22
$MgSO_4$	200	21
CaC12 + $NaHCO_3$	50 + 250	19
MS-222	5	17
KC1	100	5

Table 3. Rank order effectiveness of transport media formulations in reducing the severity of the secondary osmoregulatory disturbances accompanying hauling stress, as judged by success in alleviating hypochloremia. Results given as percent decrease in area under the blood chloride response curve relative to control fish tested in untreated water.

Transport media formulation	Concentration (ppm)	Decrease in hypochloremia relative to control fish (%)
MS-222	25	59
MS-222 + CaC12	10 + 50	46
MS-222 + CaC12 + $NaHCO_3$	10 + 50 + 250	45
MS-222 + $NaHCO_3$	25 + 250	42
MS-222	10	38
$NaHCO_3$	250	34
MS-222 + $CaCl_2$	25 + 250	31
MS-222 + NaHC03	10 + 250	22
MS-222 + CaC12 + MgS04	10 + 50 + 200	22
$CaCl_2$	50	22
CaC12	100	16
MS-222 + NaHC03 + CaC12	25 + 250 + 50	15
NaCl	500	9
$MgSO_4$	100	3

Table 4. Rank order effectiveness of candidate transport media formulations in reducing the overall severity of the stress response in juvenile spring chinook salmon.

Transport media formulation	Concentration (ppm)	Benefit-cost ratio	Decrease in overall stress response relative to controls[a] (%)
MS-222	25	0.6	53
MS-222 + $NaHCO_3$	25 + 250	0.5	52
MS-222 + $CaCl_2$	10 + 50	1.1	48
MS-222	10	1.1	47
MS-222 + $CaCl_2$	25 + 50	0.3	36
MS-222 + CaC12 + $NaHCO_3$	10 + 50 + 250	0.5	35
NaHC03	250	1.7	29
MS-222 + $CaCl_2$ + NaHC03	25 + 50 + 250	0.2	23
MS-222 + NaHC03	10 + 250	0.5	23
KC1	100	2.4	23
$CaCl_2$	50	1.0	22
MS-222 + $CaCl_2$ + MgS04	10 + 50 + 200	0.2	22
CaC12	100	0.4	20
NaCl	500	0.4	16
CaC12 + NaHC03	50 + 250	0.2	8

[a] Average of percent decrease in hyperglycemia (Table 2) and in hypochloremia (Table 3).

further testing.

Hauling stress challenge tests were next conducted to evaluate the protection afforded by10 ppm MS-222 to pre-stressed spring chinook hauled at loadings above the normally used 0.5 lb/gal. For comparative purposes, tests with steelhead trout and a formulation of 10 ppm MS-222 + 250 ppm NaHC03 were included. Tests were conducted at loadings of 0.5, 1.0, and 2.0 lbs/gal (termed standard, intermediate, and high). As expected, 10 ppm MS-222 substantiallly reduced (by 55%) the stress response of juvenile chinook challenged at 0.5 lb/gal, the usual hauling density (Table 5). At 1.0 and 2.0 lbs/gal, protection against stress was correspondingly reduced but was still evident. For the transport medium formulation of 250 ppm NaHC03 + 10 ppm MS-222 , the degree of stress mitigation was only about 33%, but protection remained fairly uniform at the higher loadings tested (1.0 and 2.0 lbs/gal).

In the case of steelhead, the results with 10 ppm MS-222 + 250 ppm NaHC03 were similar to those obtained with chinook salmon; a modest, but roughly comparable degree of protection at standard, intermediate, or high hauling densities. The results with MS-222 at10 ppm were not unlike those obtained for chinook.

The protection afforded by selected transport media during mixed species hauling was next evaluated. Mixed groups of chinook salmon and steelhead were held together in 4-foot circular tanks for 18 hours at a loading density of 0.05 lb/ft^3 or less prior to testing. The primary transport medium formulation selected for testing was 10 ppm MS-222. For comparison we conducted a few tests with 10 ppm MS-222 + 50 ppm CaC12, and with 50 ppm CaC12 alone.

The increase in the stress response of chinook salmon due to interspecific interactions with steelhead was evaluated as a function of steelhead size,

Table 5. Percent reduction in physiological stress response (hyperglycemia and hypochloremia) in pre-stressed spring chinook smolts during hauling stress challenge tests with 10 ppm MS-222 and 10 ppm MA-222+250 ppm $NaHCO_3$ at the indicated fish loadings. Results for steelhead trout given as a species comparison.

Fish species and transport media formulation	Fish loadings (lbs/gal): 0.5	1.0	2.0
Chinook salmon			
10 ppm MS-222	60	47	25
10 ppm MS-222 + 250 ppm $NaHCO_3$	33	29	27
Steelhead trout			
10 ppm MS-222	55	43	27
10 ppm MS-222 + 250 ppm $NaHCO_3$	14	20	24

and chinook:steelhead ratio, at fish loadings of 0.05 lb/gal.

In general, the tests showed that a mild increase in the physiological stress repsonse does occur in spring chinook when steelhead smolts are present (Table 6). For a 1:1 species ratio, the maximum increase was about 5 and 12% during a 6- and 24-hour holding period respectively. Surprisingly, the stress was not increased when the steelhead were larger rather than smaller than the chinook. However, the influence of steelhead numbers was more predictable. increasing the salmon to steelhead ratio from1 :1 to 1:2 resulted in an increase in the stress response of about 12 and 31% respectively over a 6- and 24-hour period.

A 6-hour haul and a 1:1 chinook - steelhead species ratio is fairly conservative in terms of Columbia River smolt hauling operations, and the 5-10% increase in the stress response that could be expected in spring chinook salmon mixed with steelhead is probably within physiological tolerance limits. Work by Congleton et al. (1984) on plasma cortisol concentrations in chinook salmon smolts resulting from mixed species holding (with steelhead) at Lower Granite and Little Goose dams, gave similar results. Although hypercortisolemia did occur in the chinook, it was judged to be relatively mild, and the conclusion was that no physiologically detrimental effects were likely because cortisol concentrations typically did not exceed 70 ng/ml. Thus the added stress on chinook salmon attributable to mixed species collection and holding at Columbia River dams, followed by a 6-hour mixed species hauling period can be expected to impose only a mild additional physiological load. The full consequences to ocean survival have not been determined, however, as discussed below, a seawater growth and survival assessment revealed no short-term adverse effects. Pending completion of tagging studies to evaluate actual ocean survival, it

Table 6. Increase in overall stress response of spring chinook salmon held with steelhead as a function of steelhead size and salmon:steelhead ratio. Results given as percent increase in hyperglycemia and hypochloremia relative to fish held singly. Chinook salmon, 23 g; small steelhead, 16 g; large steelhead 42 g; loading 0.05 lb/gal.

Species ratio and relative fish size	Holding period (hours)	
	6	24
1:1 Species Ratio		
Chinook + small steelhead	5	12
Chinook + large steelhead	0.5	3
1:2 Species Ratio		
Chinook + small steelhead	12	31
Chinook + large steelhead	not tested	not tested

would probably be prudent to continue to follow existing procedures for the collection, separation, holding, and transport of migrating spring chinook salmon and steelhead.

Tests of the effectiveness of selected transport media in mitigating the stress response during mixed species hauling challenge tests showed that, as in single species tests, 10 ppm MS-222 was the most effective at fish loadings of both 0.5 and 2.0 lbs/gal (Table 7). The protection afforded by 10 ppm MS-222 was also beneficial to the steelhead. The overall stress response was reduced by about 72% at 0.5 lb/gal and 15% at 2.0 lbs/gal.

Tests of the benefits of temperature decreases (hypothermia) and of reduced light intensity in mitigating hauling stress were also conducted using single or mixed species conditions at a loading rate of 1.0 lb/gal. Reducing the water temperature from ambient (10-12°C) to 4-5°C during the stress challenge tests reduced the severity of the primary stress response (hyperglycemia) in spring chinook salmon by about 30%. However, hypothermia offered no protection against the accompanying osmoregulatory disturbances (hypochloremia) which would limit its usefulness as a practical stress-mitigation technique for Columbia River fish transport operations.

The evaluation of the effect of light intensity showed that both hyperglycemia and hypochloremia were reduced in severity under darkened conditions as compared with bright lighting. Specifically, spring chinook challenged at 1.0 lb/gal in darkness showed a reduction in the overall stress response of about 25% as compared with fish challenged under a light intensity of 24 ft-candles. Thus, steps taken to reduce light intensity during all stages of Columbia River barging and trucking operations would be expected to be beneficial.

Table 7. Effectiveness of selected transport media in mitigating the stress response in juvenile spring chinook salmon during mixed species challenge tests. Chinook:steelhead ratio, 1:1; 6-hour haul, 18-hour recovery period. NT denotes not tested.

Fish loading rate and transport media formulation	Percent decrease in: Hyperglycemia	Percent decrease in: Hypochloremia	Decrease in overall stress response (%)
0.5 lbs/gal			
10 ppm MS-222	55	31	43
10 ppm MS-222 + 50 ppm CaC12	47	27	37
50 ppm $CaCl_2$	60	0	30
2.0 lbs/gal			
10 ppm MS-222	60	38	49
10 ppm MS-222 + 50 ppm CaC12	NT	NT	NT
50 ppm $CaCl_2$	13	11	12

Field Evaluations--Single and Mixed Species

Test hauls conducted with pre-stressed hatchery spring chinook smolts revealed several drawbacks to the use of 25 ppm MS-222 as a transport medium that were not apparent in the tests conducted under laboratory conditions. Columbia River hatchery spring chinook pre-stressed before transport by collection and holding procedures at the hatchery (designed to approximate those at the dams) could not maintain their equilibrium during hauling, and sank to the bottom of the hauling tanks. This would create a potential for problems with recirculating pump intakes in hauling trucks using this type of equipment. Although gill ventilation seemingy was not affected, the recovery of swimming ability required about10 minutes after the smolts were stocked from the truck into fresh water. Although this is a relatively short time period, even this small increase in susceptibility to predation would probably be undesirable. In addition, blood chemistry analyses revealed that these fish developed a significant (p = 0.05) hyperglycemia and undesirable secondary osmoregulatory changes (hypochloremia). Activation of the pituitary-interrenal axis was indicated by the fact that average values for cortisol, which initially averaged about 53 $\pm$ 13 ng/ml ($\bar{X} \pm$ SD)--well within the estimated normal range for this species--increased to 223 + 14 ng/ml after an 18-hour pre-stress, followed by loading into the hauling truck. After a 6-hour test haul to MFS, the cortisol levels had decreased substantially but were still moderately higher in the smolts hauled in 25 ppm MS-222: 129 $\pm$ 15 ng/ml, compared with 100 $\pm$ 27 ng/ml in the controls. As would be expected from the hypercortisolemia, the hyperglycemia in the post-transport smolts was also substantial--blood glucose values averaged 201 mg/100 ml in both groups compared with 79 mg/100 ml before the haul.

Although smolt survival during transport was marginally better in the groups hauled in 25 ppm MS-222 (Table 8), the difference was not significant ($p = 0.05$). There was no delayed (post-hauling) mortality during a 5-day freshwater holding period in either test or control groups. There were also no benefits of transport in 25 ppm MS-222 to seawater tolerance, growth, or survival (Table 9). The survival of the test and control groups during a 48-hour acclimation to full-strength seawater, and their survival, growth, and development during a 30-day holding period at a salinity of 28°/oo was not significantly different. During the second month in sea water, an idopathic mortality began in both groups and by the fourth month, it was significantly higher in the test fish than in the controls. No pathogens, such as _Vibrio_ or _Aeromonas salmonicida_ could be isolated. Thus the survival rate during the first month in sea water is probably the most indicative of what the actual ocean survival could be expected to be. Based on this, it appears that the use of 25 ppm MS-222 as a transport medium for juvenile spring chinook salmon offers no substantive benefits in terms of decreased hauling loss or increased early marine survival and growth.

As the next step in the field trials, an evaluation of 10 ppm MS-222 as a transport medium was conducted during routine Corps of Engineers (COE) Columbia River trucking operations. Because of low run sizes, permission to work with migrating spring chinook smolts could not be obtained and trials with fall chinook were substituted.

The muscle adenylate energy charge (AEC) was chosen as a measure of stress mitigation achieved because the small size of the fall chinook made blood sampling and analyses difficult. The AEC--defined as ATP + 0.5 ADP/(ATP + ADP + AMP)--can be used as an index of the amount of energy that is metabolically available to an organism in the form of adenine nucleotides, and thus as an

Table 8. Effectiveness of 25 ppm MS-222 in reducing immediate and delayed mortality in spring chinook smolts hauled from Winthrop NFH and held for five days in fresh water at MFS. The hauling mortality was marginally reduced by the use of 25 ppm MS-222 but the difference was not significant ($p = 0.05$).

Treatment group	Hauling loss (%) Immediate	Hauling loss (%) Delayed (5 days)
Hauled in MS-222	0.2	0
Hauled in untreated water	0.3	0

Table 9. Seawater growth and survival of spring chinook salmon smolts following transport at 0.5 lbs/gal in 25 ppm MS-222 (test), or untreated water (control). (*) indicates significant difference from controls (p = 0.05).

Months in seawater	Group	Growth: Monthly weight ($\bar{X} \pm$ SD)	Growth: Cumulative weight gain (%)	Mortality: Monthly (%)	Mortality: Cumulative (%)
1	Test	35.1 ± 12.0	14.0	1.5	1.5
	Control	38.0 ± 11.4	15.8	1.5	1.5
2	Test	39.0 ± 15.4	26.6	17.8	19.0
	Control	44.0 ± 13.2	34.1	9.6	11.0
3	Test	46.8 ± 20.2	51.9	24.7	39.0
	Control	50.7 ± 16.5	54.5	23.0	31.5
4	Test	49.6 ± 22.9	61.0	17.5	53.0*
	Control	53.0 ± 19.7	61.6	11.7	39.5

index of energy drains due to environmental stress (Atkinson 1968, 1977; Reinert and Hohreiter 1984).

By its definition, the AEC may range in value from 0 to 1. Values of 0.8 to 0.9 are considered favorable and are associated with non-limiting, non-stressful environmental conditions (Chapman et al. 1971; Ridge 1972; Ching et al. 1975; Wijsman 1976). AEC values between 0.5 and 0.7 have been measured in many organisms under stressful or limiting environmental conditions. Examples among fishes are gulf killifish, Fundulus grandis, (MacFarlane 1981), Florida pompano, Trachinotus carolinus, (Vetter and Hodson, 1982), common carp, Cyprinus carpio (Dreidzic and Hochachka, 1976) goldfish, Carassius auratus (Van den Thillart et al. 1980), and the flounder Platichthys flesus (Jorgensen and Mustaf a, 1980). AEC values of less than 0.5 are considered unfavorable and are associated with severely limiting environmental conditions and life-threatening stress (Ridge 1972; Ivanovici 1974, 1979).

The AEC results from the test haul of fall chinook salmon in 10 ppm MS-222 from McNary to the normal release site below Bonneville are presented in Table 10. No differences could be detected in the AEC or in the concentrations of ATP, ADP, or total adenylates between either the test or control groups. The favorable AEC, and high ATP and total adenylate concentrations measured both before and after transport are indicative of a lightly stressed organism. The AMP concentration did decline significantly in both test and control groups after transport but there was no significant between-group difference ($p = 0.05$). Thus, it appears that the presently used COE trucking procedures for fall salmon chinook at a nominal fish loading of 0.5 lb/gal do not impose a stress severe enough to be detected as shifts in white muscle tissue adenylate concentrations.

Table 10. Effect of hauling fall chinook in 10 ppm MS-222, on muscle adenylates and energy charge during COE trucking, McNary to Bonneville: 0.5 lb/gal. Post-transport muscle AMP levels were significantly decreased ($p = 0.05$) in both test and control groups but there were no benefits to muscle ATP, ADP or AEC levels. Values are given as $\bar{X} \pm SD$.

Test group	ATP	ADP	AMP	Total	AEC
control, pre-transport	6.6 ± 0.45	0.71 ± 0.20	0.29 ± 0.17	7.5 ± 0.50	0.92 ± 0.02
control, post-transport	6.6 + 0.72	0.69 ± 0.16	0.15 + 0.10•	7.5 ± 0.67	0.93 ± 0.02
test, post-transport	6.3 + 0.84	0.70 ± 0.09	0.10 ± 0.08*	7.1 ± 0.83	0.93 ± 0.02

*Significantly less than control, pre-transport ($p = 0.05$).

It is possible that the adenylate pool is too fluid and transient to be a useful parameter in measuring the degree of stress associated with fish hauling operations. Monitoring liver or muscle glycogen levels concurrently with adenylates would probably give a more nearly complete picture of energy reserves.

A second group of migrating fall chinook was collected at McNary Dam and hauled to MFS in a transport medium of 10 ppm MS-222 for a seawater survival and growth assessment. As shown in Table 11, if only the immediate benefits to survival during hauling are considered, no differences occurred. However, there was a delayed mortality during the 14-day freshwater holding period of about 42% in fall chinook hauled in untreated Columbia River water as compared with only 14% in those hauled in 10 ppm MS-222. The seawater growth and survival evaluation showed that during the first month, some benefits accrued to smolts transported in 10 ppm MS-222 (Table 12). Although the differences were only significant at the 90% level, a small increase in growth and survival occurred. During the second through fourth months, mortalities of unknown etiology became extensive in both test and control groups obscuring further results. Growth of surviving smolts, however, still remained better in the test groups hauled in 10 ppm MS-222 and this benefit persisted for the full 4-month period (Table 12). As occurred in the previously discussed seawater trials with hatchery spring chinook hauled in 25 ppm MS-222, the unexpectedly low seawater survival in the fall chinook groups was apparently unrelated to the test or control treatments.

For the field evaluations of the effectiveness of MS-222 for stress mitigation during mixed species hauling, spring chinook and steelhead smolts were transported from the Columbia River Eagle Creek NFH to MFS using a loading rate of 0.5 lb/gal and a species ratio of 1:1. The benefit to spring

Table 11. Effectiveness of 10 ppm MS-222 in reducing immediate and delayed mortality in fall chinook salmon transported from McNary Dam to MFS and held for 14 days in freshwater. A significant ($p = 0.05$) delayed mortality of 41.8% occurred in chinook hauled in untreated Columbia River water.

Conditions	Mortality rates (%) of fish hauled in: 10ppm MS-222	Untreated water
Test haul	0.0	0.0
Holding period (days)		
2	7.2	32.0
3	0.9	0.0
7	1.3	0.6
8	0.9	0.6
9	0.0	0.0
10	2.2	1.2
11	0.5	2.4
14	1.4	5.0
Total	14.4%	41.8%*

*Denotes significant difference ($p = 0.05$) from test fish.

Table 12. Effect of hauling in 10 ppm MS-222 on the seawater survival of McNary fall chinook salmon held 4 months at MFS.

Months in Seawater	Group	Growth: Monthly weight (g: $\bar{X} \pm$ SD)	Growth: Cumulative weight gain (%)	Mortality: Cumulative (%)
1	Test	13.3 ± 3.4	7.2	2.0
	Control	13.2 ± 2.7	6.4	4.5
2	Test	14.6 ± 4.1	17.7	40.0
	Control	14.0 ± 3.6	12.9	21.5
3	Test	18.0 ± 6.0	45.2	63.0
	Control	18.4 ± 4.8	48.4	58.0
4	Test	23.7 ± 7.6*	91.1*	83.5
	Control	20.3 ± 5.0	63.8	72.5

* Significantly different from controls (P=0.05)

chinook of hauling in 10 ppm MS-222 was primarily in reducing the delayed hauling mortality (Table 13). The mortality rate during the 6-hour haul itself was low and equal in both test and control groups (0.80). However, a delayed mortality of 15.3% occurred during the 14-day freshwater holding period in the smolts hauled in untreated Columbia River water as compared to only 6% with 10 ppm MS-222 as a transport medium.

The physiological test results showed that a substantial hypercortisolemia occurred during the 18-hour mixed species holding period in the raceways. However, it appeared to be slightly moderated by the use of MS-222 during hauling, and recovery to baseline levels occurred in both test and control groups within 24-hours after transport (Table 14). The hyperglycemia pattern was generally similar to the cortisol results except that recovery from hauling required about 48-hours (Table 15). Again, the blood sugar disturbances may have been mitigated somewhat in the spring chinook hauled in MS-222. The blood chloride analyses showed that only a moderate hypochloremia developed in both the test and control groups. Again, full recovery required about 48 hours (Table 15).

Mortality rates during the 4-month holding period in seawater were undesirably high, but seawater survival seemed to be slightly, but again not significantly better in the groups hauled in 10 ppm MS-222 (Table 16). Growth of surviving fish tended to be somewhat slower in the control groups, but again, the difference was not significant. Thus, although the use of 10 ppm MS-222 during mixed species hauling at 0.5 lb/gal had a beneficial effect on the delayed mortality in freshwater (14 days), any benefits to seawater growth and survival were minimal.

Additional field trials of 10 ppm MS-222 were conducted during COE

Table 13. Effectiveness of 10 ppm MS-222 in reduing immediate and delayed mortality (%) in spring chinook smolts hauled with steelhead at 0.5 lb/gal and held for 14 days in freshwater.

Conditions	Mortality (%) of fish hauled in: 1 Oppm MS-222	Untreated water
Six-hour haul	0.8	0.8
Holding period (days)		
2	0.8	0.8
3	1.2	1.6
5	1.6	2.5
6	0.0	0.8
7	0.0	0.9
8	0.4	1.7
9	0.0	2.2
12	0.8	1.8
14	0.4	2.2
Total mortality	6.0%	15.3%*

* Significantly different from test fish ($p = 0.05$).

Table 14. Effectiveness of 10 ppm MS-222 in mitigating hypercortisolemia in spring chinook salmon smolts during and after mixed species hauling, (0.5 lb/gal; chinook-steelhead, ratio, 1:1; Eagle Creek NFH to MFS; 10 hours). Values given as $\overline{X}$ + SD.

Conditions	Test (ng/ml)	Control (ng/ml)
Baseline	39 ± 21	39 ± 21
After 18-hour pre-stress	174 ± 47*	174 ± 47*
Days after transport	133 ± 60*	149 ± 56*
1	36 ± 35	36 ± 21
2	47 ± 28	29 ± 16
5	25 ± 21	38 ± 31
7	34 ± 34	75 ± 103
9	16 + 31	12 ± 11

*Denotes significant difference from baseline values ($p = 0.05$); no differences between test and control groups were significant.

Table 15. Effectiveness of 10 ppm MS-222 in mitigating hyperglycemia and hypochloremia in spring chinook smolts during mixed species hauling, 0.5 lb/gal; chinook-steelhead ratio, 1 :1 ; Eagle Creek NFH to MFS, lo-hours. There were no significant differences between test and control groups.

	Blood glucose (mg/100 ml)		Blood chloride (meq/100 ml)	
Conditions	Test ($\bar{X}$ ± SD)	Control ($\bar{X}$ ± SD)	Test ($\bar{X}$ ± SD)	Control ($\bar{X}$ ± SD)
Baseline	71 + 4	n + 14	122 ± 2	122 ± 2
After 18-hour pre-stress	123 ± 16	123 + 16	112 ± 5	112 ± 5
Days after transport	122 + 54	166 + 56	107 + 15	105 ± 6
1	137 + 65	123 ± 35	112 ± 10	114 ± 5
2	90 T 22	87 ± 31	125 ± 4	124 ± 3
5	61 ± 12	62 ± 12	126 ± 3	126 ± 2
7	54 ± 6	57 ± 13	126 ± 8	127 ± 2
9	61 ± 9	57 + 13	129 ± 3	129 ± 3

Table 16. Seawater growth and survival of spring chinook smolts following mixed species hauling at 0.5 lbs/gal in 10 ppm MS-222 (test) or untreated water (control). Chinook/steelhead ratio, 1:1, Eagle Creek NFH to MFS, 10 hours. There were no significant differences between test and control groups.

		Growth		Mortality	
Month	Group	Mean weight (g)	Cumulative weight gain (%)	Monthly (%)	Cumulative (%)
0	Test	47.4	--	--	--
	Control	53.6	--	--	--
1	Test	not measured		3	3
	Control	not measured		2	2
2	Test	70.2	48.1	24	26
	Control	80.8	50.7	11	13
3	Test	75.3	58.9	15	37
	Control	83.7	56.2	14	25
4	Test	87.0	83.5	15	46
	Control	92.0	71.6	9	32

trucking of Columbia River fall chinook from McNary Dam to Bonneville and by trucking in U.S. Fish and Wildlife Service (FWS) equipment from McNary Dam to MFS for seawater growth and survival evaluations.

In contrast to the situation in the earlier trials, blood samples were taken from about 50 fall chinook salmon that had been collected and held in a raceway at McNary Dam for about 48-hours, before they were hauled. The smolts were loaded at a nominal 0.5 lb/gal in both the FWS and COE hauling trucks. Upon arrival at the normal release site below Bonneville Dam (COE) or at MFS, post-transport blood samples were again taken for cortisol analysis. The results showed that MS-222 had no beneficial effects in mitigating hypercortisolemia in fall chinook salmon and, indeed, may have stimulated cortisol production somewhat (Table 17). This phenomenon was not apparant during the longer (lo-hour) test haul from McNary Dam to MFS.

The immediate and delayed mortality occurring as a result of hauling from McNary to MFS and holding the fall chinook for 14 days in freshwater is illustrated in Table 18. Data from a similar haul of hatchery spring chinook are included for a species comparison. The major benefit to fall chinook of transport in 10 ppm MS-222 was a significant reduction in immediate mortality; 7.4% compared with 29% in untreated water. The delayed mortality was the same in test and control groups of fall chinook salmon but was significantly reduced in the spring chinook hauled with MS-222. Most of the delayed mortality occurred within the first 24 hours, but it continued at a low rate during the entire two-week period prior to the time of sea water conversion. In the case of the hatchery spring chinook, most of the mortality was delayed rather than immediate but again, 10 ppm MS-222 gave significant protection ($p = 0.05$).

To initiate the four-month seawater growth and survival evaluations,

Table 17. Effect of transport in 10 ppm MS-222 on plasma cortisol of migrating fall chinook salmon collected at McNary Dam and trucked in COE equipment at a nominal 0.5 lb/gal to the usual release site below Bonneville or to MFS for seawater tolerance testing.

	Plasma cortisol (ng/ml; $\bar{X} \pm SD$)		
Trucking conditions	Pre-loading (raceway)	Post-loading (hauling truck)	Post-transport (release site)
McNary to Bonneville (4 hrs)			
Test	123 ± 58	186 ± 59*	202 ± 44*
Control	123 ± 58	167 ± 41	169 ± 65
McNary to MFS (10 hrs)			
Test	126 ± 28	198 ± 33*	242 ± 43*
Control	126 ± 28	179 ± 29*	262 ± 48*

*Denotes significant difference from pre-loading (raceway) values ($p = 0.05$).

Table 18. Effects of hauling in 10 ppm MS-222 on immediate and delayed mortality in fall chinook test-hauled from McNary Dam to MFS (10 hrs) and held for 14 days in freshwater. Data for a similar test haul of spring chinook are included as a species comparison. MS-222 significantly reduced hauling mortality.

Species and Origin	Mortality (%) in fish hauled in: Untreated water	Mortality (%) in fish hauled in: 10 ppm MS-222
Columbia River Fall chinook		
Immediate (10 hours)	29.0	7.4*
Delayed (14 days)	7.4	7.4
Hatchery Spring chinook		
Immediate (10 hours)	0.9	0.9
Delayed (14 days)	23.1	8.8*

*Indicates significant difference from fish hauled in untreated water (p = 0.05).

test and control groups were stressed by being anesthetized, weighed, and measured. The salinity was increased to 28°/oo over a 48-hour period, and the fish held in full-strength seawater for four months as before. The effects of hauling in 10 ppm MS-222 on the seawater growth and survival of migrating fall chinook collected at McNary Dam are summarized in Table 19. There were no significant benefits to either the growth or survival of test or control fish during the first month in seawater. An unexplained mortality that began in both groups during the second month obscured further results.

Table 19. Effects of hauling in 10 ppm MS-222 or untreated water on seawater growth and survival of fall chinook salmon; McNary Dam to MFS (10 hr), loading of 0.5 lb/gal.

Month	Group	Growth: Monthly weight (g) ($\bar{X} \pm$ SD)	Growth: Cumulative weight gain (%)	Mortality: Monthly (%)	Mortality: Cumulative (%)
0	Control	7.4 ± 3.8	--	0	0
	Test	6.7 ± 2.9	--	0	0
1	Control	10.0 ± 4.5	34.9	10	10
	Test	9.3 ± 3.2	38.8	20	20
2	Control	11.7 ± 5.0	58.7	46	51
	Test	9.4 ± 3.6	89.8	25	40
3	Control	15.3 ± 9.9	106.2	80	90
	Test	10.3 ± 4.0	53.1	92	95
4	Control	24.9 ± 19.0	236.5	80	98
	Test	11.8 ± 2.3	76.2	60	98

Benefits and Consequences: The Problem of Equipment Corrosion

The biological benefits of transport media containing dissolved substances such as MS-222 or electrolytes in mitigating stress and improving smolt survival must be balanced against the undesirable consequences of increased potential for fish handling and hauling equipment corrosion problems. However, predicting the actual likelihood that corrosion problems would probably result from the use of the transport media tested in the present study is difficult because of the many variables that affect the susceptibility of metals used in the hauling trucks and barges. For example, adding oxygen to water containing electrolytes accelerates corrosion in direct proportion to the dissolved oxygen concentration. Iron does not rust in water without dissolved oxygen, nor in air without water vapor present. Maintaining the dissolved oxygen concentration near saturation is a biological requirement of fish hauling, but in practice the percent saturation of oxygen may fluctuate substantially. Temperature and pressure would be expected to play only a small role in trucking equipment corrosion but the occurrence of stresses and strains in the metal of the hauling tanks may well be a significant factor.

Dissolved electrolytes in hauling tank water will especially tend to accelerate corrosion where dissimilar metals are in contact with each other. This would be the case with pump and refrigeration fittings made with ferrous or non-ferrous metals which are in contact with the stainless steel usually used in fish hauling tanks.

Factors influencing the potential of the transport media tested for accelerating the corrosion of fish hauling equipment can thus be divided into primary and secondary effects. Primary factors are those inherent in the physical and chemical properties of the metals used in the equipment and control measures would only be feasible during the design stage. Chief among these is the electrode potential. When dissimilar metals used in fish hauling trucks are wetted by transport media containing electrolytes such as Na+ and Cl-, the metal with the lowest electrode potential (in the electrochemical series) corrodes as a result of the voltage developed by any other metal with a higher electrode potential. The secondary factors are enviromental in nature and include the kind and ionic strength of any transport media added, the pH, dissolved oxygen concentration, water temperature, flow rate (if the hauling tank water is circulated), and any stresses and strains in the metals resulting from the use of the equipment. Since many of the secondary factors are a function of the biological requirements of fish hauling, and can be modified only within certain limits, close control at the equipment design stage would seem to offer the most viable alternative for corrosion prevention.

Conclusions

We tested many transport media formulations in this study, most of which were combinations of mineral salts that would tend to promote corrosion. However, the results of the laboratory and field evaluations indicated10 ppm MS-222 to be the most effective of the transport formulations in reducing stress and improving the survival of juvenile spring chinook salmon during single or mixed species hauling. Transport media formulations containing $NaHCO_3$ or CaC12 were also promising, but the biological benefits of their regular use would probably be outweighed by the possibility of corrosion. Insofar as can be determined, MS-222 used alone will not contribute to equipment corrosion problems. Since a transport medium consisting of 10 ppm MS-222 reduced fish activity levels (potential for scale loss), did not stimulate oxygen consumption, and was effective in reducing stress, it is recommended as the alternative of choice for Columbia River spring chinook transport operations whether or not equipment corrosion problems continue to be seen as a major constraint.

Acknowledgments

The assistance of the U.S. Army Corps of Engineers (COE) was very valuable and greatly appreciated in both the initial study design, and final field evaluation stages of this project. Their cooperation during their smolt hauling operations from McNary Dam is particularly appreciated. All COE personnel involved in the project were very helpful, but we especially thank Mr. Jim Athern, Walla Walla District for his help with the field testing and Dr. Doug Arndt, Portland District for this help during the experimental design stage.

Ms. Theresa Barilla, our initial BPA project officer, provided very valuable assistance in getting the project underway, and her successors Mr. J. Dugoni and Ms. Kathleen J. Anderson also deserve thanks for their assistance in resolving problems, reviews of reports, and general helpful attitude. Mr. Don Park, National Marine Fisheries Service, also provided helpful advice at several stages of the project.

Finally, we thank Dr. Rowan Gould, Mr. Carl Christenson, and Mr. Lee Burger (former project biologists), and Mr. S. Smith Smith, biologist-in-charge of the Marrowstone Island Field Station.

References

Atkinson, D.E. 1968. Citrate and the citrate cycle in the regulation of energy metabolism. Biochem. Soc. Symp. 27:23-40.

Atkinson, D.E. 1977. Cellular Energy Metabolism and its Regulation. Academic Press, New York. 293 pp.

Carmichael, G., G. Wedemeyer, J. McCrearen, and M. Millard. 1983. Physiological effects of hauling and handling stress on smallmouth bass (Micropterus dolomieui). Prog. Fish-Cult. 45:110-113.

Chapman, A.G., L. Fall, and D.E. Atkinson. 1971. Adenylate energy charge in Escherichia coli during growth and starvation. J. Bacteriol. 108:1072-1086.

Ching, T.M., S. Hedtke, S.A. Russell, and H.J. Evans. 1975. Energy state and dinitrogen fixation in soybean nodules of dark-grown plants. Plant Physiol. 55:796-798.

Congleton, J.L., T.C. Bjornn, C.A. Robertson, J.L. Irving, and R.R. Ringe. 1984. Evaluating the effects of stress on the viability of chinook salmon smolts transported from the Snake River to the Columbia River estuary. Tech. Rep. 84-4, Idaho Coop. Fish. Res. Unit, University of Idaho, Moscow.

Dreidzic, W.R., and P.W. Hochachka. 1976. Control of energy metabolism in fish white muscle. Am. J. Physiol. 230:579-580.

Faupel, R.P., H.J. Seitz, W. Tarnowski, V. Thiemann, and C.H. Weiss. 1972. The problem of tissue sampling from experimental animals with respect to freezing technique, anoxia, stress and narcosis. Arch. Biochem. Biophys. 148:509-522.

Haswell, M.S., G.J. Thorpe, L.E. Harris, T.C. Mandis, and R.E. Rauch. 1982. Millimolar quantities of sodium salts used as prophylaxis during fish hauling. Prog. Fish-Cult. 44:179-183.

Hattingh, J., F. Fourie, and J.H.J. Van Vuren. 1975. The transport of freshwater fish. J. Fish Biol. 7:447-449.

Holm-Hansen, O., and D.M. Karl. 1978. Biomass and adenylate energy charge determination in microbial cell extracts and environmental samples. Methods Enzym. 57:73-85.

Ivanovici, A.M. 1974. Pyrazus ebeninus, responses to salinity and measurement of stress. Aust. Mar. Sci. Bull. 47:10.

Ivanovici, A.M. 1979. Adenylate energy charge: potential value as a tool for rapid determination of toxicity effects. Pages 241-255 in Proc. Fifth Annual Aquat. Toxicol. Workshop, Hamilton, Ontario. November 7-9, 1978. Fish. Mar. Serv. Can. Tech. Rep. 862, pp. 241 -255.

Ivanovici, A.M. 1980. Adenylate energy charge: an evaluation of applicability to assessment of pollution effects and directions for future research. Rapports et Proces-verbaux des Reunions, Conseil International Pour l'Exploration de la Mer 179:23-28.

Jorgensen, J.B., and T. Mustafa. 1980. The effect of hypoxia on carbohydrate metabolism in flounder (Platichthys flesus L.)--11. High energy phosphate compounds and the role of glycolytic and gluconeogenic enzymes. Comp. Biochem. Physiol. 67B:249-256.

Long, C.W., J.R. McComas, and B.H. Monk. 1977. Use of salt (NaCl) water to reduce mortality of chinook salmon smolts (Oncorhynchus tshawytscha) during handling and hauling. Mar. Fish. Rev. 39(7):6-9.

Macfarlane, R.B. 1981. Alterations in adenine nucleotide metabolism in the Gulf killifish (Fundulus grandis) induced by low pH water. Comp. Biochem. Physiol. 68B:193-202.

McFarland, W., and K. Norris. 1958. The control of pH by buffers in fish transport. Calif. Fish Game 44:291-310.

Nikinmaa, M., A. Soivio, T. Nakari, and S. Lindgren. 1983. Hauling stress in brown trout (Salmo trutta): physiological responses to transport in fresh water or salt water, and recovery in natural brackish water. Aquaculture 34:93-99.

Park, D.L. 1980. Transportation of chinook salmon and steelhead smolts 1968-80 and its impact on adult returns to the Snake River. National Marine Fisheries Service, Processed Report. Northwest and Alaska Fisheries Center, Seattle, Washington.

Park, D.L., G.M. Matthews, J.R. Smith, T.E. Ruehle, J.R. Harmon, and S. Achord. 1984. Evaluation of transportation of juvenile salmonids and related research on the Columbia and Snake rivers, 1983. National Marine Fisheries Service, Processed Report, Northwest and Alaska Fisheries Center, Seattle, Washington.

Pickering, A.D. 1981. Introducton: the concept of biological stress. Pages l-9 in A. Pickering, ed. Stress and Fish. Academic Press, London.

Piper, R.G., I.B. McElwain, L. Oreme, J.P. McCrearen, L.G. Fouler, and J.R. Leonard. 1982. Transportation of live fishes. Pages 348-371 in Fish Hatchery Management. U.S. Dept. Interior, Fish Wildl. Serv., Washington, D.C.

Raymond, H., and C.W. Sims. 1980. Assessment of smolt outmigration and passage enhancement studies for 1979. Report to U.S. Army Corps of Engineers, National Marine Fisheries Service, Processed Report. Northwest and Alaska Fisheries Center, Seattle, Washington.

Reinert, R.E., and D.W. Hohreiter. 1984. Adenylate energy charge as a measure of stress in fish. Pages 151-162 in V. Cairns, P. Hodson, and J. Nriagu, eds. Contaminant Effects on Fisheries. John Wiley and Sons, Toronto, Canada.

Ridge, J.W. 1972. Hypoxia and the energy charge of the cerebral adenylate pool. Biochem. J. 127:351:355.

Schreck, C.D. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. Pages 295-317 in A. Pickering, ed. Stress and Fish. Academic Press, London.

Specker, J.L., and C.B. Schreck. 1980. Stress responses to transportation and fitness for marine survival in coho salmon (Oncorhynchus kisutch) smolts. Can. J. Fish. Aquat. Sci. 37:765-769.

Van den Thillart, G., F. Kesbeke, and A. van Waarde. 1980. Anaerobic energy-metabolism of goldfish, Carassius auratus (L.). Influence of hypoxia and anoxia on phosphorylated compounds and glycogen. J. Comp. Physiol. 136:45-52.

Vetter, R.D., and R.E. Hodson. 1982. Use of adenylate concentrations and adenylate energy charge as indicators of hypoxic stress in estuarine fish. Can. J. Fish. Aquat. Sci. 39(4):535-541.

Wedemeyer, G.A. 1972. Some physiological consequences of handling stress in the juvenile coho salmon (Oncorhynchus kisutch) and steelhead trout (Salmo gairdneri). J. Fish. Res. Board Can. 29:1780-1783.

Wedemeyer, G.A. 1976. Physiological response of juvenile coho salmon (<u>Oncorhynchus kisutch</u>) and rainbow trout (<u>Salmo gairdneri</u>) to handling and crowding stress in intensive fish culture. J. Fish. Res. Board Can. 33:2699-2702.

Wedemeyer, G.A., and E. Casillas. 1985. Physiological consequences of scale loss in coho salmon smolts. (In preparation).

Wedemeyer, G.A., and D.J. McLeay. 1981. Methods for determining the tolerance of fishes to environmental stressors. Pages 247-275 <u>in</u> A. Pickering, ed. Stress and Fish. Academic Press, London, England.

Wedemeyer, G.A., D.J. McLeay, and C.P. Goodyear. 1984. Assessing the tolerance of fish and fish populations to environmental stress: the problems and methods of monitoring. Pages 164-195 <u>in</u> V. Cairns, P. Hodson, and J. Nriagu, eds. Contaminant Effects on Fisheries. Wiley and Sons, Toronto, Canada.

Wijsman, T.C.M. 1976. Adenosine phosphates and energy charge in different tissues of <u>Mytilus edulis</u> under aerobic and anaerobic conditions. J. Comp. Physiol. 107:129-140.

Appendix 1. Annual average values (ppm unless otherwise indicated) for water chemistry characteristics in the Columbia River below McNary Dam at Umatilla, Oregon, 1975-1980 (J. Sainsbury, Environmental Protection Agency, personal communication).

Characteristic	Year					
	1975	1976	1977	1978	1979	1980
Conductivity (umho)	172.2	160.7	184.1	161.7	176.4	156.8
Calcium	19.0	20.0	23.0	17.0	18.5	--
Magnesium	5.5	4.1	5.9	4.8	4.8	--
Sodium	19.0	4.9	7.5	5.95	5.5	--
Potassium	1.7	1.2	1.4	1.2	0.9	--
Chloride	4.0	2.7	3.6	3.5	3.0	--
Sulfate	17.0	13.7	16.8	12.8	14.5	--
Bicarbonate	95.0	76.8	84.2	71.8	70.0	--
Dissolved oxygen	10.4	12.0	11.3	11.3	10.8	11.1
Alkalinity	78.0	63.0	59.0	58.8	57.5	--

Appendix 2. Expected seasonal averages (May-August) for Columbia River water chemistry characteristics at McNary Dam. (A. Novotny, National Marine Fisheries Service, personal communication).

Characteristic	Expected seasonal average (ppm)
Conductance (umho)	152
Calcium	18
Magnesium	4.9
Phosphate	0.07
Sodium	2.8
Dissolved oxygen	11.0
Alkalinity	53.5
Hardness (as $CaCO_3$)	59.5
pH (units)	8.1

Appendix 3. Physicochemical characteristics of water used in the hauling stress challenge tests at Marrowstone Field Station, 1982-1983. Values given as ppm unless otherwise noted (U.S. Fish and Wildlife Service, unpublished data).

Characteristic	Year	
	1982	1983
Alkalinity	--	74.3
Conductivity (umhos)	238	78
Hardness (as $CaCO_3$)	99.3	48
pH (units)	8.12	8.08
Temperature (°C)	15.8	8.5
Ammonia-N	--	0.037
Nitrite-N	--	<0.002
Cadmium	<0.0006	CO.0006
Calcium	20.4	13.0
Copper	0.0025	0.0017
Iron	<0.06	<0.06
Lead	<0.005	<0.005
Magnesium	11.9	3.8
Manganese	0.002	0.001
Potassium	1.9	0.39
Sodium	24.0	3.8
Zinc	0.01	0.015

Appendix 4. Yearly average physicochemical characteristics of water used during freshwater holding and seawater growth and survival tests at Marrowstone Field Station, 1982-83 (U.S. Fish and Wildlife Service, unpublished data).

Characteristic and unit	Fresh water	Sea water
Temperature (^{o}C)	12.0	11.5
pH	8.2	7.9
Dissolved oxygen (ppm)	10.0	8.8
Supersaturation* (%)	102	103
Salinity ($^{o}/oo$)	--	28.5
Conductance (μmho)	150	--
Hardness (ppm as $CaCO_3$)	90	--

*Total dissolved gas (% saturation)